PHYSIO-BIOCHEMICAL AND MOLECULAR CHARACTERIZATION FOR DROUGHT TOLERANCE IN RICE GENOTYPES AT EARLY SEEDLING STAGE

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ABSTRACT

The aim of the current investigation was to study the influence of drought-stressed by using PEG on some rice genotypes at seedling stage. The performance was judged by growth, physiological, biochemical and molecular constituents at seedling stage. The results of study suggested that growth attributes were reduced under different drought stress (70 and 140 g/L PEG) in most of the cases as compared with control. Among various tested genotypes IRAT 259, Line 7 and Line 8 exhibited the lowest reduction values of relative water content, chlorophyll content and membrane stability index at 70 and 140 drought levels. The Line 8 produced the highest amount of proline under stress conditions which is indicating its highest tolerance to drought stress. The antioxidant enzymes such as catalase, peroxidase and polyphenol oxidase were induced by the drought levels. The growing expressions of antioxidant enzymes assist the plant for adaptation of plant under environmental conditions and tolerate stress. The IRAT 259 has highest increase percentage in antioxidant enzymes under stress. Total sixteen SSR primers examine for characterizing the power of each SSR primer by calculating polymorphic information contents and a total of 41 alleles were amplified using 16 SSR primers. The variation in number of amplified alleles per primer ranged from one allele as for wmc27 to five alleles for wmc179 and wmc 215, with an average of 2.56 alleles. The highest value was 100% polymorphism belonged to

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13 out of the 16 primers. Phylogenetic analyses per primer were ranged from 0.00 to 0.794 with an average of 0.427. Average observed heterozygosity ranged from 0.00 to 0.670 with an average of 0.45. It was found the value of heterozygosity was 0.00 to 0.670 and the mean value of heterozygosity was 0.45. On the basis of phenotypic and genetypic (reaction with markers) performances under drought stress conditions, the Line 8 and the Line 7 can be recommended as a drought tolerant and a drought sensitive, respectively. This result can be acclaimed the important source for genetic diversity of rice in future breeding programs.

1 Introduction

Rice (*Oryza sativa* L.) is considered the most essential food crops and it needs huge amount of water as compare to other crops during growth life cycle (Wang et al., 2012). Rice plays a major role as a staple food which providing nutrition to more than three billion people and comprising 50-80% of their daily calorie intake (Khush, 2005). Rice crop plays an important role in Egypt for strengthen self-sufficiency of food and for maximizing the export of rice as strategic crop. Furthermore, the average yield of rice has to be increased by 25 – 30 % to face the demands of the increase of population growth rate (RRTC, 2013). Identifying rice genotypes and breeding lines with high levels of tolerance to drought to use as donors in breeding and gene discovery is one of the most challenges for rice research (Serraj & Atlin, 2008).

Drought is one of the most important environmental stresses that influence the growth and development of plants and it is also an important challenge to agricultural researchers and plant breeders.Water stress causes severe threat in production of rice and it affects morphological, physiological, biochemical and molecular characteristics of rice crops along with its productivity. Hence, with decline worldwide water availability for agriculture there is a need for improving drought adaptation in rice and screening of drought tolerance genotypes becoming necessary. Drought tolerance is complex phenomenon which depends on the combined function of different morphological, physiological, biochemical and molecular properties. The mechanisms associated with the tolerance to water-stress and the systems that regulate adaptation of plant to water stress in rice have been completed studied (Pandey & Shukla, 2015).

Drought stress influence the expressions of antioxidant in plants, osmotic modification, chlorophyll and transpiration reduction and inhibition of growth (Gupta & Huang, 2014). The different stages of growth exhibited catalase and peroxidase activity and with the growing drought intensity accumulation of proline also increases with high levels of stress (Mahdi et al., 2007). Biotic and abiotic stresses conditions also have negative impact on cell due to accumulation reactive oxygen species (Vaidyanathan et al., 2003).

According to Pandey & Shukla (2015) development and selection of drought tolerant rice varieties depend on the understanding of the different mechanisms that manage the productivity of rice under water stress condition. Molecular characterization of the available genotypes is beneficial for the evaluation of the genetic potential of the rice crops and help in stop erosion which can justified here as a reduction of genetic diversity in time (Manifesto et al., 2001). Therefore, the objectives of the present study were: (1) to evaluate and screen the available rice genotypes for the drought tolerance and to develop comprehensive understanding of the mechanism of plants response against drought stress with the help of integrated approach of combining mechanisms based on growth, morphological, physiological and biochemical related to drought tolerance and (2) estimate genetic diversity of six rice genotypes using molecular markers technique.

2 Materials and Methods

The present investigation was carried out at the Cell and Tissue Culture Laboratory of the Agronomy Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt. Six rice genotypes including the three varieties viz., IRAT 170 (check), IRAT 259 (drought-tolerant) and Giza 182 (drought-sensitive) as well as the lines (Line 7, Line 8 and Line 9) were tested for drought tolerance. Mature rice seeds, were husked manually, and washed for 2-5 min with sterile distilled water. Seeds were then cultured on; a modified MS medium (Murashige & Skoog, 1962) supplemented with various levels of polyethylene glycol 6000 (0, 70 and 140 g/L PEG). The basal medium was supplemented with 30 g/L sucrose and 6 g/L agar. The cultures were incubated at $28 \pm 2°C$ with 16 h photoperiod for 20 days. All the treatments were laid in a complete randomized design and replicated five times and the replicate was included 10 seeds. An analysis of variance was predestined for all traits according to Steel et al. (1996) to define the significant differences between genotypes.

2.1 Growth, physiological and biochemical traits measured

The performance of rice genotypes was studied under drought stress conditions at the early seedling stage; Root and shoot lengths (cm) and fresh and dry weights (g) were measured. Relative water content was measured by the method described by Schonfeld et al. (1988) with some modification. Proline was measured according to Bates et al. (1973). Chlorophyll content (SPAD unit), was measured on three leaf seedlings taken from each replicate by chlorophyll meter (SPAD-502, Soil- Plant Analysis Department (SPAD) section, Minolta camera Co., Osaka, Japan) by Minolta (1989).

Membrane stability index (%) is a measure phenomenon of drought resistance and the level of MSI was calculated by modifying Sairam et al. (2002), leaves of control and drought-stressed plants were collected and thoroughly washed with distilled water. 100 mg of leaf sample was placed in 10 ml of double distilled water at 40ºC for 30 min and thereafter, electric conductivity (C1) was measured with conductivity meter. It was followed by calculation the electric conductivity (C2) the same samples were settled on boiling water bath (100 ºC) for 15 min. The MSI was calculated using following formula:

$$MSI = [1 – (C1/ C2)] \times 100$$

For antioxidant enzymes analysis, fresh leaf leaves samples (0.2 g) were ground in liquid nitrogen and homogenized in an ice-bath in 4 ml homogenizing solution containing 50 mM potassium phosphate buffer and 1% (w/v) polyvinylpyrrolidone) (pH 7.8). The homogenate was centrifuged at 14000 rpm at 4°C for 10 min and the resulting supernatant was used as enzyme source for catalase, peroxidase and polyphenol oxidase assays.

Assay of catalase, the assay mixture in total volume of 3 mL contained 1.5 mL of 100 mM phosphate buffer (pH 7.2), 0.5 mL of (v/v) H_2O_2 and 0.03 ml of enzyme. The final volume was made 3 ml by adding distilled water. The reaction was started by adding enzyme and change in optical density was measured at 240 nm at 60s. The enzyme activity was showed by calculating the magnitude of decomposed H_2O_2 according to Aebi (1984).

For assay of peroxidase (POD), determined by spectrophotometer at 420 nm, according to Chance & Maehly (1955) and Assay of polyphenol oxidase (PPO), determined according to Duckworth & Coleman (1970) through spectrophotometrically at wave length 420 nm at 25°C.

2.2 Molecular characterization

2.2.1 DNA extraction and PCR amplification

A 500 mg sample of frozen young leaves of the six rice genotypes were ground to powder in a mortar and a pestle in the presence of liquid nitrogen. The DNA extraction was done using the cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof et al., 1984). Twenty SSR primers were used in the present investigation. The PCR products were electrophoreses in 2% agarose gels stained with ethidium bromide and visualized under UV light or were separated via capillary electrophoresis using a QI Axcel Advanced system device.

2.2.2 Data handling of SSR marker

SSR data was registered based on the existence of the amplified Products for each primer To investigate the discriminatory power of each SSR primer, the polymorphic

information content (PIC) was measured according to Smith et al. (2000). As well as, Heterozygosity (Ho) was measured according to (Hormaza, 2002).

3 Results and Discussion

3.1 In *vitro* screening of rice genotypes for drought tolerance

As screening technique, the survival ability of the six rice genotypes was evaluated by culturing mature seeds on MS medium supplemented by three doses of PEG-6000 (0, 70 and 140 g/L)during germination stage. Highly significant differences were recorded among two-way interaction (genotypes and drought levels) (P≤ 0.01) for all studied traits, revealing the presence of genetic diversity in the material used (Figure 1 and 2).

3.2 Growth traits as affected by drought stress

In all genotypes, the seedlings growth decreased with increasing the levels of stress (Figure 1). Among various tested genotypes, lowest reduction of shoot length (0.00%) was observed in IRAT 259 while the highest reduction (36.62%) was reported from the genotype Giza 182 at 70 g/L PEG induced stress. On the other hand, the reduction percentages were 19.44 and 67.83 for IRAT 259 and Line7 genotypes under 140 g/L PEG stress treatments respectively. In the present study, IRAT 259 was followed by the Line 9 genotypes and registered as the lowest means reduction than other genotypes under level 70 g/L PEG this thing is indicating that these genotypes are more tolerant and relatively showing small decreases in shoot length under drought stress (Figure 1).The present findings are in line with Lum et al. (2014) and in agreement with these findings, a previous study (EL Sabagh et al., 2015a).

Concerning shoot fresh weight, reduction percentages of all genotypes ranged from 23.38 to 41.67% (average 29.58%) and 43.18 to 75.86% (average 57.14%) under 70 and 140 g/L PEG drought stress, respectively (Figure 1). Shoot fresh weight of the genotypes Giza 182 and Line 7 were more adversely affected than other genotypes by the 70 and 140 g/L PEG stress treatments, respectively. The reduction range of shoot dry weight was 5.42 to 43.99% at 70 g/L drought stress while the maximum reduction of shoot dry weight (63.77%) was recorded in genotype IRAT 170 and it was followed by IRAT 259 (60.75%) at 140 g/L PEG induced drought stress (Figure 1). The lowest reduction of mean shoot dry weight (less than 15%) was recorded at the Line 8 genotype under 70 g/L PEG followed Giza 182 and Line 9 genotypes.

Roots play great role in plant existence under stress conditions. It was observed that under stress conditions a significant reduction was reported in root length. The lowest reduction in root length (8.29 and 14.85%) was recorded at the genotypes of Line 8 and IRAT 259 while the highest reduction (30.73 and 57.14%) was observed at IRTAT 259 and Line 8 under 70 and 140 g/L PEG induced stresses, respectively (Figure1).
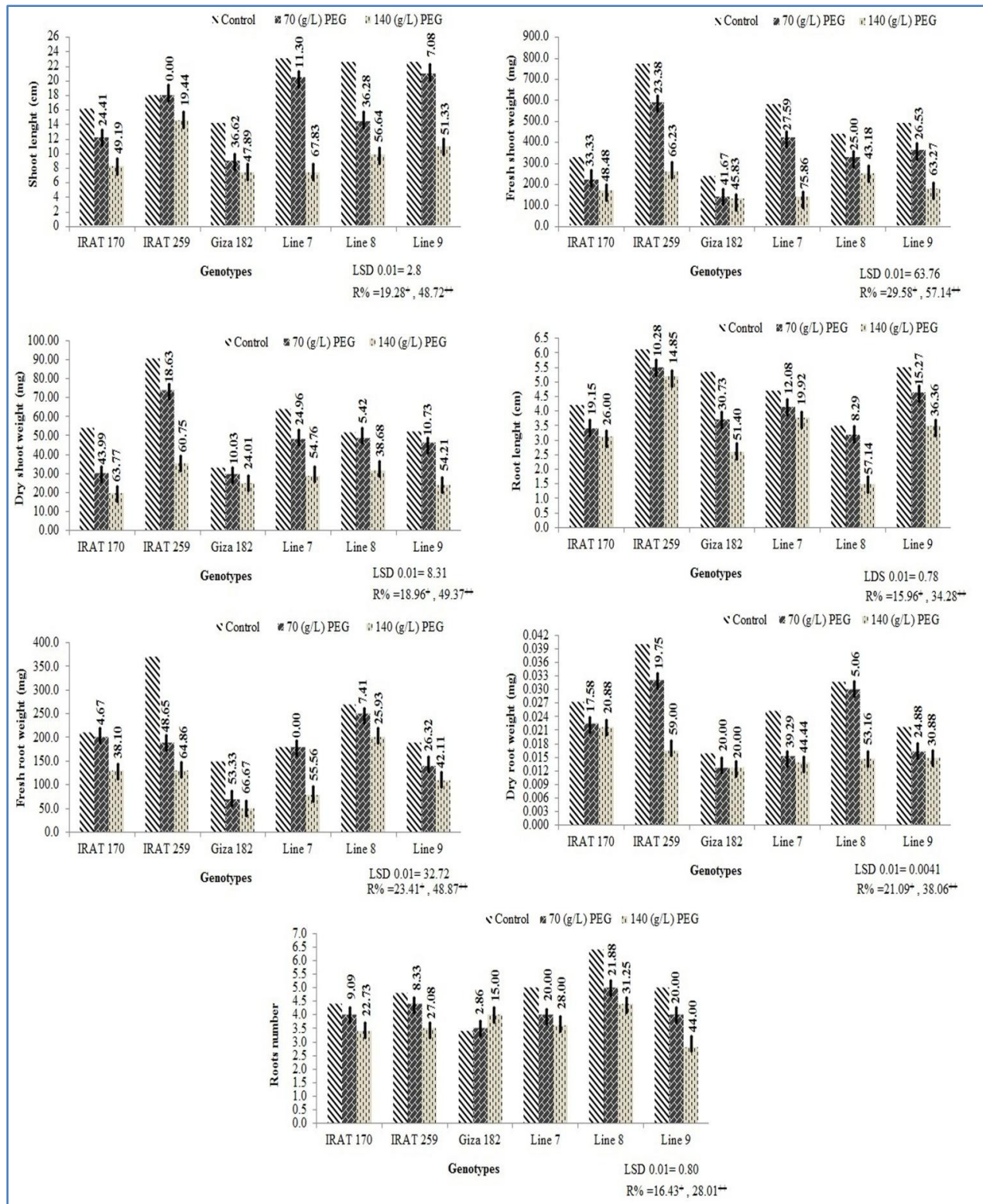
Figure 1 Mean of six rice genotypes of seven growth traits under (0, 70 and 140 g/L) polyethylene glycol. NB: Numerical values above bars showed the percentage reduction in the trait relative to the control; R%= reduction percentage; [+,++]Grand mean of reduction percentage at 70 and 140g/L PEG, respectively.

Table 1 Size of DNA fragments generated from SSR analysis of six rice genotypes.

Markers	Product size (bp)	Genotypes					
		Giza 182	IRAT 170	IRAT 259	Line 7	Line 8	Line 9
Xwmc27	400	-	-	+	-	+	+
Xwmc147	110	-	+	-	-	-	-
	170	-	-	+	-	+	+
Xwmc149	190	-	+	+	+	+	-
	205	-	-	-	-	-	+
	240	-	-	-	-	+	-
	270	-	+	-	-	-	-
Xcfd1	225	+	-	+	+	+	+
	250	-	-	+	-	+	-
	275	-	-	-	-	+	-
Xwmc179	100	-	+	-	-	+	-
	110	+	-	+	+	-	-
	150	-	+	-	-	-	+
	170	-	-	+	-	+	-
	270	-	-	+	-	+	+
Xwmc215	100	+	-	-	+	-	+
	120	-	-	+	-	-	-
	170	-	-	+	+	-	-
	240	+	-	+	+	+	+
	290	-	-	+	-	-	-
Xwmc233	270	-	-	+	-	+	+
	300	-	-	-	-	+	-
Xgwm249	70	+	-	+	-	-	-
	90	-	+	-	-	-	-
	150	-	-	+	-	+	-
Xwmc387	160	-	-	-	-	+	-
Xwmc169	145	-	+	-	-	+	+
	155	-	-	+	-	+	-
	170	-	-	+	-	+	+
Xwmc44	220	+	+	+	-	+	-
Xwmc14	90	-	-	-	-	+	-
	250	-	-	-	-	+	-
Xwmc18	70	+	+	+	+	+	+
	270	-	-	-	-	+	+
Xwmc31	60	+	+	+	+	+	+
	150	-	-	-	-	+	-
Xwmc327	120	-	-	-	-	+	+
	130	-	-	-	-	+	-
Xwmc8	70	+	+	+	+	+	+
	110	-	-	+	-	+	+
	140	-	-	-	-	+	+

(+) means present, (-) means absent

The genotype IRAT 259 maintained lower mean reduction (10.28 and 14.85%) under 70 and 140 g/L PEG levels, respectively. As shown in (Figure 1), root fresh weight was reduced significantly by media moisture deficit. Reduction percentages of genotypes ranged from 0.00 to 53.33% and 25.93 to 66.67% was recorded under 70 and 140 g/L PEG drought stress, respectively. Further, higher reduction in root fresh weight was reported, which averaged 23.41 and 48.87% relative to the control under 70 and 140 g/L PEG stress levels, respectively. Three genotypes (IRAT170, Line 7 and Line 8) showed low means reduction (less than 15%) under 70 g/L PEG level, this thing is suggesting that these genotypes are less sensitive to drought stress as compared to the three other genotypes. Similar type of findings was reported by Fraser et al. (1990).

Regarding root dry weight, the reduction mean values of genotypes ranged from 5.06 for Line 8 to 39.29 % for Line 7, and 20.00 for Giza 182 to 59.00 % for IRAT 259 under 70 and 140 g/L PEG stress, respectively with an averages of 21.09 and 38.06% overall genotypes under treatments, respectively (Figure 1). The Line 8 registered the lowest means reduction than other genotypes under level 70 g/L PEG which is indicating that this genotype is tolerant to drought. With respect to the number of root, all genotypes exhibited means reduction in number of root under both treatments relative to the control treatment with same genotypes, except the Giza 182 registered increased under 70 and 140 g/L PEG levels, which gave 2.86 and 15.00%, respectively (Figure 1). Two genotypes registered low means reduction (less than 15%) under 70 g/L PEG in number of root were IRAT 170 (9.09%) and IRAT 259 (8.33), indicating that these genotypes are more tolerant under this level of drought stress, but most genotypes recorded moderate means reduction under water stress treatment (140 g/L PEG).

It is evident from the results that PEG treatments had inhibitory effect on all the growth attributes of rice accessions. The genotypes which sensitive to drought stress are exhibited more decline in biomass as compared to the resistant genotypes. Similar observations were reported by Jiang & Lafitte (2007) and Lum et al. (2014). Based on the results, a variation in drought tolerance was reported among various genotypes during seedling growth stage. So, results of study suggested that most drought-tolerant variety is IRAT 259 while the highest drought sensitive variety is Giza 182 (Figure 1). The major rice genotypes showed a significant decrease in shoot length at the various drought levels as compared with control in major traits. These results were in compliance with those of Mohammadkhani & Heidari (2008). A significant reduction in root length for all genotypes was reported higher at high levels of drought as compare to control (Figure 1). These results were in compliance with those of Fraser et al.(1990) and Ahmad et al. (2013).

3.3 Physiological and biochemical traits as influenced by drought stress

Effect of drought on certain physiological and biochemical traits related to crop productivity in rice included relative water content, chlorophyll content, proline content, membrane stability index and antioxidant enzymes activities measured for three stress levels of 0, 70 and 140 g/L PEG (Figure 2). Result of study suggest that the relative water content, chlorophyll content and membrane stability index are depressed by drought stress, while the level of proline content and antioxidant enzymes activities (polyphenol oxidase,peroxidase and catalase) increased when plants subjected to drought stress. The differences among the genotypes in response to drought stress for final germination were highly significant (Figure 2).

A significant variation was found in terms of the relative water content (RWC) in the leaves of plants due to drought stress in major genotypes compared the control (Figure 2). The data

were agreement with those obtained by Halder & Burrage (2003). Among the genotypes IRAT 170, Giza 182 and Line 9 were maintained the highest means reduction of RWC, which gave 8.61, 12.40 and 9.81% under 70 g/L PEG and 22.57, 19.22 and 17.38% under 140 g/L PEG, respectively. These genotypes lost high amount of leaf water when subjected to drought, and consequently they are considered sensitive to drought stress compared to the genotypes IRAT 259, Line 7 and Line 8, illustrating that these genotypes retained more water in leaf tissue under same drought stress. The average reduction was as much as 6.63 and 14.32% at 70 and 140 g/L PEG, demonstrating that the genotypes are tolerant, similar results was reported by earlier researchers Alizade (2002) and Islam et al. (2011).

In this study, chlorophyll synthesis in plant was reduced due to drought stress conditions. The reduction percentages of chlorophyll content were 4.84 and 10.58% under 70 and 140 g/L PEG drought stress of all genotypes, respectively (Figure 2).The result suggests that this trait is more tolerant to drought stress. Reduction percentages under 70 g/L PEG treatment ranged from 0.09% for the Line 8 to 9.12% for the Line 9 and from 1.80% for the Line 8 to 13.24% for the IRAT 170 under 140 g/L PEG, indicating that these genotypes are more tolerant to drought stress. Evain et al. (2004) suggested that the lowest values of stomatal conductance, photosynthesis and relative water content cloud be due to stress conditions. Similar types of results were reported by Seemann & Sharkey (1986) and Barutçular et al. (2016).

Membrane stability index decreased with increasing moisture stress and there were significant differences among the genotypes (Figure 2). The mean reduction of this trait was 3.65 and 7.51% under 70 and 140 g/L PEG treatments respectively; this thing is suggesting that this trait is more tolerant to drought stress. The reduction mean values of genotypes ranged from 1.96 for Line 8 to 6.44 % for IRAT 170 and 3.38 for Line 7 to 14.64 % for IRAT 170 under 70 and 140 g/L PEG stress, respectively. Hence, the approach of tolerance of these genotypes to drought stress were reflected by its higher value of membrane stability index. These results are correspond with Munns (2002); Al-Ashkar& El-Kafafi (2014) and EL Sabagh et al.(2015b).

Proline was accumulated in leaf of rice plants subjected to water stress and the highest accumulation was recorded in the severe stress treatment (Figure 2). Average proline content increased 23.90 and 45.93% under drought stress treatments of 70 and 140 g/L PEG, respectively (Figure 2). The genotype IRAT 259 accumulated the highest proline content of 44.08 and 69.21% while Line 8 recorded 37.15 and 74.72% under 70 and 140 g/L PEG, respectively, these results are in agreements with Pireivatloum et al. (2010); Lum et al. (2014) and EL Sabagh et al. (2016). Besides, acts as an excellent osmolyte; proline plays three major roles during drought stress, i.e., as a metal chelator, an antioxidative defence molecule and a signaling molecule (Hayat et al., 2012).
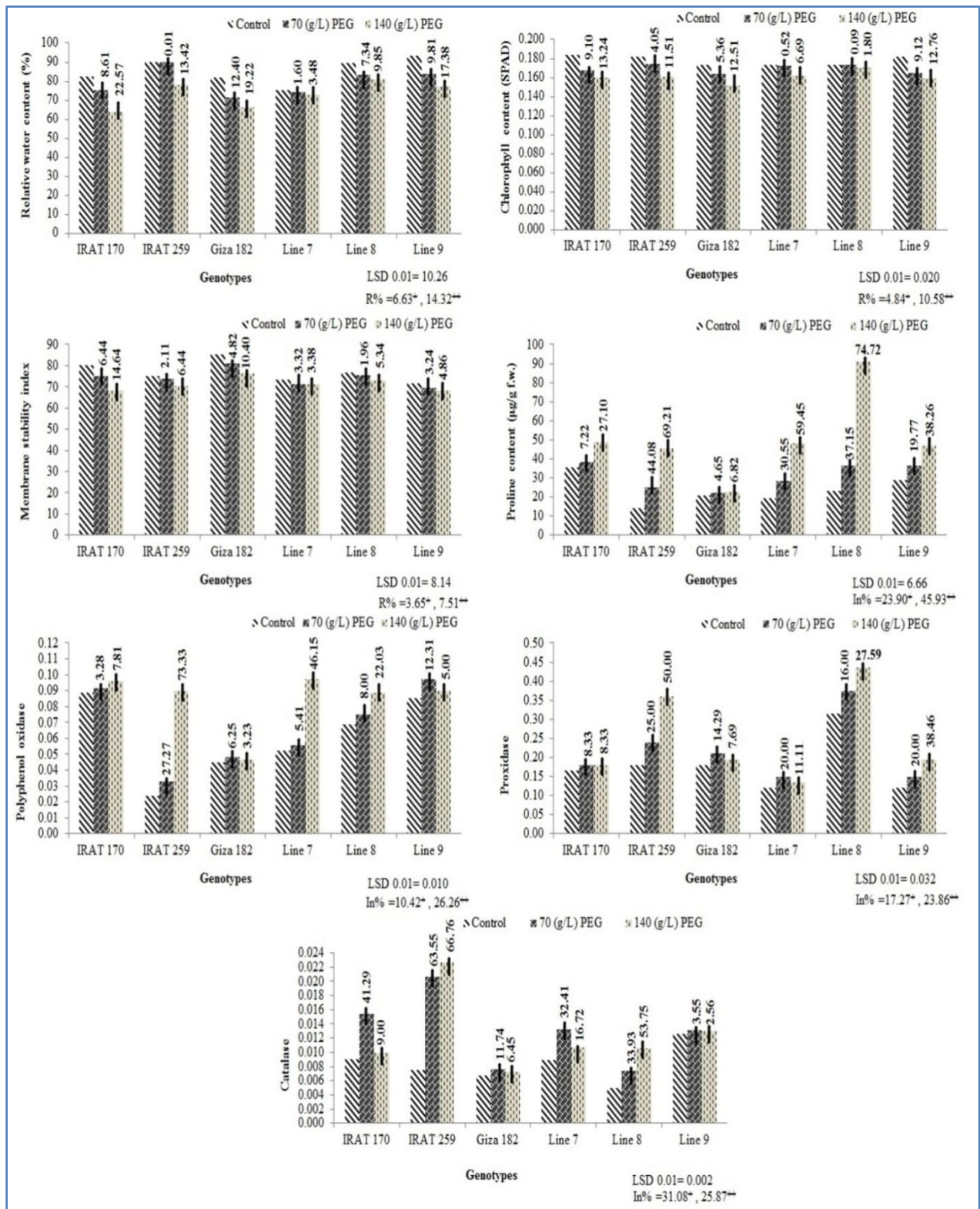
Figure 2 Mean of six rice genotypes of seven physiological and biochemical traits under (0, 70 and 140 g/L) polyethylene glycol; NB: Numerical values above bars showed the percentage reduction or increase in the trait relative to the control; R%= reduction percentage; In%= increase percentage; [+, ++] Grand mean of percentage reduction or increase at 70 and 140 g/L PEG, respectively.

Proline accumulation might promote plant damage repair ability by increasing antioxidant activity during drought stress. In plants under water stress, proline content increases more than other amino acids, and this effect has been used as a biochemical marker to select varieties aiming to resist such conditions (Fahramand et al., 2014). Thus, proline content can be used as criterion for screening drought tolerant rice varieties.

The results of this study clearly showed that drought stress treatments of 70 and 140 g/L PEG increased polyphenol content of 10.42 and 26.26%, respectively (Figure 2). The genotypes IRAT 259 and Line 9 recorded highest increase percentage in phenol accumulation reached 27.27 and 12.31% under 70 g/L PEG while the genotypes IRAT 259 and Line 7 recorded highest increase percentage in phenol accumulation under 140 g/L PEG, which gave 73.33 and 46.15% respectively. High values of phenol indicated high stress tolerance, IRAT 259 and Line 9 genotypes showed more tolerance to drought stress than other genotypes. Agastian et al. (2000) observed that, polyphenol content was increased in various tissues of plants undera biotic stress conditions. A common effect of drought stress is the disturbance between the generation and quenching of reactive oxygen species (ROS) (Subhashini & Reddy, 1990; Zheng & Wang, 2001; Faize et al., 2011).

Peroxidase activity (POD) was significantly increased when plants of different genotypes were subjected to water stress. The average POD was 17.27 and 23.86% in all genotypes under 70 and 140 g/L PEG, respectively (Figure 2). Catalase activity (CAT) also significantly increased under drought stress as compared with control for all the genotypes. Catalase activity increased 31.08 and 25.87% for all genotypes under drought stress treatments (70 and 140 g/L PEG), respectively (Figure 2). The genotypes IRAT 259 recorded highest increase percentage in catalase activity (63.55 and 66.76%) under 70 and 140 g/L PEG respectively, indicating its tolerance to drought stress. The genotype Giza 182 recorded the catalase activity (11.74 and 6.45%) under 70 and 140 g/L PEG respectively, indicating it's sensitive to drought stress. The plants are tolerance to drought-stress has higher levels of antioxidant systems and substrates (Athar et al., 2008).

In this research increased value of CAT and POD activities was reported in drought tolerant varieties, but, this value decreased in the sensitive varieties. These results are best way to protect the plants against H_2O_2. So, the growing of POD action could effectively withstand the oxidative stress that caused by stress (Mandhania et al., 2006). The tolerance genotypes against environmental stresses has been associated with higher activities of antioxidant enzymes and produced as protective defence system to counteract the oxidative injury that caused by drought stress in rice. The activities of antioxidants can effectively diminish the ROS, and cloud decrease passive effect of drought stress (Lum et al., 2014).

The results of reduction percentages for studied traits can be classified into three categories according to Farag (2005) these are (i) Drought tolerant traits included relative water content, chlorophyll content and membrane stability index reduced less than 15% under 70 and 140 g/L PEG suggesting that these traits could be used as selection criteria for screening to drought resistant genotypes in rice; (ii) Moderately tolerant traits included growth traits, i.e. shoots and roots under 70 g/L PEG reduced more than 15% ; and (iii) Drought susceptible traits like shoots and roots under 140 g/L PEG reduced more than 30%. It is worthy to note that the breeder should be taken into consideration to increase positive percentages in proline content and antioxidant enzymes activities (polyphenol oxidase, peroxidase and catalase) occurred under drought stress, which related to tolerance plant under these conditions (Farag, 2005).

3.4 Genetic diversity analysis based on SSR markers technique

3.4.1 SSR analysis

Twenty SSR primers were screened for their ability to amplify the genomic DNA from six rice genotypes. The number of amplification bands per primer varied from 1.0 as for primer Xwmc 27 to 5.0 for primer Xwmc 215 depending on the primer and the DNA sample. Among these 41 amplified fragments, 8.38% were not polymorphic while 91.62% was polymorphic among the six rice genotypes (Table 2). The highest value was 100% polymorphism belonged to 13 out of the 16 primers. An example of polymorphism with wmc147, wmc149, wmc179 and wmc215 are shown in Figure 3.The highest levels of polymorphism were recorded for wmc179 and wmc215 while the lowest levels of polymorphism were recorded for wmc27, wmc387, wmc18 and wmc31 (Table 1).

Using potential markers generated in the present research to improve drought tolerance–associated DNA markers are showed in Table 1. Specific DNA bands generated from analysis SSR primers here some rice genotypes reported to be drought tolerant/sensitive (on the basis of data performance/pedigree) were used. The results from Figure 3 and Table 1 revealed that DNA band at 120, 170 and 290 bp are present in IRAT259 as drought tolerant, but not in Giza 182 as drought sensitive, when primer wmc215 is used. On the other hand, specific DNA bands at about 100 bp is present in Giza 182 as drought sensitive but not in the IRAT259 and IRAT170 as drought tolerant, when primer Xwmc215 was used. Moreover, specific DNA bands, generated from SSR primers (Table 1), could be used to characterize between IRAT259 (drought tolerance) and Giza 182 (drought sensitive).

Table 2 Levels of genetic information generated by sixteen SSR primers on six rice genotypes.

Primer	Sequence of primer (5' -3')	No. of amplification products	No. of polymorphic products	Polymorphism (%)	PIC	Ho
Xwmc27	F= AATAGAAACAGGTCACCATCCG R= AGAGCTGGAGTAGGGCCAAAG	1	1	100	0.00	0.00
Xwmc147	F= AGAACGAAAGAAGCGCGCTGAG R= ATGTGTTTCTTATCCTGCGGGC	2	2	100	0.320	0.00
Xwmc149	F= ACAGACTTGGTTGGTGCCGAGC R= ATGGGCGGGGGTGTAGAGTTTG	4	4	100	0.390	0.33
Xcfd1	F=ACCAAAGAACTTGCCTGGTG R= AAGCCTGACCTAGCCCAAAT	3	3	100	0.532	0.33
Xwmc179	F= CATGGTGGCCATGAGTGGAGGT R= CATGATCTTGCGTGTGCGTAGG	5	5	100	0.794	0.67
Xwmc215	F= CATGCATGGTTGCAAGCAAAAG R= CATCCCGGTGCAACATCTGAAA	5	5	100	0.720	0.50
Xwmc233	F= GACGTCAAGAATCTTCGTCGGA R= ATCTGCTGAGCAGATCGTGGTT	2	2	100	0.375	0.16
Xgwm249	F= CAAATGGATCGAGAAAGGGA R= CTGCCATTTTTCTGGATCTACC	3	3	100	0.640	0.16
Xwmc387	F= CATTTTGACACCCACACTCG R= CTGGATCCCCTCTTCGCTAT	1	1	100	0.00	0.00
Xwmc169	F= TACCCGAATCTGGAAAATCAAT R= TGGAAGCTTGCTAACTTTGGAG	3	3	100	0.658	0.50
Xwmc44	F= GGTCTTCTGGGCTTTGATCCTG R= TGTTGCTAGGGACCCGTAGTGG	1	1	100	0.00	0.00
Xwmc14	F= ACCCGTCACCGGTTTATGGATG R= TCCACTTCAAGATGGAGGGCAG	2	2	100	0.500	0.16
Xwmc18	F= CTGGGGCTTGGATCACGTCATT R= AGCCATGGACATGGTGTCCTTC	2	1	50	0.625	0.33
Xwmc31	F= GTTCACACGGTGATGACTCCCA R= CTGTTGCTTGCTCTGCACCCTT	2	1	50	0.245	0.16
Xwmc327	F= TGCGGTACAGGCAAGGCT R= TAGAACGCCCTCGTCGGA	2	2	100	0.445	0.16
Xwmc8	F= CACGCGCACATCTCGCCAACTAA R= CGTGGTCTAGTCCGCGTTGGGTC	3	2	66	0.595	0.50
Total		41	39			
Mean		2.56		91.62	0.427	0.25

PIC = Polymorphic information content; Ho = Observed heterozygosity.

There reducibility of these variety-specific markers was confirmed in SSR analyses for which DNA isolation, PCR amplification, and gel electrophoresis were carried out separately. The technique of molecular marker has provided and helping in identification and genetic characterization of QTLs with positive effects on stress tolerance during various plant stages (Foolad, 2005). Comparatively, however, a limited research has been conducted to identify genetic markers associated with drought tolerance in different plant species. In the current research, genotypes analyzed were mainly identified according to various traits under water stress conditions. In this regard, the molecular characterization was more efficient in the generation of an unbiased picture of diversity than an agronomic approach.

3. 4 .2 Levels of genetic information generated by SSR primers

A total of 41 alleles were amplified of sixteen SSR among rice genotypes. The variation in number of amplified alleles per primer ranged from one allele as for wmc27 to five alleles for wmc179 and wmc215, with an average of 2.56 alleles (Table 2). The amplified alleles varied from 70 to 400 bp in sizes (Table 2). Polymorphism information content (PIC) provides information on allele diversity and frequency among genotypes. PIC differed greatly for sixteen SSR markers studied in six rice genotypes used in this study. Overall, the mean value of the PIC was (0.000 to 0.794) and the average was (0.427) (Table 2). The lowest value was recorded for three SSR viz. wmc27, wmc387 and wmc44.While the maximum PIC value was 0.794 for primer wmc179.
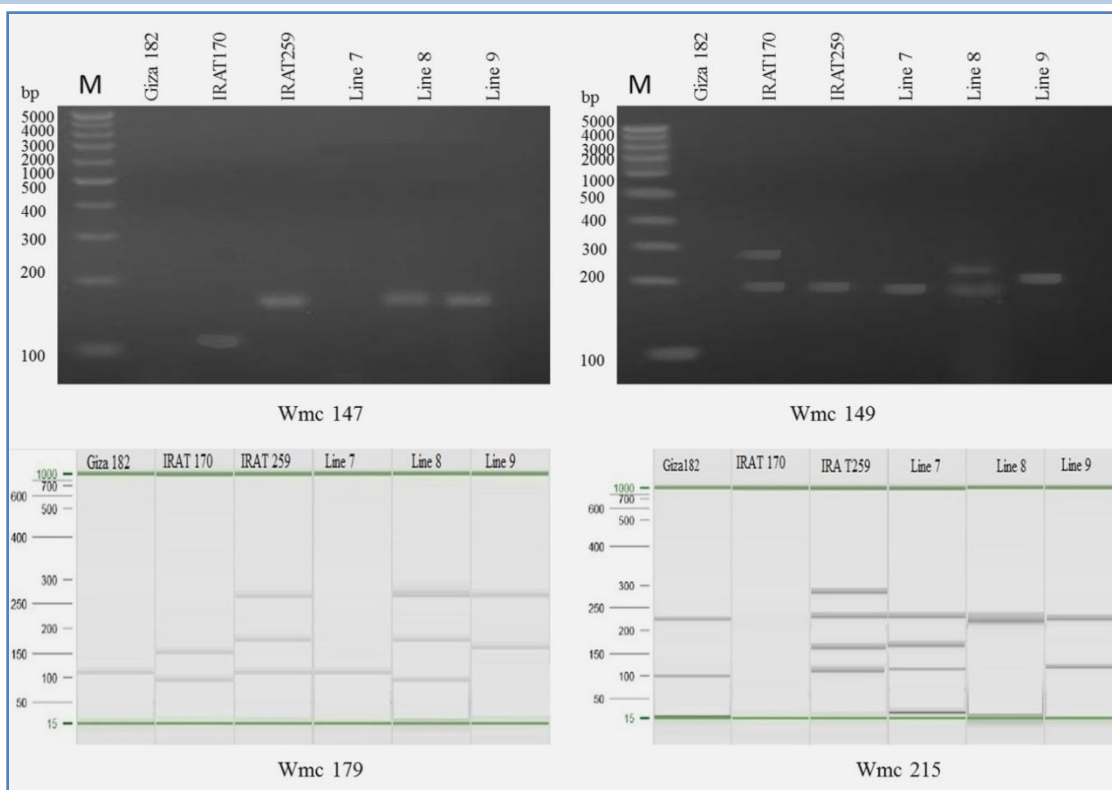
Figure 3 Examples of the SSR fingerprinting produced by Wmc147, Wmc149, Wmc179 and Wmc215 primers in the six rice genotypes.

It was observed, a significant and positive correlation (0.797) between PIC values and number of amplified alleles per primer. The value of heterozygosity were (0.00 to 0.67) and the mean value of heterozygosity were (0.25) (Table 2). The lowest Ho value was recorded (0.00) for four SSR such as wmc147 primer, while the highest value recorded (0.67) for primers wmc179 (Table 2).

The results indicated that the total number of amplified alleles (41) as well as the average number of amplified alleles per primer (2.56) was relatively lower compared to previous results. These results agree with Babu et al. (2014) and Choudhary et al. (2013). These differences could be attributed to differences in genotypes as well as SSR primers. While, in this study, the mean value of (Ho) was higher (0.25) compared to the 0.01 recorded by Kyung et al. (2015) using 49 SSR primers. Only previously reported polymorphic SSR primers were employed in the present study. The low mean value of PIC (0.427) showed the presence of high genetic homogeneity among genotypes as well as additional polymorphic SSR primers to achieve successful characterization for entails improvement of rice genotypes. The mean value of PIC was (0.58) among 76 Korean rice varieties (Kyung et al., 2015)

Conclusion

Considering the results of this study, Significant differences were observed among rice genotypes in respect of all measurements (morphological, physiological and biochemical traits) and molecular analysis. Based on phenotypic and genotypic (reaction with markers) performances under drought stress conditions, the Line 8 and the Line 7 can be recommended as a drought tolerant and a drought sensitive, respectively. This result can be acclaimed the important source for genetic diversity of rice in future breeding programs.

Acknowledgments

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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