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Research Article

Comparative Physiological Study on Six Egyptian Rice Cultivars Differing in their Drought Stress Tolerance

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Abstract

Rice (*Oryza sativa* L.) is a semi-aquatic plant, grow well in tropical, subtropical and temperate regions and it is highly affected by water shortage. The current experiment was conducted to compare the impact of drought stress on growth and physiology of six Egyptian rice cultivars (Giza177, Giza178, Giza179, Giza182, Sakha101 and Sakha106). Rice cultivars were planted in summer season 2015/2016 in botanical garden, Faculty of Science, Ain Shams University under normal condition (80% water holding capacity) or drought stress (40% water holding capacity). Results clearly showed that drought stress induced oxidative damage to all the studied rice cultivars as indicated by increased lipid peroxidation, membrane electrolyte leakage (EL) and reduced membrane stability index (MSI). The most tolerant cultivar was Giza 179 (G179) and the most sensitive one was Sakha 101 (Sk101). Under drought stress, G179 showed the highest dry weight of root, proline contents as well as antioxidant activity. Meanwhile, malondialdehyde (MDA) content, EL, MSI, chlorophyll and relative water (RWC) contents were least affected by drought in cultivar G179 as compared with other cultivars. Generally, the detected amounts of proline and total antioxidant activity in rice cultivars were found to be positively related to the plant ability to cope with drought stress as indicated by better growth and low oxidative damage.

Keywords: Antioxidant Activity; Chlorophyll Content; Electrolyte Leakage; Lipid Peroxidation; Membrane Stability Index; *Oryza sativa* L.; Proline

Introduction

Rice is one of the most important crops around the world; it is considered staple food for more than half of the world population. Rice comes after wheat among the highest consuming cereal worldwide and followed by maize [1,2]. Moreover, rice provides 50 - 80% of daily calorie [3], as its nutritional value represents carbohydrate fat, protein, vitamins (such as thiamine, riboflavin and niacin) and minerals (such as calcium, iron, magnesium, phosphorus, potassium, zinc and manganese) [4].

Total area cultivated by rice in the world was 160 million hectares (M ha) in 2015/2016 with yield 481.5 million metric tons (MMT). These cultivated area are distributed in Asia, Africa and South America [5] and consuming 34 - 43% of the total global irrigation water [6]. In Egypt, rice considered strategic crop not only as essential traditional diets but also used as exported crop for increase nation outcome and land reclamation. It is represent 22% of total cultivated area in Egypt and consume around 20% of total water available for irrigation [7].

Rice suffer from several biotic and a biotic stress that adversely affect crop yield. Biotic stress represented in insect and microbial diseases (such as stem borer, blast, brown spot, etc). While a biotic stress such as drought, salinity, high and low temperature and flooding [8]. Rice crop considered one of the major water consuming crops where continuous submerging is the only irrigated method used. Where one hectare of rice consume about 17000 m^3 water. Thus, drought stress considered the most serious stress to rice production nowadays [9]. Where about one third of rice cultivated area suffer from water shortage, and rice crop exposed to drought condition one or more time during growth stages [8,10].

Drought affect biochemical, physiological and morphological status of plant [11,12]. Response of plant depends on plant genotype, developmental stage, stress severity and duration [13]. Drought adversely affect cell water potential, turgidity, membrane stability and photo-oxidation of chlorophyll leading to accumulation of reactive oxygen species (ROS), generated mostly in chloroplast, mitochondria and peroxisome, causing oxidative stress [14]. ROS molecules such as singlet oxygen (1/2 O_2), superoxide anion radical (O_{2^-}), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2) is highly active molecules that react with biological molecules such as lipid, carbohydrate, protein, chlorophyll and nucleic acid, unlimited oxidation of cellular component lead to cell death [15]. Therefore, 50% of rice crop loss annually by drought [2].

Increasing water shortage threatened rice productivity worldwide [6]. As well as more rice will be required in near future due to increasing population, so more effort has been made to increase rice yield by 40% to cover population requirements [16,17].

Aim of the Study

The aim of the present work was to study the effect of drought stress on growth, photosynthetic pigments, relative water content (RWC), electrolyte leakage (EL), membrane stability, proline and total antioxidant activity of six Egyptian rice cultivars to assign the most tolerant cultivar to drought stress. This comparative study may also be helpful in developing a better understanding and provide additional information about the mechanisms underlying drought stress tolerance in rice.

Materials and Methods

This experiment was carried out in botanical garden, Faculty of Science, Ain Shams University on summer season of 2015/2016. Six rice cultivars (G177, G178, G179, G182, Sk101 and Sk106) were planted in plastic pots (12 cm in diameter 10 cm long), each pot filled with 1K of soil (clay: sand) (2:1 w/w). Ten grains were sown per pot and irrigated with water to 80% of soil water holding capacity and exposed to normal daylight and temperature. Ten days old seedlings were irrigated with Hoagland's nutrient solution [18], once a week, and water holding capacity of each pot was kept at 80% till 21 days old seedlings, each pot set was divided to two groups one of them was irrigated with 80% water holding capacity (control), other irrigated with 40% water holding capacity (drought stressed seedlings) for 7 days (until leaf rolling symptoms were observed as a drought stress indicator). At the end of experiment, 28 days old seedling were collected and growth criteria of different cultivars was measured including seed germination percentage, percentage of survived plant, shoot and root length, root/shoot ratio, fresh and dry weights for shoot, root and whole plant, leaf area and number of leaves per plant. In addition, photosynthetic pigments, leaf relative water content, lipid peroxidation (as evaluated by malondialdehyde content), electrolyte leakage, membrane stability index, proline and antioxidant activity were determined.

Growth parameters

Ten seedlings from each treatment were sampled, shoots and roots were separated. Lengths and leaf area were measured, number of leaves per plant were counted and fresh weights (f.wt) of shoots and roots were recorded. For dry weight (d.wt) determination, samples were oven dried at 80°C for 72h and then weighed three times till constant weight.

Determination of relative water content (RWC)

Fresh weight of the second young leaf were weighed (f.wt.) and then cut to 1cm in length, and placed in 10 ml distilled water over night to rehydrate, the 2nd weight were recorded, called turgid weight (t.wt.), after that leaves were dried in oven for two days at 80°C and reweighed as dry weight (d.wt.) according to Basnayake., *et al* [19]. RWC was calculated according to the following equation

$$RWC = [f.wt. - d.wt.]/[t.wt. - d.wt.] \times 100$$

Where f.wt. = fresh weight of leaves, d.wt. = dry weight of leaves, t.wt. = turgor weight of leaves

Determination of electrolyte leakage (EL)

Electrolyte leakage is the measuring total leakage of inorganic ions from leaf cells by using method described by Sullivan and Ross [20].

Youngest leaves of plant seedlings were washed with deionized water, cut into twenty sections (1 cm in length) and were placed in 10 ml deionized water. Then heated twice in water bath at 45°C and 55°C for 30 minutes for each, each time the electrical conductivity (EC) was measured with a conductivity meter (ME977-C, Max Electronics, India). (ECa) and (ECb) were measured respectively, Consequently, the content were autoclaved for 10 minutes at 100°C and (ECc) was recorded. Finally, the following equation was used in electrolyte leakage calculations.

$$\text{Electrolyte leaakage (\%)} = \frac{(ECb-ECa)}{ECc \times 100}$$

Membrane stability index (MSI)

Youngest leaves tissues (0.2g) were washed by deionized water, cut into sections (1 cm length), placed in 10 ml deionized water and heated at 40°C in a water bath for 30 minutes, then the electrical conductivity (EC1) was measured by using a conductivity meter (ME977-C, Max Electronics, India). Subsequently, the content was boiled for 10 min in a boiling water bath (100°C) and the conductivity (EC2) was measured. Finally, by using formula described by Premchandra., *et al.* [21] and modified by Sairam [22], Membrane stability index (MSI) was calculated.

MSI = [1 - (EC1/EC2)] ×100

Extraction and determination of photosynthetic pigments

Leaf chlorophyll (a and b) and carotenoids content were determined by Spectrophotometric method recommended by Metzner., *et al* [23]. Fresh weight of leaves (1g) was homogenized in 85% aqueous acetone for 5 minutes. The homogenate was centrifuged and the supernatant was made up to 100 ml with 85% aqueous acetone. The absorbance was measured against a blank of pure 85% aqueous acetone at 3 wave lengths of 663, 644 and 452.5 nm using Spectrophotometer (Spectronic 601, Milton Roy Company). The concentration of chlorophyll a, chlorophyll b and carotenoids were determined respectively, as µg/ml using these equations:

Chlorophyll a = 10.3 E663 - 0.918 E644

Chlorophyll b =19.7 E644 - 3.870 E663

Carotenoids = 4.2 E452.5 - (0.0264 chlorophyll a + 0.426 chlorophyll b)

Finally, the pigment contents were calculated as µg/g f.wt of leaves.

Determination of lipid peroxidation as measured by malondialdehyde (MDA) content

The level of lipid peroxidation was quantified in terms of malondialdehyde (MDA) by using the method of Heath and Packer [24]. A (200 mg) fresh weight of plant sample was homogenized in 2.5 ml of water. The homogenate was mixed with the same volume of a 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) trichloroacetic acid. The mixture was heated at 95°C for 30 minutes and then quickly cooled in an ice-bath and centrifuged at 3000×g for 5 minutes and the absorbance of the supernatant was measured at 532 and 600 nm using Spectrophotometer (Spectronic 601, Milton Roy Company). The MDA concentration was determined by dividing the difference in absorbance (A532 - A600) by its molar extinction coefficient (155 mM^{-1} cm^{-1}) and the result was expressed as nmol/g fresh weight.

Determination of proline

Free proline was determined according to the method described by Bates., *et al* [25]. Half gram fresh weight of plant tissue was homogenized in 10 ml of 3% sulfo-salicylic acid and the homogenate was filtered through Whatman No.1 filter paper and the filtrate was made up to 10 ml. Two ml of the filtrate were taken with 2 ml ninhydrin reagent and 2 ml glacial acetic acid. The mixture was kept in boiling water bath for one hour. The mixture was cooled in ice bath. After that, the reaction mixture was extracted with 4 ml of toluene, shacked vigorously for 15 - 20 seconds. The mixture was left to separate in a separating funnel. The upper phase was taken at room temperature and the absorbance was measured at 520 nm using Spectrophotometer (Spectronic 601, Milton Roy Company). Toluene used as a blank. Proline concentration was determined from a standard curve of proline and calculated as µg/g fresh weight of the plant

Determination of total antioxidant activity (radical-scavenging activity)

The radical-scavenging activity of plant samples was determined using 1, 1, diphenyl-2-picryhydrazyl (DPPH) as a free radical [26].

Fresh plant samples (1g) were homogenized in methanol, then filtered through filter paper Whatman No. 2. and complete total volume up to 5 ml. 0.5 ml of methanolic solution of DPPH (0.1 mM) was added to 1.5 ml of methanolic extract, the mixture was shaken and allowed to stand for 30 min in the dark at room temperature. A decrease in absorbance of these extracts was measured at 517 nm using a Spectrophotometer (Spectronic 601, Milton Roy Company). Blank is DPPH solution without extract.

DPPH free radical scavenging ability (%) was calculated by the following formula:

$$\% \text{ scavenging activity} = 100 \times \frac{(\text{A control} - \text{A sample})}{(\text{A control})}$$

Statistical analysis

The experimental results were analyzed with the Statistical Package for Social Sciences (SPSS) v 20.0 software (SPSS Inc., Chicago, Illinois, USA), and analysis of variance (ANOVA) was used for statistical analysis. The means of the various results were tested for level of significance by Duncan's test. Statistical significance was accepted at $p < 0.05$.

Results

Shoot length of all cultivars was significantly decreased under drought stress. The least pronounced decrease was recorded in cultivar G182 and evaluated by 3.67% below the control, followed by Sk106, G179, G178 and G177 with 7.55%, 8.9%, 14.57% and 21.83%, below the corresponding control, respectively. While Sk101 recorded the most sever decrease in shoot length (34.5%) as compared with control (Figure 1a). Root length showed different responses in different cultivars. The highest increase in root length induced by drought was obtained in G178 (60%), followed by G179, G177 and then G182 (29.8%, 22.6% and 8.14%, respectively). In contrast, stressed Sk101 and Sk106 showed a significant decrease in root length (14.5% and 43.3%, respectively) (Figure 1b). Moreover, leaf area adversely affected by drought. Here, cultivars arranged in ascending manner from less to most affected cultivars: G182, G178, Sk106, G179, G177 and Sk101 with 38.1%, 39.95%, 42.5%, 51.3%, 52.4% and 63.4%, respectively as compared with control (Figure 1c). Numbers of leaves per plant also negatively affected by drought treatment, the results also represented in Figure (1d). G179 and Sk106 come in front in terms of less affected by drought (7.28% and 7.6%, respectively). While G177, G182, Sk101 and G178 comes in next order with 18.7%, 31.14%, 33.4 and 35.3% decrease, respectively.

In addition, fresh weight of rice seedlings showed significant decrease in all cultivars as compared with control (Figure 1e). G179 record the lowest decrease (32.56%), followed by G178, G182, G177 and sk106 (47.37%, 53.3%, 56.6% and 61.9%) respectively. Otherwise, Sk101 record (77.78%) the most decrease in fresh weight of shoot system. Drought stress also had negative effect on dry weight of shoot system in all cultivars (Figure 1f). The least cultivar affected by drought is G178 and record (12.5%) as compared to control. G182, G179, Sk106, G177 comes after it in arrangement (14.29%, 22.2%, 25% and 28.57%) respectively, and Sk101 come in the last order with (50%) decrease in dry weight of shoot system comparing with control. Fresh weight of root of different cultivars showing conflicting reactions; Giza 178, G179, G177 record significant increase (133.3%, 80%, 13.3% respectively) as compared with control. While treated G182 not changed comparing with control. Conversely, Sk101 and Sk106 negatively affected by drought treatment and decreased by (25% and 50%) respectively (Figure 1g). As well as, dry weight of root of rice cultivars that Also showing different responses to drought, where stressed cultivars (Giza 178, G179, G177 and G182) show enhancement in their dry weight of root by percent (300, 33.3, 20 and 16.67%) respectively. On the other hand, Sk101 and Sk106 show significant decrease in their root dry weight by percent (40 and 60%, respectively) as compared with control (Figure 1h).

Figure (2a-2d) demonstrated the changes in photosynthetic pigments content in the leaves of *Oryza sativa* plants responding to drought treatment. Generally, significant decrease in chlorophyll a and chlorophyll b was observed in leaves of all cultivars. G179 show slightly decrease from the control where chlorophyll (a) and chlorophyll (b) record (15.2 and 16.3% respectively), while Sk101 recorded (50.9 and 48.7% respectively), is the most inhibition in chlorophyll (a) and (b) content among all cultivars. Moreover, the ratio of chlorophyll (a/b) show significant increase in most cultivars except G179 does not show changes comparing to control. Whereas Sk106 record (29%), the highest increase in this ratio among all cultivars. In contrast, carotenoids show significant increase in all cultivars comparing with control. Among six cultivars, G177 record (1.75%) less increase in carotenoid content. While top result (27.1%) recorded by Sk101.

Drought stress had negative effect on R.W.C of treated cultivars, and these results showed in figure 2e. G179 is less affected cultivar by 22.4% below the control. Next, G178, G177, G182, Sk106 and Sk101 by 22.85%, 24.1%, 24.4%, 43.4% and 50% respectively. Sk101 is the most affected cultivar comparing with control plants. Moreover, MDA contents show highly significant increase in response to drought stress in treated cultivars except G179 that increased by 58.14% as compared with control and consider less affected cultivar. While other cultivars G178, G177, Sk101, G182 and Sk106 recorded (226.3%, 333.3%, 381.7%, 437.3% and 458.67%, respectively) comparing with control (Figure 2f). While more, Electrolyte leakage show significantly increase in all cultivar in response to drought treatment and these results presented in figure 2g. The less responded cultivar was (G179) which record (22.7%) increase in EL comparing with control. Followed by G178, G177, G182, Sk106 and Sk101 in last order, by 53.9, 74.8, 76.07, 106.4 and 150.8% increase over the unstressed control, respectively. While MSI decrease significantly in rice seedling exposed to drought comparing with control. Treated G179 record the nearest value to control (only 4.2% decrease in MSI compared to control). whereas the other cultivars; G178, G177, G182, Sk106 and Sk101 record 11.85%, 15.4%, 18.83%, 42.95% and 51.4%, respectively (Figure 2h). In addition, proline content increased significantly in all drought treated cultivars. Where G179 record the maximum increase in proline content (210.35% over the control), followed by G178, G182, G177, Sk106 and Sk101 come in last order by 149%, 130%, 111.5%, 38.7% and 28.6%, respectively above the control

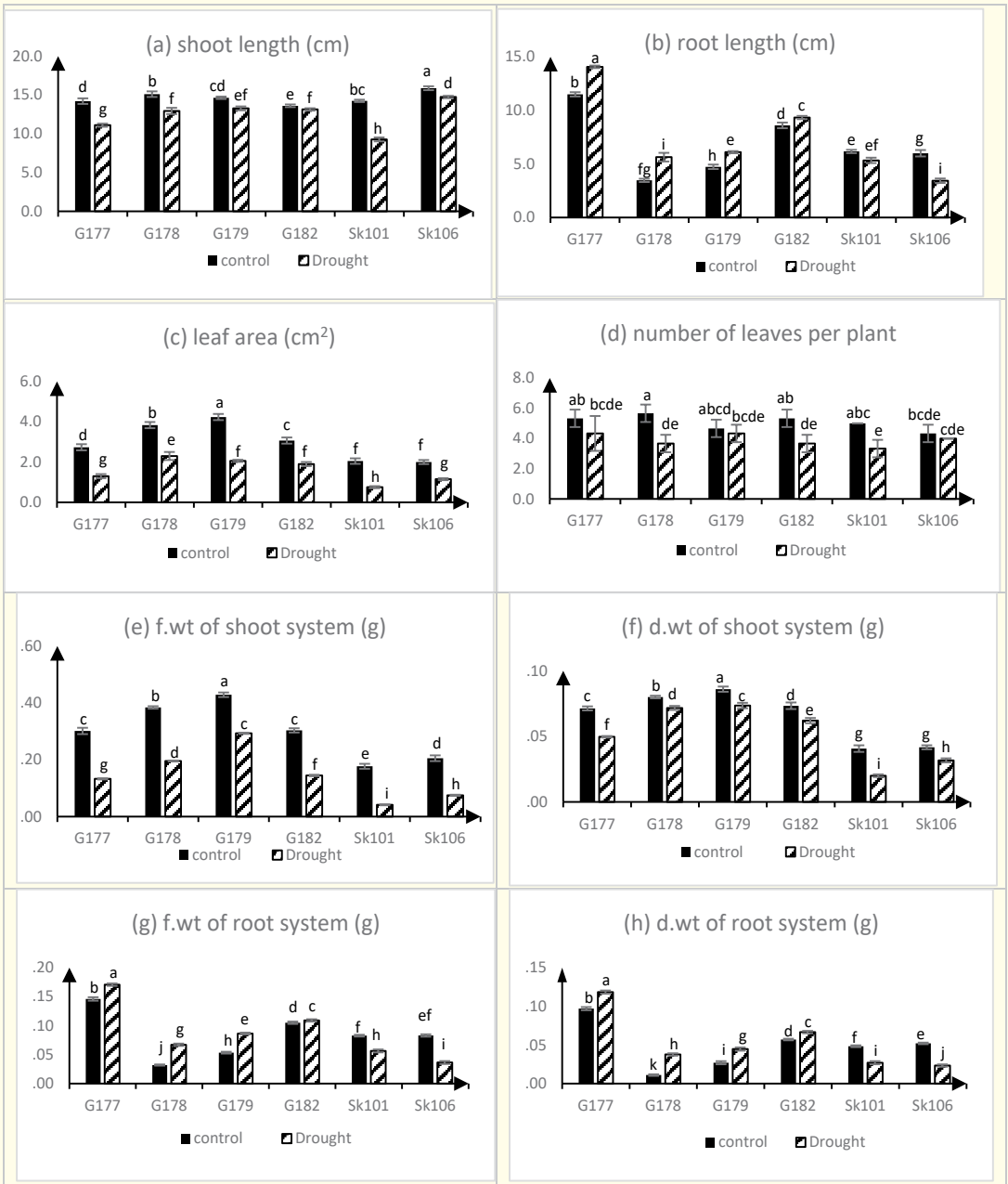

Figure 1: Changes in shoot length, root length, leaf area and number/plant, fresh and dry weight for shoot and root systems in response to drought treatment (40% WHC) for 7 days of six rice cultivars differing in drought tolerance.

value (Figure 2i). Total antioxidant activity of treated plants also show significant increase as compared with control. G179 ranked first with 213.75% over the control, followed by G178, G182, G177, Sk106 and in last order Sk101 by 104.5%, 101.5%, 94.48%, 53.55% and 49.89% increase, respectively (Figure 2j).

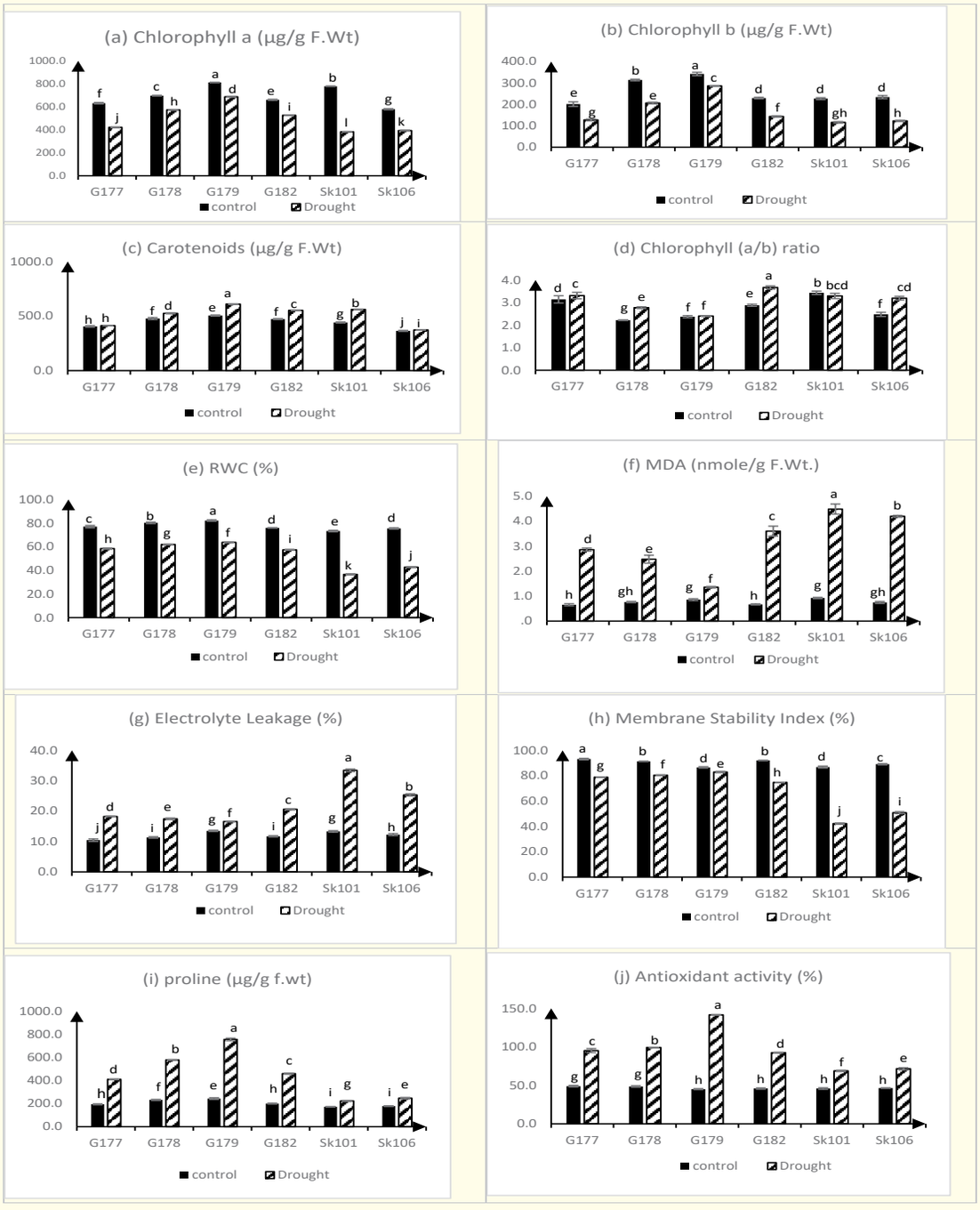

Figure 2: Changes in chl. a, chl. b, carotenoids, chl. (a/b), RWC, MDA, Electrolyte leakage, Membrane stability index, proline and anti-oxidant activity in response to in response to drought treatment (40% WHC) for 7 days of six rice cultivars differing in drought tolerance.

Discussion

In this work, we analyzed several physiological parameters in six Egyptian rice cultivars differing in their drought tolerance. As shown from the above results, rice cultivars have variant responses to abiotic stress due to complicated interaction between stress and molecular, biochemical and physiological responses of plant and its effect on growth and development [27].

All cultivars under study showed significant decrease in their RWC, the least affected cultivar is G179, while Sk101 considered the most affected one. These results suggested that drought stress

imposed more negative impact on water balance of plant through close of stomata which lead to decrease absorption of water and minerals from soil, reduce water potential and turgor pressure of cells, resulting in impair cell elongation and cell division [12,28]. Therefore, morphological criteria also adversely affected by drought, and this clearly observed from experiment results, only two cultivars (G178 and G179) show enhancement in root length and dry weight. These results are consistent with Larkunthod., *et al.* [12] who observe that all rice cultivars under study showed reduction in fresh and dry weight, when exposed to 20% PEG 6000 (polyethylene glycol) for 7 and 14d.

Chlorophyll is the most important components of photosynthesis, as photosynthetic rate has been proved to be linearly correlated to chlorophyll content [29]. In the studied rice cultivars, chlorophyll content exhibited considerable sensitivity under drought stress in all cultivars. Cultivar G179 was the least affected one, as it recorded 15.27% decrease in chlorophyll a, 16.36% decrease in chlorophyll b, and 20.8% increase in carotenoids content, as compared with control. Moreover, Sk101 recorded 50.8% decrease in chlorophyll a, 48.74% decrease in chlorophyll b, and 27.1% increase in carotenoids content, comparing with control. The main reason beyond negative impact of drought on chlorophyll is ABA accumulation in guard cells which induce stomatal closure, leading to limited gas exchange, declined CO_2 assimilation resulting in photosynthesis restriction or ceases [2]. Consequently, photo-oxidation rate increased, reactive oxygen species (ROS) (such as O_2^- and H_2O_2) produced and reacted with chloroplast membrane [30], leading to lipid peroxidation, destruction of chloroplast and lost viability, the end result is conversion of leaf color from green to yellow, resulting in further reflectance of the incidence radiation [31,32]. In our experiment, carotenoids content was increased in all cultivars under study, the highest value obtained by Sk101 whereas G177 recorded the least increase. This negative effect on photosynthesis leading to decline in plant nutrient, and decreasing in plant height, leaf area and yield of plant [33]. Moreover, Dalal and Tripathy [34] found that PEG-induced drought stress decreased chlorophyll content of rice seedling. Endang., *et al.* [35] reported that total chlorophyll declined with increasing water deficit stress, total chlorophyll decrease by percent (4.2 - 93.6%) comparing with plants grown under saturated condition.

ROS not only disrupt chloroplast membrane but also any membrane in cell (such as plasma membrane, mitochondrial and ribosomal membranes). Therefore, lipid peroxidation and permeability increased as well as cell lost its integrity [36]. Results of this experiment revealed that membrane integrity of G179 is well protected against drought stress where membrane stability index decreased by percent 4.2% comparing with its control and this considered the least decrease among all cultivars. Electrolyte leakage of treated G179 record 22.7% increase and this also considered the best value among all cultivars under study. Where electrolyte leakage value reflect the plasma membrane damage by stresses. MDA content reflects the lipid peroxidation level resulting from oxidative stress, considered decomposition product of polyunsaturated fatty acids [33], G179 record the less increase among other cultivars. On the other hand, Sk101 show the most affected cultivar where record the highest increase in electrolyte leakage and decrease in membrane stability index by as compared with control. As well as increase in malondialdehyde by percent 381% comparing with control, these results agreed with those obtained by Larkunthod., *et al* [12].

One of the most important mechanisms to counteract damage resulting from oxidative stress in tolerant plants is developing enzymatic and non-enzymatic free radical scavenger molecules, which react with ROS and converted it into safe molecules to cells [37]. Generally, increase in antioxidant activity enhance the stability of plant to survive against adverse effect of oxidative stress [38]. In this study, all cultivars show increase in antioxidant activity, the most pronounced value was recorded in G179, while the least value recorded in Sk101 as compared with their control. This in consistent with Endang., *et al.* [35] who reported that as compared with plant grown under saturated condition, drought stress cause an increase in antioxidant activity.

The ability of rice plant to maintain turgor pressure is another important tolerant mechanism against drought stress, and this occur when osmotic potential decrease by enhancement of compatible solutes accumulation such as sugars, amides, amino acid, especially proline [39]. Proline considered one of the most common osmolytes, act as an osmoprotectant, defend macromolecules from denaturation [40], also act as antioxidant scavenging molecules [41], which reduces cell acidity [42]. As well as, proline protect plant against stress via enhancement expression of some genes which play important role in plant protection and defense, modulation of mitochondrial function and its effect in cell propagation [13]. In our study, proline show enhancement in all cultivars, G179 record the most enhancement value while, Sk101 record the least increase comparing with control plants. These results agreed with those obtained by Endang., *et al*. [35] who reported that proline content increased under drought stress in leaves of rice cultivars under study.

Conclusion

In conclusion, drought stress caused oxidative damage to all the studied rice cultivars that affect their growth, photosynthetic pigments content and membrane stability. Based on the present study, G179 is a highly drought tolerant cultivar that showed better performance among all the studied rice cultivars, and Sk 101 is the most sensitive one. The amounts of drought -induced proline and total antioxidant activity were found to be directly related to drought tolerance in rice. Further metabolomic studies are required to highlight the differential defense mechanisms induced in sensitive and tolerant rice cultivars against drought stress.

Bibliography

1. Anis G., *et al*. "Evaluation of promising lines in rice (Oryza sativa L.) to agronomic and genetic performance under Egyptian condition". *International Journal of Agronomy and Agricultural Research* 8.3 (2016): 52-57.

2. Moonmoon S., *et al*. "Effect of Drought Stress on Grain Dry Weight, Photosynthesis and Chlorophyll in Six Rice Genotypes". *Scholars Journal of Agriculture and Veterinary Sciences* 4.1 (2017): 13-17.

3. Mishra B and Chaturvedi G. "Flowering Stage Drought Stress Resistance in Upland Rice in Relation to Physiological, Biochemical Traits and Yield". *International Journal of Current Microbiology and Applied Sciences* 7.2 (2018): 71-82.

4. Gina K and Barbara B. "Analysis of food composition data on rice from a plant genetic resources perspective". *Food Chemistry* 80.4 (2003): 589-596.

5. IRRI. "Bringing hope, improving lives-strategies. Plan 2007-2015". International Rice Research Institute, Los Banos (2006): 61.

6. RKB. Rural knowledge Bank International Rice Research Institute, Los Banos (via internet) (2015).

7. Omar M and Moussa A. "Water management in Egypt for facing the future challenges". *Journal of Advanced Research* 7.3 (2016): 403-412.

8. Hassan HM., *et al*. "Performance of some rice genotypes under both salinity and water stress conditions in Egypt". *Journal of Plant Production* 4.8 (2013): 1235-1257.

9. Farid M., *et al*. "Genetical and molecular studies on salinity and drought tolerance in rice (Oryza sativa L.)". *Journal of Agricultural Research* 42.2 (2016): 1-23.

10. Abd Allah A., *et al*. "Response of some root and yield traits to water stress for some rice varieties". *Journal of Agricultural Research* 42.3 (2016): 352-364.

11. Chen W., *et al*. "Silicon Alleviates Drought Stress of Rice Plants by Improving Plant Water Status, Photosynthesis and Mineral Nutrient Absorption". *Biological Trace Element Research* 142.1 (2011): 67-76.

12. Larkunthod P., *et al*. "Physiological Responses under Drought Stress of Improved Drought Tolerant Rice Lines and their Parents". *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 46.2 (2018): 679-687.

13. Maisura Muhamad A., *et al*. "Some physiological character responses of rice under drought conditions in a paddy system". *Journal of ISSAAS* 20.1 (2014): 104-114.

14. Lum MS., *et al*. "Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice". *Journal of Animal and Plant Sciences* 24.5 (2014): 1487-1493.

15. Maria H. "Drought stress and reactive oxygen species (Production, scavenging and signaling)". *Plant Signaling and Behavior* 3.3 (2008): 156-165.

16. Bhuiyan M., *et al*. "Parental line selection for yield contributing character of rice (Oryza sativa)". *International Journal of Agricultural Research, Innovation and Technology* 7.1 (2017): 46-50.

17. Simon S and Korukkanvilakath S. "Screening for Osmotic Stress Responses in Rice Varieties under Drought Condition". *Rice Science* 24.5 (2017): 253-263.

18. Hoagland DR and DI Arnon. "The water culture method for growing plant without soil". California Agricultural Experiment Station. Cir. No. 347. University of California Berkley Press, CA (1950): 347.

19. Basnayake J., *et al*. "Genotypic variation of osmotic adjustment and desiccation tolerance in contrasting sorghum inbred lines". *Field Crops Research* 35.1 (1993): 51-62.

20. Sullivan CY and ROSS WM. "Selecting for drought and heat resistance in grain sorghum". In H Mussell and R Staples (eds). Stress physiology in crop plants. John Wiley and Sons. Inc (1979): 263-281.

21. Premchandra GS., *et al*. "Cell membrane stability an indicator of drought tolerance as affected by applied nitrogen in soybean". *Journal of Agriculture Sciences* 115.1 (1990): 63-66.

22. Sairam RK. "Effect of moisture stress on physiological activities of two contrasting wheat genotypes". *Indian Journal of Experimental Biology* 31 (1994): 594-597.

23. Metzner H., *et al*. "Untersuchungen zur Synchronisierbarkeit einzelner Pigmentmangel Mutanten von Chlorella". *Planta* 65.2 (1965): 186-194.

24. Heath RL and Packer L. "Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation". *Archives of Biochemistry and Biophysics* 125.1 (1968): 189-198.

25. Bates L., *et al*. "Rapid determination of free proline for water-stress studies". *Plant and Soil* 39.1 (1973): 205-207.

26. Brand-Williams W., *et al*. "Use of a free radical method to evaluate antioxidant activity". *LWT Food Science and Technology* 28.1 (1995): 25-30.

27. Meena KK., *et al*. "Abiotic Stress Responses and Microbe-Mediated Mitigation in Plants: The Omics Strategies". *Frontiers in Plant Science* 8 (2017): 172.

28. Amini H., *et al*. "Effect of water deficiency on seed quality and physiological traits of different safflower genotypes". *Turkish Journal of Biology* 38.2 (2014): 271-282.

29. Li Rong-hua., *et al*. "Evaluation of Chlorophyll Content and Fluorescence Parameters as Indicators of Drought Tolerance in Barley". *Agricultural Sciences in China* 5.10 (2006): 751-757.

30. Lisar S., *et al*. "Water stress in plants: causes, effects and responses". In Water Stress; Rahman M, Hasegawa H, Eds.; In-Tech: Rijeka, Croatia (2012): 1-14.

31. Ahmadikhah A and Amir M. "Effect of reduced plant height on drought tolerance in rice". *Biotech* 6.2 (2016): 221.

32. Schlemmer MR., *et al*. "Remotely Measuring Chlorophyll Content in Corn Leaves with Differing Nitrogen Levels and Relative Water Content". *Agronomy Journal* 79 (2005): 106-112.

33. Zain N., *et al*. "Impact of cyclic water stress on growth, physiological responses and yield of rice (Oryza sativa L.) grown in tropical environment". *Ciência Rural* 44.12 (2014): 2136-2141.

34. Dalal V and Tripathy B. "Modulation of chlorophyll biosynthesis by water stress in rice seedlings during chloroplast biogenesis". *Plant, Cell and Environment* 35.9 (2012): 1685-1703.

35. Endang DP., *et al*. "Growth, yield and physiological characters of three types of Indonesian rice under limited water supply". *Asian Journal of Plant Sciences* 16.2 (2017): 101-108.

36. Gaschler MM and Stockwell BR. "Lipid peroxidation in cell death". *Biochemical and Biophysical Research Communications* 482.3 (2017): 419-425.

37. Hojati M., *et al*. "Responses of growth and antioxidant systems in Carthamus tinctorius L. under water deficit stress". *Acta Physiologiae Plantarum* 33.1 (2011): 105-112.

38. Kamel M., *et al*. "Effect of drought on safflower natural dyes and their biological activities". *EXCLI Journal* 13 (2014): 1-18.

39. Nikolaeva MK., *et al*. "Effect of Drought on Chlorophyll Content and Antioxidant Enzyme Activities in Leaves of Three Wheat Cultivars Varying in Productivity". *Russian Journal of Plant Physiology* 57.1 (2010): 94-102.

40. Sharma SS and Dietz KJ. "The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress". *Journal of Experimental Botany* 57.4 (2006): 711-526.

41. Liang X., *et al*. "Proline mechanisms of stress survival". *Antioxidants and Redox Signaling* 19.9 (2013): 998-1011.

42. Kishor P., *et al*. "Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance". *Current Science* 88.3 (2005): 424-438.

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