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SALT AND DROUGHT STRESS IN WHEAT AND THE ROLE OF ABSCISIC ACID

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Abstract

The effects of different concentrations (150 mM and 300 mM) of NaCl alone or in combination with drought stress were determined on six wheat varieties. Among them, four varieties *viz.* E-38, E-37, E-2 and E-30 were newly released, whereas Lu-26 and Pasban–90 were commercial varieties. The plants were grown in pots under natural conditions with protective measures against rain. Drought was induced for 7d to salt stressed plants at three-leaf stage by withholding the supply of salt/water (in case of control). The response of salt and drought were studied on growth parameters and yield of wheat. The proline contents of leaves and the concentration of abscisic acid and giberrellic acid were also measured at three leaf stage. The increasing concentration of NaCl had a significant inhibitory effect on the growth parameters. Relatively sensitive varieties *i.e.* Pasban-90 and E-38 were markedly affected, whereas, the relatively tolerant varieties Lu-26 and E-30 were the least affected by either concentration of NaCl and performed better with respect to both yield parameters and biochemical characteristics. The combined effect of salt and drought was more inhibitory than salt alone. Both the proline and ABA contents were greater in Lu-26 and in E-30 treated with 300 mM NaCl. The role of ABA and proline in salt and drought stress has been discussed.

Introduction

Wheat (*Triticum aestivum* L.) is a major crop of rainfed agriculture in Pakistan. It is a moderately salt resistant crop contributing as food for more than 1/3rd of the total population. Recent research has shown that root responses to drought include a number of metabolic changes which can be interpreted as signals passing from root to shoot (Bahrun *et al.*, 2002). Vapor pressure deficit and the rate of soil water loss have been shown to modify the intensity of the signals and the sequence of events (Andreasson *et al.*, 2001). Physiologists have observed that individual plant respond to most stress by changing their hormonal balance frequently producing more ABA and less gibberellin (GA) and cytokinin (Bano *et al.*, 1993; Ali *et al.*, 1999; Bahrun *et al.*, 2002). Some studies on the wheat and other grain crops have shown that plant water deficit during grain filling substantially affects grain weight (Rahman & Yoshida, 1985), due to early plant senescence, cessation of grain filling (Hossain *et al.*, 1990) and shortening of the grain filling period (Aspinall, 1965; Brooks *et al.*, 1982; Egli 1998; Royo *et al.*, 2000). Osmotic adjustment has been considered one of the crucial processes in plant adaptation to drought because it sustains tissue metabolic activity. Aspinall & Paleg (1981) and Voetberg & Sharp (1991) reported that plant responses to environmental stress include ABA and proline accumulation

The present investigation was aimed to evaluate the performance of the newly released varieties and the commercial cultivars to different concentrations of NaCl (150 mM and 300 mM). The drought is also induced to a group of salt stressed plants with the aim to simulate the natural condition in barani areas where a year of drought may result

in combined effect of salinity and drought stress. Since ABA is thought to have important functional roles in plants under stress, it was reported to act as root-to-shoot signal under drought stress (Davies & Zhang, 1991) as well as salt stress (Hartung & Jeschke (1999), hence the endogenous level of ABA and GA were also monitored under the salt and drought stress. The effect of NaCl alone and in combination with drought were determined on growth biochemical contents and yield of plants.

Materials and Methods

Seeds of six wheat varieties (4 preliminary yield trials, PYT and two established varieties were obtained from National Agricultural Research Center, Islamabad. The cv. LU- 26, Pasban-90, E-38, E-30, E-37 and E-3 were of 1:3 and grown under natural condition. Surface sterilized seeds of six varieties were planted in pots containing mixture of soil and sand in the ratio 3:1.

***Treatments made*:** Control (untreated), NaCl (150 mM), NaCl (300 mM), NaCl (150 mM + drought) and NaCl (300 mM+ drought)

Induction of drought and salt stress: Aqueous solution of NaCl was given to seedlings 10d after sowing. The treated plants were irrigated with NaCl till maturity, whereas the control plants received irrigation with tap water. At three leaf stage drought was applied by withholding the supply of water for 7d. Thereafter, the drought stressed plants were treated with NaCl. Plants were harvested at 50 % flowering for the measurement of growth and yield parameters.

***Chlorophyll content of leaves*:** The chlorophyll content of leaves was determined by the method of Arnon (1949) as modified by Kirk (1968). The crude preparation (1 mL) was mixed with 4 mL of 80 % (v/v) acetone and allowed to stand in the dark at room temperature for ten minutes. It was centrifuged at 2000 rpm for 5 min. to clear the suspension. Supernatant, which contained soluble pigment, was used for the determination of chlorophyll. Absorbance of the solution was read at 645 nm (chlorophyll a) and at 663 nm (chlorophyll b) on spectrophotometer against 80 % (v/v) acetone blank.

Total chlorophyll was determined for the equation given by Arnon (1949).

Total chlorophyll (mg/L) = (20.2xA645) + (8.02xB663)

***Proline estimation of leaves*:** Free proline content of leaves was determined following the method of Bates *et al.* (1973). Fresh plant material (0.1 g) was homogenized wit 5 mL Sulfosalicylic acid (3.0 %) in mortar. Samples were centrifuged at 2000 rpm for 5 min. Supernatant was adjusted to 5 mL of distilled water, % 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 toluene layer was recorded at 520 nm. A calibration series of 10, 20, 30, 40, 50, 60, 70, 80 90, 100 μg of proline (DL) was also run and a standard curve was plotted and the concentration of unknown sample was calculated for the proline content with reference to the standard curve.

***Extraction and analyses of hormones from leaves*:** Extraction for ABA and GA was made following the method of Kettner & Doerffling (1980) and analyses were made with the help of HPLC according to the procedure of Li *et al.* (1994). Fresh leaf samples were ground in 80% methanol with butylated hydroxy toulene (BHT) (10mg/l). The extraction of leaves was made at 4°C in the dark for 72 with frequent change in solvent after 24 h. The sample was centrifuged at 300 rpm for 10 min. The centrifugate was reduced to aqueous phase using rotary thin film evaporator (RFE). The pH of the aqueous phase was adjusted to 9 with 0.1 N NaOH and partitioned with ethyl acetate 3 times. The pH of the aqueous was readjusted to 2.5-3.0 and partitioned three times with 1/3 rd volume of ethyl acetate. The ethyl acetate phase was dried down at 35°C using RFE. The dried samples was redissolved in 1ml of methanol (100%)and analyzed for the presence of GA and IAA using HPLC (Shimadzu, Japan) equipped with C18 cartridge column measuring 3.9x 150 mm. The HPLC was operated at 300C with methanol and 1% acetic acid (2.3) as mobile phase at flow rate of 1ml/min. The detection was made with variable wave length detector at 254 nm and 278 nm for ABA and GA respectively (Li *et al.*, 1994).

***Protein estimation of seeds*:** Seed protein was estimated following the procedure of AOAC (1982). Proteins contents of the seeds measured by Kjeldahl method.

$$\% \text{ Crude protein} = \frac{(Vi\text{-}V2)N}{1000w} \times 14 \times 6.25 \times 100$$

While

V1= Sample titer (in mL)
V2= Blank titer (in mL)
N= Normality of standarized H_2SO_4
W= Weight of sample

***Statistical analyses of the data*:** The data were analyzed statistically by Analysis of Variance technique and comparison among treatment means was made by "Duncan's Multiple Range Test" (DMRT) using MSTAT-C version 1.4.2.

Results

***Shoot height*:** The ANOVA and DMRT (Tables 1a, b) of shoot height· plant^{-1} showed significant differences among all the treatments ($p<0.05$). Low concentration (150 mM) of NaCl used alone and in combination with drought did not exhibit any significant effect except in Pasban-90. At high concentration (300 mM) NaCl used either alone or in combination with drought, showed maximum inhibition in E-37 but Lu-26 was least affected.

***Shoot dry weight*:** The ANOVA and DMRT (Tables 2a, b) of shoot dry weight· plant^{-1} showed significant differences among all the treatments ($p<0.05$). Significant decrease in shoot dry weight was observed in E-37 under the combined effect of salt and drought stresses. Pasban-90 showed maximum and highly significant decrease in shoot dry weight plant^{-1} at 300 mM NaCl used either alone or in combination with drought. The Lu-26 was least affected. Noteworthy, E-30 and E-2, did not exhibit any significant difference with the increase in the concentration of NaCl.

Table 1a. ANOVA of shoot height in 6 wheat varieties under different concentrations of NaCl and drought stress.

Source	D.F.	S.S.	M.S.	F	p
Replication	2	0.294	0.147	0.3036	n.s.
Variety (V)	5	215.942	43.188	89.1879	p<0.001
Salinity (S)	4	583.559	145.89	301.275	p<0.001
V x S	20	51.919	2.596	5.3609	p<0.001
Error	58	28.086	0.484		
Total	**89**	**879.801**			

Table 1b. DMRT of mean shoot height in wheat under different concentrations of NaCl and drought stress.

Salinity (mM)	0	150	150 + D	300	300 + D
Variety					
LU-26	23.3B	19.87CD	21.07C	17.27HIJ	17.7FGHI
Pasban90	22.03FGH	16.03JK	16.6IJK	13.63L	14.03L
E-38	22.73B	17.7FGHI	18.77DEFG	15.47K	15.63K
E-37	23.53B	17.53GHI	19.33DE	15.87K	14.2L
E-30	24.83A	18.93DEF	20.77C	18.23EFGH	18.1EFGH
E-2	25.17A	20.97C	19.9CD	16.23JK	16.23JK

Table 2a. ANOVA of shoot dry weight (g) under different concentration of NaCl and drought stress.

Source	D.F.	S.S.	M.S.	F	p
Replication	2	0.083	0.041	1.9625	n.s.
Variety (V)	5	7.47	1.494	70.7	p<0.001
Salinity (S)	4	12.255	3.064	144.9821	p<0.001
V x S	20	3.149	0.157	7.4513	p<0.001
Error	58	1.226	0.021		
Total	**89**	**24.183**			

Table 2b. DMRT of shoot dry weight (g) under different concentration of NaCl and drought stress.

Salinity (mM)	0	150	150 + D	300	300 + D
Variety					
LU-26	2.44ABC	2.06EFG	2.22CDE	2.01EFGH	1.9FGHI
Pasban90	1.79GHIJ	1.79GHIJ	1.54JKL	0.84N	0.71N
E-38	2.37BCD	2.13DEF	2.03EFG	1.21M	1.27M
E-37	2.57AB	1.83GHI	1.70IJK	1.39LM	1.74HIJ
E-30	2.68A	2.66A	2.24CDE	2.02EFG	1.46KLM
E-2	2.56AB	2.18CDE	2.29CDE	1.86FGHI	1.27M

All such mean values under a category which share a common letter are not different, otherwise they differ at p<0.05.

***Chlorophyll contents of leaves*:** All varietal mean values (Tables 3a, b) differed from each other significantly ($p<0.05$). The plants treated with 150 mM NaCl and 300 mM NaCl alone and in combination with drought showed maximum and highly significant decrease in chlorophyll contents of leaves in Pasban-90 as compared to its control. While E-30 and Lu-26 showed least decrease.

***Proline content of leaves*:** The DMRT (Tables 4a, b) of proline contents of leaves plant^{-1} showed significant differences among all the treatments ($p<0.05$). At low concentration (150 mM) of NaCl, leaf proline was significantly greater in Lu-26 even under drought stress but at high concentration (300 mM) NaCl used alone or under drought, the maximum increase was observed in E-30 .The cv E-38 and Pasban 90 showed least increase under 300 mM NaCl treatment. The amount of proline was more accumulated under the treatment of NaCl alone.

***Abscisic acid content of leaves*:** The ABA content of wheat plant (Fig. 1) had increased significantly under the NaCl stress made alone. The susceptible variety, Pasban-90 showed least increase in ABA under 300 mM NaCl. There was significant increase in the endogenous level of ABA in E-30 under 300 mM NaCl made alone and in combination with drought.

***Gibberrellic acid content of leaves*:** The data presented in Fig. 2 indicated that cv. E 30 showed marked decline in the GA_3 content with increase in NaCl concentration. In all the varieties the combined effect of salt and drought was more inhibitory. Pasban-90 having lower level of basal ABA exhibited maximum decrease in GA_3 content under salt and drought stress .The tolerant variety Lu-26 showed initial decrease due to 150 mM NaCl but at 300 mM NaCl there was no decrease, possibly the variety required an initial adjustment stage with the slinity stress. The NaCl (300 mM) accompanied by drought showed significant decrease in GA.

***Number and weight of 100 seeds*:** The ANOVA and DMRT (Tables 5a, b) of number of seed per plant and weight of 100 seeds (Tables 6a, b) showed significant differences among all the treatments ($p<0.05$). The results of treatment means of number of seed plant^{-1} revealed that plants treated with 300 mM of NaCl followed by drought stress showed maximum and highly significant (35 %) decrease in number of seeds plant^{-1} in Pasban-90 as compared to its control. While minimum decrease (30 %) was observed in Lu-26 and in E-30 treated with 300 mM NaCl followed by drought. The plants treated with 300 mM NaCl alone showed maximum decrease (31 %) in number of seeds in Pasban-90, while least decrease (22 %) was observed in E-2 under 300 mM salt stress. Pasban-90 showed maximum decrease in 100 seed weight both at 150 mM as well as at 300 mM NaCl alone and in presence of drought. The cv. Lu-26 exhibited the least decrease.

***Protein content of seeds*:** The result (Fig. 3) showed that seed protein increased under stress condition. The tolerant varieties Lu-26 and E-30 had higher protein content of seed under NaCl treatment made alone or in combination with drought stress,but the sensitive variety, Pasban 90 has little increase in protein content. Even at higher concentration of NaCl. Beltrano *et al.* (1999) reported that total protein content was higher in stressed plant as compared to those plants which were unstressed.

Table 3a. ANOVA of chlorophyll content of leaves in 6 wheat varieties under different concentrations of NaCl and drought stress.

Source	D.F.	S.S.	M.S.	F	p
Replication	2	17.933	8.966	10.4524	n.s.
Variety (V)	5	744.54	148.908	173.5874	p<0.001
Salinity (S)	4	4397.552	1099.388	1281.596	p<0.001
V x S	20	307.558	15.378	17.9265	p<0.001
Error	58	49.754	0.858		
Total	**89**	**5517.336**			

Table 3b. DMRT of mean chlorophyll content of leaves in wheat under different concentrations of NaCl and drought stress.

Salinity (mM)	0	150	150 + D	300	300 + D
Variety					
LU-26	73.97A	62.73DE	61.5EF	59G	53.83IJ
Pasban90	68.53C	63.67D	52.7JK	50.7L	43.27N
E-38	73.7A	61.93EF	57.17H	58.53GH	48.33M
E-37	70.87B	63.67D	55.4I	58.33GH	51.37KL
E-30	74.57A	68.23C	60.77F	63.77D	58.73GH
E-2	73.2A	62.73DE	61.7EF	58.33GH	53.97IJ

Table 4a. ANOVA of proline contents of leaves in 6 wheat varieties under different concentrations of NaCl and drought stress.

Source	D.F.	S.S.	M.S.	F	p
Replication	2	0.44	0.22	1.1323	n.s.
Variety (V)	5	47.735	9.547	49.0834	p<0.001
Salinity (S)	4	470.912	117.728	605.2714	p<0.001
V x S	20	42.362	2.118	10.8898	p<0.001
Error	58	11.281	0.195		
Total	**89**	**572.731**			

Table 4b. DMRT of mean proline contents of leaves in 6 wheat varieties under different concentrations of NaCl and drought stress.

Salinity (mM)	0	150	150 + D	300	300 + D
Variety					
LU-26	0.25G	3.283D	0.5033G	7.857A	5.16B
Pasban90	0.2067G	0.82FG	0.47G	4.697BC	2.42E
E-38	0.23G	2.37E	0.4833G	4.347C	2.777DE
E-37	0.2433G	1.457F	0.4567G	5.107BC	3.387D
E-30	0.28G	3.323D	0.5133G	8.04A	5.227B
E-2	0.31G	2.697DE	0.5667G	8.353A	4.583BC

All such mean values under a category which share a common letter are not different, otherwise they differ at p<0.05.

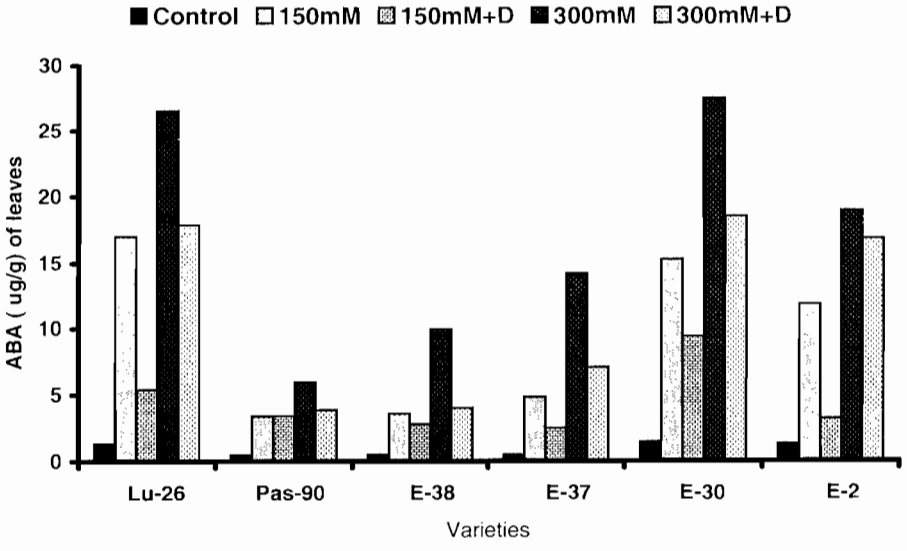

Fig 1. Abscisic acid (µg/g) content of leaves under NaCl stress.

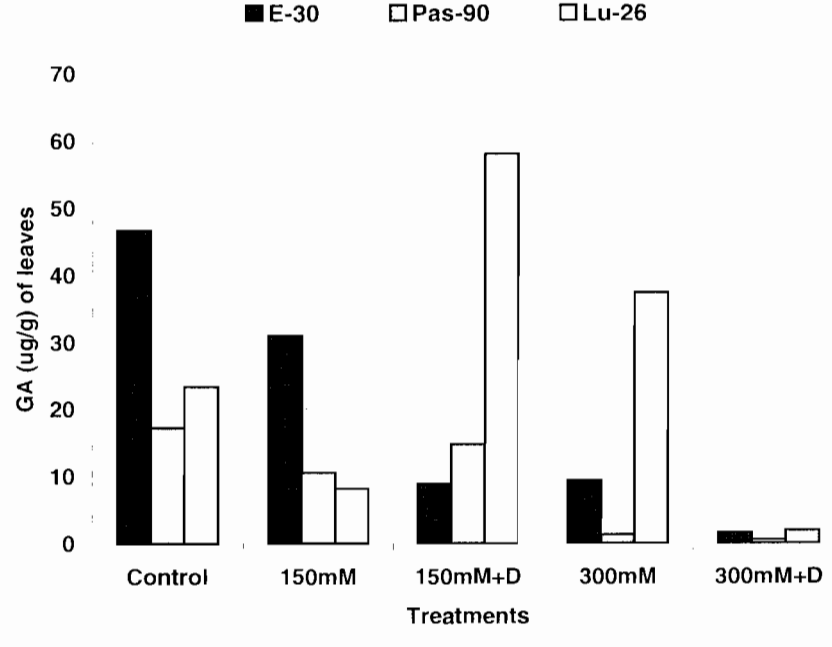

Fig. 2. The GA (µg/g) content of leaves under NaCl stress.

Table: 5a. ANOVA of number of seeds in 6 wheat varieties under different concentrations of NaCl and and drought stress.

Source	D.F.	S.S.	M.S.	F	p
Replication	2	104.067	52.033	1.4138	n.s.
Variety (V)	5	11030.9	2206.18	59.9449	p<0.001
Salinity (S)	4	36668.78	9167.194	249.0852	p<0.001
V x S	20	2280.156	114.008	3.0977	p<0.001
Error	58	2134.6	36.803		
Total	**89**	**52218.5**			

Table 5b. DMRT of mean number of seeds in 6 wheat varieties under different concentrations of NaCl and drought stress.

Salinity (mM)	0	150	150 + D	300	300 + D
Variety					
LU-26	183.7B	162.7DE	145.7FG	134HIJ	127LMNO
Pasban90	163.7FGH	125.3JKLM	117LMNO	111.3NOP	105.7OP
E-38	162DE	154EF	133.3HIJ	127.7JKL	108OP
E-37	164.7CDE	141.7GHI	121KLMN	116.3LMNO	104P
E-30	193.7A	174BC	146.3FG	139.3IJK	133.3JKLM
E-2	165.7CD	154EF	125JKLM	128.7JK	114.3MNOP

Table: 6a. ANOVA of 100 seed weight in 6 wheat varieties under different concentrations of NaCl and drought stress.

Source	D.F.	S.S.	M.S.	F	p
Replication	2	0.031	0.016	1.3296	n.s.
Variety (V)	5	28.043	5.609	476.7074	p<0.001
Salinity (S)	4	24.194	6.048	514.1011	p<0.001
V x S	20	1.568	0.078	6.6639	p<0.001
Error	58	0.682	0.012		
Total	**89**	**54.518**			

Table 6b. DMRT of mean 100 seed weight in 6 wheat varieties under different concentrations of NaCl and drought stress.

Salinity (mM)	0	150	150 + D	300	300 + D
Variety					
LU-26	5.56B	5.38BC	5.23CD	4.63FGH	4.107J
Pasban90	4.51GH	4.143J	4.097J	3.323KL	2.637M
E-38	4.453HI	4.247J	4.1J	3.47K	3.147L
E-37	5.397BC	5.233CD	4.97E	4.583FDH	4.193J
E-30	6.14A	5.523B	5.117DE	4.67FG	4.647FGH
E-2	5.39BC	5.13DE	4.727F	4.283IJ	4.18J

All such mean values under a category which share a common letter are not different, otherwise they differ at p<0.05.

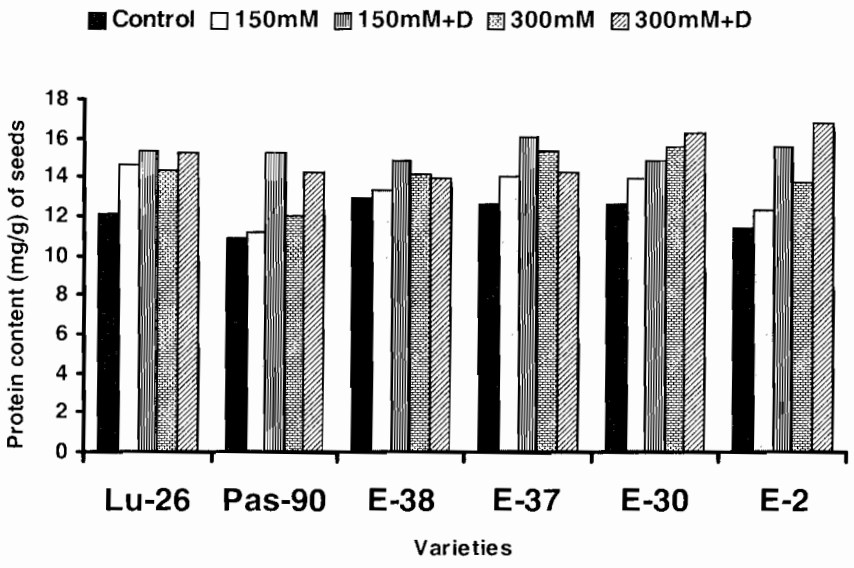

Fig. 3. Protein content (mg/g) of seeds under NaCl stress.

Discussion

Salt stress markedly affected the growth and yield potential of wheat cultivars, particularly in the sensitive lines as investigated through the related physiological parameters. However, some lines of *Triticum aestivum* (L.) posses varying tolerance to salinity in a much better way than the others.

***Plant height and shoot dry weight*:** The reduction in plant height can be attributed to the adverse effects of salinity on the cell elongation and cell expansion. Zidan *et al.*, (1990) showed that 100mM NaCl in the growth medium caused reduction in the length of epidermal cell and in the rate of apparent cell production. Salt stress markedly affected the growth and yield potential of wheat cultivars, E-30 was least affected by the salinity and drought. The combined effect of salt and drought was more severe in Pasban-90 and E-38. Low concentration of NaCl (150mM) has less detrimental effect as compared to high concentration of salt (300mM) used in combination with drought stress. Mansoor (1994) reported salinity induced decrease in the growth of shoot and root of two wheat varieties and salinity induced decrease in the growth of shoot and root of two wheat varieties.

***Chlorophyll content*:** The reduction in chlorophyll content has previously been reported. Asharf *et al.* (1994) observed reduction in chlorophyll (a, b and total) in *Triticum aestivum* under the stress. Jaing *et al.* (1994) reported chlorophyll degradation in rice seedling induced by osmotic stress might be due to the formation of more active OH hydroxyl radical. There was less decrease in the chlorophyll content in E-30 under the treatment of NaCl in combination with drought.

Proline content: The accumulation of proline and some other organic solutes associated with stress may serve as a compatible solute in order to maintain the osmotic balance between the cytoplasm and vacuole (Flowers & Yeo, 1989). Proline increased more in 300 mM NaCl in E-30 than the lower concentration of NaCl (150 mM). The amount of proline was more accumulated under the treatment of NaCl alone. The increased accumulation of proline under drought may be either due accelerated rate of synthesis of proline or due to inhibition of its oxidation resulting in large accumulation of proline in water stressed tissue (Stewart *et al.*, 1977; Rhodes *et al.*, 1986; Mumtaz *et al.*, 1995).

The amount of proline was more in E-30 under stress condition than other varieties and wheat variety Pasban-90 had least increase in stress condition. Possibly, the drought susceptible genotype is incapable of the regulation of production of proline as osmoregulant as has been previously reported. The over production of proline may lead to increase tolerance against water stress and among the selected varieties of cultivated plants the resistance is higher in varieties which accumulate more proline (Martinez *et al.*, 1995). Khan *et al.* (1993) and Santos-Diaz & Alejo (1994) also reported that proline was many fold greater in resistance than susceptible cultivars. (Yang *et al.*, 1995). Sanada *et al.* (1995) noted that proline has bifunctional role in salt tolerance. It acts as osmoregulant in light while in dark it serve as substrate to supply energy to compartmentalize ion into vacuole.

Abscisic acid: Perhaps feedback mechanism operates for ABA accumulation under combined treatment of salt + drought. There is increase in ABA production with increase in salt concentration (150mM to 300mM). There was less amount of ABA in Pasban-90 and E-38 in all salt treatments alone and in combination with drought. Bano *et al.* (1993) concluded that the concentration of ABA, PA and their conjugate forms increase during water stress. Roeb *et al.* (1992) and Sultana *et al.* (2000) reported an increase in ABA production under NaCl salinization. ABA may induce resistance when plants are exposed to several stresses (Talanova & Titov 1994; Newman & Smith, 1997; Hansen & Doerffling, 2003).

Protein content of seed: From present investigation it was obvious that seed protein increased in stress condition. The variety Lu-26 and E-30 had increased maximum seed protein under the treatment of NaCl alone.

Yield parameters: The salt stress had adverse effect on the weight and number of seeds, the reduction being more severe at 300 mM NaCl applied in combination with drought than the 300 mM NaCl alone. The adverse effect of drought on the yield was previously reported by the Blum & Johnson (1992) that water stress was inversely related to the plant size and there is reduction of shoot biomass. Akber *et al.* (1972) demonstrated that salinity severely reduced panicle length, panicle and seed weight, thereby reducing the grain yield. Aldesuquv *et al.* (1998) found that NaCl significantly reduced all growth parameters.

In conclusion there is circumstancial evidence that ABA is involved in salt tolerance of plants, particularly through survival protection at high salinity level also reported by Cramer (2002). ABA is also involved in the synthesis of solutes for osmotic adjustment (Netting 2000). It is inferred from the present investigation that out of preliminary yield trail E-30 performed better under the treatment of NaCl and drought stress. This is

reflected in greater proline production and higher accumulation of endogenous ABA. Whereas, both ABA and GA were minimum in sensitive variety. The combined effect of salt and drought was more inhibitory than salt alone. Salt and drought stress were reported to have differential effect (Sunker-Ramanjulu *et al.*, 1999). Changes in the proline content and ABA/GA ratio in leaves of wheat may be taken as biochemical indicators for screening of varieties for salt tolerance. Preliminary yield trail E-38 has very less growth and yield under salt and drought stress. Out of established varieties, Lu-26 performed better with respect to growth and yield parameters, than the Pasban-90.

References

Aldesuquv, S.H., A.T. Mankarios and H. A. Awad. 1998. Effect of two antitranpirants on growth and productivity of salt treated wheat plants. *Egyp. J. Physiol Sci.*, 22: 189-211.

Akber, M., T. Yabuno and S. Nakao. 1972. Breeding for the saline resistant varieties of rice. Variability of salt tolerance among some rice varieties. *Jpn. J. Breed.*, 22: 272-284.

Ali, M., C.R. Jensen, V.O. Mogensen, M.N. Andersen and I.E. Henson. 1999. Root signalling and osmotic adjustment during intermittent soil drying sustain grain yield of field grown wheat. *Field Crop Res.*, 62: 35-52.

Andreassen, A., F. Asch and R. Jensen. 2001. Intensity of the soil drying alters the response of upland rice genotypes to drought events. In: *Proceedings Deutsher Tropentag*. p. 9-11.

AOAC, 1982. Official methods for the determination of nitrogen and protein. In: *Association Of Official Analytical Chemist* (Ed.): Washington DC. p. 788-789.

Arnon, D.J. 1949. Copper enzyme in isolated choroplast polyphenol oxidase *in Beta vulgaris. Plant Physiol.*, 24: 1-15.

Asharf, M.Y., A.R. Azmi, A.H. Khan and S.A. ALA. 1994. Effect of water stress on total phenol peroxidase activity and chorophyll content in wheat (*Triticum aestivum* L.). *Acta Physiol. Plant*, 16: 185-191.

Aspinall, D. 1965. The effect of soil moisture stress on the growth of barley. 11. Grain growth. *Aust. J. Agric. Res.*, 16: 265-275.

Aspinall, D. and L.G. Paleg. 1981. Proline accumulation: Physiological aspects. In: *Physiology and Biochemistry of drought resistance in plants.* (Eds.): Paleg, L.G. and D. Aspinall. Sydney: Academics Press, 243-249.

Bano, A., K. Dorffling, D. Bettin and H. Hahn. 1993. Abscisic acid and cytokinin as possible root to shoot signal in xylem sap of rice plants in dying soil. *Aust. J. Plant Physiol.*, 20: 109-115.

Bahrun, A., C.R. Jensen, F. Asch and V.O. Mogensen. 2002. Drought induced changes in xylem pH, ionic composition and ABA concentration act as early signals in field grown maize (*Zea mays* L.). *J. Exp. Bot.*, 53: 251-263.

Bates, L.S., R.P. Waldern and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil*, 39: 205-207.

Beltrano, J., M.G. Ranco and E.R. Montaldi. 1999. Drought stress syndrome in wheat is provoked by ethylene evolution imbalance and reversed by rewatering, aminoethoxyvinylglycine, or sodium benzoate. *J. Plant Growth. Regu.*, 18(2): 59-64.

Blum, A. and J.W. Johnson. 1992. Transfer of water from root to dry soil and the effect on wheat water relation and growth. *Plant and Soil*, 145: 141-149.

Brooks, A., C.F. Jenner and D. Aspinall. 1982. Effect of water deficit on endosperm starch granules and on grain physiology of wheat and barley. *Austral. J. Plant Physiol.*, 9: 423-436.

Buhl, M.D. and C.R. Stewart. 1983. Effect of NaCl on the proline synthesis and utilization in excised barley leaves. *Plant Physiol.*, 72: 664-667.

Cramer, G.R. 2002. Response of abscisic acid mutants of *Arabidopsis* to salinity. *Funct Plant Biology*, 29: 561-567.

Davies, W.J. and J. Zhang. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annu. Rev. Plant Physiol. and Plant Mol. Biol.*, 42: 55-76.

Egli, D.B. 1998. Seed biology and the yield of grain crops. (CAB International:Oxford.).

Flower, T.J. and A.R. Yeo. 1989. Effects of salinity on plant growth and crop yield. In: *Cherry J. Environmental Stress in Plants*. Biochemical and Physiological mechanisms. Springer. Verlan, p. 101-119.

Hartung, W. and D. Jeschke. 1999.Abscisic acid: A long-distance stress signal in salt –stressed plants.In: *Plant responses to environmental stresses*. (Ed.): H.R. Lerner. Marcel Decker, Inc. New York. Basel., p. 333-349.

Hansen, H. and K. Doerffling. 2003. Root-derived trans-Zeatin riboside and absciscic acid in drought stressed and rewatered sun floers plants: Interaction in the control of leaf diffusive resistance? *Funct. Pl. Biol.*, 30: 365-373.

Hossain, A.B.S, R.G. Sears, T.X. Cox and G.M. Paulsen. 1990. Dessication tolerance and its relationship to assimilate partitioning in winter wheat. *Crop Sci.*, 30: 622-627.

Jaing, Y., W.Y. Yang., Xu. Jiang and C. Qiaoyon. 1994. Active oxygen demand and effect on chlorophyll degradation in rice seedling under osmotic stress. *Acta Botanica Sinica*, 36: 289-295.

Kettner, J. and K. Doerffling. 1995. Biosynthesis and metabolism of abscisic acid in tomato leaves infected with *Botrytis cinerea*. *Planta*, 196: 627-634.

Khan, A.H., M.Y. Asharf and A.R. Azmi. 1993. Osmotic adjustment in wheat: A response to water stress. *Pak. J. Sci. Ind. Res.*, 36(4): 151-155.

Kirk JTO. 1968. Studies on the dependence of chlorophyll synthesis and protein synthesis in *Euglena gracilis* together with a monogram for the determination of chlorophyll concentration. *Planta*, 78: 200-207.

Li, J.C.J. Shi, X.L.Zhao, G.Wang, F.H. Yu, Y.J. Ren and H. Fenxi. 1994. Separation and determination of three kinds of plant hormones by high performance liquid chromatography. *J. Chromato.*, 22(8): 801-804.

Mansoor, M.M.F. 1994. Changes in growth, Osmotic potential and cell permeability of wheat cultivars under salt stress. *Biologia plantarum* (Prague). 36: 429-434.

Martinez, C.A., C. Gurrero and V.D. Mareno. 1995. Fluctuation of carbon exchange rate proline content and osmotic potential in two water stressed patato hybrid. *Revista Brasil. Fisiolog. Veggetal.*, 7(1): 27-33.

Mumtaz, S., S.S.M. Naqvi, A. Shareen and M.A. Khan. 1995. Proline accumulation in wheat seedling subjected to various stresses. *Acta Physiolog. Plantarum.*, 17(1): 17-20.

Netting, A.G. 2000. pH, abscisic acid and the integration of metabolism in plants under stressed and non-stressed conditions. Cellular responses to stress and their implications for plant water relations. *J. Exp. Bot.*, 51: 147-158.

Newman, D. and B.A. Smith. 1997. The influence of leaf water status and ABA on growth and stomata of Phaseolus seedling with hypoxic root. *J. Exp. Bot.*, 42: 1499-1506.

Rahman, M.S. and S. Yoshida. 1985. Effect of water stress on grain filling in rice. *Soil Sci. Plant Nutr.*, 31: 497-511.

Roeb, G.W., J. Wieneke and F. Fuehr. 1992. Effect of high sodium chloride concentration in the nutrient medium on transpiration, abscisic acid, cytokinin and proline content of 2 soyabean (Glycine max) cultivar. *Z. Pflanzenernaehr. Bodenkd.* 145: 103-116.

Rhodes, D., S., Handa and R.A. Bressan. 1986. Metabolic changes associated with adaptation of plant cell to water stress. *Plant Physiology*, 81: 890-903.

Royo, C., M, Abaza, R., Blanco and D.G.F. delMorat. 2000. Triticate grain growth and morphometry as affected by drought stress late sowing and stimulated drought. *Aust. J. Plant Physiol.*, 27: 1051-1059.

Sanada, Y., H. Ueda, K., Kuribayashi, T. Anadoh, F. Hayashi, N. Tamai and K. Wada. 1995. Novel Light-dark changes f proline level in halophyte(*Mesembryanthemum crystallinum* L.) and glycophytes (*Hordeum vulgare* L. and *Triticum aestivum* L.) leaves and roots under salt stress. *Plant cell Physiol.*, 36: 965-970.

Santos-Diaz, M.S. and N.O. Alejo. 1994. Effect of water stress on growth, osmotic potential and solute accumulation in cell cultures from chili pepper (a mesophyte) and creosote bush (a xerophyte). *Plant Sci.* (Limerick), 96: 21-29.

Stewart, G.R., S.F. Boggess, D. Aspinall and L.G. Paleg. 1997. Inhibition of proline estimation by water stress. *Plant Physiol.*, 59: 930-932.

Sultana, N., T. Ikeda and M.A. Kashem. 2000. Amelioration of NaCl stress by gibberellic acid in wheat seedling. *Bulletin of the Faculty of Agriculture*, 52: 71-76.

Sunker-Ramanjulu, W. Kaiser, K.J. Dietz and S. Ramanjulu. 1999. Salt and drought stress differentially affect the accumulation of extracellular proteins in barley. *Biosci.*, 54: 337-347.

Talanova, V.V. and A.F. Titov. 1994. Endogenous abscisic content in cucumber leaves under the influence of unfavorable temperatures and salinity. *J. Exp. Bot.*, 45: 1031-1033.

Voetberg, G.S. and R.E. Sharp. 1991. Growth of the maize primary root at low water potentials. III. Role of increased proline deposition in osmotic adjustment. *Plant Physiol.*, 96: 1125-1130.

Yang, J.C., Z.Q. Wang and Q.S. Zhu. 1995. The relationship between free proline accumulation and drought resistance in rice under different soil moisture status. *Chinese J. Rice Sci.*, 9: 92-96.

Zidan, I., H. Azaizeh and P.M. Newmann. 1990. Does salinity reduce growth in maize root epidermal cell by inhibiting their cell dividing capacity. *Plant Physiol.*, 93: 7-11.