

# Mechanism of Salinity Tolerance in Selected Wheat (*Triticum aestivum* L.) Cultivars

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An experiment in NaCl solution culture was conducted to observe the mechanism of salinity tolerance in selected three wheat (Triticum aestivum L.) cultivars, [BWIR7 (Mexico), Gutha (Australian) and KRL19 (Indian)] differing in salinity tolerance. Fifteen days old seedlings of each cultivar grown in half strength Hoagland nutrient solution were subjected to incremental salt stress until the required salinity levels (0, 180-mol m-3) were obtained. Dry matter yield (DMY) at 30 days, indole acetic acid (IAA), abscisic acid (ABA) and total free amino acid (AA) contents of leaves/root at 29 days and dehydrogenase activity of roots at 34 days were determined after initiation of the salinity stress. Shoot and root weight (DMY), dehydrogenase of root and IAA contents both in leaves and root of all the cultivars decreased whereas, ABA and AA contents increased. At saline treatment, cultivar, KRL19 produced maximum shoot DMY and BWIR7 minimum. Whereas, in case of roots Gutha produced maximum and KRL 19 minimum DMY. Dehydrogenase activity of root BWIR7 was more affected followed by Gutha and KRL19. The extent of decrease of IAA content in leaves and roots of Gutha was more pronounced than the other cultivars. Increase in ABA contents of shoots and roots were more in KRL 19 followed by BWIR7 and Gutha respectively. The extent of increase of AA contents was more in the leaves of KRL 19. From this study, it was concluded that cultivar KRL 19 has the salinity inclusion and Gutha salinity exclusion mechanism. The salinity tolerance cultivars were in order of KRL 19 > Gutha> BWIR7.

Key words: Salinity, dehydrogenase activity; IAA, ABA, AA

Salinity has created an alarming situation in India. Saline and sodic soil conditions reduce the value and productivity of crops. The poor crop growth in saline soil is throught to be due to such caues as imbalance in mineral nutrition, reduction in water uptake and direct toxicity of salt to plants. Depending upon a number of soil and climatic factors the salt accumulation in soil varies which in turn affects plant growth to different degrees (Bernstein, 1975). The problem of soil salinity is of frequent occurrence in irrigated areas of the world (Shannon, 1984). In India about 10 million hectares of land are affected by soil salinity and alkalinity (Bhargava, 1989). Development of salinity tolerant cultivars is a possible alternative to expensive engineering approach to utilize the waste land.

Salinity tolerance in wheat has been and is being extensively researched in India and elsewhere in the world, but still efforts to improve salinity tolerance has been hampered by a number of factors, particularly the lack of understanding of the mechanism of salinity tolerance and interaction of salinity with various environmental factors with regards to plant growth. Wheat tolerance to salinity varies with stage of plant growth, nature and level of salinity, duration of stress etc. (Qureshi *et al.*, 1990) and is affected by soil moisture, climate, nutrition and management practices (Mass and Hoffman, 1977). Different physiological traits such as selectivity for potassium, exclusion and / or compartmentation of sodium and chloride ions, osmotic adjustment by accumulation of organic solutes have all been related to salinity tolerance of crop plants (Wyn Jones and Storey, 1981). In this study, an attempt has been made to study the effect of NaCl salinity on yield, dehydrogenase activity, indole acetic acid (IAA) contents, abscisic acid (ABA) content and total free amino acid contents (AA) and their relationship with salinity tolerance of cultivars under consideration.

#### **Materials and Methods**

This experiment was conducted in Division of Crop Improvement at Central Soil Salinity Research Institute, Karnal-132001(Haryana) with artificial day / night temperature of 18/8 °C, respectively. Sufficient healthy seeds of three wheat cultivars [BWIR 7 (Mexico), Gutha (Australian) and KRL 19 (Indian)] were soaked in 0.3% fungicide solution for 20 h. After draining fungicide solution, the seeds were washed thrice with tap water. The seeds were sown in quartz sand in iron trays. The condition in trays kept moist with water and trays remained covered

until the sprout come out and kept waited for nine days. Thirteen days old 20 seedlings of each cultivars were transferred to 1 cm plugged holes in wooden covers over 32 L half strength Hoagland and Arnon (1950) nutrient solution in plastic containers. Ten holes were used for each cultivar and each hole having two seedlings. Fifteen days old seedlings were subjected to incremental salinity stress. Salt concentration was increased by 25 mol m-3 after every 12 h by adding NaCl to nutrient solution until the required salinity level of 180 mol m-3 was obtained in respective container. Thirty five days old seedlings were subjected to full strength Hoagland nutrient solution. Solution were renewed after every 7 days and pH (6.0-6.5) was daily maintained and loss of water made regularly. Solutions were aerated for 9 h every day with air pump by splitting into three equal parts and intervals. Twelve plants were harvested 30 days after salinization. The plants were washed for five minutes in running tap water followed by a quick rinse in distilled water. The plant tissue dried at 70 °C was weighed. Abscisic acid and Indole acetic acid were determined 29 days after salinization by the method given Weiler et al. (1986), total free amino acid after 29 days by the method given by Wrigley and Bietz (1988). Dehydrogenase activity of the roots was determined after 34 days salinization using the method as given below:

One gram root sample was taken in a test tube. A volume of 5 mL 0.4% TCC and 5 mL 0.M. Phosphate buffer solution were added. Then the sample was incubated at  $37^{\circ}$ C. After 3 hours, samples were immediately taken out from incubator and ground. 2 mL 2 NH<sub>2</sub>SO<sub>4</sub> was added for stopping the enzyme reaction. Then roots in pestle mortar with ethyl acetate and volume was made up were ground to 50 mL. The reading was taken at 485 nm wavelength on spectrophotometer and calculated as under.

Reduced TTC (g.g.<sup>1</sup>. F. wt.  $h^{-1}$ ) = 50 x reading of sample/weight of sample x time (in h.)

Where TTC = 2, 3, 5- triphenyl tetrazolium chloride. Statistical analysis was done by the method give by Panse and Sukhatme (1985).

### **Results and Discussion**

The result of present study indicated that salinity decreased the DMY of shoots and roots,

dehydrogenase activity of roots and IAA content of leaves and roots, whereas an increase in ABA and AA contents was observed in all the cultivars in leaves and roots. Data in Table-1 revealed that saline treatment gave 63% DMY in both of shoots and roots. The cultivar KRL 19 produced higher DMY of shoot (67% of control) with 27% coefficient of variation followed by Gutha and BWIR 7. However Gutha showed superiority in roots (67% of control) with 28% coefficient of variation followed by BWIR 7 and KRL 19. Reduction of DMY under saline environment is in agreement with those of Almansouri et al. (1999).

Data in Table-2 revealed that saline treatment decreased and gave 67% dehydrogenase activity. The root dehydrogenase activity was higher (74% of control) in KRL 19 with 19% coefficient of variation and lowest (56% of control) in BWIR7 with 38% coefficient of variation. The root dehydrogenase activity had positive correlation with shoot potassium content and negative with root potassium content under saline treatments as well as on cumulative means of two treatments (Pervaiz et al., 2002). This finding is disagreement with the observation of increased content of dehydrogenase in case of KRL 19 and Gutha which might have caused an enhanced synthesis of glycerol through the process of synthesis or dissimilation which might act as an osmoregulator, resulted in an increased salt tolerance than as descried in case of Dunaliella tertiolect (Ghoshal and Goyal, 2002). In the present study analysis of glycerol was not determined but its possibility to increase salt tolerance may not be ruled out. However, it needs further investigation.

Data in Table-3 revealed that salinity decreased

and gave 64 and 9.52% IAA contents both in leaves and roots respectively. The decrease in IAA content is in agreement with Wright (1978), Guinin and Brummet (1987), Ikeda *et al.* (1989) and Prakash and Prathapasenan (1990). The IAA content of leaves were higher (93% of control) in BWIR 7 with 5% coefficient of variation and lowest (10% of control) in Gutha with 12% coefficient of variation. In case of roots regarding IAA content, the highest (22.80% of control) in KRL 19 with 85% coefficient of variation and lowest (2.45% of control) in Gutha with 133% coefficient of variation were found.

Data in Table-4 revealed that salinity increased the ABA contents in both leaves and roots of all the

Table1. Effect of sodium cl	hloride salt on dr	v matter vield	d of shoot and root o	f three cultivars

NaCl		Dry weight of shoot (g)				Dry weight of root (g)			
(molm <sup>-3</sup> )	BWIR7	Gutha	KRL 19	Average	BWIR7	Gutha	KRL 19	Average	
0	1.30	1.52	1.42	1.41	0.49	0.44	0.53	0.49	
180	0.73	0.89	0.92	0.84	0.31	0.29	0.31	0.30	
	(60)	(61)	(68)	(63)	(62)	(67)	(59)	(63)	
Average	1.02	1.21	1.17		0.40	0.37	0.42		
C.V. (%)	36	34	27		31	28	36		

Table 2. Effect of sodium chloride salt on the dehydroenase activity of roots of three wheat cultivars

Nacl (molm <sup>-3</sup> )	Dehydrogenase activity (TTC gg <sup>-1</sup> . Fresh weight. h)							
(	BWIR7	Gutha	KRL 19	Average				
0	519	475	480	491				
180	29.2 (56)	334(70)	344(74)	323(67)				
Average	405	404	412	323				
C.V. (%)	38	23	19					

cultivars. The ABA contents of leaves were highest (945% of control) under KRL 19 with 16% coefficient of variation and lowest (125% of control) in Gutha with 16% coefficient of variation. As well as

concerned to roots regarding ABA contents, the highest (335% of control) in KRL 19 with 79% coefficient of variation and lowest (204% of control) in Gutha with 50% coefficient of variation were found. The data is in agrement with Verma (1980), Clipson et al. (1988) and Cammue et al. (1989). The probable cause in increase ABA contents might have been due to the accumulation of Na<sup>+</sup> contents, which caused the deficiency of water in plant tissue. Inconsistent pattern was observed in between ABA contents and salinity tolerance of cultivars on dry weight basis. Accumulation of more DMY in case of KRL 19 might have been due to the stimulation of protein synthesis from glutamic acid/ or accumulation of more Na<sup>+</sup> in shoot and survival under adverse situation is the genetic character of

Table 3. Effect of sodium chloride salt	on indole acid content of leaves ar	nd roots of three wheat cultivars.

NaCl (molm <sup>-3</sup> )	Indole acetic acid content (P mol g <sup>-1</sup> . fresh weight)								
		Roots							
	BWIR7	Gutha	KRL 19	Average	BWIR7	Gutha	KRL 19	Average	
0	850	2250	970	1457	253	274	80	202	
180	790	272	844	648	8.40	6.80	19.50	11.50	
	(93)	(10)	(90)	(64)	(3.30)	(2.45)	(22.80)	(9.52)	
Average	820	1411	927		131	140	50		
C.V. (%)	5	12	7		130	133	85		

this genotypes. Accumulation of more ABA in the excised leaves of drought tolerance line of both maize and sorghum than susceptible has been reported by Larque - Saavedra and Wain (1976) and ABA as responsive elements in salt tolerance indica rice by Sudhiranjian *et al.* (1998). But this review is not in agreement with the above result of this

experiment because at 180 mol m-<sup>3</sup> salinity in Gutha showed moderate relative shoot DMY and least ABA contents, whereas BWIR 7 least relative shoot DMY and moderate ABA contents in its leaves. The results of these experiments are in agreement with the hypothesis of Pitman *et al.* (1974) who reported that effect of ABA depends on the species, growth

Table 4. Effect of sodium chloride salinity on abscisic acid contents of leaves and roots of three wheat cultivars

NaCl (molm <sup>-3</sup> )			Abscisic a	acid content (	P mol g <sup>-1+.</sup> Fre	esh weight)		
		Roots						
	BWIR7	Gutha	KRL 19	Average	BWIR7	Gutha	KRL 19	Average
0	52318	47482	1383	37894	3485	5936	11528	6983
180	81689	60897	134598	92395	7683	12677	34097	18419
	(155)	(125)	(9450)	(408)	(227)	(204)	(335)	(255)
Average	67003	54185	74240		5584	6204	22813	
C.V. (%)	31	16	16		57	50	79	

condition and temperature. Salinity increased the AA contents in both leaves and roots of all the cultivars except in roots of BWIR 7 (Table-5). The AA content of leaves was the highest (148% control) in KRL 19 with 26% coefficient of variation and lowest (119% of control) in BWIR 7 with 12% coefficient of variation. With regard to roots, AA content was higher (115% of control) in Gutha with 10% coefficient of variation and lowest (90% of control) was recorded in BWIR 7 with 12% coefficient of variation. Increased content of amino acid might be act as salt stress

protectant which helps plant to maintain favourable internal tissue water under salinity stress condition on (Shah *et al.*, 1990).

In addition to this increased content of free amino acid with increased salinity might have due to either retarded protein synthesis or due to arrest of proteiosynthetases and / or salinity may induce an inhibitory effect on polysomes formation. In the present study, protein synthesis and polysomes formation was not determined but possibility that

NaCl (molm <sup>-3</sup> )			Tota	al free amino a	acid content (	ppm)		
		Roots						
	BWIR7	Gutha	KRL 19	Average	BWIR7	Gutha	KRL 19	Average
0	3765	3036	2752	3184	2177	2312	2189	2226
180	4218	3846	3914	3993	1912	2536	2363	2270
	(119)	(133)	(148)	(133)	(90)	(115)	(112)	(105)
Average	3991	3441	3333		2045	2424	2276	
C.V. (%)	12	21	28		12	10	9	

Table 5. Effect of sodium chloride salinity on total free amino acid contents of leaves and roots of three wheat cultivars.

\*C.V. =Coefficient of Variation

inhibition of protein synthesis and polysomes formation in salinity exposed plant may not be ruled out (Ghoshal and Goyal 2002). The increase in AA contents is in agreement with those of Gorham et al. (1984) and Mattioni et al. (1997). Decrease in AA contents due to salinity was reported by Gorham et al. (1985) in the Thinopyurum bessarbium. The roots relative AA contents at 180 mol m-3 salinity also showed consistent pattern with root DMY. Generally, increase in AA contents in leaves was more than in roots. However, individual character of each cultivars, it was noted that KRL 19 has the inclusion mechanism because of production of higher DMY in the presence of higher quantity of sodium and chloride in its shoots (Pervaiz et al., 2002), ABA and AA contents in leaves and even higher root dehydrogenase activity which are the salinity tolerance/ salt inclusion mechanism. The Gutha showed salt exclusion mechanism, because of production of moderate DMY among the cultivars under consideration and accumulation of least sodium and chloride in its shoots (Pervaiz et al., 2002). However smallest content was recorded in ABA and IAA, whereas a reverse trend was noted in case of root dehydrogenase activity (Gupta and Kaur, 1970). The cultivar BWIR 7 was found sensitive due to minimum shoot DMY, minimum and maximum reduction in IAA content of leaves and roots dehydrogenase activity (Gupta and Kaur, 1970), respectively and moderate accumulation of sodium and chloride content in shoots.

The cultivars KRL 19 and Gutha showed salt inclusion and exclusion mechanism, respectively, and found salt tolerance cultivars. BWIR 7 was salt sensitive due to its low DMY/ root dehydrogenase activity and accumulation of more sodium and chloride contents in its shoots as compared to Gutha.

#### References

- Almansouri, M., Kinet, J.M. and Lutts, S. 1999. Compared effect of sudden and progressive imposition of salt stress in (Triticum three durum wheat durum Desf.) cultivars. *J Plant Physiol.* **154**: 743-752.
- Bernstein, L. 1975. Effect of salinity and sodicity on plant growth. Ann. Rev. Phytopathol. **13**: 295-312.

- Bhargava, G.P. 1989. Salt affected soil of India, A source of book. Oxford and BH Publishing Company, New Delhi p. 261.
- Cammue, B.P.A., Broekaert, W.F., Kellens, J.T.C., Raikhel, N.V. and Peumans, W.J. 1989. Stress induced accumulation of wheat germ agglutinin and abscisic acid in root of wheat seeding. *Plant Physiol.* **91**: 1432-1435.
- Clipson, N.J.W., Lachno and Flower, T.J. 1988. Salt tolerance in the halophyte,Suaeda maritime L. Dum:Abscisic acid concentration in response to content and altered salinity. *J Expt. Bot.* **39**: 1381-1388.
- Ghoshal, D. and Goyal, A. 2002. Review in Plant Biochemistry and Biotechnology Vol. 1 pp. 21-32.
- Gorham, J.E., McDonnell and Wyn Jones, R.W. 1985. Salt tolerance in the Triticeae; Growth and solute accumulation in leaves of Thinpyrum berssarabium. *J. Expt. Bot.* **36**: 1021-1031.
- Gorham J,E. McDonnell & Wyn Jones R.G. 1984. Pintol and other solute stressed *Sesbania aculeata Z. Pflanzen Physiol.* Bd. **114**: 173-175.
- Guinin, G. and Brummett, D.L.1987. Concentration of abscisic acid and indole acetic acid in cotton fruit and their abscission. *Plant Physiol.* 83: 199-202.
- Gupta, U.S. and Kaur, P. 1970. The relation between salt tolerance and dehydrogenase activity in *Piscum sativum* L. verities. *Sci. Cult.* **36**: 293-294.
- Hoagland, D.R. and Amon, D.I. 1950. The water culture method for growing plants without soil. *Calif. Agri. Exp. Stn. Ciric.* **347**: 39.
- Ikeda, J., Kobayashi, M. and Takahashi, E. 1989. The effect of salt on growth, IAA productivity and extracellular polysaccharides productivity of *Rhizobium* spp. *Japanease J Soil Sci.* and *Plant Nut.* **60**: 41-46.
- Imam-ul-Haq, S.M. and Larher, 1985. Osmoragulation in higher plant. New Phytol. 93: 209-216.
- Larque-Saavedra, A. and Wain, R.L. 1976. Abscisic acid as a gentic character related to drought tolerance. *Ann. Appl. Biol.* **83**: 291-297.
- Mass, E.V. and Hoffman, G. 1977. Crop salt tolerance. J. Irri. Drainage Div. Amer. Soc. Civil Engg. 103: 115-134.
- Panse,V.G. and Sukhatme, P.V. 1985. Statistical Methodfor agricultural Research, New Delhi (23<sup>rd</sup> edition). Publication and Information Division, Indian Council of Agriculture Research, New Delhi p.
- Pervaiz, Z., Afzal, M., Xiaoe, S., Xi, Y. and Ancheng, L. 2002. Physiological parameters of salt tolerance in wheat. Asian J. Plant Sci., 1: 478-481.

- Pitman, M.G., Luttge, U., Lauchli, A. and Ball, E. 1974. Action of abscisic acid on ion transport as affected by root temperature and nutrient status. *J. Expt. Bot.*, 25: 147-155.
- Prakash, L. and Prathapasenan, G. 1990. Sodium chloride and Gibberellic acid induced changes in the content of auxin and activates of and cellulose and pectin layse during leaf growth in rice. *Ann. Bot.*, **65**: 251-257.
- Qureshi, R.H, Rashid, A. and Ahmad, N. 1990. In: Genetic Aspect of Plant Mineral Nutration Elbsam, N., M. Damborth and BC Laughman (eds), pp, 315-324. Kluwer Acad Pub., Dordecht, Netherland.
- Shah, S.H., Wainwright, S.J. and Merrut. 1990. Physiology and biochemistry of higher plant. New Phytok. 116: 37-39.
- Shannon, M.C. 1984. Salinity Tolerance in Plant. Strategies for Crop Improvement pp. 231-254. Wiley, New York, U.S.A.
- Sudhiranjan, G., Chattopadhyay, M.K., Chatterijee, P., Ghos, B. and Gupta, S. 1998. Expression of abscisic acid-

responsive element-binding protein in salt tolerance Indica rice. *Plant Mol. Biol.*, **37**: 629-637.

- Verma, S.K. 1980. Specific ions effect on accumulation abscisic acid in wheat. J. Plant Nutr., 2: 537-546.
- Weiler, E.W., Eberle, J., Mertens, R., Atzom, R., Feyerabend, M., Joundan, P.S., Amsheidt, A. and Wieczorek, 1986.
  In: Immunology in Plant Science. Wang, T.I. (ed) pp. 27-58. Cambridge University Press, Cambridge, U.K.
- Wright, S.T.C. (1978). In: Phytohormones and Related Compound. A Comprehensive Treatise Vol. 2, D.S. Letham, P.B. Goodwin and T.J.V. Higgins (eds) pp 495-536. Elsevier North-Hollan Biomedical Press, Amsterndam
- Wrigley, C.W. and Bietz, J.A. 1988. Protein and amino acids. In: Wheat Chemisty and Technology 3<sup>rd</sup> ed. Y. Porneranz ed. Am. Assoc. Cereal Chem., St. Pant. M.N.I. 159:275.
- Wyn Jones, R.G. and Stroey. 1981. In: Physiology and Biochemistry of Drought Tolerance. Paieg. L.G. and D Aspinal (eds) pp. 204. Academic Press, Sydney.

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