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EFFECT OF SALINITY AND ABA APPLICATION ON PROLINE PRODUCTION AND YIELD IN WHEAT GENOTYPES

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Abstract

The present experiment was conducted at Agricultural University Peshawar, Khyber Pukhtun Khwa Pakistan, to study the response of 6 wheat genotypes to foliar ABA applications and induced salinity stress for their proline production using completely randomized design (CRD) with three replications. Shoot proline contents were significantly ($p \leq 0.05$) affected by genotypes, salinity levels, ABA application and their all possible interactions. Maximum proline content and yield attributes were produced by SR-40 when compared with other genotypes under study. High salinity stress (10 dSm^{-1}) had a positive effect on proline synthesis of wheat genotypes. Exposure of plants to both salinity stress and foliar application of ABA resulted in elevated levels of proline and better yield when compared with other treatments.

Introduction

Salinity is one of the important abiotic stress affecting plant growth and productivity (Shafi *et al.,* 2009; 2010 a, b; Din *et al.,* 2011; Shazma *et al.,* 2011; Yousaf *et al.,* 2011). In Pakistan, wheat production has been deficient in recent years. Yield losses of wheat in moderately saline areas of Pakistan are estimated to be 65% (Quayyum & Malik, 1988). If varieties of wheat are capable of giving high yields on slight to moderately salt-affected soils, then the productivity of such lands could be increased manifold which might also allow expansion of agriculture into more marginal lands. There is a pressing need to develop appropriate techniques for the screening of wheat cultivars/lines for salt tolerance. The study of ion transport and regulation within the intact plant tissues of wheat will also increase our understanding of the mechanisms of salt tolerance in this species and allow the development of selection markers valuable to the breeders. The recognition of selection criteria for salinity stress will be a step towards the urgent need of developing wheat varieties with better ability to grow and produce grain in salt affected areas where wheat is either grown inefficiently or not at all today.

Researchers have suggested that selection is more convenient and applicable if the plant species possesses distinctive indicators of salt tolerance at the whole plant, tissue or cellular levels (Munns, 2002; Ashraf, 2002). Despite a great deal of research into salinity tolerance of plants, the metabolic sites at which salt stress damages plants and the adaptive mechanisms utilized by plants to survive under saline stress are still not well understood. The main problem is due to the lack of well defined plant indicators for salinity tolerance that could practically be used by plant breeders for improvement of salinity tolerance in a number of important agricultural crops. This is partly due to the fact that the mechanisms of salt tolerance are very complex and variation occurs not only amongst the species but in many cases, also among cultivars within a single species (Ashraf, 2002; Ashraf & Harris, 2004; Khan *et al.,* 2006; Shafi *et al.,* 2011 a, b; Bakht *et al.,* 2006; 2011). Nonetheless, comparisons have been drawn between different biochemical indicators and plant tolerance to salt stress (Garg *et al.,* 2002; Hsu *et al.,* 2003; Yamada *et al.,* 2003; Yang *et al.,* 2003).

A comprehensive knowledge of the biochemical basis of salt tolerance of different genotypes will aid in the identification of suitable salt tolerant species for salt affected areas. A number of criteria including proline accumulation have been suggested to screen for salt tolerance in plants (Mutlu & Buzcuk, 2007; Cha-um & Kirdmanee, 2008; 2009b; Bakht *et al.,* 2011). Petrusa & Winicov (1997) reported that salt tolerant alfalfa plants rapidly increase their proline contents in their roots, compared with salt sensitive plants where the increase was slow. Similar results were also reported in alfalfa by Fougrere *et al.,* (1991). Ahmad *et al.,* (1981) observed that salt tolerant ecotypes of *Agrostis stolonifera* accumulated more proline in response to salinity than did salt sensitive ecotypes. Comparatively, salt tolerant plants of *Brassica juncea* showed higher degree of osmotic adjustment in the leaves and a higher critical point concentration of NaCl, at which the endogenous level of free proline rose sharply, than the relatively salt sensitive genotypes (Jain *et al.,* 1991). Higher proline accumulation was found in salt tolerant *B. juncea* plants with better plant growth than the control (Kirti *et al.,* 1991). Madan *et al.,* (1995) concluded that the activities of proline biosynthetic enzymes (pyrroline-5-carboxylate reductase and ornithine aminotransferase) increased considerably in *B. juncea* in tolerant lines under salt stress. In contrast activity of the proline degrading enzyme (proline oxidase) decreased under salt stress in the leaf tissues of *B. juncea.*

Materials and Methods

The present experiment was conducted to study the response of 6 wheat genotypes to ABA applications and induced salinity stress for their proline production and yield attributes. For this purpose pot experiments were conducted at Khyber Pukhtun Khwa Agricultural University Peshawar, Pakistan, using completely randomized design (CRD) with three replications. Twenty seeds of each genotype were planted in cemented pots (50

x 40 cm) lined with polyethylene sheet containing 20 kg of well dried and 2 mm meshed soil collected from the surface (0-15 cm) of a normal field . Seeds of each variety were sown in each pot at uniform depth. Four weeks after emergence the desired quantity of salinity (0, 4, 6, 10 dSm^{-1}) and $CaCl_2$ (2:1 molar ratio) was introduced gradually. Fifteen days after emergence, the plants were thinned out to 10 pot^{-1} and N, P and K was applied @ 135:120:60 kg ha^{-1}. Plants were irrigated according to their requirements.

Abscisic acid application: Foliar application of ABA (10^{-4} M) was carried out 4 weeks after emergence before induction of salinity stress. Foliar spray was done at 10-12 h of daylight for optimum uptake of the ABA and the soil in the pots was covered with aluminum foil to avoid contamination of soil with the applied ABA. A few drops of non-ionic detergent (NP-40) were added to the solution before spray for sticking the solution to the foliage.

Determination of proline concentration: Proline concentration in leaves was determined according to the method of Bates *et al.,* (1973). Briefly, fresh leaf materials (0.1g) were homogenized with 5 ml sulfosalicylic acid (3.0%) w/v with a mortar and pestle. Samples were centrifuged at 2000 rpm for 5 min. Supernatant was adjusted to 5 ml with distilled water, 5 ml glacial acetic acid and 5 ml acidic ninhydrin (0.1% in acetone) were added. Reaction mixture was shaken and heated in water bath for 30 min. Mixture was cooled and then extracted with 10 ml toluene in separating funnel. Absorbance of the toluene layer was recorded at 520 nm. A calibration series of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg ml^{-1} of proline was also run and a standard curve was plotted and the concentration of the unknown sample was calculated for the proline content with reference to the standard curve. In the present research project attempts were made investigate the effect of a phytohormone (abscisic acid, (ABA)) under salinity stress in laboratory condition.

Statistical analysis: All data are presented as mean values of three replicates. Data were analyzed statistically for analysis of variance (ANOVA) following the method described by Gomez & Gomez (1984). MSTATC computer software was used to carry out statistical analysis (Russel & Eisensmith, 1983). The significance of differences among means was compared by using Duncun's Multiple Range test (DMRT) (Steel & Torrie, 1997).

Results

Shoot proline contents were significantly ($p<0.05$) affected by various genotypes, different salinity levels, ABA application and all their possible interactions, 3, 6 and 9 weeks after foliar spray of ABA and salinity exposure (Tables 1-3). Shoot proline contents were maximum (769.25 µg g^{-1} fresh weight) in genotype SR-40, whereas minimum (632.29 µg g^{-1} fresh weight) in local genotype 3 weeks after exposure to salinity. Shoot proline contents increased by 60.65, 69.51 and 74.79% at 4, 6 and 10 dSm^{-1} salinity stress respectively compared with control. Foliar application of ABA to control (0 dSm^{-1}) had a stimulatory effect on proline contents (*ca.* 290.89 *vs* 248.28) with increase of 14.64% when compared with untreated control (-ABA). Salt treated plants when applied with ABA, resulted in an increase of 5.65, 9.57 and 17.32% in shoot proline contents at 4, 6 and 10 dSm^{-1} salinity levels respectively compared with untreated plants (-ABA; Table 1). Various interactions indicated that maximum (1295.67 µg g^{-1} fresh weight) shoot proline contents were produced by SR-40 when sprayed with ABA at high salinity level (10 dSm^{-1}), while minimum (239.67 µg g^{-1} fresh weight) was produced in local genotype without the foliar application of ABA at control.

Maximum (925.08 µg g^{-1} fresh weight) shoot proline contents were produced by SR-40, while minimum (682.62 µg g^{-1} fresh weight) in local genotype 6 weeks after foliar spray of ABA and salinity treatment (Table 2). Exposure of plants to different salinity stress (4, 6 and 10 dSm^{-1}) increased shoot proline contents by 62.40, 70.70 and 76.84 respectively when compared with control. Foliar spray of ABA to control (+ ABA; 0 dSm^{-1}) showed positive correlation with shoot proline contents (*ca.* 293.50 *vs* 263.89) with an increase of 10.08% compared with untreated control (-ABA). Salt exposed plants when sprayed with ABA, resulted in an increase of 11.25, 13.24 and 23.64% in their shoot proline contents at 4, 6 and 10 dSm^{-1} salinity levels respectively when compared with untreated plants (-ABA; Table 2). In case of interactions, maximum (1665.33 µg g^{-1} fresh weight) shoot proline contents were observed in SR-40 when sprayed with ABA and exposed to high salinity level (10 dSm^{-1}), whereas minimum (252.67 µg g^{-1} fresh weight) in local genotype without the application of ABA at control (Table 2).

Table 1. Shoot proline contents (µg g^{-1} fresh weight) of various wheat genotypes, 3 weeks after ABA application and salinity stress.

Genotypes	Salinity levels (dSm^{-1})								Means
	0		4.0		6.0		10.0		
	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	
SR-23	289.67	249.33	699.67	649.67	939.67	849.67	1269.67	1049.67	749.63b
SR-40	300.33	272.33	719.33	670.33	940.33	882.33	1295.67	1073.33	769.25a
SR-20	286.67	239.67	671.67	613.00	906.67	841.67	1242.67	1009.67	726.46d
SR-22	290.33	245.33	690.33	625.33	917.33	852.33	1254.33	1019.33	736.83c
Local	288.00	239.67	610.33	594.67	789.67	724.67	931.67	879.67	632.29f
SR-25	290.33	243.33	622.33	633.67	798.67	735.33	943.33	892.33	644.92e
Mean	290.89g	248.28h	668.94e	631.11f	882.06c	814.33b	1156.22a	987.33b	

DMRT value for interactions at $p<0.05 = 49$

Means of the same category followed by different letters are significantly different using DMRT test ($p<0.05$)

Table 2. Shoot proline contents (µg g^{-1} fresh weight) of various wheat genotypes, 6 weeks after ABA application and salinity stress.

Genotypes	Salinity levels (dSm^{-1})								Means
	0		4.0		6.0		10.0		
	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	
SR-23	292.00	265.00	805.33	714.67	1127.67	977.67	1649.67	1259.67	886.46b
SR-40	305.33	280.33	819.33	926.33	1140.33	990.33	1665.33	1273.33	925.08a
SR-20	289.67	259.67	740.00	651.67	1033.67	934.67	1616.33	1211.67	842.16d
SR-22	298.33	269.33	750.33	665.33	1045.33	944.33	1627.33	1221.00	852.67c
Local	286.33	252.67	659.67	624.67	868.67	782.67	1053.67	932.67	682.62f
SR-25	289.33	256.33	665.33	629.33	875.33	785.33	1060.33	938.33	687.46e
Mean	293.50g	263.89h	740.00e	702.00f	1015.17c	902.50d	1445.44a	1139.44b	

DMRT value for interactions at $p<0.05 = 88$

Means of the same category followed by different letters are significantly different using DMRT test ($p<0.05$)

Table 3. Shoot proline contents (µg g^{-1} fresh weight) of various wheat genotypes, 9 weeks after ABA application and salinity stress.

Genotypes	Salinity levels (dSm^{-1})								Means
	0		4.0		6.0		10.0		
	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	
SR-23	295.67	269.67	933.67	799.67	1375.67	1133.67	2161.67	1536.67	1063.29b
SR-40	300.33	275.33	939.33	807.33	1382.33	1140.33	2165.33	1543.33	1069.21a
SR-20	289.67	262.67	828.67	704.67	1188.67	1009.67	1954.67	1393.67	954.04d
SR-22	295.33	268.33	835.33	709.33	1195.67	1015.33	1960.33	1400.33	960.00c
Local	284.67	256.67	719.67	662.67	964.67	852.67	1189.67	997.67	741.04f
SR-25	288.33	260.33	725.33	666.33	970.67	859.67	1195.33	1003.67	746.21e
Mean	292.33g	265.50h	830.33e	725.00f	1179.61c	1001.89d	1771.17a	1312.56b	

DMRT value for interactions at $p<0.05 = 127$

Means of the same category followed by different letters are significantly different using DMRT test ($p<0.05$)

Shoot proline contents (µg g^{-1} fresh weight) of various genotypes, 9 weeks after foliar spray of ABA and salinity levels are presented in Table 3. Again, maximum shoot proline contents of 1069.21 µg g^{-1} fresh weight were noted in SR-40, while minimum (741.04 µg g^{-1} fresh weight) in local genotype. Shoot proline contents increased by 63.37, 73.50 and 85% with increasing salinity stress i.e., 4, 6 and 10 dSm^{-1} respectively compared with control. Plants maintained at control when sprayed with ABA (+ ABA; 0 dSm^{-1}) had a stimulatory effect on proline contents (*ca.* 292.33 *vs* 265.50) with increase of 9.17% compared with untreated control (-ABA). Salt exposed plants when applied with exogenous ABA, resulted in an increase of 14.35, 17.59 and 28.90% in their shoot proline contents at 4, 6 and 10 dSm^{-1} salinity levels respectively when compared with untreated plants (-ABA; Table 3). Maximum (2165.33 µg g^{-1} fresh weight) shoot proline contents were observed in SR-40 when sprayed with ABA at high salinity level (10 dSm^{-1}), whereas minimum (256.67 µg g^{-1} fresh weight) were recorded in local genotype with out the application of ABA at control (Table 3).

Various genotypes, different salinity levels, ABA application and all their possible interactions had a significant ($p<0.05$) effect on number of spikes $plant^{-1}$, grains $spike^{-1}$ and hundred grain weight (Tables 4-6). Number of spikes $plant^{-1}$ was maximum (3.21) in SR-40, whereas minimum was observed in local genotype. The data further suggested that number of spikes $plant^{-1}$ were reduced by 33.21, 40.98 and 60.77% at 4, 6 and 10 dSm^{-1} salinity stress respectively compared with control. Application of ABA to control (0 dSm^{-1}) plants (compared with control (-ABA)) had an adverse effect on number of spikes $plant^{-1}$ (*ca.* 2.83 *vs* 2.44) with the reduction of 13.78%. Application of ABA (+ABA) to salt exposed plants resulted in an increase of 20.50, 16.50 and 41% in number of spikes $plant^{-1}$ at 4, 6 and 10 dSm^{-1} salinity levels respectively when compared with untreated plants (-ABA). In case of interactions, maximum spikes $plant^{-1}$ were observed in SR-40 with or without the application of ABA at control (Table 4).

Maximum grains $spike^{-1}$ (35.71) were produced by SR-40, while minimum by local genotype. Number of grains $spike^{-1}$ was reduced by 27, 49.91 and 65.61% at 4, 6 and 10 dSm^{-1} salinity levels respectively when compared with control. Foliar application of ABA to control plants (0 dSm^{-1}) reduced grains $spike^{-1}$ by 38% compared with untreated control (-ABA). Application of ABA (+ABA) to salt treated plants resulted in an increase of 16, 11.30 and 7.2% in number of grains $spike^{-1}$ at 4, 6 and 10 dSm^{-1} salinity levels respectively compared with untreated plants (-ABA).

Hundred grains weight was maximum (2.83 g) in SR-40, whereas minimum (2.27 g) was noted in local genotype. Similarly, 100 grains weight was reduced by 18, 34.44 and 62.83% at salinity stress of 4, 6 and 10 dSm^{-1} respectively compared with control. Salt exposed plants when sprayed with ABA, resulted in an increase of 11.78, 1040 and 15.30 % in 100 grains weight at 4, 6 and 10 dSm^{-1} salinity levels respectively when compared with untreated plants (-ABA; Table 6).

Table 4. Spikes plant^{-1} of various wheat genotypes as affected by ABA application and salinity stress.

Genotypes	Salinity levels (dSm^{-1})								Means
	0		4.0		6.0		10.0		
	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	
SR-23	3.00	4.00	3.00	3.00	3.00	3.00	3.00	2.00	3.00a
SR-40	4.00	4.00	4.00	2.67	3.00	3.00	3.00	2.00	3.21a
SR-20	2.00	2.00	2.33	1.67	2.00	1.00	1.67	1.00	1.71b
SR-22	2.00	3.00	2.00	2.00	2.00	1.00	1.67	1.00	1.83b
Local	1.67	2.00	1.00	1.00	1.00	1.00	1.00	0.33	1.13c
SR-25	2.00	2.00	2.00	1.00	1.00	1.00	1.00	0.33	1.29c
Mean	2.44ab	2.83a	2.39ab	1.89c	2.00bc	1.67c	1.89c	1.11d	

DMRT value for interactions at $p<0.05 = 0.36$

Means of the same category followed by different letters are significantly different using DMRT test ($p<0.05$)

Table 5. Grains spike^{-1} of various wheat genotypes as affected by ABA application and salinity stress.

Genotypes	Salinity levels (dSm^{-1})								Means
	0		4.0		6.0		10.0		
	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	
SR-23	44.67	46.00	41.67	35.00	26.00	26.00	23.00	21.33	33.00b
SR-40	47.33	49.33	43.33	38.33	29.33	28.33	26.33	23.33	35.71a
SR-20	42.67	45.00	40.00	32.00	25.33	23.67	22.00	19.00	31.21c
SR-22	45.33	46.33	43.33	34.33	27.33	26.33	23.33	20.33	33.33b
Local	41.67	42.67	38.67	28.67	24.67	20.67	20.00	5.33	27.79e
SR-25	43.33	44.33	41.33	31.33	26.33	23.00	21.33	5.67	29.58d
Mean	44.17a	45.61a	41.39b	33.28c	26.50d	24.67e	22.67f	15.89g	

DMRT value for interactions at $p<0.05 = 4.10$

Means of the same category followed by different letters are significantly different using DMRT test ($p<0.05$)

Table 6. Hundred grains weight (g) of various wheat genotypes as affected by ABA application and salinity stress.

Genotypes	Salinity levels (dSm^{-1})								Means
	0		4.0		6.0		10.0		
	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	+ABA	-ABA	
SR-23	3.30	3.36	2.80	2.47	2.50	2.24	1.96	1.60	2.53b
SR-40	3.52	3.60	3.36	3.01	2.70	2.48	2.18	1.80	2.83a
SR-20	3.13	3.22	2.05	2.60	2.34	2.10	1.85	1.48	2.47b
SR-22	3.20	3.30	3.10	2.75	2.36	2.18	1.90	1.55	2.55b
Local	3.08	3.15	3.00	2.50	2.25	2.97	1.79	0.45	2.27c
SR-25	3.14	3.21	3.05	2.55	2.31	2.05	1.86	0.49	2.33c
Mean	3.23a	3.31a	3.00b	2.70c	2.41d	2.17e	1.92f	1.23g	

DMRT value for interactions at $p<0.05 = 0.53$

Means of the same category followed by different letters are significantly different using DMRT test ($p<0.05$)

Discussion

Our results indicated that genotypes responded differentially to various levels of salinity for their endogenous proline content and yield characteristics. Results indicated that endogenous proline concentration was significantly affected by different genotypes, salinity exposure and foliar ABA application. The data indicated that among different wheat genotypes, SR-40 and SR-23 performed better when compared with the other genotypes. Similarly, foliar application of ABA had a significant effect on proline content. ABA when applied to the foliage increased endogenous proline contents which are considered necessary and beneficial for providing salinity tolerance. Genotypes SR-40 and SR-23 produced maximum proline and better yield, while local genotype had minimum of these parameters compared with other genotypes under study. Enhanced synthesis of proline is among the few markers used for assessing salinity tolerance of a particular plant species. The present study also revealed that when different genotypes were exposed to salinity and ABA application, a marked increase in endogenous proline concentration and yield was observed. Genotypes SR-40 and SR-23 were on the top of the list for producing more proline and yield compared with other genotypes exposed to various levels of salinity. Sarwar & Ashraf (2003) reported that genetic variability exits in some primitive breed wheat varieties under salt stress. Similarly, ABA application to the foliage also increased endogenous proline levels of the various genotypes under salinity stress. From these results it is clear that proline is involved in the protection of plant against salinity stress as evident from the better performance of genotypes SR-40 and SR-23 in term of yield parameters under various salinity levels (Tables 4-6). Shaheen & Hood-Nowotny (2005) reported diversity in salt tolerance at intra-specific level in a number of plant species.

Osmotic adjustment is considered a crucial process in plant adaptation to salinity as it maintains tissue metabolic activities and helps in re-growth upon removing the stress, however, this response varies among genotypes. Osmotic adjustment is usually a slow process and is triggered by the peoduction of osmotic compounds like proline (Samuel *et al.,* 2000; Hamilton & Heckathorn, 2001; Mutlu & Buzcuk, 2007; Cha-um & Kirdmanee, 2008; 2009b; Bakht *et al.,* 2011) and methylated quaternary ammonium compounds eg., glycine betaine and alanine betain (Rathinasabapthi *et al.,* 2001; Sakamoto & Murata, 2002). In addition to decreasing cell osmotic potential, these solutes may protect the cell membrane under dehydration.

It has been reported that over expression of a gene encoding for moth bean P5CS in transgenic tobacco plants resulted in accumulation of proline up to 10-18 fold over control plants and better growth under dehydration stress (Kavi *et al.,* 1995). The same gene the control of an ABA stress inducible promoter in transgenic rice resulted in the accumulation of up to 2-5 folds more proline than control plants under stress condition (Zhu *et al.,* 1997). Proline is one the major compatible solutes, which may help to maintain relatively high water content necessary for plant growth and cellular function. Proline has been reported to alter the permeability and fluidity characteristics of membrane (Slocum *et al.,* 1984). It has also been reported that proline may act as a buffer protecting the cells from large changes in cytosolic pH, which may accompany cell desiccation (Slocum *et al.,* 1984). Proline also regulates the accumulation of useable N, is osmotically very active, contributing to membrane stability and reduce the adverse effects of NaCl on cell membrane (Gadallah, 1999; Mansour, 1998). Maggaio *et al.,* (2004) are of the view that proline may act as a signaling/regulatory molecule able to activate multiple responses that are component of the adaptation process.

Exogenous application of proline decreased shoot Na^+ and Cl^- accumulation and thereby enhanced growth under saline conditions in cultured barley embryos (Lone *et al.,* 1987). Petrusa & Winicov (1997) reported that salt tolerant alfalfa plants rapidly doubled their proline content in their roots, whereas in salt sensitive plants the increase was slow. Relatively, salt tolerant plants of *Brassica juncea* showed higher degree of osmotic adjustment in the leaves and a higher critical point concentration of NaCl, at which the endogenous level of free proline rose sharply, than did the relatively salt sensitive genotypes (Jain *et al.,* 1991). Higher proline accumulation was found in salt tolerant *B. juncea* plants with better growth than the control (Kirti *et al.,* 1991).

Conclusions

From the present study it can be concluded that different salinity levels, wheat genotypes, foliar spray of ABA and their all possible interactions had a significant effect on shoot proline content and yield. Different genotypes of wheat responded differentially to salinity stress and foliar application of ABA. Genotypes SR-40 and SR-23 produced more endogenous shoot proline content and better yield when exposed to different salinity stress and sprayed with ABA compared with other wheat genotypes under trial.

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