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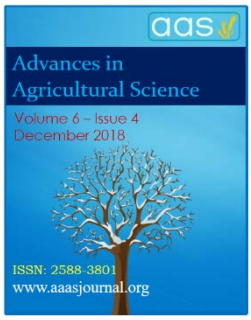

Research Article Open Access

Determination of Genetic Variation and Expression Pattern of *DHNs* and *HSPs* in Some Rice Genotypes under Water Stress

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ABSTRACT

The present study aimed to investigate the different responses of sensitive and tolerant rice genotypes under water stress conditions induced by using poly ethylene glycol (PEG 6000). The studied genotypes were Giza177, Giza181, Giza182 and Sakha103 (sensitive), Sakha104 (moderate) and Orabi2 (tolerant). The present investigation included the determination of germination %, fresh and dry weight, shoot and root length, cell membranes stability as rate of electrolyte leakage (EL) and lipid peroxidation as MDA content. Semi-quantitative reverse transcriptase polymerase chain reaction (sqRT-PCR) for some protective proteins such as heat stable proteins (*HSPs*) and dehydrins (*DHNs*) standardized on actin transcript amounts were carried out to investigate the changes in the expression pattern of those genes. All determinations were carried out for all studied genotypes under both control and drought conditions. The obtained results revealed that water stress tolerance of tolerant genotypes was accompanied with decreasing of electrolyte leakage rate and low content of MDA comparing with sensitive ones. sqRT-PCR analysis for expression pattern of studied genes showed increasing in the expression of *HSP-13*, *HSP-12*, *HSP-9* and *DHN-2* in the seedlings of tolerant genotypes comparing with sensitive ones. The present study pointed to the participation of those genes in the acquisition of drought tolerance in tolerant genotypes.

Introduction

Rice plays a major role as a staple food, supporting more than three billions people and comprising 50% to 80% of their daily calorie intake (Khush, 2005). Drought stress severely impairs its production. Worldwide, drought affects approximately 23 million hectares of rainfed rice (Serraj *et al.*, 2009). Rice occupies about 22% of the total cultivated area in Egypt during summer and it consumes about 20% (about 16500 m3/ha) of the total water resources (Aboulila, 2012) which is liable to decrease year after year (Mahassen *et al.*, 1999). The unpredictability of drought patterns and the complexity of the response mechanism involved have made it difficult to characterize component traits required for improved performance. Oxidative damage is one of the major causes of plant injury under drought stress (Zhou *et al.*, 2007). Cell membranes are one of the first targets of many stresses and it's their membrane stability has been widely exploited as an indicator of stress tolerance (Blum and Ebercon, 1981; Morsy *et al.*,

2005, Omar *et al.,* 2009, 2015). The degree of cell membrane injury induced by stress may be easily estimated through measurements of electrolyte leakage from the cells (Bajji *et al.,* 2002). Free malondialdehyde (MDA), as an oxidative stress marker, is thought to be involved in deterioration of various biological functions due to its attachment to biomolecules such as proteins and nucleic acids in stressed plants (Yamauchi *et al.,* 2008, Wu N. *et al.,* 2011,Omar *et al.,*2015). Crops exposed to drought conditions showed differential expression of stress related genes. Functional proteins such as Heat stable proteins (HSP) and Dehydrins (DHNs) showed overproduction as a response to water deficient experiments (Sato and Yokoya, 2008, Close, 1996, Allagulova *et al.,* 2003, and Omar *et al.,* 2013). In rice, more than 5,000 genes are up-regulated and more than 6,000 are down-regulated by drought stress (Maruyama *et al.,* 2014). The proper evaluation of genetic responses of different rice genotypes to water stress is becoming an acute issue and provides the theory basis for rice drought tolerance. This can help molecular breeder to improve drought tolerant considering different genotypes. The present study aimed to investigate physiological responses of some rice genotypes to drought conditions induced with PEG and assessment the expression pattern of some *HSP*s and *DHN*s genes with acquisition of drought tolerance.

Materials and Methods

Plant Material and Growth Conditions

Six rice (*Oryza sativa L.*) genotypes (Table 1) were obtained from Rice Research and Training Center (RRTC) at Kafr El sheikh and kindly from prof. Dr. Said Soliman, Genetics Dept, Faculty of Agriculture, Zagazig University,Egypt for the genotype Orabi2. Forty seeds of each genotype were germinated in petri dishes (10cm) on wet layer of filter paper. Drought stress treatments were induced using polyethylene glycol (PEG 6000) (dissolved in distilled water). Solution by various concentrations (0, 5, 10, 15 % W/V) of PEG are equal to 0, –0.05, –0.15 and –0.3 MPa, respectively according to Burlyn and Kaufmann (1973). Drought stress treatments were carried out by overlaying the solution (25ml) of each solution onto petri dishes. Plats were irrigated with Hoagland solution up to 25ml to save the concentration of PEG treatment. Plats were kept for growth of seedlings up to 15 days at 29± 1˚C. Thirteen /eleven h light/dark system was used at incubator. The experiments used Randomized Complete Design (RCD) with three replicates.

Germination percentage

Germination percent was determined by counting the number of germinated seeds after 4 days. Rice seeds were considered as germinated when the radical visibly protruded from the seed coat by at least 2 mm.

Fresh and dry weights

Seedling fresh weight (FW) and dry weight (DW) were measured for each genotype grown under control and drought conditions at 15-day-old seedlings. Dry weight (DW) was determined by reweighting the whole seedling after drying at 105˚C for 3 h.

Evaluation of lipid peroxidation product

Lipid peroxidation was evaluated as the concentration of thiobarbituric acid (TBA) reactive products equated with malondialdehyde (MDA), as described by (Anjum *et al.,* 2012) with some modifications. One half gram of plant tissues was homogenized in 5% (w/v) trichloroacetic acid (5 mL), centrifuged at 4000 rpm at 5˚C for 10 min. The chromogen was formed by mixing 2 mL of supernatant with 3 mL of reaction mixture containing 20 % (w/v) trichloroacetic acid (TCA), 0.5% (w/v) 2-thiobarbituricacid (TBA). The

Table 1. Rice genotypes; name, pedigree and type

Genotype Name	Pedigree	type
Giza177	Giza171/Yomji No.1//Pi No.4	*Japonica*
Giza 181	IR28/IR22	*Indica*
Giza 182	Giza181l/IR39422-161-1-3//Giza181	*Indica*
Sakha103	Giza177/Suweon349	*Japonica*
Sakha 104	GZ 4096-8-1/GZ4100-9-1	*Japonica*
Orabi-2	New developed rice variety by Dr.Said Soliman, Genetic Dept. fac of agriculture, Zagazig univ., Egypt.	*Indica*

Table 2. RAPD primer: codes, sequences and annealing temperatures

Primer codes	**Sequence 5 ---------- 3**	**Annealing temperature**
OPA-05	AGGGGTCTTG	32 ˚C
OPA-11	CAATCGCCGT	32 ˚C
OPB-10	CTGCTGGGAC	36 ˚C
OPC-02	GTGAGGCGTC	36 ˚C
OPD-07	TTGGCACGGG	36 ˚C
OPA-01	CAGGCCCTTC	36 ˚C
OPB-07	GGTGACGCAG	36 ˚C
OPC-05	GATGACCGCC	36 ˚C
OPF-14	TGCTGCAGGT	32 ˚C
OPL-03	CCAGCAGCTT	32 ˚C
OPA-04	AATCGGGCTG	32 ˚C
OPA-10	GTGATCGCAG	32 ˚C

mixture was heated at 100˚C for 15 min; the reaction was then stopped by rapid cooling in ice-water bath, followed by centrifugation at 4000 rpm at 5˚C for 10 min. The amount of MDA was then measured colorimetric using spectrophotometer (UV1901PC) at 532 nm and correction for unspecific turbidity was done by subtracting the absorbance at 450 and 600 nm. The TBA-reactive products (MDA) were expressed as nmol.g^{-1}DW.

Measurement of electrolyte leakage (EL)

Electrolyte leakages (EL) of individual seedlings were measured using a conductivity meter (Adwa-AD32). Three replicates were used for each seedling which was placed in a vial containing 20 mL of de-ionized water with gently shaken then the conductivity of the solution was measured immediately. The conductivity of the solution ($\mu S \cdot cm^{-1}.FW \cdot h^{-1}$) was measured once again after 1 h. Finally, each vial was placed in boiling water for 1 h, then cooled to room temperature (with gently shaken) and the total conductivity was measured. Leakage rate of electrolytes (was calculated as the net conductivity of the solution with seedlings immersed for 1 h divided by the total conductivity after boiling according to (Omar, *et al.*, 2012).

DNA extraction and RAPD analysis

Total genomic DNA was extracted from seedlings by the easy extraction kit (EZ-10 Spin Column Genomic DNA Mini-preps Kit, plant) followed by an RNase-A treatment. The quantification and

Table 3. Selected genes, accession numbers, primer name, primer sequence and annealing temperature

Gene name	Accession no.	Primer name	Primer sequence 5 ---------- 3	Annealing temperature
HSP1	M80186	Mhsp1	F-CCGATCGTTCCACCTCCAAA R-CATGCATGACACGCCTTGAC	55˚C
HSP4	DQ180746	Mhsp4	F-GGAGGAGTCGTGCAAGTACC R-TCATCACATCGCATACGGCA	54˚C
HSP9	GU120341	Mhsp9	F-AACGTGTTCGACCCCTTCTC R-TAGCCGGTAACCTGGATGGA	54˚C
HSP12	GU120338	Mhsp12	F-AACGTGTTCGACCCCTTCTC R-CATGCATGACACGCCTTGAC	54˚C
HSP13	AY034057	Mhsp13	F-CGGTGGTCATTTCCTTCCCA R-GAAGGGGTCAAACACGTTGC	54˚C
DHN1	U60097	Mdhn1	F-GGAATGGGAAGGACGACGAA R-ACTGGAGAACGCCATCACAC	55˚C
DHN2	AY786415	Mdhn2	F-AAAGAAGGAGGAGCACCACG R-TTGTGGTAACCGGGCAGTTT	56˚C
DHN3	EF576194	Mdhn3	F-TGGAGGAGTTCGTAGCAGGA R-GACGCCAGGATAATACACATCA	54˚C
DHN4	EF444533	Mdhn4	F-CACTAGCTGGAACACTTGGGT R-CTGGTTGTTGCCCTTGTTGC	54˚C
Actin	X16280	RAc1	F-CATGCTATCCCTCGTCTCGACCT R-CGCACTTCATGATGGAGTTGTAT	55˚C

qualification of the extracted DNA was determined on 0.8 % agarose gel. Random amplified polymorphic DNA (RAPD) was used to characterize genetic variations of the used genotypes. A set of twelve10-mer oligonucleotides was used for RAPD-PCR (Table 2). Primers were selected for their association with water stress tolerant genotypes from some previous studies (Youssef *et al.,* 2010; Ullah *et al.,* 2013). PCR amplification reactions were carried out in 25 µl reaction volume according to instruction supporting with GoTaq® Green master Mix, 2x (Promega). The amplification runs through four min at 94 ˚C and then 35 cycles of 1 min at 94˚C, 2 min at 32 or 36 ˚C (according to the primer) and 1min at 72 ˚C, followed by a final extension at 72 ˚C for 5 min. using (MyGene®MG96G) programmable thermal cycler. Fifteen µl of PCR amplified product were loaded into 2 % agarose gel supplemented with ethidium bromide. The TBE buffer 1X was used as a running buffer and 100bp DNA ladder was used to estimate the molecular size of the amplified fragments. Electrophoresis was conducted at 60 Volts for 3 h. Gels were then visualized and photographed under UV-trans illuminator by digital camera with UV filter adaptor.

Data analysis and phylogenetic tree construction

Separated bands were scored for each RAPD marker based on the presence and absence of bands, generating a binary data matrix of 1 and 0 for each marker system. The presence / absence matrix for amplified DNA fragments was analyzed using the PAST program, version 1.90 (Hammer *et al.,*

2001). The data matrix was used to calculate genetic similarity based on Jaccord`s similarity coefficients to establish genetic relationship among the genotypes based on unweighted pair group method of arithmetic averages (UPGMA) and sequential agglomerative hierarchical nested (SAHN) clustering.

Total RNA extraction and cDNA Synthesis

Total RNA was extracted from seedlings of control and drought treated plants of different genotypes using EZ-10 Spin Column Plant RNA Mini-Preps Kit (BIO BASIC CANADA INC). For quality control the RNA was analyzed in 1 % agarose gel with RNase-free devices. Gel stained with ethidium bromide (EtBr; 10 mg. ml^{-1}) Gels were then visualized and photographed under UV-trans illuminator by digital camera with UV filter adaptor. For quantification of the RNA content the samples were diluted 1:500 in DEPC-treated water. The absorption of the samples was measured at 260 and 280 nm using the spectrophotometer (UV1901PC). One A260 corresponds to 40µg.ml^{-1} RNA. The purity of RNA sample was calculated from the A260/A280 ratios. Values higher than 1.8 the RNA purity was considered acceptable. First-strand cDNA was synthesized using 5µg of total extracted RNA for each sample according to the protocol supported by GoScript ™ reverse transcription Kit using Oligo $(dT)^{15}$ primer.

Semi-quantitative reverse transcriptase polymerase chain reaction (sqRT-PCR)

Polymerase chain reaction (PCR) was used to amplify the number of copies of specific cDNA sequences in vitro. All primers used for semi quantitative RT-PCR is listed in Table3. They were designed based on sequencing data of expressed sequence tags (ESTs) data base of selected genes on the website of National Center for Biotechnology Information (NCBI). Primers were designed using the Primer Primer 5 software following the manufacturer's guideline for primer design. Primers were ordered from BIOSEARCH TECHNOLOGIES COMPANY. Samples of cDNA were standardized on actin transcript amount. Actin cDNA (accession no. X16280) used as an internal constitutively expressed control (reference gene). Gene specific primers (Table3) used to amplify different related genes. For typical PCR reaction, 1µl cDNA was used as template in 25 µl reaction volume according to instruction supporting with GoTaq® Green master Mix, 2x (Promega USA). PCR program for sqRT-PCR was optimized for each gene to yield optimal contrast between samples in the fluorogrammes of subsequently performed EtBr-agarose gel electrophoresis. The general program was; 94°C for 5 min, followed by cycle of 94°C for 1 min,54-56°C for 1 min, and 72°C for 1 min, and last extension step of 72°C for 7 min.

Results and Discussions

The present study was undertaken to get better understanding of the morphological and physiological basis of water stress resistance in respect to genotypic differences. That could be used to select or create new varieties standing for water deficit to obtain a better productivity under water stress conditions.

Germination percentage (G %) and seedling growth

Germination percentage (G %) showed gradually decreased with increasing of PEG concentrations in G177 (Figure 1A), while for other genotypes it was fairly static (with control, 5% and 10% PEG) and showed slight decrease (with 15% PEG). Figure 1B summarized that water stress condition caused losing in germinability of 18-35% of tested seeds in G177, G182, and SK104 comparing with control condition. On the other hand, other genotypes lost the germinability of less than 10% of their seeds. Responses of different genotypes to water stress as

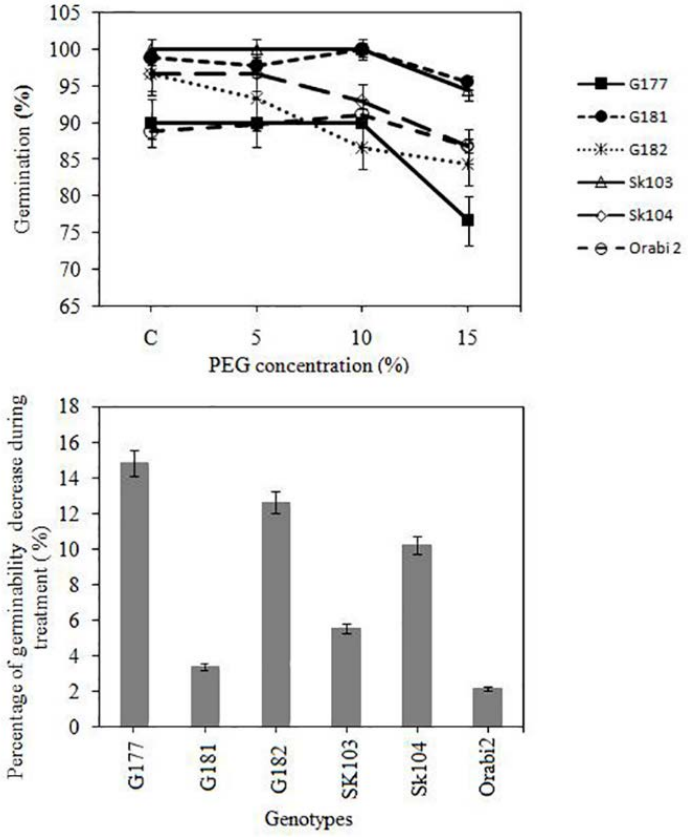

Figure 1. Changes in germination % of all studied genotypes under control and PEG treatments (A), and the percentage of decrease in germination % of all studied genotypes as a result of treatment with 20% PEG (B).

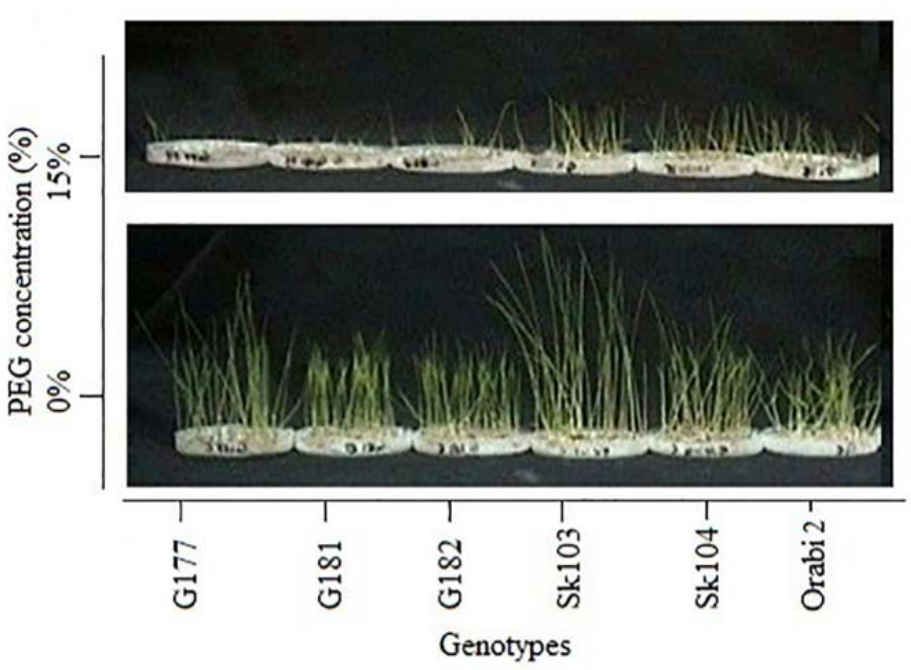

Figure 2. Seedling of studied genotypes under control (0%) and drought treatments (15%) after two weeks of planting.

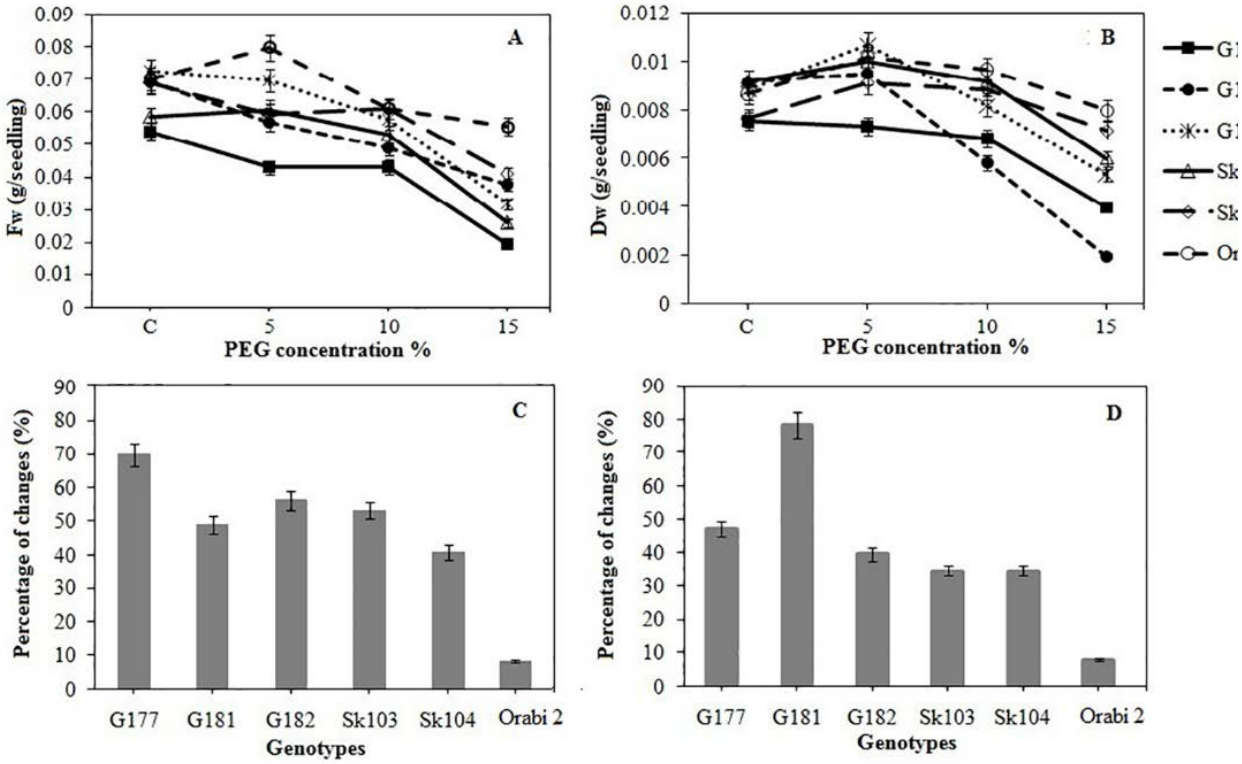

Figure 3. Changes in FW(A), DW(B) of all studied genotypes under control and PEG treatments and the percentage of decrease in FW(C) and (DW) of all studied genotypes as a result of treatment with 20%PEG.

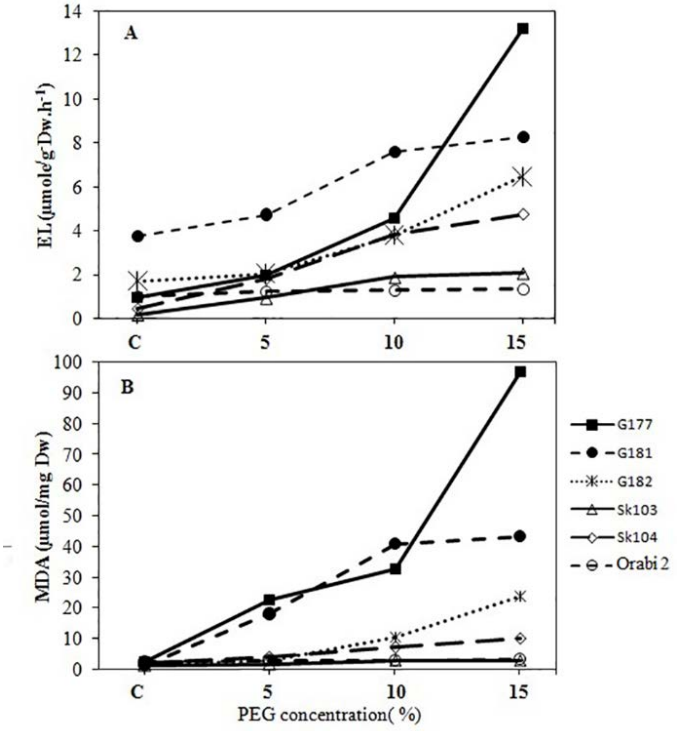

Figure 4. Changes in electrolyte leakage (EL) (A) and MDA content (B) of all studied genotypes under control and PEG treatments

seedling growth after two weeks of treatment (15% PEG), showed the severe effects of water stress on growth of sensitive genotypes (G177, G181 and G182) comparing with moderate and tolerant ones (Sk103, Sk104 and orabi2) (Figure 2). Germination and seedling development under laboratory conditions have been accepted as suitable growth stages for testing the response to abiotic stresses (Sharif-zadeh and Mohsen, 2008) and thus it was employed to evaluate the drought sustaining character of studied genotypes. Under abiotic stress conditions, delayed seed germination or inhibition of seed germination has been reported in almost all the crop plants (Jeannette *et al.*, 2002; Khan and Gulzar, 2003; Sosa *et al.*, 2005). The systematic sequence of complex physiological and biochemical events of successive processes which lead to embryonic axes emergence involved in seed germination appear to be variously affected by abiotic stress such as drought, salinity or ionic stress in the external environments (Fadzilla *et al.*, 1997). Germination of seeds under stress conditions differs from one crop to the other and even a significant variation is recorded amongst the different cultivars of the same crop (Zeng and Shannon, 2000). The reduction in seed germination may be due to the less availability of free water to the seeds during early hours of imbibition, thus leaving the hydrolytic enzymes inactive (Shah and Loomis, 1975; Hadas, 1976). Also, inhibition of germination at higher osmotic potential may possibly be attributed to moisture deficit in the seed below the threshold requirement for germination (Everiff, 1983). The correlation between germination percentage in PEG initiated drought and other drought sustaining characters at old plant showed good correlation to PEG treatment at different concentrations (Chutia and Borah, 2012). Thus the determination of germination index can be used just as an easy and reliable parameter for measuring drought sustenance among the traditional rice cultivars.

Seedlings Fresh and dry weight determination

Results of FW indicated that seedlings of all genotypes showed a gradually decrease in FW values with increasing PEG concentrations. On the other hand, Orabi2 seedlings treated with 5% PEG showed an increase in FW value by 11% comparing with its control and gradually decreased with increasing of PEG concentrations (Figure 3A). Under water stress condition, G177 seedlings lost about 70% of their FW comparing with control condition, while G181, G182, SK103 and SK104 seedlings lost about 50% of their FW comparing with control condition (Figure 3B). On the other hand, Orabi2 seedlings lost about 10% of their FW under control condition. The seedlings of all genotypes except G177 treated with 5% PEG showed an increase in DW values comparing with DW values of seedlings under control condition (Figure 3A). A sharp decrease in DW values of G177, G181,G182 and SK103 seedlings with increasing PEG concentration was noticed while SK104 and Orabi2 seedlings were fairly static with 5% and 10% and showed a slight decrease with increasing PEG concentration (Figure 3B). Seedlings of G181 lost about 80% of their DW at 15% PEG treatment while seedlings of G177, G182, SK103 and SK104 lost about (35% - 45%) of their DW. On the other hand, Orabi2 seedlings lost less than 10% of their DW comparing with their values under control treatments. The decrease in growth rate as fresh and dry weight under PEG stress is related to the reduction in photosynthetic rate caused by osmotic stress generated by polyethylene (Zhang and Kirkham 1996) as a reduction in Chlorophyll-a and Chlorophyll-b under water stress conditions (Chutia and Borah 2012). The genotype which success to keep high value for FW has the ability to maintain tissue water status and avoid the drought induced damages (Abdel-Nasser and Abdel-Aal, 2002). Keeping high value of DW pointed to the ability of genotype to maintain photosynthesis process under water stress conditions that causes continuously accumulation of metabolites (Werner *et al.*, 2001).

Table 4. Number of total fragments, monomorphic fragments, polymorphic fragments, unique fragments and percentage of polymorphism obtained per each RAPD primer for all tested genotypes

Primers	Range of fragment sizes (bp)	Total No. of fragments	Monomorphic fragments	Polymorphic Fragments	Unique Fragments	Polymorphism %
OPA-05	362-1534	9	4	5	0	**55.55**
OPA-11	222-1014	8	2	3	3	**37.50**
OPB-10	306-1289	10	3	2	5	**20.00**
OPC-02	269-1014	13	6	3	4	**23.07**
OPD-07	321-1027	12	2	4	6	**33.33**
OPA-01	526-1190	8	2	5	1	**62.5**
OPB-07	287-1215	9	3	5	1	**55.55**
OPC-05	297-1115	14	2	8	4	**57.55**
OPF-14	314-1705	18	3	8	7	**44.44**
OPL-03	186-1156	11	6	3	2	**27.27**
OPA-04	298-1446	12	3	5	4	**41.66**
OPA-10	267-1663	12	4	5	3	**41.66**
Total	222-1705	136	40	56	40	
Average		11.33	3.33	4.66	3.33	**41.17**

Table 5. the similarity coefficient values among all tested genotypes based on band polymorphism generated by RAPD-PCR primers.

Genotypes	G177	G181	G182	Sk103	Sk104	Orabi2
G177	1	0.48598	0.49541	0.52885	0.62105	0.43119
G181		1	0.77011	0.54639	0.55914	0.58065
G182			1	0.55556	0.58511	0.57292
Sk103				1	0.72619	0.5
Sk104					1	0.49474
Orabi2						1

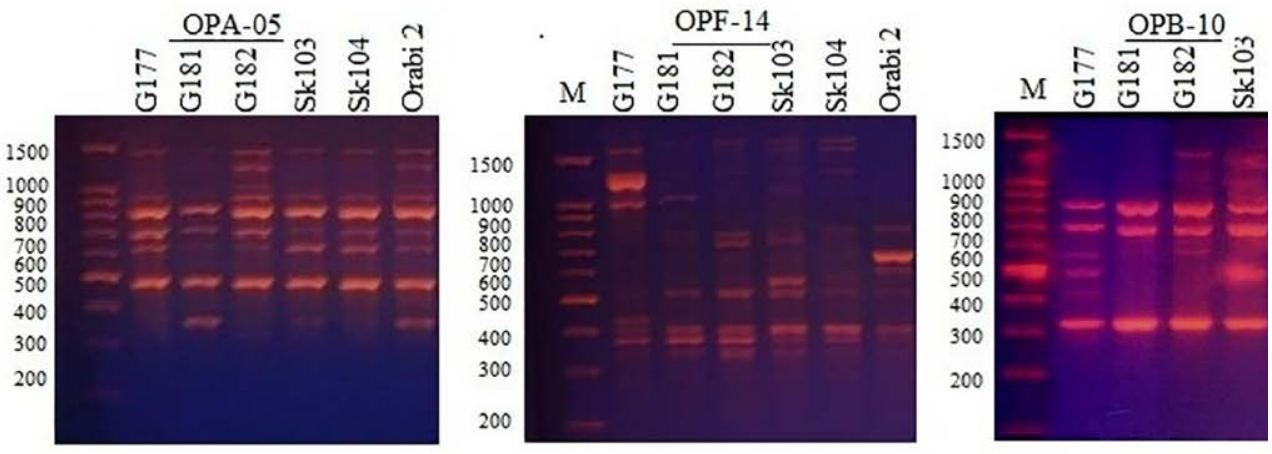

Figure 5. An example of DNA polymorphism of six rice genotypes amplified by some RAPD primers (OPA-05, OPF-10 and OPB-10) where: M - 100bp DNA marker.

Rate of electrolyte leakage and MDA content

Analysis of the rate of electrolyte leakage (EL) and MDA content for all genotypes shown in (Figure 4 A and B) showed dramatically increase in EL and MDA content of G177 and G181 seedlings with increasing water stress. Trends of increasing in EL and MDA values of G182, SK103 and SK104 genotypes along with stress were less than that of G177 and G181. On the other hand, seedlings of Orabi2 showed semi constant values for both EL and MDA contents along with increasing water stress. Considering that Lipid peroxidation is a biochemical marker for the free radical mediated injury where it reported to be increased under sever water stress condition (Verma and Dubey, 2003 and Baisak *et al.,* 1994). Lower values of electrolyte leakage and MDA in genotype Orabi2 than other genotypes indicated that Orabi2 is better equipped with efficient free radical quenching system that offers protection against oxidative stress. Thus the enhancement of cellular membrane stability may contribute to the increase of stress tolerance in rice.

RAPD analysis of studied genotypes

The use of RAPDs for comparative purpose relies on the assumption that similarity of fragment size is a dependable indicator of homology (Rieseberg, 1996). The twelve RAPD-PCR Primers that were used to determine the genetic diversity of genotypes under study selected for their association with water stress tolerant genotypes from some previous studies (Youssef *et al.,* 2010; Ullah *et al.,* 2013). RAPD marker profiles generated in all studied genotypes by OPA-05, OPF-14 and OPb-10 random primer as examples are shown in Figure 5. The total numbers of amplified bands produced by 12 RAPD primers were 136 bands. The size of generated bands ranged from 222 to 1705 bp and the average of amplified bands per primer was 11.33. The primers OPA-01, OPC-05, OPA-05 and OPB-07 presented the highest percentage of RAPD polymorphism (62.5%, 57.55%, 55.55% and 55.55 % respectively. While OPB-10, OPC-02 and OPL-03 primers presented the lowest percentage of RAPD polymorphism (20%, 23.07 and 27.27%, respectively (Table 4). Unique bands generated by OPB-10 primer with tolerant (Orabi2) and moderate genotypes (SK103 and ,SK104) presented in Figure (5) can be considered as potential markers to identify drought tolerant lines or may even be more useful when converted into a simple-sequence PCR based marker that can be used for large-scale drought tolerance screening of cultivars. The similarity coefficient values among all genotypes based on band polymorphisms generated by RAPD-PCR (Table 5) indicated that the highest similarity value (0.77) was found between G181 and G182 and the lowest value (0.43) was found between Orabi 2 and G177. Cluster analyses using UPGMA method were used to group studied genotypes and construct a dendrogram based on RAPD markers (Figure 6). The resulted dendrogram separated the 6 genotypes into 3 main clusters. The first cluster contained only Orabi 2 genotype which was the most physiologically distinct with its tolerant performance under drought condition during the experiment. The second group divided to two sub-cluster, first sub-cluster had two genotypes SK103 and SK104 which showed moderate drought tolerance. And the second sub-cluster includes the most drought sensitive genotype G177 in recent study. This relationship is indicative of plant derived from interspecific hybridization (Marsolais *et al.,* 1993) where G177 is one of SK103 parent (Table 1). The third cluster contained two genotypes G181 and G182 genotypes. Orabi 2 is the most distinct genotype as a genetic background and physiological response to water stress, was separated from all the remaining studied genotypes suggesting that some of the genotype-specific RAPD markers could possibly be associated with drought responses. Similar separation of tolerant cultivars using cluster analysis occurred in indian rice cultivars under salt stress (Kanawapee *et al.,* 2011). Based on the results of the similarity coefficient and UPGMA cluster analysis of studied

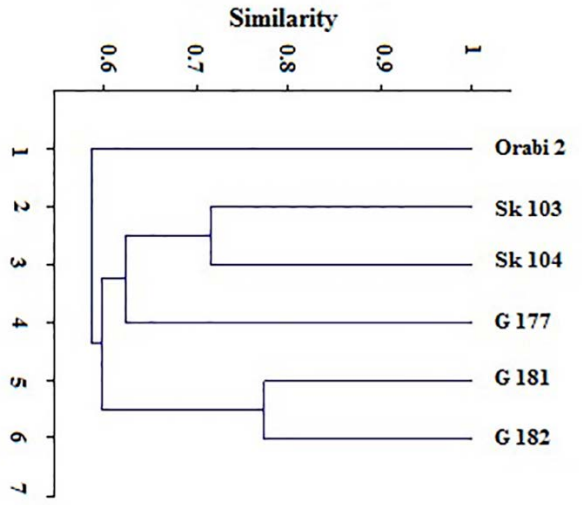

Figure 6. UPGMA dendrogram representing the genetic relationships among the six studied genotypes based on 12 RAPD primers.

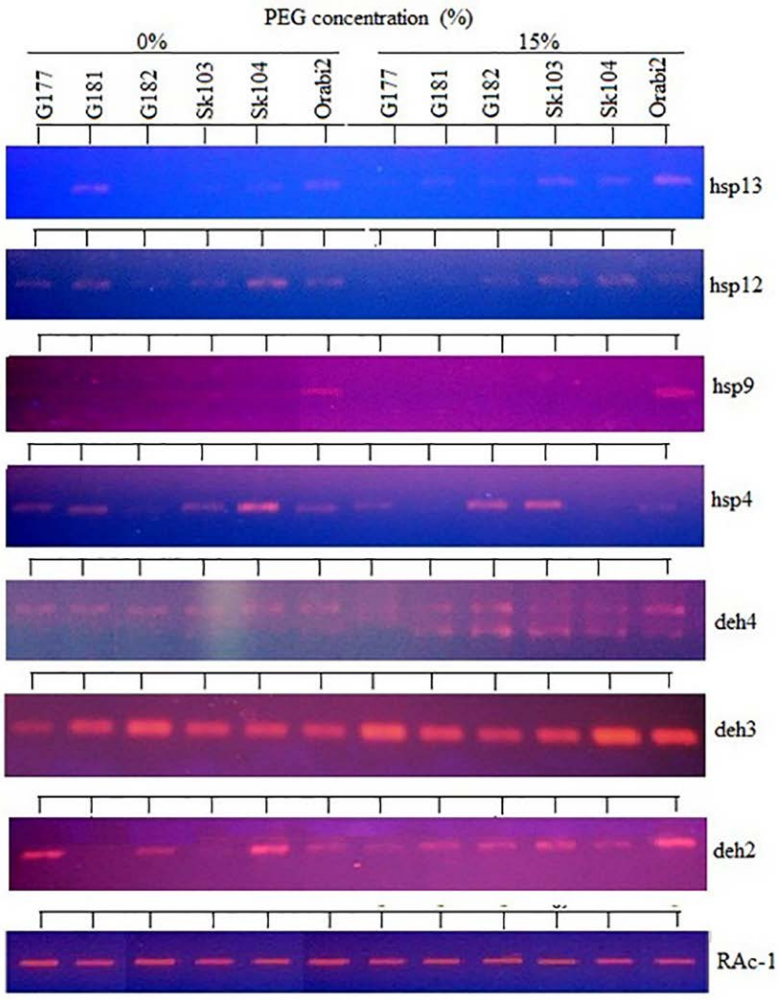

Figure 7. Semi quantitative RT-PCR analysis of transcription pattern of DHNs and HSPs genes of studied genotypes under control and drought induced conditions. Transcripts were amplified from cDNA samples standardized on actin transcript amount. The number of amplification cycles was optimized for each gene (23 cycles for *actin*, 33 cycles for *DHN-2* and *DHN-4*, 25 cycles for *DHN-3*, 26 cycles for *HSP-4* and *HSP-12*, 30 cycles for *HSP-9* and 33cycle for *HSP-13*) to yield optimal contrast between samples in EtBr-agarose gel electrophoresis.

genotype indicated that drought tolerant and sensitive genotypes were dissimilar to each other. The results were in covenant with their response to drought stress. Our results were in good harmony with Naceur et al. (2012), Mariey et al. (2013), Khatab and Mariey (2013) and El-Akhader et al. (2016).

Semi Quantitative analysis of some specific genes

Semi quantitative analysis of some selected genes showed a wide range of expression pattern between studied genotypes under both control and PEG stress treatments. Transcription profiles shown in Figure 7 illustrated that the transcript amounts of *HSP-9* were undetectable at all sensitive and moderate tolerant genotypes G177, G181, G182, SK103 and SK104 in both control and PEG treated seedlings. While it was detected on Orabi 2 genotype in control and PEG treated seedlings with a noticeable increase in the transcript amount under PEG treatment. Absence of any transcript for *HSP-13* and *DHN-2* noticed in all sensitive genotypes under controlled condition, while week appearance of transcripts of both genes were recorded in SK104 and Orabi2 genotypes. PEG treatment induced a week expression of *HSP-13* and *DHN-2* in G181, G182 and SK103 and noticeable increase in transcript level in Sk104. High expression of *DHN-2* induced as a result of PEG treatment in Orabi2 treated seedlings. Among all studied genotypes, Sk104 and Orabi2 showed the highest amount of *HSP-12* transcripts under control condition. PEG (15%) treatment induced losing of expression of *HSP-12* transcript in G177 and G181 seedlings, while sharp increase occurred as a response to PEG treatment at Orabi2 seedling. Losing the expression of some genes after PEG treatment occurred with *HSP-4* transcript at G181, SK104 and Orabi2. The amount of *DHN-3* and *DHN-4* transcripts showed similar patterns among all studied genotypes under controlled and stressed condition. Considering the expression pattern of these genes and performance of G177 as a sensitive genotype and Orabi2 as tolerant genotype we conclude that tolerance of water stress in Orabi2 genotype was accompanied with induced expression of *HSP-13*, *HSP-9*, *DHN-4*, *DHN-3* and *DHN-2*, while bad performance of G177 genotype under stress condition was accompanied with absence or losing the expression of *HSP*-12, *HSP*-9, *HSP*-4, *DHN-4* and *DHN-2*. Considering this pattern of genes expression and the role of *HSPs* in enhancing cellular membrane stability in transgenic plants under stresses (Ahn and Zimmerman, 2006; Wang *et al.*, 2005), and its function as a "membrane stabilizing factor" by increasing the membrane physical order to protect membranes from stress damage (Torok *et al.*, 2001; Tsvetkova *et al.*, 2002, Fedoroff, 2006; Harndahl *et al.*, 1999; Omar *et al.*, 2011). The presented result may propose that expression of *HSP-13*, *HSP-12*, *HSP-9* and *DHN-2* in seedlings of drought tolerant genotypes in controlled condition and increasing their transcripts level under stressed condition may play a role in acquisition of drought tolerance in tolerant and moderate tolerant genotypes. Kumar et al (2014) reported that transgenic rice plants over expressing *OsDhn1* showed higher tolerance to drought and salt stress probably via ROS scavenging and reducing the oxidative damage, and confirmed that *OsDhn1* is an important gene for drought and salt stress-tolerance in rice. Because of most DHN's functions as molecular chaperons. Considering that drought and salt are the most significant factor limiting world agricultural production. Based on the evidence that drought resistance is an inducible process, identification of drought responsive genes from tolerant rice genotypes comparing with sensitive ones may help to further clone drought resistant genes in plants. The constitutive expression of sHSP was proposed that sHSP may cause enhancement of cellular membrane stability and contribute to the increase of stress tolerance in rice.

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