Breeding Sciences 00: 000-000

Research Paper

Evaluation of some rice varieties and landraces cultivated in Nigeria for drought tolerance based on phenotypic traits and their association with SSR polymorphisms

Celestine A. Afiukwa*[1], Julius O. Faluyi†[2], Christopher J Atkinson†[3], Benjamin E. Ubi†[1,4], David O. Igwe†[1] and Richard O. Akinwale†[5]

[1] *Department of Biotechnology, Faculty of Sciences, Ebonyi State University, Abakaliki, Nigeria*

[2] *Department of Botany, Faculty of Biological Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria*

[3] *Department of Agriculture, Health and Environment, Natural Resources Institute, University of Greenwich, Kent, ME4 4TB, UK*

[4] *Molecular Breeding Laboratory, Arid Land Research Center, Tottori University, 1390 Hamasaka, Tottori 680-0001, Japan;*

[5] *Department of Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria*

Corresponding author (email: afiukwa@yahoo.com)

Running title: *Breeding for rice drought tolerance using markers*

Abstract

Breeding for drought tolerance based on direct selection for high grain yield under drought has been hindered by the complex nature of drought tolerance mechanisms and the approaches used. Molecular marker-based approaches are a promising alternative. In this study, 30 rice (*Oryza sativa* L.) accessions cultivated in Nigeria were screened in a greenhouse for drought tolerance based on morpho-physiological traits and assessed for DNA polymorphisms using SSR markers for possible marker – trait associations. Our results showed that five Nigerian rice landraces (IJS-02, IJS-09, IK-PS, IK-FS and Lad-f) and three improved varieties (FARO-44, IR-119 and IWA-8) were highly drought tolerant. Sixteen of 20 markers tested yielded amplified products and generated 221 alleles (4 – 5 alleles per marker) with PIC values ranging from 0.24 - 0.95 per marker. Although, none of the markers were present in all the accessions that were found to be highly drought tolerant with respect to any particular morph-physiological trait, some of the markers (RM252, RM331, RM432, RM36, RM525, RM260 and RM318) amplified alleles unique to nearly all the tolerant Nigerian landraces (IJS-02, IJS-09, IK-PS, IK-FS) and FARO-11, a drought tolerant control. These markers may be usefully exploited for molecular breeding of rice for drought tolerance.

Key Words: Nigeria, climate change, rice, drought stress, drought tolerance, SSR markers, molecular breeding.

Introduction

Rice is recognized as one of the most important staple food crop, accounting for more than half of human caloric intake globally. It is generally valued for its high nutritional benefits apart from being rich in calories, it is high in fibre, vitamins and minerals and low in cholesterol and sodium, suggesting it is a healthy source of energy. Asia is the largest producer and consumer of rice (Sellamuthu *et al.* 2011, Khush 2005). In 2009, Nigeria was ranked 12th in the world's list of rice-consuming countries, while it is ranked 17th globally, third in Africa and first in West Africa, as producers of rice (FAO 2011). However, Nigerian rice production does not meet current demand or have the capacity to cope with an expanding population. Production is also suggested to be declining due to effects of climate change particularly through drought, heat, flooding and pests and diseases (Rosenzweig *et al.* 2000).

Drought is recognized as a major abiotic stress that limits rice productivity and adversely affects grain quality in rain-fed and upland ecosystems (Bimpong *et al.* 2011, Tao *et al.* 2006, Yang 2008). Rice is most sensitive to drought stress during reproductive development at which time moderate water shortages can result in a significant reduction in grain yield (O'Toole 1982, Venuprasad *et al.* 2008). The extent to which drought affects yield varies depending on the intensity and the time of occurrence of the stress within the crop growth cycle (Srividhya *et al.* 2011). Yield losses ranging from 15 to 50% have been reported (Srividhya *et al.* 2011, Pandey and Bhandari 2009). The situation becomes more serious with increasing global climate change. Hence, the development of high-yielding and drought- tolerant varieties for rain-fed regions is a major goal of rice breeding.

Plant responses to drought are well known and believed to be complex involving numerous changes at the physiological, biochemical and molecular levels (Braga *et al.* 2015, Atkinson and Urwin 2012, Xu *et al.* 2010). Tolerance to drought stress is therefore the result of expression of a number of traits over the stress time period. Thus, no single trait is likely to improve crop productivity, in isolation, in response to water- deficits (Kamoshita *et al.* 2008, Farooq *et al.*, 2009). Various traits associated with rice performance under drought stress, including root morphology, root penetrability and distribution of roots; leaf rolling; reduced leaf area; early flowering and early seed maturity; osmotic adjustment (accumulation of compatible solutes such as proline and soluble sugars); and increased production of ABA and stomatal closure, have been reported (Price *et al.* 2000, Bimpong *et al.* 2011). Selection and use of these traits in breeding programmes could lead to sustainable production in drought prone regions (Nguyen *et al.*, 1997). The wild species of rice, though phenotypically inferior in agronomic traits, are important reservoirs of many useful genes, especially genes for tolerance to major biotic and abiotic stresses, and can be used to improve the cultivated species for these desired traits through breeding (Sanchez *et al.* 2014, Ali *et al.* 2010). Genes from *O. glaberrima* were used to develop NERICA lines with improved yield, earliness, weed competitive ability and tolerance to abiotic stresses, by interspecific hybridization with *O. sativa* (Sanchez *et al.* 2014).

Complex responses to drought coupled with often unreliable and labour-intensive conventional phenotyping have made it difficult to breed rice varieties with improved drought tolerance (Ingram *et al.* 1994). To overcome this problem, molecular markers have been utilized to identify genotypes having traits directly related to drought tolerance and the strategy is already well developed and known to be more efficient than conventional variety improvement. Development of molecular markers and their use for the genetic dissection of agronomically-important traits has become a powerful approach for studying the inheritance of complex plant traits such as drought tolerance (Suji *et al.* 2011). The use of molecular markers for the selection of complex breeding traits offers greater selection accuracy with less labour and time inputs, and enables assemblage of different target traits into a single cultivar. Hence, use of molecular markers to detect QTLs controlling drought tolerance related traits has the potential to accelerate breeding for drought tolerance and will ultimately contribute to reducing the problem of food security aggravated by changing climatic conditions.

Substantial efforts have been made towards the identification of QTLs underlying traits associated with drought tolerance in rice chromosomes using molecular markers. Zheng *et al*. (2000) identified two QTLs for root penetration ability and root thickness that co-localizes with rice SSR markers RM252 on rice chromosome 4 and RM60 on chromosome 3. Rice QTLs for root growth rate and root penetration ability have also been mapped using RFLP and AFLP markers (Price and Tomas, 1997, Price *et al.* 2000). The co-location of QTLs for root traits with those of yield under drought, has allowed combined selection of both traits (Salunkhe *et al*. 2011). Warburton *et al*. (2011) reported 131 SNP-trait associations for drought-related traits in rice. Deshmukh (2012), found 12 SSR markers that were strongly associated with root traits under drought and 14 SSR markers that were significantly associated with yield and its components under drought. Several other studies of molecular markers associated with drought related traits have also been reported in the literature, indicating that these markers could be usefully utilized in the molecular breeding of rice for improved drought tolerance.

The objectives of this study are to evaluate some of the SSR markers reportedly linked to drought tolerance traits in rice varieties and landraces cultivated in Nigeria as part of the development of a protocol for marker-assisted breeding for drought tolerance for sustainable rice production in the face of increasing climate change.

Materials and Methods

Plant Materials

Thirty *O. sativa* accessions from different regions of Nigeria including a drought tolerant control and a susceptible control as well as some improved varieties were used (Table 1). Seeds of the local rice accessions selected based on popularity were obtained from farmers in the Nigeria States of Ebonyi, Enugu, Ekiti and Osun, while the improved varieties were obtained from the AfricaRice and the Alliance for Green Revolution in Africa (AGRA) through the Biotechnology Research and Development Centre, Ebonyi State University, Abakaliki, Nigeria.

Drought screening

Drought screening was conducted in a greenhouse of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria where temperature ranged between 23 – 39^{o}C during April and October, 2015. Rice seeds were germinated in plastic pots containing sandy-loam soil. At the 3-leaf stage corresponding to around 2-weeks after sowing, seedlings were transplanted (one plant per pot) into polyethylene bag growing pots measuring 30 x 20 cm with a volume of about 9,420cm^3. Drainage holes were provided at the bottom and the pot plants arranged in a completely randomized block design with ten replicates. The plants were irrigated for 45 days after sowing (DAS) by daily watering to slightly above soil saturation and thereafter five replicates each were assigned to one of two treatments – the control (well-watered) and those exposed to drought by withholding of water. Adequate irrigation was maintained for the control treatment, while irrigation was withheld for 8 days in the drought stressed treatment during which the soil volumetric moisture content (SVMC measured using ASTM D-2216, 2014) declined from 19.5 ± 0.7% to 2.2 ± <0.1%.

The physical and chemical properties of the soil used are shown in Table 2. Compound fertilizer (NPK 15–15–15) was applied at the rate of 4.4 g pot^{-1} corresponding to 200 kg ha^{-1} in two applications (2 weeks after transplanting and at panicle initiation stage).

The plants were kept weed-free throughout the period of the experiment by regular hand weeding. Irrigation was resumed on the drought-stressed group after the 8 days and continued till maturity at the same rate as that in the control treatment (Ndjiondjop *et al.* 2010).

Agro-botanical traits measurements

Data on plant height, leaf length and width, panicle length, number of primary branches, number of spikelet per panicle, spikelet fertility, panicle density, grain weight per plant and 1000 seed weight (adjusted to 14% moisture content) were collected using the procedures specified in *Standard Evaluation System for Rice* (SES) (IRRI, 1996) and those of Deshmukh (2012). After grain harvest, plants were harvested and the soil was washed off and the shoots and roots separated and wrapped in aluminium foil for oven drying at 80°C to a constant weight. Shoot and root dry weights were recorded and used to calculate root/shoot ratios (Deshmukh 2012). Grain length and breadth were measured using Vernier callipers. Days to 50% booting and heading or flowering and days to maturity were also recorded using the SES procedures (IRRI, 1996).

Measurement of leaf water potential (Ψ)

Leaf water potential (LWP) was measured on the youngest fully developed leaf on the main tiller using *WP4C Dewpoint psychrometer* (Decagon Devices, Inc., USA) following the procedures used by Xiong *et al.* (2015).

Genotyping using SSR markers

Genomic DNA was extracted from two week old leaves of the rice accessions grown in greenhouse using Zymo Research plant/seed DNA extraction kit (Vos *et al.* 2007). Twenty SSR primers were tested to find polymorphisms among the rice accessions. The SSR markers were chosen based on previous reports of their association with drought tolerant traits in rice (Deshmukh 2012, Temnykh*et al.* 2001, Zheng *et al.* 2000). The list of SSR primers used for the study is shown in Table 3.

The PCR mixture was composed of 2.0 µl of DNA template (50 ng 25 μl^{-1}), 1.0 µl each of the forward and reverse primers (5 µM), 1.5 µl of $MgCl_2$ (50 mM), 2.0 µl of 10 X Taq buffer, 0.4 µl of 2.5 mM dNTP mix, 1.0 µl DMSO (dimethyl sulfoxide), 0.1 µl of 5 units Taq DNA polymerase and made up to 25.0 µl with nuclease-free water. The PCR profile was 94°C for 2 min followed by 30 cycles of 94°C for 30 sec, 55°C for 45 sec and 72°C for 45 sec with a final extension at 72°C for 5 min. The amplification products were resolved on 8% denaturing polyacrylamide gel and the DNA fragments were revealed by silver staining and captured using a gel imager. The presence or absence of specific amplification bands were scored and used to determine number of alleles per primer and polymorphism information content (PIC) value of each microsatellite locus and to generate a dendrogram of the 30 rice accessions.

Statistical analysis

Analysis of Variance (ANOVA) was performed using SAS software version 9.0, on the morpho-physiological trait values to compare the performances of the accessions under the imposed drought; percentage changes in the mean values of the traits due to stress or drought tolerance index (DI) and stress index (I_s) based on a combination of traits values were also computed using the formulae shown below and were used to rank the performances of the accessions under the drought; principal components analysis was also performed on the drought tolerance index values to determine traits that contributed most to the observed differences in drought performance among the rice accessions. Population genetic structure was determined using a dendrogram of the SSR data by the UPGMA method using

Numerical Taxonomy System (NTSYSpc) version 2.02, while association analysis was carried out by physically comparing the population clustering pattern based on individual SSR marker data and the pattern of phenotypic traits depressions. Number of alleles per primer and polymorphism information content (PIC) of each SSR locus was also recorded from the software output.

Drought tolerance index (DI) = $(X_{\text{control}} - X_{\text{drought}})/X_{\text{control}} \times 100$

Where X_{control} is the measured trait mean value under well-watered conditions and X_{drought} the mean values under drought stress induced by 8 days of withholding water (Reyniers *et al.* 1982), while

Stress index (I_s) = $1 - Y_{\text{drought}}/Y_{\text{control}}$,
Where Y_{drought} is the sum of means of trait values under drought stress condition and Y_{control} the sum of means under irrigated condition (Fischer and Maurer, 1978). The closer to 1 the greater the negative effect of drought on yield, while the closer to zero the greater the tolerance to drought.

Results

Effect of 8 days drought on the rice accessions

Water withholding for 8 days decreased SVMC from 19.5 - 2.2% (≈ 88.6% reduction). Almost all the measured growth, yield and physiological parameters were significantly affected ($P<0.05$) and the accessions responded differently to the drought treatment (Tables 4, 5 and 6).

Growth and yield: Only 2 of the rice accessions (AGW-PS and UPIA-1) did not show reductions in plant heights whereas around 65% showed significant ($P<0.05$) height reduction of between 4.5 and 23%, with the largest reduction observed in AGW-55 and FARO-11. Around 44% of the accessions had significant reductions in leaf length (LL) on the main tillers of between 6.6 – 23% with largest reduction occurring in UPIA-1, IFW-13 and AGW-PS, while IWA-10, NERI-34, FARO-44, IR-119, FARO-19, UPIA-2, IK-PS and IJS-02 were not affected. Some 61% of the accessions showed significant reductions in leaf width (LW) between 8 and 29% with AGW-PS and IR-119 having the largest reductions but some accessions such as FARO-19, NERI-34 and FARO-11 were not affected. Panicle length (PL) was significantly reduced from 5 to 28% in 56% of the accessions with IHEK and FARO-19 showing the largest reductions. Sixty one percent of the accessions showed significant reductions of between 5.6 to 32% in the number of primary branches per panicle (NPBPP) on the main tillers, with largest effect on AGW-PS, IFW-07 and FARO-11, while 26% including FARO-19, IJS-09, Lad-f, NERI-34 and FARO-44 did not show any effect. The drought treatment significantly reduced number of spikelet per panicle on the main tillers in 78% of the accessions, but had no effect on FARO-44. Reduction ranged from 1.9 to 40%. FARO-44, IJS-02 and IR-119 exhibited the lowest reductions while IHEK, IWA-10, IFW-07 and IFW-13 had the largest reductions. Reductions in spikelet fertility (SF) between 3.7 to 20% were observed in 70% of the accessions. The largest reduction occurred in IR-119 and NERI-34 whereas in IWA-10, AGW-PS and FARO-19 there were no effects. Panicle density (PD) was depressed in all the accessions, ranging from 5.8 – 38% except in FARO-44, FARO-19, NERI-34 and IK-FS. Reductions were significant ($P<0.05$) in 70% of the accessions from 9.5 – 38%. PD was most depressed in IWA-10, FARO-57, IFW-13 and IFW-07. With the exception of IJS-02, seed weight per plant (SWPP) decreased in all the accessions with the reduction ranging from 0.4% in IJS09 to 44% in IHEK. Reductions (9.4 – 44%) were significant in 78% of the accessions. The reduction in 1000 seed weight (1000SW) varied from 0.8% in IFW-13 to 15% in IHEK, while there was no reduction for FARO-19. Shoot dry weight (SDW) decreased significantly in 78% of the accessions, with reductions ranging from 10.5% in IR-119 to 46% in UPIA-2, but no reduction was observed with Lad-f and IWA-8. Root dry weight (RDW) depression occurred in almost all accessions varying from 2.3% in IWA-to 44% in AGWU-116. Reductions were significant in 56.5% of the accessions (7 – 44%), the exceptions were IJS-02, FARO-44, AGW-PS and IWA-8. Root shoot dry weight ratio was significantly depressed (from 1.5 – 27%) in about 39% of the accessions ($P<0.05$). Significant increases in RSDWR of around 6.8 to 76% were apparent in many accessions (47%), but there were no effects in 13% of the accessions. The greatest reduction was seen with Lad-f, FARO-57 and FARO-19 while the largest increase was with AGW-PS, AGW-55, FARO-44, IJS-09, IWA-10 and UPIA-1. The effect of drought on grain lengths and widths showed that only about 30% of the accessions had significant reductions ($p<0.05$) in grain lengths of between 3 – 9%, while significant reductions in grain width of between 5.9 and 11% occurred in 22% of the accessions. The most reduced growth and yield traits were shoot dry weight, seed weight per plant and root dry weight that were respectively decreased up to 46%, 44% and 44%.

The effects of withholding water, on all of the traits, were combined in Stress Index (Is) for each accession and the values were used to rank the accessions in order of drought tolerance. Based on this, FARO-44 and the Nigerian landraces (Lad-f, IJS-02, IJS-09, IK-FS and IK-PS) followed by IR-119 showed lower depressions in growth and yield traits due to withholding water, while IHEK and IFW-07 exhibited the largest growth depressions (Figure 1).

Earliness: Booting, flowering and maturity dates were significantly delayed in almost all accessions ($P<0.05$). Delays ranged from 0 – 21 days for days to 50% booting, 1 to 21 days for days to 50% flowering and 1 to 22 days for delays to maturity were observed (Table 5). Accessions IK-PS, IK-FS, FARO-44 and FARO-57 were delayed the least, while IFW-13 followed by FARO-19 and FARO-11 had the longest delays to booting and flowering dates. Stress index based on earliness traits indicated that IK-PS, FARO-44 and IK-FS were more drought tolerant while FARO-11 followed by IFW-13 and FARO-19 exhibited higher sensitivity to drought (Figure 2).

Leaf water status: Leaf water potential (LWP) was the most significant drought affected metric (Table 6). Reduction ranged from 144 to >4,000%. The Nigerian landraces (IJS-09, IJS-02, IK-PS and IK-FS followed by the improved varieties IR-119 and FARO-44 exhibited lower reductions in LWP, while the accessions (AGW-102, AGW-PS and AGW-116) recorded larger reductions. Ranking using Is based on LWP also showed the landraces IJS-09, IJS-02, IK-FS followed by IR-119, IK-PS and FARO-44 to have lower reductions in LWP, while accessions AGW-102, AGW-PS and AGW-116 were reduced the most (Figure 3).

Principal components analysis of trait depression values

The percentage depressions in trait values were subjected to principal components analysis to determine those most responsible for the observed differences in the accessions responses to withholding water. Eight (8) components were used but only 4 were significant (Table 7). The result of the PCA indicate that reduction in PL, number of spikelet per panicle and PD, as well as, the delay in maturation provided the greatest contributions to the observed differences in the performance of the accessions under drought.

SSR polymorphism and population structure of the accessions

To access the level of genetic diversity in the population studied, a total of 20 SSR primers were used to study DNA polymorphism among the rice accessions. Of the 20 primers, 16 produced scorable amplification bands used in the analysis while 4 primers failed to amplify any of the rice DNA. The 16 SSR primers amplified a total of 221 alleles. Number of alleles per primer ranged from 4 to 25 with a mean of 13.8 while the PIC values spanned from 0.24 to 0.95 with an average value of 0.77. Table 8 shows the major allele frequency, number of alleles and PIC of each of the microsatellite loci in the studied accessions.

A dendrogram of the 30 rice accessions using UPGMA procedure clustered the accessions into 6 major groups almost in accordance with their source locations (Figure 4). Nwad, a landrace from Ebonyi State, formed a distinct group (Group 1) suggesting that it is distantly related from the rest of the accessions. Group 2 included all accessions from Ebonyi State with only 2 accessions (Nwad and Lad-f) falling outside this group. Group 3 was a large cluster with distinct sub-groups. Accessions from Enugu State (AGW-PS, AGW-116 and AGW-102) are clustered together with only 1 (AGW-55) outside the sub-group but still showing a significant relationship. Accessions from Ife in Osun State (IFW-55, IFW-07 and IFW-13) are clustered together, the FARO lines (FARO-19, FARO-44 and FARO-57) except FARO-11 are grouped together while the improved varieties (UPIA-2, NERI-34, IR-119 and IR-184) are clustered together. Group 4 contained the single accession (Lad-f) also from Ebonyi State. Group 5 was comprised of accessions from Ekiti State (IJS-02, IJS-09, IK-PS and IK-FS) and FARO-11, whereas group 6 was another cluster of improved varieties including IWA-8, IWA-10 and UPIA-1).

Analysis of marker-trait association under drought-stress

The pattern in which each of the SSR markers clustered the accessions was compared with the pattern of individual trait depression due to the drought. Our result show that none of the markers clearly grouped the accessions according to the pattern of trait depression but few of the markers amplified alleles common only to accessions IJS-02, IJS-09, IK-PS, IK-FS and FARO-11. With the exception of FARO-11, these accessions are among the first five accessions that exhibited greater depressions in LWP (Figure 3) and also among the first seven accessions that showed lower drought depressions in overall growth and yield traits (Figure 1). Furthermore, they are among the first ten accessions that exhibited the least delay in flowering and maturation. FARO-11(OS6) is a known drought tolerant cultivar and is used here as a drought tolerant control. RM252 amplified about 100 bp fragment, RM331 amplified about 80 bp, RM432 amplified about 90 bp, RM36 produced about 80 bp, RM525 produced about 70 bp, 72 bp and 75 bp fragments in these accessions (IJS-02, IJS-09, IK-PS, IK-FS and FARO-11), whereas RM260 amplified as short 30 bp fragment in the accessions including ARUB. RM318 amplified about 70 bp fragment in the landraces IJS-02, IJS-09, IK-PS, IK-FS and ARUB but not in FARO-11 (Figure 5).

Discussion

Screening of rice for growth and yield performance under drought stress

Drought tolerance generally denotes the ability of a crop plant to survive, grow and yield satisfactorily under water-limited conditions (Turner 1979, Delphine *et al.* 2010). In this study, some rice varieties cultivated in Nigeria, including landraces and improved varieties, were screened in the greenhouse for their growth and yield performance under complete withholding water for 8d when at a late vegetative stage in their development (45 DAS). A number of vegetative and reproductive traits were used to characterize the accessions under drought. The rice accessions exhibited large differences in their responses to withholding water. Although the duration of withholding of water was short, the adverse effects (Fig. 6) were actually severe probably due to the low water retention capacity of the soil used (74%

sand; Table 2), with SVMC declining by 88% of field capacity and drought stress developing more rapidly due to the soils low water holding capacity. It was observed that no single accession showed either the greatest or least depressions in all the traits measured. For instance, AGW-PS followed by UPIA-1, FARO-44 and IJS-09 were the most drought tolerant accessions based on reductions in plant height, whereas FARO-44, IJS-02, IWA-8 and IJS-09, respectively, were the most tolerant using panicle length depression. Similarly, FARO-44 followed by IJS-02, IR-119, IJS-09 and IK-FS were the most tolerant in terms of depression in spikelet number on the main panicle while IJS-02 followed by IJS-09, IWA-8 and IWA-10 was the most drought tolerant in terms of grain yield per plant (SWPP) (Table 4). This type of response can be linked to the complex nature of drought tolerance involving mechanistic interactions between an array of morphological, physiological, biochemical and genes and their expression (Li and Xu 2007, Price *et al.* 2002, Mitra 2001) and the differential responses of different rice accessions to drought (Deshmukh 2012).

To unambiguously rank the rice accessions based on their overall growth and yield performance under the imposed water withholding conditions and to select the most drought tolerant accessions, a stress index (I*s*) was used which relies on a combination of trait values under withholding water and well-watered (Fischer and Maurer 1978) was used. Based on this procedure, FARO-44, Lad-f, IJS-02, IJS-09, IR-119, IK-FS and IK-PS, in decreasing order, were the most tolerant of the 23 accessions screened in relation to growth and yield, while IHEK followed by IFW-07, FARO-57, AGW-55, IFW-13 and IWA-10 were the most susceptible. It is worth noting that 5 of the 7 most tolerant accessions here are Nigerian landraces (Lad-f, IJS-02, IJS-09, IK-FS and IK-PS). These accessions, especially IJS-02, IJS-09, IK-FS and IK-PS, were found to be the earliest maturing of the 23 accessions (91-104 days; data not shown), which is an important late season drought avoidance strategy (Jongdee *et al.*, 2002; Araus *et al.* 2002). These landraces also recorded the highest 1000 seed weight

(39 to 42 g). However, they have a very low tiller number (3-5) which requires improvement to fully exploit their drought avoidance potential. Alternatively, increasing the sowing density of these accessions may adequately compensate for the lower tiller number, considering their high seed quality (1000 seed weight). Furthermore, planting them in this way may not lead to an unacceptable level of competition for photosynthetically active radiation, but would amount to effective utilization of space and soil resources, since the accessions are not of an 'open plant' type. These accessions can be promising breeding material for improvement of higher yielding genotypes for enhanced drought tolerance in Nigeria and other similar situations and locations. However these genotypes have been somewhat neglected, by farmers, owing to their low tillering and yields, but as landraces, they appear better adapted to the Nigerian environment and are potential reservoirs of adaptability genes including those for drought and other abiotic stress tolerance (Camacho *et al.* 2005, Friis-Hansen and Sthapit 2000). Furthermore, these landraces performed better than FARO-11 which was used here as drought tolerant control (Ubi *et al.* 2011), while sharing several similar phenotypic features such as tiller number, height, culm morphology, grain shape and size with FARO-11. Principal components analysis revealed depressions in panicle lengths, number of grains per panicle, panicle density and delays in maturity date as the most important traits determining variations in rice performance under drought.

Screening for ability to maintain leaf water status

Si analysis based on LWP also indicated that same landraces (IJS-09, IJS-02, IK-FS and IK-PS) among the 5 most drought tolerant accessions by their ability to maintain higher LWP under water withholding conditions. This suggests that these accessions may be using drought avoidance mechanism to cope with the stress of a water shortage. Drought tolerance is frequently apparent as increased capacity to maintain a higher LWP relative to a reduction in SVMC (Fukui *et al.* 1999, Kato *et al.*, 2001, 2006, Mitra 2001). By so doing they are able to extract water from the soil as its water potential falls thereby minimizing the yield losses (Singh *et al.* 2012). Of the 23 accessions screened, these accessions (IJS-09, IJS-02, IK-FS and IK-PS) also maintained the highest root to shoot ratio (0.23 – 0.28; data not shown) which can enhance root soil exploitation to extract more of the available soil moisture to maintain root and leaf tissue turgor and therefore growth under drought (Wang *et al.* 2006, Samson *et al.* 2002, Blum *et al.* 1989).

Screening for SSR polymorphism and their association with phenotypic drought traits

Genetic improvement of rice for drought tolerance through conventional breeding is slow due to the spatial and seasonal variations in drought timing and severity, the complex nature of drought tolerance itself and the difficulty in selecting for combinations of traits which best suit combating drought induced yield reductions (Courtois *et al.* 2003, Khush, 2001). Among the factors accounting for the slow progress in developing drought tolerant rice is the low heritability, multiple gene control, epistatic gene interaction, high incidence of genotype x environment interactions, etc. which could seriously influence 'actual' yields (Atlin and Lafitte 2002, Cattivelli *et al.* 2008). The use of molecular markers to select accessions possessing genes and genomic regions that control target traits can fast-track the progress in breeding for drought tolerant rice, because molecular markers are transmitted faithfully from generation to generation and are not subject to environmental influences (Senior *et al.* 1998, Gurta, *et al.* 1999, Korzun, *et al.* 2001, Crouch and Ortiz 2004).

SSRs are a DNA marker system of choice for genetic analysis in rice because of their abundance in the rice genome, high level of polymorphism and high but simple reproducible assays involved (Powell *et al.* 1996, Singh *et al.* 2010). The 16 SSR primers used here generated 4 to 25 alleles per primer with polymorphism information content (PIC) values ranging from 0.24 to 0.95. As high as 11 out of the 16 markers (≈69%) produced PIC values between 0.76 and 0.95 reflecting the high discriminating powers of the markers used. The genetic dendrogram, based on the SSR data clustering, was highly effective in reflecting the source locations of the accessions. The accessions from Ebonyi State (IHEK, Mass, AGRE, Arub, Ogbe and R-P) were grouped almost entirely together with only 2 accessions (Nwad and Lad-f) outside the cluster. This analysis also separately grouped accessions from Enugu State (AGW-PS, AGW-116,AGW-102 and AGW-55) and accessions from Osun State (IFW-55, IFW-07 and IFW-13) and showed that these 2 groups were closely related, which was supported by their poor performances under water withholding. The dendrogram also revealed a close linkage of the Enugu (AGW-) and Osun (IFW-) accessions with the FARO lines except FARO-11. It was noted that all accessions from Ekiti State (IJS-02, IJS-09, IK-PS and IK-FS) were grouped with FARO-11 and that all members of this group exhibited highly similar phenotypic features, but the landraces were much more drought tolerant than FARO-11 (a drought tolerant control). The improved varieties other than the FARO lines (UPIA-2, NERI-34, IR-119, IR-184, IWA-8, IWA-10 and UPIA-1) were clustered into two separate groups. It can be deduced from this study that the FARO lines used here originated from Nigeria and that the markers were able to partition the accessions in line with their source locations reflecting the robustness of SSR markers to dissect the population genetic structure and demographic history of domestication (Akkaya *et al.* 1992, Cho *et al.* 2000, Garris *et al.* 2005).

The patterns of accession clustering of individual SSR markers when compared with that of the individual trait depressions caused by drought, was used to determine marker-trait associations for drought tolerance. Although none of the markers typically clustered the accessions absolutely in accordance with the pattern of trait depressions, some of the markers (RM252, RM331, RM432, RM36, RM525, RM260 and RM318) each amplified alleles unique to accessions IJS-02, IJS-09, IK-PS, IK-FS and FARO-11. Four of these accessions (IJS-02, IJS-09, IK-PS and IK-FS) are landraces from Ekiti State and are among the most drought tolerant accessions found here based on their capacity to maintain LWP, and grow and yield satisfactorily under the imposed drought. It is important to note that though FARO-11(OS6) is a known drought tolerant cultivar (Ubi *et al.* 2011) and used as a drought tolerance validation, it did not really perform in this study as expected. Although the reason for its poor performance under withholding water here is not apparent, it could be due to the prevailing environmental conditions.

Acknowledgements

This research is supported by funding from the Department for International Development (DfID) under the Climate Impact Research Capacity and Leadership Enhancement (CIRCLE) programme.

Reference

Akkaya, M.S., A.A. Bhagwat, and P.B. Cregan, (1992) Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132: 1131-1139.

Ali, M.L., P.L. Sanchez, S.Yu, M. Lorieux, and G.C. Eizenga (2010) Chromosome segment substitution lines: a powerful tool for the introgression of valuable genes from *Oryza* wild species into cultivated rice (*O. sativa* L.). Rice 3(4): 218–234.

Araus, J.L., G.A. Slafer, M.P. Reynolds, and C. Royo (2002) Plant breeding and water relations in C3 cereals: what should we breed for? Annals of Botany 89: 925–940.

Atkinson, N.J. and P.E. Urwin, (2012) The interaction of plant biotic and abiotic stresses: from genes to the field. Journal of Experimental Botany 63(10): 3523-3543.

Atlin, G.N. and H.R. Lafitte, (2002) Marker-assisted breeding versus direct selection for drought tolerance in rice. *In*: Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice. 208. Saxena, N. P. and O'Toole, J. C., Eds., Proceedings of International Workshop on Field Screening for Drought Tolerance in Rice, Patancheru, India. 11–14 Dec 2000. ICRISAT, Patancheru, India, and The Rockefeller Foundation, New York.

Bargaz, A., M. Zaman-Allah, M. Farissi, M. Lazali, Drevon, R.T. Maougal and G. Carlsson (2015) Physiological and molecular aspects of tolerance to environmental constraints in grain and forage legumes. International Journal of Molecular Science 16(8): 18976–19008.

Bimpong, I.K., R. Serraj, J.H. Chin, J. Ramos, J. Mendoza, E. Hernandez, M.S. Mendioro, and D.S. Brar (2011) Identification of QTLs for drought-related traits in alien introgression lines derived from crosses of rice (*Oryza sativa* cv. IR64) × *O. glaberrima* under lowland moisture stress. Journal of Plant Biology 54: 237-250.

Blum, A., L. Shpiler, G. Golan, and J. Mayer (1989) Yield stability and canopy temperature of wheat genotypes under drought stress. Field Crop Research 22: 289–296.

Camacho, V., C. Tiana, N. Maxted, M. Scholten and B. Ford-Lloyd (2005) Defining and identifying crop landraces. Plant Genetic Resources 3(3): 373-384.

Cattivelli, L., F. Rizza, F.-W. Badeck, E. Mazzucotelli, A.M. Mastrangello, E. Francia, C. Mare, A. Tondelli and M. Stanca, (2008) Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. Field Crops Research 105: 1-14.

Cho, Y.G., T. Ishii, S. Temnykh, X. Chen, L. Lipovich, S.R. McCouch, W.D. Park, N. Ayres, and S. Cartinhour (2000) Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa*L.) Theoretical and Applied Genetics 100: 713-722.

Courtois, B., L. Shen, W. Petalcorin, S. Carandang, R. Mauleon and Z. Li (2003) Locating QTLs controlling constitutive root traits in the rice population IAC165/ Co39. Euphytica 134: 335–345.

Crouch, J.H. and R. Ortiz (2004) Applied genomics in the improvement of crops grown in Africa. African Journal of Biotechnology 3 (10): 489 – 496.

Delphine, F., S. Jefferies, H. Kuchel, and P. Langridge (2010) Genetic and genomic tools to improve drought tolerance in wheat. Journal of Experimental Botany 61(12): 3211-3222.

Deshmukh, V.V. (2012) Genome wide association mapping of drought resistance traits in rice (*Oryza sativa* L.). M. Sc. Dissertation, Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University. Pp 165.

FAO (Food and Agriculture Organization) (2011) http://faostat.fao.org/

Farooq, M., N. Kobayashi, A.Wahid, O. Ito and S.M.A. Basra (2009) Strategies to produce more rice with less water. In: Advances in Agronomy Vol.101 pp: 351–388.

Fischer, R.A., R. Maurer (1978) Drought resistance in spring wheat cultivar: I- Grain yield response. Australian Journal of Agricultural Research 29: 897-912.

Friis-Hansen, E. and B. Sthapit (2000) Participatory approaches to the conservation of plant genetic resources. International Plant Genetic Resources Institute, Italy, Rome, Pp 199.

Fukai, S., G. Pantuwan, B. Jongdee and M. Cooper (1999) Screening for drought resistance in rain-fed lowland rice. Field Crops Research 64 (1-2): 61-74.

Garris, A., T.H. Tai, J. Coburn, S. Kresovich and S. McCouch (2005) Genetic structure and diversity in *Oryza sativa* L. Genetics 169: 1631-1638.

Gurta, P.K., R.K. Varshney, P.C. Sharm and B. Ramesh, (1999) Molecular markers and their application in wheat breeding. Plant Breeding 118: 369 –390.

Ingram F.D., K.T. Bueno, O.S. Namuco, E.B. Yambao and C.A. Beyrouty (1994) Rice root traits for drought resistance and their genetic variation. *In*: Kirk, G.J.D. (Ed.), Rice Roots: Nutrient and Water Use. International Rice Research Institute, Manila, Philippines.

International Rice Research Institute (IRRI) (1996) Standard Evaluation System for Rice. 4th ed., International Network for Genetic Evaluation of Rice, Genetic Resource Center, Los Baños. The Philippines.

Jongdee, B., Fukai, S. and Cooper, M. (2002) Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. Field Crop Research 76: 153-163.

Kamoshita, A., R.C. Babu, N.M. Boopathi, and S. Fukai (2008) Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. Field Crop Research 109: 1–23.

Kato, Y., J. Abe, A. Kamoshita and J. Yamagishi (2006) Genotypic variation in root growth angle in rice (*Oryza sativa* L.) and its association with deep root development in upland fields with different water regimes. Plant and Soil 287: 117–129.

Khush, G.S. (2001) Green revolution: the way forward. Nature Reviews 2: 815-822.

Khush, G.S. (2005) What it will take to Feed 5.0 Billion Rice consumers in 2030. Plant Molecular Biology 59: 1-6.

Korzun, V., S. Malyshev, A.V. Voylokov and A. Borner (2001) A genetic map of rye (*Scale cereale* L.) combining RFLP, isozyme, microsatellite and gene loci. Theoretical and Applied Genetics 102: 709 – 717.

Li, Z.K. and J.L. Xu. (2007) Breeding for drought and salt tolerant rice (*Oryza sativa* L.): progress and perspectives. Advances in Molecular Breeding toward Drought and Salt Tolerant Crops Pp 531-564.

Mitra, J. (2001) Genetics and genetic improvement of drought resistance in crop plants. Current Science 80(25): 758-763.

Ndjiondjop, M., F. Cisse, K. Futakuchi, M. Lorieux, B. Manneh, R. Bocc and B. Fatondji, (2010) Effect of drought on rice (*Oryza* spp.) genotypes according to their drought tolerance level. Second Africa Rice Congress, Bamako, Mali, 22–26 March 2010: Innovation and Partnerships to Realize Africa's Rice Potential, Pp 151-158.

Nguyen, H.T., R.C. Babu and A. Blum (1997) Breeding for drought tolerance in rice: physiology and molecular genetics considerations. Crop Science 37: 1426–1434.

O'Toole, J.C. (1982) Adaptation of rice to drought-prone environments. *In*: Drought Resistance in Crops, with Emphasis on Rice. International Rice Research Institute, Manila, Philippines.

Pandey, S. and H. Bhandari, (2009) Drought: coping mechanism and poverty, Insights from rainfed rice farming in Asia. Asia and the pacific division. International Fund for Agricultural Development (IFAD). Pp 5-43.

Powell, W., G.C. Machray and J. Provan (1996) Polymorphism revealed by simple sequence repeats. Trends in Plant Science 1: 215–222.

Price, A.H. and A.D. Tomos (1997) Genetic dissection of root growth in rice (*Oryza sativa* L.) II: mapping quantitative trait loci using molecular markers. Theoretical and Applied Genetics 95: 143-152.

Price, A.H., J.E. Cairns, P. Horton, H.G. Jones, and H. Griffiths (2002) Linking drought-resistance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. Journal of Experimental Botany 53: 989-1004.

Price, A.H., K.A. Steele, B.J. Moore, P.B. Barraclough and L.J. Clark (2000) A combined RFLP and AFLP linkage map of upland rice (*Oryza sativa* L.) used to identify QTLs for root-penetration ability. Theoretical and Applied Genetics 100: 49–56.

Reyniers, F.N., B. Truong, L. Jacquinot and R. Nicou (1982) Breeding for drought resistance in dryland rice, In: Drought Resistance in Crops with Emphasis on Rice. IRRI, Los Baños, Philippines pp. 273–292.

Rosenzweig, C., A. Iglesias, X.B. Yang, P.R. Epstein and E. Chivian (2000) Climate change and U.S. agriculture: The impacts of and extreme weather events on productivity, plant diseases, and pests. Center for Health and The Global Environment Harvard Medical School (http://www.med.harvard.edu/chge/), Pp 47.

Salunkhe, A.S., R. Poornima, K.S. Prince, P. Kanagaraj, J.A. Sheeba, K. Amudha, K.K. Suji, A. Senthil and R.C. Babu. (2011) Fine Mapping QTL for Drought Resistance Traits in Rice (*Oryza sativa* L.) using bulk segregant analysis. Molecular Biotechnology 49: 90-95.

Samson, B., M. Hasan and L. Wade, (2002) Penetration of hardpans by rice lines in the rain-fed lowlands. Field Crop Research 76: 175-188.

Sanchez, P.L., R.A. Wing, and D.S. Brar (2014) The Wild Relative of Rice: Genomes and Genomics. *In*: Q. Zhang and R.A. Wing (eds.), Genetics and Genomics of Rice, Plant Genetics and Genomics: 9 Crops and Models 5, Springer Science+Business Media, New York, Pp 9-25.

Sellamuthu, R., G.F. Liu, C.B. Ranganathan and R. Serraj (2011) Genetic analysis and validation of quantitative trait loci associated with reproductive-growth traits and grain yield under drought stress in a doubled haploid line population of rice (*Oryza sativa* L.). Field Crops Research, 124: 46-58.

Senior, M.L., J.P. Murphy, M.M. Goodman, C.W. Stuber (1998) Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. CropScience 38: 1088-1098.

Singh, N., T.T. Dang, G.V. Vergara, D.M. Pandey, D. Sanchez, C.N. Neeraja, E.M. Septiningsih, M. Mendioro, E.M. Tecson-Mendoza, A.M. Ismail, D.J. Mackill, and S. Heuer (2010) Molecular marker survey and expression analyses of the rice submergence-tolerance gene SUB1A. Theoretical and Applied Genetics 121: 1441-1453.

Singh, S., S. Pradhan, A. Singh, and O. Singh (2012) Marker validation in recombinant inbred lines and random varieties of rice for drought tolerance. Australian Journal of Crop Science 6: 606-612.

Srividhya, A., L.R. Vemireddy, S. Sridhar, M. Jayaprada, P.V. Ramanarao, A.S. Hariprasad, H.K. Reddy, G. Anuradha, and E. Siddiq (2011) Molecular mapping of QTLs for yield and its components under two water supply conditions in rice (*Oryza sativa* L.). Journal of Crop Science and Biotechnology 14 (1): 45-56.

Suji, K.K., K.R. Biji, R. Poornima, K.S. Prince, K. Amudha, S. Kavitha, S. Mankar, and R.C. Babu (2011) Mapping QTLs for plant phenology and production traits using Indica Rice

(*Oryza sativa* L.) lines adapted to rain-fed environment. Molecular Biotechnology 52(2): 151-60.

Tao, H., H. Brueck, K. Dittert, C. Kreye, S. Lin and B. Sattelmacher (2006) Growth and yield formation for rice (*Oryza sativa* L.) in the water-saving ground cover rice production system (GCRPS). Field Crops Research 95(1): 1-12.

Temnykh, S., G. DeClerck, A. Lukashova, L. Lipovich, S. Cartinhour and S. McCouch, (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.) frequency, length variation, transposon associations, and genetic marker potential. Genome Research 11: 1441-52.

Turner, N.C. (1979) Drought resistance and adaptation to water deficits in crop plants. *In*: Mussell H, Staples CR, eds. Stress physiology in crop plants. John Wiley and Sons, New York, Pp 343–372.

Ubi, B.E., A.A. Efisue and O.H. Oselebe (2011) Diversity of drought stress tolerance response in rice cultivars and breeding lines at the vegetative stage. Journal of Agriculture, Biotechnology and Ecology 4(3): 70-89.

Venuprasad, R., M.T. Cruz, M. Amante, R. Magbanua, A. Kumar and G.N. Atlin, (2008) Response to two cycles of divergent selection for grain yield under drought stress in four rice breeding populations. Field Crop Research 107(3): 232-244.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Vandelee, M. Homes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau, (2007) Development of polymorphic microsatellites markers in sesame (S*esamum indicum L.*). Molecular Ecology 5: 736-739.

Warburton, M., J. Yan, and J. Crouch (2011) Association mapping for enhancing maize (L.) genetic improvement. Crop Science 51: 433-449.

Wang, H., Y. Inukai, and A. Yamauchi (2006) Root development and nutrient uptake. Critical Reviews in Plant Sciences 25: 279–301.

Xiong, D., T. Yu, T. Zhang, Y. Li, S. Peng and J. Huang (2015) Leaf hydraulic conductance is coordinated with leaf morpho-anatomical traits and nitrogen status in the genus *Oryza*. Journal of Experimental Botany 66(3): 741–748.

Xu, Z., G. Zhou, and H. Shimizu, (2010) Plant responses to drought and rewatering. Plant Signal Behaviours 5(6): 649–654.

Yang, J.C., Liu, K., Zhang, S.F., X.M. Wang, Q. Zh, X.M. Wang and L.J. Liu (2008) Hormones in rice spikelets in responses to water stress during meiosis. Acta Agronomica Sinica 34(1): 111-118.

Zheng, H., R.C. Babu, M.S. Pathan, L. Ali, N. Huang, B. Courtois and H.T. Nguyen (2000) Quantitative trait loci for root-penetration ability and root thickness in rice: comparison of genetic backgrounds. Genome 43: 53-61.

Table 1. *Oryza sativa* L. accessions used in the development of drought response traits and markers

S/N	Name	Source	Status	Code	Remark
1	Nwadende*	Ebonyi	Landrace	Nwad	-
2	Ihenkiri	Ebonyi	Landrace	Ihek	-
3	Lady’s finger	Ebonyi	Landrace	Lad-f	-
4	Agreement*	Ebonyi	Landrace	Agre	-
5	Arubus*	Ebonyi	Landrace	Arub	-
6	Room and palour*	Ebonyi	Landrace	R-P	-
7	Mass*	Ebonyi	Landrace	Mass	-
8	Ogbese*	Ebonyi	Landrace	Ogbe	-
9	IJ$_{\text{SLLW}}$FS-02	Ekiti	Landrace	IJS-02	Faluyi & Nwokocha
10	IJ$_{\text{SLLW}}$ FS-09	Ekiti	Landrace	IJS-09	Faluyi & Nwokocha
11	IKph^{+}PS	Ekiti	Landrace	IK-PS	Faluyi & Nwokocha
12	IKph^{+}FS-217	Ekiti	Landrace	IK-FS	Faluyi & Nwokocha
13	AWGU I Pr^{++}PS	Enugu	Landrace	AGW-PS	-
14	AWGU I FS-116	Enugu	Landrace	AGW-116	-
15	AWGU II FS-55	Enugu	Landrace	AGW-55	-
16	AWGU III FS-102	Enugu	Landrace	AGW-102	-
17	IFW Pr^{++}FS-55*	Osun	Landrace	IFW-55	Faluyi & Nwokocha
18	IFW FS-07	Osun	Landrace	IFW-07	Faluyi & Nwokocha
19	IFW FS-13	Osun	Landrace	IFW-13	Faluyi & Nwokocha
20	FARO 11 (or OS6)	AfricaRice	Improved variety	FARO-11	Tolerant check
21	FARO 19	AfricaRice	Improved variety	FARO-19	Susceptible check
22	FARO 44	AfricaRice	Improved variety	FARO-44	-
23	FARO 57	AfricaRice	Improved variety	FARO-57	-
24	IWA 8	AGRA	Improved variety	IWA-8	-
25	IWA 10	AGRA	Improved variety	IWA-10	-
26	UPIA 1	WARDA	Improved variety	UPIA-1	-
27	UPIA 2	WARDA	Improved variety	UPIA-2	-
28	NERICA 34	WARDA	Improved variety	NERI-34	Interspecific hybrid
29	IR06N 184	WARDA	Improved variety	IR-184	-
30	IR06A 119	WARDA	Improved variety	IR-119	-

*Accessions lost during the experiment.

Table 2: Properties of the soil (sandy loam) used to grow *Oryza sativa* L. accessions to assess phenotypic responses during withholding water

Property	Value
pH in water	6.70
pH in $CaCl_2$	6.40
Phosphorus concentration (µg/kg)	17.6
Potassium concentration (mg/kg)	0.30
Nitrogen concentration (%)	1.26
Organic carbon content (%)	0.94
Organic matter content (%)	1.61
Sand (%)	73.7
Silt (%)	9.3
Clay (%)	17.0

Table 3. List of Primer sequences used to study marker-trait associations in *Oryza sativa* L. accessions induced by withholding water

S/N	Primer name	Forward primer (5' – 3')	Reverse primer (5' – 3')	Source
1	RM38	ACGAGCTCTCGATCAGCCTA	TCGGTCTCCATGTCCCAC	Srividhya *et al*., 2011
2	RM331	GAACCAGAGGACAAAAATGC	CATCATACATTTGCAGCCAG	Srividhya *et al*., 2011
3	RM60	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC	Vikram *et al*., 2011
4	RM252	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	McCouch *et al*., 2002
5	RM170	TCGCGCTTCTTCCTCGTCGACG	CCCGCTTGCAGAGGAAGCAGCC	Yue *et al*., 2005
6	RM318	GTACGGAAAACATGGTAGGAAG	TCGAGGGAAGGATCTGGTC	Srividhya *et al*., 2011
7	RM279	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	Samuel *et al*., 2010
8	RM7390	CTGGTTAACGTGAGAGCTCG	GCAGATCAATTGGGGAGTAC	McCouch *et al*., 2002
9	RM432	TTCTGTCTCACGCTGGATTG	AGCTGCGTACGTGATGAATG	Vikram *et al*., 2011
10	RM5367	AGTACCTCTCACTCGCCTGC	TGTCAGCTGTGAGTGAAGTCG	McCouch *et a*l., 2002
11	RM5423	ATCCCACTTGCAGACGTAGG	ACAGCAGCAAGGTGCCTC	McCouch *et al*., 2002
12	RM15850	ATACACAGATGACGCACACG	TTAGGTGTGTGAGCGTGGC	McCouch *et al*., 2002
13	RM36	CAACTATGCACCATTGTCGC	GTACTCCACAAGACCGTAC	Brondani *et al*., 2002
14	RM3558	ACGAGAGATCTTCTTTGCAG	CCTCTATTTATGCCTCTACGC	McCouch *et al*., 2002
15	RM517	GGCTTACTGGCTTCGATTTG	CGTCTCCTTTGGTTAGTGCC	Hong *et al*., 2005
16	RM6130	GGCAGAGAGAGCTGCATCTC	GACGACGACGAACCCAAC	McCouch *et al*., 2002
17	RM583	AGATCCATCCCTGTGGAGAG	GCGAACTCGCGTTGTAATC	Swamy *et al*., 2011
18	RM1141	TGCATTGCAGAGAGCTCTTG	CAGGGCTTTGTAAGAGGTGC	McCouch *et al*., 2002
19	RM260	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG	McCouch *et al*., 2002
20	RM525	GGCCCGTCCAAGAAATATTG	CGGTGAGACAGAATCCTTACG	McCouch *et al*., 2002

Table 4. Percentage depressions in trait values in *Oryza sativa* accessions induced by 8 days of withholding water

Accession	Height	LL	LW	PL	NPBPP	NSPP	SF	PD	SWPP	1000 SW	SDW	RDW	RSDWR	GL	GW
AGW-102	19.2	10.2	3.3	5.4	3.2	24.8	2.6	19.8	29.4	8.8	11.3	-8.4	-22.3	5.0	1.5
AGW-116	2.4	6.6	12.4	3.9	15.0	20.0	5.2	16.6	37.2	4.7	40.2	43.6	5.8	-1.1	8.9
AGW-55	23.4	2.2	20.7	8.5	3.7	27.1	6.1	20.3	11.8	2.6	39.9	6.5	-55.6	0.6	0.5
AGW-PS	-4.3	16.9	29.4	3.3	32.4	18.8	0.2	16.6	17.0	3.9	27.2	-28.5	-76.4	-1.1	-6.0
FARO-11	22.0	8.8	0.0	9.6	23.9	22.2	6.5	13.8	17.7	7.7	13.9	12.3	-1.8	-1.7	0.8
FARO-19	2.3	-7.4	-10.8	18.7	-14.6	14.6	0.3	-5.1	3.3	-0.7	25.6	40.2	19.5	-2.6	-1.5
FARO-44	1.6	-26.2	3.5	-9.9	0.0	-27.8	2.2	-16.3	25.2	2.1	21.4	-28.7	-63.6	6.8	1.4
FARO-57	11.4	12.4	14.0	5.1	0.0	34.0	10.1	30.5	13.9	3.0	3.7	26.6	23.8	-8.0	2.5
IFW-07	11.5	16.1	15.2	12.2	31.7	37.3	6.3	28.4	40.0	5.8	5.8	10.1	4.6	-5.7	3.7
IFW-13	5.6	18.2	20.4	9.2	14.1	36.8	2.8	29.9	9.4	0.8	11.0	5.0	-6.8	2.4	-1.0
IHEK	17.4	9.6	11.4	28.6	7.1	40.1	13.9	14.5	43.7	14.7	36.0	37.0	1.5	-1.9	11.3
IJS-02	3.8	-1.7	12.0	-9.0	10.9	1.9	3.7	10.2	-1.7	1.7	15.0	16.0	1.2	3.0	6.5
IJS-09	2.0	4.7	1.1	-6.3	-4.3	4.0	7.4	9.5	0.4	2.1	3.4	-34.4	-39.2	-2.7	-3.9
IK-FS	3.2	6.6	3.0	7.2	9.1	6.3	4.4	-1.1	9.5	2.7	29.0	27.6	-1.9	-7.7	-2.5
IK-PS	6.2	-2.6	1.0	5.3	5.7	10.1	5.6	5.8	18.3	1.8	22.1	7.2	-19.2	1.6	2.5
IR-119	12.6	-20.2	22.4	-5.2	8.1	1.9	20.4	6.5	14.7	5.8	10.5	14.5	4.5	2.1	-2.7
IR-184	20.8	4.6	21.4	-1.2	10.3	9.9	13.6	11.3	18.9	3.4	11.3	12.8	1.7	1.5	-1.6
IWA-10	11.7	-27.7	8.1	3.8	15.9	40.0	-0.3	37.9	2.8	7.1	27.9	2.3	-35.5	2.9	-1.0
IWA-8	8.7	2.3	3.5	-7.6	7.6	9.3	8.0	15.2	2.1	14.2	-7.2	-15.7	-7.9	2.0	5.9
Lad-f	4.5	12.2	9.8	9.7	-3.7	15.6	10.1	6.5	10.3	3.1	-47.4	-7.9	26.8	7.6	3.3
NERI-34	13.4	-27.6	-1.2	11.1	-1.7	7.8	15.1	-3.5	17.1	9.1	32.0	33.2	1.7	6.5	7.9
UPIA-1	-0.4	23.1	11.7	9.5	1.8	21.9	12.9	13.4	14.9	6.4	19.0	-14.2	-40.9	-1.1	-7.4
UPIA-2	10.9	-7.1	15.1	0.6	8.3	21.6	1.7	20.8	25.1	10.8	45.7	36.5	-16.8	9.4	-0.5

NB: LL = leaf length, LW = leaf width, PL = panicle length, NPBPP = number of primary branches per panicle, NSPP = number of spikelet per panicle, SF = spikelet fertility, PD = panicle density, SWPP = seed weight per plant, 1000 SW = 1000 seed weight, SDW = shoot dry weight, DRW = root dry weight, RSDWR = root-shoot dry weight ratio, GL = grain length, GW = grain width.

Table 5. Delays in booting, heading and maturity dates in *Oryza sativa* L. accessions induced by 8 days of withholding water (drought stress) in a greenhouse

Accession	Delay in days to 50% booting	Delay in days to 50% heading date	Delay in days to maturity
AGW-102	10	13	10
AGW-116	11	14	19
AGW-55	10	16	17
AGW-PS	8	9	9
FARO-11 (OS6)	14	20	18
FARO-19	17	17	14
FARO-44	1	2	3
FARO-57	3	2	18
IFW-07	11	11	19
IFW-13	21	21	18
IHEK	4	4	5
IJS-02	7	8	4
IJS-09	9	4	3
IK-FS	1	2	2
IK-PS	0	1	1
IR-119	9	6	8
IR-184	8	7	6
IWA-10	11	12	16
IWA-8	7	7	3
Lad-f	11	16	11
NERI-34	9	14	12
UPIA-1	6	4	22
UPIA-2	4	10	6

Fig.1. Ranking of rice accessions using drought stress index based on growth and yield traits. Bars represent I*s* values. The closer the value is to zero, the more drought resistant is the rice accession (8.5 cm x 12.5 cm)

Fig. 2. Ranking of rice accessions using drought stress index based on earliness traits (booting, heading and maturity dates). Bars represent I*s* values. The closer the value is to zero, the more drought resistant is the rice accession (8.5 cm x 12.5 cm).

Table 6. Effect of 8 days of withholding water on leaf water potential (LWP) in *Oryza sativa* accessions

Accession	Unstressed (Mpa)	Stressed (Mpa)	% Change in LWP
AGW-102	-0.70	-33.12	-4631
AGW-116	-0.88	-26.19	-2876
AGW-55	-1.12	-26.66	-2280
AGW-PS	-0.76	-25.37	-3238
FARO-11	-0.78	-16.26	-1985
FARO-19	-0.88	-19.97	-2169
FARO-44	-0.87	-3.20	-268
FARO-57	-1.15	-21.60	-1778
IFW-07	-0.71	-3.53	-397
IFW-13	-0.97	-26.70	-2653
IHEK	-1.16	-4.38	-278
IJS-02	-0.70	-1.74	-149
IJS-09	-0.69	-1.69	-145
IK-FS	-0.19	-0.49	-158
IK-PS	-0.43	-1.53	-256
IR-119	-1.39	-3.95	-184
IR-184	-1.17	-21.66	-1751
IWA-10	-1.18	-10.92	-825
IWA-8	-0.87	-4.26	-390
Lad-f	-0.71	-4.93	-59
NERI-34	-1.03	-21.95	-2031
UPIA-1	-1.22	-16.53	-1255
UPIA-2	-0.93	-16.27	-165

Fig. 3. Ranking of rice accessions using drought stress index based on leaf water potential (LWP). Bars represent I*s* values. The closer the value is to zero, the more drought resistant is the rice accession (8.5 cm x 12.5 cm).

Table 7. Eigenvectors and eigenvalues of the phenotypic traits of *Oryza sativa* accessions induced by 8 days of withholding water

Phenotypic trait	PC1	PC2	PC3	PC4
Height	0.199	0.245	0.045	0.174
LL	0.221	-0.178	0.147	-0.394
LW	0.141	-0.204	0.365	-0.011
PL	*0.299*	0.206	-0.077	-0.107
NPBPP	0.196	-0.172	0.366	0.074
NSPP	*0.421*	-0.003	0.097	-0.091
SF	-0.021	0.293	0.092	-0.269
LWP	-0.262	0.168	0.110	-0.297
PD	*0.335*	-0.156	0.202	-0.039
SWPP	0.177	0.241	0.313	0.137
1000 SW	0.099	0.288	0.260	0.192
SDW	0.061	0.085	0.187	0.414
RDW	0.189	0.387	-0.107	0.022
RSDWR	0.129	0.342	-0.266	-0.339
GL	-0.117	0.030	-0.140	0.471
GW	0.075	0.424	-0.012	0.083
50% BD	0.275	-0.185	-0.398	0.043
50% HD	0.298	-0.102	-0.392	0.226
MD	*0.354*	-0.129	-0.132	-0.057
Eigenvalue	24	16	12	10
Cumulative proportion	24	40	52	62

*LL (leaf length), LW (leaf width), PL (panicle length), NPBPP (number of primary branches per panicle), NSPP (number of spikelet per panicle), SF (spikelet fertility), LWP (leaf water potential), PD (panicle density), SWPP (seed weight per plant), 1000 SW (1000 seed weight), SDW (shoot dry weight), RDW (root dry weight), RSDWR (root to shoot dry weight ratio), GL (grain length), GW (grain width), BD (date to 50% booting), 50% HD (date to 50% heading), MD (maturity date).

Table 8. Detected genetic diversity indices in rice accessions using SSR markers

S/N	SSR marker	Major allele frequency	Allele number	PIC
1	RM170	0.10	22	0.94
2	RM60	0.20	11	0.86
3	RM38	0.17	20	0.92
4	RM36	0.13	17	0.92
5	RM279	0.10	25	0.95
6	RM260	0.60	4	0.52
7	RM318	0.10	21	0.94
8	RM331	0.60	7	0.57
9	RM432	0.63	5	0.52
10	RM517	0.07	25	0.95
11	RM525	0.57	7	0.61
12	RM583	0.87	5	0.24
13	RM1141	0.20	18	0.9.0
14	RM5423	0.30	9	0.80
15	RM15850	0.33	15	0.84
16	RM6130	0.40	10	0.76
	Mean	0.34	13	0.77

Fig. 4. A dendrogram of 30 rice accessions from 20 SSR markers based on UPGMA. Numbers 1 – 6 represent separate clusters (12.2 cm x 16.2 cm).

Fig. 5. Gel photos of some SSR markers that amplified fragments common in size to rice accessions IJS02, IJS09, IK-PS, IK-FS and FARO-11 corresponding to lanes 9, 10, 11, 12 and 20. Numbers 1 – 30 represent each of the rice accessions, A = RM525, B = RM432, while M = 50bp DNA ladder (3.28 cm x 15.24 cm).

Fig.6. *Oryza sativa* L. plants subjected to withholding of water after 4 and 8 days of exposure to drought in a greenhouse. Fig. 6A shows the plants at four days drought stress, while Fig. 6B shows the plants at eight days drought stress (6.23 cm x 5.78 cm).