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Genotypic difference in response of chlorophyll content and peroxidase activities to salt stress in triticale

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Abstract

The goal of the present study was to determine the salinity resistance of 5 triticale genotypes. Salt-stress experiments were carried out under tissue culture conditions. Regenerated seedlings of five genotypes were exposed to 0 and 100 mM NaCl. Peroxidase activity and pigment content were determined for seedling applied with salt for 7, 14, and 21 days. Peroxidase activity was observed in the triticale seedlings of salt stress in in-vitro conditions. Melez 2001 and Mikham 2002 were determined to be more responsive to stress severity than the other genotypes in terms of chlorophyll content. Our results showed that there was a positive correlation between chlorophyll content and 100 mM concentration salt stress in salt-resistant triticale genotypes.

Keywords: Genotypic difference, Chlorophyll content, Peroxidase activities, Triticale.

Introduction

Salinity is one of the most significant abiotic factors decreasing the development and crop yield of agronomically important plants (1). To better improve cultivars against salt stress rely on the concerted efforts by different factors such as tissue physiology, gene transformation, and breeding. The use of new cell biology tools for elucidating the regulation functions of salt-stress resistance is based on the control of specific stress-related mechanisms (2). Hence, tissue culture is a very useful technique for improving salt stress-resistant plants. When a plant is exposed to salt severity, numerous transcription factors are activated, resulting in promoted degrees of several biomolecules and secondary metabolites (3). Salinity can causes serious injury and subsequently results in oxidative damage of enzymatic activities. NaCl stress resistance requires activation of physiological and metabolic structures to protect the intact cell from damaging mechanisms (4). Many types of research have been

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conducted on changes in enzyme activity in seedlings during stress conditions such as salt severity. Nevertheless, most of these studies have focused on salt-susceptible crops such as rice (5), chickpeas (6), or maize (7). A few reports are present on alters in peroxidase activity and pigment prediction during salt resistance in vitro, and its role in the improvement of salt resistance is not fully known. The goal of the present work was to determine the peroxidase activity and total pigment content of five triticale genotypes in terms of salt-stress resistance.

Material and Method

Salt-stress treatment: Seeds of 5 genotypes of triticale were provided by the East Anatolia Agricultural Research Institute, Erzurum. Mature seeds were sown in dishes (15 cm) containing the autoclaved half-strength MS medium and 0.6% (w/v) Phytagel (Sigma-Aldrich). Petri dishes (3 per treatment) contained 25 seeds each in growth chambers fluorescent light with 62 µmol m-2 s-1 and 16 h : 8 h light:dark cycle at 26 ± 1 °C. To determine seed germination in response to salt, seeds were sown in media containing 0 (control), and 100 mM NaCl. Seedlings were evaluated for accumulation of total pigment estimation and peroxidase enzyme activity.

Enzyme Activity: The POX activity was measured by monitoring the increase in absorbance at 470 nm in 50 mM phosphate buffer (pH 5.5) containing 1mM guaiacol and 0.5 mM H_2O_2 (8). One unit of POX activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01/min.

Pigment Estimation: Leaf chlorophylls (Chl) were extracted in 80% acetone and absorbance at 663 and 645 nm were measured. Chl a, Chl b, and total Chl contents were then calculated.

Statistical Analysis: Each experiment was repeated three times. Analysis of variance was conducted using the one-way ANOVA test using SPSS 13.0 and means were compared by the Duncan test at the 0.05 level of confidence.

Results

Peroxidase assay: Peroxidase increased in callus of all triticale cultivar under salt stress. A continuously increase in peroxidase was determined in stress time in all genotypes. The highest peroxidase value in 21 days was found in 'Tatlıcak' followed by 'Alper Bey', 'Mikham 2002', 'Melez 2001' and Ümran Hanım. Alper Bey and Ümran Hanım genotypes displayed a more increase in peroxidase dosage in 14. day, compared to 21. day (Figure 1). The highest peroxidase amount in 14 days, was also found in 'Tatlıcak', followed by 'Alper Bey', 'Ümran Hanım', 'Mikham 2001', and Melez 2001. Peroxidase values displayed a middle range of variation between genotypes in 7 days, ranging from 4.5 to 6.25 nmol g-1 FW (Figure 1). The lowest value of peroxidase in 7 days, was found in 'Ümran Hanım', and the lowest in both 'Mikham 2002' and 'Melez 2001'.

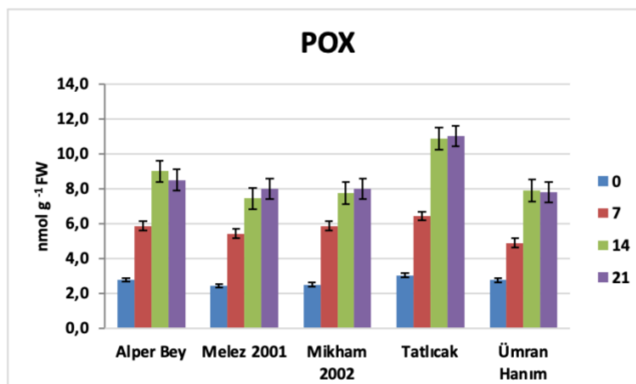

Figure 1. Changes of POX in five triticale genotypes treated with 100 mM salt stress in in vitro conditions.

Pigment Estimation: Chlorophyll content was different among triticale cultivars at salt stress. At salt stress, the highest chlorophyll content was obtained in Ümran Hanım, whereas the lowest was obtained in Mikham 2002 and Melez 2001 after 21 days at cold acclimation. Similarly, the same cultivars had the highest chlorophyll content at the salt stress for 7 and

14 days (Figure 2, 3, and 4).

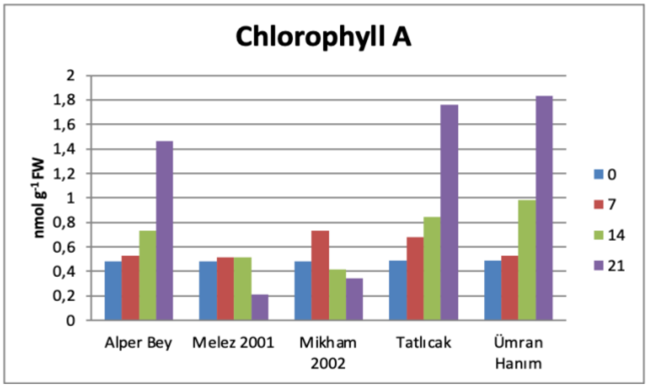

Figure 2. Changes of chlorophyll A in five triticale genotypes treated with 100 mM salt stress in in vitro conditions.

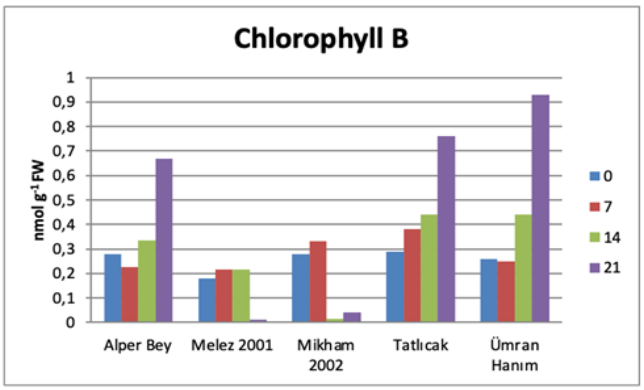

Figure 3. Changes of chlorophyll B in five triticale genotypes treated with 100 mM salt stress in in vitro conditions.

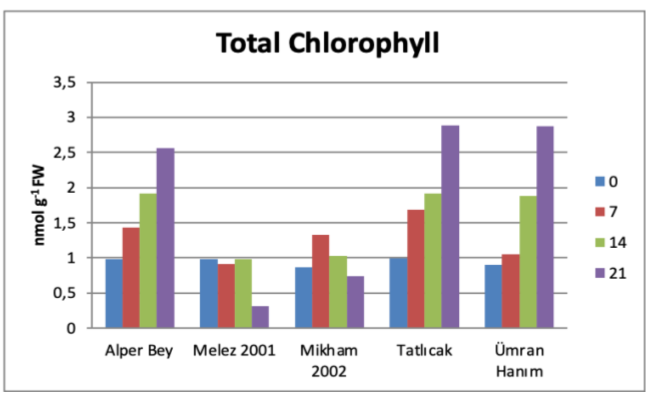

Figure 4. Changes of total chlorophyll in five triticale genotypes treated with 100 mM salt stress in in vitro conditions.

Discussion

In terms of salt stress mechanism, most of the studies reported so far have been conducted at the whole plant stage (6) however, this is the first showing the determination of salinity in triticale. Peroxidase enzymes play a significant role in the defense responses of crops to biotic or abiotic stresses (9). In this experiment, a considerable increase in the peroxidase activity could be determined in the seedlings of salt stress in in vitro conditions. Pujari and Chanda et al., (2002) (10) indicated that a salt-stressed seedling of Vigna unguiculata retained more peroxidase than that of unstressed seedling when both were applied with higher salt stress. Moreover, the activity of peroxidase in several species has been well documented in response to abiotic stress. The effects of stress severity on the peroxidase enzyme are proved to be linked to the salt-resistant ability (11). Our results displayed the highest degree of peroxidase in the high concentration salt-treated group. Thus it seems that this property is an effective indicator of salt-resistant in seedlings derived from Triticale cultivars. Chlorophyll is the basic factor for green pigments and is present in chloroplasts as components in all photosynthetic plant tissue. In this report, pigment estimation in seedlings was also influenced by NaCl (Figure 2, 3, and 4), and this effect depends on the genotypes. Compare to control groups, chlorophyll a, b and carotenoid content in two triticale (Melez 2001 and Mikham 2002) genotypes decreased (Figures 2, 3, and 4), whereas the other three triticale (Ümran Hanım, Alper Bey and Tatlıcak) genotypes increased after 1 week. The effect of inhibition of chlorophyll under stress conditions plays a critical role in photoinhibition or ROS formation (12). The decrease in photosynthesis under stress can also be attributed to a reduction in chlorophyll values. However, salinity decreases the chlorophyll content in salt-sensitive genotypes and promotes it in salt-resistant genotypes. In conclusion, these results displayed that there is a positive correlation between chlorophyll content and low concentration salt stress in salt-resistant triticale genotypes.

Declaration of Interest: No potential conflict of interest relevant to this article was reported.

Authors' Contributions: PU and SA contributed to the study conception and design. PU and SA contribution to laboratory work. Literature research (PU and SA), Writing the article (PU and SA). All authors read and approved the final manuscript. PU: Pınar Uysal, SA: Sinan Ata

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