BIOLOGIA PLANTARUM 43 (4): 615-619, 2000

BRIEF COMMUNICATION

Combination effect of NaCl salinity and nitrogen form on mineral composition of sunflower plants

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Abstract

The effect of two N-forms (NH_4^+ and NO_3^-) and NaCl on pattern of accumulation of some essential inorganic nutrients was examined in sunflower (*Helianthus annuus* L.) cv. Hisun 33. Eight-day-old plants of were subjected for 21 d to Hoagland's nutrient solution containing 8 mM N as NH_4^+ or NO_3^-, and salinized with 0, 60, or 120 mM NaCl. N-form and addition of NaCl to the growth medium had no significant effect on total leaf N. However, root N of NH_4-supplied plants decreased significantly with increase in NaCl concentration, whereas that of NO_3-supplied plants remained unaffected. There was no significant effect of NaCl on leaf or root P, but the NO_3-supplied plants had significantly lower concentration of leaf P than that of NH_4-supplied plants at varying salt treatments. Salinity of the rooting medium also did not show any significant effect on Na^+ concentrations of leaves or roots of plants subjected to two different forms of N. NH_4-treated plants generally had greater concentrations of Cl^- in leaves and roots and lower K^+ content in leaves than NO_3-supplied plants. Ca^{2+} concentrations of leaves and roots and Mg^{2+} concentrations of leaves decreased in NH_4-supplied plants due to NaCl, but they remained unaffected in NO_3-treated plants.

Additional key words: ammonium, *Helianthus annuus*, mineral nutrients, nitrate, salt stress, sunflower.

Of the essential mineral nutrients, nitrogen is required in the greatest amounts by plants and is considered to be a major limiting factor to plant growth (Mengel and Kirkby 1987, Bloom 1988, Marschner 1995). NO_3^- and NH_4^+ ions are the two dominant forms of nitrogen absorbed by all types of plants (Haynes and Goh 1978, Mengel and Kirkby 1987, Salsac *et al.* 1987, Marschner 1995).

There are many studies in which it has been found that plants differ in their abilities to acquire NH_4^+ and NO_3^- from the growth medium. For example, bromegrass, maize, soybean, and sorghum differ widely in their capacity to absorb NO_3^- (Warncke and Barber 1974). Differences for NO_3^- acquisition within species have been observed in barley (Smith 1973), maize (Barber *et al.* 1992, Schortemeyer *et al.* 1993, Schortemeyer and Feil 1996) and spring wheat (Brunetti *et al.* 1972, Cox and Reisenauer 1973, Feil 1994). On the other hand, it has been shown (Ravindra and Pandey 1978, Peterson *et al.* 1988) that rice grew well with all N as NH_4^+.

Interaction of different N-forms with other ions is evident from many earlier reports. For instance, it was found that Ca, Mg and K contents in pea and cucumber were low when NH_4^+ was the sole N-source (Barker and Maynard 1972, Haynes and Goh 1978), whereas P and S concentrations were increased in maize plants relative to those in plants grown with only NO_3^--N (Blair *et al.* 1970). Many workers observed a strong interaction between uptake of Cl^- and NO_3^- (Smith and Fox 1977, Kafkafi *et al.* 1982, Glass and Siddiqi 1985).

There is considerable evidence that NO_3^- enhances translocation of cations (Wilcox *et al.* 1973, Alyemeni 1997) and NH_4^+ inhibits cation translocation (Polizotto *et al.* 1975). Tomato plants fed with NH_4^+-N were found to have reduced shoot and root contents of Ca, Mg, K, P and NO_3^-. Similarly, a depression of leaf and stem K in tomato plants was also observed (Barker and Ready 1989) when they were fed with NH_4^+. Other workers (Barker and Maynard 1972, Barker *et al.* 1966) also reported that plants grown with NH_4^+-N were lower in Ca, Mg and K than those grown with NO_3^--N.

Received 22 February 2000, *accepted* 15 April 2000.

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This depression of cation accumulation was suggested to be due to a competition between NH_4^+ and other cations.

It is now evident that salt stress has a significant effect on N nutrition in plants. For example, Heikal (1977) found that total N content of the leaves of wheat and radish was decreased significantly by salinity, whereas that of sunflower leaves was increased. Hummadi (1977) also found a decrease in total N and NO_3^- uptake with increasing soil salinity in a Mexican wheat. By contrast, some authors reported an increase in N content in plants subjected to salt stress. Lal and Singh (1973) observed depressed uptake of N, P, K and Cl but increased N and Na contents of grain and straw with increasing salinity. Similarly, Helal and Mengel (1979), working with barley, found that total N content of roots decreased with increasing salinity of the rooting medium, whereas that of the shoot increased. There is some evidence that N supplied to plants shows a considerable interaction with salinity tolerance of plants. This was ascribed to the fact that uptake and assimilation of NO_3^- and NH_4^+ have different energy requirements and to interaction between NaCl and nitrogen uptake (Leidi *et al.* 1991, Hawkins and Lewis 1993).

These contrasting reports were the basis of our hypothesis of the present study that different N-forms, NH_4^+ and NO_3^-, and salt stress have an antagonistic effect on the uptake and accumulation of N and other essential nutrients in sunflower.

The seeds of sunflower (*Helianthus annuus* L.) cv. Hisun 33 obtained from a local seed supplier were surface sterilized in 5 % sodium hypochlorite solution for 8 min. The experiment was carried out during March - May 1998 in the Botanic Garden of the Bahauddin Zakariya University, Multan (30°11'N and 71°28'E). Plants were grown in a glasshouse with natural sunlight for 11 to 13.5 h. The irradiance measured at noon ranged from 760 to 1660 μmol m^{-2} s^{-1}. The day/night temperature was 31 ± 6 and 2 3± 4 °C, respectively. Relative humidity during the day ranged from 31.5 to 46.5 %. Eight-day-old seedlings (at the first leaf stage) were transplanted into a plastic pot with aerated full strength Hoagland's nutrient solution. The experiment was arranged in a completely randomized design with four replicates, three NaCl treatments (0, 60, and 120 mM), and two nitrogen forms (8 mM NO_3^- or NH_4^+ applied as KNO_3 or $(NH_4)_2SO_4$); pH of the treatment solution was maintained at 6.4. The concentrations of K, Ca, and SO_4 were maintained uniform in treatment solutions of both N-sources. The NaCl and N-form treatments were begun 10 d after sowing. The NaCl concentration increased stepwise in aliquots of 30 mM every day until the appropriate treatment concentration was attained. The solutions were changed every other day for following 21 d and then the plants were harvested. The plants were separated into shoots and roots, washed with distilled water and blotted dry before recording the fresh masses. All plant parts were dried at 65 °C for one week, and their dry masses were measured.

Contents of Na, K, Ca, Mg, N and P in plant tissue were determined by the methods described by Allen *et al.* (1986) in fully expanded youngest leaves. Ground dry leaf and root samples (100 mg) were digested in 2 cm^3 of sulfuric peroxide mixture until a clear and almost colourless solution was obtained. After complete digestion, the volume of each sample was brought to 100 cm^3 of deionized water. Na and K were determined with a flame photometer (*PFP7, Jenway*, Dunmow, UK) and Ca and Mg with an atomic absorption spectrophotometer (*Analyst 100, Perkin Elmer*, Beaconsfield, Bucks, UK). Total P and N contents were determined by the methods described by Allen *et al.* (1986). Ground dry plant material (100 mg) of each of the two different plant parts was also extracted in 10 cm^2 of distilled deionized water at 80 °C for 4 h and Cl^- concentration in the extracts was determined with a chloride analyzer (*Model 925, Corning*, Halstead, UK). The data for all the measured parameters were subjected to analysis of variance using *COSTAT* computer package (*Cohort Software*, Berkeley, USA). The mean values were compared with least significance difference test following Snedecor and Cochran (1980).

NO_3-supplied non-salinized sunflower plants had significantly greater dry masses of shoots and roots than NH_4-supplied plants. Addition of NaCl to the growth medium caused more marked reduction in dry masses of shoots and roots in NO_3- than NH_4-supplied sunflower plants (Table 1). However, Na^+ concentrations (Table 1) in leaves and roots of both NO_3- and NH_4-fed plants increased similarly with increase in NaCl concentration of the growth medium. NH_4-supplied plants had a greater concentrations of Cl^- (Table 1) in leaves than NO_3-supplied plants at 0 and 60 mM NaCl, whereas this difference was masked at the highest NaCl concentration. No consistent pattern of Cl^- accumulation was observed in the roots of both NH_4- and NO_3-supplied plants.

While there was a large difference between NO_3- and NH_4-supplied control plants in root K^+ content, these plants did not differ significantly at NaCl treatments. Statistically significant decrease in K^+ content was induced 120 mM NaCl in roots of NH_4-supplied plants (Table 1).

Ca^{2+} concentrations in the leaves and roots (Table 1) of NH_4-supplied plants decreased significantly with increase in external NaCl concentration, whereas those in NO_3-supplied plants remained unaffected. NH_4-supplied plants had significantly lower Ca^{2+} in leaves and roots than that of NO_3-supplied plants at 60 and 120 mM NaCl.

Mg^{2+} concentration (Table 1) in the leaves of NH_4-supplied plants decreased significantly with the addition of NaCl to the rooting medium, whereas that of NO_3-supplied plants remained unaffected. NH_4-supplied plants had significantly lower leaf Mg^{2+} than that of NO_3-supplied plants at 120 mM NaCl. There was no significant effect of NaCl on root Mg^{2+} of sunflower plants, but there was an overall difference between NO_3-supplied and NH_4-supplied plants in this variable.

There was no significant effect of external NaCl on the leaf N content (Table 1) of sunflower plants, and NH_4-supplied and NO_3-supplied plants also did not differ significantly in this variable. Root N of NH_4-fed plants decreased significantly with increase in NaCl concentration of the growth medium, whereas that of NO_3-supplied plants remained unchanged. NO_3-supplied and NH_4-supplied plants differed significantly in root N only at the control treatment.

There was no significant effect of NaCl of the rooting medium on leaf P (Table 1). However, NO_3-supplied plants had significantly lower leaf P than that of NH_4-supplied plants at all external NaCl concentrations. NaCl did not affect the root P content, and NO_3- and NH_4-supplied plants did not differ significantly in this variable.

Table 1. Shoot and root dry masses [g plant^{-1}] and concentrations of different ions [g kg^{-1}(d.m.)] in leaves and roots of sunflower plants when subjected for 21 d to 0, 60 or 120 mM NaCl in Hoagland's nutrient solution containing 8 mM N as NH_4^+ or NO_3^-. Means with the same letters in each column (a-b) and each row (x-z) do not differ significantly at the 5 % level. Means without letters had LSD 5 % non-significant.

Parameter	N-forms/NaCl	Shoot/leaf			Root		
		0	60	120	0	60	120
Dry mass	NO_3-N	4.28 ± 0.59ax	1.68 ± 0.21ay	1.06 ± 0.06ay	1.31 ± 0.22ax	0.79 ± 0.06ay	0.44 ± 0.02az
	NH_4-N	2.16 ± 0.30bx	2.15 ± 0.12ax	1.41 ± 0.21bx	0.36 ± 0.02bx	0.26 ± 0.02bx	0.31 ± 0.02ax
Na^+	NO_3-N	11.76 ± 1.88	17.07 ± 0.38	23.33 ± 1.29	18.35 ± 2.47	30.70 ± 1.54	29.15 ± 1.91
	NH_4-N	15.35 ± 1.78	18.71 ± 0.66	23.11 ± 2.31	16.56 ± 1.44	20.87 ± 6.86	32.94 ± 2.63
Cl^-	NO_3-N	16.30 ± 1.93	24.96 ± 0.93	41.14 ± 5.45	18.81 ± 2.91ax	41.15 ± 4.34ay	43.45 ± 3.45ay
	NH_4-N	27.40 ± 3.69	36.11 ± 1.87	41.68 ± 4.83	27.56 ± 2.20ax	24.33 ± 9.52bx	65.28 ± 3.41by
K^+	NO_3-N	48.40 ± 5.95	52.18 ± 2.53	39.68 ± 0.91	36.93 ± 1.05ax	24.13 ± 3.02ay	21.45 ± 3.67ay
	NH_4-N	48.80 ± 3.63	40.95 ± 2.48	27.76 ± 2.78	46.33 ± 3.94bx	26.24 ± 1.71ay	17.93 ± 0.70az
Ca^{2+}	NO_3-N	70.86 ± 9.58ax	70.76 ± 9.41ax	76.24 ± 3.52ax	72.32 ± 7.66ax	82.35 ± 2.41ax	73.20 ± 7.54ax
	NH_4-N	72.34 ± 7.01ax	55.81 ± 3.92bx	38.12 ± 1.33by	64.19 ± 6.91ax	67.72 ± 4.59bx	37.19 ± 4.65by
Mg^{2+}	NO_3-N	5.31 ± 0.26ax	4.83 ± 0.08ax	5.26 ± 0.24ax	4.01 ± 0.18	3.93 ± 0.13	3.81 ± 0.47
	NH_4-N	5.17 ± 0.33ax	4.47 ± 0.26ay	3.90 ± 0.29by	3.07 ± 0.16	4.20 ± 0.53	2.17 ± 0.08
N	NO_3-N	22.90 ± 0.80	20.79 ± 3.20	24.15 ± 2.16	18.2 ± 3.28ax	15.50 ±1.55ax	16.30 ± 3.60ax
	NH_4-N	21.20 ± 1.01	24.69 ± 0.80	25.82 ± 4.41	26.2 ± 2.11bx	15.10 ± 4.66ay	15.75 ± 1.21ay
P	NO_3-N	3.35 ± 0.26ax	2.01 ± 0.05ay	3.05 ± 0.33ax	6.80 ± 0.23	7.33 ± 0.71	7.02 ± 0.92
	NH_4-N	4.32 ± 0.33bx	4.51 ± 0.40bx	3.91 ± 0.24bx	5.71 ± 0.29	7.11 ± 0.36	6.96 ± 0.63

Significant interaction between NaCl and N-forms were found for growth and contents of different ions. This can be related to the evidence that N form supplied to plants has a considerable interaction with salinity tolerance of plants (Leidi *et al.* 1991, Hawkins and Lewis 1993). This was ascribed to the phenomenon that uptake and assimilation of NO_3 and NH_4 have different energy requirements and to interaction between NaCl and N uptake.

In this study NaCl or different N-forms had no significant effect on total N of leaves, whereas root N of NH_4-supplied plants decreased significantly with increase in external NaCl concentration, and these plants had significantly higher total N in roots than NO_3-supplied plants under non-saline conditions. The results for leaf N cannot be explained in the light of earlier studies. For instance, Heikal (1977) found a decrease in total N of the leaves of wheat and radish due to salt stress, whereas the reverse was true in sunflower. Hummadi (1977) also found a depression in total N in the leaves of Mexican wheat due to salt stress. The higher total N in the roots of NH_4-supplied plants as compared with that of NO_3-supplied plants under non-saline medium may have resulted due to the fact that NH_4 is mainly absorbed passively like other monovalent cations, whereas NO_3 absorption is an active and energy requiring process (Swader *et al.* 1975, Mengel and Kirkby 1987). Ammonium, once absorbed, is rapidly assimilated into organic compounds but the assimilation of NO_3 costs a large amount of energy (Salsac *et al.* 1987). In addition, Lewis and Chadwick (1983) reported that the shoot is the main organ of NO_3 assimilation and the root is the major organ of NH_4 assimilation in barley plants. Similar results were found in maize (Murphy and Lewis 1987).

Despite N, other ions determined in the present study also show a considerable interaction with different N-forms of the growth medium. For instance, it is evident that K, Ca and Mg contents in leaves decreased considerably in NH_4-supplied plants, whereas these contents remained almost unchanged in NO_3-supplied plants under NaCl treatments. By contrast, the pattern of accumulation of P in leaves was reverse from that of

K, Ca and Mg in NH_4-supplied plants. These results are in close conformity with earlier studies in which K, Ca and Mg contents in pea and cucumber plants were low when they were fed with only NH_4-N (Barker and Maynard 1972, Haynes and Goh 1978), whereas P and S contents were increased in maize relative to those in plants grown with only NO_3-N (Blair *et al.* 1970). However, the reduction in cation uptake in NH_4-supplied plants can be explained in view of the findings that NH_4 inhibits the translocation of cations (Polizotto *et al.* 1975, Mengel and Kirkby 1987). Cl^- concentrations in the leaves or roots of NO_3-supplied plants were lower than that in NH_4-supplied plants under non-saline conditions, and the highest NaCl treatment of the growth medium caused greater accumulation of Cl^- in the roots of NH_4-supplied plants than in that of NO_3-supplied plants. These results can be partly explained in the light of some earlier studies in which a considerable interaction between uptake of Cl^- and NO_3^- ions was noted (Smith and Fox 1977, Kafkafi *et al.* 1982, Glass and Siddiqi 1985). They suggested that NO_3^- and Cl^- ions are mutually inhibitory for each other.

In conclusion, there was a considerable interaction between two N-forms and salt stress in relation to growth and accumulation of different inorganic nutrients.

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