EFFECT OF GIBBERELLIC ACID AND KINETIN ON NITROGEN CONTENT AND NITRATE REDUCTASE ACTIVITY IN WHEAT UNDER SALINE CONDITIONS

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Received on 24 Feb., 2000, Revised on 10 Oct., 2000

SUMMARY

Increasing levels (0 to 12 dSm^{-1}) of chloride and sulphate salinity decreased the leaf area, straw and grain yield of wheat (*Triticum aestivum* L.); the magnitude of decrease being less in tolerant cultivar WH-533 as compared to the sensitive C-306. Pre-sowing seed soaking treatments with gibberellic acid (0.4 mM), kinetin (0.4 mM) and gibberellic acid (0.4 mM) + kinetin (0.4 mM) alleviated the deleterious effects of chloride as well as sulphate salinity in both the cultivars. The combined treatment of gibberellic acid (0.4 mM) + kinetin (0.4 mM) was most effective. It was further observed that the plant growth regulator induced salinity stress alleviation was due to a concomitant increase in the tissue N content and nitrate reductase activity.

Key words : **GA, kinetin, nitrate reductase, nitrogen content, wheat.**

INTRODUCTION

Soil salinity is an enormous problem adversely affecting growth and development of crop plants and results into low agricultural production (Agarwal *et al.* 1979, Maas 1990, Garg and Gupta 1997). Salinity also affects nitrogen metabolism through reduced nitrogen uptake and its accumulation, nitrate reductase activity and degradation of several nitrogen macromolecules (Feigin 1985, Garg *et al.* 1990, Grattan and Grieve 1994). Nitrate reductase (NR) enzyme is highly sensitive to all types of stresses including salinity stress in different crop plants (Garg *et al.* 1993, Lahiri *et al.* 1996). Concerted attempts have been made to mitigate the harmful effects of salinity by exogenous application of plant growth regulators (Khan and Ungar 1985, Taneja *et al.* 1992, Malibari 1993, Datta *et al.* 1998). However, pertinent information with regard to regulation of nitrogen accumulation and associated activity of enzymes like nitrate reductase during such plant growth regulator-mediated salinity stress alleviation is not available. Therefore, the present investigation was carried out to examine the effect of gibberellic acid and cytokinins alone as well as in combination, on the regulation of growth, yield attributes, nitrogen accumulation and nitrate reductase activity in two cultivars of wheat under chloride and sulphate salinity.

MATERIALS AND METHODS

Experimental studies were conducted in earthen pots (30 cm dia.) using two cultivars of wheat (WH-533, salinity tolerant and C-306, salinity sensitive), two types [Chloride- (Cl) and Sulphate (SO_4-) dominated] and four levels (S_0=control, S_1=6 dSm^{-1}, S_2=9 dSm^{-1} and S_3=12 dSm^{-1} ECe) of salinity. Each pot was filled with 6 kg of dry and sieved yellow dune sand and salinized with 2 l of desired saline solution. Saline solutions were prepared by adding mixture of salts (NaCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$ for Cl-salinity and Na_2SO_4, $MgCl_2$, $MgSO_4$ and $CaCl_2$ for SO_4-salinity) in Hoagland's solution (Hoagland and Arnon, 1950). The ratio of different cations and anions were maintained on milliequivalent basis like Na^+: (Ca^{2+} + Mg^{2+}) = 1:1, where Ca^{2+}: Mg^{2+}=1:3 and Cl^-:SO_4^{-2}=7:3 for Cl-salinity and 3:7 for SO_4-salinity.

Pre-sowing seed soaking treatments of distilled water (control), gibberellin A_3 0.4 mM (GA), kinetin 0.4 mM (CK) and gibberellin A_3 0.4 mM + kinetin 0.4 mM (GA+CK) were given for 6 h. Four healthy seeds were sown in each pot at uniform depth and distance after appropriate moisture level of sand was attained. Thinning was done 10 d after emergence and only two plants of comparable growth were maintained in each pot. Eight plants per treatment were maintained in a completely randomised design. Leaf area per plant taken as a measure of growth, was measured by portable leaf area meter (Model LI-3000, LI-COR, USA). Plant samples for estimating the nitrogen content (Linder, 1944) and nitrate reductase activity (Jaworski, 1971) were taken 65 days after sowing (DAS) from 3rd leaf from top. The data on straw and grain yield were recorded at 135 DAS, at the time of harvesting.

RESULTS AND DISCUSSION

Leaf area was not affected significantly at the lower level of salinity (S_1) but thereafter a significant decrease was observed with increasing levels of (S_2 and S_3) salinity as compared to the control (S_0) (Table 1). SO_4-salinity was observed to be less depressive than Cl-salinity in both the cultivars. Within the cultivars C-306 was more sensitive as it showed significantly lower leaf area at S_2 and S_3 levels of salinity compared to WH-533. Seed soaking with plant growth regulators produced significantly more leaf area per plant under both types of salinity and in both the cultivars. However, the efficiency of the combination treatment GA+CK was better than rest of the treatments in improving the leaf area under non-saline (S_0) as well as saline conditions (S_1, S_2 and S_3).

Results presented in Table 2 shows that N content of the leaves decreased gradually with increasing levels of salinity from S_1 to S_3 compared with the controls in both the cultivars and under both types of salinity. It may be noted that N content did not vary significantly in the two salinity types. However, higher N content was observed in tolerant WH-533 than in sensitive C-306 under the S_0 control as well as S_1 to S_3 salinity treatments. Among seed soaking treatments GA+CK was most effective in improving the N content in leaves under both the salinity levels and it was more in the cv. WH-533 as compared to

Table 1. Effect of different seed soaking treatments on the leaf area (cm^2 $plant^{-1}$) in two cultivars of wheat under different levels of Cl- and SO_4-salinity

Cultivars	Salinity levels	Seed soaking treatments and salinity type									
		Control		GA		CK		GA+CK		Mean	
		Cl	SO_4	Cl	SO_4	Cl	SO_4	Cl	SO_4	Cl	SO_4
WH-533	S_0	136.8	136.8	140.4	140.4	147.2	147.2	149.4	149.4	143.5	143.5
	S_1	118.2	147.0	125.4	153.4	133.4	160.8	136.4	170.6	128.4	158.0
	S_2	70.8	84.4	81.3	96.4	85.8	104.8	92.0	107.8	82.5	98.4
	S_3	42.4	51.8	50.4	56.7	53.0	63.4	56.9	68.5	50.7	60.1
	Mean	92.1	105.1	99.4	111.1	104.3	119.1	103.7	124.1	101.2	114.9
C-306	S_0	153.8	153.8	161.1	161.3	170.2	170.2	175.8	175.8	165.3	165.3
	S_1	139.1	150.2	152.9	167.3	161.3	168.3	159.2	171.6	153.1	164.4
	S_2	72.5	86.3	79.5	95.3	84.7	101.4	89.8	107.3	181.7	97.6
	S_3	31.3	39.8	41.3	43.8	50.7	56.9	53.9	61.9	44.3	50.6
	Mean	99.2	107.5	108.8	116.9	116.7	124.2	119.7	129.2	111.1	119.5

CD at 5% LS : Cultivars = 4.4; Salinity treatments = 4.4; Salinity levels = 6.2; Seed soaking treatments = 6.2

Table 2. Effect of different seed soaking treatments on the nitrogen content (µmol g^{-1} dry weight) in two cultivars of wheat under different levels of Cl- and SO_4- salinity

Cultivars	Salinity levels	Control Cl	Control SO_4	GA Cl	GA SO_4	CK Cl	CK SO_4	GA+CK Cl	GA+CK SO_4	Mean Cl	Mean SO_4
WH-533	S_0	1149	1149	1096	1096	1125	1125	1152	1152	1131	1131
	S_1	1071	1109	1031	1081	1045	1079	1117	1127	1091	1099
	S_2	894	911	927	947	913	925	935	963	917	937
	S_3	638	660	685	697	669	710	706	721	675	697
	Mean	935	957	935	955	938	960	978	991	953	966
C-306	S_0	1118	1118	1112	1112	1086	1086	1110	1110	1107	1107
	S_1	1047	1069	1065	1046	1035	1094	1094	1083	1060	1073
	S_2	780	829	844	869	857	873	897	854	844	856
	S_3	568	632	605	673	591	660	618	694	596	664
	Mean	878	912	907	925	892	928	930	935	902	925

CD at 5% LS : Cultivars = 20.9; Salinity treatments = N.S.; Salinity levels = 29.7; Seed soaking treatments = N.S.

its respective controls but in case of C-306, it was effective only under Cl-salinity. Other treatments were found to be ineffective in this respect. Helal *et al.* (1975) also observed that in spring barley salinization with NaCl impaired growth and uptake of labelled N. In contrast, Helal and Mengel (1979) observed little effect of NaCl salination on N uptake in winter barley but found that its incorporation into protein fraction was impaired. Aslam *et al.* (1984) also found that Cl- and SO_4-salts severely inhibited uptake of NO_3^- in barley seedlings, the former inhibited more than the latter.

The NR activity was reduced significantly with rising salinity levels (S_1 to S_3) as compared to the non-saline conditions (Table 3). This trend was observed under both types of salinity. Cl-salinity was more depressive than SO_4-salinity in both the cultivars while WH-533 exhibited lesser reduction in NR activity. Aslam *et al.* (1984) reported reduction in nitrate reductase activity in barley seedlings under NaCl salinity. Khan *et al.* (1996) also observed salinity-induced reduction in the activity of nitrate reductase in soybean plants. Seed soaking with growth regulators (GA, CK, GA+CK) caused a slight but significant increase in NR activity in both the cultivars and in both the salinity types. The effect of GA+CK treatment was most pronounced. Enhancement in the activity of NR with GA_3 is attributed to increased enzyme protein synthesis in *Vigna mungo* (Srivastava and Mathur 1981, Srivastava *et al.* 1981). Devi (1998) observed an increase in the activity of NR in *Parkia javanica* plants treated with GA_3 and kinetin and suggested that GA_3 enhances the synthesis of enzyme protein while cytokinin stimulates enzyme activity or retards enzyme degradation. Earlier, Sachar *et al.* (1975) suggested that cytokinin stimulated NR activity by operating at transcriptional level.

Straw and grain yield remained unaltered at the S_2 as compared to S_0 control but decreased at the higher salinity levels (S_3 and S_4) in both the cultivars and salinity types (Table 4 and 5). However, reduction was higher under Cl- than SO_4-salinity. Cultivar C-306 produced more straw yield than WH-533 but reverse trend was observed in case of grain yield under non-saline conditions. Grain yield reduction was more severe as compared to straw yield in both under Cl- and SO_4-salinity in C-306 than WH-533. Seed soaking with GA and CK caused well marked increase in both straw and grain yield, though the combined treatment of GA+CK emerged as most promising in this regard in both the cultivars and salinity types.

Table 3. Effect of different seed soaking treatments on the activity of nitrate reductase (μmol NO_2^- cm^{-2} h^{-1}) in two cultivars of wheat under different levels of Cl- and SO_4- salinity

Cultivars	Salinity levels	Seed soaking treatments and salinity type									
		Control		GA		CK		GA+CK		Mean	
		Cl	SO_4	Cl	SO_4	Cl	SO_4	Cl	SO_4	Cl	SO_4
WH-533	S_0	117.9	117.9	115.5	115.5	118.2	118.2	116.7	116.7	117.1	117.1
	S_1	120.4	118.2	117.1	117.5	121.1	119.7	118.1	119.3	119.2	118.7
	S_2	102.7	105.4	108.9	109.9	107.6	108.9	110.8	114.5	107.5	109.7
	S_3	76.9	80.5	80.9	84.2	81.2	85.1	83.5	88.6	80.6	84.6
	Mean	104.5	105.5	105.6	106.8	107.1	108.0	107.3	109.3	106.1	107.5
C-306	S_0	131.8	131.8	128.0	128.2	128.2	128.2	130.3	130.3	129.6	129.6
	S_1	127.2	124.4	129.8	130.5	132.6	126.8	129.3	128.6	129.8	127.6
	S_2	101.7	104.4	107.3	110.5	108.4	112.9	110.3	113.3	106.9	110.3
	S_3	68.7	76.2	78.4	84.6	76.1	82.8	79.6	85.3	75.7	82.3
	Mean	107.4	109.2	110.9	113.4	111.3	112.6	112.4	114.4	110.5	112.4

CD at 5% LS : Cultivars = 0.908; Salinity treatments = 0.980; Salinity levels = 1.382; Seed soaking treatments = 1.382

Table 4. Effect of different seed soaking treatments on straw yield (g $plant^{-1}$) in two cultivars of wheat under different levels of Cl- and SO_4- salinity

Cultivars	Salinity levels	Seed soaking treatments and salinity type									
		Control		GA		CK		GA+CK		Mean	
		Cl	SO_4	Cl	SO_4	Cl	SO_4	Cl	SO_4	Cl	SO_4
WH-533	S_0	2.340	2.340	2.321	2.321	2.327	2.327	2.368	2.368	2.339	2.339
	S_1	2.304	2.450	2.467	2.516	2.368	2.279	2.404	2.322	2.386	2.392
	S_2	1.839	1.430	1.948	3.043	1.938	1.951	2.113	2.141	1.960	2.016
	S_3	1.108	1.222	1.212	1.397	1.285	1.538	1.318	1.423	1.231	1.395
	Mean	1.898	1.985	1.987	2.069	1.979	2.024	2.051	2.064	1.979	2.036
C-306	S_0	2.621	2.621	2.585	2.585	2.568	2.568	2.566	2.566	2.585	2.585
	S_1	2.563	2.618	2.552	2.573	2.548	2.524	2.620	2.623	2.571	2.585
	S_2	1.822	1.924	1.929	2.044	2.018	1.984	2.162	2.222	1.983	2.043
	S_3	1.022	1.115	1.148	1.227	1.224	1.250	1.252	1.335	1.162	1.232
	Mean	.007	2.070	2.054	2.103	2.089	2.082	2.150	2.186	2.075	2.111

CD at 5% LS : Cultivars = 0.036; Salinity treatments = 0.036; Salinity levels = 0.50; Seed soaking treatments = 0.50

Table 5. Effect of different seed soaking treatments on grain yield (g plant^{-1}) in two cultivars of wheat under different levels of Cl- and SO_4- salinity

Cultivars	Salinity levels	Control Cl	Control SO_4	GA Cl	GA SO_4	CK Cl	CK SO_4	GA+CK Cl	GA+CK SO_4	Mean Cl	Mean SO_4
WH-533	S_0	1.539	1.539	1.548	1.548	1.534	1.534	1.533	1.533	1.539	1.539
	S_1	1.525	1.557	1.495	1.507	1.524	1.536	1.544	1.600	1.532	1.550
	S_2	1.277	1.246	1.315	1.287	1.310	1.345	1.332	1.354	1.308	1.308
	S_3	0.897	0.963	1.000	1.033	1.008	1.058	1.066	1.069	0.993	1.031
	Mean	1.310	1.326	1.340	1.344	1.344	1.368	1.369	1.389	1.340	1.357
C-306	S_0	1.452	1.452	1.440	1.440	1.433	1.433	1.451	1.451	1.444	1.444
	S_1	1.440	1.437	1.509	1.458	1.471	1.417	1.419	1.465	1.460	1.444
	S_2	1.110	1.104	1.195	1.208	1.177	1.247	1.269	1.304	1.188	1.216
	S_3	0.676	0.745	0.813	0.847	0.784	0.856	0.834	0.890	0.777	0.835
	Mean	1.170	1.185	1.240	1.238	1.216	1.239	1.243	1.278	1.317	1.235

CD at 5% LS : Cultivars = 0.015; Salinity treatments = 0.015; Salinity levels = 0.021; Seed soaking treatments = 0.021.

Present findings are similar to several reports (Garg *et al.* 1990, Garg *et al.* 1996, Lahiri *et al.* 1996) where a decline in N level and NR activity was reported with concomitant decline in shoot biomass, pod weight, seed weight and yield of wheat, clusterbean, mungbean and mothbean due to salinity stress. These studies further suggested that amelioration of salinity induced inhibition of growth and development is due to enhanced N status and NR activity mediated through plant growth regulators like gibberellins and cytokinins.

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