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Effects of Salicylic Acid on Triticale under Salt Stress

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Abstract: The goal of this study was to determine the effect of salicylic acid (50 µM) on triticale grown under salt stress. In this study, we investigated shoot and root lengths, malondialdehyde, proline, ion leakage, relative water content, chlorophyll content. The results indicated that salicylic acid is quite effective to deal with salt stress. Anatomically shoot (3%) and root lengths (4%), as well as relative water content (12%) and chlorophyll content (9%), were increased by salicylic acid under salt toxicity by comparison to merely the salt application. Moreover, according to the only salt stressed plant, malondialdehyde, proline, and ion leakage were decreased 7%, 26%, 23% respectively by the application of salicylic acid in shoot tissues under salt stress. Similarly, SA decreased malondialdehyde, proline and ion leakage in root tissues under salt stress. Overall our results indicated that salicylic acid can be used for agricultural production of triticale under salt stress.

Anahtar kelimeler: Triticale, salt toxicity, salicylic acid, malondialdehyde.

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1. INTRODUCTION

Salinity is one of the factors affecting productivity in cultivated areas. Saline-alkaline soils are defined as soils that contain both salt and sodium at a level that prevents the usual growth and development of cultivated plants (Kanber et al., 2005). Soil salinity reduces water uptake and root growth as well as transpiration and respiration of the plant. As a result, the hormonal balance is destroyed, photosynthesis decreases, protein synthesis decreases as a result of the decrease in nitrate intake and the plant height decreases. As this affects the wet and dry weight of the plant, it reduces the number of flowers and causes a decrease in yield (Karım et al., 1993; Kanber et al., 2005). In areas where soil salinity cannot be controlled, plants with high salt resistance that can provide economic efficiency should be cultivated. Koebner and Martin (1996) identified triticale as the primary salt-resistant grain in their study, in which they examined high-level salt-resistant grains. However, even triticale can become unsuitable for agriculture after a certain salinity level.

In recent years, many studies have been carried out on the synthetic production of some growth regulating substances and their effects on the improvement of plant product quality and yield (Sarinana-Aldaco et al., 2020; Yadav et al., 2020). Salicylic acid (SA), which is commercially produced acetylsalicylic acid (ASA), is synthesized by higher plants and some microorganisms, and also stimulates systemic acquired resistance (SAR) against bacterial, fungal and viral infections in the plant and nitrate reductase in some plants (Abdelkhalek and Al-Askar, 2020; Huang et al., 2020). It is a growth regulator that causes physiological effects such as increasing the activity and dry matter amount.

SA is formed by the catalysis of many chemical reactions by a large number of enzymes. SA synthesis pathway starts with shikmic acid and is completed by three different pathways. Benzoic acid and ortho-coumaric acid are present in the reaction before salicylic acid synthesis (Khan et al., 2015). It was determined that salicylic acid stimulates flowering promotes, shoot formation, stimulates rooting (El-Kinany, 2020), prevents leaf fall, blocks ethylene synthesis (Lee and Yoon, 2020), and increases grain yield (Yadav et al., 2020). They reported that foliar application of salicylic acid in corn and soybean increased pore density and transpiration, as well as leaf area and plant dry weight, but did not affect plant height and root length (Khan et al., 2003). Salicylic acid (SA) is a substantial key molecule in the signal transduction pathway of biotic and abiotic stress factors. SA could be a promising compound for the

decrease of the abiotic stress sensitivity of crops (Horva´th et al., 2007; Khan et al., 2015) SA has been found to alleviate the detrimental effects of various stress factors in plants. Recent investigations have shown that SA alleviated salt stress damage in various plants (Khan et al., 2014; Khan et al., 2015; Nazar et al., 2015).Salicylic acid also increases the tolerance of triticale plants under abiotic stress conditions such as salinity (Yanyan et al., 2005), high and low temperature, frost (Gołębiowska-Pikania et al., 2019), heavy metal (Talebi et al., 2014) water and drought (Shanazari et al., 2018) stress. SA concentration is important for effective protection and growth, in the present research 50 µM SA used in accordance with literature (Kováčik et al., 2009; Shakirova et al., 2016).Besides, a preliminary experiment was conducted to determine SA concentration.

With the rapid increase in the world population, the decrease in natural resources has intensified efforts to develop new plant species and varieties resistant to stress conditions. One of the most successful products obtained as a result of plant breeding and genetic studies is triticale (Müntzing, 1979). Triticale, which is the result of the hybridization of wheat and rye; while it gets the yield potential comes from durum wheat, its resistance to cold, drought and marginal soil conditions comes from rye. It is also known to show resistance to most diseases and pests (Varughese et al., 1996; Lelley, 2006; Lonbani and Arzani, 2011). During salinity in plants, excessive amounts of reactive oxygen species (ROS) are produced in cell organelles and this causes oxidative damage (Foyer and Shigeoka, 2011). It was reported that triticale cultivars incresed antioxidative responses to varying amounts of NaCl (Demirbas and Balkan, 2020).

Present investigation has been designed to understand role of SA on triticale under salt toxicity. The result of this work is very important for sustainable agriculture and future investigation.

2. MATERIALS and METHODS

2.1. Growth Conditions and Stress Treatments

Triticale (Triticosecale) cv. Umranhanım seeds were kindly sourced from The East Anatolian Agricultural Research Institute of Turkey. To prevent contamination, the seeds were the first surface sterilized with 8% sodium hypochlorite, then rinsed with sterile autoclaved water four to five times. The seedlings were grown in plastic pots filled with perlite at 24±1°C in a growth chamber under controlled conditions, 8 hours dark and 16 hours light (400 µmol m^{-2} s^{-1}) photo-cycle.

The experiment was first started as seed germination with ½ Hoagland (pH 5.8) solution (Hoagland and Arnon, 1950). Germinated seeds reached a sufficient size after 14 days of growth, subsequently the determined salt stress (200 mM sodium salt) were applied into ½ Hoagland solutions for 3 days (Table 1).

The seeds containing the control set were regularly irrigated with ½ Hoagland's solution. Salicylic acid (50 µM) was added as from the initiate of seedling germination (Table 1). Moreover in overall, four individual treatments were given as Control, NaCl, SA, SA and NaCl. Plants were removed from their perlites at the end of 17 days to performed analyzes. Experiments were carried out in 3 replicates for each analysis.

Table 1. Treatments, time and medium parameters

Treatments Duration (24ºC)	Medium first 14 days	Medium 14-17 days
Control	½ Hoagland	½ Hoagland
Salt	½ Hoagland	½ Hoagland + Salt (200 mM)
SA	½ Hoagland + SA (50 µM)	½ Hoagland + SA (50 µM)
SA+Salt	½ Hoagland + SA (50 µM)	½ Hoagland + SA (50 µM)+ Salt (200 mM)

2.2. Growth parameters

Triticale shoots and root pieces were removed from the perlite after the 17th days of growth and the lengths of the tissues were measured in cm.

2.3. Determination of malondialdehyde (MDA) content

Malondialdehyde (MDA) content was used to determine lipid peroxidation by the method of Ohkawa and the research group (Ohkawa et al., 1979). Fresh tissues were triturated with liquid nitrogen and then treated with 5% trichloroacetic acid (TCA). The lysed tissues were centrifuged at 12000 rpm for 15 minutes. Thiobarbituric acid was added to Trichloroacetic acid (1/4 w / w) at the specified ratio then supernatant in the same amount was incubated 25 minutes at 96ºC on a dry heat block. The samples were centrifuged at 10000 rpm for 5 minutes in order to obtain a supernatant. Supernatant absorbances were evaluated at 532 nm subsequently to determine substrate to nonspecific turbidity absorbance was read at 600 nm. MDA contents were calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.4. Determination of proline amounts

Proline contents were determined by the methods of Bates and friends (1973). Firstly 0.2 g of tissue was dissected with the help of liquid nitrogen and then treated with 3% sulfosalicylic acid. The 0.1 ml supernatant was then mixed with 0.1 ml of 3% sulfosalicylic acid, 0.2 ml of acid ninhydrin and 0.2 ml of 96% acetic acid. These mixtures were incubated at 96°C for 1 hour then 1 ml of toluene was added and centrifuged. The absorbance value of the supernatants were evaluated at 520 nm to determine proline amounts.

2.5. Determination of cell ion leakage

Thermo Scientific Orion 013016MD conductivity meter was used to measure electrical conductance. Membrane damage of shoot and root tissues was determined with the help of the method developed by Nanjo and friends (1999). Tissues were incubated in mannitol at falcon tubes for 3 hours and then EC_1 values were measured. Afterwards incubated them at boiling water for 10 minutes then cooling at room temperature, EC_2 value was obtained. After these processes, the ion leakage was calculated using the formula $(EC_1/EC_2) \times 100$ as % EC.

2.6. Determination of relative water content

Relative water content was calculated using the method developed by Smart and Bingham (1974). Leaf explants was used to acquire relative water content (RWC) values. Firstly, the leaf weights were measured and the plants were immersed in water for 24 hours at room temperature. Subsequently turgid mass was obtained by weighed to hydrated tissues. Tissues were afterward dried in a warm (60^oC) incubator at 48 hours later weighed for the evaluation of dry masses.

2.7. Determination of chlorophyll (SPAD) content

Konica-Minolta SPAD-502PLUS Chlorophyll meter was used for obtaining SPAD value. SPAD-502PLUS device was calibrated according to the manufacturer's instructions. Each leaf SPAD value was acquired at least 15 readings and then chlorophyll determinations were performed.

2.8. Data analysis

Experiments were carried out in 3 replicates per analysis. The importance of the implementation effect was determined at the 5% prospect level by utilized the Tukey test of one-way ANOVA with the assistance of SPSS 15 (Statistical Package for Social Sciences, SPSS Inc., IL, USA).

3. RESULTS and DISCUSSION

A present investigation was examined by shoot lengths, MDA, proline, ion leakage, relative water content (RWC), and chlorophyll content on triticale under salt stress. Our findings were proving that SA is a promisingly beneficial effect on triticale production under salt stress.

The production of salicylic acid protects plants against environmental stresses with the increased enzyme activity of SA in the biosynthetic pathway. Salicylic acid has played a role in many plants in improving salinity stress tolerance mechanisms (Khan et al., 2015). The investigation was explained that salt causes oxidative stress, peroxidation of membrane lipids, and membrane damage (Ye et al., 2000). The relevant literature was clearly signified that SA in low dose increases the seed germination by reducing oxidative damage under salt toxicity (Rivas-San Vicente and Plasencia, 2011). Therefore probably plant shoot lengths were improved with the application of SA under high salinity stress (Table 2). Moreover in salt toxicity, SA was provided shoot lengths as much as control plants. Similarly, root lengths were increased by SA under salt stress, although not as much as control plants (Table 3).

Former investigations were stated as lipid peroxidation and membrane permeability increased under salt stress. A recent study was explaining that salt stress causes a significant increase in MDA content on maize (Torğut and Akbulut, 2020). However, lipid peroxidation was decreased by the application of SA (Gunes et al., 2007; Horva´th et al., 2007; Azooz, 2009). Our findings indicating that SA was played an important role in membrane damage prevention (Table 2 and Table 3). MDA content was decreased in both shoot and root tissues on triticale under salt toxicity by SA treatments. Control and SA treated plants statistically the same importance. However, SA has decreased MDA contents as statistically important under high saline conditions.

Plants benefit proline as an osmoprotectant to overcome and combat the negative factors created by abiotic stresses. Plants were exposed to stress, synthesize osmotic regulators and deposit them in their tissues (Chookhampaeng, 2011; Mohamed et al., 2018). Proline content probably rises to protect plants when plants deal with stress. The recent research demonstrated that SA was reduced proline content under salt toxicity (Shahba et al., 2010). Present research proline results were statistically similar on both shoot and root tissues (Table 2 and Table 3). Moreover, proline contents were relatively high at salt toxicity. However, SA was reduced proline content on shoot and root tissues under salt stress. These findings were clarifying that SA decreased salt stress, therefore proline content comparatively reduced (26%)according to only salt-treated samples.

Cell membrane stability is widely used to determine stress tolerance. Membrane leakage, which is an indicator of cell membrane integrity and increment comparatively with damage (Palta et al., 1982; Premachandra et al., 1992). The amount of ions leaking from the cytoplasm into the apoplastic fluid due to the functional disorders in the cell membrane that occur as a result of the damage (Eugenia et al., 2003). In various investigations, membrane leakages were decreased with the help of SA under salt stress (El-Tayeb, 2005; Azooz, 2009; Karlidag et al., 2009). Similarly, SA was reduced ion leakage level both shoot and root in present investigations (Table 2 and Table 3). Statistical differences were observed among the treatments. In accordance with MDA content, SA was provided membrane stability by decreasing ion leakage levels.

Relative water content (RWC) is an important component to understand plant water retaining capability. Many researchers defined that RWC enhanced under salt stress by SA treatments (El-Tayeb, 2005; Azooz, 2009; Karlidag et al., 2009). In a similar manner, SA was increased RWC (12%) under salt stress. Even SA was increased RWC according to control plants at salt-free samples (Table 3).

The investigation was reported that salt stress decreased the chlorophyll amount of cucumber seedlings, but treatment with SA increased the chlorophyll content (Yildirim et al., 2008). Plant protection against salt toxicity and photosynthesis regulation were providing by used of SA (Khan et al., 2010; Nazar et al., 2011; Syeed et al., 2011).

In salt toxicity, plant chlorophyll (SPAD) content similarly was increased by SA treatments. Therefore our findings explain that SA was importantly in maintaining chlorophyll content (9% increased according only salt applicated plant) under salt stress (Table 3).

Table 2. Performed analysis on shoot tissues on triticale under salt stress

Shoot tissues	Shoot lengths (cm)	MDA (nmol g^{-1})	Proline (µmol g^{-1})	Ion leakage %	RWC %	Chlorophyll (SPAD)
Control	29.05±0.15^{a}	4.41±0.11^{c}	27.31±1.36^{c}	4.40±0.03^{c}	87.10±0.44^{b}	36.42±0.52^{a}
Salt	27.84±0.30^{b}	5.58±0.04^{a}	61.51±2.11^{a}	7.20±0.04^{a}	71.91±0.28^{d}	28.06±0.63^{c}
SA	29.28±0.24^{a}	4.40±0.07^{c}	21.30±1.20^{c}	4.24±0.06^{c}	90.45±0.64^{a}	34.96±1.50^{ab}
SA+Salt	28.84±0.04^{a}	5.18±0.08^{b}	45.18±1.93^{b}	5.51±0.07^{b}	81.90±0.52^{c}	30.96±0.46^{bc}

Treatments represent in order Control (normal growth), Salt (200 mM salt application), SA (50 µM salicylic acid), simultaneous application of SA and Salt (50 µM salicylic acid and 200 mM salt) respectively. MDA and RWC stands for malondialdehyde and relative water content respectively. The values appointed by distinct characters are meaningfully different on 5% significance level.

Table.3 Performed analysis on root tissues on triticale under salt stress

Root tissues	Root lengths (cm)	MDA (nmol g^{-1})	Proline (µmol g^{-1})	Ion leakage %
Control	17.18±0.10^{ab}	3.01±0.23^{c}	52.92±3.42^{c}	17.09±0.69^{c}
Salt	15.80±0.15^{c}	6.29±0.08^{a}	95.87±2.83^{a}	30.90±0.88^{a}
SA	17.46±0.18^{a}	2.74±0.13^{c}	48.62±2.70^{c}	17.07±0.72^{c}
SA+Salt	16.56±0.24^{bc}	4.79±0.09^{b}	70.27±2.60^{b}	25.03±0.83^{b}

Treatments represent in order Control (normal growth), Salt (200 mM salt application), SA (50 µM salicylic acid), simultaneous application of SA and Salt (50 µM salicylic acid and 200 mM salt) respectively. MDA and RWC stands for malondialdehyde and relative water content respectively. The values appointed by distinct characters are meaningfully different on 5% significance level.

4. CONCLUSIONS

Present research on triticale revealed that SA was highly effective to provide tolerance to salt stress. The significant system components shoot and root tissues such as MDA, ion leakage, proline contents were decreased by SA applications under salt toxicity. Furthermore, shoot and root lengths, RWC, chlorophyll content were increased by SA under salt stress. Overall based on all performed analyses SA is a pretty good solution to tackle with salt stress. Therefore, our research will guide future studies on triticale production and the subject.

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