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Residual Effect of Boron and Sodium Chloride Applied to Wheat on Growth and Biochemical Parameters of Green Gram (*Vigna radiata*) Grown in Subsequent Season

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ABSTARCT

Background: Experiment was conducted in pots to examine the residual effects of different levels of soil applied boron and sodium chloride (NaCl) to wheat during *rabi* on growth and biochemical parameters of green gram sown in following *kharif*.

Methods: Boron or NaCl stress, which were applied individually or in combinations to wheat, had significant residual effects on biochemical processes and yield attributes of green gram.

Result: At rising levels of NaCl or B, the content of sugar, starch, as well as free amino acids in leaves decreased, while the content of proline was increased. Under boron and NaCl stress, the MDA level increased dramatically due to lipid peroxidation. There were noteworthy residual toxic effects on growth, development, biochemical parameters, yield and yield attributes of green gram sown in the following *kharif* season.

Key words: Biochemical parameter, Boron, Green gram, Residual effect, Salinity.

INTRODUCTION

Pulse being a major source of proteins (three times more than cereals) contains low fat and is rich in fibre; they also contain vitamins and minerals like iron, potassium and folate. It is also rich in sulphur, calories and vitamins especially B-complex. It has important role in human diet as well as in farm economy of our country (Swaminathan *et al*., 2021). They contribute in maintaining the soil fertility as well as mitigating climate change through atmospheric nitrogen fixation. India is the leading producer of pulses in the world accounting for the production of 22.40 million tons in an area of 29.28 million ha (Adhana and Yadav, 2019). India stands first in the production of green gram, contributing about 75% of world's production (Taunk *et al.,* 2012). Green gram is one of the major *kharif* pulse crop covering 31.15 lakh ha (Pavithra *et al.,* 2021). It contains 24-25% protein in seeds. It is generally grown in rainy and summer seasons in central part of India.

Boron (B) is an essential nutrient and its availability in soil and irrigation water is a key determinant of agricultural production (Nable, 1988). Boron toxicity affects a wide range of processes in vascular plants, including distorted metabolism, decreased root cell division, reduced leaf chlorophyll contents and photosynthetic rates (Nable *et al.,* 1997). Availability of boron is greatly influenced by the soil characteristics like pH, electrical conductivity, organic matter, cation exchange capacity, calcium carbonate and texture of soil (Evans and Sparks, 1983). Sources of high B include over-fertilization and irrigation with water containing high concentrations of B (Nable *et al.,* 1997).

Sodium chloride induced salinity affects the growth of plants. It has an effect on nitrogen metabolism and the urea cycle; as a result, the leaf may turn yellow due to an accumulation of urea in the leaf, which is known as urea burning. Simultaneous boron toxicity and salinity stress can occur when plants are grown in soils with naturally high levels of salts and boron, which is prevalent in semi-arid and arid regions with low annual precipitation and inadequate drainage (Nable *et al.,*1997).

Once salinity or boron toxicity develops in field, their effects persist to subsequent crops. Current study aims to study the effect of both on morpho-physiological and biochemical parameters of green gram.

MATERIALS AND METHODS

The experiments were conducted in pots during the *kharif* 2018 at the Agricultural farm of the Institute of Agricultural Sciences Banaras Hindu University, Varanasi, India. During *rabi* 2017-18 wheat variety HUW 234 was grown in earthen

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pots containg .10 kg soil missed with FYM (4:1). Pots were supplied with following 9 treatment combinations: T_1: Normal soil, T_2: Normal soil+2.0 mg B kg^{-1} soil, T_3: Normal soil+3.5 mg B kg^{-1} soil, T_4: Normal soil+2.50 g NaCl kg^{-1} soil, T_5: Normal soil+5.0 g NaCl kg^{-1} soil, T_6: Normal soil+2.0 mg B kg^{-1} + 2.50 g NaCl kg^{-1} soil, T_7: Normal soil+3.5 mg B kg^{-1}+2.50 g NaCl kg^{-1} soil, T_8: Normal soil+2.0 mg B kg^{-1} +5.0 g NaCl kg^{-1} soil, T_9: Normal soil+3.5 mg B kg^{-1} + 5.0 g NaCl kg^{-1} soil. There was no drainage in the pots. After harvesting wheat, in the same pots green gram variety HUM-2 was grown in triplicate by adopting the completely randomized block design (CRD) in the subsequent *kharif* under recommended package of practices.

Shoot length, root length, leaves $plant^{-1}$, leaf area $plant^{-1}$ and dry weight $plant^{-1}$ were determined following standard practices at regular intervals after sowing. Using these data relative growth rate (RGR), net assimilation rate (NAR)(Radford, 1967) and leaf area ratio (LAR) (Watson, 1952) were calculated.

Chlorophyll a, b, total chlorophyll and carotenoid contents (Hiskox and Israelstam, 1979), soluble sugars and starch (Dubios *et al.,* 1956), free amino acids (Yemm *et al.,* 1995), proline (Bates *et al.,*1973) and malondialdehyde (MDA) content (Heath and Packer 1968) were determined in first fully expanded leaves from top at 20, 40, 60 days after sowing. At harvest total seed weight $plant^{-1}$, number of pods $plant^{-1}$, number of seeds $plant^{-1}$, totals seeds pod^{-1}, test weight (100 seed weight) and harvest index (Nichiporovitch, 1960) were determined. All biochemical parameters were measured in first fully expanded leaf from top.

Results and Discussion

Effect of different levels of boron and NaCl on shoot length (cm), root length (cm), number of leaves $plant^{-1}$, leaf area (cm^2 $plant^{-1}$) and dry weight (g $plant^{-1}$) was recorded at different stages of growth (Table 1). At different stages of growth, the response of plant to different treatments in terms of shoot length was significant and at 45 DAS the highest shoot length was recorded in plants under treatment T_6 (40.97 cm), which was at par with T_5 and T_1. At 45 DAS the highest root length was recorded in plants under treatment T_1, which was at par with T_2, T_4, T_7 and T_8. T_1 (39.40 $cm^2/plant^{-1}$) again registered the maximum leaf area $plant^{-1}$. The dry weight $plant^{-1}$ was noted maximum at T_4 (1.75 g/$plant^{-1}$), which was at par with T_3 and T_2. Shoot-root length decreases with increased concentrations of salt and boron (Lata *et al*., 2017). Although due to strong stimulation of defense sometimes plant shows improved dry weight as compared to control under stress.

Effect of different levels of boron and NaCl on amino acids, proline, soluble sugars, starch and malondialdehyde was recorded at different stages of growth (Table 2). At 60 DAS, plants under T_3 (4.931 mg g^{-1} fresh weight) contained significantly higher amount of amino acids. The maximum amount of proline was recorded in plants under T_3 upto 40 DAS and at 60 DAS T_9 (0.214 mg g^{-1} fresh weight) showed significantly higher amount. Samet and Çikili (2019) found significant proline accumulation under excess B, which indicates that B is contributing to the osmotic stress. However, Mann *et al*. (2019) observed increase in proline content with increasing levels of salinity. Plants under T_1 (32.67 mg g^{-1} fresh weight) showed significantly higher amount of soluble sugars and the minimum amount in plants under T_9 (24.38 mg g^{-1} fresh weight) at 40 and 60 DAS. The amount of starch content at 60 DAS under T_1 (102.4 mg g^{-1} fresh weight) showed significantly higher and minimum amounts in plants under T_9. A similar result has been reported by Wang *et al*. (2021). The maximum MDA content was found in plants under T_8 (2.636 µ mole g^{-1} fresh weight) and minimum amount of MDA was found in the plants under T_1. Similar result is reported by Samet and Çikili (2019).

It is reported that under salinity or boron toxicity, photosynthetic rate is reduced and one of the reasons for reduction in photosynthetic rate is due to reduction in the

Table 1: Effect of different levels of boron and NaCl on shoot length (cm), root length (cm), number of leaves $plant^{-1}$, leaf area (cm^2 $plant^{-1}$) and dry weight (g $plant^{-1}$) in green gram variety HUM-2 at different stages of growth during *kharif* 2018.

Treatment*	Shoot length			Root length			Number of leaves $plant^{-1}$			Leaf area			Dry weight		
	Days after sowing			Days after sowing			Days after sowing			Days after sowing			Days after sowing		
	15	30	45	15	30	45	15	30	45	15	30	45	15	30	45
T_1	9.50	18.90	38.23	11.03	14.23	18.43	8.00	13.00	15.33	3.23	11.57	39.40	0.198	0.325	1.396
T_2	10.43	17.03	34.60	12.83	10.93	17.23	8.00	12.67	14.67	2.93	10.90	38.33	0.154	0.301	1.614
T_3	9.47	13.53	35.13	13.53	10.50	15.67	7.00	12.67	15.00	3.07	10.27	34.90	0.116	0.249	1.718
T_4	9.77	17.53	33.57	12.73	7.20	17.20	6.00	10.67	13.33	2.97	9.63	34.23	0.169	0.243	1.751
T_5	9.63	17.50	39.43	10.90	7.80	14.03	7.33	11.33	15.00	2.90	9.23	34.77	0.159	0.245	1.579
T_6	9.90	15.93	40.97	11.63	9.87	13.93	6.67	11.00	16.00	2.90	8.77	32.37	0.126	0.172	1.502
T_7	11.93	17.00	35.53	11.20	11.30	17.70	6.67	9.67	11.67	2.64	8.10	36.33	0.129	0.167	1.318
T_8	10.60	16.50	32.33	10.73	9.77	17.03	7.00	9.00	15.00	2.93	8.77	33.00	0.119	0.217	1.237
T_9	9.40	15.77	32.03	9.43	8.70	14.97	6.00	9.67	13.00	2.80	7.97	32.83	0.107	0.193	1.219
CD at 5%	1.234	2.273	4.231	1.034	1.095	2.103	N/S	N/S	1.981	0.310	1.271	3.189	0.035	0.059	0.305
SE(m)±	0.616	0.759	1.413	1.206	1.293	0.702	0.609	1.139	1.759	0.104	0.424	1.065	0.012	0.020	0.102

Table 2: Effect of different levels of boron and NaCl on amino acids (mg g^{-1} fresh weight), proline (mg g^{-1} fresh weight), soluble sugars (mg g^{-1} fresh weight), starch (mg g^{-1} fresh weight) and malondialdehyde (µ mole g^{-1} fresh weight) in first fully mature leaf from top in green gram variety HUM-2 at different stages of growth during *kharif* 2018.

Treatment*	Amino acids			Proline			Soluble sugars			Starch			Malondialdehyde
	Days after sowing			Days after sowing			Days after sowing			Days after sowing			Days after sowing
	20	40	60	20	40	60	20	40	60	20	40	60	60
T_1	1.209	1.341	4.841	0.028	0.033	0.043	22.68	28.88	32.67	41.79	59.36	102.4	0.269
T_2	1.207	1.627	4.718	0.038	0.041	0.053	22.56	26.98	31.87	34.89	56.21	99.84	0.530
T_3	0.659	1.457	4.931	0.058	0.061	0.077	19.13	25.76	28.97	41.89	46.85	87.07	0.709
T_4	0.538	1.542	4.331	0.039	0.053	0.085	18.03	25.57	29.77	34.68	45.52	75.02	0.769
T_5	0.720	1.044	2.193	0.020	0.037	0.163	19.28	23.14	29.37	40.67	49.93	65.91	0.631
T_6	0.784	2.198	3.424	0.036	0.045	0.119	20.23	24.79	27.99	37.66	45.25	78.81	0.637
T_7	0.849	2.723	3.027	0.042	0.049	0.139	18.34	24.73	26.13	30.45	42.51	80.46	1.648
T_8	0.997	1.552	2.199	0.043	0.051	0.171	16.85	22.68	25.12	31.99	41.70	79.08	2.636
T_9	0.858	0.997	1.644	0.045	0.059	0.214	17.19	21.54	24.38	32.89	47.91	55.65	2.232
CD at 5%	0.295	0.705	0.901	0.017	0.011	0.034	1.388	1.472	1.333	N/S	N/S	12.65	0.436
SE(m)±	0.098	0.236	0.301	0.006	0.004	0.009	0.655	0.736	0.604	19.29	5.489	10.90	0.146

Table 3: Effect of different levels of boron and NaCl on chlorophyll a (mg g^{-1} fresh weight), chlorophyll b (mg g^{-1} fresh weight), total chlorophyll (mg g^{-1} fresh weight), chlorophyll a /chlorophyll b and carotenoids (mg g^{-1} fresh weight), in first fully mature leaf from top in green gram variety HUM-2 at different stages of growth during *kharif* 2018.

Treatment*	Chlorophyll a			Chlorophyll b			Total Chlorophyll			Chlorophyll a /Chlorophyll b			Carotenoids		
	Days after sowing			Days after sowing			Days after sowing			Days after sowing			Days after sowing		
	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
T_1	0.441	0.662	0.869	0.323	0.339	0.461	0.924	1.001	1.330	1.365	1.953	1.885	0.064	0.081	0.098
T_2	0.280	0.909	0.878	0.220	0.443	0.509	0.860	1.352	1.387	1.273	2.052	1.725	0.067	0.076	0.117
T_3	0.268	0.868	0.528	0.363	0.442	0.576	0.771	1.310	1.104	0.738	1.964	0.917	0.083	0.056	0.167
T_4	0.258	0.568	0.708	0.335	0.287	0.328	0.993	0.835	1.166	0.770	1.979	2.159	0.051	0.048	0.292
T_5	0.293	0.744	0.859	0.259	0.338	0.407	0.852	1.082	1.266	1.131	2.201	2.111	0.057	0.068	0.203
T_6	0.519	0.729	0.872	0.309	0.335	0.403	0.828	1.064	1.275	1.680	2.176	2.164	0.061	0.056	0.289
T_7	0.214	0.424	0.513	0.202	0.319	0.333	0.546	0.743	0.846	1.059	1.329	1.541	0.058	0.072	0.375
T_8	0.135	0.446	0.584	0.139	0.358	0.388	0.470	0.804	0.972	0.971	1.246	1.505	0.059	0.083	0.116
T_9	0.142	0.461	0.551	0.144	0.279	0.432	0.388	0.740	0.983	0.922	1.652	1.275	0.052	0.065	0.078
CD at 5%	0.171	0.156	0.115	0.248	0.118	0.152	0.272	0.144	0.138	0.179	0.172	1.154	NS	NS	0.016
SE(m)±	0.057	0.052	0.038	0.083	0.039	0.051	0.025	0.841	0.955	0.166	3.096	6.009	0.021	0.005	0.006

level of photosynthetic pigments (Carillo *et al*., 2011). Hegazi *et al*. (2018) reported that mild concentrations of boron can increase the photosynthetic pigments. The amount of chlorophyll a, chlorophyll b, their ratio, total chlorophyll and carotenoids in first fully mature leaf from top was measured at 20, 40, 60 DAS (Table 3). The chlorophyll a and b content was high in T_2, which was at par with T_1, however the ratio was high in T_6 (2.164). The carotenoids content was higher in T_7 (0.375 mg g^{-1} fresh weight).

The total dry weight $plant^{-1}$, total seed weight $plant^{-1}$, test weight, number of pods $plant^{-1}$, total number of seeds pod^{-1} and total number of seeds $plant^{-1}$ showed significant difference between the treatments (Table 4). The entire above mentioned yield parameters were observed maximum in plants under T_1. The harvest index obtained showed significant difference between the treatments. The maximum harvest index was observed in plants under T_2, which is at par with T_6. Salinity and/or nutrient toxicity result in reduction in yield and yield attributes of crop (Mauromicale, 2010).

The RGR, NAR, LAR were measured among different stages (Table 5). Between 15-30 days stage the plants under T_3 showed highest RGR. When RGR was calculated between 45 DAS and harvest stage, plants under T_1 had significantly higher RGR. The NAR measured was found higher under T_2 between 15-30 DAS and T_4 between 30-45 DAS. The LAR showed the highest value under T_6 between 15-30 days stage. Between 30-45 DAS it was significantly higher under T_7. Major effect of salinity and boron toxicity has been reported to decrease photosynthetic processes of plant (Lovatt, 1984).

Table 4: Effect of different levels of boron and NaCl on total seed weight plant^{-1} (g), total dry weight (g), test weight (g 100^{-1} seeds), harvest index (%), pods plant^{-1}, seeds pod^{-1} and seeds plant^{-1} in green gram at harvest stage during *kharif* 2018.

Treatment*	Total dry weight	Total seed weight plant^{-1}	Test weight g 100^{-1} seeds	Harvest index	Pods plant^{-1}	Seeds pod^{-1}	Seeds plant^{-1}
T_1	6.78	2.17	2.39	32.01	9.33	9.79	91.33
T_2	5.41	2.01	2.29	37.15	9.33	10.29	96.00
T_3	4.26	1.27	1.95	29.81	6.67	10.30	68.67
T_4	5.22	1.39	1.83	26.63	8.00	9.50	76.00
T_5	4.27	1.42	2.04	33.26	7.67	9.69	71.67
T_6	4.80	1.68	2.21	35.00	8.67	8.77	76.00
T_7	4.32	1.19	1.74	27.55	7.33	9.45	74.33
T_8	4.41	1.13	1.65	25.62	8.00	9.38	75.00
T_9	4.03	1.10	1.69	27.30	7.01	9.61	67.33
CD at 5%	0.435	0.766	0.105	2.589	1.929	1.729	12.43
SE(m)±	0.145	0.256	0.014	4.376	0.577	9.207	5.820
C.V.	9.451	11.98	11.15	10.63	10.23	11.50	9.18

Table 5: Effect of different levels of boron and NaCl on relative growth rate (RGR) (g g^{-1} fortnight^{-1}), net assimilation rate (NAR) (g cm^{-2} fortnight^{-1}) and leaf area ratio (LAR) (cm^2 g^{-1}) in green gram variety HUM-2 at different stages of growth during *kharif* 2018.

Treatment*	RGR			NAR		LAR	
	Days after sowing			Days after sowing		Days after sowing	
	15-30	30-45	45-H#	15-30	30-45	15-30	30-45
T_1	0.496	1.458	1.581	0.019	0.047	25.51	30.91
T_2	0.670	1.680	1.210	0.024	0.060	27.66	27.90
T_3	0.764	1.932	0.908	0.022	0.073	34.24	26.47
T_4	0.363	1.975	1.093	0.013	0.078	27.79	25.40
T_5	0.432	1.864	0.995	0.016	0.069	27.49	26.90
T_6	0.311	2.167	1.162	0.009	0.074	35.89	29.45
T_7	0.258	2.066	1.187	0.008	0.061	33.09	33.76
T_8	0.601	1.741	1.271	0.018	0.056	32.66	31.20
T_9	0.590	1.843	1.196	0.017	0.058	33.90	31.55
CD at 5%	0.141	0.012	0.201	0.005	0.008	3.784	1.918
SE(m)±	0.018	0.187	0.022	0.001	0.003	4.866	1.251

*T_1: Normal soil, T_2: Normal soil+2.0 mg B kg^{-1} soil, T_3: Normal soil+3.5 mg B kg^{-1} soil, T_4: Normal soil+2.50 g NaCl kg^{-1} soil, T_5: Normal soil+5.0 g NaCl kg^{-1} soil, T_6: Normal soil+2.0 mg B kg^{-1}+2.50 g NaCl kg^{-1} soil, T_7: Normal soil+3.5 mg B kg^{-1}+2.50 g NaCl kg^{-1} soil, T_8: Normal soil+2.0 mg B kg^{-1}+5.0 g NaCl kg^{-1} soil, T_9: Normal soil+3.5 mg B kg^{-1}+5.0 g NaCl kg^{-1} soil.

#: Harvest.

CONCLUSION

It has been found that differing amounts of boron or NaCl stress, either used singly or in combination, have a substantial impact on plant growth and development by changing several biochemical processes, ultimately lowering the economic yield. When treated to *rabi* planted wheat, the 2.0 mg B kg^{-1} soil, 2.5 g NaCl kg^{-1} soil, 3.5 mg B kg^{-1} soil and 5.0 g NaCl kg^{-1} soil, or their combinations, showed substantial residual toxic effects on growth, development, biochemical parameters, yield and yield characteristics. As a result, it is advised that on soils that are somewhat saline or where boron is present in higher amounts in the soil or when boron is sprayed for enhancing wheat yield during *rabi*, green gram cultivation may be used in the following *kharif* season.

Conflict of interest

There is no conflict of interest among the authors.

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