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Pyramiding and Functional Expression of Drought Tolerance Genes in Egyptian Rice

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LIST OF ABBREVIATIONS

Abbreviation		Description
APX	:	Ascorbate Peroxidase
bZIP	:	basic leucine zipper
CAT	:	Catalase
CTAB	:	Cetyl Tri Methyl Ammonium Bromide
DREBs	:	Dehydration-responsive element binding proteins
EDTA	:	Ethylenediaminetetraacetic acid
Fig.	:	Figure
Fv/Fm	:	variable fluorescence / maximum fluorescence
G. V	:	Genetic variance
G.C. V	:	Genetic coefficient of variance
HSP	:	Heat shock proteins
LEA	:	Late embryogenesis abundant
MABC	:	Marker assisted backcrossing
MAS	:	Marker assisted selection
MDA	:	Malondialdehyde.
OsCPK	:	Rice calcium dependent protein kinase
P. V	:	Phenotypic variance
P.C.V	:	Phenotypic coefficient of variance
PCR	:	Polymerase chain reaction
PIC	:	Polymorphic information content
PS II	:	photosystem II
QTL	:	Quantitative trait loci
RM	:	Rice marker
SOD	:	Super oxide dismutase
SSR	:	Simple sequences repeats.
TE	:	Tris EDTA

TILLING   : Targeting Induced Local Lesions in Genomes

TSS   : Total soluble sugars.

3.2.4. Molecular markers analysis:

The DNA of 12 selected rice genotypes was extracted using the cetyl-tetramethyl ammonium bromide (CTAB) method as described by Murray and Thompson (1980).

3.2.4.1 Reagents and solutions:

A. 1M TRIS – HCL (pH 8.0): 121.1 g of TRIS base were dissolved in 800 ml of d.d. H_2O, then the pH was adjusted to 8.0 using concentrated HCL. The volume was adjusted to 1L and sterilized using an autoclave.

B. 0.5 M EDTA (pH 8.0): 186g of EDTA was dissolved in d.d. H2O then the volume was brought up to 1L and the pH was adjusted to 8.0 and autoclaved.

C. 5 M NaCl: 292.2 g of NaCl were dissolved in 800 ml d.d. H2O and the volume was adjusted to 1L then autoclaved.

D. CTAB extraction buffer:

    100 mM tris–HCl (pH 8).

     20 mM EDTA (pH 8).

     CTAB 2% w/v

     1.4 M NaCl.

     0.2 % Beta mercaptoethanol (20 µl per 10 ml solution) was added just before use, and the volume was brought up to 100 ml using double distilled water.

E. Chloroform: Isoamyle alcohol (24:1 v/v) freshly prepared.

F. TRIS- EDTA buffer (pH 8): 10 mM Tris-HCl and 1mM EDTA.

G. Isopropanol.

H. **Ethidium bromide stock solution**: 1g ethidium bromide was dissolved in 100 ml d.d. H2O and stored in room temperature in a dark bottle.

I. 50X TAE buffer: 242 g of tris base were dissolved in 800 ml d.d. H2O then 57.1 ml glacial acetic acid and 100 ml of 0.5 M EDTA were added, and the volume was adjusted to 1 L.

3.2.4.2. DNA extraction:

100 -150 mg of fresh seedling leaves were disrupted by a TissueLyser II (Qiagen; Manchester, UK). A total of 700 µl of CTAB buffer were added and well homogenized, and then the samples were incubated in a water path at 65°C. for 30- 60 min. with occasional and gentle swirling. 700 µl chloroform: isoamyl alcohol (24:1) were added and the tubes were inverted slowly several times then centrifuged at 15000 rpm for 15 min at 4°C. the aqueous layer was transferred to a new 1.5ml Eppendorf tube, 0.6 volume of precooled Isopropanol was added and mixed gently and incubated at -20°C for 30 min. DNA pellet was precipitated at 10000 rpm for 10 min at 4°C, then washed three times in 70% ethanol. Well-dried DNA pellet was dissolved in 100 µl TE buffer. DNA was quantified using a NanoDrop (thermo scientific), and the DNA of all samples was approximately adjusted to concentration of 15 ng/μl which is good for PCR reaction.

3.2.4.3. SSR protocol and the polymerase chain reaction (PCR):

Thirteen simple sequence repeats primers (SSR) linked to drought tolerance in rice were selected and used in the present study for molecular characterization of 12 selected rice genotypes. All the SSR primers were introduced from Sigma Aldrich Company, Germany. The nucleotide sequence, annealing temperature and the chromosome number of the 13 SSR primers used in this investigation are presented in Table 5.

Table (5): Forward and reverse sequences, annealing temperature and chromosome number of the 13 used SSR primers.

Marker name	Forward sequence 5`-------`3`	Reverse sequence 3`--------`5`	Repeat motif	Annealing temperature	*Ch No.
RM22	GGTTTGGGAGCCCATAATCT	CTGGGCTTCTTTCACTCGTC	(GA)22	55	3
RM 319	ATCAAGGTACCTAGACCACCAC	TCCTGGTGCAGCTATGTCTG	(GT)10	55	1
RM60	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC	(AATT)5AATCT(AATT)	55	3
RM201	CTCGTTTATTACCTACAGTACC-	CTACCTCCTTTCTAGACCGATA-	(CT)17	55	9
RM157B	CCTCCTCCTCACGAATCCCGCC	GGGCTTCTTCTCCGCCGGCTTC	(CT)11(TC)10	55	3
RM260	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG	(CT)34	55	12
RM324	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	(CAT)21	55	2
RM525	GGCCCGTCCAAGAAATATTG	CGGTGAGACAGAATCCTTACG	(AAG)12	55	2
RM328	CATAGTGGAGTATGCAGCTGC	CCTTCTCCCAGTCGTATCTG	(CAT)5	55	9
RM472	CCATGGCCTGAGAGAGAGAG	AGCTAAATGGCCATACGGTG	(GA)21	55	1
RM228	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC	(CA)6(GA)36	55	10
RM175	CTTCGGCGCCGTCATCAAGGTG	CGTTGAGCAGCGCGACGTTGAC	(CCG)8	60	3
RM3805	AGAGGAAGAAGCCAAGGAGG	CATCAACGTACCAACCATGG	(GA)19	55	6

*Ch. No: chromosome number.

Polymerase chain reaction (PCR) was conducted in a volume of 15 µl reaction mixture using iNtRON's Master mix Solution (i-TaqTM). the mixture contained 2 µl of 15 ng/µl genomic DNA, 1µl of each of forward and reverse primers (10 pmol), 7.5 µl of 2X Master mix solution and the final volume was made up to 15 µl with double distilled water.

The PCR amplifications were executed in a thermal cycler (Biometra and Applied Bio systems) following this profile: initial denaturation step at 94°C for 5 min, followed by 35 cycles each cycle includes the following steps, denaturation at 94°C for 1 min, annealing (according to

the primer) for 1 min and extension at 72°C for 1 min. a final extension step of 7 min at 72°C was given.

3.2.4.4. PCR products analysis:

The amplified PCR products were separated by electrophoresis in 3% high resolution agarose gel (Sigma Aldrich, USA), stained with ethidium bromide and following the manufacturer protocol. A known DNA ladder (50 and100 bp DNA ladders were used, 0.5 μg /μl, H3 RTU) was run against the PCR products. Gels were visualized under UV illumination and photographed using Biometra gel documentation unit (BioDoc, Biometra, Germany). The molecular size of the amplified bands for the different studied SSR markers was calculated using gel analysis software (AlphaEaseFC 4.0, USA) based on the migration of band relative to the standard molecular size of DNA marker. Individual alleles (variation in molecular size of amplified product for individual primer pairs) for the SSR markers were scored to prepare a 1/0 matrix based on the presence (1) and absence (0). Heterozygosity was calculated according to formula described by Nei (1978) as follow:

$$H_{e=1-\sum P_i^2}$$

Where P_i is the frequency of the ith allele.

Effective number of alleles per locus (A_e) was measured as described by Weir (1989)

$$A_{e=1/((1-H_e)} = 1/\sum_1 P^2$$

suggesting a general and a critical role of these genes in drought stress response and signaling in rice. In addition, the expression levels of these genes were induced in a greater extent in the tolerant genotypes, indicating its crucial role in drought tolerance as a significant positive correlation among the expression level, physiological measurements, and better rice performance under drought stress condition. Also, these genes could be utilized as candidate genes and functional markers in marker assisted selection (MAS), allele mining and gene pyramiding in more efficient breeding programs to develop rice cultivars better adapted to adverse environment.

Conversely, the *OsMYB6, OsCDPK7* and *OsDREB2B* genes were repressed in response to drought stress. Additionally, *OsDREB2E* and *OsCPK4* were up regulated in the sensitive genotype IR64. This implies that these genes may not have a central role in drought tolerance in the studied genotypes and different genes from their gene families may be involved. Furthermore, *OsZIP23, OsDREB1A, OsWRKY13* and *OsDREB1C* genes were differentially expressed among the different genotypes, and this may indicate intrinsic transcriptomic responses to dehydration stress and genotype specific drought responsive genes.

In addition to the physiological parameters and enzymatic activities described earlier in this study, the enhanced drought tolerance may be attributed to a strongly induced expression of drought stress responsive genes (Guo et al., 2006; Zhu et al.,2018; Tang et al., 2019). Similarly, in our study the candidate line was superior under field evaluation and had a higher yield under drought stress condition and maintained higher relative water content, Fv/Fm values, SOD activity and proline content than the other genotypes. The advanced candidate line surpassed its two parents and alleviated the damage caused by the adverse conditions more effectively. Gene expression patterns analysis indicates that the candidate line has a higher capacity to modulate a higher expression

5. SUMMURY

The field experiment of the current investigation was carried out at the Research Farm of the Rice Research and Training Centre, Sakha, Kafr Elshiekh, Egypt and the molecular and biochemical studies were conducted at the molecular biology and biotechnology, University of Sheffield, U.K. The major objectives of this study were:

- Evaluation of some rice genotypes under drought stress condition.
- Detection of the differences in physiological and biochemical responses among drought susceptible and drought tolerant rice varieties.
- Determining the functional expression of drought responsive genes in the most highly candidate tolerant lines for drought using quantitative qRT-PCR.
- Validating the QTLs associated with drought tolerance in rice.
- Development of superior drought stress tolerant varieties by pyramiding of multiple drought genes from different sources.

This study could be classified into two main parts:

The first part: Evaluation of the response and performance of the tested rice genotypes to drought stress under field condition.

This part investigated the field screening of 160 rice genotypes *(Oryza sativa L.).*, including 148 advanced breeding lines, seven introduced varieties and five commercial Egyptian varieties under both normal and water deficit conditions. Water deficit condition was imposed via irrigation every 12 days without keeping standing water.

The studied characteristics were; (A) growth traits i.e., plant height (cm), days to heading (days), number of tillers per plant, chlorophyll content (SPAD),

panicle length (cm), flag leaf area (cm^2) and (B) yield and its attribute traits i.e., number of panicles per plant, sterility percentage (%), 100- grain weight (g), harvest index (%), grain yield per plant (g), drought susceptibility index (DSI), drought tolerance index (DTI), tolerance Index (TOL), drought tolerance efficiency (DTE),geometric mean productivity (GMP), harmonic mean (HM), yield stability Index (YSI) and yield Index.

The experiments were designated in alpha lattice design with three replications under both normal and drought conditions. However, the efficiency of lattice design was not higher than randomized complete block design (RCBD), therefore the RCBD analysis was used when the block mean square is greater than the residual mean square. The analysis of variance (ANOVA) was used to test the significances of the differences among the tested rice genotypes, then the combined analysis was employed to examine the interaction of the tested rice genotypes with the environments and years in addition to the interaction between years with environments.

The second part: physiological, biochemical, and molecular studies:

This part was carried out at the department of Molecular biology and biotechnology, university of Sheffield, U.K. The main aims of this part were to understand and detect the differences in physiological and biochemical responses of drought susceptible and drought tolerant rice genotypes, determining the functional expression of drought related genes in the most highly candidate tolerant lines for drought using quantitative qRT-PCR and validating the QTLs associated with drought tolerance in selected rice genotypes.

This part of study was done using the top candidate advanced breeding line (RBL112) and its two parents Giza 178 and IR60080-46A and the susceptible check IR64. The advanced breeding line RBL112 was selected

based on its tolerance and high yielding under drought stress condition in the field evaluation.

The seeds of RBL112, IR60080-46A, Giza 178 and IR64 were directly planted in 13D pots (0.88 l), containing soil consisting of 70% Kettering Loam, 23% Vitax John Innes No. 3, 5% silica sand and 2% Osmocote Extract Standard 5–6 month slow-release fertilizer by volume saturated with water. Plants were grown in controlled-environment growth champers at 13h 30°C : 11 h 24°C light : dark cycle, PAR 1000 µmol m-2 s-1 and 60% relative humidity, with a constant supply of water to the pot base and watering from the top once a week unless otherwise stated. The plants were grown up to 29 days in a big tray flooded with water, subsequently we discard the water and prevent watering the plants to apply the drought treatment. Control (well-watered) plants and treated plants were randomized within the growth chambers.

The following physiological and biochemical indices were measured: proline content, SOD activity, MDA content, TSS content, relative water content, Fv/Fm Values, and stomatal density, as well as the functional expression profiles of 22 stress-related genes in the four rice genotypes were studied under drought and well-watered conditions using qRT-PCR.

Marker validation of the QTLs linked to drought tolerance in rice was done using 12 rice genotypes (seven advanced breeding lines; five highly tolerant and two moderately tolerant along with their parents). Those lines were selected based on their performance under field condition. 13 SSR markers linked to drought tolerance in rice were implemented.

The most prominent results could be summarized as follow:

First part: Field evaluation for 160 rice genotypes (*Oryza sativa*. L) under well-watered and drought conditions.

A. analysis of variance:

The combined analysis of variance revealed that the year's mean squares were highly significant for chlorophyll content, flag leaf area, sterility percentage, and grain yield per plant, while it was non-significant for the rest of the traits under study.

Environments mean squares were a highly significant for all the studied characters and this means that there was significant difference among the environments under both of normal and drought conditions and the performances of the tested genotypes varied from one environment to another.

Highly significant interaction among the genotypes and the environments was observed for all studied traits, this means that the genotypes under investigation behaved differently from one environment to another and can ranked differently in each environment. Furthermore, the non-significant interaction between the years and the genotypes which was found in all the studied traits indicating that the tested materials performed independently from the seasonal changes hence we can recommend the superior lines.

The interactions among the genotypes ×years × environments were non-significant for all studied characters which reflect a non-changing performance for the rice genotypes in each environment in the different years.

B. Mean performances of the tested genotypes:

The most preferred mean values for growth characters under well-watered condition were obtained from RBL29, RBL118 and RBL119 genotype for plant height; Sakha103, RBL130, RBL19 and RBL2 for days to heading; RBL106 and RBL 129 for number of tillers per plant; RBL11, RBL1 and RBL 144 for panicle length; RBL1, WAB881SG9 and RBL30 for chlorophyll content.

The most desirable mean values for growth characters under water -deficit stress condition were observed in Morobrekan and RBL22 for plant height;

Sakha 105, RBL103, RBL102 and RBL104 for days to heading; RBL9, RBL1 and RBL106 for number of tillers per plant; RBL44, Morobrekan and RBL144 for panicle length; RBL44, WAB881 and RBL1 for chlorophyll content.

The most preferable mean values for yield and its attributes under non-stress condition were obtained from RBL106, RBL1 and RBL29 for panicles number per plant; morobrekan, RBL35 and RBL143 for 100-grin weight; RBL 16, RBL36 and RBL26 for sterility percentage; RBL112, RBL44 and RBL1 for harvest index; RBL106, RBL1 and RBL48 for grain yield per plant.

The most favourable mean values for yield and its attributes under drought stress condition were found in IR6008046A, RBL106 and RBL9. for panicles number per plant; Morobrekan, RBL35 and RBL10 for 100-grain weight; RBL80, RBL112 and RBL44 for sterility percentage; RBL112, RBL44 and RBL10 for harvest index; RBL112, RBL7, RBL9 and RBL44 for grain yield per plant.

It is possible to draw a conclusion that the following advanced breeding lines RBL112, RBL44, RBL7, RBL9, RBL10 and RBL35 performed well under water deficit stress condition and showed superiority of most of the secondary traits linked to drought tolerance, hence they can be recommended for further screening and could be planted in the areas affected by water shortage.

C. Phenotypic correlation:

Under drought stress condition, grain yield per plant had consistently positive correlation with each of number of tillers per plant, panicle length, chlorophyll content, number of panicles per plant, harvest index and 100- grain weight.

D. Genetic parameters:

The estimates of the genotypic variance (GV) were higher under normal condition than the drought condition in all the studied characters, except for days to heading, sterility percentage and 100- grain weight. The same trends also were observed in the estimates of phenotypic variation (PV), but the corresponding values were higher under both environments.

Phenotypic coefficient of variance (PCV %) values were found to be high for number of tillers per plant, flag leaf area, number of panicles per plant, sterility percentage, grain yield per plant and harvest index under both control and drought stress conditions, while days to heading scored the lowest PCV % values. Genotypic coefficient of variation (GCV %) exhibited the same trend for the same traits under both environments.

In general, heritability in broad sense values were moderate to high under the two environments. These values ranged from 85.80 to 97.79 % under non stress conditions, whereas the range under drought stress was from 73.95 to 96.05 %.

E. Stress tolerance indices:

The Following indices: drought susceptibility index (DSI), drought tolerance index (DTI), tolerance index, drought tolerance efficiency (DTE), geometric mean productivity (GMP), yield stability index (YSI) and the Yield index were measured. It could be concluded that, the following advanced breeding lines RBL 112, RBL 44, RBL9, RBL10 and RBL7 were identified as promising high yielding and drought tolerant cultivars as they exhibited lesser reduction in mean performance and showed relative stability in grain yield production under drought stress conditions in compared to well-watered condition.

Second part: physiological, biochemical, and molecular studies:

A. Physiological and biochemical responses:

SUMMURY

The proline content was greatly increased in all studied genotypes under the drought treatment in compare to the control condition. However, the increase was significantly higher in both the advanced candidate line RBL112 and the parent IR6008046A than the sensitive check IR64 and the Egyptian variety Giza178.

Drought stress significantly increased the SOD activity in all genotypes (the increase was about 55.13%, 47.35%, 42.87% and 37.15 % for the candidate line RBL112, IR608046A, Giza 178 and IR64, respectively) in comparison with non-stress condition. Furthermore, there was a remarkable increase in total soluble sugars under drought stress condition in all the tested genotypes compared to the normal conditions.

MDA content was markedly increased in the leaves of the four rice genotypes under drought stress conditions compared to control plants. Drought induced a highly significant increase in MDA concentration in IR64 in contrast the candidate line RBL112 and the tolerant parent IR6008046A displayed the lowest MDA content under drought stress condition.

Compared to well-watered condition, drought stress remarkably decreased the RWC in all genotypes, however the candidate line RBL112 and the tolerant parent IR6008046A exhibited higher values of RWC and showed a considerable ability to maintain a high level of water in their leaf tissues.

Maximum quantum yield for primary photochemistry (Fv / F_m), values of dark adapted Fv/Fm were nearly the same in all the four genotypes under flooded condition, however a gradual decline in Fv/Fm values was observed in the four genotypes three days of drought onset. The selected advanced candidate line showed a higher Fv/Fm ratio and maintained Fv/Fm levels for at least one day longer than the other genotypes.

There were no significant differences in stomata density among the studied four genotypes under drought stress condition.

B. Functional Gene expression profiles of stress-related genes in rice under drought and well-watered conditions:

1. Expression profiles of *OsAHL1, OsLG3* and *OsSKIPa*:

The expression of *OsAHL1* gene was remarkably induced in the four genotypes under drought conditions, however the expression level of *OsAHL1* was pronounced in the candidate line RBL112 (3.58-fold) and surpassed its two parents IR60080-46A (2.87-fold) and Giza 178 (3.45fold).

The transcript level of *OsLG3* in response to drought was upregulated in the leaves of the four genotypes, the extent of the induction was the highest in the candidate line RBL112 (2.4-fold), followed by IR60080-46A (1.8fold) under drought stress in comparison with non-stress condition, while it was slightly induced in IR64 (1.1-fold) due to drought stress.

The expression level of *OsSKIPa* in response to drought stress was not altered in the leaves of IR64 and Giza178, while it was highly expressed in IR60080-46A (1.79-fold change) and the candidate line (1.67-fold change).

The transcript level of CDPK7 was downregulated in the leaves of all genotypes with a slight reduction in the candidate line RBL112 in response to drought stress.

The transcription levels of *OsCPK4* were slightly up regulated in IR64 (0.19-fold change), whereas it was repressed in the tolerant parent IR6008046A (2.43-fold change) and slightly down regulated in the candidate line and Giza 178 due to drought stress.

The sensitive variety IR64 exhibited the highest transcription levels of *OsZIP23*, while it was poorly expressed in the other genotypes under normal

growth conditions. Meanwhile, the transcript levels of *OsZIP23* were up regulated in the four genotypes due to drought treatment.

OsMYB6 gene was highly repressed in the candidate line and the tolerant parent IR6008046A, slightly downregulated in the susceptible check IR64 but it was none significantly induced in the Egyptian parent Giza178 under drought condition compared to control condition.

In comparison with control condition, *OsWRKY13* was strongly induced in the four genotypes in response to drought stress. The candidate line RBL112 scored the highest induction level (2.14-fold change), then IR6008046A (1.5-fold change), Giza178 (1.6-fold change) and IR64 (1.4-fold change).

NAC1 gene displayed a highly upregulation in the leaves of the four genotypes studied under drought stress condition, the induction extent was significantly higher in the candidate line RBL112, IR60080-46A and Giza 178 in compared to IR64.

2. Expression profiles of drought functional genes:

Drought caused high induction in the transcription level of the stress responsive gene *OsRab21* in the leaves of IR60080-46A (23.2fold) and the candidate line RBL112 (15.5fold) while it was slightly repressed in the leaves of the Egyptian variety Giza178 with no significant change in IR64.

OsP5CS gene was highly induced in the leaves of the four genotypes under drought stress condition, the expression level was much higher in the drought tolerant genotypes than the sensitive check IR64 and the moderate tolerant parent Giza 178.

The *APX2* transcripts were found to be accumulated in a higher degree in the leaves of the tolerant and moderately tolerant genotypes (The candidate line

RBL112 (64.99-fold), Giza 178 (61.14-fold) and IR6008046A (34.63-fold) than the sensitive check IR64 (18.51-fold), under drought stress conditions.

The transcript level of *OsCATA* gene was strongly enhanced in the leaves of all genotypes studied due to drought treatment, however the expression level of *OsCATA* was significantly higher in the candidate line (15.9-fold), IR6008046A (15.7- Fold) and Giza 178 (13.9-fold) compared to IR64 (6.6-fold).

Under drought stress condition, there was a massive induction in the transcript level of *OsLEA3* in the leaves of the four genotypes in compared to control conditions. However, the expression level was significantly higher in the leaves of the candidate line RBL112.

Inhibition of rice heat shock protein gene *OsHSP101* expression was observed in the leaves of the sensitive check IR64 under water- deficit conditions, while it was upregulated in the leaves of the other genotypes. The international parent IR6008046A scored the highest induction level (2.61-fold change).

3. The expression profiles of *Os04g55710 and Os1g64660*:

The transcription level of Os04g55710 in response to drought treatment was down regulated in IR64 and Giza 178 genotypes. On the other hand, it was slightly induced in IR60080-46A and remained unchanged in the candidate line.

Os1g64660 was highly induced in RBL112, IR6008046A and Giza 178, while it was slightly repressed in IR64 under drought stress condition.

4. The expression profiles of dehydration responsive element binding proteins (DREBs):

DREB2A gene was induced in response to drought stress in the leaves of all genotypes. The transcript level was the highest in RBL112 under both normal

(2.9-fold) and drought conditions (7.00-fold), followed by IR6008046A (4.1-fold under drought condition.

OsDREB1A gene was upregulated in the leaves of IR64 by drought treatment, while it was strongly repressed in the rest of the genotypes.

The transcript level of *OsDREB1C* was slightly induced in IR64 and Giza 178; moreover, it was strongly induced in IR60080-46A (6.96- fold change) and RB112 (4.28-fold change) in response to drought stress. *OsDREB2E* was differentially expressed among the four genotypes; however, it was poorly expressed under both conditions. The expression level of *OsDREB2E* was slightly induced in the sensitive check IR64, remained unchanged in Giza 178 and the candidate line with non-significant reduction in IR6008046A.

OsDREB2B gene was downregulated in all genotypes studied under dehydration conditions, plus it was weakly expressed in the four genotypes under non stress and stress condition.

5. Hierarchical cluster analysis of the studied genotypes and the studied genes under both normal and drought conditions:

The transcripts level of drought tolerance related genes was able to classify the studied genotypes according to their drought tolerance nature into drought sensitive, moderately tolerant, and tolerant ones.

C. Marker validation of QTLs related to drought tolerance in rice:

Eleven out of the 13 SSR markers were found to be polymorphic. Altogether 28 alleles were detected. The number of alleles amplified by a single marker varied from 2 (RM22, RM319, RM201, RM472, RM228 and RM328) to 4 (RM3805) with an average of 2.15 alleles per locus.

Overall size of the amplified fragments ranged from 70 bp (RM175) to 300 bp (RM472). The effective number of alleles (Ae) per locus ranged from 1.18

(RM472) to 3.78 (RM3805) with an average of 2.05. Heterozygosity (He) varied from 0.153 for RM472 to 0.757 for RM3805 with an average of 0.466.

Polymorphic information content (PIC) values ranged from 0.141 in the case of RM 472 to 0.736 in the case of RM3805 with an average of 0.369 across the primers. There were three highly informative markers RM3805, RM525 and RM324 (PIC > 0.50), six informative markers RM22, RM319, RM228, RM328, RM260 and RM175 (0.50 < PIC > 0.25) and two slightly informative markers RM472 and RM201 (PIC < 0.25).

It can be concluded that the RM 472, RM3805, RM260 and RM175 are more efficient in discriminating among rice germplasm based on their tolerance to drought stress. Hence these markers can be utilized in marker assisted breeding procedures in more efficient way in selecting the individuals harboring the QTLs linked to drought tolerance, pyramiding and introgressing those QTLs in one elite local variety.