DEVELOPMENT OF NATIVE SPECIES OF CRETE IN THE URBAN LANDSCAPE: AN INVESTIGATION OF SYSTEMS AND PLANT REPRODUCTION, ADAPTABILITY AND FITNESS OF WILD SPECIES IN COMMERCIAL HORTICULTURE

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DEDICATION

To

My husband Nick

And

My granddaughter Anna

ABSTRACT

The flora of Crete in its multifarious micro-landscapes offers the possibility of selecting native species with high ornamental value for use in urban landscapes and commercial horticulture. To assess this potential eight species were selected: *Staehelina petiolata, Ptilostemon chamaepeuce, Otanthus maritimus, Prasium majus, Convolvulus dorycnium, Campanula pelviformis, Sternbergia sicula and Colchicum macrophyllum.* The biological cycle *in situ*, sexual and asexual propagation as well as their cultural requirements and adaptability in urban landscape were studied.

In addition to identifying the light and temperature requirements for seed germination of the species, other factors were important including after-ripening for *P. chamaepeuce* and *S. petiolata*, scarification for *P. majus* and *C. dorycnium* and imbibition in 20°C for *C. macrophyllum* and *S. sicula.* Propagation by stem cuttings was influenced by mist, addition of IBA, the season and the substrates. IBA solutions at 4000, 2000 ppm and 2000 ppm in powder promoted on rooting of *S. petiolata, P. chamaepeuce and P. majus* stem tip cuttings with the highest rooting level of 50, 70 and 72%, respectively; while both IBA at 0 or 2000 ppm on *Otanthus maritimus* produced 100% rooting. Best substrates were peat-moss+vermiculite, vermiculite, sand and vermiculite and best seasons autumn, spring or late autumn, early summer, and spring for *Staehelina, Ptilostemon, Otanthus and Prasium*, respectively. Cross-cutting and BA on the base of *Sternbergia sicula* increased the bulblets from 1-3.5 to 12.8 and 13.5 per bulb yearly, respectively. *Colchicum macrophyllum* had a very low asexual reproductive activity producing one corm per year. The wounding of the replacement bud stimulated development of the reserve bud so that each corm could produce two or more cormlets. Morphogenesis was achieved *in vitro* by the affect of BA; callogenesis and rhizogenesis by NAA, and rhizogenesis by IBA on *S. petiolata and P. chamaepeuce* seedlings. Adventitious bulblets and plantlets formed on scales and clumps of shoots of *S. sicula* by the addition of BA and NAA producing over 20 bulblets per bulb. 2,4-D, BA, NAA and combinations produced callus, shoots and new corms on buds of *C. macrophyllum*.

All studied species were established successfully in pots and in an urban landscape. The ornamental characteristics and use of eight species was determined and a master plan was designed using indigenous plants of Crete.

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS

2,4-D	2,4-dichlorophenoxyacetic acid
2iP	6-(γ-γ-Dimethylalylamino)purine
ABA	abscisic acid
APG	Angiosperm Phylogeny Group
BA	6-benzylaminopurine
ca.	Circa
cv.	Cultivar
CW	coconut water
E	Endemic
EC	Electrical contactivity
GA_3	gibberillic acid
G	Gram
H	Hour
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
L	litre
LD	long day
M	metre
M	mole
mg	milligram
ml	millilitre
min	minute(s)
mS	milli-Siemens
MS	Murashige and Skoog medium
MSS	MS plus 3% sucrose at pH=5.8
ND	neutral day
NAA	α-naphthaleneacetic acid
°C	degree Celsius
pp.	page(s)
ppm	part per million
RBG	Royal Botanic Garden
R.H.	Relative Humidity
rpm	revolutions per minute
s	second(s)
sd	standard deviation
SE	somatic embryogenesis
SD	short day
spp.	species
T_{50}	time to germinate the 50% of the final germinated seeds
TDZ	thidiazuron
Tween 20	polyoxyethylene-sorbitan monolaurate
UV	ultra violet

v/v	volume in volume
W	watts
WL	Water loss
WPM	McCown's Woody Plant Medium
w/v	weight in volume
μM	micromole

GLOSSARY

Accrescent: increasing abnormally in size with age
Achene: A small, usually single-seeded, dry, fruit that stays closed at maturity.
Acute: Coming sharply to a point at the apex
Apex: The end of a leaf or growing tip of a shoot
Ascending: Rising somewhat diagonally and curving upward
Axil: The angle formed by a stem with a branch, leaf stalk, or flower stalk growing from it.
Axillary: Growing from an axil.
Auline: Relating to or growing on a stem.
Caespitose: Tufted; growing in small clumps.
Capitulum: A dense head of flowers
Capitate: Head-like; collected in a dense cluster.
Capsule: A pod or seed vessel made of two or more cells, which becomes dry and splits open when mature to release its seeds
Caudex: The swollen water-storage tissue, usually consisting of both the root and stem of a succulent or woody plant.
Cormel: A smaller, secondary corm produced by an old corm.
Corolla: The inner whorl of the perianth of the flower composed of petals.
Coriaceous: Of leaves which are thick and stiff, like leather.
Colymb: A raceme whose lower stalks are longer than the upper ones, so the inflorescence has a flat top.
Corymbose: Having flowers in corymbs.
Cyme: An inflorescence in which the terminal flowers open first, other flowers being borne on branches arising below it.
Endemic: of taxa that are found only in one particular place or area.
Endocarp: The innermost layer of tissue in fruit surrounding the seeds.
Entire: Undivided and without teeth.
Flora: The total of plant species in a particular area
Florets: A single flower in a head of many flowers.
Glabrescent: Becoming glabrous.
Glabrous: Hairless; smooth. Also glabrate.
Hirsute: Having coarse, rigid hairs
Hilum: The scar on a seed marking its point of attachment.
Hispid: Having stiff, bristly hairs or tiny spines
Hysteranthus: Leaves appear after the flowers
Imbricate: Overlapping in regular order, as the scales on a snake.
Indigenous: Native; not exotic.
Involucre: A collection of whorls of small leaves or bracts beneath a flower or an inflorescence
Lanate: Woolly; covered with long, fine, curly hairs.
Lanceolate: Long and thin and broadest below the middle, tapering to a point like a lance; lance-shaped
Mucronate: Terminated by a sharp tip.
Oblong: Longer than broad, and mostly parallel-sided, such as an oblong leaf.
Obtuse: Blunt or rounded at the extremity, as an obtuse leaf, sepal, or petal.
Ovary: The enlarged, rounded, *ovule*-producing base of the female portion (the *pistil*) of a flower that eventually develops into a fruit after being fertilized.
Ovate: The leaf is egg-shaped; the broadest part is below the middle.
Ovule: An outgrowth of the *ovary*, which upon fertilization becomes the seed.

Panicle: A loosely branched, pyramidal flower cluster
Pappus: A group of fine hairs on a small dry fruit, which helps in dispersal by wind, e.g. in the family Compositae
Pedicel: The stalk of a flower. Also pedicle.
Peduncle: A botanical term used to describe the stalk of a single flower, as in the Tulip, or of a cluster of flowers, as in the Geranium.
Perianth: The term for that part of a flower which surrounds the *pistil* and *stamens*; it generally consists of *sepals* and petals.
Periderm: Tissue forming part of the bark, consisting of pheloderm, phellogen and phellem (cork).
Pericarp: The wall of a fruit that is fleshy, as in berries, or hard, as in nuts.
Petiole: The stalk of a leaf.
Phenology: The study of organisms and their activities in relation to the seasons of the year.
Pinnate: Resembling a feather in structure with the parts arranged on both sides of an axis; A *compound* leaf in which the leaflets grow in a row on each side of the midrib.
Pinnatifid: Cut or sectioned to form a feather shape, as the fronds of many ferns
Polymorphism: The occurrence of two or more forms of a species in the same population or habitat.
Population: A group of individuals of the same species living in the same place or area close enough to breed together.
Pulp: The succulent part of a fruit.
Raceme: A kind of inflorescence with a central axis bearing flowers along its length.
Revolute: Rolled or curled backward or downward, as the edges of the leaves of some plants.
Scape: A leafless flower stalk.
Sepal: A leaf or division of the calyx.
Sericeous: Silky
Serrate: Leaves and other plant parts that have sharp "teeth" along their edges pointing toward the tip.
Sessile: Leaves and other plant parts that have no stalk, but instead grow directly from the stem of the plant
Stellate: Star-shaped.
Stellate leaves: Three or more leaves that surround the stem in a whorl, or radiate like the spokes of a wheel or the points of a star.
Stone: the hard endocarp of a drupe containing the seeds
Stone fruit: A fruit with a hard endocarp enveloped in a pulp, as the peach, cherry, and plume; a drupe or drupelet.
Substrate: The medium on which an organism lives, as soil or rock.
Thickets: Group of bunches.
Tomentose: Covered with thickly matted, woolly hairs.
Trigonous: Triangular in cross-section.
Xerophyte: A plant that lives in a desert or other dry habitat.
Whorl: A group of three or more organs of the same kind, arising at the same level on a stem and arranged in a circle, e.g. the petals of a flower or the branches of a horsetail.

1 GENERAL INTRODUCTION

Observing the natural landscape of Crete it is difficult to realise the big variety of native plants in relation to texture, colour, the perfume, the form and size, as well as blossoming all round the year. These elements, adapted to the Mediterranean climate, lend a particular character to the landscape of Crete. Observing at the same time the urban landscape in the bigger urban centres and the ornamental plants in the public and private gardens and parks it is apparent that very little indigenous plants of Crete have been used as ornamentals. This becomes more obvious on the slopes of national roads where the newly organised plantings are different from the adjoining natural landscape. This results in a change of the ecological landscape and the local character of the region as well as bigger difficulties in the management of plants with different climatic and other requirements (e.g. water, pest and diseases and salinity of soil).

This situation is due to the fact that very few plants from the local flora have been studied and developed as ornamental plants for gardens or other amenity uses. Consequently their production and distribution from local nurseries is not possible. However, in recent years there has been a drive to study and exploit many of the indigenous types in Greece aiming mainly at their use in the commercial floriculture and landscape architecture. Mediterranean countries generally and more specifically Crete, Cyprus and the Aegean islands, which have a large number of the endemic and indigenous plants, could constitute important sources of "new ornamental plants". These plants are more easily adaptable to the dry and warm conditions that prevail in these regions.

The plants that were selected for the present study are indigenous or endemic plants of Crete. However, there is need to understand and assess their reproductive biology and to develop systems of propagation that remove the need to source plants from natural habitats, often in fragile ecological areas. Equally there is a need to determine the ability of the plants to produce acceptable floral and commercial characteristics that include adaptation to urban and other artificial landscapes. Although some of them thrive also in other regions and countries there

are no references from other researchers on studies and exploitation for ornamental horticulture.

The objectives of this research are to select some native (endemic or indigenous) species and:

- to investigate their biological cycle *in situ*;
- to develop methods of propagation;
- to assess their adaptation in the urban environment and their suitability for their use as "new ornamental plants" in floriculture and landscape architecture.

1.1 NEW ORNAMENTAL PLANTS

"New ornamental plants" according to Von Henting (1995) can be described as:

1. plants that were known previously but because of cultivation or other problems or they were not popular, were no longer cultivated. After years of problem solving or a change in tastes, their production has then risen and the forgotten species becomes a "new ornamental plant";
2. developing new forms of products such as mini-pot plants, hanging baskets, hydro pots or developing perennials and small woody ornamentals into potted and container plants giving them the name of "new ornamental plants" instead of "new ornamental products";
3. genuinely "new ornamental plants", which have not been previously cultivated in the region into which they become introduced, nor had they previously been on the market there. There are differences between those that were made already known (through literature, botanical gardens or through local wild flora) and those that had remained unknown up until the time they were introduced.

The search for and development of genuinely "new ornamental plants" is important where one has a high economic potential, a large and receptive market and where an increasing demand and desire for change and large number coincide. Native plants are easier to handle and introduce than foreign ornamental plants, which often present problems. The greater the distance between place of discovery and

the place of development, and the more the climate and other conditions differ, the more difficult the adaptation and research for cultivation can be.

A large number of species from the wild have no ornamental value in any way, but still many species can be exploited directly or after some selection and breeding work, and besides many species can contribute to the renewal of already cultivated crops. Mostly, new crop research aims to broaden the market or to stimulate the use of plants of the native flora. A close co-operation between research and industry is desirable and has many advantages. Research can solve problems connected with the production of a new crop and the industry can do the introduction and marketing. Sometimes protection of an endangered plant species may be the aim of developing new crops and means of propagation. When such a plant is multiplied by tissue culture, cuttings or seeds collected from cultivated plants, the production of these plants or flowers can eliminate the need to collect them in the wild and thereby minimize the risk of extinction (Vonk Noordegraaf, 2000).

1.1.1 History

The origin of the collection and use of ornamental plants in living spaces and work places in Europe lies in the quite distant past, in the 17th and 18th centuries. Particularly the latter was dubbed "the golden century of botany" by Tyler Whittle in his famous book "The Plant Hunters" as cited by Von Henting (1995). Both centuries were characterized by distant overland journeys and sea voyages which made it possible to discover and acquire large tracts of territory whose flora and fauna were new and strange. Along with ornamental plants, medicinal herbs and 'smelling' plants, as well as plants with agricultural and industrial uses were among these "new' plants which greatly changed Europe over the course of the centuries.

The history of plant collection and the development of ornamental plants also advanced in the 19th and 20th centuries. Gardeners and scientists have taken part in plant searches up until today. In recent times, after a long pause during and after the Second World War, an intensive collection and development of "new ornamental plants" began in Europe. Production was built up once more and an ever-increasing demand led to high levels of expansion, cultural methods and

techniques. Parallel to this, consumer interest in ornamental plant products in general, and for "new ornamental plants" in particular, has risen greatly. Consequently in recent years there have been many efforts to introduce new plant species into the market as ornamental plants all over the world.

1.1.2 Current situation and native plants of Greece

The world flora is comprised of about 250,000 flowering plant species, belonging to 544 families and 12,000 fern species (Good, 1974). Less than 1% of these species are used for the production of ornamentals (Hennipman, 2000). Plants that come from South Africa and Australia are widely spread as ornamentals for indoor or outdoor use. The Mediterranean area represents an important area of biodiversity (Greuter, 1991; Heywood, 1995) but there is a lack of understanding of their reproductive biology and ability to adapt outside of the range of their normal environment. This has important repercussions in *ex-situ* plant conservation and with increasing efforts to make use of new plant species in commercial markets such as ornamental plants.

However, our knowledge of most of these species is scarce, which limits their potential as a natural resource for floricultural use.

In recent years much research has been conducted on native species of Greece in order to investigate the potential for their exploitation as ornamental plants for indoor and outdoor places or as cut flowers. Many of these investigations have studied methods of propagation by seeds, cuttings or by tissue culture as well as the impact of the environment on their growth, flowering and cultivation (Papafotiou *et al.*, 2000; Vlahos and Dragassaki, 1995). In Crete, one important factor to be considered is the ability of these plants to adapt to low water supplies and high salt concentrations in the soil during and after cultivation in nurseries due to the fact that these problems are becoming dramatically worse.

Native species of Greece (whose flora comprises 5,700 taxa according to Strid, 1995) on which relevant research associated with their exploitation has already started are: *Ebenus cretica* (Vlahos and Dragassaki, 1995; Vlahos and Dragassaki, 2000; Vlahos, 1996; Lydaki and Vlahos, 2000; Σύρος and Οικονόμου, 2001a, b),

Arbutus andrachne (Μπερτσουκλής *et al.*, 2003 and 2004a), *Arbutus unedo* (Λιμνιάτης, *et al.*, 2001; Βαλασκόπουλος *et al.*, 2004), *Phlomis fruticosa* (Παπαφωτίου *et al.*, 2003), *Lilium chalcedonicum* (Παπαφωτίου *et al.*, 2001; Papafotiou and Rappou, 2003), *Cistus incanus, Thymelaea hirsuta, Euphorbia characias, Euphorbia acanthothamnos* and *Genista acanthoclada* (Papafotiou *et al.*, 2000a; Papafotiou *et al.* 2000b; Παπαφωτίου και Μήκος 2004; Καρτσώνας και Παπαφωτίου, 2004), *Thymus mastichina, Lotus cytisoides, Centranthus ruber, Limonium pestinatu* and *Limonium sinense* (Maloupa *et al.*, 2000), *Lavantula stoechas* (Maloupa *et al.*, 2000; Papafotiou *et al.*, 2000), *Cistus creticus* (Pela *et al.*, 2000), *Quercus euboica* (Καρτσώνας *et al.*, 2001), *Glopularia alypum* (Μπερτσουκλής *et al.*, 2000; Bertsouklis *et al.*, 2003), *Ilex aquifolium, Juniperus excelsa* (Ριφάκη *et al.*, 2001), *Pancratium maritimum* (Dragassaki *et al.*, 2003), *Myrtus communis* (Χατζηαντωνίου *et al.*, 2004), *Consolida ajacis, Chaerophyllum coloratum, Asphodelus fistulosus, Malcolmia flexuosa, Delphinium staphisagria, Nigella damascena* (Μαλούπα *et al.*, 2004) and the flora of Cyprus (Χειμωνίδου, 2004).

1.1.3 Natural character and flora of Crete.

Crete is the fifth largest island in the Mediterranean Sea. It is situated between 34°50′ - 35°40′ N and 23°30′ - 26°20′ E, covering an area of 8300 km^2. Its maximum length is 260 km and the maximum and minimum widths are 60 and 12 km, respectively. Its geographical location is between three continents Europe, Asia and Africa (Kypriotakis, 1998). It has an extremely irregular outline and great variation in temperature and rainfall. So a rich native, naturalized and cultivated flora has developed. Plains, mountains, coasts, sand dunes, gorges and cliffs provide a home for many indigenous and endemic species. The vegetation of Crete consists of typical Mediterranean types, coniferous, maquis and garrigue vegetation, while hundreds of ornamental species, introduced at varying times, have been planted in the inhabited areas and have been naturalized.

Information on the plants of Crete is found for the first time in the work of ancient classic Greeks (Theophrastos, Dioskouridis, etc.). However the first explicit reports on the flora of Crete come from the Venetian Buondelmonti (1415), the French Belon (1553) and the British J. Ray (1694, cited by Kypriotakis, 1998).

The last author included in a single list the known plants of Crete that reached 208 in number. Later reports of many researchers followed with the numbers of species altered continuously. In 1993, Turland *et al.* recorded 1700 species while by 1995 Jaahn and Schonfelder and Montmolin and Iatrou reported 1800 and 1820 taxa (species and sub-species), respectively. According to Montmolin and Iatrou (1995) from the 1820 taxa of the flora of Crete 180 taxa are endemic plants i.e. 9.9% (as cited by Kypriotakis, 1998).

Many genera of the wild flora of Crete include species with ornamental value but we do not know them and have not exploited them up to now. There are also many species with very attractive flowers or other parts that are not cultivated because of their difficult propagation, slow growth, and poor keeping quality or for other reasons.

1.1.4 Trends and requirements in floriculture developments

The trends in international research are, apart from the search of native plants for their utilization in floriculture, the capability of using them as medicinal or aromatic plants, the preservation of biodiversity through their cultivation, and the sustainability and the protection of rare or endangered species of plants, which are threatened by extinction.

It is very difficult to predict which trends will be dominating in the near future. To know current and possible future trends, one must look about and carefully study popular magazines for the home and garden. For several years trends such as "country style" and "natural" have been popular in many countries. In such trends there is place for native flowers, which do not have a too artificial image. Trends cannot be changed easily; therefore it is better to follow them with appropriate crops (Vonk Noordegraaf, 1998). On the contrary there is a great risk of investing (e.g. starting a research and breeding programme) in a 'trendy' crop if such a crop does not possess strong characteristics for good quality and different possibilities in colour and forms, to make it less dependent on an existing trend and capable of going with another trend (Vonk Noordegraaf, 1998).

1.1.5 Strategy of evaluation

The issue of selecting plants for new floricultural crop programmes is complex and several authors have presented various strategies (Armitage, 1998; Hodge, 1996; Vonk Noordegraaf, 1998; Roh and Lawson, 1998; and Johnston *et al.*, 2000). Hodge (1996) gave five points for successful new crops development in Australia: market-led assessment, funding provided by government, development of multi-disciplinary research teams, key industry sector champion and agronomic suitability and sustainability. Armitage (1998) discussed the issue of selection of taxa for the new programme at the University of Georgia and considered that species with colourful flowers or foliage ease of propagation, lack of pest and disease problems and short production time, as desirable plants for the floricultural industry. Vonk Noordegraaf (1998) suggested the drawing up of an inventory of 'showy' species that are not currently used as a strategy towards the solution of the problems with present-day knowledge. Johnston *et al.* (2000) said that the strategy for the introduction of plants used in their programme in Australia relied on collaboration with commercial partner and market research. They, as a team, managed to overcome the barriers of the propagation and production of some recalcitrant native plants.

1.1.6 Criteria to select new ornamental crops

Von Henting (1998) mentioned that in the period of the seventies the first criteria of collectors were often "love at the first moment", which stimulated them to undertake further activities. It was often for example an unusual form of the plant, the special size or colour of the flowers, which inspired further steps of taking this ornamental plant under cultivation. Later they took under consideration the other main criteria like propagation, the reaction during the further stages of growth, the resistance again insect, diseases and extreme growth conditions, the durability, the ability for transport, and the acceptance of the market, etc.

Nowadays when searching for new plants more details are considered according to Von Henting (1998):

- first, the total impression of the plant e.g. more aesthetic reasons;
  the characteristic(s) of the plant or plant parts, which may indicate a possible later use;

- the details of the habitat which can give indications for later cultivation methods;
- the growth rhythm of the newly discovered plant.

In addition, there are criteria for evaluation of further stages including the experimental phase, the production phase, preparation for the market, the possible storage, and transport, etc.

According to the needs of urban landscape design main attention is paid to the growth parameters, decorative foliage, duration of blossoming and flower quality of bedding plans. The landscaping value of bedding plants is also determined including the time the plant needs for growing up to its decorative form, how fast the gaps between planted plants disappear and full flowering starts, and how outside factors influence the plants.

Other criteria are taken into consideration, which until now have not been considered nearly as important, including the competitiveness of the new plants with others, the suitability for highly technical methods of cultivation, genetic possibilities, the possible necessity for post-harvest treatments, the health of consumers, etc. Correspondingly, the methods of evaluation of ornamental plants in general and of new ornamental plants in special are changing constantly.

1.2 SEXUAL PROPAGATION

Producing plants from seed is the most important propagation method for agronomic, forestry, vegetable, and flowering bedding plants. Methods vary from field seeding operation to very sophisticated greenhouse transplant production systems. Seedling propagation involves careful management of germination conditions and knowledge of the requirements of individual kinds of seeds. Success depends on the good quality of seeds, manipulating seed dormancy and supplying a proper environment to the seeds and resulting seedlings (Hartmann, *et al.*, 2002).

1.2.1 Factors that affect the germination of the seeds

The germination of a seed and the installation of the seedling are the most critical stages of plants survival. Contrary to other stages of development, this process is not reversible and consequently germination in nature at the wrong time or in adverse soil conditions can lead to the death of the plant. Most seeds are located in the surface layer of the soil: according to Kigel (1995) 80 - 90% of seeds are found in the first 2 cm of the ground.

Three conditions must be fulfilled to initiate germination (Jann and Amen, 1977; cited by Hartmann *et al.*, 2002):

1. seeds must be viable; that means the embryo must be alive and capable of germination;
2. the seed must be subjected to the appropriate environmental conditions;
3. any primary dormancy condition present within the seed must be overcome.

Many factors interact with each other in the germination of seeds including the availability of water, temperature, light, salinity, pH, and the oxidation of substrate. According to Hartmann *et al.* (2002), the factors in the environment that impact upon the properties of germination include temperature, water, gases and light.

1.2.1.1 Water

Seed germination has three phases of water uptake:

- imbibition phase with the rapid increase in water uptake;
- lag phase, which follows imbibition and is a period of time when there is active metabolic activity but little water uptake, and
- radicle protrusion, which results from a second period of fresh weight gain driven by additional water uptake (Hartmann *et al.*, 2002).

The availability of water is the only factor limiting germination at suitable temperatures for seeds without a dormancy condition. The water imbibition of seeds depends on the water relationship between the seed and its germination medium (Weston *et al.*, 1992). Water moves from areas of high water potential to areas of low water potential. The water potential of the seed is more negative than

moist germination medium, so water moves into the seed. The more the fine-texture of the substrate as well as close compaction to the seed surface the easier a uniform moisture supply. Excess soluble salt (high salinity) in the germination medium may exert strong negative pressure and counterbalance the water potential of the seeds. Salts may also produce specific toxic effects that inhibit germination and reduce seedling stands.

Water stress can reduce germination percentage of some seeds. Germination of some seeds, particularly those with dormancy problems, is inhibited as moisture levels are decreased. Seeds of other species, when exposed to excess water, produce extensive mucilage that restricts oxygen supply to the embryo, reducing germination. In these cases germination improves with less moisture. Moisture content of the medium can also impact upon germination percentage during plug production (Carpenter and Williams, 1993).

1.2.1.2 Gases

Seeds of different species vary in their ability to germinate at very low oxygen levels, as occurs under water (Bewley and Black, 1994). Seeds of some water plants (e.g. rice) germinate readily under water, while germination is inhibited in air. Carbon dioxide (CO_2) is a product of respiration and under conditions of poor aeration, can accumulate in the soil. At lower soil depths increased CO_2 may inhibit germination to some extent but probably plays a minor role.

1.2.1.3 pH

Low or high pH can reduce the germination rate. The germination of seeds is generally achieved in pH from 4 until 9 but a lot of plants have special requirements. For a lot of ornamentals the optimum pH range is from 5.5 to 7.5 (Farthing and Ellis, 1990; Shoemaker and Carlson 1990). Deviations from this rule are often reported in the literature and concern mainly wild plants.

1.2.1.4 Temperature

Temperature is the most important environmental factor that regulates the timing of germination. Species have different minimum, optimum and maximum temperature requirements, depending on their climatic origin. Seeds of some

species that adapt to a Mediterranean climate require low temperatures and fail to germinate at temperatures higher than about 25°C. Species of this group tend to be winter annuals in which germination is prevented in the hot summer, but takes place in the cool autumn when winter rains commence. Alternating variations of day/night temperatures often give better results than constant temperatures for both seed germination and seedling growth. Use of fluctuating temperatures is a standard practice in seed testing laboratories. The fluctuation should be about 10 °C. Seeds of some species will not germinate at all at constant temperatures. It has been suggested that one of the reasons imbibed seeds in the soil do not germinate is that soil temperature fluctuations disappear with increasing soil depth (Lang, 1987).

1.2.1.5 Light

Recent research demonstrates that light acts in both dormancy induction and release and is a mechanism that adapts plants to specific niches in the environment, often interacting with temperature. Two characteristics of the light affect seed germination: the quality (wavelength) and the photoperiod (duration).

Seeds can be distinguished in two categories regarding their reaction to the light - light sensitive and insensitive (indifferent). For photosensitive seeds the action of light can promote or inhibit germination and both of these reactions to light can be seen proportionally in seeds of the same species (Frankland, 1986). The photo-insensitive seeds constitute a special category, in which germination is not influenced by the presence or absence of light. However the photo-sensitivity can be revealed only under extreme conditions such as low osmotic potential or unfavourable temperatures (Thanos and Mitrakos, 1979; Delipetrou, 1996). Photo-inhibition of germination has been reported in a lot of coastal types including: *Glaucium flavum* (Thanos *et al.*, 1989), *Allium staticiformae*, *Brassica tournefortii*, *Cacile maritime*, *Otanthus maritimus* (Thanos *et al.*, 1991), and *Matthiola tricuspidata* (Thanos *et al.*, 1994).

Delipetrou (1996) distinguished four types of reaction of the 32 coastal seeds taxa studied under continuous white light:

Inhibition; with higher final germination and lower T_{50} (the time required for a seed lot to reach 50 % germination) in the dark than in the light;
Delay; with equal final dark and light germination but significantly lower T_{50} in the dark;
Indifference, with no significantly different final germination and T_{50} between dark and light; and
Promotion, with higher final germination in the light than in the dark.

In 15 taxa (from the 32 taxa studied) inhibition was observed and in 6 taxa delay was observed.

1.2.2 Seed dormancy

According to Desai *et al.* (1997) the term "seed dormancy" has been used to describe two inactive conditions: one is resulting from unfavourable environmental conditions and the other due to internally imposed germination blocks. Seed germination may be delayed, for example, by inadequate water supply or unfavourable temperatures. In some seeds, germination may be prevented by the presence of blocking mechanisms within the seed, which must be removed before germination can occur.

According to Hartmann *et al.* (2002) dormancy is a condition where seeds will not germinate even when the environmental conditions (water, temperature, and aeration) are favourable for germination. Seed dormancy prevents immediate germination but also regulates the time, conditions and place that germination will occur. The length and nature of dormancy differ among various species and cultivar of crops. In nature different types of primary dormancy have evolved to aid the survival of species by programming the time of germination, for particular favourable times in the annual seasonal cycle.

There are two major categories of dormancy: primary and secondary dormancy. Primary dormancy is a condition that exists in the seed as it is shed from the plant. In contrast secondary dormancy occurs in seeds that were previously non-dormant when the environment was unfavourable for germination.

Types of seed dormancy, causes and conditions to break the dormancy recorded by Crocker (1916), Nikoleva (1977) and further modified by Baskin and Baskin (1985) (cited by Hartmann, *et al.*, 2002) are presented in table 1.1 Major categories include:

1. Primary dormancy;
    - Exogenous dormancy;
    - Endogenous dormancy;
    - Combination (double) dormancy.
2. Secondary dormancy:

- Thermodormancy;
- Conditional.

Table 1.1 Categories of dormancy (Source from Hartman *et al.* 2002)

Types of Dormancy	Causes of Dormancy	Conditions to Break Dormancy	Representative Genera
1.Primary dormancy			
a. Exogenous dormancy	Imposed by factors outside the embryo		
Physical	Impermeable seed coat	Scarification	*Baptisia, Convolvulus, Gleditsia, Lupinus*
Chemical	Inhibitors in seed coverings	Removal of seed coverings (fruits) Leaching seeds	*Beta, Iris*
b. Endogenous dormancy	Imposed by factors in the embryo		
Morphological	The embryo is not fully developed at the time the seed sheds from the plant	Warm or cold stratification	
Rudimentary	Small developing embryo	Cold stratification and potassium nitrate	*Anemone, Ranunculus*
Linear	Small differentiated embryo less than $^1/_2$ size of seed	Warm stratification and gibberellic acid	*Daucus, Cyclamen, Viburnum*
Physiological			
Non-deep	Factors within embryo inhibit germination	Red light	*Lactuca, Primula*
	Positively photodormant (requires light)	Darkness	*Cyclamen, Nigella*
	Negatively photodormant (inhibited by light)	Short period of dry storage	*Cucumis, Impatiens*
Intermediate	After ripening	Moderate periods (up to 8 weeks) of cold stratification.	*Aconitum, Cornus, Pinus*
Deep	Embryo germinates if separated from the seed coat. Often responds to gibberellic acid.	Long periods (>8 weeks) of cold stratification.	*Dictamnus, Euonymus, Prunus, Rhodotypos*
	Embryo does not germinate when removed from seed coat or will form a physiological dwarf.		
c. Combinational dormancy			
Morphophysiological	Combinations of different dormancy conditions that must be satisfied sequentially.	Cycles of warm and cold stratification.	*Asimina, Helleborus, Ilex, Magnolia, Mertensia*
Epicotyl	Combination of underdeveloped or rudimentary embryo and physiological dormancy.	Warm followed by cold stratification	
	Radicle begins growth when temperature and water permit, but epicotyl remains dormant.		*Asarum, Paeonia*
Epicotyl and radicle (double dormancy)	Radicle and epicotyl require chilling stratification but radicle is released during first year and then epicotyl is released after a second chilling	Cold stratification followed by warm followed by a second cold stratification	*Convallaria, Trillium*
Exo endodormancy	Combinations of exogenous and endogenous dormancy conditions. Example: physical (hard seed coat) plus intermediate physiological dormancy	Sequential combinations of dormancy-releasing treatments. Example: scarification followed by cold stratification.	*Cercis, Tilia*
2. Secondary dormancy	After primary dormancy is relieved, high temperature induces dormancy	Growth regulators or stratification	*Apium, Lactuca, Viola*
a. Thermodormancy			
b. Conditional dormancy	Change in ability to germinate related to time of the year	Chilling stratification	*Many species with endogenous dormancy display conditional dormancy*

1.2.2.1 Growth regulator control of dormancy and germination

The mechanisms of dormancy and its release involves the action of inhibitors and growth-promoting hormones which occur commonly in plants and have specific physiological and biochemical effects on induction, maintenance and release of seed dormancy. The main growth regulators that impact on the dormancy and the germination of seeds are:

- **Gibberellins**

Gibberellins comprise the class of hormones most directly implicated in control and promotion of seed germination. They release the dormancy in seeds of several plant species and even accelerate germination of non-dormant seeds (Desai *et al.*, 1997). They occur at relatively high concentrations in developing seeds but usually drop to a lower level in mature dormant seeds, particularly in dicotyledonous plants.

Seed germination depends on the presence of endogenous (or exogenously applied) gibberellins and may be inhibited by the presence of growth retardants such as ABA (Karseen, 1995). Gibberellins appear to play a role in two different stages of germination. One occurs in the initial enzyme induction and the second is in the activation of reserve food-mobilizing systems. Their synthesis and perception are affected by numerous environmental signals that also influence release from dormancy. These include light, temperature (including stratification) and nitrate levels (Hartmann *et al.*, 2002). It is generally accepted that growth retardants such as abscisic acid compete against the action of gibberellins. This competition is based mainly on the direct inhibition of the synthesis of the gibberellins and not on the inhibition of the action of the growth regulator (Πασπάτης, 1998). Gibberellins applied commercially as gibberellic acid (GA3) or GA4+7 can relieve certain types of dormancy, including non-deep and intermediate physiological dormancy, photodormancy and thermodormancy (Hartmann *et al.*, 2002). It has been suggested that seed dormancy is controlled by the balance of the relative levels of these promoters and inhibitors (Desai *et al.* 1997).

- **Abscisic acid (ABA)**

Abscisic acid is an important naturally occurring growth regulating compound not only in seed germination but also in plant growth in general. ABA plays a pivotal role during the development of primacy dormancy. It is specifically involved with the onset and maintenance of dormancy. During seed development ABA is involved in the regulation of a number of processes, such as suppression of precocious germination, induction of reserve proteins, development of desiccation tolerance and induction of dormancy (Hilhorst and Karssen, 1992). ABA increases with maturation of the fruit and may induce primary dormancy. It has been isolated from the seed coats of many dormant seed species (peach, apple, walnut and plum) but it usually decreases during dormancy-releasing processes such as stratification. Application of ABA can inhibit germination of non-dormant seeds and offset the effects of applied gibberellic acid. In general, inhibition is temporary and disappears when seeds are shifted to an ABA-free solution (Hartmann *et al.* 2002).

- **Cytokinins**

Cytokinins activity tends to be high in developing fruits and seeds but decreases and becomes difficult to detect as the seeds mature. The application of cytokinins can overcome dormancy in a number of species, for example the dormancy of lettuce seeds could be overcome by supplying kinetin (Miller, 1956). However kinetin is found to be effective only in presence of low levels of light. In complete darkness kinetin and other cytokinins could break the dormancy of only a small percentage of lettuce seeds (Desai *et al.* 1997). According to Thomas (1977), cytokinins act by counteracting the effect of inhibitors, especially ABA, when applied simultaneously. The dormancy-breaking effect of gibberellic acid is prevented by ABA, whose inhibition is overcome by kinetin. Cytokinins are thus thought to have a "permissive" role, allowing a second promoter (gibberellins) to act.

- **Ethylene**

Ethylene plays a regulatory role in the release of some types of seed dormancy (Kepczynski and Kepczynska, 1997). In some seeds that develop primary

dormancy, ethylene synthesis can be among the prerequisites for breaking dormancy.

The use of inhibitors of ethylene biosynthesis or its action has provided data implicating an ethylene requirement for seed dormancy or germination in some species. However, the role of ethylene in germination remains controversial. Some authors hold that gas production is consequence of the germination process, while others contend that ethylene production is a requirement for germination. Furthermore among the seeds that require ethylene, some are extremely sensitive to the gas, while others require relatively high levels to trigger germination (Matilla, 2000).

For some species, a strong correlation has been shown between treatments that overcome dormancy and the ability of the seed to produce ethylene. There are also examples where exogenous ethylene application as either the gas or ethephon (an ethylene-releasing compound) alleviates seed dormancy without additional dormancy-breaking treatments (Kepczynski and Kepczynska, 1997).

Control of a corn weed called witchweed (*Striga hermonthica*) is based on the effect of ethylene on the germination of its seeds before the germination of the grain seeds occur (Logan, 1991). Ethylene production and application has also been implicated as a mechanism to alleviate thermodormancy of germination for chickpea, lettuce and sunflower (Hartmann *et al.*, 2002).

According to the generally accepted hormonal theory of seed dormancy, the sensitivity to endogenous hormone is imposed and maintained by inhibitors, which may decline during disappearance of dormancy. Promoters are responsible for the release of dormancy. Thus, light, chilling and other factors may break seed dormancy by causing a drop in the inhibitor level and a rise in the level of promoters (Bewley and Black, 1982).

1.2.3 Seed storage behaviour

The classification used by Royal Botanical Garden (2003) is based on the 'Hong' publication (1998) on seed storage behaviour. A summary of the various storage categories used in Seed Information Database {SID) is given below:

Orthodox seeds can be dried without damage to low moisture contents, usually much lower than those that would normally be achieved in nature. Over a wide range of storage environments their longevity increases with reduction in both moisture content and temperature, in a quantifiable and predictable way.

Recalcitrant seeds do not survive drying to any large degree and are thus not amenable to long term storage, although the critical moisture level for survival varies among species. In this database this category includes those seeds, of some aquatic species in particular, described as viviparous.

Intermediate seeds are more tolerant of desiccation than recalcitrants, though that tolerance is much more limited than is the case with orthodox seeds and they generally lose viability more rapidly at low temperature. They do not conform to all the criteria defining orthodox seeds, especially in respect of the quantification and predictability of the relationship between longevity and both drying and cooling.

1.3 VEGETATIVE (ASEXUAL) PROPAGATION

Vegetative or asexual propagation is used to produce a plant identical in genotype with the mother plant. The conventional methods of asexual propagation are by cuttings (stem or leaves), by budding and grafting, by layering and specialized vegetative structure (bulbs, corms, tubers, tuberous roots and stems and pseudobulbs). These methods have gradually expanded to include micropropagation, in which new plants are produced from very small structure (embryos, shoot tips, meristems) or cells, protoplasts, callus or other tissues, in aseptic culture. Associated with this technology are novel methods of plant propagation and breeding, including the ability to isolate and transfer fragments of DNA representing specific genes from one vegetative cell to another or to combine two somatic cells together outside of the normal plant reproductive processes. Theses procedures are categorized as genetic engineering and the science as biotechnology (Hartmann *et al.*, 2002).

1.3.1 Propagation by cuttings

Cutting propagation is the most important means for clonal regeneration of many horticultural crops. Adventitious root formation is a prerequisite to successful cutting propagation. Adventitious roots are of two types:

- preformed or latent roots, and
- wound-induced roots.

Preformed roots initiate naturally on stem while they are still attached to the parent plant and roots may emerge prior to the stem piece being severed from the plant. They usually lie dormant until the stem is cut into cuttings and placed under favourable environmental conditions for further development and emergence of the primordia of adventitious roots. Such preformed root initials occur in a number of easily-rooted genera, such as *Salix, Populus, Coleus* and others (Hartmann *et al.*, 2002).

Wound-induced roots are developed only after the cuttings are made, in response to wounding of the prepared cuttings. They are considered to be formed *de novo*. Any time living cells at the cut surfaces are injured and exposed, a response to wounding begins (Davies *et al.*, 1982). The subsequent wound response and root regeneration process includes three steps (Hartmann *et al.*, 2002):

- The outer injured cells die, a necrotic plate forms, the wound is sealed with a corky material and the xylem may plug with gum. This plate protects the cut surfaces from desiccation and pathogens.
- Living cells behind this plate begin to divide after a few days and a layer of parenchyma cells form callus which develops into a wound periderm.
- Certain cells in the vicinity of the vascular cambium and phloem begin to divide and initiate *de novo* adventitious roots.

According to Hartmann *et al.* (2002) stages of *de novo* adventitious root formation can generally be divided into four stages:

- Dedifferentiation of specific differentiated cells;
- Formation of root initial from certain cells near vascular tissue;
- Subsequent development of root initials into organized root primordia, and

- Growth and emergence of the root primordia outward through other stem tissue plus the formation of vascular tissue between the root primordia and the vascular tissue of the cutting.

The time for development of root initials and visible roots after cuttings are placed in the propagating bed varies widely. In juvenile cuttings of creeping fig (*Ficus pumila*) root primordia are formed more quickly than in mature plants under optimal auxin treatments (Davies *et al.*, 1982). This was also reported by White and Lovell (1984) for *Agathis australis* where primordia formation was variable in cuttings from different-aged stock plants.

Adventitious roots usually originate on herbaceous plants just outside and between the vascular bundles, but the tissue involved at the site of origin can vary widely depending upon plant species and propagation technique (Altamura *et al.*, 1991; cited by Hartmann *et al.*, 2002).

Adventitious roots in stem cuttings of woody perennial plants usually originate from living parenchyma cells and secondary phloem but sometimes in vascular rays, cambium phloem, callus or lenticels (Harbage *et al.*, 1994). Two patterns of adventitious root formation emerge: direct root formation of cells in close proximity to the vascular system (e.g. generally more easy-to-root species); and indirect root formation, where non-directed cell divisions, included callus formation, occur before cells divide in an organized pattern to initiate adventitious root primordia (e.g. generally more difficult-to-root species) (Geneve, 1991).

Callus is an irregular mass of parenchyma cells in various stages of lignification that commonly develops at the basal end of a cutting placed under environmental conditions favourable for rooting. Roots frequently emerge through callus. In easy-to-root species the formation of callus and the formation of roots are independent of each other, even though both involve cell division. Their simultaneous occurrence is due to their dependence upon similar internal and environmental conditions. In some species, callus formation is a precursor of adventitious root formation, while in other species excess callusing may hinder rooting. Origin of adventitious roots from callus tissue has been associated with

difficult-to-root species (Cameron and Thomson, 1969; Bhella and Roberts, 1975; Geneve, 1991).

1.3.1.1 Effect of bud and leaves on adventitious roots formation and seasonal variation in propagation success.

Auxin produced from axillaries buds is transported down the shoot and is important in subsequent root formation at the base of the cutting. Bud removal from cuttings in certain species will stop root formation, especially in species without preformed root initials. In some plants, if the tissue exterior to xylem is removed just below a bud, root formation is reduced, indicating that some root-promoting compounds travel through the phloem from the bud to the base of the cutting. If hardwood deciduous cuttings are taken in midwinter when the buds are in the rest period, this has either no effect or can inhibit rooting (Fadl and Hartmann, 1967; Howard, 1965). But if the cuttings are made in early autumn or in the spring when the buds are active and not at rest, they show a strong root-promoting effect. Conversely with cuttings of apple and plum rootstocks, the capacity of shoots to regenerate roots increases during the winter, reaching a high point just before bud-break in the spring (Howard, 1968): this is believed to be associated with a decreasing level of bud dormancy following winter chilling.

According to Day and Loveys (1998) *Boronia megastigma* cuttings propagated poorly in winter when flowers were developing on stock plants. Conversely adventitious rooting of *Hypocalymma angustifolium* had greater success when cutting were taken in winter during flower development. He suggests that seasonal variation in propagation success may be mediated through changes in the endogenous plant growth regulator or carbohydrate concentrations.

The present of leaves on cuttings exert a strong stimulating influence on rooting. Cuttings of difficult to root cultivars of Avocado under mist soon shed their leaves and die, whereas leaves on the cutting of cultivars that have rooted are retained as long as nine months. While the presence of leaves can be important in rooting, leaf retention is more a consequence of rooting than a direct cause of rooting (Reuveni and Raviv, 1981; cited by Hartmann *et al.*, 2002).

Carbohydrates transferred from the leaves are important for root development. However the strong root-promoting effects of leaves and buds are probably due to other, more direct factors such as producing and transportation of auxin from the apex to the basal areas of cuttings.

1.3.1.2 Effect of plant growth regulators on adventitious root and shoot formation.

All classes of growth regulators as well as grown retardants/inhibitors, polyamines and phenolics influence root initiation either directly or indirectly (Davis and Haissig, 1972).

Auxins

Auxins generally promote adventitious roots while inhibiting adventitious bud and shoot formation. **Indolo-3-acetic acid (IAA)** was identified as a naturally occurring compound having considerable auxin activities (Haissig and Davis, 1994). Since 1935 investigators demonstrated the practical use of IAA in promoting root formation on stem segments cuttings. About the same period it was shown that two synthetic auxins **indolo-3-butyric acid (IBA)** and **α-naphthalene acetic acid (NAA)** were even more effective than naturally occurring or synthetic IAA for rooting. Today, IBA and NAA are still the most widely used auxins for rooting stem cuttings and for rooting tissue culture-produced micro-cuttings. It has been repeatedly confirmed that auxin is required for initiation of adventitious roots on stem. It has been also shown that division of the first root initial cells is dependent upon either applied or endogenous auxin (Gasper and Hofinger, 1989; Haissig, 1972; Stromquist and Hansen, 1980 as cited by Hartmann *et al.*, 2002; Mohameed and Karam, 2001; Graves, 2002).

Cytokinins

Cytokinins alone inhibit adventitious root formation and promote adventitious bud and shoot formation. The combination of cytokinins with auxins in various concentrations can give different effects. Generally a high auxin/low cytokinin ratio favours adventitious root formation and a low auxin/high cytokinin ratio favours adventitious bud formation. Cytokinins seem to play an indirect rather than direct role on rooting (Van Staden and Harty, 1988). Generally the main plant

growth regulator used commercially to enhance rooting of cuttings is auxin, while cytokinins are used to stimulate adventitious bud formation.

Other growth regulators play a minor role in root and bud formation:
Gibberellins inhibit both adventitious root and buds formation.
Ethylene can enhance, reduce, or have no effect on adventitious root formation but does not promote buds and shoots.
ABA inhibits rooting but in combination with auxin can promote rooting in some species.

1.3.1.3 The biochemical basis for adventitious root formation; biotechnological advances.

According to Hartmann *et al.* (2002) the biochemical basis for root formation implies that there are root-promoting and root-inhibiting substances produced in plants and their interaction is thought to be involved in rooting. Therefore this theory considers that difficult-to-root cuttings either lack the appropriate root-promoting substances or are high in root inhibiting substances.

Despite very active research on the biology and manipulation of cuttings, the primary chemical stimulus for dedifferentiation (the most critical step of adventitious root formation) and root initial formation remains unknown (Davies and Hartmann, 1988; Davis and Haissig, 1990; Haissig, 1994).

Rooting potential is complex and likely controlled by many genes with unknown modes of action and inheritance. Molecular studies are essential to reveal the basic mechanism of rooting. Poor rooting limits the commercial production of potentially important clones of many woody plant species. Today, difficulties in rooting *in vitro* and *ex vitro*, developing successful tissue culture multiplication systems and transformation systems for rooting limit the production of transgenic woody plants (fruits, nuts, wood, and landscape ornamentals). Some difficult-to-root woody species have been genetically "transformed" to easy-rooters. Rooting of kiwi (*Actinidia deliciosa*) cuttings were improved by introducing genes from the root-inducing bacterium *Agrobacterium rhizogens* (Rugini *et al.*, 1991; Rugini, 1992; cited by Hartmann *et al.*, 2002).

1.3.1.4 Manipulation of plants to maximize cutting propagation

Plants can be divided into three classes with regard to growth regulation effects on rooting: easy-to-root, moderate easy-to-root and recalcitrant (Hartmann *et al.*, 2002). Cuttings of recalcitrant (difficult-to-root) plants can be rooted only if specific influencing factors are taken into consideration and if they are maintained at the optimum condition. These factors include:

- selection and maintenance of source material that are easy to root (juvenile, rejuvenated, etiolated);
- manipulating the environmental conditions of stock plants (water, temperature, light, carbohydrates and other);
- selection of type of cuttings and seasonal timing;
- treatments of cuttings (auxins, wounding, etc.);
- environmental manipulation during rooting (water, humidity, temperature, light).

1.3.2 Propagation by specialized vegetative structures.

Bulbs, corms, tubers, rhizomes, tuberous roots and pseudobulbs are specialized vegetative structures that function primarily in the storage of food and water during extreme environmental conditions. Herbaceous perennials in which the shoots die down at the end of the growing season form such modified plant parts These plants, called **geophytes,** survive in the ground as dormant fleshy organs that bear buds to produce new shoots the next growing season (Hartmann *et al.*, 2002).

A **bulb** can be described as an organ consisting of a short stem bearing a number of swollen fleshy leaf bases or scale leaves, with or without a tunic, with the whole enclosing the next year's bud. This definition suffers from the limitation that it gives little indication of the geographical or botanical occurrence of the habit or the diverse types of bulbs found in nature (Rees, 1972).

There are three basic bulb structures, defined by type of scales and growth pattern. The Amaryllis (*Hippeastrum*) is an example of one type in which the expanded bases of leaves are used for food storage. There are no scale leaves in this type of bulb. Tulip (*Tulipa*) is an example of the second type of bulb. This type of bulb

has only true scales, with leaves produced on a vegetative shoot. A third type, which includes the daffodil (*Narcissus*) has both expanded bases of leaves and true scales.

Meristems develop in the axil of the scales producing underground miniature bulbs, known as **bulblets**. These bulblets when grown to full size are known as **offsets.** Bulblets and offsets provide a method of vegetative propagation. Despite the advantages of the propagation from offsets, there are many species that produce few numbers of them. Vijverberg (1981) reported that from 215 examined varieties of *Hippeastrum* only eight produced over 10 offsets per bulb, while some others produced an average of only 0.1 offsets per bulb. Very little production of offsets was ascertained in Iris (3 - 4 offsets/bulb yearly) by De Munk and Schipper (1993), in some varieties of daffodil (1.6 offsets/bulb yearly) by Rees (1969) and in *Nerine* (1 - 4 offsets/bulb yearly) by Van Brenk and Benschop (1993).

According to Hartmann *et al.* (2002) and De Hertog and Le Nard (1993) the following methods of vegetative propagation of the bulbs have been developed:

- **scaling** - a propagation procedure in which individual scales are removed and placed under appropriate conditions to cause the production of adventitious bulblets;
- **bulb cutting** (or **bulb chipping**) - a method of propagation in which a bulb is cut into fragments of three or four bulb scales attached at basal pate: a variation of this method called **twin-scaling** involves dividing bulbs into segments, each containing a pair of bulb scales and a piece of basal plate;
- **scooping** - the basal plate of a mature bulb is scooped out with a special curve-bladed scalpel;
- **scoring** (or **cross-cutting**) - three straight knife cuts are made across the base of the bulb, each deep enough to go through the basal plate and the growing point.

The scooping, cross-cutting and scoring, techniques lead to the removal of apical dominance and result in callusing of the exposed scale tissue.

A **corm** is a unique geophytic structure characteristic of certain important ornamentals, such as *Gladiolus*, *Crocus*, *Freesia*, *Colchicum* and others. The

nutrient-holding body is a solid stem structure and not a series of scales (as bulbs), and the **tunic** is made up of the dry leaf bases from the previous season. This covering (tunic) protects corms against injury and water loss. Another difference from the bulbs is that a corm lasts just one year. When active, the food store is depleted and the corm starts to shrivel. At the same time one or more new corms start to develop on the old one. These new corms form the next year's planting material. A few species form small cormlets, such as gladiolus, but most of them produce one (scarcely two) corms every year without forming any cormlets, such as Colchicum (Hessayon, 1999).

Although bulbs and corms have special reproductive features and they can propagate easily by conventional methods, most suffer from several important propagation problems, according to Hartmann *et al.* (2002):

- some cultivars are very vulnerable to systemic viruses;
- multiplication rates by natural division tend to be low, and
- seedlings have a long juvenile period before flowering.

The slow rate of multiplication by bulbs and corms can be increased by **micropropagation** using axillary shoot proliferation, adventitious shoot formation bulblet induction on scales and other methods.

1.3.3 In vitro propagation

1.3.3.1 Tissue culture and micro-propagation of ornamental plants

Tissue culture is the ability to establish and maintain plant organs (shoots, roots, embryos and flowers) and plant tissue (cells, callus, and protoplasts) in aseptic culture. Micro-propagation is a form of tissue culture used to regenerate new plants and is used as an accelerated form of clonal propagation. It is the propagation method of choice to propagate plants that are slow to multiply or those that cannot be clonally propagated by any other way. It is also used to regenerate plants that have been genetically modified through biotechnology (Hartmann *et al.*, 2002).

Ever since Murashige (1974) highlighted the potential of plant tissue culture for mass multiplication of horticultural crops there have been many examples of the application of organogenesis and somatic embryogenesis being used for this purpose (Hartmann *et al.*, 2002). The advantages of plant tissue culture for mass multiplication are several but the main ones are that cloning can be extremely rapid and propagation work can be carried out all the year round (George, 1993). The use of tissue culture techniques enables scientists to segregate cells, tissues, and organs from the parent organism for subsequent study as isolated biological units. The attempts to reduce an organism to its constituent cells, and subsequently to study these cultured cells as elementary organisms, is therefore of fundamental importance (White, 1954). In addition, the cryopreservation of plant tissue cultures and the establishment of *in vitro* gene banks have attained considerable interest (Dodds and Roberts, 1982).

A great number of ornamental plants are nowadays produced in Europe. The most frequently cloned ornamental plants in Europe are: Ficus, Syngonium, Spathiplyllum, Gerbera, Rosa, Philodendron, Saintpaulia, Nephrolepis, Cordiline, Anthurium, Calathea, Cymbidium, Dieffembachia and Rhododendron (Pierik, 1991).

Many native plants of Greece, which are threatened or have the potential to be used as medicinal or ornamental plants, are difficult to propagate by conventional methods. Mass propagation of these native plants can play an important role for their survival, phytoremediation and ornamental landscaping.

The most common micropropagation systems for ornamental plants are (Pierik 1998):

- Single-node culture - this method is the simplest, most natural and safe method (no problem with mutations) with plants which elongate, forming a stem with leaves and buds in their axils but is difficult with rosette plants;

- Axillary branching - axillary buds have their dormancy broken by breaking apical dominance with cytokinin. This method has become the most important propagation method, being simple and safe as well as preserving genetic stability. However, mutation can occur when adventitious buds are formed as result of high cytokinin levels.

- Regeneration of adventitious buds/shoots - this method includes the formation of adventitious buds/shoots on explants from leaves, petioles, stems, scales, and floral stems. However, the percentage of plant species that can regenerate adventitious buds is relatively small. The chances of obtaining mutations are much higher with this method than the first two described.

- Regeneration of plants from callus, cells and protoplast. The greatest difficulty with callus cultures is their genetic instability. This class of culture system has been used extensively in the production of new and novel genotypes. Genetic variation induced in *in vitro* culture plants is known as "somaclonal variation". Variation may either be natural or may be induced by mutagenic treatments or through specific genetic engineering techniques (Hartmann *et al.*, 2002).

1.3.3.2 Developmental stages in micropropagation

Four distinct stages are recognized for most plants:

Stage I. Establishment – placing tissue into culture and having it initiate micro-shoots.

Stage II. Multiplication – including multiple shoot production.

Stage III. Root formation – initiating roots on micro-cuttings.

Stage IV. Acclimatization – gradually moving plants to open-air conditions.

1.3.3.3 *In vitro* seed culture

Many plant species can be successfully cultured *in vitro* by making use of vegetative explants (nodes, leaf pieces, stem segments, anthers, ovules, apical tips, etc.). However, if the use of these explants leads to the destruction of a rare plant, or if the vegetative explants are difficult to sterilise because of the hairy or sticky

nature or the explant is not appropriate for the envisaged (future) experiments, then seeds can sometimes be used as a source of explants.

Although there may be disadvantages in using seedlings (from a genetic fidelity point of view), their tissues often provide suitable explants; zygotic embryos can be dissected out of developing (embryo rescue) or mature seeds (embryo culture), or the seed can be allowed to germinate *in vitro* thus producing a microbial-free source of young nodal and apical tip tissues for micropropagation. Adventitious shoots can often be induced to form directly on cotyledons, hypocotyls or young leaves, or these explants may be used to initiate callus growth. The use of seed for micropropagation can be useful for homozygous plant species (e.g. wheat) but for heterozygous plant species (e.g. grapevine) there is no use for this technique unless it forms part of a plant breeding project. Seedling shoot apices can also be used to start shoot cultures. Shoots obtained from aseptically cultured seeds are also normally free of systemic diseases (Kandasamy and Mantell, 1995 as cited by Tsoktouridis, 2000).

1.3.3.4 The propagation of plants from axillary shoots

Axillary shoot cultures have proved to be the most generally applicable and reliable methods of *in vitro* propagation particularly from the point of view of the genetic fidelity of propagated materials. Shoot culture and single or multiple nodes culture are the main methods used. Both depend on stimulating precocious axillary shoot growth by overcoming the dominance of shoot apical meristems (George, 1993).

Shoot culture is now preferred for cultures started from explants bearing an intact shoot meristem, whose purpose is shoot multiplication by the repeated formation of axillary branches. Shoot apex and meristem tip culture has been of interest particularly to plant pathologists who have recognised its value for producing pathogen-free plants (particularly for eradicating systemic virus infections). Shoot culture was conventionally initiated from meristem or shoot tips, but it has become increasingly popular to use larger explants such as 1 cm shoot apices or stem section carrying lateral buds. Such explants can sometimes be more readily disinfected and are frequently more easily established *in vitro* (George, 1993).

The method of propagation of plants from axillary shoots has been utilised increasingly for commercial plant propagation. The advantages of this method in comparison to other tissue culture methods are:

- the method can be applied to a wide range of different plant species using the same principles and basic methods;
- viruses can be controlled simultaneously as multiplication is achieved;
- there is a general uniformity and 'trueness to type' of regenerated plants;
- the relatively high rates for mass propagation are possible in many species within a few months.

1.3.3.5 Culture media

There are numerous tissue culture media described in the literature and many of them can be supplied commercially and are used for a wide variety of plant species. A nutrient medium usually consists of the following major groups of ingredients: inorganic macronutrients (N, P, K, Ca, Mg and Fe); inorganic micronutrients (Mn, Cu, Zn, B, Na, Cl, I, S, Mo, Co, AI and Ni); vitamins (nicotinic acid, pyridoxine and thiamine); organic nitrogen sources (glycine and inositol); sugar (sucrose); growth regulators (e.g. auxins, cytokinins, gibberellic acid); optional organic compounds (casein hydrolysate, yeast extract, coconut water); and optional gelling agent (agar, phytagel, gelrite) (Gamborg *et al.*, 1976).

1.3.3.6 Plant growth regulators

Besides a nutritional source, plant tissue cultures require some compounds which are generally active at very low concentrations and which are known as plant growth regulators. Growth and morphogenesis *in vitro* are regulated by the interaction and balance between the growth regulators supplied in the medium and the growth substances produced endogenously. The effects of growth regulators are generally not absolute and specific. The responses of cells, tissues and organs *in vitro* can vary according to cultural conditions, the type of explant and the plant genotype (George, 1993).

There are five broad classes of growth regulators that are of known importance in tissue culture. They are the auxins, cytokinins, gibberellins, ethylene and abscisic acid. A common feature of auxins is the property of inducing cell division and

stimulating root initiation. The compounds include 2,4-Dichlorophenoxyacetic acid, indolo acetic acid, naphthalene acetic acid and also 2,4,5-Trichlorophenoxyacetic acid and picloram. Cytokinins are adenine derivatives which have an important role in shoot induction. The most frequently used cytokinins are kinetin, benzyladenine, zeatin and isopentenyladenine. There are also now available other types of compounds, e.g. phenylureas such as thidiazuron (TDZ), with cytokinin-like activities which can be successfully used for micropropagation. Auxins and cytokinins are by far the most important for regulating growth and morphogenesis in plant tissue and organ cultures (Gaspar *et al.*, 1996).

1.3.3.7 Effect of cytokinins and auxins in tissue cultures

In tissue cultures, cytokinins appear to be necessary for plant cell division. It has been suggested that cytokinins might be required to regulate the synthesis of proteins involved in the formation and function of the mitotic spindle apparatus (Jouanneau, 1970, 1975). For instance, subculture of *in vitro* tissues onto a medium containing a cytokinin can cause the cells to divide synchronously after a lag period (Jouanneau, 1971).

They are very effective in promoting direct or indirect shoot initiation in combination with auxins. A balance between auxin and cytokinin normally gives the most effective organogenesis. The formation of adventitious shoots directly from explanted tissues or indirectly from callus is regulated by an interaction between auxins and cytokinins. One or more cytokinins added to the culture media encourages the growth of axillary buds and reduces apical dominance in shoot cultures of broad-leafed plants. High concentrations of cytokinins induce many small shoots which fail to elongate and the leaves in some species have an unusual shape and/or become hyperhydric (George, 1993).

High concentrations of cytokinins generally inhibit or delay root formation (Schraudolf and Reinert, 1959) and also prevent root growth and the promotive effects of auxins on root initiation in *Phaseolus vulgaris* (Humphries, 1960). But there are cases where low rates of cytokinins induce or promote root growth (Fries, 1960) or adventitious root formation (Nemeth, 1979).

The specificity of action of cytokinins on tissue or organ cultures can vary according to the particular compound used (Vieitez and Vieitez, 1980), the type of culture (Paek *et al.*, 1987), the variety of plant which it was derived (Boxus and Terzi, 1988) and whether the explant is derived from juvenile or mature tissues (Messeguer and Melé, 1987; cited by Tsoktouridis, 2000).

1.4 AIMS AND OBJECTIVES OF THE STUDY

The main aim of this study is to investigate and exploit (the possibility to introduce) native plant species of Crete as "new ornamental plants" in the direction of commercial floriculture and urban landscape plantings. Eight species were chosen which have not been investigated previously because of their potential ornamental value:

- three woody evergreen shrubs from the Asteraceae family: *Staehelina petiolata* (L.), Hillard & Burt, *Ptilostemon chamaepeuce* (L.), Less and *Otanthus maritimus* (L.) Hoffmanns & Link;
- two geophytes from the Amaryllidaceae and Colchicaceae families: *Sternbergia sicula* Tineo ex Guss and *Colchicum macrophyllum* B. L. Burtt respectively;
- two small woody perennial of the Lamiaceae and Convolvulaceae families: *Prasium majus* L. and *Convolvulus dorycnium* L., respectively and
- a biannual of the Campanulaceae family: *Campanula pelviformis* Lam.

This study involved multi-disciplinary research, with the following specific objectives:

- to locate native plants species, to classify, explore and select those which may be suitable to be investigated as ornamental plants;
- to investigate their phenological and biological life cycle during the year and describe their habitat and characteristics;
- to assess seed propagation and systems to overcome the problems from dormant seeds;
- to determine the capacity for asexual conventional propagation by cuttings, bulbs and corms;

- to establish a rapid reliable protocol for *in vitro* multiplication of the selected species via shoot cultures, callus culture and/or embryogenesis;
- to assess flowering characteristics;
- to investigate growth requirements for cultivation and adaptability in an urban landscape;
- to determine plant establishment either from seeds or asexual propagules;
- to design a small garden using the experimental plants in combination with other native plants of Crete as a case study.

2 GENERAL MATERIALS AND METHODS

2.1 PLANT MATERIAL

The plant material that was used for this research came from natural populations and was collected from the regions of observation (*in situ*) of the plant species. A big collection of plants was also maintained in the greenhouses and installations of the farm at the Technological Educational Institute of Crete. This constituted the available source of plant material for the experiments.

Seeds were collected from the spring until the autumn of the years 2002 - 2005 from mature fruits. Afterwards the seeds were left in the ambient environment for 10 - 30 days, depending on the species, until they were dry. Then the seeds were cleaned, weighed and maintained in glass or plastic containers at room temperature until their use. The cuttings for the rooting and *in vitro* experiments were collected one or two days before the experiments were conducted. They were placed in plastic bags and maintained in the refrigerator (5 - 7°C) until their use.

Bulbs and corms were collected periodically all through the year from the regions of observation and their weight and size were recorded. Afterwards they were planted in the greenhouse or in the park for acquisition and maintenance of new plant material for their use in future experiments.

2.1.1 Selected species and the observation areas

Surveys of native plants were conducted in their natural habitats. A great number of native species with potential use as ornamental plants were found in many places in Crete from 0 to 700 m altitude. The criteria that were considered for their selection were:

- the potential ornamental value of their flowers and/or foliage;
- their resistance to the unfavourable conditions of the arid climate;
- minimal problems from pests and diseases;
- the absence of previous research on their cultivation as ornamental plants;
- personal preferences and sentiment value;

- the possibility of finding and collecting appropriate plant material for the research;
- the distance and access to the observation area: it was necessary to be able to visit the sites of the plants regularly and frequently to undertake the surveys.

The initial survey was undertaken over the island of Crete. Finally eight species were selected as examples, in order to examine their potential use for the floriculture sector. The selected species and observation areas are described below (Table 2.1).

Table 2.1 Selected species, families and the places where they were observed.

Number	Species	Family	Location of observation areas
1	*Staehelina petiolata (L.)* Hilliard & Burt. Notes R. Bot. Gdn Edinb. 32:384 (1973)*	ASTERACEAE (COMPOSITAE)	Archanes district (15 km to the South of Heraklion)
2	*Ptilostemon chamaepeuce* (L.) Less., Gen. Cynaroceph. Spec. Arctot. 5 (1832)*	ASTERACEAE (COMPOSITAE)	Archanes district (15 km to the South of Heraklion)
3	*Otanthus maritimus* (L.) Hoffmanns & Link., Fl. Port. 2: 365 (1834)*	ASTERACEAE (COMPOSITAE)	North coast of Hersonissos (20 km to the East of Heraklion)
4	*Convolvulus dorycnium* L., Syst. Nat. Ed. 10, 2: 923 (1759) **	CONVOLVULACEAE	North coast of Hersonissos (20 km to the East of Heraklion)
5	*Prasium majus* L., Sp. Pl. 601 (1753)*	LAMIACEAE (LABIATAE)	TEI district at Heraklion
6	*Campanula pelviformis* Lam., Encycl. Meth. Bot. 1: 586(1785)*	CAMPANULACEAE	Exo Mouliana (15 km to the West of Sitia)
7	*Colchicum macrophyllum* B. L. Burtt, Kew Bull. 5: 433 (1951)*	COLCHICACEAE (LILIACEAE)	Exo Mouliana (15 km to the West of Sitia)
8	*Sternbergia sicula* (Tineo ex Guss.) D. A. Webb, Bot. Jour. Linn. Soc.76: 358(1978)*	AMARYLLIDACEAE	Zaros district (45 km to the South. of Heraklion)

* Cited by Tutin *et al.* (1964 - 1980) in Flora Europea.

** Cited by Davis (1965 - 1988) in Flora of Turkey and the East Aegean Islands.

2.2 THE CLIMATE OF CRETE

The climate of Crete is differentiated depending on the geographic site (location), the altitude and the bas-relief, which results in the creation of microclimatic conditions in the various regions of the island. It is mild in the coastal areas and becomes relatively more severe further into the hinterland, both in temperature and rainfall.

The medium annual rainfall in all Crete is 893 mm (Μάρκου-Ιακωβίδου, 1979). The annual rainfall in the coastal regions is between 483 and 656 mm (Πέννας 1977) while in the mountain it exceeds 2000 mm. The coldest months of the year are January and February and the hottest months are July and August. The annual medium temperature ranges from 13°C to 20°C. The annual medium relative humidity (R.H.) of air ranges between 64% and 75%. The amount of sunlight in Crete is between 2800 - 3000 hours and it is 600 hours higher than that of North Greece. The regions of Southern Crete have the highest sunlight in the island (Πέννας, 1977).

The climatic conditions in the observation areas are similar to those that are described above for Crete. The medium annual rainfall in the north coast of Heraklion districts for the last three years, 2003 – 2005, was 527.8 mm as recorded at the airport of Heraklion (Figure 2.1, Appendix 1). Maximum and minimum temperatures for the same period fluctuated between 8 and 30°C (Figure 2.2, Appendix 1). The medium R.H. ranged from 58% to 70% (Figure 2.3, Appendix 1) and the sunlight from 90.4 h in December to 394.8 h in July (Figure 2.4, Appendix 1).

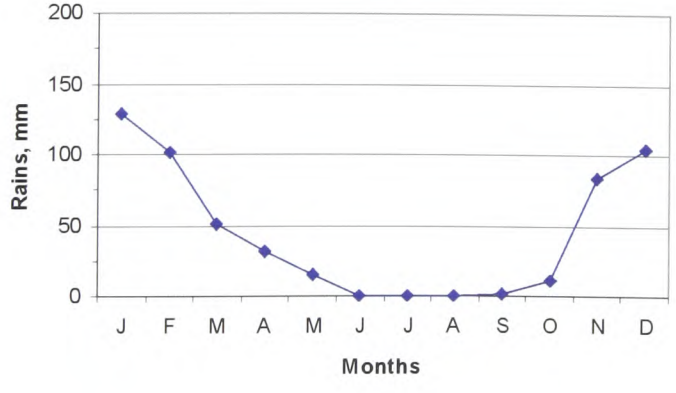


Figure 2.1 Mean rainfalls (mm) per month for the years 2003 - 2005 at the airport in Heraklion.

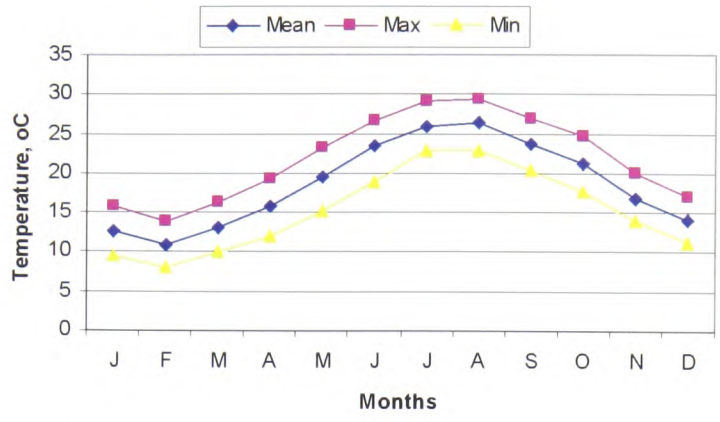


Figure 2.2 Medium, maximum and minimum temperatures in the airport of Heraklion. Means of the years 2003 - 2005.

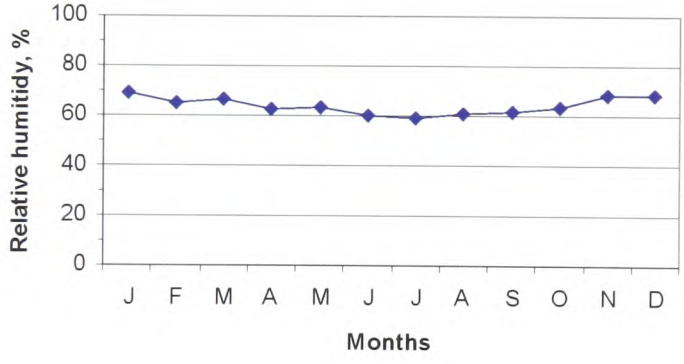


Figure 2.3 Relative humidity (%) per month at the airport of Heraklion. Means of the years 2003 - 2005.

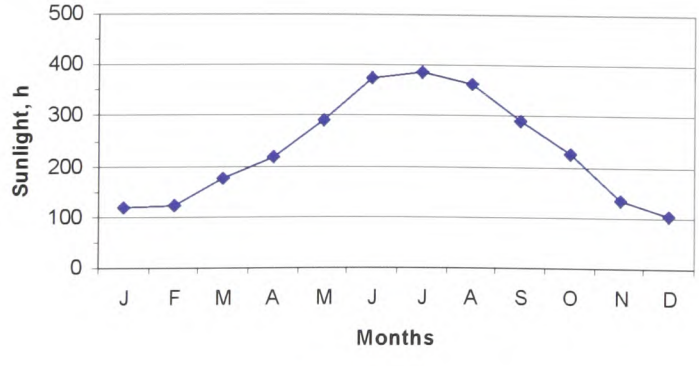


Figure 2.4 Sunlight per month at the airport of Heraklion. Means of the years 2003 - 2005.

Climatic data taken from the airport of Sitia, near to the observational areas for the examined species of *Campanula pelviformis* and *Colchicum macrophyllum*, are presented in Appendix 1 and in Figures 2.5 – 2.8. Mean rainfall in the district of Sitia for the three last years was 528.9 mm. Minimum and maximum temperatures for the same period fluctuated between 5.2 and 34.3°C while R.H. ranged between 58% in June and 80% in December. The sunlight ranged from 92.5 h in February to 387.7 h in July.

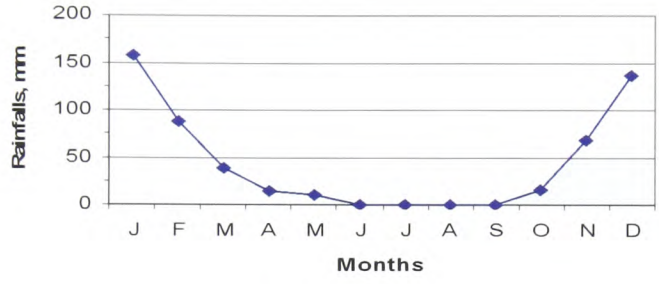


Figure 2.5 Mean rainfall (mm) per month for the years 2003 - 2005 at the airport in Sitia.

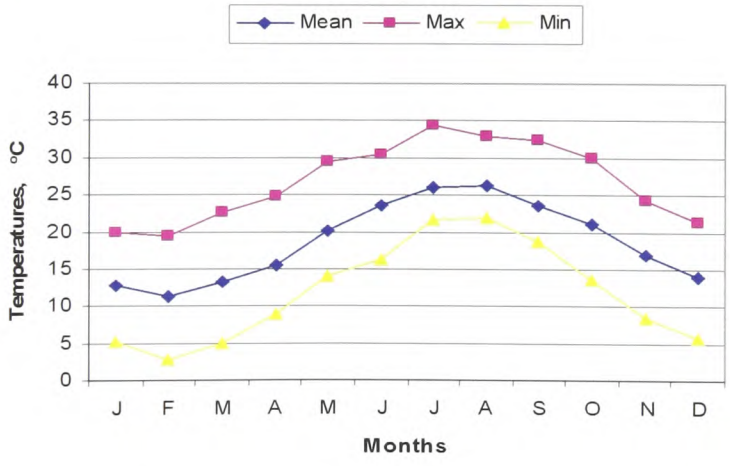


Figure 2.6 Medium, maximum and minimum temperatures at the airport of Sitia. Means of the years 2003 - 2005.

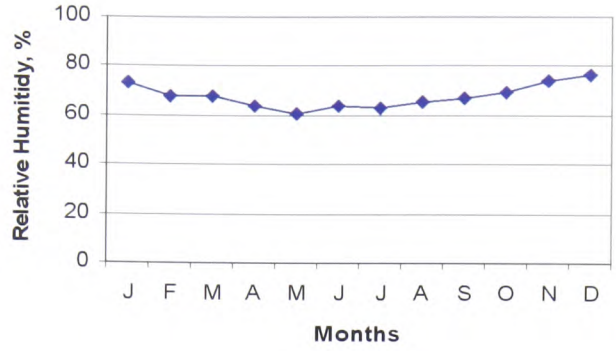


Figure 2.7 Relative humidity (%) per month at the airport of Sitia. Means of the years 2003 - 2005.

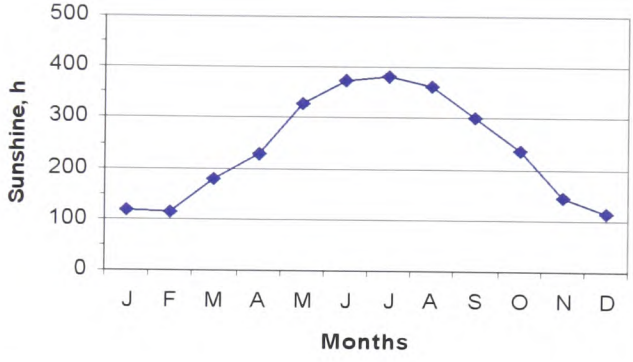


Figure 2.8 Sunlight in hours per month at the airport of Sitia. Means of the years 2003 - 2005.

2.3 FACILITIES AND EQUIPMENTS

2.3.1 Experimental Greenhouse

The experiments of propagation by cuttings were conducted in an intermittent mist compartment of a glasshouse in the farm of Technological Educational Institute of Crete in Heraklion. There was an electric heating system beneath the cuttings.

Both the seed germination experiments and plant growth experiments were conducted in a second compartment of the greenhouse where the pots were placed on metal tables. Micro-logs (Fourier) data loggers were put in both compartments.

2.3.2 Growth chambers

All the experiments for seed germination were conducted in the following growth chambers and rooms:

A Conviron EF7, Canada chamber (125 x 60 x 110cm). The light source was at the top of the chamber and comprised 4 fluorescent lamps (Sylvania cool white 60 W, F48T12/CW/HO). The temperature in this chamber was adjusted to 20°C and the photoperiod was 16 hours. An irradiance of 36 μmol m^{-2} s^{-1} was recorded at the surface of the seeds.

Two identical chambers, where the light source comprised 4 fluorescent lamps (Philips TLD 58/54CE) at the two sides of the chamber. The temperature in one chamber was maintained at 15°C and in the second at 10°C and the photoperiod was 16 hours. An irradiance of 36 μmol m^{-2} s^{-1} was recorded at the surface of the seeds.

An Astell Scientific (Tactical 308) chamber where the temperature was maintained at 5 or 10°C depending on the experiments and photoperiod of 16 hours.

A growth room with temperature set at 24 ± 2°C, photoperiod 16 hours and light (36 μmol m^{-2} s^{-1}) from fluorescent lamps.

An oven with temperature at 30°C without light.

A refrigerator at 5 – 8°C.

A heater (Unitronic 320 OR. P. SELECTA).

2.4 PROPAGATION BY SEED

The experiments for seed germination were conducted by sowing the seeds directly in pots, which were kept in the unheated greenhouse of TEI Crete. Plastic pots, 19 x 9 cm, were used and the seeds were sown at a depth of 1 - 2 cm in the substrate, which consisted of mixed peat moss, perlite and trade compost.

Physical and chemical characteristics of the substrates used for all experiments in the thesis are described bellow:

Trade compost for ornamental foliage plant compost: Florabella

Electrical Conductivity: 60mS/m (+/-25%)

pH value (H_2O): 5.5-6.5

Amount of added fertilizer (NPK fertilizer, 14:16:18): 1.5kg/m^3

Recommended use: Potting substrate for foliage plants

Produced by: Klasman-Deilmann GmbH, Germany

White Peat Moss

Electrical Conductivity: 10 mS/m

pH value (H_2O): 5.5-6.0

Type: Peat

Cation Exchange Capacity: 110-130 meq/100g

Degree of decomposition: H_2-H_5

Composition: Peat without additives

Designation of origin: Lithuanian

Produced for: Klasmann-Deilmann GmbH, Germany

Perlite (inorganic and sterile)

Trade name: Perloflor

pH: 6.5-7.5

Particle sizes: 1-5 mm

Specific gravity: 80-110 kg/m^3

Water capacity: 250-300 lt water/m^3

Cation Exchange Capacity: 1.5 meq/100g

Designation of origin: Milos Greece

Produced by: ISOCON A.E. (Perlite Institute Member) Greece

Vermiculite (inorganic and sterile)

Trade name: Agra-Vermiculite

pH: neutral

Particle sizes: 1-3 mm

Specific gravity: 80 kg/m^3

Cation Exchange Capacity: 100-150 meq/100g

Produced by: Agra-Vermiculite, Germany

For the experiments investigating the effect of temperature and light on the germination of seeds, chambers with controlled conditions were used. Plastic Petri

dishes (9 cm in diameter) were used with either wet blotting paper or 0.8 - 2% agar in deionized water as recorded in the individual experiments. For *in vitro* germination of seeds plastic Petri dishes and/or glass test tubes (28 x 20 mm) were used.

After seeds had been placed in Petri dishes or test tubes all handling and observations were carried out under green safety lamps (General electric F1 5T.8 G-6 15W, Green Photo USA).

2.5 PROPAGATION BY CUTTINGS, BULBS AND CORMS

The cuttings after pre-treatment with various concentrations of growth regulators were stuck in multiple pot discs full of the suitable substrate and placed under mist.

Bulbs and corms were collected from their habitat and planted in plastic pots in the greenhouse. All the experiments on the effect of growth regulators on bulbs and corms took place in the same compartment in the greenhouse and various substrates and pots as recorded in the individual experiments.

2.6 *IN VITRO* PROPAGATION

All the tissue culture studies described in this thesis were carried out in the Botanical laboratory of the School of Agriculture Technology of TEI of Crete.

2.6.1 Plant material

The source plants used in this study were obtained from native plants in their habitat or from stock plants in the greenhouse. Seeds collected from the plants in the wild were used as a source of seedlings for *in vitro* regeneration of *Staehelina petiolata* and *Ptilostemon chamaepeuce*. *Sternbergia sicula* bulbs and *Colchicum macrophyllum* corms were extracted both from the soil in their habitat or from the pots of the greenhouse stock in order to use them as initial material for *in vitro* experiments as described in Chapters 9 and 10.

2.6.2 Culture media

The following culture media were used for all the *in vitro* studies:

The Murashige and Skoog (MS) (Murashige and Skoog, 1962) basal salt mixture (Sigma M.5524) was used for all seed germination for *in vitro* cultures (Table 2.2).

The Murashige and Skoog (MS) (Murashige and Skoog, 1962) basal medium with vitamins (Sigma M.5519) was used for the *in vitro* studies of all species. The plain MS medium with vitamins and 3% sucrose hereafter referred to as **MSS**, was the basis for most of the plant tissue culture media used in this study (Table 2.2).

McCown's Woody Plant Basal Salt Mixture Medium (Sigma M6774) with the addition of MS vitamins (Myo-inositol 100 $mg.l^{-1}$, Nicotinic Acid 0.5 $mg.l^{-1}$, Pyridoxine 0.5 $mg.l^{-1}$, Thiamine 0.1 $mg.l^{-1}$) was used in some cases for woody *S. petiolata* and *P. chamepeuce* as referred to in the individual experiments (Table 2.2).

The appropriate amounts of plant growth regulators (BA, NAA and 2,4-D) were supplemented at various levels prior to pH adjustment depending on the objective of the individual experiment. All media were made with deionized water. The gelling agent, 0.8% (w/v) agar (Fluka, Biochemika), was added to the medium after pH measurement. The pH of the medium was always adjusted to 5.8, with either NaOH or HCl, measured by a temperature-compensated combination electrode and a digital pH meter (InoLab WTW or Crison micro-pH 2001).

All media were autoclaved for 20 min at 121°C in a STERILCLAY-75 autoclave. Technical agar was supplied by Fluka Biochemika and the rest of growth regulators, powder nutrient elements and coconut water used in tissue culture studies were supplied by Sigma Chemical Co.

Table 2.2 Plant cell culture basal salt mixtures and media (mg/l)

COMPONENT	MS (Sigma M5519)	MS (Sigma M5519)	McCown's WPM (Sigma M6774) + Vitamins
Ammonium Nitrate	1650.0	1650.0	400.0
Boric Acid	6.2	6.2	6.2
Calcium chloride anhydrous	332.2	332.2	72.5
Calcium Nitrate			386.0
Cobalt Chloride-$6H_2O$	0.025	0.025	
Cupric Sulphate-$5H_2O$	0.025	0.025	0.25
Na_2 EDTA	37.26	37.26	37.3
Ferrous Sulphate-$7H_2O$	27.8	27.8	27.8
Magnesium Sulphate	180.7	180.7	180.7
Manganese Sulphate-H_2O	16.9	16.9	22.3
Molybdic Acid (Sodium Salt)-$2H_2O$	0.25	0.25	0.25
Potassium Iodide	0.83	0.83	
Potassium Nitrate	1900.0	1900.0	
Potassium Phosphate Monobasic	170.0	170.0	170.0
Potassium Sulphate			990.0
Zinc Sulphate-$7H_2O$	8.6	8.6	8.6
Glycine	2.0		
Myo-inositol	100.0		100.0
Nicotinic-Acid (Free acid)	0.5		0.5
Pyridoxine-HCL	0.5		0.5
Thiamine-HCL	0.1		0.1
Grams of Powder to prepare 1 litre	4.4	4.3	2.3

2.6.3 Culture vessels

Flat-bottomed rimless glass tubes (30 mm diameter x 100 mm height) and glass test tubes (28 mm diameter x 200 mm height) were used for plant regeneration and shoot culture in a solid medium. Conical Erlenmeyer flasks of 250 ml were used for embryogenetic callus in liquid medium. After the explants were cultured the vessels were closed tightly using hydrophobic cotton plugs wrapped in 10 x 10 cm sections of cloth and covered with aluminium foil or with cork wrapped with cling-film, under sterile conditions maintained inside a laminar air flow cabinet.

2.6.4 Aseptic techniques

Inner surfaces of laminar flow cabinets (Microflow) used for aseptic manipulations were sprayed and swabbed down with 95% ethanol using cotton tissue and left running for ca. 15 min before use. Petri dishes were used as a sterile clean smooth surface for dissections. Dissection instruments were wrapped in aluminium foil and sterilised by placing in a dry heat oven at 120 °C for 120 min. Continuous sterility of instruments during culture work was ensured by frequent dipping in 95% ethanol followed by burning over a gas flame.

2.6.5 Glassware preparation

The glassware was autoclaved at 120°C for 25 min and washed three times with detergent followed with tap water and a rinse with deionised water. After rinsing, the vessels were put in metal or plastic baskets for culture work use.

2.6.6 Culture incubation

Culture incubation was carried out in a growth room maintained at temperatures of 24 ± 2°C in 16 h light/8 h dark. Illumination was provided by Phillips TLD 58W/54CE fluorescent tubes providing an average irradiance of 36 μmol m^{-2} s^{-1} at culture level.

Treatments of cultures using liquid plant tissue culture medium were maintained on a KL2 shaker (Edmund Buhler7400 Tubingen) set at 130 rpm to ensure the optimal dispersion of the medium during incubation.

2.6.7 Transfer of microplants to glasshouse conditions

Well-rooted microplants of *Staehelina petiolata* and *Ptilostemon chamaepeuce* were removed from culture tubes and any culture medium around their roots gently washed away under a stream of running tap water. The microplants were then transferred into plastic pots, which contained a mixture of 1:1:1 (v/v/v) potting compost, perlite and peat moss. Pots were placed in a Styrofoam cubicle in the greenhouse under natural ambient conditions. During summer when there were high temperatures inside the glasshouse the rooted microplants were planted in rock-wool plugs (diameter: 2 cm) and were maintained in the same growth room. At the end of summer they were transferred into plastic pots.

2.7 GROWTH AND ADAPTATION

In order to study the growth and adaptability of the produced selected species, an open flat field 160 m^2, in the park of TEI, was used. Six species were planted at the beginning of March and observations were taken at different periods depending on the species. Two rows of each species were planted about two meters away from each other (Appendix 4). Irrigation system was applied from PVC pipes (0.5 inch diameter) with individual drips on each plant.

To observe the growth of species the height of a plant from the ground to the highest point and the diameter at the widest point of the plant were measured after 6 months of culture or before the above-ground part of plants had been dried (3 months for *Campanula pelviformis*). Blossoming was recorded for the flowering species during the period of observation.

2.8 USE IN THE LANDSCAPE

A garden design was planned (laid out) using indigenous and endemic plants of Crete. Information about the characteristics and aesthetic value of the species were based on the existing literature and experiences obtained during the tour to the observation areas and other places in Crete during the three last years. This garden will be constructed in the near future in a sloping place of the Park of TEI.

2.9 STATISTICAL ANALYSIS

The Microsoft Excel 2003 (Microsoft) statistical programme was used for calculating the standard deviation and to make the graphs for all experiments. The SPSS-11 statistical programme was used to calculate the means, the P- value, analysis of variance, standard errors of means and Tukey's Honestly Significant Difference (HSD) of means at 5% level of significance for all data collected (Petersen, 1985).

3 *STAEHELINA PETIOLATA* (L.) HILLIARD & BURT

3.1 INDRODUCTION – LITERATURE REVIEW

The name Staehelina is given in honour of the Switzerland Botanist Staehelin. The genus according to Tutin *et al.* (1964 - 1980) comprises the following five species in Europe which differ from each other in their leaf and flower characteristics:

1. *S. fruticosa* thrives in the Aegean region comprising Crete.

2. *S. uniflosculosa* is endemic of southern and western parts of the Balkan Peninsula.

3. *S. baetica* is endemic of southern Spain.

4. *S. dupia* thrives in south west Europe, extending to central Italy, and

5. *S. arborea* (syn. *S. arborescens* L.) is endemic to Crete.

The last one is also synonymous with *S. petiolata* (Meikle, 1985).

Two species are indigenous in Crete: *S. petiolata* (endemic) and *S. fruticosa*.

Taxonomy according to Royal Botanic Gardens, Kew (http://www.Kew.org) for the genus *Staehelina* is the following:

APG Clade: EUDICOTS - CORE EUDICOTS - ASTERIDS - EUASTERIDS II
APG Order: Asterales
APG Family: ASTERACEAE
Kew Family: COMPOSITAE
Genus: Staehelina
Species Epithet: petiolata (syn. *Gnaphalium petiolatum*)

There are also records for 25 more species of *Staehelina* all over the world:

S. pungens an annual species in the Mediterranean region (Scirus web) and 24 world-wide species recorded by the Royal Botanic Garden, Kew (http://www.Kew.org): *S. alpine, S. apiculata, S. arborescens, S. centauroides, S. chamaepeuce, S. corymbosa, S. cretica, S. elegans, S. fascicuiata, S. fimbriata, S. gnaphaloides, S. hastata, S. ilicifolia, S. imbricate, S. kurdica, S. lobelii, S. pinnata,*

S. rosmarinifolia, S. sintenisii, S. solidaginoides, S. spinosa, S. squarrosa, S. syriaca, and *S. tenuifolia.*

S. petiolata is an evergreen shrub up to one metre tall (Tutin *et al.*, 1964 - 1980). The branches are silvery, with rosettes of leaves at the apices. Its leaves are ovate, entire, obtuse or sub-obtuse, dark green and glabrescent above and silvery-sericeous beneath, crowded near stem tips. Rosette-leaves are 50 - 80 x 35 - 55 mm and the petiole 25 - 40 mm. Its capitula (heads) are in terminal simple or compound corymbose cymes and their length is 25 – 35 mm. Involucre is 15 - 20 x 5 – 7 mm and involucral bracts are glabrous to sericeous, brown. Corolla is pink to purple, tubular. Its seeds are achenes, 4 x 2 mm, glabrous while the pappus is ca. 15 mm in length.

S. petiolata thrives in limestone cliffs and at an altitude of 0 to 1450 m. (Tutin *et al.*, 1964 - 1980) all over the island of Crete in small populations (Kypriotakis, 1998).

3.2 VEGETATIVE CHARACTERISTICS OF *S. PETIOLATA*

3.2.1 Material and methods

Iouktas Mountain, near Archanes village at 550 – 570 m altitude, where *S. petiolata* thrives, was selected as the area of observation. This area was visited once a month regularly all through the year and three or more times a month for the last three years during the periods of collecting cuttings and seeds. The growth of the plant, the size of the leaves, the size and number of the flowers and seeds and the aesthetic value of plant were recorded regularly during the suitable season.

Plant material (cuttings, leaves, flowers, fruits and seeds) were collected from the east side of Iouktas Mountain in Archanes village where there is the greatest number of *Staehelina* plants. The samples of vegetative parts of the plants were put in plastic bags in the refrigerator (5 - 7°C) for three days (maximum) until their use in the experiments.

The climatic features of the observation area are typically Mediterranean with hot, dry summers and mild, wet winters (Appendix 1). The analysis of the soil in the observation area, undertaken by the Soil Science Laboratory of the School of Agriculture Technology, is presented in appendix 2. The main characteristics of this soil are that it is a sand loam with a low percentage of CaCO3 and neutral to slightly alkaline pH.

3.2.2 Results and discussion

3.2.2.1 Distribution

In the observation area, maquis plants and in more open spaces garrigue (phrygana) plants dominate. *S. petiolata* was found to thrive among cliffs (Plate 3.1) and other shrubs such as *Cistus creticus*, *Salvia officinalis*, *Thymus capitatus*, *Pistacia lentiscus*, *Calycotome* sp., *Ebenus cretica*, *Quercus coccifera*, *Phlomis* sp. *Euphoria* sp. And *Genista acanthoclada*, as well as other grassy, bulbous and annual plants (Plate 3.2).

Very few mature plants of *Staehelina* were observed in areas where there were dense populations of other plant species. Most of them are in more open spaces where there is not much competition from other species.

During spring there are several seedlings around the mature plants but during summer most of them become desiccated because of the dry weather and the absence of rainfall as well as due to the fact that other more resistant plants are dominant and successfully compete for available water. Large quantities of seeds of *S. petiolata* are released from August to September. The wind takes them off the heads to land mostly around the adult plants or a small distance away. At the end of spring the seedlings appear under the mother plants in relatively small quantities in comparison with the large number of the released mature seeds. In a square, 2 x 2 m around a mother plant, 35 seedlings were counted at the observation sight. Where other more prevailing plants cover the soil, these are not favourable conditions for seeds of *S. petiolata* to germinate, grow up and become established.

As a result there are very few mature plants in the observation area as well as in the other districts of Crete. In the densest part in the observation area in a square of 5 x 5 m, 55 young plantlets were counted. There was one more spot with small established plants in the observation area and some mature spreading plants in an area of 20,000 m^2.

3.2.2.2 Botanical description

S. petiolata according to Tutin *et al.* (1964 – 1980) is an evergreen shrub up to one metre tall. In the observation area the height of *S. petiolata* mature plants was not more than 0.80 m and its canopy diameter from 1.0 to 1.6 m.

The branches are silvery, with rosettes of leaves at the apices. Its leaves are ovate, entire, obtuse or sub-obtuse, dark green and glabrescent above and silvery-sericeous beneath, crowded near stem tips. Each rosette comprised 9 – 17 mature leaves, which were 5 – 10 cm in length and 3.2 – 5.5 cm wide (Table 3.1). The length of the petioles was 2.5 – 6.3 cm. The lowest leaves of the rosettes have the longest petioles.

Its capitula (heads) are in simple or compound corymbose cymes and their length was 2.5 – 3.5 cm. (Plate 3.3). Involucres were 1.5 – 1.7 cm x 0.5 – 0.7 cm and involucral bracts glabrous to sericeous, brown. Corolla light pink. Its seeds are achenes, 0.8 x 0.2 cm, (Plate 3.4) glabrous and the pappus was ca. 1.5 cm in length. The number of capitula per inflorescence and the number of total and mature seeds were 5 – 19, 5 – 9 and 0 – 6, respectively (Table 3.2). There were 97 seeds per g. (after drying for 30 days in open air on a bench of the laboratory during August and then stored in a sealed glass vase).

Table 3.1 Number and size of mature leaves and petioles in rosettes and inflorescences of *Staehelina petiolata* (n = 30).

	Maximum	Minimum	Average	Sd
No of mature leaves per rosette	17	9	11.66	2.42
Length of mature leaves of rosette (cm)	10	5	6.9	1.58
Width of mature leaves of rosette (cm)	5.5	3.2	4.76	0.93
Length/Width	1.8	1.2	1.45	0.17
Length of petioles of rosette leaves (cm)	6.3	2.5	3.97	1.53
Length of leaves of inflorescence stems (cm)	2.8	1.2	2.1	0.64
Width of leaves of inflorescence stems (cm)	1.6	0.7	1.12	0.37

Table 3.2 Number of capitula per inflorescence of *Staehelina petiolata* (n = 50), number of total and mature seeds per head (n = 270) and weight of seeds (n = 150).

	Maximum	Minimum	Average	Sd
No. of capitula per inflorescence	19	5	10.46	3.31
No. of seeds per head	9	5	6.09	0.61
No. of mature seeds per head	6	0	3.07	1.41
Weight per seed (mg)	16	6	10.5	1.8

3.2.2.3 Biological cycle

During the second ten days of March for the years 2004 and 2005 the first new shoots sprouted from axils of the upper mature leaves in each rosette formed during the previous year. The new rosettes (1 – 4 on each old rosette) developed until the end of May. Simultaneously from March the stems of inflorescences appeared on the upper part of last year's rosettes while the upper new rosettes continue to grow (Plates 3.5 and 3.6).

Anthesis lasts from May to July. The mature seeds appeared at the beginning of August and were released from the plants from the end of August until the middle of September (it depended on the date of anthesis and the moisture of the soil).

During autumn their leaves became light green and the growth of the stem stopped. In winter the plants remained in a dormant condition. After the rains in autumn and winter the plants absorb water and during spring the new rosettes sprouted again.

3.2.2.4 Utilization

There are no references for the propagation and the utility of *S. petiolata* species. From the observations made it is apparent that the ornamental value of its foliage and flowers, as well as its resistance to drought, make it a suitable garden plant in the event that an effective way of propagation is developed and its potential to adjust to the urban landscape is investigated as reported below.

3.3 PROPAGATION BY SEED

3.3.1 Introduction

There are no references about the conditions necessary for seed germination of *S. petiolata*. From the observations in their habitat, the seeds germinate in large quantities after a period of rain and many seedlings are established in spring. The experiments that follow were conducted in order to investigate the conditions which affect the germination of *S. petiolata* seeds, including temperature, light and soil medium in conditions *ex vitro* and *in vitro* and the percentage germination one year after the seeds were released from the fruits. Mature seeds were collected from native plants in the observation area. Two lots of seeds were used to conduct these experiments:

1. seeds which were collected during August 2003, and
2. seeds which were collected during August 2004.

3.3.2 Germination of *S. petiolata* seeds in different substrates from November 2003 to September 2004 in an unheated glasshouse.

Material and methods

Seeds from seed lot 1 (August 2003) were used for this experiment. They were cleaned from their calyx and dried in the open air for a month. Then they were weighed, placed in a plastic container and stored in a dry place for 75, 105, 180, 240, 320 and 377 days at room temperature.

Underdeveloped or dead seeds (observed by naked eye) were discarded and the well-developed seeds were leached under tap water for 30 min before each sowing. They were sown during November, December, February, May, July and September, in 19 cm pots (30 seeds per pot and three pots per treatment) in peat moss and perlite (1:1, v/v) or trade compost (Florabella) at a depth of 1 cm. The pots were placed in an unheated greenhouse. Seed germination was recorded every one or two days for a period of 40 days. The seeds were considered as germinated when their cotyledons were seen on the surface of the potting media. The percent germination and the number of days to 50% germination (T_{50}) were recorded.

The germination percentage data were transformed in angular values before statistical analysis. Treatment means are presented in table 3.3 without transformation. Data was analysed by analysis of variance and the differences in the treatment means were compared by Tukey's test.

Result and discussion

The weight of the seeds ranged from 6 to 16mg per seed. Seeds with weights less than 6 mg were empty or had been attacked by insects.

The germination properties of the seeds sown in two potting medium, and at different dates is presented in table 3.3 and figures 3.1 – 3.7 (Plate 3.7). The shortest time to attain T_{50} (8.4 days) was observed in compost during May and the longest (20.5 days) in peat moss and perlite during July. There were no significant differences among the other treatments (soil medium or time from sowing).

The highest germination rate (92.5 %) of *S. petiolata* was obtained by seeds sown in compost, 180 days (during February) after the seeds were collected and the lowest (10 %) by seeds sown in peat moss and perlite 377 days (during September 2004) after collection. There are no significant differences in germination in compost between November and February and among November December and May (Table 3.3; Fig. 3.7). The germination rate in peat moss: perlite (1:1) decreased gradually from November to the next September, from 85.6% to 10%. From spring (May) towards autumn (September) a decrease of the germination rate

was observed in both substrates, with the decline being greater in peat moss: perlite. This probably is a sign of the seeds' decay after summer and/or that the higher temperatures during summer and autumn were an obstacle for seed germination (Fig. 3.7).

Table 3.3 Percent germination and T_{50} of *S. petiolata* seeds in two potting media sown at different times through year. Means compared by Tukey's test.

Months	Potting medium	% germination ± sd	T_{50} in days
November -03	Peat moss+Perlite (1:1)	85.6 ± 1.9ef*	11.2ab*
	Compost	78.9 ± 5.1ef	11.1ab
December -03	Peat moss+Perlite (1:1)	71.7 ± 2.4cde	11.7ab
	Compost	73.3 ± 0cde	12.5ab
February -04	Peat moss+Perlite (1:1)	70 ± 0cde	15.3ab
	Compost	92.5 ± 3.5f	12.1ab
May -04	Peat moss+Perlite (1:1)	56.7 ± 12.6bcd	17.3ab
	Compost	76 ± 5.8de	8.4a
July -04	Peat moss+Perlite (1:1)	16.7 ± 3.3a	20.5b
	Compost	46.7 ± 8.8b	15.8ab
September-04	Peat moss+Perlite (1:1)	10 ± 5.8a	17.3ab
	Compost	52.2 ± 1.9bc	11.5ab

* Values followed by the same letter are not significantly different ($P = 0.05$)

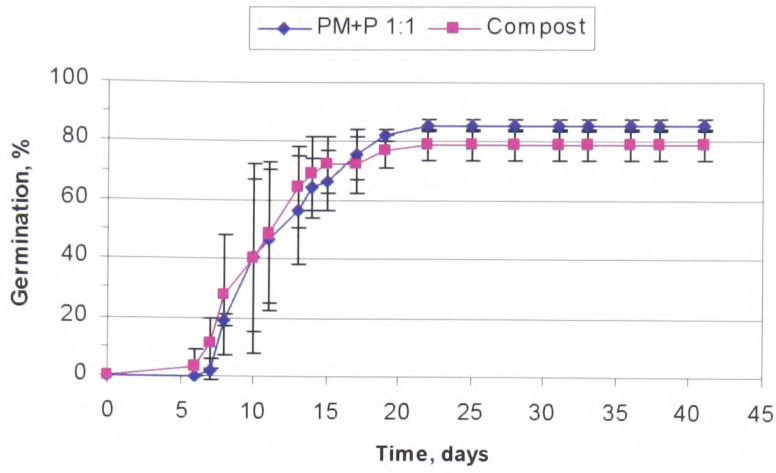


Figure 3.1 Effect of the potting medium on the germination of *S. petiolata* seeds under unheated greenhouse conditions 75 days after collection (during November), mean ± sd.

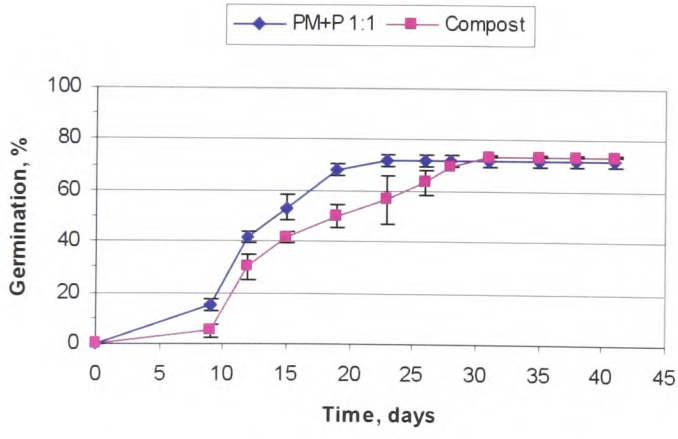


Figure 3.2 Effect of the potting medium on the germination of *S. petiolata* seeds under unheated greenhouse conditions 105 days after the seeds were collected (during December), mean ± sd.

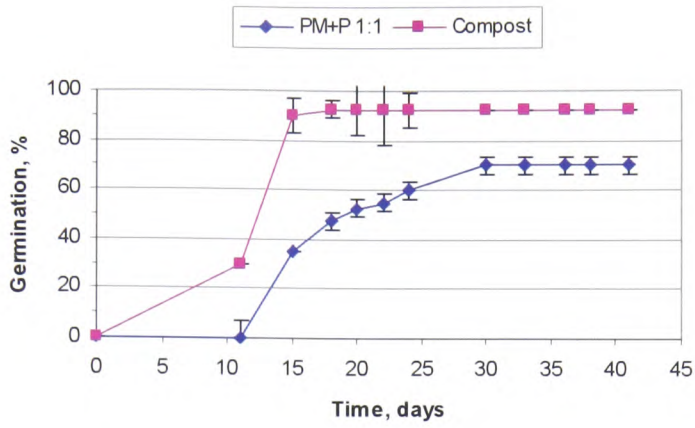


Figure 3.3 Effect of the potting medium on the germination of *S. petiolata* seeds under unheated greenhouse conditions 180 days after collecting the seeds (during February), mean ± sd.

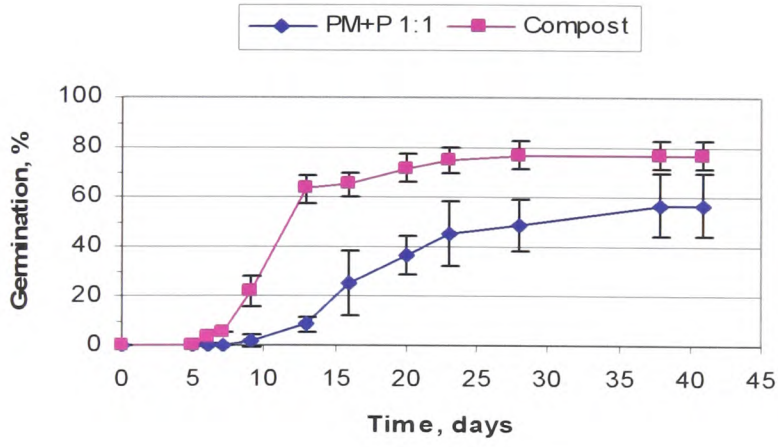


Figure 3.4 Effect of the potting medium on the germination of *S. petiolata* seeds under unheated greenhouse conditions 240 days after collection (during May), mean ± sd.

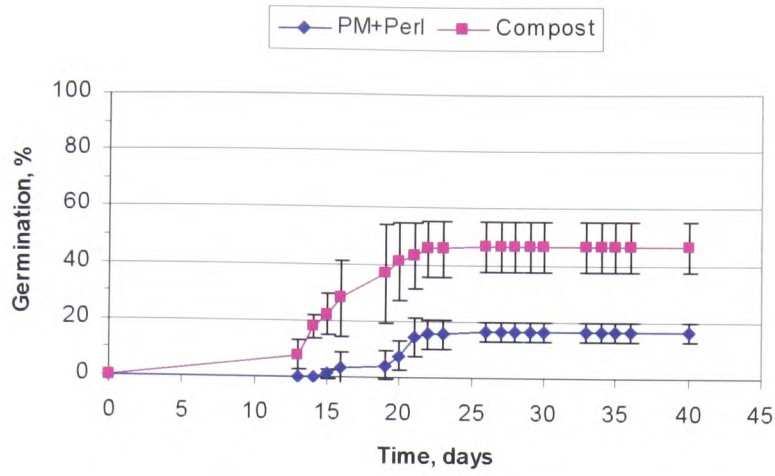


Figure 3.5 Effect of the potting medium on the germination of *S. petiolata* seeds under unheated greenhouse conditions 320 days after collection (during July), mean ± sd.

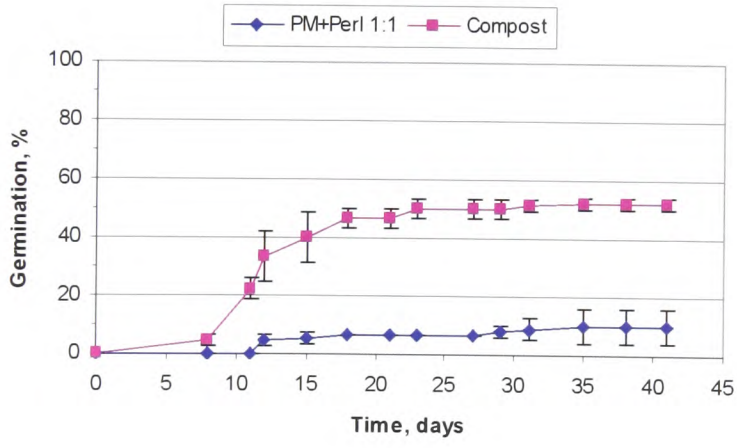


Figure 3.6 Effect of the potting medium on the germination of *S. petiolata* seeds under unheated greenhouse conditions 377 days after collection (during September), mean ± sd.

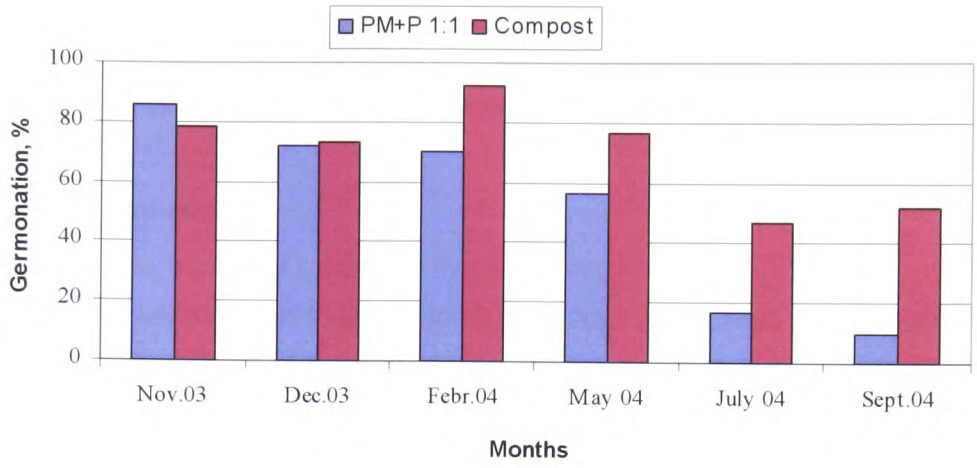


Figure 3.7 Final mean germination levels of *S. petiolata* seeds sown from November 2003 to September 2004 in two potting media, peat moss + perlite (1:1, v/v) and trade compost. Seeds were collected in August 2003 from the observation area.

3.3.3 Effect of the time of storage and the potting medium of two seed lots of *S. petiolata* seeds, on the germination in an unheated greenhouse.

Material and methods

Two seed lots were used to conduct this experiment:

1. Seeds which were collected during August 2003 (same seed lot as the previous experiment), and
2. Seeds which were collected during August 2004.

Both seed lots were collected from the observation area and were cleaned, dried and stored as described in the previous experiment. Then they were leached under tap water for 30 min before each sowing. Two potting media were used (peat moss: perlite 1:1 v/v and trade compost), 30 seeds per pot and 3 pots per treatment. Sowings were undertaken in September and October 2004. The results acquired from the previous experiment for the seed lot 1 in September 2004 were used also to this experiment. Data was analysed by ANOVA and the differences in the treatment means were compared by Tukey's test.

Results and discussion

Best germination was observed in the compost for both seed lots and the two months September and October. However significant differences were not observed in the final germination level among seeds that were collected a year before sowing and those that were collected the same year for both the months September and October if the seeds were sown in the same substrates (Figures 3.8 - 3.12; Table 3.4). However, the germination rate of *S. petiolata* seeds collected in August 2004 is slower than seeds collected in August 2003 when the seeds were sown in the same substrate for both the months September and October (Table 3.4). The delay in germination between the two seed lots may be due to the need for after-ripening of the seeds after they are separated from the mother plant. This type of after-ripening exists in many fleshy harvest seeds of herbaceous plants and disappears later before the growers sow the seeds in autumn (Hartmann *et al.*, 2002). The low percent germination of seed lot 1 may be due to the climate or to the storage conditions.

The higher germination in compost compared to the peat moss + perlite may be due to the better contact of the seeds with the substrate or to the fact that the compost contains more nitrogen which can promote *S. petiolata* seed germination.

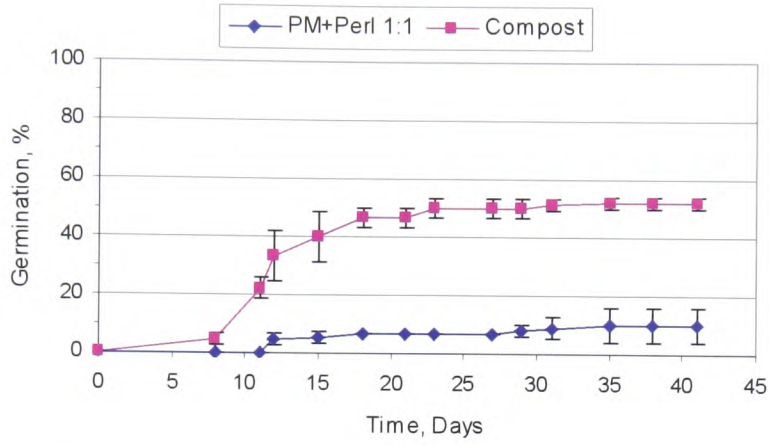
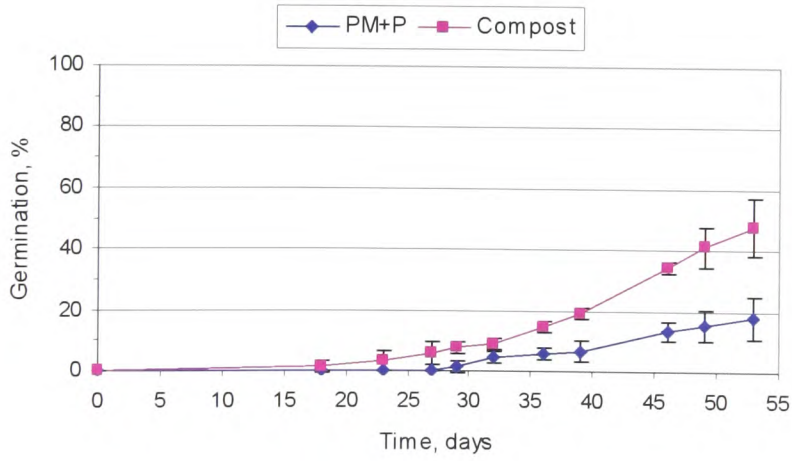
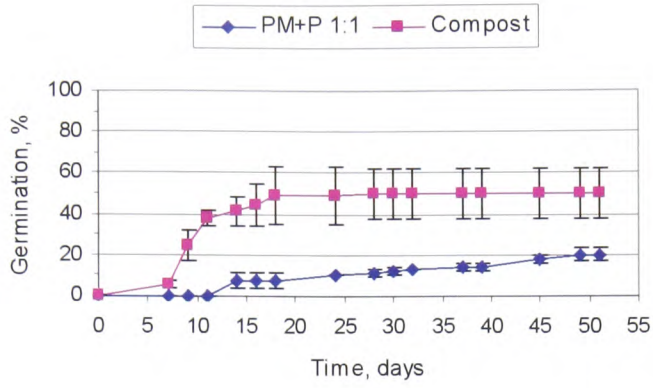


Figure 3.8 Germination time course of *Staehelina petiolata* seeds in peat moss + perlite 1:1 and compost in an unheated greenhouse (sown during September 2004). Seed lot 1 (August 2003). The vertical bars represent sd.



3.9 Germination time course of *Staehelina petiolata* seeds in peat moss + perlite 1:1 and compost in an unheated greenhouse (sown during September 2004). Seed lot 2 (August 2004). The vertical bars represent sd.



3.10 Germination course in time of *Staehelina petiolata* seeds in peat moss+ perlite 1:1 and compost in an unheated greenhouse (sown during October 2004). Seed lot 1 (August 2003). The vertical bars represent sd.

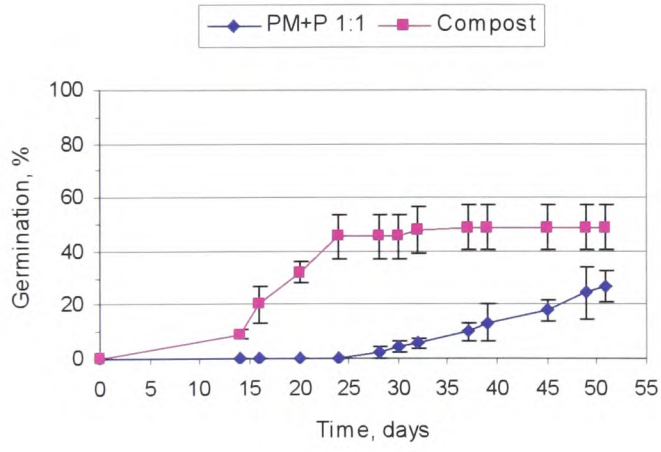


Figure 3.11 Germination time course in time of *Staehelina petiolata* seeds in peat moss+ perlite 1:1 and compost in an unheated greenhouse (sown during October 2004). Seed lot 2 (August 2004). The vertical bars represent sd.

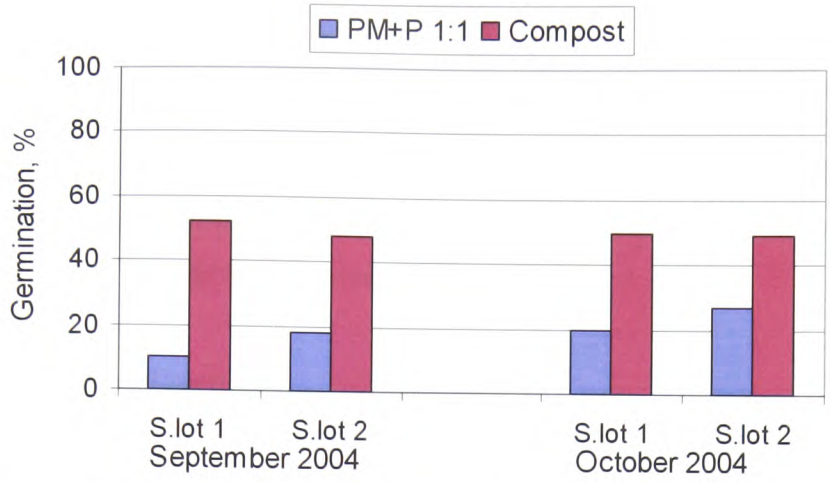


Figure 3.12 Cumulative germination of two lots of *Staehelina petiolata* seeds in two substrates (PM+P and Compost) Seed lot 1: collected in August 2003; Seed lot 2: collected in August 2004.

Table 3.4 Effect of the potting medium (PM+P and Compost) and the seed lot on the germination of *S. petiolata* seeds. PM+P = Peat moss:perlite 1:1. Seed lot 1: collected in August 2003; Seed lot 2: Collected in August 2004. Sowing months: September and October 2004.

Sowing month	Seed lot	Substrates	Germination, %	T_{50}
September 2004	1	PM+P	10.0 ± 5.8a	11.7 ± 0.5a
		Compost	52.2 ± 1.9b	11.3 ± 0.6a
	2	PM+P	17.8 ± 6.9a	39.3 ± 5.4b
		Compost	47.8 ± 9.6b	41.2 ± 3.2b
October 2004	1	PM+P	20.0 ± 3.3a	27.2 ± 4.3c
		Compost	50.0 ± 12.0b	9.1 ± 0.5a
	2	PM+P	26.7 ± 5.8a	39.8 ± 1.6d
		Compost	40.0 ± 8.4b	17.4 ± 0.7b

Means with the same letters are not significantly different at L=0.05 according to Tukey's test.

3.3.4 Effect of temperature and lighting on germination of *S. petiolata* seeds *in vitro*.

The previous experiments highlighted the effect of media and storage on seed germination in the greenhouse environment. Further, more controlled experiments were undertaken to determine the temperature dependence and the effect of white light on *in vitro* germinated seeds.

Material and methods

Seeds of *S. petiolata* were collected from the observation area in August 2003. Undeveloped, diseased or damaged seeds were discarded. The mature seeds were dried in open air for a month and were stored in an air-proof container in a dry place until use.

In May 2004, the *Staehelina* seeds were surface sterilized by using 80 % (v/v) ethanol for 15 sec and 0.75% sodium hypochlorite (NaOCl), with a few drops of Tween 20, for 15min. Seeds were then rinsed three times using sterilized distilled water. All handling procedures were carried out under aseptic condition in the laminar air flow cabinet with sterilized instruments. Seeds then were cultured in plastic Petri dishes on half strength MS basal salt mixture medium (Murashige and Skoog, 1962) with 0.8% agar adjusted in pH 6.0 and sterilized at 121°C for 25 min. Twenty five seeds were placed in each Petri dish and six dishes were placed in each chamber at 10, 15, 20 and 25°C with light (light/dark - 16/8 h) and dark conditions, and in a chamber at 30°C only in dark conditions. The Petri dishes were wrapped with aluminium foil in order to obtain the dark conditions for half of the plastic Petri dishes. The seeds were observed daily and considered germinated when the radicals pierced the seed coats. The treatment design for this experiment was factorial and completely randomized. The germination percentage data were transformed in angular values before statistical analysis. Data was analysed by ANOVA and the differences in the treatment means were compared by Tukey's test.

Result and discussion

The highest germination percentage (99.3%) of *S. petiolata* seeds was recorded at 15°C in light/dark (L/D) and dark (D) conditions 10 and 9 days after sowing the

seeds, respectively (Table 3.5 and Figures 3.13 - 3.15). The lowest germination was observed at 30°C in dark and 25°C in light conditions. Better results at 25°C were obtained in darkness than under lighting. There were no significant differences in percentage germination among the temperatures of 10, 15 and 20°C in L/D and D conditions. Days to germinate half of the final germinated seeds (T_{50}) were least at 20°C than the lower temperatures (Table 3.5; Plate 3.8). All the seedlings were planted in 5 cm pots and had achieved 100 % survival three months later.

Table 3.5 Germination percentage and T_{50} (in days) of *S. petiolata* seeds *in vitro* at 10, 15, 20 and 25°C under light (light/dark, 16/8 h) or darkness and 30°C under darkness.

Temperature °C	Light/Dark	% germination	T_{50} in days
10	Light/Dark	98cd *	6.7cd
10	Dark	97.3cd	6.3bcd
15	Light/Dark	99.3d	6bc
15	Dark	99.3d	5.6bc
20	Light/Dark	98.6cd	3.5a
20	Dark	92.6bc	3.8a
25	Light/Dark	62.6a	3.6a
25	Dark	87.3b	7.6 d
30	Dark	49.7a	4.9ab

*Values followed by the same letter are not significantly different (P= 0.05)

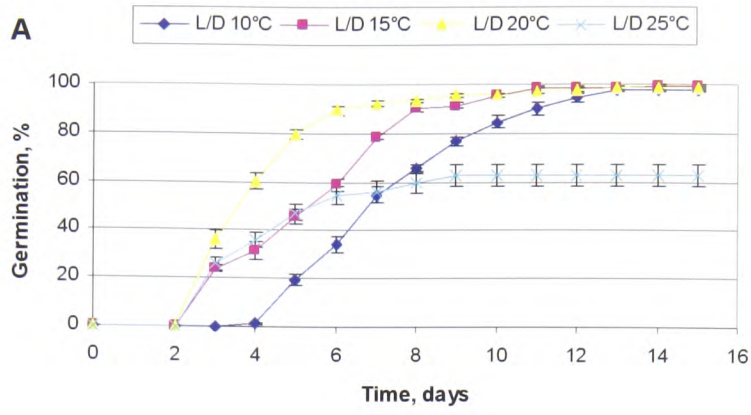


Figure 3.13 Germination time courses of *S. petiolata* seeds cultivated *in vitro* at different temperatures under light. Mean ±sd.

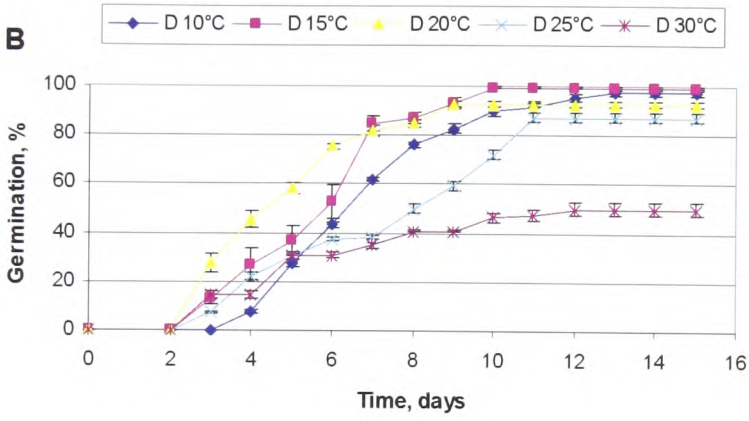


Figure 3.14 Germination time courses of *S. petiolata* seeds cultivated *in vitro* at different temperatures in darkness. Mean ±sd.

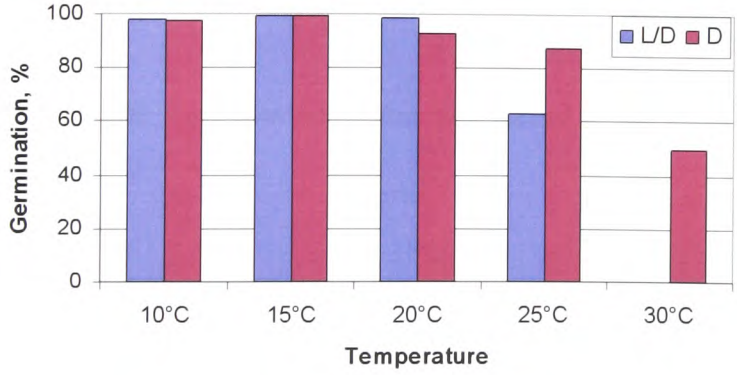


Figure 3.15 Total percentage germination of *S. petiolata* seeds using *in vitro* cultures under different temperature and light regimes.

3.3.5 Conclusion

Based on the results of germination seeds of *S. petiolata* presented in this chapter, the germination rate of the seed in compost was faster and more uniform than the germination in the peat moss: perlite mixture. Another important feature of the compost's superiority is the growth rate of the seedlings after germination in comparison with that in the peat moss and perlite mixture (Plate 3.7).

The most favourable period for sowing is from autumn to spring (November to May) reaching 92.5% in February. It appears that seeds of *S. petiolata* need at least two months after-ripening to release them from the primary dormancy and to increase germination levels. This dormancy period ensures that seeds germinate when the environment is more suitable for seedling survival (Hartmann *et al.*, 2002). Seasonal temperatures from autumn until spring in the natural environmental in Crete are more suitable for seed germination than temperatures during summer and early in autumn. For most cultivated crops non- deep physiological dormancy may last for one to six months and disappears with dry storage during normal handling procedures (Geneve, 1998).

Germination of surface-sterilized seeds on MS medium at standard temperature conditions is better and faster than germination in compost or peat moss: perlite in greenhouse conditions. Temperatures from 10 to 20°C induce higher germination percentages (up to 100% at 15°C) although seeds can germinate also at higher temperatures (30°C). The germination of seeds over a wide range of temperatures is characteristic of non-dormant seeds (Hartmann *et al.*, 2002).

Seedlings that were planted in pots and placed in the greenhouse achieved 100% survival.

3.4 PROPAGATION BY CUTTINGS

3.4.1 Introduction

The discovery that auxins promote root initiation in cuttings was first mentioned in 1934 and has provided practical benefits for vegetative propagation. Most of the investigations have involved studies of: the optimal growth regulator concentrations for cuttings, the most effective methods of application, the relative efficacy of different auxins and the applications at different times of year (Smith and Thorpe, 1976).

Results from my research during the years 1999 - 2002, showed that cuttings of *S. petiolata* from west Crete, a different site than the thesis collection area, are able to root under mist (Antonidaki *et al.*, 2004). The application of the auxin IBA affected the rooting and the number of roots positively. IBA at 1000, 2000, 4,000 and 8000 ppm induced 69%, 90%, 22% and 0% rooting, respectively. The high concentrations of hormone affected the rooting in a negative way but they did not affect the number of roots on the rooted cuttings.

The experiments reported here were carried out in order to further investigate the effect of the concentration of auxins and substrates on the rooting of *S. petiolata* cuttings under mist taken during different seasons of the year. All the cuttings that were used for these experiments came from the observation area (Archanes, Heraklion) and were collected one day before the experiments took place. They came from the tips of the previous year's growth. All stem tip cuttings, 5 – 15 cm in length, had 4 - 5 mature leaves from the rosette. The lower part of the stem was bare from leaves so it was not possible to use it as segment cutting in order to germinate under mist.

3.4.2 Effect of IBA on rooting of *Staehelina petiolata* stem cuttings during spring (April 2003).

Material and methods

Softwood tip cuttings, 5 – 6 cm long, were collected from adult plants in their natural habitat in April 2003. Their lower leaves were removed and 2 - 3 mature

leaves were left on the cuttings. The cut of the lower section of the cuttings was renewed and the cuttings were dipped in 50% (v/v) ethanol solution containing 0, 1000, 2000, and 4000 ppm IBA for 30 sec. The treated cuttings, after drying, were placed in perlite in multiple pot discs and were kept under intermittent mist. The temperature at the base of the cuttings was adjusted to 24 ± 3°C. After 40 days the cuttings were assessed for rooting.

The experiment design was a randomized complete block with 10 cuttings per replication and 3 replications per treatment. Total cuttings used were 120.

Result and discussion

During the experiment contamination of the cuttings was observed and many were discarded. After 40 days none of the remaining healthy cuttings had rooted. These results are probably due to very soft cuttings that tend to have lower endogenous carbohydrates (Hartmann *et al.*, 2002) and are more susceptible to diseases, indicating that cuttings collected in April are not suitable for propagation.

3.4.3 Effect of IBA on rooting and callus formation of stem tip cuttings of *Staehelina petiolata* during early summer (June 2003).

The previous experiment had not proved successful in producing rooted cuttings which may have been due to both the age of the cutting and their susceptibility to infection. Therefore, the experiment was repeated using cuttings from older plant material.

Material and methods

In June 2003, stem tip leafy cuttings were collected from the observation area in order to examine the effect of different concentrations of ethanol solutions of IBA (0, 500, 1000, 2000, 4000, and 8000 ppm) and powder IBA (0.06% and 0.2%) on the rooting of *S. petiolata* cuttings during summer season. Cuttings were 10 – 15 cm long and the same method was repeated as the previous experiment apart from the dipping time in the solution of IBA, which was 1min, due to the fact that the cuttings were harder than the spring cuttings. Rooting medium was perlite. After 50 days the number of rooted cuttings and the number of roots from each cutting or the cuttings with calluses were recorded.

The experiment design was a randomized complete block with 10 cuttings per replication and 5 replications per treatment (total cuttings 400).

Result and discussion

The highest rooting percentage (30%) was recorded in the treatment with IBA powder at 2000 ppm (Figure 3.16). There were significant differences in percentage rooting between solution and powder of IBA. Callus formed on those cuttings that did not root. The highest percentage of cuttings that formed callus on the base occurred with the control and as the IBA solution concentration increased the presence of callusing decreased. As the concentration of the solution or powder IBA increased more cuttings were rooted and less callus was formed. However, the highest concentration of IBA in solution (8000 ppm) inhibited root and callus formation. The greatest number of roots per rooted cutting occurred under the influence of IBA in the powder form (Figure 3.17). There were no significant differences between the treatments in 2000, 4000 ppm in solution and 600 and 2000 ppm in powder.

In some species, callus formation is a precursor of formation of roots, while in other species excess callusing may hinder rooting (Hartmann *et al.*, 2002). The initiation of adventitious roots from callus tissue in woody plants has been associated with difficult to root species or the mature phase of the plants (Davis and Rotter, 1985; Geneve, 1991).

The number of rooted cuttings in every treatment were far fewer than from those which were collected from other districts in west Crete in experiments conducted during the same period (early summer) in 2002 (data not shown). Factors such as different place and growth conditions of the plants from the collection areas, different temperature and water supply as well as different clones and hardness of the cuttings were probably some of the causes of the lower rooting percentage. On the other hand, it is worth mentioning that in the 8000 ppm IBA no cuttings rooted as observed in the experiments in 2002.

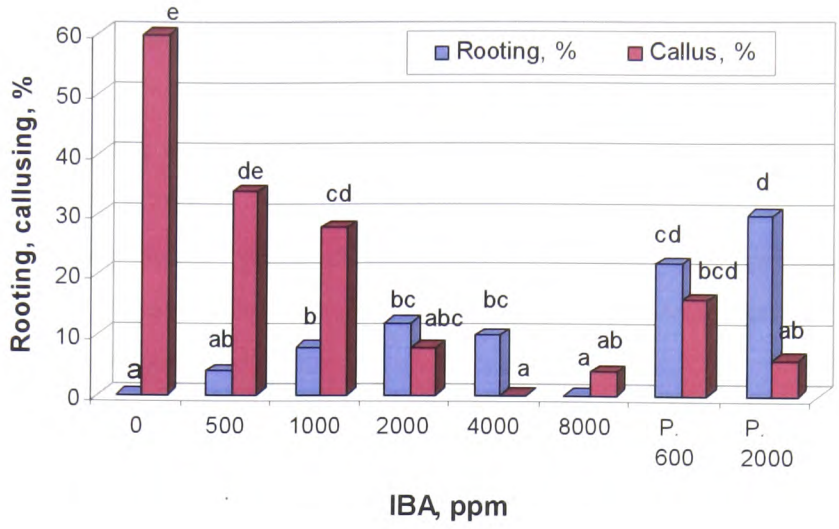


Figure 3.16 Effect of solutions of IBA at 0, 500, 1000, 2000, 4000 and 8000 ppm and IBA powder at 600 and 2000 ppm on the rooting and the formation of callus of *S. petiolata* stem tip cuttings during summer (June). Means with the same letters are not significantly different at $P = 0.05$ according to Tukey's test.

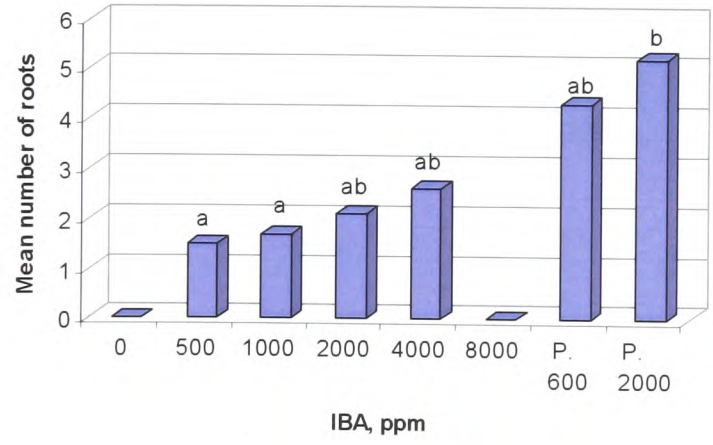


Figure 3.17 Effect of solutions of IBA at 0, 500, 1000, 2000, 4000 and 8000 ppm and IBA powder at 600 and 2000 ppm on the mean number of primary roots of per rooted cutting of *Staehelina petiolata* taken during summer (June). Means with the same letters are not significantly different at $P = 0.05$ according to Tukey's test.

Cuttings with callus were put in a perlite medium after they had been disinfected with improdione (Rovral 50, Rhone Poulenc) and then treated with IBA powder at 2000 ppm (0.2% RADICIN). After three months, 47.5% of the cuttings produced

roots. During this period the leaves of the cuttings remained healthy green and no abscission (collapse) was observed.

3.4.4 Effect of IBA on rooting of stem tip cuttings of *S. petiolata* during autumn (November 2003).

The previous experiment indicated that cuttings collected in June had low percentage germination. Therefore, the experiment was repeated using cuttings collected late in autumn.

Material and methods

In November 2003, stem tip leafy cuttings were collected from wild plants from the observation area after the rains had started and the plants had absorbed water. Cuttings, 10 – 15 cm in length, were treated using the same methods as in the two previous experiments. The concentrations of IBA were 0, 500, 1000, 2000, 4000, and 8000 ppm and the time of dipping 1 min. The temperature at the base of the cuttings in the mist was adjusted to 23 ± 3°C. After 50 days the number of rooted cuttings and the number of roots from each cutting were recorded.

The experimental design was a randomized complete block with 7 cuttings per replication and 4 replicates per treatment (total cuttings 168).

Results and discussion

The auxin IBA promoted the rooting of the *S. petiolata* cuttings with the percentage increasing gradually from 500 to 4000 ppm IBA (Figure 3.18), with the highest significant (P=0.05) percentage rooting achieved at 4000 ppm IBA (53.5%). At the concentration of 8000 ppm IBA, no plants rooted as in all previous experiments. The rooting percentages in all treatments with plants from the observation area in Archanes Heraklion were lower than those involving plants from other districts in west Crete in experiments conducted during the same period (autumn) in 2002 (Antonidaki *et al.*, 2004).

The number of roots per rooted cutting was significantly greater in the concentration of 4000 ppm than in the other treatments (Figure 3.19).

These results agree with the results from other researchers who reported that high concentrations of IBA prevent rooting but favour the differentiation of the root primordia (Kralik and Psota, 1985; Lomax *et al.*, 1995).

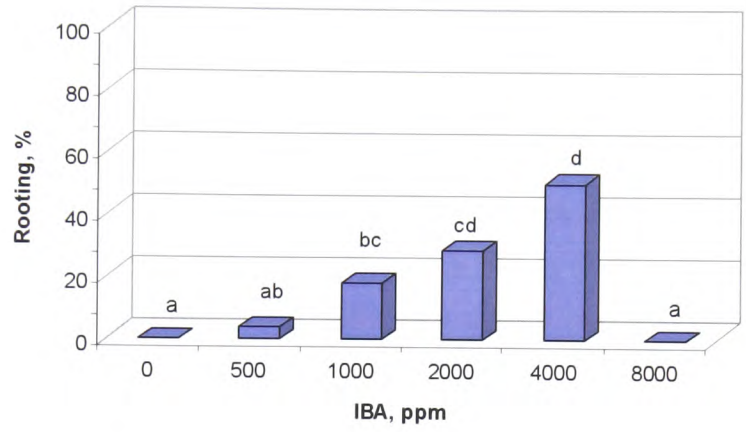


Figure 3.18 Effect of solutions of 0, 500, 1000, 2000, 4000 and 8000 ppm IBA on the rooting of *Staehelina petiolata* stem tip cuttings during November. Means with the same letters are not significantly different at $P = 0.05$ according to Tukey's test.

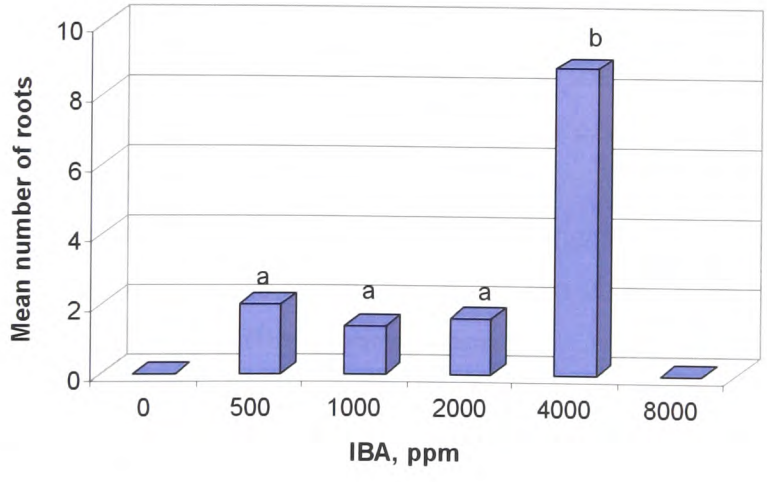


Figure 3.19 Effect of solutions of 0, 500, 1000, 2000, 4000 and 8000 ppm IBA on the number of primary roots of *Staehelina petiolata* stem tip cuttings during November. Means with the same letters are not significantly different at $P = 0.05$ according to Tukey's test.

3.4.5 Effect of different substrates on rooting of stem tip cuttings of *Staehelina petiolata*

In order to further optimize the propagation of *Staehelina petiolata*, the effect of rooting medium was studied, using optimal levels of IBA in powder to induce rooting.

Material and methods

On 10 June 2003, cuttings 10 – 15 cm in length with 4 - 6 leaves were taken from plants in the observation area and dipped in an aqueous solution of 1% pilazin (carpendazim). Then the cuttings were treated with 0.2% IBA powder at the base of the stem. The treated cuttings were inserted in seven different rooting media: peat moss, sand, perlite, vermiculite, peat moss + perlite (1:1, v/v), peat moss + sand (1:1, v/v) and peat moss + vermiculite (1:1, v/v) and were put in a mist environment. After 50 days the number of rooted cuttings and the number of roots from each cutting were recorded.

The experimental design was a randomized complete block with 5 cuttings per replication and 5 replicates per treatment (total cuttings 175).

Result and discussion

The best rooting percentage (60%) was observed in peat moss and peat moss+sand followed by the rooting medium vermiculite (52%) peat moss + vermiculite (52%) and sand (Figure 3.20). The lowest percentage rooting was recorded in perlite (40%) and peat moss + perlite (36%). Peat moss alone or with sand improved the rooting percentage of the cuttings of *S. petiolata* and significant differences were observed. These results are similar with the results during June for the perlite as substrate and the effect of IBA in powder form.

As far as the root number is concerned there is a statistically significant difference between the substrates of perlite alone or perlite + peat moss and the peat moss + vermiculite mixture, while no significant differences were observed among the other treatments (Figure 3.21). Therefore peat moss, vermiculite and sand or their mixtures were better substrates for rooting the *S. petiolata* cuttings than the perlite (Plate 3.9).

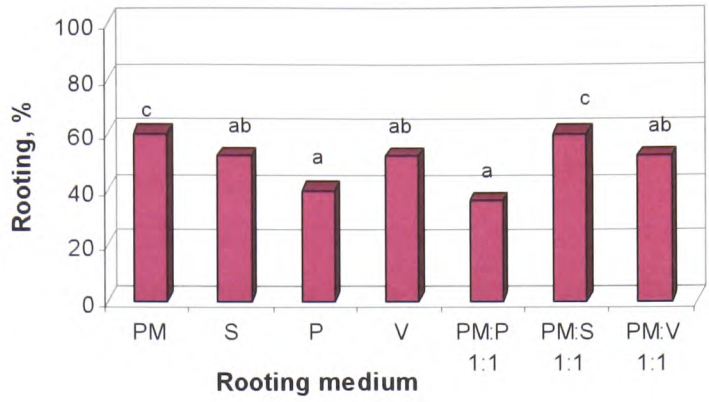


Figure 3.20 Effect of different substrates on the rooting of *Staehelina petiolata* stem tip cuttings. PM = Peat moss, S = Sand, P = Perlite, V = Vermiculite. Means with the same letters are not significantly different at $L = 0.05$ according to Tukey's test.

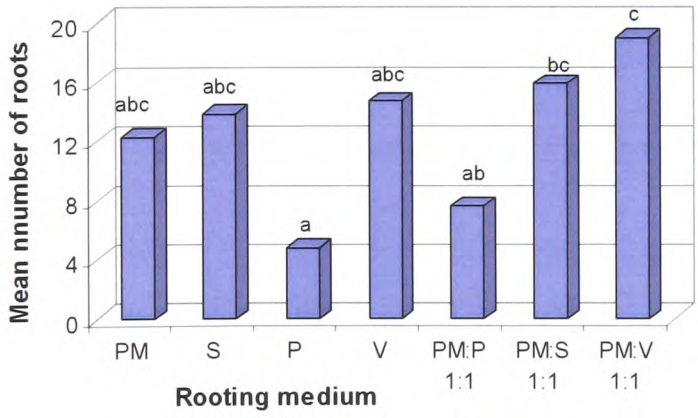


Fig. 3.21 Effect of different substrates on the number of primary roots per rooted cutting of *Staehelina petiolata*. PM = Peat moss, S = Sand, P = Perlite, V = Vermiculite. Means with the same letters are not significantly different at $L = 0.05$ according to Tukey's test.

3.4.6 Conclusion

Based on the results presented in this chapter and the previous experiments from 2001 and 2002, the propagation of *S. petiolata* by leafy stem tip cuttings is possible, but the use of IBA is essential for the promotion of rooting as none of the cuttings rooted without auxins.

Factors such as seasonal variation, origin and growth conditions of the plants, the concentration of IBA and the rooting medium affected the percentage rooting and the number of primary roots of rooted cuttings. The highest rooting percentage and the most numbers of roots per rooted cutting were obtained with material taken during autumn (November) compared with spring (April) and summer (June).

Concerning the substrates, peat moss, sand, vermiculite and their mixes were better than perlite or perlite and peat moss mix, regarding both the rooting percentages and the number of roots per rooted cutting. This may due to the fact that peat moss and vermiculite provide more moisture to the cuttings than perlite. Generally an appropriate propagation medium depends on the species, cutting type, season and propagation system (Hartmann *et al.*, 2002).

3.5 *IN VITRO* REGENERATION OF *STAEHELINA PETIOLATA*

3.5.1 Introduction

The aim of the *in vitro* regeneration experiments on *Staehelina petiolata* is to develop micropropagation techniques that will lead to the production of a plentiful supply of plant material from a small number of mother plants as well as the creating the potential for an approach for a breeding programme of the species. Thus suitable material will be produced in sufficient quantities for use as ornamental garden plants and there will be limited impact on the wild population and thus prevent its possible extinction.

3.5.2 *In vitro* propagation of *Staehelina petiolata* using vegetative stem explants

Experiments that follow have been conducted at least two times.

3.5.2.1 Effect of the growth regulators BA and NAA on *in vitro* proliferation of vegetative stem explants of *Staehelina petiolata.*

Experiment 1

Material and methods

Shoots of *Staehelina petiolata* growing in the area of Archanes were collected in June from two individual healthy plants. They were utilized as plant material for the study of *in vitro* proliferation, two days after their collection. The leaves were removed from their base and terminal and nodal stem segments from the shoots were used as explants to establish the *in vitro* culture.

Sterilization and explants preparation:

The shoot explants (1 - 1.5 cm long) were pre-washed with soapy water using commercial detergent. Then they were disinfected with 95% (v/v) ethanol for 10 sec. The surface disinfection with alcohol was followed by surface-sterilization with 10 % commercial bleach (containing 5% sodium hypochlorite) with Tween 20 (3 - 4 drops /100 ml solution) for 10 min and followed by rinsing three times with deionized sterilized water. The same process was repeated twice. After sterilization, stem nodal and inter-nodal segments with one or two buds and shoot tips were isolated using a sterile surgical knife and forceps in a laminar air-flow cabinet.

Media and culture procedure

Explants were cultured *in vitro*, to explore the possibility of developing a protocol for propagation via organogenesis. After sterilization the explants were transferred onto MSS (Chapter 2.6.2) supplemented with 0, 0.5, 1, or 2 mg/l BA and 0 or 1 mg/l NAA in all possible combinations. The pH of the media was adjusted to 5.8 prior to autoclaving. All media were autoclaved at 121°C for 15 min and stored in a media storage room until utilized. Explants were placed on the surface of the sterilized media and kept at a temperature of 24 ± 2°C and 16/8 h light/dark.

A total of 320 explants were used, 160 from each individual plant and twenty explants per treatment.

Experiment 2

Material and methods

Shoots were removed from one plant from the area of Archanes in July. The shoots were rinsed with tap water and were put in a vase with deionized water at room temperature for one week. The leaves were removed and segments from the tip and the middle of the shoots were used as explants to establish the *in vitro* culture.

The explants, 1 - 1.5 cm with 3 - 4 buds, were washed with soapy water and disinfected with 73% (v/v) ethanol for 30 sec., followed sterilization with 5% CaO_2Cl_2 for 20 min. Then the explants were rinsed three times with deionized sterilized water for 3, 3 and 30 min.

After the sterilization the explants were transferred onto MSS (MS with 3% sucrose) supplemented with 0, 1, 2, or 4 mg/l BA and 0 or 0.5 mg/l NAA in all possible combinations. The pH of the media was adjusted to 5.8 prior to autoclaving. All media were autoclaved at 121°C for 15 min and stored in a media storage room until utilized. Explants were placed on the surface of the sterilized media and kept at 24 ± 2°C temperature and 16/8 h light/dark.

A total of 80 explants were used, ten explants per treatment.

Experiment 3

Material and methods

The plant materials, in this experiment were shoot tips removed at the end of July from one year old seedlings grown in pots in the greenhouse. First the explants were surface sterilized with 95% ethanol for 30 sec and then with 10% CaO_2Cl_2 for 15, 20 or 30 min. The same substrates as in the previous experiment were used and 10 explants per treatment. A total of 80 explants were used, ten explants per treatment.

Experiment 4

Material and methods

In the middle of December stem tip cuttings from one year old seedlings were used as explants. They were disinfected with 70% ethanol for 1 min and 10% household bleach (final concentration NaOH 0.5%) with some drops of Tween 20, for 10 min. This sterilization procedure was repeated one more time. The MSS substrate was supplemented with BA at 0, 1, 2, or 4 mg/l and NAA at 0, 0.5, 1 or 2 mg/l in all possible combinations. Ten explants per treatment (160 explants) were embedded on each test tube contained 12.5-15 ml substrate.

Experiment 5

Material and methods

Two kinds of plant material were used in this experiment at the end of January:

- Shoots from 18 month old seedlings raised in pots in the greenhouse and
- Apical tips from 3 month old seedlings from pots in the greenhouse.

Apical segments of shoots with 2 - 4 buds were used as explants. The shoot explants were sterilized with 70% ethanol for 30 second and 10% CaO_2Cl_2 (plus 3 - 4 drops of Tween 20/100ml) for 15 min. The apical tips were sterilized with 70% ethanol for 30 second, followed by 5% CaO_2Cl_2 for 10 min (plus 3 - 4 drops of Tween 20 per 100 ml solution), both with agitation using a magnetic stirrer (SBS A160). The explants were rinsed three times for 3, 3 and 30 min with distilled sterilized water. From the base of each explant a small part was incised and discarded. After that, because of the exudation problem, each explant was put in distilled sterilized water for 2 - 3 min and then embedded in the substrate.

Modified McCown Woody Plant Medium (WPM) (McCown and Lloyd, 1981) with 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine-HCl and 0.1 mg/l thiamine-HCl, supplemented with BA at 0, 1, 2 or 4 mg/l and NAA at 0, 0.5, 1 or 2 mg/l, in all possible combinations, was used for *in vitro* cultures. Eight explants were used per treatment.

A completely randomized design was used in all five experiments.

Five weeks later the percentage uninfected and those explants which had responded in each treatment were recorded. A subculture followed on using the same modified WPM and six weeks later the proliferated explants were transferred onto MSS medium supplemented with BA at 1, 2 or 4 mg/l and BA/NAA 1/0.5 mg/l.

Results and discussion

The results regarding the sterilization of the explants are presented for all of the experiments in Table 3.5a.

Experiment 1 and 2

All of the explants were contaminated and those that were not infected showed no response within six weeks.

Experiment 3

A low percentage (2.5%) uninfected explants were left when the disinfection 10% CaO_2Cl_2 for 15 min was used with but no response to the media was recorded.

Experiment 4

Following double sterilization with NaOCl at 0.5% for 10 min a percentage explant survival of 3.75% was recorded. These explants came from the treatments BA/NAA 1/0.5 and 2/0.5 and produced 1 to 2 shoots per explant. After three subcultures in the same medium and one transfer to medium containing BA at 2 mg/l, 10 to 15 shoots were developed in each tube (data not shown).

Experiment 5

The highest percentage of uninfected explants was recorded with the 3-month old seedlings when they were disinfected with 5% CaO_2Cl_2 for 10 min and the best response on the 18 months seedlings, which were sterilized with 10% CaO_2Cl_2 for 15 min. All the proliferated explants produced callus at all combinations of BA/NAA, formed on the base of the explants and the surface of cut petioles (Plate 3.10). The largest amount of callus was observed on 4/1 and 4/2 mg/l BA/NAA. No response was recorded in the control and BA or NAA alone. After the subculture in the same culture medium (WPM) with the same concentrations of BA

or NAA no shoots developed after six weeks. The same results were recorded after the transfer to MSS medium after six more weeks.

Conclusions

Despite the fact that the number of surviving explants was small some useful results can be noted:

- Uninfected and proliferated explants were recorded only from the pot-grown plants in the greenhouse aged from 3 to 18 months. None of the explants from wild plants responded to the treatments.
- The optimal sterilizing chemical and concentrations were CaO_2Cl_2 at 5% for 10 min or 10% for 5 min, followed by the double sterilization with 0.5% NaOCl for 10 min.
- Best results regarding the disinfection were observed during winter.
- In all treatments with WPM containing no plant growth regulators or BA or NAA alone no proliferation was observed.
- Largest amount of callus was observed with 4/1 and 4/2 mg/l BA/NAA (Plate 3.10)
- No regeneration occurred from callus after two subcultures in WPM or MSS medium with or without growth regulators.
- Uninfected explants from one-year old plants, after double sterilization with 0.5% NaOCl for 10 min., produced shoots on MS medium supplemented with 1 or 2 mg/l BA and 0.5 mg/l NAA.

Table 3.5a Chemical disinfection and methods for sterilization vegetative explants, the percentage of uninfected explants and the response % of uninfected explants of *Staehelina petiolata*.

	Sub-strate	Explants	Alcohol		NaOCl		CaO_2Cl_2		Uninfected explants (%)	Response of explants (% of uninfected)
			%	Time sec	%	Time min	%	Time min		
1st Experiment	MSS	Shoots from wild plants	95	10	0.25	10	-	-	none	None
2nd Experiment	MSS	Shoots from wild plants	73	30	-	-	5	20	none	None
3rd Experiment	MSS	1 year seedlings	95	30	-	-	10	15	2.5	None
3rd Experiment	MSS	1 year seedlings	95	30	-	-	10	20	none	None
3rd Experiment	MSS	1 year seedlings	95	30	-	-	10	30	none	None
4th Experiment	MSS	1 year seedlings	70	60	0.5	10	-	-	3.75	100% produced shoots
5th Experiment	WPM	18 months seedlings	70	30	-	-	10	15	45.5	53.3% produced callus
5th Experiment	WPM	3 months seedlings	70	30	-	-	5	10	84.8	35.7% produced callus

MSS: MS plus 3% sucrose and 0.8% agar at pH=5.8

3.5.3 *In vitro* propagation of *Stehelina. petiolata* using seedlings

Despite the fact that many wild plants can be successfully cultured *in vitro* by making use of vegetative explants as stem and apical segments, leaves, parts of flowers, etc. (Dhar *et al.*, 2000; Nobre, *et al.*, 2000; Monteuuis and Bon, 2000; Sudhersan *et al.*, 2001) vegetative explants from *Staehelina petiolata* are difficult

to sterilise while uninfected explants have a low proliferation. In these cases as well as if the use of these explants leads to the destruction of a rare plant, then seeds can sometimes be used as a source of disease-free explants. The seeds can be germinated *in vitro* producing a microbial-free source of young apical tips or nodal tissue for micropropagation. Cotyledon, hypocotyls or young leaves can also produce directly adventitious shoots or initiate callus growth. Seedling shoot apices can be used to start shoot cultures.

3.5.3.1 Effect of growth regulators (BA and NAA) as well as the light on the proliferation of *Staehelina petiolata* cotyledons and hypocotyls explants.

Because of the very slow growth of seedlings of *S. petiolata*, cotyledons and hypocotyls were used as explants from 6 – 8-weeks old seedlings, in which the apical shoot had not yet appeared. Previous experiments (Antonidaki *et al.*, 2004) showed the effect of cytokinins and auxins on the differentiation of *Staehelina petiolata* tissue of seedlings. The aim of this experiment is to find out the optimum concentrations of BA and NAA as well as the conditions (light /dark) which promote callogenesis and/or organogenesis.

Materials and methods

Previous *in vitro* experiments with explants taken from field or greenhouse-grown plants resulted in highly infected or damaged explants. Therefore, seedlings from seeds derived from a single *Staehelina petiolata* plant were used as a source of explant material.

Seeds pre-washed with soapy water, were surface sterilized by using 80% ethanol for 15 sec and 0.75% NaOCl with a few drops of Tween 20 for 15 min. Seeds were then rinsed three times with sterilized distilled water for 5 minutes. Seeds were cultured on half strength MS basal salt mixture medium in glass tubes. The medium was stabilized with 8 g/l agar and the pH was adjusted to 6.0 prior to autoclaving. The seeds were incubated at 20 ± 2°C in 16 h photoperiod.

Cotyledons and hypocotyls were taken from six-week old seedlings from the above mentioned seeds. Cotyledons were cut by longitudinal sections on each of their side and the central core of each cotyledon was placed on the culture medium.

Hypocotyls with the apical bud were put upright on the medium after roots and cotyledons were removed.

All explants were cultured onto MSS basal medium supplemented with 0, 1, 2, or 4 mg/l BA and 0, or 0.5 mg/l NAA in all possible combinations and solidified with 0.8% agar adjusted to pH 5.8 prior to autoclaving. Half of the tubes were kept at 24 ± 2°C under 16h photoperiod and 36 μmol m^{-2} s^{-1} from fluorescence lamps and half covered by aluminium foil to eliminate the light. Cultures in the dark were moved to the light after 8 weeks. The produced shoots were subcultured on MSS medium with 1 mg/l indolobutyric acid (IBA).

Results and discussion

Seeds germinated *in vitro* after ten days at 20°C. Six weeks later no shoots were developed from the main bud. Seedlings without visible microbial contaminations were chosen as explant material.

Proliferation under light

Friable soft callus formation, white to cream, was recorded three to four weeks after embedding both hypocotyl and cotyledon explants in all cultures containing NAA. The hypocotyls alone produced shoots in all of the media treatments, with the presence of compact callus (Table 3.6). The shoots from MSS medium without growth regulators produced leaves typical of the species, while the leaves from the treatments with BA had different characteristics. Their leaves were smaller with a light green stripe around the leaf margin. Roots were formed on hypocotyls in MSS medium .with 0.5 mg/l NAA or without growth regulators while on cotyledons in MSS medium only with 0.5 mg/l NAA (Table 3.6). Roots were formed also from shoots subcultured in 1mg/l IBA after 10 days (Plate 3.10 and 3.11).

Proliferation in darkness

The bases of hypocotyls and cotyledons cultured on MSS medium supplemented with BA became fleshy. Callus, white to creamy in colour, was produced by all treatments containing NAA. This further proliferated after the first subculture reaching up to 2 cm in diameter while its central region turned black, but no

differentiation was observed. Best growth of callus was recorded on MSS with 2/0.5 mg/l BA/NAA

All explants rooted on MSS supplemented with 0.5mg/l NAA (Table 3.7). In the dark, the sprouting of the main tip buds was delayed and they only sprouted when they had been moved to the light after 8 weeks.

Table 3.6 Effect of BA and NAA on the *in vitro* induction of shoots, callus and roots from *S. petiolata* hypocotyls and cotyledons explants under 16h photoperiod, after 6 weeks. (N=12).

Substrates	Hypocotyl explant			Cotyledon explant		
BA/NAA	Shoots %	Callus %	Roots %	Shoots %	Callus %	Roots %
0/0	83.3	0	14.3	0	0	0
1/0	90.9	0	0	0	0	0
2/0	90.0	0	0	0	0	0
4/0	72.7	0	0	0	0	0
0/0.5	63.6	100	72.7	0	100	50
1/0.5	80.0	100	0	0	100	0
2/0.5	83.3	100	0	0	100	0
4/0.5	83.3	100	0	0	100	0

Table 3.7 Effect of BA and NAA on the *in vitro* induction of shoots, callus and roots from *S. petiolata* hypocotyls and cotyledons explants in dark in six weeks. (N=12).

Substrates	Hypocotyl explant			Cotyledon explant		
BA/NAA	Shoots %	Callus %	Roots %	Shoots %	Callus %	Roots %
0/0	0	0	0	0	0	0
1/0	0	0	0	0	0	0
2/0	0	0	0	0	0	0
4/0	0	0	0	0	0	0
0/0.5	0	100	100	0	100	100
1/0.5	0	100	0	0	100	0
2/0.5	0	100	0	0	100	0
4/0.5	0	100	0	0	100	0

3.5.3.2 Effect of BA and NAA on the number and length of shoots and roots induced from apical buds *Staehelina petiolata* seedlings.

Materials and methods

Six-week old seedlings were used to initiate tissue cultures. The roots and cotyledons were removed to leave the hypocotyls with the apical bud of the seedling. This was used as the explant and was put upright in the culture media.

Plant tissue culture media were developed from MSS medium supplemented with 0, 1, 2 or 4 mg/l BA and NAA at 0 or 0.5 mg/l in all possible combinations. Twelve replicates were used for each treatment and the explants were subcultured six weeks after transplanting to the culture medium. The produced shoots were transferred to MSS medium with 1 mg/l IBA.

The number and length of shoots as well as the number of roots were recorded twelve weeks after establishing the experiment. All cultures were incubated at 24 ± 2°C and 16 h photoperiod. Rooted shoots were transferred for acclimatization (Stage IV see: Chapter 1.1.3.3) in intermittent mist in greenhouse.and compost using as soil medium.

Results and discussion

Two to three weeks after culture initiation, shoots appeared from the tip of the explants while adventitious bud and shoots formed within five weeks.

The commonly observed cytokinin effects of shoot proliferation i.e. reduction of stem growth and inhibition of root formation were observed in *S. petiolata*. No shoot proliferation was observed in the explants on MSS medium without plant growth regulators (BA or NAA). The addition of BA, NAA or both in the MSS basal medium stimulated the proliferation of multiple shoots (Table 3.8 and Figure 3.22). Treatments containing 2 and 4 mg/l BA produced the higher number of shoots as well as white to green nodules but with reduced length of shoots (Figure 3.23). No significant differences in number of shoots were recorded between 2 and 4 mg/l BA. Treatments containing 1 or 2 mg/l BA produced lower number of shoots than those of 4 mg/l but the shoots had a higher growth rate. The addition of NAA reduced the level of shoot proliferation compared to treatments with BA alone. Root induction was recorded in the treatment with 0.5 mg/l NAA and without growth regulators. Roots were formed from shoots subcultured in 1mg/l IBA ten days after they were embedded in the medium. Rooted micro-shoots were acclimatized successfully in rock wool and low intensity lighting at 24 ± 2°C and in greenhouse (Plates 3.10 - 3.12).

Table 3.8 Effect of BA and NAA on the number and length of shoots and number of roots using bud explants from *in vitro* seedlings of *Staehelina petiolata*,

Treatment BA/NAA	Mean number of shoots ± sd	Mean length of stem ± sd (mm)	Mean number of roots ± sd
0/0	1.0 ± 0.0	6.3 ± 2.4	5.5 ± 2.2
1/0	6.2 ± 2.3	4.4 ± 1.2	0.0 ± 0.0
2/0	11.6 ± 6.2	3.1 ± 0.6	0.0 ±0.0
4/0	12.3 ± 4.0	2.11 ± 1.0	0.0 ± 0.0
0/0.5	2.4 ± 1.0	3.7 ± 1.4	8.3 ± 3.1
1/0.5	1.7 ± 1.2	2.8 ± 1.5	0.0 ± 0.0
2/0.5	1.5 ± 1.4	2.8 ± 2.4	0.0 ± 0.0
4/0.5	1.8 ± 1.6	1.7 ± 1.4	0.0 ± 0.0

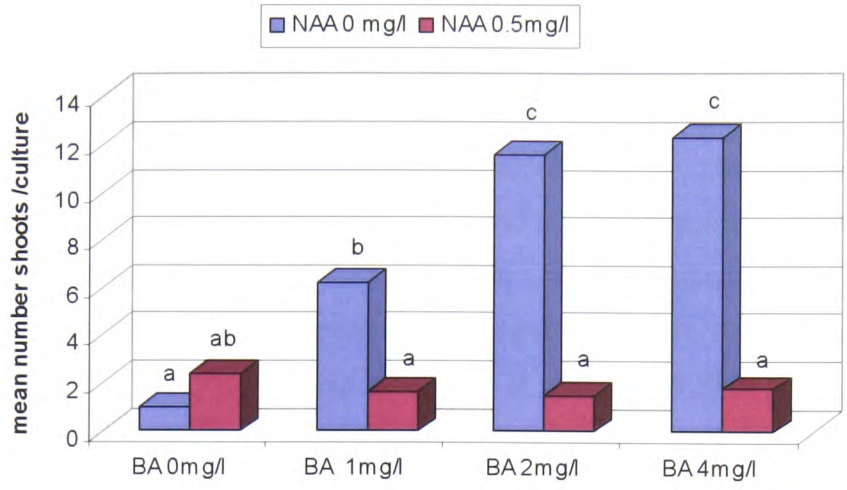


Figure 3.22 Effect of BA and NAA on the number of shoots of *Staehelina petiolata in vitro*, using seedling bud explants on MSS medium. Means with the same letters are not significantly different at $P = 0.05$ according to Tukey's test.

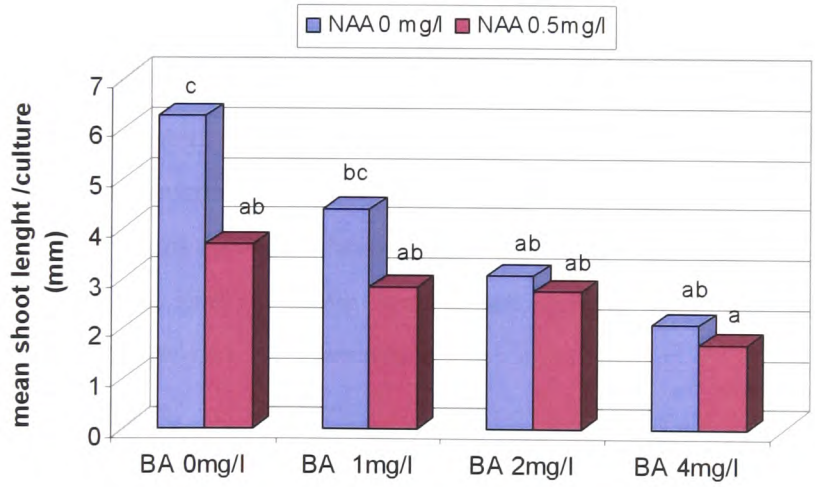


Figure 3.23 Effect of BA and NAA on the mean length of shoot of *Staehelina petiolata in vitro*, using seedling bud explants on MSS medium. Means with the same letters are not significantly different at $P = 0.05$ according to Tukey's test.

3.5.3.3 Shoot differentiation from callus induced from cotyledons and hypocotyls of *Staehelina petiolata* seedlings.

Callus is, in principle, a non-organized and little differentiated tissue; however differentiated tissue can be present especially in larger clumps of callus tissues. An exogenous supply of regulators is often recommended to initiate callus formation

on an explant. The exogenous regulator requirement depends on the genotype and endogenous hormone content. Two types of regulators are generally used: auxin and cytokinin alone or in combination (Pierik 1998). Auxin at a moderate to high concentration is the primary growth substance used to produce callus. The principal auxins include IAA, NAA and 2,4-D in increasing order of effectiveness. Cytokinin is supplied also if there is not an adequate source within the explant. Many other factors are important for callus induction, including genotype, composition of nutrient medium and physical growth factors (temperature, light, etc.).

Callus is produced on explants *in vitro* as a response to wounding and growth substances present either within the tissue or supplied in the medium. Explants from almost any part of the plant can be excised, disinfected and induced to form callus. Organogenesis begins with dedifferentiation of parenchyma cells to produce centres of meristematic activity.

Callus tissue originating from juvenile and/or herbaceous material generally regenerates much better than material from adult and/or woody plants. The regeneration capacity of cells and callus tissue may reduce or be completely lost if growth is continued for too long. Some plants are only capable of forming adventitious organs and no embryos from callus whereas others behave entirely the opposite (Pierik 1998). Some species are capable of regenerating by either adventitious shoots or adventitious embryos.

Callus is usually more capable of regenerating adventitious roots than adventitious shoots. Root formation generally takes place in a medium containing auxins and low cytokinin concentration. On the contrary, adventitious shoot formation can appear in callus tissue if there is a low auxin concentration and a high cytokinin concentration. BA is the most effective cytokinin for inducing the formation of adventitious shoots. Many other factors can promote the induction of adventitious shoots on callus such as a high salt and ammonium concentration.

Callus and cell cultures are useful as methods for commercial propagation because of the high rate of multiplication and the possibility industrialization. In practice,

these methods have not been used directly, however, because significant amounts of genetic and/or epigenetic aberrations tend to develop during cell multiplication (Hartmann, *et al.*, 2002). This genetic variation induced in plants produced by *in vitro* culture methods is known as somaclonal variation.

Somaclonal variation is particularly interesting in plants which naturally show little variation or in which variation is difficult or impossible to induce. Plant breeders have used somaclonal variation in a few plant species (sugar cane, potato, and tomato). In recent years researchers have directed increasing effort towards the isolation of mutants from plant tissue culture produced as a result of somaclonal variation (Pierik, 1998).

Material and methods

Seeds of *S. petiolata* were aseptically germinated *in vitro*. After six weeks of growth in 16 h light or in continuous darkness, cotyledons and hypocotyls from each seedling were sectioned and put in the same tube (one seedling in each tube). They were cultured on MSS medium supplemented with 2/0.5 mg/l BA/NAA. The medium was stabilized with 8 g/l agar and the pH adjusted to 5.8 prior to autoclaving.

After four weeks all cultures produced callus which was subcultured on to the same medium. Within four weeks callus covered all the surface of the medium in the tubes. Five weeks later callus was transferred on to MSS medium free of growth regulators. Half of the cultures were incubated under light/dark (16/8h light/dark) at 25 ± 2°C and half of them in continuous dark. A third transfer followed after five weeks on MSS with BA at 0.1, 0.5, 1, 2 or 4 mg/l. Shoot number was recorded six weeks after the last transfer as well as the percentage of proliferation.

Results and discussion

Callus was produced from all hypocotyls and cotyledons. The more the BA the medium contained the more hard the callus produced while the callus turned green. Green nodules appeared on the surface of the callus which later formed shoots. The best regeneration and number of shoots was observed in the 4 mg/l BA treatment (Table 3.9). Callus produced and maintained in the dark became grey to black and no regeneration from callus was observed.

Table 3.9 Effect of BA concentration on shoot regeneration from callus induced from cotyledons and hypocotyls of *Staehelina petiolata* seedlings under L/D conditions.

BA concentration	Regeneration of explants (%)	Mean number of shoots
0.1 mg/l	0	0
0.5 mg/l	66.6	0.9
1 mg/l	73.3	1.1
2 mg/l	75.5	1.5
4 mg/l	100	1.7

3.5.3.4 Somatic embryogenesis

Introduction

Somatic embryogenesis (SE) is the development of embryos from vegetative cells and tissues within *in vitro* systems in which the haploid or diploid somatic cells develop into differentiated plants through characteristic embryological stages without fusion of gametes (Sharp *et al.*, 1980; Raghavan, 1986). Somatic embryos differ from sexually derived zygotic embryos in general significant ways. Somatic embryos are clones or copies of the mother plant, while zygotic embryos show diversity from the fusion of sexual gametes (Hartmann *et al.*, 2002).

Direct and indirect embryogenesis are the two ways of obtaining somatic embryos in culture. Direct embryogenesis in culture proceeds from cells which are already programmed for embryogenic development prior to explanting, i.e. they are pre-embryogenic determined cells requiring only growth regulators or favourable conditions to allow their release into cell division and expression of embryogenesis. By contrast, indirect embryogenesis requires re-determination of differentiated cells, callus proliferation and the development of the embryogenetically determined state (Sharp *et al.*, 1980).

Germination, the process in which the embryo is awakened, is necessary for getting complete plants and it requires specific physical conditions. Similarly, the somatic embryos, although developed artificially need precise cultural conditions for

initiation and development of shoots and roots leading to complete plantlet formation (George, 1993/6).

Factors affecting somatic embryogenesis are:

Genotype

SE of cultured tissues and organs is more influenced by genotype than by any other factor. There are numerous examples in the literature in which the results of during SE studies have varied from one variety of plant to another. It is probably true to say that effects of genotype impose one of the greatest constraints to successful SE as much as to the tissue culture and micropropagation of plants (George, 1993/6).

Explant material

The choice of the explant material appears to be one of the most critical determinants for SE initiation. For the indirect SE of dicotyledons, young leaves are generally suitable for culture as well as epidermal and pith tissue. In the case of indirect SE, certain regions of the plant body can be chosen for their abilities to respond to cultural conditions and competence to produce embryogenic calli and embryoids (Novak and Konecna, 1982). Callus tissue originating from juvenile and/or herbaceous material generally regenerates much better than material from adult and/or woody plants (Pierik, 1998)

Nutrient media

The choice of nutrient media for the particular culture stages is of key importance for the induction of somatic embryos (George, 1993/1996). A survey of the literature shows that a large number of different, but specific, *in vitro* nutrient media have been used for the induction of SE in individual plant species. MS is the most often used for SE (Mathew and Philip, 2003; Pindo *et al.* 2002). Many of these media are common and have been used with only slight modifications. For many species, one medium is used for the initial callusing phase and for maintenance of the callus or suspension cultures; a second medium is then used for somatic embryo maturation; and in many cases a third is then employed to allow the normal growth of SE structures into plants (Steward *et al.*, 1967).

Hormones

Auxins are supplied in media for the induction of embryogenic callus, mainly at the first stage of SE. The most commonly reported auxin used in media to culture monocotyledons is 2,4-Dichlorophenoxy acetic acid. More recently, auxins such as IAA and NAA, have been used as more effective in some dicotyledons species (George, 1993/6). Cytokinins have been very important, mostly in dicotyledons, where they are applied in low concentrations (0.1 - 10μM). Zeatin, kinetin and BA, either singly or in combination have mostly been used.

Natural extracts Coconut water milk is one of the most commonly used natural extracts in plant tissue culture.

Material and methods

Plant material for callus induction

The explant material used for callus induction experiments was the following:

- whole seedlings 1-3 weeks old (apart from the roots) from seeds germinated *in vitro*;
- cotyledons from 2-3 weeks seedlings of seeds germinated *in vitro*, and
- hypocotyls from 2-3 weeks seedlings of seeds germinated *in vitro*.

Tissue culture media and growth regulators for callus induction

The basal MSS medium supplemented the following auxins with various concentrations of cytokinins to induce callogenesis were studied:

- 0.5, 1 or 2 mg/l NAA, and in combination with BA at 1/0.5 or 2/0.5 mg/l BA/NAA;
- 1, 2 or 4 mg/l 2,4-D, with or without the addition of Coconut Water;
- 1, 2 or 4 mg/l 2,4-D, with the combination of 0.1 mg/l BA.

Tissue culture media and growth regulators for somatic embryo germination

The follow culture media were used in all possible combinations:

1. MSS solidified with 6.5 g/l agar, containing BA at 0, 0.5 alone or in combination with NAA at 0, or 0.5 mg/l.

2. MS with 20 g/l sucrose solidified with 6.5 g/l agar, containing either BA at 0, 0.1, 0.2 and 0.5 mg/l alone or in combination with NAA at 0, 0.1, 0.2 or 0.5 mg/l and coconut water (5%).
3. Liquid MS with 20 g/l sucrose containing either BA at 0, 0.1, 0.2 and 0.5 mg/l alone or in combination with NAA at 0, 0.1, 0.2 or 0.5 mg/l with or without coconut water (5%).

Treatments of cultures using liquid plant tissue culture medium were maintained on a KL2 shaker (Edmund Buhler7400 Tubingen) set at 130 rpm to ensure the optimal dispersion of the medium during incubation (Plate 3.13). The cultures were incubated either in the dark or in the light under 36 μmol m^{-2} s^{-1} and 16 h photoperiod.

Results and discussion

Effect of different tissue culture media and growth regulators on callus induction

The effect of 2.4-D to induce callogenesis was negative for all types of explants used. That part of the explant in contact with the medium with 2.4-D became swollen but no callus was induced. The cotyledons and hypocotyls as explants responded well to NAA in all concentrations and combinations with BA. Callus induced from NAA alone was soft and friable, its colour remained white to pale cream and its growth was quick (Plate 3.10). Callus induced from the combination of BA/NAA was harder and slower growing than with NAA alone. No essential differences were recorded between callus induced in light or dark.

Effect of plant tissue culture media, growth regulators and other substances on somatic embryogenesis.

None of the explants on liquid or solidified medium, with growth regulators or other substances, under light or in dark induced somatic embryogenesis.

3.5.3.5 Conclusions for *in vitro* culture of *S. petiolata*

A variety of *in vitro* techniques was examined to propagate *S. petiolata* from vegetative explants with varying degrees of success. A considerable amount of

effort was devoted to producing sterile explant material. The best source of vegetative explants for the *in vitro* propagation of *S. petiolata* came from one to one half year old plants maintained in a healthy state in the greenhouse. Sterilization of the explants proved difficult but success was achieved with succeeding with 5 and 10% CaO_2Cl_2 for 5 - 10 min or 0.5% NaOCl at for 10 min during winter.

The best results for producing shoots and roots were obtained from culturing in MSS with the addition of 1 - 2 mg/l BA and 0.5 mg/l NAA, respectively. Stem explants from vegetative plants produced callus but did not induce axillary shoots on the WPM medium.

Adventitious and auxiliary shoots could be obtained by the addition of BA and NAA on seedling apical buds. The inclusion of BA on explants induced shoots but reduced stem growth and inhibited the root formation. NAA at 0.5 mg/l and 1 mg/l IBA promote the induction of the roots in shoots produced in vitro from cotyledons and hypocotyls. Adventitious shoots, derived from callus produced by the effect of 2/0.5 mg/l BA/NAA, can be induced after 4 - 5 months under light and the affect of 0.5 – 4 mg/l BA. However, the shoots produced by the affect of high concentration BA formed smaller leaves which reverted to the typical form of the species after culturing on medium free of growth regulators (Plate 3.12). Importantly, rooted micro-shoots were readily acclimatized in intermittent mist in greenhouse (Plate 3.12).

It was not possible to induce somatic embryos via callus. According to Thomas and Davey (1975) as cited by Pieric (1998), some plant species are capable of regenerating by either adventitious shoots or adventitious embryos. This issue should be investigated further more using new plant material as well as growth medium and growth regulators.

3.6 GROWTH AND ADAPTATION

3.6.1 Culture requirements

Having observed the growth of *Staehelina petiolata* during the last three years in its habitat as well as the results of propagation experiments, there appears to be a problem with its low growth rate. The next experiment was designed to investigate this problem and to know the relationship between the plant development and the growing media and fertilization, measuring several growth parameters. This area of research into the culture requirements for *S. petiolata* has not been covered in any published references.

3.6.1.1 Effect of the soil medium and the fertilization on the growth rate of *S. petiolata.*

The present work is a study on the growing media and fertilization needed for producing an appealing pot plant with a quick growth rate suitable for commercial use in landscape architecture.

Material and methods

Four-month old seedlings, produced from the seed propagation experiments, were used as plant material. The seeds were sown at the end of November in plastic pots filled with trade compost (Florabella).

Uniform seedlings were re-potted in one litre (15 cm) plastic pots (one plant per pot) in the last week of March with three different soil media: Peat Moss:Perlite 1:1 (v/v), Peat Moss:Perlite: 2:1 (v/v) and Trade Compost (Florabella)

Trade compost had a pH of 6.0, while for the two media containing peat moss and perlite it was necessary to adjust the pH to 6.0 - 6.2 through the addition of limestone.

Two fertilization treatments were applied to the above soil media: 1 and 2 g/l N-P-K (20-20-20). Fertilization started two weeks after transplanting. Half of the plants of each medium were fertilized with 1 g/l and the other half with 2 g/l. Fertilizers were supply in water solution (120 ml per each pot) once a week.

In total six treatments resulting from all combinations of three media and two concentrations of fertilizers were applied. The experiment was conducted using 4 randomized blocks of six plots each containing 7 plants per treatment and block. Three of the blocks were placed in separate benches in an unheated glasshouse and one under a shading frame. The experiment was composed of 168 plants.

After one, three and six months from planting, the plant height (the length from the surface of the soil to the higher upper point of the plant), the canopy diameter and the number of lateral shoots were measured. The significance of the results was tested by two way analysis of variance (F test) and the treatment means were compared using Tukey's test (HSD, Honest Significant Difference) at the 5% level of probability (P=0.05).

Results and discussion

Compost promoted significantly the elongation of the shoots and canopy diameter at both rates of fertilization after six months of cultivation (Tables 3.10 and 3.11). Fertilization at 1 g/l produced plants with significantly more lateral shoots than the fertilization at 2 g/l after three and 6 months while medium did not effect on the number of shoots (Table 3.12). There were significant differences for the number of leaves among the compost combined with 1g/l fertilizer and the other medium after 3 and 6 months (Table 3.13; Plates 3. 14 - 3. 16).

In conclusion, the best results came from the treatment with compost and the application of 1g/l N-P-K fertilizer (20-20-20) for all parameters examined. This was shown also by the better aesthetic value of the plants in this treatment. In contrast, the growth rate of the plant continued to be slow, which is a disadvantage for ornamental plants to promote to the market. More efforts have to be made to select more suitable material and to investigate further the environmental conditions which could promote growth.

Table 3.10 Effect of potting medium (m) and fertilization (f) on the height of *Staehelina petiolata* plants. Means of 4 replicates (7 plants per replicate and treatment). f_1=fertilization at 1g/l, f_2=fertilization at 2g/l, m_1=compost, m_2=peat moss:perlite 1:1, m_3=peat moss:perlite 2:1.

Mean increase of the plant height in 1, 3 and 6 months after re-potting (cm)			
Treatment	After 1month	After 3months	After 6months
$f_1 \times m_1$	1.88a	3.85a	6.025c
$f_1 \times m_2$	1.31a	2.38a	2.705ab
$f_1 \times m_3$	2.36a	3.90a	4.320bc
$f_2 \times m_1$	2.56a	4.16a	5.263c
$f_2 \times m_2$	1.55a	2.50a	2.502ab
$f_2 \times m_3$	1.66a	1.95a	1.952a
F_{fxm}	3.267^{NS}	4.895*	4.515*
f_1	1.85a	3.38a	4.350b
f_2	1.93a	2.87a	3.239a
F_f	0.106^{NS}	2.368^{NS}	13.236**
m_1	2.22b	4.01b	5.644 b
m_2	1.43a	2.44a	2.604 a
m_3	2.01ab	2.93ab	3.136 ab
F_m	4.390^{*}	7.998**	37.692***

^{NS}=not significant, *=significant at $P \leq 0.05$, **=significant at $P \leq 0.01$, ***=significant at $P \leq 0.001$. Mean separation in column by HSD at $P=0.05$.

Table 3.11 Effect of potting medium (m) and fertilization (f) on the mean increase of the diameter of *Staehelina petiolata* plants. Means of 4 replicates (7 plants per replicate and treatment). f_1=fertilization at 1g/l, f_2=fertilization at 2g/l, m_1 =compost, m_2=peat moss:perlite 1:1, m_3=peat moss:perlite 2:1.

Mean increase of the canopy diameter in 1, 3 and 6 months after repotting (cm)			
Treatment	After 1month	After 3months	After 6months
$f_1 \times m_1$	1.775a	6.011 b	7.393 b
$f_1 \times m_2$	1.821a	2.511 a	2.661 a
$f_1 \times m_3$	2.589a	3.800 ab	4.221a
$f_2 \times m_1$	2.221a	5.307 b	6.868 b
$f_2 \times m_2$	1.614a	2.714 a	2.714 a
$f_2 \times m_3$	1.993a	2.246 a	2.246 a
F_{fxm}	3.090^{NS}	1.609^{NS}	2.132^{NS}
f_1	2.062a	3.504a	4.758a
f_2	1.943a	3.423a	3.943a
F_f	0.473^{NS}	2.930^{NS}	3.896^{NS}
m_1	1.998a	5.659b	7.130b
m_2	1.718a	2.613a	2.688a
m_3	2.291a	3.023a	3.234a
F_m	3.656^{NS}	22.784***	45.850***

^{NS}=not significant, *=significant at $P \leq 0.05$, **=significant at $P \leq 0.01$, ***=significant at $P \leq 0.001$. Mean separation in column by HSD at $P=0.05$.

Table 3.12 Effect of potting medium (m) and fertilization (f) on the number of lateral shoots of *Staehelina petiolata*. Means of 4 replicates (7 plants per replicate and treatment). f_1=fertilization at 1g/l, f_2=fertilization at 2g/l, m_1=compost, m_2=peat moss:perlite 1:1, m_3=peat moss:perlite 2:1.

Mean number of lateral shoots of the plants in 1, 3 and 6 months after repotting			
Treatment	After 1month	After 3months	After 6months
$f_1 \times m_1$	0.536a	2.607a	3.214a
$f_1 \times m_2$	0.536a	2.321a	2.250a
$f_1 \times m_3$	0.679a	2.107a	2.321a
$f_2 \times m_1$	0.571a	1.429a	1.571a
$f_2 \times m_2$	0.786a	1.607a	1.607a
$f_2 \times m_3$	0.321a	1.036a	1.036a
F_{fxm}	0.722^{NS}	0.140^{NS}	0.511^{NS}
f_1	0.583a	2.345b	2.595b
f_2	0.560a	1.357a	1.405a
F_f	0.013^{NS}	6.926*	8.461*
m_1	0.554a	2.018a	2.393a
m_2	0.661a	1.964a	1.929a
m_3	0.500a	1.571a	1.679a
F_m	0.204^{NS}	0.562^{NS}	$1,046^{NS}$

^{NS}=not significant, *=significant at $P \leq 0.05$, **=significant at $P \leq 0.01$, ***=significant at $P \leq 0.001$. Mean separation in column by HSD at $P=0.05$.

Table 3.13 Effect of potting medium (m) and fertilization (f) on the number of leaves of *Staehelina petiolata*. Means of 4 replicates (7 plants per replicate and treatment). f_1=fertilization at 1g/l, f_2=fertilization at 2g/l, m_1 =compost, m_2=peat moss:perlite 1:1, m_3=peat moss:perlite 2:1.

Mean number of the leaves of plants in 1, 3 and 6 months after repotting			
Treatment	After 1month	After 3months	After 6months
$f_1 \times m_1$	9.429a	13.464b	18.107c
$f_1 \times m_2$	8.607a	7.214a	8.143a
$f_1 \times m_3$	10.000a	7.714a	8.786ab
$f_2 \times m_1$	9.143a	10.071ab	14.107bc
$f_2 \times m_2$	8.964a	8.857a	8.571ab
$f_2 \times m_3$	9.357a	8.536a	8.357ab
F_{fxm}	0.547^{NS}	3.740*	5.097**
f_1	9.464a	9.46a	11.679a
f_2	9.155a	9.16a	10.345a
F_f	0.232^{NS}	0.147^{NS}	1.942^{NS}
m_1	9.286a	11.768b	16.11b
m_2	8.786a	8.038a	8.357a
m_3	9.679a	8.125a	8.571a
F_m	1.707^{NS}	9.293**	28.382***

^{NS}=not significant, *=significant at $P \leq 0.05$, **=significant at $P \leq 0.01$, ***=significant at $P \leq 0.001$. Mean separation in column by HSD at $P=0.05$.

3.6.2 Adaptation and survival of *Staehelina petiolata* in urban landscape

The aim of this experiment is to investigate the adaptation and survival of the species that have been produced either by the means of sexual or asexual reproduction in the greenhouse reported above.

An experimental plan was designed for the six herbaceous and woody native plants investigated in this research using part of the trial park of TEI in Heraklion. The allocated land for these experiments was 385m^2 located in the east part of the park (Appendix 10).

Material and methods

Plants of *S. petiolata*, 1.5 years old, which were derived from seed germination and cultivated in pots in an unheated greenhouse, were planted in the experimental park of Technological Educational Institute in Heraklion in two rows. Distance between the plants was 1 m and between the rows 2.60 m. Twenty six plants were planted in the first week of March 2005.

Water was supplied one or two times a week depending on the needs. Frequent watering was required the two first months until the plant were established and during the hot months of summer. Fertilization was supplied with the watering, once a month, with N-P-K (20-20-20) and additional iron. From the results of soil analysis conducted by the Soil Science Laboratory of the School of Agriculture Technology it appeared that the planting area has a Loam soil with neutral to slightly alkaline pH (pH=7.3), of 2.1mS/cm electro-conductivity, with high percentage of $CaCO_3$ (41%) and minimum organic substances, and sufficiency of K and P (Appendix 2).

A mixed soil medium was prepared containing peat moss : perlite : soil - 2 : 1 : 2 (v/v) in which 1.5 kg ammonium nitrate, 0.5 kg potassium nitrate and 3 kg slow release fertilizer (Ferti-Feed 18N-6P-12K) was added per m^3. During the planting 5 litres of soil medium was added individually in each planting hole in order to increase the organic matter of the soil. Six months after the planting, the number of survived plants, the height, diameter and number of lateral shoots were measured

Results and discussion

From the 26 plants, 23 survived (88.5%) six months after the date they were planted. The morphological characteristics of their leaves such as size and colour were similar with those in their natural habitat. The extent of the growth of the plants, recording height and diameter, were similar to those in the pots in the glasshouse for the same time of six months (Table 3.14). In contrast, the number of shoots was larger in the ground than in the pots. During the cultivation in the plot no problems from pests and diseases were observed (Plate 3.17). Thus, *Staehelina petiolata* can adapt easily in pots and in artificial landscape and no problems of pests or diseases occur during cultivation in the gardens.

Table 3.14 Mean increase in height, canopy diameter and number of shoots of *Staehelina petiolata* after 6 months cultivation in the experimental plot in the park of TEI. Mean ± sd. N=29.

	Initial means	6 months means	Increase
Height (cm)	8.90 ± 1.33	14.09 ± 1.81	4.83 ± 1.94
Diameter (cm)	9.34 ± 2.74	23.74 ± 3.77	14.68 ± 3.94
Number of shoots	1.16 ± 1.02	6.96 ± 2.16	6.00 ± 1.93

3.7 CONCLUSION

Staehelina petiolata an endemic plant of Crete has those characteristics and the aesthetic value that could be exploited as an ornamental landscape plant. Based on this research, it can be seen that propagation by seed is easier 2 - 3 months after the release of the seeds in winter in trade compost (reaching up to 92.5%), than in summer and in mix peat moss + perlite. Germination *in vitro* was maximum (up to 99%) at 10, 15 and 20°C under light or dark conditions. However at 25°C better germination was recorded in darkness than under light.

The rooting of the cuttings was affected by the season of the year, the genotype, the substrate and the concentration of IBA. Best results were acquired in autumn after the rainfalls at 4000 ppm IBA (rooting up to 53.5%). Peat moss, vermiculite, sand

and their mixes proved better substrates than perlite in June with 2000 ppm IBA in powder form (rooting up to 60%).

The sterilization of the vegetative explants was not very successful. Adventitious and auxiliary shoots could be obtained by the addition of BA and NAA on seedling apical tips. More shoots (12.3 / explant) were produced at 4 mg/l BA but better growth was observed at 1 and 2 mg/l BA. Adventitious shoots produced from callus by the affect of BA/NAA at 2/0.5 mg/l after 4-5 months under light. NAA at 0.5 mg/l and IBA at 1mg/l promote the induction of the roots in shoots produced *in vitro* while fewer shoots were rooted in MS without hormones. Rooted micro-shoots were acclimatized successfully in rock-wool in the growth room as well as in intermittent mist in the greenhouse.

Despite the aesthetic value and the fact that *S. petiolata* is adapted easily in urban landscape its slow growth rate remains a disadvantage for exploiting as an ornamental and garden plant. Further studies are required on the environmental conditions and management which could promote growth.

4 *PTILOSTEMON CHAMAEPEUCE* (L.) LESS

4.1 INTRODUCTION – LITERATURE REVIEW

Ptilostemon chamaepeuce, known as "shrubby ptilostemon", is described as a spineless evergreen shrub due to the fact that most of the other species in the same genus have spines (Blamey and Grey-Wilson, 1993).

The genus *Ptilostemon* according to Tutin *et al.* (1964 - 1980) comprises the following nine species in Europe:

1. *P. chamaepeuce* which thrives in East Mediterranean comprising Greece (the Aegean island and Crete) and Cyprus.

2. *P. afer* which thrives in the mountains of the Balkan peninsula including Greece.

3. *P. gnaphaloides* in Greece including Crete and southern Italy

4. *P. niveus* in Italy

5. *P. echinocephalus* in Russia.

6. *P. hispanicus* in the Iberian peninsula.

7. *P. strictus* in the Balkan peninsula comprising Greece and Italy

8. *P. stellatus* is an annual plant, in the Mediterranean comprising Crete.

9. *P. casabonae* in the Iberian Peninsula and Italy.

There are also recorded 10 more world-wide species of *Ptilostemon*: *P. abylensis, P. abbendiculatum, P. alpini* var. *camptolepis, P. diacanthus, P. diacantha subsp. turcicus, P. dyricola, P. leptophyllus, P. muticum, P. polycephalus* and *P. rhiphaeus.*

For the P. chamaepeuce (syn. Cirsium chamaepeucae and *Serratula chamaepeucae)* the following varieties have been recorded: *P. chamaepeuce* var. *camptolepis, P. chamaepeuce* var. *cyprius* and *P. chamaepeuce* var. *elegans.*

P. chamaepeuce is a spineless evergreen dwarf shrub, 30 – 100 cm in height, with white-downy stems, especially when it is young. Leaves are linear acute, densely white-downy beneath and light green on the upper surface, with recurved margins. Flower-heads purple 14 – 24 mm, in lax spreading, few-flowered, clusters or solitary and florets 20 – 25 mm long. It flowers from May to July. Its habitats are cliffs, on various rock types in the east Mediterranean including Greece, Crete, Aegean islands and Cyprus (Blamey and Grey-Wilson, 1993).

Taxonomy according to Royal Botanic Gardens, Kew (http://www.Kew.org) for the genus *Staehelina* is the following:
APG Clade: EUDICOTS - CORE EUDICOTS - ASTERIDS - EUASTERIDS II
APG Order: Asterales
APG Family: ASTERACEAE
Kew Family: COMPOSITAE
Genus: Ptilostemon
Species Epithet: chamaepeuce (L.) Less

There are no references for the propagation, use and management of *P. chamaepeuce* as an ornamental plant.

4.2 VEGETATIVE CHARACTERISTICS OF *PTILOSTEMON CHAMAEPEUCE*

4.2.1 Material and methods

The area where the survey was undertaken and the plant material of *P. chamaepeuce* collected was the northeast slopes of the Youchtas Mountain near Archanes town at altitudes from 400 – 600 m. This area was visited once a month regularly all the year round and more times as needed during the periods of collecting cuttings and seeds for the propagation experiments. A second district, 30 km to the South of Heraklion (San Thomas) and the same altitudes as Archanes, was observed to compare the number of flowers and seeds. Plant material of cuttings, flowers, fruits and seeds were collected from the observation areas in different periods during 2003 and 2004 to conduct the vegetative experiments.

4.2.2 Results and discussion

4.2.2.1 Distribution

In the observation area, maquis plants and in more open spaces garrigue plants dominate. *P. chamaepeuce* thrives among cliffs and other shrubs, grassy, bulbous and annual plants. In some places it dominates over other shrubs and on uncultivated slopes large numbers of *Ptilostemon* plants are commonly almost the only species present (Plate 4.1). A large population of the plant exists all over the observation slope. In spring, seeds germinate near and at long distances from the mother plants because of the feather pappus that spread the seeds. The seedlings can survive if there are appropriate conditions in the soil and sufficient water.

4.2.2.2 Botanical description

On the northeast slopes of the Iouktas Mountain, the height of *P. chamaepeuce* is more than one metre and in some cases it reaches up to 1.5 m. Stems are white due to the fact that they are covered with a thick layer of very short hairs.

Leaves are linear acute white-tomentose beneath and light green on the upper surface, with recurved margins, densely on the stem. The lower leaves in the stem dry during the dry periods of the year.

Flowering occurs on tomentose branches up to 80 cm in length with leaves as long as those on the main stems. Flower-heads are purple, 20 – 30 mm in total length and 20 – 25 mm wide during anthesis or 25 – 30 mm wide during the dispersal of the seeds. Number of heads per branch may reach up to 30 in Archanes district but only 13 in San Thomas, in clusters or solitary (Table 4.1). Between the two places examined there were differences between the numbers of heads per flowering branch. Probably this is due to the different soil or microclimate conditions and/or the different genotype.

Seed achenes are 3.5 – 5 mm with a pappus of 14 – 16 mm (Plate 4.2). Mature seeds per head numbered up to 19 in Archanes and to 23 in San Thomas (Table 4.1). The weight of the seeds ranges from 4 – 22 mg. each (Table 4.1). Seeds that weigh less than 13 mg are hollow.

Table 4.1 Number of flowers (inflorescences) per flowering branch and mature seeds in well-developed capitula (heads) of *P. chamaepeuce* in two districts of Heraklion.

	Average	Sd.	Max	Min
Number of flower heads per flowering branch (Archanes) N = 50	10.9	6.0	30	8
Number of flower heads per flowering branch (San Thomas) N = 80	5.7	1.7	13	3
Number of mature seeds per head (Archanes) N = 30	14.3	2.8	19	8
Number of mature seeds per head (San Thomas) N = 50	15.0	4.2	23	8
Weight per seed in mg. (Archanes) N = 100	13.9	4.6	22	4
Weight per seed in mg. (S. Thomas) N = 100	12.9	4.6	21	4

4.2.2.3 Life cycle

P. chamaepeuce is a xeroscape species that fits well in the Mediterranean climates, although long dry summers create stress and its ornamental value is downgraded. After the rains during winter and the increase of temperature in spring, new shoots sprout at first from the upper part of the previous year's stems. In the beginning of April the shoots are already about 10 – 20 cm long. By the end of April the longest shoots are 40 – 45 cm long and during the first 10 days of May the flower buds are visible on the tips of shoots. Anthesis starts from the middle until the end of June and lasts about one month. The first mature seeds are dispersed from the middle of August until the end of September. Life form can be classified according to the system of Raunkiaer (as modified by Govaerts *et al.*, 2000) in the nanophanerophytes because they are woody with persistent stems up to 1m in length.

4.2.2.4 Utilization

There are no references about the utilization of this plant. It is grown in some gardens near Athens receiving no water. It looks good all year round and the dried flower bracts are very beautiful. As a result of the survey and observations of *P. chamaepeuce* plants through the last three years, it appears that they have the potential to be used as garden plants for hedges alone or in combination with other shrubs, for mass plantings and rock gardens. They could also be used as pot plants, but a management system would have to be developed for the production of compact plants with many flowers. It may be suitable for cut flowers and dry flowers because of the long stems of its inflorescence and the large number of heads on them.

4.3 PROPAGATION BY SEED

Seeds of *P. chamaepeuce* that were used in the germination experiments were derived from their native habitat at Archanes. They were collected from the heads (capitula) of the plants during the period of their dispersal in the summer of 2003 and 2004. Underdeveloped, diseased or damaged seeds, visible to the naked eye, were discarded. Then they were dried in the open air for about one month and then put in plastic pots at room temperature until their use.

The seeds collected from the observation had various characteristics. Their colour ranged from cream to dark brown with light brown stripes. A large number of them were empty and damaged from insects. Their weight ranged from 4 to 22 mg (Table 4.1). From a sample of 50 seeds from the observation area, examined by cutting (cross-section) under a stereoscopic microscope, 66% were fully developed, 28% had been attacked by insects or damaged and 6% were empty. All seeds that weighed less than 10 mg were empty while seeds from 10 to 13 mg were damaged or attacked by insects. Seeds over 14 mg were well developed and suitable for sowing. Using a floating test in water and in 95 % ethanol the following were observed:

1. Approximately 75 % of the seeds floated in the water at different depths from the surface to the bottom, which made it difficult to separate the

floated from the sunken seeds. The weight of the floating seeds ranged from 6 mg to 18 mg.

2. Seeds that sunk to the bottom of the water were more than 14 mg.
3. Seeds floating in ethanol were less than13 mg. Half of them were less than 10 mg and empty, and half of them were 10 - 13 mg and damaged or attacked by insects.
4. Seeds that sunk in ethanol were all more than 14 mg and well developed with no damage or attack by insects or diseases.

The aim of the following experiments was to investigate:

- the suitable time for natural propagation after the releasing of the seeds from the heads;
- the appropriate substrate for germination of the *P. chamaepeuce* seeds in the greenhouse;
- optimum germination temperature;
- the effect of light on the germination of the seeds, and
- the aseptic conditions for producing seedlings to be used for *in vitro* regeneration of *P. chamaepeuce.*

4.3.1 Effect of the substrates and the time after ripening on the germination of *Ptilostemon chamaepeuce* seeds in the greenhouse

Seeds that had been collected from the wild during autumn 2003 were used to conduct a preliminary germination test. They were sown in 19-cm pots in two different rooting media, peat moss + perlite (1:1, v/v) and compost in an unheated greenhouse on 12th November, 10th December 2003 and on 26th February 2004 in order to study the natural germination of the seeds.

The germination of the seeds was poor and the germination percentage were 22.2% and 22.2% for November, 25% and 30% for December and 37.5% and 45% for February in peat moss + perlite or compost, respectively (data not shown).

The low germination percentage was due probably to the fact that insects had damaged most of the seeds. It was not possible to select the attacked seeds by visual observation before sowing.

Further experiments were undertaken the next year (2004) as described below.

4.3.1.1 Material and methods

Seeds collected on 23 August 2004 were examined under stereoscopic microscope (without dissecting them) and the well-developed were selected for these experiments. They were left in the open air to dry. Before sowing they were rinsed under tap water and then soaked for 30 min in tap water. The seeds were sown on 3rd September, 15th October, 15th November, 15th December 2004 and 13th January 2005 in 3 pots (19 cm in diameter and 9 cm in height) per treatment and 30 seeds per pot at a depth of 1 – 1.5 cm. Trade compost (pH = 6.0) and perlite: peat moss (1:1, v/v; pH = 6.2) were the two media used.

The germination percentage data were transformed to angular values before statistical analysis. Treatment means are presented in Table 4.2 without transformation. The statistical analysis of the data was based on analysis of variance and the means were compared by Tukey's test.

4.3.1.2 Results and discussion

The percentage germination of the seeds increased from September to January in both media (Table 4.2 and figures 4.1 - 4.5). The germination during the first months of autumn was very slow and not uniform. Ten days after the seeds' collection (3rd September) low germination occurred in both media as well as in October (53 days later) in peat moss + perlite. This is probably due to the after-ripening period after the maturing of the seeds. After-ripening is the type of endogenous non-deep primary physiological dormancy that exists in many freshly harvested seeds of herbaceous plants and disappears during dry storage (after-ripening) so that it is generally gone before the grower sows the seeds. For most flower crops it may last for one to six months and disappears with dry storage during normal handling procedures (Hartmann *et al.*, 2002). If the percentage of damaged seeds from insects (ca. 20 - 30%) is taken into account for the months December and January the real germination of the health seeds can reach to 100%. From the statistical analysis it was clear that there are significant differences among the time after collection from September to January and between the two substrates in November (Appendix 3; Table 4.2; Plates 4.3 and 4.4).

These results lead to the conclusion that the best season for sowing seeds of *P. chamaepeuce* by natural methods is the period after December when the season's temperatures are suitable for germination and the seed dormancy has been released. In nature seeds that begin to germinate in low temperatures (5°C) can later continue their growth when the temperatures become more favourable. In many areas of low and medium altitude in Mediterranean climates the temperature during late autumn, winder and early spring is not different from the requirements for germination of *P. chamaepeuce* seeds. In contrast, when the temperatures go up to 15 or 20°C seeds do not germinate (it depends on the species) even though there is sufficient water preventing the death of seedlings the following summer.

Table 4.2 Percent germination and T_{50} of *P. chamaepeuce* seeds collected in August 2004 and sown monthly in two soil media from September 2004 to January 2005.

Month of sowing	Soil medium	% germination ± sd	T_{50} in days ± sd
September 2004	Peat moss+Perlite (1:1)	13.3 ± 12a*	33.2 ± 13.7c*
	Compost	16.7 ± 8.8ab	32.5 ± 13.4c
October 2004	Peat moss+Perlite (1:1)	16.7 ± 10ab	36.7 ± 1.2abc
	Compost	41.1 ± 5bc	25.3 ± 2.5abc
November 2004	Peat moss+Perlite (1:1)	48.9 ± 8.4cd	25.8 ± 2.7ab
	Compost	51.1 ± 6.9cd	24.3 ± 4.7bc
December 2004	Peat moss+Perlite (1:1)	61.1 ± 8.4cde	22.8 ± 0.2abc
	Compost	71.1 ± 5.1de	22.6 ± 0.7abc
January 2005	Peat moss+Perlite (1:1)	75.6 ± 1.9de	14 ±1.0 abc
	Compost	81.1 ± 6.9e	13.31 ± 1a

* Values followed by the same letter are not significantly different ($P = 0.05$)

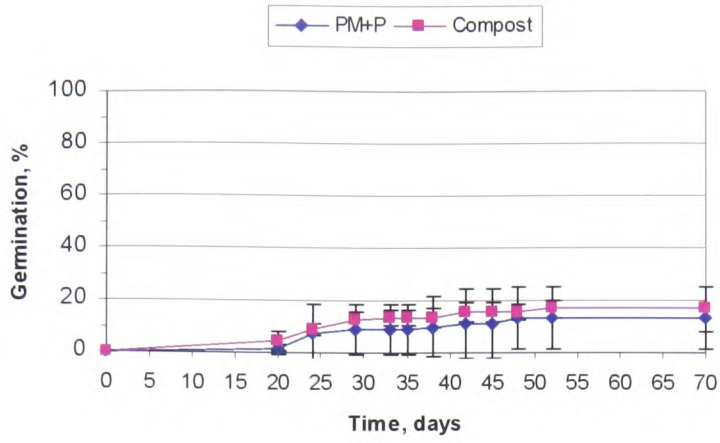


Figure 4.1 Effect of substrate on the germination of *P. chamaepeuce* seeds in the greenhouse during September 2004.

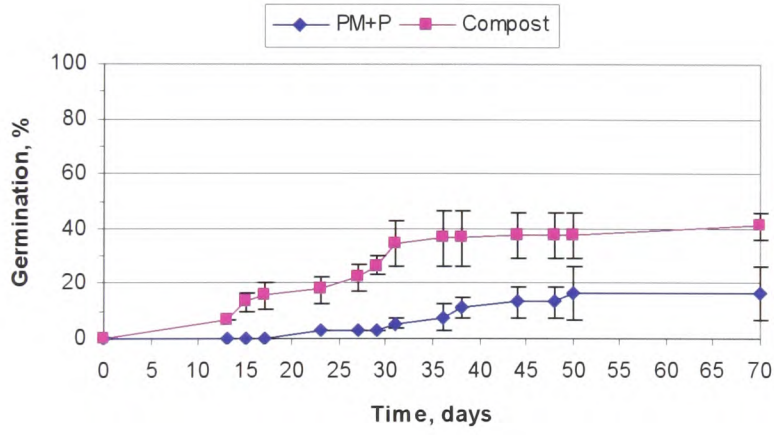


Figure 4.2 Effect of substrate on the germination of *P. chamaepeuce* seeds in the greenhouse during October 2004.

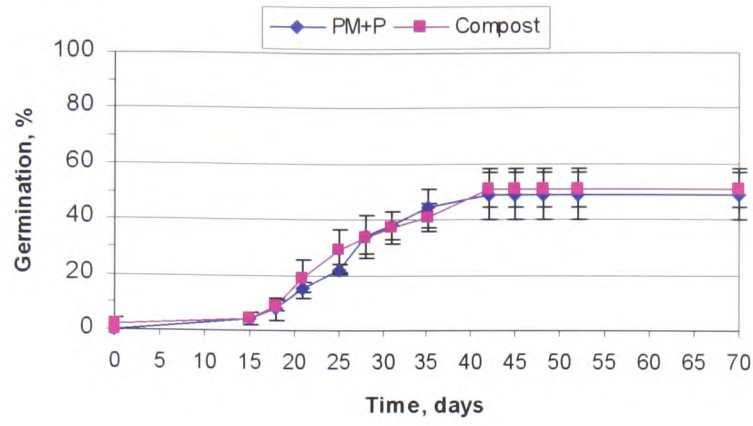


Figure 4.3 Effect of substrate on the germination of *P. chamaepeuce* seeds in the greenhouse during November 2004.

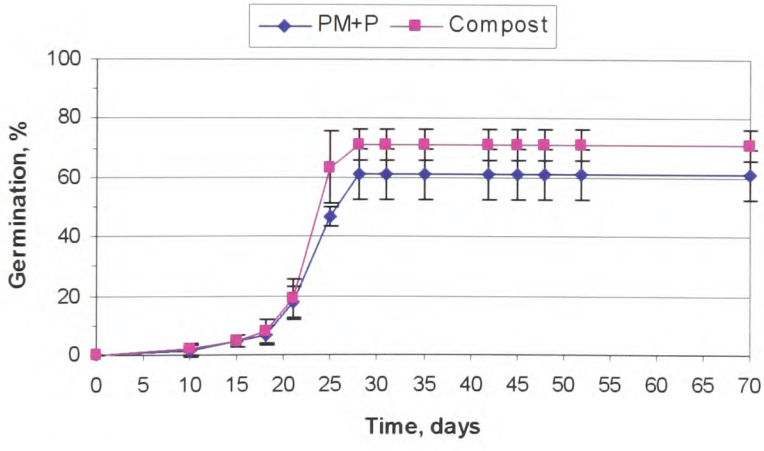


Figure 4.4 Effect of substrate on the germination of *P. chamaepeuce* seeds in the greenhouse during December 2004.

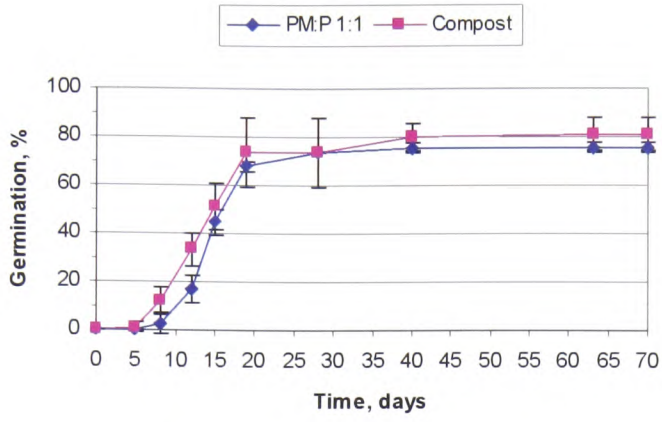


Figure 4.5 Effect of substrate on the germination of *P. chamaepeuce* seeds in the greenhouse during January 2005.

4.3.2 Effect of temperature and light on the germination of *P. chamaepeuce* seeds in controlled chambers.

The previous experiment identified the importance of timing in germination of *P. chamaepeuce* seeds. Further experiments were undertaken to determine the influence of light and temperature in increasing germination.

4.3.2.1 Material and methods

Seed, collected in August 2004, were selected by visual observation for this experiment. Germination percentage was checked at 5, 10, 15 and 20°C under light/dark or dark conditions. Three plastic Petri dishes with absorbent cotton and paper were used for each treatment. The cotton and paper were soaked with deionized water and 30 non-disinfected seeds were put in each Petri dish on 29th December 2004. The germinated seeds were assessed every two or three days for a period of 27 days. In the end the infected, empty or dead seeds as well as the non-germinated seeds were counted in each Petri. All handling and observations for seeds germinating in the dark were carried out under green safety lamps (General electric F15T8 G-6 15W Green-Photo USA).

Light /dark conditions were set for 16/8 photoperiod and the light was provided by fluorescent lamps (36 μmol m^{-2} s^{-1}).

The germination percentage data were transformed to angular values before statistical analysis and treatment means are presented in Table 4.4 without transformation. The statistical analysis of the data was based on analysis of variance and the means were compared by Tukey's test.

4.3.2.2 Results and discussion

The germination of the seed began from its narrowest part, which is the point of its attachment on the head. The cover of the seed was open in two parts and the radicle appeared first while the cotyledons remained covered for 2 to 4 days more depending on the temperature.

Germinated, non-germinated and infected, empty or dead seeds are presented in table 4.3. Of the seeds examined, 69.7% were well-developed and healthy (germinated and non-germinated) while the remainders were infected, empty or dead (Table 4.3). These results are close to the results from the first examined seeds under stereoscopic microscope (66% and 34%) and show that a high percentage of *P. chamaepeuce* seeds in the population of the observation area are not suitable for the sexual reproduction of the species.

Highest and fastest rates of germination were recorded at 10 and 15°C, with 100% germination after deduction for damaged seeds (Figure 4.8). No significant differences in germination were observed among the affect of dark and light conditions at the examined temperatures. In contrast there is a delay in the germination of the seeds under light at 15°C (Figures 4.6 and 4.7 and Table 4.4).

Table 4.3 Germinated, non-germinated and infected and/or empty seeds at different temperature and light after 27 days of incubation. L = Light; D = Dark.

	5°C L/D	5°C D	10°C L/D	10°C D	15°C L/D	15°C D	20°C L/D	20°C D	Mean
% germi-nated	50.0	57.8	64.4	71.1	66.7	66.7	63.3	65.6	63.2
% non-germinated	7.8	10	0	0	0	0	17.8	16.6	6.5
% infected/ empty	42.2	32.2	35.6	28.9	33.3	33.3	18.9	17.8	30.3

Table 4.4 Percent germination and time germination in days (T_{50}) of *P. chamaepeuce* in different temperatures under light/dark or dark conditions.

	Germination, %		T_{50} (days)	
	Light/Dark	Dark	Light/Dark	Dark
5°C	50 ± 13.3a*	57.8 ± 1.9a*	17.4 ±0.12d*	16.0 ± 0.56d*
10°C	64.4 ± 17.1a	71.1 ± 16.8a	3.8 ± 0.0a	3.9 ± 0.1a
15°C	66.7 ± 3.3a	66.7 ± 13.3a	7.9 ± 0.2b	4.1 ± 0.8a
20°C	63.3 ± 10.0a	65.6 ± 11.7a	9.3 ± 0.7bc	10.7 ± 2.3c

*Values followed by the same letter are not significantly different (p = 0.05) Mean ± sd

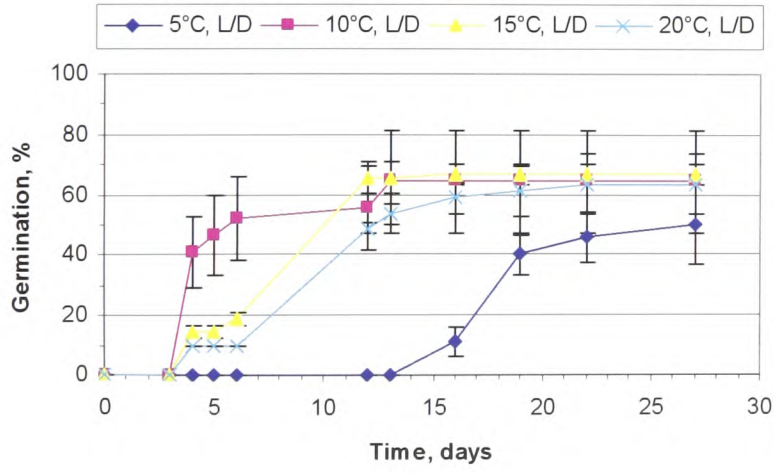
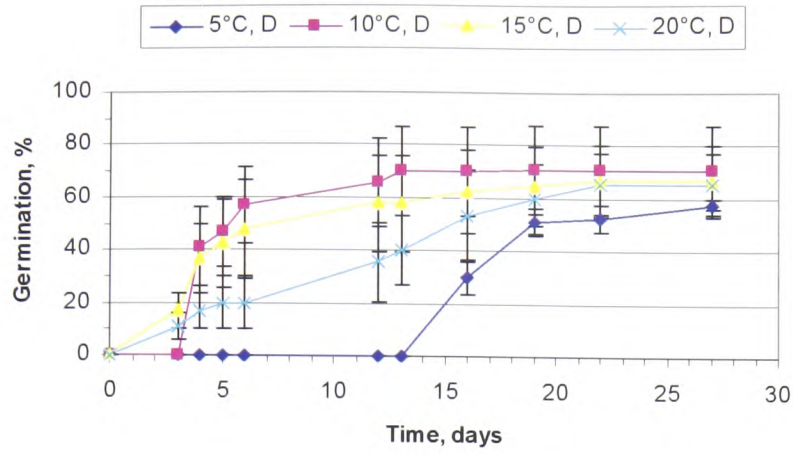


Figure 4.6 Germination time course of *P. chamaepeuce* at different temperatures under light/dark 16/8h.



Figures 4.7 Germination time course of *P. chamaepeuce* at different temperatures in dark conditions.

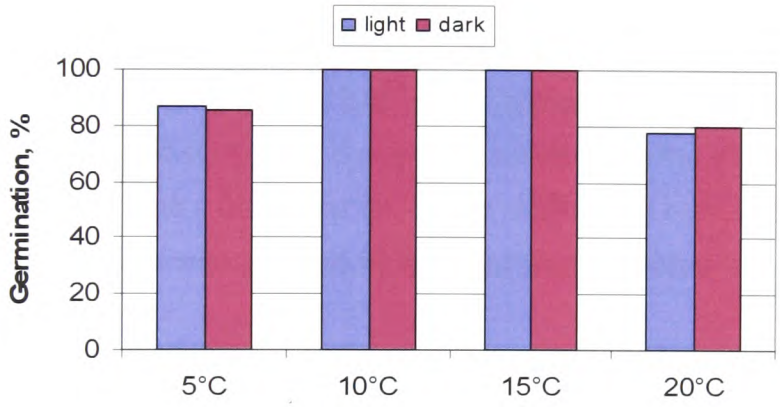


Figure 4.8 Cumulative percentage germination after deduction of the infected, dead and empty seeds of *P. chamaepeuce* under different temperature and lighting.

4.3.3 Germination of *P. chamaepeuce* seeds *in vitro*

The following series of experiments were conducted in order to acquire plant material for *in vitro* regeneration of *P. chamaepeuce* from seedlings. These results can also be compared with the results from previous experiments for seed germination of *P. chamaepeuce* as there are no infestations from fungi and bacteria.

4.3.3.1 Material and methods

Seeds were collected from the wild during the summer in the years 2003 (seed lot **I**) and 2004 (seed lot **II**) and used as plant material. All seeds were selected by visual observation and washed before disinfection under tap water using household detergents.

For the disinfection of the seeds the standard methods with ethanol and NaOCl or CaO_2Cl_2 in different concentrations were used. The disinfection was followed by three rinsing with deionized and sterilized water for 3, 3 or 15 min (unless other cited).

Two culture media used:

1. the MS salt medium (Sigma M5524) as described in the chapter 2.6.2 for *in vitro* seed germination solidified with 8g/l agar,

2. deionized water with 10 g/l agar.

Both were sterilized as described in the Chapter 1 and the pH was adjusted to 6.0. Gibberellic acid (GA_3) was dissolved in absolute (100%) ethanol and applied into the medium with a filter, after the culture medium had been sterilized. Continuous dark conditions were achieved by wrapping the Petri dishes with aluminium foil.

Materials are described for each experiment:

1. On 2nd June 2004 seeds from seed-lot I were disinfected with 95% ethanol for 30 sec and 0.75% NaOCl for 15 min following by three rinses with deionized sterilized water for 3, 3 and 15 min. These were cultured in Petri dishes with half strength MS salt medium (1). Two dishes for each treatment with 20 seeds per each dish were placed at 10, 15, and 20°C and light/dark (16/8h) or dark conditions (total seeds 240).

2a. On 28th July 2004 (ten days after collecting) seeds from seed-lot II were disinfected with 95% ethanol for 2 min and 10% CaO_2Cl_2 for 20 min following by 3 rinsing with deionized sterilized water for 3, 3 and 15 min and then cultured in Petri dishes with half stretch MS salt medium (1). Three dishes for each treatment

with 20 seeds per each dish were placed at 10, 15, 20, and 25°C in light/dark (16/8h) or dark conditions and at 5 and 30°C in dark conditions (total seeds 600).

2b. In parallel a pre-experiment 500 ppm GA_3 was added to the agar medium (2). One dish with 20 seeds was placed at 10, 15, 20, and 25°C in light/dark (16/8h) or dark conditions and at 5 and 30°C in dark conditions (total seeds 200).

3. On 5th August 2004 seeds from the seed-lot II were disinfected with 95% ethanol for 2 min and 10% CaO_2Cl_2 for 20 min following by three rinses with deionized sterilized water for 3, 3 and 15 min. These were cultured in 82 glass tubes with half strength MS salt medium (1) and 1 or 2 seeds per each tube. Half of them were placed at 10°C and half at 22 ± 2°C under light/dark conditions (total seeds 110).

4. On 4th October 2004 seeds from the seed- lot II were disinfected with 95% ethanol for 2 min and 10% CaO_2Cl_2 for 20min following by three rinses with deionized sterilized water for 3, 3 and 15 min and cultured in 90 glass tubes with half strength MS salt medium (1) at pH 6.5 and 2-3 seeds per each tube. They remained for 7 days at 22 ± 2°C under light/dark conditions and then were transferred (30 tubes per temperature) to 10°C, 15°C and ambient temperatures (in the greenhouse) (total seeds 230).

5. On 21 October 2004, 30 seeds from seed-lot II were scarified using glass paper and 30 remained intact. Then they were disinfected with 95% ethanol for 2 min and 0.75% NaOCl for 15 min following by three rinses with deionized sterilized water for 3, 3 and 15min. Both groups were cultured in 60 test tubes with half strength MS salt medium (1) and 1 seed per tube (total seeds 60).

6a. On 16 November 2004 seeds from seed-lot II were disinfected with 95% ethanol for 1 min and 1% NaOCl for 20 min following by three rinses with deionized sterilized water for 3, 3 and 15min and were cultured in plastic Petri dishes with half strength MS salt medium (1). Three dishes per each treatment with 25 seeds each dish were placed at 10, 15, 20, and 25° C and light/dark (16/8h) or dark conditions as well as at ambient natural temperature (in greenhouse) (total seeds 1000).

6b. As 6a, but after their disinfection the seeds were soaked in an aqueous deionized sterilized solution of 1000 ppm GA_3 for 24 hours. Next day the seeds

were placed in 12 petri dishes with the same medium, temperatures and light/dark conditions (total seeds 300).

6c. A third and fourth group from above mentioned seeds (6a) were placed in the agar medium (2) with the addition of 500 ppm or 1000 ppm GA_3. Fifteen dishes were used for each medium and 3 per treatment with 25 seeds in each dish (total seeds 750).

7. Non-sterilized seeds from seed-lot II were pre-treated for 35 days on wet filter paper in plastic bags placed at 5°C, 20°C and room temperature. During this period the room temperature in the laboratory fluctuated between 16 to 26°C. Forty days after pre-treatment (26 November 2004) the bags were opened and the germinated seeds were measured. Non-germinated seed were disinfected and incubated at different temperatures (5, 10, 15 and 20°C and two (1) and (2) media (total seeds 1,500).

8. On 28 January 2004 seeds from seed-lot II were tested for 8 different sterilizations methods at 15°C L/D. Five test tubes with 5 seeds per tube with agar culture medium (2) were used per treatment (total seeds 200). The sterilization methods were:

a. Washing under tap water with household detergent as control.

b. Washing under tap water with household detergent plus 95% ethanol for 1 min and three rinses for 3, 3 and 15 min.

c. Washing under tap water with house detergent plus 95% ethanol for 15 sec and 2.5% CaO_2Cl_2 for 10 min and three rinses for 3, 3 and 15 min.

d. Washing under tap water with household detergent plus 70% ethanol for 15 sec and 5% CaO_2Cl_2 for 10 min and 3 rinsing for 3, 3 and 15 min.

e. Washing under tap water with household detergent plus 70% ethanol for 15 sec and 10% CaO_2Cl_2 for 10 min and three rinses for 3, 3 and 15 min.

f. Washing under tap water with household detergent plus 2% CaO_2Cl_2 for 5 min and 3 rinsing for 3, 3 and 15 min.

g. Washing under tap water with household detergent plus 5% CaO_2Cl_2 for 5 min and three rinses for 3, 3 and 15 min.

h. Washing under tap water with household detergent plus 10% CaO_2Cl_2 for 5 min and three rinses for 3, 3 and 15 min.

4.3.3.2 Results and discussion

Applying the standard methods with ethanol and NaOCl or CaO_2Cl_2 in different concentrations and time exposed in the first six experiments (1 - 6) no germination of the *Ptilostemon chamaepeuce* seeds was recorded apart from the experiment 2 where low germination occurred in both media and at 5, 10 and 15°C (Table 4.5). Higher percentage germination (25%) was recorded by the effect of GA_3 in 15°C but it was not taken into consideration due to the small number of seeds left healthy.

Forty days after establishing the seventh experiment, the level of seed germination at 5°C, 20°C and room temperature was 66.8%, 0% and 5.2%, respectively. High temperature appears to delay or inhibit germination.

Results from the eighth experiment showed that in all treatments using ethanol no germination occurred, but high germination (84 - 100%) was recorded in all treatments without ethanol and the percentage infected seeds (8 - 20%) was low (Table 4.6; Plate 4.5). Non-germinated seeds, from the treatments using ethanol, were examined under stereoscopic microscopic and it was observed that their embryos and cotyledons were damaged and had died and turned blue-green.

Table 4.5 Effect of temperature and gibberellic acid (GA_3) on *in vitro* seed germination of *P. chamaepeuce* using sterilization with 95% ethanol for 2 min and CaO_2Cl_2 for 20 min (experiment 2).

	Germination %										
Temperatures (°C) and lighting	5 D	10 L/D	10 D	15 L/D	15 D	20 L/D	20 D	25 L/D	25 D	30 L/D	30 D
MS 1/2 strength	1.6	6.6	8.3	1.6	8.3	0	0	0	0	0	0
Agar 1% + GA3 (500ppm)	0	15	0	25	10	0	0	0	0	0	0

(D=dark, L=light)

Table 4.6 Effect of different sterilization methods on *in vitro P. chamaepeuce* seed germination (Experiment 8)

	Methods of sterilization							
	A*	B	C	D	E	F	G	H
Germinated (%)	100	0	0	0	0	96	92	84
Infected (% of germinated seeds)	80	100	100	100	100	20	12	8

*a= control (no disinfection), b= 95% ethanol, c=95% ethanol + 2.5% CaO_2Cl_2, d=70% ethanol + 5% CaO_2Cl_2, e=70% ethanol + 10% CaO_2Cl_2, f=2% CaO_2Cl_2, g=5% CaO_2Cl_2, h=10% CaO_2Cl_2.

4.4 PROPAGATION BY CUTTINGS

4.4.1 Introduction

Previous research had indicated that the propagation by cutting of *P. chamaepeuce* was difficult and irregular because of inconsistent and poor rooting results (Antonidaki, unpublished). For this reason a series of sixteen experiments were conducted in order to investigate the reason for the poor and irregular rooting and to maximize propagation by cuttings. The aim of this research was to find out the:

- effect of IBA concentrations and duration of absorption on the rooting and the number of roots on leaf cuttings;

- fluctuation of the rooting potential throughout the year;
- effect of the type of rooting medium;
- effect of the type of cutting and the clone;
- effect of juvenility after pruning the plants;
- effect of manipulation of plants such as re-juvenility, rinsing, using warm water, active charcoal, etc.

Each experiment was repeated two or more times. Results in some of them were negative and in some circumstances inconsistent. Three of them are described below and some information and comments are underlined for the others in the conclusion.

4.4.2 Effect of IBA in solution and powder form, the rinsing of the base of the cuttings and the application of warm water on the rooting of *P. chamaepeuce* leaf stem tip cuttings during spring 2004

Material and methods

Leaf stem tip cuttings, 10 – 15 cm long, were collected from adult plants in their natural habitat (the observation area) in April 2004. Their lower leaves were removed and their base was disinfected with 1 g/l Vitavax (37.5% Carboxin, and 37.5% Captan) and Benlate (50 % Benomyl). The cut of the lower section of the cuttings was renewed and the cuttings were dipped in ethanol solution (50%, v/v) of 0, 500, 1000, 2000, 4000, 8000 ppm IBA for 30 sec, or were dusted with 600 and 2000 ppm IBA (powder formula). Two more treatments were applied: the base of the cuttings was dipped and rinsed under tap water for 2 hours or dipped in warm tap water at 40°C for 1 hour in a heater (Unitronic 320 OR. P. SELECTA). Cuttings from both of these treatments were dusted with 600 ppm powder IBA. The treated cuttings, after their moist bases had been dried, were inserted in perlite, in multiple-pot trays and were placed under mist (Plate 4.6). The temperature at the base of the cuttings was maintained at 24 ± 4°C. Forty three days after preparing the cuttings, the percentage of rooting and callus percentage, the root number and the root length of the cuttings were recorded.

Forty five terminal cuttings per treatment were arranged in a randomized compete block design with 15 cuttings per replication and 3 replicates per treatment (total cuttings 450).

Results and discussion

Only treatments involving the use of 600 ppm IBA in powder stimulated root development of stem tip cuttings of *P. chamaepeuce*. Cuttings treated with 600 ppm IBA powder + warm water (40°C for 1h) produced a significantly higher percentage rooting (24.4%), than the other treatments (Table 4.7; Figures 4.9 - 4.11; Plate 4.7). No root development was induced by solutions of IBA as well as by the control treatments. However, callus was formed in control and low concentration IBA. Neither roots nor callus were induced in high concentrations of IBA (4000 and 8000 ppm). Haissig (1972) proposed that the lack of adventitious root formation in response to auxin may due to the lack of essential active enzymes, presence of enzyme inhibitors or lack of substrate phenolics for the synthesis of auxin-phenolic conjugates. The treatment with warm water may rinse the inhibitors or disinfect the base of the cuttings (Hara *et al.*, 1994). Despite that, Loach (1988) reported that dipping methods are more effective in rooting response than powder treatment methods, in contrast to these results for *Ptilostemon chamaepeuce*.

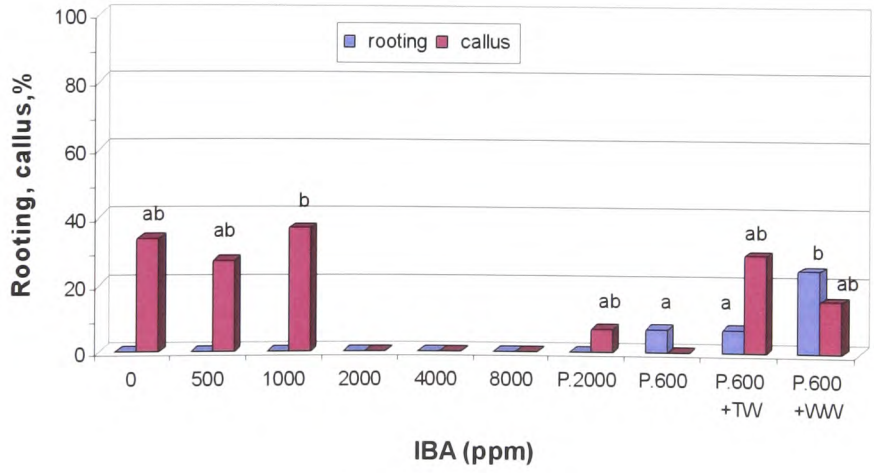


Figure 4.9 Effect of IBA, rinsing with tap water and application of warm water at the base of the cutting on the rooting and callus induction of *Ptilostemon chamaepeuce* leaf stem tip cuttings.

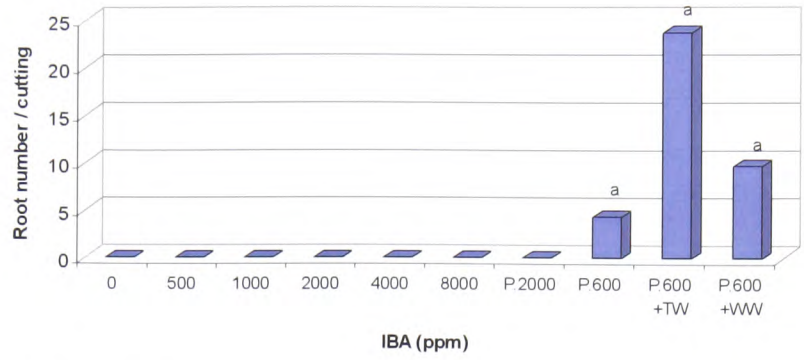


Figure 4.10 Effect of IBA, rinsing with tap water and application of warm water at the base of the cutting on the root number of *Ptilostemon chamaepeuce* leaf stem tip cuttings.

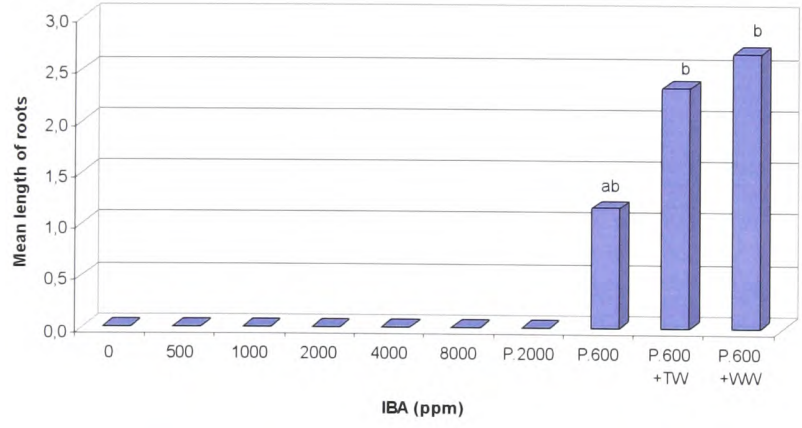


Figure 4.11 Effect of IBA, rinsing with tap water and application of warm water at the base of the cutting on the length of roots of *Ptilostemon chamaepeuce* leaf stem tip cuttings.

Table 4.7 Effect of IBA, rinsing with tap water and application of warm water at the base of the cutting on the rooting of *Ptilostemon chamaepeuce* leaf stem tip cuttings.

IBA (ppm)	Rooting (%)	Callus (%)	Number of roots/cutting	Length of roots/cutting (cm)
0	0.0 ± 0.0	33.4 ± 23.1ab*	0.0 ± 0.0	0.0 ± 0.0
500	0.0 ± 0.0	26.7 ± 0.0ab	0.0 ± 0.0	0.0 ± 0.0
1000	0.0 ± 0.0	36.7 ± 28.5b	0.0 ± 0.0	0.0 ± 0.0
2000	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4000	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
8000	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Powder 2000	0.0 ± 0.0	6.7 ± 6.7ab	0.0 ± 0.0	0.0 ± 0.0
Powder 600	6.7 ± 0.0a	0.0 ± 0.0	4.3 ± 5.8a	1.2 ± 0.9ab
Powder 600+T.W.	6.7 ± 0.0a	28.8 ± 3.9ab	23.7 ± 25.1a	2.3 ± 2.0b
Powder 600+W.W.	24.4 ± 7.7b	15.5 ± 10.1ab	9.7 ± 4.5a	2.7 ± 4.5b

T.W. = tap water; W.W. = warm water, mean ± sd
* Values followed by the same letter are not significantly different ($P = 0.05$).

4.4.3 Effect of concentration of IBA, source of material, rinsing the base of the cuttings and the application of warm water on the rooting of *P. chamaepeuce* leaf stem tip cuttings during winter 2004

The aim of this experiment is to investigate the differences in rooting potential between two different source plants and the effect of IBA treatments.

Material and methods

Two wild plants (designated A and B) were selected and leaf stem tip cuttings, 10 - 15 cm long, were taken from their natural habitat (the observation area) in December 2004. Their lower leaves were removed and the base was disinfected with 1 g/l Vitavax (37.5% Carboxin, and 37.5% Captan) and 1g/l Rovral (50% Improdion). The cut of the lower section of the cuttings was renewed and the cuttings were dipped in ethanol solutions of 0, 1000, 2000, 4000, and 8000 ppm IBA for 1 min, or were dusted with 600 and 2000 ppm powder IBA. Two more

treatments were applied: in the first of them the cuttings were rinsed under tap water for 1 hour and in the second the base of the cuttings was dipped in warm water at 40°C for 20 min. Cuttings from both of these treatments were dusted with 600 ppm powder IBA. The treated cuttings were inserted in perlite, in multiple-pot discs and were placed under mist (Plate 4.6A).

The temperature on the base of the cuttings was maintained at 24 ± 4°C. After 40 days the number of rooted cuttings, the cuttings producing callus and the number and length of roots per rooted cutting were recorded.

The experimental design was a randomized complete block with 5 cuttings per replication and 4 replicates per treatment in factorial arrangement (total cuttings 180 from each plant): In this experiment all treatments appear equally in each replicate block.

Results and discussion

Although the highest percentage rooting (30%) was recorded from cuttings taken from plant A treated with the IBA powder at 2000 ppm (Table 4.8; Figure 4.12), statistical analysis of the results showed that there were no significant differences between either the plants or the concentration of IBA and other treatments on the percentage rooting.

In the case of primary root production there are significant differences between the two plants, but not among the IBA treatments (Figure 4.13). Callus induction on the base of the cuttings occurred only in the control and with low IBA concentrations in both the plants. Similar results have been recorded in *Staehelina petiolata* for callus induction.

Cuttings that were rooted in 8000 ppm IBA had the greatest length of primary roots as well as more secondary roots (Table 4.8). The length of primary roots and the number of secondary roots are also significantly higher in the plant A than the plant B. Consequently the source of rooting material affected rooting potential regarding the number and length of primary and secondary roots but not on the percentage rooting of the cuttings.

Table 4.8 Effect of IBA, the clone, the rinsing with tap water and the application of warm water at the base of the cutting on the rooting of *Ptilostemon chamaepeuce* leaf tip cuttings.

Plant A IBA ppm	Rooting (%0	Callus (%)	Number of PR/RC	L/RC (cm)	Number of SR/RC
0	0.0a	45.0bc	00a	0.0a	0.0a
1000	15.0a	0.0a	7.5a	7.5a	37.8a
2000	10.0a	0.0a	13.5a	6.8a	14.0a
4000	20.0a	0.0a	5.3a	7.8a	29.0a
8000	5.0a	0.0a	10.0a	12.0a	70.0a
Powder 600	10.0a	10.0ab	2.5a	8.6a	19.0a
Powder 2000	30.0a	0.0a	7.4a	8.9a	22.5a
Powder 600+T.W.	20.0a	0.0a	4.0a	1.5a	0.0a
Powder 600+W.W.	25.0a	0.0a	6.5a	6.5a	38.5a
Plant B					
IBA ppm					
0	0.0a	50c	0.0a	0.0a	0.0a
1000	10.0a	0a	2.0a	2.3a	2.3a
2000	10.0a	0a	6.0a	6.1a	2.5a
4000	10.0a	0a	4.5a	7.1a	5.5a
8000	0.0a	0a	0.0a	0.0a	0.0a
Powder 600	0.0a	0a	0.0a	0.0a	0.0a
Powder 2000	5.0a	0a	2.0a	12.0a	1.0a
Powder. 600+T.W.	20.0a	0a	2.3a	1.2a	0.0a
Powder 600+WW	15.0a	5a	1.5a	3.8a	4.0a

T.W. = tap water; W.W. = warm water. PR = primary roots; RC = rooted cutting; L = length; SR = secondary roots.

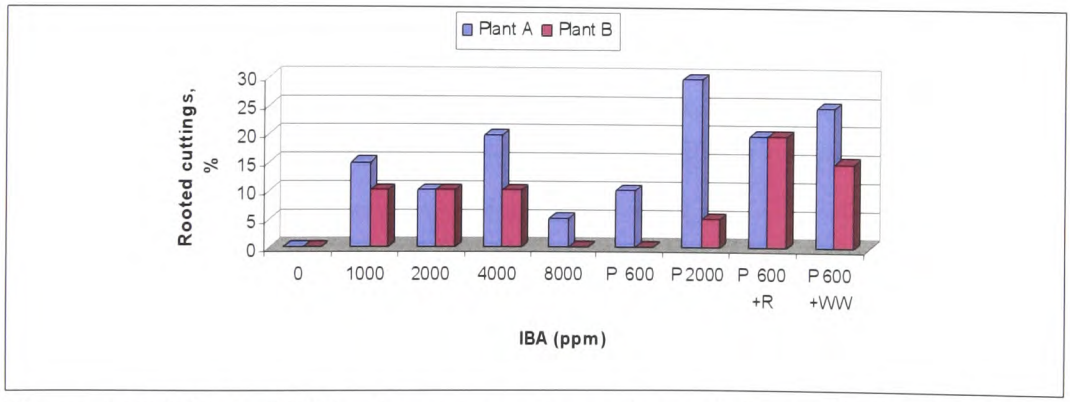


Figure 4.12 Effect of IBA, plant source, the rinsing and the application of warm water at the base of the cutting on the rooting of *Ptilostemon chamaepeuce* leaf tip cuttings.

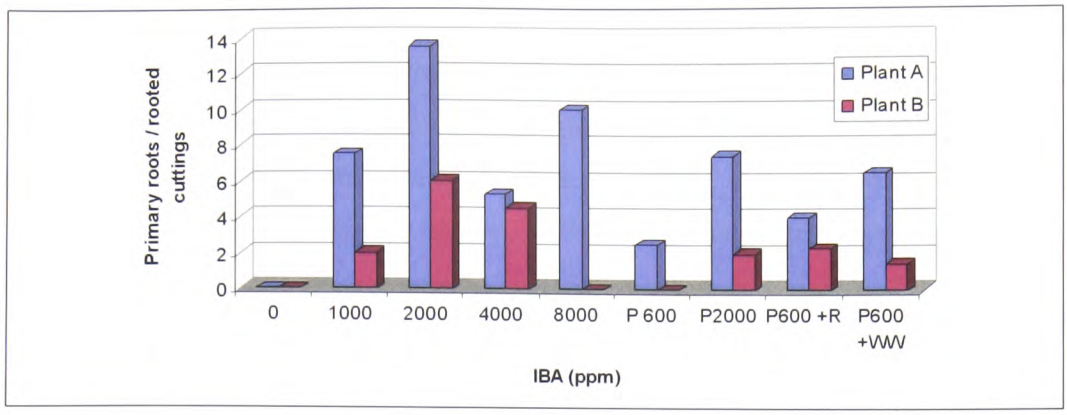


Figure 4.13 Effect of IBA, plant source, rinsing and application of warm water at the base of the cutting on the number of roots of *Ptilostemon chamaepeuce* leaf tip cuttings.

4.4.4 Effect of different substrates on the rooting of *P. chamaepeuce* leaf stem cuttings during May 2003 and 2004.

In previous experiments using various concentrations IBA and application methods the rooting of *P. chamaepeuce* cuttings was relatively low. Therefore the following experiment was conducted to examine the effect of different types of rooting medium.

Material and methods

Stem tip cuttings, 10 – 15 cm in length, were taken from plants in the observation area in May 2003 and 2004. Their lower leaves were removed and the bases of the cuttings were treated with 600 ppm powder IBA. The treated cuttings were inserted into seven different rooting media: peat moss, perlite, sand, vermiculite, peat moss + perlite (1:1, v/v), peat moss + perlite + soil (1:1:1, v/v/v) and perlite + soil (1:1, v/v) and placed in a mist environment (Plate 4.6B).

The following year the experiment was repeated using the following rooting media: peat moss, perlite, sand, vermiculite, peat moss + perlite (1:1, v/v), peat moss + sand (1:1, v/v) and vermiculite + perlite (1:1, v/v).

After 45 days the number of rooted cuttings and the number and length of roots per rooted cutting were recorded.

The experimental designs were randomized complete block with 5 cuttings per replication and 5 replicates per treatment (total cuttings 175 for each experiment). The rooting percentage data were transformed to angular values before statistical analysis. Treatment means are presented in Table 4.9 and 4.10 without transformation. The statistical analysis of the data was based on analysis of variance and the means were compared by Tukey's test.

Results and discussion

A significantly higher level of rooting of *P. chamaepeuce* was achieved with vermiculite as the rooting medium, producing 84% and 64% success for 2003 and 2004, respectively (Tables 4.9 and 4.10). Moreover, in vermiculite, cuttings produced 9.7 and 11.7 roots per cutting and the root length was 2.3 cm and 2.9 cm, respectively for the years 2003 and 2004. However when rooted in perlite only 32% and 12% of cuttings rooted. However, when perlite was mixed with sand and/or peat moss rooting was higher (40%). The cutting of *P. chamaepeuce* failed to root in the peat moss, which could be attributed to its low pH. This problem was eliminated when peat moss was mixed with Perlite and soil. Cuttings rooted nearly as well (40%) when soil was mixed with perlite and/or peat moss producing 7 and 9.4 roots respectively.

These results suggest that rooting of cuttings depends on the rooting media properties that may relate to different levels of water holding capacity, porosity and pH (Hartmann *et al.*, 2002).

Table 4.9 Effect of different substrates on the rooting of *P. chamaepeuce* leaf stem cuttings during May 2003

Substrates	Rooting (%)	Mean number of roots	Mean length of roots (cm)
PM	0a	0.0a	0.0a
Perl.	32bc	2.9ab	1.2abc
Sand	8ab	1.8ab	0.7ab
Verm	84d	9.7c	2.3bc
PM+Per	8ab	1.9ab	0.2a
PM+Per+Soil	40c	7.0bc	2.4c
Per+Soil	40c	9.4c	1.5abc

PM = peat moss, Perl. = perlite, Verm = vermiculite

Table 4.10 Effect of different substrates on the rooting of *P. chamaepeuce* leaf stem cuttings during May 2004

Substrates	Rooting (%)	Mean number of roots	Mean length of roots (cm)
P.M.	0a	0a	0.0a
Perl.	12a	2.2ab	1.2bcd
Sand	12a	3.2ab	0.9abc
Verm	64b	11.7c	2.9e
PM+Per	8a	2ab	0.3ab
PM+Sand	16a	4.4ab	2.1de
Per+Verm.	24ab	7.7bc	1.8cd

4.4.5 Conclusions

From these experiments conducted over two years the following conclusions can be drawn:

- The better season for the rooting of *P. chamaepeuce* cutting was spring and late in the autumn or early winter (November-December). Cuttings failed to root when they were taken for rooting from the beginning of summer until the middle of autumn before the rains start. Seasonal variation in propagation success has been highlighted by many researchers and may be mediated through changes in the endogenous plant growth regulator or carbohydrate concentrations (Bhella and Roberts, 1975; Day and Loveys, 1998).
- Rooting was improved by using IBA in powder rather than solution. The duration of dipping for 10, 30 or 60 sec did not improve the rooting of the plant. This may due to the different amount of hormone taken up by the cuttings which depends on the environment and the condition of the cutting (Loach, 1988).
- Stem segment leaf cuttings did not root at all using IBA, as well as thin and thick hard leafless cuttings after scarring on their base.
- Vermiculite used as a rooting medium stimulated more roots and growth of roots and led to higher percentage rooting. Propagation medium also affect rooting response of stem cuttings of *Arbutus andranchne* (Al-Salem and Karam, 2001).

- There are differences arising from source of cuttings, especially in number and growth of roots but not in the overall rooting percentage.
- Rooting was not improved if using cuttings from re-juvenated plants after pruning or if active charcoal was mixed with rooting medium.
- The treatment with warm water 40°C on the base of the cuttings increased the rooting of the cuttings.

4.5 *IN VITRO* PROPAGATION OF *P. CHAMAEPEUCE* (L) Less

The aim of *in vitro* propagation of *Ptilostemon chamaepeuce* was to develop the potential for mass propagation of selected clones or the possibility to acquire new healthy clones with better characteristics than those from the wild to be used as ornamental plants.

No published data was found regarding the micropropagation of *Ptilostemon chamaepeuce*. The results of disinfestations of vegetative explants of reported by Chimonidou *et al.* (2004) were negative.

Experiments were conducted with explants derived both from mature and juvenile plants that originated either from wild or greenhouse stock and from seeds collected from isolated plants in the wild or the experimental garden of TEI. Different culture media and different concentrations of growth regulators and chemical sterilants (bleach) were used to investigate the proliferation of explants.

4.5.1 Node and shoot *in vitro* cultures from vegetative explants

4.5.1.1 Effect of BAP and NAA on the *in vitro* culture of *Ptilostemon chamaepeuce* using explants from the stems of wild plants.

1st experiment

Material and methods

Plant material

Shoots, 10 - 15 cm in length, were collected from wild plants of *P. chamaepeuce* and utilized as plant material for the study of *in vitro* proliferation. The leaves

were cut at their base and discarded. Two shoot segments, 1.5 to 2.0 cm in length, were cut from the shoots, one from the tip of the shoot and the second from the central part. The latter consisted of two or more nodes. The tip of the shoots had two or more nodes and the apical meristem. In total, eighty shoot tips and the same number of intermediate parts from two healthy plants of selected plants from the wild were used as explants to establish *in vitro* cultures.

Sterilization and explants preparation

A routine method was used to sterilize the explants. The shoot explants (1.5 – 2 cm long) were pre-washed with soapy water. Then they were disinfected with 73% (v/v) ethanol for 30 seconds. The surface disinfection with ethanol was followed by surface-sterilization with 10% (v/v) commercial chloride (containing 5% sodium hypochlorite) with Tween 20 (4 drops per 100 ml), for 10 min and then rinsing three times with deionized sterilized water for 5, 10 and 30 min. The disinfestation was repeated twice. After the surface sterilization stem nodal segments (with two or more bud) and shoot tips were isolated using a sterile surgical knife and forceps in a laminar air flow cabinet.

Media and culture procedure

Explants were cultured *in vitro*, to explore the possibility of developing a protocol for propagation via organogenesis, using standard tissue culture methods (Ammirato, 1990). After sterilization the explants were transferred onto MSS (Chapter 2.6.2) medium supplemented with 0, 0.5, 1, or 2 mg/l BA and 0 or 1 mg/l NAA in all possible combinations as well as 5 g/l active carbon to absorb phenol exudation. The pH of the media was adjusted to 5.8 – 6.0 prior to normal autoclaving. Explants were placed on the surface of the sterilized media in test tubes and kept at 24 ± 2°C temperature and 16/8 h light/dark from fluorescent tubes providing an average irradiance of 36 μmol m^{-2} s^{-1} at culture level.

Each experiment contained 20 explants per treatment.

Results and discussion

Most of the explants were infected by fungi and bacteria. The uninfected explants became brown to black and were destroyed. Only 1.25% of the explants were not

infected and no response to the media occurred within six weeks or after subculture in the same treatment.

4.5.1.2 Effect of different disinfections, culture media, growth regulators and plant material on the *in vitro* proliferation of *Ptilostemon chamaepeuce*

After the poor results from the previous experiment, using plant material from the wild, five more experiments were undertaken with different disinfection methods, media, growth regulators and source of plant material.

Material and methods

Plant material

2nd experiment: Shoot tips and central segments from wild plants

3rd experiment: Shoot tips and central segments from pruned plants from wild as well as stems from plants in the garden of TEI.

4th experiment: Shoot tips and central segments, from 1 year old seedlings in the greenhouse.

5th experiment: Shoot tips and central segments from 1 year old seedlings in the greenhouse.

6th experiment: 1 year old seedlings in the greenhouse.

Culture media

The following culture media were used:

2nd experiment: MSS supplemented with BA at concentrations of 0, 0.5, 1 or 2 mg/l and NAA at 0 or 0.5 mg/l.

3rd experiment: MSS supplemented with BA at concentrations of 0, 1, 2 or 4 mg/l and NAA at 0 or 0.5 mg/l.

4th experiment: MSS supplemented with BA at 0, 1, 2 or 4 mg/l and NAA at 0, 0.5, 1 or 2 mg/l.

5th experiment: **McCown** WPM (Sigma M6774) with the addition of Myo-inositol 100 mg/l, Nicotinic Acid 0.5 mg/l, Pyridoxine 0.5 mg/l, Thiamine 0.1 mg/l and supplemented with BA at 0, 1, 2 or 4 mg/l and NAA at 0, 0.5, 1 or 2 mg/l.

6th experiment: MSS medium (Chapter 2.6.2).

Sterilization of the explants.

Three different chemicals were used to sterilize the explants derived from the wild or greenhouse stock: Trade bleach (5% NaOCl), Calcium hypochlorite (CaO_2Cl_2) and antimycotic-antibiotic suspension in different concentrations and time treatments (Table 4.11). Four drops from Tween 20 per100 ml were added to each disinfectant solution.

The sterilization process was repeated twice in some cases. After the sterilization the explants were rinsed thoroughly three times with deionized sterilized water. After the leaves were removed, the shoots were further washed with ethanol and detergents and rinsed with water, as follows.

2nd experiment: Shoot tips: 73% ethanol for 30 sec, 2.5% CaO_2Cl_2 for 10 min. Shoot central segments: 73% ethanol for 30 sec, 5% CaO_2Cl_2 for 10 min. This was followed by three rinses with deionized sterilized water for 5, 5 and 30 min. This sterilization process was repeated for one more time.

3rd experiment: Shoot tips: 95% ethanol for 2 min, 10% CaO_2Cl_2 for 20 min. Shoot central segments: 95% ethanol for 2 min, 10% CaO_2Cl_2 for 30 min. This was followed by three rinses with deionized sterilized water for 3, 3 and 15 min.

4th experiment: 95% ethanol for 1 min, 0.5% NaOCl for 10 min. This was followed by three rinses of 3 min with deionized sterilized water.

5th experiment: Different concentration of Calcium hypochlorite and duration, as follows:

- 95% ethanol for 30 sec, 5% CaO_2Cl_2 for 5 min.
- 95% ethanol for 30 sec, 5% CaO_2Cl_2 for 10 min.
- 95% ethanol for 30 sec, 10% CaO_2Cl_2 for 7 min.
- 95% ethanol for 30 sec, 10% CaO_2Cl_2 for 15 min.

All explants were rinsed 3 times with deionized water for 3, 3 and 15 min.

6th experiment:

- 0.5% NaOCl for 7 min.

- 95% ethanol for 1 min, 0.5% NaOCl for 7 min.
- 5% Antibiotic antimycotic suspension (Sigma A5955) for 2 hours.

Results and discussion

None of the disinfection procedures was satisfactory in producing suitable uninfected material for *in vitro* culture. Most of the explants were infected and those that were not infected became brown to black. A very low percentage of the explants remained green (Table1) but there was no response to the media recorded within 6 weeks.

No differences were observed between 1-year-old seedlings and stem tips/segments explants from mature plants.

The same number of one year old seedling explants from the greenhouse pots was used to study the differences between mature and juvenile response, but no positive results were achieved. The high levels of infection may have been caused by the hairy nature of the stems and leaves of *Ptilostemon chamaepeuce*, which make their explants difficult to sterilize (Chimonidou *et a.l*, 2004).

Table 4.11 Percentage non-infection following different methods of chemical disinfection and sterilization of vegetative explants of *Ptilostemon chamaepeuce*.

	Alcohol		NaOCl		CaO_2Cl_2		Sigma		Uninfected
	%	Time sec	%	Time min	%	Time Min	%	Time h	(green) explants %
1st Experiment	73	30	0.5	10	-	-	-	-	1.25 (160:2)
2ndExperiment	73	30	-	-	2.5	10	-	-	0
3rd Experiment	95	60	-	-	10	10	-	-	3.75 (80:3)
4thExperiment	95	60	0.5	10	-	-	-	-	0
5th Experiment	95	30	-	-	5	5	-	-	2.5(40:1)
5th Experiment	95	30	-	-	5	10	-	-	5 (40:2)
5th Experiment	95	30	-	-	10	7	-	-	2.5(40:2)
5th Experiment	95	30	-	-	10	15	-	-	2.5(40:1)
6th Experiment	-	-	-	-	5	7	-	-	0
6th Experiment	70	60	-	-	5	7	-	-	0
6th Experiment	-	-	-	-	-	-	5	2	0

4.5.2 In vitro propagation of *Ptilostemon chamaepeuce* from seedlings

Since the vegetative explants are very difficult to sterilize, experiments were conducted with explants derived from seedlings that had grown from sterilized seeds.

4.5.2.1 Effect of BA and NAA on adventitious and axillary shoot formation of *Ptilostemon chamaepeuce* using as explants *in vitro* produced micro-shoots from seedlings.

Material and methods

Plant material

Seeds derived from one isolated plant of *Ptilostemon chamaepeuce* were collected on July 2003 and 2004 and dried for 30 days in the open air at room temperature. The seeds then were stored in a plastic container under room temperature until their use. The seeds were germinated *in vitro* on half strength MS salt medium as described in this chapter 4.3.3. The shoot tips of 2-week old seedlings were excised and cultured on MSS medium supplemented with 0.5 mg/l BA. After three subcultures when a large number of shoots had been acquired they were subcultured for a further 10 days on MS medium without hormones. The apical tips of these shoots, containing the apical bud plus 2 - 3 axillary buds, were used as explants for investigating the effect of growth regulators on *Ptilostemon chamaepeuce*.

Cultures were incubated in 36 μmol.m^{-2}.s^{-1} cool-white fluorescent light and 16 h photoperiod at 24 ± 2°C.

Culture media

The explants were culture on MSS media supplemented with 0, 1, 2 or 4 mg/l BA and 0, 0.5, 1 or 2 mg/l NAA in all possible combinations and in a factorial arrangement. One explant was put in each test tube. Twenty explants were used in each concentration.

After 5 weeks the number of shoots per explant and the mean length of shoots and roots per culture were recorded. The statistical analysis of the data was based on analysis of variance and the means were compared by Tukey's test.

Results and discussion

Two weeks after culture adventitious shoots began to appear on the base of the explants in all treatments except control and NAA (no BA) treatments (Plate 4.8). After five weeks incubation, all of the treatments produced adventitious shoots to a varying degree (Figure 4.14). The least number of shoots was produced in media with NAA alone. The addition of BA increased significantly the number of shoots, particularly when no NAA was present. Increasing the concentration of NAA with BA tended to reduce the number of shoots, except at the highest concentration of BA.

The shoots grew longer on basal media without growth regulators (Figure 4.15). As the concentration of NAA increased, shoot growth was reduced. The addition of BA (even without NAA) reduced shoot growth compared to the control treatment.

At high concentrations of BA more adventitious shoots and axillary branches were induced on the base and stem of the explants. When the treatment was BA alone (no NAA) more axillary branches were observed on the axils of explants than adventitious shoots on the base of the shoots, while in the combinations BA/NAA more adventitious shoots were formed on the callus and the base of the shoots (Plate 4.9).

Nodules formed on the base and the nodes of the explants, mainly in high concentrations of BA, which produced shoots after 2 or 3 subcultures (Plate 4. 10).

Root formation was observed with the addition of NAA alone in the medium. The rooting percentages at 0.5, 1, and 2 mg/l NAA were 41, 45 and 75% respectively. None of the explants rooted in treatments containing BA. Callus formation was observed on all the treatments with NAA or on combinations of BA/NAA. Callus was soft and friable when it came from NAA treatments and hard when it came

from the combinations BA/NAA. At high concentrations of BA harder callus and more nodules were produced.

The vitrification phenomenon was recorded in the high concentrations of BA and BA/NAA or when explants remained in culture for more than five weeks.

According to George (1996) growth regulators must be selected not only to give the highest rate of shoot multiplication, but also to provide shoots of good quality, which will root and grow satisfactorily. Satisfactory shoot quality can usually be obtained by reducing the level of cytokinin used at each subculture or before shoots are harvested for rooting (George 1996). For this reason it is recommended that lower concentrations of hormones are used despite the fact that high concentrations of BA promote the induction of more adventitious shoots.

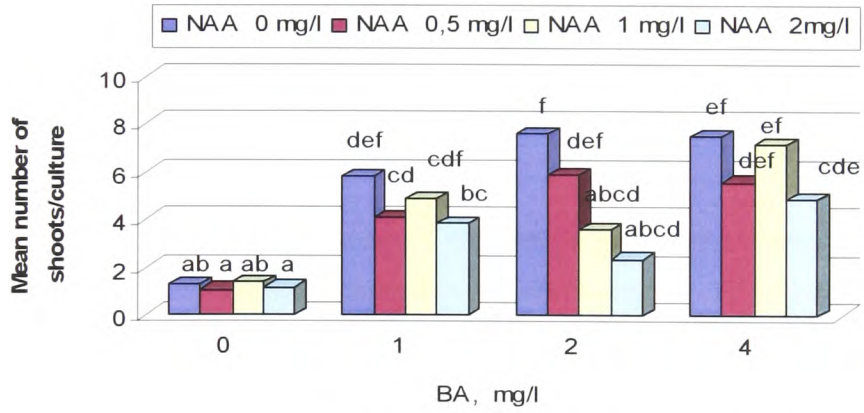


Figure 4.14 Effect of different combinations of BA/NAA on the number of adventitious shoots of *Ptilostemon chamaepeuce*, using micro-shoot explants from *in vitro* produced seedlings, after 5 weeks incubation at 24 ± 2°C and 16 h photoperiod. Values followed by the same letter are not significantly different (P= 0.05).

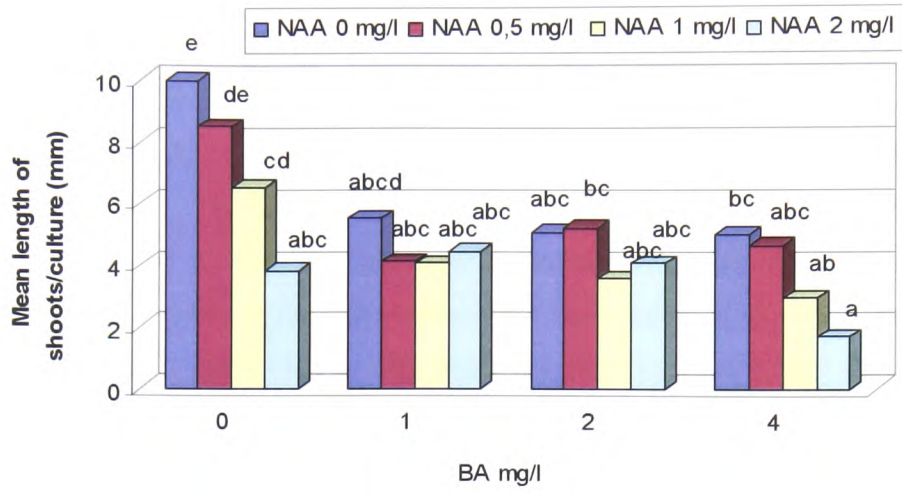


Figure 4.15 Effect of different combinations of BA/NAA on the length of shoots of *Ptilostemon chamaepeuce*, using micro-shoot explants from *in vitro* produced seedlings, after 5 weeks incubation at 24 ± 2°C and 1 6h photoperiod. Values followed by the same letter are not significantly different (P= 0.05)

4.5.2.2 Effect of NAA and IBA on the rooting of *Ptilostemon chamaepeuce* micro-shoots.

Material and methods

The same source of explants as the previous experiment was used to investigate the potential of rooting of *Ptilostemon chamaepeuce* micro-shoots. The medium was MSS supplemented with 0, 0.5, 1 or 2 mg/l NAA and 1, 2, 4 mg/l IBA. Fifteen explants were used in each treatment and one explant was embedded in each test tube. After 5 weeks the percentage of rooting, the numbers of roots per micro-shoot and the mean length of roots per micro-cutting were recorded.

Some of the rooted micro-cuttings were planted in pots with trade compost and perlite (4:1 v/v) and left in the greenhouse. Some were planted in Styrofoam poly-pots with rock wool in the growth room at 24 + 2°C. The latter were watered with 10 % Hoagland's Nutrient Solution in deionized water twice a week. Two months later they were transferred to the greenhouse in pots containing compost: perlite 4:1 (v/v). Data was analysed by ANOVA and the means were compared by Tukey's test.

Results and discussion

Ten days after the initiation of the culture the first roots were visible from the formed callus on the base of some explants. In four weeks the rooting was completed (Plate 4.11). None of the explants rooted in MSS medium without auxins (Figure 4.16). The best percent rooting was recorded in 2 mg/l NAA (80%) and it was followed by the IBA treatment at 4mg/l. However there were not statistical differences among the other treatments. The number of roots was greater at 4 mg/l IBA and 2 mg/l NAA and the mean length of roots at 2 mg/l IBA and NAA but there were no significant differences among the treatments (Figure 4.17 and 4.18). The roots from micro-cuttings at 0.5 mg/l NAA originated directly from the base of the stem without forming callus. The formed callus on the base of the rooted micro-cuttings and the high temperature in the greenhouse inhibited the direct establishment of the rooted shoots (*ex vitro*) and none of the rooted micro-shoots was established. In contrast, all of the rooted micro-cuttings which had been planted first in the Styrofoam poly-pots were established later in the greenhouse (Plate 4.12 and 4.12a).

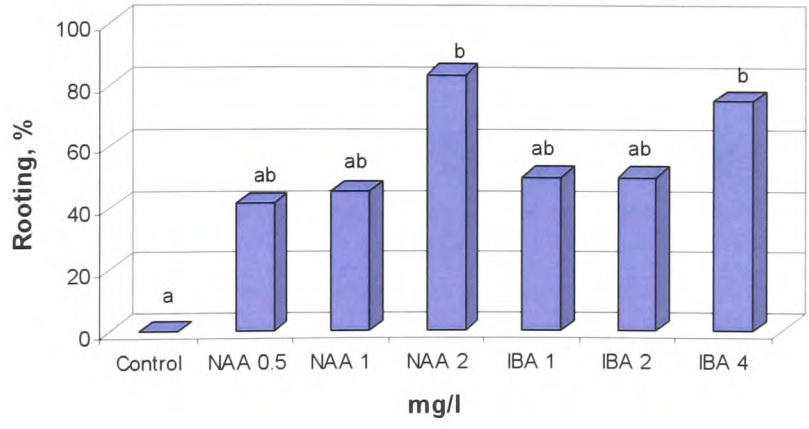


Figure 4.16 Percentage rooting of *Ptilostemon chamaepeuce* micro-shoots. Values followed by the same letter are not significantly different ($P = 0.05$)

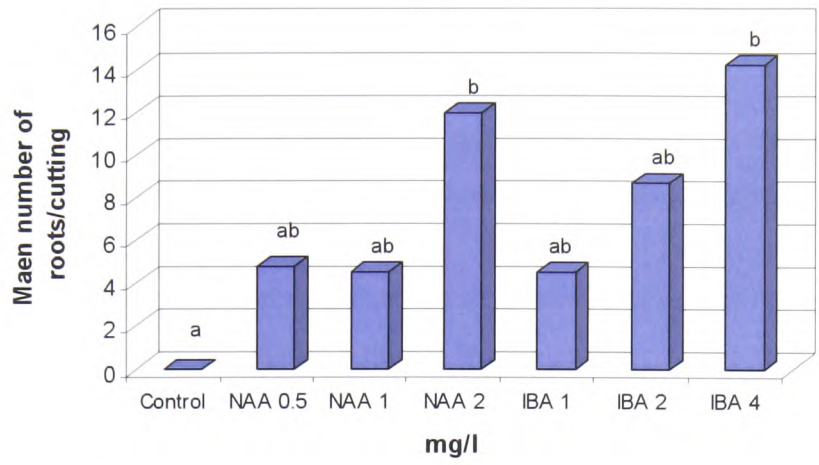


Figure 4.17 Effect of NAA and IBA on the number of roots of *in vitro* produced micro-shoots of *Ptilostemon chamaepeuce*. Values followed by the same letter are not significantly different ($P = 0.05$)

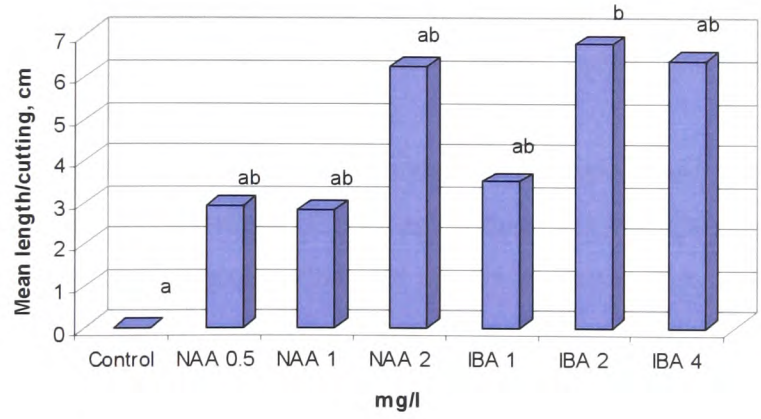


Figure 4.18 Effect of NAA and IBA on the length of roots of *in vitro* produced micro-shoots of *Ptilostemon chamaepeuce*. Values followed by the same letter are not significantly different ($P = 0.05$)

4.5.2.3 Effect of BAP and NAA on the regeneration of shoots and roots of *in vitro* culture of *Ptilostemon chamaepeuce* seedlings using McCown Woody Plant Medium.

This experiment aimed to investigate the effect of growth regulators on the proliferation of *Ptilostemon chamaepeuce* shoots from seedlings cultured on McCown Woody Plant Medium (WPM). Propagation of some woody plants has been found to be more successful on media with lower levels of nitrogen than that found in MS medium. Media used for woody plants, such as McCown Woody Plant Medium contain on average more PO_4^{3-}, SO_4^{2-}, and Mg^{2+}, but less NO_3^- and NH_4^+ than those used for herbaceous plants (George, 1996).

The use of WPM is recommended for some woody plants and increases the proliferation compared with MS medium (McCown and Lloyd, 1981; Romano, 1992; Μπερτσουκλής *et al.*, 2004).

Material and methods

Plant material

Three-week old seedlings were used for *in vitro* establishment of *Ptilostemon chamaepeuce* on WPM. The seeds were sterilized and germinated *in vitro* as described in the section 3.3 of this chapter.

The roots from each seedling were cut under the cotyledons and were discarded. The rest of the shoot (cotyledons with the apical bud or shoot) was used as explants for *in vitro* culture. Each explant was put upright in the medium, one in each test tube.

Culture media

McCown WPM was used as the culture medium (McCown and Lloyd, 1981), adding 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine-HCl and 0.1 mg/l thiamine-HCl and supplemented with BA at 0, 1, 2 or 4 mg/l and NAA at 0, 0.5, 1 or 2 mg/l in all possible combinations, in a factorial arrangement. Cultures were incubated in 38 $\mu mol.m^{-2}.s^{-1}$ cool-white fluorescent light and 16h photoperiod at 24 ± 2°C. All data were analyzed by analysis of variance and the means were compared by Tukey's test.

Results and discussion

Supplements of BA or BA and NAA in combination stimulated the proliferation of highest mean numbers of shoots compared to the control or NAA alone (i.e. no BA) treatments (Table 4.12; Figure 4.19). The effect of BA and NAA was the same as recorded in the previous experiment. The higher the concentration of BA used, the larger the mean numbers of shoots produced per culture after 6 weeks incubation. NAA alone produced no more than one shoot or less per culture even after 6 weeks. NAA had an inhibitory effect on shoot proliferation in cultures with BA.

The optimal concentrations of cytokinins in this experiment were 2 and 4 mg/l, stimulating the highest number of proliferated shoots. This was followed by the combinations of BA/NAA at 4/0.5 mg/l, BA at 1 mg/l and BA/NAA at 1/0.5, 2/2 and 4/1 mg/l, without significant differences among them.

In the BA treatments alone more axillary than adventitious shoots (branches) were observed while in the combination BA/NAA treatments more adventitious than axillary shoots were induced.

Three weeks after the initiation of the culture drying was observed on the tip of leaves and shoots and some shoots were destroyed while some others stopped growing (Plate 4.13). The more severe drying was observed on the explants treated with high concentrations of BA and NAA (BA/ NAA: 4:2 and 4:1). After six weeks many of the produced shoots were dry or had stopped growing in these treatments.

The mean length of the total shoots produced *in vitro* was lower after 6 months than those after 3 months because of more shoots produced with less length and the growth of the formed buds was suspended (Figure 4.20). In the treatments with NAA at 0.5, 1 and 2 mg/l 50%, 55% and 70% of the explants were rooted, respectively and the first roots were visible from the second week from the initiation of the culture (data not showed). No explants were rooted from the

control and from those treatments in which the media contained BA alone or in combination with NAA.

In order to acquire a reasonable length of shoots with a medium number of shoots (3 - 4 per explant) without vitrification problems, the following concentrations of BA and NAA are recommended:

- BA at 1 or 2 mg/l ,alone or
- the combination of BA at 1 or 2 mg/l and NAA at 0.5 or 1 mg/l.

Table 4.12 Effect of different combinations of BA/NAA on the number and the length of shoots of *Ptilostemon chamaepeuce* using WPM and seedlings as explants after 3 and 6 weeks incubation at 25 ± 2°C and 16h photoperiod.

BA/NAA	Number of shoots		Length of shoots (mm)	
	3 weeks – Number of shoots	6 weeks - Number of shoots	3 weeks – Length of shoots	6 weeks - Length of shoots
0/0	1ab*	1ab	5abc	4.5abcde
0/0.5	0.6a	0.5a	2.8ab	2.5abc
0/1	0.7a	0.7ab	2.6a	2.2ab
0/2	0.8a	0.7ab	2.1a	1.7a
1/0	3.2e	4cde	6.9abc	5.2abcde
1/0.5	2.8de	3bcde	7.4abc	4.6abcde
1/1	1.2ab	2.2abcd	12.5c	6.2de
½	1.6abcd	1.9abc	3.2ab	2ab
2/0	3.4e	4.5de	8.4bc	6.6e
2/0.5	2.4bcde	2.5abcd	4.6abc	6.1cde
2/1	1.3abc	2.6abcde	12.4c	7.2e
2/2	1.3abc	3bcde	6abc	4.8abcde
4/0	2.7cde	4.9e	8.4bc	5.4bcde
4/0.5	2.4bcde	4.5de	5.3abc	5.3abcde
4/1	1.3abc	2.9bcde	5.9abc	4.2abcde
4/2	0.5a	0.5a	3.5ab	2.7abcd

* Values followed by the same letter are not significantly different ($P = 0.05$)

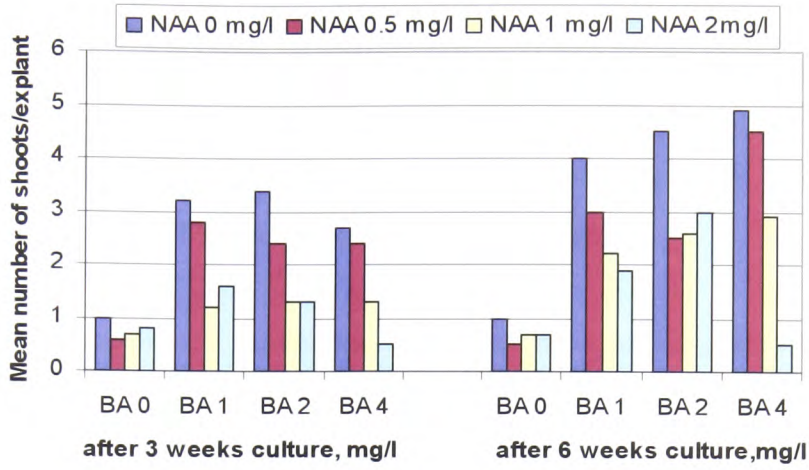


Figure 4.19 Effect of different combinations of BA/NAA on the number of shoots of *Ptilostemon chamaepeuce* using seedlings as explants after 3 and 6 weeks incubation at 25 ± 2°C and 16 h photoperiod.

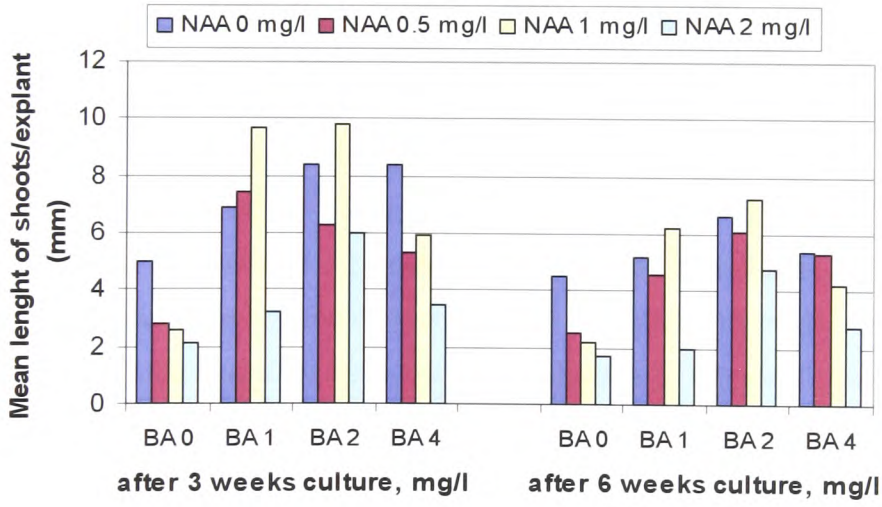


Figure 4.20 Effect of different combinations of BA/NAA on the length of shoots of *Ptilostemon chamaepeuce* using seedlings as explants after 3 and 6 weeks incubation at 25 ± 2°C and 16 h photoperiod.

Conclusion

The results described in this section demonstrated that *Ptilostemon chamaepeuce* can be propagated *in vitro* using shoots from seedlings with shoot and axillary bud

proliferation methods. Using MS medium explants produced more shoots (6 - 8 per culture) than in McCown WPM medium (3 - 5 shoots per culture). More shoots were acquired by the effect of 2 or 4 mg/l BA alone or in combination with 0.5 or 1 mg/l NAA. However using 1 or 2 mg/l BA alone or in combination with 0.5 or 1 mg/l NAA produced shoots which were longer without vitrification problems. The rooting of the shoots was obtained in NAA at 0.5, 1, 2 mg/l and in BA at 1, 2, and 4mg/l but not without rooting hormones.

Rooted cuttings planted in pots and transferred direct to the greenhouse had very small percentage establishment. In contrast shoots that had been previously acclimatized in rock wool and low intensity lighting achieved 100% establishment in pots under greenhouse conditions.

4.6 *PTILOSTEMON CHAMAEPEUCE* AS A CUT FLOWER

Due to the large length of *Ptilostemon chamaepeuce* flower stems (up to 80cm) and the abundant number of its inflorescences (heads) in the same stem (mean 10, max. 30), the plant could be used for cut flowers either alone or as accompanying in bouquets. The aim is the investigation of this possibility.

A particular emphasis of cut flowers is extended maintenance in the vase. After cutting, flowers decline for various reasons. The main reason for decreasing the vase life of the most flowers is the rapid reduction of the water potential of their flowering stem after their harvest. This phenomenon may usually be due:

- to the decreased absorption of water, because of the obstruction of the vessels from bacteria or the creation of bubbles of air;
- to the high osmotic potential of water in which flowers are maintained;
- to the collection of the flowers in earlier stages than the recomented (regular), and
- to the increased loss of water with the transpiration because the inadequate post-harvest handling.

For these reasons the water in which the flowers are maintained should be of low salinity and acidity (pH = 4 - 5), because it creates an unfavourable environment for the growth of bacteria that causes obstruction of vessels of water transport (Marousky, 1971). Also, a small percentage of carbohydrates that are acquired during production can decrease the vase life and the production of ethylene either from the plants themselves or from diseases that coexist with the plants (Halevy and Mayak, 1981b). On the other hand the addition of sugar replaces the rapidly exhausted store of carbohydrates through respiratory metabolism of the cut flowers and at the same time it contributes in the increase and maintenance of the osmotic pressure of the flowers petals, the faculty of absorption of water and the maintenance turgidity (Παπαδημητρίου, 1995).

The use of preservative substances in the water that the cut flowers are maintained is an important post-harvest handling treatment that aims to increase their maintenance in the consumer's vase. Most preservatives contain usually sucrose, an antimicrobial factor and occasionally they also include certain other components as inhibitors of ethylene action, wetting agents or even growth regulators (Halevy, 1976; Durkin, 1985).

4.6.1 Effect of different preservative solution on the improvement of vase life of *Ptilostemon chamaepeuce* flowers.

A carbohydrate and an inhibitor of ethylene are usually used in the preservative solutions. As a bacteriacide, citric acid has been used as well as sulphuric or citric salts of hydroxyquinoline combinations that release chloride in the water such as dichloroisocyanuric acid (Marousky, 1971; Van Doorn *et al.*, 1986b). The addition of dichloroisocyanuric acid (DICA) as a bacteriostatic agent has improved considerably the flower's vase life in other species of cut flowers (Van Doorn *et al.*, 1990).

The senescence of petals in a lot of species is connected with the production and the action of ethylene. Flowers differ both in the production and their sensitivity in ethylene. There are flowers that present a climacteric increase in production of ethylene and are sensitive to the effect of ethylene such as the carnation and

petunia. Others neither produce nor are they sensitive to the effect of ethylene e.g. genus of the Compositae family (*Chrysanthemum*, *Gerbera*) and many monocots such as iris, tulip and narcissus (Reid, 1989). Silver thiosulphate (STS) has been very widely used as an inhibitor of ethylene and although its effectiveness is important in a lot of species cut flowers, it is harmful for people and its replacement is sought. Methylcyclopropene (l-MCP), a cyclic carbohydrate, is effective in the inhibition of ethylene action in many flowers and flowerpot plants (Serek *et al.*, 1994; Παπαδημητρίου *et al.*, 2004).

As for carbohydrates, sucrose is commonly used as it promotes the growth of buds while simultaneously retarding the symptoms of senescence (Halevy and Mayak, 1981). Ichimura (1998) showed that the application of 2% of sucrose in the vase water improved considerably the maintenance of the flowers. The same researcher and his collaborators (Ichimura *et al.*, 1998) found that Lisianthus produces ethylene and that the flowers were not sensitive to the effect of exogenous ethylene in the stage of commercial harvest but their sensitivity is increased as senescence progresses.

Material and Methods

Flower stems selected from native plants having a length 30 – 35 cm with 4 - 10 flowers (heads) per flower stem were harvested from the wild. One of these heads in each flower stem was open (stage 5) while the rest were in the 1 - 4 stages of flowering (Plates 4.15). Heads that were larger than 1 cm diameter (stages 2, 3, 4 and 5) were counted. Flowers that were found in the 1st stage were not calculated in the experiment.

Glass bottles were used instead of vases for the experiments (Plate 4.14). A flower stem with 200 ml from each solution was placed in each bottle. Then the bottle's opening was covered with plastic film in order to avoid water evaporation. Each bottle with the flower stem and the solution was weighed at the initiation of the experiment and after 2, 5, 8, 12 and 14 days and the amount of the water loss was recorded. Fifty bottles, 10 per treatment were used for the solutions.

The effect of the bacteriostatic dichloroisocyanuric acid (DICA) was studied with 20 ppm solution added in all the treatments apart from the control (that contained only distilled water). The following treatments were set up:

- Distilled water (DW)
- DW + 20 ppm DICA
- DW + 20ppm DICA + 1% Sucrose
- DW + 20 ppm DICA + 50 ppm Ethephon
- DW + 20 ppm DICA + 10 ppm Agral

The vases were placed in a chamber set at 20 ± 1°C, 60 - 70% humidity and lighting at 36 μmol.m^{-2}.s^{-1} from fluorescent lamps with a photoperiod of 12 hours.

For the evaluation of the effect of the treatments above, the vase life (longevity), the blossom of the flowers and the absorption of water were measured. The statistical analysis was based on the analysis of variance and the comparison of means of the treatments became with Tukey's test on the level of P= 0.05.

Results and discussion

The application of DICA considerably increased the total and medium vase life of the flowers stems of *Ptilostemon chamaepeuce* in all the treatments in comparison with the control, apart from the treatment that contained ethephon (Table 4.13). The addition of sucrose significantly increased even further the total duration of life compared to all the other treatments. However, there were no statistically significant differences in the mean life duration of each flower (head) between the treatments.

The percentage inflorescence blossoming (with the diameter bigger than 1 cm) was highest in sucrose (97.2%) followed by the treatment with DICA (94%) and the DICA and Agral treatment (91.7%) without significant differences from the control. None of the buds at the 1st stage (Plate 4.15) opened in any treatment.

The addition of the bacteriocide DICA, alone or in combination with the wetting agent Agral, (Figure 4.21) increased the water loss of the flowers and is related to

increased flower maintenance in these treatments. The addition of Ethephon in the conservation solution caused senescence of the petals and inflorescences in a few days after the application and few heads blossomed. The vase life of the heads, which had already flowered before the application of the treatment with Ethephon, were reduced.

The results above show the sensitivity of *Ptilostemon chamaepeuce* flowers to the action of ethylene as well as the positive effect of sucrose, DICA and Agral in the maintaining the duration of the flowers life for approximately two weeks.

Table 4.13 Effect of different preservative solution on the lifetime of heads and flower stems of *Ptilostemon chamaepeuce* flowers.

Treatments	Head opening (%)	Mean vase life of each head (Days)	Vase life of flower stems (Days)	Vase life of half of the heads of the stems (Days)
Distilled water (DW)	76.8 ± 11.0b *	5.8 ± 1.4a	20.6 ± 0.8b	12.2 ± 1.8b
DW + 20 ppm DICA	94.0 ± 15.2b	6.4 ± 2.1a	22.4 ± 0.7c	14.1 ± 1.4bc
DW + 20ppm DICA + 1% Sucrose	97.2 ± 21.8b	7.1 ± 1.6a	24.0 ± 0.9d	15.4 ± 3.8c
DW + 20 ppm DICA + 50 ppm Ethephon	3.7 ± 7.8a	5.3 ± 0.4a	6.0 ± 1.0a	4.1 ± 0.3a
DW + 20 ppm DICA + 10 ppm Agral	91.7 ± 18.0b	5.9 ± 1.4a	20.6 ± 1.1b	16.4 ± 2.8c

*Values followed by the same letter are not significantly different ($P = 0.05$)

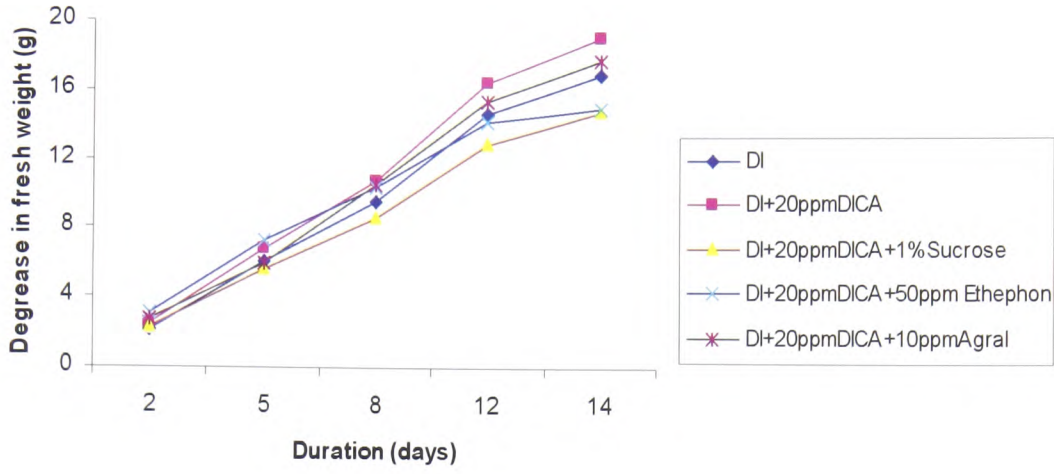


Figure 4.21 Effect of different preservative solutions on water loss from *Ptilostemon chamaepeuce* inflorescences.

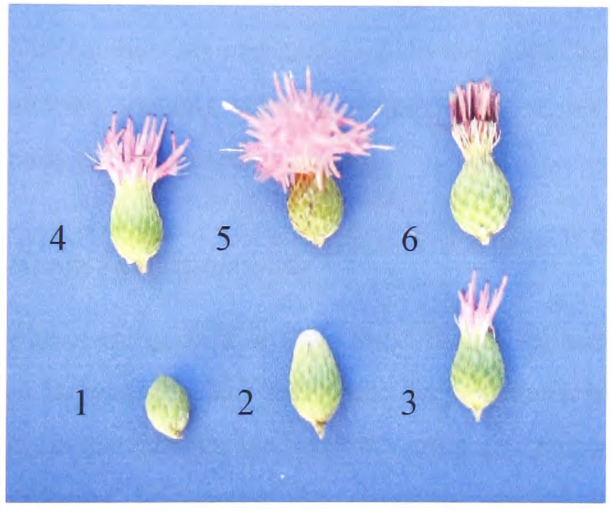


Plate 4.15 Stages of development of *Ptilostemon chamaepeuce* head flowers.

4.6.2 Effect of different harvest stage of the flowers on head opening and vase life of *Ptilostemon chamaepeuce* flowers.

This experiment was conducted in order to investigate the more suitable stage of harvest for opening more buds and increasing longevity during their maintenance in the vase.

Materials and methods

From native plants 15 flower stems, having the length of approximately 30 cm with 4 - 11 flowers (heads) per stem were harvested. The heads that were more than 1 cm in diameter (stages 2, 3, 4 and 5) were selected and counted. Five flower stems did not have any flower (head) at the stage of full blossoming, five had one head at this stage and five flower stems had 3 heads in full blossoming (stage 5). The flower stems were placed in solution of 20 ppm DICA in deionized water. Vases, as in the first experiment, were placed in a chamber with the same conditions. During their maintenance in the vase, buds that opened (apart from those that had already opened), were measured as well as the time until the drying of half of the buds that had already opened.

Results and discussion

The opening of buds from those flower stems that had no open flowers on the day of harvest, reached 90.5% and for the flower stems in which one flower had been open the rate declined to 67% although the two treatments were not significantly different. In contrast, the rate of the opening heads in the flowering stems with 3 heads opened at harvest (29.3%) was significantly different from these two treatments (Table 4.14). The mean vase life in the first treatment (0 opened heads) was 16.6 days, significantly different from the third treatment (3 opened heads) that lasted 9.8 days but not from the second with 15 days (Table 4.14). These results show that the more suitable stage of harvest is when the most of the heads of the inflorescences are closed and in stages 2, 3 or 4.

Table 4.14 Effect of different stage of the flowers during collection on the opening of the heads and the preservation of *Ptilostemon chamaepeuce* flowers in the vase.

Open flowers	Head opening (%)	Vase life of half of the heads of the stems (Days)
0	90.5b*	16.6b
1	67.0b	15ab
3	29.3a	9.8a

*Values followed by the same letter are not significantly different (P= 0.05)

4.7 GROWTH AND ADAPTATION

4.7.1 Growth rate of *Ptilostemon chamaepeuce* in greenhouse

Having observed the growth rate of *Ptilostemon chamaepeuce* in the wild and during the propagation experiments in greenhouse it would be important to assess its potential to adapt easily and grow rapidly as an ornamental pot plant.

Seedlings derived from autumn sowing were cultivated in pots in three potting media: peat moss: perlite (1:1 and 2:1, v/v) and trade compost in the greenhouse. They were fertilized with 1 g/l of N-P-K (20-20-20) once a week. They were also pruned three times at six week intervals. After six months cultivation in all soil media the seedlings had a mean height of 30 – 35 cm from the surface of the substrate and 6 - 10 branches each. This means that they grow rapidly in all the examined potting media and no problems from pests and diseases were observed.

Seedlings from November which were not pruned and had only one stem reached up to 80 cm in height and blossomed next June i.e. 8 - 9 months from sowing. Plants from cuttings also flowered in 8 to 12 months after rooting in the greenhouse.

4.7.2 Adaptation and survival of *Ptilostemon chamaepeuce* in the landscape

The preliminary studies during the cultivation of seedlings and rooted cuttings in the greenhouse were successful and showed that *Ptilostemon chamaepeuce* can be cultivated as a pot plant without special problems in order to be used in commercial floriculture. The aim of this experiment was to find out if the produced plant in

pots in the greenhouse can survive in the open spaces in Mediterranean climates using the experimental park of TEI.

Material and methods

The experimental plan contained 32 plants of *Ptilostemon chamaepeuce* which were set in two rows (Appendix 10). The distance between the plants was 1 m and between the rows 3.20 m. One year old plants from seedlings were planted in the first week of March 2005. The same conditions and methods were used as described in chapter 3.6.2.

Six months after the planting, the plants that failed to get established, the height, diameter and number of lateral shoots were measured.

Results and discussion

Thirty plants (93.8%) survived six months after the date they were planted. The phenological characteristics of their leaves as size and colour were similar to those in their natural habitat.

The plants increased their height by 58%, their diameter 66% and their axillary shoot number by 22% in 6 months after planting (Table 4.15), which was much more growth than in pots. During the cultivation in the plot no problems from pests and diseases were observed.

Table 4.15 Mean increase of height, diameter and number of shoots of *Ptilostemon chamaepeuce* after 6 months cultivation in the experimental plot in the park of TEI. Mean ± sd. N=6.

	Initial means	6 months means	Increase means	Increase %
Height (cm)	30.3 ± 3.5	47.9 ± 6.1	17.6 ± 8.7	58
Diameter (cm)	32. 2± 7.7	53.6 ± 9.0	21.4 ± 7.6	67
Number of shoots	6 ± 2.4	7.3 ± 2.1	1.32 ± 0.8	22

4.7.3 Effect of pruning on the growth and flowering of *Ptilostemon chamaepeuce.*

Pruning of landscape ornamental plants is essential and is undertaken for various purposes. Two of the main goals are to give the plants a suitable shape for better aesthetic value or to induce them to flower. However, each species may respond differently to pruning and therefore it is important to determine how each species responds to pruning, training or pinching. There is no information on the response of *Ptilostemon chamaepeuce* to pruning and, hence, this experiment addresses this aspect.

Material and methods

Thirty plants that had survived after planting in the plot in the experimental park of TEI were used as plant material to investigate the effect of pruning on the growth and flowering of 1.5 year old *Ptilostemon chamaepeuce.*

One month after the planting the following treatments were applied:

1. Six plants were used as control in which no pruning was applied.
2. In six plants the lower shoots were removed and 4 - 5 upper shoots were left intact.
3. The lower shoots of six plants were pinched and the upper 4 - 5 shoots were left intact.

4. The upper shoots from six plants were pinched in one third (1/3) of their height and the lower ones were left without pruning.
5. The remaining six plants were trimmed to form a hedge.

The irrigation and fertilization was applied as detailed in chapter 3.6.2.

The response of the plants to pruning was recorded over the following six months in relation to the number of flowering plants, the number of flower stems per plant and the number of heads per flower stem.

Results and discussion

The foliage of the plants was well developed apart from those of treatment 5 that became yellowish and shrunken. This was due to the fact that microbes (bacteria and/or fungi) attacked them on the cut shoots and leaves after trimming. The plants of treatments 2 and 3 in which the lower shoots were removed or pinched flowers from the middle of July to the middle of August. Plants in all other treatments did not flower. These results indicate that the dominance of the tip bud was strengthened in the treatment 2 and 3 by pruning the lower shoots. Consequently the upper shoots were more elongated, producing the flowers at the top.

In contrast, in treatments 4 and 5 the dominance of the upper shoots was limited by pruning them and more axillary shoots were produced that could not reach the suitable height to flower. In the control treatment, the growth of the plants was as in the previous experiment but the length of the shoots reached during the flowering season (June to August) was not enough to induce flowering. As a result of the above pruning treatments three types of landscape plants were formed:

- the early flowering type with long flowering stems (treatments 2 and 3);
- the normal type as they are thrive in nature (1, control treatment) i.e. going to flower the next year, and
- the thick bush type (treatments 4 and 5) that produce plants suitable for hedges.

Conclusion

The growth rate of *Ptilostemon chamaepeuce* is one of the most important characteristics that make it a possible new ornamental plant that can adapt easily to pots and to landscape. It also can be cultivated to produce cut flowers because of the large number of inflorescences, the colour and the steady (hard) and long flowering stems. During cultivation in pots and in gardens no severe problems of pests or diseases occurred. Its aesthetic value in its habitat in the observation area was retained through the year apart from the hot months during summer until the rains start when the leaves turn light green. Its cultivation in the garden showed that it can survive and retain without special cultivation problems and without losing its ornamental value if an irrigation programme can be applied during summer producing various types of plants for different uses and with high aesthetic value. These characteristics make it a possible new landscape plant especially in dry Mediterranean climates.

4.8 CONCLUSION

The results of the present research showed that *Ptilostemon chamaepeuce* plants of East Mediterranean adapted in the Mediterranean climate, have those characteristics that render possible their choice for exploitation in commercial floriculture and landscape architecture. Its foliage, flowers and form constitute important aesthetic characteristic and initial criteria of choice for further research (Von Henting, 1998). During the experimental research it was possible to achieve fast rhythms of growth, resistance to pest and diseases as well as the capacity to survive unfavourable conditions of the local environment.

Regeneration in nature is achieved by the seeds that are released late the autumn after the rains. Germination of the seeds in greenhouse conditions showed that the seed needs a period of after-ripening during the summer. The need for after-ripening in the dry period is considered as an additional mechanism to avoid germination in high temperatures. This prevents the germination in the case of accidental rain during the dry conditions in summer. The requirement for after-ripening that has been recorded in many species, apart from determining the period

of germination contributes also in the creation of a 'bank' of seeds in the ground (Delipetrou 1995).

Highest germination was achieved at 10 and 15°C, in both light and dark, characteristic for the germination of Mediterranean origin species (Thanos *et al.*, 1994).

Asexual propagation by stem tip cuttings of *Ptilostemon chamaepeuce* was irregular and in some cases inconsistent. According to Loach (1988) propagation by cuttings of many species usually give inconsistent results for the following reasons:

- the different amount of hormone taken up by the cuttings, either as powder or solution, which depends on the condition of the cutting as well as on the environment;
- the applied auxins works alongside an undetermined background level of endogenous IAA, which vary with the type of cutting and stock plant condition, and environment;
- auxins interact with other endogenous growth regulators which also vary in concentration;
- different clones' response to applied regulators.

From the experiments the best seasons for rooting were early in winter and in spring, which was further enhanced by IBA powder, warm water and vermiculite, while in summer cuttings failed to root. However, rooting was not improved by using cuttings from rejuvenated plants after pruning despite the fact that many researchers have produced best results in many species applying this method (Davies and Joiner, 1980; Davies *et al.*, 1982; Davies, 1984; Maynard and Bassuk, 1987; Davies and Hartmann, 1988; Graves, 2002).

There were differences in number and growth of roots of *Ptilostemon chamaepeuce* but not in the overall rooting percentage between the two individual plants from the wild. Genotype usually influences the rooting of some ornamental species or cultivar (Henry *et al.*, 1992; Hilaire *et al.* 1996).

Node stem explants failed to be sterilized for *in vitro* propagation of *Ptilostemon chamaepeuce* because of the thick hair covering the shoots. It was propagated *in vitro* using shoots from seedlings germinated in aseptic conditions with shoot and axillary bud proliferation method. The results indicated that the addition of BA increased the number of shoots but decreased the growth of the produced shoots compared with the control. Increasing the concentration of NAA in the treatments with the combination of BA tended to reduce the number of shoots. Using 1-2 mg/l BA alone or in combination with 0.5-1mg/l NAA produced shoots of best quality without vitrification problems. Microcuttings in MS medium grow better than in WPM where most leaves and shoots dried. NAA at 2mg/l and IBA at 4mg/l produced rooted microcuttings up to 80% and 75% correspondingly, while without auxins no rooting was achieved. These results compared with the rooting experiments results *ex-vitro* show that the rooting of microcuttings is easier to be achieved in aseptic conditions and more controlled environment for *P. chamaepeuce*, a difficult to root species.

Rooted cuttings that had been previously acclimatized in rock wool plugs and low intensity lighting were established 100% in pots under greenhouse conditions.

Ptilostemon chamaepeuce has the potential to be exploited as a cut flower. Present studies in this chapter demonstrated the positive effect of sucrose, DICA and Agral in maintaining the duration of flower life for approximately two weeks. As an ornamental landscape plant can easily adapt and survive in Mediterranean gardens growing rapidly and blossoming. Applying adequate pruning *Ptilostemon chamaepeuce* could produce tree types of landscape plants: early flower shrubs, free nature form or bush type plants suitable for hedges. It can also withstand the adverse climate conditions without losing its ornamental value.

5 *OTANTHUS MARITIMUS* (L.) HOFFMANNS & LINK

5.1 INTRODUCTION

Otanthus maritimus (L.) Hoffmanns & Link is a small perennial shrub which thrives on coastal habitats of stabilized shingle and sand dunes. It is of Mediterranean and Atlantic origin and its distribution extends from the Mediterranean Sea to the west European coasts northwards to south east Ireland (Tutin *et al.*, 1980).

It belongs to the Anthemideae tribe of Asteraceae (Compositae) family (Oberprieler, 2001). According to Tutin *et al* (1980) the synonym *Diotis maritime* (Desf. ex Cass.) is known but on the web more synonyms are recorded: *Diotis candidissima*, *Filago maritima* and *Neesia maritima*.

5.2 OBSERVATION AREA

The observation area as well as the place of collection of all plant material (foliage and seeds) was the north coast of Crete near Hersonissos town, growing on the seaward side of sand dunes (Plate 5.1). Along the riverside and in the estuary of the Aposselemis stream there are a few plants of *O. maritimus* from which all plant material was collected, mainly cuttings and seeds.

5.3 DESCRIPTION

The life form of *O. maritimus* according to Govaerts *et al.* (2002) belongs to the chamaephytes with persistent woody stems ascending, stout, branched above. Shoots are short, white-woolly evergreen spreading perennial up to 50 cm.

Leaves are alternate, oblong to oblong-lanceolate 5 - 17 mm, untoothed to blunt toothed, unstalked, fleshy (Blamey and Grey-Wilson, 1993). Flower-heads are yellow 7 – 10 mm, globose button like, without rays, borne in close, flat-topped

clusters. Flower bracts white-woolly. Fruits are achenes ca. 4 mm without pappus, curved (Tutin *et al.*, 1980; Plate 5.3).

In the observation area plants of *O. maritimus* have the same characteristics (Plate 5.1). It blossoms from May to August on the north coast of Crete.

5.4 UTILIZATION

Otanthus maritimus is reputed among the Bedouins to be an effective antiasthmatic drug and it is used for treating asthmatic bronchitis (Sabri *et al.*, 1983; Jacuponic, 1987). Sequiterpene hydrocarbons have been exacted from its roots (Pascual *et al.*, 1981) and guaianolides, monoterpene diols and flavone glycoside from its aerial parts (Jakuponic *et al.*, 1988), as well as cis-chrysanthenyl acetate (30.4%), camphor (12.9%) and artemisia alcohol (12.6%) (Tsoukatou *et al.*, 2000).

There are no references about the use of *Otanthus maritimus* as ornamental plant. It has a medium ornamental value based mainly on the white colour of foliage that makes contrast against other green plants in the gardens and the small yellow flowers that blossom all through the summer. In contrast it withstands the unfavourable conditions of the coastal areas and the high salinity of the soil in sand dunes. This makes it suitable to be utilized as a small frame plant or in rock gardens in coast residential areas where no other ornamental plants can survive (Plate 5.2). Nowadays its use is limited in the gardens of few beach hotels on the north coast of Heraklion district in sites very close to the sea where few plants can survive.

5.5 PROPAGATION BY SEED

Mature seeds of *O. maritimus* were observed from August to November but a very high percentage of the seeds were empty or immature. Seeds of *O. maritimus* are dormant and did not germinate after six months or two years under darkness or light on continuous or fluctuating temperatures (Delipetrou, 1996). The release of

dormancy (to levels of 90%) was achieved after 4 weeks chilling at 5°C for seeds collected from Crete (Chania). Seeds released from dormancy, germinate in the dark at 15°C or at alternating temperatures (20/13°C) and in natural conditions in April. However, seeds collected from the Peloponessos area did not germinate with or without chilling (Delipetrou, 1996).

The followed experiment was conducted to investigate further the germination of *O. maritimus* using seeds collected from a natural habitat and sown in different soil substrates.

Material and methods

Seeds of *O. maritimus* were collected from the north shore of Hersonissos town in September 2003. Due to the fact that the most of the seeds were immature or empty, only the most vigorous and well developed of them were chosen under stereoscopic visual observation. They were sown in pots in two different substrates at a depth of 1cm in an unheated greenhouse on 12th November and 12th December 2003. The two substrates were compost and peat moss + perlite (1:1, v/v). Three pots per treatment and 30 seeds per pot were used for this experiment.

Results and discussion

None of seeds germinated two months after the first sowing in November. From the second sowing in December only a low percentage (21 %) of the seeds germinated, 32 days after sowing in the compost substrate and none in the perlite + peat moss. Many of the germinated seeds produced abnormal seedlings, small and thin (data not showed). These results confirm the previous results reported by Delipetrou (2006) that the seeds of *Otanthus maritimus* can only be released from dormancy under certain conditions and that there exists variability associated with the location of collection. Germination of *O. maritimus* seeds was not achieved also by Thanos *et al.* (1991).

5.6 PROPAGATION BY CUTTINGS

Because of the bad quality of the seed of *Otanthus maritimus*, alternative methods of propagation were investigated using stem tips and segment cuttings in different substrates and time periods of year.

5.6.1 Effect of the concentration of IBA on the rooting of *Otanthus maritimus* stem tip and segment cuttings in spring.

Materials and methods

In March 2003, stem tip and segment cuttings 5 – 7 cm in length of *O. maritimus* were treated with 0, 500, 1000, 1500 and 2000 ppm IBA in ethanol solution and 0.06% IBA powder, planted in perlite and placed in the mist (Plate 5.4). The temperature under the cuttings was justified at 24 ± 2°C. After 45 days the number of rooted cuttings and the number of roots from each cutting were counted. The experiment design was a randomized complete block with 10 cuttings per replication and 5 replications per treatment (total cuttings 300). Rooting percentages were subjected to arcsin transformation and then analysed by ANOVA test. Treatment means are presented in Table 5.1 without transformation.

Results and discussion

Best rooting percentage in both stem tip and segment cuttings came from the effect of 0.06% IBA (62% and 65%, respectively) on the base of the cuttings. This treatment also produced the highest number of roots (14.6 and 18, respectively) which differ significantly from all other treatments. The control treatment without using rooting hormones, was equally as effective in inducing rooting (68% and 60% for stem tip and segment cuttings, respectively). Both treatments had significant differences in percentage rooting from all other treatments with solution IBA.

The results were presented during the 21st Conference of the Hellenic Society for Horticultural Sciences in 2003 (Αντωνιδάκη *et al.*, 2004).

Table 5.1 Effect of the concentration of IBA on the rooting of *Otanthus maritimus* stem tip and segment cuttings in March.

	Rooting cuttings (%)		Number of roots of cuttings	
IBA, ppm	Stem tip	stem segments	stem tips	Stem segments
0	68d	60c	6.2c	7.2ab
500 solution	32c	34b	5.4bc	12.4bc
1000 solution	12b	24ab	3.2ab	6.4a
1500 solution	8ab	14ab	1.2a	3a
2000 solution	6a	14a	1.8a	2.4a
2000 powder	62d	65c	14.6d	18c

*Means with the same letters are not significantly different at P=0.05 according to Tukey's test.

5.6.2 Effect of seven substrates on the rooting of *O. maritimus* stem tip cuttings.

The experiments continued in order to investigate which substrate is better for rooting and the effect of auxins on the rooting of cuttings taken in autumn.

Material and methods

On 22nd October 2003, stem tip cuttings, 5 - 7 cm in length, were taken from plants in the observation area and dipped in a solution of 1g/l of Benlate (50% benomyl) in water. Then the cuttings were treated with 0.2% IBA powder at the base of the stem. The treated cuttings were inserted into seven different rooting media: peat moss, perlite, sand, vermiculite, peat moss + perlite (1:1, v/v), peat moss + vermiculite (1:1, v/v) and peat moss + sand (1:1, v/v) and then put in a mist environment (Plate 5.4).

After 35 days the amount of rooting and the number of rooted cuttings from each cutting were recorded. The experimental design was a randomized block with 5 cuttings per replication and 5 replicates per treatment (total cuttings 175). Rooting percentage were subjected to arcsin transformation and then analysed by ANOVA test. Treatment means are presented in Table 5.2 without transformation.

Result and discussion

The best rooting percentage was observed in sand (72%) followed by rooting in peat moss + sand (56%) and peat moss + vermiculite (52%) but there were no significant differences between these treatments (Table, 5.2). The lowest percentage of rooting was recorded in peat moss (20%). Sand alone or with peat moss improved the rooting percentage of the cuttings of *O. maritimus*. However, the highest number of roots per rooted cutting was produced in vermiculite. Therefore, overall sand and vermiculite were the best substrates for rooting *O. maritimus* cuttings.

Table 5.2 Effect of different substrates on the rooting of *Otanthus maritimus* stem tip cuttings.

Substrates	Rooting (%) ± sd	No of roots per cutting ± sd
Peat moss	20 ± 14.1a*	1.7 ± 1.2a
Perlite	40 ± 14.1ab	3.9 ± 2.2ab
Sand	72 ± 11.0c	4.8 ± 2.3ab
Vermiculite	44 ± 8.9b	6.7 ± 0.6c
Peat moss + Perlite	32 ± 11.0ab	1.9 ± 1.3a
Peat moss + Vermiculite	52 ± 11.0bc	5.1 ± 3.9ab
Peat moss + Sand	56 ± 16.7bc	4.2 ± 2.4ab

*Means with the same letters are not significantly different at P=0.05 according to Tukey's test.

5.6.3 Effect of the concentration of IBA on the rooting of *Otanthus maritimus* stem segment cuttings in June.

This experiment was carried out in order to confirm the results from the first experiment and to investigate the effect of auxins (IBA) on the cuttings on June when the cuttings are harder than in spring. In addition, the cuttings were taken from the middle of the stems rather than the stem tip.

Material and methods

In June 2004, shoots of *O. maritimus* were cut from plants close to the sea in the observation area. Stems cuttings, 10 – 12 cm in length, were taken from the middle part of the shoots. The leaves from the lower (ca. 1.5cm) portion of the cuttings were removed. Then the cuttings were dipped in solutions of IBA in 50% ethanol

for 30 sec at concentrations of 0, 500, 1000, 2000 or 4000 ppm. One more treatment was the dusting of the cutting base with 0.06% IBA powder (Radicin). After the point of the cut was dried, the cuttings were embedded in sand rooting medium based on the results of previous experiment. The cuttings were placed in intermittent mist. A maximum temperature of 33°C and a minimum 17°C was recorded during this period. Thirty six days after the starting of the experiment the number of rooted cuttings and the number of the roots per cutting were assessed.

The experimental design was a randomized block with 5 cuttings per replication and 4 replicates per treatment (total cuttings 120). The rooting percentage data were transformed to angular values before statistical analysis. Treatment means are presented in Table 5.3 without transformation.

Results and discussion.

The highest percentage rooting of the cuttings was recorded with the control (no auxins) and the treatment with 0.06% IBA powder. In both cases the rooting of the cuttings was 100% and differed significantly from all other treatments (Table 5.3). The rooting of the cuttings decreased gradually as the concentration of IBA was increased (Plate 5.5). The number of the roots per rooted cutting did not differ statistically among all treatments.

The higher rooting results obtained in this experiment compared to the two previous experiments could be due to the sand substrate and to the increased hardness of the cuttings which were taken in June.

Table 5.3 Effect of the concentration of IBA on the rooting of *Otanthus maritimus* stem tip cuttings in June.

IBA ppm	Rooting (%) ± sd	Number of roots per cutting ± sd
0	100 ± 0.0c*	25.5 ± 10.2b
500 solution	45 ± 30.0b	9.8 ± 3.8ab
1000 solution	15 ± 10.0ab	20.5 ± 18.6ab
2000 solution	10 ± 11.5a	4.3 ± 5.1ab
4000 solution	0 ± 0.0a	0.0 ± 0.0a
600 powder	100 ± 0.0c	21.8 ± 6.7ab

*Means with the same letters are not significantly different at P=0.05 according to Tukey's test.

5.6.4 Conclusion

The results of these experiments suggest that both stem tip cuttings and stem segment cuttings of *O. maritimus* can be easily propagated without using growth regulators. Stem segment cuttings produced more rooted cuttings than from stem tips. Dusting the base of the cuttings with 0.06% IBA in powder formula produced rooting up to 100%, but the use of IBA in solution decreased the level of rooting and in the 4000 ppm the rooting was zero. Sand alone or mixed with peat moss as a rooting medium improved the percentage rooting while the vermiculite increased the number of the roots per cutting. Better results were obtained early in summer than in spring and autumn perhaps because of the hardness of the cuttings or the different environmental conditions.

5.7 SURVIVAL AND ADAPTABILITY IN URBAN LANDSCAPE

Seedlings of *O. maritimus* which were derived from seed germination experiments were cultivated in pots with compost + perlite (2:1, v/v) in an unheated glasshouse. After two months, 70.1% of the seedlings had survived and any abnormal seedlings died a month later while rooted cuttings were survived 80% six months later in the same potting medium. The rooted cuttings had a higher growth rate than the seedlings in pots (Plate 5.6).

Six of the seedlings six months' aged were planted in the experimental park of TEI in one row (Appendix 4) and cultivated in the same way that has been described in

3.6.2 chapter. Three months after the planting only 4 seedlings had survived. They had a mean height of 25 cm but they did not have any axillary shoots (Plate 5.7). Adaptability was better after planting in the sandy soil present of two private gardens providing low frequency watering. It was observed that plants that were planted in gardens close to the sea in poor and saline soil survived and grew better than those plants in rich and well watered gardens (Plate 5.8).

5.8 CONCLUSION

Based on the results of the propagation research of *Otanthus maritimus* could one say that propagation by cuttings using 0.06% IBA powder or without auxins in sand in June is more favourable than propagation by seed because of the poor quality of the seed and abnormality of the seedlings as well as the better growth rate and adaptability in pots of the rooted cuttings. Because of its ease of adaptability and good performance in poor soils it is ideal in areas with degraded soils, coastal areas and saline soils.

6 *PRASIUM MAJUS* L.

6.1 INTRODUCTION

Prasium majus L. belongs to the Lamiaceae (Labiatae) family. It is a small woody perennial shrub, which thrives in dry places among bushes or rocks, field boundaries, olive groves, beside old walls, roadsides, and rock crevices at altitudes of 0 – 650 m throughout the Mediterranean region (Tutin *et al.*, 1980; Blamey and Grey-Wilson, 1993). *Prasium majus* is the only species of the genus *Prasium*.

The classification according to Royal Botanic gardens Bulletin, Kew is as follows:

APG Clade: EUDICOTS- CORE EUDICOTS- ASTERID-EUASTERID I

APG Order: Lamiales

APG Family: LAMIACEAE

Kew Family: LABIATAE

Genus: Prasium

Species Epithet: majus

Species Author: L.

Life form: cham. or nanophan.

The life form can be classified according to the system of Raunkiaer (as modified by Govaerts *et al.*, 2000) in the nanophanerophytes because some shoots in special conditions can arrive up to 4 m in height or as a chamaephyte as the main body of the plant, especially in high altitudes, is not usually more than 50 cm in height.

6.2 DESCRIPTION

The morphological characteristics of *Prasium majus* in the observation area are close to that described by Tutin *et al.*, (1980), Blamey and Grey-Wilson, (1993):

much-branched shrub to 4 m, though often only 50 cm, with rather erect branches, hairless or slightly hairy.

Leaves 2 - 5 x 0.8 – 2 cm, shiny, dark green, oval to lanceolate, toothed, the lower with a heart-shaped base the upper more truncated, all stalked. The length of petioles is 10 – 18 mm. Bracts leaf-like but smaller. Flowers white or pale lilac, 17 - 20 mm long, borne in terminal racemes, Corolla is 2-lipped with the upper lip oblong and the lower with the middle lobe largest (Plate 6.1). Calyx is up to 25 mm in fruit. Nutlets are 1 - 4 mm shiny-black when ripe, ovoid-trigonous. Fruit have a succulent part (pulp) outside the seeds (Plate 6.2).

6.3 LIFE CYCLE

New shoots sprout during autumn after the first rains. The leaves of the plants dried during the summer in the observation area and new shoots sprout again after the rains in autumn. They remain on the shoots during the summer if the plants are watered and put under shade or low temperature but their colour becomes yellow-green. They blossom from January to May in their natural habitat. The flowering continues if it is cultivated in pots all through the year. Mature seeds from 1 to 4 in each lobe were found from March (Plate 6 2). Plants in cultivation can produce mature seeds earlier from January.

6.4 UTILIZATION

It is used in Crete as an edible plant among other wild ones to make pies or cooked food because of its aromatic leaves. The seeds of *Prasium majus* contain 15 % oil and 8 % proteins (Hagemann *et al.*, 1967). It could be used as a garden plant in dry or fertile soils, among other edible or aromatic plants or in rock gardens. In summer it can survive if it is watered regularly and low temperature is maintained.

6.5 PROPAGATION BY SEED

Preliminary studies during the years 2001 and 2002 in the greenhouse of TEI showed that the seeds of Prasium begin to germinate 12 - 14 days after the sowing

time in winter but no special experiments on the germination of *P. majus* seeds were conducted. There are no references for the propagation of *P. majus* by sexual or asexual methods.

Seeds were collected from the wild in 2003 and 2004 and dried, after their pulp was removed, for 15 days in open air on a bench in the laboratory. Cleaned seeds were weighed and it was found that 1 g contains 165 seeds (1000 seeds weight 6.1 g). According to Hagemann *et al.* (1967) 1000 seeds, without removing their pulp, weigh 7.2 g. All seeds of *Prasium majus* used for the germination experiments were dried without the pulp.

6.5.1 Germination of *Prasium majus* seeds in two substrates in an unheated greenhouse.

Material and methods

Seeds of *Prasium majus* collected in June 2004 were dried and placed in a plastic container and stored in a dry place in room temperature until January when the experiment started.

From the examination of the seeds it was ascertained that many of them were empty or attacked by insects. For this reason seeds were subjected to a flotation test and undeveloped, dead or attacked seeds were discarded. Two germination media were prepared: trade compost and peat moss and perlite 1:1 (v/v). Seeds were sown in January 2005 in 19 x 8 cm pots (20 seeds per pot and 2 pots per medium). The pots were placed on a bench of an unheated greenhouse. Seed germination was recorded every 3 or 4 days for a period of 35 days. The seeds were considered as germinated when their cotyledons were seen on the surface of the medium.

Results and discussion

Higher percentage germination was recorded in compost (55%) after 31 days while in peat moss and perlite 43% germination was reached in the same incubation time (Figure 6.1). In addition, the T_{50} were not significantly different (Table 6.1).

The seedlings in the pots developed better in the compost and two months later most of them were transplanted into individual pots. Growth in compost was faster than in peat moss and perlite. All the transplanted seedlings survived (Plate 6.3)

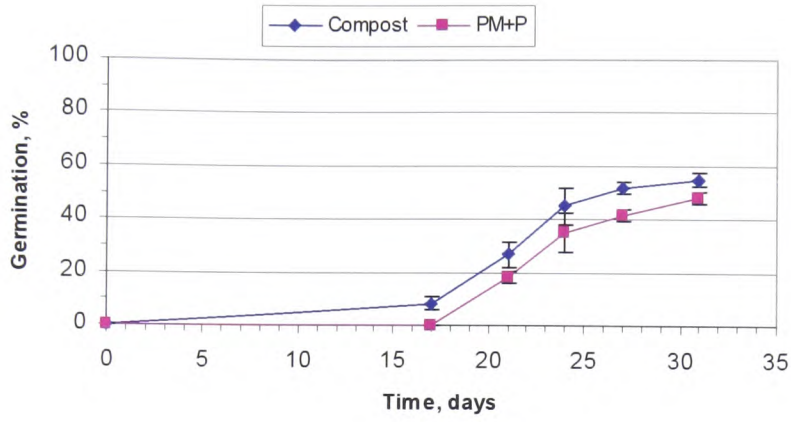


Figure 6.1 Germination time course of *Prasium majus* seeds in compost and peat moss + perlite (1:1, v/v) in an unheated greenhouse after 31 days of incubation.

Table 6.1 Mean percent germination and T_{50} of *Prasium majus* seeds in two germination media in an unheated greenhouse after 31 days of incubation ± sd.

Germination medium	Germination, %	T_{50}
Compost	55.0±3.4a*	21.1±1.1a
Peat Moss+Perlite (1:1, v/v)	48.3±2.4a	22.2±1.0a

*Means with the same letters are not significantly different at P=0.05 according to Tukey's test.

6.5.2 Effect of temperature and lighting on the germination of *P. majus* seeds.

Although compost proved the better potting medium for seed germination, only a 50% germination rate was achieved. Therefore, it was decided to investigate the effect of temperature and light on germination.

Material and methods

Dried and pre-washed for 30 minutes, seeds of *Prasium majus* were subjected to flotation to remove non viable seeds. After discarding undeveloped and insect-attacked seeds, the healthy ones were placed in 9-cm Petri dishes on blotting paper saturated with deionized water. Petri dishes were placed in growth chambers at 10, 15 and 20°C under light/dark (16/8h from fluorescent lamps at 36 μmol/m^2/sec) or dark conditions. All Petri dishes were wrapped with plastic transparent foil to avoid water evaporation. Darkness was achieved by wrapping the Petri dishes with aluminum foil.

Three Petri dishes were used per treatment and 20 seeds per dish (360 seeds in total).

A completely randomised design was used. The germination percentage data were transformed in angular values before statistical analysis. Treatment means are presented in table 6.2 without transformation. The data was analysed by analysis of variance and the means were compared by Tukey's test.

Results and discussion

In all treatments seeds start to germinate after 9 - 11 days and the germination continued up to 48 days after which the experiment ceased (Figure 6.2). The percentage germination ranged from 61.7% at 10°C and light/dark conditions to 46.7% at 10°C and 20°C and darkness, although there were no significant differences among the treatments (Table 6.2; Figure 6.3; Plate 6.4).

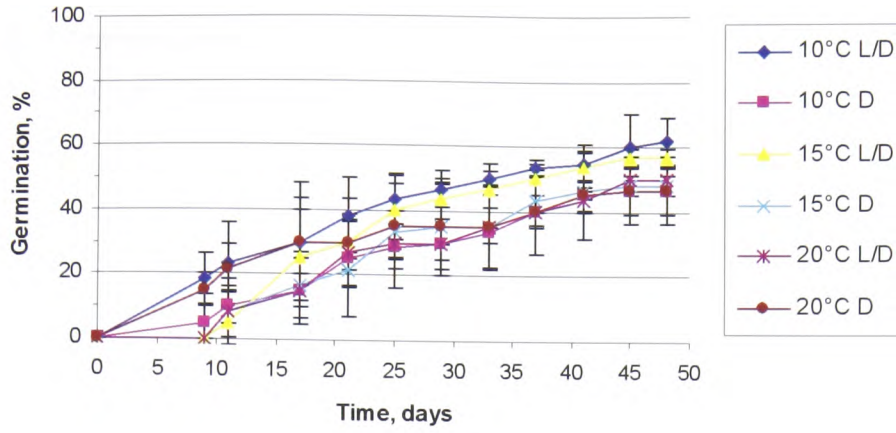


Figure 6.2 Germination time course of *P. majus* seeds at 10, 15 and 20°C and light/dark (16/8h) or dark conditions.

Table 6.2 Effect of temperature and lighting on the % germination and T_{50} of *Prasium majus* seeds. Mean ± sd.

	10°C L/D	10°C D	15°C L/D	15°C D	20°C L/D	20°C D
Germination %	61.7 ± 7.6a*	46.7 ± 7.6a	56.7 ± 2.9a	48.3 ± 2.9a	50.0 ± 5.0a	46.7 ± 10.4a
T_{50}	16.5 ± 5.8a	16.5 ± 5.8a	18.8 ± 1.7a	19.6 ± 4.1a	22.7 ± 9.0a	18.6 ± 2.9a

*Means with the same letters are not significantly different at P=0.05 according to Tukey's test.

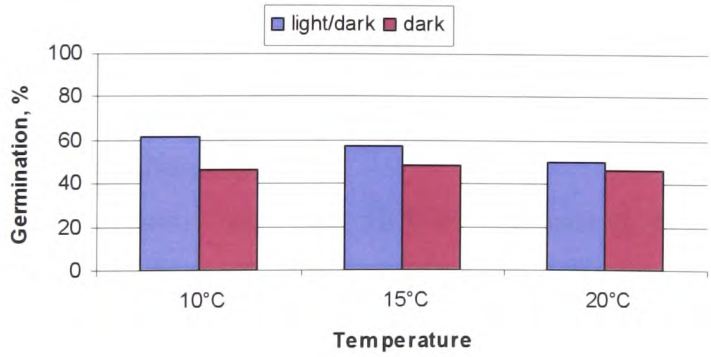


Figure 6.3 Accumulative germination by the effect of temperature and lighting (light/dark: 16/8h) on *P. majus* seeds.

A large number (up to 50%) of the seeds of *Prasium majus* from the collected area were immature, empty or attacked by insects. These seeds floated on the water. The endocarp of the seeds is hard and it is difficult to imbibe water. This may be the reason that the germination is prolonged and relatively low.

Further treatments were examined to improve the germination rate.

6.5.3 Effect of temperature, boiling water, acids and scarifying on the germination of *P. majus* seeds.

Material and methods

A second seed lot was used to check the germination after the following pre-treatments:

1. Scarified by 98% sulphuric acid (H_2SO_4) for 10 min, 50% for 20 min, or 20% for 30 min and then washed with abundant deionized water.
2. Scarified with 37% HCl for 30 min, 7.5% for 30 min or 5 hours and 15% for 30 min. After treatments the seeds were washed with deionized water.
3. Immersed into boiling water for 30 sec or 1 min.
4. Exposed to 100°C for 1, 5 or 10 min in an oven,
5. Scarified by cracking the seed coats.

After the pre-treatments the seeds were placed in Petri dishes with saturated filter papers and incubated at 15°C temperature in light/dark and dark conditions. Two Petri dishes were used per treatment and 20 seeds per dish (total seeds 520).

Results and discussion

Seeds pre-treated with H_2SO_4, HCl, boiling water or 100°C failed to germinate 30 days after placing in Petri dishes. They became brown to black and their embryo and endosperm was destroyed. Seeds that had scarified by cracking the seed coats achieved 100% germination both in light/dark and darkness (data not shown) after 14 days.

These results indicate that seeds of *Prasium majus* have a primary exogenous dormancy (or seed coat dormancy). Exogenous dormancy of many other species is

imposed upon the seed from factors outside the embryo including the seed coat and/or parts of the fruits. The tissue enclosing the embryo can impact upon germination by inhibiting water uptake, by modifying gas exchange (e.g. limiting oxygen to the embryo), by preventing leaching of inhibitors from the embryo and by supplying inhibitors to the embryo. In nature various mechanisms exist for softening hard seeds such as fluctuating temperatures, by freezing, by fire or by microorganisms. In cultivation any method to break, soften or remove the seed covering is immediately effective for improving imbibition and germination (Hartmann *et al.*, 2002).

Germinated seeds were transferred in pots in the environment of an unheated greenhouse and all had survived six months later.

6.6 PROPAGATION BY CUTTINGS

From preliminary experiments conducted during 2002 and 2003 it was established that *P. majus* cuttings could root using auxins in concentrations depending on the hardness and the type of the cuttings (Antonidaki, unpublished data). Further experiments were conducted to increase the level of rooting through use of auxin.

6.6.1 Effect of different concentration of IBA on the rooting of *P. majus* cuttings

Material and methods

On the 1st of March 2004, stem tips and stem segment cuttings, 5 – 8 cm in length, were collected from wild and were treated with 0, 500, 1000, 1500, 2000, 4000 ppm IBA, (diluted in 50% ethanol and 50% distilled water) and 0.2% IBA in powder form. They were placed in plastic multiple pot discs with perlite as substrate and were put under intermittent mist (Plate 6.5).

Heating was applied to produce a temperature of 25 ± 3°C at the base of the cuttings for the first eight days. The following days the heating was stopped because of the high environmental temperatures. Thirty two days later the cuttings were extracted from the pots and the number of the rooted cuttings as well as the number and length of the roots from each cutting were counted.

The experimental design was a randomized complete block with 5 stem cuttings and 5 segment cuttings per treatment in 3 blocks.

Results and discussion

The best results of rooting percentage were recorded with stem tip cuttings in IBA powder (72%) (Table 6.3). However, there was considerable variability and there were no significant differences between the treatments apart from those of 500 ppm IBA solution and IBA powder. Regarding the segment stem cuttings, the highest rooting percentage was recorded both in 2000 ppm solution and the powder. However there were no statistically significant differences apart from those among the control and the 1500 and 2000 ppm both in solution and powder form treatments.

Most roots were formed in segment stem cuttings (13.2/cutting) with 2% IBA powder (Plates 6.6). In relation to the number of roots in tip stem cuttings and the length of roots there were no statistical differences among all treatments (Table 6.3; Plate 6.6).

Table 6.3 Effect of different concentration of IBA on the rooting, number and length of roots of *P. majus* stem tip or segment cuttings. Each value is the mean of 3 replications (5 cuttings/replication).

	Rooting, (%)		Number of roots per cutting		Mean length of roots (cm)	
IBA, ppm	tip cutting	segment cutting	tip cutting	segment cutting	tip cutting	segment cutting
0	16.7ab*	5.6a	1.7a	0.3.a	0.8a	0.1a
500	5.6a	33.3ab	1.7a	1.2a	1.5a	0.9a
1000	22.2ab	27.8ab	2.3a	2.8ab	1.8a	1.2a
1500	16.7ab	44.4 b	1.7a	8.6ab	0.8a	0.8a
2000	44.4ab	50.0 b	7.4a	2.0ab	1.7a	1.3a
4000	38.9ab	22.2ab	4.6a	2.8ab	1.4a	1.0a
2000 powder	72.2b	50.0 b	9.2a	13.2b	1.4a	2.0a

*Means with the same letters are not significantly different at P=0.05 according to Tukey's test.

6.6.2 Effect of seven substrates on the rooting of *Prasium majus* stem cuttings

Rooting in perlite was relatively low so more substrates were tested to increase the percentage rooting of *P. majus*.

Material and methods

On 22nd of March 2004, cuttings 5 – 8 cm in length were taken from plants in the observation area and dipped in a solution of 1 g/l of Benlate (50% benomyl) in water. Then the cuttings were treated with 0.2 % IBA powder at the base of the stem. The treated cuttings were inserted into seven different rooting media: peat moss, perlite, sand, vermiculite, peat moss + perlite (1:1, v/v), peat moss + vermiculite (1:1, v/v) and peat moss + sand (1:1, v/v) and put in a mist environment.

After 50 days the presence of rooting and the number of rooted cuttings from each cutting were recorded.

The experimental design was a randomized complete block with 5 cuttings per replication and 6 replicates per treatment (total cuttings 210). Rooting percentages were subjected to arcsin transformation and then analysed by ANOVA test. Treatment means are presented in Table 6.4 without transformation.

Result and discussion

The best rooting percentage was observed in vermiculite (83.3%) followed by rooting in peat moss and perlite (53.3%) and perlite (40%) (Table 6.4). Additionally, in vermiculite more roots per rooted cutting were induced, but no statistically significant differences were recorded between vermiculite and perlite or peat moss + perlite. Sand completely inhibited cuttings to root.

Therefore vermiculite as well as perlite and mixture perlite + peat moss were the best substrates for rooting of *Prasium majus* cuttings from the observation area and under the described conditions (Plate 6.7).

Table 6.4 Effect of different substrates on the rooting and number of roots of *Prasium majus* stems cuttings.

Substrate	Rooting, %	Number of roots/cutting
Peat moss	3.3ab*	1.0ab
Perlite	40.0bcd	6.8cd
Sand	0.0a	0.0a
Vermiculite	83.3d	9.4d
Peat moss+Perlite	53.3cd	5.4bcd
Peat moss+Vermiculite	33.3abc	2.7abc
Peat moss+Sand	33.3abc	2.3abc

*Means with the same letters are not significantly different at $P = 0.05$ according to Tukey's test.

6.7 SURVIVAL AND ADAPTATION OF *PRASIUM MAJUS* IN URBAN LANDSCAPE

The rooted cuttings derived from the above mentioned experiments were planted in pots in a mixture of compost + perlite + peat moss (1:1:1, v/v) and maintained in a greenhouse for one year. A percentage of 85% of the rooted cuttings survived in

pots where they flowered after two or three months depending on the vigour of the cuttings, the development of their rooting system and the environmental conditions (Plate 6.8). .The aim of this research is to investigate the possibility of adaptation and survival of *Prasium majus* that have been produced by asexual reproduction by cuttings in the greenhouse.

Material and methods

Twenty one plants of *Prasium majus* derived from vegetative propagation one year after the initiation of rooting were planted in the experimental park in two rows at a distant of 2.7 m between the rows and 1 m between each plant (Appendix 4).
The plants were planted and cultivated in the same way as described in chapter 3.6.2. Six months after the planting, the number of survived plants, the height spread and number of lateral shoots were measured.

Results and discussion

Nineteen plants survived (90.5%) and adapted in the outdoors environmental conditions. During the three summer months June, July and August the *P. majus* plants were stressed and their foliage became yellowish because of the high seasonal temperatures. During the same period the foliage of *P. majus* plants in the wild had already entirely dried. In the park their foliage was alive but the leaves were yellow and the quality was poor.

In September when the temperatures decreased the plants recovered and their quality improved (Plate 6.9). Some of the plants continued flowering all through the six months of cultivation in the park.

The extent of the growth of the plants recording height and diameter is presented in the table 6.5. The recorded growth was acquired mainly during the first three months after the planting and during the summer less development occurred.
During the six months of cultivation in the plot no problems from pests and diseases were observed.

Table 6.5 Mean increases in height and spread of shoots of *Prasium majus* after six months cultivation in the experiment plot in the park of TEI.

	Initial mean	6 months mean	Mean increase
Height, cm	13.7 ± 3.3	23.1 ± 5.7	8.4 ± 4.6
Spread, cm	20.6 ± 4.0	32.4 ± 8.5	10.7 ± 7.5

In conclusion *Prasium majus* can adapt in an artificial landscape without special problems apart from the yellowish foliage during the summer. This problem in an aesthetic garden can be managed by pruning the plants at their base in June and planting annual summer plants near them. Next autumn new shoots will sprout from the buds on the bases of the remaining woody branches.

6.8 CONCLUSIONS

Prasium majus, a woody Mediterranean perennial plant, has a long period of anthesis and dark green aromatic leaves. It can be used as a rock garden plant for many types of soil and climate conditions. At the same time it could be utilized as traditional edible plant for the local people. The seeds of *P. majus* have "seed coat" dormancy and need scarification for quick germination. After cracking the hard pericarp, seeds were germinated at 15°C under light or darkness up to 100% in 15 days in compost. It can be propagated easily by stem tip and segment cuttings using 2000 ppm IBA in solution or powder form. Better substrates for rooting and best root development were proved vermiculite and mix of peat moss + perlite 1:1 (v/v). The rooted cuttings were established in the garden environment and had a rapid growth and anthesis especially in winter (Plate 6.10). Plant survival and prolonged quality can be achieved all year by managing the watering and temperature and through pruning.

7 *CONVOLVULUS DORYCNIUM* L.

7.1 INTRODUCTION

The genus *Convolvulus* belongs to the Convolvulaceae family and comprises approximately 100 species worldwide with the greatest diversity found in the Mediterranean region (Carine, 2001).
Classification according to Royal Botanic gardens Kew (http://www.hew.org) for the genus Campanula is as follows:

APG Clade: EUDICOTS - CORE EUDICOTS - ASTERIDS - EUASTERIDS I
APG Order: Solanales
APG Family: CONVOLVULACEAE
Kew Family: CONVOLVULACEAE
Genus: Convolvulus
Species Epithet: dorycnium
Species Author: L.

Convolvulus dorycnium is a woody shrub that thrives in dry rocky places, scrub and roadsides usually near the sea. It is a widespread shrub which extends to the East Mediterranean and Anatolia up to west Iran, south Greece, Crete, Aegean Islands, Cyprus and Tunisia (Davis, 1965 – 1988; Blamey and Grey-Wilson, 1993). Eight species of the genus *Convolvulus* thrive in Crete: *C. althaeoides*, *C. argyrothamnos*, *C. arvensis*, *C. dorycnium*, *C. elegantissimus*, *C. libanoticus*, *C. oleifolius*, and *C. siculus* (Meikle, 1985). Most of them are weeds that are difficult to eliminate from cultivated crops.

7.2 DESCRIPTION

Convolvulus dorycnium is an upright multi-branched shrub 50 - 100 cm in height. Its mature stems and braches are woody, rigid, densely hairy light green. The new leaves that sprout in the wild in early spring (February) are hairy oblong to ovate. Flowers are pale pink, 20 - 30 mm, in branched inflorescences but solitary at each

branch tip. Corolla is funnel-shaped, pleated, closed in the night and expanding in daylight. During flowering, leaves are not present on the flower stems. *Convolvulus dorycnium* flowers from May to July in the observation area producing a whole spherical to oval ball shape consisting of bare flowers from leaves stems and abundant flowers (Plate 7.1). The upper ground part becomes dry during the summer, which sprouts again early in the spring from the rhizome that remains under the soil surface.

7.3 LIFE CYCLE

The life form of *Convolvulus dorycnium* according to the system of Raunkiaer, as modified by Govaerts *et al.* (2000), is classified as a hemicryptophyte. They have stems that die-back during unfavourable seasons and surviving buds are formed just below soil level. This group includes many biennial and perennial herbs, including those in which buds grow from a basal rosette.

The leaves of *Convolvulus dorycnium* appear on the soil surface at the end of winter or early in spring after the rains have penetrated the soil to reach root depth and the temperature has increased. Leaves form rosettes and more than one rosette sprouts from the buds that are just below the soil level. During the spring multi-brunched stems are produced from rosettes, which develop upright to a length of 50 – 100 cm. On the each tip of the branches the flower buds are solitary (Plate 7.1). Flowering takes place from May to July depending on the temperature and the available water. Seeds in single seeded capsules mature from July and stay on the plant until late in autumn although the branches have already dried (Plate 7.2 and 7.3). The whole of the above ground parts of the plant dry up in summer in the Mediterranean climate where no rains occur in summer.

7.4 UTILIZATION

There are no references about its use. It could be used as a garden plant in dry and barren soil and in coastal residential areas. Its ornamental value would be due to the abundant pink flowers in branched inflorescences without leaves. Another advantage is that *Convolvulus dorycnium* survives and flowers during the dry

period of the year (June and July) at a time when few wild plants blossom in dry places without watering.

7.5 PROPAGATION BY SEED

Seeds of *Convolvulus dorycnium* collected from observation area (North sea-side area of Heraklion) were cleaned from the capsule material and dried in open air for 20 days and then weighed. Their weight ranged from 6 to 17 mg/seed and the mean weight was found 13.2 ± 3.1 mg per seed. Most of the seeds, ranging from 60 to 65%, were attacked by an insect of the Bruchidae family.

From the preliminary studies in the previous years it was ascertained that the *Convolvulus dorycnium* seeds had a low and irregular germination (Antonidaki, unpublished data). This was as a result of two factors: on the one hand the seeds are attacked by insects and on the other hand that some of the seeds continue to be hard for many days even if they were placed in water for imbibition.

The following experiments were conducted to investigate natural germination in soil in the greenhouse and in stable controlled temperatures in growth chambers.

7.5.1 Germination of *Convolvulus dorycnium* seeds in two substrates in an unheated greenhouse.

Material and methods

Seeds collected in August 2004 from the wild were subjected to flotation and undeveloped or attacked by insects seeds were rejected. Then the remaining seeds were rinsed under tap water for 30 min in order to absorb water and remove possible inhibitor substances.

Two germination media were prepared for evaluation: trade compost and peat moss with perlite (1:1, v:v). Three separate lots of seed were sown, in November and December 2004 and in February 2005 in 19 x 8 cm pots (30 seeds per pot and 3 pots per medium). The pots were placed on a bench in an unheated greenhouse. Seed germination was recorded every 3 or 4 days for a period of 47 days. The

seeds were considered as germinated when their cotyledons were seen on the surface of the medium.

A completely randomised block design was used. The germination percentage data were transformed in angular values before statistical analysis. Treatment means are presented in table 7.1 without transformation. The statistical analysis of the data was based on analysis of variance and the means were compared by Duncan's test.

Results and discussion

Seeds began to germinate 7 - 9 days after sowing in all treatments (Figures 7.1 - 7.3). Highest percentage germination was recorded in February (62.2% and 60% in peat moss+perlite and compost, respectively) which was significantly different from December (Table 7.1). There are no statistically significant differences between the two germination media with regard to percent germination as well as the speed of germination (Table 7.1).

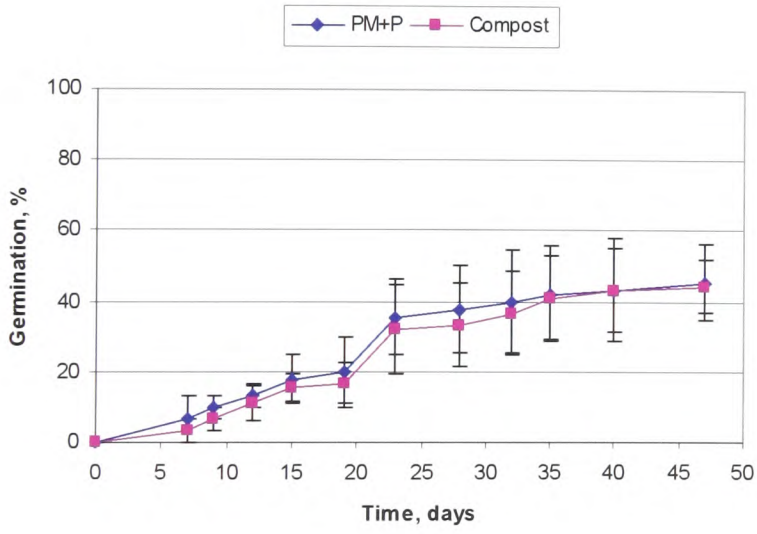


Figure 7.1 Time course germination of *Convolvulus dorycnium* seeds in compost and peat moss + perlite (1:1, v/v) in an unheated greenhouse during November 2004.

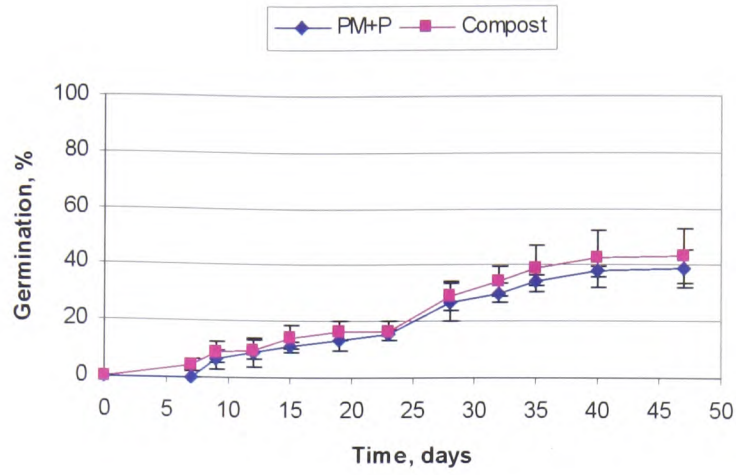


Figure 7.2 Time course germination of *Convolvulus dorycnium* seeds in compost and peat moss + perlite (1:1, v/v) in an unheated greenhouse during December 2004.

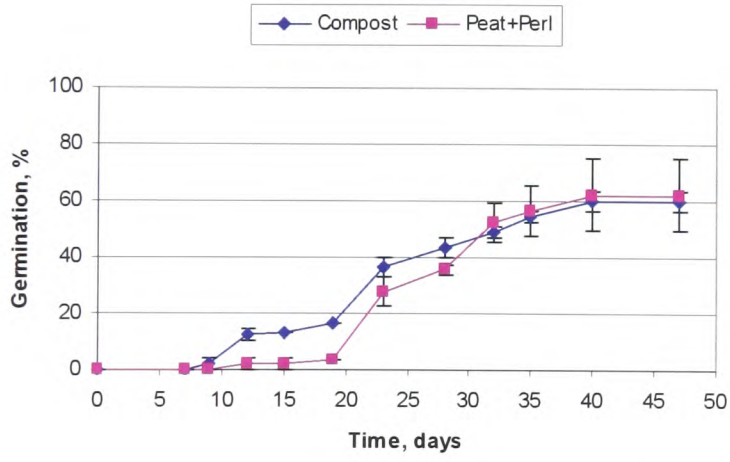


Figure 7.3 Time course germination of *Convolvulus dorycnium* seeds in compost and peat moss + perlite (1:1, v/v) in an unheated greenhouse during February 2005.

Table 7.1 Germination % and T_{50} of *Convolvulus dorycnium* seeds in peat moss + perlite (1:1, v/v) and compost germination medium in an unheated greenhouse in November 2004, December 2004 and February 2005.

Germination medium	Germination %		T_{50}	
	PM+P	Compost	PM+P	Compost
November 2004	45.6 ± 10.7abc*	44.4 ± 7.7ab	19.3 ± 2.1a	20.8 ± 1.1a
December 2004	38.9 ± 6.9a	43.3 ± 10.0ab	25.6 ± 3.8a	22.1 ± 6.2a
February 2005	62.2 ± 3.3c	60.0 ± 12.6bc	25.3 ± 3.1a	21.7 ± 0.4a

*Means with the same letters are not significantly different at P=0.05 according to Duncan's test.

7.5.2 Effect of temperature and light on the germination of *Convolvulus dorycnium* seeds.

Material and methods

Convolvulus dorycnium seeds, collected in August 2004, were dried and stored in a plastic container in a dry place until use. In January 2005 they were subjected to flotation in order to sort and discard the undeveloped and insect-attacked seeds. The seeds remained in water for a further 30 min to absorb water and/or remove possible inhibitor substances. After that seeds were placed in 9-cm Petri dishes with filter paper saturated with deionized water. Petri dishes were placed in growth chambers at 10, 15, 20 and 25°C under light/dark (16/8h from fluorescent lamps at 36 μmol/m^2/sec) or at 5, 10, 15, 20 and 25°C in dark conditions. All Petri were wrapped with plastic transparent foil to avoid water evaporation. Darkness was achieved by wrapping the Petri dishes with aluminum foil. Seed germination was recorded every 3 or 4 days for a period of 40 days. Three Petri dishes were used per treatment and 20 seeds per dish (540 seeds in total).

The germination percentage data were transformed to angular values before statistical analysis. Treatment means are presented in table 7.2 without transformation. The statistical analysis of the data was based on analysis of variance and the means were compared by Tukey's test.

Results and discussion

In all treatments seeds started to germinate after 4 - 6 days except for incubation at 5°C where germination started after 11 days (Figures 7.4 and 7.5). The time needed to germinate 50% of the seeds (T_{50}) was less at 25°C with statistically significant differences between 5°C in dark and 10°C in light/dark condition but not from the other treatments (Table 7.2).

Best germination was obtained by seeds incubated at 20°C and dark conditions (90%) 40 days after sowing. However there were no significant differences between germination at 20°C in light/dark conditions (61.7%) and 15°C in darkness (66.7%), (Table 7.2). Light did not affect the speed of germination. Also, there were no statistical differences between germination for seeds incubated in light or darkness at all temperatures.

Seeds, which had been attacked by insects or their hard pericarp had been destroyed, imbibed water as indicated by a softening and swelling of the seed and germinated earlier than the intact seeds. The intact seeds absorbed water more gradually. As soon as a seed absorbed water it germinated immediately. All seeds that had not germinated by the end of the experiment remained hard (Plate 7.5).

Generally speaking *Convolvulus dorycnium* seeds germinate over a wide range of temperatures with the best results at 20°C and the worse at 5°C. This indicates that if the seed pericarp is removed or scarified, seeds are released from dormancy and germinate quickly. The difficulty is for seeds to absorb water, which may due to the presence of macrosclereid cells in the seed coat which prevent water uptake in seeds with exogenous physical dormancy (seed coat dormancy). This suggests that germination can be induced by any method that can soften or scarify the covering. Physical dormancy is a genetic characteristic found in species from at least 15 plant families including the Convolvulaceae family (Hartmann *et al.*, 2002).

Theses results are similar with the results from RBG Kew for seeds from Jordan that achieved 100% germination for scarified seeds at 15°C, 8/16h lighting in 1% agar medium (http://www.kew.org, 2005). Small differences in germination rate

may due to a combination of the degree of scarification of the seeds and different light conditions or incubation time.

The seedlings were transplanted in individual pots and recorded a high survival rate (92%) (Plate 7.4)

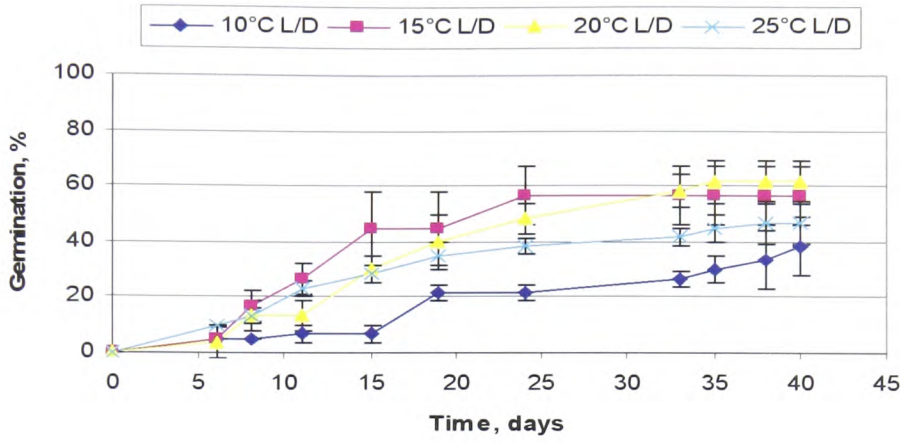


Figure 7.4 Germination time course of *Convolvulus dorycnium* seeds at 10, 15, 20 and 25°C and light/dark (light/dark: 16/8h) conditions.

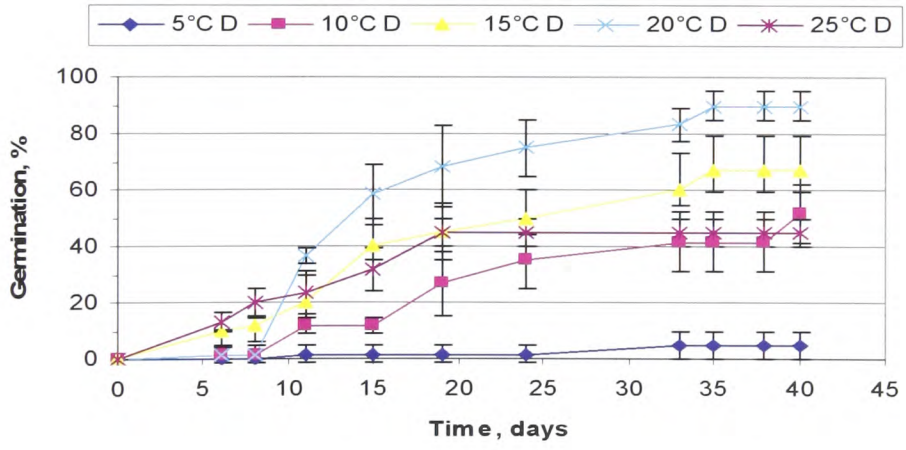


Figure 7.5 Germination time courses of *Convolvulus dorycnium* seeds at 5, 10, 15, 20 and 25°C and dark conditions.

Table 7.2 Effect of temperature and light (light/dark: 16/8h) on percent germination and T_{50} of *Convolvulus dorycnium* seeds.

Temperature	Germination%		T_{50}	
	Light/dark	Dark	Light/dark	Dark
5°C	0	5a	-	26.3c
10°C	38.3b*	51.7b	22.6bc	19.4abc
15°C	56.7b	66.7bc	10.7a	14.0ab
20°C	61.7bc	90.0c	15.6abc	12.4ab
25°C	46.7b	45.0b	10.6a	8.5a

*Means with the same letters are not significantly different at P=0.05 according to Tukey's test.

7.6 ASEXUAL PROPAGATION

The shoots of *Convolvulus dorycnium* elongate from the rosettes, producing soft small stems which form directly flower stems without leaves. Consequently this is not suitable material for propagation of *Convolvulus dorycnium* by cuttings. Preliminary studies on the rooting of newly sprouted stems during spring 2003 showed that none of them rooted despite the addition of various IBA concentrations.

Trying to propagate *Convolvulus* by root cuttings also failed due to lack of shoot development.

7.7 SURVIVAL AND ADAPTATION OF *CONVOLVULUS DORYCNIUM* IN URBAN LANDSCAPE

The aim of this research is to investigate the possibility of adaptation and survival of *Convolvulus dorycnium* that have been produced by means of sexual reproduction in the greenhouse.

The seedlings derived from the above seed germination experiments were planted in pots in a mixture of compost+perlite+peat moss (1:1:1, v/v) and maintained in the greenhouse for five months. They were then transferred to the experimental plot in the park of TEI

Material and methods

Sixteen four-month old seedlings of *Convolvulus dorycnium*, derived from the above propagation by seed experiment, were planted in the experimental park in two rows at a distance of 2.8 m between the rows and 1.15 m between each plant (Appendix 4). The seedlings had formed one rosette with 5 - 6 leaves each. The plants were planted and cultivated in the same way as described in chapter 3.6.2.
Six months after planting, the number of survived plants, the height, plant diameter and number of lateral shoots were measured.

Results and discussion

Fourteen plants survived (87.5%) and adapted in the outdoor environmental conditions after six months cultivation. Four months after planting, flower branches had formed and flower buds appeared on the tips of the branches. A limited number of the buds blossomed late in July and August but most of them did not flower (Plate 7.6). This may due to the fact that the flowering period of *Convolvulus* plants in nature had passed before inflorescences have been formed. A second reason may be the difference in soil type with regard to texture of the soil and/or the content of nutrients such as $CaCO_3$ (Appendix 2).

The extent of the growth of the plants regarding height and diameter is represented in table 7.3. The recorded growth is confined to those stem flowers without any leaves. During the six months of cultivation in the plot no serious problems from pests and diseases were observed. New rosettes of *Convolvulus dorycnium* appeared from the buds on stems just below the soil level the following December after the onset of rains (Plate 7.7).

Table 7.3 Mean dimensions in height, diameter as well as the number of flower brunch of *Convolvulus dorycnium* after six months cultivation in the experiment plot in the park of TEI.

	Growth in 6 months ± sd
Height, cm	42.1 ± 6.4
Canopy diameter, cm	60.3 ± 12.2
Number of branches	3.4 ± 1.2

Convolvulus dorycnium can survive in an artificial landscape maintaining its good appearance during summer if moderate watering is undertaken during the summer months. During the summer periods all the over-ground parts of the plants of *Convolvulus dorycnium* are dried after flowering in the wild. More research is required to investigate the affect of watering on the seasonality of flowering and floral characteristics as well as on the form of the plants.

7.8 CONCLUSION

Convolvulus dorycnium is a woody shrub that is wide spread to the east Mediterranean and Anatolia and thrives in dry rocky places, scrub, roadsides and near the sea. It has a very intensive presence in the wild from May to July due to the abundant flowers. Seeds have imposed exogenous physical dormancy (seed coat dormancy) and the germination is limited by the hard seed coat that is impenetrable to water. Germination can be induced by any method that can soften or scarify the covering. Seeds germinate in a wide range of temperatures with best results at 20°C and the worse at 5°C.

Asexual propagation by stem or root cutting was not possible for *Convolvulus dorycnium*. Five-month old seedlings could be established outdoors with a high survival rate and some flowering. More research is required to investigate the cultivation needs for grown and flowering in pots and the landscape.

8 *CAMPANULA PELVIFORMIS* LAM.

8.1 INTRODUCTION

Campanula pelviformis Lam. (syn. *Campanula corymposa* Desf.) is an endemic species of central and east Crete, which belongs to the Campanulaceae family. The genus Campanula containing about 300 species extends in the North hemisphere to temperate and Mediterranean climates (Meikle, 1985). It is known as bellflowers because they have large and showy flowers shaped like a bell.

Classification according to Royal Botanic Gardens Kew for the genus Campanula is as follows:
APG Clade: EUDICOTS - CORE EUDICOTS - ASTERIDS - EUASTERIDS II
APG Order: Asterales
APG Family: CAMPANULACEAE
Kew Family: CAMPANULACEAE
Genus: Campanula
Species Epithet: sp.

In Greece there are about 40 species of Campanulas. Twelve species are indigenous in Crete and 8 of them are endemic (E): *C. aizoides*, *C. erinus*, *C. cretica* (E), *C. creutzburgii* (E), *C. hierapetrae* (E), *C. jaquiinii* (E), *C. laciniata*, *C. pelviformis* (E), *C. saxatilis ssp. saxatilis* (E), *C. spatulata ssp. spatulata* (E), *C. trichocalycina, and C. tubulosa* (E) (Meikle, 1985).

Campanula pelviformis belongs to the quinqueloculares section of the genus *Campanula* in group B2 as proposed by Phitos and Danboldt (1978). Other species in the same group are *C. tubulosa* Lam. and *C. carpatha*. These three species are indigenous to Crete and Karpathos. There is polymorphism in the Aegean species of the genus Campanula of the section quinqueloculares. A large part of the total variation is found between the populations within species (Eddie and Ingrouille, 1999).

Campanula pelviformis thrives on stony slopes, thickets, road side and olive groves in east Crete at altitudes from 0 to 950 m (Tutin *et al.*, 1965 - 1980).

8.2 OBSERVATION AREA

The observation area was a slope near the village of Exo Mouliana, 15 km to the west of Sitia. All plant material (foliage, flowers and seeds) were collected from an altitude of 350 – 370 m. In this place the Campanula plants are interspersed among shrubs or under olive trees (Plate 8.1).

8.3 DESCRIPTION

According to Tutin *et al.*, (1965 - 1980), *Campanula pelviformis* is a biennial plant with stems 20 – 30 cm tall ascending simple or branched, hispid. Its leaves are hispid, ovate, acute and serrated, with or without stalk. Corolla is ca. 30 mm broadly campanulate, blue-lilac or white (Plate 8.2 and 8.3). Calyx with teeth three times longer than ovary. Seeds are born in capsules (Plate 8.4).

In the observation area (Exo Mouliana, Sitias) the development of *Campanula pelviformis* plants in the wild showed much variation. Their inflorescences height varied from ca.10 up to 50 cm and the canopy diameter from ca. 20 to 65 cm. Largest plants are grown in well cultivated soil under olive trees while small ones with poor growth are found on rocky and stony slopes. From the rosette of each plant develop up to 9 flower stems and from each stem 5 - 15 secondary branches. A flower stem contained up to 55 flowers. Measurements of the floral characteristics were made from 10 flowers derived from 5 plants (Table 8.1).

Table 8.1 Characteristics of the *Campanula pelviformis* flowers in wild. N = 10

Calyx tube length (mm)	Calyx teeth length(mm)	Corolla length (mm)	Corolla diameter (mm)	Corolla tube diameter (mm)
7.6 ± 1.3	14.3 ± 0.8	26.7 ± 4.0	36.9 ± 7.9	21.4 ± 3.2

Fruits are capsules and each capsule contained ca. 500 to 850 seeds (Plate 8.4). One thousand air-dried seeds weighed 0.07g.

8.4 LIFE CYCLE

Campanula pelviformis life form according to the system of Raunkiaer, as modified by Govaerts *et al.* (2000), is classified as hemicryptophytic. They have stems that die-back during unfavourable seasons and surviving buds form on (or just below) the soil surface. This group includes many biennial and perennial herbs, including those in which buds grow from a basal rosette.

The seeds began to germinate in the end of February until the beginning of March. In the end of March the seedlings have 5 - 6 leaves each and until the end of June up to 16 and a well developed under ground rootstock (caudex) with a well branching root system. During summer the leaves and flowers dry and next autumn after the onset of rains new leaves and shoots sprout from the caudex. The first flowers appear at the end of April and a full anthesis during May. Flowering lasts 1 - 1.5 months and depends on the spring rains. Most of the *Campanula pelviformis* plants persist and flower more than two years in nature producing shoots from the caudex of the thick roots (Plate 8.5). In cultivation in the pots in greenhouse as well as in outdoor cultivation, plants have been maintained for at least three years. So generally we can say that *Campanula pelviformis* is a perennial rather than a biennial plant.

Fruits mature from May to June and the capsules open to disperse the seeds during summer when the whole of the foliage dries.

8.5 UTILIZATION

There are many Campanula species that are used in trade floriculture as garden or pot plants, but there are no references about *Campanula pelviformis*. Their abundant blue bell-like flowers make it an attractive plant that could be used as a garden plant as well as pot plant in a compact form. They can also cultivate in dry and barren soils without irrigation.

8.6 PROPAGATION BY SEED

Sexual propagation is the natural means of propagation of *C. pelviformis*. Two lots of seeds, which were collected in June 2003 from the observation area, were sown during November and December 2003 in pots with trade compost in the greenhouse (Plate 8.6). Percentage germination reached about 90%. It is obvious that *C. pelviformis* seeds are easy to propagate in the greenhouse conditions during this period. The follow experiment was carried out to investigate the germination of *C. pelviformis* seeds in different temperatures and lighting.

8.6.1 Effect of temperature and lighting on the germination of *Campanula pelviformis* seeds

Material and methods

Seeds of *C. pelviformis* were collected in May 2003 and stored in a dry place at room temperature until January 2004 when the germination experiment was carried out. Seeds were placed in Petri dishes on 1% agar in deionized water in controlled chambers at 10, 15, 20 and 25°C under light/dark (16/8h) or dark conditions. The dishes were wrapped with plastic transparent foil to avoid water evaporation. Complete darkness was achieved by wrapping the Petri dishes with aluminum foil. Seed germination was recorded every 3 or 4 days for a period of 40 days.

Three Petri dishes were used per treatment and 50 seeds per dish (1200 seeds in total). The germination percentage data were transformed to angular values before statistical analysis. Data was analysed by ANOVA and the means were compared by Tukey's test.

Results and discussion

In all treatments seeds began to germinate within 7 - 10 days after their placement in Petri dishes apart from these at temperatures 20 and 25°C in the dark that needed 4 days more. Germination was completed in ca. 30 days (Figures 8.1 and 8.2; Plate 8.6). The germination percentage reached up to 96% at 15°C in light/dark conditions, but there were no statistical significant differences in final germination levels between 10°C and 15°C either under dark or light/dark conditions (Table 8.2;

Figure 8.3). However, there was a significant decline in germination as the temperature increased to 25°C and the lowest percentage germination (8.7%) was recorded at 25°C in the dark.

These results indicate that optimum temperatures for germination of *C. pelviformis* seeds are 10 and 15°C under light/dark condition while higher temperatures inhibit germination. Light affects the germination only at high temperatures (20 and 25°C) that higher percent germination was recorded under light than in darkness. Seeds were germinated faster in 15°C in light or darkness and in 20°C in light (Table 8.2).

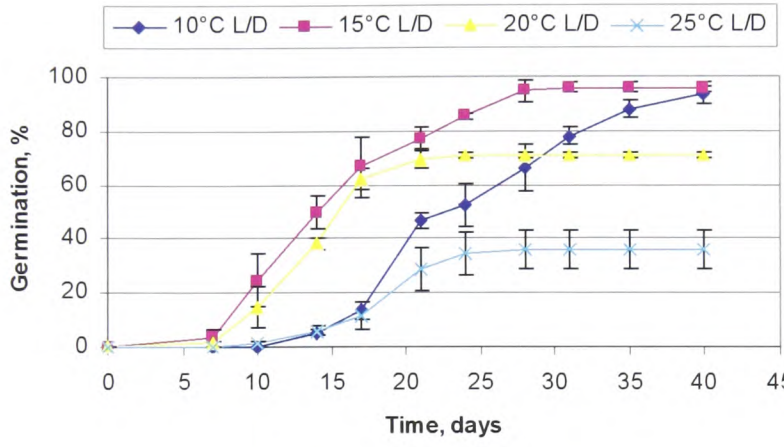


Figure 8.1 Time course germination of *Campanula pelviformis* seeds at different temperatures in light/dark conditions (16/8h) on 1% agar as substrate.

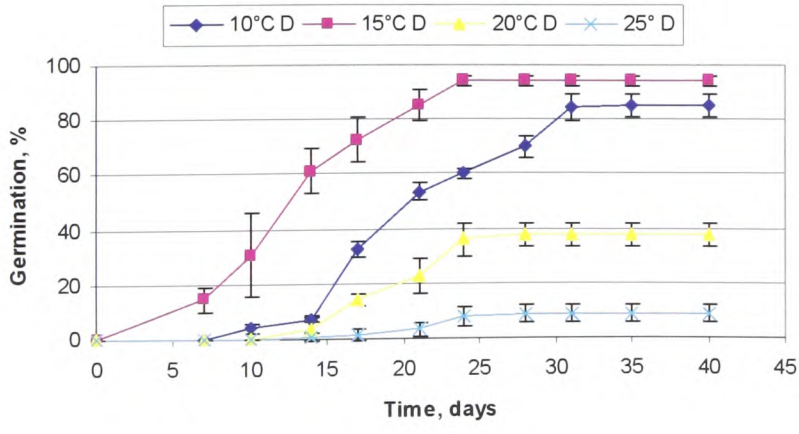


Figure 8.2 Time course germination of *Campanula pelviformis* seeds at different temperatures in dark conditions on 1% agar substrate.

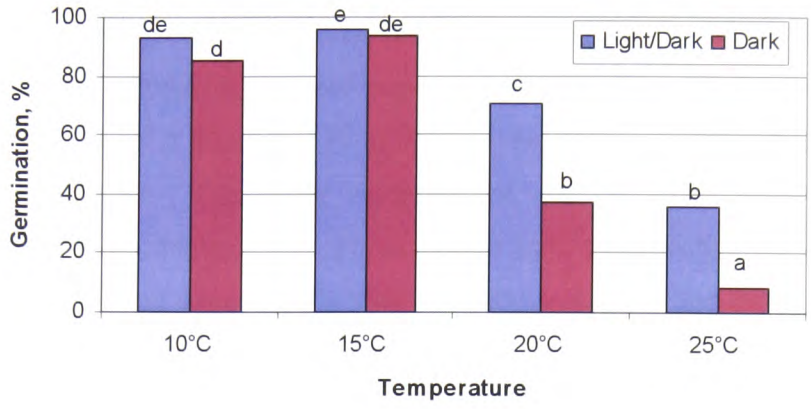


Figure 8.3 Cumulative germination of *Campanula pelviformis* seeds at different temperatures in light/dark (16/8h) and dark conditions on 1% agar substrate.

Table 8.2 Effect of temperature and light/dark (16/8h) or dark conditions on the germination and T_{50} of *Campanula pelviformis* seeds in 1% agar. Mean ± sd.

Temperature °C	Germination, %		T_{50}	
	Light/Dark	Darkness	Light/Dark	Darkness
10	93.3 ± 3.1de*	85.3 ± 4.2d	22.4 ± 2.4c	18.8 ± 0.5b
15	96.0 ± 2.0e	94.0 ± 2.0de	13.9 ± 0.9a	11.9 ± 1.6a
20	70.7 ± 1.2c	37.7 ± 4.2b	13.5 ± 0.3a	19.5 ± 0.9bc
25	36 ± 7.2b	8.7 ± 3.1a	18.5 ± 0.8b	21.7 ± 0.8bc

*Means with the same letters are not significantly different at P=0.05 according to Tukey's test.

8.7 SURVIVAL AND ADAPTATION OF *CAMPANULA PELVIFORMIS* IN URBAN LANDSCAPE

The aim of this research was to investigate the possibility of adaptation, survival and flowering of *Campanula perviformis* that had been produced by sexual reproduction in the greenhouse. The seedlings derived from the above mentioned seed germination experiments were planted in pots in a mixture of compost+perlite+peat moss (1:1:1, v/v) and maintained in the greenhouse for eight months (Plate 8.7). Plants in the greenhouse flower producing high quality pot plants (Plates 8.8 and 8.9). Eight months old seedlings were transferred to the experimental plot of TEI in March 2005.

Material and methods

Twenty seven seedlings of *Campanula pelviformis*, eight months old, derived from the above propagation by seed experiments were planted in the experimental park in two rows at a distance 2.30 m between the rows and 0.5 m between each plant (Appendix 4). The plants were planted and cultivated in the same way as described in Chapter 3.6.2. Three months after the planting, the surviving plants, their height and diameter, the number of inflorescences and the number of flowers per inflorescences were measured.

Results and discussion

Twenty two plants survived (81.5%) from the 27 planted and adapted in the outdoors environmental conditions after four months cultivation. A spectacular flowering occurred early in May and lasted for ca. 20 days. All the over-ground parts of the plants dried in June.

The growth of the plants, recording height and diameter and flowering (number of flowering stems and number of flowers), are presented in the table 8.3. During the four months of cultivation in the plot no problems from pests and diseases were observed. Eight months after planting, new growth developed from the underground rootstock (caudex) and up to 40 shoots were counted per plant (Plate 8.10).

Table 8.3 Mean height, diameter and the number of inflorescences and flowers of *Campanula pelviformis* after three months cultivation in the experiment plot in the park of TEI

	Growth and flowering in 3 months ± sd
Height, cm	30.5 ± 4.5
Diameter, cm	49.7 ± 5.0
Number of inflorescences	7.3 ± 1.5
Number of flowers/inflorescence	28.4 ± 3.3

8.8 CONCLUSION

Campanula pelviformis an endemic species of central and east Crete has a high aesthetic value suitable to be used both as pot or garden plant. Population studied was in the east Crete (Sitia area) in 350 - 370 m altitude. Inflorescences up to 50 cm in height are well branched and have up to 55 blue flowers per stem. They are biennial or perennial in wild due to the well developed under ground rootstock (caudex) with a well branching root system, which remains under the ground during the dry period of the year. Seeds are released in summer and germinate the next spring. The first year the vegetative phase is competed and the next year they flower.

Germination in peat moss in greenhouse reached up to 90% in trade compost the next autumn after the release of the seeds from mother plants. Best germination was achieved at 10 and 15°C in light or darkness (up to 96%). There was interaction between light and temperature so that germination under light is higher than those in darkness in 20 and 25°C. Seedlings were established and developed in pots producing flowers 10 months after seeding. Eight month old seedlings were established successfully in the garden with high quality flowers.

9 *STERNBERGIA SICULA* TINEO EX GUSS.

9.1 INTRODUCTION – LITERATURE REVIEW

The genus Sternbergia belongs to the Amaryllidaceae family, which contains eight species that are widespread extending all the way from south-western Europe into south-western Asia. The recorded species are: *S. colchiciflora*, *S. fischeriana*, *S. clusiana*, *S. lutea*, *S. sicula* (Acik *et al.*, 1970), *S. pulchella* (Mathew, 1993), *S. candida* (Mathew, 1979) and *S. greuteriana* (Kamari and Artelari, 1990). In the south Aegean area the genus Sternbergia is represented by three species: *S. greuteriana* (an endemic species) *S. lutea* and *S. sicula* (Kamari and Artelari, 1990). These three species of Sternbergia thrive also in Crete.

There is confusion over the taxonomic classification, which was based initially on morphological characteristics. *S. sicula* (Tineo ex Guss) is recorded as a subspecies of *S. lutea* (Tutin *et al.*, 1980; Kamari and Artelari, 1990; Blamey and Grey-Wilson, 1993), with a more delicate form and leaves 3 – 5 mm wide compared to leaves of *S. lutea*, which are 3 – 15 mm in width. In the flora of Turkey (Davis, 1984) *S. lutea* and *S. sicula* are classified as two different species. The character used to define them in species categorisation was limited to the width of leaves which is 7 – 12 mm in *S. lutea* and 2 – 6 mm in *S. sicula*. However they are confused with each other because some mid-forms are also detected in nature.

Examining the chromosomes, *S. lutea* was found to be diploid (2n = 22) as well as triploid (2n = 3x =33), whereas *S. sicula* proved to be diploid with 2n = 22 + 0-1B chromosomes and a karyotype similar to those of *S. lutea* and *S. greuteriana* (Kamari and Artelari, 1990). Yuzbasioglu *et al.* (1996) using Giemsa C-banding analysis determined that there are morphologic and cytological similarities between *S. lutea* and *S. sicula* and suggest that *S. sicula* should be a subspecies of *S. lutea*.

The same species (*S. sicula*) has been recorded in the Melambes district of Crete and was named as *S. minoica* by Ravenna (in Onira Bot. Leafl. 5:39, 2001 as cited by Chilton and Turland, 2002). This taxon was described as having dark green leaves, with a paler central stripe throughout their length and 4 – 8 mm wide

(Chilton and Turland, 2002). In this district (Melambes) about 40 km away to the west from the observation area (Zaros) there are probably similar populations as the morphological characteristics of the two distinct species resemble each other.

Regardless of whether *S. sicula* is a species or subspecies of *S. lutea,* in this work the morphological characteristics in the observation area were examined as an ornamental plant and the species will be referred to as *S. sicula.* Nevertheless morphological differences among *S. lutea* and *S. sicula* are very distinct in the observation area (Plates 9.1) and in pot cultivation (Plates 9.2 and 9.3).

They are geophytes with hysteranthous foliage that flower in autumn before their leaves emerge. *S. sicula* is one of the first autumn flowering bulbs whose leaves can be hysteranthous (leaves appear after the flowers have opened) or synanthous (leaves appear at the same time as the flowers) depending on the timing of the autumn rains.

9.2 GEOGRAPHICAL DISTRIBUTION

S. sicula is widespread in eastern Mediterranean from southern Italy and Sicily to southern Greece and the Aegean region (Tutin *et al.*, 1980; Blamey and Grey-Wilson, 1993). It thrives among other perennial, annual, and grassy plants, or under bushes, shrubs and olive trees at an altitude of 100 to 1000 m on gravel and rocky ground.

In the island of Crete they have expanded from the observation area to the south west, 50 km away in a rocky elevation with soil belts and soil pockets in which quantities of alluvium accumulate. They are also found under olive trees if the ground under them is not tilled. They are located also near the village of Gouves 25 km to the east of Heraklion, in Chora Sphacion in Chania, in the plateau of Lasithi and in the island of Dia (personal observation or local information).

9.3 OBSERVATION AREA

The observation area as well as the area of collection of every plant material (bulbs, flowers and seeds) was the south foot of Ides Mountain, 50 km south from Heraklion near the village of Zaros at 450 - 500 m altitude (Plate 9.4). The plants were observed, material collected and measurements made over a period of three years. Collected bulbs were sown and the plants cultivated during 2003 and 2004 in an unheated greenhouse, from which morphological measurements were taken. Using various experimental and observational techniques and sampling in the vegetative stages from the Ides plant populations of *Sternbergia sicula,* the phenology, life cycle, sexual and asexual propagation and survival in pots and the garden were studied.

9.4 PLANT DESCRIPTION

9.4.1 Plant life-form

The life-form of the mature plant is classified according to the system of Raunkiaer (1934) as modified by Govaerts *et al.* (2002). According to this system the species of the genus Sternbergia are perennial geophytes, which have stems that die back during unfavourable seasons, with the plant surviving as underground bulbs.

Some geophytes of different genera and families flower without bearing leaves at the end of summer (September and October). These are known as species with hysteranthous foliage. The leaves appear only after the first showers or after the snow has melted in the Mountains. When foliage and flowers appear simultaneously, this is symptomatic of synanthous foliage (Dafni *et al.*, 1981). There are two types of hysteranthous geophytes according to Dafni *et al.* (1981): the *Urginea* type occurs in *Urginea*, *Scilla*, *Narcissus* and *Pancratium* and the *Crocus* type comprises *Crocus*, *Merendera*, *Colchicum* and *Sternbergia*.

In the *Urginea* type the ovary is situated at above ground level, in the Crocus type it is subterranean. In the Crocus type species fruit is kept below the ground level during the winter and is protected from winter damage while seed dispersal starts in spring and the first chance for germination is the following autumn. Members of

the Urginea type disperse their seeds before the first winter rains and germination is immediate without dormancy. In contrast, species of the crocus type exhibit after-ripening of seeds during the winter and seed dormancy of up to one year.

Sternbergia sicula plants grow in clusters of 3 to 23 bulbs which are the result of vegetative propagation or both vegetative and sexual propagation (Table 9.5). In the most densely populated sites, 10-12 clusters per m^2 (ca 80-100 bulbs) were found during the three last years of observations.

9.4.2 Leaves

The leaves of *S. sicula*, as described by Blamey and Grey-Wilson (1993) and Tutin *et al.* (1980), are deep green, strap-shaped and finely notched 3 – 5 mm in width. Other authors (Yuzbasioglu *et al.*, 1997; Ravena, 2001 as cited by Chilton and Turland, 2002) found that the width of the leaves was 2 - 6 or 4 – 8 mm. It is evident that in each habitat, plants have different characteristics due to genetic differences or to the acquired characteristics in response to environmental stimuli.

9.4.2.1 The leaves of *Sternbergia sicula* in the observation area

In the observation area *S. sicula* presents large variation in the structure and size of its leaves. Leaves appear on the surface of the ground a few days after the autumn rains at the same time as the flowers (synanthous) or after the flowers (hysteranthous). In the observation area for the three last years, the first leaves have appeared from the end of October until the middle of November and they were well developed until the middle of December.

Sampling from the study site it was found that more leaves were produced in November (Table 9.1). This may due to the fact that leaves appearing at the end of November are derived from the main bulb, which enclosed bulblets and lateral bulbs, while later in December and January many new bulblets (off-set, lateral bulblets) have separated from the main bulbs. These new bulbs produce fewer leaves per bulb than the mother bulb and the mean number of leaves of the total bulbs was found to be fewer. A second reason may be that each individual sampling (clusters of bulbs) differs in the proportion of small and large bulbs.

Table 9.1 Number of leaves per bulb in three samples during November, December and January from the natural habitat in the observation area. Mean ± sd., N = 80

Date of sampling	Mean number of leaves per bulb
30 November	6.0 ± 1.8
30 December	4.8 ± 1.9
30 January	4.5 ± 2.0

The width of the leaves was measured at 1.5 to 6 mm at the end of December 2004 when they were fully developed and the length of the leaves was 4 cm to 19 cm. Maximum number of leaves was 9 and minimum 1 (Table 9.2).

Table 9.2 Minimum, maximum and average number, width and length of leaves of *Sternbergia sicula in situ* during December 2004.

	Average ± sd	Maximum	Minimum
Number of leaves/bulb	4.84 ± 1.96	9	1
Width of leaves (mm)	3.26 ± 1.26	6	1.5
Length of leaves from the surface of the ground (cm)	13.11 ± 3.91	19	4
Length of the under ground part of leaves (cm)	7.31 ± 3.77	14	1

In flowering bulbs with growing fruits, the leaves and fruits grew downwards parallel to the surface of the ground (Plate 9.5). This may happen in order to have the fruit closer to the ground for release of seeds to germinate later. In contrast, those bulbs which did not produce flowers or where fruit were not produced grew upwards in an upright position.

The phase of vegetative development of the leaves in the wild depends on the timing of the rains or the availability of water in controlled environments such as pots. Leaves do not emerge above ground unless the soil is wet. This behaviour is common in geophytes and hemicryptophytes in the Negev (Guttermann, 1981; Boeken and Guttermann, 1989). From the end of March to the end of April every aerial part of the plants was dry in all sites in Crete where *Sternbergia* was found.

This means that *Sternbergia* belongs to the summer dormant type of bulbs which is the second type of the bulb dormancy according to Le Nard and De Hertog (1993). The first type comprises bulbs without dormancy (as in the species of Hippeastrum and Nerine) and the third type of bulbs contains bulbs with winter dormancy as found in Lilium, Alium and other species. In Crete, most of the bulbs are summer dormant such as Crocus, Gladiolus, Muscary, Narcissus, Pancratium and other species (Dragassaki, 2002).

9.4.2.2 Leaves of the *Sternbergia sicula* in pot cultivation

Material and methods

In October 2002, 70 bulbs of *S. sicula* were collected from the wild after anthesis had been completed. Each bulb was separated from each other from their clusters and was planted in 9 cm pots full with potting medium of peat moss + perlite (2:1, v/v). During the growing period they were watered once or twice a week (depending on their needs) and were fertilised every month with 2 g/l N-P-K fertilizer (20-20-20). During summer watering was stopped and started again in October. They were maintained for two years in the greenhouse and the dates of anthesis and the number of flowers per plant during autumn 2003 was recorded. The pots with different leaf characteristics were marked in the spring of 2004. In September 2004, before anthesis or leaf development, the newly produced bulbs were extracted from the pots and their number, weight, height and diameter were measured.

Results and discussions

It was observed that in the study pots the leaves of some bulbs appear as two distinct morphological types: the leaves of the first type are 3 – 6 mm wide dark green with a paler central stripe throughout their length, and the leaves of the second are 2 – 3 mm wide dark green without a pale stripe in the centre (Plate 9.6). Each pot contained the same type of leaves and bulbs.

The bulbets were separated from their mother bulb and their parameters were recorded. The number of flowers per bulb, recorded in autumn of 2003, was almost the same in the two categories of leaf type. The number, weight, height and

diameter of the bulblets varied among the two types of leaves (Table 9.3). Bulbs with wider leaves had less bulblets but larger bulbs while bulbs with narrower leaves had formed more bulblets which were smaller. The length of leaves reached up to 38 cm in the both categories as observed in spring 2004. The fact that the larger the bulbs the wider leaves were produced may be due in part to genetic reasons, to endogenous hormonal effect on the bulbs or environmental factors.

9.4.3 Roots

The roots usually begin to elongate as the autumn rains start in October or November. Roots were observed on the 2nd of October 2004 in natural populations of *Sternbergia sicula* in shaded sites under olive trees, five days before the first flowers sprouted and before the rains started. The temperature during October was lower than usual. This may suggest that roots emerge when the temperature decreases and the relative humidity increases. This agrees with similar findings of Boeken and Guttermann (1986) for *Sternbergia clusiana* in Israel. However, in contrast some plants of *S. sicula* kept in pots in greenhouse during the summers of 2003 and 2004 kept their leaves and roots if they were watered regularly.

Table 9.3 Number of *S. sicula bulbs and* bulblets, weight, height and diameter per bulb after two years cultivation (2002 – 4) and flowering bulbs %, number of flowers per bulb and number of flowers per flowering bulb after one years cultivation, for bulbs having 3 – 6 and 2 – 3 mm leaves' width. N (for leaves' width 3 – 6 mm) = 39; N (for leaves' width 2 – 3 mm) = 23.

	Leaf width 3-6 mm (Average ± sd)	Leaf width 2-3 mm (Average ± sd)
Number of total bulbs and bulblets	2.1 ± 1.2	7.0 ± 6.6
Mean weight of bulbs and bulblets(g)	4.3 ± 2.0	0.7 ± 0.4
Height of bulbs and bulblets (mm)	22.0 ± 5.6	15.4 ± 4.3
Diameter of bulbs and bulblets (mm)	20.2 ± 5.7	9.1 ± 2.5
Flowering bulbs (%)	35.0	30.4
Number of flowers/bulb/year	0.5	0.6
Number of flowers/flowering bulb/year	1.6	1.8

9.4.4 Bulbs

The bulb of *Sternbergia sicula* is a perennial tunicate (having outer dry and membranous scales) with a circumference of up to 9 cm and belongs to the Narcissus bulb type according to Rees (1972). These bulbs are made up of both scales and leaf bases in contrast to the Hippeastrum type whose bulb is composed entirely of leaf bases and the Tulip type which is entirely of scales. The mature large bulb of the Narcissus type is a branching system made up of a number of annual bulb units. Each apical meristem produces one bulb unit annually and a unit comprises two or more scales and two or more leaves with an inflorescence (Plate 9.7).

In each Sternbergia bulb there are usually 1 – 6 bulb units among the scales and/or up to 7 lateral bulblets attached on the base of the bulbs that usually split away and form a new bulb (Plate 9.8). Each bulb unit can produce up to four flowers yearly (Plate 9.9). However in the same bulb (with its inner bulb units) up to 3 bulb units can flower in the same year. A semi-transparent membrane, distal of the scales, extends above the soil and encloses the leaves early in the growing season (Plate 9.10).

Bulbs are globular or ellipsoid due to the pressure from other bulbs. They are reproduced by a new bulb unit (bulblet) in the inner of bulbs or lateral bulb unit (bulblet, offset, daughter bulb) that appears in the axils of the outer dry scales and is attached on the basal plate (the short, thickened stem of a bulb).

9.4.4.1 Bulbs of *Sternbergia sicula in situ*

During April 2004 after the leaves of *Sternbergia sicula* were dry, 30 clumps (groups) of bulbs from the observation area were collected. Two hundred and ninety two bulbs were separated from each other and sorted with regard to their circumference in 4 groups: circumference © > 5 cm, C = 4 – 5 cm, C = 3 – 4 cm and C <3 cm. Then the length and the weight of each bulb were measured.

The results revealed that from the examined bulbs 7.5 % had their circumference over 5 cm, 31.9 % between 4 – 5 cm, 32.9 % between 3 – 4 cm and 27.7 % smaller than 3 cm (Table 9.4). These different sizes of bulbs indicate that they were asexually reproduced in different years and suggest that there is much *in situ* variation in size. The length and the weight of the bulbs are shown in Table 9.4.

Table 9.4 Circumference, length and the weight of the *S. sicula* bulbs in their habitat during April 2004, after the leaves were dry, and percentage frequency of different circumference of bulbs. N=292

Circumference groups	% occurrence in each group	Mean Circumference (cm) ± sd.	Mean Length (cm) ± sd.	Mean Weight (g) ± sd
Circumference: > 5 cm (n = 22)	7.5	5.54 ± 0.62	2.59 ± 0.32	3.34 ± 0.93
Circumference: 4-5 cm (n = 93)	31.9	4.62 ± 0.73	2.32 ± 0.37	1.96 ± 0.98
Circumference: 3-4 cm (n = 96)	32.9	3.50 ± 0.31	1.94 ± 0.28	0.93 ± 0.23
Circumference: < 3 cm (n = 81)	27.7	2.04 ± 0.65	1.50 ± 0.28	0.35 ± 0.17

In November 2003 when anthesis was completed and the fruit began to grow, 10 clumps with a total of 76 various size bulbs were collected from the wild. The bulbs were separated and circumference, number of leaves, flowers and fruits were counted. A second sample of 13 clumps was collected in January 2004 which contained 126 bulbs and was used to compare against the first sample. During this period the fruits had completed their development and some of the seeds had already matured. The same parameters were counted and the number of flowers was estimated as the total number of fruits.

Number of bulbs per cluster varied from 7.6 in November to 9.6 in January while the circumference of the bulbs in November was bigger than January (Table 9.5). A possible reason for the smaller size of the bulbs in January is that during seed development, nutrients from the mother bulb are transported to the seeds for their growth and development.

The bigger circumference as well as greater number of leaves, flowers and fruits per bulb in November's sample may also due to the fact that each mother bulb contained bulblets in the inner or on the basal plate of bulbs and some of them sprouted flowers and gave fruits, even before being split from the mother bulbs

(Table 9.5). In January some bulblets in the outer scales of the mother bulb separated and became new unit bulbs.

Table 9.5 Number of bulbs of *S. sicula* per cluster (groups) at two sampling dates during November 2003 and January 2004, as well as their circumference, number of leaves, flowers and fruits per bulb (n = 13 for cluster, n = 130 for bulbs)

	Sampling in November			Sampling in January		
	Average ± sd	Max	Min	Average ± sd.	Max	Min
No of bulbs / cluster	7.6 ± 2.6	13	3	9.6 ± 5.2	23	4
Circumference (cm)	5.9 ± 1.4	10	3.3	4.4 ± 1.6	8.2	0.8
No of leaves / bulb	6 ± 1.8	10	2	4.5 ± 2	9	1
No of flowers / bulb	1.7 ± 1.2	5	0	0.5 ± 0.7	5	0
No of fruits / bulb	0.7 ± 0.9	4	0	0.4 ± 0.6	2	0

9.4.4.2 Bulbs of *Sternbergia sicula* under cultivation in greenhouse

On the basis of the results described in 9.4.2.1 the bulbs that come from the plants cultivated in the greenhouse show variation in their size, the weight and the number of the bulblets produced. We can distinguish two types of bulb whose characteristics vary according to the leaf types. The bulbs of the first type of leaf are heavier and larger than the bulbs of the second type, while they produce less bulblets than the second type's bulbs (Table 9.3). The bulbs collected first from the wild had some variation. This variation became more distinct under cultivation in different conditions in the greenhouse. This is evidence that the phenological changes of *Sternbergia* may be due both to genetic and environment impact.

9.4.5 Flowers

The flowers of *S. sicula* are solitary in each scape (the leafless flower stalk that grows from the ground), 1 - 5 per mother bulb. In the first stage of development, they look like crocus flowers but later turn to star-shaped (Plate 9.11). It is possible to further distinguish them because they have six stamens compared to three in flowers of crocus. The flower is golden-yellow, erect, 25 – 50 mm long and 20 – 50 mm spread (in its full anthesis). Tepals are oblong, 7 – 15 mm wide

the outer and 4 – 9 mm the inner ones. Stamens are 20 – 30 mm long and the anthers 5 mm.

The above dimensions were measured from a large sample from the observation area. They are not always equal to those that have been recorded by other researchers (Tutin *et al.*, 1980; Blamey and Grey-Wilson, 1993). The number of flowers per plant (generative and vegetative bulbs) was recorded from 0.5 to 1.7 (Table 9.3 and 9.5). Generative bulbs can produce up to 5 flowers per bulb. The mother bulb blossoms first producing up to 3 flowers and then the lateral plant units follow. In this way the blossom lasts *in situ* about a month, which is very important attribute for using Sternbergia as an ornamental plant.

Flowering lasted from 20 - 40 days *in situ* and after cultivation in greenhouse (Table 9.6). The longevity of a single flower is 4 - 8 days depending on the temperature. From the beginning of October until the beginning of December observations were made *in situ* once a week to ascertain the start and the end of *S. sicula* anthesis. For the three years 2002, 2004 and 2005, the start day of anthesis was in the first ten days of October while in 2003 there was a delay of a month. This delay may have occurred due to the high temperature and low rainfall in autumn 2003 (personal observation, because there is not a meteorological station in this area) compared with the usual conditions of the season. Two km to the south (altitude 250 m) where the temperatures are usually higher than those in the observation area the flowering of *S. sicula* is delayed by ca 10 days. These are personal observations during the last three years. According to Guttermann (1991) low temperatures (below 25°C) and long days can also accelerate the flowering of *S. clusiana* and induce higher percentage of flowering. Temperature also affects flower formation in saffron (*Crocus sativus* L.). The dormancy of corms can be released by high summer temperature but a long hot summer delays flower emergence which occurrs in late autumn as the temperature falls. So by controlling the temperature the flowering of corms can be delayed from early September to mid-December (Molina *et al.*, 2004 and 2005).

Table 9.6 Start and end of flowering of *S. sicula* in its habitats in the south foot of Ides Mountain during the four last years in relation to flowering in pots in an unheated greenhouse in Heraklion.

	2002		2003		2004		2005	
Flowering	Start* date	End* date	Start date	End date	Start date	End date	Start date	End date
In nature. Altitude: 420 - 450m	5 - 10 Octob	25 - 30 Octobe	8 - 12 Novem	3 - 8 Decem.	5 - 10 Octob.	10 - 15 Novem.	8 - 12 Octob.	20 - 25 Novem.
In greenhouse: Altitu 10 m	-------	--------	6 October	2 Novemb	20 Septem	31 October	14 October	10 Novem

*Start flowering day is the day that the first flower blossoms and the end flower date the first day that every flower withers.

Relationship between the circumference of the bulbs, the leaves and the flowers.

From samples taken in *situ* in November 2003, 90 bulbs were selected according to their circumference: 30 bulbs over 5 cm, 30 bulbs 3 – 5 cm and 30 bulbs smaller than 3 cm. The number of leaves and flowers were counted in each bulb as well as the number of scales. More leaves (7.3/bulb) and flowers (2.2/bulb) were formed in the bulbs over 5 cm in circumference while 4.4 and 2.1 leaves and 0.6 and 0.1 flowers were recorded in the bulbs with circumference of 3 - 4 and <3 cm, respectively (Table 9.7). For bulbs over 5 cm in circumference, 86.6 % produced more than 2 flowers each, while 53.3 % from those of 3 – 5 cm blossomed and only 13.3 % from the category lower than 3 cm.

In the literature the relation between the size of the bulb and their flowering has been reported for other species, for example *Hippeastrum* (Rees, 1972; Ocubo, 1993), *Narcissus* and *Hyacynthus* (Rees, 1972), *Ornithogalum* (Jansen van Vuuren, 1997). The size of the bulb is probably related to the extent of storage food in the scales. However this is not the main factor that affects anthesis as Le Nard (1980) expresses the opinion that the activity of the roots affects the induction of the flowering for some bulbs. Han (2001) also reports that the number of flowers of

some species of *Brodiaea* per scape increase and the forcing time decreased as the size of the mother corm increased. Bulbs of *Sternbergia* smaller than 3 cm are usually in the juvenile (vegetative) stage and hence would not flower.

Table 9.7 Relation between the circumference of bulbs, and the number of leaves and flowers

Bulb circumference	Number of leaves	Number of flowers	Flowered bulbs (%)
More than 5 cm	7.3 ± 1.6	2.2 ± 1.3	86.6
3 – 5 cm	4.4 ± 1.0	0.6 ± 0.6	53.3
Less than 3 cm	2.1 ± 0.7	0.1 ± 0.3	13.3

9.4.6 Fruits and Seeds

Fruits are sub-globose with three-part capsules as found for most species of the Amaryllidaceae family. Maturing fruits bend down to the ground and the seeds disperse on the surface. Seeds later may be carried by ants or other insects or remain on the surface of the ground and are covered by the soil after the last showers in spring. At the end of spring whole capsules covered by soil were found. Their fresh weight, the height and the circumference of capsules as well as the number of seeds per capsule were recorded during the sampling period at the end of February 2004 in the wild and in pots in the greenhouse (Table 9. 8). There were no significant differences between wild and pot plants in the characteristics measured. The capsules in the pots because they have more weight contain less seeds and are larger in circumference and smaller in length. These may due to higher water content in the tissues. In contrast, the pot-grown seedlings produce capsules with fewer seeds but more capsules per flowering bulb (Table 9. 8).

As mentioned above, the plant is myrmecochorous in that the seeds are dispersed by ants. The seeds are globular Seeds are 1 – 3 mm long, light to dark brown when matured with a white aril on the site opposite the point from which start to germinate (Plate 9.12). The aril is an outer covering or appendage of the seed which is usually fleshy that often aids seed dispersal by providing food to the disperser. Species of the genus *Sternbergia* with hysteranthous leaves in Israel have a subterranean ovary, myrmecochorous seed dispersal and a preference for

high mountains (Dafni, *et al.*, 1981). Seeds in the observation area have a well developed aril, which probably is used as food by the ants in order to disperse the seeds away to their ant-heap or elsewhere.

Seed dispersal starts in spring and the first chance for germination is during the following autumn. Species of the crocus type exhibits after-ripening of seeds during the winter and seed dormancy of up to one year (Dafni, *et al.*, 1981). In the observation area we located seedlings during spring (7 March 2005) in groups of 10-15. They were probably derived from fruit, whose seeds had not been dispersed but the capsules were buried in the ground. Seeds collected the previous year and sown in pots germinated during the same period as in nature (i.e. spring).

Table 9.8 Mean weight, length and circumference of fresh capsules of *S. sicula* and the number of seeds per capsule collected at the end of February 2003 (N = 70).

	In wild (Average ± sd.)	In pots (Average ± sd.)
Weight of capsule (g)	0.4 ± 0.2	0.5 ± 0.2
Length of capsule (cm)	1.4 ± 0.3	1.1 ± 0.3
Circumference of capsule (cm)	2.8 ± 0.5	2.7 ± 0.3
Number of seeds per capsule	11.7 ± 6.1	8.6 ± 4.2
Capsules, % of flowering bulbs	83.3 %	90 %

9.4.7 Life cycle

S. sicula has a biological life cycle comparative to other geophytes with hysteranthous foliage. According to Amots Dafni (Dafni, *et al.*, 1981), Sternbergia belongs to the Crocus type, which comprises also Crocus, Merentera and Colchicum.

The above-ground phase of *S. sicula* starts during autumn (October – November) when the flowers appeared on the bare surface of the soil and ends during spring (March – April) when the dispersal of the seeds has been completed and the leaves have dried. From late spring to autumn the plants remain under the surface of the

ground as bulbs. During this phase many morphological changes take place in their subterranean organs that there are not visible by observing above ground events.

During the years 2002, 2003, 2004 and 2005 in the observation area the anthesis started from the beginning until the middle of November and was completed from the end of October until the end of November (Table 9.6). The duration of anthesis was from 20 – 40 days in the densest populated places and started from the places of the higher altitude (in which it rains earlier and temperature is lower) towards the lower ones in the same latitudes.

The leaves appear after rainfall in autumn, usually from November to December. The mature seeds are dispersed from the end of February until the end of April depending on the date of the anthesis and water supply. The earlier the anthesis the earlier the seeds become mature. Some years, however, the rainfall stops very early in spring and the leaves dry before the seeds become mature.

9.5 UTILIZATION

Sternbergia species are known to possess Amaryllidaceae-type alkaloids (tazettin, lycorin, galanthamin, belladin, etc.), some of which have already been shown to possess antibacterial and antifungal properties. Extracts of both the *S. lutea* and *S. sicula* species showed significant antibacterial/antifungal activity (Evidente *et al.*, 1983; Weniger *et al.*, 1995).

S. lutea is widely used as a garden plant in open (or slightly shaded) places. Less use has been recorded for *S. sicula* because their flowers are relatively smaller than *S. lutea* and this flower bulb is unknown to consumers. There is no propagation material in the market and sometimes people collect the bulbs from the wild to use in their garden. In some countries it is forbidden to collect and export many geophytes from the wild (Ekim *et al.*, 1996).

In order to resolve the problems of biodiversity degradation, it will be important to develop appropriate methods of propagation which was the subject of a number of experiments reported below.

9.6 PROPAGATION BY SEED

9.6.1 Introduction

As mentioned before, most of the seeds remain in groups on the surface of the ground where they are covered with soil by the showers in spring. The seeds remain there until the rain falls during autumn and winter of the next year until there are suitable temperatures and dark conditions for the seeds to germinate.

During some preliminary studies on the germination of seeds of *S. sicula* during 2002 it was observed that the seeds, which were sown soon after the collection or some months later (during summer), had not germinated. It has also been recorded that species of the Crocus-type geophyte exhibit after-ripening of seeds during the winter and seed dormancy of up to one year (Dafni *et al.*, 1981).

The experiments that follow were conducted in order to investigate the optimal conditions that affect the germination of *S. sicula* seeds such as temperature, light and the time after their dispersal.

9.6.2 Germination of *S. sicula* seeds in an unheated greenhouse during November 2003.

Previous experiments for seed germination four months after the seeds were matured and dispersed from their capsules indicated that seeds could not germinate in constant or in alternating fixed temperatures (in greenhouse) 40 days after sowing (Antonidaki, unpublished). This experiment aimed to ascertain its potential for germination in greenhouse conditions 11 months after dispersal, as observed in nature.

Material and methods

Seeds of *S. sicula,* which were collected during April 2003 from the observation area, were stored for 11 months at room temperature in a dry place until use. They

were leached under tap water for 30 min and then were sown at a depth of 1cm in pots with trade compost. Seed germination was recorded every one or two days for a period of 30 days. The seeds were considered as germinated when the first leaf could be observed on the surface of the soil medium.

Result and discussion

The first seedlings appeared six days after sowing, and germination reached 73 % of the seeds sown and was completed in 18 days (Fig. 9.1; Plate 9.13). It indicates that germination in pots under natural conditions is possible after the primary dormancy had been released.

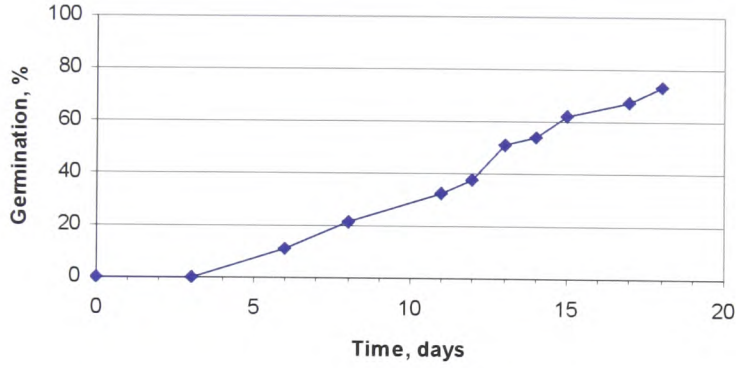


Figure 9.1 Percent germination of *S. sicula* in trade compost in an unheated greenhouse 11 months after seed collection.

9.6.3 Effect of temperature and light on the germination of fresh seeds of *S. sicula*

It is known that seeds of the genus Sternbergia are dormant and they germinate one year after their dispersal from the mother plants (previous experiment and Dafni *et al.*, 1981). This experiment is to investigate if fresh seeds just before capsules have opened are dormant and the optimum temperature for germination.

Material and methods

Seeds of *S. sicula* were collected at the end of February from the just open capsules. The seeds were fresh and the myrmecochorous (aril) were damp and visible. Half of them were placed in open air and half in the refrigerator at 5 – 7°C.

One month later (end of March) the seeds were surface-sterilized by using 95 % ethanol and 0.75 % sodium hypochlorite (NaOCl) with a few drops of Tween 20, for 15 min. Seeds were then rinsed three times using sterilized distilled water and were put in plastic Petri dishes on a substrate, which contained half strength MS macro- and micro-nutrients (2.15 g/l) and 8 g/l agar. The pH was adjusted to 6.0. Twenty seeds were placed in each Petri dish and 10 Petri dishes were placed in each chamber at 10, 15 and 23°C under light (light/dark 16/8 h) and dark conditions.

The seeds were observed every week and considered germinated when the radicals pierced the seeds.

Result and discussion

A very low germination percentage (5%) was observed at 15°C and dark conditions from the seed lot dried in open air (data not presented). The germination started two months after sowing and was completed within 40 days. None of the seeds germinated in any other of the temperature and light or dark conditions.

This lack of germination after one month's storage compares unfavourable to the higher germination rate (73 %) achieved in the first experiment when seeds had been stored for 11 months.

9.6.4 Effect of the medium, light, temperature and GA_3 on the germination of four month-old seeds of *S. sicula*

Material and methods

Taking into consideration that in many circumstances gibberellins has been shown to substitute for the chilling requirement for seed germination in other species, GA_3 in 500 and 1000 ppm was added in the seed germination medium. Gibberellic acid (GA_3) was dissolved in absolute (100%) ethanol and added to the medium by injection through a Sartorius filter (0.2μm pore diameter), after the culture medium had been sterilized.

Seeds, four months after maturing, were dried and used to investigate germination on four germination media: 2% agar (A); 2% agar plus 500 ppm GA_3 (B); 2% agar plus 1000 ppm GA_3 (C), and half strength MS salts which were solidified with 0.8% agar (D). In all media pH was justified to 6.0. Seeds were sterilized using 95% ethanol for 2 min, 1.25% NaOCl for 25 min and 3 rinses of 5, 5 and 30 min with sterilized deionized water. Seeds were incubated at 10, 15 and 20°C in light/dark (16/8 h at 36 μmol $m^{-2}s^{-1}$) or dark conditions and three Petri dishes (with 20 seeds in each) were used per treatment. The continuous darkness conditions were achieved by wrapping the Petri dishes with aluminium foil.

Results and discussion

Germination was inhibited for all treatments exposed to the light and in all treatments in the dark at 10°C. The seeds began to germinate 30 days after incubating in the chamber at 15°C on medium agar (A) or MS (D) (Table 9.9), but germination levels were relatively low. At 20°C only 2.5 % seeds germinated in the medium with 500 ppm GA_3 after 40 days.

The low germination after four months from collecting the seeds indicates that seeds continue to be dormant and GA_3 did not manage to induce germination.

Table 9.9 Effect of the medium, light and GA_3 on % germination of Sternbergia seeds four months after maturing.

	10°C				15°C				20°C			
	A	B	C	D	A	B	C	D	A	B	C	D
L/D	0	0	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	9.1	0	0	13.3	0	2.5	0	0

A = 2% agar, B = 2% agar plus 500 ppm GA_3, C = 2% agar plus 1000 ppm GA_3, D = 1/2 strength MS; L/D = light/dark, D = dark

9.6.5 Effect of temperature and light on the germination of eight month-old seeds of *S. sicula*

Material and methods

The germination rate of *S. sicula* seeds was examined for seeds that have matured for eight months. The seeds were dried after collection and placed in an air-proof plastic container and stored at room temperature in a dry place until use. The seeds were disinfected with 95% ethanol for 1min and 1% NaOCl for 20 min and rinsed with deionized sterilized water for 5, 5 and 30 min. The disinfected seeds were immersed for five more hours in the deionized sterilized water in the laminar flow cabinet in order to imbibe more water. The seeds were placed on sterilized medium of 2% agar (pH = 6.5) in plastic Petri dishes and incubated at 10, 15 and 20°C in light/dark (16/8 h at 36 μmol $m^{-2}s^{-1}$) or dark conditions and three Petri dishes (with 20 seeds in each) were used per treatment. The continuous darkness conditions were achieved by wrapping the Petri dishes with aluminium foil. Seeds were also placed on the agar medium under ambient conditions of the greenhouse.

The germination percentage data were transformed in angular values before statistical analysis. Treatment means are presented in table 9.10 without transformation. Data was analysed by ANOVA and the means were compared by Tukey's test.

Results and discussion

As for the previous experiment, none of the seeds germinated under light conditions in all the applied temperatures and suggests that light generally inhibits seed germination of *S. sicula*. Better percentage germinate (43.3%) was recorded at 15°C and under the fluctuating natural temperatures in the greenhouse (Table 9.10). Lower rates of germination were achieved at 10°C and 20°C, but none of the seeds germinated at 25°C. Germination started on the 21st day after sowing at 15°C, the 24th at 10°C and the 29th at 20°C and the greenhouse. In all treatments, germination was completed in 40 days (Figure 9.2). This germination rate (43.3%) of seeds eight months after their collection was less than the natural germination after storage for 11 months (73%).

Table 9.10 Effect of temperature on the germination of eight months *S. sicula* seeds in dark conditions.

Temperature (°C)	Germination (%)
10	16.7 ± 7.6ab
15	43.3 ± 5.8c
20	13.3 ± 13.3a
25	0 ± 0a
Fluctuating (Greenhouse)	33.3 ± 11.6bc

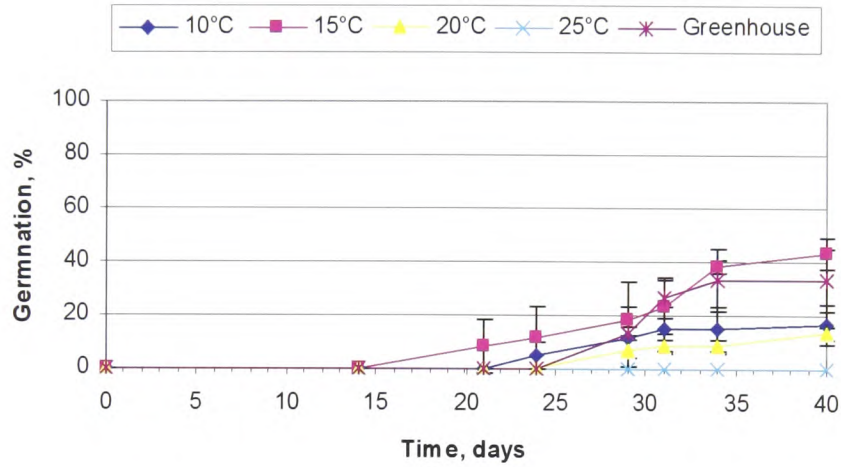


Figure 9.2 Time course of *S. sicula* seed germination in the dark at various incubation temperatures.

9.6.6 Effect of temperature and light on *S. sicula* seed germination after imbibitions for one month at two different temperatures

Material and methods

Seven month old seeds of *S. sicula* were separated into two lots of 250 seeds each. Both seed lots were placed in pots with wet vermiculite in the dark. One of them was placed at 5°C and the second at 20°C.

One month later seeds were rinsed under tap water and disinfected with 95 % ethanol for 1 min and 1% NaOCl for 10 min and then rinsed with sterilized deionized water for 5, 5 and 30 min. The disinfected seeds were placed on a substrate of 2 % agar (pH = 6.5) in Petri dishes, and incubated at 10, 15, 20°C or

under fluctuating temperatures in the greenhouse. Three Petri with 20 seeds in each were used for each treatment and they were placed either in dark or light/dark (16/8h) conditions. Forty days later seed germination rates were recorded.

Results and discussion

Seeds from the lot incubated for one month at 5°C did not germinate. Embryos were examined from sections of these non-germinated seeds and observed to be very small in the centre of the seed and undeveloped. This indicates that low temperature inhibits the growth of the embryo which fails to penetrate the endosperm and coat of the seed. This type of dormancy, known as morphological dormancy where the embryo is immature or not fully developed at time of fruit maturity, has been observed in many other species (Hartmann *et al.*, 2002). Warm temperatures near 20°C or chemical additives favour germination

Table 9.11 shows the results obtained from seeds placed in light and darkness after imbibition in vermiculite at 20°C. The optimal germination temperatures were 15 and 10°C with 70 and 66.2% germination within 35 days, respectively (Plate 9.14). Germination became more difficult at 20°C and alternating temperatures in the greenhouse.

Lighting strongly inhibited germination at all temperatures tested. This was also observed in the previous experiments of *S. sicula* seed germination. Seeds that germinated at 15, 20°C and alternating temperatures started to form small bulbs instead of seedlings after two months maintenance in the Petri dishes (Plate 9.15).

Table 9.11 Effect of temperature and light on *S. sicula* seeds germination after imbibitions in vermiculite for one month at 20°C.

Temperature (°C)	Germination (%)	
	Light/dark	Dark
10	0	66.2b
15	0	70.0b
20	0	23.9a
Fluctuating (Greenhouse)	0	26.3a

9.6.7 Conclusion

Sternbergia sicula seeds are dormant and they germinate in nature only after 11 months of after-ripening. *In vitro* germination experiments suggest that they germinate only within a narrow temperature range and germination is strongly inhibited by irradiation with white light. The optimum temperature for germination is 15°C in darkness. Gibberellins do not affect germination. Temperatures of 20°C accelerate the germination even before the 11 months of after-ripening. This probably is due to the accelerating the growth of the embryo. Highest levels of germination (73.2%) were recorded under natural propagation 11 months from dispersal of the seeds from capsules and after eight months storage and pre-treatment at 20°C for one month (70%). Fresh seeds have a very low germination rate (5%) at 15°C and four month-old seeds at 15°C (13.3%).

9.7 PROPAGATION BY BULBS

9.7.1 Introduction

Propagation by bulbs is the main natural reproductive process for *S. sicula*. After the dispersal of the seeds within small distances from the mother plant (from a few cm to a few meters) the seeds germinate and in this way the first bulb is created. If seeds remain in groups covered with soil more than one seedling develops and more initial bulbs are formed in the cluster. From the initial bulbs and through the annual continual bulb production, clusters with a significant number of bulbs are created.

Various techniques have been developed to increase the number of bulblets per mother bulb, mainly for species that produce very few bulbs. Some of the techniques (see chapter 1.1.3.2) lead to the removal of apical dominance and result in callusing of the exposed scale tissue. In this research a number of techniques for bulb propagation were examined in order to determine their capacity to increase the number of bulblets per mother plant.

9.7.1.1 Number of bulblets produced naturally

In order to investigate the number of bulblets produced in nature, bulbs were collected during autumn of the year 2002 (see chapter 9.4.2.2) and cultivated in pots in an unheated greenhouse. After two years (in September 2004) the bulbs were taken out of the pots and the new bulblets were recorded. The total mother and daughter bulbs produced over a two-year period numbered from 2 to 7 (1 - 3.5 daughter bulbs yearly depending on the type of the species) (Table 9.3).

9.7.1.2 Effect of the size of the bulbs and cross-cutting on lateral bulbs production

Cross-cutting is one of the techniques to maximize the number of bulblets per bulb which has been applied in many other species bulbs (Hartmann *et al.*, 2002).

Material and methods

Bulbs, which were collected at the end of April 2003 from the study area, were stored at room temperature until use. On 14 September 2004 before anthesis started, 120 bulbs were separated in three equal groups: bulbs with circumferences bigger than 5 cm, those that were 3 – 4 cm and bulbs smaller than 3 cm in their circumference. Half of the bulbs of each group were cross-cut on their base in two vertical sections until the main bud in the centre of the bulbs had been destroyed. The remaining bulbs were kept whole as a control. After the cutting treatment the bulbs were planted in 24 pots (5 bulbs/pot and 4 pots/treatment). The potting soil was composed of equal quantities of peat moss, perlite and trade compost. The pots were watered 2 - 3 times a week depending on the conditions. Seven months later when the leaves of the bulbs were dried, the bulbs were removed from the pots and the new bulbs separated from the mother plants and counted and weighed. The experimental design for these experiments was complete randomized.

Results and discussion

The cut-crossing affected positively the formation of new daughter bulbs from the mother bulbs in all the circumference size categories producing 12.8, 11.3 and 3.0 daughter bulbs respectively for the three size of bulbs (Table 9.12; Plates 9.16 and 9.17). However there were no differences in weight among the daughter bulbs after cross-cutting. Bulbs over 5 cm in circumference are an appropriate size for

the market and would not be used for cross-cutting, but bulbs of 3 - 4 cm in circumference could be used for exploiting and producing new bulbs since they produce as many daughter bulbs as the larger bulbs.

Table 9.12 Effect of the size and cross-cutting of the mother bulbs on the production of daughter bulbs.

Circumference of bulbs	Mean number of bulbs (± sd)		Mean bulb weight (g ± sd)	
	Control	Cross cutting	Control	Cross cutting
> 5 cm	1.3 ± 0.5a*	12.8 ± 2.5b	4.6 ± 0.4d	0.8 ± 0.1ab
3 – 4 cm	1.0 ± 0.8a	11.3 ± 2.8b	1.2 ± 0.1b	0.4 ± 0.0a
< 3 cm	0.0 ± 0.0a	3.0 ± 2.0a	2.1 ± 0.2c	0.7 ± 0.1a

* Values followed by the same letter are not significantly different (P= 0.05)

9.7.1.3 Effect of BA on daughter bulbs production

In order to improve further the number of the daughter bulblets produced by bulbs of *S. sicula* the effect of BA on the mother bulbs bigger than 4 cm in circumference was examined.

Material and methods

Bulbs, which were collected at the end of April 2003 from the study area, were stored at room temperature until use. On 15 September 2004, 80 healthy bulbs bigger than 4 cm in circumference were selected and the following four treatments were applied: **a**. 20 bulbs, which were used as a control, were immersed in water for 4 hours;

b. 20 bulbs were cross-cut (as described in the previous experiment) and then put in water for 4 hours;

c. 20 bulbs were immersed in a solution of 100 ppm BA for 4 hours;

d. 20 bulbs were immersed in a solution of 200 ppm BA for 4 hours.

BA was dissolved first in 5 ml 1N NaOH and then in distilled water. After the treatment the bulbs were planted in 16 pots (5 bulbs/pot) with four replicates. The potting soil was composed of equal quantities of peat moss, perlite and trade

compost. The pots were watered 2 - 3 times a week depending on the conditions. The pots were placed on a bench in an unheated greenhouse. Seven months later at the end of the cultivation period the bulbs were taken out of the pots and the new bulbs separated from the mother plants and counted and weighed.

Results and discussion

All three treatments produced significantly more bulblets than the control, although there were no significant differences among these treatments (Table 9.13). All treatments, cross-cutting and immersion in 100 or 200 ppm BAP for four hours produced more than 10 bulblets per bulb. The weight of the bulblets differed between the control and the three treatment but not among the cross cutting treatment and the two concentrations of BA (Table 9.13; Plate 9.18)

These two methods can practically be used to exploit *Sternbergia sicula* although the use of BA has no significant advantage over cross-cutting.

Table 9.13 Effect of cross cutting at the base of the bulb and two BA concentrations on bulblet production. Means ± sd of 4 replications (5 bulbs/replicate).

	Control	Cross cutting	100 ppm BA	200 ppm BA
Number of bulbs	1.3 ± 0.5a*	12.8 ± 2.5b	10.5 ± 3.7b	13.5 ± 1.3b
Mean weight of bulbs (g)	4.6 ± 0.4b	0.8 ± 0.1a	0.9 ± 0.1a	0.8 ± 0.0a

* Values followed by the same letter are not significantly different ($P = 0.05$)

9.7.1.4 Propagation by chipping

The method has been used for the reproduction of other members of the Amaryllidaceae. The bulbs are cut in "chips" which are slices of the bulb cut lengthwise. Each slice has a portion of the basal plate. The slices are incubated in wet vermiculite or sand at a temperature of 20 to 28°C, for 6 to 12 weeks (De Hertog, 1994.)

In a preliminary experiment bulbs of uniform size were surface disinfected with 95 % (v/v) ethanol for 10 sec, followed by 1% NaOCl for 20 min and rinsing with deionized sterilized water, and then cut into eight slices each. The slices, with 5 ml of water, were put in 9-cm Petri dishes, which were put in loosely closed plastic bags and incubated for 8 weeks at 23°C in darkness. The slices produced 1 - 2 new bulblets each.

Materials and methods

a. The same experiment was repeated using bulbs which had been stored at room temperature or at 5 – 7°C for 3 months. Both were sterilised using 95% ethanol for 1min and 1% NaOCl for 20 min and rinsed with deionized sterilized water for 3, 5 and 30 min. Each bulb was cut into 4 slices and a total of 32 slices for each treatment were placed in 9-cm Petri dishes (4 slices in each dish) and incubated at 23 ± 2°C in dark conditions. Eight weeks later the number of the newly-produced bulblets and their weight were recorded.

b. The effect of BA (0, 100, 200 and 400 ppm) was evaluated on the chips of *S. sicula* bulbs in the greenhouse. Slices were placed in the solutions of BA for 30 min and then were planted in pots with trade compost and placed on a bench in the greenhouse. One hundred slices were prepared from 20 bulbs over 5 cm in circumference and 40 slices from 20 bulbs smaller than 3 cm in circumference were used. In each pot were planted 5 - 7 slices.

Results and discussion

a. Most of the bulbs from those that had been stored at room temperature had dried and very few bulblets were formed inside the scales of the mother bulbs (Table 9.14. Plate 9.19). In contrast, a large number of bulblets were produced from the bulbs which were stored in 5 – 7°C. Each slice produced a mean of 4.1 bulblets, which means 16.4 bulblets per bulb, significantly more than those formed by the effect of cross-cutting or BA application on whole bulbs.

Table 9.14 Production of bulblets by chipping of stored bulbs. (N = 32)

	Number of bulblet/chip (± sd)	Mean weight of bulblet in mg (± sd)
Bulbs stored at 5 – 7°C	4.1 ± 3.6	78.8 ± 56.0
Bulb stored at room temperature	0.3 ± 0.2	22.1 ± 17.3

b. Most of the slices treated in BA solutions became rotten after planting in soil and therefore no meaningful results were produced. However, it was observed that from those surviving slices more bulblets were induced from the treatment with 400 ppm BA.

9.8 IN VITRO REPRODUCTION OF STERNBERGIA SICULA

9.8.1 Literature review

The conventional propagation method from seed is possible but it takes many years to develop plants capable of flower production. It was observed that after three years of seedling development the formed bulbs are too small to induce flower. Moreover, collection in the wild is difficult providing relatively small amounts of seed. The production ratio of bulblets are generally higher (2 – 7 per mother bulb) than other bulbs but ineffective in producing plenty of healthy plant material from selected clones for commercial use. The application of *in vitro* culture techniques may allow rapid propagation of sufficient numbers of uniform plants for field culture.

There are no references in the literature for the *in vitro* culture of *Sternbergia sicula* or other species of the genus *Sternbergia*. A method has been developed by Mirisi *et al.* (1995) for bulblet regeneration *in vitro* from immature embryos of endangered *Sternbergia fischeriana*. Other species of the Amaryllidaceae family have been cultured *in vitro* using the method of the simple, twin or three scales culture such as *Pancratium maritimum* (Dragassaki *et al.*, 2003), *Hipeastrum hybridum* (Huang *et al.*, 1990a, 1990b), *Narcissus bulbocodium* (Santos *et al.*, 1998) and other species of the genus *Narcissus*. *In vitro* micro-propagation has been reported for many other geophytes, a range of explants such as bulb scales,

stem nodes, leaves, seeds are usually taken from bulbs, either fresh or after storage and desiccation (Amaki *et al.*, 1984; Bach, 1992).

One of the main factors in plant tissue culture is the origin and size of explants and their polarity and pre-treatment. Many sources of tissue explants are used to establish the initial step of bulb culture, including bulb scales, leaf, stem and various parts from the inflorescence. Organogenesis depends also on the position in the bulb. The yield of hyacinth bulblets increased more in the basal part of the bulb of leaf scales and leaf segments than in the distal parts (Bach, 1992).

The aim of this work is to develop a method for *in vitro* mass production of selected clones of *Sternbergia sicula* in order to use it as ornamental bulbs for landscaping. The potential for maximum bulblets yield, in different seasons of the year, before or after storage and using different concentrations of growth regulators is examined in this study.

9.8.2 Effect of BA and NAA on bulblets production from bulbs during October.

Preliminary studies were conducted to investigate *in vitro* proliferation of *Sternbergia sicula* using the twin scaling method (Antonidaki *et al.*, 2004).

Material and methods

Explants were derived from *Sternbergia sicula* bulbs 4 – 5 cm in circumference which had been collected from the observation area during October 2003. The dry scales and the roots were removed and then the bulbs were washed with household detergent and tap water. The outer surface of the bulbs was disinfected with 70% ethanol for 1 min and 1% NaOCl for 20 min and then was rinsed with deionized sterilized water for 3, 5 and 30 min.

According to the method of twin scaling, the bulbs are first cut in longitudinal slices and then cut further across the slice in smaller pieces comprising one pair of scale parts still attached at the base with a small part of basal plate tissue. The explants were 0.5 x 0.5 cm consisting of two to four scales. Each explant was embedded into the substrate with the part of the basal plate in the medium while the

scales remained outside. Twenty explants were used for each treatment in a completely randomized design.

The culture medium comprised MS basal salts and vitamins, sucrose (30 g/l), agar (7 g/l) and the growth regulators benzyladenine (BA), at concentrations of 0, 1, 2, 4 mg/l alone, or in combination with 1 mg/l napthaleneacetic acid (NAA). All the explants were kept at 24 ± 2°C in the dark. The number of bulblets produced per explant was evaluated after 12 weeks. The newly-produced bulblets were weighed after decapitation.

Results and discussion

The results from this experiment showed that BA produced more bulblets than the control treatment (Figure 9.3). Each explant produced a mean of 3.5 to 4 bulblets when cultured on MS with the addition of BA alone. The number of bulblets produced per explant was reduced when NAA was included in the medium, or without any addition of growth regulators. NAA alone did not produce any bulblets but only callus. Twelve weeks after the culture the new bulblets were counted and weighed. The addition of NAA increased the final weight of the bulblets. The highest weight was produced in the medium supplemented with BA at 4 mg/l + NAA at 1mg/l (Figure 9.4). At 4/1 mg/l BA/NAA fewer bulblets were produced than all other treatments but they were significantly heavier than the other treatments. The larger bulbs (about 100 mg) were transferred and planted to pots. All the bulblets survived in the greenhouse six months later producing small bulbs.

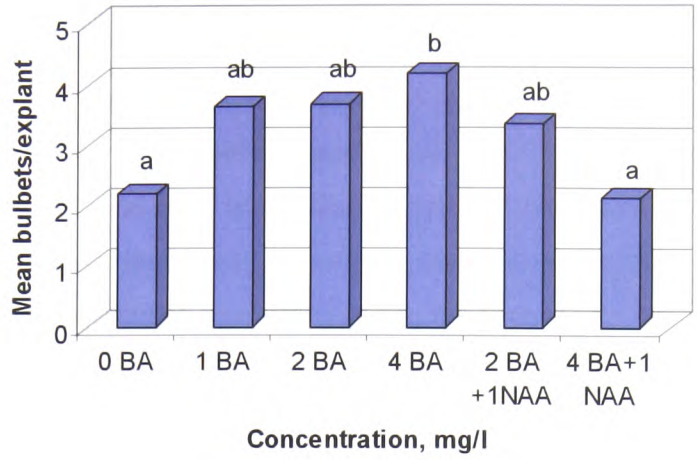


Figure 9.3 Effect of BA and NAA on the mean number of *Sternbergia* bulblets per scale explant produced *in vitro*.

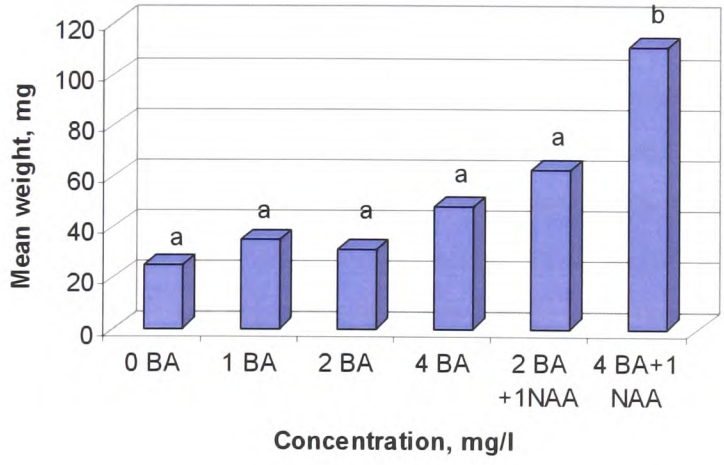


Figure 9.4 Effect of BA and NAA on *Sternbergia sicula* bulblet weight produced by the *in vitro* culture of scale explants.

9.8.3 Effect of BA and NAA on bulblet production *in vitro* of *S. sicula* bulbs after storage.

The previous experiment looked at the *in vitro* culture of explants from freshly-harvested bulbs. This experiment looks at the effect of storage on the *in vitro* propagation of *S. sicula* explants.

Material and methods

Bulbs were collected from the observation area at the end of April 2004 after the end of growing season when the aerial part of the plants was senescent. Some of the bulbs were stored at room temperature (20 – 25°C) in darkness and others were cooled to 7 – 9°C and some were planted in pots in an unheated greenhouse. Bulbs from each of these three storage conditions were used to initiate the *in vitro* culture after eight to nine months from the time that they were collected. *S. sicula* explants were taken from bulbs of 4-5 cm in circumference

Disinfection and explants preparation

The following disinfection method was applied on three sets of bulbs to produce clean explants for *in vitro* culture.

Bulbs with the papery scales and roots removed were washed with household detergent rinsed with tap water and disinfected according to the following scheme: placed into 95% ethanol for 1 minute, then immersed for 20 minutes into 25% household bleach (final concentration 1.25% in NaOCl) with the addition of 4 drops per 100 ml solution of Tween 20. Then they were rinsed three times for 3, 3, and 20 minutes with sterilized deionized water. After this the bulbs are first cut in four longitudinal slices and then cut further across the slices in half pieces to obtain explants formed by 2 – 4 segments of scales joined by a thick segment of basal plate tissue (Plate 9.20). From each explant (1 x 0.5 cm) the inner main bud and the outer scale were discarded. The basal plate was embedded into the medium.

Culture media

9.8.3.1 Effect of BA and NAA on *Sternbergia sicula* bulbs stored at room temperature.

The MSS culture medium supplemented with 0, 1, 2 or 4 mg BA and 0, 0.5, 1 or 2 mg/l NAA in all possible combinations was used to initiate and establish the explants of *S. sicula*. All cultures were placed in culture room at 24 ± 2°C and darkness. After 14 weeks culture, large rooted bulblets (over 100 mg) were planted in pots containing trade compost:perlite (4:1 v:v) and put in the greenhouse (Plate 9.21). Unrooted bulblets were transferred to MSS medium with IBA at 1, 2 and 4

mg/l, NAA, BA or combinations of BA/NAA in culture room at 24 ± 2°C in 16/8 h photoperiod and 36 μmol $m^{-2}s^{-1}$ white light. After 11 weeks the number and length of shoots as well as the number and the weight of bulblets were evaluated.

Test tubes with clumps of shoots were divided and sub-cultured two more times every three months. The number of shoots was recorded 10 months after the initiation of the culture.

9.8.3.2 Effect of BA and NAA on pre-chilled bulbs of *Sternbergia sicula*

The same MSS medium with the addition of 0, 1, 2, 4 mg/l BA or 0, 0.5, 1 and 2 mg/l NAA was used. Cultures were placed at 24 ± 2°C in darkness. After 11 weeks, explants were divided and a second culture was maintained on the same medium as well as on combinations of BA/NAA at concentrations of 2/0.5, 2/1 and 4/1 mg/l in light/dark conditions at 24 ± 2°C.

Test tubes with clumps of shoots were divided and sub-cultured two more times every three months. The number of shoots was recorded 10 months after the initiation of the culture.

9.8.3.3 Effect of BA and NAA on fresh bulbs of *Sternbergia sicula* from greenhouse storage during February.

MSS medium supplemented with 0, 1, 2 or 4 mg/l BA and 0, 0.5, 1 or 2 mg/l NAA in all possible combinations was used as culture media.

After 11 weeks the explants were transferred to MSS medium supplemented with IBA at 1, 2 or 4 mg/l and NAA at 0.5, 1 mg/l. The number and the weight of the bulblets were recorded at the time of the transfer. Test tubes with clumps of shoots were divided and sub-cultured two more times every three months. The number of shoots was recorded 10 months after the initiation of the culture.

All the explants were kept at 24 ± 2°C in darkness for 11 weeks, after which the number and length of shoots, the number of bulblets and the rooted plantlets were recorded.

9.8.3.4 Effect of BA and NAA on *Sternbergia sicula* bulbs stored at room temperature.

All the explants were successfully disinfected apart from the treatment 0/2 mg/l of BA/NAA with one third becoming infected (Table 9.15). Six weeks after inoculation explants started to swell and all of the non-infected explants began to respond to the media producing shoots, roots, callus or bulblets. All the treatments containing NAA alone produced callus with the amounts of callus increasing with higher levels of NAA.

All of the treatments produced shoots to some degree with the highest number occurring in all treatments with BA at 4 mg/l alone or with the combination of 0.5 or 2 mg/l NAA (Table 9.16). However, there was little growth of the shoots in these treatments. There was no apparent relationship between the growth of the shoots and the addition of BA and NAA, but the best growth of shoots was recorded with 1/0.5 mg/l BA/NAA. In the combinations of BA/NAA at 2/0.5, 4/0.5, 4/1 and 4/2 amorphous achlorophyllus tissues were produced at the base of the explants and the epidermal and hypodermal surface of scales (Plate 9.22). These tissues turned into shoot clumps after 10 -12 weeks (Plate 9.23).

In all of the treatments containing NAA alone the explants achieved 100% rooting, with the number of roots declining with increasing levels of NAA. Explants also rooted in BA/NAA treatments at 1/0.5, 2/0.5 and 4/0.5 mg/l or in the medium without hormones with 27.3, 33.3, 50 and 33.3 % rooting success, respectively.

Each explant produced 1 - 4 bulblets when cultured on MSS medium with or without the addition of growth regulators. The largest number of bulbs was produced by the addition of 4 mg/l BA.

The division and sub-culturing of the clumps of shoots induced a proliferation of shoots totalling 21.8 shoots per explant after 10 months and two transfers (data not shown).

The rooted bulblets were transferred to the greenhouse and were established successfully (100%).

Table 9.15 Effect of BA and NAA on *Sternbergia sicula* bulb scale explants stored for eight months at room temperature on the shoot number, bulblets and root induction 14 weeks after the initiation of the culture. (N = 6)

BA/ NAA (mg/l)	% non infected	% response	Mean number of shoots	Mean length of shoots (cm)	% callus	Mean number of bulblets	% clumps of shoots	% rooting	Mean number of roots	Mean length of roots (cm)
0/0	100	100	1	0.2	0	1.2	0	33.3	1	2.5
0/0.5	100	100	2	0.7	100	1	0	100	6.5	0.5
0/1	100	100	1.66	4.1	100	1.6	0	100	3.5	0.7
0/2	66.6	100	1.5	0.4	100	1.5	0	100	2.1	0.6
1/0	100	100	2	0.6	0	2	0	0	0	0
1/0.5	100	100	2.5	4.6	33.3	2.3	0	27.30	3.3	0.5
1/1	100	100	1.5	1.6	0	0.5	0	0	0	0
1/2	100	100	6	0.45	0	1	0	0	0	0
2/0	100	100	3.5	0.5	0	2.5	0	0	0	0
2/0.5	100	100	3	0.4	33.3	2.3	33.3	33.3	2.2	1.5
2/1	100	100	5.5	0.9	0	1.5	0	0	0	0
2/2	100	100	3	0.5	0	1	0	0	0	0
4/0	100	100	6.5	0.45	0	3.5	0	0	0	0
4/0.5	100	100	6.5	0.3	33.3	2.5	50	50	2	1.5
4/1	100	100	4.5	0.4	0	2.5	50	0	0	0
4/2	100	100	6.5	0.35	0	2	50	0	0	0

Un-rooted bulblets were transplanted on to MSS medium and continued to grow producing new shoots and bulblets but no roots were initiated (Table 9.16; Plates 9.24 and 9.25). The largest weight of bulblets was produced by the effect of 1 mg/l IBA (Table 9.16).

Table 9.16 Effect of BA and NAA on bulblets of *Sternbergia sicula* bulbs transplanted in different growth regulators after 11 weeks from the subculture.

Substrate mg/l	No of shoots	Mean length of shoots (cm)	Mean number of bulblets	Mean weight of bulblets (mg)
Control	2.85	1.29	2.29	119.6
IBA 1	1.33	3.17	1.67	348.6
IBA 2	0.67	1.33	1.0	318.0
IBA 4	2.0	2.93	1.67	299.5
NAA 1	2.67	1.0	2.0	195.3
BA/NAA 1/0,5	2.5	3.1	2.5	313.0
BA 4	3.4	0.9	1.4	112.0

9.8.3.5 Effect of BA and NAA on pre-chilled bulbs of *Sternbergia sicula*

A low percent (40 %) of explants were successfully disinfected despite the fact that the same disinfection procedure was used as for the stored bulbs (Table 9.17). Almost all of the surviving, non-infected explants responded to the media producing shoots, callus or bulblets; however, none of the cultures produced roots. All of the treatments with BA and the control produced shoots and bulblets, the larger number of shoots and bulblets were produced with the supplement 4 mg/l BA. In all treatments with NAA, explants produced callus and clumps of shoots.

Table 9.17 Effect of BA and NAA on scale explants from pre-chilled bulbs of *Sternbergia sicula.* (N = 8)

BA/NAA	% not infected	% responded	Mean number of shoots	Mean length of shoots(cm)	% with callus	% with rooting	Mean number of bulblets	% with clumps of shoots
0/0	33.3	100	3.5	0.5	0	0	3.5	0
0/0,5	42.8	100	0	0	100	0	0.3	100
0/1	42.8	100	0	0	100	0	0	100
0/2	42.8	100	2	0.5	100	0	2	100
1/0	40	100	5.5	0,2	0	0	3.5	0
2/0	28.6	100	5	0.2	0	0	4	50
4/0	50	66.6	6.5	0.2	0	0	6.5	0

After 11 weeks, the explants were divided and from the subsequent sub-culture the following were observed:

- amorphous tissue was produced and formed shoots, with the best shoot production occurring in media with NAA and particularly in combination with BA (Table 9.18);
- show growth was optimal without the addition of growth regulators;
- all treatments with NAA alone or with BA produced clumps of shoots;
- rooting was limited to two treatments, with a low percentage of rooting in the control and 1mg/l NAA;
- most treatments produced bulblets but the greatest weight of bulblets was recorded in the combination 2 mg/l BA with 0.5 or 1 mg/l NAA;
- amorphous tissue in BA stopped growing and dried.

The two-fold sub-culturing of the clumps of shoots produced 18 shoots per explant after 10 months (data not shown).

Table 9.18 Effect of BA and NAA on scale explants from pre-chilled bulbs of *Sternbergia sicula* after sub-culturing.

BA/NAA (mg/l))	Mean number of shoots	Mean length of shoots (cm)	% with callus	% rooting	% with clumps of shoots	Mean number of bulblets	Mean weight of bulblets (mg)
0/0	7.6	4.1	0	33.3	0	1	85
0/0.5	2.6	0.23	100	0	100	2	73
0/1	6.25	2.75	100	25	100	0.5	84
0/2	4	3	100	0	100	2	88.5
1/0	4	1	0	0	0	2	32
2/0	0	0	0	0	0	0	0
4/0	0	0	0	0	0	0	0
2/0.5	17.5	0.4	100	0	100	2	158
2/1	10	0.8	100	0	100	2.5	114
4/1	10	2.3	100	0	100	1.8	97

9.8.3.6 Effect of BA and NAA on fresh bulbs from greenhouse storage during February.

A high percentage (73.1 %) of contamination was recorded in fresh bulbs despite the fact that the same disinfection as used successfully for the stored bulbs was used (Table 9.19). A lower percentage of explants responded to the treatments (55.9 %) compared to the pre-chilled (97.9 %) and the stored bulbs (100 %). The presence of BA was necessary to form a significant number of shoots with the highest number of shoots on the 2 mg/l BA treatment and the best growth of shoots on the combination of 2 mg/l BA with 2 mg/l NAA. Rooting and production of bulblets were non-existent or very low in all treatments. All explants in treatments containing NAA produced callus and those on BA/NAA 2/0.5, 4/0.5, 4/1 and 4/2 formed amorphous tissue, which after they were sub-cultured produced clumps of shoots.

Table 9.19 Effect of BA and NAA on scale explants of fresh bulbs from greenhouse stock of *Sternbergia sicula* after 11 weeks culture. (N=20)

BA/ NAA (mg/l)	% non infected	% responded	Mean number of shoots	Mean length of shoots (cm)	% with callus	% with rooting	Mean number of bulblets	Clumps of shoots
0/0	15	33.3	0.33	0.4	0	0	1	0
0/0.5	15	33.3	0	0	100	0	0	0
0/1	25	20	0	0	100	0	0	0
0/2	20	20	0	0	100	0	0	0
1/0	25	40	1.4	0.45	0	0	0	0
1/0.5	55	72.7	1.45	0.43	45	9.1	0	0
1/1	30	16.6	0.16	0.33	33.3	0	0	0
1/2	10	50	0	0	100	0	0	0
2/0	10	100	2.5	0.2	0	0	0	0
2/0.5	35	85.7	2.42	0.34	42.8	0	1	14.3
2/1	40	37.5	0.88	1.33	12.5	0	0	0
2/2	10	100	2	2	50	0	1	0
4/0	30	66.6	1.3	0.22	0	0	0	0
4/0.5	40	100	1.8	1.5	25	0	0	12.5
4/1	40	87.5	2.13	0.98	50	0	0	12.5
4/2	30	66.6	1.5	1.95	50	0	0	12.5

Eleven weeks after the explants were transferred to MSS medium supplemented with IBA or NAA the number of the bulblets and their weight were recorded. The 1 mg/l IBA treatment gave the best result according to the number of bulblets while the best weight of bulblets derived from the treatment of IBA at 2 mg/l (Table 9.20). None of the bulblets rooted. The sub-culturing of the clumps of shoots produced 10.7 shoots per explant after 10 months (data not shown).

Table 9.20 Effect of BA and NAA on scale explants of fresh bulbs from greenhouse stock of Sternbergia *sicula*, after 11 weeks subculture and photoperiod 16/8 h.

Substrate mg/l	Mean number of bulblets	Mean weight of bulblets (mg)	% rooting
Control	1.1	113	0
IBA 1	3.1	134.9	0
IBA 2	2.3	375.5	0
IBA 4	2.5	202	0
NAA 0.5	1.5	49.7	0
NAA 1	0.66	16	0

Discussion and conclusion

In general, propagation of *Sternbergia sicula* is possible using *in vitro* culture of bulbs in MSS medium and the growth regulators BA and NAA. The *in vitro* regeneration of *S. sicula* was achieved with adventitious formation of bulblets or plantlets originating directly from explants or indirectly from previously formed callus.

The best results were achieved with desiccated bulbs (following long-term storage) which had much less infections than the fresh bulbs (those from greenhouse or chill storage) despite the fact that the same disinfestations procedures were used. This suggests that the microflora present on fresh bulbs is reduced with dry storage and not reduced by low temperature storage. The desiccated bulbs also produced more shoot clumps than those from the greenhouse.

Amaki *et al.* (1984, as cited by Bach, 1992) described a method for producing numerous and large bulblets from hyacinth bulb by desiccation with silica gel. In these experiments using desiccated bulbs for *in vitro* culture did not reduce the production of bulblets and plantlets, as Bonnier (1994) noticed in *Lilium* species that lost the ability to produce bulblets after a long storage period.

More bulblets were formed in pre-chilled bulbs (up to 6.5 per explant) than those that had storage in room temperature (3.5 per explant) and fresh bulbs (1 per explant) after 11-14 weeks in culture. No differences were noticed in fresh bulbs of the two different seasons of the year October and February.

Amorphous achlorophylous tissue was formed in the callus and on the cut surface of the scales in the treatments of BA / NAA at 2/0.5, 4/0.5, 4/1 and 4/2 mg/l on both the fresh and room storage bulbs. The same tissue was formed on the pre-chilled bulbs in the treatments with 0.5, 1 and 2 mg/l NAA as well as in the treatment with 2 mg/l BA (no treatments there were in the combinations of BA/NAA). These tissues formed a large number of adventitious shoots, which continued to grow better in the combinations of BA/NAA. Similar results were presented by Bach (1992) for hyacinth and by Santos (1998) for *Narcissus bulbocodium*. Using this method a large number of bulblets can be produced from one bulb, particularly after two subcultures.

Clumps of shoots were further proliferated after two subcultures in 10 months producing a total of 21.8 shoots per explant from the desiccated bulbs, 18 shoots from pre-chilled bulbs and 10.7 from fresh bulbs.

Two types of bulblets were produced:

- from the inner of the twin scales of the explants produced by the effect of BA, and
- from the growth of shoots formed from the amorphous tissue by the effect of the combination of BA/NAA.

Bulblets over 100 mg weight with or without roots were established successfully in pots in the greenhouse.

In conclusion we can say that it is possible to propagate *Sternbergia sicula* using in *vitro culture* methods using desiccated and to a lesser extent pre-chilled bulbs.

9.9 CULTIVATION IN POTS

This experiment aimed to investigate the adaptability of *S. sicula* bulbs in pots as well as the effect of watering on the growth and flowering of bulbs.

9.9.1 The effect of irrigation on *Sternbergia*'s growth and flowering.

Material and methods

Bulbs, which had been collected from the observation area from January 2004 after their flowering had completed were replanted in pots (one bulb per pot) and kept in the greenhouse. A mixture of potting soil with perlite and peat moss (1:1:1, v/v) was used as the substrate. Plants were watered regularly from January to May and then the watering stopped during summer. On 20 September 2004 the bulbs were exposed to three treatments: **a**. frequent irrigation (three times a week); **b**. once a week; and **c**. without irrigation. Each week observations were made on growth, foliage development and anthesis.

Results and discussion

All plants grew well in the pots apart from the plants without irrigation which had shorter leaves and shrunken bulbs after three months. There were no significant differences in the characteristics between the plants of **a** and **b** categories. However some bulbs from **a** category were rotten because of the excess wetting of the soil. The leaves of **a** and **b** category were longer (up to 38 cm) than the leaves in the wild (up to 17 cm) as well as the scapes (flowers stalk) (up to 15 cm) compared to the scapes of the flowers in the wild (up to 3 cm). The time of the anthesis was not significantly different among the three treatments and was on the 9^{th}, 15^{th} and 13^{th} of October for **a**, **b** and **c** categories, respectively. This indicates that the plants blossom independently of the water status of the plant. This is due to the fact that flower primordia were formed from the previous year and it is the cultivation conditions experienced during flower formation that will effect the differentiation of the next year flowering. The leaves of **a** category were 100% synanthous. Some of the leaves of **b** category were synanthous and some of them hysteranthous. The leaves of the **c** category appeared 17 - 25 days after the flowers (hysteranthous) and this may due to the relative humidity in the greenhouse

and/or the reserve of scales. In a similar way, the bulbs from dry storage at room temperature flowered the same period with those that were planted in the pots and their leaves developed slowly (up to 7 cm in length after one month) after flowering (Plate 9.26).

In the pots in the greenhouse very few plants with leaves survived through the summer if a moderate watering programme and lower temperature are applied. These plants flower before plants in the wild or under cultivation in the greenhouse but the flowers are smaller and fewer. *Sternbergia sicula* bulbs were planted as dry bulbs or as plants with leaves and flowers all through the year in various urban gardens and the park of TEI. In all cases plants were established without any problems and they blossomed well.

9.10 CONCLUSION

Sternbergia sicula is a perennial geophyte with hysteranthous foliage that flower in autumn before their leaves emerge. In cultivation it can be synanthous or hysteranthous depending on the water supply. *S. sicula* is widespread in the east Mediterranean from southern Italy and Sicily to southern Greece and the Aegean region and presents large variation in the structure and size of its leaves and bulbs. Very few nurseries cultivate these plants but many amateurs collect them to use in their gardens causing destruction of their habitat (Boyce, 1995).

Up to 9 leaves are produced per bulb, with a width from 1.5 to 6 mm and length from 4 cm to 19 cm. Each bulb can produce one to four flowers yearly, which are yellow and solitary in each scape. Flowering lasted 20 - 40 days both *in situ* and after cultivation in greenhouse. Bulbs, 7 - 10 per cluster, are perennial tunicate with a circumference up to 9 cm and belong to the Narcissus bulb type. In each generative *Sternbergia* bulb are formed usually 1 - 6 bulb units among the scales and up to 7 lateral bulblets attached on the base of the bulbs that usually split away and form a new bulb.

Sternbergia sicula seeds are dormant and germinate only within a narrow range of temperature and germination is strongly inhibited by irradiation with white light.

Optimum germination was recorded at 15°C in darkness. Wet temperatures of 20°C accelerate the germination. This probably is due to the quick growth of the embryo in high temperatures. Higher germination (73.2%) was recorded by natural propagation 11 months after the dispersal of seeds from capsules and 70% after eight months and pre-treatment at 20°C for one month.

Mean number of bulblet per mother bulb are 1 - 3.5 yearly under cultivation in greenhouse. Applying the cross-cutting method of propagation 12.8 bulblet per bulb are formed and by the effect of BA (200 ppm) on bulbs 13.5 bulblets are produced. The number of bulblet per bulb increases using the twin scale method *in vitro* up to 16 - 22 per bulb. Using various systems and pre-treatments to propagate by tissue culture clumps of shoots can be produced that lead to a large number of bulblets per mother bulb.

The Sternbergia genus is very important to be used as a landscape plant especially in the arid Mediterranean climates where the dry summer and the mild winter are ideal for this species and this makes it easy to cultivate. In addition to this very few bulbs have its flowering during autumn to complete the decorative of the beds. Further more, attractive species, which come from northern areas, are susceptible to high temperature and they usually fail to have a good appearance (presentation) in the gardens.

From these experiments, methods were developed which demonstrate that these plants are easily to cultivate with or without watering in most places in Crete from 10 to 1000 m altitude.

10 *COLCHICUM MACROPHYLLUM* B. L. BURTT

10.1 INTRODUCTION

The genus *Colchicum* belongs to the Liliaceae family (Tutin *et al.* 1980), which later was renamed as the Colchicaceae family (Royal Botanic Gardens Bulletin, Kew). According to the classification of Stefanoff (Stefanoff, 1926) as cited by Feinbrun (1958) the *Colchicum* species belong to four sections of the Colchicaceae family: Luteae in which belong the species *C. luteum* and *C. kesselringii*; Cupaniae with 10 species; Bulbocodiae with 24 species and Autumnales with 28 species. Recently new species have been described by botanists so as today about 75 species of the genus *Colchicum* are known, although some of them are confused with each other (Tan and Iatrou, 1995; Persson, 1999; Persson, 2001).

Species of *Colchicum* have expanded from Europe to western and central Asia and to the north of Africa. Five of the *Colchicum* species thrive in Crete: *C. macrophyllum*, *C. cretense* (endemic), *C. cousturieni* (endemic), *C. cupanii* and *C. pusillum*. Many other species are endemic in Greece (Tan, 1995; Persson, 1999; Kadereit, 2004).

Colchicums are known as autumn crocus, meadow saffron or naked ladies and are geophytes that form subterraneous corms. Their leaves are in clusters and appear with or after the flowers. There are both autumn and spring flowering species. Most species of *Colchicum* are very poisonous to livestock. Accidental poisoning has also been reported with autumn crocus and meadow saffron (Gabrscek, 2004; Sannohe, 2002).

Colchicum macrophyllum belongs to the Autumnales section in which anthesis takes place during autumn. It is a hysteranthous (leaves appear after the flowers) geophyte.

Taxonomy according to Royal Botanical gardens Bulletin, Kew:

Class: ANGIOSPERMAE

APG Clade: MONOCOTS

APG Order: Liliales

APG Family: COLCHICACEAE

Kew Family: COLCHICACEAE

Genus: Colchicum

(Species: Epithet: macrophyllum (B. L. Burtt))

Life Form: geophyte

The name Colchicum comes from the ancient region of Colchis, today a part of the Georgian Republic (Burgest, 1988). The name macrophyllum comes from the Greek word «μακρύ φύλλο» that means "long leave" because of its very long and wide pleated leaves (the longest in the genus Colchicum).

10.2 GEOGRAPHICAL DISTRIBUTION

Colchicum macrophyllum is distributed to the south Aegean (S .E. Greece and S. W. Turkey) at altitudes from 200 to 750 m (Tutin *et al.* 1980). In the island of Crete it is found on many hills over 200 m altitude in Sitia, Heraklion (Vianos), Chania and elsewhere. It thrives on many uncultivated slopes that face all directions and on plains in gravel soil as well as under shrubs and trees, such as near the trunk of olive trees where fields are not ploughed. Other plants that thrive near *C. macrophyllum* are mostly weeds and grassy plants while shrubs or other maquis plants are rarely found. It is the first autumn flowering corm that appears every year at the same period (September) before the rains start.

10.3 OBSERVATION AREA

The observation area as well as the place of collection of all plant material (corms, foliage, flowers and seeds) was in Sitia district near Exo Mouliana village on a slope facing north-west near a streamlet at an altitude of 340 m (Plates 10.1 and 10.2). At this place there is about 500 m^2 of uncultivated ground where *C.*

macrophyllum dominates other plants. The plants were observed in this site and material collected, mainly corms and seeds, over a period of three years. Experiments were conducted using this material only from this site in order to investigate the life cycle, sexual and asexual propagation and survival in the pots and the garden.

The climatic features are typically Mediterranean with hot, dry summers and mild, wet winters (Appendix 1).

The analysis of the soil in the observation area, undertaken by the Soil Science Laboratory of the School of Agriculture Technology, is presented in appendix 2. The main characteristics of this soil are that it has high organic matter in relation to the usual soils in Crete, low percentage of $CaCO_3$ and neutral to slightly alkaline pH.

10.4 PLANT DESCRIPTION

10.4.1 Plant life-form

The life form of *C. macrophyllum*, according to Govaerts *et al* (2002) system, is a perennial geophyte, which has stems that die back during unfavourable seasons, with the plant surviving as underground corms.

It belongs to the Crocus type and according to Dafni *et al.* (1981) is a hysteranthous geophyte. This type exhibits after-ripening of seeds during the winder and seed dormancy of up to one year. *C. macrophyllum* plants grow in clusters (groups) of different size corms which are the result of vegetative propagation or both vegetative and seed propagation (Plate 10.3).
In the observation site a mean of 3.5 clusters, with 4 - 5 corms in each cluster were counted per m^2. Seedlings were found under the leaves of the adult plants in groups of 10 - 20 at the end of March in 2005 (Plate 10.4). These seedlings derived from the seeds that accumulated *en masse* near the generative plants after their dispersal from capsules.

10.4.2 Leaves

C. macrophyllum leaves are in clusters of 4 - 5 forming a rosette and are developed after anthesis (hysteranthous). They are light green in colour, strongly plicate, ovate to elliptic-ovate, acute to acuminate, and glabrous (Plate 10.5). The leaves appear aboveground after the rains from the end of December until the end of January depending on the temperatures and amount of rain. They have a length up to 47 cm and width up to 15.5 cm, reaching their maximum size during April (Tables 10.1). The increase in number and length of leaves during the year are shown in table 10.2 and figure 10.1. Maximum length of leaves is acquired by April carrying the fruits out of the ground (Plate 10.6).

Table 10.1 Length and width of the *C. macrophyllum* leaves in nature during April 2004. N = 20.

	Leaves*	Means ± sd
Length (cm)	1st leaf	34.4 ± 4.1
	2nd leaf	37.7 ± 5.9
	3rd leaf	38.6 ± 4.7
	4th leaf	39.8 ± 3.4
	5th leaf	36.2 ± 4.5
Width (cm)	1st leaf	8.4 ± 3.6
	2nd leaf	9.2 ± 4,3
	3rd leaf	9.4 ± 3.6
	4th leaf	9.1 ± 3.3
	5th leaf	5.1 ± 3.2

*Leaves positioned in the row that they appear aboveground

Table 10.2 Number and length of leaves of the mother corms and of the reserve cormlets of *C. macrophyllum*, measured during January, March and May 2003 in pots after one year cultivation in an unheated greenhouse. N = 20, Mean ± sd.

		January 2003	March 2003	May 2003
Corms	Number of leaves	2.6 ± 1.0	2.7 ± 0.9	2.9 ± 0.7
	Length of leaves cm	8.0 ± 3.1	22.1 ± 5.9	31.7 ± 5.6
Cormlets	Number of leaves	1.5 ± 0.9	1.6 ± 0.7	1.6 ± 0.7
	Length of leaves cm	5.1 ± 1.9	18.4 ± 5.6	27.9 ± 7.2

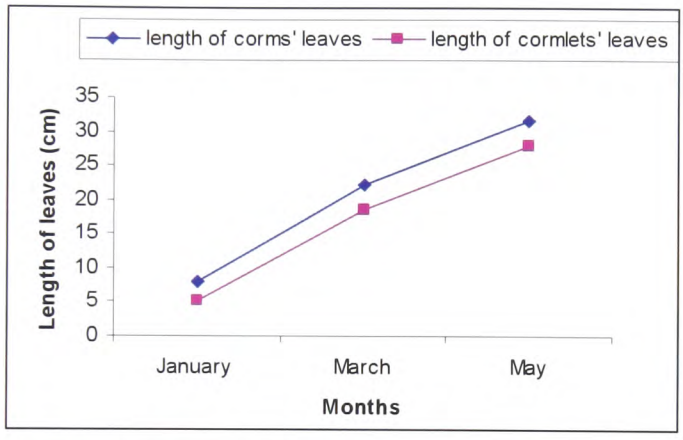


Figure 10.1 Increase in length of leaves per corm and cormlet in pot cultivation from January to May 2003.

10.4.3 Corms

The corms of *C. macrophyllum* are found during summer under ground at a depth of 10 - 30 cm in the dormant stage of growth. A corm is covered by an outer dark brown tunic which extends to a tube (cataphyll tube) containing the remains of last year's dry tissues. The cataphyll tube is ca.10 - 25 cm in length depending on the depth of the corm in the soil and just reaches the surface of the ground. At the lower part of one side of the corm there is the replacement bud (main bud) and the remaining dry roots of the previous year's growth are behind it. On the opposite point on the top part of the corm is situated the reserve bud on the axil of the second leaf of the previous year's growth (Plate 10.7). Each mother corm produces one new corm in one year from the main bud and a new bulblet from the reserve bud while the old one shrinks (Plate 10.8). It is very unusual to form more than one cormlet per mother bulb (Plate 10.9). Corms from the wild grow up to 5 cm in

diameter, 17 cm in circumference and 55 g fresh weight by the end of October after anthesis (Table 10.3). Corms which were cultivated in pots became longer and thinner than those in wild.

Table 10.3 Weight, circumference and diameter of *Colchicum macrophyllum* regenerative corms after the anthesis at the end of October 2003 from wild (N= 30)

	Average ± sd.	Maximum
Weight (g)	29.31 ± 13.81	55
Circumference (cm)	11.49 ± 2.09	17
Diameter (cm)	3.8 ± 0.69	5

10.4.4 Flowers

There are 1 - 5 and rarely 6 flowers in each corm (Plate 10.10). Some dimensions of the flowers were measured in the study area in October 2004. The length of the scape (perianth-segment + hypanthium tube, the total length from the base of the corm to the upper point of the flower) has a mean length of 25 cm while the stalk plus perianth-segment (the stalk with the flower from the surface of the ground) a mean length 12.7 cm. There are six tepals, three outer and three inner. The outer tepals are longer and wider than the inner ones. The spread of the flowerers reach 11 cm and have a mean of 8.7 cm one of the largest flowers among the geophytes (Table 10.4). Stamens are six, filaments from 2.2 to 3 cm and anthers 8 – 10 mm, purple. Styles three ca. 5 cm, shortly curved at the apex.

Table 10.4 Flower dimensions of *Colchicum macrophyllum*. N=35.

	Mean ± sd.
Scape length (cm)	25.0 ± 6.5
Stalk + perianth-segment (cm)	12.7 ± 3.3
Spread of flower (diameter) (cm)	8.7 ± 1.4
Outer tepal length (cm)	6.4 ± 0.7
Inner tepal length (cm)	5.9 ± 0.7
Outer tepal width (cm)	2.2 ± 0.1
Inner tepal width (cm)	1.7 ± 0.1

C. macrophyllum is the first flowering geophyte in the observation area. From the end of August until September the replacement bud starts to grow and the second ten days (in the observation area) the flowers of the new corm appear above the soil surface. Flowers appear during the second ten days of September without leaves (hysteranthous). Flowering lasts for about 40 days both in nature and pots (Table 10.5). The date of flowering of *C. macrophyllum* remains constant from year to year. This occurs also in other species of Colchicum and other geophytes. According to Guttermann and Boeken (1988) day length and temperature affect the timing of flowering of *Colchicum tunicatum*. It was reported that shorter day length (SD) of 9 hours accelerated the flowering of *C. tunicatum* with regard to neutral day (ND) and long day (LD) treatments (12, 15 and 18h) if the minimum and maximum soil temperature did not differ significantly among the treatments. Leaves appear aboveground from 2 to 3 months after the anthesis depending on the rains.

Table10.5 Start and end of flowering of *C. macrophyllum* in its habitat at Exo Mouliana Sitias during the four last years in relation to flowering in pots in an unheated greenhouse in Heraklion.

Year	In habitat		In pots - in garden	
	Start*	End*	Start*	End*
2002	13-17/9	25-30/10		
2003	15-20/9	1-5/11		
2004	12-15/9	24-28/10		
2005	10-15/9	25-30/10	15/9	20/10

*Start of flowering date is the day that the first flower blossoms

*End of flowering date the first date that every flower withers.

The correlation between the size of the corms and the number of the flowers produced were investigated by planting 10 corms of 15 – 18 cm in circumference and 10 corms of 12 - 14 cm in circumference (all mother corms) in pots at the end of August 2004 (before anthesis started). The corms were derived from wild and the primordia of the flowers had been differentiated before the experiment conducted. The main bud had already started to lengthen.

Results showed that the larger corms produced more flowers (up to 6 per each corm) than the smaller ones but there are no statistically significant differences (Table 10.6). The duration of the total days of anthesis of each corm ranged from 10.8 days for the smaller corms to 15.7 days for the larger ones. These results are consistent with the presence of more flowers for flowering in the lot with the larger corms. Life span of the flowers range from 5.5 days for the smaller corms up to 7.9 days for the larger corms. The longer life span is probably due to the larger food reserve contained in the larger corms.

Table 10.6 Correlation between the circumference of the corms and the number of flower, the total days of the anthesis of the corms and the life span of the flowers. N = 5. Mean ± sd.

Circumference of corms (cm)	Number of flowers	Days of anthesis	Life span of flower (days)
15 - 18	4 ± 1.2a*	15.7 ± 4.4b	7.9 ± 1.6b
12 - 14	3 ± 0.9a	10.8 ± 4.1a	5.5 ± 1.1a

*Means with the same letters are not significantly different at L=0.05 according to Tukey's test.

10.4.5 Fruits and seeds

From March, the green fruits appear in the centre of the leaf rosettes above ground (Plate 10.6). Seeds are in capsules located in the centre of the leaf-tufts. At the end of growing season the capsules turn yellow and open to release the seeds. Release starts in nature from May-June and lasts until autumn. Many of the capsules do not open to release the seed and the seeds with part of the capsule remain near the plants. They germinate one year later in spring making a cluster of seedlings if the capsules are buried in the soil and the environmental conditions are favourable.

Each corm produce up to 4 capsules (rarely 5 or 6) and each three-part capsule contains from 8 to 82 seeds (Table 10.7; Plate 10.11). The weight of seeds ranges from 14 to 84 mg and 100 seeds weight 3730 mg.

Table 10.7 Number of fruit per regenerative corm, seeds per capsule and weight of seeds.

	Mean ± s.d.	Maximum	Minimum
Number of capsules/regenerative corm (N = 76)	2.7 ± 0.9	5	1
Number of seeds/capsule (N = 132)	29.1 ± 13.9	82	8
Weight of seeds in mg. (N = 100)	37.3 ± 11.8	84	14

10.5 LIFE CYCLE

In summer, the subterranean corms of *C. macrophyllum*, with the remains of the corms from the previous year, are below the soil surface.

Flowering begins in the middle of September, long before the rainy season, and lasts until the end of October. The flowering of the first autumn corm remains the same from year to year, before the rains start. During the last four years (2001, 2002, 2003 and 2004) corms begin to flower during the second 10 days of September in the observation area. Flowers, usually numbering one to five and rarely six from each corm, appear above the soil surface before the appearance of the leaves. Anthesis in the observation area usually lasts about 40 - 50 days.

During winter from November to December, depending on precipitation, the new roots of the corms develop, and later from the end of December the first leaves appear above the soil surface. The new corms have already widened and elongated, while the corms from the previous year are shrinking. The leaves above the soil surface and the stem continue to elongate, carrying the developing fruits and the inner leaves upwards. In spring (March - April) the fruits appear above the soil surface as the stem and the outer leaves elongate further.

The seeds mature until the end of May and are dispersed from the beginning of June until the autumn. During the same period, the leaves and roots dry. The new

corm is at its maximum size and the previous year's dried corms remain on one side of the corm. During summer the corms fall into the dormant state until August when flower primordia are visible in the bud of the corms.

10.6 UTILIZATION

Corms, seeds and other parts of the plants contain alkaloid colchicines and phenolics of pharmaceutical interest and some species are used to obtain extracts (Fayyad, 2002). This has resulted in the overexploitation of the plants in their habitats (Atul, 2001). Colchicine is used against gout attacks and in experimental chromosomal studies colchicoside has been shown to have anti-inflammatory and analgesic activities.

Some of them are also used as garden plants because of the large flowers that sprout without leaves during autumn and the foliage from December to the next April as a ground-cover plant. *C. macrophyllum* is reported as a rare and little seen species with magnificent pleated foliage and large clusters of deeply chequered lilac-purple to rosy purple flowers that would be suitable as a garden plant (Christian, 2003).

10.7 PROPAGATION BY SEED

During preliminary experiments in growth chambers and the greenhouse the seeds of *C. macrophyllum* did not germinate during the first two months after sowing. According to Dafni *et al.* (1981) *C. macrophyllum* belongs to the Crocus type of hysteranthous geophytes. This type exhibits after-ripening of seeds during the winder and seed dormancy of up to one year.

The germination of the seeds in their natural habitat is also very low and irregular and very few seedlings survive (personal observations). For this reason a series of experiments were carried out to investigate the type of dormancy and the way to overcome it by conducting experiments that took place in the greenhouse and in growth chambers at stable temperatures.

10.7.1 Germination of *C. macrophyllum* seeds in an unheated greenhouse: effect of the substrate on the germination of the seeds

Material and methods

Seeds of *C. macrophyllum* that were collected in June 2003 in the region of the observation area were dried for 15 days in open air and then maintained at room temperature (20 ± 5°C) until their use. On the 18th November after soaking for five hours and leaching for one hour under tap water, the seeds were planted to a depth of 1 cm in three pots with peat moss:perlite (1:1, v/v; pH = 6.2) and 7 pots with trade compost (pH = 6). Twenty seeds were sown at 1 cm depth in each pot. The temperatures that were recorded during this experiment were: minimum 13°C and maximum 25°C.

The measurements of seed germination took place every week over a period of four months and germinated seeds were considered to be those that had developed a leaf of more than 5 mm in length above the surface of the germination medium. After finishing the experiment, the seedlings were replanted in pots in order to measure their viability and the size of the bulblets that were developed.

Results and discussion

The maximum percentage germination of *C. macrophyllum* seeds was 43.6% in compost and 10% in Peat moss+Perlite after four months of incubation (Figure 10.2). The first germinated seed was recorded 55 days after sowing in compost and 93 days in peat moss and perlite, while the T_{50} is 82 and 97 days, respectively for the two substrates. The differences in germination may be due to the different absorption of water or substances contained in them.

It is obvious that the rate of *C. macrophyllum* seed germination is very low and irregular and they show a primary dormancy. All the germinated seedlings that survived produced small cormlets.

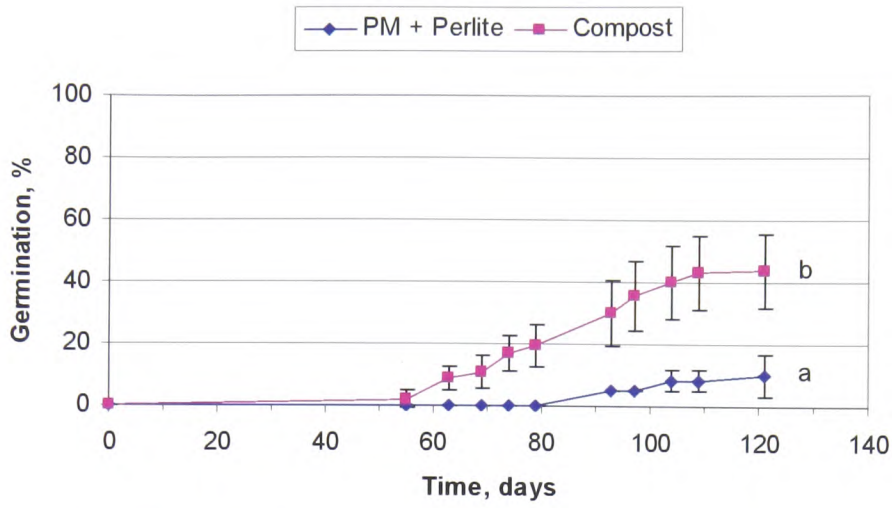


Figure 10.2 Germination of *C. macrophyllum* seeds in peat moss and perlite (1:1, v/v) and compost in an unheated greenhouse during November 2003.

In order to ameliorate the poor germination and investigate how to overcome the problems of dormancy of *C. macrophyllum* seeds, the following experiments on temperature and light were undertaken.

10.7.2 Effect of temperature and light on the germination of *C. macrophyllum* seeds.

Seeds of *C. macrophyllum* collected in June 2003 from plants in the observation area were dried for 15 days in open air and then maintained at room temperature for eight months. In March 2004 the seeds were put in metal boxes with wet vermiculite, after they had been soaked for five hours and leached under tap water. Each box contained 500 seeds. Three boxes were put in a chamber at 5°C and three at room temperature (20 ± 5°C). After one (A), two (B) and three (C) months, each lot of the seeds was tested for germination at 10, 15 and 20°C under light or darkness.

Result and discussion

After one month imbibition of seeds in wet vermiculite at 5°C very low germination (2.6% and 4%) was recorded when they were incubated in darkness or under light at 10°C (Figure 10.3). Moreover no seeds germinated at 15 and 20°C after two or three month's imbibition of seeds at 5°C.

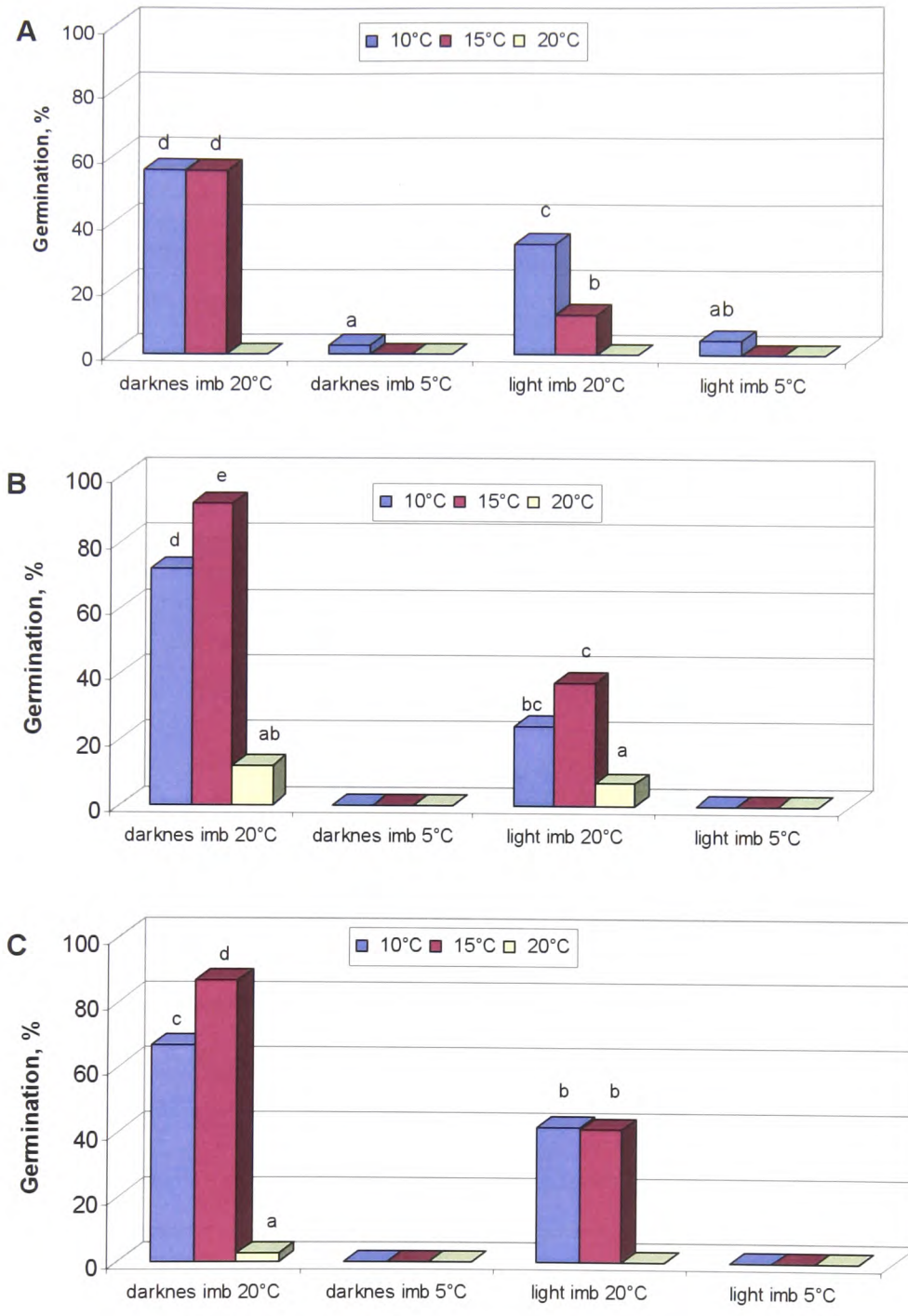
The best results were acquired after imbibition at room temperature and incubation in darkness at 10 and 15°C (56%), (Plate 10.12). Germination in the light is less than that in darkness at 10 and 15°C in all three temperature treatments. It is an indication that light inhibits the germination of *C. macrophyllum* seeds.

This indicates that chilling did not stimulate seeds to germinate compared with the seeds which were wetted at room temperature and that relatively high temperatures approaching 20°C strongly inhibit germination. Overall, the highest percentage germination (92%) was recorded at 15°C in darkness after two months imbibition of seeds in wet vermiculite at room temperature (20 ± 5°C).

Germination time course of *C. macrophyllum* seeds is presented in figure 10.4. After one month of imbibition the seeds in all treatments, apart from at 20°C, started to germinate after the 22nd day (Figure 10.4 A), while more rapid germination was achieved after two and three months imbibition (Figure 10.4, B and C).

All of the produced seedlings were established in pots in the greenhouse and continued to be cultivated after two years.

The same experiment was repeated using the next year seeds with similar results.



Values followed by the same letter are not significantly different (P=0.05).

Figure 10.3 Accumulative germination of *Colchicum macrophyllum* seeds under light or darkness at 10, 15 and 20°C after imbibition at 5°C and room temperature (20 ± 5°C) for (A) one month, (B) two months and (C) three months.

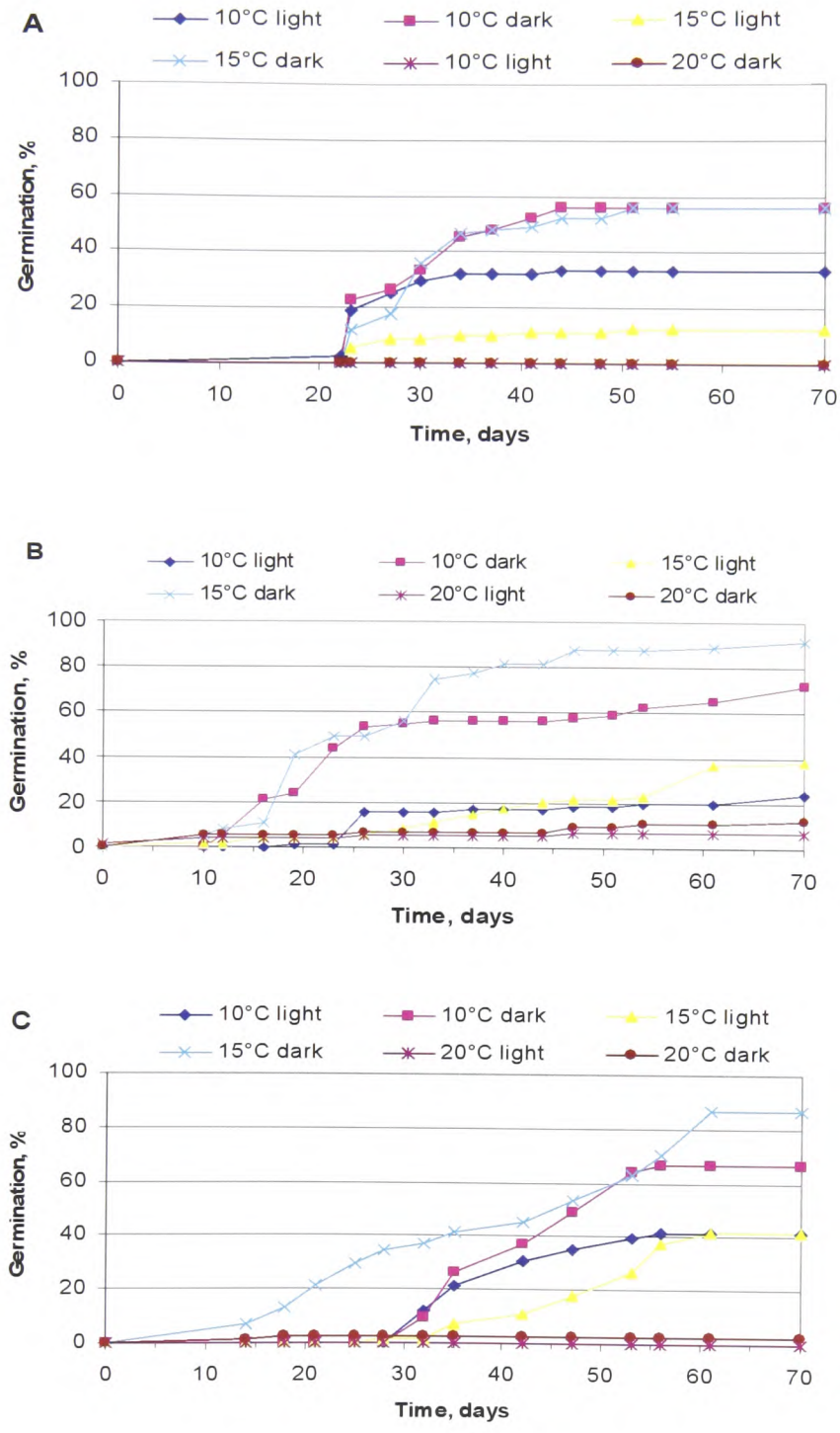


Figure 10.4 Germination course in time of *C. macrophyllum* seeds under light or darkness at 10, 15 and 20°C after imbibition at room temperature (20 ± 5°C) for (A) one month, (B) two months and (C) three months.

Table 10.8 Time germination in days of 50 % of *C. macrophyllum* seeds (T_{50}) at 10 and 15°C under light/dark or dark conditions after one (A) two (B) or three (C) months imbibition of seeds in wet vermiculite at room temperature.

	Light/Dark		Dark	
	10°C	15°C	10°C	15°C
A. Imbibition 1 month	23.4ab*	25.2ab	27.5abc	29.6abcd
B. Imbibition 2 months	29.7abcd	44.3ef	22a	23.6ab
C. Imbibition 3 months	34.9bcde	49.6f	39.7def	38.1cdef

*Values followed by the same letter are not significantly different (P=0.05).

Conclusion for germination of *C. macrophyllum* seeds

C. macrophyllum seeds are dormant and their germination in nature is late and irregular. Applying wet temperatures close to 20°C it is possible to stimulate the seeds to germinate earlier and increase the germination rate. The application of a cold wet temperature of 5°C strongly inhibited subsequent germination in all treatments of light and temperature. After the development of the embryo, the seeds of *C. macrophyllum* need a narrow range of temperature to germinate of 10 – 15°C, preferable under dark conditions.

C. macrophyllum seeds have a primary endogenous dormancy (embryo dormancy). It is possible that the embryo is not fully developed at the time of seed dispersal and a warm temperature favours germination. This category of dormancy includes many other species and families of the Apiaceae, Ericaceae, Primulaceae and Gentianaceae as well as tropical species, many of which are monocots (Hartmann *et al.*, 2002).

10.8 VEGETATIVE PROPAGATION BY CORM

The vegetative propagation of Colchicums is achieved by corms. Each mother corm produces one corm from the replacement bud while the old one shrinks. The replacement bud, which is formed in the axil of the first leaf, is located on the base of the mother corm. One or two other buds, depending on the species, are formed in the axil of the leaf two and three leaf on the upper point of the mother corm.

These one or two buds are the vegetative reserve buds. The vegetative reserve buds usually sprout only after the replacement bud has been destroyed.

C. autumnale and *C. steveni* have only one vegetative reserve bud in the axil of the second leaf in contrast to *C. tunicatum* which has two additional corm buds in the axil of the second and third foliage leaves associated with corm internodes (Guttermann and Boeken, 1988). In *C. autumnale* the axillary bud of the third foliage leaf, under special conditions, can also produce an independent sprout instead of a flower (Troll, 1967).

C. macrophyllum is found in the observation area in clusters comprising ca. 5 - 10 regenerative or vegetative corms of various sizes (Plate 10.3). Each corm has the replacement bud on the base of the corm opposite the root disk and one reserve bud on the upper point of corm on the other side than the replacement bud (Plate 10.7). The priority is the development of the new corm, using the food reserve from the old bud, to a full size so as to replace the old one, which shrinks. In most cases the reserve bud on the upper point of the corms can also produce, at the same time as the replacement bud, an independent cormlet which becomes disconnected from the mother corm (Plate 10.13). Consequently from each mother corm up to one generative and one vegetative corm will be produced yearly in nature. The vegetative cormlet has to be cultivated for a further period, depending on its size, in order to become a generative corm that is able to flower.

Observations were conducted during the last four years and samples of bulbs, leaves and flowers were taken in order to understand the natural propagation of *C. macrophyllum* in its habitat while experiments were carried out to investigate new methods to improve vegetative propagation. Corms were also planted in pots to support this research. There is no published work on this approach to propagation of *C. macrophyllum*.

10.8.1 Effect of wounding and the size of the *Colchicum macrophyllum* corms on the number of produced cormlets/corm.

This experiment was conducted to investigate **a**. the potential of *C. macrophyllum* corms to produce new corms or cormlets in relation to their size and **b**. the effect of wounding the main bud on the sprout and number of newly-produced corms.

Material and methods

Corms derived from pots where they had been cultivated for two years were divided in two lots on 7 September 2004: those that weighed over 10 g each and those that were less than 10 g. Half of each lot was wounded by cutting the base of their replacement bud and half remained intact. Both lots were planted in peat moss:perlite:compost (1:1:1, v/v/v) in pots in the greenhouse. Four corms per pot and 4 pots per treatment were used to conduct this experiment. The pots were irrigated once or twice a week depending on their needs. At the end of growing season the corms were exacted from the pots and the new corms and cormlets were counted.

Results and discussion

The size of the corm affected significantly the number of newly-produced corms or bulblets only if they were wounded, producing on average 2.5 new corms per mother corm, up to a maximum of four (Table 10.9; Plate 10.14). The reserve bud in most corms sprouted first, followed by the injured bud. Many of the smaller corms which were wounded were attacked by diseases and rotted.

Generally four types of formation of new cormlets from *Colchicum macrophyllum* mother corm were recorded: 2 corms from the two buds of *C. macrophyllum*, one corm from the replacement bud, two corms from the replacement bud or one corm from the reserve bud (Plate 10.15).

Table 10.9 Effect of wounding and the size of *Colchicum macrophyllum* corms on the number of newly-produced corms and cormlets. N = 4 (mean of 4 repeats).

		Number of cormlets/corm	Maximum	Lowest
Intact corms	Corms >10gr.	1.7 ± 0.5b*	2	1
	Corms <10gr.	1.1 ± 0.6b	2	0
Cut corms	Corms >10gr.	2.5 ± 0.9c	4	1
	Corms <10gr.	0.3 ± 0.5a	1	0

*Values followed by the same letter are not significantly different (P=0.05).

10.8.2 Effect of BA on large and small size of *Colchicum macrophyllum* corms for producing new cormlets.

Material and methods

Corms were exacted from the pots and were sorted in two lots. The corms of one lot had circumferences from 9 to 14 cm and weighed 12 – 54 g while the corms of the second lot had circumferences from 2 to 9 cm and weighed 2 – 12 g. Half of the corms of both the lots were immersed in 200 ppm BA solution for 4 hours. Then they were planted in pots with potting soil containing peat moss, perlite and compost (1:1:1, v/v/v). Three days after planting, watering started and continued twice a week. At the end of the growing period the corms were extracted from the pots and counted.

Results and discussion

Most of the corms decayed and the production of new corms was poor in all treatments. Corms over 12 g formed more corms than the same weight corm with the BA treatment (Table 10.10). However the BA treatment improved the growth of the produced corms.

Table10.10 Effect of BA and the size of *Colchicum macrophyllum* corms on the number of produced cormlets. N = 4 (mean of 4 repeats).

	Weight (g).	Number of cormlets/corm	Maximum	Lowest
Control	12 - 54	0.8	1	0
	2 - 12	0.5	1	0
BAP, 200 ppm	12 - 54	0.4	1	0
	2 - 12	0.3	1	0

10.9 *IN VITRO* REGENERATION OF *COLCHICUM MACROPHYLLUM*

10.9.1 Introduction

C. macrophyllum has a very low rate of asexual propagation. Only one corm (seldom two) is formed yearly before the mother corm disappears. By wounding the main bud up to 2.5 new bulbs could be produced. Additionally propagation by seed produces small corms that need many years to develop regenerative corms.
In order to ameliorate this poor reproduction the following *in vitro* culture experiments were conducted.

Colchicums are difficult to regenerate by *in vitro* culture. Only one reference has been published (Ellington, 1997) which reported that the growth of the cormlet of *C. autumnale* was achieved only by using paclobutrazol. Callus, shoots and roots were obtained also on corm explants of C. *luteum* cultured on MS medium containing various growth regulators (Ghauri, 1995). There are no published references for *in vitro* culture of *C. macrophyllum*.

Preliminary studies were conducted during June 2002 and different strategies have been followed in order to investigate the possibility for *in vitro* culture of *C. macrophyllum*. In one experiment a number of different media were evaluated containing: 5.1 g/l MS basal medium, 30 g/l sucrose, 8 g/l agar and BA/NAA at 0/0, 0/2, 0.2/2, 2/0.2 and 2/2 mg/l. Glass tubes 30 x 100 mm were used for all cultures and the number of explants per treatment was 20.

In a second experiment Nitsch and Nitsch basal salt mixture at 2.1g/l, Nitsch and Nitsch vitamin solution at 1 ml/l, 30 g/l sucrose and 8 g/l agar were used supplemented with BA/NAA at 0/0, 5/0.5 and 0.5/5 mg/l each. In both experiments the pH was justified to 6. Plastic Petri dishes with 10 explants in each dish and 7 dishes per treatment were used. The main buds were used as explants, extracted or cut with part of their bases depend on the size of the buds. The explants, after they were washed, were disinfected with 95% ethanol for 30 sec. and household bleach (final concentration 0.5% in NaOH) for 10 min. Then they were rinsed with deionized water for 5, 15 or 30 min respectively.

Most of the explants were infected and those that not infected did not respond to the treatments. The experiments were continued as detailed below with different medium and growth regulators as well as in a different season of the year.

10.9.2 Material and methods

10.9.2.1 Plant material, disinfection and manipulation

A. At the beginning of September 2004, corms of *C. macrophyllum* were collected from the observation area. During this period the main buds of the corms had already initiated and shoots up to 25 mm had been formed: the larger the corms the larger the shoots developed. Buds with shoots up to 15 mm were selected for *in vitro* culture.

The collected buds were separated into three categories (Plate 10.16):

a. 24 corms with a weight from 22 to 50 g/corm (diameter 35 - 50 mm) - their main buds had already a length from 6 to 15 mm.

b. 24 corms of with a weight from 8 to 21 g/corm (diameter 21 - 33 mm) and bud length up to 5 mm, and

c. 24 corms with a weight from 0.9 to 7 g/corm (diameter 10 - 27 mm) without any main bud growth.

The main and the reserve buds from all the above corms were used as explants. The main buds from the two first categories were extracted from their base by hand

while the rest were cut with a part of the corm attached to them. The explants were washed with liquid household detergents and rinsed under tap water. Then they were disinfected with 95% ethanol for 1 min and 15% household bleach (final concentration 0.75%) for 15 min. Then they were rinsed three times with deionized sterilized water for 3, 5 and 30 minutes.

B. Two and half month-old seedlings from seeds germinated in the dark at 10°C were used as explants (Plate 10.21). The root system and seeds were discarded aseptically and a part ca. 2 cm with the apical meristem was used as the explant and was embedded in the medium. Ten replicates were used per each treatment.

10.9.2.2 Culture medium

A. For *in vitro* cultures of corms:

1. MS half strength medium (2.2 g/l) 30 g/l sucrose and 8 g/l agar supplemented with: BA at 0, 0.1, 0.5 or 1 mg/l and 2,4-D at 0 or 0.5 mg/l in all possible combinations. The pH was adjusted at 6.5. The cultures were put in darkness at 10 ± 0.5°C.

2. Seven weeks (46 days) after the first culture the explants were transferred onto the follow medium:

4.4 g/l MS, 30 g/l sucrose and 8 g/l agar supplemented with 2,4-D/BA at 0/0, 1/0.5, 2/0.5 and 4/0.5 mg/l. The pH was adjusted at 6. These cultures also were put in dark at 10 ± 0.5°C.

3. 65 days later the developed shoots were transferred to 4.4 g/l MS, 30 g/l sucrose and 8g/l agar with BA at 0.1 and 0.5 mg/l and pH 6 in 16/8 photoperiod and 24 ± 2°C.

4. Shoots and corms from the above cultures were transplanted to MSS medium (pH: 6) without growth regulators after their leaves had been dried (41 days later) and were put in dark at 24 ± 2°C.

5. 59 days later half of the cultures were transferred to MSS medium plus IBA at 1, 2 or 4 mg/l and half of them subcultured in the same medium. Both were put in the dark at 24 ± 2°C and 4 weeks later in light at 16/8 h photoperiod.

New transfers took place for explants, which had produced glassy callus (Plate 10.20) onto MSS medium (pH 6) supplemented with 2.4-D at 1, 2 and 4 mg/l and transplanted into MSS medium with growth regulators BA and NAA at 0.1 or 0.5 mg/l, BA/NAA at 1/0.5 and 2/0.5 mg/l as well as into MSS medium (pH 6) with or without coconut water in order to examine the potential for the production of cormlets or somatic embryos.

Medium was distributed in glass test tubes 28 x 200 mm or 30 x 100 mm, 12 and 15 ml, respectively. The basal parts of the buds were embedded in the medium.

B. *In vitro* cultures of seedlings:

MSS medium (pH: 6) supplemented with BA at 0, 1, 2, 4 mg/l and NAA at 0, 0.5, 1, and 2 mg/l in all possible combinations.

10.9.3 Results and discussion

A. *In vitro* cultures of *C. macrophyllum* corms

All the explants from the main buds of **a** and **b** categories that had been extracted from their bases by hand had a successful disinfection (ca 90% clean) while the secondary buds produced only a mean of 18.6% non-infected explants (Table 10.11).

Table 10.11 Percentage healthy explants from different corms

	Corms 22 - 50 g.	Corms 8 - 22 g.	Corms 0.9 - 8g	Mean
Main buds	91.7	95.8	73.9	87.1
Reserve buds	20.8	17.4	17.6	18.6
Mean	56.2	56.6	45.7	

Seven weeks from the initial culture the base of the explants from the main buds of the heaviest corms (**a** category) in the treatments with 0.5 mg/l BA were swollen slightly and the buds started to grow. Shoot growth was observed from most of the explants from the main buds of all categories (**a**, **b** and **c**) and only from some of the reserve buds from category **a** on all treatments with BA without 2,4-D as well

as the control.. None of the explants from the reserve buds of **b** and **c** category rooted or responded until the time that they were transferred. In all treatments with 2,4-D explants were deformed slightly on their bases.

After seven weeks the explants from each treatment were transferred onto the second medium. Ten weeks later the length of shoots and the diameter of the formed callus were measured (Table 10.12). A high percentage (over 80%) of the explants sprouted shoots on all treatments, while callus formed in all treatments with 2,4-D, ranging from 47.2 - 69.4% (Plate 10.17). Very few explants from the control formed callus. This may due to the fact that in the first culture the medium in some treatments contained 2,4-D. Bud explants may become habituated after exposure to 2,4-D for seven weeks and they continued to proliferate after they transferred to the medium without growth regulators (Hartmann *et al.*, 2002).

Table 10.12. Effect of growth regulators on % sprouting and growth of shoots and callus.

Growth regulators: 2,4-D/BA	% shoots	% callus	Shoot length, (cm) Mean ± sd	Callus diameter (cm) Mean ± sd
0/0	85.2	6.3	2.5 ± 1.6	0.3 ± 0.1
1/0.1	86.1	69.4	2.0 ± 0.8	1.0 ± 0.4
2/0.1	86.1	52.8	1.9 ± 1.1	0.8 ± 0.3
4/0.1	83.3	47.2	2.4 ± 1.6	1.4 ± 0.8

Produced shoots (Plate 10.18) were transferred to the third medium where they stayed for 6 weeks until the leaves of the shoots dried. Small corms on the bases of the shoots formed (Plate 10.19, A). These corms were transferred to the fourth medium (MSS without hormones) in the dark. Half of them were transferred after eight weeks into the fifth medium.

Both treatments (no growth regulators in the medium and medium containing IBA) stimulated new shoots from the top of the newly formed corms five months after the pre-corms were transplanted (Plate 10.19, C, D). Callus produced by the

impact of 2,4-D (Plate 10.20) did not grow further to form any cormlets or somatic embryos in any medium with or without applying growth regulators.

B. *In vitro* cultures of *C. macrophyllum* seedlings.

None of the seedlings responded to the treatments.

10.10 ADAPTATION IN URBAN LANDSCAPE

Corms from their habitant were replanted in the rock garden of the farm of TEI. Despite their ease of establishment, only a few flowers were produced and only after the second year. This is probably due to the soil quality of the farm of TEI which has high $CaCO_3$, low organic substances and not very good drainage compared to the soil in *C. macrophyllum* habitats (Appendix 2). Corms of *C. macrophyllum* which grew in pots and continued to be watered during summer became longer than those in the soil and did not flower.

Although the garden is at a low altitude (only 10 m) while its habitat altitude is over 200 m, this is not a factor since normal flowering occurred with Colchicum stock plants in the greenhouse (Plate 10. 22, A).

Generally, planting large corms in August in well drained soil with enough organic matter leads to good establishment and flowering in an artificial landscape (Plate 10. 22, B).

10.11 CONCLUSION

Colchicum macrophyllum, a hysteranthous geophyte, is distributed in the south Aegeon at altitudes from 200 to 750 m. In the island of Crete, it thrives on gravel soil and uncultivated slopes over 200 m altitude. Its large pleated leaves and the lilac-purple abundant flowers make it a potential ornamental plant. Anthesis starts from the 10 - 20 September (in the observation area for the last four years) and lasts for one to one half months. Larger corms produce more flowers and the anthesis and the life span of the flowers is longer. Each regenerative corm can produce up to 6 capsules and each capsule contains up to 80 seeds at maximum.

Despite the relatively large number of produced seeds very few seedlings can be observed in spring in the habitat. In addition the seeds are dormant and germinate after one or more years in nature if there are appropriate conditions. In the greenhouse seeds germination rates were relatively low (43.6%) which could be improved upon by pre-treatments at room temperature in wet vermiculite followed by incubation at 15°C in the dark. In contrast pre-chilling the seeds at 5°C strongly inhibited seed germination.

These results indicate that *Colchicum macrophyllum* may have a dormancy known as endogenous morphological with an embryo of rudimentary, linear or undifferentiated type that needs to be developed in high temperatures or other treatments before it germinates (Hartmann *et al*,. 2002).

The vegetative propagation of Colchicum is achieved by corms but it has a very low multiplication through asexual propagation as each mother corm produces only one to two new corms yearly from the only two buds presents (replacement and reserve). This could be increased by wounding the replacement (main) but growth regulator (BA) had no stimulatory impact.

Propagation *in vitro* of Colchicums proved very difficult and this study showed that:

- most appropriate explants were the replacement buds from the corms over 8 g which were disinfected easily and have quick growth;
- optimum time for initiation of *in vitro* cultures is the beginning of the lengthening of the buds at the end August to the beginning of September;
- replacements buds that were used as explants were grown on MS medium in all the treatments with BA at 0, 0.1, 0.5 or 1 mg/l and 0 or 0.5 mg/l 2,4-D producing plantlets, while all treatments with 2,4-D produced callus;
- shoot formation could be initiated from the first-produced corms after three subcultures on MS with lower concentrations of BA or without hormones - in turn, theses shoots produced new corms;
- callus produced by the affect of 2,4-D did not grow further to form any cormlets or somatic embryos in any medium.

Adaptation in pots and outdoor was achieved using seedlings or corms produced from the experiments. No special problems were observed during the four years cultivation in greenhouse and in the garden as long as good soil drainage was achieved.

11 INDIGENOUS PLANTS AND THEIR USE IN URBAN LANDSCAPES: DESIGN AND CREATION OF A DEMONSTRATION GARDEN PLOT

11.1 INTRODUCTION

The term indigenous is defined as *originating naturally in a region* and is often applied to individual species that are native (i.e. have not been introduced by human activity) to a particular geographical area.

Nowadays urban parks are being maintained through high energy input. The amount of chemicals and energy use, for example in water engineering, in urban space management is in total contrast to the principles of frugal sustainable environments. Urban green spaces should become models of recycling, low energy and chemical use, good water management and nature conservation. Native Mediterranean plants save water and require limited chemical use as they are relatively more resistant to dry climates as well as to local pests and diseases. Their maintenance regime fits in with the sustainable principles.

The importance of the use of indigenous plants in urban landscape has been highlighted nowadays by many researchers. Indigenous plants in urban and suburban gardens contribute to biodiversity and sustainability while preventing the extinction of rare or endangered plant species. In addition such urban gardens attract those tourists that have botanic and ecological interests as well as pupils and students in education and nature conservation.

The uncontrolled growth of the tourism industry in the form of large hotel units in many Greek tourist destinations has caused significant alterations to the natural and cultural landscape of many coastal areas (Gkoltsiou *et al.*, 2005). Most of the open spaces in these large tourism units are occupied by turf lawn. Lawns contribute to environmental problems, including high energy use, high chemical input use (such as pesticides, herbicides, and fertilizers), health problems related to those chemicals, solid waste, high water usage, water pollution, and decreased biodiversity (Bormann *et al.*, 1993; Templeton, *et al.*, 1999). Alternative designs

using native plants may require less external inputs and support more biodiversity (Diekelmann and Schuster, 2002), both flora and fauna.

Innovative landscapes that incorporate ecologically beneficial land cover patterns have been designed in multiple scales for private lands (Nassauer, 1993). However ecologically healthy landscapes are not always considered attractive. If alternative landscape designs do not satisfy people's views of attractive landscapes, they will not install or support them. However other research has shown that yards using these plants can be designed in ways that people find attractive and gardens composed with native plants may have ecological benefits. The property occupied by private gardens is relatively small but a mosaic of environmentally beneficial yards can in the aggregate contribute to ecological health (Nassauer, 1997).

Interest in landscaping with native plants has grown rapidly in recent decades, but there is also a rise in 'native only' policies where attempts are made to exclude introduced and exotic species from landscape schemes. This is due to the weakness of the local authorities to supply adequate management and the inability of open spaces to satisfy the needs and requirements of the modern user. In contrast, some researchers have developed innovative systems of landscape planting using both native and non-native species in urban parks. This system involves ecologically-based plant communities of both native and exotic species that can be both rich ecologically and aesthetically and managed via crude non selective techniques such as cutting and burning (Hitchmouth, 2004).

Lately in Greece the planting in urban environments in both public and private spaces mostly consisting of exotic plants, whose availability in nurseries and shops is stable and continuous. Additionally they adapt to the local climate and their aesthetic value makes them attractive to the responsible municipality and the consumers. Plants that had been naturalised many years ago are nowadays unquestioned decorative elements of green spaces, completely adjusted to the site conditions and without causing the usual problems of invasion and change of the area's character. However the fully or largely replacement or substitution of native plants could lead to the eventual destruction of the Mediterranean character

particularly of South Greece where these plants appear to adjust easily and could increase the demands on water and other inputs.

In spite of the fact that in Greece and especially in Crete there is a large number of native species, very few of these are used in ornamental horticulture and urban landscaping. I personally believe that this may due to the absence of research for propagation and cultivation techniques. Consequently their production and promotion by nurseries is limited despite the fact that the indigenous and spontaneous species are easier to adapt to the local ecosystems than the 'aliens'.

11.2 LANDSCAPING WITH INDIGENOUS PLANTS

The aim of designing and constructing the demonstration plot with native plants of Crete is to enhance the education, knowledge and techniques of the students in School of Agriculture Technology in Horticultural and Landscape Architecture. The design and selection of indigenous species is the result of the participation activities of the students of ornamental and landscape horticulture.

11.2.1 Material and methods

Site description

The educational park for students in Horticultural and Landscape Architecture of the School of Agriculture Technology is situated in the North part of the TEI farm in Heraklion Crete and occupies 6000m^2. It consists of thematic gardens with hard and soft surfaces, various constructions (amphitheatre, pool, and roads) and experimental plots (Antonidaki and Giatromanolaki, 2006).

The site analysis started with a topographical diagram and the plotting of the existing plants in the allotment. The allotment of the indigenous horticultural garden plants is approximately 500m^2 and is situated in the south-western part of the park. In the centre of this plot a small amphitheatre is going to be installed (Figure 11.1). To the north of the site there are the steps of the newly constructed park and to the south the road from the entrance of the farm. There is a 10% inclination from west to the east. On the rest of the plot were scattered trees and

shrubs as well as some herbaceous aromatic species in a random arrangement. The lack of roads and paths inside discourages movement and access throughout the plot for the students or other users.

The soil of the plot is characterized as loam with slight alkaline pH and high percentage of free $CaCO_3$ (Appendix 2). Water could be made available from the municipality of Heraklion.

Development plan

From the site analysis that was made it was apparent there is need that the allotment should be created in smaller parts with lines, paths and indigenous vegetation according to the basic design principles in Landscape Architecture. The creation of new desired lines would provide comfortable and easy movement through the plot for the student and the visitors and adequate access from all sides to the amphitheatre. Existing indigenous species that have high aesthetic value and lie in a suitable place in the allotment are going to be maintained.

Native species selection

Apart from the eight species examined in the thesis, selection of more native plants began during the tours of the observation areas throughout Crete. Samples of native species were brought also by students during the five last years. Selections were guided also by bibliographic survey (Tutin, *at al.* 1964-1980; Σβήκας, 1987; Blamey and Grey-Wilson, 1993; Kypriotakis and Altelari, 1998; Hessayon, 1999; Γεωργίου και Δεληπέτρου, 2001) and personal communication with other researchers (Κυπριωτάκης, Γεωργίου κ.ά.). The plants were classified and their characteristics were recorded as well as the time of anthesis and the colour of the foliage and flowers.

Criteria that were used to select the desired plant species for designing the allotment were the following:

- Aesthetic value of the plants is affiliated to people's perceptions of indigenous plants. The size, the shapes, the forms, the foliage, the flowers, the colour of foliage and flowers are the most interesting characteristics.

- The seasonal changes of the plants that cause agreeable feelings to the observer.
- The life span of the native plants and their flowers.
- Their possible use as aromatic or edible wild plants.
- The easy propagation and cultivation without special problems of pests and diseases.
- The possible use for xeriscaping. The problem of the lack of water that appears more intense in the last years, make necessary the use of plants with less need for irrigation.
- Sustainability - the ability using low energy, inputs and water as well as the ability for self-reproduction.

11.2.2 Results and discussion

Records of native species

From the personal survey, the results from bibliographic survey, and the personal communication with other researchers, indigenous plants of Crete satisfactory for using in landscape Architecture were recorded. They were sorted according to their size and life span in groups. The time of flowering and the colour of flowers were used as dominant characteristics as well as their main decorative parts (Tables 11.1 to 11.6).

The designing of the plot

The development of the design was realized according to the basic landscape designing principles in order for the plot to be unified with the surrounding sites, to increase creativity, imagination and visual satisfaction as well as to stimulate the interest of the visitors. (Ingels, 1997; Carpenter and Walker, 1990).

At the western boundary of the plot, which is the upper point of the indigenous garden, small trees or shrubs up to 2 m will be planted (Table 1 and 2; Appendix 5). In this way hypsometrical difference between the lower and the higher point will be increased and high plants will create visual control to the passage behind the road. On the two sides of the amphitheatre, groups of climbing plants will be situated to provide shade (Table 11.4; master plan, 4). Creeping plants will be

situated on the most gradient places (Table 11.4; Apendix 5) of the indigenous garden. Herbaceous perennial plants (Table 11.3) will be planted on numbered 3 and 6 parts on the master plan and annual and biannual species (Table 11.5) will be alternated with geophytes (Table 11.6) on the remaining parts (master plan 5 and 7) among the paths so that the soil will be covered with plants all through the year.

From the available tables a free choice of the acceptable species should be selected using as criteria the height, growth, the anthesis, the colour of the foliage and the flowers, the seasonal changes and other characteristics. A useful point is also the possible availability of the plants from nurseries because there is a limited amount of indigenous vegetation available.

Generally the aims of this design are the following:

- Dense planting, which will inhibit the growth of weeds and help sustainability.
- The free choice by the students of the species. This will help the students to know the species, their functions and growth as well as an understanding of the opportunity to replace some species by others. The only limitation is that all the plants must be visible from the points of the paths. The most desirable species are those that can self-propagate.
- Encouraging decorative and spontaneous plants that have randomly grown and remain in the plot.
- Planting the most species that could establish in various places in the plot so as to increase biodiversity.
- The aesthetic visual value of the total indigenous garden.

This type of planting is suited more to the semi-naturalistic approach to design than the conventional formal or informal planting. According to Millard (2004) the concepts of indigenous and spontaneous vegetation are considered as subsets of the broader category, semi-natural vegetation. Personally I believe that it is the semi-controlled approach with natural way vegetation for small scale private and educational gardens which will enrich the biodiversity and encourage sustainability.

The identification of the degree of visual preference and the educational results need further research.

Table 11.1 Native trees and shrubs of Crete over 2m in height for landscaping

Genus	Species	Family	Time of flowering	Colour of flowers	Decorative parts
Acer	*sempervirens* (D)	Aceraceae	NS	NS	L, Fr
*Arbutus**	*adrachne* (E)	Ericaceae	Autumn	White-Cream	L, F, Fr, Tr
Ceratonia	*siliqua* (E)	Leguminosae	NS	NS	L, Fr
Juniperus	*oxycedrus* (E)	Cupressaceae	NS	NS	L, Fr
*Laurus**	*nobilis* (E)	Lauraceae	Spring	White-green	L, F, Fr, Ar.
*Myrtus**	*communis* (E)	Myrtaceae	Spring	White	L, F, Fr, Ar
*Nerium**	*oleander* (E)	Apocynaceae	Summer	Multi-coloured	L, F
Phoenix	*theophrastii*	Palmae	Spring	Yellow	L, Fr
Pistacia	*lentiscus* (E)	Anacardiaceae	NS	NS	L, Fr
Pistacia	*terebinthus* (D)	Anacardiaceae	NS	NS	L, Fr
*Punica**	*granatum* (D)	Punicaceae	May-June	Red	L, F, Fr, Ed
Quercus	*ilex* (E)	Fagaceae	NS	NS	L, Fr
*Vitex**	*agnus-castus* (E)	Verbenaceae	May-July	Purble, rosy, white	L, F

*Proposed for the garden with indigenous plants. (D) = Deciduous, (E) = Evergreen, L = Leaves, F = Flowers, Fr = Fruits, Tr = Trunk, Ar = Aromatic leaves or flower, Ed = Edible, NS=Non showy.

Table 11.2 Native shrubs of Crete up to 2m in height for landscaping

Genus	Species	Family	Time of flowering	Colour of flowers	Decorative parts
*Arbutus**	*unedo*	Ericaceae	Autumn	White-cream	L, F, Fr, Ed
Berberis	*cretica*	Berberidaceae	May-June	Yellow	L, F, Fr
*Cistus**	*incanus ssp. creticus*	Cistaceae	March-May	Rosy-purple	L, F
*Cistus**	*salvifolius*	Cistaceae	April-May	White	L, F
*Convolvulus**	*dorycnium*	Convolvulaceae	May-June	Rosy	F
Convolvulus	*oleoides*	Convolvulaceae	April-May	White-Rosy	L, F
*Daphne**	*sericea*	Thymelaeaceae	April-May	Rosy-purple	L, F, Fr
Daphne	*oleoides*	Thymelaeaceae	May-July	White-cream	L, F, Ar
*Ebenus**	*cretica*	Leguminosae	April-May	Purple-white	L, F
*Erica**	*arborea*	Ericaceae	May-June	White-pink	L, F
*Erica**	*manipuliflora*	Ericaceae	September-November	Rosy-purple	L, F
Euphorbia	*acanthothanmos*	Euphorbiaceae	March-May	Yellow	L, F, Fr
Euphorbia	*dendroides*	Euphorbiaceae	March-May	Yellow	L, F, Fr
Helianthemum	*nummularium*	Cistaceae	April-June	Yellow	L, F
Hypericum	*ssp.*	Guttiferae	May-July, September	Yellow	L, F
Lavantula	*stoechas*	Labiatae	Feb.-April	Rosy-Purple	L, F, Ar
*Otanthus**	*maritimus*	Compositae	June-September	Yellow	L, F
Phlomis	*fruticosa*	Labiatae	March-May	Yellow	L, F
*Prasium**	*majus*	Labiatae	Feb-April	White	L, F, Ar, Ed
*Ptilostemon**	*chamaepeyce*	Compositae	May-July	Pink-purple	L, F
Rosa	*ssp*	Rosaceae	April-June	Rosy-white	L, F
*Rosmarinus**	*officinalis*	Labiatae	May-June, September	Blue	L, F
*Ruta**	*chalepensis*	Rutaceae	April-May	Yellow	L, F
*Staehelina**	*petiolata*	Compositae	May-June	Pink-White	L, F
*Styrax**	*officinalis*	Styracaceae	April-May	White	L, F
Thymelaea	*hirsuta*	Thymelaeaceae	Spring	Yellow	L, F

*Proposed for the garden with indigenous plants. L = Leaves, F = Flowers, Fr = Fruits, Ar = Aromatic leaves or flowers, Ed = Edible.

Table 11.3 Herbaceous perennial species native to Crete with decorative flowers

Genus	Species	Family	Time of flowering	Colour of flowers
Achilea	*cretica*	Compositae	April-May	White
Alyssum	*ssp*	Cruciferae	Spring	Yellow
Asperula	*pubescens*	Rubiaceae	Spring	Rosy
Aster	*creticus*	Compositae	April-May	Purble-yellow
*Bellis**	*perennis*	Compositae	February-June	White-yellow
Capparis	*spinosa*	Capparidaceae	May-September	White-rosy
*Centranthus**	*ruber*	Valerianacae	April-May	Rosy-red
*Centranthus**	*sieberi*	Valerianacae	May-June	Rosy-purple
*Dianthus**	*xylorrhisus*	Caryophylaceae	Spring-summer	White
*Dianthus**	*juniperinus*	Caryophylaceae	Spring-summer	White, rosy, red
Helichrysum	*orientale*	Compositae	April-May	Yellow
Helichrysum	*doefleri*	Compositae	May-June	Yellow
*Hyoscyamus**	*albus,aureus*	Solanaceae	Spring-Autumn	Yellow
Limonium	*ssp.*	Plumbaginaceae	June-September	Purple-yellow
*Mentha**	*ssp.*	Labiatae	Summer-Autumn	Pink-purple
*Origanum**	*dictamnus*	Labiatae	May-June	Pink-purple
Saturejia	*thymbra*	Labiatae	April-June	White-pink
*Sedum**	*ssp*	Crassulaceae	Spring-Summer	White, yellow, pink
Senecio	*gnaphalodes*	Compositae	May-June	Yellow
Serratula	*cichoraceae*	Compositae	April-June	Rosy-purple
Thymus	*capitatus*	Labiatae	May-June	Pink-purple
Verbascum	*spp.*	Scrophulariaceae	Spring-Summer	Yellow

*Proposed for the garden with indigenous plants

Table 11.4 Native perennial climbing /creeping plants of Crete for landscaping

Genus	Species	Family	Time of flowering	Colour of flowers	Decorative parts
*Clematis**	*cirrhosa* (E)	Ranunculaceae	November-March	White-yellow	L, F, Fr, Ar
*Capparis***	*spinosa*(E)	Capparidaceae	May-September	White-rosy	L, F
*Convolvulus***	*argyrothamnus* (E)	Convolvulaceae	May-June	White-rosy	L, F
*Ephedra***	*fragilis* (E)	Ephedraceae	NS	NS	Fr
*Hedera**	*helix* (E)	Araliaceae	NS	NS	L, Fr
*Hyoscyamus***	*albus,aureus* (H)	Solanaceae	Spring-Autumn	Yellow	L, F
*Lonicera**	*etrusca* (E)	Caprifoliaceae	May-June	White-pink-yellow	L, F, Ar
*Otanthus***	*maritimus*(E)	Asteraceae	June-Sept	Yellow	L, F
*Origanum***	*Dictamnus*(E)	Labiatae	June-Sept	Pink	L, F, Ar
*Vinca***	*minor*(E)	Apocynaceae	Febr-May	Blue, pink, purblish	L, F

*Proposed climbing for the garden with indigenous plants.

** Proposed creeping for the garden with indigenous plants

(D) = Deciduous, (E) = Evergreen, H = Herbaceous, L = Leaves, F = Flowers, Fr = Fruits, Ar = Aromatic leaves or flowers.

Table 11.5 Annual and biannual species for landscaping native to Crete

Genus	Species	Family	Time of flowering	Colour of flowers
Alyssum	*ssp.*	Cruciferae	Spring	White-violet
Anthemis	*ssp.*	Compositae	March-May	White-
Antirrhinum	*majus*	Scrophulariaceae	All year round	Many colours
Bellardia	*trixago*	Scrophulariaceae	April-May	Many colours
Campanula	*cretica*	Campanulaceae	April-May	Blue
Campanula	*laciniata*	Campanulaceae	April-June	Blue
Campanula	*pelviformis**	Campanulaceae	April-May	Blue
Campanula	*tubulosa*	Campanulaceae	April-May	Blue
Centaurea	*redempta*	Compositae	May-July	Purple (dark)
Centaurium	*redempta*	Gentianaceae	May-July	Purplish
Centaurium	*erythrea*	Gentianaceae	April-May	Rosy
Glaucium	*flavum**	Papaveraceae	May-September	Yellow
Linum	*arboreum*	Linaceae	April-May	Yellow
Lupinus	*ssp.*	Leguminosae	March-April	Blue-white
Malcolmia	*ssp.*	Cruciferae	Febr.-April	Violet
Matthiola	*incana*	Cruciferae	March-May	Pink, purple, white
Onobrychis	*sphaciotica*	Leguminosae	May-June	Purple
Papaver	*ssp.*	Papaveraceae	Spring-Summer	Rose,red
Petromarula	*pinnata*	Campanulaceae	April-May	Light blue
Primula	*vulgaris*	Primulaceae	March-April	White
Viola	*odorata*	Violaceae	March-April	Purple

*Found to survive in habitat for more than two years.

Table 11.6 Geophytes native to Crete for landscaping use

Genus	Species	Family	Time of flowering	Colour of flowers
Allium	*Ssp.*	Liliaceae	March-May	White-rosy
Androcymbium	*Rechingery*	Liliaceae	Winter	Rosy-white
Anemone	*hortensis ssp.heidreichii*	Ranunculaceae	Spring	Purble-rose, white, red, yellow
Arum	*Creticum*	Araceae	Spring	Yellow
Asphodeline	*Lutea*	Liliaceae	April-May	Yellow
Asphodelus	*Aestivus*	Liliaceae	April-May	White
Bellevalia	*brevipedicellata*	Liliaceae	March-May	White-green
Bellevalia	*Sitiaca*	Liliaceae	March-May	White
Biarum	*Davisii*	Araceae	Autumn	Brown-purble
Colchicum	*macrophyllum*	Liliaceae	Septemp-October	Rozy-purplish
Crocus	*Sieberi*	Iridaceae	Spring	White-yellow-purblish
Crocus	*oreocreticus*	Iridaceae	Autumn	Purble-purblsh
Cyclamen	*graecum*	Primulaceae	September-December	white-rosy
Cyclamen graecum	*Creticum*	Primulaceae	March-April	Rosy
Dracungulus	*Vulgaris*	Araceae	Spring	Brown-purble
Fritilaria	*Graeca*	Liliaceae	March-May	Brown-purple, Black-brown
Fritilaria	*messanensis*	Liliaceae	May-June	Brown-purple
Gagea	*ssp.*	Liliaceae	Spring	White, yellow
Gladiolus	*ssp.*	Iridaceae	Spring	Purplish
Iris	*Cretica*	Iridaceae	January-March	Purplish-white
Iris	*unguicularis*	Iridaceae	January-March	Blue-purplish
Iris	*Cretensis*	Iridaceae	January-April	Blue-purplish
Muscari	*Commisum*	Liliaceae	April-May	Brown-blue
Narcissus	*Tazeta*	Amarylidaceae	Febr.-March	White-yellow
Ornithogalum	*creticum*	Liliaceae	July-September	White
Orchis	*ssp.*	Orchidaceae	Spring	Multicoloured
Ophrys	*ssp.*	Orchidaceae	Spring	Multicoloured
Paeonia	*clusii*	Ranunculaceae	April-May	White-rosy
Pancratium	*maritimum*	Amarylidaceae	August-September	White-green
Ranunculus	*asiaticus*	Ranunculaceae	March-Apr	white, red, yellow
Sternbergia	*lutea*	Amarylidaceae	September-November	Yellow
Sternbergia	*sicula*	Amarylidaceae	September-November	Yellow
Tulipa	*Ssp.*	Liliaceae	Winter	Multicolour
Tulipa	*cretica*	Liliaceae	Winter	White-pink
Urginea	*maritima*	Liliaceae	Sept.-October	White
Valeriana	*asarifolia*	Valerianaceae	April-May	Pink

12 GENERAL CONCLUSION

12.1 CONCLUSION

The use of native plants of a geographic region in the local floriculture and landscape architecture lends itself to a combination of advantages including:

- enhancement of biodiversity in the urban landscape;
- rescue of plant species that are in danger of disappearing;
- maintenance of local character of the region;
- better adaptation in the soil and climate conditions for xeriscape regions and consequently,
- decreased intensity of cultivation and need for inputs.

Additionally the use of new ornamental plants in the commercial floriculture sector stirs the interests of researchers and attracts consumers and scientific tourists. The natural landscape of Crete offers the possibility of a wide choice of native species with high potential ornamental value because of the big number of endemic and Mediterranean plants that are indigenous in their multifarious micro-landscapes. However, to realise this potential requires a greater and detailed understanding of the morphological and reproductive biology of these species as well as their ability to adapt to more man-made environments. There have been very few studies in this area and the research presented in this thesis is aimed at beginning to fill this knowledge gap.

Eight species from the flora of Crete were selected to conduct this study: *Staehelina petiolata, Ptilostemon chamaepeuce, Otanthus maritimus, Prasium majus, Convolvulus dorycnium, Campanula pelviformis, Sternbergia sicula* and *Colchicum macrophyllum.* A number of observational studies and experiments were undertaken, to a varying degree, on each of these species covering:

- field observations *in situ*;
- observations on a range of plant material collected from the field and grown under artificial conditions such as the greenhouse;

- *in vitro* reproductive mechanisms of selected species;
- their basic cultivation requirements, fitness and adaptation in an artificial urban landscape.

The observations *in situ* of the biological cycle and phenological stages of plants as well as the soil and climate conditions, provided an understanding of the biological parameters and factors to be studied in the subsequent *ex situ* research programme.

A common aspect of the research for all of the species was investigating the propagation by seed in substrates (trade compost and mix of peat moss + perlite) in the greenhouse and controlled chambers including the investigation of temperature and light dependence of seed germination and the dormancy state. In the cases that the seeds were characterized as dormant research was carried out on dormancy releasing factors. From these studies it was possible to determine for each species their temperature dependence, the white light effect, the time of germination and the special pre-treatments for releasing from dormancy (Table 12.1).

Table 12.1 Proposed protocols for seed propagation of studied plant species.

Species	Temperature optimum (°C)	L/D preference	Time (days)	Special pre-treatments
Staehelina petiolata	15; 10; 20	L; D	12	2-3 months after-ripening
Ptilostemon chamaepeuce	10; 15	D; L	10	2-3 months after-ripening
Prasium majus	10; 15; 20	D; L	14	Scarification
Convolvulus dorycnium	15; 20; 10	D; L	8	Scarification
Campanula pelviformis	15; 10	L	24	
Sternbergia sicula	15; 10	D	40	1 month imbibition in wet vermiculite at 20°C
Colchicum macrophyllum	15; 10	D	44	2 months imbibition in wet vermiculite at 20°C

Species specific features were observed. The substrates affected the germination of seeds and the growth of seedlings of *Staehelina petiolata* and *Otanthus maritimus* (best results in compost) but not the other species. *Staehelina petiolata* and

Ptilostemon chamaepeuce seeds need one period of after-ripening and their germination in constant temperatures enhances germination, particularly from January to February, showing characteristic low temperature dependence (10-15°C) for Mediterranean plants. The white light does not appear to affect significantly, positively or negatively on the germination of seeds of *Prasium majus, Convolvulus dorycnium, Ptilostemon chamaepeuce* (at all temperatures) and *Staehelina petiolata* (at 10, 15 and 20°C).

Seed coat dormancy caused by the hard and impenetrable seed coat that prevents the absorption of water was recorded for *Prasium majus* and *Convolvulus dorycnium* which achieved high germination rates after scarification. The germination of *Sternbergia sicula* and *Colchicum macrophyllum* seeds was achieved only in a temperature range of 10 and 15°C, while germination decreased significantly in white light. We could say that according to the proposed classification by the Hartmann *et al.* (2002) that the two species possess morphophysiological dormancy.

The reduced germination of *Campanula pelviformis* seeds at 20 and 25°C may be caused by thermodormancy, which requires red light or temperatures lower than 20°C for release (Delipetrou, 1996, Hartman *et al.*, 2002). *Otanthus maritimus* seed germination is minimal at low temperatures without cold stratification which was used by the Delipetrou (1996) to release dormancy. According to the same author the germination of seeds is suspended by the white light as a mechanism of germination avoidance on the surface of dunes and the regulation of the time.

All the plant studied produce orthodox seeds in that they can be dried without damage after their maturation to a water content lower than that found in nature. Their longevity increases with the reduction of moisture content and storage temperature in a predictive way (Hartman *et al.*, 2002); therefore they can be conserved *ex situ* in a seed bank.

Asexual propagation was achieved by stem cuttings for four species and optimal conditions are summarised in Table 12. 2.

Table 12.2 Proposed rooting media, IBA concentration and timing for rooting of four species using stem cuttings.

Species	Rooting medium	IBA requirement	Timing
S. petiolata	Peat moss, Sand, Vermiculite,	4000 ppm solution	November
P. chamaepeuce	Vermiculite	2000 ppm powder	Early winter Spring
O. maritimus	Sand	2000 ppm powder None	June
P. majus	Vermiculite, Peat moss, Perlite, Peat moss + Perlite	2000 ppm powder 2000 ppm solution	March

Sternbergia sicula and *Colchicum macrophyllum* had other forms of asexual propagation. *Sternbergia sicula* produced bulbs and bulblets but only at low rates (1 - 3.5 per bulb yearly) after cultivation in the greenhouse. However, this was enhanced considerably by applying the cross-cutting method of propagation (12.8 bulblets/bulb/season) or the addition of BA (13.5 bulblets/bulb/season). *Colchicum macrophyllum* has a very low asexual reproductive activity producing one corm per year. The wounding of the replacement bud stimulates development of the reserve bud so that each corm could produce two or more cormlets.

In vitro culture (proliferation) was investigated for the species, *Staehelina petiolata, Ptilostemon chamaepeuce, Sternbergia sicula* and *Colchicum macrophyllum* as a tool of vegetative propagation, and future mass production or breeding of the species for commercial floriculture. As far as the author is aware this is the first study which describes the micropropagation work on these four species. The factors studied in this Ph.D. thesis that affected proliferation and rooting of *in vitro* cultures were the type of plant material used, the light or dark condition, the culture media and the addition of growth regulators.

Sterilization of the vegetative explants of *Staehelina petiolata* proved to be difficult but success was achieved with CaO_2Cl_2 (5-10% for 5-10 min) or NaOCl (0.5% for 10 min) during winter. Optimal proliferation was achieved with shoot tips (derived

from 3-month old seedlings grown in greenhouse) cultured on MSS medium supplemented with 1 or 2 mg/l BA and 0.5 mg/l. Shoots were produced under the influence of NAA and BA but only from hypocotyls with the apical bud. BA induced adventitious shoots but reduced stem growth and inhibited root formation. NAA at 0.5 mg/l and IBA at 1mg/l promoted the induction of roots on the shoots produced *in vitro* from hypocotyls. Alternatively, shoots could be regenerated from hard callus (produced from cotyledons and hypocotyls under light/dark or dark conditions by the affect of BA/NAA) after 4-5 months and the addition of BA.

The hairy nature of the stems and leaves of *Ptilostemon chamaepeuce* prevented the successful disinfection of explants and their use for *in vitro* culture. However, shoots derived from *in vitro* culture of seedlings, cultured in MS or WPM medium, produced 6 – 8 or 3 - 5 shoots per explant respectively when influenced by BA alone or in combination with NAA. Increasing the concentration of BA without using NAA induced more shoots. However using BA alone or in combination with NAA produced shoots which were longer without vitrification problems. No rhizogenesis was recorded without applying rooting hormones NAA or IBA, although BA inhibited this process.

Rooted micro-shoots of the two species were acclimatized in plugs with rock-wool under intermittent mist in the greenhouse.

The *in vitro* regeneration of *S. sicula* was achieved with BA and NAA using the twin scaling method resulting in adventitious bulblets or plantlet formation originating directly from explants or indirectly from previously formed callus. The best results were achieved from desiccated bulbs (following long-term storage in room temperature) in comparison with those fresh from greenhouse or from chill storage because of their low infection. More bulblets were formed in pre-chilled bulbs (up to 6.5/explant) than those that had been stored at room temperature (3.5/explant) and fresh bulbs (1/explant).

Amorphous achlorophylous tissue was formed in the callus and on the cut surface of scales and pre-chilled bulbs with BA / NAA in both the fresh and storage bulbs. Clumps of shoots were further proliferated after two subcultures in 10 months

producing a total of 21.8 shoots per explant from the desiccated bulbs, 18 shoots from pre-chilled bulbs and 10.7 from fresh bulbs. The remaining explants continued to form new bulblets after subculturing.

Replacement and reserved buds from *C. macrophyllum* corms can initiate *in vitro* cultures producing shoots from the buds in all treatments with 2,4-D, BA or NAA as well as in control. 2,4-D produced small corms on the base of shoots which later formed new shoots on media without growth regulators or with IBA which subsequently induced new small corms.. Best period for proliferation is the end of August to the first of September when the buds begin to grow.

All of the studied species were established successfully both in pots in the greenhouse and in the environment of Technological Education Institute educational park. The ornamental characteristic and potential use of the studied species is summarised in Table 12.3. Using these species and other species from the flora of Crete a plot with indigenous plants of Crete was designed for the educational park of TEI, which is now under construction.

Botanical name	Category	Maximum height (m)	Optimal spacing (m)	Time with leaves and/or flowers	Time of flowering	Colour of flowers	Comments (Advantages-disadvantages, uses)
Staehelina petiolata	Shrub	0.5-0.8	1-1.5	Evergreen	May-June	Pink, White	Slow growth, use as landscape plant.
Ptilostemon chamaepeuce	Shrub	0.8-1	1-1.5	Evergreen	May-July	Pink-purple	Fast growth, use as cut and landscape plant.
Otanthus maritimus	Shrub	0.4-0.6	1-1.5	Evergreen	June-September	Yellow	Use in sand salinity soil (near sea).
Prasium majus	Shrub	0.5-1	0.8-1	October-April	February-April	White-pale lilac	Edible leaves and shoots, in rock garden.
Convolvulus dorycnium	Shrub	0.5-1	1-1.5	February-July	April-June	Rosy	Use in dry, sand and salinity soil (near sea).
Campanula pelviformis	Biannual–perennial	0.2-0.4	0.3-0.5	December-June	April-May	Blue	Use as pot and landscape plant.
Sternbergia sicula	Geophytes (bulbs)	0.7-0.12	0.1-0.2	October-April	October-December	Yellow	Use as pot and bed plant.
Colchicum macrophullum	Geophytes (corms)	0.3-0.4	0.3-0.4	Sept-October, January-May	September-October	Rosy-purplish	Use as pot and bed plant, poisonous.

12.2 SUMMARY OF FUTURE STUDIES

This study investigated the biology, propagation and adaptability of some species of Crete in urban landscape. Although it was possible to achieve satisfactory reproduction by sexual and asexual propagation techniques there still remained a number of problems and challenges for some species that require the development of different approaches or techniques. .

The use of alternative day and night temperatures in chambers should be investigated to accelerate and increase the percentage germination of the seeds. Some species are recalcitrant in propagation by cuttings having inconsistent results (e.g. *Ptilostemon chamaepeuce*) and/or low percentage rooting. These species need more research to understand the conditions and factors controlling rooting including assessing the temperature and humidity requirements (e.g. using fog systems), the role of different auxins and concentration of auxins, and creating more uniform and stabilised environmental conditions.

The slow growth of the *Staehelina petiolata in vitro* culture seedlings and shoots need more research examining the various concentrations of sucrose or other nutrients substrates and environmental conditions. Problems of sterilization of vegetative material were a significant constraint for *in vitro* culture of *Staehelina petiolata* and *Ptilostemon chamaepeuce*. Further research using different chemicals (e.g. $HgCl_2$) and/or sterilisations techniques may improve the results and increase the source uninfected explants.

Mass propagation and appropriate cultivation technologies are essential for the exploitation of new ornamental plants which requires closer collaborative research with the nursery industry. The examined species can be exploited directly as garden plants. However if they are going to be used as pot plants or cut flowers more fundamental research by multi-disciplinary research teams should be undertaken for selection and breeding work, postproduction care and handling, transport and market assessment.

Although these findings achieved some success in assessing the establishment and adaptability of the examined species in local artificial landscapes, further research is also necessary for their adaptability in different environmental sites. The design and construction of more than one small garden, with native plants in permanent exhibitions in Universities and garden centres, would be also useful, for attracting the consumer and stimulating the interesting of the producers.

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PLATES OF *STAEHELINA PETIOLATA*

Plate 3.1 *Staehelina petiolata* in natural habitat among cliffs

Plate 3.2 *Staehelina petiolata* in natural habitat among other shrubs

Plate 3.3 Flowers of *Staehelina petiolata*

Plate 3.4 Dry seeds of *Staehelina petiolata*

Plate 3.5 *Staehelina petiolata* in nature when the inflorescences began to grow in March

Plate 3.6 The new rosettes and the inflorescences begin to grow at the same point on the top of the previous year shoots

Plate 3.7 Seedlings of *Staehelina petiolata* in compost (A) and in peat moss + perlite (B) four months after seeding

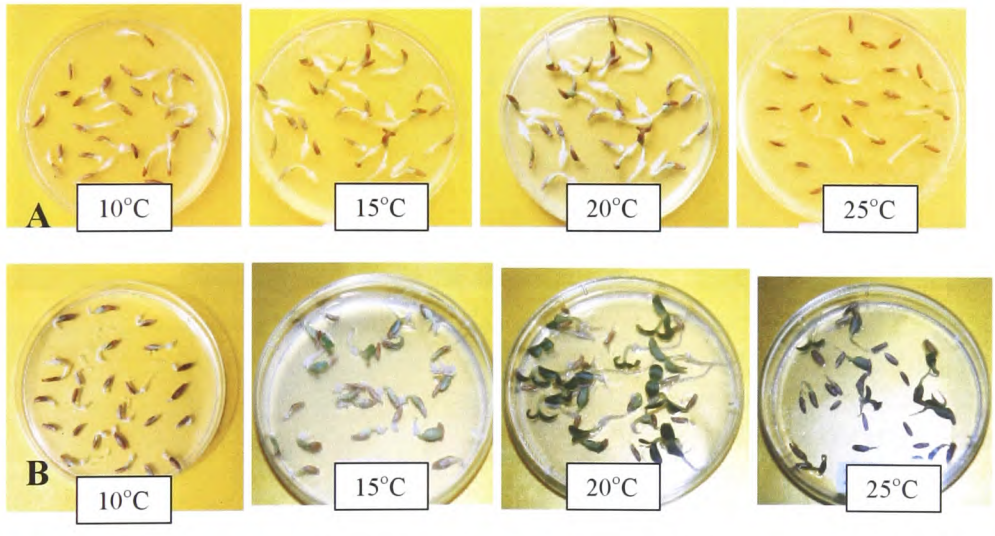


Plate 3.8 *In vitro* germination of *Staehelina petiolata* seeds in 10, 15, 20 and 25°C in darkness (A) or in light (B) after 10 days of incubation

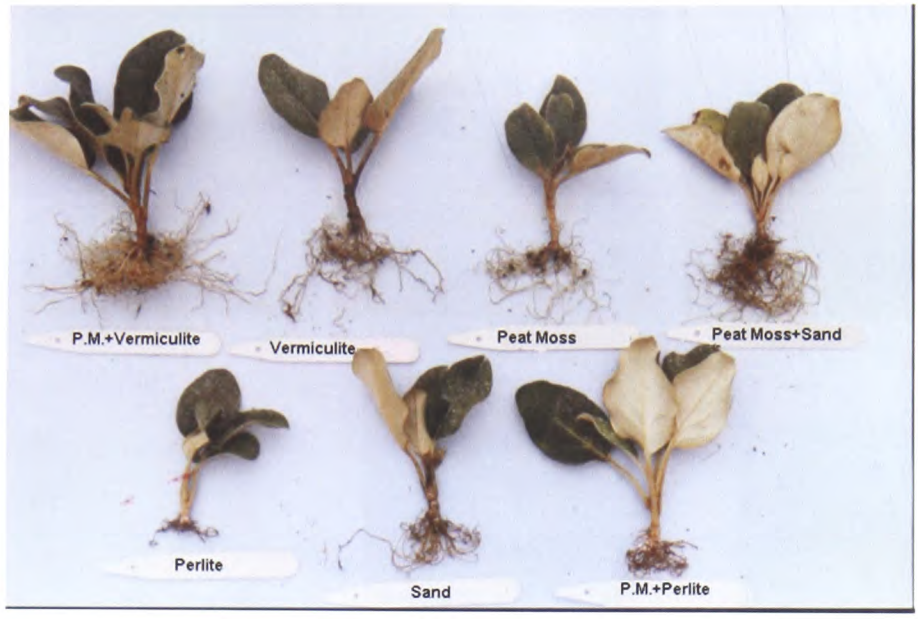


Plate 3.9 Rooting of *S. petiolata* stem tip cuttings in different substrates

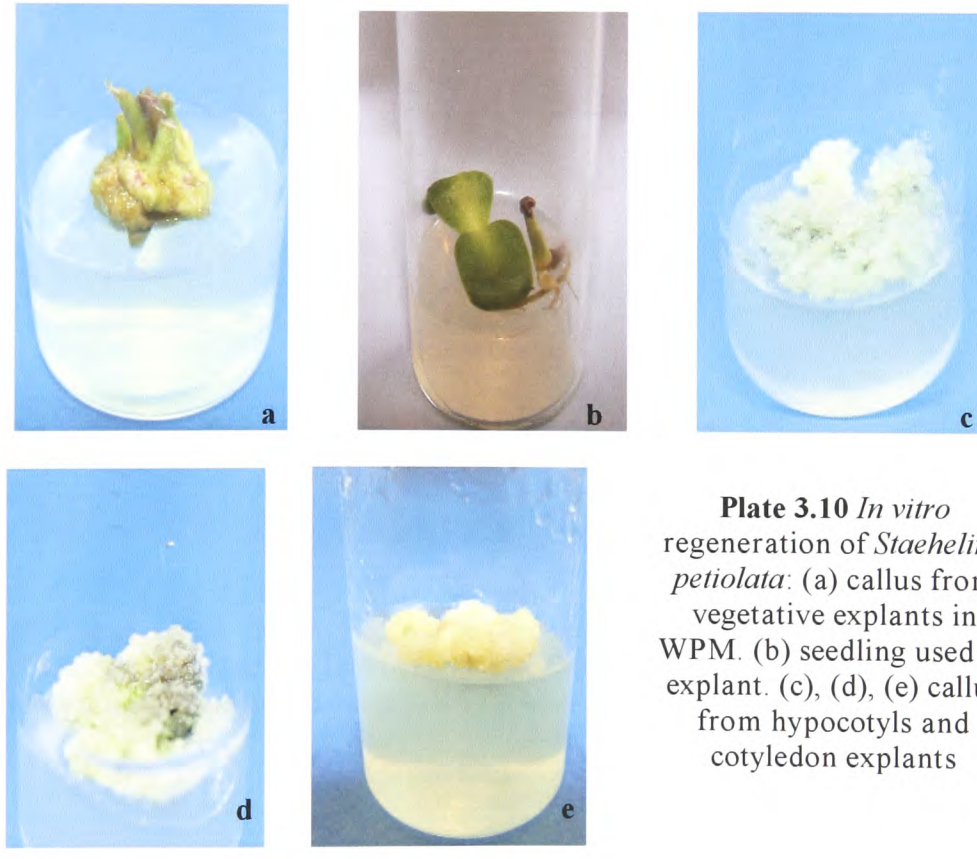


Plate 3.10 *In vitro* regeneration of *Staehelina petiolata*: (a) callus from vegetative explants in WPM. (b) seedling used as explant. (c), (d), (e) callus from hypocotyls and cotyledon explants

Plate 3.11 Callus and roots from *in vitro* culture of cotyledons (a, b) at 0.5mg/l NAA; shoots induction from callus (c); shoot induction from hypocotyls (d, e); rooted microcuttings at 1 mg/l IBA (f) and 0.5 mg/l NAA (g, h) in 5 weeks

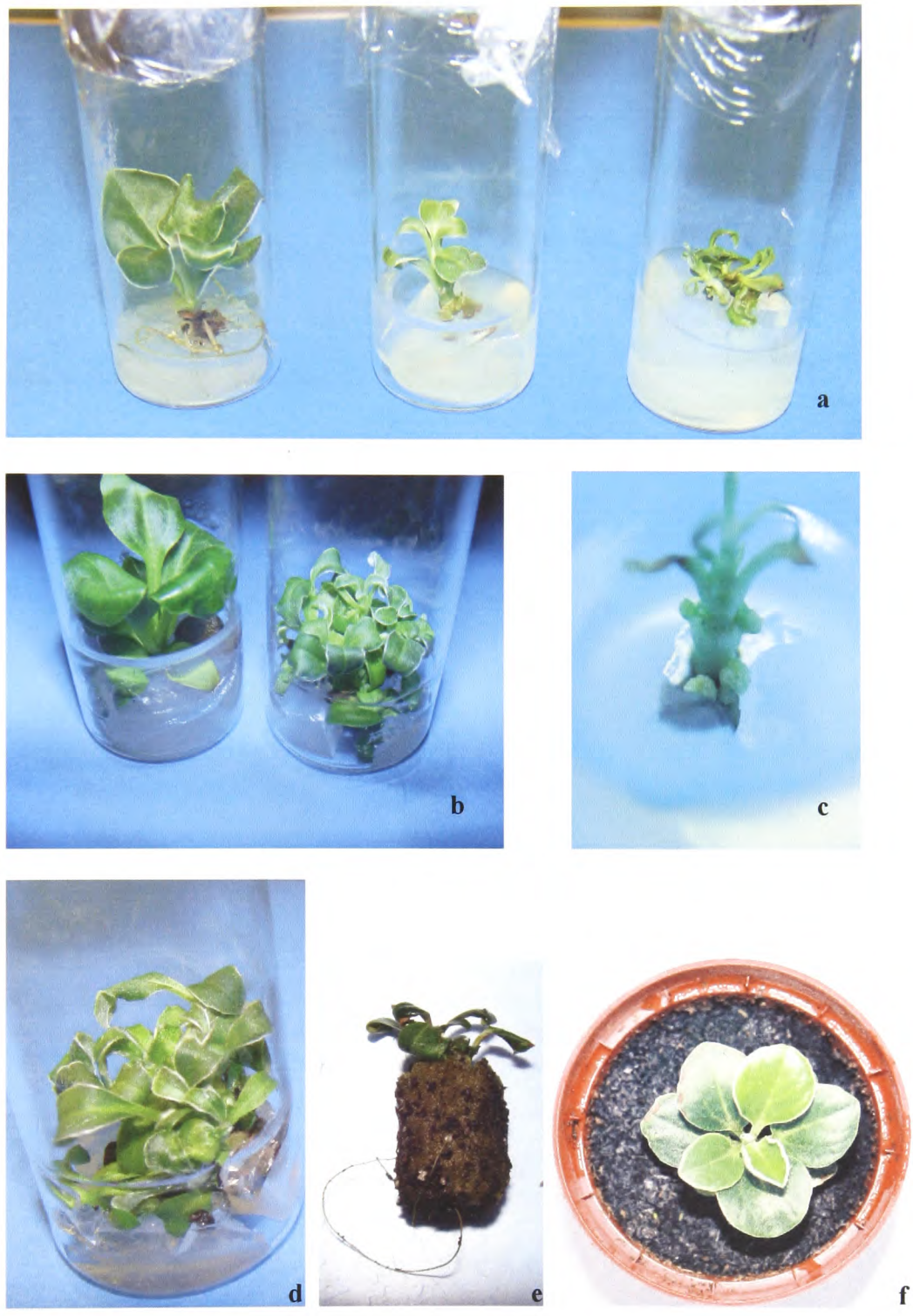


Plate 3.12 *In vitro* proliferation of shoot tips of *Staechelina petiolata* at 0, 1 and 2 mg/l BA (a) and at 0 and 4 mg/l BA (b); nodules on the base of the shoots and leaves by the affect of BA (c); leaves with light green stripes in leaf margin (d); microshoot acclimatized plantlet in rock wool and low intensity lighting (e); acclimatized plantlet in greenhouse (f)

Plate 3.13 KL2 shaker (Edmund Buhler7400 Tubingen) set at 130 rpm to ensure the optimal dispersion of the medium for embryogenesis

Plate 3.14 Five months seedling in pots with compost (A); effect of the soil medium and the fertilization on the growth rate of *S. petiolata* (B), in compost (a), in peat moss : perlite: 2:1 (b), in peat moss : perlite 1:1 (c)

Plate 3.15 Experimental design of *S. petiolata* in three different substrates and two fertilizations

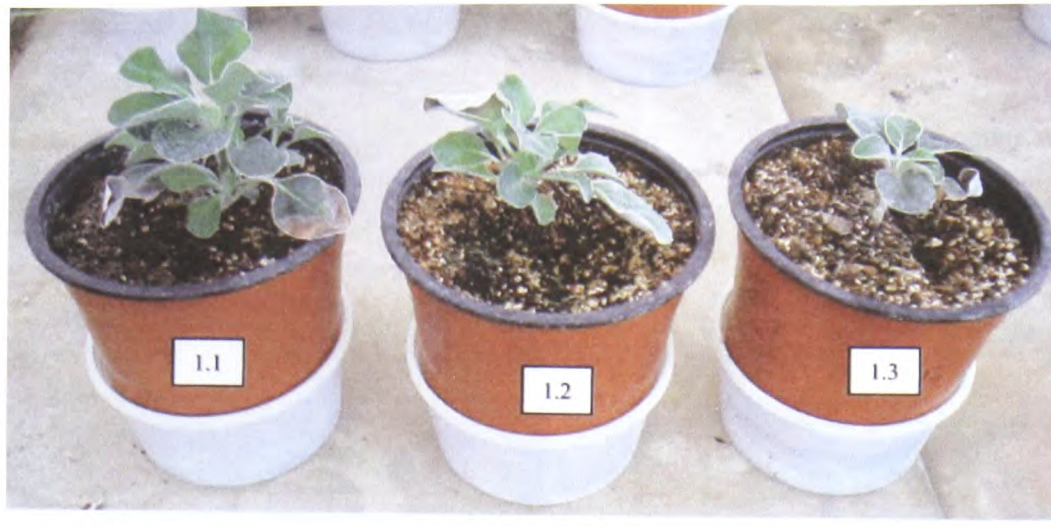


Plate 3.16 Growth of *S. petiolata* seedlings in compost (1.1), in peat moss : perlite 2:1 (1.2) and peat moss : perlite 1:1 (1.3)

Plate 3.17 Growth of *S. petiolata* in the garden six months after planting

PLATES OF *PTILOSTEMON CHAMAEPEUCE*

Plate 4.1 *Ptilostemon chamaepeuce* in natural habitat with flower's details in Iouktas Archanes Heraklion in the end of June.

Plate 4.2 Fruits, dry seeds and seed with pappus of *Ptilostemon chamaepeuce*.

Plate 4.3 Seedlings of *Ptilostemon chamaepeuce* in trade compost (A) and peat moss: perlite (v/v 1/1) (B) 60 days after seedings (in October).

Plate 4.4 Seedlings of *Ptilostemon chamaepeuce* in trade compost 30 days after seeding (in December).

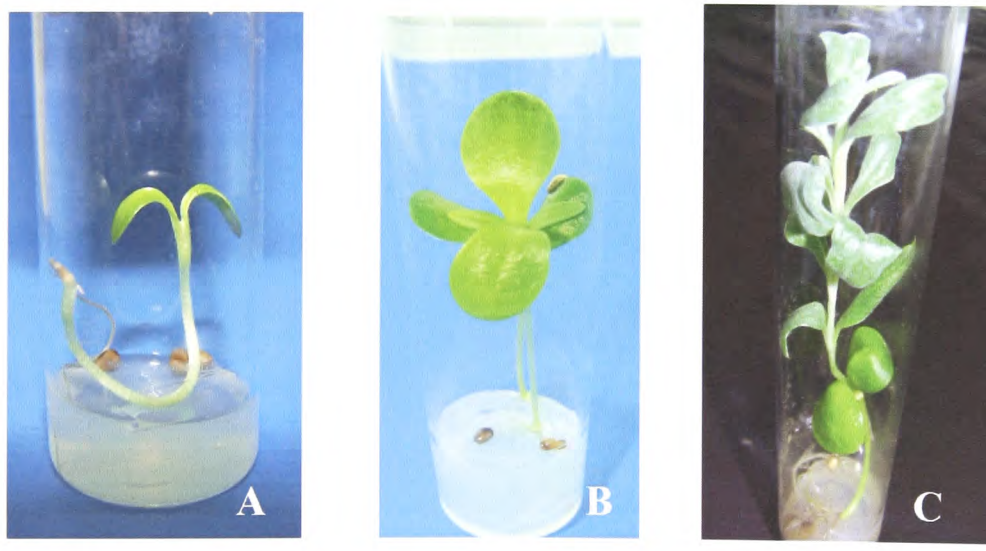


Plate 4.5 *In vitro* germination of *Ptilostemon chamaepeuce* seeds on half strength MS salt medium disinfected with 10% CaO_2Cl_2 for 5 min at 15°C. A: in one week, B: in two weeks, C: in four months.

Plate 4.6 Rooting of *Ptilostemon chamaepeuce* stem tip cuttings on perlite (A) and various substrates (B) under intermittent mist.

Plate 4.7 Rooting of *Ptilostemon chamaepeuce* stem tip cuttings on perlite : at 0.06% IBA powder (A), at 0.06% powder + rinsing under tap water (B) and at 0.06% IBA powder + warm water 40°C for 1h (C).

Plate 4.8 Adventitious shoots formation from explants derived from seedling shoots tips on MS medium and 1/0.5 mg/l BA/NAA after two and five week's

Plate 4.9 Adventitious shoots formation from explants derived from seedling shoots tips on MS medium at 0/0, 2/0.5, 4/1 and 4/0 mg/l BA/NAA after five week's incubation (from left to right).

Plate 4.10 Nodules' formation (arrow) on the base and the nodes of the seedlings explants in high concentrations of BA (4 mg/l), which produced shoots after 2 or 3 subcultures.

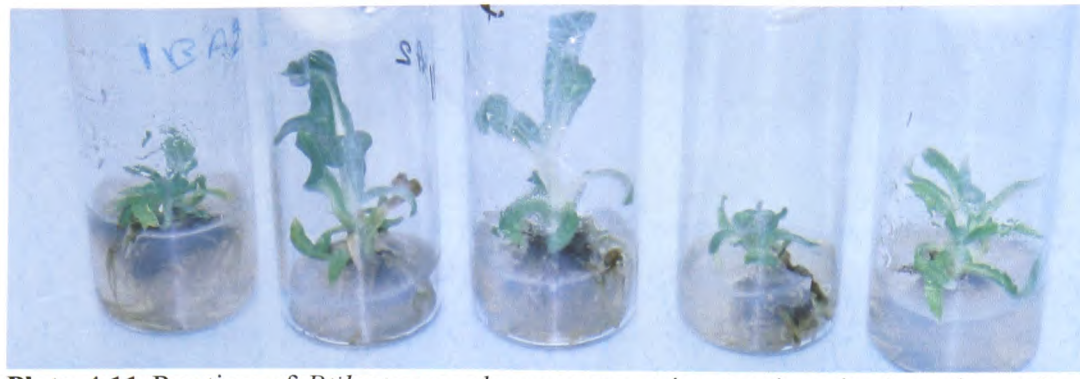


Plate 4.11 Rooting of *Ptilostemon chamaepeuce* microcuttings in 4 weeks at 2 and 1mg/l IBA, and 2, 1 and 0.5 mg/l NAA (from left to right).

Plate 4.12 Establishment of *Ptilostemon chamaepeuce* rooted microcuttings in plugs with rock wool after 1 (a), 4 (b) and 8 (c, d) weeks.

Plate 4.12a Establishment of *P. chamaepeuce* plant produced *in vitro*.

Plate 4.13 *In vitro* shoots formation of seedlings tips explants on WPM medium. Drying of the tip of leaves and shoots was caused three weeks after the initiation.

Plate 4.14 Effect of different preservative solutions on the *P. chamaepeuce* flowers vase life.

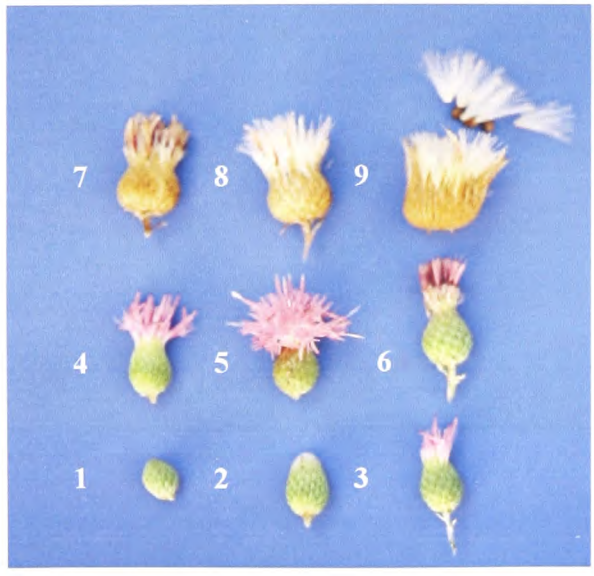


Plate 4.15 Stages of development of *Ptilostemon chamaepeuce* head flowers

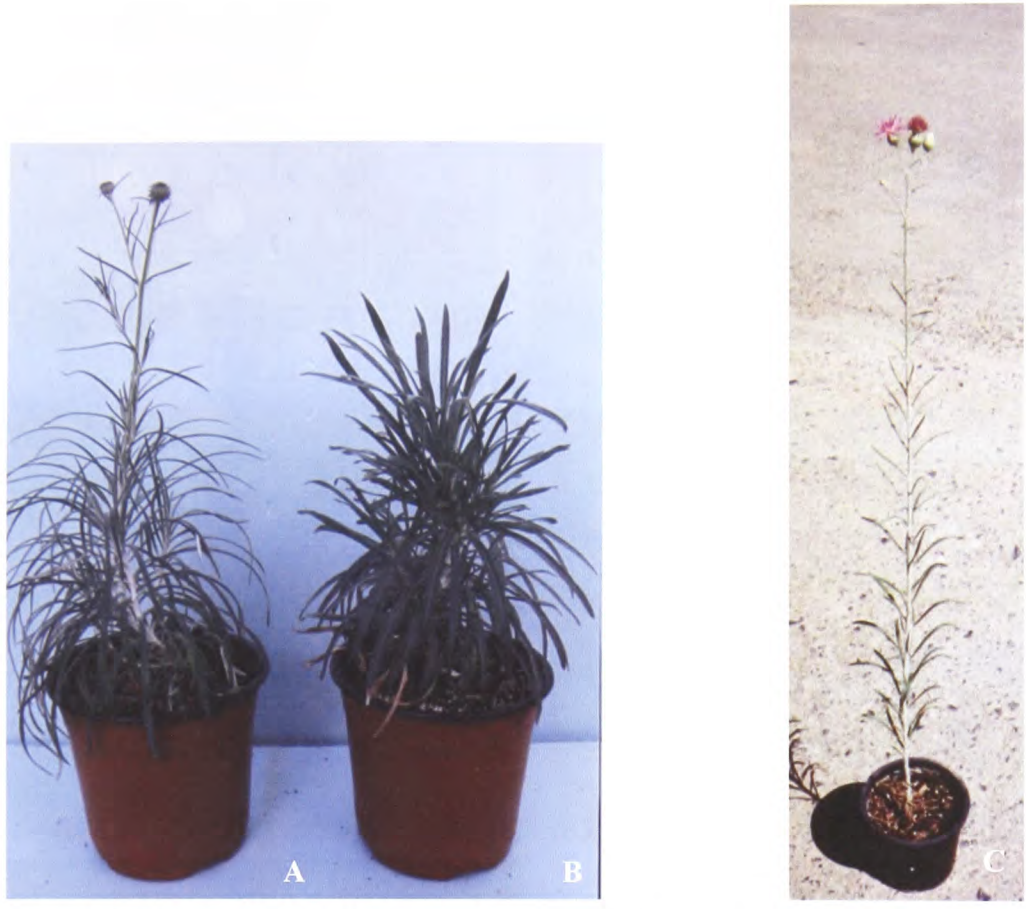


Plate 4.16 Growth of *Ptilostemon chamaepeuce* in pots: A: five months' aged plant without pruning. B: five months' aged plant after two pinching. C: Flowering of rooted stem cutting after four months from the rooting.

Plate 4.17 Adaptation and growth of *Ptilostemon chamaepeuce* in the experimental plot in garden four months after the installation

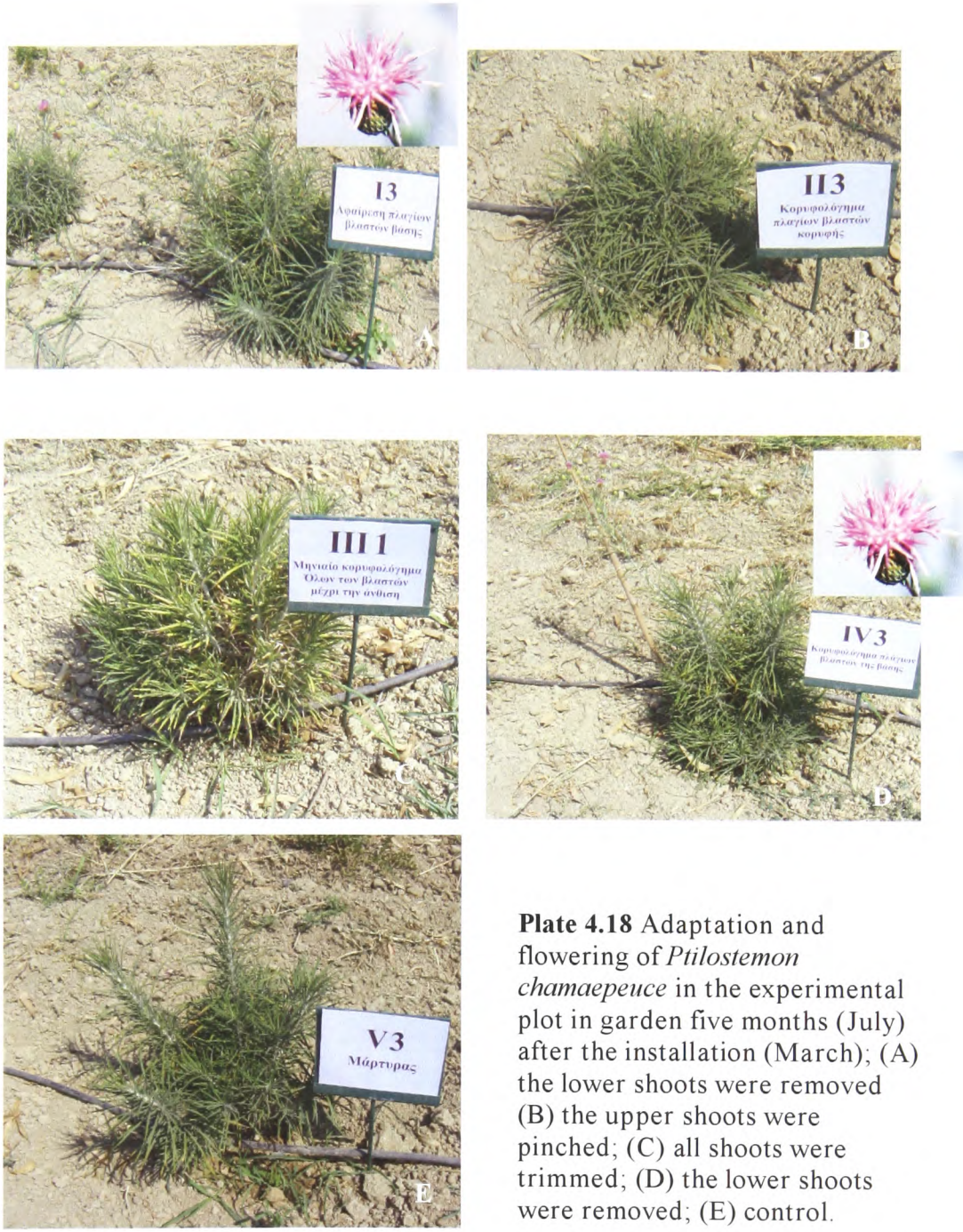


Plate 4.18 Adaptation and flowering of *Ptilostemon chamaepeuce* in the experimental plot in garden five months (July) after the installation (March); (A) the lower shoots were removed (B) the upper shoots were pinched; (C) all shoots were trimmed; (D) the lower shoots were removed; (E) control.

PLATES OF *OTANTHUS MARITIMUS*

Plate 5.1 *Otanthus maritimus* in natural habitat.

Plate 5.2 *Otanthus maritimus* in rock garden among other ornamental plants.

Plate 5.3 Mature seeds of *Otanthus maritimus*

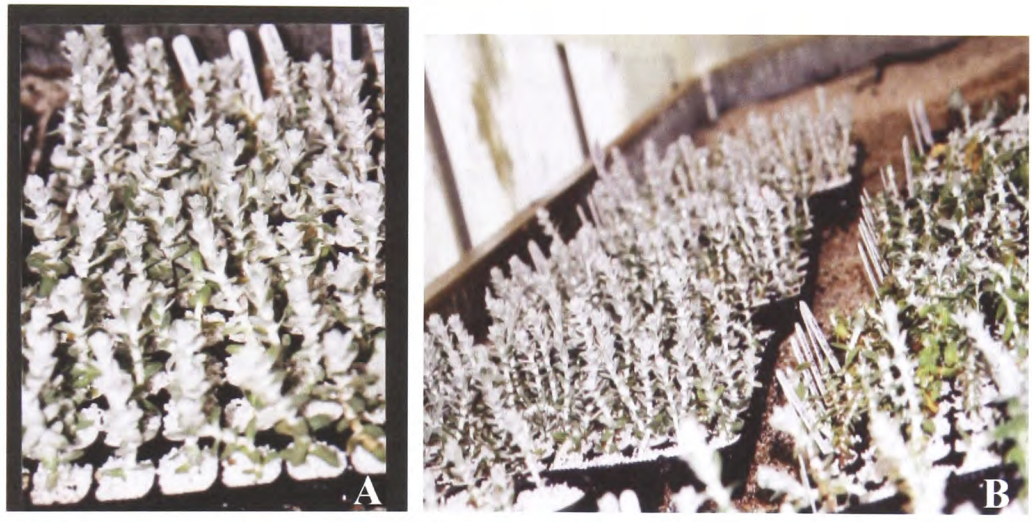


Plate 5.4 *Otanthus maritimus* cuttings in perlite (A) and different substrates (B) under intermittent mist.

Plate 5.5 Rooting of *Otanthus maritimus* cuttings on sand after treatment in IBA solution at 0, 500, 1000, 2000 or 4000 ppm and 0.06% IBA powder (from left to right) under intermittent mist for 36 days.

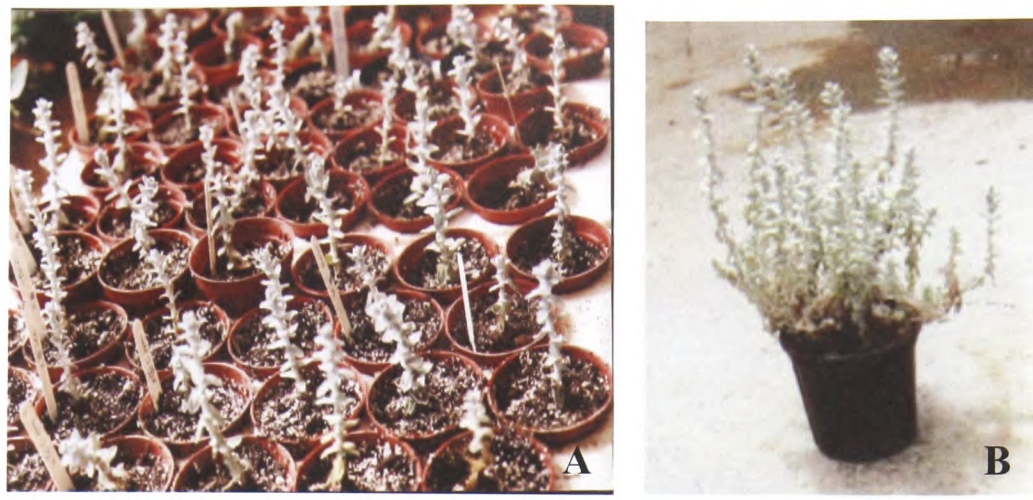


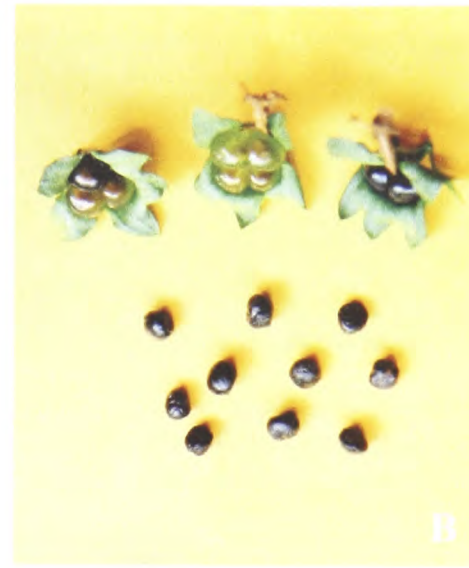
Plate 5.6 Rooted cuttings of *Otanthus maritimus* (A) transferred to pots in mix peat moss + perlite (2:1, v/v), (B) in pots eight months from the rooting.

Plate 5.7 Three months' seedlings of *Otanthus maritimus* transferred to the ground.

Plate 5.8 Plants of *Otanthus maritimus* adapted in garden (A), with flowers' details (B).

PLATES OF *PRASIUM MAJUS*

Plate 6.1 *Prasium majus* with flowers in nature habitat.



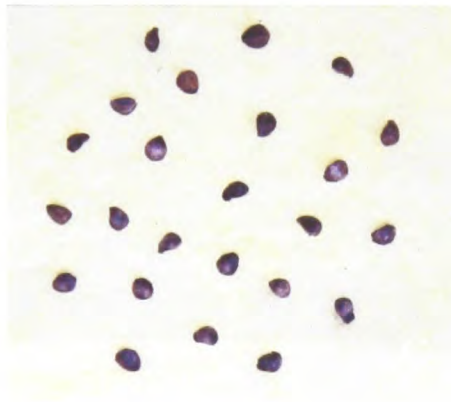


Plate 6.2 *Prasium majus* with 1 - 4 fruits in each lobe (A, B). Seeds after their pulp were removed (C).

Plate 6.3 Seedlings of *Prasium majus* three months after seeding in compost.

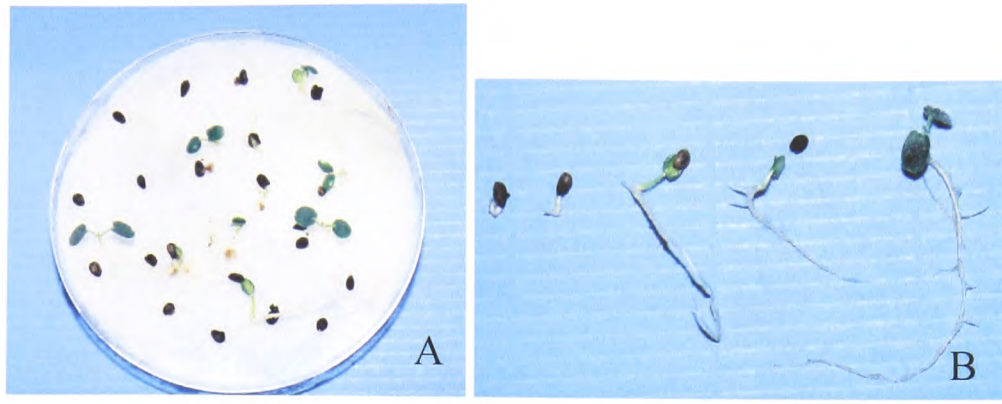


Plate 6.4 Germination of *Prasium majus* seeds in plotter paper at 15°C after 48 days of incubation under light 16/8 h (A). Stages of seedlings development (B).

Plate 6.5 *Prasium majus* stem cuttings in perlite under intermittent mist.

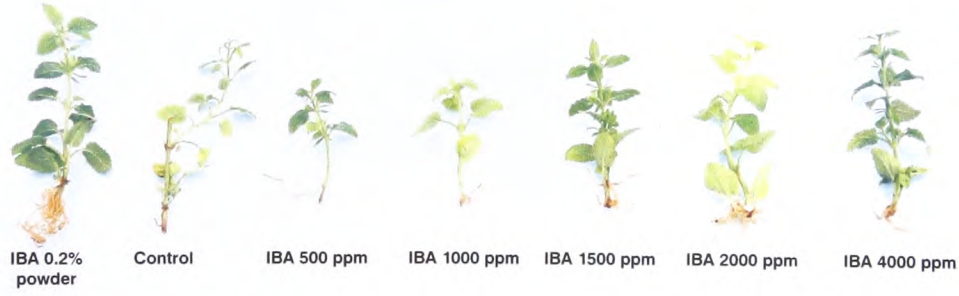


Plate 6.6 Rooting of *Prasium majus* stem cuttings in perlite treated with 0.2% IBA powder or 0, 500, 1000, 1500, 2000 and 4000 ppm IBA in solution after 32 days under intermittent mist.

Plate 6.7 Rooting of *Prasium majus* stem cuttings treated with 0.2% IBA powder in six different substrates after 32 days under intermittent mist.

Plate 6.8 *Prasium majus* plant in pot during summer.

Plate 6.9 *Prasium majus* plant in experimental plot during summer (A) and in autumn (B).

Plate 6.10 Instalment of *Prasium majus* plants in the garden of TEI in Crete

PLATES OF *CONVOLVULUS DORYCNIUM*

Plate 7.1 *Convolvulus dorycnium* in natural habitat.

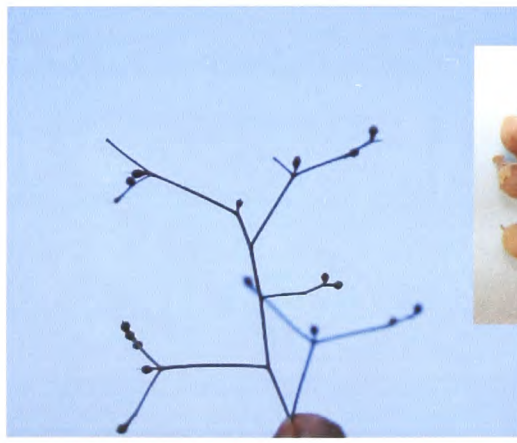


Plate 7.2 Seeds of *Convolvulus dorycnium* are in single seeded capsules

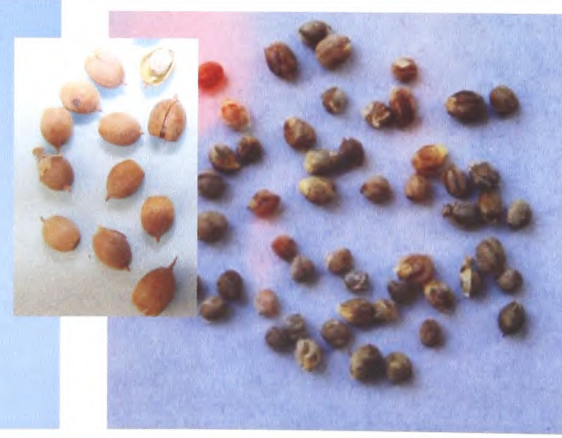


Plate 7.3 Dry seeds of *Convolvulus dorycnium*.

Plate 7.4 The seedlings after transplanting in individual pots.

Plate 7.5 Seed germination of *Convolvulus dorycnium* at 20°C under light. No germinated seeds (in blank colour) did not imbibed water.

Plate 7.6 Established plant of *Convolvulus dorycnium* in the experimental plot has formed inflorescences three months after its

Plate 7.7 New rosettes of *Convolvulus dorycnium* in December from the buds that are on stems just below the soil level.

PLATES OF *CAMPANULA PELVIFORMIS*

Plate 8.1 Plants of *Campanula pelviformis* in natural habitat under trees or on the road' side.

Plate 8.2 Details of leave and flower of *Campanula pelviformis*.

Plate 8.3 Inflorescences of *Campanula pelviformis*.

Plate 8.4 Capsules and seeds of *Campanula pelviformis*.

Plate 8.5 Rosettes are produced from the caudex of *Campanula pelviformis* thick roots the second year from the seeding.

Plate 8.6 Four months' aged seedlings of *C. pelviformis* in compost

Plate 8.7 Five months' aged seedlings of *C. pelviformis* in individual pots with a mixture of compost + perlite + peat moss 1:1:1 (v/v).

Plate 8.8 Flowering of *Campanula pelviformis* in the greenhouse.

Plate 8.9 Pot plants of *Campanula pelviformis*.

Plate 8.10 New shoots nine months after planting from the under ground rootstock of *Campanula pelviformis* in the experimental plot.

Plate 9.1 Anthesis of *Sternbergia sicula* (A) and *Sternbergia lutea* (B) in natural habitats.

Plate 9.2 Cultivation of *Sternbergia sicula* (Aa, Ba) and *Sternbergia lutea* (Ab, Bb) in pots in the greenhouse.

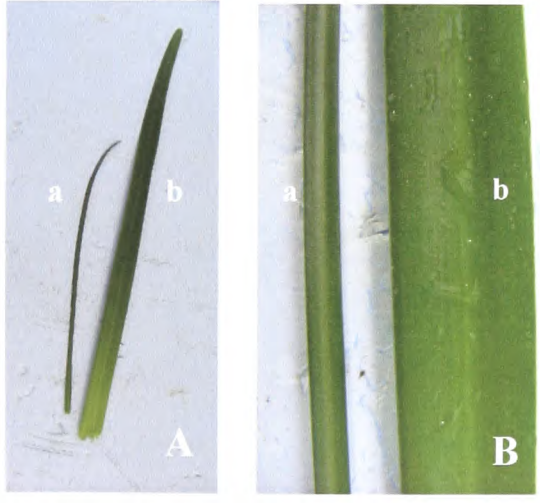


Plate 9.3 Leaves of *Sternbergia sicula* (Aa, Ba) and *Sternbergia lutea* (Ab, Bb) in pots in the greenhouse.

Plate 9.4 Natural habitat of *Sternbergia sicula* (A); groups of bulbs (B).

Plate 9.5 Fruits and leaves of *Sternbergia sicula* bend downwards to the ground.

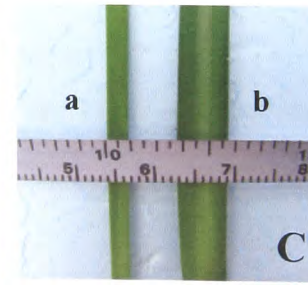


Plate 9.6 Two types of leaves of *Sternbergia sicula*: (a) with thin leaves and more daughter bulbs and (b): with wider leaves and less bulbs (A,B,C).

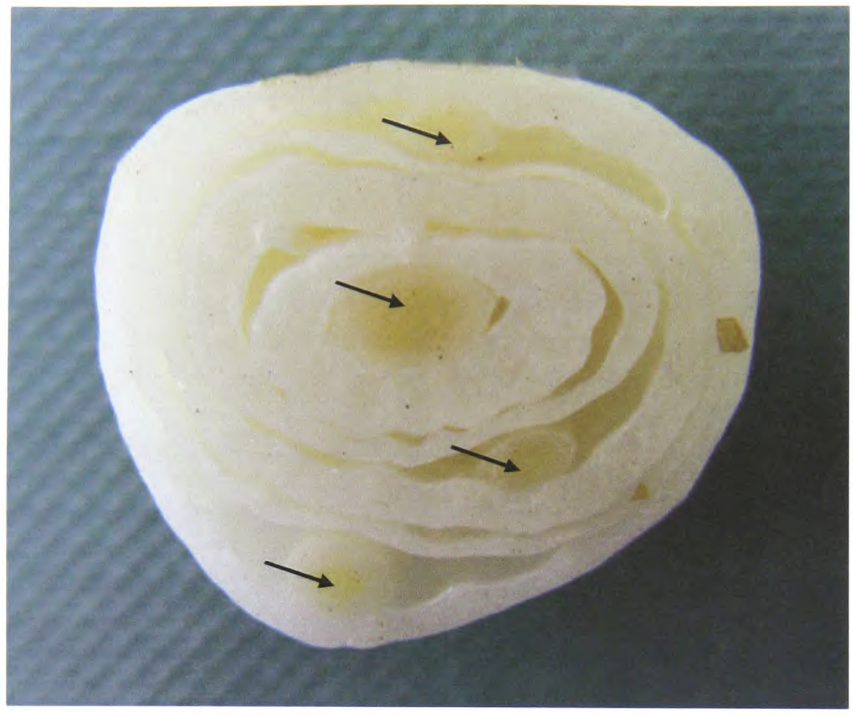


Plate 9.7 *Sternbergia sicula* bulb type is a branching system made up of a number of annual bulb units among the scales (arrows).

Plate 9.8 *Sternbergia sicula* lateral bulblets attached on the outer point of the base disc of the bulbs.

Plate 9.9 Each bulb unit of *Sternbergia sicula* can produce up to four flowers yearly plus the flowers produced from the daughter bulbs.

Plate 9.10 The semi-transparent membranous (arrow), which covers the leaves and the flowers of *Sternbergia sicula* before they appear on the surface of the ground.

Plate 9.11 Flower of *Sternbergia sicula.*

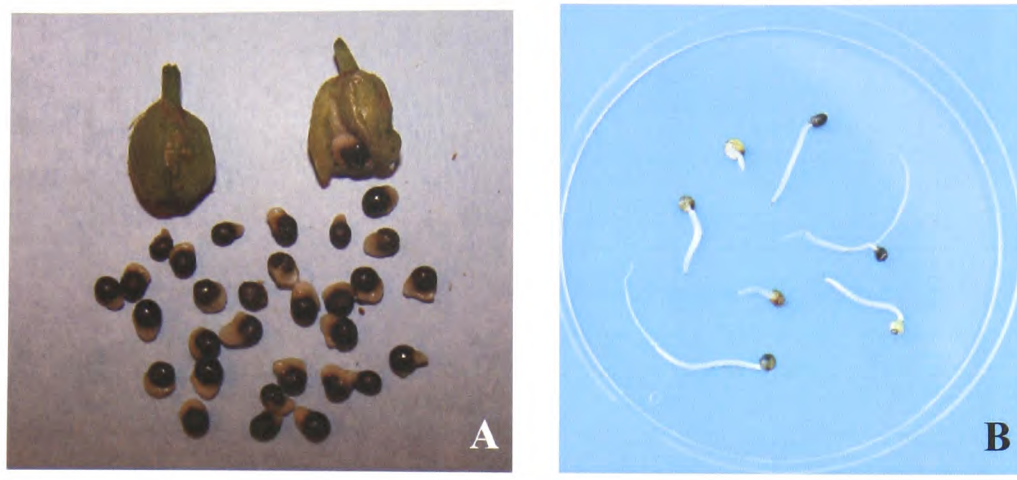


Plate 9.12 Fruits (capsules) and seeds of *Sternbergia sicula* with myrmecochorous (aril) in the one side of the seeds (A); Dry seeds in germination (B).

Plate 9.13 Germination of *Sternbergia sicula* seeds 18 days after seeding in compost.

Plate 9.14 Germination of *Sternbergia sicula* seeds at 15°C in darkness after storage in wet vermiculite at 20°C for one month.

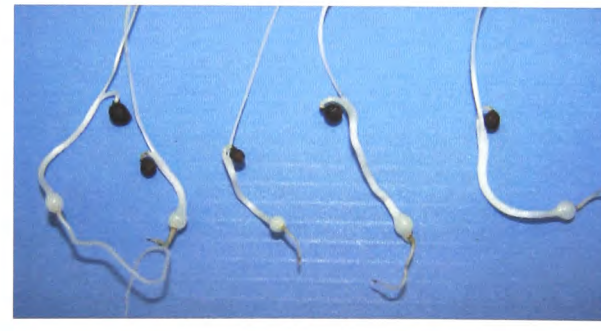


Plate 9.15 Seedlings of *Sternbergia sicula* sometimes produce directly bulbs.

Plate 9.16 Effect of the cross cutting of *Sternbergia sicula* bulbs on the production of bulblets; (a) bulb 3 - 4 cm in circumference control (no cutting), (b) 3 - 4 cm after cutting, (c) <3 cm control and (d) <3 cm after cutting.

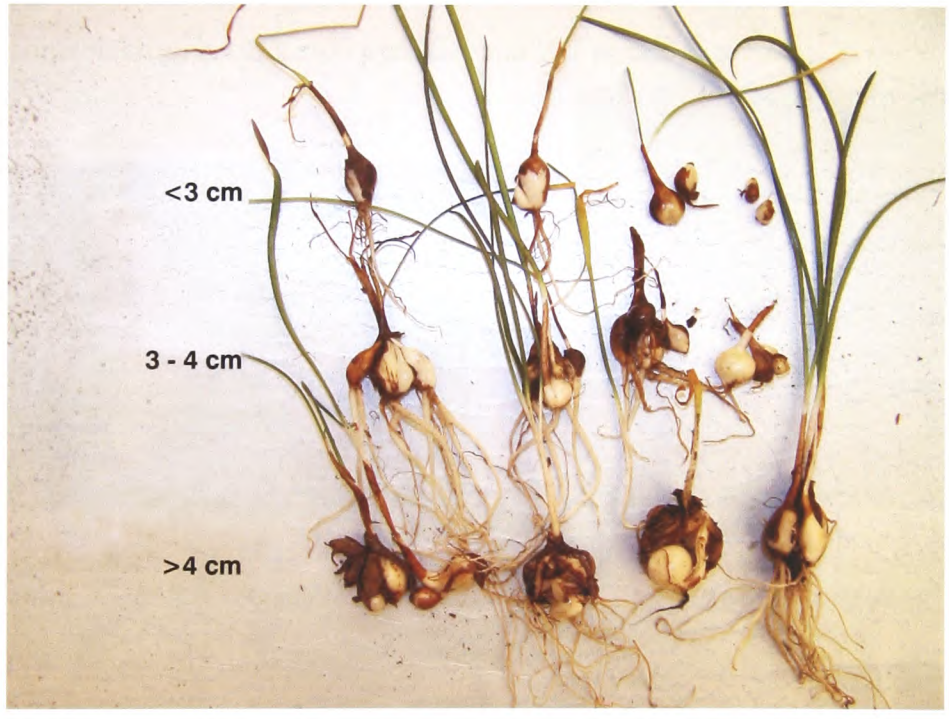


Plate 9.17 Effect of the cross cutting of the bulbs of *Sternbergia sicula* on the production of bulblets; Upper row: cross cutting on bulbs smaller than 3 cm in circumference. Middle row: cross cutting on bulbs 3-4 cm in circumference. Low row: cross cutting on bulbs bigger than 4 cm in circumference.

Plate 9.18 Effect of the cross cutting and BA on the bulblets production of *Sternbergia sicula* bulbs more than 4 cm in circumference; from left to right: control, cross cutting, 100 ppm BA and 200 ppm BA.

Plate 9.19 Bulblets produced by chipping of *Sternbergia sicula* bulbs.

Plate 9.20 Cutting of *Sternbergia sicula* bulb to obtain explant (bottom right) for *in vitro* culture.

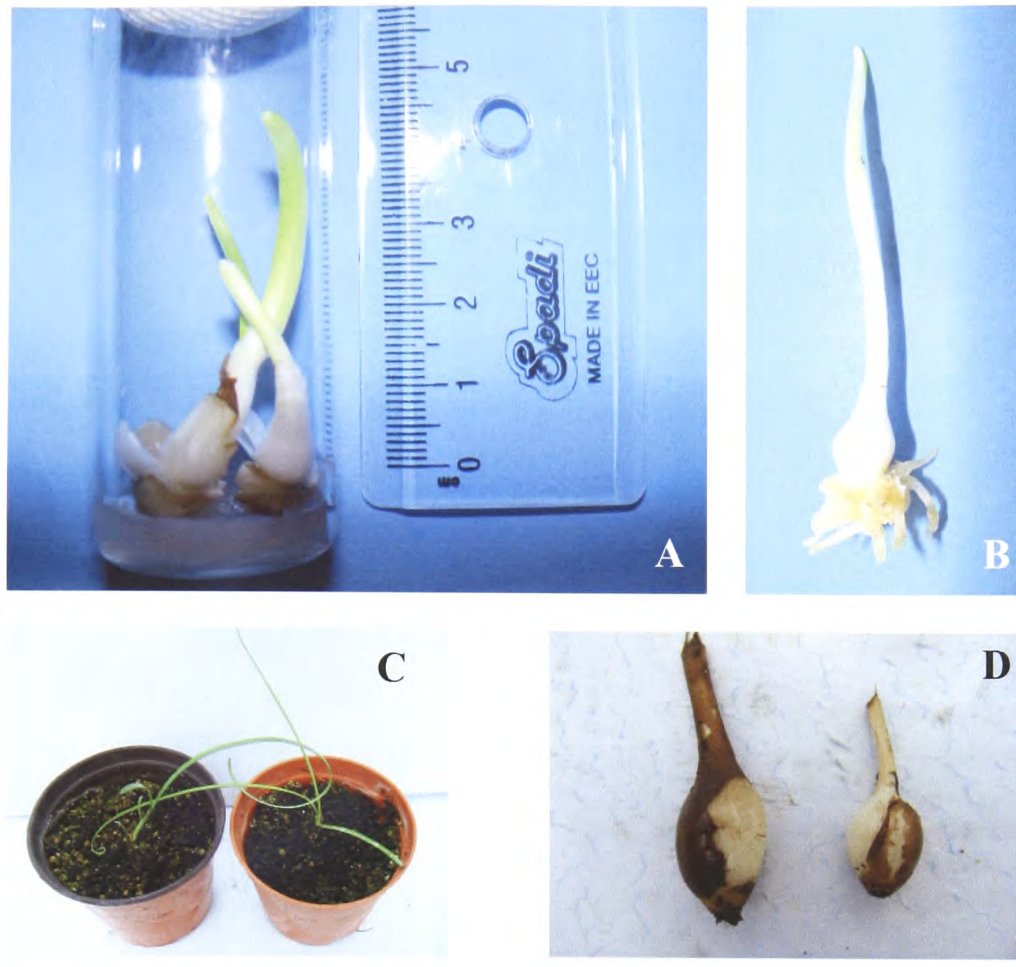


Plate 9.21 Well developed bulblets produced *in vitro* by the effect of 1mg/l NAA (A) and 0.5 mg/l NAA (B) were transferred to individual pots in greenhouse for cultivation (C); bulbs after 5 months cultivation in pots (D).

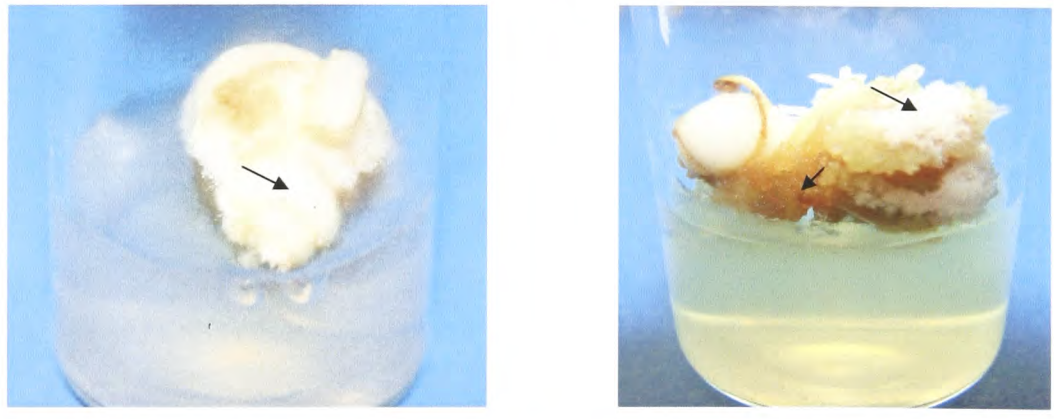


Plate 9.22 Amorphous achlorophyllous tissue formed from desiccated bulbs of *Sternbergia sicula* on the base and the cut point (arrows) of the explants in MS medium with BA/NAA at 4/1 and 4/2, 11 weeks after initiation.

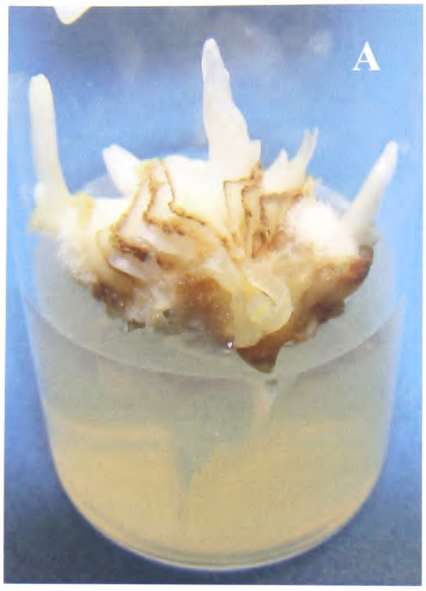


Plate 9.23 Amorphous achlorophyllous tissue and plantlets formed at the same time on explants on MS medium with BA/NAA at 4/0.5, (A) in 11 weeks (darkness), (B) in 16 weeks (darkness), (C) in 10 months (darkness) and in (D) 10 months in light/dark 16/8 h after initiation.

Plate 9.24 Bulblets grown *in vitro* after sub-culturing on MS medium with 1mg/l IBA.

Plate 9.25 Different sizes of bulblets produced by *in vitro* culture of stored *Sternbergia sicula* bulbs after 10 months from initiation of cultures.

Plate 9.26 *Sternbergia sicula* in pot cultivation (A) after two watering per week (synanthous), (B), (C) after one watering (synanthous + hysteranthous), (D) flowering during storage in room temperature, (E) leaves after flowering (a) from plants which were watered ones a week (back row) and (b) from plants without watering (front row).

PLATES OF *COLCHICUM MACROPHYLLUM*

Plate 10.1 *Colchicum macrophyllum* in natural habitat during anthesis in September (A) under olive tree; (B) on gravel soil.

Plate 10.2 *Colchicum macrophyllum* in the observation area in spring.

Plate 10.3 *Colchicum macrophyllum* grown in clusters of different size corms which are the result of vegetative propagation.

Plate 10.4 Seedlings (arrow) of *Colchicum macrophyllum* under the leaves of the adult plants in groups of 10 - 20 at the end of March.

Plate 10.5 Leaves of *Colchicum macrophyllum*

Plate 10.6 Fruits of *Colchicum macrophyllum* with one (A) and six capsules (B)

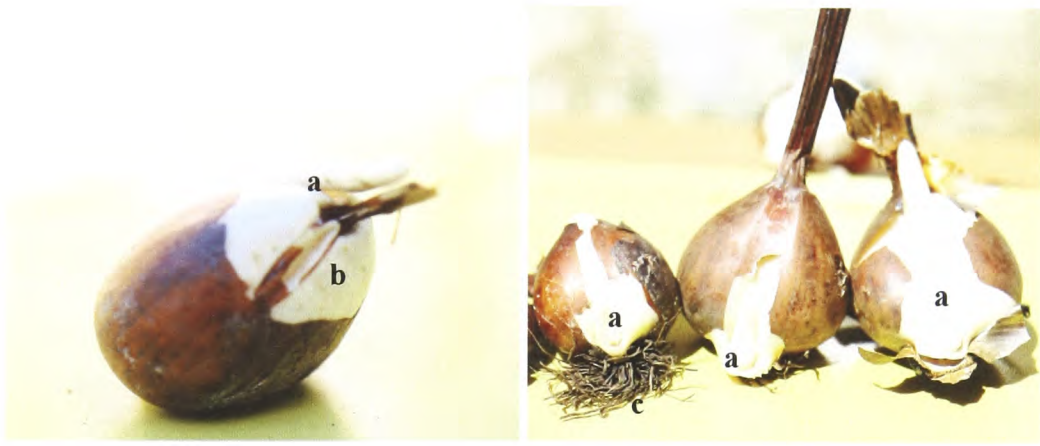


Plate 10.7 Corm of *Colchicum macrophyllum*; (a) the replacement bud. (b) the reserve bud; (c) the dry roots of the previous year.

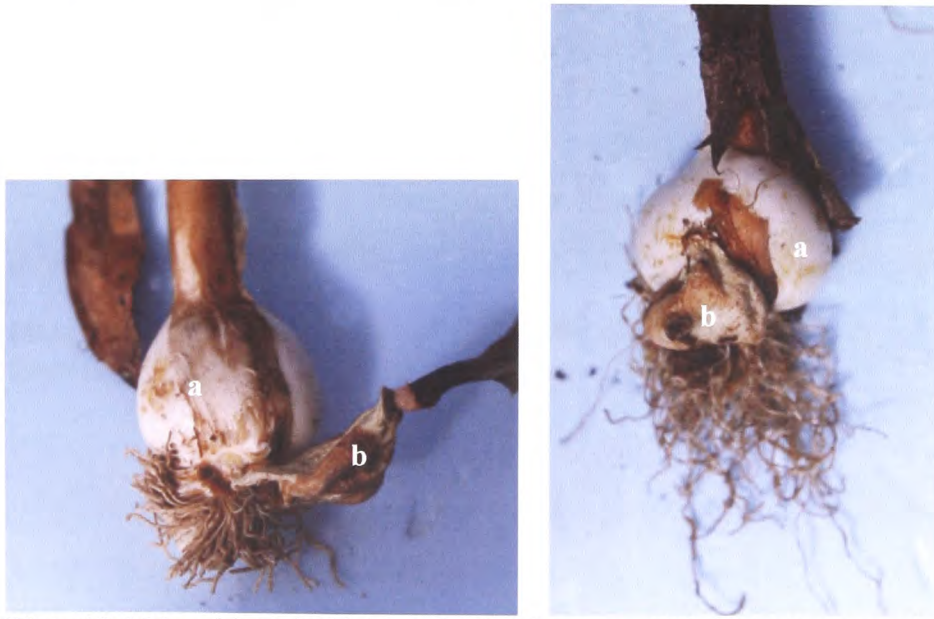


Plate 10.8 New (a) and old (shrinked) (b) corms of *Colchicum macrophyllum.*

Plate 10.9 In the natural habitat, it is rare for the mother corm (c) to produce simultaneously two daughter corms; one from the replacement bud (a) and one

Plate 10.10 From each corm of *Colchicum macrophyllum*, 1 - 5 and rarely 6 flowers are produced.

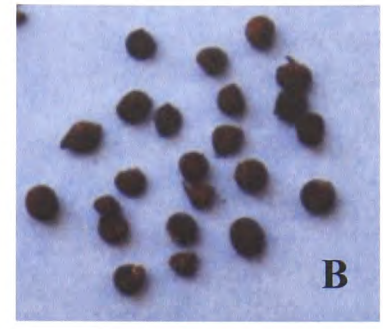


Plate 10.11 Capsules (A) and seeds (B) of *Colchicum macrophyllum.*

Plate 10.12 Germination of *Colchicum macrophyllum* seeds at 15°C in darkness after imbibition in wet vermiculite for two months at room temperature.

Plate 10.13 Simultaneous formation of new corms from the replacement (a) the reserve (b) buds in large (left) and small old (c) corms (right) of *Colchicum macrophyllum* after cultivation in pots.

Plate 10.14 Formation of new corms-cormlets after wounding of the replacement bud of *Colchicum macrophyllum*: one larger corm from the reserve bud (a) and two (right) to three (left) smaller cormlets from the replacement bud (b).

Plate 10.15 Four types of formation new corms from *Colchicum macrophyllum* mother corm (from left to the right): 2 corms from the two buds of *C. macrophyllum*, one corm from the replacement bud, two corms from the replacement bud or one corm from the reserve bud.

Plate 10.16 Three size corms of *Colchicum macrophyllum* that were used for *in vitro* regeneration: (a) 22 – 50 g/corm, (b) 8 – 21 g/corm and (c) 0.9 - 7 g/corm.

Plate 10.17 *In vitro* culture of *Colchicum macrophyllum* buds (a) on MS medium with 4 mg/l 2,4-D and 0.5 mg/l BA used as explant replacement bud and (b) on MS medium without growth regulators used as explant reserve bud, after 10 weeks culture.

Plate 10.18 Callus formation and shoot development after three subcultures using different growth regulators.

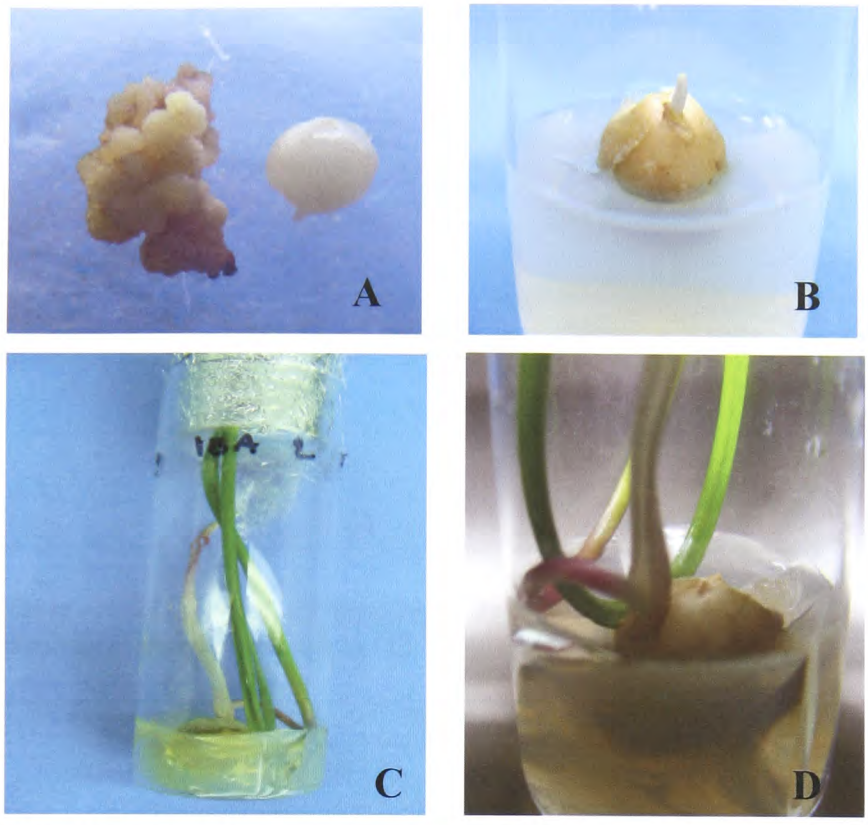


Plate 10.19 Small pre-corms (A) on the bases of the produced *in vitro* shoots were formed, which after transferred to IBA at 1, 2 or 4 mg/l (B, C, D) a new generation of shoots and cormlets were produced.

Plate 10.20 Callus produced by the impact of 2,4-D in all concentrations

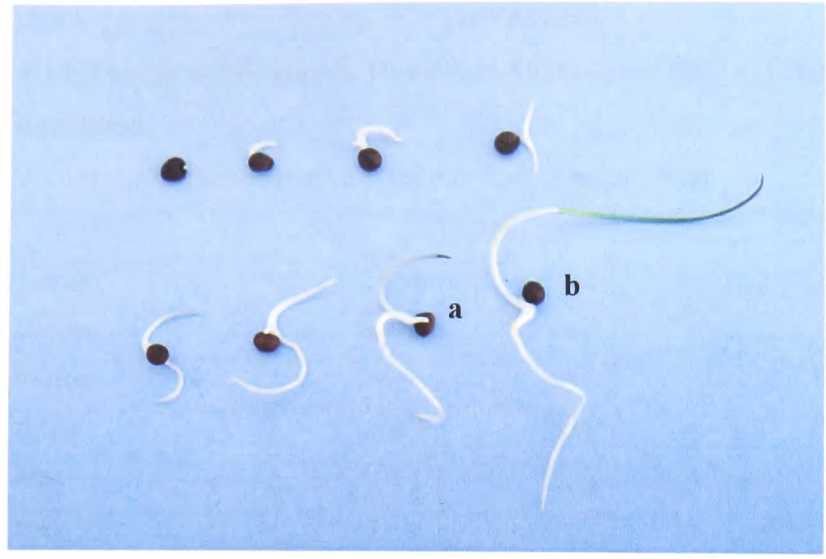


Plate 10.21 Stages of seedlings developments. Explant used for *in vitro* proliferation of seedlings (a, b).

Plate 10.22 *Colchicum macrophyllum* cultivated in pot (A) and in rock garden (B)

APPENDIX 1

1.1 Climatic conditions in Heraklion Crete from 2003 to 2005 at the airport of heraklion

Table1.1.1 Rainfall (mm) per month from 2003 to 2005

	2003	2004	2005	Mean 2003-2005
January	65.5	196.4	123.2	128.4
February	169.7	64.7	71.4	101.9
March	92	17.5	40.7	50.1
April	60.2	24.7	9.7	31.5
May	5.9	22.2	18.6	15.6
June	0	0.1	0	0.0
July	0	0	0.1	0.0
August	0	0	0	0.0
September	0	0	2.2	0.7
October	0.6	7	27.2	11.6
November	52.7	140.7	58.6	84.0
December	172.8	83	56.1	104.0
Total/year	**619.4**	**556.3**	**407.8**	**527.8**

Table 1.1.2 Medium, maximum and minimum temperature (°C) from 2003 to 2005

	Mean			Maximum			Minimum		
	2003	2004	2005	2003	2004	2005	2003	2004	2005
January	14.0	11.3	12.2	17.2	14.8	15.4	110	8.1	8.9
February	9.7	11.9	11.1	12.1	15.5	14.1	7.5	8.4	8.1
March	11.7	14.0	13.7	14.5	17.1	17.4	8.8	10.8	10.0
April	14.8	16.5	15.8	17.8	20.3	19.3	11.3	12.8	11.5
May	19.9	18.9	19.8	23.6	22.6	23.5	15.4	14.5	15.3
June	23.8	23.4	22.8	27.3	26.9	25.8	19.2	19.2	17.9
July	26.8	25.8	25.3	29.7	28.5	29.3	23.2	22.4	22.5
August	26.7	26.1	26.1	29.4	29.1	29.2	23.5	22.2	22.4
September	23.6	23.4	23.7	26.7	26.4	27.3	20.1	20.3	20.3
October	22.7	21.5	19.3	26.5	25.2	22.3	18.8	17.9	16.1
November	17.2	17.2	16.2	20.7	20.6	18.9	14.2	13.9	13.4
December	13.4	13.9	14.5	16.4	16.8	17.5	10.6	11.0	11.6

Table 1.1.3 Mean relative humidity and sunlight from 2003 to 2005

	Relative humidity (%)			**Sunlight (h)**		
	2003	**2004**	**2005**	**2003**	**2004**	**2005**
January	69	69	70	1208	1137	1222
February	67	65	63	900	1406	1335
March	68	65	67	1478	1674	2082
April	70	59	59	1922	2206	2432
May	65	62	62	3222	3185	2351
June	59	60	60	3670	3755	3772
July	58	59	60	3948	3831	3800
August	58	62	62	3734	3546	3636
September	60	63	63	2898	2887	2904
October	57	67	66	2120	2492	2148
November	72	68	65	1474	1369	1183
December	71	69	66	904	983	1169

1.2 Climatic conditions in Sitia Crete from 2003 to 2005 at the airport of sitia

Table 1.2.1 Rainfall (mm) per month from 2003 to 2005

	2003	2004	2005	Mean 2003-2005
January	53.1	240.1	181.6	158.3
February	139	56.3	65.6	87.0
march	76.4	8.4	30.9	38.6
April	27.7	3.7	8.2	13.2
May	32.6	0	0	10.9
June	0	0	0	0
July	0	0	0	0
August	0	0	0	0
September	0	0	0	0
October	0.7	13.5	31.2	15.1
November	31.3	126.8	48.9	69.0
December	220.5	149.1	41	136.9
Total/year	**581.3**	**597.9**	**407.4**	**528.9**

Table 1.2.2 Medium. maximum and minimum temperature (°C) from 2003 to 2005

	Mean			Maximum			Minimum		
	2003	2004	2005	2003	2004	2005	2003	2004	2005
January	14.1	11.3	12.7	21.6	18	20	8.2	1.2	6.2
February	10	12.2	11.3	17.6	22.4	18	2.8	0.2	5
march	12.1	13.9	13.9	20	24	24	1	8	5.8
April	14.9	15.7	16	21.8	27	25.5	7.6	9.8	8.8
May	20.6	19.2	20.6	27.4	29.8	31	14.5	13.2	14
June	24.3	23.6	22.9	30	33.5	27.8	16	15.4	17
July	26.5	25.6	26.2	36.6	32.6	33.6	22	21.4	21.2
August	26.4	26.2	26.1	32.4	35.4	31.2	22.4	22.8	20
September	23.3	23.4	24.1	31.4	33.8	32.2	18	18	20
October	22.2	21.6	19.4	33	29.8	27.2	12.6	15	13.2
November	17.2	17.2	16.5	23	25.6	24	11	7.4	6.4
December	13.6	14.3	14.2	20	19.4	24.8	3.4	7.6	6

Table 1.2.3 Mean relative humidity and sunlight from 2003 to 2005

	Humidity (%)			**Sunlight (h)**		
	2003	**2004**	**2005**	**2003**	**2004**	**2005**
January	75	73	72	121.2	119.7	115.3
February	65	72	65	92.5	133	121.2
march	67	67	69	151.1	171.6	213.2
April	72	63	57	199	234.7	248.3
May	62	59	61	327.2	320.1	341
June	58	64	69	370.4	380.1	372.5
July	60	66	63	378.1	387.7	383.3
August	63	65	69	363.7	360.7	361.7
September	64	75	62	295	306	298.1
October	64	73	72	214.6	257.8	238.9
November	74	74	73	158.1	129.1	143
December	70	80	78	98.1	115.7	134

APPENDIX 2

2.1 Soil analysis in the experimental plot of the farm of TEI in Heraklion and the observation area for *Prasium majus*.

Characteristics of the soil:

Sand: 39%
Clay: 23%
Loam: 38%
Characterization: L.
pH: 7.3
E.C. 2.1mS/cm
Total $CaCO_3$: 41%
Organic substance: 1.5%
Available phosphorus: P 40 ppm (Olsen method)
Available potassium: K 30mg/100gr soil (method of ammonium acetate)

2.2 Soil analysis in the observation area for *Convonvulus dorycnium* and *Otanthus maritimus* in the North coast of Hersonisos Heraklion.

Characteristics of the soil:

Sand: 76%
Clay: 8%
Loam: 16%
Characterization: S.L.
pH: 6.1
E.C. 16.4mS/cm
Total $CaCO_3$: 0%
Organic substance: 1.3%
Available phosphorus: P 90 ppm (Olsen method)
Available potassium: K 34 mg/100g soil (method of ammonium acetate)

2.3 Soil analysis in the observation area for *Colchicum macrophyllum* and *Campanula pelviformis* in Exo Mouliana Sitia.

Characteristics of the soil:

Sand: 59%
Clay: 19%
Loam: 22%
Characterization: S.C.L.
pH: 7.4
E.C. 0.89 mS/cm
Total $CaCO_3$: 0.6%
Organic substance: 8 %
Available phosphorus: P 55 ppm (Olsen method)
Available potassium: K 36 mg/100g soil (method of ammonium acetate)

2.4 Soil analysis in the observation area for *Sternbergia sicula* in Zaros Heraklion.

Characteristics of the soil:

Sand: 53%
Clay: 27%
Loam: 20%
Characterization: S.C.L.
pH: 7.1
E.C. 0.42 mS/cm
Total $CaCO_3$: 3%
Organic substance: 2%
Available phosphorus: P 80 ppm (Olsen method)
Available potassium: K 24 mg/100g soil (method of ammonium acetate)

2.5 Soil analysis in the observation area for *Staehelina petiolata* and *Ptilostemon chamaepeuce* in Archanes Heraklion.

Characteristics of the soil:

Sand: 66%
Clay: 16%
Loam: 18%
Characterization: S.L.
pH: 7.5
E.C. 1.2 mS/cm
Total $CaCO_3$ and $MgCO_3$: 40%
Organic substance: 2.5%
Available phosphorus: P 75 ppm (Olsen method)
Available potassium: K 14 mg/100g soil (method of ammonium acetate)

APPENDIX 3

Results of statistical analysis on experiments described in chapter 3

Table 3.1

Analysis of variance on percent germination of *Staehelina petiolata* seeds in two different substrates at different times through year (Table 3.3). The germination percentage data were transformed in angular values before statistical analysis.

Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
Substrate	,355	1	,355	75,694	,000
Time	1,943	5	,389	82,901	,000
Substrate * Time	,323	5	6,466E-02	13,797	,000
Error	9,373E-02	20	4,687E-03		
Total	27,512	32			

Table 3.2

Analysis of variance on T_{50} of *Staehelina petiolata seeds* in two different substrates at different times through year (Table 3.3).

Dependent Variable: T_{50}

Source	Sum of Squares	df	Mean Square	F	Sig.
Substrate	102,617	1	102,617	10,254	,004
Time	173,947	5	34,789	3,476	,020
Substrate * Time	87,464	5	17,493	1,748	,170
Error	200,148	20	10,007		
Total	6700,190	32			

Table 3.3

Analysis of variance (one way) on mean percent germination and T_{50} showing effect of substrate and time of *Staehelina petiolata* seed germination (Table 3.3).

		Sum of Squares	df	Mean Square	F	Sig.
Germination	Between Groups	2,655	11	,241	51,507	,000
	Within Groups	,094	20	,005		
	Total	2,749	31			
T_{50}	Between Groups	386,626	11	35,148	3,512	,007
	Within Groups	200,148	20	10,007		
	Total	586,775	31			

Table 3.4

Analysis of variance on percent germination of two seed lots of *Staehelina petiolata* seeds in two different substrates in September (Table 3.4). The germination percentage data were transformed in angular values before statistical analysis.

Dependent Variable: germination

Source	Sum of Squares	df	Mean Square	F	Sig.
Lot	3,333E-03	1	3,333E-03	,487	,505
Substrate	,521	1	,521	76,034	,000
Lot * Substrate	1,920E-02	1	1,920E-02	2,803	,133
Error	5,480E-02	8	6,850E-03		
Total	4,612	12			

Table 3.5
Analysis of variance on T_{50} of two seed lots of *Staehelina petiolata* seeds in two different substrates in September (Table 3.4).
Dependent Variable: T_{50}

Source	Sum of Squares	df	Mean Square	F	Sig.
Lot	2471,070	1	2471,070	248,536	,000
Substrate	1,763	1	1,763	,177	,685
Lot * Substrate	4,083	1	4,083	,411	,540
Error	79,540	8	9,942		
Total	10595,820	12			

Table 3.6
Analysis of variance (one way) on mean percent germination and T_{50} showing effect of substrates and seed lots on *Staehelina petiolata* seed germination in September (Table 3.4).

		Sum of Squares	df	Mean Square	F	Sig.
Germination	Between Groups	,543	3	,181	26,441	,000
	Within Groups	,055	8	,007		
	Total	,598	11			
T_{50}	Between Groups	2476,917	3	825,639	83,041	,000
	Within Groups	79,540	8	9,942		
	Total	2556,457	11			

Table 3.7
Analysis of variance on percent germination of two seed lots of *Staehelina petiolata* seeds in two different substrates in October (Table 3.4). The germination percentage data were transformed in angular values before statistical analysis.
Dependent Variable: germination

Source	Sum of Squares	df	Mean Square	F	Sig.
Lot	7,008E-03	1	7,008E-03	1,200	,305
Substrate	,261	1	,261	44,692	,000
Lot * Substrate	3,008E-03	1	3,008E-03	,515	,493
Error	4,673E-02	8	5,842E-03		
Total	5,349	12			

Table 3.8
Analysis of variance on T_{50} of two seed lots of *Staehelina petiolata* seeds in two different substrates in October (Table 3.4).
Dependent Variable: T_{50}

Source	Sum of Squares	df	Mean Square	F	Sig.
Lot	327,607	1	327,607	60,148	,000
Substrate	1234,241	1	1234,241	226,605	,000
Lot * Substrate	13,867	1	13,867	2,546	,149
Error	43,573	8	5,447		
Total	8185,330	12			

Table 3.9
Analysis of variance (one way) on mean percent germination and T_{50} showing effect of substrates and seed lots on *Staehelina petiolata* seed germination in October (Table 3.4).

		Sum of Squares	df	Mean Square	F	Sig.
Germination	Between Groups	,271	3	,090	15,469	,001
	Within Groups	,047	8	,006		
	Total	,318	11			
T_{50}	Between Groups	1575,716	3	525,239	96,433	,000
	Within Groups	43,573	8	5,447		
	Total	1619,289	11			

Table 3.10
Analysis of variance on percent germination in vitro of *Staehelina petiolata* seeds at 10, 15, 20 and 25°C under light and darkness and 30°C under darkness. (Table 3.5). The germination percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
Temperature	3,417	4	,854	56,543	,000
Light	8,333E-04	1	8,333E-04	,055	,815
Temperature * Light	,384	3	,128	8,475	,000
Error	,695	46	1,511E-02		
Total	97,635	55			

Table 3.11
Analysis of variance on T_{50} of *Staehelina petiolata* seeds germination in vitro at 10, 15, 20 and 25°C under light and darkness and 30°C under darkness (Table 3.5).
Dependent Variable: T_{50}

Source	Sum of Squares	df	Mean Square	F	Sig.
Temperature	56,130	4	14,033	21,341	,000
Light	9,541	1	9,541	14,510	,000
Temperature * Light	39,977	3	13,326	20,266	,000
Error	30,248	46	,658		
Total	1692,590	55			

Table 3.12
Analysis of variance (one way) on mean percent germination and T_{50} showing effect of temperature and lighting on *Staehelina petiolata* seed germination (Table 3.5).

		Sum of Squares	df	Mean Square	F	Sig.
Germination	Between Groups	4,016	8	,502	33,226	,000
	Within Groups	,695	46	,015		
	Total	4,711	54			
T_{50}	Between Groups	102,517	8	12,815	19,488	,000
	Within Groups	30,248	46	,658		
	Total	132,764	54			

Table 3.13
Analysis of variance on percent rooting of *Staehelina petiolata* stem tip cuttings in different concentration of IBA (Figure 3.16). The rooting percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Rooting

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	1,484	7	,212	19,442	,000
Block	,491	4	,123	11,262	,000
Error	,305	28	1,090E-02		
Total	4,759	40			

Table 3.14
Analysis of variance on percent callusing of *Staehelina petiolata* stem tip cuttings in different concentration of IBA in June (Figure 3.16). The rooting percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Callus

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	3,187	7	,455	18,332	,000
Block	,519	4	,130	5,219	,003
Error	,695	28	2,484E-02		
Total	9,552	40			

Table 3.15
Analysis of variance (one way) on mean number of roots per rooted cutting showing effect of IBA on *Staehelina petiolata* stem tip cuttings in June (Figure 3. 17).
Number of roots

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	86,101	5	17,220	6,102	,000
Within Groups	104,411	37	2,822		
Total	190,512	42			

Table 3.16
Analysis of variance on percent rooting of *Staehelina petiolata* stem tip cuttings in different concentration of IBA in November (Figure 3.18). The rooting percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Rooting

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	2,116	5	,423	27,463	,000
Block	,255	3	8,507E-02	5,519	,009
Error	,231	15	1,541E-02		
Total	4,807	24			

Table 3.17
Analysis of variance (one way) on mean number of roots per rooted cutting showing effect of IBA on *Staehelina petiolata* stem tip cuttings in November (Figure 3. 19).
Number of roots

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	389,325	3	129,775	10,179	,000
Within Groups	331,475	26	12,749		
Total	720,800	29			

Table 3.18
Analysis of variance on percent rooting of *Staehelina petiolata* stem tip cuttings in different substrates (Figure 3.20). The rooting percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Rooting

Source	Sum of Squares	df	Mean Square	F	Sig.
Substrate	,285	6	4,749E-02	4,922	,002
Block	,585	4	,146	15,158	,000
Error	,232	24	9,649E-03		
Total	22,850	35			

Table 3.19
Analysis of variance on percent rooting of *Staehelina petiolata* stem tip cuttings in different substrates (Figure 3.21).
Dependent Variable: Number of roots

Source	Sum of Squares	df	Mean Square	F	Sig.
Substrate	721,828	6	120,305	4,843	,002
Block	380,221	4	95,055	3,827	,015
Error	596,186	24	24,841		
Total	7303,072	35			

Table 3.20
Analysis of variance on the effect of BA and NAA on the number of shoots of *Staehelina petiolata in vitro*, using seedling bud explants on MS medium. (Figure 3.22).Dependent Variable: Shoots

Source	Sum of Squares	df	Mean Square	F	Sig.
BA	1328,215	3	442,738	53,011	,000
NAA	35,340	1	35,340	4,231	,043
BA * NAA	104,913	3	34,971	4,187	,009
Error	576,278	69	8,352		
Total	3816,000	77			

Table 3.21
Analysis of variance on the effect of BA and NAA on the number of shoots of *Staehelina petiolata in vitro*, using seedling bud explants on MS medium. (Figure 3.23).
Dependent Variable: Length

Source	Sum of Squares	df	Mean Square	F	Sig.
BA	116,496	3	38,832	14,851	,000
NAA	27,104	1	27,104	10,366	,002
BA * NAA	3,291	3	1,097	,419	,740
Error	180,417	69	2,615		
Total	1239,580	77			

Table 3.22
Analysis of variance (one way) on mean number of shoots and length of shoots per explant showing effect of BA and NAA on *Staehelina petiolata* seedlings explants (Figures 3. 22 and 3.23).

		Sum of Squares	df	Mean Square	F	Sig.
Shoots	Between Groups	1480,969	7	211,567	25,332	,000
	Within Groups	576,278	69	8,352		
	Total	2057,247	76			
Lenght	Between Groups	149,901	7	21,414	8,190	,000
	Within Groups	180,417	69	2,615		
	Total	330,318	76			

Table 3.23
Analysis of variance on the effect of soil medium and fertilization on the height of *Staehelina petiolata* plants (Table 3.10)
Dependent Variable: Height in the 1st month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	3,227E-02	1	3,227E-02	,106	,749
Medium	2,676	2	1,338	4,390	,032
Block	2,137	3	,712	2,337	,115
Fert * Med	1,992	2	,996	3,267	,066
Error	4,573	15	,305		
Total	97,141	24			

Table 3.24
Analysis of variance on the effect of soil medium and fertilization on the height of *Staehelina petiolata* plants (Table 3.10)
Dependent Variable: Height in the 3rd month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	1,520	1	1,520	2,366	,145
Medium	10,279	2	5,139	7,998	,004
Block	9,216	3	3,072	4,781	,016
Fert * Med	6,290	2	3,145	4,895	,023
Error	9,638	15	,643		
Total	271,068	24			

Table 3.25
Analysis of variance on the effect of soil medium and fertilization on the height of *Staehelina petiolata* plants (Table 3.10)
Dependent Variable: Height in the 6th month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	7,404	1	7,404	13,236	,002
Medium	42,167	2	21,084	37,692	,000
Block	5,530	3	1,843	3,295	,050
Fert * Med	5,051	2	2,526	4,515	,029
Error	8,390	15	,559		
Total	414,115	24			

Table 3.26
Analysis of variance on the effect of soil medium and fertilization on the diameter of *Staehelina petiolata* plants (Table 3.11)
Dependent Variable: Diameter in the 1st month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	8,503E-02	1	8,503E-02	,473	,502
Medium	1,315	2	,657	3,656	,051
Block	2,497	3	,832	4,631	,017
Fert * Med	1,111	2	,555	3,090	,075
Error	2,696	15	,180		
Total	103,933	24			

Table 3.27
Analysis of variance on the effect of soil medium and fertilization on the diameter of *Staehelina petiolata* plants (Table 3.11)
Dependent Variable: Diameter in the 3rd month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	2,811	1	2,811	2,930	,108
Medium	43,724	2	21,862	22,784	,000
Block	6,466	3	2,155	2,246	,125
Fert * Med	3,089	2	1,544	1,609	,233
Error	14,393	15	,960		
Total	410,667	24			

Table 3.28
Analysis of variance on the effect of soil medium and fertilization on the diameter of *Staehelina petiolata* plants (Table 3.11)
Dependent Variable: Diameter in the 6th month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	3,990	1	3,990	3,896	,067
Medium	93,919	2	46,960	45,850	,000
Block	9,118	3	3,039	2,967	,066
Fert * Med	4,368	2	2,184	2,132	,153
Error	15,363	15	1,024		
Total	581,023	24			

Table 3.29
Analysis of variance on the effect of soil medium and fertilization on the number of *Staehelina petiolata* shoots (Table 3.12)
Dependent Variable: Shoots in the 1st month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	3,401E-03	1	3,401E-03	,013	,911
Medium	,107	2	5,357E-02	,204	,818
Block	2,306	3	,769	2,927	,068
Fert * Med	,379	2	,190	,722	,502
Error	3,939	15	,263		
Total	14,571	24			

Table 3.30
Analysis of variance on the effect of soil medium and fertilization on the number of *Staehelina petiolata* shoots (Table 3.12)
Dependent Variable: Shoots in the 3rd month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	5,858	1	5,858	6,926	,019
Medium	,951	2	,475	,562	,582
Block	18,125	3	6,042	7,144	,003
Fert * Med	,236	2	,118	,140	,871
Error	12,686	15	,846		
Total	120,102	24			

Table 3.31
Analysis of variance on the effect of soil medium and fertilization on the number of *Staehelina petiolata* shoots (Table 3.12)
Dependent Variable: Shoots in the 6th month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	8,503	1	8,503	8,461	,011
Medium	2,102	2	1,051	1,046	,376
Block	22,925	3	7,642	7,604	,003
Fert * Med	1,027	2	,514	,511	,610
Error	15,075	15	1,005		
Total	145,633	24			

Table 3.32
Analysis of variance on the effect of soil medium and fertilization on the number of *Staehelina petiolata* leaves (Table 3.13)
Dependent Variable: Leaves in the 1st month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	,218	1	,218	,232	,637
Medium	3,204	2	1,602	1,707	,215
Block	23,112	3	7,704	8,207	,002
Fert * Med	1,027	2	,514	,547	,590
Error	14,082	15	,939		
Total	2095,143	24			

Table 3.33
Analysis of variance on the effect of soil medium and fertilization on the number of *Staehelina petiolata* leaves (Table 3.13)
Dependent Variable: Leaves in the 3rd month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	,575	1	,575	,147	,707
Medium	72,553	2	36,276	9,293	,002
Block	16,374	3	5,458	1,398	,282
Fert * Med	29,196	2	14,598	3,740	,048
Error	58,554	15	3,904		
Total	2257,265	24			

Table 3.34
Analysis of variance on the effect of soil medium and fertilization on the number of *Staehelina petiolata* leaves (Table 3.13)
Dependent Variable: Leaves in the 6th month

Source	Sum of Squares	df	Mean Square	F	Sig.
Fertilization	10,667	1	10,667	1,942	,184
Medium	311,721	2	155,861	28,382	,000
Block	38,350	3	12,783	2,328	,116
Fert * Med	22,068	2	11,034	2,009	,169
Error	82,374	15	5,492		
Total	3375,469	24			

Table 3.35
Analysis of variance (one way) on mean height, canopy diameter, number of shoots and leaves increased after 1, 3 and 6 months showing effect of soil medium and fertilization on *Staehelina petiolata* growth (Tables 3.10-3.13)).

Parameter		Sum of Squares	df	Mean Square	F	Sig.
Month 1	Between Groups	4,701	5	,940	2,522	,067
	Within Groups	6,710	18	,373		
	Total	11,410	23			
Months 3	Between Groups	18,089	5	3,618	3,454	,023
	Within Groups	18,854	18	1,047		
	Total	36,943	23			
Months 6	Between Groups	54,622	5	10,924	14,126	,000
	Within Groups	13,920	18	,773		
	Total	68,542	23			
Diameter1	Between Groups	2,510	5	,502	1,740	,176
	Within Groups	5,194	18	,289		
	Total	7,704	23			
Diameter3	Between Groups	49,624	5	9,925	8,564	,000
	Within Groups	20,859	18	1,159		
	Total	70,483	23			
Diameter6	Between Groups	102,277	5	20,455	15,040	,000
	Within Groups	24,481	18	1,360		
	Total	126,758	23			
Shoot 1	Between Groups	,490	5	,098	,282	,917
	Within Groups	6,245	18	,347		
	Total	6,735	23			
Shoots 3	Between Groups	7,045	5	1,409	,823	,549
	Within Groups	30,811	18	1,712		
	Total	37,856	23			
Shoots 6	Between Groups	11,633	5	2,327	1,102	,394
	Within Groups	38,000	18	2,111		
	Total	49,633	23			
Leaves 1	Between Groups	4,449	5	,890	,431	,821
	Within Groups	37,194	18	2,066		
	Total	41,643	23			
Leaves 3	Between Groups	102,323	5	20,465	4,916	,005
	Within Groups	74,929	18	4,163		
	Total	177,252	23			
Leaves 6	Between Groups	344,456	5	68,891	10,272	,000
	Within Groups	120,724	18	6,707		
	Total	465,180	23			

Results of statistical analysis ov experiments described in chapter 4

Table 4.1

Analysis of variance on mean percent germination on *Ptilostemon chamaepeuce* seeds in two soil media from September 2004 to January 2005 (Table 4.2). The germination percentage data were transformed in angular values before statistical analysis.

Dependent Variable: Germination

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Substrate	1,532	1	1,532	149,345	,000
Months	,592	4	,148	14,421	,000
Substrate*Months	6,182E-02	4	1,546E-02	1,506	,238
Error	,205	20	1,026E-02		
Total	19,326	30			

Table 4.2

Analysis of variance on T_{50} on *Ptilostemon chamaepeuce* seeds in two soil media from September 2004 to January 2005 (Table 4.2).

Dependent Variable: T_{50}

Source	Sum of Squares	df	Mean Square	F	Sig.
Substrate	61,633	1	61,633	1,490	,236
Months	1383,778	4	345,945	8,361	,000
Substrate*Months	135,823	4	33,956	,821	,527
Error	827,473	20	41,374		
Total	21188,720	30			

Table 4.3

Analysis of variance (one way) on mean germination and T_{50} of *Ptilostemon chamaepeuce* seeds in two soil media from September 2004 to January 2005 (Table 4.2).

		SumofSquares	df	MeanSquare	F	Sig.
Germination	BetweenGroups	2,186	9	,243	23,673	,000
	Within Groups	,205	20	,010		
	Total	2,391	29			
T_{50}	BetweenGroups	1581,235	9	175,693	4,246	,003
	Within Groups	827,473	20	41,374		
	Total	2408,708	29			

Table 4.4

Analysis of variance on germination of *P. chamaepeuce* in different temperatures under light/dark or dark conditions (Table 4.4).

Dependent Variable: Germination

Source	SumofSquares	df	MeanSquare	F	Sig.
Lighting	1,260E-02	1	1,260E-02	,728	,406
Temperature	3,871E-02	3	1,290E-02	,745	,541
Lighting * Temperature	5,301E-02	3	1,767E-02	1,020	,410
Error	,277	16	1,732E-02		
Total	20,935	24			

Table 4.5

Analysis of variance on time germination in days (T_{50}) of *P. chamaepeuce* in different temperatures under light/dark or dark conditions (Table 4.4).

Dependent Variable: T_{50}

Source	SumofSquares	df	MeanSquare	F	Sig.
Lighting	4,860	1	4,860	6,034	,026
Temperature	576,283	3	192,094	238,503	,000
Lighting * Temperature	22,370	3	7,457	9,258	,001
Error	12,887	16	,805		
Total	2625,740	24			

Table 4.6
Analysis of variance (one way) on germination and T_{50} of *Ptilostemon chamaepeuce* seeds in different temperatures under light/dark or dark conditions (Table 4.4).

Parameter		SumofSquares	df	MeanSquare	F	Sig.
Germination	BetweenGroups	,104	7	,015	,861	,556
	Within Groups	,277	16	,017		
	Total	,381	23			
T_{50}	Between Groups	603,513	7	86,216	107,045	,000
	Within Groups	12,887	16	,805		
	Total	616,400	23			

Table 4.7
Analysis of variance (one way) on effect of IBA, rinsing with tap water and application of warm water at the base of the cutting on the rooting of *Ptilostemon chamaepeuce* leaf stem tip cuttings (Table 4.7). The rooting percentage data were transformed in angular values before statistical analysis.

		Sum of Squares	df	Mean Square	F	Sig.
Rooting	Between Groups	1630,895	9	181,211	30,733	0,000
	Within Groups	117,927	20	5,896		
	Total	1748,822	29			
Callus	Between Groups	6312,780	9	701,420	4,653	0,002
	Within Groups	3014,973	20	150,749		
	Total	9327,754	29			
Number of roots	Between Groups	1591,367	9	176,819	2,587	0,037
	Within Groups	1367,180	20	68,359		
	Total	2958,547	29			
Length of roots	Between Groups	30,342	9	3,371	6,141	0,000
	Within Groups	10,980	20	0,549		
	Total	41,322	29			

Table 4.8
Analysis of variance on the effect of concentration of IBA, source of material, rinsing the base of the cuttings and the application of warm water on the rooting of *P. chamaepeuce* leaf stem tip cuttings during winter 2004 (Table 4.8). The rooting percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Rooting

Source	Sum of Squares	df	Mean Square	F	Sig.
Plants	855,913	1	855,913	3,491896	0,067201
IBA	4562,747	8	570,3434	2,326849	0,032034
Plants * IBA	887,2233	8	110,9029	0,452454	0,883496
Error	12991,05	53	245,1141		
Total	30585,4	71			

Table 4.9
Analysis of variance on the effect of concentration of IBA, source of material, rinsing the base of the cuttings and the application of warm water on the primary roots of *P. chamaepeuce* leaf stem tip cuttings during winter 2004 (Table 4.8).
Dependent Variable: Number of primary roots

Source	Sum of Squares	df	Mean Square	F	Sig.
Plants	69,620	1	69,620	5,714	,020
IBA	137,584	8	17,198	1,412	,213
Plants * IBA	54,172	8	6,772	,556	,809
Error	657,930	54	12,184		
Total	1175,440	72			

Table 4.10
Analysis of variance on the effect of concentration of IBA, source of material, rinsing the base of the cuttings and the application of warm water on the length of primary roots of *P. chamaepeuce* leaf stem tip cuttings during winter 2004 (Table 4.8).
Dependent Variable: Length of roots

Source	Sum of Squares	df	Mean Square	F	Sig.
Plants	59,587	1	59,587	4,192	,046
IBA	150,924	8	18,866	1,327	,250
Plants * IBA	35,754	8	4,469	,314	,957
Error	767,638	54	14,216		
Total	1365,470	72			

Table 4.11
Analysis of variance on the effect of concentration of IBA, source of material, rinsing the base of the cuttings and the application of warm water on the number of secondary roots of *P. chamaepeuce* leaf stem tip cuttings during winter 2004 (Table 4.8).
Dependent Variable: Number of secondary roots

Source	Sum of Squares	df	Mean Square	F	Sig.
Plants	1974,014	1	1974,014	9,544	,003
IBA	1432,090	8	179,011	,865	,551
Plants * IBA	832,674	8	104,084	,503	,849
Error	11169,375	54	206,840		
Total	18866,500	72			

Table 4.12
Analysis of variance on the effect of concentration of IBA, source of material, rinsing the base of the cuttings and the application of warm water on the callus of *P. chamaepeuce* leaf stem tip cuttings during winter 2004 (Table 4.8). Dependent Variable: Callus

Source	Sum of Squares	df	Mean Square	F	Sig.
Plants	3,335E-03	1	3,335E-03	,085	,772
IBA	3,968	8	,496	12,670	,000
Plants * IBA	,144	8	1,799E-02	,460	,879
Error	2,114	54	3,915E-02		
Total	6,951	72			

Table 4.13
Analysis of variance (one way) on the effect of concentration of IBA, source of material, rinsing the base of the cuttings and the application of warm water on the rooting of *P. chamaepeuce* leaf stem tip cuttings during winter 2004 (Table 4.8). The rooting percentage data were transformed in angular values before statistical analysis.

		SumofSquares	df	MeanSquare	F	Sig.
Rooting	BetweenGroups	6312,311	17	371,312	1,515	,125
	Within Groups	12991,047	53	245,114		
	Total	19303,358	70			
Primary roots	BetweenGroups	261,376	17	15,375	1,262	,252
	Within Groups	657,930	54	12,184		
	Total	919,306	71			
Length of roots	BetweenGroups	246,266	17	14,486	1,019	,454
	Within Groups	767,638	54	14,216		
	Total	1013,903	71			
Number of secondary roots	BetweenGroups	4238,778	17	249,340	1,205	,292
	Within Groups	11169,375	54	206,840		
	Total	15408,153	71			
Callus	BetweenGroups	4,115	17	,242	6,183	,000
	Within Groups	2,114	54	,039		
	Total	6,229	71			

Table 4.14
Analysis of variance (one way) on the effect of different substrates on the rooting of *P. chamaepeuce* leaf stem cuttings during May 2003 (Table 4.9). The rooting percentage data were transformed in angular values before statistical analysis.

		Sum of Squares	df	Mean Square	F	Sig.
Rooting	Between Groups	17638,203	6	2939,701	16,347	,000
	Within Groups	5035,344	28	179,834		
	Total	22673,547	34			
Number of roots	Between Groups	520,807	6	86,801	11,122	,000
	Within Groups	218,516	28	7,804		
	Total	739,323	34			
Length of roots	Between Groups	29,035	6	4,839	7,595	,000
	Within Groups	17,840	28	,637		
	Total	46,875	34			

Table 4.15
Analysis of variance (one way) on the effect of different substrates on the rooting of *P. chamaepeuce* leaf stem cuttings during May 2004 (Table 4.10). The rooting percentage data were transformed in angular values before statistical analysis.

		Sum of Squares	df	Mean Square	F	Sig.
Rooting	Between Groups	2,887	6	,481	6,691	,000
	Within Groups	2,014	28	,072		
	Total	4,901	34			
Number of roots	Between Groups	477,786	6	79,631	8,546	,000
	Within Groups	260,900	28	9,318		
	Total	738,686	34			
Length of roots	Between Groups	31,791	6	5,298	20,081	,000
	Within Groups	7,388	28	,264		
	Total	39,179	34			

Table 4.16
Analysis of variance on the effect of different combinations of BA/NAA on the number of adventitious shoots of *Ptilostemon chamaepeuce*, using micro-shoot explants from *in vitro* produced seedlings (Figure 4.12).
Dependent Variable: Number of shoots

Source	Sum of Squares	df	Mean Square	F	Sig.
BA	1009,644	3	336,548	68,246	,000
NAA	209,540	3	69,847	14,164	,000
BA * NAA	184,232	9	20,470	4,151	,000
Error	1277,231	259	4,931		
Total	7436,000	275			

Table 4.17
Analysis of variance on the effect of different combinations of BA/NAA on the length of adventitious shoots of *Ptilostemon chamaepeuce*, using micro-shoot explants from *in vitro* produced seedlings (Figure 4.13).
Dependent Variable: Length of shoots

Source	Sum of Squares	df	Mean Square	F	Sig.
BA	5,552	3	1,851	26,807	,000
NAA	3,470	3	1,157	16,754	,000
BA * NAA	2,016	9	,224	3,244	,001
Error	17,879	259	6,903E-02		
Total	101,158	275			

Table 4.18
Analysis of variance (one way) on the effect of different combinations of BA/NAA on the number and length of adventitious shoots of *Ptilostemon chamaepeuce*, using micro-shoot explants from *in vitro* produced seedlings (Figure 4.12 and 4.13).

		Sum of Squares	df	Mean Square	F	Sig.
Shoots	Between Groups	1386,823	15	92,455	18,561	,000
	Within Groups	1290,137	259	4,981		
	Total	2676,960	274			
Length	Between Groups	11,558	15	,771	11,170	,000
	Within Groups	17,867	259	,069		
	Total	29,426	274			

Table 4.19
Analysis of variance (one way) on effect of NAA and IBA on the rooting of *Ptilostemon chamaepeuce* micro-shoots (Figure 4.14, 4.15, 4.16).

		Sum of Squares	df	Mean Square	F	Sig.
Rooting	BetweenGroups	5,186	6	,864	4,303	,001
	Within Groups	14,061	70	,201		
	Total	19,247	76			
Number of roots	BetweenGroups	1700,132	6	283,355	3,849	,002
	Within Groups	5153,661	70	73,624		
	Total	6853,792	76			
Length of roots	Between Groups	432,213	6	72,035	2,781	,017
	Within Groups	1813,320	70	25,905		
	Total	2245,532	76			

Table 4.20
Analysis of variance on the effect of BAP and NAA on the regeneration of the number of shoots of *in vitro* culture of *Ptilostemon chamaepeuce* seedlings using McCown Woody Plant Medium after 3 months incubation (Table 4.12).

Dependent Variable: Number of shoots

Source	Sum of Squares	df	Mean Square	F	Sig.
BAP	50,650	3	16,883	19,083	,000
NAA	65,650	3	21,883	24,735	,000
BAP * NAA	23,900	9	2,656	3,002	,003
Error	127,400	144	,885		
Total	730,000	160			

Table 4.21
Analysis of variance on the effect of BAP and NAA on the lenght of shoots of *in vitro* culture of *Ptilostemon chamaepeuce* seedlings using McCown Woody Plant Medium after 3 months incubation (Table 4.12).

Dependent Variable: Length of shoots

Source	Sum of Squares	df	Mean Square	F	Sig.
BAP	459,319	3	153,106	11,753	,000
NAA	314,369	3	104,790	8,044	,000
BAP * NAA	172,856	9	19,206	1,474	,163
Error	1875,900	144	13,027		
Total	8263,000	160			

Table 4.22
Analysis of variance (one way) on the effect of BAP and NAA on the number and length of shoots of *in vitro* culture of *Ptilostemon chamaepeuce* seedlings using McCown Woody Plant Medium after 3 months of incubation (Table 4.12).

		Sumof Squares	df	Mean Square	F	Sig.
Number ofshoots	BetweenGroups	140,200	15	9,347	10,565	,000
	Within Groups	127,400	144	,885		
	Total	267,600	159			
Length of shoots	Between Groups	946,544	15	63,103	4,844	,000
	Within Groups	1875,900	144	13,027		
	Total	2822,444	159			

Table 4.23
Analysis of variance on the effect of BAP and NAA on the number of shoots of *in vitro* culture of *Ptilostemon chamaepeuce* seedlings using McCown Woody Plant Medium after 6 months of incubation (Table 4.12).

Dependent Variable: Number of shoots

Source	Sum of Squares	df	Mean Square	F	Sig.
BA	165,325	3	55,108	24,387	,000
NAA	93,225	3	31,075	13,752	,000
BA * NAA	79,825	9	8,869	3,925	,000
Error	325,400	144	2,260		
Total	1634,000	160			

Table 4.24
Analysis of variance on the effect of BAP and NAA on the length of shoots of *in vitro* culture of *Ptilostemon chamaepeuce* seedlings using McCown Woody Plant Medium after 6 months of incubation (Table 4.12).

Dependent Variable: Length of shoots

Source	Sum of Squares	df	Mean Square	F	Sig.
BA	238,250	3	79,417	14,643	,000
NAA	158,150	3	52,717	9,720	,000
BA * NAA	62,200	9	6,911	1,274	,256
Error	781,000	144	5,424		
Total	4408,000	160			

Table 4.25
Analysis of variance (one way) on the effect of BAP and NAA on the number and length of shoots of *in vitro* culture of *Ptilostemon chamaepeuce* seedlings using McCown Woody Plant Medium after 6 months of incubation (Table 4.12).

		Sum of Squares	df	Mean Square	F	Sig.
Shoots	Between Groups	338,375	15	22,558	9,983	,000
	Within Groups	325,400	144	2,260		
	Total	663,775	159			
Length	Between Groups	458,600	15	30,573	5,637	,000
	Within Groups	781,000	144	5,424		
	Total	1239,600	159			

Table 4.26
Analysis of variance (one way) on the effect of different preservative solution on the lifetime of heads and flower stems of *Ptilostemon chamaepeuce* flowers (Table 4.13).

		Sum of Squares	df	Mean Square	F	Sig.
Flower Opening %	Between Groups	60772,222	4	15193,056	67,832	,000
	Within Groups	10079,167	45	223,981		
	Total	70851,389	49			
Flower life	Between Groups	18,130	4	4,532	2,095	,097
	Within Groups	97,375	45	2,164		
	Total	115,505	49			
Vase life of inflorescence	Between Groups	1880,400	4	470,100	555,236	,000
	Within Groups	38,100	45	,847		
Half vase life of inflorescence	Total	1918,500	49			
	Between Groups	968,120	4	242,030	43,881	,000
	Within Groups	248,200	45	5,516		
	Total	1216,320	49			

Table 4.27
Analysis of variance (one way) on the effect of different stage of the flowers during collection on the opening of the heads and the preservation of *Ptilostemon chamaepeuce* flowers in the vase (Table 4.14).

		Sum of Squares	df	Mean Square	F	Sig.
Opening	Between Groups	9522,223	2	4761,111	11,550	,002
	Within Groups	4946,797	12	412,233		
	Total	14469,020	14			
Life time	Between Groups	126,400	2	63,200	6,427	,013
	Within Groups	118,000	12	9,833		
	Total	244,400	14			

Results of statistical analysis ov experiments described in chapter 5

Table 5.1
Analysis of variance on the effect of the concentration of IBA on the rooting of *Otanthus maritimus* stems tip cuttings in March (Table 5.1). Rooting percentage were subjected to arcsin transformation
Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	2,977	5	,595	110,035	,000
Block	,529	4	,132	24,460	,000
Error	,108	20	5,411E-03		
Total	12,442	30			

Table 5.2
Analysis of variance on the effect of the concentration of IBA on the number of roots of *Otanthus maritimus* stems tip cuttings in March (Table 5.1).
Dependent Variable: Number of roots

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	603,600	5	120,720	56,062	,000
Block	166,533	4	41,633	19,334	,000
Error	43,067	20	2,153		
Total	1688,000	30			

Table 5.3
Analysis of variance on the effect of the concentration of IBA on the rooting of *Otanthus maritimus* stems segment cuttings in March (Table 5.1). Rooting percentage were subjected to arcsin transformation.
Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	1,781	5	,356	19,668	,000
Block	,347	4	8,669E-02	4,787	,007
Error	,362	20	1,811E-02		
Total	13,522	30			

Table 5.4
Analysis of variance on the effect of the concentration of IBA on the number of roots of *Otanthus maritimus* stems segment cuttings in March (Table 5.1).
Dependent Variable: Number of roots

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	892,967	5	178,593	21,621	,000
Block	15,200	4	3,800	,460	,764
Error	165,200	20	8,260		
Total	3107,000	30			

Table 5.5
Analysis of variance on the effect of the concentration of IBA on the rooting of *Otanthus maritimus* stems tip cuttings in March (Table 5.2). Rooting percentage were subjected to arcsin transformation
Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	2,977	5	,595	110,035	,000
Block	,529	4	,132	24,460	,000
Error	,108	20	5,411E-03		
Total	12,442	30			

Table 5.6
Analysis of variance on the effect of the concentration of IBA on the number of roots of *Otanthus maritimus* stems tip cuttings in March (Table 5.2).
Dependent Variable: Number of roots

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	603,600	5	120,720	56,062	,000
Block	166,533	4	41,633	19,334	,000
Error	43,067	20	2,153		
Total	1688,000	30			

Table 5.7
Analysis of variance on the effect of the concentration of IBA on the rooting of *Otanthus maritimus* stems segment cuttings in March (Table 5.2). Rooting percentage were subjected to arcsin transformation.
Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	1,781	5	,356	19,668	,000
Block	,347	4	8,669E-02	4,787	,007
Error	,362	20	1,811E-02		
Total	13,522	30			

Table 5.8
Analysis of variance on the effect of the concentration of IBA on the number of roots of *Otanthus maritimus* stems segment cuttings in March (Table 5.2).
Dependent Variable: Number of roots

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	892,967	5	178,593	21,621	,000
Block	15,200	4	3,800	,460	,764
Error	165,200	20	8,260		
Total	3107,000	30			

Table 5.9
Analysis of variance (one way) on the effect of different substrates on the rooting of *Otanthus maritimus* stems tips cuttings (Table 5.3). The rooting percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Rooting

Source	Sum of Squares	df	Mean Square	F	Sig.
Substrate	1,114	6	,186	9,026	,000
Block	,169	4	4,236E-02	2,059	,118
Error	,494	24	2,057E-02		
Total	20,292	35			

Table 5.10
Analysis of variance (one way) on the effect of different substrates on the number of roots of *Otanthus maritimus* stems tips cuttings (Table 5.3).
Dependent Variable: Number of roots.

Source	Sum of Squares	df	Mean Square	F	Sig.
Substrate	96,835	6	16,139	3,039	,023
Block	11,801	4	2,950	,556	,697
Error	127,451	24	5,310		
Total	811,390	35			

Table 5.11
Analysis of variance (one way) on the effect of different concentration of IBA on the rooting of *Otanthus maritimus* stems tip cuttings in June (Table 5.4). The rooting percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Rooting

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	9,366	5	1,873	58,759	,000
Block	,208	3	6,929E-02	2,174	,134
Error	,478	15	3,188E-02		
Total	23,225	24			

Table 5.12
Analysis of variance (one way) on the effect of different concentration of IBA on the number of roots of *Otanthus maritimus* stems tips cuttings in June (Table 5.4).
Dependent Variable: Number of roots.

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	2166,633	5	433,327	4,304	,013
Block	91,383	3	30,461	,303	,823
Error	1510,237	15	100,682		
Total	8218,180	24			

Results of statistical analysis ov experiments described in chapter 6

Table 6.1
Analysis of variance (one way) on the germination of *Prasium majus* seeds in two substrates in an unheated greenhouse after 31 days of incubation (Table 6.1). The germination percentage data were transformed in angular values before statistical analysis.

		Sum of Squares	df	Mean Square	F	Sig.
T_{50}	Between Groups	1,210	1	1,210	1,071	,409
	Within Groups	2,260	2	1,130		
	Total	3,470	3			
Germination	Between Groups	,000	1	,000	1,071	,409
	Within Groups	,000	2	,000		
	Total	,001	3			

Table 6.2
Analysis of variance on the effect of temperature and lighting on the percent germination of *Prasium majus* seeds (Table 6.2). The germination percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
Lighting	3,736E-02	1	3,736E-02	7,846	,016
Temperature	1,101E-02	2	5,506E-03	1,156	,347
Lighting * Temperature	1,034E-02	2	5,172E-03	1,086	,368
Error	5,713E-02	12	4,761E-03		
Total	11,796	18			

Table 6.3
Analysis of variance on the effect of temperature and lighting on the T_{50} of *Prasium majus* seeds (Table 6.2).
Dependent Variable: T_{50}

Source	Sum of Squares	df	Mean Square	F	Sig.
Lighting	3,209	1	3,209	,105	,752
Temperature	56,023	2	28,012	,916	,426
Lighting * Temperature	29,781	2	14,891	,487	,626
Error	366,987	12	30,582		
Total	6728,000	18			

Table 6.4
Analysis of variance (one way) on the effect of temperature and lighting on the germination and T_{50} of *Prasium majus* seeds (Table 6.2). The germination percentage data were transformed in angular values before statistical analysis.

		Sum of Squares	df	Mean Square	F	Sig.
Germination	Between Groups	,059	5	,012	2,466	,093
	Within Groups	,057	12	,005		
	Total	,116	17			
T_{50}	Between Groups	89,013	5	17,803	,582	,714
	Within Groups	366,987	12	30,582		
	Total	456,000	17			

Table 6.5
Analysis of variance on the effect of different concentration of IBA on the rooting of *Prasium majus* stem tip cuttings (Table 6.3). The germination percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Rooting of stem tip cuttings

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	1,603	6	,267	2,623	,073
Block	4,738E-02	2	2,369E-02	,233	,796
Error	1,222	12	,102		
Total	9,380	21			

Table 6.6
Analysis of variance on the effect of different concentration of IBA on the rooting of *Prasium majus* stem segment cuttings (Table 6.3). The germination percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Rooting of stem segment

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	,954	6	,159	3,780	,024
Block	5,669E-02	2	2,834E-02	,674	,528
Error	,505	12	4,208E-02		
Total	8,756	21			

Table 6.7
Analysis of variance on the effect of different concentration of IBA on the number of roots of *Prasium majus* stem tip cuttings (Table 6.3).
Dependent Variable: Number of root of stem tip cuttings

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	174,759	6	29,127	3,362	,035
Block	29,261	2	14,630	1,689	,226
Error	103,952	12	8,663		
Total	657,710	21			

Table 6.8
Analysis of variance on the effect of different concentration of IBA on the number of roots of *Prasium majus* stem segment cuttings (Table 6.3).
Dependent Variable: Number of root of stem segment cuttings

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	395,170	6	65,862	4,240	,016
Block	68,454	2	34,227	2,203	,153
Error	186,399	12	15,533		
Total	1061,880	21			

Table 6.9
ANOVA on the effect of different concentration of IBA on the length of roots of *Prasium majus* stem tip cuttings (Table 6.3).
Dependent Variable: Length of roots of stem tip cuttings

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	2,763	6	,460	,183	,976
Block	1,261	2	,630	,250	,783
Error	30,266	12	2,522		
Total	73,240	21			

Table 6.10
Analysis of variance on the effect of different concentration of IBA on the length of roots of *Prasium majus* stem segment cuttings (Table 6.3).
Dependent Variable: Length of roots of stem segment cuttings

Source	Sum of Squares	df	Mean Square	F	Sig.
IBA	5,890	6	,982	1,148	,393
Block	1,390	2	,695	,813	,466
Error	10,258	12	,855		
Total	40,273	21			

Table 6.11
Analysis of variance (one way) on the effect of different concentration of IBA on the rooting, number and length of roots of *P. majus* stem tip or segment cuttings (Table 6.3). The germination percentage data were transformed in angular values before statistical analysis.

		Sum of Squares	df	Mean Square	F	Sig.
Rooting of tip cuttings	Between Groups	1,603	6	,267	2,946	,045
	Within Groups	1,270	14	,091		
	Total	2,872	20			
Rooting of segment cuttings	Between Groups	,954	6	,159	3,965	,016
	Within Groups	,562	14	,040		
	Total	1,516	20			
Number of roots of tip cuttings	Between Groups	174,759	6	29,127	3,061	,040
	Within Groups	133,213	14	9,515		
	Total	307,972	20			
Number of roots of tip cuttings	Between Groups	395,170	6	65,862	3,618	,022
	Within Groups	254,853	14	18,204		
	Total	650,023	20			
Length of roots of tip cuttings	Between Groups	2,763	6	,460	,204	,970
	Within Groups	31,527	14	2,252		
	Total	34,290	20			
Length of roots of segment cuttings	Between Groups	5,890	6	,982	1,180	,371
	Within Groups	11,648	14	4,832		
	Total	17,538	20			

Table 6.12
Analysis of variance effect of different substrates on the rooting and number of roots of *Prasium majus* stems cuttings (Table 6.4). The germination percentage data were transformed in angular values before statistical analysis.
Dependent Variable: Rooting

Source	Sum of Squares	df	Mean Square	F	Sig.
Substrate	6,782	6	1,13	9,367	0
Block	0,894	5	0,179	1,481	0,225
Error	3,62	30	0,121		
Total	24,244	42			

Table 6.13
Analysis of variance effect of different substrates on the number of roots of *Prasium majus* stems cuttings (Table 6.4).
Dependent Variable: Number of roots

Source	Sum of Squares	df	Mean Square	F	Sig.
Substrate	407,486	6	67,914	9,139	,000
Block	28,774	5	5,755	,774	,576
Error	222,926	30	7,431		
Total	1307,400	42			

Results of statistical analysis ov experiments described in chapter 7

Table 7.1

Analysis of variance on T_{50} of *Convolvulus dorycnium* seeds in peat moss + perlite (1:1, v/v) and compost germination medium in an unheated greenhouse in September 2004, October 2004 and February 2005 (Table 7.1). The germination percentage data were transformed in angular values before statistical analysis.

Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
Months	,141	2	7,044E-02	8,043	,006
Substrate	3,405E-05	1	3,405E-05	,004	,951
Months * Substrate	4,178E-03	2	2,089E-03	,239	,791
Error	,105	12	8,757E-03		
Total	11,100	18			

Table 7.2

Analysis of variance on T_{50} of *Convolvulus dorycnium* seeds in peat moss + perlite (1:1, v/v) and compost germination medium in an unheated greenhouse in September 2004, October 2004 and February 2005 (Table 7.1).

Dependent Variable: T_{50}

Source	Sum of Squares	df	Mean Square	F	Sig.
Months	53,208	2	26,604	2,343	,138
Substrate	16,245	1	16,245	1,431	,255
Months * Substrate	25,003	2	12,502	1,101	,364
Error	136,240	12	11,353		
Total	9320,710	18			

Table 7.3

Analysis of variance (one way) on mean germination and T_{50} showing effect of time seeding and substrates of *Convolvulus dorycnium* seeds (Table 7.1).

		Sum of Squares	df	Mean Square	F	Sig.
Germination	Between Groups	,145	5	,029	3,314	,041
	Within Groups	,105	12	,009		
	Total	,250	17			
T_{50}	Between Groups	94,456	5	18,891	1,664	,218
	Within Groups	136,240	12	11,353		
	Total	230,696	17			

Table 7.4

Analysis of variance on % germination of *Convolvulus dorycnium* in different temperatures under light/dark or dark conditions (Table 7.2). The germination percentage data were transformed in angular values before statistical analysis.

Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
Temperature	1,856	4	,464	40,028	,000
Lighting	,125	1	,125	10,761	,004
Temperature * Lighting	,105	3	3,485E-02	3,007	,057
Error	,209	18	1,159E-02		
Total	19,099	27			

Table 7.5
Analysis of variance on T_{50} of *Convolvulus dorycnium* seed germination in different temperatures under light/dark or dark conditions (Table 7.2).
Dependent Variable: T_{50}

Source	Sum of Squares	df	Mean Square	F	Sig.
Temperature	702,706	4	175,676	12,421	,000
Lighting	9,754	1	9,754	,690	,418
Temperature * Lighting	43,615	3	14,538	1,028	,405
Error	240,438	17	14,143		
Total	6953,160	26			

Table 7.6
Analysis of variance (one way) on mean % germination and T_{50} showing effect of temperature and lighting of *Convolvulus dorycnium* seed germination (Table 7.2).

		Sum of Squares	df	Mean Square	F	Sig.
Germination	Between Groups	1,960	8	,245	21,145	,000
	Within Groups	,209	18	,012		
	Total	2,169	26			
T_{50}	Between Groups	748,166	8	93,521	6,612	,001
	Within Groups	240,438	17	14,143		
	Total	988,605	25			

Results of statistical analysis ov experiments described in chapter 8

Table 8.1
Analysis of variance on percent germination of *Campanula pelviformis* in different temperatures under light/dark or dark conditions (Table 8.2). The germination percentage data were transformed in angular values before statistical analysis.

Source	Sum of Squares	df	Mean Square	F	Sig.
Temperature	2,941	3	,980	333,274	,000
Lighting	,284	1	,284	96,489	,000
Temperature * Lighting	,100	3	3,342E-02	11,359	,000
Error	4,707E-02	16	2,942E-03		
Total	26,090	24			

Table 8.2
Analysis of variance on time germination in days (T_{50}) of *Campanula pelviformis* in different temperatures under light/dark or dark conditions (Table 8.2).

Source	Sum of Squares	df	Mean Square	F	Sig.
Temperature	233,951	3	77,984	55,096	,000
Lighting	4,950	1	4,950	3,497	,080
Temperature * Lighting	89,741	3	29,914	21,134	,000
Error	22,647	16	1,415		
Total	7725,810	24			

Table 8.3
Analysis of variance (one way) on the effect of temperature and lighting on the germination and T_{50} of *Campanula pelviformis* seeds (Table 8.2). The germination percentage data were transformed in angular values before statistical analysis.

		Sum of Squares	df	Mean Square	F	Sig.
Germination	Between Groups	3,325	7	,475	161,484	,000
	Within Groups	,047	16	,003		
	Total	3,372	23			
T_{50}	Between Groups	328,643	7	46,949	33,170	,000
	Within Groups	22,647	16	1,415		
	Total	351,290	23			

Results of statistical analysis ov experiments described in chapter 9

Table 9.1

Analysis of variance (one way) on the effect of temperature on the germination of *Sternbergia sicula* seeds in dark conditions (Table 9.10). The germination percentage data were transformed in angular values before statistical analysis.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,912	4	,228	35,654	,000
Within Groups	,064	10	,006		
Total	,976	14			

Table 9.2

Analysis of variance (one way) on the effect of temperature and light on *Sternbergia sicula* seeds germination after imbibitions in vermiculite for one month at 20°C (Table 9.11). The germination percentage data were transformed in angular values before statistical analysis.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,612	3	,204	29,933	,000
Within Groups	,055	8	,007		
Total	,666	11			

Table 9.3

Analysis of variance (one way) on the effect of the size of the bulbs and the cross cutting on daughter bulbs production (Table 9.12).

		Sum of Squares	df	Mean Square	F	Sig.
Bulblets	Between Groups	632,375	5	126,475	40,472	,000
	Within Groups	56,250	18	3,125		
	Total	688,625	23			
Weight	Between Groups	49,022	5	9,804	262,146	,000
	Within Groups	,673	18	,037		
	Total	49,695	23			

Table 9.4

Analysis of variance (one way) on the effect cross cutting at the base of the bulb and two BA concentrations on bulblet production (Table 9.13).

		Sum of Squares	df	Mean Square	F	Sig.
Bulblets	Between Groups	382,500	3	127,500	23,359	,000
	Within Groups	65,500	12	5,458		
	Total	448,000	15			
Weight	Between Groups	42,226	3	14,075	297,060	,000
	Within Groups	,569	12	,047		
	Total	42,794	15			

Table 9.5

Analysis of variance (one way) on the effect of the BA and NAA on the mean number of *Sternbergia sicula* bulblets and the weight of bulblets per scale explant produced *in vitro* (Figures 9.3 and 9.4)

Number of bulblets

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	83,468	5	16,694	4,352	,001
Within Groups	655,865	171	3,835		
Total	739,333	176			

Weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,272	5	,054	9,298	,000
Within Groups	2,218	379	,006		
Total	2,490	384			

Results of statistical analysis ov experiments described in chapter 10

Table 10.1

Analysis of variance (one way) on the correlation between the circumference of the *Colchicum macrophyllum* corms and the number of flowers, the total days of the anthesis of the corms and the life span of the flowers (Table 10.6).

		Sum of Squares	df	Mean Square	F	Sig.
Flower	Between Groups	4,235	1	4,235	3,529	,080
	Within Groups	18,000	15	1,200		
	Total	22,235	16			
Anthesis	Between Groups	97,243	1	97,243	5,385	,035
	Within Groups	270,875	15	18,058		
	Total	368,118	16			
Life span	Between Groups	31,450	1	31,450	16,936	,001
	Within Groups	27,855	15	1,857		
	Total	59,305	16			

Table 10.2

Analysis of variance (one way) on the effect of temperature on the germination of *C. macrophyllum* seeds in peat moss and perlite (1:1, v/v) and compost in an unheated greenhouse during November 2003 (Figure 10.2). The germination percentage data were transformed in angular values before statistical analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,190	1	,190	5,635	,049
Within Groups	,236	7	,034		
Total	,425	8			

Table 10.3

Analysis of variance on the effect the temperature and lighting on the germination of *Colchicum macrophyllum* seeds after imbibition in 5°C and room temperature (20±5°C) for one month (Figure 10.3). The germination percentage data were transformed in angular values before statistical analysis

Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
Lighting	0,171	1	0,171	14,349	0,003
Temperature	2,104	2	1,052	88,464	0,000
Lighting * Temperature	0,215	2	0,108	9,045	0,004
Error	0,143	12	1,19E-02		
Total	6,572	18			

Table 10.4

Analysis of variance on the effect the temperature and lighting on the germination of *Colchicum macrophyllum* seeds after imbibition in 5°C and room temperature (20±5°C) for two months (Figure 10.3). The germination percentage data were transformed in angular values before statistical analysis

Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
Lighting	0,761	1	0,761	34,131	0,000
Temperature	1,695	2	0,848	38,033	0,000
Lighting * Temperature	0,252	2	0,126	5,644	0,019
Error	0,267	12	0,02228		
Total	10,868	18			

Table 10.5
Analysis of variance on the effect the temperature and lighting on the germination of *Colchicum macrophyllum* seeds after imbibition in 5°C and room temperature (20±5°C) three months (Figure 10.3). The germination percentage data were transformed in angular values before statistical analysis
Dependent Variable: Germination

Source	Sum of Squares	df	Mean Square	F	Sig.
Lighting	0,302	1	0,302	29,944	0,000
Temperature	2,927	2	1,464	145,324	0,000
Lighting * Temperature	0,134	2	6,72E-02	6,676	0,011
Error	0,121	12	1,01E-02		
Total	10,768	18			

Table 10.6
Analysis of variance (one way) on the effect of the temperature and lighting on the germination of *Colchicum macrophyllum* seeds after imbibition in 5°C and room temperature (20±5°C) for one two or three months (Figure 10.3). The germination percentage data were transformed in angular values before statistical analysis

		Sum of Squares	df	Mean Square	F	Sig.
1 Month	Between Groups	3,801	11	0,346	39,287	0
	Within Groups	0,211	24	0,009		
	Total	4,012	35			
2 Months	Between Groups	6,654	11	0,605	54,292	0
	Within Groups	0,267	24	0,011		
	Total	6,921	35			
3 Months	Between Groups	6,681	11	0,607	135,138	0
	Within Groups	0,108	24	0,004		
	Total	6,789	35			

Table 10.7
Analysis of variance (one way) on the effect of the temperature and lighting on T_{50} of *C. macrophyllum* seeds germination after one two or three month's imbibition of seeds in wet vermiculite in room temperature (Table 10.8).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2655,488	11	241,408	15,108	0
Within Groups	383,5	24	15,979		
Total	3038,988	35			

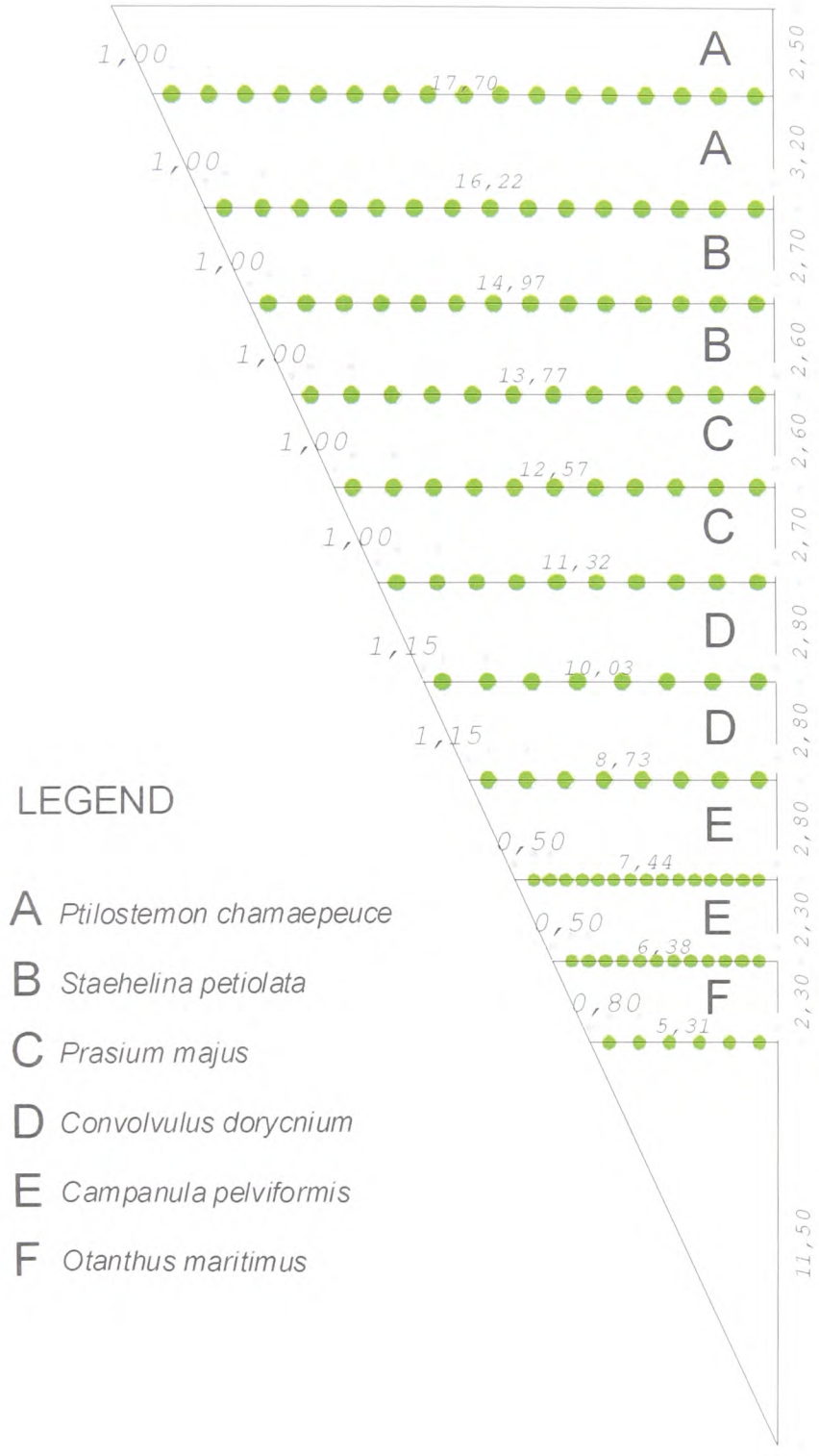
Table 10.8
Analysis of variance (one way) on the effect of wounding and the size of the *Colchicum macrophyllum* corms on the number of produced bulblets (Table 10.9).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	40,813	3	13,604	33,147	0
Within Groups	24,625	60	0,41		
Total	65,438	63			

APPENDIX 4

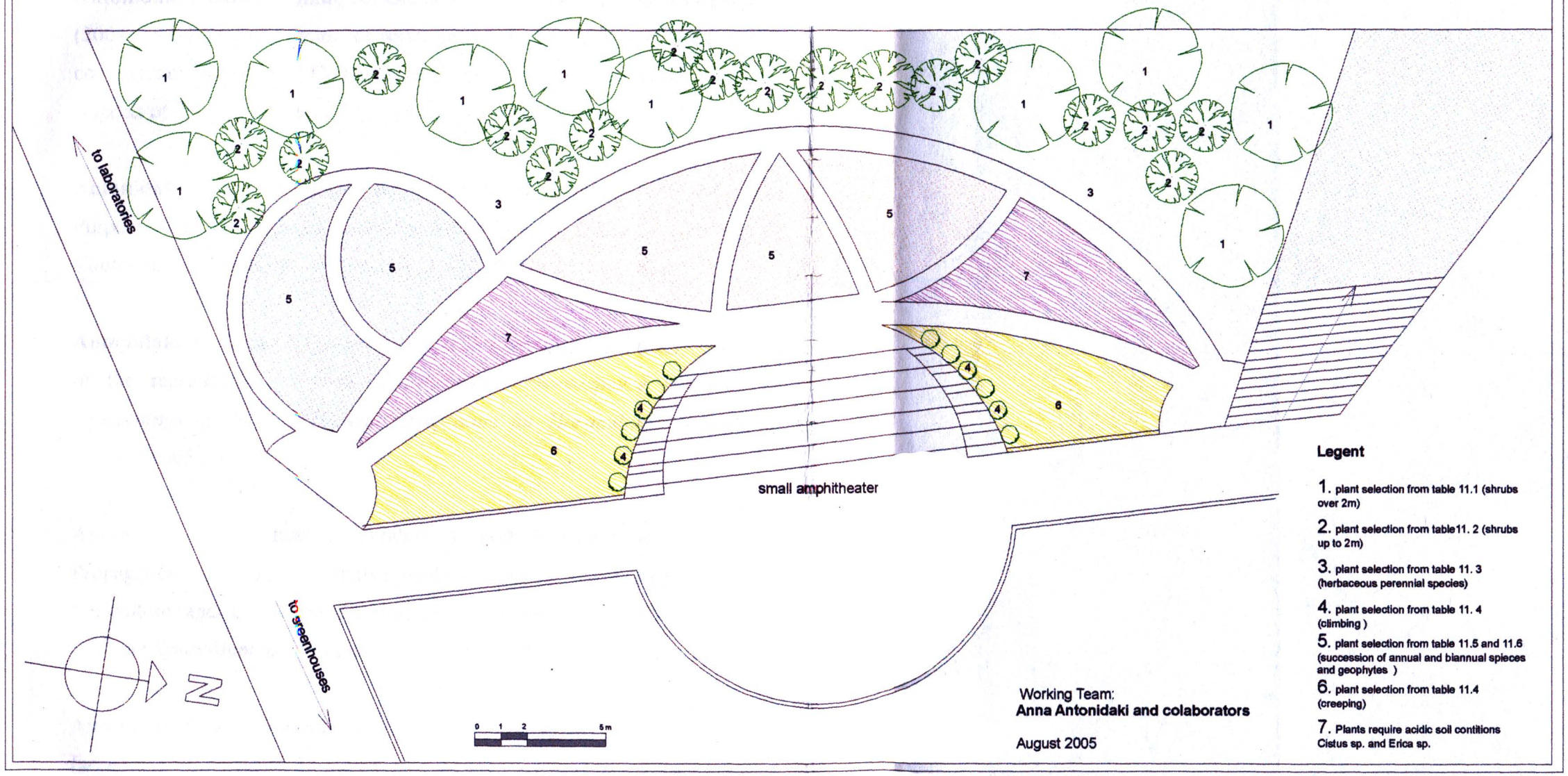
DESIGN OF THE EXPERIMENTAL PLOT IN TEI

Experimental design in the farm of TEI Crete in Heraklion for six native plants of Crete.



APPENDIX 5
MASTER PLAN

EDUCATIONAL PARK OF TEI CRETE IN HERAKLION
THEMATIC GARDENS:
1. NATIVE PLANTS OF CRETE



Publications from the Research Results of the Thesis

Antonidaki-Giatromanolaki, A., Papadimitriou, M. and Aliferis, A. (2004). The propagation of the plants *Otanthus maritimus* L. and *Staehelina petiolata* L. by stem cuttings (in Greek): *Proceedings of the 21st Hellenic Symposium for Horticultural Science,* 8-10 October 2003. Volume **11**(2) pp. 401- 404.

Antonidaki-Giatromanolaki, A., Orchard, J. E., Vlahos, J. E. and Dragassaki, M. (2004). In vitro regeneration of some native plants of Crete for possible use in commercial floriculture. Competence Gene Transfer and Expression. Special Aspects of micropropagation WG1. *COST 843* TEI of Crete.

Antonidaki, A. and Giatromanolaki, M. (2006). Designing a Park for Educational Purposes in Floriculture and Landscape Architecture (in Greek). *Proceedings of the Conference in Landscape Architecture*, 11-14 May 2005. Volume IV. 272-273.

Antonidaki A., Orchard, J., Vlahos, J. and Dragassaki, M. (in press). Investigation of the reproduction *in vitro* of *Ptilostemon chamaepeuce* (L) (in Greek). *Proceedings of the 22nd Hellenic Symposium for Horticultural Science,* 19-21 October 2005.

Antonidaki A., Orchard, J., Vlahos, J. and Papadimitriou, M. (in press). Propagation studies on some native plants of Crete for possible use in commercial floriculture and Landscape Architecture (in Greek). *Proceedings of the 22nd Hellenic Symposium for Horticultural Science,* 19-21 October 2005.

Antonidaki-Giatromanolaki, A., Orchard, J. E., Dragassaki, M. and Vlahos, J. C. (accepted). Propagation of *Sternbergia sicula*, by Seed and Tissue Culture. 27 International Horticultural Congress: 13-19 August, 2006, Seoul, Korea.