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ROLE OF ABSCISIC ACID AND PROLINE IN SALINITY TOLERANCE OF WHEAT GENOTYPES

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Abstract

Wheat genotypes were evaluated for salinity tolerance under 3 diverse environments of Yar Hussain, Baboo Dehari (District Swabi KPK Pakistan) and Khitab Koroona (District Charsadda KPK Pakistan). Eleven genotypes (Local, SR-24, SR-25, SR-7, SR-22, SR-4, SR-20, SR-19, SR-2, SR-23 and SR-40) were tested for their salinity tolerance. These locations had different salinity profile (i.e. Yar Hussain, EC. 3-3.5 dS m^{-1}; Baboo Dehari, EC. 4-4.5 dS m^{-1} and Khitab Koroona, EC. 5-5.30 dSm^{-1}). Different locations and wheat genotypes had a significant ($p < 0.05$) effect on endogenous shoot proline, shoot ABA (3, 6 and 9 weeks after emergence) and straw yield. Maximum endogenous shoot proline and ABA levels (3, 6 and 9 weeks after emergence) were recorded in genotype SR-40 followed by genotype SR-23. The results further indicated that minimum endogenous shoot proline and ABA concentrations (3, 6 and 9 weeks after emergence) were recorded at Yar Hussain. Maximum endogenous shoot proline and ABA concentration (3, 6 and 9 weeks after emergence) were observed at Khitab Koroona.

Introduction

Although the amount of salt-affected land is imprecisely known, its extent is sufficient to pose a threat to agriculture (Munn, 2002; Munn *et al.,* 2006). Salt stress affects physiology of plant at both whole plant and cellular levels through osmotic and ionic stress (Hasegawa *et al.,* 2000; Ranjbarfordoei *et al.,* 2002). The response of plants to various abiotic stresses has been an important subject of physiological studies (Bajaj *et al.,* 1999; Hasegawa *et al.,* 2000). Increased salt tolerance of crops is needed to sustain food production in many regions of the world. In irrigated agriculture, improved salt tolerance of crops can lessen the leaching requirement and thus lessen the costs of an irrigation scheme, both in the need to import fresh water and to dispose of saline water (Pitman & Lauchli, 2002). In dry land agriculture, improved salt tolerance can increase yield. In areas where the rainfall is low and the salt remains in the subsoil, increased salt tolerance will allow plants to extract more water. Salt tolerance may have its greatest impact on crops growing on soils with natural salinity when all the other agronomic constraints have been overcome (e.g., disease resistance and nutrient deficiency); subsoil salinity remains a major limitation to agriculture in all semi-arid regions. Even where clearing of land in higher rainfall zones has caused water tables to rise and salt to move, improved salt tolerance of crops will have a place. The introduction of deep-rooted perennial species is necessary to lower the water table, but salt tolerance will be required not only for the de-watering species, but also for the annual crops that follow as salt will be left in the soil when the water table is lowered. One way of increasing productivity in

stressful environment is to breed crops that are more tolerant to stress. However, success in breeding for tolerance has been limited because (a). Tolerance to stress is controlled by many genes, and their simultaneous selection is difficult (Flowers *et al.,* 2000); (b) Tremendous efforts is required to eliminate undesirable genes that are also incorporated during breeding and (c) There is a lack of efficient selection procedures particularly under field conditions. However, evaluating field performance under saline condition is difficult because of variable salinity within field (Daniells *et al.,* 2001). An incomplete understanding of the complex physiological determinants of yield under salinity has hindered the utilization of indirect selection for traits involved in the adaptive response of plants to salinity. If the tolerance of our cultivated crops to salinity could be improved, a wide variety of strategies would be available to farmers resulting in more efficient and economical practices. In this study, attempts were made under field conditions to study the response of various genotypes of wheat exposed to salinity stress.

Materials and Methods

Field experiments were conducted at 3 different locations in Khyber Pakhtunkhwa Province (Districts Swabi and Charsada) Pakistan, to study the performance of 11 wheat genotypes (Local, SR-24, SR-25, SR-7, SR-22, SR-4, SR-20, SR-19, SR-2, SR-23 and SR-40) for their salinity tolerance. These locations included Yar Hussain (EC. 3-3.5 dSm^{-1}; District Swabi), Baboo Dehari (EC. 4-4.5 dSm^{-1}; District Swabi) and Khitab Koroona (EC. 5-5.30 dSm^{-1}; District Charsada). These experiments were laid out in randomized complete block design with three replications. Experimental plots measured 4 m x 1.8 m with row to row spacing of 30 cm were maintained during the present study. A basal fertilizer dose of 135 kg N, 120 kg P_2O_5 and 60 kg K_2O ha^{-1} was applied to all treatments. Half dose of N and full does of P and K was applied at the time of sowing while the remaining half dose of N was given to all experimental plots with 2nd irrigation. Recommended agronomic practice, i.e., weeding hoeing, thinning, irrigation and plant protection measures were carried out at appropriate times. Before sowing a composite soil was collected for phyisco-chemical analysis (Table 1).

Procedures for data recording: Proline concentration in leaves was determined according to the method of Bates *et al.,* (1973). Briefly, fresh leaf materials were homogenized with 3% sulfosalicylic acid. 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.

ABA was isolated according to the method of Parry & Horgan (1991) with certain modifications. Ten g of fresh shoot and root tissue were homogenized in 80% acetone (10 ml g^{-1} of tissue) containing 0.1 M acetic acid and 1 mM BHT at 4^{o}C. Twenty KBq of *cistrans*-ABA (^{3}H) ± enantomer mixture Amersham) was added to the homogenate as an internal standard. The samples were centrifuged @ 5,000 g for 30 minutes at 4^{o}C. The supernatant was then filtered and the volume reduced to dryness using a rotavapour (Bucchi, Switzerland). The dry materials were dissolved in 50 mM sodium phosphate buffer pH 8.0 and extracted three times with an equal volume of Diethyl ether. This was then filtered and the pH adjusted to 2.5. The organic phase was then extracted again three times with diethyl ether, dried down and re-dissolved in methanol and streaked on to pre-

made 0.5 mm F254 silica gel TLC plate (Cam Lab Macherey Nagel, Germany) along with authentic ABA standard and developed in toluene/ethyl acetate/acetic acid (50:30:4, v/v). The co-migrating ABA marker spot was removed from the TLC plate and eluted with 2 ml water saturated ethyl acetate and reduced to dryness. The ABA was then methylated. Trans-ABA was added as a GC internal standard before methylation. After methylation the samples were dried under nitrogen and re-dissolved in appropriate amount of methanol for GC-FID (Packard model 430) analysis using 25m long Qudrex OV-1 column (0.32 mm i.d x 0.5 µl film thickness).

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 & Gomaz (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 locations and their interactions 3, 6 and 9 weeks after emergence (Table 2, 3 and 4). Maximum shoot proline contents were noted in SR-40 followed by SR-23 while minimum (467µg g^{-1} fresh weight) in genotype local. The same trend of proline levels in SR-40 was also observed 6 and 9 weeks after emergence (Table 3 and 4). Similarly, maximum shoot proline content of 628.2 µg g^{-1} fresh weight was obtained at Khitab Koroona when compared with Yar Hussain (473.27 µg g^{-1} fresh weight). Again, proline contents were more at Khitab Koroona 6 and 9 after emergence (Table 3 and 4). In case of interaction, maximum proline contents (724.33 µg g^{-1} fresh weight) were produced by SR-40 at Khitab Koroona while minimum of 412 µg g^{-1} fresh weight was recorded in genotype local at Yar Hussain.

Data concerning shoot ABA contents (µg g^{-1} fresh weight) 3, 6 and 9 weeks after emergence is shown in Table 5, 6 and 7. Analysis of the data showed that shoot ABA contents were significantly ($p<0.05$) affected by various genotypes, different locations and their interactions. Maximum shoot ABA contents were observed in SR-40 followed by SR-23. Minimum shoot ABA contents of 0.841 µg g^{-1} fresh weight were noted in genotype local which was statistically at par with SR-24 and SR-25. Our results also suggested that ABA contents were more in SR-40 when the data was recorded 6 and 9 weeks after emergence. Between locations, maximum shoot ABA contents were produced by plants at Khitab Koroona, 3, 6 and 9 weeks after emergence. Genotypes x locations interaction showed that SR-40 produced maximum ABA contents at Khitab Koroona, 3, 6 and 9 weeks after emergence when compared with other interactions (Tables 5, 6 and 7).

Table 1. Physio-chemical properties of the soil from three different experimental locations.

Characteristics	Yar Hussain	Baboo Dehari	Khitab Koroona
Electric conductivity (dSm^{-1})	3-3.5	4-4.5	5-5.30
K ($mg\ kg^{-1}$)	108	122	124
N (%)	0.057	0.064	0.087
P ($mg\ kg^{-1}$)	9.3	8.2	9.3
Clay (%)	23.15	25.15	24.50
Silt (%)	32.10	30.90	31.20
Sand (%)	44.78	42.45	45.15
Textural Class	Loamy	Loamy	Loamy

Table 2. Shoot proline (µg g^{-1} fresh weight) 3 weeks after emergence of wheat as affected by locations of different salinity level.

Genotypes	Yar Hussian	Baboo Dehari	Khitab Koroona	Mean
Local	412.00	459.00	530.00	467.00d
SR-24	415.33	446.33	541.67	467.78d
SR-25	416.00	474.00	531.67	473.89d
SR-7	448.00	533.00	608.67	529.89c
SR-22	464.33	536.67	603.67	534.89c
SR-4	451.67	530.67	611.33	531.22c
SR-20	460.00	543.33	604.00	535.78c
SR-19	534.00	680.67	726.67	647.11ab
SR-2	535.00	688.33	703.33	642.22b
SR-23	532.33	694.67	724.67	650.56ab
SR-40	537.33	695.67	724.33	652.44a
Means	473.27c	571.12b	628.18a	

DMRT value for interactions at $p<0.05=40.10$

Table 3. Shoot proline (µg g^{-1} fresh weight) 6 weeks after emergence of wheat as affected by locations of different salinity level.

Genotypes	Yar Hussian	Baboo Dehari	Khitab Koroona	Mean
Local	547.00	615.67	670.00	610.89d
SR-24	551.00	617.67	672.00	613.56d
SR-25	552.33	617.00	674.67	614.67d
SR-7	653.33	647.33	809.33	703.33c
SR-22	660.33	751.33	819.00	743.56b
SR-4	665.33	746.67	816.00	742.67bc
SR-20	662.00	748.00	812.33	740.78bc
SR-19	791.33	920.00	1119.00	943.44a
SR-2	794.00	818.67	1119.33	910.67a
SR-23	795.33	922.33	1119.00	945.56a
SR-40	795.33	924.67	1119.67	946.56a
Means	678.85c	757.21b	886.39a	

DMRT value for interactions at $p\leq0.05 = 50.20$

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

Table 4. Shoot proline (µg g^{-1} fresh weight) 9 weeks after emergence of wheat as affected by locations of different salinity level.

Genotypes	Yar Hussian	Baboo Dehari	Khitab Koroona	Mean
Local	560.67	729.00	784.67	691.44c
SR-24	555.00	730.33	779.67	688.33c
SR-25	556.33	735.00	785.67	692.33c
SR-7	778.33	851.67	920.33	850.11b
SR-22	780.33	850.00	923.67	851.33b
SR-4	783.00	848.33	921.33	850.89b
SR-20	782.00	853.33	921.00	852.11b
SR-19	907.33	1012.67	1348.00	1089.33a
SR-2	904.33	1011.33	1355.00	1090.22a
SR-23	909.00	1016.67	1351.00	1092.22a
SR-40	915.00	1021.33	1356.67	1097.67a
Means	766.49c	878.15b	1040.64a	

DMRT value for interactions at $p \leq 0.05 = 110.20$

Table 5. Shoot ABA levels (µg g^{-1} fresh weight) 3 weeks after emergence of wheat as affected by locations of different salinity level.

Genotypes	Yar Hussian	Baboo Dehari	Khitab Koroona	Mean
Local	0.700	0.827	0.997	0.841d
SR-24	0.703	0.837	1.000	0.847d
SR-25	0.703	0.833	1.013	0.850d
SR-7	0.897	0.947	1.110	0.984c
SR-22	0.890	0.943	1.120	0.984c
SR-4	0.907	0.940	1.107	0.984c
SR-20	0.893	0.950	1.117	0.987c
SR-19	0.960	1.110	1.187	1.086b
SR-2	0.943	1.130	1.207	1.093ab
SR-23	0.970	1.120	1.193	1.094ab
SR-40	0.960	1.117	1.230	1.102a
Means	0.866c	0.979b	1.116a	

DMRT value for interactions at $p \leq 0.05 = 0.110$

Table 6. Shoot ABA levels (µg g^{-1} fresh weight) 6 weeks after emergence of wheat as affected by locations of different salinity level.

Genotypes	Yar Hussian	Baboo Dehari	Khitab Koroona	Mean
Local	0.750	0.870	1.053	0.891c
SR-24	0.757	0.873	1.050	0.893c
SR-25	0.760	0.883	1.063	0.902c
SR-7	1.010	1.027	1.117	1.051b
SR-22	1.003	1.040	1.127	1.057b
SR-4	1.007	1.030	1.123	1.053b
SR-20	1.010	1.053	1.223	1.062b
SR-19	1.040	1.113	1.220	1.124a
SR-2	1.050	1.123	1.223	1.132a
SR-23	1.040	1.143	1.220	1.134a
SR-40	1.043	1.130	1.227	1.133a
Means	0.952c	1.026b	1.141a	

DMRT value for interactions at $p \leq 0.05 = 0.100$

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

Table 7. Shoot ABA levels (µg g^{-1} fresh weight) 9 weeks after emergence of wheat as affected by locations of different salinity level.

Genotypes	Yar Hussian	Baboo Dehari	Khitab Koroona	Mean
Local	0.794	1.000	1.185	0.993c
SR-24	0.783	1.007	1.190	0.995c
SR-25	0.798	1.003	1.200	1.000c
SR-7	1.061	1.041	1.263	1.121b
SR-22	1.0531	1.061	1.275	1.130b
SR-4	1.057	1.051	1.279	1.129b
SR-20	1.071	1.065	1.379	1.162b
SR-19	1.092	1.128	1.389	1.200a
SR-2	1.110	1.137	1.387	1.211a
SR-23	1.119	1.171	1.402	1.230a
SR-40	1.121	1.181	1.409	1.237a
Means	1.019	1.077	1.210	

DMRT value for interactions at $p \leq 0.05 = 0.111$

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

Discussion

Genotypes responded differentially to various levels of salinity for proline contents. Proline contents were significantly affected by different genotypes and salinity exposure at different locations. Genotypes SR-40 and SR-23 had maximum proline, while genotype local and SR-24 had minimum proline contents compared with other genotypes under study when exposed to salinity 3, 6 and 9 weeks after emergence. Proline is among the few markers used for assessing salinity tolerance of a particular plant species. Although use of ions for osmotic adjustment may be energetically more favorable than biosynthesis of organic osmolyte under osmotic stresses, many plants accumulate organic osmolytes to tolerate osmotic stresses. These osmolytes include proline, betaine, polylols, sugar alcohols and soluble sugars (Samuel *et al*., 2000; Chen & Murata, 2000; Hamilton & Heckathorn, 2001; Rathinasabapthi *et al.,* 2001; Sakamoto & Murata, 2002; Aziz & Khan, 2003; Chinnusamy *et al.,* 2003; Waheed *et al.,* 2006; Gurmani *et al.,* 2007). Genes involved in osmoprotectants biosynthesis are up-regulated under salt stress and concentrations of accumulated osmoprotectant correlate with osmotic stress tolerance (Zhu, 2002). Osmotic adjustment has been considered a crucial process in plant adaptation to salinity, because it sustains tissue metabolic activities and enables re-growth upon removing the stress but varies among genotypes. However, in terms of crop yield there are not many field studies showing a consistent benefit from osmotic adjustment (Quarrie *et al*., 1999), presumably because turgor maintenance in cells is often associated with slow growth (Serraj & Sinclair, 2002). Nevertheless, osmotic adjustment is important in roots enabling their sustained growth under decreasing water availability in the soil. Proline regulates the accumulation of useable N, is osmotically very active, contributing to membrane stability and mitigates the effect of NaCl on cell membrane disruption (Gadallah, 1999). 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.

Results also indicated that ABA concentrations were significantly affected by different genotypes and salinity exposure of different locations. Among the tested genotypes, SR-40 and SR-23 produced maximum ABA in their tissue while genotype

local had minimum of this compound compared with other genotypes under study when stressed for 3, 6 and 9 weeks after emergence. ABA is considered to be a stress hormone and relates closely with the adaptation of plants to stressful conditions and therefore protect plants against salinity and water stress (Bano & Aziz, 2003; Zhu, 2003; Gurmani *et al.,* 2007). Analyses of the expression of these stress inducible genes in *Arabidopsis* have indicated that ABA-dependent and independent signal pathways function in the induction of stress inducible genes. These indicate the existence of complex regulatory mechanisms between perception of abiotic signals and gene expression (Zhu, 2003). The basis for ABA as an important signal is that both salt and water deficit can induce rapid and massive accumulation of ABA in plant tissues. This process itself is cellular cascade, in which the perception of salt or water deficit signal or the initial triggering for ABA accumulation is the most important step. While many studies have tried to explore the triggering mechanism for water deficit induced ABA accumulation (Jia *et al*., 2001), much less is known about the triggering mechanism for salt stress induced ABA accumulation. Earlier studies have related the triggering mechanism to the changes in cellular water relations parameters and/or cell volume (Zhang, 2000). However, salt stress has more than its dehydration effect on plant cells. Many salt induced plant responses suggest that roots in soil must have evolved some mechanisms to detect a developing salt stress at its initial stage before a serious dehydration occurs. An osmosensor is perhaps one of these mechanisms.

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