Zagazig J. Agric. Res., Vol. 42 No. (4) 2015
http:/www.journals.zu.edu.eg/journalDisplay.aspx?JournalId=1&queryType=Master

GENOTYPIC DIFFERENCES IN ANTIOXIDANT ACTIVITIES AS A RESPONSE TO WATER STRESS IN TWO RICE GENOTYPES

Samar A. Omar*, M.H. Abdel-Fattah and M.E. Eldenary

Genet. Dept., Fac. Agric., Tanta Univ., Egypt

ABSTRACT

The present study aimed to investigate changes accompanied with acquisition of water stress tolerance in two rice varieties: Giza181 (G181) and Orabi 2.G181 and Orabi2 were selected as a sensitive and tolerant to drought stress respectively. The present investigation included determination of germination rate, fresh and dry weight, shoot and root length, electrolyte leakage, lipid peroxidation as MDA content, total protein analysis and native staining for superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) antioxidant enzymes. All determinations were carried out for both studied genotypes under control and water stress conditions. The obtained results revealed that tolerance of Orabi2 seedlings to water stress was accompanied with decreasing electrolyte leakage and low content of lipid peroxidation product (MDA). That indicates the perfect scavenging for reactive oxygen species associated with induced water stress. All stained SOD isoforms showed an increase in their activity along with water stress increasing in both studied genotypes. Participation of CAT and APX isoforms under stress condition in Orabi2 seedlings and losing their activity in G181 appears to be the main reason of water stress severe effects on G181 seedlings comparing with Orabi2 seedlings. CAT and APX appear to play a main role in scavenging H_2O_2 free radicals produced by increasing activity of SOD. Moreover some RAPD primers, indicated the presence of proliferate bands in the tolerant genotype, which were absent in the sensitive one.

Key words: Genotypic differences, antioxidant activity, water stress, rice genotypes, lipid peroxidation, electrolyte leakage.

INTRODUCTION

Rice is one of the most important food crops in the world. It makes up quality food for almost half of the world's population. In Egypt, Rice is one of the major water consuming crops and continuous flooding is the only method for irrigation. Rice occupies about 22% of the total cultivated area in Egypt during summer season and it consumes about 20% of the total water resources. Due to the limited water resources in Egypt, in addition to population increasing, the total water requirement for the rice crop is caused a problem (Aboulila, 2012). Recently, with water limitation we expect decrease in rice production. The cultivated varieties require irrigation water about 16500 m3/ha. As well as about 15.20% from rice areas was suffering a decreasing of yield due to short of water (Mahassen *et al.*, 1999). It is thus imperative to make the plants ready for the water deficit or drought. However, this all requires better understanding of the mechanism of tolerant and adaptation to drought (Farooq *et al.*, 2009).

Abiotic stresses are the major impediments restraining plant growth and resulting in significant reductions in crop productivity (Wang *et al.*, 2003). Plants respond to these adverse conditions by developing a series of physiological and biochemical strategies.

Drought stress leads to accumulation of reactive oxygen species (ROS), generated mostly in chloroplast and to some extend in mitochondria, causing oxidative stress. Major ROS molecules are single oxygen, superoxide anion radicals,

***Corresponding author: Tel. :** +201146210988
E-mail address: samar_omar5@yahoo.com

hydroxyl radicals and hydrogen peroxide (H_2O_2). ROS oxidize DNA, RNA, proteins and lipids and disturb plant cellular functions (Gill and Tuteja, 2010). Many stress related genes are induced under drought conditions, including functional proteins such as membrane proteins that maintain water movement through membranes. Transcription factors, Osmotic compounds, protective proteins and reactive oxygen species (ROS) scavengers, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and Ascorbate peroxidase (APX) have the ability to protect cells from stress-induced damage (Thomashow, 1999; Shinozaki *et al.*, 2003). Drought tolerance depends on different mechanisms. Moreover, the genotypes differ their tolerant according to in combination of mechanisms which it have. SODs are considered as the first defense against ROS, being responsible for the dismutation of O_2- to H_2O_2 and O_2. CAT, APX, POD are enzymes that catalyze the conversion of H_2O_2 to water and O_2 (Gratão *et al.*, 2005). Over-expression of these genes improved drought tolerance in various plants (Umezawa *et al.*, 2006). In rice, many studies have reported that over expression of stress- related genes had successfully increased drought tolerance to some extent (Jeong *et al.*, 2010; Xu, *et al.*, 1996). Genetic markers are useful in breeding programs for assessment of genetic variation between genotypes (Hillel *et al.*, 1992; Kahraman, 1999). Molecular markers help in characterizing and evaluation of genetic variability to identify varieties (Chao, 2006). Among several molecular markers, RAPD markers have been proved to be an efficient tool used in genetic research due to its simplicity, low cost and less performing time, ease of essay by PCR and no prior knowledge about genome is required (Williams *et al.*, 1990; Rekha *et al.*, 2011; Rabbani *et al.*, 2008). RAPD markers have been successfully employed in rice for identification and classification of cultivars (Choudhury *et al.*, 2001), identifications of hybrids (Hashemi *et al.*, 2009), genetic diversity analysis (Saker *et al.*, 2005; Kanawapee *et al.*, 2011; Ogunbayo *et al.*, 2005).

The aim of this work was to identify the biochemical and molecular markers that act in the tolerant genotype comparing to the sensitive one. Thus to point out the effective scavenger for water stress severes to use it in further studies for over expression experiment aiming to improve drought tolerance in some rice genotypes.

MATERIALS AND METHODS

Plant Materials

Giza181, variety developed by Rice Research division, ARC. as along grain variety, was used as a drought sensitive. Orabi2, new developed rice variety as a drought tolerance by Dr. Said Soliman, Genetics Dept, Faculty of Agriculture, Zagazig University, Egypt, certificate no70, 2011. Two rice varieties were selected to grown in controlled conditions at 29±1°C at incubator. Thirteen /11 hr., light/dark system was used. Forty seeds (three replicates) of both varieties were germinated in each petridishes (10 cm) on wet layer of filter paper.

Drought treatments

Drought stress was induced by polyethylene glycol 6000 (PEG 6000) with different concentrations of 0 , 5 , 10 and 15% (*W/V*) equal to control, -0.05, -0.15 and -0.3 MPa, respectively. Plates were kept for growth of seedlings up to 15 days. The experiments used RCB Design with three replicates.

Seedling length

Shoot and root lengths were measured in 15-day-old seedlings from each genotype grown under control, 5%, 10% and 15% PEG treatment conditions.

Fresh and dry weights

Whole seedlings (shoot and root) were weighted to determine fresh weight (FW). Dry weight (DW) was determined by reweighting after oven drying at 105°C for 3 hr.

Evaluation of lipid peroxidation product

Lipid peroxidation was evaluated as the concentration of TBA (thiobarbituric) -reactive products, equated with malondialdehyde (MDA), as originally described by (Anjum *et al.*, 2012), with slight modifications as in (Hendry and Grime, 1993). Plant tissues (0.5 g) were 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 mixture was heated at 100°C for 15 min., the reaction was then stopped by rapid cooling in an ice-water bath, followed by centrifugation at 4000 rpm at 5°C for 10 min. The absorbance was then read at 532 nm and correction for unspecific turbidity was done by subtracting the absorbance of the same at 450 and 600 nm. The TBA-reactive products (MDA) were expressed as nmol. g^{-1} DW.

Electrolyte leakages (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. After the vials were shaken slightly, the conductivity of the solution was measured immediately. The conductivity of the solution was measured again after 1 hr. Finally, each vial was placed in boiling water for 1 hr., cooled to room temperature (about 20±2°C) and then shaken, after which total conductivity was measured. Leakage rate of electrolytes (expressed in μS·cm−1.FW·h−1) was calculated as the net conductivity of the solution with seeds immersed for 1 hr., divided by the total conductivity after boiling according to (Omar *et al.*, 2012).

Determination of total protein content

A half gram of plant tissue was ground to a fine powder in liquid nitrogen. Ground powder was homogenized on ice with a mortar and pestle in 1.0 ml of cold extraction buffer containing, (100mM Tris Hcl pH 8, 2% SDS, 5 mM NaCl, 10 mM 2-mercaptoethanol) according to (Dure *et al.*, 1981) with slight modification. The protein extract was recovered by centrifugation of the homogenate at 4°C for 10 min at 8000 rpm. The resulting supernatant was transferred to new tubes in 50-100μl aliquots. The extracts were either directly used or stored at –20°C. Concentration of extracted proteins was determined according to the Bradford protocol (1976) using bovine serum albumin as a standard.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

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 onto the gel. Samples standardized on protein amount (15 μg proteins) were loaded on the gel. BLUeye pre-stained molecular protein Ladder (GeneDirex) was used.

Enzymes Extraction

The extraction for the enzymes was done as suggested by (Anjum *et al.*, 2012). Frozen samples (0.5 g) were ground in liquid-nitrogen with a mortar and pestle and homogenized in 1 ml 50mM sodium phosphate buffer (pH7.0) containing 1 mM EDTA-Na2 and 2% (*W/V*) polyvinylpyrrolidine-40 (PVP-40). The homogenate was centrifuged at 8000 rpm for10 min at 4°C. The supernatant was aliquated and stored at - 20°C till the assay of enzyme activity. Protein concentrations were determined according to Bradford (1976), using bovine serum albumin as a standard.

Native PAGE and enzymes activity staining

Native PAGE was performed on protein extracts from plant tissues. Separating gel containing 7.5% (for CAT), 10% (for APX) or 12% (for SOD) acrylamide. An equal amount of protein (enzyme extract), as determined by Bradford assay (Bradford, 1976), was loaded into each lane (60 μg protein for SOD and APX, 15 μg protein for CAT. Electrophoretic separation of (SOD, CAT, APX) was performed by native PAGE according to (Weydert and Cullen, 2010).

SOD activity

SOD isoforms on the gels were detected by nitrobluetetrazolium (NBT) reduction by superoxide radicals that were generated photo chemically (Beauchamp and Fridovich, 1971). After electrophoresis, the gels were covered with a solution that contained 0.25 mg·mL^{-1}NBT and 0.1 mg·mL^{-1} riboflavin, and then exposed to light. The two types of SOD (Mn-SOD and Cu/Zn-SOD) were identified using inhibitors. Mn-SOD was diagnosed by its

insensitivity to 5 mM H_2O_2 and 1 mM KCN, while Cu/Zn-SOD was identified by its sensitivity to 1 mM KCN (Navari-Izzo *et al.*, 1998).

CAT activity

CAT activity in native PAGE gels was determined using the methodology of (Woodbury *et al.*, 1971). Gels were incubated in 0.003% H_2O_2 for 20 min and developed in a 1% $FeCl_3$ and 1% $K_3Fe(CN_6)$ solution for 10 min.

APX activity

APX isoforms activity was evaluated according to (Lee and Lee, 2000), with some modification according to (Omar *et al.*, 2012). A running buffer with 2 mM AsA added was used for pre-running (30 min). After electrophoretic separation, the gels were equilibrated with 50 mM Na-phosphate buffer (pH 7.0) containing 2 mM AsA for 30 min, and then incubated in the same buffer+4 mM AsA+2 mM H_2O_2 for 20 min. H_2O_2 was added to the solution just before gel incubation. Gels were then incubated with 50 mM Na-phosphate buffer (pH 7.8), 28 mM TEMED and 2.45 mM NBT for 10-20 min with gentle agitation.

Genetic diverisity

Random amplified polymorphic DNA (RAPD) was used to characterize genetic variations of the used genotypes.Total genomic DNA was extracted from seedling by the easy extraction kit (EZ-10 Spin Column Genomic DNA Minipreps Kit, plant) followed by an RNase-A treatment. The quantification and qualification of the extracted DNA was determined on 0.8 % agarose gel.

RAPD amplification and assay

A set of twelve 10-mer oligonucleotides was analyzed for RAPD-PCR Primers names, sequences and annealing temperature were listed in Table1.

Primers were selected for their relation with water stress tolerant genotypes at 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 40 cycles of 1 min at 94°C, 2 min at 32 and 36°C (according to the primer), 1 min at 72°C, followed by a final extension at 72°C for 5 min. in a (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 100 bp DNA ladder was used to estimate the molecular size of the amplified fragments. Electrophoresis was conducted at 60 Volts for 3 hr. Gels were then visualized and photographed under UV-trans illuminator by digital camera with UV filter adaptor.

Table 1. Sequence and annealing temperature of the RAPD primers used in the study

Primer code	Sequence (5' to 3')	Annealing temperature
M 1	AGG GGT CTT G	32 °C
M 2	CAA TCG CCG T	32 °C
M 3	CTG CTG GGA C	36 °C
M 4	GTG AGG CGT C	36 °C
M 5	TTG GCA CGG G	36 °C
M 6	CAG GCC CTT C	36 °C
M 7	GGT GAC GCA G	36 °C
M 8	GAT GAC CGC C	36 °C
M 9	TGC TGC AGG T	32 °C
M 10	CCA GCA GCT T	32 °C
M 11	AAT CGG GCT G	32 °C
M 12	GTG ATC GCA G	32 °C

RESULTS AND DISCUSSION

Fresh and Dry Weights

Results shown in Fig. 1 indicate that, G181 seedlings showed a gradually decrease in FW values with increasing water stress. 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 (Fig. 1A).

Under water stress condition, G181 seedlings lost about 50% of their FW comparing with control condition, while Orabi2 seedlings lost about 10% of their FW under control condition (Fig. 1C). Seedlings of both genotypes treated with 5%.PEG showed an increase in DW values comparing with DW values of seedlings under control condition (Fig. 1B). A sharp decrease in DW of G181 seedlings with increasing PEG concentration was noticed. While Orabi2 seedlings showed a slight decrease with increasing PEG concentration.

Fig. 1C showed that seedlings of G181 lost about 80% of their DW at 15% PEG treatment while Orabi2 seedlings lost less than 10% of their DW comparing with their values under control treatments.

Shoot and Root Lengths

For both genotypes, water stress caused an increase in whole plant length as a result of increasing of root length (Fig. 2). G181 seedlings showed a decrease in shoot length along with increasing water stress (Fig. 2A). A decrease in Orabi2 seedlings shoot length was occurred only at the treatment with 15% PEG (Fig. 2B).

Rate of Electrolyte Leakage and MDA Content

Analysis of rate of electrolyte leakage (EL) and MDA content for both genotypes showed dramatically increase in EL and MDA content of G181 seedlings with increasing water stress. On the other hand, seedlings of Orabi2 showed low and constant values for both EL and MDA contents along with increasing water stress (Fig. 3).

Analysis of Total Protein

Electrophoresis analysis of total protein fractions of G181 and Orabi2 seedlings under control and water stress condition showed series of changes. Fig. 4 showed that water stress treatments induced expression for some protein bands which were absent in the control treatment in both genotypes (indicated by circular zone 2, 3) with molecular weight of approximately 18 -19 KDa. Although increasing of water stress by PEG concentration of 15% caused losing of expressed bands comparing with control and other PEG concentrations in G181 seedlings (circular zone 1) with molecular weight of approximately 100 to 180 KDa. Orabi2 seedlings showed new bands with molecular weights of 240, 35 and 18 KDa and increasing in expression level of other bands according to bands intensity (indicated by arrows) under water stress treatments.

Antioxidant Enzyme Activities

Changes in the activity of antioxidant enzymes including SOD, CAT and APX in G181 and Orabi2 during treatment are illustrated in Fig. 5.

SOD

Staining for SOD activity on non-denaturing PAGE revealed the changes in the pattern of isoenzyme activity in the genotypes under study (Fig. 5A). Two SOD isoenzyms, Mn-SOD and Fe-SOD isozymes were detected in the seedlings by SOD activity staining. Fig. 5A showed the presence of three isoforms for Fe-SOD isoenzyme in G181 and one isoform for Mn-SOD isoenzyme while Orabi2 contained only two isoforms for Fe-SOD isoenzyme. Analysis of stained gel using Gel-analyzer software (Fig. 6C) showed that in general all stained isoenzymes as aband intensity showed an increase in activity with water stress increasing in both genotypes.

CAT

Fig. 5B illustrate the presence of two isoform for CAT isoenzyme in two genotypes under study. Analysis of stained gel using Gel-analyzer software (Fig. 6B) showed that activity of enzymes in G181 seedlings was increased with increasing water stress to 5%. After that activity.

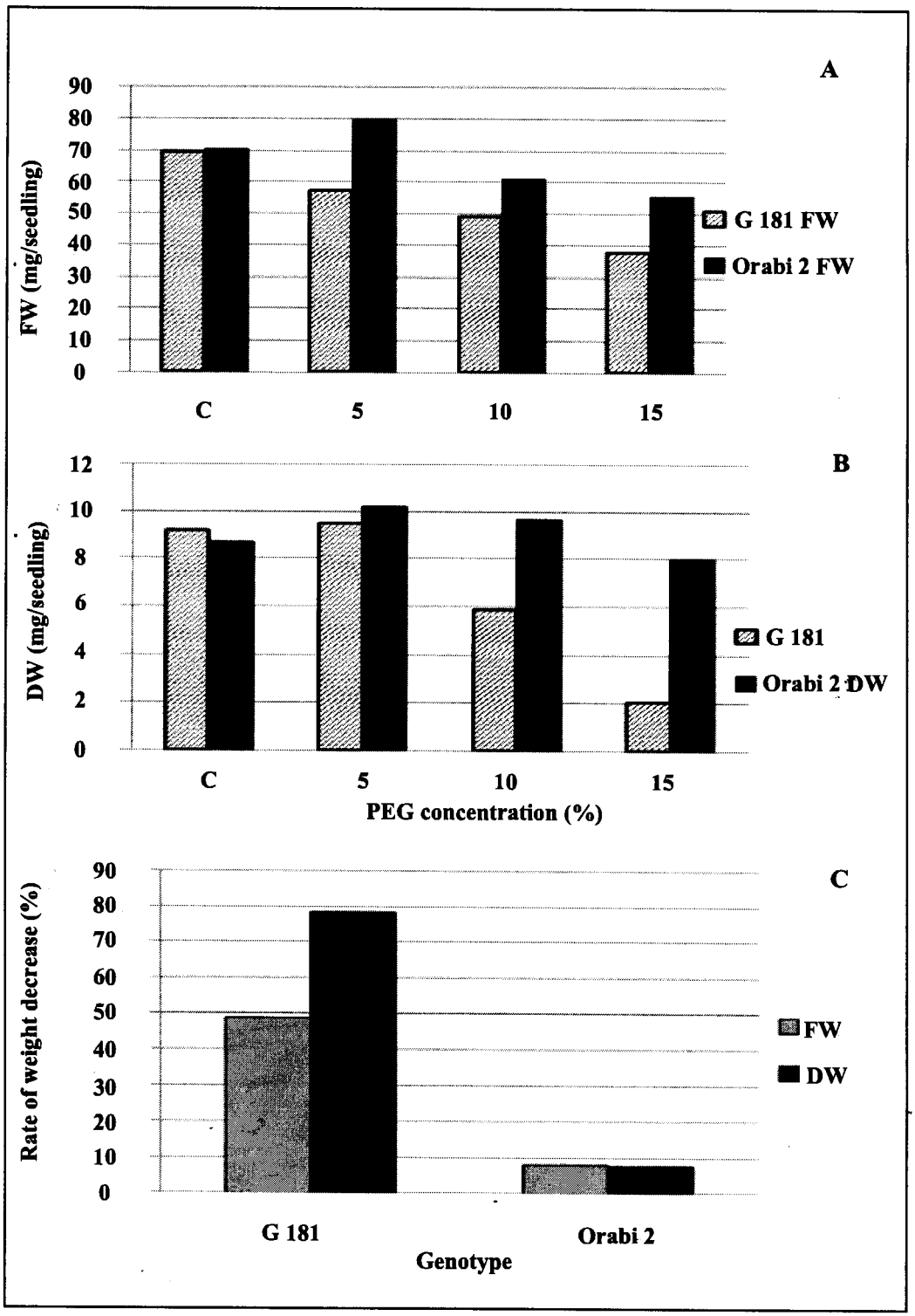

Fig. 1. Changes in FW [A], DW [B] and rate of weight decrease [C] for G181and Orabi2 genotypes under different PEG treatments

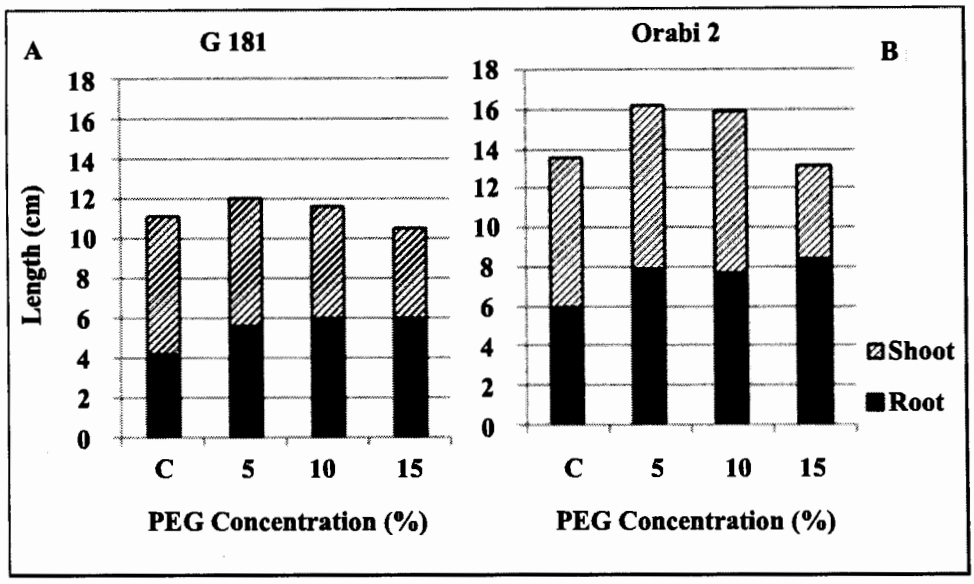

Fig. 2. Changes in shoot/root lengths of G181 [A] and Orabi2 [B] at different PEG concentrations

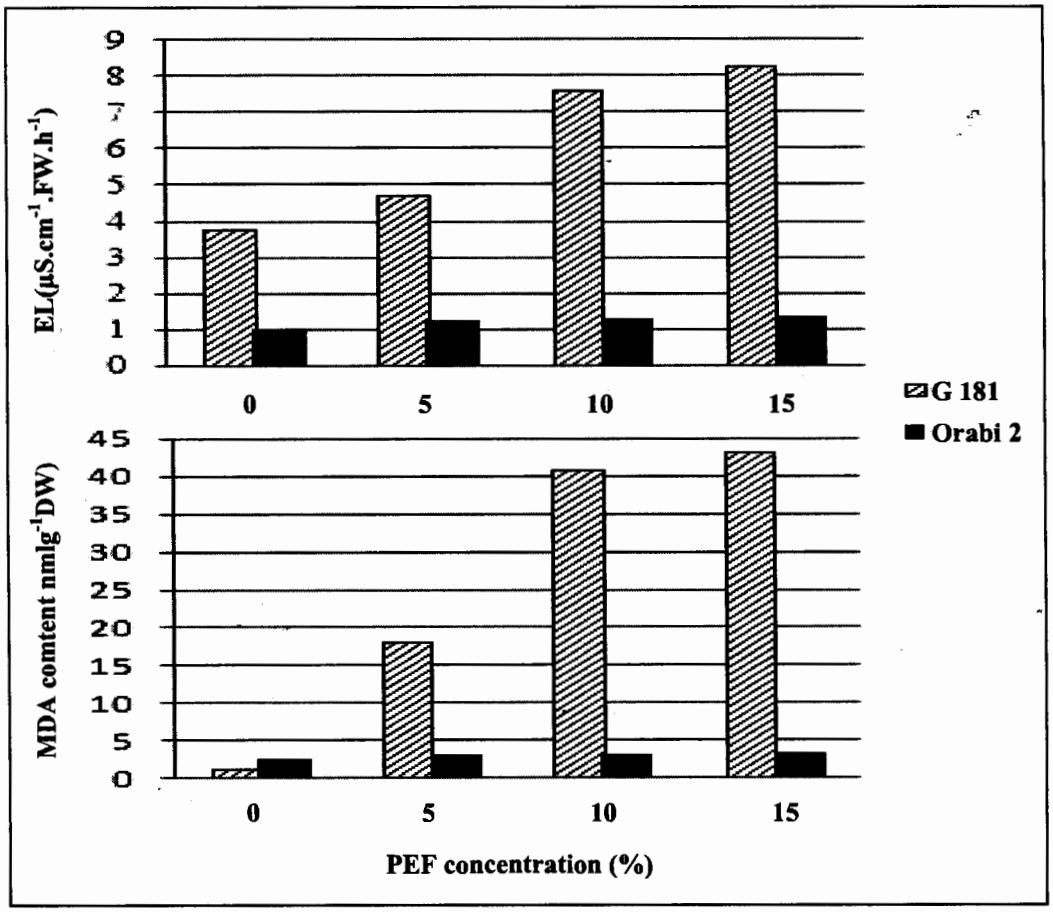

Fig. 3. Changes in rate of electrolyte leakage [A] and MDA content [B] for both G181 and Orabi2 genotypes

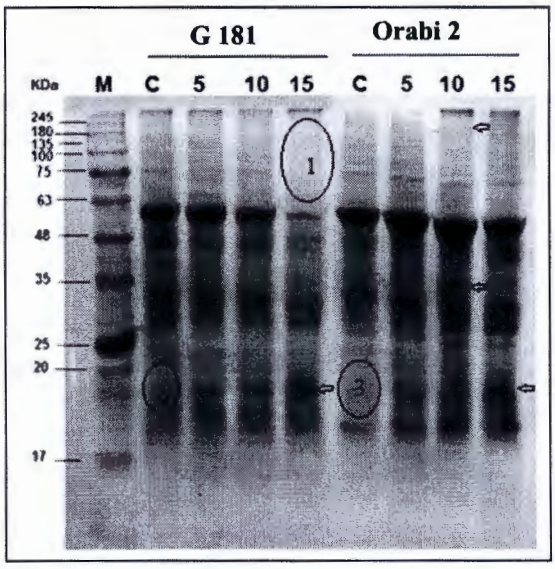

Fig. 4. SDS-PAGE analysis of total protein extracted from G181 and Orabi2 Seedlings treated with different PEG concentrations. Arrows pointed to the protein fractions which were induced or changed during PEG treatments

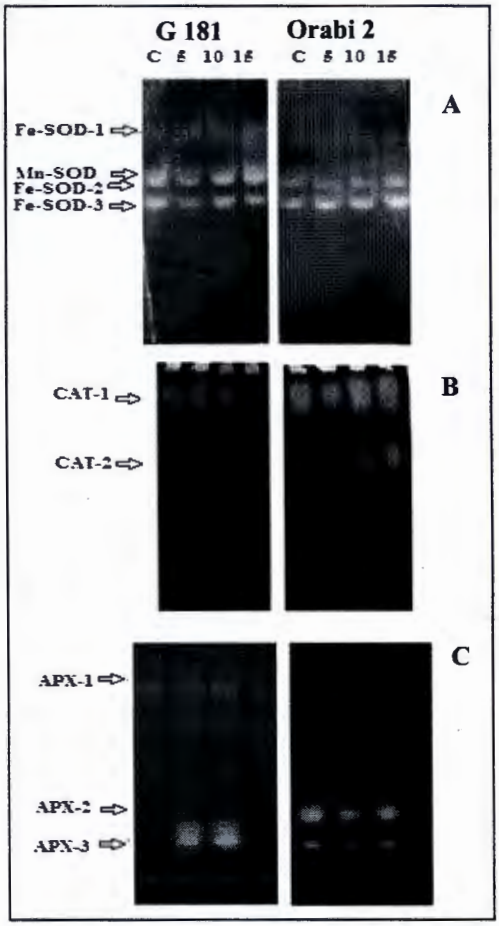

Fig. 5. Native gel staining for SOD [A], CAT [B]and APX [C] activities in control and PEG treated Seedlings. Numbers from 1 to 3 in [A] and [C] and 1 to 2 in [B] indicate different isoforms. Equal amounts of enzymes extracts (60 µg for SOD and APX and 15 µg for CAT) were loaded in each lane of staining gels

decreased along with stress increase. CAT activity in Orabi2 seedlings showed continuously increase in stained activity as water stress increased. In both genotypes, the activity of high molecular weight isoform (CAT-1) was higher than the (CAT-2).

APX

Fig. 5C show the presence two of isoforms for APX in G181 seedlings (APX-1 and APX-3) and three isoforms in Orabi2 seedlings (APX-1, APX-2 and APX-3). Analysis of stained gel using Gel-analyzer software (Fig. 6C) showed that APX-1 was decreased with water stress increasing in G181 seedlings. In contrast, it is increased with water stress increasing in Orabi2 seedlings. The second isoform (APX-2) did not appear in G181 seedlings, while it was stained in Orabi2 and showed semi stability during control and the first two PEG concentrations. With increasing water stress at PEG15% treatment, APX-2 showed loss of its activity. APX-3 induced by 5% and 10% PEG treatment in G181 and lost their activity at 15% PEG treatment. As APX-2, APX-3 showed the same pattern of activity in Orabi2 seedlings where they lost their activity at the third concentration of PEG.

RAPD Analysis

The tested twelve RAPD primers showed variations between the two genotypes under study by about 57% similarity. While three primers showed some DNA bands in Orabi2 which were absent in G181 (Fig. 7). In Orabi2 genotype, Primer M1 showed the presence of four bands (ranging from approximately 1000 to1500bp) Primer M3 illustrated presence one band with approximately 480 bp and Primer M9 showed presence of two bands with approximately 600 and 650 bp.

This study was conducted to analyze the mechanism of water stress tolerance in two rice genotypes. Both genotypes responded differently to water stress as was observed by morphological, physiological and biochemical changes. PEG treatments (Fig. 1 C) proved that genotype G181 showed a great reduction (40 and 80%) of FW and DW, respectively. On the other hand, genotype Orabi2 lost only 10% of its FW and DW (Figure 1C). 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 point to its ability to maintain photosynthesis process under water stress conditions (Werner *et al.*, 2001). Induction of new protein fractions or increasing the expression level of induced proteins in Orabi2 genotypes (Fig. 5 and Table 2) 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).

According to the obtained results, water stress tolerance was accompanied with the ability of Orabi2 genotype to maintain its membrane properties as show in low values of electrolyte leakage and MDA (Fig. 3). Lower values of electrolyte leakage and MDA in genotype Orabi 2 than genotype G181 indicated that Orabi2 is better equipped with efficient free radical quenching system that offers protection against oxidative stress. Lipid peroxidation is a biochemical marker for the free radical mediated injury (Verma *et al.*, 2003). enhanced lipid peroxidations have been reported under severe water stress (Baisak *et al.*, 1994) Because plants make use of common pathways and components in exhibiting tolerance to drought stress and oxidative stress, tolerance to oxidative stress also confers tolerance to drought stress. This phenomenon is termed cross-tolerance.This has been confirmed by higher activities fortested antioxidant enzymes (Figs. 5A, 6A).

SOD enzymes are responsible for the dismutation of O_2– to H_2O_2 and O_2 (Gratão *et al.*, 2005). Increasing of SOD isoforms along with increasing water stress in the both genotypes G181 and Orabi 2 (Figs. 5, 6) was not enough to acquire the tolerance for water stress. Increasing of SODs activities was associated with high production of H_2O_2 which conceder a long-lived molecule (half-life of 1ms), that can diffuse some distances from its production site (Bhattacharjee, 2005). Decreasing or losing the activity of CAT and APX isoforms (Figs. 5B, 6B) with increasing water stress appear to be the main reason for losing drought tolerance in the genotype G181. CAT and APX, are enzymes that catalyze the conversion of H_2O_2 to water and

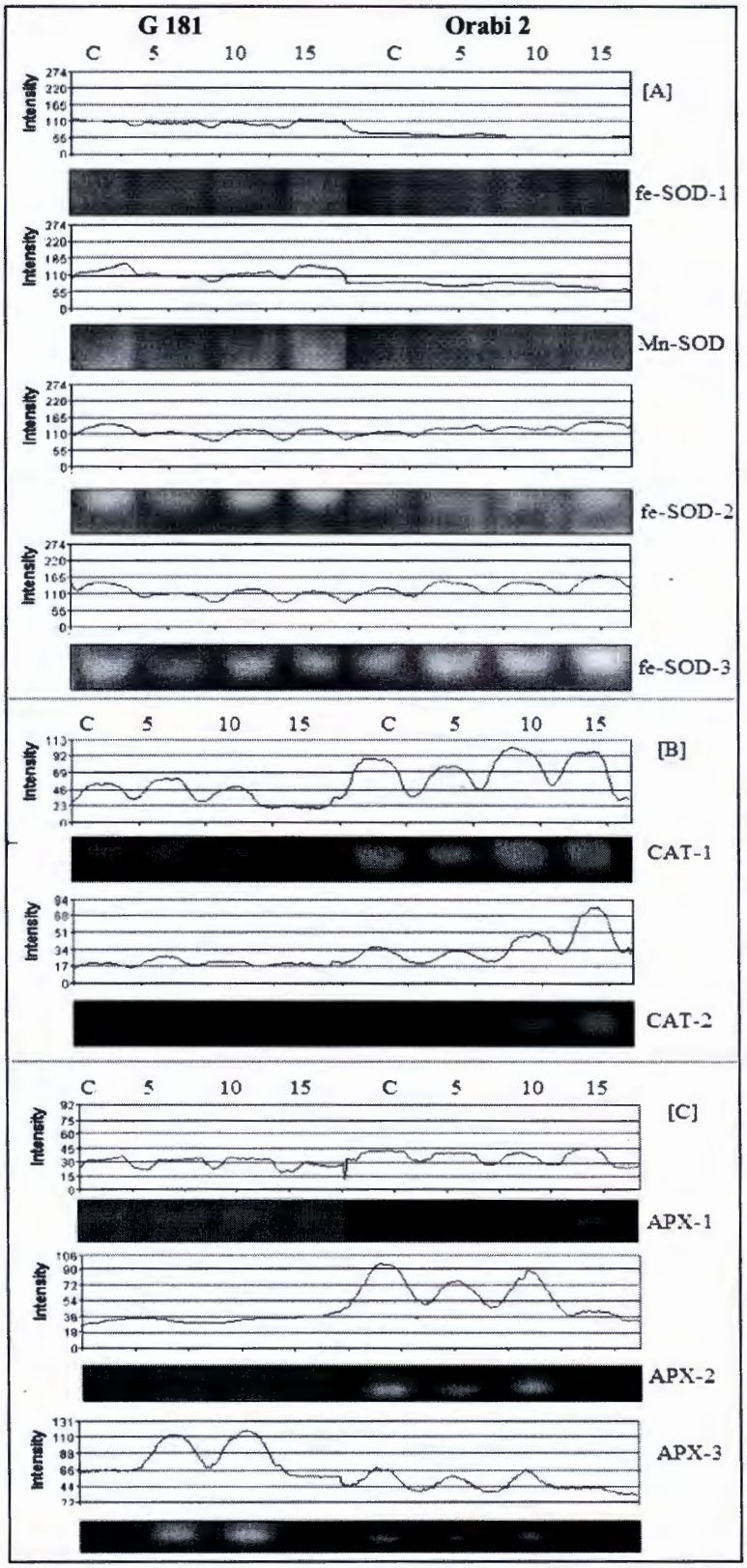

Fig. 6. Analysis of stained native gel of enzymes SOD [A], CAT [B] and APX [C] to detect enzyme activity using Gel-analyzer software

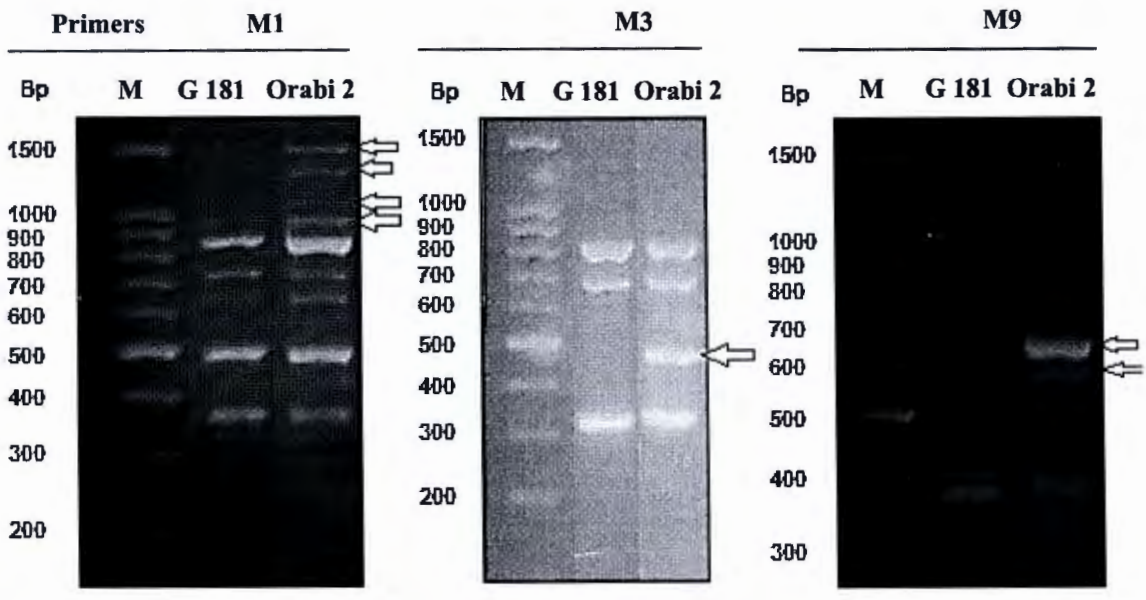

Fig. 7. DNA profiles of two rice genotypes amplified by three primers. Lane M: Molecular size marker (GeneDirex 100 bp DNA Ladder). M1, M3 and M9 (primer code)

Table 2. Changes in protein fraction numbers during water stress

Treatment	Giza 181				Orabi 2			
	Control	5%	10%	15%	Control	5%	10%	15%
No. of bands	14	15	15	12	23	24	25	24

O_2 (Gratão *et al.*, 2005). Increasing activity of CAT and APX with increasing water stress in Orabi2 seedling (Figs. 5 B, C and 6 B, C) were responsible for keeping tolerance till concentration of 10% PEG. Losing the activity of CAT and APX isoforms caused losing for the tolerance at the PEG concentration 15%. This study reveals that degree of oxidative stress and antioxidant activity has been described to be closely associated with the resistance/ susceptibility of a genotype to water stress (Mittler, 2002). Activation of antioxidant system helps the plants to avoid stress induced damages (Noctor *et al.*, 2000).

In conclusion, Activation of antioxidant enzymes and decrease in membrane damage enabled the genotype Orabi2 to better resist water stress induced damages. From the obtained biochemical and molecular data of the tolerant genotype (Orabi2) comparing to the sensitive one (G181), we can suggest that oxidative stress tolerance is a trait whose heredity is relatively less complex and is, therefore,suitable for a breeding objective that involves water stress tolerance.

REFERENCES

Abdel-Nasser, L.E. and A.E. Abdel-Aal (2002). Effect of elevated CO_2 and drought on proline metabolism and growth of safflower (*Carthamus mareoticus* L.) seedlings without improving water status. Pak. J. Biol. Sci., 5: 523–528.

Aboulila, A.A.M. (2012). Molecular genetic studies on drought tolerance in the rice (*Orzya sativa* L.) using SSR DNA marker. Ph. D. Thesis. Fac. Agric., Kafer El-Sheikh Univ., Egypt.

Anjum, S.A., M. Farooq, X.Y. Xie, X.J. Liu and M.F. Ijaz (2012). Antioxidant defense system and proline accumulation enables hot pepper to perform better under drought. Sci. Hort., 140: 66-73

Baisak, R., D. Rana, P.B. Acharya and M. Kar (1994). Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. Plant and Cell Physiol., 35 (3):489-495

Beauchamp, C. and I. Fridovich (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical biochemistry 44 (1):276-287 Bhattacharjee S (2005) Reactive oxygen species and oxidative burst:roles in stress, senescence and signal transduction in plants. CurrSci India 89:1113–1121 Colloids Surf. B: Biointerfaces, 59: 141–149.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem., 72 (1): 248-254

Bhattacharjee (2005). Reactive oxygen species and oxidative burst. Roles in stress, senescence and sigal transduction in plants.

Chao, S. (2006). Application of molecular marker technologies on cereal crops improvement. In: American Oat Workers Conf., 23-25.

Choudhury, P.R., S. Kohli, K. Srinivasan, T. Mohapatra and R. Sharma (2001). Identification and classification of aromatic rices based on DNA fingerprinting. Euphytica, 118 (3):243-251.

Dure, I.I.I.L., S.C. Greenway and G.A. Galau (1981). Developmental biochemistry of cottonseed embryogenesis and germination: changing messenger ribonucleic acid populations as shown by *in vitro* and *in vivo* protein synthesis. Biochem., 20 (14): 4162-4168.

Eva, S., M.M. Rubio-Wilhelmi, M. Luis, C. Begon˜a Blasco, J.J. Rios, M.A. Rosales, L. Romero and J.M. Ruiz (2010). Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant Sci., 178 : 30–40.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra (2009). Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev., 29: 185–212.

Gill, S.S. and N. Tuteja (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. and Biochem., 48 (12):909-930

Gratão, P.L., A. Polle, P.J. Lea and R.A. Azevedo (2005). Making the life of heavy metal-stressed plants a little easier. Functional Plant Biol., 32 (6):481-494

Hashemi, S.H., S.A.M. Mirmohammadi-Maibody, G.A. Nematzadeh and A. Arzani (2009). Identification of rice hybrids using microsatellite and RAPD markers. African J. Biotechnol., 8 (10).

Hendry, G.A. and J.P. Grime (1993). Methods in comparative plant ecology: a laboratory manual. Springer Sci. and Business Media.

Hillel, J., E. Dunnington and P. Siegel (1992). DNA markers in poultry breeding and genetic analyses. Poul. Sci. Rev.

Jeong, J.S., Y.S. Kim, K.H. Baek, H. Jung, S-H. Ha, Y. Do Choi, M. Kim, C. Reuzeau and J-K. Kim (2010). Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions. Plant Physiol., 153 (1):185-197

Kahraman, A. (1999). Genetics of flowering in lentil. In: Proc. 7th Conf. on Plant and Animal Genome, 17-21.

Kanawapee, N., J. Sanitchon, P. Srihaban and P. Theerakulpisut (2011). Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD and SSR markers. Electronic J. Biotechnolo., 14 (6):2-2.

Kohtaro, I.A., K. Hommaa, A.T. Shiraiwa, B.B. Jongdee and B.P. Mekwatanakarn (2014). The effects of cross-tolerance to oxidative stress and drought stress on rice dry matter production under aerobic conditions. Field Crops Res., 163 : 18–23.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nat., 227 (5259):680-685

Lee, D.H. and C.B. Lee (2000). Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. Plant Sci., 159 (1):75-85

Mahassen, S., S. Sayed-Ahmed, S. Soliman and A.F.A Maksoud (1999). Genetic analysis for some milling quality characters in rice under drought conditions. Zagazig J. Agric. Res., 26 (6):101-110.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7: 405– 410.

Noctor, G., S. Veljovic-Jovanovic and C.H. Foyer (2000). Peroxide processing in photo-synthesis: antioxidant coupling and redox signaling. Philos. Trans. R. Soc. Lond. Ser., 355:1465–1475.

Navari-Izzo, F., M.F. Quartacci, C. Pinzino, F.D. Vecchia and C.L. Sgherri (1998). Thylakoid-bound and stromal antioxidative enzymes in wheat treated with excess copper. Physiologia Plantarum, 104 (4):630-638

Ogunbayo, S., D. Ojo, R. Guei, O. Oyelakin and K. Sanni (2005). Phylogenetic diversity and relationships among 40 rice accessions using morphological and RAPDs techniques. African J. Biotechnol., 4 (11).

Omar, S.A., N.I. Elsheery, H.M. Kalaji, Z-F. Xu, S. Song-Quan, R. Carpentier, C-H. Lee and S.I. Allakhverdiev (2012). Dehydroascorbate reductase and glutathione reductase play an important role in scavenging hydrogen peroxide during natural and artificial dehydration of Jatropha curcas seeds. J. Plant Biol., 55 (6):469-480

Passioura, J.B. and R.J. Stirzaker (1993). Feedforward responses of plants to physically inhospitable soil. In: Buxton, D.R., Shibles, R., Forzberg, R.A., Blad, B.L., Asay, K.H., Paulsen, G.M., Wilson, R.F. (Eds.), International Crop Science I. Crop Sci. Soc. Amer., Madison, WI, 715–719.

Rabbani, M.A., Z.H. Pervaiz and M.S. Masood (2008). Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. Electronic J. Biotechnol., 11 (3):52-61.

Rekha, T., K.P. Martin, V. Sreekumar and J. Madassery (2011). Genetic diversity assessment of rarely cultivated traditional indica rice (*Oryza sativa* L.) varieties. Biotechnol. Res. Int.

Saker, M.M., S.S. Youssef, N.A. Abdallah, H.S. Bashandy and A.M. El Sharkawy (2005). Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. African J. Biotechnol., 4: 9.

Shinozaki, K., K. Yamaguchi-Shinozaki and M. Seki (2003). Regulatory network of gene expression in the drought and cold stress responses. Current Opinion in Plant Biol., 6 (5): 410-417.

Thomashow, M.F. (1999). Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Ann. Rev. Plant Biol., 50 (1): 571-599

Ullah, S.S., M.M. Hossain, M.F. Miah and S.H. Prodhan (2013). Assessment of genetic diversity among moderately drought tolerant landraces of rice using RAPD markers.

Umezawa, T., M. Fujita, Y. Fujita, K. Yamaguchi-Shinozaki and K. Shinozaki (2006). Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. Current opinion in Biotechnol., 17 (2):113-122

Verma, S. and R. Dubey (2003). Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci., 164 (4):645-655

Wang, W., B. Vinocur and A. Altman (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, 218 (1): 1-14.

Weydert, C.J. and J.J. Cullen (2010). Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nature Protocols, 5 (1):51-66

Williams, J.G., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey (1990). DNA

polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18 (22):6531-6535.

Woodbury, W., A. Spencer and M. Stahmann (1971). An improved procedure using ferricyanide for detecting catalase isozymes. Analytical Biochem., 44 (1):301-305

Xu, D., X. Duan, B. Wang, B. Hong, T-H.D. Ho and R. Wu (1996). Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol., 110 (1):249-257.

Youssef, M., A. Mansour and S. Solliman (2010). Molecular markers for new promising drought tolerant lines of rice under drought stress *via* RAPD-PCR and ISSR markers. J. American Sci., 6 (12):355-363.

Werner T, Motyka V, Strnad M, Schmülling T (2001) Regulation of plant growth by cytokinin. Proc Natl Acad Sci USA 98: 10487–10492.

الاختلافات الوراثية في نشاط مضادات الاكسدة كاستجابة للاجهاد المائي لبعض التراكيب الوراثية فى الأرز

سمر عبدالعزيز عمر - محمد حسين عبدالفتاح - مدحت عراقي الديناري

قسم الوراثة- كلية الزراعة- جامعة طنطا - مصر

تهدف هذه الدراسة إلى التحقق من التغيرات المرتبطة بتحمل الإجهاد المائي في إثنين من التراكيب الوراثية للأرز هما جيزة ١٨١(كتركيب حساس للاجهاد) وعرابي٢ (كتركيب متحمل للاجهاد) تم اختيارهم من ضمن سبعة تراكيب اجريت عليهم الدراسة، وتشتمل الدراسة علي تقدير بعض القياسات الخضرية مثل الوزن الرطب والجاف للبادرات وكذلك طول المجموع الجذري والخضري كما اشتملت على بعض التقديرات الفسيولوجية مثل تقدير معدل الإنبات ومعدل الإرتشاح واكسدة الدهون كما اشتملت الدراسة علي بعض التحليلات البيوكيماوية مثل التقدير النوعي للبروتين الكلي على الجل وكذلك تقدير نشاط انزيمات مضادات الاكسدة على الجل مثل انزيم السوبراكسيد دسميوتز وإنزيم الكتاليز وإنزيم الاسكوربيك بيروكسيداز، كذلك عمل تحليل لاختلاف التراكيب الوراثية علي المستوي الجزيئي باستخدام تكنيك الرابد، كل التقديرات شملت التراكيب الوراثية تحت الدراسة في الظروف الطبيعية (الكنترول) وفي ظروف الاجهاد المائي، وقد اظهرت النتائج أن التركيب الوراثي عرابي ٢ المتحمل للاجهاد المائي يتميز بقلة معدل الارتشاح وكذلك قلة اكسدة الدهون وهذا يعكس نشاط وفاعلية انزيمات مضادات الأكسدة والمرتبطة بتحمل الإجهاد، بالنسبة لانزيم السوبر أكسيد دسميوتاز اظهر زياده في النشاط مع زيادة الإجهاد في كلا التركيبين الحساس والمتحمل، وزيادة في النشاط لإنزيم الكتاليز والاسكوربيك بيروكسيديز مع زيادة الإجهاد في عرابي ٢ فى حين لوحظ فقد في نشاط كلا الانزيمين في جيزة ١٨١ مع زيادة الإجهاد وهذا يظهر السبب الرئيسي في تاثير الإجهاد المائي على جيزة ١٨١ مقارنة بعرابي٢ حيث يقوم كلا الانزيمين بالدور الرئيسي في الحماية من زيادة فوق أكسيد الهيدروجين الناتج عن زيادة نشاط انزيم السوبر اكسيد دسميوتيز. كما اظهرت التحليلات الجزيئية وجود اليلات مميزه لعرابي ٢ ولم تظهر مع جيزة ١٨١ وذلك مع بعض بوادئ الرابد ضمن ١٢ بادئ تم استخدامهم.

المحكمون :

١- أ.د. سعيد عبدالسلام درة أستاذ الوراثة – كلية الزراعة – جامعة كفر الشيخ.

٢- أ.د. سعيد سعد سليمان أستاذ الوراثة – كلية الزراعة – جامعة الزقازيق.