Screening and Selection of Twenty Iranian Wheatgrass Genotypes for Tolerance to Salinity Stress during Seed Germination and Seedling Growth Stage

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Abstract. Desert wheatgrass (*Agropyron desertorum* L.), tall wheatgrass (*Agropyron elongatum* L.), and crested wheatgrass (*Agropyron cristatum* L.) are native cool-season grass species that exhibit potential as a low-input turfgrass. An increased understanding of the biochemical and physiological responses of wheatgrass species and genotypes to salt stress conditions is important for developing genotypes with enhanced tolerance to salinity. The objective of this study was to characterize the physiological and antioxidative properties in 20 Iranian wheatgrass genotypes and to observe their responses to salinity stress during seed germination and seedling growth stage. A completely randomized factorial design was used with two types of factors, four levels of salinity (0, 50, 100, and 150 mM of NaCl), wheatgrass genotypes, and three replicates. In this experiment, the results demonstrated that salinity limits the germination of Iranian wheatgrass genotype seeds. The result of this study showed that among the wheatgrass genotypes, 'AD1', 'AD3', 'AC6', and 'FA' took the shortest average time to germinate. Higher levels of final germination percentage (FGP) were observed in 'AD2', 'AD3', and 'AE5' under salinity stress than other genotypes throughout the experiment. During a prolonged period of study, 'AD1' had greater rate of germination (GR) than other genotypes. Out of the 21 genotypes, five genotypes ('AD1', 'AD2', 'AD3', 'AE5', and 'FA' genotypes) were in the range of "salinity tolerant genotypes" cluster. The 'AD1', 'AD2', 'AD3', 'AE5', and 'FA' genotypes generally performed better than other genotypes under salinity conditions, mainly through maintaining higher enzymatic activities such as superoxide dismutase (SOD) (EC 1.15.1.1), catalase (CAT) (EC 1.11.1.6), ascorbate peroxidase (APX) (EC 1.11.1.11) and peroxidase (POD) (EC 1.11.1.7), and nonenzymatic antioxidant activities by glutathione (GSH). The 'AD1', 'AD2', 'AD3', 'AE5', and 'FA' genotypes also had higher proline levels and more of total nonstructural carbohydrates (TNC) content, lower malondialdehyde (MDA) content, and lower hydrogen peroxide content (H₂O₂).

regions (Sekmen et al., 2012). Different levels of salinity stress can affect turfgrass adversely. These may include a series of morphological, physiological, and biochemical metabolic disorders caused either by an osmotic stress or by an ion toxic effect (Hasegawa et al., 2000).

Salinity stress can stimulate the formation of reactive oxygen species (ROS), such as singlet oxygen (¹O₂), hydrogen peroxide (H₂O₂), and hydroxyl radicals (OH*) (Fan et al., 2013). These ROS are known as molecular factors that damage the cellular components of proteins, enzymes, membrane lipids, pigments, and nucleic acids (Hameed et al., 2014). Plant survival under adverse conditions is a feedback of advanced changes in metabolism which can be detailed as the accumulation of protective compounds such as compatible osmolytes and antioxidants (Hu et al., 2012b). ROS can accumulate when the stage of seed germination is occurring and when seedling growth happens under stress conditions (Simlata et al., 2016).

According to the role of antioxidant defense system in cleaning the ROS levels during germination, these enzymes are mainly important for the thorough occurrence of germination (Yan, 2015). Plants are often characterized by developing enzymatic and nonenzymatic defense systems for ROS scavenging to avoid these oxidative damages (Etemadi et al., 2015). Enzymatic antioxidants include SOD, CAT, POD, and APX. Nonenzymatic antioxidants are GSH, glutathione reductase, and phenolic compounds (Jianga et al., 2012).

Turfgrass management under saline conditions is becoming a serious concern in arid and semiarid regions of the world (Dai et al., 2009). Using salt-tolerant turfgrass is deemed an efficient method to alleviate salinity problems. Grass species and genotypes vary in their responses to salinity stress, which contains changes in morphological, physiological, and biochemical aspects (Zhang and Rue, 2011; Zhang et al., 2012). This indicates identification and screening of turfgrass for genetic improvement of salinity tolerance can be an acceptable strategy (Mittova et al., 2003). The first step in the program of identification and screening for salinity tolerance in grasses is to conduct a germination examination (Serena et al., 2012).

Desert wheatgrass (*Agropyron desertorum* L.), tall wheatgrass (*Agropyron elongatum* L.), and crested wheatgrass (*Agropyron cristatum* L.) are cool-season perennial grasses with strong tolerance to abiotic stress and accordingly, they are capable of growing in arid and semiarid climates (Bayat et al., 2016; Gunnell et al., 2010). This genus of wheatgrass is potentially a low-input turf because it is strongly tolerant against drought, salinity, low temperature, disease, and pests (Shana and Liagna, 2010; Sheikh-Mohamadi et al., 2017a). Until now, no information exists about Iranian wheatgrass genotypes regarding their potentials of tolerance to salinity during seed germination and seedling growth stage. Understanding the physiological and biochemical mechanism of turfgrass

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The germination phase is an important and vulnerable stage in the life cycle of plants because establishment of the seedling and plant growth can be partly defined and seriously influenced by salinity (Hu et al., 2012b). Salinity stress is becoming a main environmental factor limiting seed germination and seedling growth in arid and semiarid

salinity tolerance is important for breeders if they are to develop salt-tolerant genotypes of turfgrass. Accordingly, practitioners can improve turfgrass quality under salinity stress if the correct genotypes are used for cultivation. Our objectives were to investigate osmotic adjustment and antioxidant capacity as indices that set an array of contrast among the

20 Iranian wheatgrass genotypes evaluated in this study. The ultimate aim was to compare their tolerance to salinity. The genotypes were initially categorized as the Iranian desert wheatgrass, tall wheatgrass, and crested wheatgrass, and the experiments were conducted during their seed germination and seedling growth stage.

Materials and Methods

Plant material and seed collection site. The experimental material was comprised of 20 native wheatgrass genotypes, including eight genotypes of Iranian desert wheatgrass (*Agropyron desertorum* L.), six genotypes of Iranian tall wheatgrass (*Agropyron*

Table 1. Geographical origin of Iranian wheatgrass genotypes and tall fescue (control).

Code	Species	Region	Longitude (E)	Latitude (N)	Altitude (m)	Avg annual temp (°C)	Annual rainfall (mm/yr)
AD1	<i>Agropyron desertorum</i> L.	Fereidan-Isfahan	50.1241°	32.9401°	2,290	9	505.5
AD2		Ardakan-Yazd	54.0086°	32.3082°	1,035	19.8	69
AD3		Shahrud-Semnan	55.0163°	36.4062°	1,380	14.1	161.5
AD4		Darab-Shiraz	54.3100°	28.5400°	1,180	20.4	176.2
AD5		Mobarakeh-Isfahan	52.5132°	31.8427°	1,690	24.8	192.6
AD6		Shahrekord-Chaharmahal & Bakhtiari	50.8769°	32.3282°	2,060	21.8	267.9
AD7		Boldaji-Chaharmahal & Bakhtiari	51.0300°	31.5600°	2,220	20.5	264.5
AD8		Malayer-Hamadan	48.8146°	34.3020°	1,725	12.2	302.1
AE1	<i>Agropyron elengatum</i> L.	Geydar-Zanjan	48.5938°	36.1203°	2,775	11.5	420
AE2		Save-Markazi	50.0136°	35.6980°	998	16.6	223.5
AE3		Aligudarz- Lorestan	49.6962°	33.4050°	2,100	11.6	264.2
AE4		Paveh-Kermanshah	46.3553°	35.0430°	1,540	12.5	674
AE5		Jiroft-Kerman	57.7372°	28.6751°	720	23.5	81
AE6		Kuhin-Qazvin	49.6598°	36.3716°	1,527	13.1	304.7
AC1	<i>Agropyron cristatum</i> L.	Hashtgerd-Alborz	50.6846°	35.9614°	1,175	14.6	377
AC2		Arak-Markazi	49.7013°	34.0954°	1,750	13.9	311
AC3		Urmia-Azerbaijan	45.0433°	37.3309°	1,330	10.6	555
AC4		Takestan-Qazvin	49.7013°	36.0721°	1,260	18.1	350
AC5		Damavand-Tehran	52.0631°	35.5721°	1,903	12.1	149
AC6		Sabzevar-Khorasan Razavi-Iran	57.6678°	36.2152°	977	18.2	330
FA	<i>Festuca arundinacea</i> L.	—	—	—	—	—	—

Fig. 1. Effect of salinity stress on mean germination time (MGT, A), final germination percentage (FGP, B), and rate of germination (GR, C) of 21 plants including 20 Iranian wheatgrass genotypes (AD1 to AC6) and tall fescue (FA).

elongatum L.), and six genotypes of Iranian crested wheatgrass (*Agropyron cristatum* L.) (Table 1). Tall fescue (*Festuca arundinacea* L.) cultivar Van Gogh (relatively stress tolerant) was used as control treatment. Wheatgrass genotypes were collected in Sept. 2015 from 20 sites in Iran. All seed samples were kept at a constant 4 °C temperature at the Turfgrass Seed Testing center, Department of Horticulture at Isfahan University of Technology, Isfahan, Iran. Seeds were disinfected following the protocol of 8% sodium hypochlorite (NaClO) for 10 min to prevent fungal attack and rinsed thoroughly with deionized water (Zhang and Rue, 2011). Preliminary investigation using different levels of light (24 h dark/24 h light, 16:8 h light/dark, and 12-h light/dark cycle) and different temperatures regimes (4, 16, 24, and 32 °C) the results indicated that optimized conditions for germination of Iranian wheatgrass genotypes seeds occurred at 24 °C and 16:8 h light/dark, with a germination percentage nearly being 94%. Before the germination test began, Iranian wheatgrass genotypes seeds were tested for seed viability, by methods of tetrazolium test (TTC test) (AOSA, 2000). Three replications of 30 seeds were used to test viability using tetrazolium solution (2, 3, 5-triphenyl-tetrazolium chloride). Accordingly, seeds were longitudinally cross-sectioned through the embryo and immersed in a 0.1% TTC solution at 30 °C for 24 h in the dark. Afterward, the cut seeds were tested for pink staining (Chanyengaa et al., 2012).

Germination and seedling growth study. Iranian wheatgrass genotypes were germinated in solutions included distilled water 0, 50, 100, and 150 mM of NaCl, corresponding to 0, 0.2, 0.4, and 0.6 MPa osmotic potential, respectively (López Colomba et al., 2013). Germination and seedling growth test were conducted in a programmed incubator under the controlled conditions: 16:8 h light/dark cycle (60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (4000 lx) each day for 20 d at temperatures of 24 °C, and petri dishes were sealed with polyethylene sheets to prevent evaporation. During testing, contamination was not found. For each treatment, 100 seeds were used with four replicates each. The number of germinated seeds was recorded three times a week for 20 d. A seed was considered as a germinated seed if the radicle or coleoptile had emerged and grown more than 2 mm in length. Indices of seed germination and seedling growth were calculated as follows: the values of final germination were obtained as the percentage of the total number of germinated seeds over the number of total seeds after 20 d (Dai et al., 2009). Final germination percentage is described by $\text{FGP} (\%) = 100 \frac{\sum ni}{N}$. The mean germination time (MGT) was calculated as $\text{MGT} = \frac{\sum (ni \times di)}{N}$; where ni is the number of seeds germinated at day i after sowing, di is the time between the beginning of the experiment and the end of a particular interval of measurement, and N is the total number of germinated seeds (Zhang, 2012). A modified Timson index of germination

velocity = $\Sigma G/t$ was used for estimating the GR, where “ G ” is germination percentage after every 48 h and “ t ” is the total germination period (20 d) (Hameed et al., 2014). Radicle length and plumule length (RL and PL) and seedling fresh (RFW and PFW) and dry weight (RDW and PDW) were recorded. The seedlings were dried at 75 °C for ≈ 48 h, and seedling dry weight was measured (Colomba et al., 2013).

Hydrogen peroxide contents (H_2O_2) and MDA content. Wheatgrass genotypes seedlings samples were ground fine using liquid N_2 in ice-chilled mortar and pestle and homogenized in 4% ice-cold trichloro acetic acid. Homogenate was then centrifuged at 15,000 g for 15 min at 5 °C. Supernatant was used for the calculated of hydrogen peroxide content (H_2O_2 ; Zhou et al., 2005) and MDA content (Heath and Parker, 1968).

Proline content, TNC, and GSH content. Proline content was determined in 90% ethanol extracts from wheatgrass genotypes seedlings samples. After centrifuged at 19,000 g for 15 min, the supernatant was stored at 5 °C and proline content determined according to the method described by Bates et al. (1973) with some modifications. TNC content was measured using the method of Fry et al. (1993) with some modifications and expressed as 0.2 g of wheatgrass genotypes seedlings samples. The reaction solution absorbance was read at 515 nm by a spectrophotometer. GSH content was measured using the method of Griffith (1980) with some modifications and expressed as 0.5 g of wheatgrass genotypes seedlings samples. Absorbance was calculated at 412 nm, and the GSH content was calculated by using the standard curve.

Antioxidant enzymes. Exactly 0.4 g of wheatgrass genotypes seedlings was ground in 5 mL of 50 mM phosphate-buffer (pH 7.6) at 5 °C then centrifuged at 14,000 g for 14 min. The supernatant was gathered for assays of enzyme activities. Measurements were directed at conducting extractions and assays of different antioxidant enzymes, including APX activity, SOD activity, CAT activity, and POD activity, which were done according to the methods described by Han et al. (2008) and Zhou et al. (2005).

Data analyses. We used a completely randomized factorial design with two types of factors, four levels of salinity, wheatgrass genotypes, and three replicates. The data were subjected to analysis of variance (ANOVA) using SAS software (SAS institute Cary, NC, 1988). In addition, the mean values of SEM were compared using least significant difference test. Cluster analysis was performed to differentiate the genotypes according to the unweighted pair group method with arithmetic mean method. The cluster analysis and principle component analysis (PCA) were conducted using SPSS software for Windows 20.0 (SPSS Inc., Chicago, IL). Heat map of the correlations was conducted with MetaboAnalyst (<http://www.metaboanalyst.ca/>).

Table 2. Analysis of variance based on some characters measured of the 20 Iranian wheatgrass genotypes and tall fescue (control) under salinity stress.

S.O.V	dF	MGT	FGP	GR	RL	RFW	RDW	PL	PL	PFW	PDW	H_2O_2	MDA	Proline	TNC	GSH	SOD	CAT	POD	APX
Salt	3	678	3,494	4,717	2,412.5	1,686	1,579	13,463	13,463	1,115	741.6	12,791	5,037	83.6	111.8	60	2,097	1,448	2,803	1,973
Genotype	20	23.4	110.1	86.1	99.9	71.7	68.4	472.98	472.98	41	22.2	419.5	229.5	30.85	40.34	27.7	1,051	781	694	609.6
G $\times$ S	60	4.38	14.37	13	20.94	15.4	0.000	14.6	14.6	8.9	6.81	114.9	45	7.63	7.77	7.73	275	181	248	180

MGT = mean germination time; FGP = final germination percentage; GR = rate of germination; RL = radicle length; RFW = radicle fresh weight; RDW = radicle dry weight; PL = plumule length; PFW = plumule fresh weight; PDW = plumule dry weight; MDA = malondialdehyde; TNC = total nonstructural carbohydrates; GSH = glutathione; SOD = superoxide dismutase; CAT = catalase; POD = peroxidase; APX = ascorbate peroxidase.
^aAll values of mean squares for salt, genotype and interaction of them are significant at 1% level.

Results and Discussion

Effect of salinity stress on seeds germination. Viability was indicated by the TTC test. The results differed significantly among the various Iranian wheatgrass genotypes (data not shown). The results showed that seeds of 'AD1', 'AE1', 'AE6', and 'FA' used in the research had a high viability percentage ($\approx 98.0\%$). Seed germination is considered as the most sensitive stage of the plant life cycle (Sidari et al., 2008). Salinity stress may affect seed germination process through its complete inhibition or delay in the initiation of the germination and seedling establishment through the oxidative stress, osmotic stress, and ion toxicity (Freitas and Costab, 2014; Zhang et al., 2012). Salinity tolerance during seed germination and seedling growth stage is critical for the establishment of plants that can grow in Saline soils (Munns and Tester, 2008). In these experiments, our results demonstrated that salinity limits the germination of Iranian wheatgrass genotypes seeds. The result of this study showed that a direct relationship was observed between MGT and the increase in salinity concentration up to 150 mM NaCl in all genotypes ($P \leq 0.01$) (Fig. 1A). When

salinity levels increased (50, 100, and 150 mM NaCl), MGT increased in all genotypes in comparison with the control condition (Fig. 1A). Among the wheatgrass genotypes, 'AD1', 'AD3', 'AE5', and 'FA' had the lowest MGT in this experiment (data not shown). A longer MGT under salinity stress was concurrent with delays in seed germination (Zeng et al., 2014). The result of ANOVA showed that there are significant differences among Iranian wheatgrass genotypes ($P \leq 0.01$) when exposed to different salinity levels for FGP (Table 2). The highest FGP was observed for 'AD2', 'AD3', and 'AE5' genotypes. Salinity had significant effect on FGP ($P \leq 0.01$), where increased levels of salinity reduced these traits in the all genotypes, and the rate of decline different between genotypes (Fig. 1B). At 50 mM NaCl, 'AD2', 'AE5', and 'FA' genotypes showed the highest FGP ($\approx 94.0\%$) (Fig. 1B). As the salinity level increased to 100 mM NaCl, FGP of 'AD2', and 'AE5' grew significantly higher ($\approx 85.0\%$) than the other grasses (Fig. 1B). The 'AD2', 'AD3', and 'AE5' indicated a high amount of FGP ($\approx 65.0\%$) when the salinity level was increased to 150 mM NaCl (Fig. 1B). The results indicate that when salinity levels are

increased, the effect can result in the reduction of GR in all of the genotypes. Nonetheless, among all, the 'AD1' was less damaged, and its GR was greater than that of the other genotypes. At 0 mM NaCl treatment, the wheatgrass genotypes, 'AD4', 'AD5', and 'AE5' had the higher GR than the other genotypes (Fig. 1C). Seedlings of 'AD1', 'AE5', and 'FA' had higher GR than other genotypes under 50 and 100 mM NaCl stress, as compared with the control (Fig. 1C). When salt concentrations were increased to 150 mM NaCl, higher rates of GR were observed in 'AD1' and 'FA' (Fig. 1C). Salinity greatly affects germination and seedling growth, and finally reduces the germination percentage and rate. Furthermore, a delay in the initiation of seed germination process and seedling establishment is also expected, but variations in adaptive mechanisms may still exist in different species and genotypes (Camberato and Martin, 2004). When stress conditions are present, changes in the enzymes and hormones are commonly observed in the seed, and they can reduce the GR and FGP (Zhao et al., 2014). The maintenance of higher levels of FGP and GR under salinity stress is often associated with better salinity tolerance in genotypes (Dai et al., 2009).

Fig. 2. Effect of salinity stress on hydrogen peroxide (H_2O_2 , **A**) content, malondialdehyde (MDA, **B**) content, and proline content (**C**) of 21 plants including 20 Iranian wheatgrass genotypes (AD1 to AC6) and tall fescue (FA).

Germination of seeds should occur uniformly for a successful establishment of turfgrass seedlings. High GR and FGP are indicators that there is a high potential for successful establishment (Lai et al., 2015; Zaher-Ara et al., 2016; Zhao et al., 2014). Prevention or delay of seed germination under salinity stress caused by an osmotic and oxidative stress and ion-toxicity effect, which limits the water uptake by seeds during germination with blocking membrane, or cytosolic antioxidants enzymes and hormones which leads to a series of physiological changes, contains changed function and structure of an enzyme and general reduction in hydrolytic capacity and metabolic activity and use of content of seeds reserve (Colomba et al., 2013; Hameed et al., 2014).

Effect of salinity stress on seedling growth. Results of this study show that fresh and dry weights and length of plumule and radicle were significantly affected by salt stress and cultivars ($P \leq 0.01$) (Table 2). The increase in severity of salinity stress reduced the amount of these traits in all genotypes. Tolerant to salinity during early seedling growth is important for the seedling establishment of grass that can grow in saline areas (Hu et al., 2012b). Of the Iranian

wheatgrass genotypes, 'AD2', 'AD3', and 'AE5' had the highest RL, radicle fresh weight (RFW) and dry weight (RDW), whereas 'AC1' had the lowest of these traits. Among the 21 grasses genotypes, 'AD1', 'AD2', 'AD3', and 'FA' genotypes showed higher levels and 'AE6' genotypes showed lower levels of PL and plumule fresh weight (PFW) and plumule dry weight (PDW) (data not shown). The length of plumule and radicle are two important parameters that correspond with salt stress. Because the radicle grows to be in direct contact with the culture medium, water is absorbed from the culture medium and then the plumule supplies it to the rest of the plant (Cavallaro et al., 2016; Laghmouchi et al., 2017). Accordingly, PL and RL provide an important instance whereby plants can be studied in how they respond to environmental stress (Mickky and Aldesuquy, 2017). The strongest possibility to grow in arid and saline areas occurs for plants that can maintain a longer plumule and radicle when exposed to stress conditions (Goatley et al., 2017).

Effect of salinity stress on physiological and biochemical traits. In an environment without stress, oxygen metabolism and oxygen toxicity occurs at a low level, and there is an ideal balance between production and

elimination of ROS (Murillo-Amador et al., 2006). The balance between the production and elimination of ROS may be perturbed by a biotic and abiotic stresses (Meloni et al., 2003). Salinity stress promotes the accumulation of ROS, including 1O_2 , H_2O_2 , and OH^* , which can cause oxidative damage to vital cellular components and cellular processes, such as membrane function, carbohydrates, proteins, enzymes, DNA, and nucleic acids (Kolenc et al., 2016; Sekmen et al., 2012), for example, ROS can affect the polyunsaturated fats and membrane lipids, leading to lipid peroxidation and MDA formation (Davey et al., 2005). The production of H_2O_2 in unstressed conditions (0 mM NaCl) remained relatively constant throughout the duration of the experiment in all genotypes (Fig. 2A). In all salinity treatments (50, 100, and 150 mM NaCl), the drastic increase in H_2O_2 content was evaluated in all genotypes, whereas the lowest increase was observed in 'AD1', 'AD2', and 'AD3' genotypes (Fig. 2A). Plants that can maintain low levels of H_2O_2 content under salinity stress exposure will have the highest possibility of continued metabolic activity (Møller et al., 2007). The reduction of oxidative stress and maintaining the physical integrity of the cell membranes under salinity stress is

Fig. 3. Effect of salinity stress on total nonstructural carbohydrates (TNC, **A**) content, glutathione (GSH, **B**) content, and superoxide dismutase (SOD, **C**) activity of 21 plants including 20 Iranian wheatgrass genotypes (AD1 to AC6) and tall fescue (FA).

considered one of the mechanisms in salinity tolerance (Hu et al., 2012a).

MDA is one of the final decomposition products when lipid peroxidation occurs in the plant cell membrane as it is caused by free radical damage and oxidative stress. Its accumulation is a sign of the extent to which oxidative damage occurs (Hernández and Almansa, 2002). The MDA has been widely used as a physiological indicator for the evaluation of plant tolerance to salinity stress, and it can be used as a tool for the differentiation of salt-tolerant and salt-sensitive genotypes (Abid et al., 2016). Previous studies have reported that genotypes that exhibit lower levels of MDA content are more tolerant to salinity stress (Filek et al., 2012). Under various levels of salinity stress, the MDA production in all genotypes

increased and the rate of increase was observed to be different between them (Fig. 2B). At 50 mM NaCl treatment, the wheatgrass genotypes, and 'AE3' had the lowest MDA content (Fig. 2B). As the salinity level increased to 100 mM NaCl, MDA production in 'AD1', 'AD2', and 'AD3' significantly increased and showed the lowest MDA content in comparison with the other genotypes (Fig. 2B). When the salinity level was increased to 150 mM NaCl, the lowest rate of MDA content was observed in 'AD1', 'AD2', and 'AD3' (Fig. 2B). The results of Filek et al. (2012) suggested that the higher salt tolerance was associated with the lower MDA content. The variation in the levels of MDA content according to the different genotypes might have resulted from the ROS-mediated membrane lipid peroxidation

calculated using MDA and differences in the roles of antioxidant protective enzymes in controlling the ROS level in seeds (Hu et al., 2012a). Stress-tolerant species and genotypes showed a better chloroplast structure under abiotic stress conditions with lower accumulation of H₂O₂ and MDA than stress-sensitive plants (Luna et al., 2008).

Mechanism of osmotic adjustment is considered as an important physiological response of stress adaptation in plant cells (Sekmen et al., 2012). It involves the accumulation of a wide range of osmotically active compounds including proline and TNC within the cell (Aranjuelo et al., 2011). Proline is an amino acid that is also considered as an important osmotically active compound. It plays an integral role in maintaining cell turgor and in protecting

Fig. 4. Effect of salinity stress on catalase (CAT, **A**) activity, peroxidase (POD, **B**) activity, and ascorbate peroxidase (APX, **C**) activity of 21 plants including 20 Iranian wheatgrass genotypes (AD1 to AC6) and tall fescue (FA).

Table 3. Mean values of the characters studied based on cluster analysis.

Cluster	MGT	FGP	GR	RL	RFW	RDW	PL	PFW	PDW	H ₂ O ₂	MDA	Proline	TNC	GSH	SOD	CAT	POD	APX
SSG (1)	12.91	63.11	28.51	34.2	0.12	0.03	41.2	0.36	0.06	47.16	22.20	3.26	4.20	3.8	1,621	1,153	16.3	25.8
SMG (2)	10.50	74.54	29.73	37	0.14	0.03	47.1	0.42	0.06	32.78	20.00	10.66	13.5	7.1	2,242	1,842	31.5	39.2
STG (3)	9.17	83.84	38.42	47.1	0.17	0.03	52.5	0.47	0.07	22.11	14.81	12.46	15.7	12	2,833	2,031	43.5	49.6

MGT = mean germination time; FGP = final germination percentage; GR = rate of germination; RL = radicle length; RFW = radicle fresh weight; RDW = radicle dry weight; PL = plumule length; PFW = plumule fresh weight; PDW = plumule dry weight; MDA = malondialdehyde; TNC = total nonstructural carbohydrates; GSH = glutathione; SOD = superoxide dismutase; CAT = catalase; POD = peroxidase; APX = ascorbate peroxidase; SSG = salinity sensitive genotypes; SMG = salinity moderate genotypes; STG = salinity tolerant genotypes.

protein and membrane structures. It is antioxidant protective and helps with osmotic adjustment (Kima et al., 2016). Among the 20 experimental genotypes used in the present study, the seedling of 'AD3' and 'AE5' had the highest proline content, and 'AE4' had the lowest proline content. There was no change in the proline content in the 'AD4' and 'AE4' under salinity stress (Fig. 2C). The proline content in four of the genotypes ('AD1', 'AD2', 'AE2', and 'AE5') increased with elevating salinity stress levels (0, 50, 100, and 150 mM NaCl) (Fig. 2C). Researchers previously showed that proline accumulation correlates with environmental stress. Increased proline content can naturally enhance plant tolerance, and such an observation has also been reported in several higher plants which, because of proline, become more tolerant to stress conditions (Pompeiano et al., 2012). TNC content in plants include water-soluble (glucose, fructose, and sucrose) and storage (starch and fructan) sugars (Qian and Fu, 2005). Accumulation of TNC content under salinity stress acts in a manner that protects the cell by causing a balance in the osmotic strength of the cytosol. It further helps to protect tissue water, cellular membranes, and sustains turgor in leaves. It also assists in energy transport and energy storage (Huang and Fu, 2000). Results showed that differences in TNC content were significant in different genotypes under different salinity level

($P \leq 0.01$) (Table 2). The highest TNC content was observed in 'AD1', 'AE5', 'AC2', and 'AC6' genotypes. TNC content in 'AD1', 'AD3', 'AD6', 'AE5', 'AC2', 'AC5', 'AC6', and 'FA' seedlings increased with increasing salinity stress levels (Fig. 3A). The accumulation of TNC content in response to environmental stress has been reported in various grass species (Richie et al., 2001). Higher content of TNC in the grass could indicate a greater tolerant to stress condition. Several reports have indicated that an increase in TNC contents is associated with the improvement in tolerant to stress (DaCosta and Huang, 2006; Sheikh-Mohammadi et al., 2017b).

As a countermeasure against free radicals, plants have gradually developed highly efficient antioxidant defense system which acts to minimize and eliminate ROS-induced oxidative stresses. These defense systems are mostly comprised of antioxidant enzymes such as SOD, CAT, POD, APX, and non-antioxidant enzymes such as GSH which contribute to the prevention of subcellular damage (Simlata et al., 2016; Solimana et al., 2012). GSH is a hydrophilic nonantioxidant endogenous antioxidants, which keeps ROS from accumulating in cells and causing oxidative damage (Guo et al., 2006). In antioxidative defense mechanisms in grass, the balance between the ROS production and eliminates that determines the amount of oxidative stress (Demiral and Turkan,

Table 4. Principal component loadings for the traits measured on the 21 plants including 20 Iranian wheatgrass genotypes and tall fescue.

Label	Characters	Principal components		
		PC1	PC2	PC3
1	FGP	0.91	-0.22	-0.14
2	MGT	-0.83	-0.32	0.05
3	GR	0.8	-0.05	0.21
4	RL	0.85	-0.36	0.35
5	RFW	0.86	-0.32	0.34
6	RDW	0.84	-0.34	0.36
7	PL	0.85	0.47	0.19
8	PFW	0.83	0.47	0.19
9	PDW	-0.9	0.42	0.29
10	MDA	-0.9	0.19	0.27
11	H ₂ O ₂	-0.88	0.17	0
12	APX	0.87	0	-0.2
13	POD	0.9	-0.06	-0.19
14	CAT	0.82	-0.03	-0.24
15	SOD	0.88	-0.09	-0.28
16	GSH	0.86	0.12	-0.27
17	Proline	0.87	0.05	-0.04
18	TNC	0.79	0.05	-0.19
Eigenvalue		13.25	1.24	1.02
% of variance		73.64	6.92	5.67
Cumulative%		73.64	80.56	86.23

MGT = mean germination time; FGP = final germination percentage; GR = rate of germination; RL = radicle length; RFW = radicle fresh weight; RDW = radicle dry weight; PL = plumule length; PFW = plumule fresh weight; PDW = plumule dry weight; MDA = malondialdehyde; TNC = total nonstructural carbohydrates; GSH = glutathione; SOD = superoxide dismutase; CAT = catalase; POD = peroxidase; APX = ascorbate peroxidase. The values higher than 0.5 are presented as bold significant.

Fig. 5. Dendrogram generated based on traits measured on the 21 plants including 20 Iranian wheatgrass genotypes and tall fescue using the unweighted pair group method with arithmetic mean method.

2005). Data in this study show that seedlings of 'AE2' and 'AE5' had higher GSH content than other genotypes when exposed to the lowest level of salinity (i.e., 50 mM NaCl) (Fig. 3B). When the stress level was increased to reach the highest level of salinity (i.e., 150 mM NaCl), the GSH content of 'AD2' and 'AE5' genotypes rose significantly to become higher than the other grasses (Fig. 3B). During the experiment, seedlings of 'AE5' Iranian wheatgrass genotypes had higher GSH content than other grasses. Higher content of GSH in the grass genotype could indicate a greater tolerant to stress condition (Taliaferro, 2003). Lu et al. (2008) found that an increase in GSH content directly correlates with increasing the drought stress in bermudagrass. SOD enzymes activity was observed to stay unchanged throughout the duration of the research in all genotypes when exposed to 0 mM NaCl (Fig. 3C). However, under the 50, 100, and 150 mM NaCl stress conditions, the

highest SOD enzymes activity were observed in 'AD3' (Fig. 3C). SOD is known as a major plant antioxidant, powerful enough to cope with ROS. It induces plant tolerant against environmental stresses (Liua and Chana, 2015). SOD causes the dismutation of O_2 to H_2O_2 and to prevent OH^* formation (Sales et al., 2013). This study revealed that there was no change in the CAT enzymes activity in the 'AD8', 'AE1', 'AC1', 'AC3', and 'AC4' under salinity stress (Fig. 4A). Results show that CAT activity in 'AD1', 'AE5', and 'FA' increased when exposed to salinity stresses of 50 and 100 mM NaCl, but then decreased at 150 mM NaCl (Fig. 4A). CAT plays an essential role in scavenging H_2O_2 toxicity, thereby acting protectively by transforming H_2O_2 to H_2O and O_2 which is subsequently followed by the elimination of ROS completely (Lu et al., 2009). At 50 mM NaCl treatment, the wheatgrass genotypes 'AD5', 'AD1', and 'AC6' had higher POD enzyme activity (Fig. 4B). As salinity

levels increase to reach 100 mM NaCl, POD activity of 'AD2', 'AD3', 'AE5', and 'FA' was significantly higher than the other grasses (Fig. 4B). When the salinity level was increased to 150 mM NaCl, the higher rate of POD enzymes activity was observed in 'AD1', 'AD2', 'AE5', and 'FA' (Fig. 4B). The activity of APX in 'AD1', 'AD3', 'AD4', and 'AD6' seedlings increased with elevating salinity stress (Fig. 4C). APX and POD are important antioxidant enzymes in the plant cycle because of their physiological role in transforming H_2O_2 to H_2O and O_2 and can scavenge or detoxify this ROS completely (Wang et al., 2009). Seed germination, seedling growth, and seedling establishment are parameters that are regularly weakened by increasing levels of environmental stress such as salinity stress (Hameed et al., 2014). Tolerance to abiotic stress or sensitivity in grass often correlates well with inherent antioxidant responses and cellular adjustments to oxidative stress

Fig. 6. Heat map of the correlations between the characters studied under drought stress of the 20 Iranian wheatgrass genotypes and tall fescue.

(Amor et al., 2006). Our results indicate that the antioxidative defense mechanism is an essential component of salt tolerance in salt-tolerant wheatgrass genotypes. By comparing the response pattern of wheatgrass genotypes to salt stress and by comparing their antioxidant enzymes activities, results indicate that there is a significant genotypic difference among the genotypes with respect to salt exposure. Tolerant grass species and genotypes generally have a higher capacity to protect themselves from environmental stresses which induce oxidative damage. This can be achieved through the increase in antioxidant enzyme activity (Liua et al., 2012; Mhadhbi et al., 2011). Maintaining a high level of antioxidative enzyme activity can be likely effective for salinity tolerance by improving the antioxidant capacity as a powerful protection mechanism against oxidative stress. This has also been reported in several higher plants which happen to be tolerant under abiotic stress conditions. Xu et al. (2013) suggested that high levels of SOD, CAT, and APX can be considered as defense mechanisms in Kentucky bluegrass (*Poa pratensis* L.) and tall fescue (*Festuca arundinacea* L.) under salinity stress conditions.

Cluster and PCA analysis. Cluster analysis was performed by considering all of the measured characteristics, which classified the genotypes into three clusters (Fig. 5). Out of the 21 genotypes, five genotypes ('AD1', 'AD2', 'AD3', 'AE5', and 'FA' genotypes) were in the "salinity tolerant genotypes" cluster. Three of the five genotypes of the "salinity tolerant genotypes" cluster came from desert wheatgrass. Therefore, desert wheatgrass genotypes were more tolerant among the wheatgrass species studied herein. The cluster exhibited the maximum values of some characteristics such as FGP, GR, RL, RFW, PL, PFW, PDW, APX, POD, CAT, SOD, proline, GSH, and TNC characters, and the minimum of MGT, MDA, and H₂O₂ characteristics (Table 3). The same condition was also observed in "salinity sensitive genotypes" cluster. Twelve genotypes were in this cluster. The mean value for RDW character in the three clusters was equal. Principle component analysis revealed that the first three components explained 86.23% of the variation in the genotypes under salinity stresses (Table 4). PC1 confirmed 73.64% of total variance. All measured characteristics correlated strongly with the first component; therefore, the PC1 was named "salinity stress." Among them, the FGP showed maximum correlation with the PC1 (0.91), followed by SDW (-0.9), MDA (-0.9), and POD (0.9). Meanwhile, the SDW, MDA, H₂O₂, and MGT had negative correlations with PC1, whereas the other characteristics exhibited positive correlations with PC1. Because many measured characteristics had high correlation with the first component, it can be said that there were many correlations between other characteristics too (Fig. 6). The PC2 and PC3 confirmed 6.92% and 5.67% of the variance among the studied genotypes, respectively.

Conclusions

The results of this study show how genetic variation can cause differences in salinity tolerance among 21 grass genotypes. We observed that some wheatgrass genotypes are tolerant to salinity stress more than others. For all genotypes, FGP and GR were reduced, and MGT increased with increasing salinity stress. However, the magnitude of these effects differed between genotypes. Studying the salinity tolerance in genotypes was done using the data of all germination and seedling characteristics at all levels of drought stress. According to the results of cluster analysis, 'AD1', 'AD2', 'AD3', 'AE5', and 'FA' showed the highest salt tolerance. Our results suggest that the osmotic adjustment and antioxidant defense mechanisms depended on grass genotypes. It can be concluded that genotype is an essential component of salinity tolerance of wheatgrass. Salt-tolerant genotypes maintained higher levels of enzymatic (SOD, CAT, APX, and POD) and nonenzymatic (GSH) antioxidants activities, which caused further to accumulate compatible osmolytes (such as proline and TNC) under salinity conditions. Finally, it may be suggested that 'AD1', 'AD2', 'AD3', and 'AE5' are the strongest wheatgrass genotypes in terms of their tolerance to salt stress. However, longer-term experiments would be needed to validate salinity tolerance differences observed between these genotypes.

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