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Differential Responses of some Rice Genotypes to Drought Stress at Different Growth Stages

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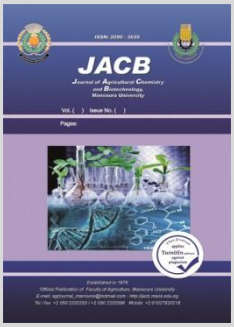

ABSTRACT

Water deficiency is one of the major abiotic stresses which adversely affect rice production. Four rice genotypes; Giza177, IR64 (sensitive), Vandana and Orabi3 (tolerant) were used to study the mechanics by which rice genotypes acquire the drought tolerance. Shoot and root lengths, fresh and dry weights, cell membranes stability as lipid peroxidation as MDA content and rate of electrolyte leakage (EL) were measured as indices for drought tolerance. Total protein analysis and differential display RT-PCR were carried out at different growth stages to study the response of the tested genotypes to drought stress. All determinations were carried out under both normal irrigation (control) and drought stress conditions. Our results indicated that drought tolerant genotypes showed different response through DD-RT-PCR than sensitive genotypes. Changes in protein profile as increasing in band number and intensity were associated with the up regulation or even induction of some RNA transcript in tolerant genotypes. Our data conclude that vegetative growth stage was the most stable under drought stress of all studied genotypes. Analysis of induced RNA followed by DD-RT-PCR to characterize genes may involve in drought tolerance mechanism need further investigation.

Keywords: Rice genotypes, drought stress, lipid peroxidation, electrolyte leakage, DD-RT-PCR.

INTRODUCTION

Rice is an essential food; it supplies more than three billion people (about 65%) of their daily calorie intake (Khush, 2005). Rice production must be increased 60% to meet the expected consumption by the year 2025 (Fageria, 2007). About 30% of freshwater for agricultural crops worldwide was used for rice cultivation. Rice uses water two to five times more than other crops such as maize or wheat. In Egypt, the competition among the limited water resources could escalate due to the large and tightly packed population. In addition to impacts of vulnerable climate changes such as increased warming and drought; subsequently the reduction in the Nile flow would further worsen Egypt's problems (Abdalla and Li, 2015).

Under water deficit, plant acts as a survival technique by stopping their growth; subsequently reducing their yield, this is a normal plant reaction to lack of water (Zhu, 2002; Manikavelu *et al*., 2006). Water deficit affects plant photosynthesis, morphological, physiological, biochemical, molecular and yield (Yang *et al.*, 2014). Membrane stability and root characters will affect by water deficiency (Gowda *et al.*, 2011; Kumar *et al.*, 2014). Water stress also changes the expression pattern of many genes as a response of control plant reaction (Omar *et al*., 2018).

This study aimed to detect the most suitable stages for water regime application and understand the mechanisms by which rice genotypes acquire the drought tolerance, by studying the differentially responses of four rice genotypes under water deficit conditions at three different developmental stages by used DD-RT-PCR, because this technique is less expensive as compared to other techniques like microarray for detecting differential gene expression (Huang *et al*., 2015).

MATERIALS AND METHODS

Plant Material and Growth Conditions

Four rice (*Oryza sativa* L.) genotypes shown in Table 1 were received from Rice Research and Training Center (RRTC) at Kafr El sheikh, the genotype Orabi3 obtained by Prof. Dr. Said Soliman, Genetics Dept., Fac. of Agric., Zagazig University. Seeds were germinated by incubation at 28ºC for three days.

Table 1. Rice genotypes; name, pedigree type and its water stress response

Genotype name	Pedigree	Water stress response	Type
Giza177	Giza 171 / Yomji No. 1 // Pi No. 4	Sensitive	*Japonica*
IR64	IR2061-465-1-5-5 × IR657-33-2-1	Sensitive	*Indica*
Vandana	C22 x Kalakeri	Tolerant	*Indica*
Orabi3	developed variety of rice by Dr. Said Soliman, Genetics Dept, Faculty of Agriculture, Zagazig University	Tolerant	*Indica*

The germinated seeds were then placed in polyvinyl chloride (pvc) pots (25 cm diameter and 35 cm height). Four seedlings per pot were maintained after seven days from planting in nine replicates, the pots were irrigated up to water holding capacity of soil. The water stress treatment begun after 10 days of planting. Soil water content (SWC) was calculated using the weight fraction:

SWC (%) = [(FW-DW)/DW]*100,

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Where FW was the fresh weight of a soil portion of the middle part of each pot and DW was the dry weight of the soil portion.

Soil drying was achieved in a hot air oven at 80 ºC for 48 h (Cha-um *et al*., 2012). Plant samples were collected at three growth stages: seedling, vegetative and flowering (20, 45 and 85 days after planting, respectively) with three replicate.

Plant weight and length

Shoots and roots weights and lengths were determined at three developmental stages (seedling, vegetative and flowering) for each genotype. Plant shoots were separated from roots for measuring the fresh and dry weights for each. Dry weight (DW) was measured after drying at 105˚C for 3 h.

Cell membrane stability index

Membrane stability was indicated by evaluation both of lipid peroxidation product and rate of membrane leakage. Lipid peroxidation was determined as the concentration of thiobarbituric acid (TBA) reactive products equated with malondialdehyde (MDA), as described by (Zedan and Omar, 2019). Rate of membrane leakage was determined by the estimation of electrolyte leakage (EL) using plant leaf. Electrolyte leakage of plants was determined using a conductivity meter (Adwa-AD32). Sample (about half gram) was placed in a glass tube containing 20 mL of de-ionized water with gently shaken, the conductivity of the solution was determined immediately (EL0). After 1 h; conductivity of the solution was measured once again (EL1). Each tube was placed in boiling water for 1 h, then sample was cooled upto room temperature and the total conductivity was determined (EL2). Leakage rate of electrolytes was calculated as [(EL1 – EL0) / (EL2 – EL0)] / weight of sample ($\mu S \cdot cm^{-1}.FW \cdot h^{-1}$) according to (Omar, *et al*., 2012).

Analysis of crude total protein

Half gram of each sample was ground to a fine powder in liquid nitrogen. Ground powder was homogenized in 1.0 ml of cold extraction buffer containing 100 mM Tris-Hcl PH 8, 2% SDS, 5 mM Nacl, 10mM 2-mercaptoethanol according to (Dure *et al* 1981). Total protein was extracted by centrifugation of the homogenate at 4˚C for 10 min at 8000 rpm. The supernatant was then transferred to 0.5 ml tubes in 50-100µl aliquots and stored at -20˚C. The concentration of extracted proteins was determined according to Bradford, (1976). SDS-PAGE was performed with a discontinuous buffer system according to Laemmli, (1970). Protein samples in 1× SDS gel loading buffer were denatured by heating at 95°C for 5 min before loading on to the gel. Samples standardized on protein amount (15 µg proteins) were loaded on the gel. BLUeye pre-stained molecular protein Ladder (GeneDirex) was used.

DD-RT-PCR analysis

Differential display reverse transcription polymerase chain reaction (DD-RT-PCR) was used to detected differential responses of studied genotypes at different developmental stages under drought stress (Manjesh *et al*., 2017). Total RNA was extracted from leaves of the studied rice genotypes using EZ-10 Spin Column Plant RNA Mini-Preps Kit (BIO BASIC CANADA INC). For quality control the obtained RNA, it was analyzed in 1 % agarose gel with RNase-free devices. The amount and the purity of RNA were carried out by using NanoDrop (BioDrop µLITE.UK). First-strand cDNA was synthetized by using 5 µg of total extracted RNA according to the protocol supported by GoScript ™ reverse transcription Kit using Oligo $(dT)^{15}$ primer. The second strand of c-DNA was synthetized and PCR amplification reactions were carried out in 25 µl reaction volume according to the pamphlet of GoTaq® Green master Mix, 2x (Promega). One µl of the first strand cDNA mix and 2µL of RAPD Primers (OPB-10: 5'-CTG CTG GGA C -3' ; OPD-07: 5'-TTG GCA CGG G-3' ; OPB-07: 5'-GGT GAC GCA G-3' ; OPF-14: 5'-TGC TGC AGG T-3' ; OPA-10: 5'-GTG ATC GCA G -3') were added. The amplification runs through four min (hold) at 94°C and then 40 cycles of (1 min at 94°C, 2 min at 32 ˚C and 36˚C, 1 min at 72°C) followed by a final extension at 72°C for 5 min (hold) in the thermal cycler (MyGene ®–MG96G). About 25 µl of PCR amplified product were loaded into 2% agarose gel supplemented with ethidium bromide.

Data analysis and phylogenetic tree construction

Separated bands were scored and analyzed based on the presence and absence of bands (0 and 1) using the PAST program, version 1.90. The genetic similarity (based on Jaccord`s formula) was detected to establish genetic relationships among the investigated genotypes based on unweighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal, 1973), using Past software (version 2.17) designed by Hammer *et al*., (2001).

Statistical analysis

The result data are presented as means ± standard errors (n=3). The data were analyzed by Tow-way ANOVA using IBM® SPSS® Statistics program version 22 (SPSS inc., il, USA) for windows at $P<0.05$ level to test the effects of irrigation and dry regime and genotype, as well as their interactions according to the following model:

$$Y_{ijk} = u + I_i + G_j + IG_{ij} + E_{ijk}$$

Where, u is the overall mean, I_i is the fixed effect of i^{th} irrigation and dry regime, G_j is the fixed effect of j^{th} plant genotype, IG_{ij} is the interaction effect and E_{ijk} is the random error. Means were tested for significant differences using Duncan's multiple range test.

RESULTS AND DISCUSSION

Shoot and root length

Presented data shows that the root length and its spread of Orabi3 and Vandana were longer compared to G177 and IR64 under control (Figure, 1). Under drought stress (as mentioned in material and method) series changes among the studied genotypes were observed (Figure, 2). All studied genotypes showed a significant decrease in shoot length under drought stress conditions comparing with control at the three studied growth stages (Figure, 2 A,B,C). At seedling stage; the reduction in root length under drought stress condition was not significant in all studied genotypes, except for Vandana seedlings which showed a significant increase in their root length about all genotypes and their control (Figure, 2D). At vegetative growth stage, there were significant decreases in root length under drought stress for all genotypes except Giza177 which showed a significant increase in their root length about their control (Figure, 2E). Drought treatment induced significant reductions in root length of Orabi3 and IR64 at flowering growth stage, while Vandana and G177 showed increasing in root length and this increasing was significant in Vandana genotype (Figure 2F). It has been affirmation that drought stress reduces

growth which reflected in biomass, plant height and other growth functions (Fischer, 1980; Kriedemann and Barrs, 1981). Reduction in development and plant growth of rice was recorded as a result of drought stress (Tripathy *et al*., 2000 and Manikavelu *et al*., 2006). Drought affects both expansion growth as well as elongation (Shao *et al*, 2008), drought inhibits cell enlargement more than cell division (Jaleel *et al*., 2009). Root length is a good indicator of rice production under drought (Fageria and Moreira, 2011 and Feng *et al*., 2012). Faster and more spread root growth is important for plant survival in complex soil conditions (De Dorlodot *et al*., 2007), our results showed that among all studied genotypes, Vandana genotype as tolerant one owned a largest and more speared root system (Figure,1) these results are agree with many studies that approved the increase in root growth as an index of the ability of plants to tolerant the drought stress for tolerant genotypes (Franco *et al*., 2006 and 2011). Greater root systems typically enable plants to extract more nutrients and water from the soil (King *et al*., 2003). Numerous studies identified many root characters that provide drought resistance. Rice genotypes that have coarse, deep roots with a high ability of penetration and branching and higher root to shoot ratio are reported as component characters of drought tolerance (Samson *et al*, 2002; Gowda *et al*, 2011). Deep rooting considered is a target trait in rice development programs under drought stress (Gowda *et al*, 2011).

Figure 1. Changes in shoot and root lengths and their spread of two genotypes (Vandana & Giza177) under control and drought stress conditions at seedling stage, as an example of obtained data.

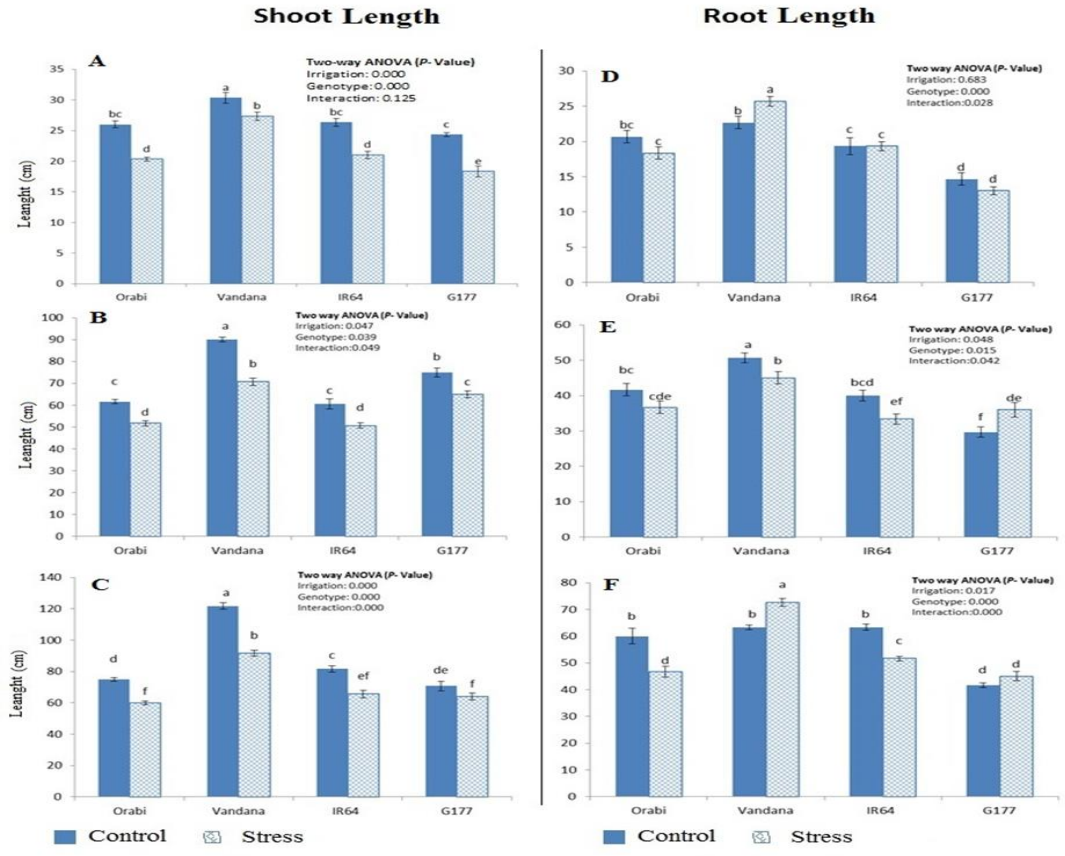

Figure 2. Changes in shoot/root lengths of all genotypes at different growth stages (seedling stage, A&D - vegetative stage, B & E and flowering stage C&F) under control and drought stress conditions.

Fresh and dry weights

Results are shown in (Figure 3) indicated changes in shoot fresh weight (FW) and dry weight (DW) at all growth stages. At seedling stage (Figures 3A and D), all studied genotypes showed a significant decrease in shoot FW and DW under drought conditions compared with control conditions, except for Orabi3 which showed non-significant reduction in DW. At vegetative growth stage (Figures, 3 B and E) all genotypes showed non-significant changes in FW, except for Vandana genotype which showed significant decrease in FW compared with control. On the other hand, all genotypes showed significant decrease in DW under drought conditions compared with control conditions. Results at flowering growth stage (Figures, 3 C and F) showed a significant decrease in FW for G177 while the other genotypes showed non-significant changes. About DW both of Orabi3 and Vandana genotypes showed non-significant decrease in their DW values, while G177 and IR64 showed a significant decrease in DW under drought condition compared with control condition. About root fresh and dry weights our results (Figures, 3 G, H, I, J, K and L) indicated that; at the seedling growth stage (Figures, 3 G and J) all studied genotypes showed a significant decrease in root FW and non-significant decrease in root DW under drought conditions compared with control conditions. At vegetative growth stage, there were non-significant changes in FW and DW for Orabi3 and G177 genotypes, while Vandana and IR64 genotypes showed significant decrease in FW and DW under drought conditions compared with control conditions (Figure, 3 H and K). At flowering growth stage (Figures, 3 I and L) both of Orabi3 and Vandana genotypes showed non-significant changes in FW, while the reduction in FW values of IR64 and G177 genotypes were recorded a high values decrease. All genotypes showed non-significant changes in root's DW under drought condition compared with control conditions. Finally, Orabi3 and Vandana genotypes showed more stable values of FW and DW for shoots and roots under drought condition at flowering stage which is one of the most important stages. Previous studies indicated significant decrease in dry and fresh weights of roots and shoots under drought stress (Centritto *et al*., 2009; Mostajeran and Rahimi-Eichi, 2009; Ji *et al*., 2012). Reduction of fresh weights of roots and shoots as well as their lengths ultimately reduction of the photosynthetic rate of biochemical and physiology processes of rice (Usman *et al*, 2013). The genotype which has ability to keep high value for fresh weight has the ability to maintain tissue water content and avoid the drought induced damages (Abdel-Nasser and Abdel-Aal, 2002). Genotype which has keeping high value of dry weight point to its ability to maintain photosynthesis process under drought stress conditions (Werner *et al*, 2001). Root dry mass is a good indicator of rice production under drought condition (Fageria and Moreira, 2011 and Feng *et al*, 2012).

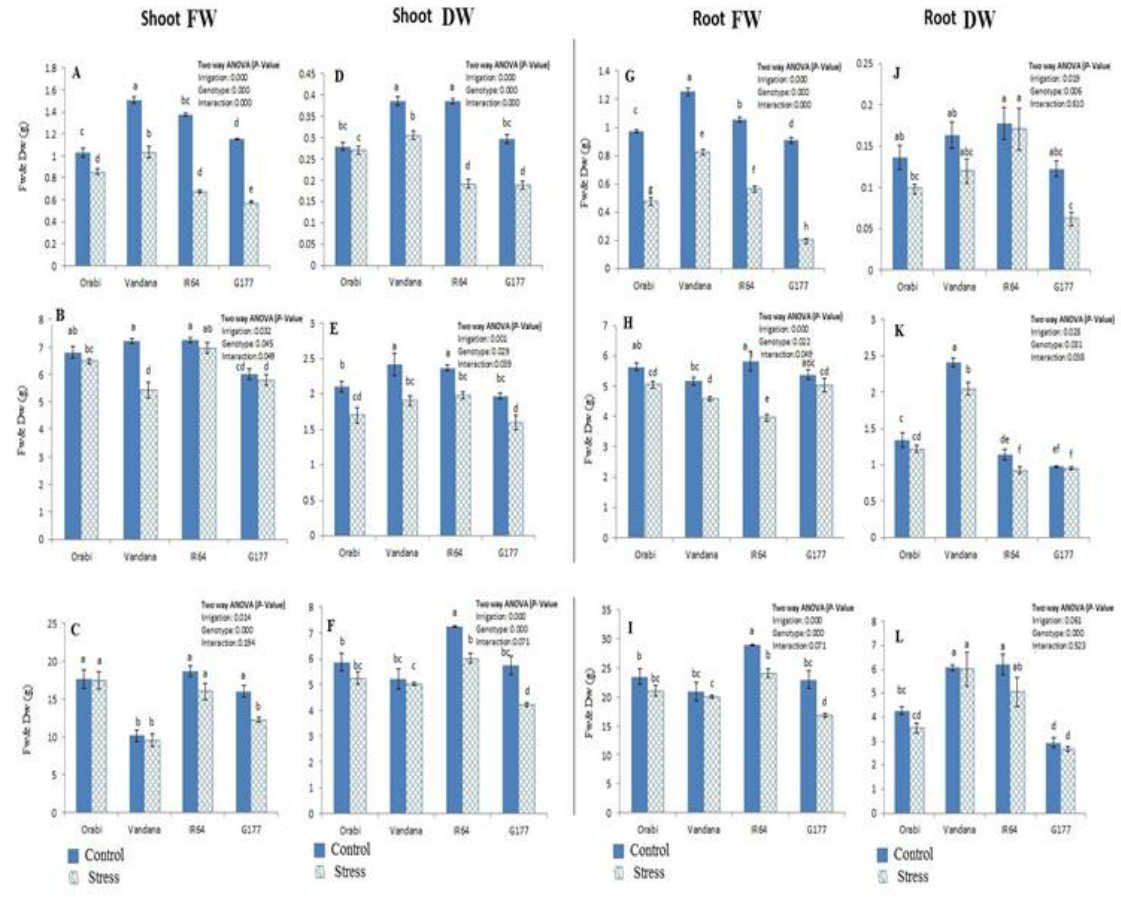

Figure 3. Changes in shoot/root fresh and dry weights of all genotypes at different growth stages (A, D,G, J-seedling stage: B, E, H and K vegetative stage: C, F, I and L-flowering stage) under control and drought stress conditions.

Rate of electrolyte leakage and malondialdehyde MDA content

Changes in malondialdehyde (MDA) content and electrolyte leakage rate (EL) are shown in (Figure 4). At the seedling growth stage, MDA content showed a significant increase under drought stress in both of Orabi3, Vandana and G177 genotypes while these changes were non-significant in IR64 genotype. In relation to EL value there are no significant change in all genotypes except G177 genotype, it showed a significant differences about control and other genotypes. (Figures 4 A and D). At vegetative and flowering growth stages, although, changes in MDA contents under drought were significant in all studied genotypes, but these increase were not enough to induce significant changes in EL at all studied genotypes, except for G177 which showed a significant increase in EL under drought stress at flowering growth stage (Figures 4 B, C, E and F). At vegetative stage, all genotypes showed non-significant changes in EL, this indicates that vegetative stage is more tolerant than other stages of both tolerant and sensitive genotypes (Figure 4 E). Lower values of EL and MDA indicate that genotypes are better equipping with efficient free radical quenching system that offers protection against oxidative stress. Sharma *et al*, (2012) reported that tolerant genotypes to drought stress have higher antioxidants activity and lower electrolyte leakage and MDA content. The cellular membranes constitute a control site of activity for many cellular processes. Furthermore, cellular membranes are subjected to change, usually with loss of integrity and increase in permeability under drought stress (Blokhina *et al*., 2000). Cell membranes are the prime targets of drought stress and due to the acquired tissue water stress, it gets extend and disrupted, of this stress can be measured by electrolyte leakage from the affected tissue (Chaturvedi *et al*., 2012).

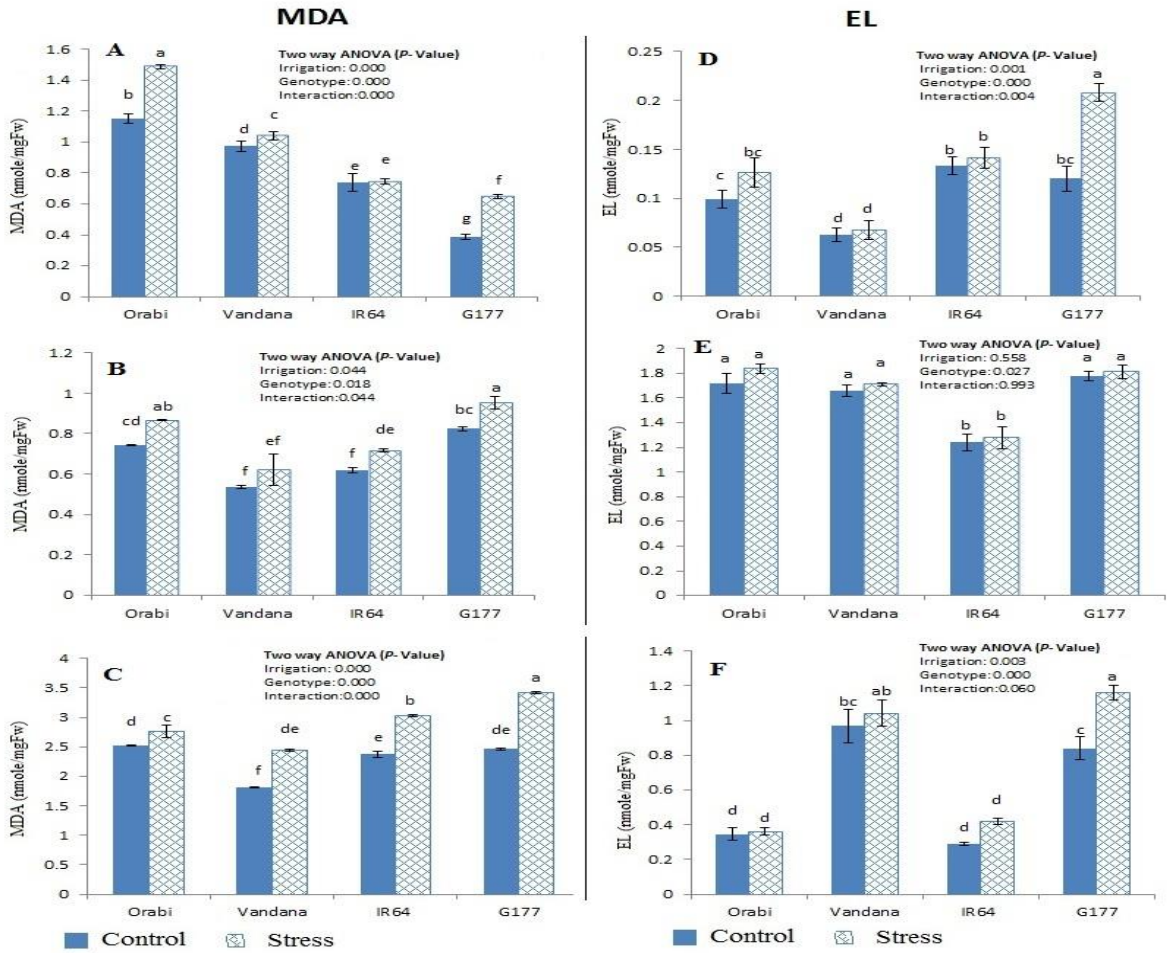

Figure 4. Changes in malondialdehyde (MDA) and (EL) electrolyte leakage of all genotypes at different stages (A-seedling stage , B-vegetative stage, C-flowering stage) under control and drought stress conditions.

Analysis of total protein

Electrophoresis analysis of total proteins fractions from shoots and roots of studied genotypes under control and drought stress condition showed series of changes. An example of changes protein pattern are shown in Figure (5). Figure (5A) showed an increased expression of some protein bands in shoot of all genotypes under drought stress (pointed with arrows), except for Orabi3 seedlings which showed reduction in the expression of protein bands with molecular weight of approximately (100 – 180 KDa). Changes in protein banding pattern of roots were clearly detected compared with changes in protein banding pattern in shoots (Figure 5B). Other changes in protein pattern were detected in the rate of expression as bands intensity under drought stress for all genotypes in different growth stages.

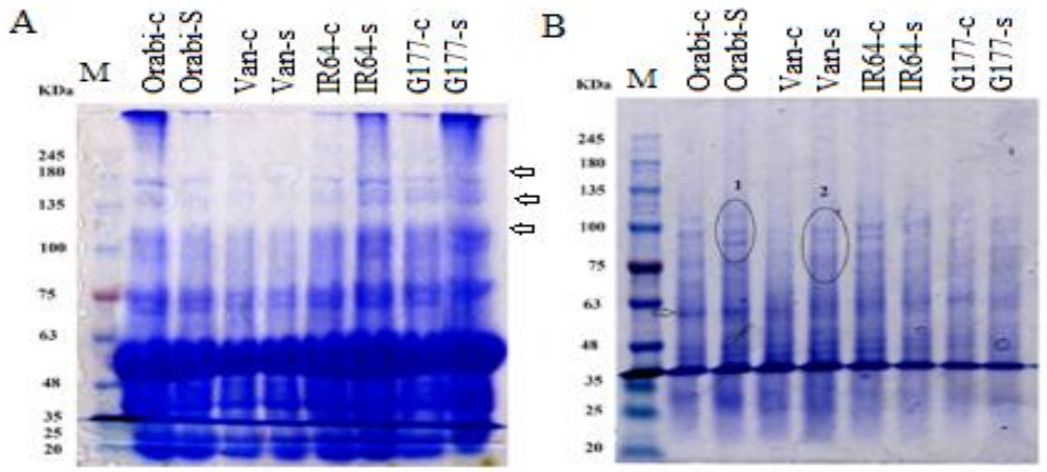

Figure 5. SDS-PAGE analysis of total protein extracted from shoot and root of studied genotypes under control and drought stress conditions at seedling stage (A- shoot, B- root).

Data are shown in the Table (2) summarize all changes in induction and inhibition of protein bands as a number of migrated bands under control and drought conditions. Data showed that Orabi3 genotype has a new band under drought stress, while G177 genotype showed reduction in the total number of bands under drought stress (Table 2). Induction of new protein fractions or increasing the expression level of induced proteins helps in maintaining of tissue water status and helps the plants to avoid the dehydration and protect enzymes from inactivation and denaturation (Passioura and Stirzaker, 1993). Constitutive expression of small heat shock proteins in transgenic plants can enhanced cellular membrane stability under drought stresse (Ahn and Zimmerman, 2006 and Wang *et al.*, 2005).

Table 2. Changes in protein bands numbers for all studied genotypes under control and drought stress conditions at different developmental stages

Developmental stage	Orabi 3			Vandana			IR64			G177		
	Total no .of bands		of% changes in bands No.	Total no. of bands		of% changes in bands No.	Total no. of bands		% of changes in bands No.	Total no. of bands		of % changes in bands No.
	C	S		C	S		C	S		C	S	
Seedling	9	10	11.1+	6	9	50+	10	7	30-	7	6	14.2-
Vegetative	4	7	75+	10	7	30-	10	9	10-	8	7	12.5-
Flowering	4	5	25+	7	6	14.3-	7	8	14.2+	10	7	30-

***% of increasing (+) or reduction (-) in band No**

Differential display reverse transcription polymerase chain reaction (DD-RT-PCR)

In DD-RT-PCR cDNA was used as template instead of genomic DNA so that cDNA-RAPD can detect the differential expression of genes. DD-RT-PCR analysis showed differential changes in RNA transcription in all studied genotypes at two growth stages (seedling and flowering) under control and drought stress conditions. The changes were shown as different amplified PCR bands on the agarose gels. Band density pointed to the level of expression for the same gene, while different band and bands number indicate to different gene expression. Bands pattern in figure 6 of obtained data (for example) showed that these changes were potentially differential up regulated as a genotype response to drought stress condition (indicated by circular zone). Some cDNAs bands showed a down regulation as a response to drought stress (indicated by rectangle) as shown in Figure (6A). Under drought stress conditions some genotypes showed new cDNA bands as plant responses to water deficit (indicated by arrows).

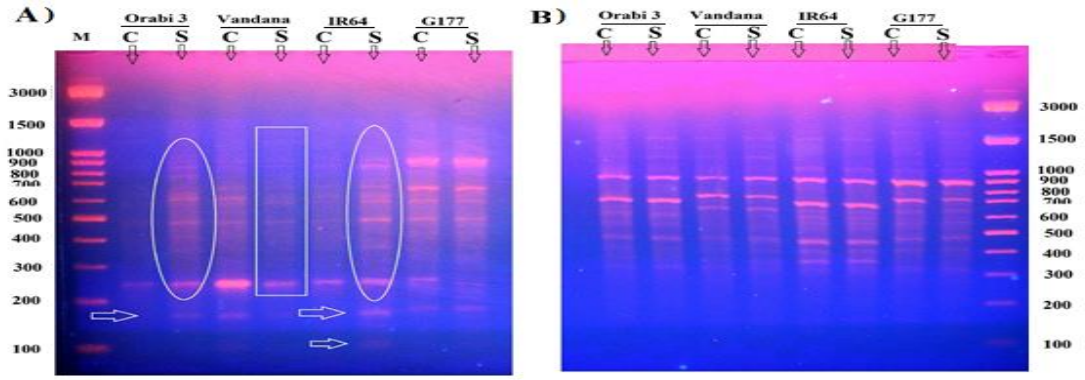

Figure 6. Differential display pattern of transcript derived fragments from all genotypes under control and stress conditions at seedling stage using primer OPD-07 (A) (A) and flowering stage using primer OPB-07 (B) as an example of obtained data.

Changing in banding pattern indicate to differential responses under stress conditions. Summarizing of total changes in induction and inhibition of DD-RT-PCR banding pattern as a number of migrated bands was shown in Table 3. Plants respond and adapt to drought stress at the molecular levels. An various of genes with diverse functions are induced or repressed by drought stress (Shinozaki *et al*., 2003; Bartels and Sunkar, 2005 and Yamaguchi-Shinozaki and Shinozaki, 2005). Most of their gene products may role in stress response and tolerance at the cellular level. Significantly, the introduction of many stress-inducible genes via gene transfer resulted in improved plant stress tolerance (Zhang *et al*., 2004 and Umezawa *et al*., 2006).

Table 3. Changes in DD-RT-PCR pattern numbers during control and drought stress conditions, percentage of induced or inhibited bands numbers

stage	Band No. and % of increasing (+) and reduction(-) in band No.											
	Primer	Orabi3		%	Vandana		%	IR64		%	G177		%
		C	S		C	S		C	S		C	S	
Seedling	OPB-10	3	10	233+	5	5	0.0	4	11	175+	12	12	0.0
	OPD-07	9	12	33+	10	12	20+	8	12	50+	15	11	26-
	OPB-07	1	7	600+	6	5	17-	5	8	60+	7	8	14+
	OPF-14	0	2	200+	3	2	33-	0	3	300+	4	8	100+
	OPA-10	7	7	0.0	7	8	14+	1	9	800+	12	10	16-
	Total	20	38	90+	31	32	3.2+	18	43	138+	50	49	2-
Flowering	OPB-10	3	6	100+	5	9	80+	7	3	57-	10	10	0.0
	OPD-07	6	6	0.0	4	5	25+	7	7	0.0	6	7	16+
	OPB-07	8	8	0.0	8	8	0.0	8	8	0.0	8	9	12+
	OPF-14	2	2	0.0	2	2	0.0	3	5	66+	3	4	33+
	OPA-10	8	8	0.0	7	7	0.0	11	10	9-	9	10	11+
	Total	27	30	11+	26	31	19+	36	33	8-	36	40	11+

The genetic similarity (GS) among tested genotypes based on the polymorphisms of their DD-RT-PCR pattern (Table 4) showed similarity between all genotypes at two growth stages (seedling and flowering) under control and drought stress conditions.

Table 4. Similarity and distance indices among four rice genotypes based on five RAPD Primers

			Orabi	Vandana	IR64	Giza177
Seedling stage	Control	Orabi	1	0.35135	0.28571	0.32692
		Vandana		1	0.41176	0.58824
		IR64			1	0.26415
		Giza177				1
			Orabi	Vandana	IR64	Giza177
	Stress	Orabi S	1	0.62791	0.73913	0.58182
		Vandana S		1	0.6087	0.55769
		IR64 S			1	0.65455
		Giza177 S				1
			Orabi3 C	Vandana	IR64	Giza177
Flowering stage	Control	Orabi3	1	0.65625	0.65789	0.53659
		Vandana		1	0.58974	0.67568
		IR64			1	0.63636
		Giza177				1
			Orabi3	Vandana	IR64	Giza177
	Stress	Orabi3	1	0.69444	0.61538	0.52174
		Vandana		1	0.52381	0.69048
		IR64			1	0.58696
		Giza177				1

Figure (7) showed the constructed phylogenetic dendrograms of studied genotypes under control and drought stress conditions in both seedling and flowering growth stages. Genotypes at the seedling growth stage under control conditions were separated into three groups; group (I) clustered Giza177 and Vandana genotypes at the same sub-clade which showed the highest similarity (0.58); group (II) contained only Orabi3 genotype which clustered separately; group (III) clustered contained only IR64 genotype. On the other hand, under drought stress conditions Orabi3 and IR64 genotypes were clustered in same sub-clade of group I which showed the highest similarity (0.73). Group (II) contained only Vandana genotype which clustered separately; group (III) contained only Giza177 genotype. At flowering growth stage plants under control conditions separated into two groups; group (I) clustered Giza177 and Vandana genotypes at the same sub-clade which showed the highest similarity (0.67); group (II) clustered Orabi3 and IR64 genotypes at the same sub-clade which showed similarity (0.65).

Plants under drought stress conditions separated into three groups; group (I) clustered Orabi3 and Vandana genotypes at the same sub-clade which showed the highest similarity (0.69); group (II) contained only Giza177 genotype which clustered separately; group (III) clustered contained only IR64 genotype. Although the rate of similarity was high between tolerant genotype (Orabi3) and sensitive one (IR64) at seedling stage under drought stress condition, the flowering stage showed a high similarity between both of Orabi3 and

Vandana as tolerant genotypes and this is consistent with the results of the production and yield character for both of Orabi3 and Vandana genotypes under drought stress (Unpublished data). Drought tolerance is considered a quantitative trait, involving the participation of a complex set of genes. When drought stress is perceived by plant, changes in pattern have been monitored, ranging from genes whose products are involved in early response such as, signal transduction, transcription and translation factors; to late response genes, such as water transport, osmotic balance, oxidative stress and damage repair (Ramanjulu and Bartels, 2002 and Shinozaki and Yamaguchi-Shinozaki, 2000; Knight and Knight, 2001 and Zhu, 2001). The products of stress inducible genes identified in rice can classified into functional proteins and regulatory proteins (Rabbani *et al*., 2003). Some of these genes in rice have been found to protect plants from desiccation through stress perception, signal transduction, transcriptional regulatory networks in cellular responses or tolerance to dehydration (Wang *et al*, 2005).

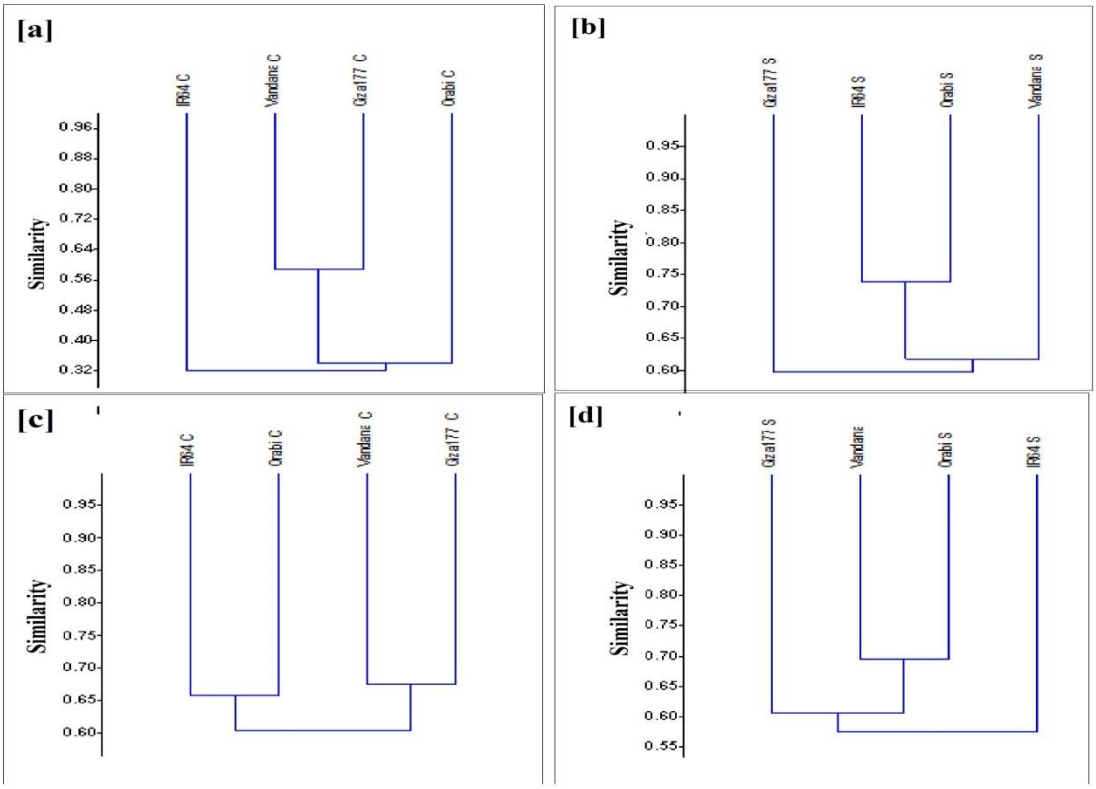

Figure 7. UPGMA phylogenetic dendrogram representing the genetic distance for all genotypes based on differential display pattern of transcript derived fragments at seedling stage (a control and b stress) and flowering stage (c control and d stress)

Our result revealed that vegetative growth stage was the most stable one under drought stress conditions of all studied genotypes therefore, irrigation water can be reduced at this stage without significant effect on plants. Orabi3 and Vandana genotypes showed more stable under drought stress conditions at flowering growth stage, which is one of the most important growth stages. Changing in banding pattern indicate to differential responses under stress condition. Considering differential changes at different developmental stages between tolerant and sensitive genotypes at our future work could detect the important of functional, structural or signal induced proteins involved in the adaptation to water deficit, identifing the differentially expressed genes remind as apoint of more detailed study in our further work.

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الاستجابات المختلفة لبعض التراكيب الوراثية للأرز لإجهاد الجفاف في مراحل النمو المختلفة

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يعد نقص المياه أحد أهم الإجهادات الغيرحيوية التي تؤثر سلبًا على نبات الأرز. تهدف الدراسة الحالية إلى فهم الآليات التي يكتسب بها نبات الأرز تحمُّل الجفاف من خلال دراسة وتوضيح الإستجابات المختلفة للتراكيب الوراثية للأرز (الحساسة والمتحملة لنقص المياه) في ثلاث مراحل نمو مختلفة: مرحلة البادرة والمرحلة الخضرية ومرحلة الأزهار. وكانت التراكيب الوراثية المدروسة هي Giza177 & IR64 (تراكيب وراثية حساسة) و Orabi3 &Vandana (تراكيب وراثية متحملة). تضمنت الدراسه تحديد الوزن الطازج والجاف ، وطول المجموع الخضري و الجذري ، ودرجة ثبات وتحمل الأغشية الخلوية من خلال معدل الإرتشاح (EL) ، وأكسدة الدهون من خلال تقدير MDA. كما تم تقدير البروتين الكلي كما" ونوعا". أيضا" تم استخدام تقنية DD-RT-PCR لتقدير التغييرات في نسخ الحمض النووي الريبوزي وذلك باستخدام شريط الـ c-DNA كقالب بدل من الـ DNA في تفاعل الـ PCR لمعرفه إستجابة التراكيب الوراثيه المستخدمه في هذه الدراسة. تم إجراء جميع القياسات للنباتات محل الدراسه تحت ظروف الري المعتاد وكذلك ظروف الجفاف. أظهرت النتائج التي تم الحصول عليها أن المرحلة الخضرية كانت الأكثر ثباتًا تحت ظروف الجفاف لجميع التراكيب الوراثية المدروسة المتحملة منها والحساسة. بينما تحت ظروف الجفاف ، أظهرت التراكيب الوراثية المتحملة (Orabi3 &Vandana) أقل تباين في قيم الوزن الطازج والجاف ، ومعدل الارتشاح ومحتوى الـ MDA. كانت التغييرات في نمط البروتين من حيث زيادة في عدد البروتينات الناتجة وكميتها مرتبطة بزيادة النسخ للحمض النووي الريبوزي في التراكيب الوراثية المتحملة. لذلك يمكن التوصية بالمزيد من الدراسه لتحليل تتابع النيوكليوتيدات لحزم الـcDNA المستحثه في الاصناف المتحمله للاجهاد لأن ذلك سوف يساعد في تحديد خصائص الجينات المرتبطة باكتساب تحمل الجفاف في نباتات الأرز.