

Assessment of some morphological and physiological traits in *Aegilops* species under salt stress conditions

A. Mahmoudi, A. Aalami, R. Beheshti, and M. Danesh Gilevaei*

Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.

*Corresponding author e-mail: daneshg_maryam@yahoo.com

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ABSTRACT

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To study the effects of salinity on different species of *Aegilops* that have salinity tolerance genes, a factorial experiment was carried out using completely randomized design with three replications in Biotechnology Laboratory of Guilan University in 2014. Morphologic (length, fresh and dry weight of shoot and root, stem diameter, and number of tillers) and physiologic (Electrolyte Leakage, RWC, Chlorophyll content and antioxidants enzymes) traits of 12 *Aegilops* genotypes from four species; *Ae. tauschii*, *Ae. crassa*, *Ae. cylindrical*, and *Ae. triuncialis* were measured under salinity stress conditions. Assessment of morphological and physiological traits showed that genotypes belong to *Ae. cylindrical* had more tolerance to salinity stress than other genotypes. Genotype 575 from *Ae. cylindrical* as tolerant genotype and genotype 675 from *Ae. crassa* as susceptible genotype were identified and used for biochemical assay. The results showed peroxidase (POD) and ascorbate peroxidase (APX) enzymes' activity increased and catalase (CAT) enzyme activity decreased under salinity stress. Following stress treatment, enzyme activity in genotype 575 was higher than 675 showing antioxidant enzyme in tolerant genotype performs more than susceptible genotype.

Key words: *Aegilops*, antioxidant enzymes, electrolyte leakage, relative water content, dry weight

INTRODUCTION

One of the major problems facing agriculture is the shortage of fresh and high quality sources of water for irrigation (Quist-Jensen *et al.*, 2015). Salt tolerant plants when grown under salinity stress conditions can adapt with different mechanisms. This adaptation includes a range of morphologic, anatomic, physiologic, and biochemical changes (Knight and Knight, 2001). Soil salinity decreases the growth of plants and in high salt concentrations, plant growth may stop. The reason for this is the reduction of water potential in the soil or the osmotic effect due to the presence of salt in the soil, which limits the absorption of water by root (Çiçek and Çakırlar, 2002).

It has been found that salinity stress reduces the stem length, stem weight and dry matter of the plant. Also, reduction of

number of leaves, tillers, and roots number, length and areas are among other salinity effects (Parida and Das, 2005). In general, positive correlation was observed between root system traits and salinity tolerance in plants (Fricke *et al.*, 2014). Osmotic stress induced by salinity increases the threshold pressure for the growth of leaf cells and ultimately leads to decrease in leaf area. Salinity stress reduced leaf area and photosynthesis rate, and consequently plant yield (Munns and Tester, 2008).

Relative water content (RWC) is widely used to describe plant water status, and is superior to cell water potential and similar to leaf water potential, and gives a strong indication of plant's response to different environmental conditions (Sade *et al.*, 2015; Schonfeld *et al.*, 1988). Studies on wheat and barley showed that though salt stress did not change the potential of

turgescence, but the relative water content decreased (Munns *et al.*, 2006). Due to the fact that early senescence of the leaves under salinity stress changes the permeability of the cell membrane, and electrolyte leakage of the membrane can indicate the degree of damage to the membrane. Therefore, electrolyte leakage is estimated to measure the salinity damage on cell membrane permeability (Demidchik *et al.*, 2014; Lutts *et al.*, 1996.). Stability of chlorophyll is an indicator of plant tolerance to stress (Modhan *et al.*, 2000). Evidence showed that with increasing salinity, the content of chlorophyll increased (Wang *et al.*, 2001).

Generally, plants encounter a wide range of environmental stresses that ultimately lead to oxidative stress in the plant (Wang *et al.*, 2008). Reactive oxygen species (ROS) can damage proteins, lipids, carbohydrates, and DNA. Tolerance to some stresses is an internal and harmonic and complex mechanism. Under stress conditions, an imbalance between the energy absorption process and its consumption by photosynthetic organs leads to the production of various reactive oxygen species which ultimately results in stress in cell membrane and symptoms caused by oxidative damage (Blokhina *et al.*, 2003).

Increase in the number of reactive oxygen radicals in the plant activates different mechanisms to reduce the toxic effects of salinity stress. Under these conditions, the level of antioxidants and enzymes that scavenge reactive oxygen species increases to reduce toxic effects. Antioxidant enzymes play a major role in reducing reactive oxygen species (ROS) levels (Blokhina *et al.*, 2003). The most important antioxidant enzymes are catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX).

Wild species are important for having useful genes for tolerance to biotic and abiotic stresses and enhancing genetic adaptation against environmental changes.

These valuable genetic resources can be incorporated into plant breeding programs to develop germplasm tolerant to biotic and abiotic stresses (Hegde *et al.*, 2002). *Aegilops* is not only a wild relative of wheat but also is one of its earliest ancestors. Wheat has inherited many of its desirable traits such as bread quality, salinity tolerance and resistance to some of the pests and diseases from *aegilops* (Jiang *et al.*, 1994). *Aegilops* has 22 species; diploid, tetraploid and hexaploid which have played major role in the development of durum and bread wheat (Matsuoka *et al.*, 2007; Kilian *et al.*, 2011). *Aegilops* as wild ancestors of wheat have a collection of valuable genes for being transferred to crop species (Arzani and Ashraf, 2016; Wang *et al.*, 2016; Masoomi-Aladizgeh *et al.*, 2015).

Different species of *Aegilops* with different genomes have been studied for salinity tolerance. Significant differences between and within species have been reported for their reaction to salinity. *Aegilops* species with DD and CD genomes showed higher tolerance to salinity (Farooq *et al.*, 1989). Different species of *Aegilops* and wheat were evaluated for salinity and drought tolerance in laboratory conditions. *Ae. cylindrical* was more tolerant to salinity and had lower electrolyte leakage than other species, and identified for having osmotic adaptation, and can limit the accumulation of sodium and chloride ions through osmotic regulation (Farooq and Azam, 2001). However, agricultural development following wheat domestication have largely focused on the selection and breeding of uniform plants with high yield, substantially limiting the gene pools of the elite cultivars (Akpınar *et al.* 2015). Therefore, utilization of *aegilops* gene pool to improve the salinity stress in a staple food crop such as wheat warrants more research.

The objective of the present study was: 1) to evaluate some morphologic and physiologic traits associated to salinity tolerance in *aegilops* species under salt

stress conditions and 2) identifying sources of salinity tolerance for being utilized in wheat breeding programs.

MATERIALS AND METHODS

Collection of plant materials and apply salt stress

In this study, 12 genotypes from different species of *Aegilops*, including four species *Ae. tauschii*, *Ae. crassa*, *Ae. cylindrical*, and *Ae. triuncial* were collected from the National Iranian Gene Bank (Table 1).

This research was carried out at the Biotechnology Laboratory of the Faculty of Agriculture at the University of Guilan, Rasht, Iran. Seeds were first disinfected in 10% sodium hypochlorite solution for 10 min. After washing the seeds in sterile distilled water in petri-contained filter paper, they germinated under controlled conditions (16 hours light for photoperiodism with a brightness of 56 micromoles per second per square meter) and temperatures (18 and 24°C, at night and day, respectively). Then the seedlings were transferred to the sandy environment with suitable drainage and irrigated with Hoagland solution (Hoagland and Arnon, 1950) for two weeks.

The experiment was conducted as factorial arrangement using completely randomized design with three replications. Factors included genotypes and salinity. Salinity treatment was applied from the day 18th. Salinity treatment included four levels of 0, 100, 200, 300 mM NaCl in four boxes each contained 36 plants (each box contained 12 genotypes with three replications and each box with 60 × 45 × 40 dimensions which correspond to length, width and depth, respectively). To keep the salinity level constant during the experiment, the temperature and pH were kept constant. Morphological and physiological traits were measured after 21 days of salinity stress.

Morphological traits

Length of roots and diameter of the

crown were measured in millimeters with the caliper. Number of tillers for all genotypes was counted. Fresh weight of shoots and the roots were measured with a precision of 0.001 with a balance. Dry weight of shoots and roots was measured after dried for 24 hours in an oven at 70 °C. Leaf area (petiole to the end of the leaf) was measured using a leaf area meter (L1-3000C model manufactured in the USA).

Physiological traits

The relative water content of the leaf was measured following Volaire *et al.* (1998).

$$RWC(\%) = \left[\frac{FW - DW}{TW - DW} \right] \times 100 \quad (1)$$

where FW is leaf fresh weight, DW is leaf dry weight, and TW is leaf turgid weight.

Greenness index (chlorophyll index) was measured with a manual chlorophyll meter (SPAD502-Minolta manufactured in Japan). Electrolyte leakage (EL) was assessed following Lutts *et al.* (1996). The amount of 0.155 g of leaves was placed in test tubes with 15 ml of twice sterilized water and then placed in an incubator shaker (Amperetablle Multiron II model) at 25 °C and 100 rpm per minute for six hours. The electrical conductivity of the solution containing the samples, EC₁ was measured by EC meter (4020 Jenway model, made in England). To destroying cell walls to releasing cellulose and electrolytes samples were placed in an autoclave at 120 °C and 130 Pa pressure for 120 minutes. After cooling the samples, EC₂ was measured in ds/m. Electrolyte leakage was calculated following Lutts *et al.* (1996).

$$EL = \frac{EC_1}{EC_2} \times 100 \quad (2)$$

The results of the experiment 1 showed that genotype 575 of *Ae. cylindrical* was resistant and genotype 675 of *Ae. crassa* were identified as susceptible. These genotypes were used next experiment. For this purpose, seeds of two plants were

Table 1. *Aegilops* genotypes and their characteristics.

No.	Species	Accession number	Collecting area	No.	Species	Accession number	Collecting area
1	<i>Ae. tauschii</i>	2207	Guilan	7	<i>Ae. triuncialis</i>	964	Kermanshah
2	<i>Ae. tauschii</i>	1770	Mazandaran	8	<i>Ae. triuncialis</i>	931	Kermanshah
3	<i>Ae. tauschii</i>	Aladozgeh	Ardebil	9	<i>Ae. triuncialis</i>	967	Kermanshah
4	<i>Ae. crassa</i>	675	Hamedan	10	<i>Ae. cylindrica</i>	591	Khorasan
5	<i>Ae. crassa</i>	598	Khorasan	11	<i>Ae. cylindrica</i>	575	Lorestan
6	<i>Ae. crassa</i>	730	Shiraz	12	<i>Ae. cylindrica</i>	622	Khorasan

cultivated and at third leaf stage, seedlings were placed in both salinity stress and control treatments. Salinity treatment was applied at 200 mM level with sodium chloride. Samples were taken at 0, 6, 24, 48 and 72 hours from the leaves for evaluation of peroxidase, ascorbate peroxidase, and catalase enzymes. Samples were immediately frozen with liquid nitrogen and transferred to a freezer of -70°C .

Extraction and assay of antioxidant enzymes

To extract the enzymes, 0.3 g of frozen leaves mixed with 3 ml phosphate buffer contained 0.68 g of KH_2PO_4 plus 0.0186 g of EDTA and 2 mg of PVPP reached to the volume of 100 ml with distilled water and centrifuged for 15 minutes at 14,000 rpm at 4°C . The supernatant was then removed and centrifuged again for 10 minutes at 10,000 rpm at 4°C (Beauchamp and Fridovich, 1971). Then, the supernatant was used to read the enzymes' activity with spectrophotometry (PG Instruments ItdT80 + UV/VIS).

To measure the kinetic activity of the enzyme peroxidase (POD), 990 μl of H_2O_2 buffer and the Guaiacol buffer were mixed in the same amount at a low temperature (ice container), then 20 μl of enzyme extraction was added and the adsorption curve at 470 nm using spectrophotometry was read. The enzyme activity was calculated following Beer Lambert's law and with the Guaiacol peroxidase power factor $\epsilon = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ (Ferreira *et al.*, 2010).

Ascorbate peroxidase enzyme (APX) activity was calculated by measuring the oxidation of ascorbate with spectrophotometry based on absorbance variation over time (OD/min) at 290 nm for

one minute. To obtain the enzymatic activity, changes in absorbance time (OD/min) are divided by constant $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$ (Asada, 1992).

Catalase activity (CAT) was assessed by measuring the H_2O_2 decomposition by spectrophotometry at 240 nm for one minute. The enzymatic reaction rate was recorded as absorbance variation over time (OD/min) at 240 nm for one minute. Finally, the enzyme activity was calculated in mMol/g FW. min following Chance and Maehly (1955).

Statistical Analysis

The data were normalized and analyzed. Mean comparison was performed using Tukey's method at the 1% probability level. All statistical analyses were performed using SAS software (version 9.0.0) and SPSS (version 11.5).

RESULTS AND DISCUSSION

Analysis of variance of data showed that significant difference between genotypes for morphological and physiological traits (Table 2) which indicates the genetic variation among *aegilops* genotypes. There was also significant difference between the salinity levels. Genotype \times salinity interaction was significant for all traits (Table 2).

Morphological traits

The highest shoot fresh weight belonged to genotype 575 from *Ae. Cylindrical* (Table 3). Reduction in fresh weight of this genotypes under different salinity levels did not show any significant difference. Genotype 675 of *Ae. crassa* had the lowest fresh weight. The highest shoot dry weight belonged to genotypes 575 and 622, and the

Table 2. Analysis of variance for morphological and physiological traits of aegilops genotypes under salt stress conditions

S. O. V.	df.	Mean Squares					
		Shoot fresh weight	Root length	Shoot length	Crown diameter	Tiller number	Leaf area
Genotype (G)	11	0.10**	73.95**	42.17*	0.34**	2.56**	80.37**
Salinity stress (SS)	3	5.36**	5021.57**	1503.70**	22.87**	19.78**	1009.72**
G × SS	33	0.03*	51.98**	44.69**	0.71**	1.05**	13.41**
Error	93	0.01	15.02	16.11	0.20	0.38	3.08

Table 2. Continued.

S. O. V.	df.	Mean Squares					
		Electrolyte leakage	RWC	Chlorophyll	Root fresh weight	Root dry weight	Shoot Dry weight
Genotype (G)	11	0.04*	327.60**	106.71**	0.500**	0.030*	0.010**
Salinity stress (SS)	3	4.98**	3630.59**	238.18**	38.240**	0.730**	0.170**
G × SS	33	0.026 ^{ns}	75.76*	43.81**	0.310**	0.010 ^{ns}	0.000**
Error	93	0.020	3.63	18.45	0.087	0.010	0.000

* and **: Significant at the 5% and 1% probability levels, respectively.
ns: Not-Significant.

lowest dry shoot weight was related to genotype 675 (Table 3). The fresh and dry weight of shoots is influenced by salinity through both vegetative growth and reduction of photosynthesis (Parida and Das., 2005).

The highest root dry weight belonged to genotype Aladizgeh and the lowest to genotype 675. However, the highest root fresh weight belonged to genotype 622 (Table 3).

Ae. tauschii genotypes distinctly had wider leaf than other genotypes. Therefore, under salinity stress conditions, the highest leaf area related to genotype Aladozgeh from *Ae. tauschii* and the lowest related to genotype 675 from *Ae. crassa* (Table 3). It is assumed that Aladozgeh and 575 genotypes with higher leaf area in different levels of salinity and also their superiority in other traits such as fresh and dry weight of roots and shoots and the number of tillers had higher photosynthetic potential. These genotypes had greater fresh and dry weight in different salinity levels. The highest tiller number, under salinity levels, belonged to genotype 2207 and the lowest to genotype 675 (Table 3). Reduction of leaf number, tiller number and dry weight of the wheat plant through increasing salinity levels has also been reported (El-Hendawy et al., 2005) which is in agreement with our findings.

Genotypes 622, 575 and Aladizgeh showed more salinity tolerant. In contrast,

genotype 675 had less salinity tolerance. The cluster analysis based on Euclidean distance and UPGMA method was performed to determine the susceptible and tolerant genotypes. The results of grouping based on dendrogram showed that the maximum distance was between genotype 575 from *Ae. cylindrica* and genotype 675 of *Ae. crassa* species (Fig. 1).

Physiological traits

Electrolyte leakage is one of the important traits that indicate cell membrane stability under stress conditions. Mean comparison showed that genotypes 622 and 575 from *Ae. cylindrica* had the lowest and genotypes 730 and 675 from *Ae. crassa* had the most electrolyte leakages (Table 3). Filek et al. (2012) reported that wheat cultivars with less electrolyte leakage were more tolerant to salinity. Damage to the cytoplasmic membrane through stress causes the leakage of the cell contents which can be determined by measuring the amount of electrolyte leakage. High levels of electrolyte leakage indicate the inability of the membrane to retain intracellular compositions, hence more electrolyte leakage from the cell membrane causes damage to it.

Genotypes 622 and 575 from *Ae. cylindrica* and Aladozge genotype from *Ae. tauschii* had the highest relative water content at all three levels of salinity (Table 3). Reduced relative water content

Table 3. Mean comparison for morphological and physiological traits of aegilops genotypes under salinity stress.

Accession No.	Shoot length (mm)	Root length (mm)	Shoot fresh weight (mm)	Relative water content (%)	Root fresh weight (mg)	Root dry weight (mg)	Shoot dry weight (mg)	Leaf area (mm ²)	Electrolyte leakage (Dc / m)	Chlorophyll content	Tiller number	Crown diameter (mm)
967	28.67ab	27.32bc	0.57abcd	71.05bc	1.01abc	0.20abc	0.14abc	6.43cd	0.47ab	36.50ab	2.45b	1.73e
675	26.53b	26.35bc	0.36e	76.89c	0.45d	0.09c	0.08e	4.46d	0.44ab	28.16d	2.00b	1.87de
730	27.88ab	28.50ab	0.45cde	76.89c	0.76cd	0.12bc	0.10cde	6.35cd	0.44ab	40.36a	2.08b	2.24cde
622	29.66ab	30.17ab	0.58abc	81.01a	1.21a	0.21abc	0.15a	10.13ab	0.33b	34.71bc	2.50b	2.45abc
575	31.52a	30.12ab	0.68a	80.94a	1.13abc	0.24ab	0.15ab	9.99ab	0.36b	34.45bc	2.50b	2.35bdc
Aladizgeh	26.89ab	28.28ab	0.50bcde	79.96a	1.14ab	0.26a	0.12cd	11.87a	0.40ab	32.68bcd	2.75b	2.55ab
964	28.39ab	32.67a	0.61ab	76.30ab	1.00abc	0.15bc	0.14ab	7.13c	0.36b	34.25bc	2.42b	2.09bcde
2207	26.25b	27.03bc	0.50bcde	72.20bc	0.84bc	0.13bc	0.12abcd	10.21ab	0.46ab	36.05ab	3.75a	2.90a
591	25.70b	27.17bc	0.41de	69.76bc	1.08abc	0.18bc	0.09de	10.12ab	0.55a	30.00cd	2.50b	2.17bcde
1770	25.14b	22.96c	0.45cde	71.89bc	0.90abc	0.17bc	0.11bde	9.68b	0.47ab	37.70ab	2.66b	2.53ab
598	26.68ab	28.50ab	0.43cde	66.97c	0.08bc	0.15bc	0.11cde	4.73d	0.43ab	34.06bc	2.083b	2.29bde
931	29.42ab	27.37bc	0.45cde	69.75bc	1.05abc	0.17bc	0.14abc	5.16cd	0.43ab	36.33ab	2.67b	1.96cde

Means, in each column, followed by at least one letter in common are not significantly different at the 0.01 probability level-using Tukey's test.

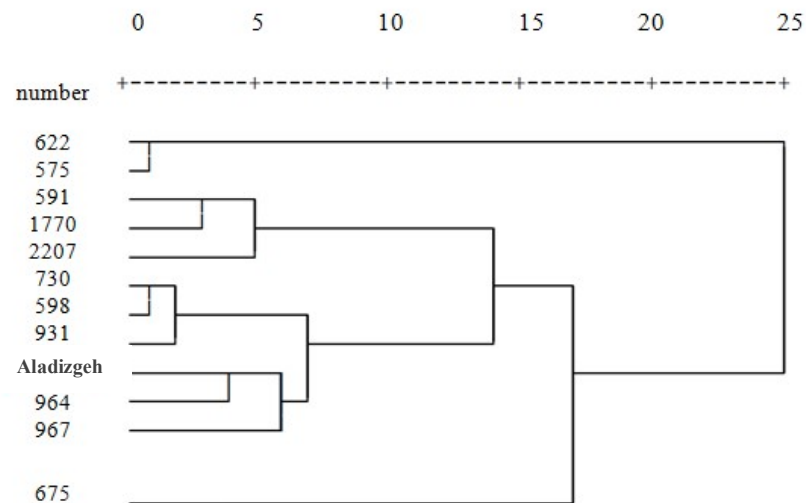


Fig. 1. Dendrogram for aegilops genotypes under salinity stress using UPGMA method.

due to salinity stress in wheat has been reported, which is due to reduced water absorption through osmotic regulation due to salinity stress (Meneguzzo *et al.*, 2000). On the other hand, increasing the accumulation of ions, especially sodium and chlorine, can reduce relative water content (Munns *et al.*, 2006). In plant cells, the volume of water and the number of soluble materials play a decisive role in regulating the intracellular solubility potential (Schonfeld *et al.*, 1988). Therefore, genotypes with more relative water content can perform more successfully through osmotic regulation. The reduction of the relative water content of aegilops genotypes under salinity stress can be attributed to the increased concentration of cellular content through osmotic regulation.

Mean comparison of chlorophyll content showed that genotype 730 of *Ae. crassa* had the highest and genotype 675 of *Ae. crassa* had the lowest chlorophyll content (Table 3). Leaf thickness increases at all salinity levels and this change in the thickness of the leaves increases the concentration of chlorophyll (Boughalleb *et al.*, 2017).

Genotypes 575 and 675 were identified as salinity tolerant and susceptible genotypes, respectively. These two genotypes were evaluated in for antioxidant enzymes.

Evaluation of antioxidant enzymes

Analysis of variance of data showed that there was significant difference between genotypes 575 and 675 for the activity of ascorbate peroxidase, peroxidase and catalase enzymes (Table 4). Ascorbate peroxidase activity increased in genotype 575 more than genotype 675 (Fig. 2a), while the catalase enzyme decreased significantly in both genotypes with extending stress duration. Hence, the highest enzyme activity observed at the time zero, and the lowest at 72 hours (Fig. 2c).

The highest activity of ascorbate peroxidase enzyme observed at 48 hours after application of salinity stress for genotype 575 and the lowest activity at the time zero for both genotypes. There was no significant difference between the time zero and 72 hours in both genotypes, but there was significant difference at other times of stress between the two genotypes. The results showed enzyme activity increased until 48 hours, and then decreased (Fig. 2a and b). Peroxidase enzyme increased in genotype 575 compared to genotype 675, though the differences were not significant. In both genotypes, the highest enzyme activity observed in six hours after application of salt stress, and then reduced as at 72 hours the least antioxidant activity was observed (Fig. 2b).

Genotype 575 had more enzymatic activity for ascorbate peroxidase, catalase, and peroxidase enzymes indicated higher antioxidant resistance of tolerant genotype under salt stress conditions. This was expected, because salinity stress typically increases the antioxidant activity (Caverzan *et al.*, 2012). We measured the antioxidant enzymes in a short time after salinity stress which indicated greater effect of osmotic stress compared with ionic stress (Munns and Tester, 2008).

Catalase and ascorbate peroxidase have a complementary role in counteracting the free radicals of hydrogen peroxide as the reduction in catalase increases the ascorbate peroxidase (Mandhania *et al.*, 2006). Such a process was clearly observed in the present study. The observed decrease in the activity of this enzyme is similar to the results of severe inactivation of the catalase enzyme in potato under salinity reported by Fidalgo *et al.* (2004). Accordingly, reducing the activity of this enzyme in salinity stress conditions may be due to the lower importance of this enzyme in the decomposition of hydrogen peroxide and is likely that aegilops genotypes 575 and 675 would use other antioxidant enzymes to reduce the damage caused by salt stress.

Table 4. Analysis of variance for catalase, peroxidase and ascorbate peroxidase enzymes activity of aegilops genotypes 575 and 675 under salt stress conditions

S. O. V.	df.	Mean Squares			
		Protein	Ascorbate Peroxidase	Peroxidase	Catalase
Genotype (G)	1	0.00024**	0.10505**	0.00008*	0.00315**
Time of salinity stress (TSS)	4	0.00007*	0.06913*	0.00023**	0.01133**
G × TSS	4	0.00005 ^{ns}	0.01975*	0.00004*	0.00024 ^{ns}
Error	20	0.00006	0.01823	0.01823	0.00031

* and **: Significant at the 5% and 1% probability levels, respectively.

^{ns}: Not-Significant

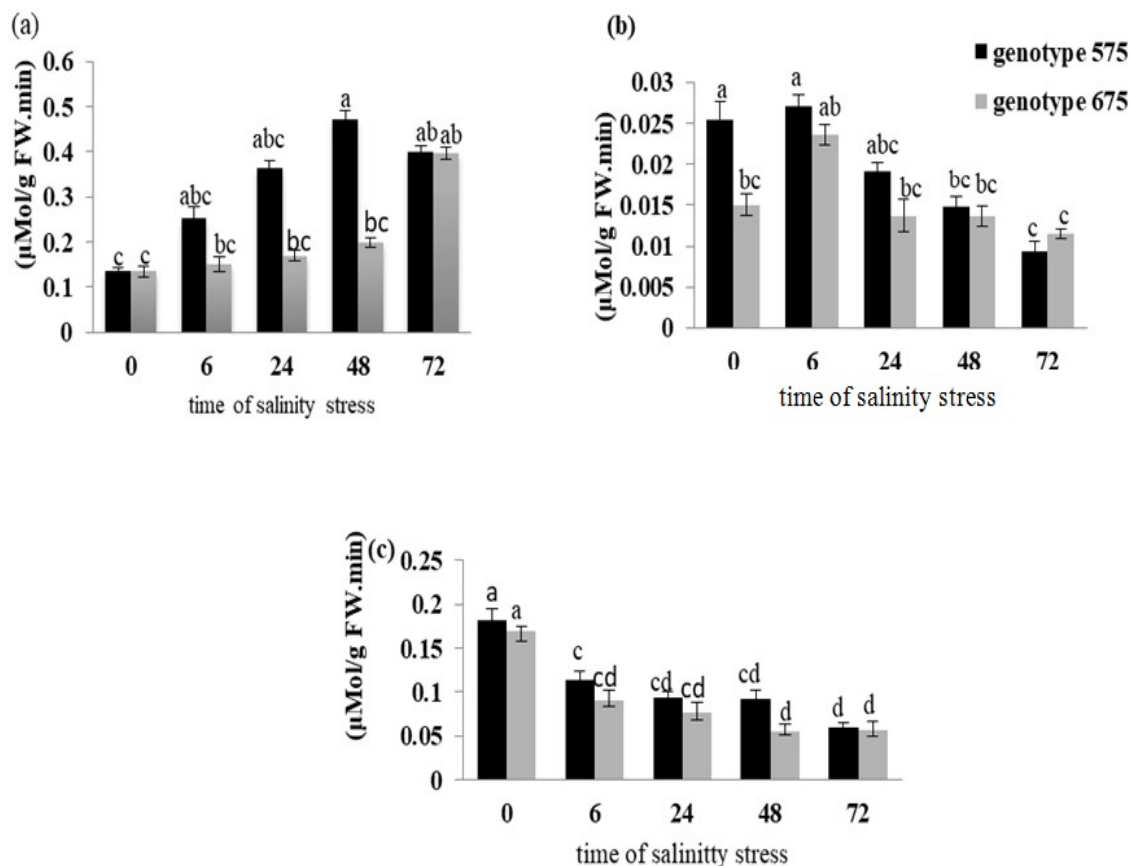


Fig. 2. Changes in: (a) ascorbate peroxidase, (b) peroxidase, and (c) catalase activity at different times in 200 mM level of salinity. Columns with at least one letter in common show not-significant at the 1% probability level.

However, there is report that catalase enzyme activity increased under salt stress conditions in rice (Demiral and Turkan, 2005).

Mudgal *et al.* (2010) reported strong correlation between tolerance to oxidative stress, caused by environmental stresses, and increase in the concentration of plant antioxidants. Increases in ascorbate peroxidase enzymes indicated that this enzyme is effective under salt stress

conditions.

Changes in the activity of antioxidant enzymes, in addition to ionic changes and osmotic regulators, can be considered as effect salinity stress on plants. The main source of ascorbate peroxidase is in chloroplasts. Ascorbate peroxidase plays an important role in stomatal activity by regulating the concentration of hydrogen peroxide in plant cells under stress, because the concentration of this compound acts as

one of the important signals in the movement of the protective stomata cells (Chen and Gallie, 2004). Therefore, the disruption of photosynthesis and the formation of active oxygen species in chloroplasts causes more ascorbate peroxidase activity to remove hydrogen peroxide (Breusgam *et al.*, 2001) and, as an electron donor, leads in decomposition of hydrogen peroxide, which is essential in reduction of sodium toxicity (Asada, 1994).

There are evidences that the activity of the peroxidase enzyme increased in some cultivars and decreased in others under salt stress conditions. Increased activity of peroxidase enzymes has been reported under salinity stress in sugar beet (Bor *et al.*, 2003), however, there is some evidence for different behavior of this enzyme in rice cultivars under salt stress conditions (Demiral and Turkan, 2005).

CONCLUSION

High genetic variation in salt tolerance was observed in morphological and physiological traits in four species. Evaluation indices showed that genotypes of *Ae. cylindrica* species were more tolerant to salinity stress than other genotypes. The results of biochemical assessments showed that salinity increased the amount of peroxidase and ascorbate peroxidase enzymes, but decreased catalase. Genotype 575 identified as salt tolerant in comparison with genotype 675 at salinity level of 200 mM. Therefore, it was concluded that salt tolerance mechanism in aegilops resistant genotype was increased antioxidant enzymes activity. Aegilops is one of the wild relatives of wheat and can be used as donor of gene(s) for salt tolerance in wheat breeding programs.

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REFERENCES

- Akpinar, B. A., Kantar, M. and Budak, H. 2015. Root precursors of microRNAs in wild emmer and modern wheats show major differences in response to drought stress. *Funct. Integr. Genomic.* 15: 587-598.
- Arzani, A., and Ashraf, M. 2016. Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. *Crit. Rev. Plant Sci.* 35: 146-189.
- Asada, K. 1992. Ascorbate peroxidase - a hydrogen peroxide-scavenging enzyme in plants. *Physiol. Plant.* 85: 235-241.
- Beauchamp, C., and Fridovich, I. 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* 44: 276-287.
- Blokhina, O., Virolainen, E., Fagerstedt, K. V., Dumas, F., Alscher, R. G., Erturk, N., Heath, L. S., Couée, I., Sulmon, C., Gouesbet, G., El Amrani, A., Laribi, B., Bettaieb, I., Kouki, K., Sahli, A., Mougou, A., Marzouk, B., Miller, G., Suzuki, N., Ciftci-Yilmaz, S., Mittler, R., Vanderauwera, S., Gollery, M. and Van Breusegem, F. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann. Bot.* 91: 179-194.
- Bor, M., Özdemir, F. and Türkan, I. 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. *Plant Sci.* 164: 77-84.
- Boughalleb, F., Abdellaoui, R., Nbiba, N., Mahmoudi, M. and Neffati, M. 2017. Effect of NaCl stress on physiological, antioxidant enzymes and anatomical responses of *Astragalus gombiformis*. *Biologia.* 72: 1454-1466.
- Breusgam, F. V., Vranove, E., Dat, J. F. and Inze, D. 2001. The role of active oxygen species in plant signal transduction. *Plant Sci.* 161: 405-414.
- Caverzan, A., Passaia, G., Rosa, S. B., Ribeiro, C. W., Lazzarotto, F. and Margis-Pinheiro, M. 2012. Plant

- responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. *Genet. Mol. Biol.*
- Chance, B. and Maehly, A. C. 1955. Assay of catalase and peroxidase. *Methods Enzymol.* 2: 764-775.
- Chen, Z., and Gallie D. R. 2004. The ascorbic acid redox state controls guard cell signaling and stomata movement. *Plant Cell* 16: 1143-1162.
- Çiçek, N., and Çakırlar, H. 2002. The Effect of salinity on some physiological parameters in two maize cultivars. *Bulg. J. Plant Physiol.* 28 (1-2): 66-74.
- Demidchik, V., Straltsova, D., Medvedev, S. S., Pozhvanov, G. A., Sokolik, A. and Yurin, V. 2014. Stress-induced electrolyte leakage: The role of K⁺-permeable channels and involvement in programmed cell death and metabolic adjustment. *J. Exp. Bot.* 65(5): 1259-1270.
- Demiral, T., and Türkan, I. 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environ. Exp. Bot.* 53: 247-257.
- El-Hendawy, S. E., Hu, Y., Yakout, G. M., Awad, A. M., Hafiz, S. E. and Schmidhalter, U. 2005. Evaluating salt tolerance of wheat genotypes using multiple parameters. *Eur. J. Agron.* 22: 243-253.
- Farooq, S., and Azam, F. 2001. Co-existence of salt and drought tolerance in Triticeae. *Hereditas* 135: 205-210.
- Farooq, S., Niazi, M. L. K., Iqbal, N. and Shah, T. M. 1989. Salt tolerance potential of wild resources of the tribe Triticeae - II. Screening of species of the genus *Aegilops*. *Plant Soil.* 119: 255-260.
- Ferreira, L. C., Cataneo, A. C., Remaeh, L. M. R., Corniani, N., Fumis, T. de F., Souza, Y. A. de., Scavroni, J. and A. Soares, B. J. 2010. Nitric oxide reduces oxidative stress generated by lactofen in soybean plants. *Pestic. Biochem. Phys.* 97(1): 47-54.
- Fidalgo, F., Santos, A., Santos, I. and Salema, R. 2004. Effects of long-term salt stress on antioxidant defence systems, leaf water relations and chloroplast ultrastructure of potato plants. *Ann. Appl. Biol.* 145(2): 185-192.
- Filek, M., Walas, S., Mrowiec, H., Rudolphy-Skórska, E., Sieprawska, A. and Biesaga-Kościelniak, J. 2012. Membrane permeability and micro- and macroelement accumulation in spring wheat cultivars during the short-term effect of salinity- and PEG-induced water stress. *Acta Physiol. Plant.* 34: 985-995.
- Fricke, W., Bijanzadeh, E., Emam, Y. and Knipfer, T. 2014. Root hydraulics in salt-stressed wheat. *Funct. Plant Biol.*: 41: 366-378.
- Hegde, S. G., Valkoun, J. and Waines, J. G. 2002. Genetic diversity in wild and weedy *Aegilops*, *Amblyopyrum*, and *Secale* species - A preliminary survey. *Crop Sci.* 42: 608-614.
- Hoagland, D. R. and Arnon, D. I. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular.* 347: 1-32.
- Jiang, J., Friebe, B. and Gill, B. S. 1994. Recent advances in alien gene transfer in wheat. *Euphytica.* 73: 199-12.
- Kilian, B., Mammen, K., Millet, E., Sharma, R., Graner, A., Salamini, F., Hammer, K. and Özkan, H. 2011. *Aegilops*. Pp. 1-76. In: C. Kole (ed.) *Wild crop relatives: genomic and breeding resources.* Springer-Verlag Berlin Heidelberg.
- Knight, H., and Knight, M. R. 2001. Abiotic stress signalling pathways: Specificity and cross-talk. *Trends Plant Sci.* 6(6): 262-267.
- Lutts, S., Kinet, J. M. and Bouharmont, J. 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars

- differing in salinity resistance. *Ann. Bot.* 78: 389-398.
- Mandhania, S., Madan, S. and Sawhney, V. 2006. Antioxidant defense mechanism under salt stress in wheat seedlings. *Biol. Plant.* 50: 227-231.
- Masoomi-Aladizgeh, F., Aalami, A., Esfahani, M., Aghaei, M. J. and Mozaffari, K. 2015. Identification of CBF₁₄ and NAC₂ genes in *Aegilops tauschii* associated with resistance to freezing stress. *Appl. Biochem. Biotechnol.* 176: 1059-1070.
- Matsuoka, Y., Takumi, S. and Kawahara, T. 2007. Natural variation for fertile triploid F₁ hybrid formation in allohexaploid wheat speciation. *Theor. Appl. Genet.* 115: 509-518.
- Meneguzzo, S., Navari-Izzo, F. and Izzo, R. 2000. NaCl effects on water relations and accumulation of mineral nutrients in shoots, roots and cell sap of wheat seedling. *J. Plant Physiol.* 156: 711-716.
- Modhan, M. M., Narayanan, S. L. and Ibrahim, S. M. 2000. Chlorophyll stability index (CSI): its impacts on salt tolerance in rice. *Int. Rice Res. Notes* 25: 38-40.
- Mudgal, V., Madaan, N. and Mudgal, A. 2010. Biochemical mechanisms of salt tolerance in plants: A review. *Int. J. Bot.* 6: 136-143
- Munns, R., and Tester, M. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59: 651-681.
- Munns, R., James, R. A. and Läuchli, A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.* 57(5): 1025-1043.
- Parida, A. K. and Das, A. B. 2005. Salt tolerance and salinity effects on plants: A review. *Ecotoxicol. Environ. Saf.* 60(3): 324-349.
- Quist-Jensen, C. A., Macedonio, F. and Drioli, E. 2015. Membrane technology for water production in agriculture: Desalination and wastewater reuse. *Desalination* 364: 17-32.
- Sade, N., Galkin, E. and Moshelion, M. 2015. Measuring Arabidopsis, Tomato and Barley leaf relative water content (RWC). *Bio-Protocol.* 5(8): 1451.
- Schonfeld, M. A., Johnson, R. C., Carver, B. F. and Mornhinweg, D. W. 1988. Water relations in winter wheat as drought resistance indicators. *Crop Sci.* 28: 526.
- Volaire, F., Thomas, H. and Lelièvre, F. 1998. Survival and recovery of perennial forage grasses under prolonged Mediterranean drought. I. Growth, death, water relations and solute content in herbage and stubble. *New Phytol.* 140: 439-449.
- Wang, D., Shannon, M. C. and Grieve, C. M. 2001. Salinity reduces radiation absorption and use efficiency in soybean. *Field Crops Res.* 69: 267-277.
- Wang, Q., Guan, Y., Wu, Y., Chen, H., Chen, F. and Chu, C. 2008. Overexpression of a rice OsDREB1F gene increases salt, drought, and low temperature tolerance in both Arabidopsis and rice. *Plant Mol. Biol.* 67: 589-602.
- Wang, Y., He, F., Bao, Y., Ming, D., Dong, L., Han, Q., Li, Y. and Wang, H. 2016. Development and genetic analysis of a novel wheat-aegilops germplasm TA002 resistant to powdery mildew. *Sci. Agric. Sin.* 49: 418-428.