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The Interactive Effect of Salinity and PGR on Certain Bio-Chemical Parameters in Wheat Seedlings

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Abstract: Wheat varieties Raj. 3777 (Salt tolerant) and Lok. 1 (Salt sensitive) were grown in seed germinator for twelve days at 25°C under 12 h light: 12 h dark photoperiod and approx. 52% relative humidity in Petri dishes containing Hoagland's solution without and with 1% NaCl and in combination with 10 or 20 ppm GA₃; 25 or 50 ppm IAA. In all there were 10 treatments for each variety and each treatment was replicated thrice. Seed germination, length of shoot and root, fresh and dry weight of shoot and root, proline, soluble sugar, soluble protein and chlorophyll content were estimated at the end of experimental period using standard methods. Analysis of data (ANOVA) revealed that salinity significantly decreased seed germination; shoot length, fresh and dry weight; root length, fresh and dry weight; increased proline content and lowered soluble sugars in shoot. All these deleterious effects of salinity were counteracted by 10 or 20 ppm GA₃ or 25 ppm IAA, while 50 ppm IAA had negative effect. These two varieties [i.e., Raj. 3777 and Lok 1] responded differently in terms of magnitude of effects of salinity and growth hormones. Further, the lower concentrations of growth hormones were more effective in ameliorating the salt stress. These results suggest that growth hormones are effective tools which could be explored for combating salinity stress in wheat.

Key words: Wheat, salinity, stress, PGR's and biochemical parameters

Introduction

Wheat (*Triticum aestivum* L.) is the staple food of majority of human race in the world and is grown in temperate, sub-tropical and tropical regions of the world. Wheat is second major food crop in India next to rice and India now ranks as the second largest wheat producer in the world. Environmental stress is the most important factor which affects the crop productivity and according to Christiansen (1982) only about 10% of world arable land may be classified into non stress category. In all arid and semi-arid region of the world, soil salinity is a major agricultural problem (Ashraf and Khan, 1993). Agricultural land is affected by salinity and this problem is further extending on account of faulty soil management practices and is enhancing due to water logging in the root zone which is leading to further salinization of soil (Ghassemi *et al.*, 1995; Gill *et al.*, 1993).

Salinity adversely affects almost all the physiological and metabolic processes of plants and the effect is combination of both osmotic and ionic effects (Ashraf *et al.*, 1991; Khan *et al.*, 1995). Plants are generally most sensitive to salinity during germination and early seedling growth. The high levels of the salinity concentration is effected partitioning of leaf area and dry matter in wheat (Iqbal, 2005). The extent of metabolic disturbance in plant by salinization of medium varies with the type of predominant ions, their concentration, physiological stage of growth at which it is exposed to stress and the plant species/variety. An understanding of the physiological and bio-chemical attributes which enable a plant to tolerate saline environments is a pre-request for creating genotypes suitable for cultivation in saline soil. Growths of both salt-tolerance and sensitive genotypes were affected by

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salinity (El-Hendawy *et al.*, 2005). In wheat, variation in ion selectivity (e.g., K⁺) among genotypes was only considered to be a secondary result of genetic variation in Na⁺ uptake (Munns and James, 2003). Similarly, James *et al.* (2002) found that differences in the rate of photosynthesis likely accounted for genotypic variation in dry matter production. Munns and James (2003) reported that several salt-tolerant wheat genotypes do indeed demonstrate very high leaf Na⁺ levels.

It has been realized that salt tolerance is a whole plant phenomenon perceptible under field conditions (Flower *et al.*, 1985). Nevertheless, selections for salt tolerance have often been carried out on germinating seeds or on very young seedlings. Some studies showed that the variety of wheat and barley sensitive to salinity at an early growth stage were found much more tolerant when exposed to salinity at a later stage (Ayers *et al.*, 1995).

The effect of GA₃ and IAA in context of the amelioration of susceptibility to salinity in wheat is not a simple reaction but it is a complex interaction between the plant, GA₃, IAA and salinity levels (El-Hendawy *et al.* 2004; Shazia Naseer, 2001) and its exploitation is affected by numerous variables relating to plant itself (genotype, growth stage and maturity period etc.). GA₃ mitigates the effects of salinity on germination and had similar effects on coleoptiles growth, shoot growth and root growth (Madan and Kumar, 1983). The present study has been undertaken to investigate the interaction of salinity and PGR's on phenological and biochemical parameters in wheat seedling.

Materials and Methods

The present study was undertaken in two genotypes (Raj-3777 and Lok-1) of wheat (*Triticum aestivum* L.) at Department of Biochemistry, Maharana Paratap University of Agriculture and Technology, Udaipur, 313 001 India during 2002. The genotypes were germinated in 10 environments created by the combinations of two levels each of salinity, GA₃ and IAA as described below:

S. No.	Treatment combination	S. No.	Treatment combination
1.	Salinity (0.0%) / Control	2.	Salinity (1.0%) NaCl
3.	Salinity (0.0%) + GA ₃ 10 ppm	4.	Salinity (1.0%) + GA ₃ 10 ppm
5.	Salinity (0.0%) + GA ₃ 20 ppm	6.	Salinity (1.0%) + GA ₃ 20 ppm
7.	Salinity (0.0%) + IAA 25 ppm	8.	Salinity (1.0%) + IAA 25 ppm
9.	Salinity (0.0%) + IAA 50 ppm	10.	Salinity (1.0%) + IAA 50 ppm

Petridishes layered with blotting paper were autoclaved (120°C, 20 psi) for 20 min. seeds were surface sterilized with 0.1% HgCl₂ solution for one minute and soaked in the treatment medium for 4 h after which 10 seeds were placed in each sterile Petri dish and irrigated with 10 mL of treatment solution. The Hoagland's (Epstein, 1972) solution was used as the nutrient medium with and without GA₃, IAA and salinity at levels as required.

The experiment was replicated three times. The Petri dishes containing seeds were placed in germinator maintained at 25°C with 12 h. light, 12 h dark photoperiod and relative humidity of 50±2%. The experiment was performed for 12 days.

The seedlings were harvested (12th day) and the following observations were recorded by standard methods. (a) Germination percentage (b) Fresh and dry weight of root (g) (c) Fresh and dry weight of shoot (g) (d) Seedling shoot and root length (cm) (e) Proline content µg g⁻¹ (Bates *et al.*, 1973) (f) Total soluble sugar (Dubois *et al.*, 1956) (g) Soluble protein (Lowry *et al.*, 1951) (h) Chlorophyll content (Moran and Porath, 1980).

Results

Germination

Analysis of variance of data for germination percent, dry and fresh weight and length of shoot and root of both varieties as affected by salinity GA₃ and IAA are present in Table 1. The germination

Table 1: Data analysis of variance for germination (%), fresh and dry wt. of shoot and root, shoot and root length

		MS						
Source of variance	df	Germination (%)	Fresh wt. of shoot	Dry wt. of shoot	Fresh wt. of root	Dry wt. of root	Shoot length	Root length
Treatments	19	278.158**	5421.167**	38.490**	1384.845**	13.993**	43.776**	67.084**
Variety (V)	1	375.000**	6211.838**	74.593**	879.368**	7.004**	74.393**	179.920**
Salinity (S)	1	2535.000**	84037.838**	473.204**	18148.204**	172.382**	477.088**	378.508**
PGR (P)	4	206.667**	1118.589**	7.226**	1209.377**	14.168**	35.766**	77.355**
VXS	1	201.667*	1176.608**	8.740**	56.260**	0.104*	7.913**	2.360**
VXP	4	50.000 ^{NS}	221.355**	1.978**	54.372**	0.830**	4.535**	12.724**
SXP	4	243.333**	1164.180**	29.353**	308.835**	4.534**	24.901**	51.207**
VXSXP	4	43.333 ^{NS}	389.849**	5.136**	234.473**	2.065**	2.886**	37.166**
Error	40	33.333	10.565	0.198	2.586	0.016	0.130	50.059
Total	59							

* Significant, ** Highly significant, NS Non-significant, CV% for germination = 6.264 CV% for dry weight of root = 2.578, CV% for fresh weight of shoot = 2.997 CV% for shoot length = 3.127, CV% for dry weight of shoot = 3.815 CV% for root length = 3.933, CV% for fresh weight of root = 3.417

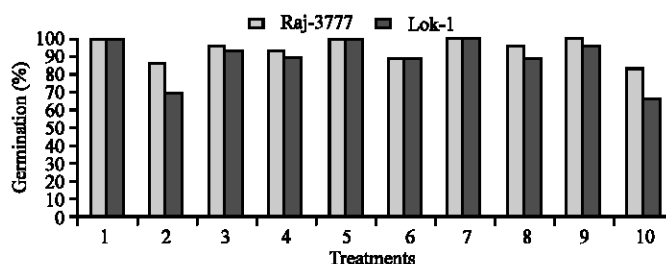


Fig. 1: Germination percent in Raj-3777 and Lok-1 varieties under various treatments

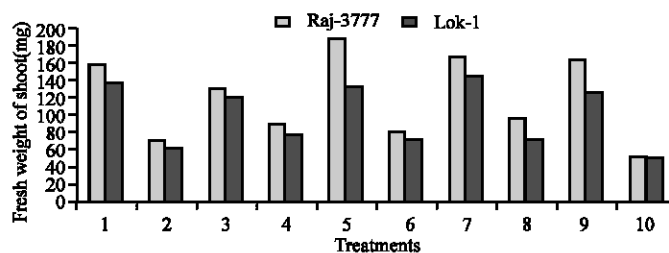


Fig. 2: Fresh weight of shoot (mg) in Raj-3777 and Lock-1 varieties under various treatments

percentage was reduced under saline treatment in both genotypes (Raj-3777 and Lok-I) as compared to control or non-saline condition (Fig. 1). The adverse effect of salinity (1%) was ameliorated by different concentration of GA₃ and IAA.

The highest percentage reduction in germination was seen in LOK-1 (30%) followed by RJ-3777 (13.33%) under salinity. However, the deleterious effect of salinity was maximally mitigated by 10 ppm GA₃, 20 ppm GA₃ and 25 ppm IAA in sensitive variety LoK-1. Fifty parts per million IAA was found to exhibit inhibitory effect thereby indicating that higher concentration of PGR may be inhibitory for plant growth and development.

Fresh Weight of Shoot

The fresh weight was reduced with saline treatment in both varieties as compared to control. The highest reduction in fresh weight was observed in Raj-3777 (56.44%) followed by LoK-1 (55.18%) (Fig. 2). The adverse effect of salinity (1% NaCl) was ameliorated by 10 ppm GA₃ (25.88%) followed

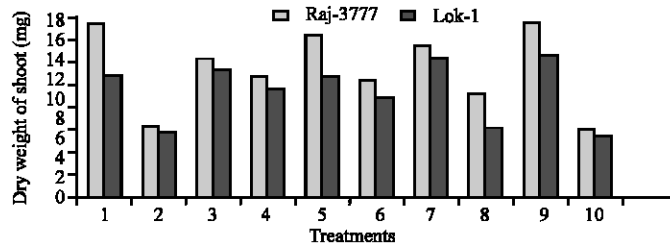


Fig. 3: Dry weight of shoot (mg) in Raj-3777 and Lok-1 varieties under various treatments

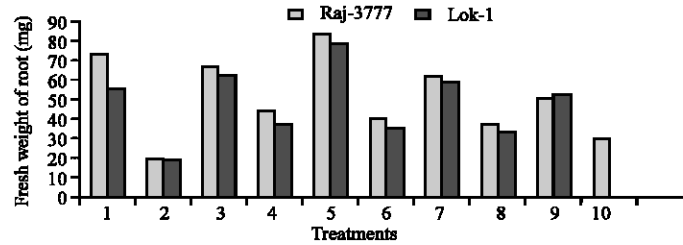


Fig. 4: Fresh weight of root (mg) in Raj-3777 and Lok-1 varieties under various treatments

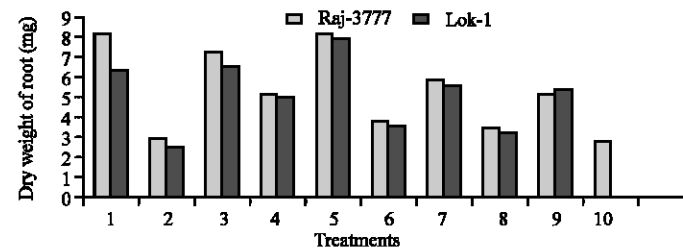


Fig. 5: Dry weight of root (mg) in Raj-3777 and Lok-1 varieties under various treatments

by 25 ppm IAA and 20 ppm GA₃ in Lok-1 variety. Likewise, the adverse effect of salinity was maximally mitigated by 25 ppm IAA followed by 10 ppm GA₃ and 20 ppm GA₃ in Raj-3777.

Dry Weight of Shoot

The dry weight of shoot was decreased under salt stress over non-saline plants. The highest decrease in fresh weight of shoot was noticed in Lok-1 (85.00%) followed by Raj-3777 (58.31%). The maximum counteraction of adverse effect of salinity was seen under 10 ppm GA₃ (68.00%) followed by 20 ppm GA₃ (51.46%) and 25 ppm IAA (8.19%) in Lok-1. 10 ppm GA₃ (67.56%) gave maximum amelioration of deleterious effects of salinity followed by 20 ppm GA₃ (63.17%) and 25 ppm IAA (45.04%) in Raj-3777 (Fig. 3).

Fresh Weight of Root

Fresh weight of root was decreased under saline environment. The highest decrease in fresh weight of root as compared to controls was observed in Raj-3777 (73.75%) followed by Lok-1 (66.12%) (Fig. 4). The deleterious effects of salinity on root fresh weight was maximum ameliorated by 10 ppm GA₃ (124.89 and 96.76) followed by 20 ppm GA₃ (111.12 and 85.10%) and 25 ppm IAA (93.16 and 77.37%) in both genotypes Raj-3777 and Lok-1, respectively.

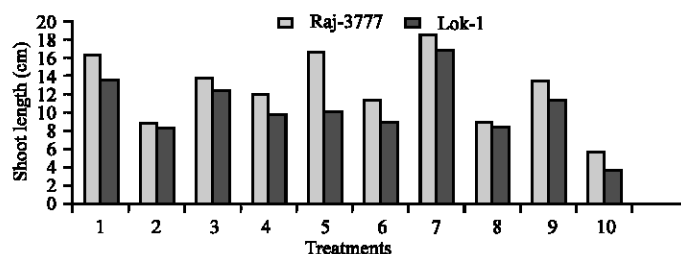


Fig. 6: Shoot length (cm) in Raj-3777 and Lok-1 varieties under various treatments

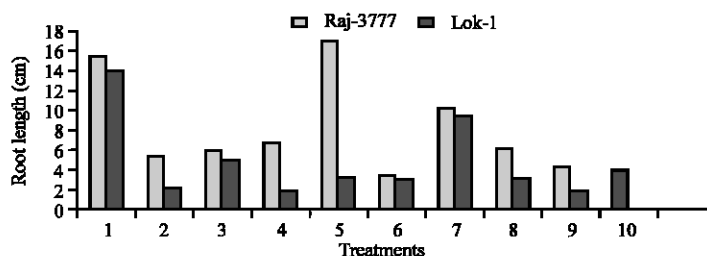


Fig. 7: Root length (cm) in Raj-3777 and Lok-1 varieties under various treatments

Dry Weight of Root

The dry weight of root was decreased under saline conditions. The maximum reduction in dry weight of root was noticed in Lok-1 (60.03%) followed by Raj-3777 (58.67%) as compared to control. The adverse effect of salinity on root dry weight was maximally counteracted by 10 ppm GA₃ (98.81 and 75.08%) followed by 20 ppm GA₃ (43.47 and 30.71%) and 25 ppm IAA (26.48 and 19.45%) in LoK-1 and Raj-3777, respectively (Fig. 5). However, the 50 ppm IAA was observed as inhibitory and in Lok-1 genotype there was no roots formation.

Shoot Length

Salinity decreased the shoot length in both the varieties (Fig. 6). The highest percent reduction of seedling under saline environment was observed in Raj-3777 (46.06%) followed by LoK-1 (36.70%). 10 ppm GA₃ (35.95 and 16.47%) exhibited maximum amelioration of the deleterious effects of salinity followed by 20 ppm GA₃ (28.08 and 7.05%) in Raj-3777 and LoK-1, respectively.

Root Length

Both the varieties showed decrease in root length under salinity (1% NaCl). Figure 7 shows that the overall root length in LoK-1 was observed less than Raj-3777 under these treatments. The highest percent reduction in root length was observed in LoK-1 (84.28%) in comparison to Raj-3777 (64.51%) than control. The adverse effect of salinity was best counteracted by 20 ppm GA₃ (36.36%) followed by 10 ppm GA₃ (21.81%) and 25 ppm IAA (12.72%) in Raj-3777, while in LoK-1 25 ppm IAA (44.09%) showed the best effect followed by 20 ppm GA₃ (40.90%). 50 ppm IAA was inhibitory and root formation did not occur in LoK-1 variety.

Biochemical Parameters

Proline

Analysis of variance of data for proline, total soluble sugars, protein and chlorophyll content of both varieties are affected by salinity GA₃ and IAA is given in Table 2. The proline content was significantly increased under salinity as compared to control. Figure 8 shows that the proline content

Table 2: Data Analysis of Variance for proline content, soluble sugar, water soluble protein and chlorophyll content

Source of variance	df	MS			
		Proline content	Soluble sugar	Water soluble protein	Chlorophyll content
Treatments	19	274939.007**	0.856**	7.474**	0.524**
Variety (V)	1	468184.334**	0.794**	2.087**	0.559**
Salinity (S)	1	2575703.204**	12.060**	88.549**	1.719**
PGR (P)	4	110941.188**	0.458**	6.356**	0.974**
VXS	1	517713.126**	0.037 ^{NS}	7.273**	0.000 ^{NS}
VXP	4	69815.350**	0.039**	1.430**	0.365**
SXP	4	165942.001**	0.326**	2.781**	0.513**
VXSXP	4	68861.577**	0.022 ^{NS}	0.458**	0.066**
Error	40	17.196	0.011	0.026	0.001
Total	59				

* Significant, ** Highly significant, NS Non-significant, CV% for proline content = 1.242 CV% for soluble sugar = 1.338, CV% for water soluble protein = 3.437 CV% for chlorophyll content = 2.157

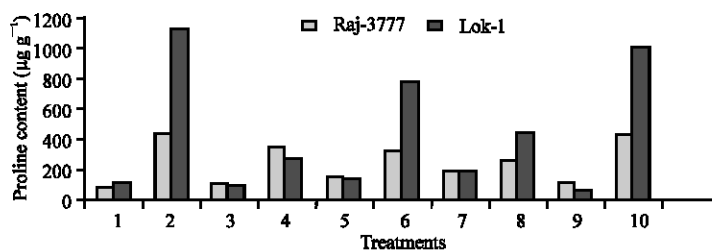


Fig. 8: Proline content ($\mu\text{g g}^{-1}$) in Raj-3777 and Lok-1 varieties under various treatments

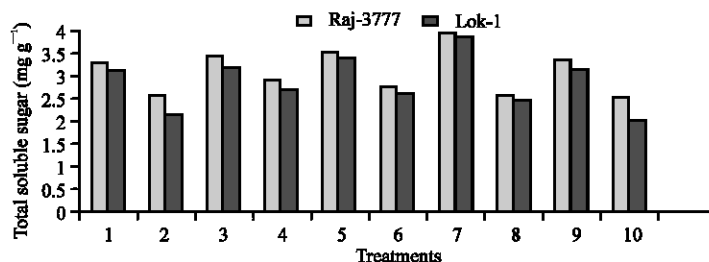


Fig. 9: Total soluble sugar (mg g^{-1}) in Raj-3777 and Lok-1 varieties under various treatments

was higher in LoK-1 than Raj-3777 with all treatments. Salinity (1%) increased the proline levels nearly 4 times, while, 10, 20 ppm GA_3 and 25, 50 ppm IAA decreased the proline content levels.

The highest percent increase in proline content was noted in Lok-1 (8.47 times) followed by Raj-3777 (3.67 times) under 1% salinity. The deleterious effect of salinity were best counteracted by 10 ppm GA_3 (75.00%) followed by 25 ppm IAA (61.12%), 20 ppm GA_3 (31.68%) and 50 ppm IAA in LoK-1. In Raj-3777 genotype, 25 ppm IAA (41.42%) showed best counteraction of the adverse effect of salinity followed by 20 ppm GA_3 (24.29%) and 10 ppm GA_3 (21.43%).

Total Soluble Sugars

The total soluble sugar was reduced under saline (1%) environment in both varieties as compared to non-saline environment. The deleterious effect of salinity (1%) was ameliorated by different concentration of GA_3 and IAA (Fig. 9). The highest percent reduction was observed in LoK-1 (31.94) followed by Raj-3777 (21.21%). The adverse effect of salinity was maximum counteracted by 10 ppm GA_3 (26.76%) followed by 20 ppm GA_3 (23.47%) and 25 ppm IAA (15.96%) in LoK-1 and similar trend was observed in Raj-3777 but 50 ppm IAA noticed inhibitory for both genotypes.

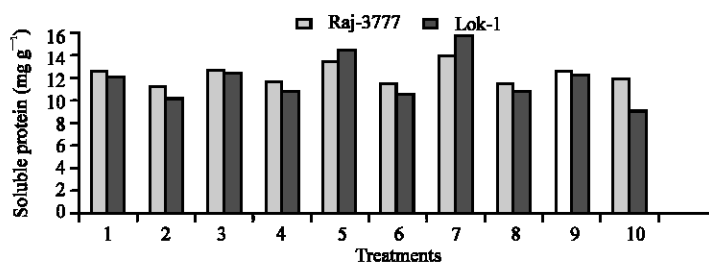


Fig. 10: Soluble protein (mg g^{-1}) in Raj-3777 and Lok-1 varieties under various treatments

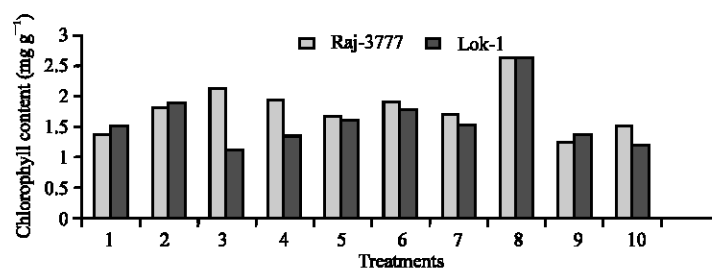


Fig. 11: Chlorophyll content (mg g^{-1}) in Raj-3777 and Lok-1 varieties under various treatments

Soluble Proteins

Figure 10 shows that the soluble protein content was decreased under saline (1%) NaCl condition in both genotypes in comparison to control. The adverse effect of salinity (1%) was ameliorated by different concentrations of GA_3 and IAA. The highest percent decrease was noticed in Lok-1 (15.56) then Raj-3777 (11.11). The effect of salinity in Lok-1 genotype was maximum overcome by 25 ppm IAA (7.09%) followed by 10 ppm GA_3 (5.61%) and 20 ppm GA_3 . Fifty parts per million IAA inhibitory for LoK-1 and these treatment highest evaluated the salinity effect in Raj-3777.

Chlorophyll Content:

Figure 11 shows the chlorophyll content in LoK-1 was significantly lower than Raj-3777 and that salinity caused a slight increased in the chlorophyll content. Amongst PGR increase in chlorophyll content was observed under the influence of 20 ppm GA_3 and 25 ppm IAA, while 50 ppm IAA decreased chlorophyll content of the shoot. In case of Raj-3777, 10 ppm GA_3 , 20 ppm GA_3 and 25 ppm IAA increased chlorophyll content. While in case of LoK-1 only 25 ppm IAA increased chlorophyll content.

Discussion

The results of the present study revealed that germination percent, dry and fresh weight and length of shoot and root, total soluble sugars, protein content and chlorophyll content of both wheat varieties decreased with increasing salinity levels of the growth medium, while proline content increased. Seed treatment with both the growth regulator proved effective in ameliorating the adverse effect of salinity on dry weights. These findings corroborates with those of Stark and Roth (1991), Maliwal (1997) and Flowers and Yeo (1989) who also found that increasing salinity in the growth medium reduced the plant growth in terms of all the above mentioned parameters. However, growth regulators treatment mitigated the adverse effect of salinity on all these parameters. The decrease in root and leaf weights due to salinity could be due to unbalance nutrition (Hagazi *et al.*, 1995; Yamanouchi *et al.*, 1995), damage of membranes and disturbed avoidance mechanism (Storey and Wyn

Jone, 1978; Ashraf and Sarwar, 2002; Ashraf *et al.*, 1992). A review by Ashraf (2004) summarized that glycophytes can use both ion exclusion and inclusion mechanism in response to saline substrates. The mechanism that is used depends on the pattern of ion distribution between the leaves and on ion compartmentation within the cell (Munns *et al.*, 2002)

Ashraf *et al.* (1991) reported that the decreased in dry matter under salinity was more in case of sensitive varieties. Aldesuquy (1998) and Shazia Naseer (2001), reported decrease in all growth parameters and that GA₃ and IAA ameliorated the effects of salinity in wheat. Roy and Srivastava (1999) reported decrease in root and shoot length. Sultana *et al.* (2000) reported seedling dry matter, shoot and root length decreased under NaCl and that GA₃ ameliorate this effect of salinity. The changes in phenological characters observed in the present study corroborates with reports available in above literature.

In the present study it was observed that salinity (1% NaCl) had adverse effects on all the parameters analyzed in both the genotypes. The effects were higher in Lok-1 variety than Raj-3777 there by suggesting that Lok-1 with variety was sensitive. El-Hendawy *et al.* (2004) reported that the different concentration of PGR's (10, 20 ppm GA₃ and 25, 50 ppm IAA) ameliorate the adverse effect of salinity and try to maintain the reducing environment due to salinity. We also observed in our study that 10, 20 ppm GA₃ and 25 ppm IAA, counteract the deleterious effect of salinity in both genotypes, however, we did also observe deleterious effects of 50 ppm IAA in both varieties. Naseer (2001) has been reported that soaking of wheat seeds in IAA is ameliorated the effect of salinity on dry matter and ion accumulation. Exogenously applied GA₃, has been reported to increase seed germination in wheat (Pfahler *et al.*, 1991).

The salt stress increased the proline content by nearly 4 times and that GA₃ and IAA lowered the same. The lowest proline was observed under 10 ppm GA₃ followed by 25 ppm IAA, 20 ppm GA₃ and 50 ppm IAA. Interaction study shows that these two varieties differ extremely in their response to salinity. The basal levels of proline were higher in Raj-3777 (Salt tolerant) variety while, it was 5-9 times in case of LoK-1 (Salt Sensitive). These results clearly demonstrate that genotypes differ in their response to stress. Gupta and Srivastava (1989) and Ashraf *et al.* (1991) also reported that differences in response exist between wheat genotypes and that proline accumulation is a suitable parameter for assessing stress levels. The total soluble sugars also higher in Raj-3777 under all these conditions. Salinity decreased the levels while PGR increased the soluble sugar content. Soluble protein was lower in Lok-1 as compared to Raj-3777 and salinity decreased soluble protein. This adverse effect of salinity was counteracted by PGR treatments. PGR treatments also caused increase in chlorophyll content in the present study. The response of chlorophyll content to salt stress depended on differences in salt tolerance among the wheat genotypes. Similar findings have also been reported in alfalfa (Winacov and Seemann, 1990) and cowpea (Murillo-Amador *et al.*, 2002), therefore, response of chlorophyll content in wheat to salinity depended on both salinity levels and the degree of salt tolerance of genotypes. Cowpea, for example, Murillo-Amador *et al.* (2002) found that the chlorophyll content of the salt tolerant genotypes was increased under salinity, whereas in salt-sensitive genotypes it was different.

Proline accumulation has been associated with abiotic stress (salinity stress) (Delauney and Verma, 1993). Lu and Yi (1992) reported increased levels of free proline in wheat exposed to salinity stress and that increased in salinity decreased proline oxidase activity. Proline accumulation is correlated with tolerance of water stress (De Ronde *et al.*, 2000; Sharma and Sharma, 2002).

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