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ALLEVIATION OF SALINITY STRESS EFFECTS ON PHOTOSYNTHESIS, NITROGEN METABOLISM AND YIELD OF INDIAN MUSTARD BY NITROGEN FERTILIZATION

B.K. GARG*, U. BURMAN AND S. KATHJU

Division of Soil-Water-Plant Relationships, Central Arid Zone Research Institute, Jodhpur 342 003

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SUMMARY

A study was undertaken to explore the possible alleviation of detrimental effects of salinity on growth, photosynthesis and nitrogen metabolism of Indian mustard through nitrogen fertilization. Experimental plants grown at two levels of nitrogen (0 and 60 kg ha^{-1}) were irrigated with waters having 0.2, 5 and 10 dS m^{-1} EC at weekly intervals from 20 DAS till maturity. Nitrogen application led to significant increase in the concentrations of N, P and K and a decrease in Na concentration in the shoot tissue, under varying salinity levels, both at vegetative and flowering stages. Observations on contents of total chlorophyll, soluble protein, free amino acids, starch, total soluble sugars, nitrate reductase activity and net photosynthetic rates at vegetative and flowering stages revealed adverse effects of salinity on photosynthesis and leaf nitrogen metabolism. However, nitrogen fertilized plants displayed higher photosynthesis and more efficient N metabolism under all levels of salinity at both the growth stages. This led to significant improvement in leaf area development, plant growth and seed yield under salt stress. It was found that seed yield of N fertilized plants even under highest salinity level was comparable with that of non-saline control plants grown without N application. Results indicated that improvement of soil nitrogen status through N application favorably modulated photosynthetic efficiency and carbohydrate metabolism of salt stressed plants besides positive effects on N metabolism which contributed to better performance of plants both under control and salinity stress.

***Key words*: Indian mustard, mineral nutrition, nitrogen metabolism, photosynthesis, salinity**

INTRODUCTION

In the semi-arid parts of Rajasthan in India, growth of Indian mustard is often restricted as the ground waters used for irrigation are mostly moderate to highly saline and soils are also nitrogen deficient. In Rajasthan salt affected soils occupy about 1 million hectare out of which nearly 70 per cent area is in arid region (Mehta *et al*. 1970). Nearly 84 per cent of the area of arid Rajasthan has ground water of more than 2.2 dS m^{-1} salinity and constitute 60 per cent of ground water source (Dhir 1977, Gupta *et al*. 1998). Salt stress conditions are well known to adversely affect N uptake and metabolism in majority of plant species (Aslam *et al.* 1984, Garg *et al.* 1990, Dubey 1998). Fertilizer application under these conditions has been reported to significantly alleviate the detrimental effects of salts in a number of crops, (Ravikovitch and Yoles 1971, Bernstein *et al*. 1974, Feigin 1985, Garg *et al.* 1990, 1993). This response, however, seems to be crop specific and is known to be mediated through elimination of nutritional deficiencies as well as nutrition induced higher metabolic efficiency (Bernstein *et al.* 1974, Garg and Vyas 1998).

Salt stress affects photosynthesis by its influence on stomatal conductance, partial pressure of CO_2 in leaves,

* Corresponding author, E.mail: bkgarg@cazri.res.in

chlorophyll content, photochemical and carboxylation action besides changes in the ultra structure of chloroplasts and increase in levels of soluble sugars in the tissues (Downton *et al.* 1985, Sharma and Hall 1991, Dubey 1996). Likewise limiting N availability during plant growth is known to affect various components of photosynthetic apparatus, plant growth and development. However, the consequences of such effects of N limitation in the face of salinity stress are not well known (Seeman and Sharkey 1986) and little information on this aspect is available for mustard crop. Therefore, present investigation was undertaken to explore the possible alleviation of salinity effects on plant growth, mineral composition, photosynthetic parameters and N metabolism through N fertilization in view of the poor nutritional status of arid zone soils.

MATERIALS AND METHODS

The investigation was carried out at the Central Research Farm of the Central Arid Zone Research Institute, Jodhpur. Plants of Indian mustard (*Brassica juncea* L cv. B10-902) were grown in glazed pots containing 40 kg loamy sand soil (Typic Camborthids) having 7.1, 5.6, 63.1 and 24.1 per cent of clay, silt, fine sand and coarse sand, respectively. The soil had 0.28 per cent organic carbon and 0.023 per cent total nitrogen, 80 kg ha^{-1} available nitrogen, 15 kg ha^{-1} available phosphorus and 120 kg ha^{-1} available potassium. The initial salinity (ECe) of the experimental soil was 0.68 dS m^{-1}. Plants were grown at two levels of nitrogen (0 and 60 kg ha^{-1} designated as N_o and N_{60}) applied through urea at the time of sowing. Two plants were maintained in each pot and there were 60 pots under each nitrogen treatment. After 15 days of sowing (DAS) these pots were further subdivided into three sets each of which received waters of 0.2, 5 and 10 dS m^{-1} EC at weekly intervals till maturity. The proportion of different salts in the synthesized saline waters was kept almost similar to that of local groundwater (55% NaCl, 15% Na_2SO_4, 10% each of $NaHCO_3$, $MgCl_2$ and $MgSO_4$). Control plants received tap water (0.2 dS m^{-1}) throughout the growing period. There were 20 pots (replicates) under each of the six treatments. The build up of salinity was ascertained at the time of harvest from measurement of the electrical conductivity of saturated soil extract (ECe) of soil samples collected from root zone of different treatments.

At the vegetative (40 DAS), and flowering stages (65 DAS), two uppermost fully expanded fresh leaves were analyzed, in quadruplicate, for the contents of total chlorophyll (Arnon 1949), soluble protein (Lowry *et al.* 1951), free amino acids (Yemm and Cocking 1955), starch and total soluble sugars (Yemm and Willis 1954). At the same time nitrate reductase activity was also estimated (Jaworski 1971) in the same set of leaves. Rates of net photosynthesis and stomatal conductance were simultaneously measured in two uppermost fully expanded leaves using LICOR-6200 portable photosynthetic system. These measurements were made between 10.00 - 11.00 a.m. in four plants under each treatment. Leaf area was measured at both the growth stages using LICOR-3000 leaf area meter. Concentrations of nitrogen, phosphorus, potassium and sodium were analyzed, in triplicate (Jackson 1973) in the representative dry shoot samples at vegetative and flowering stages. Data on seed yield and shoot dry matter was recorded at the time of harvest and observations were based on 10 replicates for each treatment. The significance of the data was ascertained through analysis of variance adopting factorial design where salinity and nitrogen constituted the two variables.

RESULTS AND DISCUSSION

Irrigation with saline water of 0.2, 5 and 10 dS m^{-1} EC led to build up of root zone salinity (ECe) of 0.85, 4.60 and 8.22 dS m^{-1}, respectively under N_0 and 0.88, 4.40 and 7.97 dS m^{-1}, respectively under N_{60} treatments. Consequently, increasing salinity due to saline water irrigation reduced seed yield and shoot dry matter progressively under both the nitrogen treatments (Table 1). On an average seed yield decreased by 16.6% and shoot dry weight by 23.9% at the highest level of salinity (10 dS m^{-1}) as compared to control plants. However, the magnitude of decrease in seed yield as well as dry matter of shoot with increasing salinity was comparatively less in nitrogen fertilized plants compared to unfertilized plants. Data obtained in the present study revealed a favourable influence of N application on plant performance at all salinity levels as also observed in

Table 1. Influence of salt stress and N fertilization on seed yield and shoot dry matter of mustard

Salinity level ($dS\ m^{-1}$)	Seed yield ($g\ plant^{-1}$)			Shoot dry weight ($g\ plant^{-1}$)		
	N_0	N_{60}	Mean	N_0	N_{60}	Mean
0	4.48	5.32	4.90	15.64	17.75	16.69
5	4.07	4.83	4.45	13.09	15.39	14.24
10	3.68	4.48	4.08	11.53	13.89	12.71
Mean	4.08	4.88	-	13.42	15.68	-
	S	N	SxN	S	N	SxN
LSD (P = 0.05)	0.33	0.27	NS	1.04	0.85	NS

S = Salinity, N = Nitrogen

several other crops (Garg *et al.* 1990, Grattan and Grieve 1994, Burman *et al.* 2002) including mustard (Garg *et al.* 1993). Chauhan *et al.* (1988) observed 37 and 80% improvement in seed yield of Indian mustard with 60 and 90 kg N ha^{-1}, respectively in comparison with 0 kg N ha^{-1}, following irrigation with saline water.

Increasing salinity progressively and significantly decreased concentrations of nitrogen, phosphorus and potassium and increased sodium in the shoot tissue at both the growth stages irrespective of the N levels (Fig. 1). However, concentrations of N, P and K were higher at the vegetative compared to the flowering stage. Notwithstanding a consistent decline in tissue concentrations of N, P and K was observed under different salinity levels but nitrogen fertilized plants maintained significantly higher concentrations of these nutrients compared to unfertilized plants at both the growth stages. In contrast, Na concentration which progressively increased with increased salinity was comparatively lower in N-fertilized than unfertilized plants (Fig.1). However, nitrogen induced gains may be relatively limited under high salinity. This was more obvious from the fact that at 10 dS m^{-1} salinity level, the average decline in concentrations of N, P and K was about 22% at the flowering stage, whereas Na concentration increased by 219% compared to control plants. The maintenance of higher concentrations of N, P, K and lower concentration of Na in fertilized plants compared to unfertilized plants probably helped the salt

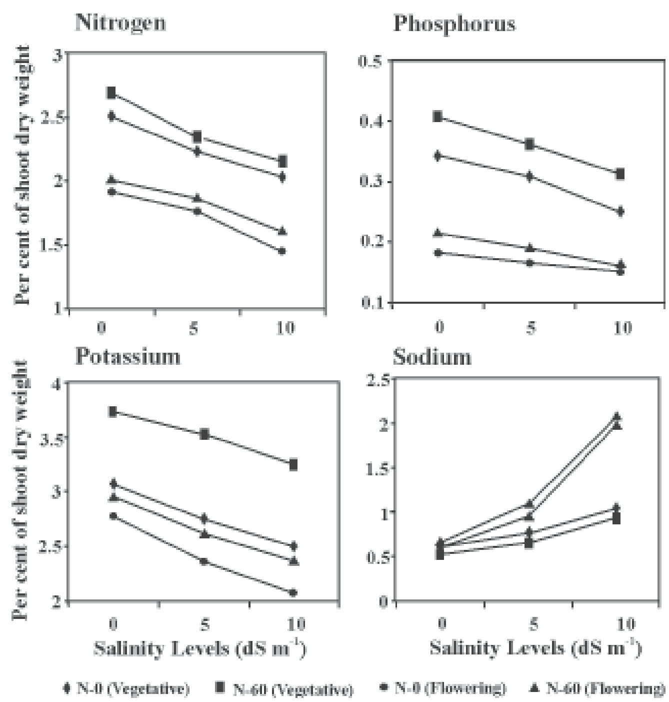

Fig. 1. Effects of salt stress and N fertilization on concentrations (per cent of dry weight) of nitrogen, phosphorus, potassium and sodium in shoot tissue of mustard plants at vegetative and flowering stages.

LSD values for nitrogen and salinity at the vegetative stage were 0.13 and 0.16 for N, 0.016 and 0.020 for P, 0.16 and 0.20 for K and 0.10 and 0.12 for Na, respectively. The corresponding LSD values at the flowering stage were 0.10 and 0.12 for N, 0.011 and 0.014 for P, 0.14 and 0.17 for K and 0.10 and 0.13 for Na, respectively. The interaction between nitrogen and salinity (N xS) was not significant in all cases.

stressed plants to overcome nutrient imbalance and carry out their normal physiological processes. This phenomenon has also been observed earlier in wheat, Indian mustard and isabgol crops grown under saline water irrigation (Garg *et al.* 1990, 1993, Burman *et al.* 2002).

Leaf area development was significantly influenced both by saline water irrigation and N fertilization. While increasing salinity significantly decreased leaf area both at vegetative and flowering stages, N application had a favourable and significant effect on leaf area at all salinity levels (Table 2). Reduction in leaf area was 30.7% in unfertilized plants and 28.6% in N fertilized plants at 10 dS m^{-1} during the vegetative stage and 32.3 and 30.0%, respectively at the flowering stage indicating positive effects of N application under saline water irrigation. On an average the N application enhanced leaf area by 35.5% at vegetative stage and 22.8% at the flowering stage. Besides promoting leaf area, N fertilization also enhanced net photosynthetic rate at both the growth stages, irrespective of salinity levels (Table 2). Furthermore salinity induced decline in net photosynthetic rate was consistently less under the N_{60} treatment at both the growth stages. The reduction in photosynthesis under salt stress was associated with a decrease in stomatal conductance particularly at 10 dS m^{-1} salinity level at both the growth stages. The detrimental effects of salt stress on photosynthesis due to stomatal and non-stomatal effects are well documented (Downton 1977, Dubey 1996, Garg *et al.* 2001). Results of the present study also indicate stomatal closure on account of salt stress in mustard particularly at the flowering stage.

A progressive decrease in the levels of total chlorophyll with increasing salinity was also observed at both the growth stages (Table 3). The magnitude of decrease in chlorophyll concentration at 10 dS m^{-1} was comparable in fertilized and unfertilized plants at the vegetative stage (18.1-18.4%) but it was relatively less in N fertilized than unfertilized plants at the flowering stage. Similarly reduction in starch content was considerably less in the N fertilized plants than in control plants (N_o) at both the salinity levels, particularly during

Table 2. Effect of salt stress and N fertilization on leaf area, net photosynthesis and stomatal conductance of mustard plants at vegetative and flowering stages

Salinity level	Leaf area (cm² plant⁻¹)			Net photosynthesis (µmol m⁻² s⁻¹)			Stomatal conductance (mol m⁻² s⁻¹)		
(dS m⁻¹)	N_0	N_{60}	Mean	N_0	N_{60}	Mean	N_0	N_{60}	Mean
				Vegetative stage					
0	456	602	529	8.75	11.40	10.08	1.03	1.32	1.18
5	378	525	452	8.25	10.57	9.41	0.84	0.96	0.90
10	316	430	373	7.43	9.91	8.67	0.73	0.86	0.79
Mean	383	519	-	8.14	10.63	-	0.87	1.05	-
	S	N	S xN	S	N	SxN	S	N	SxN
LSD (P=0.05)	54	44	NS	1.15	0.95	NS	0.16	0.13	NS
				Flowering stage					
0	567	691	629	10.13	12.48	11.31	1.04	1.28	1.16
5	455	587	521	9.58	11.631.	10.60	1.00	1.09	1.04
10	384	480	432	9.46	9.64	8.55	0.42	0.53	0.47
Mean	469	586	-	9.06	11.25	-	0.82	0.97	-
	S	N	SxN	S	N	SxN	S	N	SxN
LSD (P=0.05)	87	71	NS	0.84	0.68	NS	0.10	0.08	NS

S = Salinity, N = Nitrogen

Table 3. Effect of salt stress and N fertilization on levels of total chlorophyll and starch in leaves of mustard plants at vegetative and flowering stages

Salinity level (dS m^{-1})	Total chlorophyll (mg g^{-1} dw)			Starch (mg g^{-1} dw)		
	N_0	N_{60}	Mean	N_0	N_{60}	Mean
			Vegetative stage			
0	13.44	15.71	14.58	185.7	209.1	197.4
5	12.47	13.75	13.11	170.2	184.1	177.1
10	11.00	12.82	11.91	139.5	160.5	150.0
Mean	12.30	14.09	-	165.1	184.6	-
LSD						
(P=0.05)	S	N	SxN	S	N	SxN
	0.70	0.57	NS	6.7	5.5	NS
			Flowering stage			
0	10.73	12.20	11.47	169.2	204.8	187.0
5	9.48	11.22	10.35	142.3	179.2	160.7
10	8.25	9.88	9.07	120.8	156.4	138.6
Mean	9.49	11.10	-	144.1	180.1	-
LSD						
(P = 0.05)	S	N	SxN	S	N	SxN
	0.47	0.39	NS	7.6	6.2	NS

S = Salinity; N = Nitrogen

the flowering stage. However, the levels of total chlorophyll and starch remained significantly higher in the N fertilized as compared to unfertilized plants irrespective of the salinity level and growth stage.

Nitrogen metabolism was modulated in mustard plants by N fertilization under saline water irrigation. A general decline in nitrate reductase activity (NRA) and soluble protein concentration with increased salinity was observed under both the nitrogen treatments but the N fertilized plants maintained a significantly higher NRA and soluble protein level than unfertilized plants at all salinity levels (Table 4). The decline in soluble protein level under salt stress was associated with an increase in free amino acids levels at both the growth stages (Table 4). However, the salinity-induced increase in free amino acids was less in N fertilized than unfertilized plants indicating less disruption in N metabolism of plants under N treatment. The reduction in soluble protein level with associated increase in free amino acids under salinity may be attributed both to disruption in protein synthesis (Kahane and Poljakoff-Mayber 1968) as well as to enhanced proteolysis (Dubey 1998). However, N application seemed to partly arrest both these detrimental effects. Similar observations have been made by several other investigators (Dubey 1998, Garg and Vyas 1998).

The observations on the impact of N fertilization on NRA and levels of soluble protein and free amino acids indicate the importance of adequate N nutrition under saline conditions. Although NRA is known to be highly sensitive to salt stress (Rao and Gnanam 1990) the results of our previous studies on wheat (Garg *et al.* 1990), Indian mustard (Garg *et al.* 1993) and isabgol (Burman *et al.* 2002) as well as of the present study indicate that higher N availability in the soil under N application possibly induced a greater NRA despite salt stress. Favourable effects of improved soil fertility on mineral nutrients and enzymes of ammonia assimilation (viz. GS, GOGAT and GDH) under varying levels of salinity in mustard reported

Table 4. Effects of salt stress and N fertilization on nitrate reductace activity and levels of soluble protein and free amino acids in leaves of mustard plants at vegetative and flowering stages

Salinity level ($dS\ m^{-1}$)	Nitrate reductase ($\mu mol\ NO_2\ g^{-1}\ dw\ h^{-1}$)			Soluble protein ($mg\ g^{-1}\ dw$)			Free amino acids ($mg\ g^{-1}\ dw$)		
	N_0	N_{60}	Mean	N_0	N_{60}	Mean	N_0	N_{60}	Mean
Vegetative stage									
0	18.08	27.23	22.66	116.3	128.0	122.2	17.49	19.46	18.47
5	16.43	24.37	20.40	112.1	124.6	118.4	18.62	20.28	19.45
10	13.22	21.62	17.42	95.8	109.3	102.5	19.74	21.59	20.56
Mean	15.91	24.41	-	108.1	120.6	-	18.62	20.98	-
	S	N	SxN	S	N	SxN	S	N	SxN
LSD (P=0.05)	1.06	0.87	NS	3.77	3.07	NS	1.12	0.92	NS
Flowering stage									
0	16.35	20.00	18.17	105.8	113.8	109.8	14.87	16.07	15.47
5	14.37	17.03	15.70	88.6	96.6	92.6	16.05	16.87	16.46
10	12.32	15.41	13.81	82.4	91.6	87.0	17.12	18.07	17.60
Mean	14.35	17.48	-	92.3	100.7	-	16.01	17.00	-
	S	N	SxN	S	N	SxN	S	N	SxN
LSD (P=0.05)	0.66	0.54	NS	1.74	1.42	2.50	0.93	0.76	NS

S = Salinity; N = Nitrogen

earlier (Garg *et al.* 1993) suggest the beneficial role of N application on N metabolism of salt affected plants.

These findings suggest that N fertilization can favourably influence the nutrient uptake, photosynthetic efficiency and carbohydrate metabolism of salt stressed mustard plants. Additionally higher N availability in the soil under N-fertilization probably can induce a higher nitrate reductase activity, associated with higher protein concentration compared to unfertilized plants. These findings have an agronomic importance and the benefits of N application can possibly be obtained in many crops grown in nitrogen deficient soils under saline conditions.

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