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NITROGEN MITIGATES EFFECT OF SALINITY ON PLANT WATER RELATIONS AND PHOTOSYNTHESIS IN INDIAN MUSTARD (*Brassica juncea*)

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The interaction between salinity (8 and 12 dS m^{-1}) and three levels (40, 80 and 120 kg ha^{-1}) of different forms of nitrogen (NO_3^-, NH_4^+ and NO_3^- + NH_4^+) were studied in *Brassica juncea* cv. RH-30. The plants were salinized with 8 and 12 dS m^{-1} at 35 and 55 days after sowing. The relative water content (RWC), water potential (ψ_w) and osmotic potential (ψ_s) exhibited a marked decline under salinity stress. The application of the combined form (NO_3^- + NH_4^+) of nitrogen (120 kg ha^{-1}) considerably improved the water status and mitigated the adverse effect of salinity on growth. The salinity-induced osmotic effect led to stomatal closure and caused a substantial reduction in net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E) at the pre-flowering and flowering stages (45 and 65 DAS). Salinity effects were considerably moderated by additional nitrogen supply, which varied with the source of nitrogen, the level of salinity/fertilizer and the stage of plant growth. The inhibition in photosynthesis was relatively greater in ammonium-fed (NH_4^+) than in nitrate-fed (NO_3^-) plants, while the transpiration rate was relatively lower in nitrate-fed plants grown either with or without saline water irrigation. The nitrate form of nitrogen @ 120 kg ha^{-1} proved best in alleviating the adverse effect of salinity on photosynthesis and transpiration at both the growth stages.

Key words: water relations, photosynthesis, salinity, nitrogen source, *Brassica juncea*

Introduction

In warm dry areas the salt concentration increases in the upper soil layer due to high water losses, which exceed precipitation (Ebert et al., 2002). Overcoming salt stress is a major issue in these regions to ensure agricultural sustainability and crop production. Salinity is a major abiotic stress reducing the yield of a wide variety of crops all over the world (Tester and Davenport, 2003). In the semi-arid parts of India, the growth of Indian mustard is often restricted as the groundwater used for irrigation is mostly moderately to highly saline. A better understanding of how salinity affects physiological processes is important if such water is to be used efficiently. Nitrogen contributes substantially to plant growth and is directly related to crop yield potential. Nitrate (NO_3^-) and ammonium (NH_4^+) are the most abundant N sources for higher plants and their availability usually constitutes a limiting factor for plant growth (Frechilla et al., 2001; Nathawat et al., 2005; 2007). Salt stress interferes with nitrate uptake in many plants species due to the Cl^- content (Khan and Srivastava, 1998; Abdelgadir et al., 2005). The nitrogen source may affect the growth and productivity of barley (Ali et al., 2001) and wheat (Cramer and Lewis, 1993). However, most studies have been conducted in the absence of salinity, and the interaction between N source and water status has been given little attention. Thus, studies on the combined effect of these growing conditions on photosynthesis and water status are generally scarce and completely lacking for oil seeds. This paper reports on the joint effect of salinity and nitrogen forms at two growth stages (pre-flowering and flowering) on the water status and gas exchange parameters in plants.

Materials and methods

Plant materials and treatments

The experiment was conducted during October 1999 and 2000 in the pot house of the College of Basic Sciences and Humanities, CCS HAU, Hisar. Mustard plants (*Brassica juncea* cv. RH-30) were raised in pots, each of which was lined with polythene bags and filled with 6 kg of dune sand. Before seed sowing, the pots were supplied with nitrogen through different nitrogen sources, $Ca(NO_3)_2$, $(NH_4)_2SO_4$ and a combination of both at three levels, i.e. 40 mg kg^{-1}, 80 mg kg^{-1} and 120 mg kg^{-1} dune sand (equivalent to 40, 80 and 120 kg ha^{-1}). Balanced doses of PKS were given at the time of pot filling. After the application of fertilizers, the pots were saturated with water and allowed to settle over night. Sowing was carried out at the field capacity of dune sand. Ten seeds were sown in each pot at a uniform depth (1.5 to 2.0 cm) and distance. Fifteen days after sowing, thinning was done and three plants of uniform size were maintained per pot. Each pot was supplied with an equal quantity of N-free nutrient solution at regular intervals of 15 days. The desired salinity levels (ECe 0, 8 and 12 dS m^{-1}) were obtained by adding Cl and SO_4 salts of Na, Ca and Mg. For each ECe level, the requisite amount of salts per litre was added to the pots on a soil saturation basis at 35 DAS (Stage I) or 55 DAS (Stage II). A non-saline control was maintained separately. Sampling was done 10 days after applying saline water irrigation, i.e. 45 and 65 DAS (corresponding to the pre-flowering and flowering stages). Three replications were maintained for each treatment during the whole course of the experiment. The total plant dry weight was recorded at 45 and 65 DAS, while plant height was measured at harvest (Stages I and II). The chlorophyll and carotenoid contents were estimated according to the method of Hiscox and Israelstam (1979) using dimethyl sulphoxide (DMSO) as solvent.

Water relations

The relative water content (RWC) was determined by weighing the 3rd leaf from the top and floating it on de-ionized water for 6 h at constant temperature in diffused light. When the leaf became fully turgid, it was re-weighed, after which it was dried and the dry weight was determined. The RWC was calculated by the following formula (Barrs and Weatherley, 1962):

$$\text{RWC (\%)} = \frac{\text{Leaf fresh weight} - \text{leaf dry weight}}{\text{Leaf fully turgid weight} - \text{leaf dry weight}} \times 100$$

Leaf water potential (ψ_w) was determined from the third fully expanded leaf in a pressure chamber (Model-3000 series, Plant Water Status Console, Soil Moisture Equipment Corp., Santa Barbara, California, USA) using the technique introduced by Scholander et al. (1965). The values of water potential were expressed as –MPa. Simultaneously, adjacent leaves were harvested and frozen in liquid N_2 for osmotic potential (ψ_s) measurements. These frozen leaves were thawed and the osmotic potential was determined for the expressed solution after measuring sap osmolarity with a model 5100-B vapour pressure osmometer (Wescor Inc., Logan, Utah, USA). A standard curve was prepared with the help of sodium chloride solution. The values of osmotic potential of the leaf sap were recorded with the help of the standard curve and expressed in –MPa.

Leaf gas exchange characteristics

Various leaf gas exchange parameters such as net photosynthetic rate (µmol m^{-2} s^{-1}), transpiration rate (mmol m^{-2} s^{-1}) and stomatal conductance (mmol m^{-2} s^{-1}) were measured separately on the upper and lower surfaces of fully expanded leaves using a portable infrared gas analyzer (PP system Model CIRAS-1). These measurements were made on the third leaves between 9.30 to 11.00 A.M.

Statistical analysis of data

The statistical evaluation of the data was done for a factorial completely randomized design [3 salinity levels × 3 nitrogen forms × 3 nitrogen levels with 3 replicates] (Raghavarao, 1983).

Results

Effect of salinity and nitrogen source on growth

The total dry weight per plant and the plant height decreased at higher salinity levels from 8 to 12 dS m^{-1} over non-saline plants in both the pre-flowering and flowering stages. However, the plants generally maintained the highest total dry weight per plant and greatest plant height when grown with the combined (nitrate + ammonium) form N and the lowest with NH_4^+-N under saline conditions (Tables 1 and 2). Table 1 indicates that the percentage reduction in total dry weight per plant (34.70% and 47.76%) was maximum with NH_4^+-N and minimum (17.12% and 26.34%) with the combined form of nitrogen under 8 to 12 dS m^{-1} salinity compared with non-saline plants at the pre-flowering stage. A similar trend was observed at the flowering stage, the corresponding figures being 28.28% and 39.23% for NH_4^+-N and 14.51% and 21.72% for the combined form of nitrogen (Table 1). At the pre-flowering stage the total dry weight per plant was consistently greater with the highest dose of nitrogen (120 kg ha^{-1}) than with the lowest dose (40 kg ha^{-1}), irrespective of the N source, but the interactive effect between salinity and different levels of nitrogen sources was non-significant. Similarly, at the flowering stage the highest level of nitrogen application led to the greatest alleviation of the deleterious effect of salinity on total dry weight except for the highest level of the ammoniacal form (Table 1).

Table 1

Effect of nitrogen source, levels and their interaction with salinity on total plant dry weight (g) in *Brassica juncea* cv RH-30

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dS m^{-1})							
		0	8	12	Mean	0	8	12	Mean
		Pre-flowering stage (45 DAS)				Flowering stage (65 DAS)			
Nitrate form (NO_3^-)	40	1.631	1.205	1.038	1.291	2.418	1.903	1.464	1.928
	80	2.124	1.715	1.519	1.786	3.154	2.565	2.384	2.701
	120	2.322	2.005	1.769	2.032	3.78	3.335	3.005	3.373
	Mean	2.026	1.642	1.442	1.702	3.118	2.601	2.284	2.668
Ammoniacal form (NH_4^+)	40	1.242	0.745	0.588	0.859	1.762	1.272	1.06	1.374
	80	1.557	1.071	0.889	1.173	2.404	1.781	1.522	1.902
	120	1.153	0.763	0.587	0.834	2.021	1.406	1.195	1.541
	Mean	1.317	0.86	0.688	0.955	2.072	1.486	1.259	1.606
Combined form ($NO_3^- + NH_4^+$)	40	1.553	1.111	1.012	1.225	2.49	2.006	1.679	2.058
	80	2.477	2.002	1.793	2.091	3.327	2.809	2.625	2.92
	120	2.471	2.275	1.982	2.243	3.958	3.543	3.35	3.617
	Mean	2.167	1.796	1.596	1.853	3.259	2.786	2.551	2.865
Overall mean		1.837	1.432	1.242		2.816	2.291	2.031	

C.D. ($P<0.05$) for NS/NL/SL = 0.03 C.D. ($P<0.05$) for NS/NL/SL = 0.027
C.D. ($P<0.05$) for NS×NL, NL×SL & NS×SL = 0.051 C.D. ($P<0.05$) for NS×NL, NL×SL & NS×SL = 0.046
C.D. ($P<0.05$) for NS×NL×SL = ns C.D. ($P<0.05$) for NS×NL×SL = 0.08
NS = Nitrogen source; NL = Nitrogen level; SL = Salinity level; ns = Non-significant

Table 2

Effect of nitrogen source, levels and their interaction with salinity on plant height (cm) in *Brassica juncea* cv. RH-30 at harvest

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dS m^{-1})							
		0	8	12	Mean	0	8	12	Mean
		Pre-flowering stage (45 DAS)				Flowering stage (65 DAS)			
Nitrate form (NO_3^-)	40	99.1	84.5	75.6	86.4	94.6	78.1	69.1	80.6
	80	113.0	99.4	91.5	101.3	111.8	94.3	85.6	97.2
	120	119.0	107.7	99.3	108.6	115.4	101.2	93.4	103.3
	Mean	110.3	97.2	88.8	98.7	107.2	91.2	82.7	93.7
Ammoniacal form (NH_4^+)	40	94.7	84.8	70.9	83.4	89	70.1	61.4	73.5
	80	113.9	95.0	87.8	98.9	108.1	91.7	83.1	94.3
	120	105.1	85.2	79.3	89.8	99.2	79.5	73.5	84
	Mean	104.5	88.3	79.3	90.7	98.7	80.4	72.6	83.9
Combined form ($NO_3^- + NH_4^+$)	40	110.3	96.3	86.1	97.5	106.4	87.1	79.3	90.9
	80	127.1	103.3	101.7	110.7	122.6	104.8	95.7	107.7
	120	128.4	114.5	106.2	116.3	126.3	110.5	101.5	112.7
	Mean	121.9	104.6	98	108.1	118.4	100.8	92.1	
Overall mean		112.0	96.0	88.0		108.1	90.8	82.4	

C.D. ($P<0.05$) for NS/NL/SL = 2.637 C.D. ($P<0.05$) for NS/NL/SL = 2.661
C.D. ($P<0.05$) for NS×NL, NL×SL & NS×SL = 4.566 C.D. ($P<0.05$) for NS×NL, NL×SL & NS×SL = 4.611
C.D. ($P<0.05$) for NS×NL×SL = 7.908 C.D. ($P<0.05$) for NS×NL×SL = ns
NS = Nitrogen source; NL = Nitrogen level; SL = Salinity level; ns = Non-significant

Plant height decreased with higher salinity levels (8 to 12 dS m^{-1}) compared with non-saline plants at harvest (Stages I and II). The detrimental effect of salinity was more pronounced in ammonium-fed plants than for nitrate or the combined form of N. The decline in plant height was maximum (15.50 and 24.11%) with the ammoniacal form and minimum (14.19 and 19.60%) with the combined form of N at the two levels of salinity at Stage I, while these figures were 18.54 and 26.44% for NH_4^+-N and 14.86 and 22.21% for the combined form of nitrogen at Stage II. The reduction in plant height induced by salinity was mitigated by the application of the highest level of N through different sources. However, the combined form of N (120 kg ha^{-1}) counteracted the adverse effect of salinity to a greater extent than the other two forms at both sampling stages (Table 2).

Effect of salinity and nitrogen source on photosynthetic pigments

A decline in photosynthetic pigments (chlorophyll and carotenoid) was observed under salinity (8 and 12 dS m^{-1}) compared with the non-saline control at the pre-flowering and flowering stages (Tables 3 and 4). At pre-flowering this reduction in chlorophyll content under saline conditions was maximum (25.32 and 38.73%) for the ammoniacal form and minimum (21.99 and 33.73%) for the combined form of nitrogen over the control plants. In the same way, the maximum (18.69 and 35.33%) and minimum (14.45 and 25.14%) reductions were observed with the ammoniacal and combined form, respectively, compared to non-saline plants at the flowering stage (Table 3). Table 4 indicates that under saline conditions the reduction in carotenoid content was highest (18.23 and 16.35%) with the ammoniacal form and lowest (11.93 and 15.34%) with the combined form of nitrogen at the pre-flowering stage. Similarly, at the flowering stage the decline was maximum (17.15 and 21.30%) for NH_4^+-N and minimum (11.76 and 17.64%) for the combined form of nitrogen as compared to the control plants. Irrespective of the nitrogen source the higher levels of nitrogen (80 and 120 kg ha^{-1}) increased the chlorophyll and carotenoid contents of the leaves significantly as compared to the lowest level of nitrogen (40 kg ha^{-1}) at both sampling stages, but the interactive effect of salinity and different levels of nitrogen sources was non-significant at both the pre-flowering and flowering stages (Tables 3 and 4).

Effect of salinity and nitrogen source on water relations

The reduction in RWC in leaves under saline conditions (8 and 12 dS m^{-1}) was greatest (17.58 and 27.95%) with the ammoniacal form and smallest (13.49 and 22.16%) with the combined form of N at the pre-flowering stage (45 DAS). Higher levels (80 and 120 kg ha^{-1}) of all the nitrogen sources (NO_3^-, NH_4^+ and NO_3^- + NH_4^+) showed a significant increase in RWC over the lowest level of nitrogen (40 kg ha^{-1}) at both the sampling dates, but the interaction between salinity and different levels of nitrogen sources was non-significant (Fig. 1A).

Table 3

Effect of nitrogen source, level and their interaction with salinity on total chlorophyll content (mg g^{-1} dry weight) in leaves of *Brassica juncea* cv. RH-30

Nitrogen sources	Nitrogen levels ($kg\ ha^{-1}$)	Salinity levels ($dS\ m^{-1}$)							
		0	8	12	Mean	0	8	12	Mean
		Pre-flowering stage (45 DAS)				Flowering stage (65 DAS)			
Nitrate form (NO_3^-)	40	18.89	13.64	10.72	14.42	14.29	11.80	8.91	11.67
	80	19.23	14.71	11.92	15.29	16.56	14.02	12.17	14.25
	120	19.43	15.76	14.25	16.48	18.35	16.39	14.70	16.48
	Mean	19.18	14.70	12.30	15.39	16.40	14.07	11.93	14.13
Ammoniacal form (NH_4^+)	40	14.33	10.21	7.75	10.76	13.51	11.02	7.95	10.83
	80	15.73	11.92	9.33	12.32	14.25	11.94	9.63	11.94
	120	16.25	12.47	11.29	13.34	14.61	11.49	9.82	11.97
	Mean	15.44	11.53	9.46	12.14	14.12	11.48	9.13	11.58
Combined form ($NO_3^- + NH_4^+$)	40	19.13	14.31	11.72	15.05	15.80	13.22	10.79	13.27
	80	20.79	16.32	13.56	16.89	16.19	13.93	12.10	14.07
	120	22.43	18.02	16.04	18.83	18.23	15.82	14.71	16.25
	Mean	20.78	16.21	13.77	16.92	16.74	14.32	12.53	14.53
Overall mean		18.49	14.15	11.84		15.75	13.29	11.20	

C.D. ($P<0.05$) for NS/NL/SL = 0.208 C.D. ($P<0.05$) for NS/NL/SL = 0.187
C.D. ($P<0.05$) for NS×NL, NL×SL & NS×SL = 0.36 C.D. ($P<0.05$) for NS×NL, NL×SL & NS×SL = 0.323
C.D. ($P<0.05$) for NS×NL×SL = ns C.D. ($P<0.05$) for NS×NL×SL= ns
NS = Nitrogen source; NL = Nitrogen level; SL = Salinity level; ns = Non-significant

Table 4

Effect of nitrogen source, level and their interaction with salinity on carotenoid content (mg g^{-1} dry weight) in leaves of *Brassica juncea* cv. RH-30

Nitrogen sources	Nitrogen levels ($kg\ ha^{-1}$)	Salinity levels ($dS\ m^{-1}$)							
		0	8	12	Mean	0	8	12	Mean
		Pre-flowering stage (45 DAS)				Flowering stage (65 DAS)			
Nitrate form (NO_3^-)	40	1.62	1.38	1.29	1.43	1.75	1.42	1.32	1.50
	80	1.74	1.51	1.49	1.58	1.86	1.64	1.53	1.68
	120	1.82	1.60	1.61	1.67	1.97	1.83	1.72	1.84
	Mean	1.72	1.50	1.46	1.56	1.86	1.63	1.52	1.67
Ammoniacal form (NH_4^+)	40	1.65	1.28	1.57	1.50	1.73	1.37	1.29	1.46
	80	1.67	1.34	1.28	1.43	1.79	1.49	1.43	1.57
	120	1.46	1.30	1.13	1.29	1.55	1.33	1.26	1.38
	Mean	1.59	1.30	1.33	1.41	1.69	1.40	1.33	1.47
Combined form ($NO_3^- + NH_4^+$)	40	1.63	1.39	1.26	1.43	1.76	1.44	1.33	1.51
	80	1.75	1.55	1.51	1.61	1.88	1.68	1.54	1.70
	120	1.89	1.71	1.68	1.76	1.98	1.84	1.74	1.85
	Mean	1.76	1.55	1.49	1.60	1.87	1.65	1.54	1.69
Overall mean		1.69	1.45	1.42		1.81	1.56	1.46	

C.D. ($P<0.05$) for NS/NL/SL = 0.071 C.D. ($P<0.05$) for NS/NL/SL = 0.025
C.D. ($P<0.05$) for NS×NL, NL×SL & NS×SL = 0.124 C.D. ($P<0.05$) for NS×NL, NL×SL & NS×SL = 0.043
C.D. ($P<0.05$) for NS×NL×SL = ns C.D. ($P<0.05$) for NS×NL×SL = ns
NS = Nitrogen source; NL = Nitrogen level; SL = Salinity level; ns = Non-significant

The decline in water potential under saline conditions (8 and 12 dS m^{-1}) was most pronounced (25.08 and 32.33%) for the NH_4^+-N and lowest (12.21 and 19.36%) for the combined form of N, over non-saline conditions at the pre-flowering stage. In the same way, at the flowering stage these reductions were greatest (27.06 and 32.41%) with ammoniacal and smallest (10.33 and 20.48%) for the combined form of N at the 8 and 12 dS m^{-1} levels of salinity. The application of N (120 kg ha^{-1}) in combined form caused a smaller decrease (6.25 and 12.50%) in water potential at salinity levels of 8 and 12 dS m^{-1} at both sampling stages (Fig. 1B).

The osmotic potential in the leaves declined appreciably with salinity at both the pre-flowering and flowering stages. Under salt stress (8 and 12 dS m^{-1}) the highest percentage reduction (25.31 and 41.89%) was observed for NH_4^+-N and the lowest (13.62 and 27.60%) for the combined form of N at the pre-flowering stage. At the flowering stage, too, the combined form of nitrogen resulted in the least reduction (18.06 and 25.73%) in osmotic potential, whilst the greatest reduction (25.21 and 44.85%) was observed with the ammoniacal form of nitrogen. The highest dose of the combined form of N (120 kg ha^{-1}) caused less reduction in the osmotic potential of the leaves than the lowest N dose (40 kg ha^{-1}) under saline conditions. In contrast, the highest rate of the ammoniacal form of N (120 kg ha^{-1}) resulted in the greatest upsurge in the osmotic potential of the leaves at both sampling stages (Fig. 1C).

Effect of salinity and nitrogen source on photosynthesis

The reduction in the photosynthetic rate was relatively lower in nitrate-fed plants grown either with or without saline water irrigation as compared to the control plants. These reductions under salinity were maximum (27.81 and 39.20%) and minimum (26.76 and 32.70%) with the application of the ammoniacal and nitrate form of nitrogen, respectively, as compared to control plants at the pre-flowering stage. Correspondingly, plants treated with the ammoniacal form of nitrogen showed the greatest reduction (20.67 and 36.23%) in the photosynthetic rate and the nitrate form the smallest reduction (13.92 and 25.55%) as compared to non-saline plants at the flowering stage (Fig. 2A). The highest level of nitrogen (120 kg ha^{-1}) in nitrate form exhibited the smallest reduction (13.92 and 27.10%) in the photosynthetic rate at salinity levels of 8 and 12 dS m^{-1}, respectively, compared to non-saline plants. This reduction was maximum (18.99 and 39.94%) at the lowest level of nitrogen application at 45 DAS. Furthermore, increasing the level of nitrogen (80 and 120 kg ha^{-1}), irrespective of the source of nitrogen, gave a considerably higher photosynthetic rate in the leaves, except for the application of the ammoniacal form at 120 kg ha^{-1}, compared to the lowest dose of nitrogen, i.e. 40 kg ha^{-1}. The interactive effect between salinity and different levels of nitrogen sources was found to be non-significant at 65 DAS (Fig. 2A).

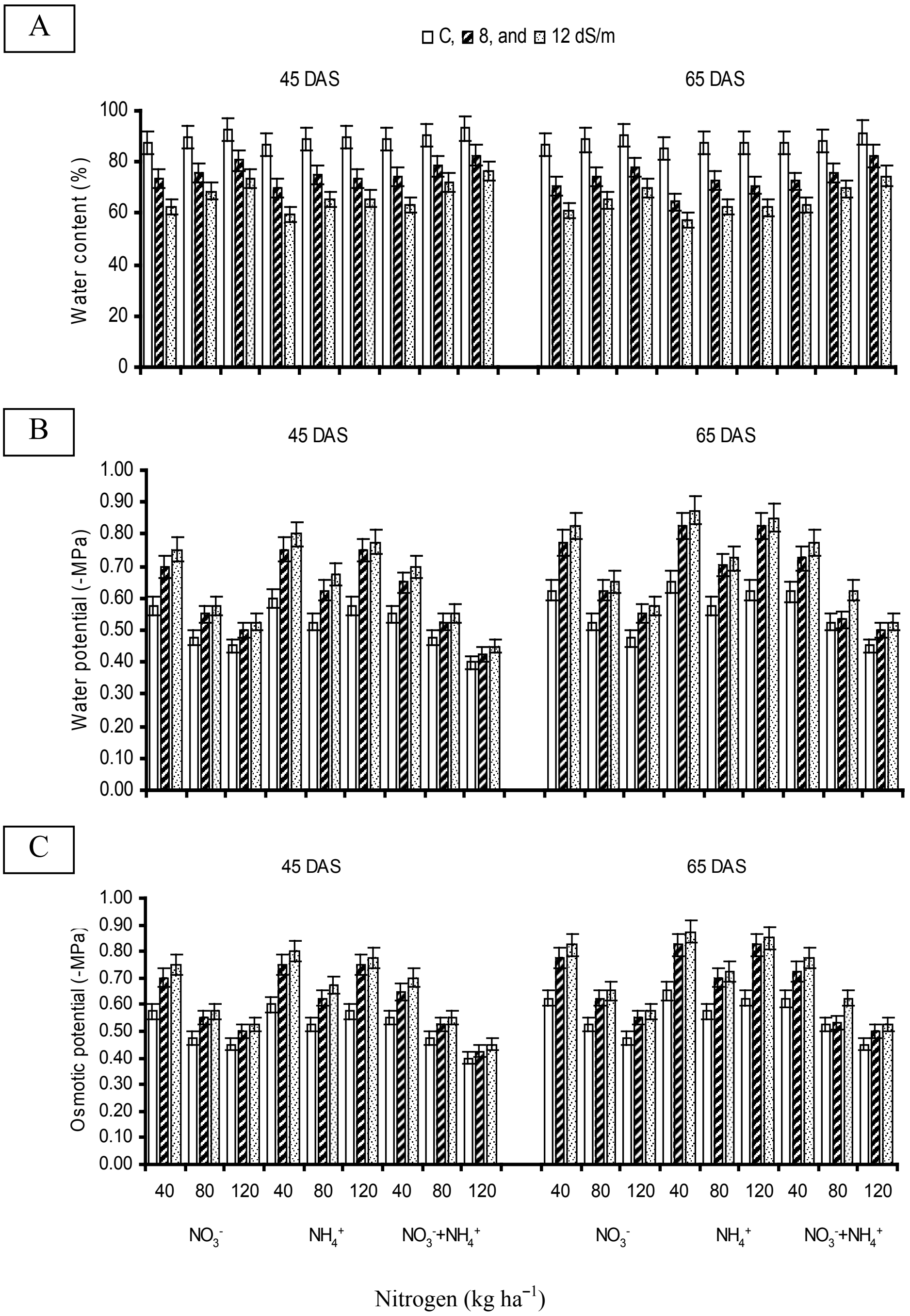

Fig. 1. Effect of nitrogen source, levels and their interaction with salinity on (A) water content (%), (B) Water potential (–MPa) and (C) osmotic potential (–MPa) in the leaves of *Brassica juncea* cv. RH-30

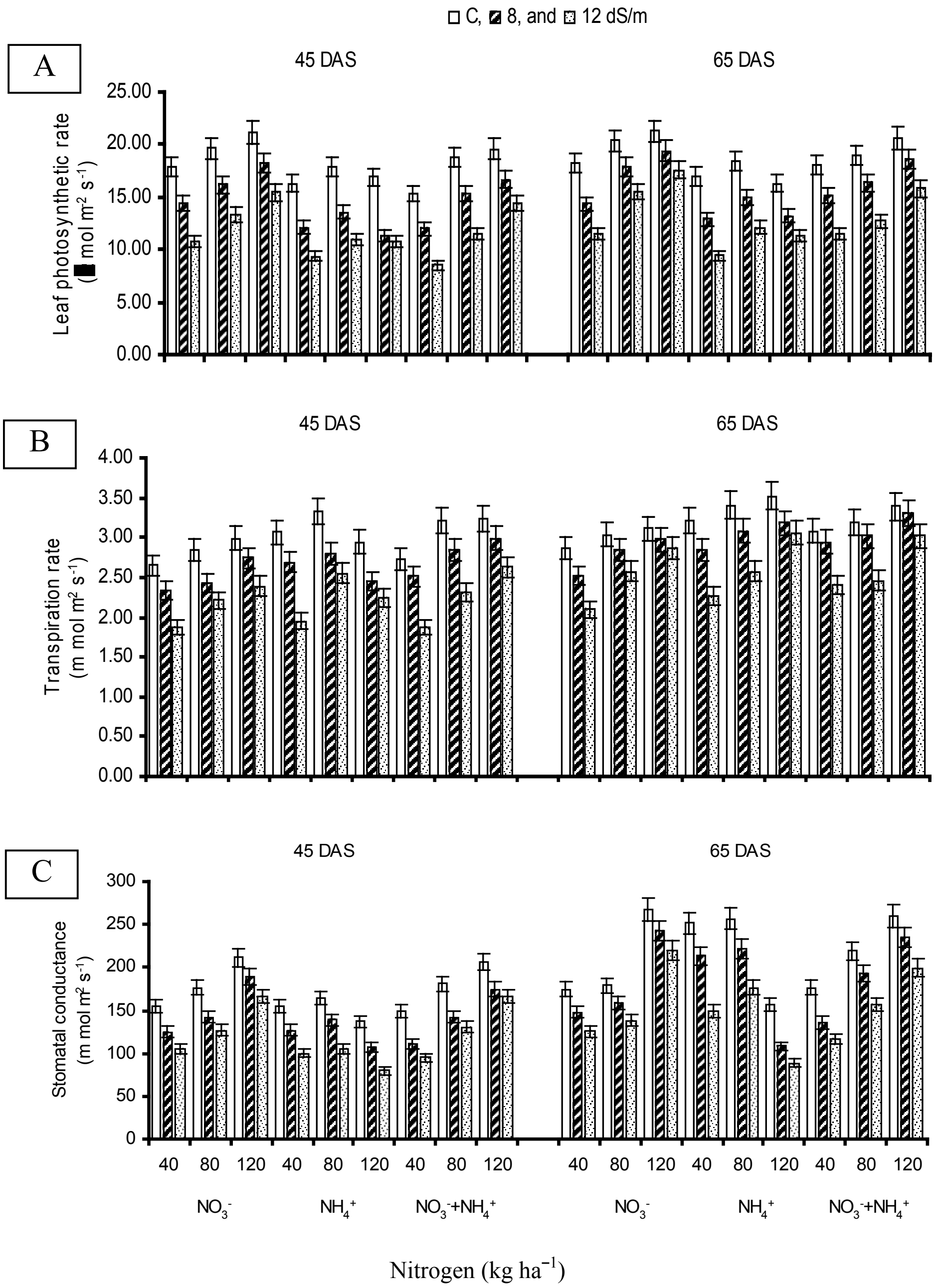

Fig. 2. Effect of nitrogen source, levels and their interaction with salinity on (A) leaf photosynthetic rate (□mol m^{-2} s^{-1}), (B) transpiration rate (mmol m^{-2} s^{-1}) and (C) stomatal conductance (mmol m^{-2} s^{-1}) in *Brassica juncea* cv. RH-30

Figure 2B illustrates that the transpiration rate decreased with increasing levels of salinity (8 and 12 dS m^{-1}). The reduction due to salinity was greatest (14.79 and 27.65% at 8 and 12 dS m^{-1}, respectively) for NH_4^+-N and smallest (11.66 and 23.67%) for NO_3^--N at the pre-flowering stage. Equally, treating plants with the ammoniacal form of nitrogen resulted in the maximum reduction (10.35 and 22.18%) in the transpiration rate, while the nitrate form of nitrogen led to a smaller reduction (7.64 and 16.61%) in comparison to non-saline plants at the flowering stage (Fig. 2B). A nitrogen dose of 120 kg ha^{-1} in nitrate form caused the least decrease (8.36 and 20.06%) in the transpiration rate at salinity levels of 8 and 12 dS m^{-1}, while this reduction was highest (12.07 and 29.05%) at the lowest level of the combined form of nitrogen, i.e. 40 kg ha^{-1} at 45 DAS. The highest level of nitrogen (120 kg ha^{-1}) in the nitrate form resulted in the least reduction (4.18 and 8.03%) in the transpiration rate at 65 DAS (Fig. 2B).

Stomatal conductance was found to decrease due to salinity stress, with the greatest reduction (18.45 and 37.85%) for plants treated with the ammoniacal form of nitrogen and the least reduction (15.86 and 26.56%) for the nitrate form, as compared to control plants at 45 DAS. The reduction at the two salinity levels was also highest (18.33 and 37.77%) for NH_4^+-N and lowest (11.68 and 22.25%) for NO_3^- -N at 65 DAS (Fig. 2C). N fertilization induced a significant rise in the stomatal conductance of stressed plants by improving the water status. The stomatal conductance was highest in plants treated with the nitrate form of nitrogen as compared to the ammoniacal form at both sampling stages. The least reduction in the stomatal conductance under saline conditions (10.54 and 21.57% at 45 DAS) was recorded at the highest rate of NO_3^- -N (120 kg ha^{-1}). At 65 DAS the least reduction (9.45 and 18.16%) in stomatal conductance was observed for the highest level of nitrate-N and the greatest reduction (15.26 and 28.05%) for the lowest level (Fig. 2C).

Discussion

Indian mustard is generally grown in arid and semi-arid regions where the groundwater used for irrigation is moderately to highly saline. Since nitrogen contributes substantially to plant growth, an understanding of the interactive effect of nitrogen forms and their combinations with a saline environment on mustard is an important practical aspect for plant growth and its relation to crop productivity. Total dry plant weight decreased at the pre-flowering and flowering stages under salinity stress. The decline in total plant dry weight was found to be greatest with the ammoniacal form and lowest with the combined form of nitrogen under saline conditions at both sampling stages (Table 1). Similar studies have shown that the dry plant weight decreased under salt stress (Abdelgadir et al., 2005). Salinity caused a clear reduction in plant biomass. The biomass production of ammonium-fed plants was lower than that of nitrate-fed plants (Rios-Gonzalez et al., 2002). The highest level of the combined form of nitrogen (120 kg ha^{-1}) significantly alleviated the deleterious effect of salt stress (8 dS m^{-1}) on total plant dry weight as compared to lower levels in the flowering stage (Table 1). It is interesting to note that the percentage reduction in dry matter production under salt stress was relatively less with the combined (NO_3^- + NH_4^+) form of nitrogen, as compared to the two individual forms. Among the different sources of N, the lowest dose (40 kg ha^{-1}) of NH_4^+-N exhibited the least ameliorating effect on growth parameters, while the highest dose (120 kg ha^{-1}) aggravated the deleterious effect of salinity (12 dS m^{-1}) at the flowering stage (Table 1). The reduction in dry matter under salinity is attributed to a decrease in metabolic activity. The impairment of the N-metabolism is one of the primary problems for plant growth under a saline environment (Nathawat et al., 2005). A decline in plant height was observed with salinity (8 and 12 dS m^{-1}) at both sampling stages, though the combined form of nitrogen (120 kg ha^{-1}) alleviated the deleterious effect of salinity on plant height at stage I (Table 2). Growth declined under saline stress but nitrate-fed plants were less sensitive to salinity than ammonium-fed plants. This different sensitivity was due mainly to the better maintenance of root growth in nitrate-fed plants (Frechilla et al., 2001).

The highest inhibitory effect of salinity (8 and 12 dS m^{-1}) on photosynthetic pigments (chlorophyll and carotenoids) was observed for the ammoniacal form and the lowest for the combined form of nitrogen at both the pre-flowering and flowering stages. The chlorophyll content of the leaves decreases in general under salt stress (Mishra et al., 2006). Significant increases in photosynthetic pigments were noticed with higher levels of nitrogen at both stages, suggesting that the adverse effect of salinity on chlorophyll and carotenoids was partially mitigated by applying the combined form of nitrogen at 120 kg ha^{-1} (Tables 3 and 4). In conformity with this, Garg et al. (1990) also reported that the levels of total chlorophyll declined under salt stress, but that nutritional improvement under both normal and saline conditions led to a higher concentration of chlorophyll.

Under saline conditions there was a substantial reduction in plant water status in terms of relative water content, water potential and osmotic potential. These parameters showed the greatest reduction in the ammoniacal form and the lowest reduction in the combined form of N as compared to non-saline plants at the pre-flowering and flowering stages (Fig. 1). N-deficiency markedly decreased the abundance of Hg-sensitive water channels (Carvajal et al., 1996), which could lower the steady-state water potential of leaves, preventing them from maintaining adequate turgor for growth (Radin and Boyer, 1982). The presence of salt in the soil solution decreased the osmotic potential of the soil, thereby resulting in water stress and making it difficult for the plants to absorb the water necessary for growth. Leaf water potential also decreased (Munns, 1993), although this decrease was accompanied by a decrease in leaf osmotic potential, so as to maintain the leaf turgor pressure of the salinised plants (Tattini et al., 1995).

It is evident from the data recorded under saline conditions that there was a decline in P_N, E and g_s as compared to the control plants. The reduction in the photosynthetic rate was relatively lower in nitrate-fed plants grown either with or without saline water irrigation compared to the ammoniacal form (Fig. 2A). Among the N forms, the increase in transpiration rate was greater for ammonium-N than for nitrate-N (Fig. 2B). A decrease in stomatal conductance was noted with increasing salinity level, showing a maximum decrease for the ammoniacal form and the least reduction for the nitrate form (Fig. 2C). Net CO_2 assimilation, stomatal conductance and transpiration rate were markedly decreased by the salt treatment (Khan et al., 1994; Tattini et al., 2002). NH_4^+-fed plants transpired more per unit leaf area and had smaller leaf mass than NO_3^--fed plants. Both facts suggest some sort of regulation of the water economy, when leaf area seems to be diminished by salinity-induced water stress and stomata continue losing water at higher rates. This phenomenon could be related to the importance of K^+ and its recirculation for N uptake and assimilation (Lips et al., 1987; Van Beusichem et al., 1988), hence the growth response and protective effect of K^+ on photosynthesis against the inhibitory effects of water stress.

Studies on the effect of improved soil fertility on RWC, water potential, osmotic potential, photosynthetic rate, transpiration rate and stomatal conductance firmly established the importance of a salinity-fertility interaction at the pre-flowering and flowering stages (Figs. 1 and 2). High values of RWC, ψ_w and ψ_s were maintained with the combined form in comparison to the ammoniacal form of N (Fig. 1). The application of N in the combined form (120 kg ha^{-1}) considerably improved the water status in terms of RWC and water potential (Fig. 1A, B). Nutritionally induced tolerance to salt stress was reported earlier in various crops (Garg et al., 1990; Khan et al., 1994). It was evident from the data that under saline conditions plants exhibited a decrease in the photosynthetic rate. The highest level (120 kg ha^{-1}) of NO_3^--N considerably improved the photosynthetic rate compared with non-saline plants, but the highest level of NH_4^+-N was found to have an inhibitory effect on the photosynthetic rate (Fig. 2A). The addition of N to plants subjected to salinity improved their growth and nitrogen-metabolizing enzymes, and thus their salt tolerance (Dubey and Pessarakli, 1995; Nathawat et al., 2005).

The transpiration and stomatal conductance decreased with increasing levels of salinity at the pre-flowering and flowering stage irrespective of the form of nitrogen (Fig. 2B, C). Among the different nitrogen forms, the transpiration rate and stomatal conductance were increased more by ammonium-N than by nitrate-N. According to Hsio and Lauchli (1986), plants showed a relatively higher transpiration rate under ammonium-N, which could be related to the depletion of other cations by the presence of an excessive concentration of ammonium (NH_4^+). This view was further strengthened in the highest ammonium-N treatment, where the plants exhibited a sharp increase in their transpiration rate (Khan et al., 1994). The nitrate form of nitrogen proved beneficial irrespective of the rate, while the ammoniacal form was beneficial up to 80 kg ha^{-1} and became inhibitory at 120 kg ha^{-1}. Besides this, the highest level of NO_3^--N (120 kg ha^{-1}) resulted in the smallest reduction in the transpiration rate and stomatal conductance under saline conditions as compared to non-saline plants (Fig. 2B, C). The present results are in accordance with other reports in which a reduction in photosynthesis was observed in plants grown in the presence of NaCl, which was attributed to stomatal resistance or to a reduction in the capacity of the photosynthetic machinery (Khan et al., 1994).

Conclusions

Plant water relations and gas exchange parameters were adversely affected by salt stress. The combined form of nitrogen (120 kg ha^{-1}) considerably improved the water status and mitigated the adverse effects of salinity. The inhibition of gas exchange parameters under salinity was partially restored with the use of different nitrogen sources. The nitrate form of nitrogen (120 kg ha^{-1}) proved better than the other two forms (combined and ammoniacal) in partially reviving the gas exchange parameters.

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