

Exploring ion homeostasis and mechanism of salinity tolerance in primary tritipyrum lines (Wheat× *Thinopyrum bessarabicum*) in the presence of salinity

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Abstract

Due to lack of water resources in irrigated agriculture, genetically improving plants to abiotic stresses such as salinity is a necessity for food and feed production. In this respect, the new third man-made amphiploid cereal, tritipyrum ($2n=6x=42$, AABBE^bE^b), is an example which is capable of tolerating a high level of NaCl. In order to determine the salinity tolerance mechanisms of this new cereal, an experiment was conducted using hydroponic technique. Ten tritipyrum lines and two wheat cultivars were tested under three levels of salinity (50, 100 and 200mM NaCl). The effect of salinity stress on Na⁺ and K⁺ concentration of leaf, shoot and root, proline and chlorophyll content were measured at 50% ear emergence and their grain yield plant⁻¹ was evaluated at physiological maturity. Leaf Na⁺ concentration in tritipyrum lines increased with increasing salinity while K⁺ concentration did not show any especial pattern. The chlorophyll and proline content in tritipyrum lines were higher than that of wheat cultivars. Despite the high sodium concentration in tritipyrum lines in comparison with wheat, the grain yield of tritipyrum lines were less affected than that of wheat. There was also a negative correlation between proline content and grain yield plant⁻¹ in tritipyrum lines. It can be concluded that mechanisms such as higher Na⁺ uptake along with appropriate ion compartmentation could be used by tritipyrum lines to combat with salt stress like some halophytes and it can make tritipyrum lines suitable for planting in saline soils and improving the salinity tolerance of wheat.

Keywords: Halophytic wild ancestors, Proline, Salt tolerance mechanism, Tritipyrum.

Abbreviations: LNa_ leaf sodium concentration; LK_ leaf potassium concentration; LK:Na or L K⁺/Na⁺_ leaf potassium to sodium ratio; SN_ stem sodium concentration; SK_ stem potassium concentration; SK:Na_ stem potassium to sodium ratio; RN_ root sodium concentration; RK_ root potassium concentration; RK:Na_ root potassium to sodium ratio; Chl a_chlorophyll a concentration; Chl b_ chlorophyll b concentration; Chl a/b_ chlorophyll a to chlorophyll b ratio; T Chl_ total chlorophyll; GY_ grain yield plant⁻¹; Pr: proline content; DW_ dry weight; FW_ fresh weight; PCA_ principle component analysis; Yp_ yield of genotype in stress condition; Yp_ yield of genotypes under control condition; Na⁺_ sodium concentration and K⁺_ potassium concentration.

Introduction

Despite the favorable effects of wheat domestication such as increasing the quality and quantity of yield, it has created some genetic bottlenecks such as genetic erosion. This by itself leads to an increased susceptibility to some environmental stresses like salinity (that currently affects around %7 of the earth's surface). Therefore, the need for another neo-domestication of wheat was felt (Faris, 2014; Peng et al., 2011). To achieve this goal and improve salinity tolerance of wheat, use of genetic resources such as its halophytic wild ancestors like tall wheat grass spp (e.g. *Thinopyrum* spp.) is suggested (Colmer et al., 2006a; Colmer et al., 2006b; Peng et al., 2011). Among tall wheat grasses, *Thinopyrum bessarabicum* (a salt-tolerant perennial wheat grass that grows on sea shores of Crimea) can survive up to 350 mM NaCl for long periods (Gorham et al., 1985). Maintaining leaf K⁺ concentration, synthesizing

glycinebetaine and regulating leaf Na⁺ and Cl⁻ concentration for osmotic adjustment are strategies that have been adopted by *Th. bessarabicum* to resist salinity (Colmer et al., 2006b). Hybridization between wheat and *Th. Bessarabicum* has shown encouraging results and has resulted in the creation of a third man-made amphiploid (tritipyrum) (King et al., 1997). It is a new product that is promising the production of a new synthetic amphiploid like triticale and it has E^b genome instead of D genome in hexaploid wheat. This amphiploid has high tolerance to salinity, wheat like growth, seed set and novel multiple seed trait in some lines and its meiosis is generally regular. Of course it has undesirable traits such as late maturity, continuous production of tillers, brittle rachis and partial meiotic instability. It is capable of becoming a new crop and it has been suggested to be used as a new cereal crop in saline soils (King et al., 1997). However, a question

that is raised about tritipyrum is that: among salinity resistance mechanisms which ones are used by tritipyrum lines?

During salinity stress, Na⁺ not only takes over Na⁺/K⁺ co-transporters, but it can also block K⁺ specific transporters and these events cause increasing sodium entree up to toxic levels, and decreasing potassium uptake until there is insufficient concentration for enzymatic reaction and osmotic adjustment (Apse and Blumwald, 2007). Also salinity stress changes photosynthetic pathway, decreases chlorophyll content and increases chlorophyll *a*/ chlorophyll *b* ratio (Parida and Das, 2005; Khan, 2003; Khatkar and Kuhad, 2000). More than one salt resistance mechanism is involved in plants and a combination of factors and strategies contributes to wheat salinity resistance (Flowers and Colmer, 2008; Mudgal et al., 2010; Munns and Tester, 2008). Tolerance to osmotic stress, Na⁺ exclusion from leaf blade and tissue tolerance were introduced as mechanisms of salinity tolerance (Munns, 2008). Compartmentation of Na⁺ and increasing accumulation of compatible solutes like proline are strategies that are used by tissue tolerance mechanisms (Munns, 2008). Some studies have shown a good relation between Na⁺ exclusion and salt tolerance (Poustini and Siosemardeh, 2004; Munns and Jame, 2003). However, in other studies no significant relation between Na⁺ exclusion and salt tolerance has been found (Genc, 2007). Accumulation of Na⁺ in the vacuole disturbs the balance of osmotic pressure of ions; hence collection of organic solutes like proline in cytosol controls this disorder (Munns, 2008). Thus, this parameter has been supported as a criterion of salt stress tolerance (Ashraf and Harris, 2004). However, some researchers have found that there is no correlation between leaf proline and salt tolerance (Poustini et al, 2007; Lutts et al., 1996).

The aims of the present study were as follows. Firstly comparing salinity resistance, ion homeostasis, proline content as well as chlorophyll content of primary tritipyrum lines with bread wheat cultivars, Secondly, what is the effect of these parameters on the resistance of primary tritipyrum lines to salinity in controlled environment?, and Thirdly, to determine whether Na⁺ exclusion or tissue tolerance cause ion homeostasis in primary tritipyrum lines.

Results

Out of 10 primary tritipyrum lines and two wheat cultivars, the wheat cultivar Gascoigne (salt sensitive) lacked enough tolerance and died due to salinity stress. Moreover, one of the primary tritipyrum lines (St/b) displayed late maturity and stayed green up to the end of experiment. Therefore, these two genotypes were eliminated from the analysis.

Na⁺ and K⁺ ion concentration in different tissues

The flag leaf Na⁺ concentration pattern over three levels of salinity (50, 100 and 200mM NaCl) was similar and an increase in flag leaf Na⁺ accumulation with increasing salinity levels was observed in all tested genotypes (Table 2). In primary tritipyrum lines except on La(4B,4D)/b, flag leaf Na⁺ concentration increased highly from 50 to 100 mM, but no tangible increase was observed from 100 mM to the highest salinity level (200 mM) (Table 2). On the other hand, Na⁺ concentration showed an intense increase in wheat cultivar Bam from 50 mM to 200 mM (Table 2). The flag leaf Na⁺ concentration in wheat cultivar Bam was lower than that of tritipyrum lines at 50 mM, but there was a 25-fold

difference in Na⁺ concentration of wheat at 50 compared to 200 mM, while the value of primary tritipyrum lines was approximately equal to 2.5-fold (Table 2). At the highest salinity level (200 mM), the flag leaf of (Ka/b)*(Cr/b), F₆ line showed the lowest, whereas La(4B, 4D)/b showed the highest Na⁺ accumulation (Table 2). In the case of stem, NaCl treatment caused an increase in Na⁺ concentration in all genotypes (Table 2). Genotypes exhibited little variation in stem Na⁺ concentration from 50 to 200 mM NaCl, with the exception of Ka/b line and wheat cultivar Bam. At 50 mM NaCl, Ka/b line had low Na⁺ concentration (0.61 mg/g DW) and (Ma/b)*(Cr/b), F₄ had high Na⁺ concentration (2.7 mg/g DW) (Table 2). At the highest level of salinity, maximum Na⁺ concentration was related to La/b and minimum value was associated with (Ka/b)*(Cr/b), F₂ (Table 2). In primary tritipyrum lines, the rate of stem Na⁺ accumulation was constant while, it was higher in wheat cultivar Bam (Table 2). Na⁺ concentration in root tissue increased at all salinity levels, but the variation among genotypes was outstanding (Table 2). At the highest salinity level (200 mM), maximum root Na⁺ concentration was related to La (4B,4D)/b and Az/b and minimum value was associated with Ka/b (Table 2).

There was no specific trend for K⁺ concentration of flag leaf in all genotypes at different salinity levels (Table 2). However, it was noteworthy that in wheat cultivar Bam, fluctuation of K⁺ concentration of flag leaf at different salinity levels was more than tritipyrum lines (Table 2). The flag leaf K⁺ concentration of (Ka/b)*(Cr/b), F₆ line with low flag leaf Na⁺ accumulation was lower than that of other varieties at the highest salinity level (Table 2). Similar to K⁺ concentration of flag leaf, there was no clear trend in stem K⁺ concentration during salinity stress (Table 2). At the highest salinity level, Ka/b had the highest leaf K⁺ concentration (Table 2). Also, there was no distinct pattern for root K⁺ concentration and wheat cultivar Bam showed the highest K⁺ concentration at all salinity levels compared to primary tritipyrum lines except at 50 mM NaCl (Table 2). Generally, both primary tritipyrum lines and wheat cultivar Bam showed a decrease in Na⁺ concentration from root to flag leaf at all salinity levels while this trend was reversed in case of potassium (Fig. 1).

The genotypes differed in flag leaf Ka⁺/Na⁺ ratio at all salinity levels (Table 2). Some primary tritipyrum lines showed higher and some showed lower Ka⁺/Na⁺ ratios than wheat cultivar Bam (Table 2). In stem at 50 mM and 100 mM NaCl, the varieties were different in terms of Ka⁺/Na⁺ ratio. But at 200 mM, there were fewer variations among the varieties. All varieties exhibited a decrease in Ka⁺/Na⁺ ratio with increasing salinity (Table 2). For all varieties under salt stress, root Ka⁺/Na⁺ ratios had decreased (Table 2). At two salinity levels (50 and 100 mM NaCl) wheat cultivar Bam had the highest Ka⁺/Na⁺ ratio (Table 2).

Salinity and photosynthetic pigment concentration

Increasing the external salinity level up to 100 mM led to an increase in chlorophyll *a* content, but it decreased at 200 mM (Table 2). In all salinity levels, wheat cultivar Bam had lower Chl-*a* than primary tritipyrum lines and (Ma/b)*(Cr/b), F₄ had higher Chl-*a* content at 100 mM NaCl (Table 2).

There was no certain pattern for chlorophyll *b* content. The highest Chl *b* content was related to (Ka/b)*(Cr/b), F₂ at 100 mM and the lowest one was related to (Ka/b)*(Cr/b), F₆ at 200 mM (Table 2). Generally primary tritipyrum lines exhibited higher chlorophyll *a* and chlorophyll *b* than wheat cultivar Bam (Table 2).

Table 1. Abbreviation, ploidy level and genomic constitution of primary tritipyrum lines and wheat cultivars in the present study.

Genotypes	Abbreviation	Ploidy	Genome
Wheat cultivars			
<i>T.aestivum</i> cv. Bam	Bam	6X	AABBDD
<i>T.aestivum</i> cv. Gascoigen	Gascoigen	6X	AABBDD
Tritipyrum lines			
(Karim/Th. Bessarabicum)*(Creso/ Th. Bessarabicum) F ₆	(Ka/b)*(Cr/b), F ₆	6X	AABBE ^b E ^b
(Karim/Th. Bessarabicum)*(Creso/ Th. Bessarabicum) F ₂	(Ka/b)*(Cr/b), F ₂	6X	AABBE ^b E ^b
(Macoun/ Th. Bessarabicum)*(Creso/ Th. Bessarabicum) F ₄	(Ma/b)*(Cr/b), F ₄	6X	AABBE ^b E ^b
(Stewart/ Th. Bessarabicum)*(Creso/ Th. Bessarabicum) F ₄	(St/b)*(Cr/b), F ₄	6X	AABBE ^b E ^b
Langdon/ Th. Bessarabicum (4B,4D)/ Th. Bessarabicum	La(4B,4D)/b	6X	AABBE ^b E ^b
Karim/Th. Bessarabicum	Ka/b	6X	AABBE ^b E ^b
Creso/ Th. Bessarabicum	Cr/b	6X	AABBE ^b E ^b
Stewart/ Th. Bessarabicum	St/b	6X	AABBE ^b E ^b
Langdon/ Th. Bessarabicum	La/b	6X	AABBE ^b E ^b
Aziziah/ Th. Bessarabicum	Az/b	6X	AABBE ^b E ^b

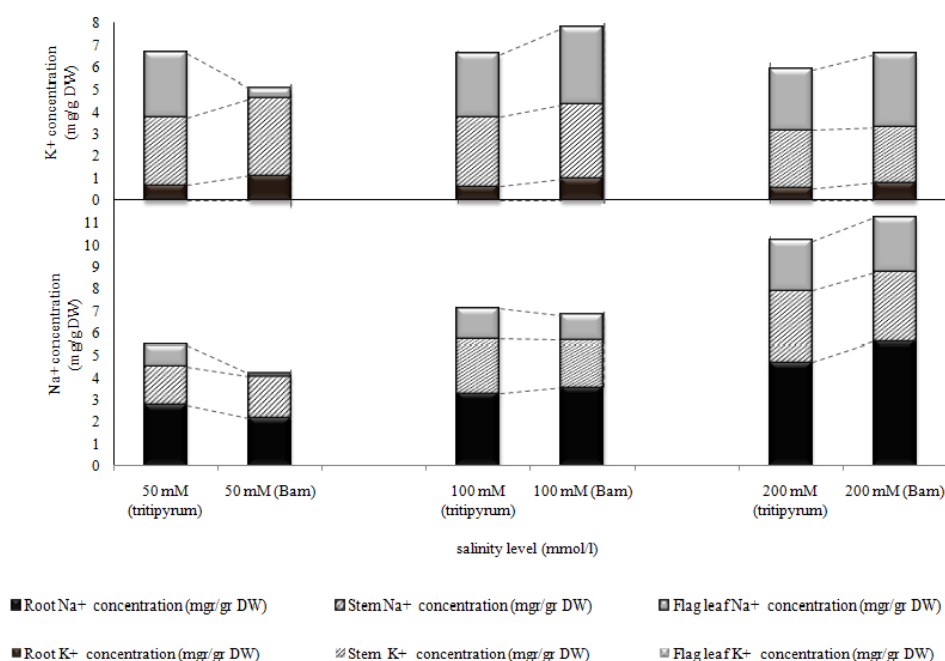


Fig 1. Na⁺ and K⁺ concentration in different tissue of tritipyrum lines and wheat cultivar Bam in different NaCl levels.

The trend for total chlorophyll content and Chl *a/b* ratio of the genotypes under salt stress was similar to Chl-*a* (Table 2). In relation to Chl *a/b* ratio, (Ka/b)*(Cr/b),F₆ had the highest amount of Chl *a/b* ratio at all salinity levels.

Proline content and salinity

Salt treatment influenced proline content of primary tritipyrum lines and wheat flag leaves (Fig. 2). Increasing salinity level caused increasing proline content of wheat cultivar Bam leaves but in tritipyrum lines, increasing salinity from 50mM to 100mM caused a decrease in proline content but it was not remarkable. However, at 200mM salinity a marked increase in proline content of tritipyrum leaves were induced (Fig. 2). In 50 mM NaCl treatment the lowest proline content belonged to wheat cultivar Bam (Fig. 2). On the average, the proline content of tritipyrum lines was approximately 1.5 fold more than its content in wheat cultivar Bam.

Grain yield under salt stress treatment

Primary tritipyrum lines could demonstrate a better performance against salt stress than wheat cultivar Bam and their grain yield was more than Bam particularly at 100 and 200 mM NaCl levels (Fig.3). At the first level of salt treatment (50 mM), grain yield of primary tritipyrum lines was similar to wheat cultivar Bam. Except on Ka/b and Az/b, other primary tritipyrum lines produced similar grain yield or even lower grain yield in comparison with wheat cultivar Bam. However, at this salt level, La(4B,4D)/b had the lowest (0.65 g/plant) and Ka/b had the highest(1.18 g/plant) grain yield (Fig.3). At the second salt treatment (100 mM), grain yield of all primary tritipyrum lines except La(4B, 4D)/b was satisfactory, but a significant decrease was observed in yield of wheat cultivar Bam. It was noteworthy that primary tritipyrum lines grain yield in 100 mM was higher than Bam grain yield (Fig. 3). At 100 mM, only two lines La (4B, 4D)/b and Cr/b produced lower grain yield than wheat cultivar Bam. The highest grain yield belonged to (St/b)*(Cr/b), F₄

Table 2. Ion concentration and K⁺/Na⁺ ratio in flag leaf, stem and root of primary tritipyrum lines and bread wheat (cv;Bam) under different salinity levels.

genotypes	Salinity level (mM)	Na ⁺ concentration (mg/g DW)			K ⁺ concentration (mg/g DW)			K ⁺ /Na ⁺ ratio			Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Chlorophyll a/Chlorophyll b	Total Chlorophyll
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Chl a	Chl b	Chl a/Chl b	Total Chl
(Ka/b)*(Cr/b), F ₂	50	0.55 ^{gh*}	0.90 ^{jk}	2.95 ^{dk}	2.70 ^{bd}	3.09 ^{ch}	0.71 ^{ch}	5.08 ^{ac}	3.46 ^b	0.24 ^{bg}	1.49 ^a	0.58 ^{ac}	2.62 ^{bc}	2.06 ^{ab}
	100	1.53 ^{dg}	1.75 ^{hj}	3.08 ^{dk}	2.64 ^{bd}	3.96 ^{ab}	0.72 ^{cg}	1.76 ^{hm}	2.31 ^c	0.24 ^{bh}	1.52 ^a	0.77 ^a	2.04 ^c	2.29 ^a
	200	2.11 ^{be}	2.34 ^{eh}	3.84 ^{cd}	2.61 ^{bd}	2.31 ^{hi}	0.34 ^{hj}	1.27 ^{jm}	1.01 ^{fg}	0.09 ^{ik}	1.45 ^a	0.56 ^{ac}	2.67 ^{bc}	2.01 ^{ab}
(Ka/b)*(Cr/b), F ₆	50	0.50 ^{gh}	1.00 ^{jk}	2.69 ^{ek}	3.01 ^{ad}	3.23 ^{be}	0.73 ^{bg}	6.33 ^a	3.25 ^b	0.27 ^{bf}	1.33 ^a	0.40 ^{bc}	3.30 ^{ac}	1.73 ^{ab}
	100	0.51 ^{gh}	1.40 ^{ik}	2.92 ^{dk}	2.91 ^{bd}	3.12 ^{cg}	0.63 ^{ci}	6.19 ^{ab}	2.27 ^c	0.22 ^{bi}	1.40 ^a	0.38 ^{bc}	3.77 ^{ab}	1.78 ^{ab}
	200	0.56 ^{gh}	2.56 ^{dh}	3.84 ^{cd}	2.06 ^{cd}	2.71 ^{ci}	0.64 ^{ci}	3.68 ^{cf}	1.06 ^{fg}	0.17 ^{ek}	1.37 ^a	0.33 ^c	4.37 ^a	1.70 ^{ab}
(Ma/b)*(Cr/b), F ₄	50	1.23 ^{eg}	2.70 ^{dg}	2.82 ^{ek}	3.91 ^{ab}	2.56 ^{di}	0.47 ^{ej}	3.18 ^{dh}	0.95 ^{fg}	0.17 ^{ek}	1.48 ^a	0.55 ^{ac}	2.86 ^{ac}	2.03 ^{ab}
	100	1.46 ^{eg}	2.84 ^{cg}	3.48 ^{cg}	3.14 ^{ad}	3.21 ^{bf}	0.30 ^{ij}	2.32 ^{em}	1.13 ^{fg}	0.09 ^{ik}	1.54 ^a	0.56 ^{ac}	2.78 ^{ac}	2.11 ^{ab}
	200	2.66 ^{bc}	3.66 ^{ac}	5.30 ^a	2.93 ^{bd}	2.50 ^{ei}	0.47 ^{ej}	1.12 ^{lm}	0.69 ^g	0.09 ^{ik}	1.26 ^a	0.45 ^{ac}	2.83 ^{ac}	1.72 ^{ab}
(St/b)*(Cr/b), F ₄	50	0.96 ^{fh}	2.37 ^{eh}	3.60 ^{ce}	2.86 ^{bd}	3.42 ^{ac}	0.52 ^{dj}	3.11 ^{di}	1.46 ^{cg}	0.15 ^{fk}	1.48 ^a	0.48 ^{ac}	3.19 ^{ac}	1.95 ^{ab}
	100	1.57 ^{cg}	2.90 ^{cg}	3.59 ^{cf}	2.71 ^{bd}	4.21 ^a	0.78 ^{af}	1.73 ^{hm}	1.47 ^{cg}	0.22 ^{bi}	1.53 ^a	0.66 ^{ac}	2.34 ^{bc}	2.19 ^a
	200	1.71 ^{bf}	3.35 ^{ad}	5.08 ^{ab}	2.61 ^{bd}	2.66 ^{ci}	0.40 ^{gj}	1.52 ^{im}	0.79 ^{fg}	0.08 ^{jk}	1.48 ^a	0.59 ^{ac}	2.58 ^{bc}	2.07 ^{ab}
La(4B,4D)/b	50	1.75 ^{bf}	1.76 ^{hj}	2.23 ^{jk}	3.13 ^{ad}	2.40 ^{fi}	0.67 ^{ci}	1.80 ^{hm}	1.37 ^{dg}	0.30 ^{bd}	1.36 ^a	0.51 ^{ac}	2.72 ^{ac}	1.87 ^{ab}
	100	1.81 ^{bf}	2.24 ^{fi}	3.31 ^{ci}	3.22 ^{ad}	2.10 ⁱ	0.71 ^{ch}	1.81 ^{hm}	0.94 ^{fg}	0.21 ^{bi}	1.42 ^a	0.42 ^{bc}	3.41 ^{ac}	1.84 ^{ab}
	200	5.40 ^a	3.20 ^{ae}	5.79 ^a	4.49 ^a	2.38 ^{gi}	0.64 ^{ci}	0.84 ^m	0.76 ^{fg}	0.11 ^{gk}	1.10 ^a	0.38 ^{bc}	2.92 ^{ac}	1.48 ^b
Az/b	50	0.61 ^{gh}	1.43 ^{ik}	2.37 ^{ik}	2.81 ^{bd}	3.01 ^{ch}	1.15 ^a	4.59 ^{bd}	2.13 ^{cd}	0.48 ^a	1.42 ^a	0.60 ^{ac}	2.39 ^{bc}	2.02 ^{ab}
	100	0.98 ^{fh}	2.07 ^{gi}	3.39 ^{ch}	2.70 ^{bd}	3.23 ^{be}	0.87 ^{ad}	2.73 ^{ek}	1.59 ^{cf}	0.25 ^{bf}	1.49 ^a	0.56 ^{ac}	2.75 ^{ac}	2.04 ^{ab}
	200	1.22 ^{eg}	3.00 ^{bf}	5.59 ^a	2.50 ^{bd}	2.68 ^{ci}	0.84 ^{ae}	2.07 ^{gm}	0.90 ^{fg}	0.15 ^{ek}	1.28 ^a	0.50 ^{ac}	2.66 ^{bc}	1.78 ^{ab}
Cr/b	50	1.39 ^{eg}	2.51 ^{dh}	2.46 ^{hk}	3.05 ^{ad}	3.12 ^{cg}	0.41 ^{fi}	2.25 ^{fm}	1.26 ^{eg}	0.17 ^{dk}	1.20 ^a	0.59 ^{ac}	2.02 ^c	1.79 ^{ab}
	100	1.57 ^{cg}	3.00 ^{bf}	3.55 ^{eg}	2.77 ^{bd}	2.82 ^{ci}	0.38 ^{gj}	1.82 ^{hm}	0.95 ^{fg}	0.11 ^{hk}	1.36 ^a	0.58 ^{ac}	2.37 ^{bc}	1.94 ^{ab}
	200	2.69 ^b	3.63 ^{ac}	5.31 ^a	2.55 ^{bd}	2.86 ^{ci}	0.25 ^j	0.98 ^{lm}	0.79 ^{fg}	0.05 ^k	1.27 ^a	0.57 ^{ac}	2.26 ^{bc}	1.84 ^{ab}
Ka/b	50	0.54 ^{gh}	0.61 ^k	2.64 ^{fk}	1.93 ^{de}	2.91 ^{ci}	0.83 ^{ae}	3.60 ^{cg}	4.88 ^a	0.31 ^{bc}	1.40 ^a	0.69 ^{ab}	2.05 ^c	2.10 ^{ab}
	100	2.08 ^{be}	2.80 ^{cg}	2.62 ^{gk}	2.70 ^{bd}	2.83 ^{ci}	0.54 ^{dj}	1.32 ^{jm}	1.01 ^{fg}	0.21 ^{cj}	1.46 ^a	0.63 ^{ac}	2.34 ^{bc}	2.08 ^{ab}
	200	2.60 ^{bd}	3.12 ^{bf}	3.02 ^{dk}	2.94 ^{bd}	3.16 ^{bg}	0.84 ^{ae}	1.14 ^{km}	1.01 ^{fg}	0.28 ^{be}	1.36 ^a	0.61 ^{ac}	2.25 ^{bc}	1.97 ^{ab}
La/b	50	1.24 ^{eg}	2.42 ^{eh}	3.14 ^{dj}	3.47 ^{ad}	3.35 ^{bd}	0.56 ^{cj}	2.83 ^{ej}	1.38 ^{dg}	0.18 ^{dj}	1.35 ^a	0.54 ^{ac}	2.59 ^{bc}	1.89 ^{ab}
	100	1.37 ^{eg}	2.95 ^{eg}	4.25 ^{bc}	3.27 ^{ad}	3.09 ^{ch}	0.40 ^{gj}	2.48 ^{el}	1.05 ^{fg}	0.09 ^{ik}	1.50 ^a	0.54 ^{ac}	2.94 ^{ac}	2.04 ^{ab}
	200	2.04 ^{bf}	4.03 ^a	5.35 ^a	2.67 ^{bd}	2.91 ^{ch}	0.41 ^{fi}	1.34 ^{jm}	0.72 ^g	0.08 ^{jk}	1.39 ^a	0.63 ^{ac}	2.23 ^{bc}	2.02 ^{ab}
Bam Wheat cv.	50	0.10 ^h	1.72 ^{hj}	2.18 ^k	0.39 ^e	3.42 ^{ac}	1.10 ^{ab}	3.87 ^{ce}	2.06 ^{ce}	0.51 ^a	1.24 ^a	0.42 ^{bc}	3.00 ^{ac}	1.66 ^{ab}
	100	1.24 ^{eg}	2.34 ^{ch}	2.69 ^{ek}	3.60 ^{ac}	3.29 ^{be}	0.93 ^{ac}	3.05 ^{di}	1.41 ^{dg}	0.35 ^b	1.24 ^a	0.45 ^{ac}	2.74 ^{ac}	1.69 ^{ab}
	200	2.69 ^b	3.87 ^{ab}	4.90 ^{ab}	3.57 ^{ac}	2.61 ^{ci}	0.75 ^{bg}	1.33 ^{jm}	0.68 ^g	0.15 ^{ek}	1.28 ^a	0.56 ^{ac}	2.30 ^{bc}	1.84 ^{ab}

*The means from experiments performed with same letters do not differ statistically by Tukey test (p < 0.05)

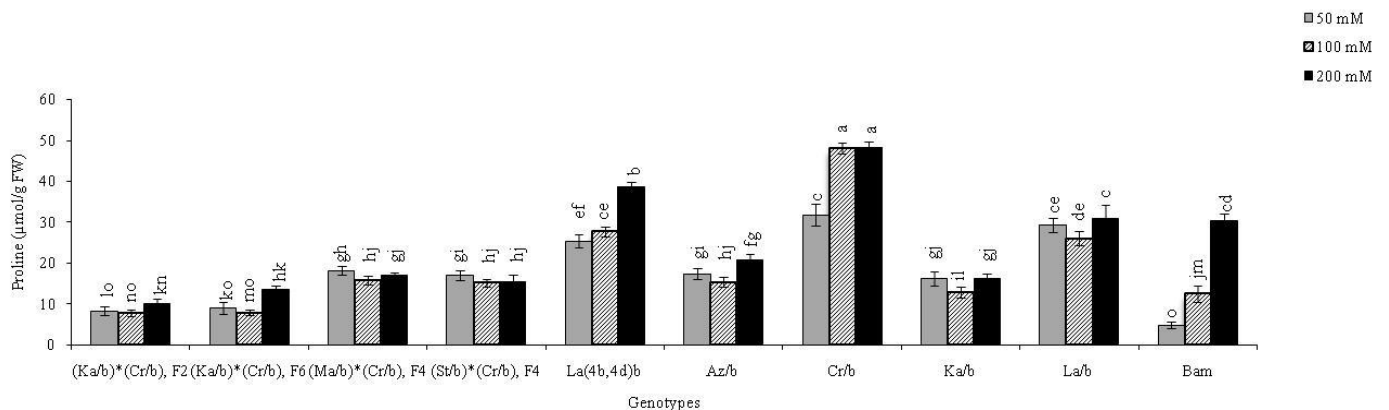


Fig 2. Effect of salinity on flag leaf proline content of primary tritipyrum lines and wheat cultivar Bam. Different letters indicate statistically different means (p < 0.05).

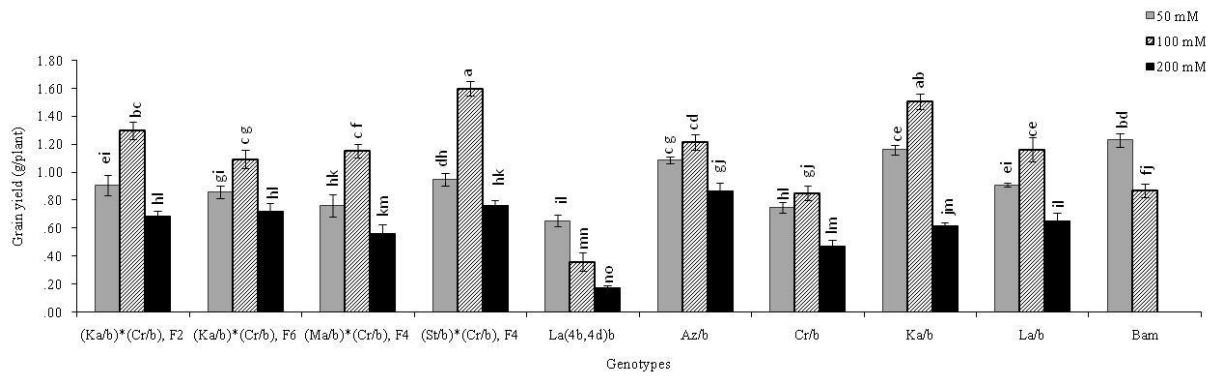


Fig 3. Response of grain yield of primary tritipyrum lines and wheat cultivar bam to salinity treatment. Different letters indicate statistically different means ($p < 0.05$).

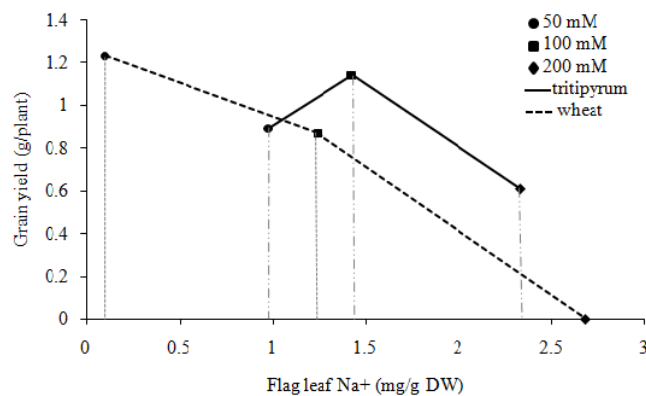


Fig 4. Relationship between flag leaf Na^+ accumulation on grain yield of tritipyrum lines and wheat cultivar Bam.

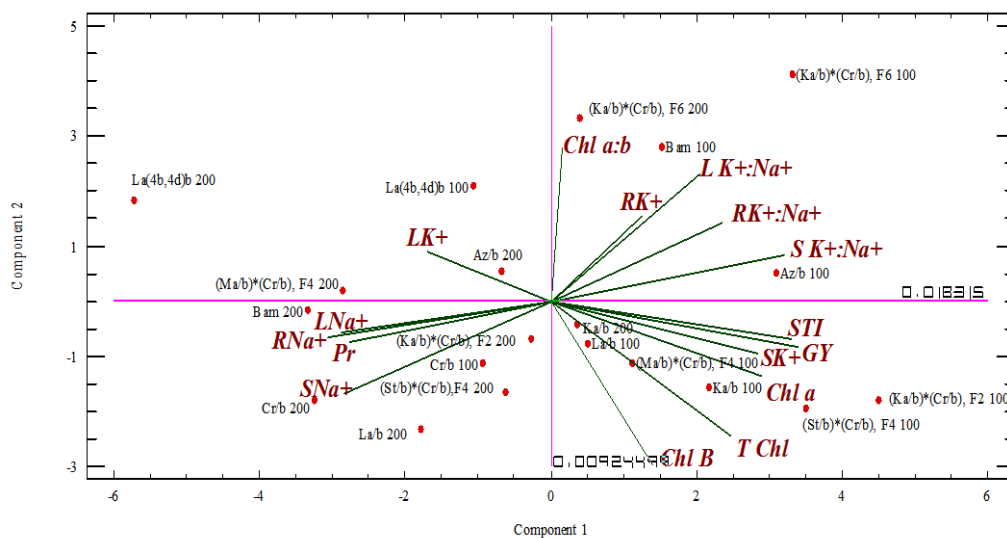


Fig 5. Biplot representation of the results of principal component analysis (PCA) based on measured characteristics of primary tritipyrum lines and wheat cultivar Bam during salinity stress. Genotypes names are beside the red point and the traits are made clear in uppercase letters. LNa: leaf Na^+ concentration. LK: leaf K^+ concentration. LK:Na: leaf K/Na ratio. SN: stem Na^+ concentration. SK: stem K^+ concentration. SK:Na: stem K/Na ratio. RN: root Na^+ concentration. RK: root K^+ concentration. RK:Na: root K/Na ratio. Chl a: chlorophyll a concentration. Chl b: chlorophyll b concentration. Chl a/b: chlorophyll a: chlorophyll b ratio. T Chl: total chlorophyll. GY: grain yield plant^{-1} . Pr: proline content.

and the lowest were related to La (4B, 4D)/b lines (Fig.3). At the highest salt level (200 mM), all primary tritipyrum lines produced grain yield but wheat cultivar Bam did not set seeds. In comparison to grain yield in 50 mM NaCl, primary tritipyrum lines and wheat cultivar Bam presented a 37% and 100% reduction in grain yield at 200 mM NaCl, respectively (Fig. 3) and at this salinity level, Az/b produced the highest grain yield, La (4B, 4D)/b had the lowest and wheat cultivar Bam had no grain yield at all (Fig.3). According to this fact that flag leaf is the major source of assimilates involving in grain filling, the relationship between Na⁺ concentration of flag leaf and grain yield was investigated (Fig. 4). The results showed that increasing salinity caused an increase in grain yield of tritipyrum lines particularly at 100 mM NaCl while they accumulated higher Na⁺ ions in their flag leaf than wheat cultivar. The stress caused an increase in flag leaf Na⁺ concentration of wheat and at the same time caused a decline in wheat grain yield and it could not produce any grain at 200 mM NaCl.

Principal Component Analysis (PCA)

To analyze the relations among genotypes characteristics, assess the patterns of variation and generally obtain further perception about the physiological mechanisms involved in increasing salt resistance of genotypes, principal component analysis (PCA) was constructed using physiological parameters and grain yield plant⁻¹(Fig. 5). The relationship among measured characteristics during salt treatments and the used genotypes is represented in Fig 5. In this Figure, the correlation coefficient between two traits was estimated by the cosine of the angle between them ($r = \cos 180^\circ = -1$, $\cos 0^\circ = 1$ and $\cos 90^\circ = 0$).

Five characteristics including K⁺:Na⁺ ratio of root, shoot and flag leaf, K⁺ concentration of root and Chl a/b ratio were located in the same quadrant and they were positively correlated to each other. Among these traits, K⁺:Na⁺ ratio of stem and root are positively correlated to salinity tolerance index (STI). These traits are related to regulation of ion accumulation and selection of other useful nutrients against Na⁺ during salinity treatment (Fig. 5). The two dimensional graph of PCA can help improve understanding the relationship among genotypes and measured characteristics. So, this graph can describe the positive role of K⁺:Na⁺ ratio of shoot and root on improving salinity tolerance of Az/b, Bam, (Ka/b)*(Cr/b), F₆ during 100 mM and (Ka/b)*(Cr/b), F₆ in 200 mM NaCl (Fig. 5). Chlorophyll a, chlorophyll b, total chlorophyll and K⁺ concentration of shoot were located in another quadrant which could suggest the effect of K⁺ ion on photosynthetic apparatus under salinity stress. Their positive relations with salinity tolerance index (STI) showed the positive effect of these traits on performance of genotypes in this quadrant (Fig. 5). Na⁺ content of leaf, shoot and root and proline content were in another quadrant. These traits showed the salinity damages are related to ion toxicity. They were positively correlated with each other but their relations were negative with salinity tolerance index (STI). These relations showed increasing proline and Na⁺ concentration with increasing salinity levels and their negative effect on plant performance of located genotypes in this quadrant (Fig. 5).

Discussion

Sodium ion is a key ion responsible for the damage caused during salt stress in plants (Munns and Tester, 2008; Tuteja, 2007). Increasing sodium concentration in plant tissues by increasing salinity levels has been observed in different

studies (Rajendran et al., 2009; Santa-Maria and Epstein, 2001). Regarding tritipyrum, the results of our experiments also indicated that salinity increased sodium concentration in various tissues of primary tritipyrum lines and the sodium concentration in different tissues of primary tritipyrum lines was more than that of wheat cultivar Bam (Fig. 1). Despite the higher accumulation of sodium, the yield of primary tritipyrum lines at different levels of salinity was higher than wheat cultivar Bam (Fig. 3). In addition, our results showed that at the second level of applied salinity stress (100 mM), leaf sodium concentration of (Ma/b)*(Cr/b), F₄ and La/b lines was approximately equal to leaf sodium concentration of wheat cultivar Bam and leaf sodium concentration of Ka/b, (St/b)*(Cr/b), F₄ and (Ka/b)*(Cr/b), F₂ lines was more than wheat cultivar Bam (Table 2). Nevertheless, the performance of these primary tritipyrum lines was greater than that of wheat cultivar Bam (Fig. 3). It is believed that the increased Na⁺ in plant tissue during salt stress reduces plant performance and there is a negative relationship between wheat leaf sodium concentration and grain yield (Poustini and Siosemardeh, 2004). Therefore sodium exclusion is mentioned as a selection criterion in relation to salt tolerance of cereal crops (Munns et al., 2006). The results of our experiment not only revealed more compatibility and tolerance of primary tritipyrum lines to salinity in comparison with wheat, but also indicated a positive relationship between increasing concentrations of sodium and performance up to 100mM salinity level. It is consistent with the results of other researchers' experiments who reported the negative effect of Na⁺ on plant performance (Dionisio-Sese and Tobita, 2000; Garthwaite et al., 2005; Munns and James, 2003; Wei et al., 2003). Thus, according to the findings of the present study, salinity tolerance is over shadowed not only by the mechanism of sodium exclusion but also by other involved physiological responses such as "tissue tolerance". In this case the compartmentation of Na⁺ into the vacuoles by a Na⁺/H⁺ anti porter may protect the cell from the harmful effect of sodium and the cell uses sodium osmotic adjustment (Blumwald et al., 2000). Primary tritipyrum lines may also have a mechanism of salt tolerance like their ancestor *Thinopyrum bessarabicum* which uses sodium for osmotic adjustment and thereby increases its tolerance to salinity (Colmer et al., 2006b).

Despite higher sodium concentration of La(4B,4D)/b primary tritipyrumin than wheat cultivar Bam at 200 mM salinity level, this line had the lowest performance while wheat cultivar Bam had no grain yield (Figs. 3). These observations may imply a strong system of tissue tolerance in La (4B/4D)/b. It is worth mentioning that D genome includes Kna1 locus on the long arm of chromosome 4d that leads to lower sodium concentration (Dubcovsky et al., 1996; Gorham et al., 1987). Nevertheless, higher concentration of sodium in La (4B/4D)/b line's leaf tissue and its lower performance than that of other primary tritipyrum lines may be the result of either negative interaction between D genome with A genome of durum wheat and E_b genome of *Th. bessarabicum* that leads to decreased expression of this trait on D genome or the other genes brought about by D genome that increase sodium when combined with the other genes of this line. Overall, the results regarding the concentration of sodium ions in primary tritipyrum lines showed a correlation between tissue tolerance and sodium exclusion mechanisms under salinity stress which might play a key role in salinity tolerance of primary tritipyrum lines.

Potassium is mentioned as an essential monovalent cation that has a positive effect on improving salt tolerance in plants (Cuin et al., 2008; Munns et al., 2006; Rascio et al., 2001).

Salt tolerance is associated with transport of potassium from root to shoot (Chen et al., 2005; Chen et al., 2007; Cuin et al., 2008).

In the present study, potassium content of different tissues of primary tritipyrum lines at different salinity levels did not follow a specific pattern, but in wheat cultivar Bam K⁺ concentration decreased with increasing salinity levels (Fig. 1). In addition, fluctuations in potassium concentration at various salinity levels were not as high as that of sodium (Fig. 1). Although the amount of potassium in different tissues and at different salinity levels of wheat cultivar Bam was higher than that of primary tritipyrum lines, its performance was more affected by salinity (Fig. 1). Unlike Na⁺ concentration, K⁺ concentration increased from root to shoot and leaves in studied genotypes. The concentration of potassium in different tissues can be a sign of other involved physiological mechanisms that, despite the accumulation of high levels of sodium and very little changes in potassium, maintains ionic balance and therefore increases salinity tolerance. It can be noted that maintaining the potassium levels while salinity is increasing may be hereditary as primary tritipyrum ancestor, *Th. bessarabicum*, that preserves K⁺ concentration in salinity conditions as another strategy to increase tolerance to salinity stress (Colmer et al., 2006b).

Several studies suggest a positive relationship between the K⁺/Na⁺ ratio and high salt tolerance. Thus, this ratio is used as another selection criterion for the selection of resistant varieties (Chen et al., 2007). According to the results in this experiment, there is a positive relationship between this ratio and stress tolerance (Fig. 5) and it seems that this ratio can be assumed as a suitable criterion for determining resistance to salt stress in this plant.

At all salinity levels, the amount of chlorophyll *a* and chlorophyll *b* of primary tritipyrum lines were higher than that of wheat cultivar Bam. However, an increase in the salinity level reduced chlorophyll *a* and *b* in both primary tritipyrum lines and wheat cultivar Bam. But this reduction was higher in wheat than primary tritipyrum lines (Table 2). In general, chlorophyll content had a positive effect on grain yield plant⁻¹ under salinity treatment (Fig. 5). Experiments reported greater drop of chlorophyll *a* and *b* in a salt sensitive wheat variety than salt tolerant variety and suggested that the increase in Chl *a/b* implied that photosystem II was affected more by salinity (El-Shintinawy, 2001; Zheng et al., 2008). Thus, it seems that the prevention of the chlorophyll biosynthesis and the degradation of chlorophyll are the main factors for this result (Husain et al., 2003; Khan, 2003; Khatkar and Kuhad, 2000).

In our experiment, there was a positive relation between proline and Na⁺ concentration in leaf, stem and root (Fig. 5). In other words, increasing Na⁺ caused increase in proline accumulation (Fig. 5). Furthermore, proline showed negative relationship with salinity tolerance index (STI) and grain yield plant⁻¹ (Fig. 5). These observations can be related to the fact that, proline accumulation can be a sign of stress injury and it cannot be a protective compound against injurious effects of high concentration of ions during salinity stress and its role in osmotic adjustment either is lower than other ions or organic solutes and maybe it does not play any role in osmotic adjustment. The reports about the role of proline on salinity tolerance are different. Some researchers indicated that there are no relations between proline content and salinity tolerance (Lutts et al. 1996; Poustini et al., 2007). Lutts et al. (1996) found no relationship between proline content and salt tolerance in rice cultivars. It was concluded that proline may not be involved in salt tolerance in wheat cultivar (Poustini et al., 2007). However, other reports

indicated that proline acts as osmoprotectant and causes salt tolerance (Ashraf and Foolad, 2007; Wagdy et al., 2002; Goudarzi and Pakniyat, 2009). In some cases, proline was introduced as a biochemical marker for increasing salt tolerance (Ashraf and Harris, 2004; Martinez et al., 1996). Tritipyrum lines may use Na⁺ ions as an osmotic adjustment agent similar to its ancestor *Thinopyrum bessarabicum* (Colmer et al., 2006b). The negative relation between proline content and grain yield plant⁻¹ signified that proline has no direct role in the salt tolerance of the used genotypes.

Materials and methods

Genetic materials

Seeds were kindly provided by Dr. Shahsavand (Shiraz University, Iran). Tritipyrum lines included (Ka/b)*(Cr/b), F₆, (Ka/b)*(Cr/b), F₂, (Ma/b)*(Cr/b), F₄, (St/b)*(Cr/b), F₄, La (4B, 4D)/b, St/b, Cr/b, Az/b, La/b, Ka/b (Table 1). *T. aestivum* cv. Bam (salt tolerant) and *T. aestivum* cv. Gascoigne (salt sensitive) were used as standard based on findings of other studies carried out on their salt tolerance (Vahabzadeh et al., 2009).

Verbalization method

Seeds that were uniform in shape and weight were selected. The selected seeds were sown in seedling growing trays (with 2 cm in diameter and 7 cm deep cells) which were filled with sand, soil and manure (2: 1: 1). Then they were vernalized for 6 weeks outside of the green house. At the end of the sixth weeks, the seedlings were at 3rd leaf stage and ready for being transplanted in planting containers (43× 34× 25 cm).

Plants growth condition

The experiment was conducted in a glasshouse in the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, with daily temperature of 25°C/ 20°C day/night and natural light without relative humidity control in a hydroponic culture system. A drain was placed in the bottom of planting containers, and then they were filled to a depth of 5 cm with gravels that had a diameter of one centimeter. Then, rest of the space in the containers was filled with washed sand to a depth of 23 cm. The containers were kept on iron benches with a height of 80 cm above ground level. Three irrigation pipes with a length of 40 cm and a diameter of 5 mm were placed in 10 cm spacing between them in each container. Holes for water withdrawal were inserted on any irrigation pipe that was buried at the depth of 2 cm below the sands in the container. The pipes in each container were connected to a main water pipe and irrigation was done through a pump attached to it. Pumps were inserted in 100 liter plastic barrels which were located on the floor near the iron bench. Healthy and similar seedlings in shape and size were selected and transferred to the containers. In each container, 4 rows of seedlings were planted according to a planting pattern with 8 cm spacing between rows and 2 cm spacing within rows. The seedlings were irrigated with half strength Hoagland solution (Hoagland 1950). After three days, the seedlings received full strength Hoagland solution and pH of the solution was maintained at 6.0 with 5N HNO₃. The electrical conductivity of the solution in each barrel was controlled twice a week. Since evaporation caused a loss in the volume of water in the barrel, the amount of water lost from the barrel was replaced by holding its volume up to 100 liters. Salt treatments were applied incrementally at the 4th leaf stage. To perform salt

treatments, NaCl was added in 3 steps by 50 mM/day to reach the intended salinity levels, i.e. 50, 100 and 200 mM NaCl. Control plants received 50mM NaCl. All plants were watered daily by pumping Hoagland's solution containing 50, 100 and 200 mM NaCl and allowed it to be drained into the holding barrels. The solution was renewed weekly.

Tissues Na⁺ and K⁺ measurement

Seven plants of each treatment were harvested at 50% ear emergence stage of each genotype. Then the root, stem and flag leaf blade of each genotype were separated and used to measure the concentration of sodium and potassium ions (Isaac and Kerber, 1971). Flag leaf blades were washed by distilled water because it was likely that the surfaces of leaves were coated with salt inadvertently. The flag leaf blades, as well as sheets were placed in small paper envelopes and dried in an oven at 60°C-70°C for 2 days.

Roots were rinsed rapidly to prevent efflux of Na⁺ from root cells. Washed roots were put into small paper envelopes and dried in an oven at 60°C-70°C for two days. Dried roots, shoots and flag leaf blade were ground by an electric mill. One gram of the milled tissue was heated in an electric furnace at a temperature of 580°C for two hours. To release cations, the produced ash was washed by 2M hydrochloric acid. The extract was diluted with distilled water and filtered with a filter paper and sodium and potassium concentrations were measured with a flame photometer.

Chlorophyll measurement

To measure the amount of chlorophyll, Lichtenthaler method (1987) was applied. 0.1 gram of leaf tissue was ground in a mortar and mixed with 4 ml of 80% acetone. The resulting solution was centrifuged for 5 minutes at 3000 rpm. To determine the amount of chlorophyll, the absorption of the supernatant was read with spectrophotometer at a wavelength of 647, 664nm. The total chlorophyll, and chlorophyll *a* and *b* were calculated by the following equations:

$$Chl_b = 21.21A_{647} - 5.1A_{664}$$

$$Chl_a = 12.25A_{664} - 2.79A_{647}$$

$$Chl_T = Chl_a + Chl_b$$

Where, A₆₄₇ and A₆₆₄ are absorbance at 647 and 664 nm wave lengths, respectively. Chl_a is chlorophyll *a*, chl_b is chlorophyll *b* and chl_T is the total chlorophyll.

Proline content of flag leaf tissue

The flag leaf of each genotype grown under salt stress conditions were sampled at 50% ear emergence stage and kept at -80°C until use. 0.5 gram of frozen leaf tissue was ground in liquid nitrogen. Proline was extracted with 10 ml 3% (w/v) sulfosalicylic acid. After centrifugation, the supernatant was used for determination of proline content as described by Bates et al (1973). Two milliliter of the extract was reacted with 2 ml glacial acetic acid and 2 milliliter ninhydrin reagent in a test tube and was incubated at 100°C for one hour. The mixture was cooled in an ice bath to terminate the reaction and then it was translocated to room temperature. 2 ml toluene was added to the reaction mixture and was mixed vigorously for 15-20 seconds. The chromophore phase containing toluene was collected and its absorbance was recorded at 520 nm using toluene as blank.

Proline concentration was determined using standard curve as µg/ml and calculated on fresh weight basis as follows:

$$\frac{[(\mu\text{g proline}) / \text{ml} \times \text{ml toluene}]/(115.5 \mu\text{g}) / (\mu\text{mole})]}{[(\text{g sample})] / 5} = (\mu\text{moles proline}) / (\text{g fresh weight})$$

Grain yield Measurement

Grain yield per plant was measured by weighing grains of all plant tillers at the maturity stage.

Salt tolerant index (STI) was calculated according to Fernandez (1992),

$$STI = ((Y_p \cdot Y_s)) / (Y_p)^2$$

Where Y_s is the yield of genotype in stress condition and Y_p is the yield of genotypes under control condition.

Experimental design and statistical analysis

Experimental design was split plot in completely randomized design with three replications. Simple statistical analysis including mean comparison and standard error were conducted by SPSS (Ver. 16.0, SPSS, Chicago, IL, USA). Mean comparisons were calculated using Tukey's test at 5% probability level. Principal component analysis (PCA) was used to obtain a multivariate view of collected variables and data. Principal component analysis (PCA) and cluster analysis were carried out using Statgraphics Centurion version 16 software. Charts were drawn using Excle and Sigmaplot 10 software.

Conclusion

The results of this experiment indicated that primary tritipyrum lines showed more salt tolerance than wheat salt tolerant cultivar Bam. It seems that after solving some agronomical problems related to this plant such as fragile rachis and improving its quantitative and qualitative traits, the plant will have the capability of being used in saline lands to improve this lands productivity. Moreover, by understanding the physiological mechanisms of salt stress tolerance in tritipyrum lines and identifying genes involved, due to the proximity of this plant to wheat, primary tritipyrum lines can be used to increase the salt tolerance of wheat varieties and enhance its performance under salt stress. Unlike wheat, primary tritipyrum lines are young and new cereal crop; therefore this preliminary study could be a prelude to a long-term project to increase salt tolerance in wheat.

Acknowledgements

The authors would like to express their appreciation to Ferdowsi University of Mashhad, Iran, for providing funds of this research.

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