

# The transcriptome response of African and South American cassava (*Manihot esculenta* Crantz) to infection by the begomovirus EACMV-UG

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## Abstract

In this research, we investigated the mechanism of resistance to cassava mosaic begomoviruses (CMBs) that causes cassava mosaic disease (CMD). This is to identify potential new sources of resistance to this economically important disease. Fourteen cassava varieties were tested for their resistance to the severe specie of CMBs: the *East African cassava mosaic virus-Uganda* (EACMV-UG). While the six African cassava varieties (72-TME 14, TME 204, TZ 130, Nase 1, Nase 3 and Nase 14) expressed no or mild disease symptoms and a contained low viral load and therefore have been classified as resistant, all South American varieties showed severe leaf symptoms and supported high viral load. Nase 3 (resistant), TZ 130 (resistant) and Ebwanateraka (susceptible) were selected and subjected to RNA-Sequencing (RNA-Seq). A time-course transcriptome analysis of the three cassava varieties identified the largest number of differentially expressed genes (DEGs) in Nase 3 (4228) with 1725 genes uniquely overexpressed upon virus infection. Further analyses revealed that resistant varieties responded earlier to the infection than susceptible varieties. Additionally, expressions of heat shock proteins and transcription factors such as ethylene-responsive transcription factor (ERFs), teosinte-like, cycloidea and PCF1 (TCPs), heat stress transcription factor and basic leucine zipper were observed to be upregulated early. Collectively, these results suggest that resistant varieties maintain a low titer of EACMV-UG by activating specific stress-response genes early. The role

25 of these genes in plants remains to be investigated but offer insights into the molecular mechanism of  
26 resistance to CMD.

27 **Keywords:** Cassava, EACMV-UG, RNA Sequencing, DEG, Resistance.

## 28 **1 Introduction**

29 Cassava (*Manihot esculenta* Crantz; Family: *Euphorbiaceae*) is an important food security crop for >450  
30 million people in Africa and its production is threatened by cassava mosaic disease (CMD) (Legg *et al.*,  
31 2015). At least 11 distinct cassava mosaic begomoviruses (CMBs) cause CMD, which can only be  
32 effectively controlled by growing resistant varieties (Legg *et al.*, 2011). One of the strains of CMB is *East*  
33 *African cassava mosaic virus-Uganda* (EACMV-UG) which is a member of the genus *Begomovirus*, family  
34 *Geminiviridae* based on the guidelines of the International Committee on Taxonomy of Viruses (ICTV) in  
35 2022. EACMV-UG is a recombinant strain that has been associated with the CMD pandemic in eastern  
36 Africa since the late 1980s (Otim-Nape, Bua and Baguma, no date; Legg *et al.*, 2006). Regardless of the  
37 virus species, symptoms range from green to yellow mosaic together with leaf deformation. Early infection  
38 of susceptible varieties causes severe stunting and reduction in yield (Alabi, Kumar and Naidu, 2011). The  
39 most sustainable way to control CMD is by growing resistant varieties, and several resistant varieties have  
40 been identified (Legg *et al.*, 2011; Nicaise, 2014), however, the mechanisms underlying the resistance have  
41 been poorly understood.

42 Transcriptome sequencing by RNA-Seq is one of the ways of understanding the molecular processes  
43 underlying disease resistance by identifying changes in gene expression during viral infections (Nicaise,  
44 2014; Stobbe and Roossinck, 2014; Fan *et al.*, 2015) By comparing susceptible and resistant varieties, RNA-  
45 Seq identifies gene expression changes during defense response and elucidates complex resistance  
46 mechanisms. Few RNA-Seq studies have been performed to evaluate the response of cassava varieties  
47 challenged with cassava viruses. In cassava, transcriptome analysis has been conducted following  
48 inoculation with a different strain of CMB; *South African cassava mosaic virus* (SACMV), a member of

49 the genus *Begomovirus*. Allie et al. (Allie et al., 2014) compared transcripts of cassava genotypes and  
50 showed that 4181 and 1008 transcripts in total were differentially expressed in response to SACMV in  
51 susceptible (T200) and tolerant (TME3) varieties, respectively. GOslim functional group demonstrated that  
52 in T200 and TME3, differentially expressed genes were overrepresented in the cellular component category  
53 of plasma membrane and nucleus. Alterations in expression of transcription factors, resistance (R) genes  
54 and histone/DNA methylation-associated genes, were observed (Allie et al., 2014). Transcriptome studies  
55 have also been conducted following inoculation with two RNA virus species; *cassava brown streak*  
56 *virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV) (Patil et al., 2015; Tomlinson et al.,  
57 2018) together known as cassava brown streak ipomoviruses (CBSIs) belonging to family *Potyviridae*,  
58 genus *Ipomovirus* which cause cassava brown streak disease (CBSD) (Maruthi et al., 2017). RNA-Seq data  
59 identified the overexpression of *PAL1*, *PAL2*, cinnamic acid and two chalcone synthase genes in a resistant  
60 variety Kaleso and silencing of *PAL1* by RNA interference led to increased susceptibility to CBSD (Kavil  
61 et al., 2021). Other defense-related genes such as NAC transcription factors and elongation factor,  
62 eIF(iso)4E were over-expressed after CBSV infection (Maruthi et al., 2014). In Kaleso, several defense  
63 response genes such as encoding LRR-containing, NB-ARC-containing, pathogenesis-related, late  
64 embryogenesis abundant, selected transcription factors, chaperones, and heat shock proteins were highly  
65 overexpressed upon UCBSV infection. Defense-related GO terms of translational elongation, translation  
66 factor activity, ribosomal subunit and phosphorelay signal transduction were also overrepresented (Amuge  
67 et al., 2017).

68 In this study, 14 cassava varieties were screened for EACMV-UG resistance by recording symptoms and  
69 quantifying the virus in the plant after virus inoculation. Three of these varieties were selected as resistant  
70 (Nase 3 and TZ 130) and susceptible (Ebwanateraka) and subjected to RNA-Seq 2, 4, 7 and 28 days after  
71 grafting (dag) to identify resistance genes. The time points up to 7 dag constituted the early infection stage  
72 and 28 days represented virus replication and movement (before foliar symptoms appeared). With this

73 approach, we aimed to identify genes that respond early to infection as well as genes modulated during  
74 virus replication and movement.

## 75 **2 Materials and methods**

### 76 **2.1 Planting materials and graft-inoculation**

77 Cassava varieties were obtained from Latin America (supplied by the International Centre for Tropical  
78 Agriculture, CIAT-Colombia), and from the National Agricultural Research Systems (NARS) of Kenya  
79 and Uganda (Table 1) (Maruthi *et al.*, 2019). For each variety, 15 virus-free cuttings of 10 cm length were  
80 planted and grown in glasshouse conditions of  $28 \pm 5$  °C and 50-60% relative humidity (RH). The resistant  
81 variety Nase 3 and susceptible variety Col 2246 were used as controls.

82 Virus inoculum was obtained from var Ebwanateraka exhibiting severe symptoms, end-point PCR using  
83 ACMV-CP/R3 and UV-AL1/F1 primers (Zhou *et al.*, 1997) confirmed the presence of EACMV-UG. At 12  
84 weeks after planting, four plants per variety were virus-inoculated by side grafting using EACMV-UG  
85 scions or healthy scions (mock-inoculated) as control (Mohammed *et al.*, 2012).

86 **Table 1. East African and Latin American cassava varieties screened for resistance to CMD.**

<b>GEOGRAPHICAL ORIGIN</b>	<b>VARIETIES</b>	<b>RESISTANCE TO CMD</b>
<b>KENYA</b>	Kibandameno	Susceptible (Tumwegamire <i>et al.</i> , 2018; Maruthi <i>et al.</i> , 2019)
<b>UGANDA</b>	72-TME 14	Resistant (Tumwegamire <i>et al.</i> , 2018)
	Ebwanateraka	Susceptible (Otim-Nape, Thresh and Shaw, 1997)
	Nase 1	Resistant (Tumwegamire <i>et al.</i> , 2018; Maruthi <i>et al.</i> , 2019)

	Nase 3	Tolerant (Tumwegamire <i>et al.</i> , 2018; Maruthi <i>et al.</i> , 2019)
	Nase 14	Resistant (Tumwegamire <i>et al.</i> , 2018; Maruthi <i>et al.</i> , 2019)
	TME 204	Resistant (Tumwegamire <i>et al.</i> , 2018; Maruthi <i>et al.</i> , 2019)
	TZ 130	Resistant (Tumwegamire <i>et al.</i> , 2018; Maruthi <i>et al.</i> , 2019)
<b>SOUTH AMERICA</b>	Col 2246	Susceptible
	PER 317	Unknown
	PER 335	Unknown
	PER 368	Unknown
	PER 415	Unknown
	PER 608	Unknown

87

## 88 **2.2 Symptom scoring and sample collection**

89 Leaf symptom severity was scored weekly on all plants using the five-point scale (Beyene *et al.*, 2016),  
90 where 0 represents no disease symptoms and five very severe mosaic on leaves affecting 50-80% of the  
91 leaf area. For enhancing symptom expression, the plants were cut back to 20 cm above soil level at 16  
92 weeks after grafting (wag) and all leaves were removed. Symptom development was assessed on newly  
93 formed leaves after four weeks using the same scale.

94 In this time series experiment, samples were collected at 1, 2, 4, 7 dag and, 2, 4, 8, 12, 16 and 24 wag from  
95 EACMV-UG and mock-inoculated plants (10 time points x 14 varieties x 3 plants x 2 treatments). For each  
96 sample, three leaf discs were collected from top, middle and bottom of each plant, but avoiding the scions.

97 Samples were frozen immediately in liquid nitrogen before storage at -80 °C. For each time point, three  
98 biological replicates were processed independently.

### 99 **2.3 Total nucleic acid extraction and real-time PCR**

100 Total nucleic acid was extracted from samples using the cetyltrimethyl ammonium bromide (CTAB)  
101 method (Abarshi *et al.*, 2010; Otti *et al.*, 2016). The pellet was suspended in 100 µl molecular grade water,  
102 and the total nucleic acid concentration was determined using a Nanodrop 2000 spectrometer (Thermo  
103 Scientific, Wilmington USA) and stored at -20 °C.

104 EACMV-UG titre was measured in each sample by qPCR in duplicate using primers and probes (Otti *et*  
105 *al.*, 2016) with a minor modification where the EACMV-specific probe contained no secondary quencher.  
106 Samples with Cq values greater than 35 were considered negative and relative quantities of positive samples  
107 calculated using  $2^{-\Delta\Delta Cq}$  method with PP2A as an internal housekeeping gene (Abarshi *et al.*, 2010).

### 108 **2.4 Data analysis**

109 Varieties were classified into three categories, resistant, tolerant, and susceptible based on foliar severity  
110 and relative virus quantity over time (Fargette *et al.*, 1996; Ogbe *et al.*, 2003). Resistant plants showed little  
111 or no foliar symptoms with low virus titre, tolerant plants expressed foliar symptoms with low virus titre  
112 and susceptible plants showed severe foliar symptoms with high virus concentrations (Kuria *et al.*, 2017).  
113 Analysis of variance was also carried out on means of all replicates for CMD leaf symptom severity at 2,  
114 4, 8, 12, 16 and 24 wag and their average score compared to the resistant control Nase 3, which is currently  
115 one of the best sources of resistance to CMD (Thresh, Fargette and W Otirn-Nape, 1994). An agglomerative  
116 hierarchical clustering tree was generated using average disease severity and virus quantities to classify the  
117 varieties (Houngue *et al.*, 2019)

## 118 **2.5 RNA-Seq and transcriptome analysis**

119 Leaf samples collected at 2, 4, 7 and 28 dag from each EACMV-UG- and mock-inoculated plants were  
120 subjected to RNA-Seq. Total RNA was isolated using the CTAB method (Maruthi *et al.*, 2002) and lithium  
121 chloride with slight modifications. Flash frozen leaf tissues (18 leaf discs) were ground in liquid nitrogen  
122 before being transferred into 1 ml of pre-heated CTAB buffer and mixed. Samples were heated at 60 °C for  
123 10 min and approximately 800 µl of sample mix was transferred to an equal volume of phenol: chloroform:  
124 isoamyl alcohol (25:24:1) before being centrifuged at 14,000 rpm for 10 min at 4 °C. The top aqueous phase  
125 was transferred to an equal volume of chloroform, mixed by inversion, and centrifuged at 14,000 rpm for  
126 20 min at 4 °C. The RNA was then precipitated from the supernatant by adding 0.3 volumes of 8M LiCl  
127 and incubated at 4 °C overnight. Samples were then centrifuged at 14,000 rpm for 10 min at 4 °C to pellet  
128 the RNA. The pellet was washed with 500 µl of 70% ethanol, followed by centrifuging at 13,000 rpm for  
129 5 min at 4 °C before being dried for 10 min at low temperature in a spin vac and resuspended in 30 µl of  
130 RNase-free water. DNA was removed using the DNase Max® kit Quick-Start protocol (Qiagen, USA).  
131 RNA quantities were measured using the NanoDrop spectrophotometer (Thermo Scientific, Wilmington  
132 USA). The RNA integrity was then confirmed using the Agilent 2100 Bioanalyzer instrument according to  
133 the manufacturer's protocol (Agilent Technologies, CA USA). cDNA libraries and RNA-Seq were  
134 performed by Macrogen (Europe) for generating 100 bp paired-end reads using the Illumina NovaSeq next-  
135 generation sequencing system.

136 Paired end reads from samples were aligned to the *Manihot esculenta* v6.1 reference genome downloaded  
137 from Phytozome 12 (<https://phytozome.jgi.doe.gov/pz/portal.html>) with HISAT2 (version 2.1.0,  
138 <https://ccb.jhu.edu/software/hisat2/manual.shtml>) (Prochnik *et al.*, 2012). Samtools (v.1.9) was used to  
139 convert and sort HISAT2 output into sorted bam files which were then assembled into genes using Cufflinks  
140 (Trapnell *et al.*, 2012). Assemblies were merged into one file using the Cuffmerge utility program with  
141 *Mesculenta\_305\_v6.1.gene.gff3.gz* file serving as a reference annotation.

142 Expression values were normalised as fragments per kilobase per million reads (FPKM) values. Cuffdiff  
143 was used to identify DEG in pairwise comparisons between control and virus-infected samples for each  
144 variety and timepoints. To reduce artefacts, genes with FPKM values  $\leq 1$  in all samples were discarded from  
145 the analysis. Genes having  $\log_2$  fold change  $\geq 1$  and  $p\text{-value} \leq 0.05$  were considered up-regulated, whereas  
146 those with fold change  $\leq -1$  and  $p\text{-value} \leq 0.05$  were deemed to be down-regulated upon EACMV-UG  
147 infection.

148 DEGs were queried against the *M. esculenta* genome database at g: Profiler to determine functional  
149 annotation. Functional enrichment analysis was performed using g: Profiler (version  
150 e98\_eg45\_p14\_ce5b097) with g: SCS multiple testing correction methods applying a significance threshold  
151 of 0.05 (Raudvere *et al.*, 2019).

## 152 **3 Results**

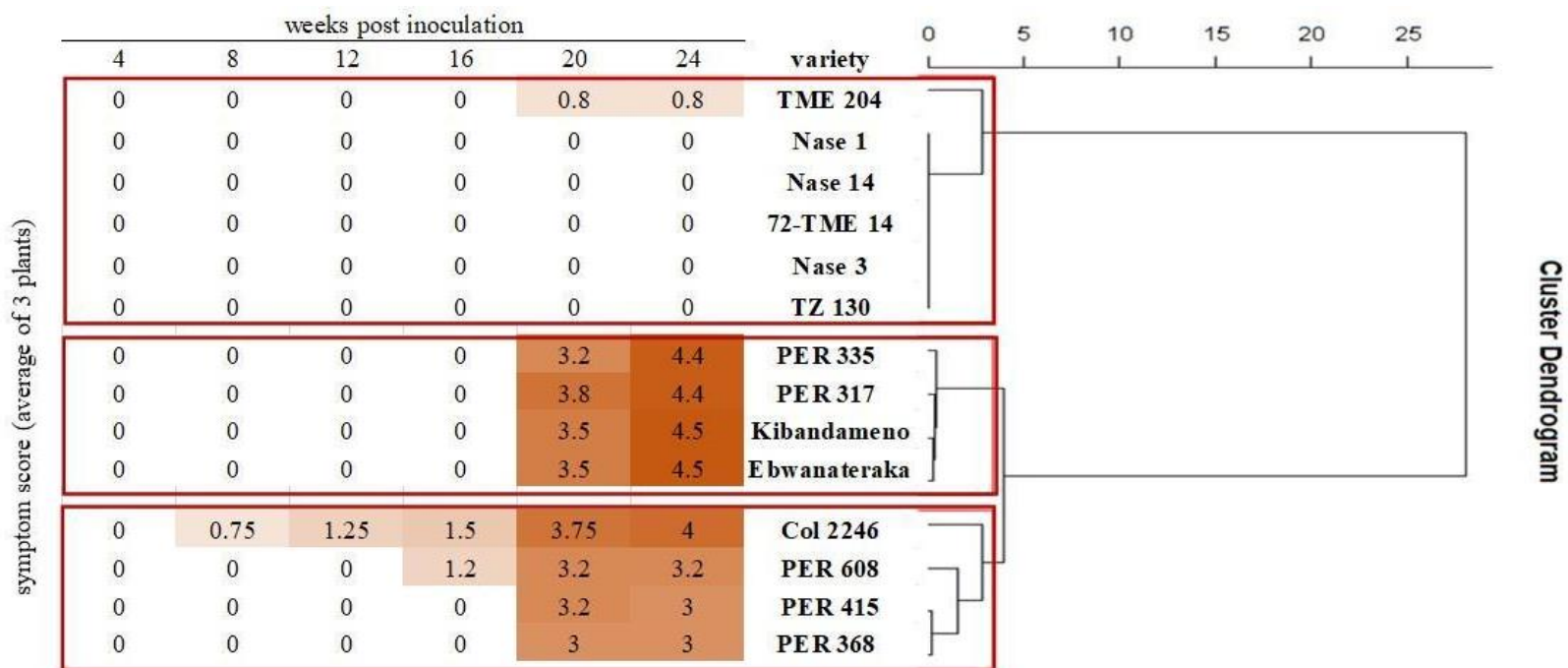
### 153 **3.1 Varieties response to virus infection**

154 About 95% of all cassava plants grafted established a successful graft union between 3 and 4 wag. Early  
155 mild symptoms (score 2) were identified on Col 2246 at 8 and 16 wag, followed by PER 608 at 16 wag and  
156 all other varieties remained symptomless. There was a significant difference between the CMD severity  
157 scores of the 14 varieties at 12 wag ( $df=13$ ;  $p < 0.0001$ ).

158 At 16 wag varieties were cut back to 20 cm above soil level and leaves were removed. After a month, newly  
159 formed leaves were evaluated for the presence of symptoms. At 24 wag (eight weeks after cutting back),  
160 six varieties inoculated with EACMV-UG remained symptomless (72-TME 14, Nase 1, Nase 3, Nase 14  
161 and TZ 130), TME 204 had a severity score between 0-2 and the other seven varieties had a severity score  
162 between 2-5 (Col 2246, Ebwanateraka, Kibandameno, PER 317, PER 335, PER 368, PER 415 and PER  
163 608) (Figs 1 and 2). PER 415, PER 335, PER 317, PER 368, PER 335, TME 204 and Kibandameno each  
164 scored greater than 3 at 24 wag after pruning.



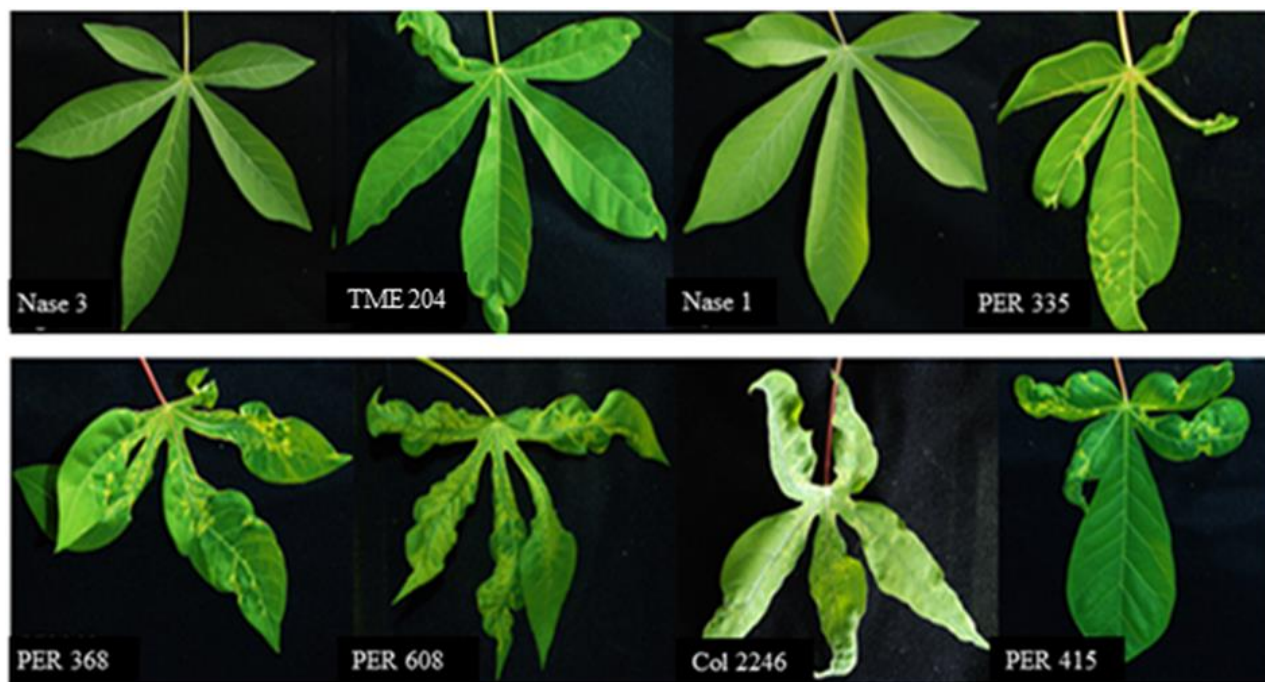




166

167 **Figure 1: Symptom recording of cassava varieties from 4 to 24 weeks post inoculation with EACMV-Ug (left) and hierarchical clustering**

168 **(right) of symptom values.**



169

170 **Figure 2: CMD symptom expression on cassava varieties at 24 wag.**

171 Cluster analysis was carried out based on foliar severity scores of the 14 varieties. Three clusters were  
 172 identified with group 1 having six varieties (72-TME 14, Nase 1, Nase 3, Nase 14, TZ 130 and TME 204),  
 173 and group 2 and 3 with eight varieties (Col 2246, Ebwanateraka, Kibandameno, PER 317, PER 335, PER  
 174 368, PER 415 and PER 608). Group 1 contained varieties with a severity score between 0-2 and were  
 175 considered resistance/tolerant (R/T). Group 2 and 3 varieties had a severity score between 3-5 and were  
 176 considered susceptible (S) (Fig 2).

### 177 **3.2 Quantification of EACMV-UG using qPCR**

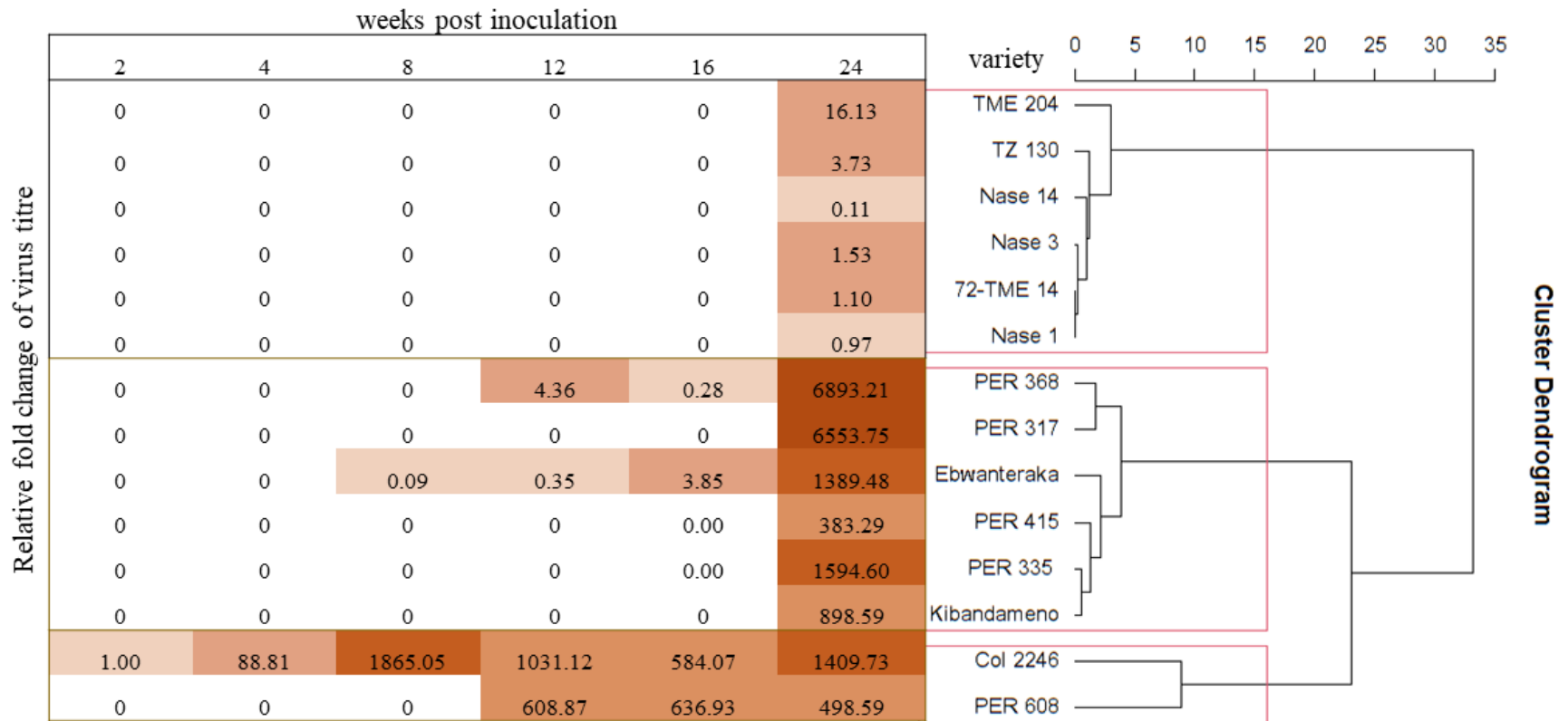
178  $\Delta Cq$  values calculated from the expression of EACMV-UG and PP2A were compared between a multiplex  
 179 and uniplex qPCR test. The Wilcoxon signed-rank test showed that multiplexing did not significantly  
 180 change the  $\Delta Cq$  value at a threshold of  $p = 0.05$ , the multiplex qPCR test was then used for the rest of the  
 181 study.

182 The virus was detected in all varieties and virus titer fold change was calculated using Col 2246 at 2 wag  
183 as a sample calibrator. The virus load was relatively low at 8 wag (1.08 fold change) in Ebwanateraka and  
184 12 wag (5.36) in PER 368. This increased to 1390.47-fold change and 6894.21-fold change (Fig 3) in  
185 Ebwanateraka and PER 368, respectively, at 24 wag. In Col 2246, the virus quantity increased to the highest  
186 level at 8 wag (1866.05 fold change) but decreased to 585.07 fold change at 16 wag before rising to 24 wag  
187 (1410.72 fold change). Varieties with low relative virus load included 72-TME 14 (2.09-fold change), Nase  
188 1 (1.9-fold change), Nase 3 (2.5-fold change), Nase 14 (1.1-fold change), TME 204 (17.1-fold change) and  
189 TZ 130 (4.7-fold change).

190 Pruning affected virus concentration at 20 and 24 wag. The virus concentration of Col 2246 and PER 608  
191 increased from 1.2 to 3.2-fold change and 1.5 to 4.4-fold change between 16 and 24 wag, respectively. The  
192 virus concentration in PER 317 and PER 368 was much higher at 24 wag than 16 wag while it was similar  
193 at 16 and 24 wag in Col 2246 and PER 608 was (Fig 3).

194 Spearman's rank correlation rho analysis showed positive correlation for Col 2246 (0.59), Ebwanateraka  
195 (0.77), Kibandameno (0.66), PER 317 (0.81), PER 335 (0.76), PER 368 (0.99), PER 415 (0.99), PER 608  
196 (0.71) and TME 204 (0.99) between virus titre and mean foliar symptom score. While 72-TME 14, Nase 1,  
197 Nase 3, Nase 14 and TZ 130 had relatively low virus loads with no foliar severity at 24 wag (Fig 3).

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199

200 **Figure 3: Time course quantification of EACMV-UG titre from 4 to 24 weeks post inoculation with EACMV-Ug (left) and hierarchical**  
 201 **clustering (right) of relative fold change of virus titre.**

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203

### 204 **3.3 Transcriptome analyses and GO term enrichment of DEGs**

205 Transcriptome analyses of Ebwanateraka (susceptible) Nase 3 and TZ 130 (resistant) produced 700 million  
206 reads generated from 67 samples. FPKM values were generated for each gene in the cassava reference  
207 genome. Approximately 70% of the reads were uniquely mapped to the genome which represent 17,000  
208 genes expressed with FPKM value  $\geq 1$  in each sample (Tables S1 and S2). DEGs were quantified between  
209 virus- and mock-inoculated samples except for Nase 3 at 2 dag, where no DEGs were identified.

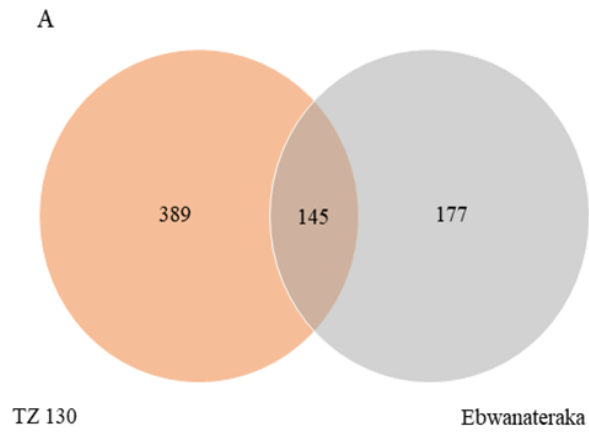
210 The total transcripts after the quality filtering of the data (fold change cut-off,  $p \leq 0.05$ ) were used for  
211 downstream analysis. Nase 3 had the highest number of DEGs (1940 genes up-regulated; 2288 genes down-  
212 regulated), followed by TZ 130 (983 genes up-regulated; 1567 genes down-regulated) when compared to  
213 Ebwanateraka (910 genes up-regulated; 333 genes down-regulated) over the 28 dag period (Table S3, Fig  
214 4). Notwithstanding the genetic background of these varieties, the highest number of DEGs were seen  
215 among TZ 130 and Nase 3 at 4 and 7 dag compared with other combinations of varieties (Nase 3 and  
216 Ebwanateraka, TZ 130 and Ebwanateraka). (Fig 4).

217 All DEGs were further assigned into three groups based on their functional annotation and gene ontology  
218 (GO) terms: molecular functions (MF), cellular components (CC) and biological processes (BP) (Table S4,  
219 Figs S1, S2 and S3). Among all the varieties, MF and BP associated with transcription were enriched.  
220 Specifically, response to abiotic stimulus BP was enriched in Nase 3 at 4 dag for up-regulated genes but  
221 not in any later time points after virus inoculation. BP was also absent in Ebwanateraka and TZ 130 DEGs  
222 at all-time points (Fig S3).

223

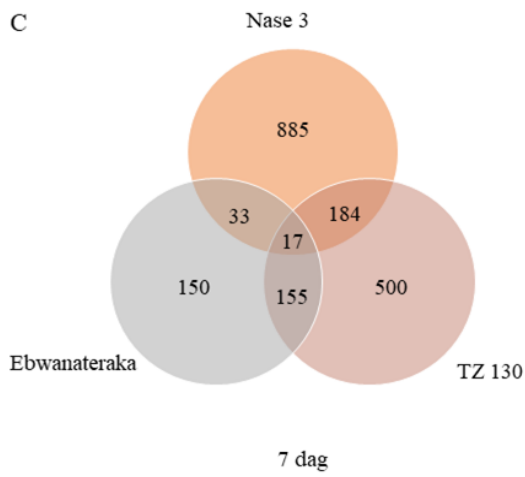
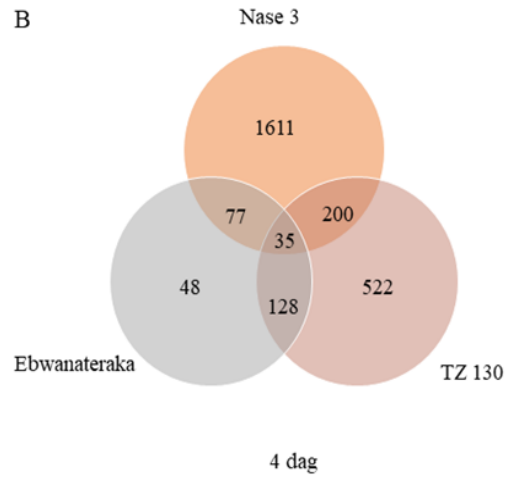
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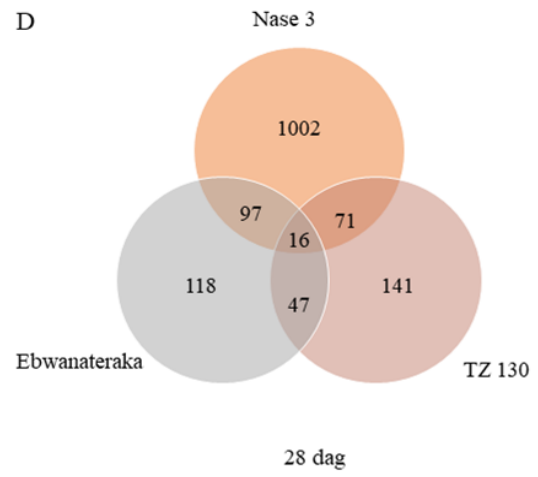


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228



229 **Fig 4. comparisons of DEGs between Ebwanateraka, Nase 3 and TZ 130 at 2 (A), 4 (B), 7 (C)**  
 230 **and 28 (D) dag.**

231

232

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### 234 **3.4 Putative CMD defence genes**

235 Several defence genes were observed to be differentially expressed across all time points in all  
236 varieties. The observed transcripts included heat shock proteins (HSPs), lactoylglutathione lyase  
237 glyoxalase I (GLO1), late embryogenesis abundant protein (LEA), histone-related genes,  
238 pathogenesis-related (PR) proteins, leucine-rich repeat (LRR) proteins and transcription factors  
239 (TFs) which are involved in stress tolerance and defence response (Table S5, S6 and S7).

240 Three *MeHSP20-like* chaperone superfamily protein (Manes.01G119000, Manes.02G124600 and  
241 Manes.02G124700) and Manes.16G083600 were up-regulated at 4 dag (fold change = 1.0–5.79)  
242 in the resistant/tolerant Nase 3 and TZ 130. Manes.16G083600 was up-regulated in Nase 3 at 28  
243 dag. These HSPs were not differentially expressed at any time point in the susceptible  
244 Ebwanateraka (Table 2).

245 Two GLO1 genes (Manes.02G101800 and Manes.01G143500) were down-regulated at 4 dag in  
246 Nase 3 and TZ 130. These were not differentially expressed in Ebwanateraka at any time point  
247 (Table 2).

248 *MeLEA\_3* (Manes.03G197100) was up-regulated in Nase 3 at 4 and 7 dag and in TZ 130 at 4 dag.  
249 This was not differentially expressed at 2 dag in Ebwanateraka (Table 2).

250 Five histone related genes (Manes.01G202800, histone *H2A* (Manes.05G070300), histone *H3*  
251 (Manes.14G013600, Manes.13G097500, Manes.12G129100) were all down-regulated in Nase 3  
252 and TZ 130. While they were not differentially expressed at all time points in Ebwanateraka (Table  
253 2).



254 Among the four *MePR* genes analysed, *PR-10f5* (Manes.15G008100), *PR-5c* (Manes.11G095900)  
255 and *PR-5h2* (Manes.06G007400 and Manes.14G170800), Manes.15G008100 were down-  
256 regulated at 4 dag in TZ 130 and Nase 3, and at 28 dag in Ebwanateraka (Table 2).

## 257 **Response of Transcription Factors to EACMV-UG infection**

### 258 **Ethylene-responsive transcription factor (ERF)**

259 Manes.13G093300 and Manes.18G069000 were up-regulated at 4 dag in Nase 3 and down-  
260 regulated in TZ 130 at 7 dag while Manes.12G087500 was up-regulated in Nase 3 and  
261 Ebwanateraka at 4 and 7 dag, respectively. Manes.16G111000 was down-regulated in  
262 Ebwanateraka and Manes.15G066800 was up-regulated in Nase 3 at 4 dag. At the same time point,  
263 Manes.07G114500 was induced in Nase 3 and TZ 130. ERF-5 (Manes.01G085200) was induced  
264 at 4 dag in Nase 3 and TZ 130 and at 7 dag in Ebwanateraka. The same gene was down-regulated  
265 in TZ 130 at 7 dag.

### 266 **Teosinte-like, cycloidea and PCF1 (TCP)**

267 Four of the five TCP genes were up-regulated only in Nase 3 at different time points: MeTCP11d  
268 (Manes.04G016700), MeTCP7 (TCP21-RELATED; Manes.11G108500), MeTCP19  
269 (Manes.06G141800) at 4 dag and MeTCP15d (TCP family transcription factor;  
270 Manes.14G077200) at 28 dag. MeTCP20a (Manes.04G088500) was up-regulated in  
271 Ebwanateraka but down-regulated in Nase 3 at 4 dag. Finally, MeTCP9a (Manes.01G263300) was  
272 down-regulated in both Nase 3 and TZ 130 at 7 dag and up-regulated in Nase 3 at 4 and 28 dag.

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275 **Heat stress transcription factor (HSF)**

276 Only six HSFs were observed in this study. Four of them were Nase 3-specific DEGs: MeHSFB-  
277 2B (Manes.01G065200), MeHSFB-1 (Manes.12G101000), MeHSFA-3 (Manes.08G020700) and  
278 MeHSFC-1 (Manes.16G116200), which were up-regulated at 4 dag while the MeHSFB-1  
279 (Manes.13G124500) was down-regulated at 28 dag in TZ 130. HSF-2A (Manes.14G027700)  
280 was up-regulated at 4 dag both in Nase 3 and TZ 130.

281 **Basic leucine zipper (bZIP)**

282 All three bZIP genes identified in this study were Nase 3-specific while Manes.18G001000 and  
283 Manes.18G005000 encoded MebZIP60. Manes.18G001000 was up-regulated at 4 dag and down-  
284 regulated at 28 dag while Manes.18G005000 was up-regulated only at 4 dag. Manes.01G227900  
285 was up-regulated at 4 and 28 dag.

286 **Nuclear factor Y (NF-Y)**

287 MeNF-YA-10-RELATED (Manes.09G044200) was up-regulated in Ebwanateraka at 2 dag, while  
288 MeNF-YA-10-RELATED, A-3-RELATED (Manes.04G142600) and ALPHA-RELATED  
289 (Manes.16G097900) were up-regulated at 4 and 28 dag. A-1-RELATED (Manes.14G003100) and  
290 ALPHA-RELATED (Manes.09G025200) were up-regulated at 4 dag and ALPHA-RELATED  
291 (Manes.10G141400) at 7 dag in Nase 3.

292 **Other Transcription Factors (NAC, MYB, bHLH and WRKY)**

293 NAC: NAC DOMAIN-CONTAINING PROTEIN 72 (MeNAC72; Manes.15G084800) was up-  
294 regulated in Ebwanateraka at 4 and 28 dag but was not differentially expressed in Nase 3 and TZ  
295 130.

296 MYB: Manes.12G085600 was up-regulated in TZ 130 and Ebwanateraka at different time points  
297 (2 and 4 dag, respectively). At 7 dag, it was down-regulated in TZ 130 but up-regulated in  
298 Ebwanateraka.

299 bHLH: MebHLH57 (Manes.05G015800) was shown to be down-regulated in TZ 130 and Nase 3  
300 (at 2 and 7 dag, respectively) and up-regulated at 4 dag in Ebwanateraka.

301 WRKY: WRKY DNA-binding domain (WRKY 70; Manes.08G170600) was up-regulated in Nase  
302 3 and not differentially expressed in TZ 130 and Ebwanateraka.

303 **Table 2. Selected DEGs that were expressed at different levels in susceptible (Ebwanateraka) and resistant (Nase 3 and TZ 130) varieties**  
 304 **at different time points.**

Gene IDs	Protein name	Variety	Gene expression fold change between virus and mock inoculated plants							
			2 dag log2 fold change	Adj. P value	4 dag log2 fold change	Adj. P value	7 dag log2 fold change	Adj. P value	28 dag log2 fold change	Adj. P value
Manes.01G119000	HSP20-like chaperone superfamily protein	Ebwanateraka	-0.06071	>0.05	0.712806	>0.05	-0.00687	>0.05	1.31916	>0.05
		TZ 130	0.626176	>0.05	1.13962	<0.05	0.582087	>0.05	0.543521	>0.05
		Nase 3	NA	NA	3.33074	<0.05	-1.7193	>0.05	-0.09432	>0.05
Manes.02G124600	HSP20-like chaperone superfamily protein	Ebwanateraka	-0.04156	>0.05	0.417014	>0.05	0.344491	>0.05	0.782273	>0.05
		TZ 130	0.358427	>0.05	1.0322	<0.05	0.320253	>0.05	-0.18567	>0.05
		Nase 3	NA	NA	5.75847	<0.05	0.196281	>0.05	0.659707	<0.05
Manes.02G124700, Manes.02G124800	HSP20-like chaperone superfamily protein	Ebwanateraka	0.236262	>0.05	0.557475	>0.05	1.55634	>0.05	0.964728	<0.05
		TZ 130	0.663816	>0.05	1.5206	<0.05	0.208954	>0.05	0.965357	>0.05

		Nase 3	NA	NA	5.79276	<0.05	0.959413	>0.05	0.415391	>0.05
Manes.16G083600	17.6 KDA CLASS II HEAT SHOCK PROTEIN-RELATED	Ebwanateraka	0.104547	>0.05	0.084419	>0.05	0.833265	>0.05	1.1471	>0.05
		TZ 130	-0.90388	>0.05	1.61178	<0.05	1.31665	>0.05	-0.15462	>0.05
		Nase 3	NA	NA	5.52934	<0.05	-0.28711	>0.05	1.57786	<0.05
Manes.01G143500	LACTOYLGLUTATHIONE LYASE GLYOXALASE I// SUBFAMILY NOT NAMED	Ebwanateraka	0.092847	>0.05	-0.33239	>0.05	0.10087	>0.05	-0.3435	>0.05
		TZ 130	-1.51369	<0.05	-1.55201	<0.05	0.109942	>0.05	0.110754	>0.05
		Nase 3			-1.10906	<0.05	1.54269	<0.05	-0.40953	>0.05
Manes.02G101800	LACTOYLGLUTATHIONE LYASE GLYOXALASE I// SUBFAMILY NOT NAMED	Ebwanateraka	NA	NA	NA	NA	NA	NA	NA	NA
		TZ 130	-1.52005	<0.05	-1.29112	<0.05	0.372692	>0.05	0.292894	>0.05
		Nase 3	NA	NA	-1.21241	<0.05	0.93428	>0.05	-0.72648	<0.05
Manes.03G197100	Late embryogenesis abundant protein (LEA_3)	Ebwanateraka	0.922234	<0.05	0.573219	>0.05	-0.19654	>0.05	0.345698	>0.05
		TZ 130	0.405275	>0.05	1.63458	<0.05	0.714648	<0.05	-0.70995	>0.05

		Nase 3			1.47171	<0.05	1.4739	<0.05	0.326894	>0.05
Manes.01G202800	Manes.01G202800.1.p - (1 of 2) PTHR23430:SF76 - HISTONE H2A VARIANT 1-RELATED	Ebwanateraka	0.71641	>0.05	0.151189	>0.05	-0.77489	>0.05	-0.60158	>0.05
		TZ 130	-1.14505	>0.05	-2.78418	<0.05	-1.68322	<0.05	0.050764	>0.05
		Nase 3	NA	NA	-0.84901	>0.05	-4.02193	<0.05	-0.88381	>0.05
Manes.05G070300	Manes.05G070300.1.p - (1 of 19) K11251 - histone H2A (H2A)	Ebwanateraka	0.92733	>0.05	-0.59825	>0.05	-0.51015	>0.05	0.529688	>0.05
		TZ 130	-2.11049	<0.05	-4.0027	<0.05	-1.99963	<0.05	0.254461	>0.05
		Nase 3	NA	NA	-1.45309	>0.05	-5.52002	<0.05	-0.08511	>0.05
Manes.14G013600	Manes.14G013600.1.p - (1 of 14) K11253 - histone H3 (H3)	Ebwanateraka	1.29511	>0.05	-0.91882	>0.05	-0.90872	>0.05	0.932617	>0.05
		TZ 130	-1.40766	<0.05	-3.08618	<0.05	-2.03717	<0.05	0.363321	>0.05
		Nase 3	NA	NA	-1.0283	>0.05	-5.07045	<0.05	0.34621	>0.05
Manes.15G008100	Pathogenesis-related protein Bet v I family (Bet_v_1)	Ebwanateraka	-0.78164	<0.05	-0.37797	>0.05	-0.55282	>0.05	-1.01783	<0.05
		TZ 130	0.192398	>0.05	-1.61064	<0.05	0.033711	>0.05	0.101362	>0.05

		Nase 3	NA	NA	-1.19987	<0.05	0.576867	>0.05	-0.14117	>0.05
Manes.11G095900	Manes.11G095900.1.p - (1 of 1) PTHR31048:SF4 - PATHOGENESIS- RELATED THAUMATIN- LIKE PROTEIN	Ebwanateraka	1.21228	<0.05	-0.30358	>0.05	-0.74074	>0.05	0.010438	>0.05
		TZ 130	-2.11106	<0.05	-0.51751	>0.05	-1.07914	<0.05	0.748967	>0.05
		Nase 3	NA	NA	0.211652	>0.05	-1.96604	<0.05	0.256043	>0.05
Manes.06G007400	Manes.06G007400.1.p - (1 of 2) PTHR31048:SF1 - PATHOGENESIS- RELATED THAUMATIN- LIKE PROTEIN- RELATED	Ebwanateraka	1.83305	<0.05	-0.0993	>0.05	-0.4168	>0.05	-0.19691	>0.05
		TZ 130	-2.05019	<0.05	-1.43309	<0.05	-2.08151	<0.05	0.878607	>0.05
		Nase 3	NA	NA	0.489769	>0.05	-2.63034	<0.05	0.011581	>0.05
Manes.15G008100	Pathogenesis-related protein Bet v I family (Bet_v_1)	Ebwanateraka	-0.78164	<0.05	-0.37797	>0.05	-0.55282	>0.05	-1.01783	<0.05
		TZ 130	0.192398	>0.05	-1.61064	<0.05	0.033711	>0.05	0.101362	>0.05
		Nase 3	NA	NA	-1.19987	<0.05	0.576867	>0.05	-0.14117	>0.05

## 306 **4 Discussion**

307 In this study we measured symptom expression and EACMV-UG relative loads over time in 14 cassava  
308 varieties and used the RNAseq method to identify DEGs between EACMV-UG-inoculated and mock-  
309 inoculated plants across time with three cassava varieties, Nase 3 (resistant), TZ 130 (resistant) and  
310 Ebwanateraka (susceptible). The results indicated Col 2246 was the first to show pronounced symptoms at  
311 8 wag, this is consistent with previous observations that susceptible varieties displayed more severe  
312 symptoms than the resistant ones (Ogbe *et al.*, 2003; Kuria *et al.*, 2017; Houngue *et al.*, 2019). Vars.  
313 Ebwanateraka, PER 317, PER 368 and PER 608 supported high virus concentration (Fig 3) as much as Col  
314 2246 but not in other varieties (72-TME 14, Kibandameno, Nase 1, Nase 3, Nase 14, TZ 130, TME 204,  
315 PER 335 and PER 415) before 16 wag. This could be attributed to their ability to restrict virus multiplication  
316 and limit symptom development. Pruning had a significant effect on symptom severity and virus quantity  
317 (df=13; p<0.0001) (21). Col 2246, Ebwanateraka, Kibandameno, PER 317, PER 335, PER 368, PER 415  
318 and PER 608 expressed severe symptoms with high virus quantities and were considered susceptible. TME  
319 204 expressed mild symptoms with lower virus quantities compared to Col 2246 (Figs 1 and 2) hence they  
320 were considered tolerant (Thresh, Fargette and W Otirn-Nape, 1994; Fargette *et al.*, 1996). The definitions  
321 of resistance, tolerance and susceptibility adopted in this work are those established earlier (Ogbe *et al.*,  
322 2003), where resistance corresponds to no or very low accumulation of the virus with no or mild foliar  
323 symptoms. Tolerance is an interaction in which viruses accumulate but with mild foliar symptom severity,  
324 while susceptibility is high virus concentration with severe disease symptoms. Based on these definitions,  
325 vars. 72-TME 14, TZ 130, Nase 1, Nase 3 and Nase 14 were considered CMD resistant. These varieties  
326 were also classified as resistant based on field screening (Tumwegamire *et al.*, 2018; Maruthi *et al.*, 2019)  
327 Col 2246, Ebwanateraka, Kibandameno, PER 317, PER 335, PER 368, PER 415 and PER 608, accumulated  
328 high viral titres and displayed severe symptoms, which grouped them as susceptible, while only TME 204  
329 grouped as tolerant with mild symptoms and low viral titres. 72-TME 14, Nase 1, Nase 3 and Nase 14 were  
330 improved cassava varieties obtained from Uganda (Otim-Nape, Thresh and Shaw, 1997) and TZ 130 was



331 an improved variety released by both Uganda and Tanzania (Sserubombwe *et al.*, 2001) These varieties  
332 can thus provide relief from the impacts of CMD in these countries.

333 Transcriptomes (RNA-Seq) of Nase 3 and TZ 130 (resistant), together with the susceptible Ebwanateraka  
334 were analysed to understand the mechanisms of resistance and to identify putative EACMV-UG resistance  
335 genes. About 80% of the sequences from all three varieties mapped to the cassava reference genome [except  
336 two Nase 3 samples which mapped poorly (51%)]. The non-alignment of the remaining 20% sequences  
337 could be due to their diverse origin (11, 12) as sequences used in this study were of African origin while  
338 the reference genome was from a partial inbred line called AM560-2 from Latin America (Otim-Nape,  
339 Thresh and Shaw, 1997; Sserubombwe *et al.*, 2001; Prochnik *et al.*, 2012). Relatively fewer DEGs were  
340 observed in the susceptible Ebwanateraka (1243) over the 28 dag period compared to the resistant Nase 3  
341 (4228) and TZ 130 (2550). The total number of genes repressed by the virus (Nase 3; 2067 and TZ 130;  
342 1176) was greater than the number of induced genes in the resistant varieties (Nase 3; 1725 and TZ 130;  
343 897) vice versa for the susceptible variety Ebwanateraka. A similar pattern was observed in the  
344 transcriptome response of cassava to SACMV (Allie *et al.*, 2014).

345 Many GO terms were enriched in Nase 3 followed by TZ 130 at 4, 7 and 28 dag. This was particularly acute  
346 at 7 dag where more defence genes were expressed in Nase 3 and TZ 130 compared to Ebwanateraka  
347 suggesting a rapid response to EACMV-UG, which can be a characteristic of resistant varieties. Five major  
348 groups of TFs (WRKY, HSF, bZIP, TCP and ERF) were up-regulated in Nase 3 and HSP was up-regulated  
349 in TZ 130. These TFs were previously reported to play crucial roles in immune responses against pathogens  
350 (Buscaill and Rivas, 2014; Phukan, Jeena and Shukla, 2016; Noman *et al.*, 2017). WRKY are largest families  
351 of DNA-binding TFs involved in plant development as well as responses to biotic and abiotic stress (Lai *et*  
352 *al.*, 2008; Birkenbihl *et al.*, 2018). WRKY DNA-binding domain (*WRKY 70*; Manes.08G170600) was up-  
353 regulated in Nase 3, which was involved in defence responses (Li *et al.*, 2006, 2013). In *Arabidopsis*,  
354 *AtWRKY70* was involved in the cross-talk between SA- and JA-mediated signalling pathways (Li *et al.*,

2006). We speculate that *MeWRKY70* identified in this study may also be involved in a similar cross-talk in cassava. Another class of transcription factor, HSF, plays a role in plant response to several biotic and abiotic stressors (Fragkostefanakis *et al.*, 2015). Four *MeHSFs* genes were up-regulated at 4 dag in Nase 3 and TZ 130, *MeHSFB-1* (Manes.13G124500) and *HSFB-2A* in particular were both up-regulated at 4 dag possibly indicating the early response by the resistant varieties. In *Arabidopsis*, *HsfB1* and *HsfB2b* suppress the general heat shock response in the absence of extreme heat but supports acquired thermotolerance (Ikeda, Mitsuda and Ohme-Takagi, 2011). Three bZIP genes were up-regulated in Nase 3 at most time points, which are also known to respond to biotic and abiotic stress in plants (Wei *et al.*, 2012; Alves *et al.*, 2013).

For BP, response to abiotic stimulus was highly expressed in Nase 3 (Fig S1). The majority of the selected DEGs were shown to be mostly PR genes, HSPs, LEA and Histone-related genes involved in basal immune and other metabolic processes. Data from all three varieties were scrutinised to identify differences between genes of resistant and susceptible varieties. PR proteins are an important part of plant immunity. In this study, *PR-10f5* was down-regulated in Nase 3 and TZ 130 at 4 dag and Ebwanateraka at a much later point (28 dag). While the *PR-5c* and *PR-5h2* were induced in Ebwanateraka and suppressed in TZ 130 at 2 dag and suppressed in Nase 3 and TZ 130 at 7 dag. Our results are similar to that of an earlier study where transcripts level of *PR-5* (thaumatin superfamily protein) increased after SACMV infection in the susceptible cassava var. T200 (Allie *et al.*, 2014). Results upon UCBSV infection (another cassava virus) showed that 012383 was up-regulated at 1 and 45 dag in Albert (susceptible) (94). *PR-5* in cassava and *Arabidopsis* were also induced upon whitefly infestation (Zarate, Kempema and Walling, 2007; Irigoyen *et al.*, 2020). Results from the different studies show that despite the induction of PR genes in susceptible var., it was incapable of preventing viral replication and lead to progressive increase in symptom severity and virus titre. The HSPs are involved in regulatory, signalling and defence pathways. In this study, *MeHSP20-like* chaperone superfamily protein (Manes.02G124600, Manes.02G124600 and Manes.02G124700) was up-regulated in Nase 3 and TZ 130 at 4 dag after virus infection, suggesting an early defence response.

380 Similarly, 14 *MeHSPs* were induced in the resistant variety Namikonga while the susceptible Albert, had  
381 only two genes induced after UCBSV infection (Amuge *et al.*, 2017). *MeHSP20-like* chaperone superfamily  
382 proteins were also induced 24 hrs after infestation by mealybug in the resistant variety AR23.1 and  
383 repressed in the susceptible variety (P40/1) (Rauwane *et al.*, 2018). LEA proteins also play an important  
384 role in abiotic stress adaptation in plants and they were expressed in both TZ 130 and Nase 3. Similarly,  
385 two LEA genes were induced in the resistant cassava varieties but remained unchanged in the susceptible  
386 variety upon infection by UCBSV (Amuge *et al.*, 2017). Histones are well known to play an important role  
387 in DNA replication and transcription regulation (Liu *et al.*, 2010; Saze *et al.*, 2012) were down-regulated  
388 (*H3* and *H2A*) in Nase 3 (at 7 dag) and TZ 130 (at 2, 4 and 7 dag) but not in Ebwanateraka. Histone *H3*  
389 plays a role in geminivirus replication and movement (Kong and Hanley-Bowdoin, 2002; Zhou *et al.*, 2011).  
390 The *H2A* was overexpressed in the susceptible cassava variety T200 and *Nicotiana benthamiana*, but not  
391 in the tolerant variety TME3 (Allie *et al.*, 2014). These consensual data on the down-regulation of *H3* and  
392 *H2A* in resistant/ tolerant plants perhaps suggests their role in the inhibition of geminivirus DNA replication  
393 in resistant/ tolerant plants. The next steps in understanding the mechanisms of resistance to EACMV-UG  
394 should include validating the role of these putative genes by gene upregulation and knockdown  
395 experiments.

## 396 **5 Conclusions**

- 397 • We confirmed that the six cassava varieties 72-TME 14, TME 204, TZ 130, Nase 1, Nase 3 and  
398 Nase 14 from Africa were resistant to the most severe strain of the CMBs, EACMV-UG,  
399 unambiguously by molecular approaches. These will make a reliable source of resistance to CMD  
400 in Africa and thus cassava can be safely cultivated and promoted for mitigating the impact of the  
401 devastating CMD pandemic in affected countries.

- 402       • All the South American cassava varieties tested were susceptible to EACMV-UG, further  
403       confirming their limited utility as a source of resistance to the begomoviruses and highlighting the  
404       dangers of ever accidentally introducing the African CMBs to South America.
- 405       • We investigated the mechanism of resistance to EACMV-UG by a time-course transcriptome  
406       analysis (RNA-Seq) of the two resistant varieties Nase 3 and TZ 130 and a susceptible var.  
407       Ebwanateraka. Early response of the resistant varieties was key for their resistance to virus  
408       infection, while the response of the susceptible variety was delayed. Also, fewer unique DEGs  
409       were expressed by the susceptible variety compared to the resistant ones while the opposite was  
410       true for the total number of DEGs identified.
- 411       • The number of GO terms and genes enriched especially HSPs and LEA were higher for Nase 3 and  
412       TZ 130 at 7 dag when compared to Ebwanateraka, suggesting a rapid response to EACMV-UG in  
413       the resistant varieties.
- 414       • TFs such as bZIP and WRKY associated with plant resistance against abiotic stress were also up-  
415       regulated in Nase 3 at early time points (up to 7 dag) but not in Ebwanateraka and TZ 130.
- 416       • The down-regulation of LRR and PR genes in Nase 3 and TZ 130 was surprising and requires  
417       further investigations.

## 418   **Abbreviations**

- 419   CMBs: Cassava mosaic begomoviruses
- 420   CMD: Cassava mosaic disease
- 421   EACMV-UG: *East African cassava mosaic virus*-Uganda
- 422   RNA-Seq: RNA-Sequencing
- 423   DEGs: Differentially expressed genes
- 424   ERFs: Ethylene-responsive transcription factor
- 425   TCPs: Teosinte-like, cycloidea and PCF1

426 SACMV: *South African cassava mosaic virus*  
427 CBSV: *Cassava brown streak virus*  
428 UCBSV: *Ugandan cassava brown streak virus*  
429 CBSIs: Cassava brown streak ipomoviruses  
430 dag: days after grafting  
431 wag: weeks after grafting  
432 RH: relative humidity

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## 442 **Declaration of competing interest**

443 The authors declare that they have no known competing financial interests or personal relationships that  
444 could have appeared to influence the work reported in this paper.

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## Supporting information

### Tables

**Table S1**

**Summary of RNA-Seq reads mapping to the cassava reference genome, GC content and quality values.**

Sample ID	Treatment	Replicate	Time point (days)	Processed reads	Mapped reads	Unmapped reads	% of mapped reads	GC (%)	Q20 (%)	Q30 (%)
<b>TZ 130</b>	Virus	1	2	32,181,822	27,853,367	4,328,455	93.91	46.95	98.10	94.65
<b>TZ 130</b>	Virus	2	2	42,080,862	38,306,209	3,774,653	93.93	46.06	98.09	94.52
<b>TZ 130</b>	Virus	3	2	46,291,560	41,657,775	4,633,785	92.92	45.64	98.04	94.33
<b>Ebwanateraka</b>	Virus	1	2	42,369,306	39,195,845	3,173,461	93.65	45.4	98.07	94.41
<b>Ebwanateraka</b>	Virus	2	2	39,837,308	36,546,746	3,290,562	93.58	45.94	98.22	94.80
<b>Ebwanateraka</b>	Virus	3	2	61,752,982	35,186,849	26,566,133	91.25	53.66	98.19	94.76
<b>TZ 130</b>	Mock	1	2	34,744,920	22,851,734	11,893,186	94.00	51.95	97.62	93.64
<b>TZ 130</b>	Mock	2	2	38,288,994	27,242,619	11,046,375	93.45	50.46	98.24	94.99

<b>Sample ID</b>	<b>Treatment</b>	<b>Replicate</b>	<b>Time point (days)</b>	<b>Processed reads</b>	<b>Mapped reads</b>	<b>Unmapped reads</b>	<b>% of mapped reads</b>	<b>GC (%)</b>	<b>Q20 (%)</b>	<b>Q30 (%)</b>
<b>TZ 130</b>	Mock	3	2	30,775,128	14,904,394	15,870,734	92.69	56.04	98.23	94.85
<b>Ebwanateraka</b>	Mock	1	2	34,600,388	17,860,720	16,739,668	93.48	56.71	98.17	94.73
<b>Ebwanateraka</b>	Mock	2	2	38,832,350	34,335,564	4,496,786	94.04	45.96	98.00	94.32
<b>Ebwanateraka</b>	Mock	3	2	55,405,314	51,587,888	3,817,426	93.46	45.22	98.24	94.85
<b>Nase 3</b>	Virus	1	4	30,435,490	26,378,439	4,057,051	65.77	46.95	98.04	94.59
<b>Nase 3</b>	Virus	2	4	38,932,554	36,257,888	2,674,666	71.15	45.34	98.16	94.66
<b>Nase 3</b>	Virus	3	4	36,999,264	28,626,331	8,372,933	48.43	48.79	98.00	94.39
<b>TZ 130</b>	Virus	1	4	34,590,884	31,190,600	3,400,284	88.42	46.01	97.97	94.27
<b>TZ 130</b>	Virus	2	4	46,661,094	42,652,906	4,008,188	93.11	45.70	98.10	94.50
<b>TZ 130</b>	Virus	3	4	40,116,560	37,296,366	2,820,194	86.67	45.35	98.18	94.60
<b>Ebwanateraka</b>	Virus	1	4	41,324,476	32,447,979	8,876,497	93.13	48.36	97.99	94.34
<b>Ebwanateraka</b>	Virus	2	4	37,551,772	25,328,670	12,223,102	77.37	52.21	97.96	94.54
<b>Ebwanateraka</b>	Virus	3	4	34,397,980	32,485,452	1,912,528	90.17	45.19	98.16	94.63

<b>Sample ID</b>	<b>Treatment</b>	<b>Replicate</b>	<b>Time point (days)</b>	<b>Processed reads</b>	<b>Mapped reads</b>	<b>Unmapped reads</b>	<b>% of mapped reads</b>	<b>GC (%)</b>	<b>Q20 (%)</b>	<b>Q30 (%)</b>
<b>Nase 3</b>	Mock	1	4	30,828,390	27,326,285	3,502,105	91.41	45.04	97.87	94.03
<b>Nase 3</b>	Mock	2	4	36,704,388	33,243,164	3,461,224	92.97	45.19	98.02	94.41
<b>Nase 3</b>	Mock	3	4	47,190,366	41,381,232	5,809,134	78.52	45.14	97.98	94.21
<b>TZ 130</b>	Mock	1	4	48,097,488	40,315,314	7,782,174	91.44	46.2	97.76	93.95
<b>TZ 130</b>	Mock	2	4	32,057,432	25,027,237	7,030,195	88.64	48.42	98.07	94.70
<b>TZ 130</b>	Mock	3	4	56,313,566	30,065,813	26,247,753	90.57	54.94	97.82	94.01
<b>Ebwanateraka</b>	Mock	1	4	36,787,862	33,465,918	3,321,944	87.69	45.65	98.13	94.61
<b>Ebwanateraka</b>	Mock	2	4	51,651,030	24,043,554	27,607,476	83.82	55.25	97.86	94.15
<b>Ebwanateraka</b>	Mock	3	4	33,699,730	30,697,084	3,002,646	78.07	45.08	98.00	94.50
<b>Nase 3</b>	Virus	1	7	32,960,034	30,755,008	2,205,026	53.39	44.43	97.95	94.24
<b>Nase 3</b>	Virus	2	7	33,499,876	30,997,435	2,502,441	90.97	44.69	98.19	94.67
<b>Nase 3</b>	Virus	3	7	37,445,146	33,992,704	3,452,442	46.55	45.69	97.97	94.33
<b>TZ 130</b>	Virus	1	7	38,384,954	34,385,242	3,999,712	91.09	45.92	97.99	94.29

<b>Sample ID</b>	<b>Treatment</b>	<b>Replicate</b>	<b>Time point (days)</b>	<b>Processed reads</b>	<b>Mapped reads</b>	<b>Unmapped reads</b>	<b>% of mapped reads</b>	<b>GC (%)</b>	<b>Q20 (%)</b>	<b>Q30 (%)</b>
<b>TZ 130</b>	Virus	2	7	36,920,644	20,343,275	16,577,369	93.31	55.48	97.97	94.54
<b>TZ 130</b>	Virus	3	7	34,977,426	31,808,471	3,168,955	92.53	45.56	98.02	94.35
<b>Ebwanateraka</b>	Virus	1	7	30,875,776	25,975,790	4,899,986	90.78	47.18	98.02	94.60
<b>Ebwanateraka</b>	Virus	2	7	37,195,682	33,185,987	4,009,695	89.58	47.07	97.97	94.39
<b>Ebwanateraka</b>	Virus	3	7	46,956,838	42,491,243	4,465,595	90.94	46.47	98.16	94.84
<b>Nase 3</b>	Mock	1	7	38,473,838	35,699,874	2,773,964	84.13	45.13	97.99	94.33
<b>Nase 3</b>	Mock	2	7	44,390,272	40,927,831	3,462,441	89.22	45.25	97.92	94.10
<b>Nase 3</b>	Mock	3	7	30,449,934	26,646,737	3,803,197	90.49	46.24	98.00	94.35
<b>TZ 130</b>	Mock	1	7	38,873,938	36,506,515	2,367,423	92.79	44.62	98.14	94.66
<b>TZ 130</b>	Mock	2	7	51,232,452	45,801,812	5,430,640	92.20	45.25	97.85	94.47
<b>TZ 130</b>	Mock	3	7	37,493,178	33,762,607	3,730,571	87.51	46.19	97.91	94.27
<b>Ebwanateraka</b>	Mock	1	7	30,207,730	26,178,019	4,029,711	89.40	47.02	98.12	94.66
<b>Ebwanateraka</b>	Mock	2	7	37,292,968	18,542,064	18,750,904	90.05	55.34	97.93	94.25

<b>Sample ID</b>	<b>Treatment</b>	<b>Replicate</b>	<b>Time point (days)</b>	<b>Processed reads</b>	<b>Mapped reads</b>	<b>Unmapped reads</b>	<b>% of mapped reads</b>	<b>GC (%)</b>	<b>Q20 (%)</b>	<b>Q30 (%)</b>
<b>Ebwanateraka</b>	Mock	3	7	33,386,220	18,055,268	15,330,952	86.66	54.41	97.96	94.34
<b>Nase 3</b>	Virus	1	28	41,729,892	27,625,189	14,104,703	49.72	51.31	97.97	94.36
<b>Nase 3</b>	Virus	2	28	58,063,310	50,242,182	7,821,128	54.08	46.52	98.29	94.93
<b>Nase 3</b>	Virus	3	28	39,391,020	36,999,985	2,391,035	66.20	44.6	97.88	94.00
<b>TZ 130</b>	Virus	1	28	32,001,428	28,602,876	3,398,552	86.53	46.02	98.14	94.53
<b>TZ 130</b>	Virus	2	28	37,945,172	33,418,313	4,526,859	89.38	46.65	98.16	94.65
<b>TZ 130</b>	Virus	3	28	40,239,228	27,990,407	12,248,821	88.07	50.71	98.10	94.68
<b>Ebwanateraka</b>	Virus	1	28	29,926,518	28,008,228	1,918,290	69.56	44.93	98.10	94.70
<b>Ebwanateraka</b>	Virus	2	28	36,444,796	33,875,438	2,569,358	93.59	45.21	98.01	94.27
<b>Ebwanateraka</b>	Virus	3	28	34,162,408	31,699,298	2,463,110	92.95	45.67	98.04	94.37
<b>Nase 3</b>	Mock	1	28	35,042,668	32,617,715	2,424,953	92.79	45.34	98.06	94.44
<b>Nase 3</b>	Mock	2	28	32,388,548	29,528,639	2,859,909	93.08	45.82	98.16	94.72
<b>Nase 3</b>	Mock	3	28	38,627,186	35,892,381	2,734,805	91.17	44.78	97.94	94.19

Sample ID	Treatment	Replicate	Time point (days)	Processed reads	Mapped reads	Unmapped reads	% of mapped reads	GC (%)	Q20 (%)	Q30 (%)
<b>TZ 130</b>	Mock	1	28	32,903,266	26,704,291	6,198,975	81.16	47.76	97.82	94.32
<b>TZ 130</b>	Mock	2	28	56,942,258	48,566,052	8,376,206	85.29	46.71	97.86	94.34
<b>TZ 130</b>	Mock	3	28	35,208,638	32,525,740	2,682,898	92.38	45.87	98.13	94.60
<b>Ebwanateraka</b>	Mock	1	28	56,389,092	47,051,058	9,338,034	83.44	47.81	97.99	94.62
<b>Ebwanateraka</b>	Mock	2	28	39,316,096	36,823,456	2,492,640	93.66	45.19	98.24	94.81
<b>Ebwanateraka</b>	Mock	3	28	30,702,166	24,365,239	6,336,927	79.36	49.31	97.80	94.12

**Processed reads:** Total number of reads after trimming; **Mapped reads:** Number of aligned reads; **Unmapped reads:** Number of unaligned reads; **GC (%):** GC Content; **Q20 (%):** Ratio of bases that have phred quality score greater than or equal to 20; **Q30 (%):** Ratio of bases that have phred quality score greater than or equal to 3.

**Table S2****Number of genes expressed from each RNA-Seq sample and gene expression range.**

<b>Varieties</b>	<b>Time point (dag)</b>	<b>Treatment</b>	<b>Genes expressed</b>	<b>FPKM&lt;1</b>	<b>FPKM&gt;1</b>	<b>FPKM&gt;10</b>	<b>FPKM&gt;100</b>	<b>FPKM&gt;1000</b>
<b>Ebwanateraka</b>	2	Mock	29759	8282	10187	10155	1042	93
		Virus	29350	7945	10202	9893	1207	103
<b>Ebwanateraka</b>	4	Mock	29615	8483	9110	10875	1072	75
		Virus	29258	8128	9231	10598	1216	85
<b>Ebwanateraka</b>	7	Mock	29680	7290	10321	10697	1278	94
		Virus	29732	7507	9908	10908	1321	88
<b>Ebwanateraka</b>	28	Mock	29620	8738	10576	9231	990	85
		Virus	29383	8384	10590	9348	966	95
<b>Nase 3</b>	4	Mock	29590	10655	12490	5896	487	62
		Virus	27003	8609	11922	5747	627	98



<b>Varieties</b>	<b>Time point (dag)</b>	<b>Treatment</b>	<b>Genes expressed</b>	<b>FPKM&lt;1</b>	<b>FPKM&gt;1</b>	<b>FPKM&gt;10</b>	<b>FPKM&gt;100</b>	<b>FPKM&gt;1000</b>
<b>Nase 3</b>	7	Mock	29294	9915	12389	6284	642	64
		Virus	27947	8690	12192	6492	502	71
<b>Nase 3</b>	28	Mock	29300	10183	12354	6181	525	57
		Virus	26932	7602	12424	6286	543	77
<b>TZ 130</b>	2	Mock	29081	8255	10177	9381	1163	105
		Virus	29514	8448	10331	9591	1047	97
<b>TZ 130</b>	4	Mock	29653	7388	10602	10425	1147	91
		Virus	29821	8334	9876	10221	1279	111
<b>TZ 130</b>	7	Mock	29829	7848	10164	10359	1352	106
		Virus	29653	7662	10077	10615	1200	99
<b>TZ 130</b>	28	Mock	29309	7579	10316	9182	1124	108
		Virus	29568	8610	10714	9000	1128	116

**Table S3****Distribution of DEGs for each cassava variety at 2, 4, 7 and 28 dag.**

Varieties	Time point (dag)	Number of DEGs with fold change $\geq 1$	Number of DEGs with fold change $\leq -1$	Total number of DEGs (p<0.05)
<b>Ebwanateraka</b>	2	237	85	322
<b>Ebwanateraka</b>	4	208	80	288
<b>Ebwanateraka</b>	7	336	19	355
<b>Ebwanateraka</b>	28	129	149	278
<b>Total number of DEGs</b>		839	315	1243
<b>Nase 3</b>	4	1033	890	1923
<b>Nase 3</b>	7	425	694	1119
<b>Nase 3</b>	28	482	704	1186
<b>Total number of DEGs</b>		1725	2067	4228
<b>TZ 130</b>	2	166	368	534
<b>TZ 130</b>	4	495	390	885
<b>TZ 130</b>	7	224	632	856

<b>TZ 130</b>	28	98	177	275
<b>Total number of DEGs</b>		897	1176	2550

**Table S4**

**Number of enriched GO terms for up- and down-regulated genes in Nase 3, TZ 130 and Ebwanateraka.**

Time (days)	GO terms	No. of up-regulated genes			No. of down-regulated genes		
		Nase 3	TZ 130	Ebwanateraka	Nase 3	TZ 130	Ebwanateraka
2	MF	-	0	4	-	3	0
	BP	-	0	0	-	5	0
	CC	-	0	9	-	9	0
	Total	-	0	13	-	17	0
4	MF	6	4	4	1	7	8
	BP	7	24	23	0	0	0

Time (days)	GO terms	No. of up-regulated genes			No. of down-regulated genes		
		Nase 3	TZ 130	Ebwanateraka	Nase 3	TZ 130	Ebwanateraka
	CC	8	0	3	17	9	9
	Total	21	28	30	18	16	17
7	MF	7	0	1	5	10	0
	BP	5	1	0	24	14	0
	CC	0	1	0	21	1	0
	Total	12	2	1	44	25	0
28	MF	2	3	3	3	7	2
	BP	0	6	4	0	0	0
	CC	0	2	0	6	0	0
	Total	2	11	7	9	7	2

MF-Molecular function; BP-Biological function and CC-Cellular component.

**Table S5**

**Expression ratios in cassava variety Ebwanateraka of selected defence response genes showing significant differential expression in at least one treatment.**

<b>Gene ID</b>	<b>Gene name</b>	<b>2 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>4 dag _ log2 fold change</b>	<b>Adj. P value</b>	<b>7 dag _ log2 fold change</b>	<b>Adj. P value</b>	<b>28 dag _ log2 fold change</b>	<b>Adj. P value</b>
<b>Manes.16G111000</b>	EREBP-like factor (EREBP)ERF family protein.	0.417027	>0.05	-1.05329	<0.05	-0.4235	>0.05	-0.46092	>0.05
<b>Manes.07G114500</b>	Manes.07G114500.1.p - (1 of 44) K09286 - EREBP-like factor (EREBP)ERF family protein	0.108404	>0.05	0.626588	>0.05	0.31582	>0.05	-0.03576	>0.05
<b>Manes.01G085200</b>	K09286 - EREBP-like factor (EREBP) ERF 5	-0.23443	>0.05	0.995581	<0.05	1.43487	<0.05	0.776767	>0.05
<b>Manes.15G066800</b>	K09286 - EREBP-like factor (EREBP)ERF family protein	0.522603	>0.05	0.03652	>0.05	-0.53238	>0.05	-0.10834	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.13G093300</b>	PTHR31657:SF9 - ETHYLENE- RESPONSIVE TRANSCRIPTION FACTOR CRF5- RELATED	-0.25582	>0.05	0.197045	>0.05	-0.19048	>0.05	0.636983	>0.05
<b>Manes.18G069000</b>	PTHR31657:SF9 - ETHYLENE- RESPONSIVE TRANSCRIPTION FACTOR CRF5- RELATED	0.008422	>0.05	-0.09817	>0.05	0.012032	>0.05	0.228224	>0.05
<b>Manes.02G154400</b>	PTHR31657:SF9 - ETHYLENE- RESPONSIVE TRANSCRIPTION FACTOR CRF5- RELATED	0.31074	>0.05	0.09569	>0.05	0.9875	>0.05	0.673212	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.12G087500</b>	PTHR31657:SF9 - ETHYLENE- RESPONSIVE TRANSCRIPTION FACTOR CRF5- RELATED	-0.11369	>0.05	0.107312	>0.05	1.10452	<0.05	-0.28626	>0.05
<b>Manes.01G119000</b>	HSP20-like chaperone superfamily protein	-0.06071	>0.05	0.712806	>0.05	-0.00687	>0.05	1.31916	>0.05
<b>Manes.16G083600</b>	17.6 KDA CLASS II HEAT SHOCK PROTEIN-RELATED	0.104547	>0.05	0.084419	>0.05	0.833265	>0.05	1.1471	>0.05
<b>Manes.02G124600</b>	HSP20-like chaperone superfamily protein	-0.04156	>0.05	0.417014	>0.05	0.344491	>0.05	0.782273	>0.05
<b>Manes.02G124700</b> <b>Manes.02G124800</b>	HSP20-like chaperone superfamily protein	0.236262	>0.05	0.557475	>0.05	1.55634	>0.05	0.964728	<0.05
<b>Manes.02G101800</b>	LACTOYLGLUTATHIO NE LYASE	0.118374	>0.05	-0.23241	>0.05	-0.18878	>0.05	-0.41656	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
	GLYOXALASE I// SUBFAMILY NOT NAMED								
<b>Manes.01G143500</b>	LACTOYLGLUTATHIO NE LYASE GLYOXALASE I// SUBFAMILY NOT NAMED	0.092847	>0.05	-0.33239	>0.05	0.10087	>0.05	-0.3435	>0.05
<b>Manes.03G197100</b>	Late embryogenesis abundant protein (LEA_3)	0.922234	<0.05	0.573219	>0.05	-0.19654	>0.05	0.345698	>0.05
<b>Manes.15G008100</b>	Pathogenesis-related protein Bet v I family (Bet_v_1)	-0.78164	<0.05	-0.37797	>0.05	-0.55282	>0.05	-1.01783	<0.05
<b>Manes.11G095900</b>	Manes.11G095900.1.p - (1 of 1) PTHR31048:SF4 - PATHOGENESIS- RELATED	1.21228	<0.05	-0.30358	>0.05	-0.74074	>0.05	0.010438	>0.05



Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
	THAUMATIN-LIKE PROTEIN								
<b>Manes.06G007400</b>	Manes.06G007400.1.p - (1 of 2) PTHR31048:SF1 - PATHOGENESIS- RELATED THAUMATIN-LIKE PROTEIN-RELATED	1.83305	<0.05	-0.0993	>0.05	-0.4168	>0.05	-0.19691	>0.05
<b>Manes.14G170800</b>	Manes.14G170800.1.p - (1 of 2) PTHR31048:SF1 - PATHOGENESIS- RELATED THAUMATIN-LIKE PROTEIN-RELATED	1.82118	<0.05	-0.20602	>0.05	-0.37133	>0.05	-0.18684	>0.05
<b>Manes.01G202800</b>	Manes.01G202800.1.p - (1 of 2) PTHR23430:SF76 - HISTONE H2A VARIANT 1-RELATED	0.71641	>0.05	0.151189	>0.05	-0.77489	>0.05	-0.60158	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.05G070300</b>	Manes.05G070300.1.p - (1 of 19) K11251 - histone H2A (H2A)	0.92733	>0.05	-0.59825	>0.05	-0.51015	>0.05	0.529688	>0.05
<b>Manes.14G013600</b>	Manes.14G013600.1.p - (1 of 14) K11253 - histone H3 (H3)	1.29511	>0.05	-0.91882	>0.05	-0.90872	>0.05	0.932617	>0.05
<b>Manes.13G097500</b>	Manes.13G097500.1.p - (1 of 14) K11253 - histone H3 (H3)	0.710506	>0.05	-1.03176	>0.05	-0.67841	>0.05	0.65626	>0.05
<b>Manes.12G129100</b>	Manes.12G129100.1.p - (1 of 14) K11253 - histone H3 (H3)	0.981131	<0.05	-0.55784	>0.05	-0.72805	>0.05	0.4865	>0.05
<b>Manes.15G084800</b>	NAC DOMAIN- CONTAINING PROTEIN 72	-0.1742	>0.05	1.43168	<0.05	0.55926	>0.05	2.58993	<0.05
<b>Manes.12G085600</b>	MYB-LIKE DNA- BINDING PROTEIN	-0.84364	>0.05	1.50261	<0.05	2.5961	<0.05	0.882663	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
	MYB // SUBFAMILY NOT NAMED								
<b>Manes.05G015800</b>	TRANSCRIPTION FACTOR BHLH57- RELATED	0.951463	<0.05	1.21036	<0.05	-0.34301	>0.05	0.812478	>0.05
<b>Manes.04G088500</b>	PTHR31072:SF4 - TRANSCRIPTION FACTOR TCP20- RELATED	-0.27213	>0.05	1.27574	<0.05	-0.12709	>0.05	0.277033	>0.05
<b>Manes.01G263300</b>	PTHR31072:SF10 - TRANSCRIPTION FACTOR TCP9	0.8737	<0.05	-0.17337	>0.05	-0.53818	>0.05	0.422898	>0.05
<b>Manes.04G016700</b>	PF03634 - TCP family transcription factor (TCP11d)	0.198783	>0.05	-0.1188	>0.05	-0.56608	>0.05	-0.37527	>0.05
<b>Manes.11G108500</b>	PTHR31072:SF7 - TRANSCRIPTION	0.118824	>0.05	-0.21006	>0.05	-0.66181	>0.05	-0.46039	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
	FACTOR TCP21-RELATED (TCP7)								
<b>Manes.14G077200</b>	PF03634 - TCP family transcription factor (TCP15d)	0.248786	>0.05	0.568693	>0.05	-0.42306	>0.05	-0.37298	>0.05
<b>Manes.06G141800</b>	PTHR31072:SF32 - TRANSCRIPTION FACTOR TCP19	0.036175	>0.05	0.136511	>0.05	1.61611	>0.05	-0.20839	>0.05
<b>Manes.14G027700</b>	PTHR10015:SF168 - HEAT STRESS TRANSCRIPTION FACTOR B-2A	0.068913	>0.05	0.149554	>0.05	-0.10221	>0.05	1.02747	>0.05
<b>Manes.01G065200</b>	PTHR10015:SF169 - HEAT STRESS TRANSCRIPTION FACTOR B-2B	0.271967	>0.05	-0.00226	>0.05	0.096168	>0.05	0.637869	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.12G101000</b>	PTHR10015:SF166 - HEAT STRESS TRANSCRIPTION FACTOR B-1	0.035133	>0.05	0.188198	>0.05	0.054677	>0.05	0.116627	>0.05
<b>Manes.16G116200</b>	PTHR10015:SF187 - HEAT STRESS TRANSCRIPTION FACTOR C-1	0.075892	>0.05	0.082368	>0.05	-0.08672	>0.05	0.757296	>0.05
<b>Manes.13G124500</b>	PTHR10015:SF166 - HEAT STRESS TRANSCRIPTION FACTOR B-1	-0.37205	>0.05	0.525624	>0.05	0.442397	>0.05	0.39469	>0.05
<b>Manes.08G020700</b>	PTHR10015:SF164 - HEAT STRESS TRANSCRIPTION FACTOR A-3	0.100081	>0.05	0.176222	>0.05	0.157739	>0.05	-0.24563	>0.05
<b>Manes.08G170600</b>	PF03106 - WRKY DNA - binding domain (WRKY)	-0.03286	>0.05	0.469992	>0.05	0.691157	>0.05	0.096889	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.18G001000</b>	PTHR22952:SF117 - BZIP TRANSCRIPTION FACTOR 60	-0.01194	>0.05	0.31893	>0.05	0.457714	>0.05	-0.77297	>0.05
<b>Manes.01G227900</b>	PTHR22952:SF146 - BZIP TRANSCRIPTION FACTOR-LIKE PROTEIN	0.109465	>0.05	0.019307	>0.05	-0.41419	>0.05	0.010588	>0.05
<b>Manes.18G005000</b>	PTHR22952:SF117 - BZIP TRANSCRIPTION FACTOR 60	-0.01666	>0.05	0.151755	>0.05	0.136504	>0.05	-0.11578	>0.05
<b>Manes.10G141400</b>	PTHR12632//PTHR12632 :SF15 - TRANSCRIPTION FACTOR NF-Y ALPHA-RELATED // SUBFAMILY NOT NAMED	-0.35891	>0.05	0.0919292	>0.05	0.176604	>0.05	0.301008	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.16G097900</b>	PTHR12632//PTHR12632 :SF8 - TRANSCRIPTION FACTOR NF-Y ALPHA- RELATED // SUBFAMILY NOT NAMED	-0.21167	>0.05	0.342293	>0.05	-0.16442	>0.05	0.735793	>0.05
<b>Manes.09G025200</b>	PTHR12632//PTHR12632 :SF15 - TRANSCRIPTION FACTOR NF-Y ALPHA- RELATED // SUBFAMILY NOT NAMED	-0.33283	>0.05	-0.12652	>0.05	0.75774	>0.05	1.08504	>0.05
<b>Manes.09G044200</b>	PTHR12632:SF7 - NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT A-10-RELATED	1.2413	<0.05	1.3145	>0.05	0.032047	>0.05	0.950623	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.04G142600</b>	PTHR12632:SF12 - NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT A-3-RELATED	0.541648	>0.05	0.166156	>0.05	-0.10488	>0.05	0.31161	>0.05
<b>Manes.14G003100</b>	PTHR12632:SF14 - NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT A-1-RELATED	0.335464	>0.05	0.172175	>0.05	0.025711	>0.05	0.423998	>0.05
<b>Manes.02G100700</b>	Manes.02G100700.1.p - (1 of 2) PTHR27000:SF169 - LEUCINE-RICH REPEAT-CONTAINING PROTEIN(Leucine-rich repeat (LRR) family protein),	0.793199	<0.05	-0.0197	>0.05	-0.22234	>0.05	0.381474	>0.05



Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.02G211400</b>	PF00069//PF08263//PF13855 - Protein kinase domain (Pkinase) // Leucine rich repeat N-terminal domain (LRRNT_2) // Leucine rich repeat (LRR_8)Leucine-rich repeat protein kinase family protein	0.763228	<0.05	-0.27597	>0.05	-0.71529	>0.05	0.050919	>0.05
<b>Manes.14G124000</b>	Manes.14G124000.1.p - (1 of 1) PTHR27000:SF160 - LEUCINE-RICH REPEAT-CONTAINING PROTEIN DDB_G0281931-RELATED	0.743537	<0.05	-0.20087	>0.05	-0.60248	>0.05	-0.03701	>0.05

**Table S6**

**Expression ratios in cassava variety TZ 130 of selected defence response genes showed significant differential expression in at least one treatment.**

<b>Gene ID</b>	<b>Gene name</b>	<b>2 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>4 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>7 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>28 dag_ log2 fold change</b>	<b>Adj. P value</b>
<b>Manes.16G111000</b>	EREBP-like factor (EREBP)ERF family protein.	-0.74221	<0.05	-0.10068	>0.05	0.946588	<0.05	-0.02271	>0.05
<b>Manes.07G114500</b>	Manes.07G114500.1.p - (1 of 44) K09286 - EREBP-like factor (EREBP)ERF family protein	-0.16059	>0.05	1.37252	<0.05	-0.62499	>0.05	-0.37838	>0.05
<b>Manes.01G085200</b>	K09286 - EREBP-like factor (EREBP) ERF 5	-0.89822	>0.05	1.77923	<0.05	-1.78643	<0.05	-1.54039	<0.05
<b>Manes.15G066800</b>	K09286 - EREBP-like factor (EREBP)ERF family protein	-0.56138	<0.05	-0.36132	>0.05	-0.35228	>0.05	0.023917	>0.05
<b>Manes.13G093300</b>	PTHR31657:SF9 - ETHYLENE-RESPONSIVE	0.228217	>0.05	0.220369	>0.05	-0.22371	>0.05	-0.80573	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
	TRANSCRIPTION FACTOR CRF5-RELATED								
<b>Manes.18G069000</b>	PTHR31657:SF9 - ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR CRF5-RELATED	-0.06191	>0.05	0.356398	>0.05	0.12929	>0.05	-0.51472	>0.05
<b>Manes.02G154400</b>	PTHR31657:SF9 - ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR CRF5-RELATED	-0.15949	>0.05	0.458963	>0.05	-1.41897	<0.05	-1.04471	>0.05
<b>Manes.12G087500</b>	PTHR31657:SF9 - ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR CRF5-RELATED	0.120874	>0.05	0.511466	>0.05	-0.9065	<0.05	-0.63155	>0.05
<b>Manes.01G119000</b>	HSP20-like chaperone superfamily protein	0.626176	>0.05	1.13962	<0.05	0.582087	>0.05	0.543521	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.16G083600</b>	17.6 KDA CLASS II HEAT SHOCK PROTEIN-RELATED	-0.90388	>0.05	1.61178	<0.05	1.31665	>0.05	-0.15462	>0.05
<b>Manes.02G124600</b>	HSP20-like chaperone superfamily protein	0.358427	>0.05	1.0322	<0.05	0.320253	>0.05	-0.18567	>0.05
<b>Manes.02G124700</b> <b>Manes.02G124800</b>	HSP20-like chaperone superfamily protein	0.663816	>0.05	1.5206	<0.05	0.208954	>0.05	0.965357	>0.05
<b>Manes.02G101800</b>	LACTOYLGLUTATHIONE LYASE GLYOXALASE I // SUBFAMILY NOT NAMED	-1.52005	<0.05	-1.29112	<0.05	0.372692	>0.05	0.292894	>0.05
<b>Manes.01G143500</b>	LACTOYLGLUTATHIONE LYASE GLYOXALASE I // SUBFAMILY NOT NAMED	-1.51369	<0.05	-1.55201	<0.05	0.109942	>0.05	0.110754	>0.05
<b>Manes.03G197100</b>	Late embryogenesis abundant protein (LEA_3)	0.405275	>0.05	1.63458	<0.05	0.714648	<0.05	-0.70995	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.15G008100</b>	Pathogenesis-related protein Bet v I family (Bet_v_1)	0.192398	>0.05	-1.61064	<0.05	0.033711	>0.05	0.101362	>0.05
<b>Manes.11G095900</b>	Manes.11G095900.1.p - (1 of 1) PTHR31048:SF4 - PATHOGENESIS-RELATED THAUMATIN-LIKE PROTEIN	-2.11106	<0.05	-0.51751	>0.05	-1.07914	<0.05	0.748967	>0.05
<b>Manes.06G007400</b>	Manes.06G007400.1.p - (1 of 2) PTHR31048:SF1 - PATHOGENESIS-RELATED THAUMATIN-LIKE PROTEIN-RELATED	-2.05019	<0.05	-1.43309	<0.05	-2.08151	<0.05	0.878607	>0.05
<b>Manes.14G170800</b>	Manes.14G170800.1.p - (1 of 2) PTHR31048:SF1 - PATHOGENESIS-RELATED THAUMATIN-LIKE PROTEIN-RELATED	-2.18517	<0.05	-1.89714	<0.05	-2.25197	<0.05	1.08695	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.01G202800</b>	Manes.01G202800.1.p - (1 of 2) PTHR23430:SF76 - HISTONE H2A VARIANT 1-RELATED	-1.14505	>0.05	-2.78418	<0.05	-1.68322	<0.05	0.050764	>0.05
<b>Manes.05G070300</b>	Manes.05G070300.1.p - (1 of 19) K11251 - histone H2A (H2A)	-2.11049	<0.05	-4.0027	<0.05	-1.99963	<0.05	0.254461	>0.05
<b>Manes.14G013600</b>	Manes.14G013600.1.p - (1 of 14) K11253 - histone H3 (H3)	-1.40766	<0.05	-3.08618	<0.05	-2.03717	<0.05	0.363321	>0.05
<b>Manes.13G097500</b>	Manes.13G097500.1.p - (1 of 14) K11253 - histone H3 (H3)	-1.87267	<0.05	-3.41095	<0.05	-1.68693	<0.05	0.384152	>0.05
<b>Manes.12G129100</b>	Manes.12G129100.1.p - (1 of 14) K11253 - histone H3 (H3)	-1.67218	<0.05	-3.07263	<0.05	-1.66397	<0.05	1.01189	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.15G084800</b>	NAC DOMAIN- CONTAINING PROTEIN 72	0.683795	>0.05	1.0249	>0.05	-0.66826	>0.05	-0.61396	>0.05
<b>Manes.12G085600</b>	MYB-LIKE DNA-BINDING PROTEIN MYB // SUBFAMILY NOT NAMED	2.80005	<0.05	0.496297	>0.05	-1.80201	<0.05	0.300044	>0.05
<b>Manes.05G015800</b>	TRANSCRIPTION FACTOR BHLH57- RELATED	-1.25632	<0.05	0.29579	>0.05	-0.97037	<0.05	0.947927	>0.05
<b>Manes.04G088500</b>	PTHR31072:SF4 - TRANSCRIPTION FACTOR TCP20-RELATED	0.57983	>0.05	0.838864	>0.05	0.981856	>0.05	0.210186	>0.05
<b>Manes.01G263300</b>	PTHR31072:SF10 - TRANSCRIPTION FACTOR TCP9	-0.78624	<0.05	-0.11711	>0.05	-1.01773	<0.05	-0.57769	>0.05

<b>Gene ID</b>	<b>Gene name</b>	<b>2 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>4 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>7 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>28 dag_ log2 fold change</b>	<b>Adj. P value</b>
<b>Manes.04G016700</b>	PF03634 - TCP family transcription factor (TCP11d)	0.052667	>0.05	0.075874	>0.05	0.006407	>0.05	-0.12601	>0.05
<b>Manes.11G108500</b>	PTHR31072:SF7 - TRANSCRIPTION FACTOR TCP21-RELATED (TCP7)	-0.2008	>0.05	0.530019	>0.05	-0.38669	>0.05	-0.12122	>0.05
<b>Manes.14G077200</b>	PF03634 - TCP family transcription factor (TCP15d)	-0.23293	>0.05	0.555711	>0.05	0.162585	>0.05	0.008667	>0.05
<b>Manes.06G141800</b>	PTHR31072:SF32 - TRANSCRIPTION FACTOR TCP19	-0.05645	>0.05	0.736111	>0.05	-0.45955	>0.05	-0.8324	>0.05
<b>Manes.14G027700</b>	PTHR10015:SF168 - HEAT STRESS TRANSCRIPTION FACTOR B-2A	-0.0866	>0.05	1.07322	<0.05	-0.21625	>0.05	-0.00415	>0.05
<b>Manes.01G065200</b>	PTHR10015:SF169 - HEAT STRESS TRANSCRIPTION FACTOR B-2B	0.000794	>0.05	-0.35378	>0.05	-0.16739	>0.05	-0.639	>0.05



<b>Gene ID</b>	<b>Gene name</b>	<b>2 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>4 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>7 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>28 dag_ log2 fold change</b>	<b>Adj. P value</b>
<b>Manes.12G101000</b>	PTHR10015:SF166 - HEAT STRESS TRANSCRIPTION FACTOR B-1	0.144119	>0.05	0.035624	>0.05	0.397869	>0.05	-0.2609	>0.05
<b>Manes.16G116200</b>	PTHR10015:SF187 - HEAT STRESS TRANSCRIPTION FACTOR C-1	-0.47418	<0.05	-0.27812	>0.05	0.093718	>0.05	-0.80139	>0.05
<b>Manes.13G124500</b>	PTHR10015:SF166 - HEAT STRESS TRANSCRIPTION FACTOR B-1	0.432079	>0.05	0.850477	<0.05	0.220963	>0.05	-1.49264	<0.05
<b>Manes.08G020700</b>	PTHR10015:SF164 - HEAT STRESS TRANSCRIPTION FACTOR A-3	-0.16138	>0.05	0.184278	>0.05	0.179533	>0.05	-0.10133	>0.05
<b>Manes.08G170600</b>	PF03106 - WRKY DNA - binding domain (WRKY)	0.682525	<0.05	-0.33423	>0.05	-0.2399	>0.05	0.210511	>0.05
<b>Manes.18G001000</b>	PTHR22952:SF117 - BZIP TRANSCRIPTION FACTOR 60	0.435308	>0.05	0.68465	>0.05	-0.43945	>0.05	-0.21111	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
<b>Manes.01G227900</b>	PTHR22952:SF146 - BZIP TRANSCRIPTION FACTOR-LIKE PROTEIN	-0.12453	>0.05	0.366918	>0.05	0.194381	>0.05	-0.1865	>0.05
<b>Manes.18G005000</b>	PTHR22952:SF117 - BZIP TRANSCRIPTION FACTOR 60	0.147663	>0.05	0.478916	>0.05	-0.13351	>0.05	0.230125	>0.05
<b>Manes.10G141400</b>	PTHR12632//PTHR12632:S F15 - TRANSCRIPTION FACTOR NF-Y ALPHA-RELATED // SUBFAMILY NOT NAMED	-0.03759	>0.05	-0.23186	>0.05	0.438989	>0.05	-1.01943	>0.05
<b>Manes.16G097900</b>	PTHR12632//PTHR12632:S F8 - TRANSCRIPTION FACTOR NF-Y ALPHA-RELATED // SUBFAMILY NOT NAMED	-0.29464	>0.05	0.197398	>0.05	-0.98481	>0.05	-1.247	>0.05
<b>Manes.09G025200</b>	PTHR12632//PTHR12632:S F15 - TRANSCRIPTION	0.136564	>0.05	0.127987	>0.05	0.831642	>0.05	-0.65383	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value	28 dag_ log2 fold change	Adj. P value
	FACTOR NF-Y ALPHA-RELATED // SUBFAMILY NOT NAMED								
<b>Manes.09G044200</b>	PTHR12632:SF7 - NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT A-10-RELATED	-0.88462	<0.05	-0.63644	>0.05	-0.97751	<0.05	-0.75839	>0.05
<b>Manes.04G142600</b>	PTHR12632:SF12 - NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT A-3-RELATED	-0.08877	>0.05	0.549376	>0.05	0.07183	>0.05	-0.48043	>0.05
<b>Manes.14G003100</b>	PTHR12632:SF14 - NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT A-1-RELATED	-0.19609	>0.05	0.446144	>0.05	0.34451	>0.05	-0.38552	>0.05

**Table S7**

**Expression ratios in cassava variety Nase 3 of selected defence response genes showed significant differential expression in at least one treatment.**

<b>Gene ID</b>	<b>Gene name</b>	<b>2 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>4 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>7 dag_ log2 fold change</b>	<b>Adj. P value</b>
<b>Manes.16G111000</b>	EREBP-like factor (EREBP)ERF family protein.	0.004404	>0.05	-0.50992	>0.05	-0.48086	>0.05
<b>Manes.07G114500</b>	Manes.07G114500.1.p - (1 of 44) K09286 - EREBP-like factor (EREBP)ERF family protein	1.74455	<0.05	0.055785	>0.05	0.04648	>0.05
<b>Manes.01G085200</b>	K09286 - EREBP-like factor (EREBP) ERF 5	1.36428	<0.05	0.105933	>0.05	-0.96043	>0.05
<b>Manes.15G066800</b>	K09286 - EREBP-like factor (EREBP)ERF family protein	1.41402	<0.05	-1.14728	>0.05	0.323514	>0.05
<b>Manes.13G093300</b>	PTHR31657:SF9 - ETHYLENE-RESPONSIVE	1.03746	<0.05	0.139058	>0.05	0.422681	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value
	TRANSCRIPTION FACTOR CRF5-RELATED						
<b>Manes.18G069000</b>	PTHR31657:SF9 - ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR CRF5-RELATED	1.10789	<0.05	0.321105	>0.05	0.183102	>0.05
<b>Manes.02G154400</b>	PTHR31657:SF9 - ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR CRF5-RELATED	1.0376	<0.05	0.263915	>0.05	-0.34752	>0.05
<b>Manes.12G087500</b>	PTHR31657:SF9 - ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR CRF5-RELATED	1.04324	<0.05	0.567546	>0.05	-0.33512	>0.05
<b>Manes.01G119000</b>	HSP20-like chaperone superfamily protein	3.33074	<0.05	-1.7193	>0.05	-0.09432	>0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value
<b>Manes.16G083600</b>	17.6 KDA CLASS II HEAT SHOCK PROTEIN-RELATED	5.52934	<0.05	-0.28711	>0.05	1.57786	<0.05
<b>Manes.02G124600</b>	HSP20-like chaperone superfamily protein	5.75847	<0.05	0.196281	>0.05	0.659707	<0.05
<b>Manes.02G124700</b> <b>Manes.02G124800</b>	HSP20-like chaperone superfamily protein	5.79276	<0.05	0.959413	>0.05	0.415391	>0.05
<b>Manes.02G101800</b>	LACTOYLGLUTATHIONE LYASE GLYOXALASE I // SUBFAMILY NOT NAMED	-1.21241	<0.05	0.93428	>0.05	-0.72648	<0.05
<b>Manes.01G143500</b>	LACTOYLGLUTATHIONE LYASE GLYOXALASE I // SUBFAMILY NOT NAMED	-1.10906	<0.05	1.54269	<0.05	-0.40953	>0.05
<b>Manes.03G197100</b>	Late embryogenesis abundant protein (LEA_3)	1.47171	<0.05	1.4739	<0.05	0.326894	>0.05
<b>Manes.15G008100</b>	Pathogenesis-related protein Bet v I family (Bet_v_1)	-1.19987	<0.05	0.576867	>0.05	-0.14117	>0.05

<b>Gene ID</b>	<b>Gene name</b>	<b>2 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>4 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>7 dag_ log2 fold change</b>	<b>Adj. P value</b>
<b>Manes.11G095900</b>	Manes.11G095900.1.p - (1 of 1) PTHR31048:SF4 - PATHOGENESIS-RELATED THAUMATIN-LIKE PROTEIN	0.211652	>0.05	-1.96604	<0.05	0.256043	>0.05
<b>Manes.06G007400</b>	Manes.06G007400.1.p - (1 of 2) PTHR31048:SF1 - PATHOGENESIS-RELATED THAUMATIN-LIKE PROTEIN-RELATED	0.489769	>0.05	-2.63034	<0.05	0.011581	>0.05
<b>Manes.14G170800</b>	Manes.14G170800.1.p - (1 of 2) PTHR31048:SF1 - PATHOGENESIS-RELATED THAUMATIN-LIKE PROTEIN-RELATED	-0.27355	>0.05	-2.50195	<0.05	0.253398	>0.05
<b>Manes.01G202800</b>	Manes.01G202800.1.p - (1 of 2) PTHR23430:SF76 - HISTONE H2A VARIANT 1-RELATED	-0.84901	>0.05	-4.02193	<0.05	-0.88381	>0.05

<b>Gene ID</b>	<b>Gene name</b>	<b>2 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>4 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>7 dag_ log2 fold change</b>	<b>Adj. P value</b>
<b>Manes.05G070300</b>	Manes.05G070300.1.p - (1 of 19) K11251 - histone H2A (H2A)	-1.45309	>0.05	-5.52002	<0.05	-0.08511	>0.05
<b>Manes.14G013600</b>	Manes.14G013600.1.p - (1 of 14) K11253 - histone H3 (H3)	-1.0283	>0.05	-5.07045	<0.05	0.34621	>0.05
<b>Manes.13G097500</b>	Manes.13G097500.1.p - (1 of 14) K11253 - histone H3 (H3)	-0.95352	>0.05	-6.31874	<0.05	0.213008	>0.05
<b>Manes.12G129100</b>	Manes.12G129100.1.p - (1 of 14) K11253 - histone H3 (H3)	-0.69231	>0.05	-5.24672	<0.05	0.549904	>0.05
<b>Manes.15G084800</b>	NAC DOMAIN-CONTAINING PROTEIN 72	0.059823	>0.05	0.879526	>0.05	-1.2401	>0.05
<b>Manes.12G085600</b>	MYB-LIKE DNA-BINDING PROTEIN MYB // SUBFAMILY NOT NAMED	0.171947	>0.05	-0.53038	>0.05	-2.25102	>0.05
<b>Manes.05G015800</b>	TRANSCRIPTION FACTOR BHLH57-RELATED	0.190056	>0.05	-2.51789	<0.05	0.431815	>0.05



<b>Gene ID</b>	<b>Gene name</b>	<b>2 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>4 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>7 dag_ log2 fold change</b>	<b>Adj. P value</b>
<b>Manes.04G088500</b>	PTHR31072:SF4 - TRANSCRIPTION FACTOR TCP20-RELATED	-2.11242	>0.05	0.936893	>0.05	-0.55163	>0.05
<b>Manes.01G263300</b>	PTHR31072:SF10 - TRANSCRIPTION FACTOR TCP9	1.65564	<0.05	-1.62762	<0.05	1.2286	<0.05
<b>Manes.04G016700</b>	PF03634 - TCP family transcription factor (TCP11d)	1.21865	<0.05	0.046543	>0.05	0.172699	>0.05
<b>Manes.11G108500</b>	PTHR31072:SF7 - TRANSCRIPTION FACTOR TCP21-RELATED (TCP7)	1.71474	<0.05	-0.38135	>0.05	0.845673	<0.05
<b>Manes.14G077200</b>	PF03634 - TCP family transcription factor (TCP15d)	0.501651	>0.05	-0.28161	>0.05	1.3691	<0.05
<b>Manes.06G141800</b>	PTHR31072:SF32 - TRANSCRIPTION FACTOR TCP19	1.2959	<0.05	0.003537	>0.05	-0.43541	>0.05

<b>Gene ID</b>	<b>Gene name</b>	<b>2 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>4 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>7 dag_ log2 fold change</b>	<b>Adj. P value</b>
<b>Manes.14G027700</b>	PTHR10015:SF168 - HEAT STRESS TRANSCRIPTION FACTOR B-2A	1.95655	<0.05	0.025577	>0.05	-0.19667	>0.05
<b>Manes.01G065200</b>	PTHR10015:SF169 - HEAT STRESS TRANSCRIPTION FACTOR B-2B	2.30593	<0.05	-0.75619	>0.05	-0.39492	>0.05
<b>Manes.12G101000</b>	PTHR10015:SF166 - HEAT STRESS TRANSCRIPTION FACTOR B-1	1.24785	<0.05	0.504082	>0.05	0.066316	>0.05
<b>Manes.16G116200</b>	PTHR10015:SF187 - HEAT STRESS TRANSCRIPTION FACTOR C-1	1.69637	<0.05	-0.37736	>0.05	0.784607	<0.05
<b>Manes.13G124500</b>	PTHR10015:SF166 - HEAT STRESS TRANSCRIPTION FACTOR B-1	2.07958	<0.05	0.78328	>0.05	-0.26695	>0.05

<b>Gene ID</b>	<b>Gene name</b>	<b>2 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>4 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>7 dag_ log2 fold change</b>	<b>Adj. P value</b>
<b>Manes.08G020700</b>	PTHR10015:SF164 - HEAT STRESS TRANSCRIPTION FACTOR A-3	1.02168	<0.05	0.397485	>0.05	0.08793	>0.05
<b>Manes.08G170600</b>	PF03106 - WRKY DNA - binding domain (WRKY)	0.409696	>0.05	1.31333	<0.05	-0.41115	>0.05
<b>Manes.18G001000</b>	PTHR22952:SF117 - BZIP TRANSCRIPTION FACTOR 60	1.18965	<0.05	0.407845	>0.05	-1.13315	<0.05
<b>Manes.01G227900</b>	PTHR22952:SF146 - BZIP TRANSCRIPTION FACTOR-LIKE PROTEIN	1.21937	<0.05	-0.13565	>0.05	1.05313	<0.05
<b>Manes.18G005000</b>	PTHR22952:SF117 - BZIP TRANSCRIPTION FACTOR 60	1.05318	<0.05	0.342463	>0.05	-0.23261	>0.05
<b>Manes.10G141400</b>	PTHR12632//PTHR12632:SF15 - TRANSCRIPTION FACTOR	0.160846	>0.05	1.74787	<0.05	0.8941	<0.05

Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value
	NF-Y ALPHA-RELATED // SUBFAMILY NOT NAMED						
<b>Manes.16G097900</b>	PTHR12632//PTHR12632:SF8 - TRANSCRIPTION FACTOR NF-Y ALPHA-RELATED // SUBFAMILY NOT NAMED	1.67435	<0.05	0.98762	>0.05	1.12135	<0.05
<b>Manes.09G025200</b>	PTHR12632//PTHR12632:SF15 - TRANSCRIPTION FACTOR NF-Y ALPHA-RELATED // SUBFAMILY NOT NAMED	2.20195	<0.05	1.21888	>0.05	0.801024	<0.05
<b>Manes.09G044200</b>	PTHR12632:SF7 - NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT A-10-RELATED	2.32675	<0.05	0.794974	>0.05	1.81213	<0.05
<b>Manes.04G142600</b>	PTHR12632:SF12 - NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT A-3-RELATED	1.51847	<0.05	0.87787	>0.05	1.37838	<0.05

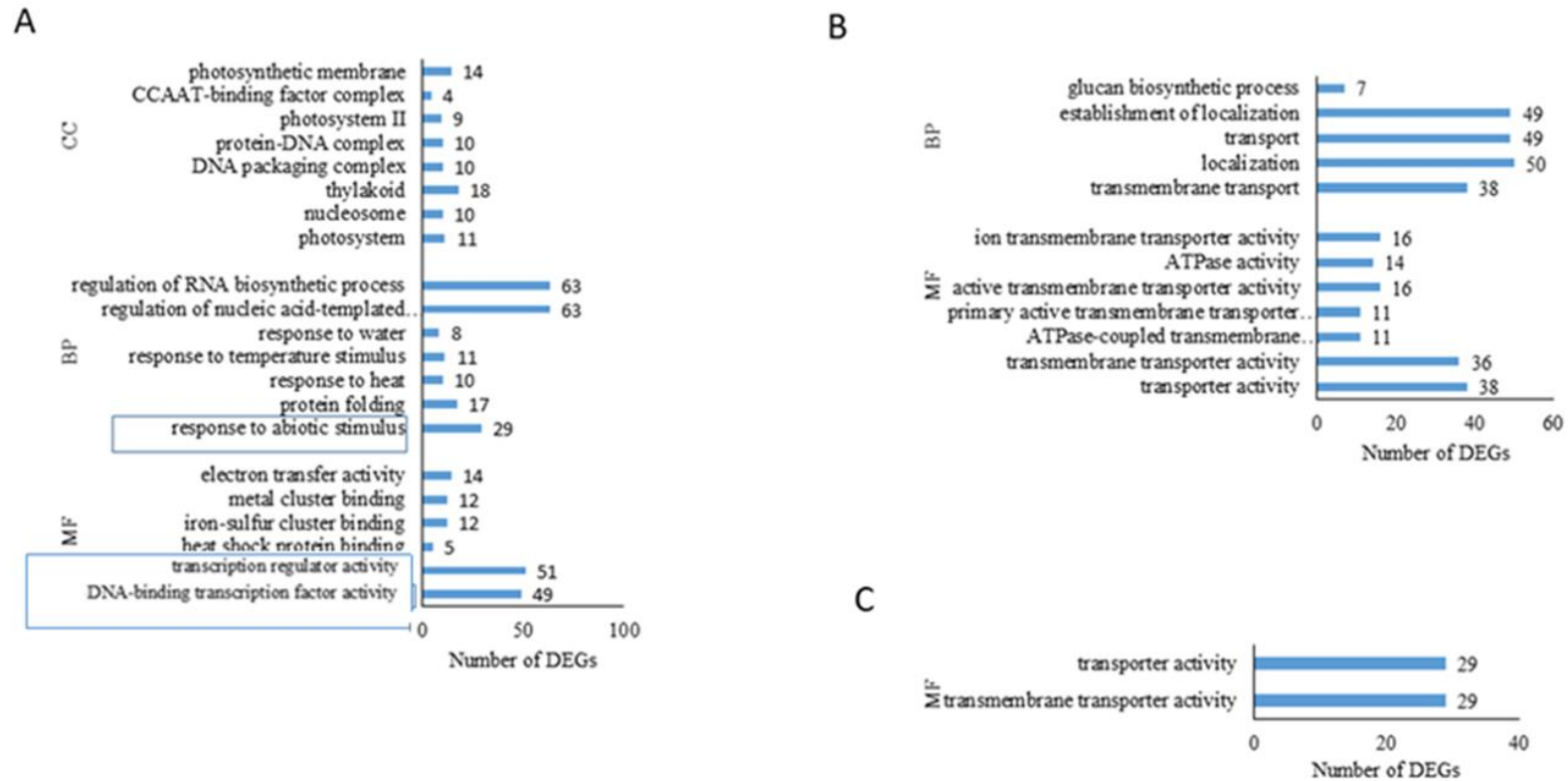
Gene ID	Gene name	2 dag_ log2 fold change	Adj. P value	4 dag_ log2 fold change	Adj. P value	7 dag_ log2 fold change	Adj. P value
<b>Manes.14G003100</b>	PTHR12632:SF14 - NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT A-1-RELATED	1.16204	<0.05	0.544981	>0.05	0.499282	>0.05
<b>Manes.02G100700</b>	Manes.02G100700.1.p - (1 of 2) PTHR27000:SF169 - LEUCINE-RICH REPEAT- CONTAINING PROTEIN(Leucine-rich repeat (LRR) family protein),	0.562826	>0.05	-2.15865	<0.05	0.057277	>0.05
<b>Manes.02G211400</b>	PF00069//PF08263//PF13855 - Protein kinase domain (Pkinase) // Leucine rich repeat N- terminal domain (LRRNT_2) // Leucine rich repeat (LRR_8) Leucine-rich repeat protein kinase family protein	0.26375	>0.05	-1.33378	<0.05	0.786188	<0.05
<b>Manes.14G124000</b>	Manes.14G124000.1.p - (1 of 1) PTHR27000:SF160 -	0.824328	>0.05	-1.99548	<0.05	0.748484	<0.05

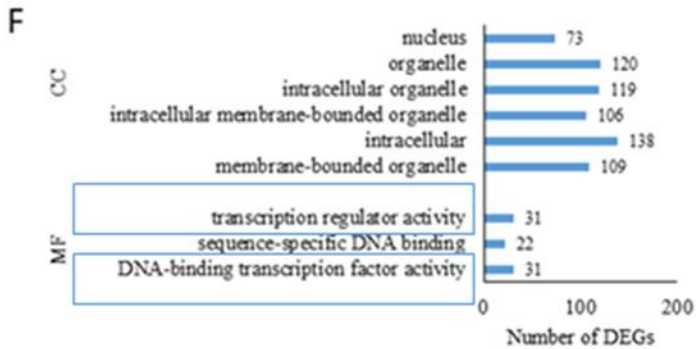
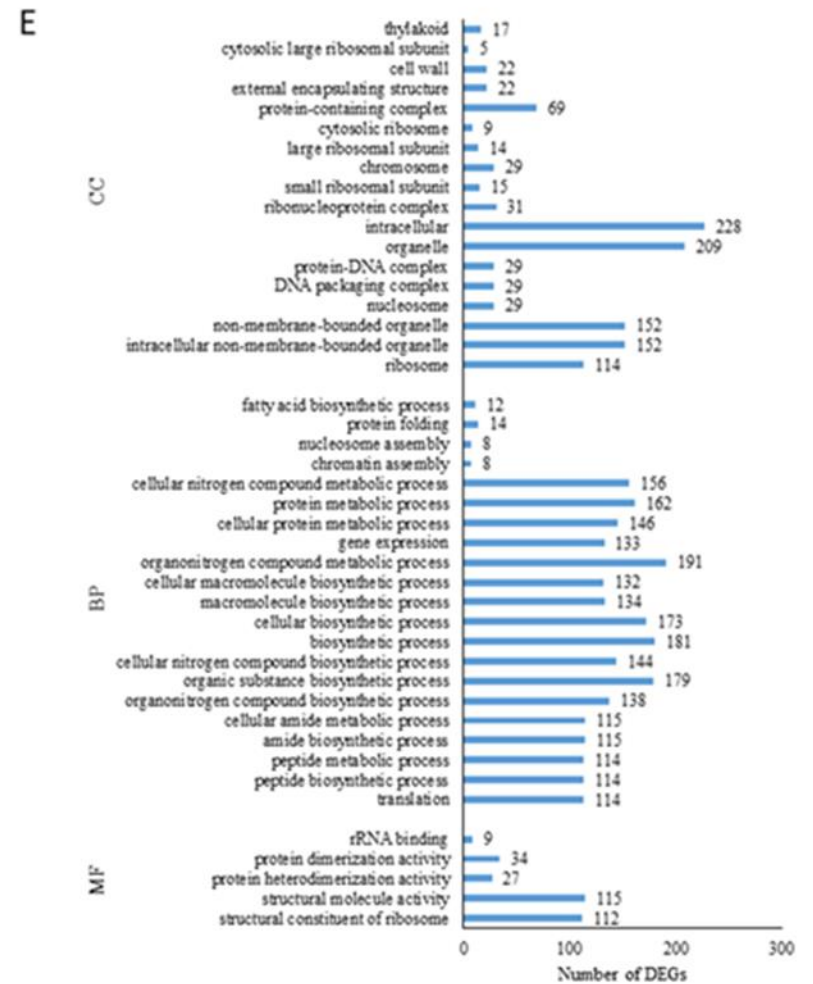
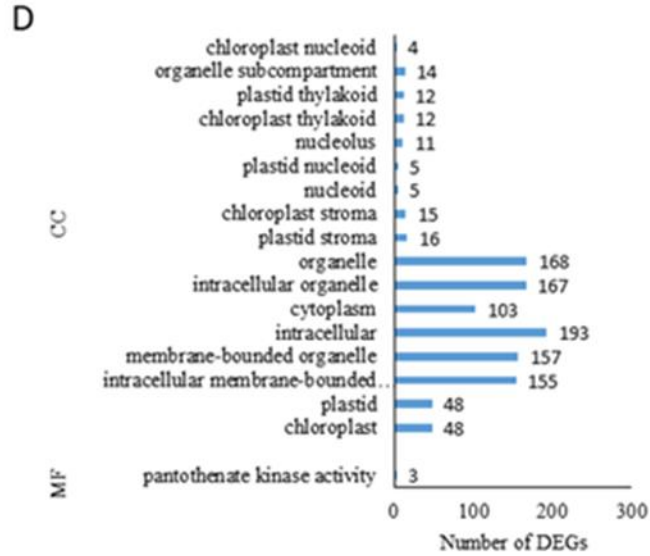
<b>Gene ID</b>	<b>Gene name</b>	<b>2 dag_ log2 fold change</b>	<b>Adj. P value</b>	<b>4 dag _ log2 fold change</b>	<b>Adj. P value</b>	<b>7 dag _ log2 fold change</b>	<b>Adj. P value</b>
	LEUCINE-RICH REPEAT-CONTAINING PROTEIN DDB_G0281931-RELATED						

# Figures

## Figure S1

### Enriched GO terms in Nase 3 for up-regulated genes



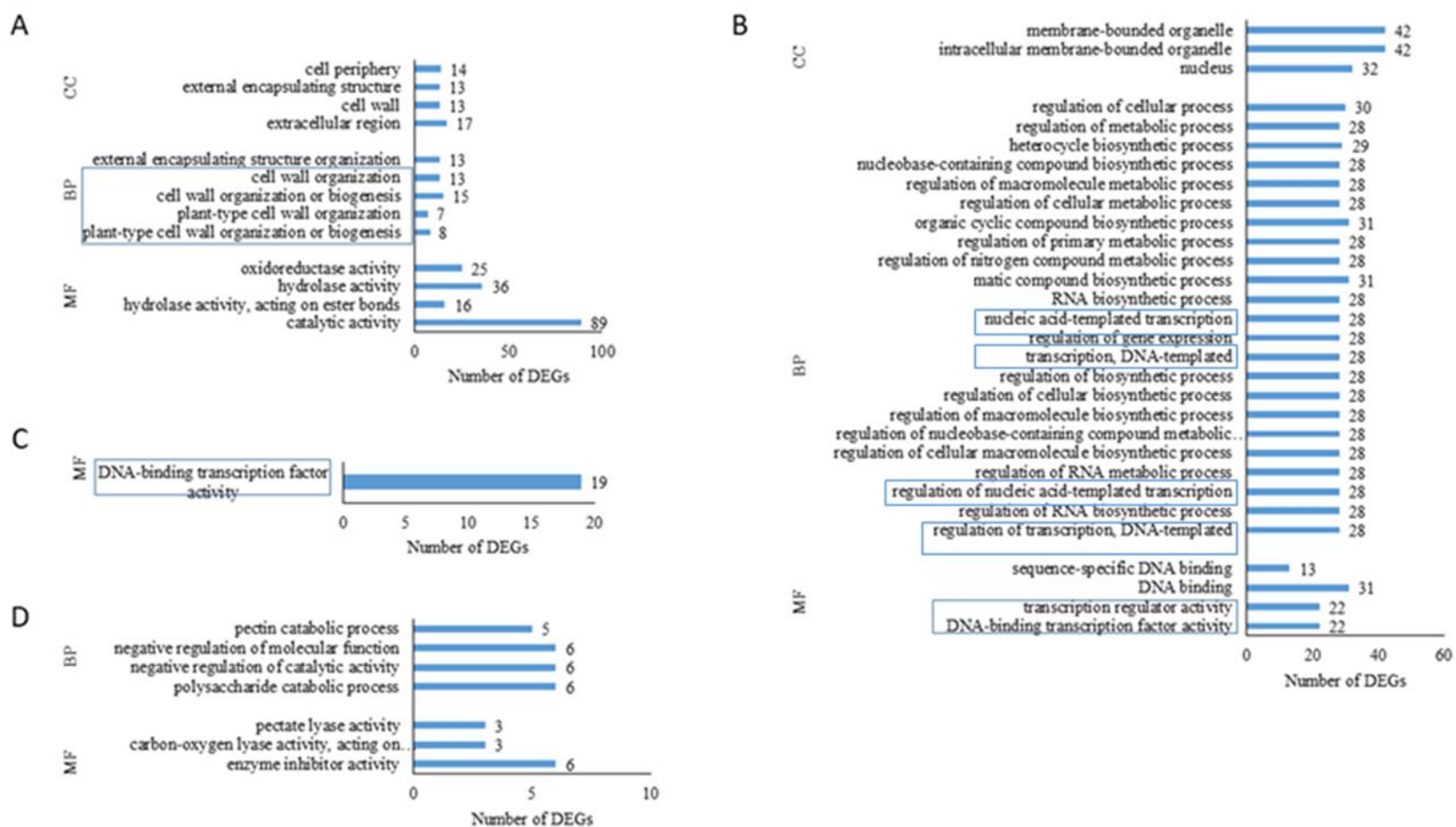


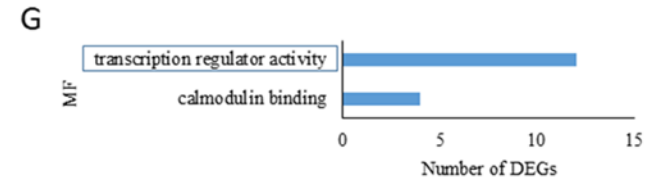
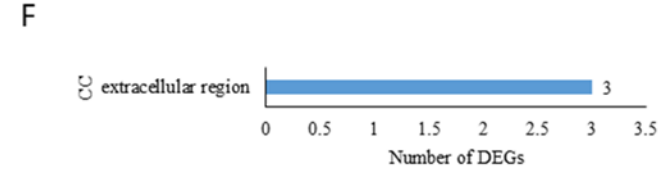
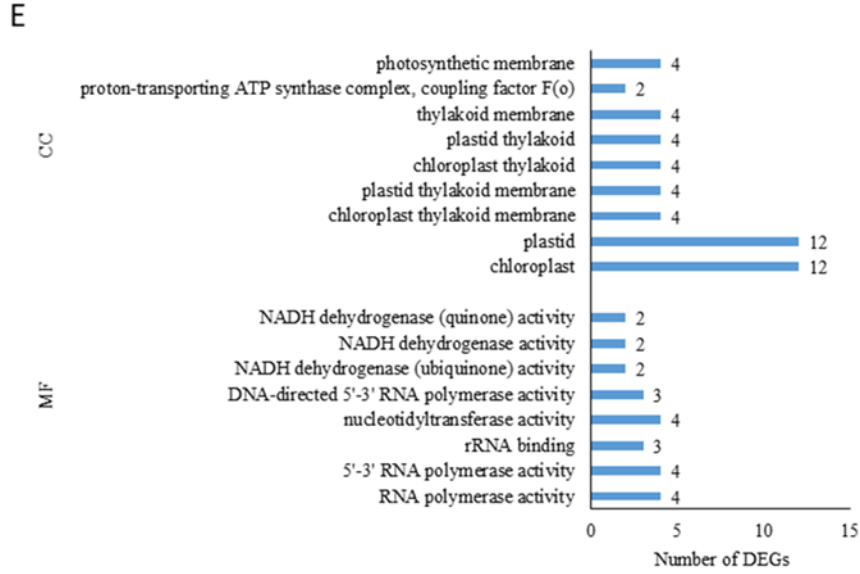
A. 4 dag, B. 7 dag, C. 28 dag and down-regulated genes D. 4 dag, E. 7 dag and F. 28 dag for CC, BP and MF categories. Blue arrows and boxes represent GO terms enriched in Nase 3 and TZ 130 but not in Ebwanateraka.



**Figure S2**

**Enriched GO terms in Ebwanateraka for up-regulated genes**

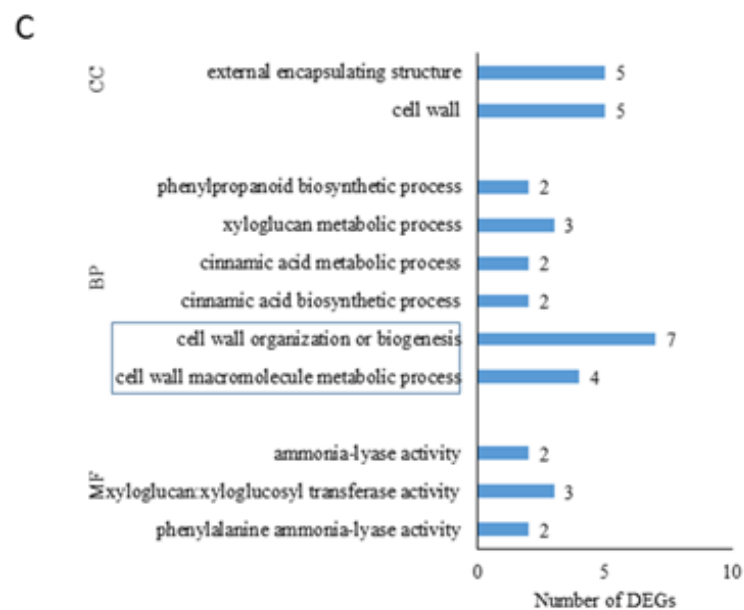
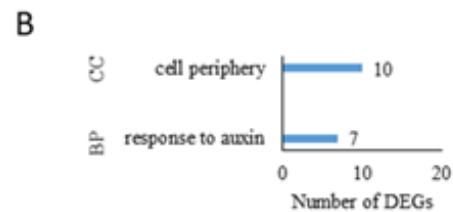
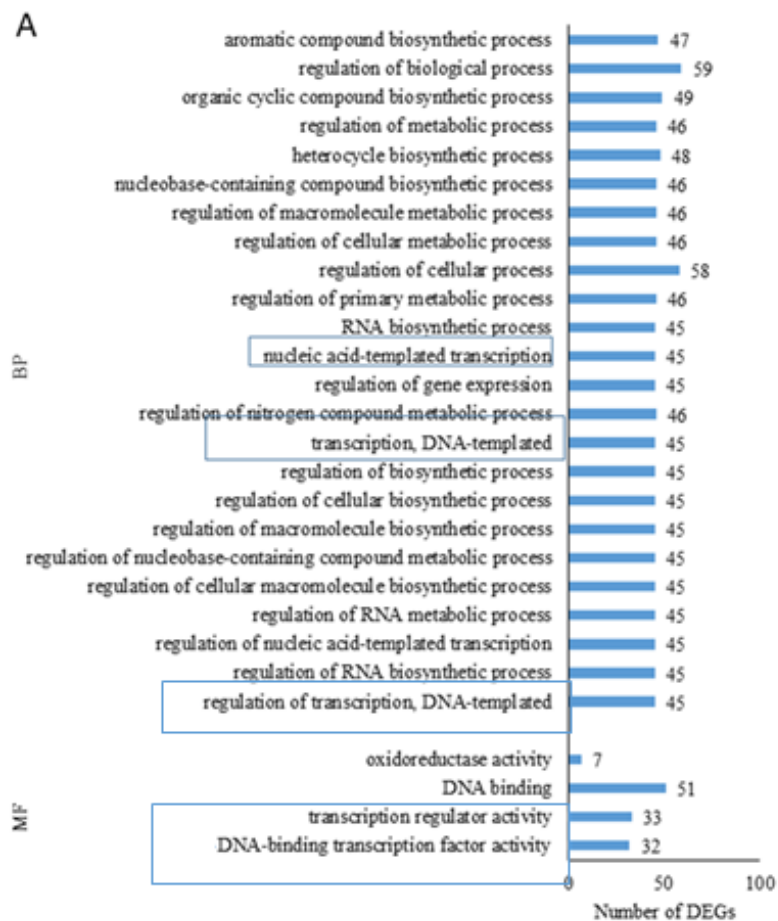


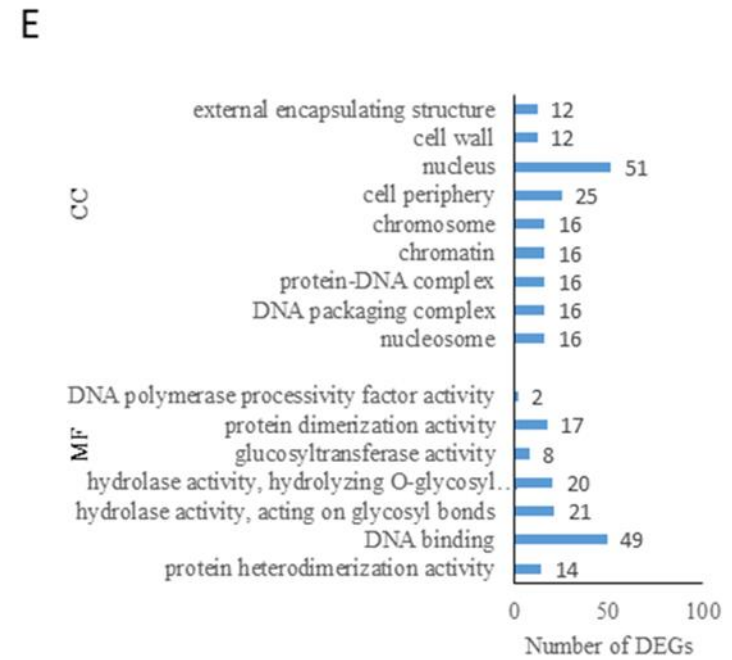
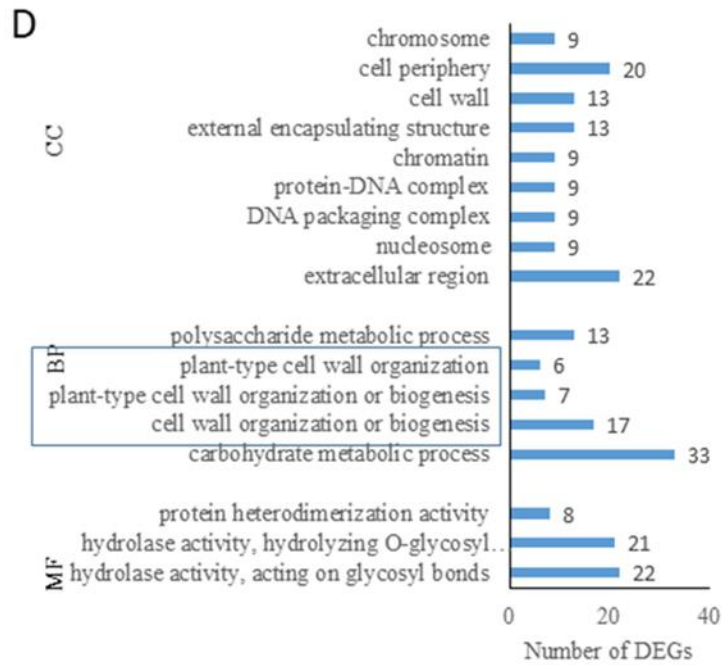


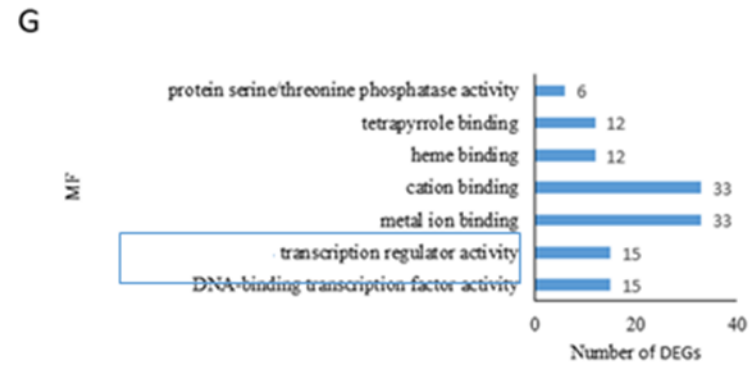
A. 2 dag, B. 4 dag, C. 7 dag and D. 28 dag and down-regulated genes E. 4 dag, F. 7 dag and G. 28 dag for CC, BP and MF categories. Blue arrows and boxes represent GO terms enriched in Nase 3 and TZ 130 but not in Ebwanateraka.

**Figure S3**

**Enriched GO terms in TZ 130 for up-regulated genes**







A. 4 dag, B. 7 dag, C. 28 dag and down regulated genes D. 2 dag, E. 4 dag, F. 7 dag and G. 28 dag for CC, BP and MF categories. Blue arrows and boxes represent GO terms enriched in Nase 3 and TZ 130 but not in Ebwanateraka