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# PHOTOSYNTHATE PARTITIONING IN WHEAT (*TRITICUM AESTIVUM* L.) AS AFFECTED BY ROOT-ZONE SALINITY AND FORM OF N

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**Abstract**

Carbon-14 pulse labeling technique was used to study the effect of rooting medium salinity and form and availability of N on growth and rhizodeposition of wheat (*Triticum aestivum* L.). Of the applied $^{14}C$ pulse, 83% was determined in plants, while 89% and 11% was determined in the shoot and root portions, respectively. Salinity showed a depressing effect on different plant parameters particularly on roots. However, $NO_3^-$-fed plants showed better growth than $NH_4^+$-fed plants at all the three salinity levels.

Presence of NaCl in the rooting medium led to a decrease in the water content of both root and shoot portions. The proportion of assimilated $^{14}C$ released into the rooting medium as rhizodeposits varied between 1.5 and 3.2%, while 8-13% was unaccounted for and assumed to be respired. Rooting medium salinity led to higher rhizodeposition and lower loss of $^{14}C$. Relatively higher proportion of $^{14}C$ was released as rhizodeposits and retained in root and shoot portions of plants fed with $NH_4^+$ or $NH_4^+$+ $NO_3^-$ than those with $NO_3^-$ while less was respired. The specific activity of the rhizodeposits was also higher under saline conditions. The rhizodeposits in $NH_4^+$-fed plants were more highly labeled as compared to $NO_3^-$-fed plants.

## Introduction

Form and availability of N are important factors governing plant growth and productivity. Although majority of the plants grow best with a mixture of $NH_4^+$ and $NO_3^-$, the former may cause growth inhibition in many species when supplied as the exclusive N source (Cramer & Lewis, 1993; Lang & Kaiser, 1994; Marschner, 1999). The inhibitory effects of $NH_4$ are more severe for plants grown under saline conditions (Lewis *et al*., 1989; Speer *et al*., 1994; Khan *et al*., 1994). However, $NH_4^+$ should generally be considered as the predominant form of N in saline soils due to inhibition of nitrification (Westerman & Tucker, 1974; Gandhi & Paliwal, 1976). The plant species capable of growing under saline conditions may have evolved both morphological and physiological adaptations for uptake and assimilation of $NH_4^+$ (Martins-Loução *et al*., 2000). The mechanism involved that not only help plants meet extra demands of energy for $NH_4^+$ assimilation but will also exert a beneficial effect on rhizospheric microbial functions including immobilization-remineralization turnover and nitrification.

In qualitative as well as quantitative terms, rhizodeposition is a function of both rhizospheric and atmospheric factors (Høgh-Jensen & Schjoerring, 2001; Shaw & Burns, 2005). Annual plants grown under arable conditions are reported to transport 30-50% of the photosynthetic C below-ground during their life cycle (Swinnen *et al*., 1994; Domanski *et al*., 2001). In fact, almost all the organic C found in soil is primarily plant-derived in the form of root/shoot residues and root exudates (Kuzyakov & Domanski, 2000 & 2002). A significant proportion of the rhizodeposits is lost through rhizospheric respiration that may represent 51 to 89% of the total $CO_2$ efflux from soil; half of this

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coming from root respiration (Kuzyakov *et al*., 1999). Kuzyakov & Domanski (2000 & 2002) reported that of the total C translocated below-ground, 7-13% is ultimately found in roots, 2-5% exuded and 7-14% used up in root respiration for the maintenance, root growth and ion uptake.

Under saline conditions, rhizodeposition is reported to increase due to salt-induced leakiness of cells (Vieira Santos *et al*., 2001). Likewise, $NH_4^+$-fed plants are also reported to exude more C into the rhizosphere (Trolldenier & von Rheinbaban, 1981; Giordano *et al*., 1994). Therefore, under saline conditions rhizodeposition may be enhanced both due to salinity and prolonged presence of $NH_4^+$. However, to our knowledge such interactions *vis-à-vis* rhizodeposition have not been reported. Our objective was to study the interactive effects of salinity and forms/availability of N on rhizodeposition under hydroponic conditions.

## Material and Methods

Wheat seeds of approximately same size were surface sterilized by 1-min., shaking in ethanol followed by 3 rinses with sterile distilled water and planted in wet autoclaved (15 minutes, 15 lb pressure) sand soaked in Hoagland solution (Arnon & Hoagland, 1940). Sand moisture content was maintained at 15% (w/w) with sterilized distilled water before transplanting the seedlings (10 days after germination) to pots containing Hoagland nutrient medium. The experimental setup consisted of 30 cm deep plastic pots 10 cm in diameter and wrapped in black paper to keep the roots in darkness, air distribution system consisting of 20-mm diameter PVC pipe with holes at regular intervals to accommodate tightly-fitting silicon tubing (3-mm diameter), and a diaphragm pump to push the air through the rooting medium. This system provided sufficient aeration in the root zone to inhibit bacterial growth. Half-strength Hoagland nutrient medium was filled in plastic pots (1 lit. $pot^{-1}$) and three 10-days old seedlings held in polystyrene discs were transferred to each of the 112 pots. This setup allowed continuous submergence of roots in the rooting medium. Before transfer, the seedling roots were washed free of any adhering sand with running tap water.

At 30$^{th}$ day after transplanting, the pots were transferred to polyethylene chamber (2.25 $m^3$) provided with $^{14}CO_2$ generator that consisted of a burette containing $^{14}C$-labelled Sodium carbonate ($^{14}C$, 140 μCi; $CO_3$-C, 470 mg), a magnetic stirrer, a 250-ml beaker containing 50-ml lactic acid and a magnet bar, a fan to facilitate mixing of newly generated $^{14}CO_2$, brass tubing through which chilled water was circulated to keep the chamber temperature at around 20-25$^o$C, a glass trough containing a sheet of filter paper to be soaked in 10% NaOH solution for trapping residual $^{14}CO_2$ and a silicone tube (the protruding end was plugged with a rubber bung) piercing through the polyethylene cover was left in the trough to be used for pouring NaOH solution after competing the pulse labeling protocol. Polyethylene chamber was tightly sealed after completing the interior setup.

For generating $^{14}CO_2$, $Na_2{}^{14}CO_3$ solution was allowed to flow through the burette (stopcock was pre-adjusted such that the liquid flowed drop-wise) into the lactic acid-containing beaker, which was continuously being stirred. Efficiency of $CO_2$ generation using such an arrangement is reported to vary between 70 and 95% (Kuzyakov & Domanski, 2002). In the present study, $Na_2CO_3$ used was sufficient to approximately double the concentration of $CO_2$ in the chamber atmosphere and was considered necessary to maintain $CO_2$ for higher efficiency of photosynthesis. After 24 hrs exposure

to $^{14}CO_2$ atmosphere, 500-ml of 10% NaOH solution was allowed to flow into the trough that contained a sheet of filter paper to increase the surface area for absorbing $CO_2$. After 4 hrs of this intervention that was assumed sufficient to absorb the entire $^{14}CO_2$, the chamber was dismantled. Filtre paper sheet was shredded and allowed to stay in NaOH solution with continuous stirring for 2 hrs. This exercise was assumed to result in a homogenized suspension a portion of which was centrifuged. Aliquot of the supernatant was subjected to scintillation counting.

Plants from four pots placed in the chamber at different positions were removed, weighed fresh, sand-witched in blotting papers, dried to a constant weight, and preserved as such for autoradiography. After autoradiography, root and shoot portions were separated, finely powdered and aliquots analyzed for total C and $^{14}C$. These determinations served as baseline for subsequent study on the distribution of initially assimilated $^{14}C$ in root and shoot portions and in the rhizodeposits. The proportion of $^{14}C$ not accounted for in these pools was considered as lost due to rhizorespiration.

The rooting medium in the remaining 108 pots was replaced with Hoagland solution such that all possible combinations (36 in total) of the 3 variables were obtained; each treatment combination being in triplicate. The variables were i) 0, 150, and 300 mM NaCl, ii) N as calcium nitrate, Ammonium sulphate, Ammonium nitrate] and iii) N in either form equivalent to 0.5, 1, 1.5, and 2.0 times the N concentration of Hoagland solution. The rooting medium was continuously aerated using the setup described above. After 7 days, green-ness of the leaves was measured using a Minolta chlorophyll meter and plants removed from the pots. Root length (longest root), and fresh weight of root and shoot portions was recorded, the material dried to a constant weight at 65$^oC$ in filter paper sandwiches and preserved for $^{14}C$ measurements. Entire volume of rooting medium was filtered, stored at -20$^oC$, freeze-dried, and aliquots analyzed for total C and $^{14}C$ and the values ascribed to rhizodeposits.

Total C was determined by a wet oxidation method (Azam & Sajjad, 2005). For the determination of $^{14}C$, appropriate amounts of freeze-dried rooting medium and plant material were combusted in a biological oxidizer followed by liquid scintillation counting using Packard Tricarb LSC-4550 (counting efficiency, 94%). Specific activity of the rhizodeposits was measured as kBq $^{14}C$ $g^{-1}$ C.

Standard error and coefficient of correlations were calculated using Microsoft Excel software. Significance of treatment mean differences was determined using the SAS statistical package (Anon., 1998).

## Results

Autoradiography was performed mainly for qualitative purposes to observe the distribution of $^{14}C$ in shoot and root portions (Fig. 1). Plants harvested immediately after pulse labeling showed a dense darkening of the leaves and roots. Shoot portion appeared to be relatively uniformly labeled. The roots showed more darkening in some regions probably due to the short-term accumulation of $^{14}C$-labelled compounds for subsequent release as rhizodeposits. Alkali, used to absorb residual $^{14}C$ in the chamber (after 24 hrs of exposing plants $^{14}CO_2$) contained *ca* 17% of the applied $^{14}C$ suggesting an incorporation of 83% in plants. Of the total plant $^{14}C$, 89% and 11% was determined in the shoot and root portions, respectively. Relative distribution of $^{14}C$ in shoot and root portions was in fair agreement with the autoradiography results where the shoot was more heavily labeled as observed by the intensity of blackening.

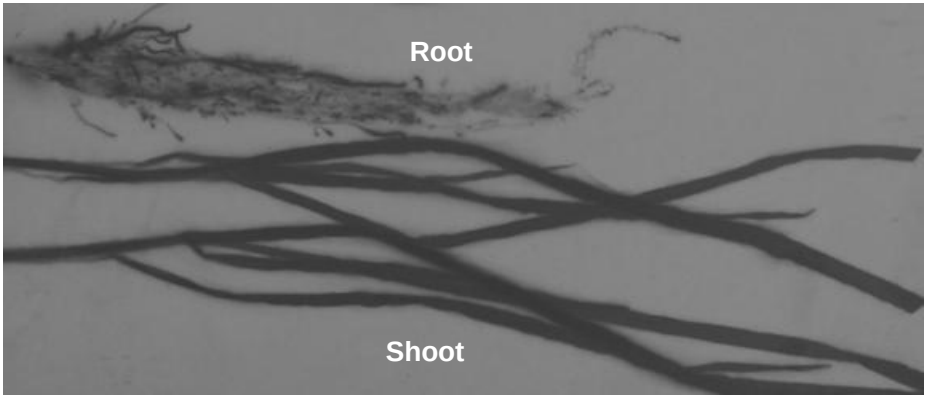


Fig. 1. Autoradiogram of root and shoot portions of wheat immediately after pulse labeling (24-h exposure to $^{14}CO_2$ environment).

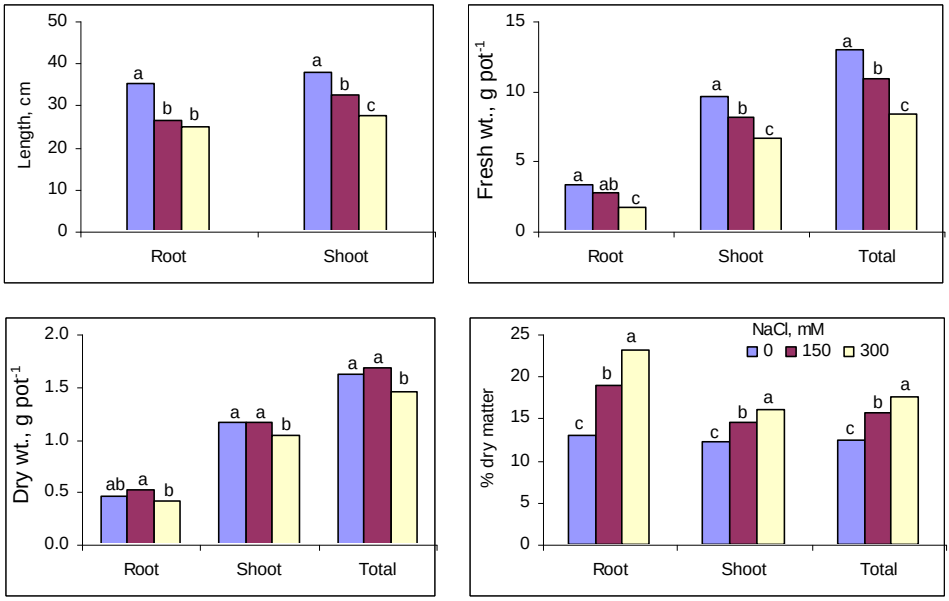


Fig. 2. Effect of NaCl salinity on different plant growth parameters. All values areaverage of different forms and levels of N. The bars within a parameter sharing similar letter are not significantly different from each other at p=0.05.

The effect of NaCl salinity on different plant parameters presented as average of different forms and concentrations of N in the rooting medium are shown in Fig 2. Both root and shoot lengths were significantly reduced under saline conditions. The data showed reductions of 29.0% and 26.7% in the root and shoot lengths respectively at 300 mM NaCl compared to untreated control. Reduction in fresh weight of both plant components (roots and shoots) due to NaCl was also significant and amounted to 46.8% and 31.2% in root and shoot portions respectively at the highest level of salinity as compared to control. Total plant biomass was 15.7% and 35.2% of the control (0 mM

NaCl) at 150 and 300 mM NaCl respectively. Changes in dry weight of root and shoot portions were not very clear but % dry matter content showed a significant increase under saline conditions and thus a decrease in water content of the plant tissues.

The effect of salinity (values averaged over different forms and levels of N) on the percent distribution of assimilated $^{14}C$ in different pools and specific activity of rhizodeposits are shown in Table 1. Relatively higher proportions of $^{14}C$ were retained in both root and shoot portions under saline conditions as compared to control and showed increasing trend with the increase in salinity level. However, the differences due to salinity were non significant. During 7 days of plant growth at 0, 150 and 300 mM NaCl, 2.4, 2.6 and 2.9% of the assimilated $^{14}C$ got accumulated in the rooting medium as rhizodeposits suggesting an increase in salinity-induced rhizodeposition. The highest increase was observed at 300 mM NaCl salinity level as compared to control (no NaCl). Loss of $^{14}C$ through respiration was 19.8, 19.1 and 15.1% at 0, 150 and 300 mM NaCl, respectively. The specific activity of the rhizodeposits (kBq $^{14}C$ $g^{-1}$ C) was also higher under saline conditions showing an average for different N treatments of 186.4, 196.6 and 251.9 kBq $g^{-1}$ C at 0, 150 and 300 mM NaCl salinity levels, respectively. The rhizodeposits showed high labeling of $^{14}C$ at 300 mM as compared to control.

The effect of form of N on the percent assimilated $^{14}C$ determined in roots, shoots, rhizodeposits, respired and specific activity of rhizodeposits are given in Table 2. Root portion showed relatively higher proportion of $^{14}C$ in $NH_4^+$- compared to $NO_3^-$-fed plants (36 *vs.* 34 % of the assimilated $^{14}C$ respectively) but the difference was non-significant. Similar was true for shoot portion that contained *ca* 44% of the assimilated $^{14}C$. Rhizodeposition was significantly more in $NH_4^+$- compared to $NO_3^-$-fed plants and showed a concomitant increase or decrease with the concentration of N. Loss of $^{14}C$ (presumably through respiration) was more in $NO_3^-$-fed plants. The effect of $NH_4^+$ was not mitigated to a significant extent by the presence of $NO_3^-$ i.e., in the rooting medium containing Ammonium nitrate. A higher proportion of $^{14}C$ seemed to have been transported from shoots to the roots with increasing amounts of $NO_3^-$ in the rooting medium; a reverse being true for $NH_4^+$ and $NH_4^+ + NO_3^-$ (data not shown). The rhizodeposits in $NH_4^+$-fed plants were more highly labeled as compared to $NO_3^-$-fed plants as the specific activity in the presence of $NH_4^+$, $NO_3^-$ and $NH_4^+ + NO_3^-$ was observed in the order of 137.9, 269.6, and 267.4 kBq $g^{-1}$ C respectively.

Promotion or inhibition of growth was observed from the effects of N concentration on length and mass of root and shoot portions. The plant parameters generally showed a significant increase with $NH_4^+$-N and a decrease with $NO_3^-$-N concentration. When averaged over different N concentrations, root length was significantly higher (37%) in $NO_3^-$-fed plants as compared to $NH_4^+$-fed plants. Shoot length was also affected positively with increased concentration of $NO_3^-$ except that at highest level of N. Increasing concentration of $NH_4^+$ showed a negative effect. Fresh weight of both root and shoot portions was higher in $NO_3^-$- compared to $NH_4^+$-fed plants. When both $NH_4^+$ and $NO_3^-$ were present, the weight was better than $NH_4^+$ but less than $NO_3^-$ treatment (Calcium nitrate in the rooting medium). In $NO_3^-$ alone treatment, concentration of N showed no effect on fresh weight of shoot but negative effect was observed for the other two N treatments i.e., $NH_4^+$ and $NH_4^+ + NO_3^-$.

Form of N had a significant bearing on different plant parameters (Fig. 3) with $NO_3^-$-fed plants showing better growth than $NH_4^+$-fed plants (averaged over NaCl levels). The roots were affected by the form of N as they were healthier in $NO_3^-$-fed plants but showed visible symptoms of deterioration in $NH_4^+$-fed plants. The leaves of $NO_3^-$-fed plants were greener as compared to those of $NH_4^+$-fed plants (data not shown).

**Table 1. Percent recovery of assimilated $^{14}C$ in different components as affected by NaCl salinity.**

| Component | Concentration of NaCl (mM) | | |
|---|---|---|---|
| | 0 | 150 | 300 |
| Root | 34.75[a] | 34.94[a] | 36.20[a] |
| Shoot | 43.08[a] | 43.37[a] | 45.81[a] |
| Respired | 19.79[a] | 19.09[a] | 15.09[b] |
| Rhizodeposits | 2.39[b] | 2.61[b] | 2.90[a] |
| Sp. activity of rhizodeposits, μCi $g^{-1}$ C | 186.4[c] | 196.6b | 251.9[a] |

Same superscripts in the same row do not differ at p=0.05

**Table 2. Percent recovery of assimilated $^{14}C$ in different components as affected by form of N in the rooting medium during 7 days of growth after pulse labeling.**

| Component | Form of N | | |
|---|---|---|---|
| | Calcium nitrate | Ammonium sulphate | Ammonium nitrate |
| Root | 33.90[a] | 36.00[a] | 35.98[a] |
| Shoot | 43.44[a] | 44.75[a] | 44.06[a] |
| Respired | 20.33[a] | 16.45[b] | 17.19[b] |
| Rhizodeposits | 2.33[b] | 2.80[a] | 2.77[a] |
| Sp. Activity of rhizodeposits, μCi $g^{-1}$ C | 137.90[b] | 269.60[a] | 267.40[a] |

Same superscripts in the same row do not differ at p=0.05

## Discussion

Under saline conditions, growth of most of the plants is inhibited albeit to a variable extent (Lewis *et al*., 1989; Speer & Kaiser, 1994). This inhibition is attributed to several factors dominant amongst which are water relations and specific ion effects. However, the reported leakiness of roots under saline conditions (Meharg & Killham, 1991; Vieira Santos *et al*., 2001) and the role of cellular material thus released in affecting rhizospheric microbial functions will have a significant influence on the plant growth and productivity. Form and availability of N further add to these effects since nitrification is retarded under saline conditions (Gandhi & Paliwal, 1976; Westerman & Tucker, 1974), leaving the plants with $NH_4^+$ as the predominant source of N. In the present study, rooting medium salinity showed in general a growth retarding effect. Besides other reasons, salinity of the rooting medium led to a decrease in water content of both root and shoot portions (and thus higher dry matter percentage). This would have affected normal physiological functioning of the plants including photosynthesis and respiration. A balance of assimilated $^{14}C$ after 7 days of plant growth in rooting medium of different salinity and N combinations showed lesser proportion of $^{14}C$ to be respired at 300 mM NaCl compared to other treatments while more was retained in the root and shoot portions. Meharg & Killham (1991) also reported a higher retention of $^{14}C$ in root and shoot portions under saline conditions. Since rooting medium was not sterilized in the present studies, lower respiration under saline conditions could be attributed partly to reduced microbial decomposition of rhizodeposits.

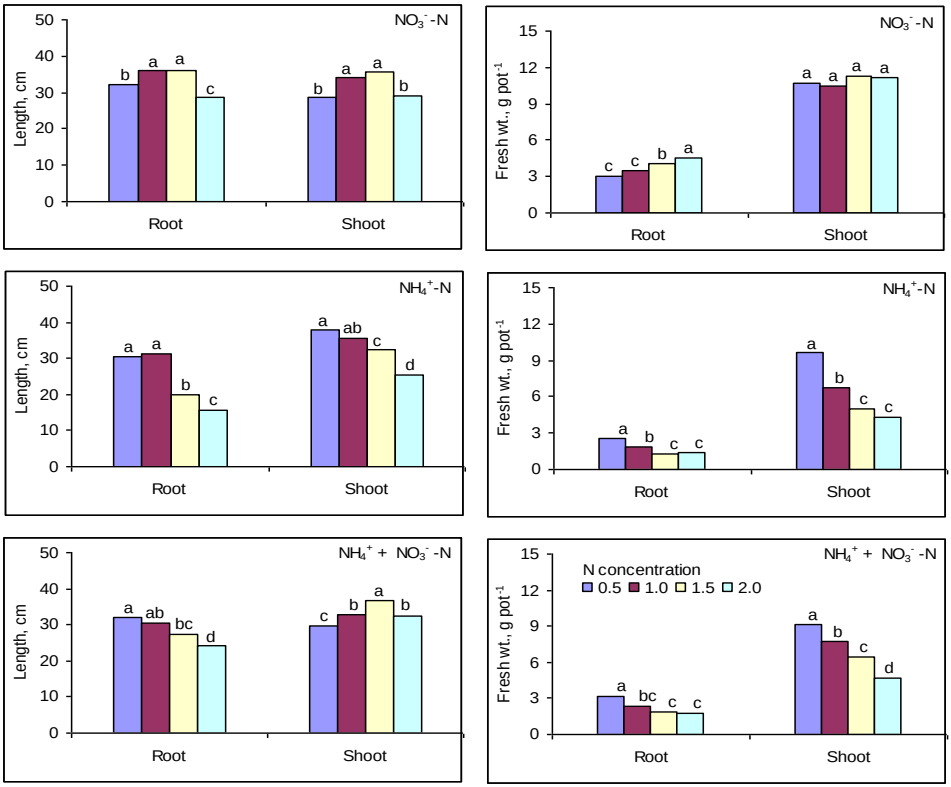


Fig. 3. Effect of form and concentration of N on different plant growth parameters. All values are average of different forms and levels of N. N concentration: half (0.5), equivalent (1.0), 1.5 times (1.5) or twice (2.0) the concentration in Hoagland nutrient solution. The bars within a parameter sharing similar letter are not significantly different from each other at p=0.05.

Negative effect of salinity on plant growth was substantiated by $NH_4^+$ in the present study, while $NO_3^-$-fed plants maintained higher growth at both the levels of NaCl. Higher amounts of $NH_4^+$ showed strong inhibitory effect on plant growth, particularly the elongation and dry matter of roots. The roots were lean and showed visible damage that could be one of the reasons for higher rhizodeposition through release of cellular material under saline and $NH_4^+$-supplemented conditions. Growth inhibition has been reported in many species receiving $NH_4^+$ as the exclusive N source (Cramer & Lewis, 1993; Lang & Kaiser, 1994; Marschner, 1999), while interactions of the two N forms with salinity are also well documented. Ammonium nutrition was found to increase the sensitivity of plants to salinity (Lewis *et al*., 1989; Speer & Kaiser, 1994), whereas $NO_3^-$ has been reported to decrease the negative effects of salinity (Khan *et al*., 1994). Similar effects observed on pea seedlings were attributed to a rapid uptake and accumulation in leaf cells of $Cl^-$ leading to a breakdown of metabolism (Speer *et al*., 1994). $NH_4^+$ may also impair uptake of essential nutrient cations, especially of $Mg^{++}$ and $Ca^{++}$. In alfalfa, photosynthesis was reduced more in $NH_4^+$- than in $NO_3^-$-fed plants in the presence of ions (Khan *et al*., 1994). In the present study, $NH_4^+$ had an inhibitory effect on tissue water content that could retard normal physiological functioning and thus a lower dry matter accumulation of $NH_4^+$-fed plants.

One of the important reasons of reduced plant growth under stress situations could be the significant increase in the rhizodeposits as observed in the present study (*ca* 21% more at 300 mM NaCl compared to control (values averaged for form and availability of N) and those reported by others (Jones & Darrah, 1995). On the short-term basis this increase in rhizodeposition may not seem substantial but would be significant when entire crop growth period is considered. The increase in rhizodeposition suggests disruption of cell walls and membranes making the root cells leaky. Salinity induced cellular leakiness has been reported (Meharg & Killham, 1991; Vieira Santos *et al*., 2001). This will not only impair the normal root functioning in terms of water and nutrient flow (Dakora & Philips, 2002), but could have implications for the rhizospheric microbiology and microbial processes under natural plant growth conditions (Breland & Bakken, 1991). In this study, up to 3% of the assimilated $^{14}C$ was deposited in the rooting medium during 7 days after applying the labelled Sodium carbonate. The cumulative values of rhizodeposits could be substantial (30-50% of the photosynthetic C) during the life cycle of annual crops (Domanski *et al*., 2001; Swinnen *et al*., 1994). In fact, within days a significant proportion of the photosynthates are transported to roots and rhizodeposits (Kuzyakov *et al*., 2001; Wlofgang *et al*., 1999). The rhizodeposits may contain sugars, organic acids and low molecular weight polysaccharides (Kraffczyk *et al*., 1984; Kuzyakov *et al*., 2002) but were not characterized in the present studies.

In the present study, $NH_4^+$-fed plants showed a higher rhizodeposition as compared to $NO_3^-$-fed plants suggesting that $NH_4^+$ had an additive effect with salinity on making the root cells leaky. Lewis *et al*., (1989) reported that $NH_4^+$-fed wheat plants allocated 36% more C to the roots than $NO_3^-$-fed plants. This may be necessary to meet the enhanced carbohydrate/energy demands at the root level for efficient $NH_4^+$ assimilation. Greater specific activity of the rhizodeposits in $NH_4^+$-fed plants suggested a quick response of plants to rooting-medium conditions in terms of reallocation of photosynthates. With cells becoming leaky, a part of the photosynthates translocated to the roots was released into the rooting medium. Under hydroponic conditions, bacterial abundance at barley roots was found to increase at higher $NH_4^+$-N levels and could be attributed to increased rhizodeposition as measured by $^{14}C$ methods (Liljiroth *et al*., 1990). Christiansen-Weniger & van Veen (1991) and Martins-Lucao *et al*., (2000) suggested that cracks developed by greater root branching and root initials in response to $NH_4^+$ were responsible for higher rhizodeposition. Under natural conditions with soil as the rooting medium, the compounds released will have a profound effect on microbial population and their activities and consequently on plants. For example, microbial immobilization of essential nutrients may be enhanced and more so as $NH_4^+$ is known to be a preferred source of N for microbes (Azam *et al*., 1993; Jansson, 1958). In some previous studies on wheat, inhibition of nitrification in soil and hence prolonged availability of $NH_4^+$ was found to cause significant inhibition of wheat growth (Lodhi *et al*., 1998). These observations were attributed mainly to changed water relations of plants as hardly any cuticular wax was observed on the leaves grown under conditions inhibitory to nitrification.

In conclusion, root zone salinity had a retarding effect on plant growth as generally reported but it caused an increase in rhizodeposition as well. A significantly higher $^{14}C$ labeling (specific activity) of the rhizodeposits under saline conditions suggested a rapid transport and release into the rhizosphere of recently assimilated C. $NH_4^+$ in the rooting medium had an inhibitory effect on plant growth that increased with the concentration but it caused a higher and rapid transport into the rooting medium of recently synthesized

photoassimilates. Root damage accompanied by increased leakiness of root cells was assumed to be the possible reason for enhanced rhizodeposition with implications for rhizospheric microbial functions and nutrient uptake by plants under saline conditions.

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