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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF RICE (*ORYZA SATIVA* L.) VARIETIES AGAINST DROUGHT STRESS

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Abstract

Physiological and biochemical responses of six different rice (*Oryza sativa* L.) varieties cultivated in Turkey and two local varieties, namely Karacadağ and Hazro to the drought were investigated. After 12 days of drought treatment, the highest and lowest OP values were observed in Osmancık-97 (-1.14 MPa) and Karacadağ (-1.55 MPa) varieties, respectively. In the same treatment, it was observed that the amount of proline increased 19.9-fold in Karacadağ and 3.6-fold in Osmancık-97. When the data obtained from all parameters were correlated with drought stress tolerance, Osmancık-97 and Beşer varieties were considered to be tolerant, while Gönen and Karacadağ varieties are considered to be more sensitive.

Introduction

Drought, one of the abiotic stress factors, is considered to be the most destructive stress factor that reduces productivity in agricultural production compared to other environmental stresses (Lambers *et al.* 2008). Under the drought conditions, plants give a series of physiological and biochemical responses to maximize water utilization in the environment as well as to retain the water present in their cells. The relation between drought stress and relative water content or osmotic potential was examined in some plant species (Zhang *et al.* 2010), who reported that the RWC and OP of plants decrease due to the drought effect. Plant cells exposed to drought try to regulate their OP by accumulating certain osmolytes in order to maintain turgor conditions (Vinod 2012). Proline, one of the best-known osmolytes, acts as a mediator in osmotic regulation to preserve the integrity of the plasma membrane and while at the same time protecting the cell from the harmful effects of reactive oxygen species (ROS) (Valliyodan and Nguyen 2006).

Rice widely cultivated tropical and subtropical climatic conditions, in South and Southeast Asia and Africa makes its place among warm climate cereals. It is one-year plant, being one of the most important cereal crops used by more than half of the world's population as a nutritional source. Rice is sensitive to the drought stress factor and shows a wide scale in tolerance to drought between species, subspecies and wild species due to genetic variations (Lafitte and Vijayakumar 2006). Studies on the development of drought-tolerant rice varieties were reported to be one of the key areas of study for the next years (Valliyodan and Nguyen 2006).

Approximately 40% of the agricultural areas on the earth are arid, semi-arid and have low precipitation regimes. It has been reported that the preference of plant species or varieties which have higher yields and use the water more effectively in areas where water availability is limited provide great economic advantages in agricultural terms (Hirt and Shinozaki 2004). So far there was no report on the drought tolerance of rice varieties grown in Turkey. Thus the physiological and biochemical responses of eight varieties of rice to drought stress tolerance investigated.

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Materials and Methods

This study was carried out in the Biological Research Laboratory of Batman University. Six varieties of rice seeds (Beşer, Paşalı, Osmancık-97, Kızıltan, Halilbey, Gönen) were obtained from the Thrace Agricultural Research Institute while Karacadağ and Hazro varieties were collected from local producers. Germination and growth of the seeds were observed in a growth chamber with photoperiod set at 25 ± 2ºC and 16 hrs light/8 hrs darkness. Seeds planted in the soil were watered according to the field capacity with ¼ Hoagland nutrient solution for 28 days. Well-irrigation plants were used as control and control plants were watered every other day to field capacity. Plants undergoing drought stress treatment were exposed to 12 day drought stress by without irrigation. Samples were harvested at the 0, 3, 6, 9, and 12th day during drought stress. The plants were harvested and the aerial and roots parts were separated from each other. The fresh weights (FW), dry weights (DW) of plants were weighed, and root lengths were measured. RWC of the plants was calculated using three values and were measured according to the formula proposed by Hu *et al.* (2010). The leaf osmotic potential of rice varieties was determined according to Ball and Oosterhuis (2005) with an osmometer (Wescor 5520). One g of leaf sample was taken and crushed in a 2 ml ependorf with glass baguette and centrifuged at 5000 rpm for 10 min. The 10 µl of the fluid accumulated at the bottom of Ependorf were used to determine leaf osmotic potential. Carotenoid content was determined according to Arnon (1949). The 0.25 g fresh leaf samples were taken and extracted with 80% acetone. The absorbances of the extracts were measured by spectrophotometer at 480 nm and recorder.

The content of proline was determined spectrophotometrically by the Acid-Ninhydrin method (Ghoulam *et al.* 2002). The 100 mg of the plant sample was homogenized with 2 ml of 40% methanol. The amount of proline was calculated with the graph using the L-proline standard.

The one-way analysis of variance (ANOVA) and Duncan's test were performed on the data obtained from the analyses using the SPSS 21.0 statistical analysis program. The $p \leq 0.05$ value was accepted as statistically significant.

Results and Discussion

The fresh weight value was found to decrease in all varieties under the effect of the applied stress factor. The rates of decrease in the FW of varieties after 12 days of treatment were the highest in Karacadağ with 80.2%, and the lowest in Osmancık-97 with 42.8%. Unlike the other varieties, a significant decrease was not observed in the FW of Osmancık-97 and Beşer varieties until the 9th day, but the 12-day treatment caused a decrease in the fresh weight of both varieties (Fig. 1A). As seen in Fig. 1B, there were no statistically significant changes in the Osmancık-97, Gönen, Halilbey and Paşalı varieties. However, there was an increase only in Beşer while the DW of Karacadağ, Hazro and Kızıltan decreased compared to the control group. The highest rate of increase in the total DW was observed in Beşer variety with 42.3%, and the highest decrease was observed in Karacadağ variety with 47.8%.

The increase of biomass in plants relates to the organic matter synthesis, in other words, the photosynthetic capacity. Under stress conditions, closure of stomata and inhibition of gas exchange cause reduction of photosynthetic capacity. As a result, it is considered that the restriction of the photosynthetic capacity of the rice varieties which grow under stress conditions causes a decrease in the DW of the plant. Additionally, water losses directly lead to a decrease in the fresh weights of plants, since approximately 80-95% of the plant consists of water. Therefore, it can be said that after the drought treatment in the present study, decreases in the FW of the varieties were associated with the water loss. The present results are more or less similar to the findings of Khoyerdi *et al.* (2016) who reported that the drought effect causes a decrease in the amount of fresh and dry weight.

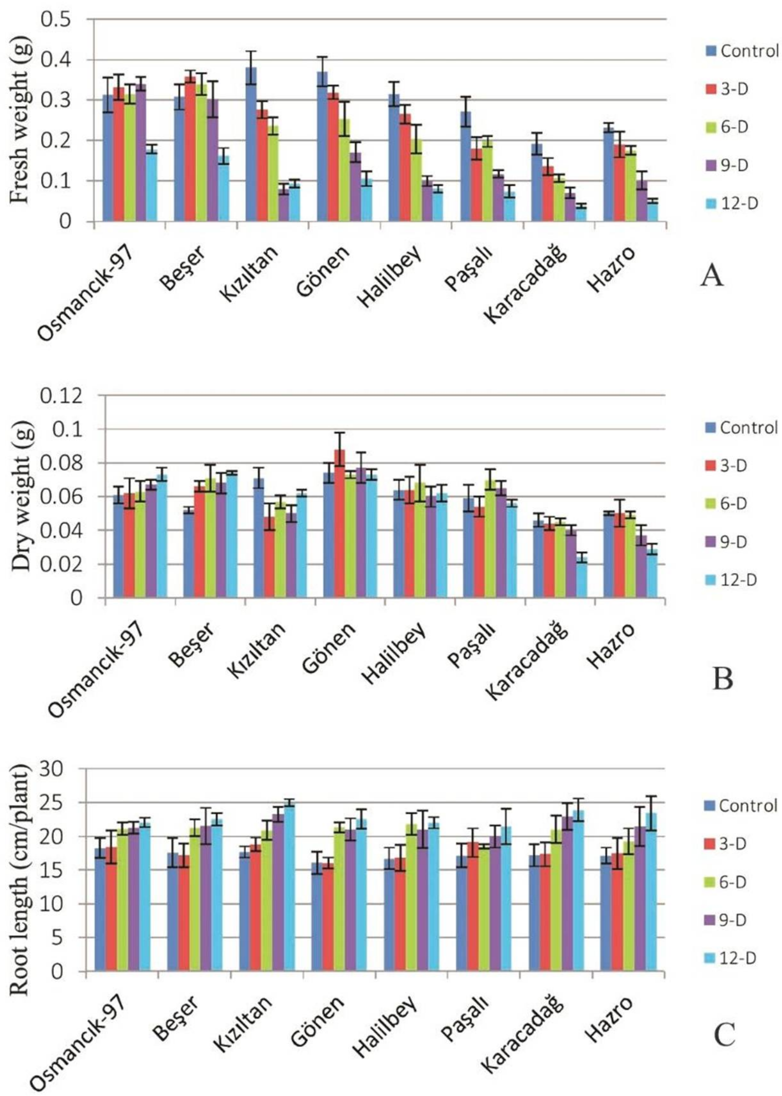

Fig. 1. Effect of drought stress on fresh weight (A), dry weight (B) and root length (C) in rice varieties. Vertical bars indicate ± SE (D-day).

It is known that plants increase their root lengths in order to effectively use the water in the soil in case of drought. In this study, it was observed that the 3-day treatment was not significantly effective on the root growth, but the root growth increased significantly in all varieties after the 3rd day depending on the increase in drought severity. In all rice varieties, the longest root length was observed in the 12-day stress groups, which were exposed to the most severe drought treatment (Fig. 1C). When the root lengths of the varieties were compared with their control groups, the highest increase rate (7.32 cm, 41.3%) was observed in Kızıltan and the lowest increase rate (3.81 cm, 20.8%) was observed in Osmancık-97. The most evident example of morphological changes is the acceleration of root growth and increased proportion of root/stem, in plants for more efficient use of soil water under drought conditions (Verslues *et al.* 2006). According to the results of the present study, the increase in root length of rice varieties is accepted as a response to the drought factor in order for plants to be able to reach the water in the depths of the soil. Similar to the results in this study, the increased root lengths in cases of water deficiency were reported by Kato *et al.* (2007) in six different rice genotypes and by Werner *et al.* (2010) in the transgenic tobacco plant. When the data obtained from the growth parameters were evaluated, it was observed that Osmancık-97 and Beşer varieties had the highest value in terms of FW and the minimum increase in terms of the root length was observed. These results suggest that Osmancık-97 and Beşer were more tolerant to drought than the other varieties. It was observed that the root length of the varieties of Kızıltan, Gönen and Karacadağ has increased more in order to use the water efficiently. This result indicates that they are more affected by the drought stress factor. Moreover, it can be said that Karacadağ rice variety is affected by drought stress more than the other varieties in terms of the FW-DW (Fig 1A, B).

In all rice varieties, when the 12-day drought treatment was compared with the control groups, the ratio of reduction in the RWC was founded 89.5% in Karacadağ, 88.1% in Gönen, 86.9% in Kızıltan, 65.6% in Beşer and 58.6% in Osmancık-97. As shown in Fig. 2, the highest RWC and the lowest value measured after the stress treatment were found in Osmancık-97 and Karacadağ varieties, respectively. The OP value of all the rice varieties exposed to stress decreased compared to the control groups. When the 12-day stress treatments were compared among themselves, the highest OP value was found in Osmancık-97 variety with -1.14 MPa, while the lowest value was in Karacadağ variety with -1.55 MPa (Fig. 3). Since the RWC and OP values are parameters directly expressing water conditions in plant tissues, in plants exposed to drought, it was to be a usual situation for the OP and RWC to decrease. Hu *et al.* (2010), Zhang *et al.* (2010), Chu *et al.* (2014) reported in their studies that the drought factor caused the RWC to

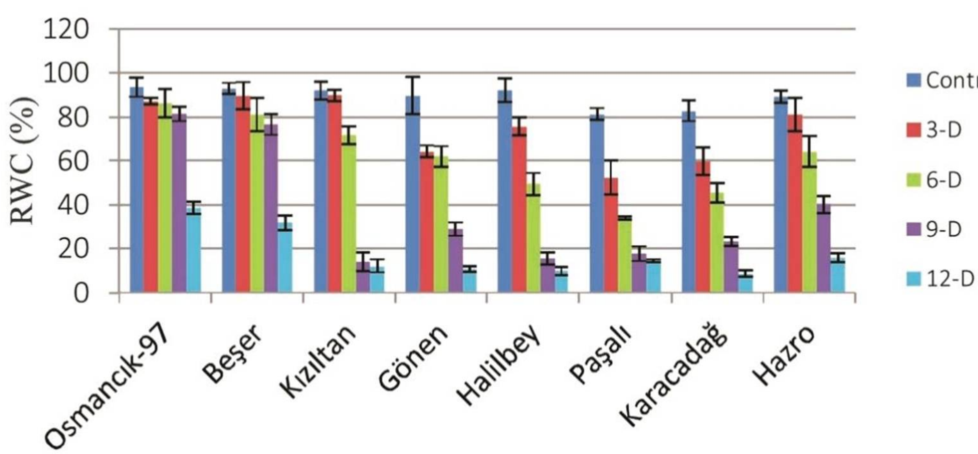

Fig. 2. Effect of drought stress on relative water content in rice varieties. Vertical bars indicate ± SE (D-day).

decrease in plants. Previously it was reported that the water content of varieties sensitive to drought was affected more in the tolerant/sensitive rice (Degenkolbe *et al.* 2009) exposed to the drought stress factor. It was also reported that plant tolerance to drought stress could be associated with different systems, including the ability to retain the high RWC (Oukarroum *et al.* 2007). In the present study, Osmancık-97 and Beşer varieties were considered tolerant since they were less affected in terms of the RWC and OP, while Karacadağ, Halilbey, and Gönen may be considered sensitive since they were the most affected varieties. When the results of the OP and RWC, which are among the analyses performed for measuring the plant's water status, were examined, it was observed that they support each other.

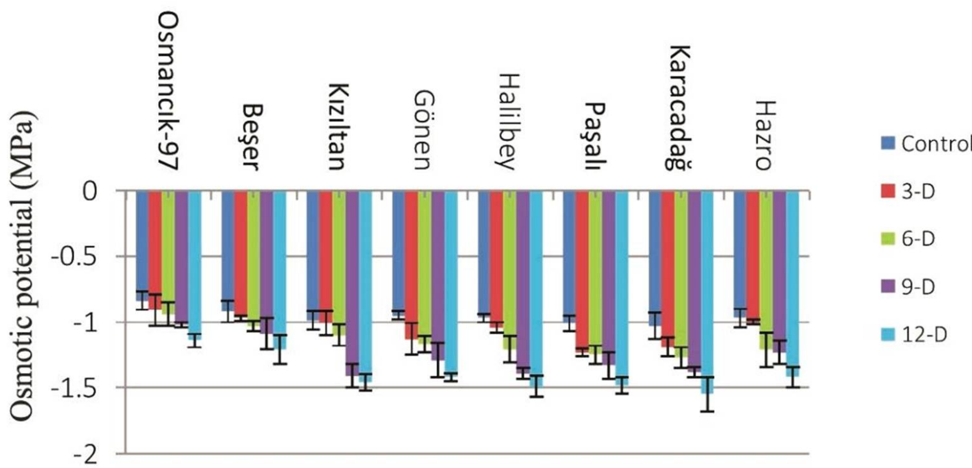

Fig. 3. Effect of drought stress on osmotic potential in rice varieties. Vertical bars indicate ± SE (D-day).

Statistically increases in the carotenoid content was observed in all varieties after the drought treatment compared to the control (Fig. 4A). The carotenoid amount (9.24, 9.73 µg/g, respectively) in Osmancık-97 and Beşer was found to be lower compared to the other varieties. The highest value in terms of carotenoid content was measured at 11.68 µg/g in Karacadağ. It is known that carotenoids constitute an important part of the antioxidant defense system because they stabilize triplet chlorophyll in stressed tissues, inhibit singlet oxygen production and protect plants from oxidative damage (Jaleel *et al.* 2009). According to the data obtained from the present study, the increase in the carotenoid amount is thought to occur to prevent oxidative stress in rice varieties. There are many studies suggesting that plants exposed to drought increased their carotenoid levels in order to cope with oxidative stress (Cai *et al.* 2005, Yang *et al.* 2015).

The drought treatment caused an increase in proline content in all rice varieties. However, in Osmancık-97 variety, it exhibited a similarity to the 3 and 6-day treatment control group. When the 12-day drought treatment of all varieties and their control groups were compared, it was observed that the proline content increased 19.9 fold in Karacadağ, 13.8 fold in Kızıltan, 9.7 fold in Gönen, 9.2 fold in Halilbey, 8.9 fold in Hazro, 8.4 fold in Paşalı, 5.5 fold in Beşer and 3.6 fold in Osmancık-97 (Fig. 4B). It can be said that the variety least affected by drought is Osmancık-97 and the variety most affected is Karacadağ.

It was reported that in some plant species under stress, proline accumulation correlates with stress tolerance and that proline concentration is higher in stress tolerant plants (Pyngrope *et al.* 2013; Orcan *et al.* 2019). However, although Osmancık-97 and Beşer were considered tolerant according to the previous parameters, their proline content was found to be low. When the RWC

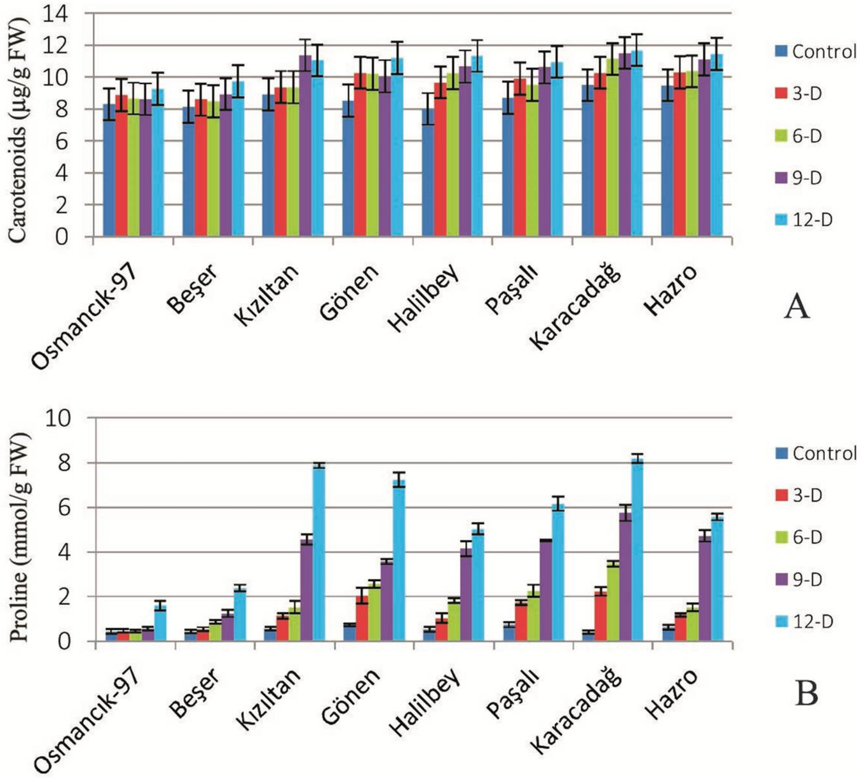

Fig. 4. Effect of drought stress on carotenoid (A), and proline content (B) in rice varieties. Vertical bars indicate ± SE (D-day).

and OP values of the varieties considered to be tolerant were examined, it was determined that both varieties did not lose much water and their water conditions were good. In this case, it is suggested that different osmolytes are accumulated instead of proline in the osmotic regulation carried out for the continuity of water intake in the plant. Results reported by Cia *et al.* (2012) on the sugar cane plant is similar to the results of the present study showing lower relative water content in varieties sensitive to drought compared to tolerant varieties, whereas proline content was higher in sensitive varieties.

From the present results it may infer that when the relative water contents of all rice varieties were compared, the highest RWC percentage was observed in Osmancık-97 and Beşer varieties, while the lowest RWC was observed in Karacadağ, Halilbey and Gönen varieties. After drought stress treatment, Halilbey, Karacadağ and Gönen varieties have the lowest osmotic potential value compared to their control groups, whereas the varieties with the highest osmotic potential value were determined as Osmancık-97 and Beşer. Moreover, as a result of drought application in Osmancık-97 and Beşer varieties, the amount of carotenoids was lower than the other varieties. The low amount of carotenoids in these varieties suggests that they are less affected by the drought stress. Minimum increase rate in terms of root length was observed in Osmancık-97 and Beşer cultivars. In addition, unlike other varieties, they had the highest value in terms of FW and an increase in DW and biomass. According to FW-DW data, it was determined that Karacadağ variety was more affected by drought stress than other varieties. When all parameters are evaluated together, Osmancık-97 and Beşer are considered to be more tolerant to drought and Karacadağ and Gönen varieties are considered to be more sensitive compared to the other varieties. Due to high paddy and rice yields, Osmancık-97 variety alone constitutes more than 80% of the total cultivation area in Turkey in recent years. The findings obtained may become a scientific reference for the development of high-yield and high-quality varieties that can adapt to climatic conditions and contribute to the creation of drought tolerant genotypes that can meet the demands of the country.

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