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Sugar exudation by roots of kallar grass [*Leptochloa fusca* (L.) Kunth] is strongly affected by the nitrogen source

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Abstract Exudation of sugars (glucose, fructose and sucrose) and that of cations and anions from intact roots of kallar grass [*Leptochloa fusca* (L.) Kunth] grown hydroponically with ammonium or nitrate (3 mM) as N source was investigated. In different experiments, plants grown on ammonium had slightly higher sugar contents than nitrate-grown plants, but their total sugar exudation during a 2-h period was up to 79-fold higher than under nitrate nutrition. Relative root exudation of inorganic anions and cations and that of amino acids (as a percentage of the internal contents exuded per time) was either similar or slightly higher from ammonium-grown than from nitrate-grown plants. Analysis of root architectural parameters revealed that ammonium-grown plants had a higher number of root tips/side roots per gram root fresh weight than nitrate-grown plants, whereas other root parameters, viz. length, diameter, volume and surface area were similar under the two N sources. A majority of the fine roots having diameter up to 0.4 mm represented up to 86% of the total root length, 64% of the total root surface area, and 35% of the total root volume; the root length and surface area per root system of that major root population were similar in ammonium- and nitrate-grown plants. Apparently, root architecture was not responsible for the different exudation rates. Within 12–24 h after shifting ammonium-grown plants to nitrate nutrition, root sugar levels and visible root architecture remained unchanged, yet the sugar exudation rate was reduced 30-fold. Short-term uptake of [^{14}C]glucose (10 μM) from the rooting medium was similar for ammonium- and nitrate-grown plants. Thus, the very different sugar exudation rates were neither related to internal root sugar concentration, nor to the different root architecture, nor to differential resorption of sugars by ammonium- versus nitrate-grown plants. Increased external Ca^{2+} did not alter sugar exudation, and decreased external pH (4.5) only slightly increased sugar exudation from roots of nitrate-grown plants kept at pH 6.5. It is suggested that the much higher sugar exudation in response to ammonium may facilitate the ecologically and economically important association of diazotrophs with kallar grass roots.

Keywords Ammonium · *Leptochloa* · Nitrate · Root (exudation, architecture) · Sugar exudation

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Introduction

The amount of primary root-generated C compounds released into the rhizosphere may comprise up to 20% of the net CO_2 assimilation of plants (Biondini et al. 1988; Wolfgang et al. 1999), and under special conditions even higher values have been reported (see below). Sugars, organic acids and amino acids are considered to be the major constituents of the low-molecular-weight root exudates, with the proportion of sugars being the highest (65%) and amino acids the lowest (2%; Kraffczyk et al. 1984). Factors governing the qualitative and quantitative release of root exudates include: the species and developmental stage of plants, soil physical stress, plant nutrition, mechanical or disease injury, herbivory, foliar-applied chemicals, and the presence of microbes (Marschner 1995; Holland et al. 1996).

The nutrient status of plants not only affects the overall growth but also the root cell-membrane permeability and levels of sugars, amino acids and inorganic ions in the roots (Marschner 1995). It is, therefore, logical to relate the plant nutritional status to the quality and quantity of root exudates. However, it is not well

understood whether exudation of different compounds is influenced by their content in roots, or due to changed root cell-membrane permeability or root architecture. In P-deficient *Pinus radiata*, loss of amide and amino acids from roots increased due to excess free amide/amino-N in the roots, whereas an opposite trend was observed under N-deficiency, indicating a concentration-dependent effect (Bowen 1969). Proteoid roots of white lupin predominantly exude citric acid (up to 23% of acquired carbon) into P-deficient soils, which helps to mobilize phosphate (Dinkelaker et al. 1989). Aluminium is known to stimulate root exudation of organic acids, which plays a key role in the avoidance strategy of Al-tolerant cultivars of wheat (Delhaize et al. 1993). Increased sugar exudation from peanut roots at low external Ca^{2+} supply (0.25 mM Ca^{2+}) was attributed to increased root cell-membrane permeability (Shay and Hale 1973). On the other hand, Rovira (1959) did not find any consistent effect on the release of amino acids from roots of tomato, clover, and canary grass grown with Ca^{2+} levels ranging from 0.5–50 mM.

Ammonium or nitrate as N source has a strong impact on the uptake of other ions, cellular pH regulation, rhizospheric pH and thus on overall cell metabolism (Marschner 1995). Although considerable information has been generated regarding the effect of N source on various aspects of plant growth and metabolism (Chaillou et. al. 1991; Cramer and Lewis 1993; Lang and Kaiser 1994; Martins-Loucao et al. 2000), reports concerning its role in carbon and nitrogen partitioning in the rhizosphere are scanty. Higher root respiration due to ammonium rather than nitrate nutrition was partly attributed to increased root exudation and hence stimulation of bacterial growth (Trolldenier and von Rheinbaban 1981). Ammonium nutrition rather than nitrate nutrition is reported to increase root exudation by wheat roots, leading to increased lesion severity of the take-all fungus *Gaeumannomyces graminis* (Brown and Hornby 1987).

There are several reasons to investigate the impact of the nitrogen source on root exudation:

i. Roots of ammonium-grown plants invariably possess higher amino acid content than nitrate-grown plants (Cramer and Lewis 1993), though sugar levels may either be lower (Matsumoto and Tamura 1981) or higher (Martins-Loucao et al. 2000). Therefore, considering the concentration-dependent effects, ammonium-grown plants may differ from nitrate-grown plants in root exudation, at least with respect to sugars and amino acids.
ii. Roots of plants grown under ammonium as the sole N source are reported to have higher branching (Martins-Loucao et. al. 2000). Considering the major sites of exudation, either root apices or the points of lateral root emergence (Frenzel 1960; Schroth and Snyder 1961; van Egeraat 1975), differences in root exudation may be expected due to changed root architecture in response to nitrogen source.
iii. Excretion of the nitrogen fixed by diazotrophs and its availability to the host plant in the form of ammonium (Christiansen-Weniger and van Veen 1991) may lead to speculation that ammonium serves as a signal in triggering the exudation from host roots to fulfil the carbon requirements of diazotrophs. However, experimental evidence for such a role of ammonium is less well documented, though increased root exudation due to inoculation with diazotrophs such as *Azospirillum brasilense* has been attributed to increased root permeability (Venkateswarlu and Rao 1985).

The present paper reports the effect of nitrogen source, either ammonium or nitrate, on short-term exudation of sugars from intact roots of kallar grass [*Leptochloa fusca* (L.) Kunth]. Kallar grass is highly tolerant to waterlogging and salinity, and is proposed as the primary colonizer for biological amelioration of salt-affected lands (Sandhu and Malik 1975). Because of the inhibitory effect of salinity on nitrification (Sethi et. al. 1993), and the presence of associative N_2-fixers around its roots (Bilal and Malik 1987; Bilal et al. 1990), ammonium may be considered as the major N source for kallar grass.

Materials and methods

Plant cultivation

Seeds of kallar grass [*Leptochloa fusca* (L.) Kunth] were collected from saline lands at the Bio-Saline Research Substation-II (Pucca Anna) of NIAB, Faisalabad. Seeds were germinated in moist sand and supplied with half-strength pre-culture nutrient solution. Seedlings about 6 weeks old were transferred on to PVC plates (four plants each) provided with 8-mm-diameter holes, which could hold seedlings with the aid of a short piece of silicone tubing. These plates were held on 1.8-l plastic pots containing half-strength pre-culture nutrient solution. The pre-culture nutrient solution consisted of 3 mM KNO_3, 2 mM $MgSO_4$, 4 mM $CaCl_2$, 4 mM KCl, 2 mM KH_2PO_4, 126 μM NaFeEDTA, and trace elements according to Johnson et al. (1957). After 10 days, the plants were transferred to 100% nutrient solutions with composition similar to the pre-culture solution except the N source, which was either 3 mM NH_4Cl ('ammonium plants') or 3 mM KNO_3 ('nitrate plants'). The initial pH values for ammonium and nitrate nutrient solutions were 6.5 and 5.5, respectively. Fluctuations in the pH were adjusted daily and nutrient solutions replaced every second day. Unless otherwise indicated, plants remained under treatments for 4 weeks. The plants were grown in a glasshouse under semi-controlled conditions with 12 h daylength and under a photosynthetic photon flux density varying from 250 to 350 μmol m^{-2} s^{-1}. The temperature ranged from 22 to 31 °C during the day and 15–25 °C during the night, whereas the relative humidity varied between 55 and 80% depending on plant age. We did not provide aeration to the rooting medium in order to avoid mechanical stress, and also since a well-developed aerenchyma facilitates air transport to the root. Indeed, continuous aeration had no visible effect on root and shoot growth of the grass.

Collection of root exudates

Root exudates were collected from intact plants 4 h into the light phase. The exudate-collection medium was a freshly prepared

complete nutrient solution (pH 5.5) with ammonium or nitrate as N source. In order to measure the exudation of inorganic ions, the exudate-collection medium comprised a solution of glycine-betaine (pH 5.5) with osmolality equivalent to that of the 3 mM ammonium nutrient solution (59 mM betaine) or 3 mM nitrate nutrient solution (63 mM betaine). Plants were carefully harvested, roots rinsed four times in the corresponding exudate-collection medium and placed in a beaker (two to three plants each), with roots dipped in the exudate-collection medium [approx. 7 ml (g FW)$^{-1}$] leaving about 2 cm of the proximal end outside the medium. Shoots were held erect with a plastic rod fixed outside the beaker by adhesive tape. The beakers were kept under the light and gently shaken to ensure a uniform contact of roots with the medium. After 2 h (if not mentioned otherwise) portions of the exudate-collection medium were sampled, passed through a 0.45-μm membrane filter (Spartan 30/B; Schleicher and Schüll), boiled for 4 min and stored at –20 °C until analysis. Under the above experimental conditions, root exudation could not be distinguished from the simultaneous resorption of the exudates. Our data, therefore, represent the net exudation, instead of total root exudation, which might have been higher if resorption were also accounted for.

[^{14}C]Glucose uptake

Uptake of ^{14}C-labeled glucose in the presence of ammonium or nitrate was studied using kallar grass plants that remained under ammonium or nitrate nutrition for 4 weeks. Experimental conditions were essentially similar to those used for collection of root exudates, except that the rooting medium (nutrient solution either with ammonium or nitrate as N source) contained 10 μM labeled glucose with 925 Bq ^{14}C per plant. At the desired time intervals, aliquots of the rooting medium were sampled into 5-ml scintillation vials containing 4 ml of scintillator (Emulsifier-Safe; Packard) to determine the remaining radioactivity in a liquid scintillation counter (Wallac 1400 DSA).

Extraction of plant material

Following collection of root exudates, plants were rinsed four times in distilled water, dried with tissue paper, weighed and quickly frozen in liquid nitrogen. Plant material was ground and the frozen powder suspended in distilled water [5 ml (g FW)$^{-1}$]. After further grinding until thawed, samples were centrifuged (14,000 *g*, 4 °C, 15 min.), the supernatant boiled for 4 min and cooled. The insoluble protein pellet was separated by centrifugation (14,000 *g*, 4 °C) and the supernatant stored at –20 °C until analysis.

Solute analyses

Major sugars (glucose, fructose and sucrose) were separated by anion-exchange chromatography (0.1 N NaOH as eluent) on a Carbopac column plus pre-column, and detected directly by pulsed amperometry (Dionex 4500 i; Dionex, Idstein, Germany). Anions (NO_3^-, Cl^-, PO_4^-, SO_4^- and malate) were determined by isocratic, continuously suppressed anion chromatography (IC 1000; Biotronik, Maintal, Germany). Cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) were measured by ICP (inductively coupled plasma emission spectroscopy; Jobin Yvon 70 plus; JSA, München, Germany). Ammonium was measured by flow injection analysis (GAT WESCAN 360; Gamma Analysentechnik, Bremerhaven, Germany). Total amino acids were measured by the ninhydrin method.

Analysis of root architecture

An image-analysis technique was employed for comparing the root architecture of kallar grass grown under ammonium or nitrate as N source. Four replicate plants were used for each treatment. From each plant, six primary roots were randomly removed at the point of origin, blotted with tissue paper, weighed, and stained for 1 min in methyl violet (0.1 g l^{-1}). Each root portion was spread out in a transparent tray on a desktop scanner (AGFA SNAP SCAN 1236), and the image recorded and analysed using WinRHIZO 4.1c software (Regent Instruments , Blain St, Quebec, Canada). Data from root portions (six) corresponding to a single replicate plant were pooled before statistical analysis.

Statistics

Statistical analysis was performed by Student's *t*-test using MSTAT-C software. Some parameters, particularly root exudation and root architecture possessed a high degree of spatial variability, with coefficients of variation up to 79%. Therefore, where necessary, the data were $\log_{10}$-transformed before statistical analysis.

Results

Plant growth

In our experiments, shoot biomass was not affected by the N-source, whereas root fresh biomass under ammonium nutrition was almost 2-fold lower than that under nitrate (data not shown). In different experiments, the light intensity and temperature in the greenhouse varied because of the external environment. Consequently, biomass yields and sugar concentration/exudation during different experiments also varied in quantitative terms. Nevertheless, the effect of N source on different parameters always followed similar trends.

Root exudation

Sugar exudation was compared using either complete nutrient solution or glycine-betaine solution as the exudate-collection medium. Glycine-betaine was used as a zwitterionic osmoticum that did not show up as a peak in HPLC-sugar or ion analyses. The two exudate-collection media did not differ with respect to sugar exudation (Table 1). However, in both types of exudate-collection medium, ammonium plants exuded within 2 h almost 40-times more sugars than nitrate plants

Table 1 Comparison of complete nutrient solution or an isotonic glycine-betaine solution as medium for collection of sugar exudates from kaller grass (*Leptochloa fusca*) roots (mean ± SD, $n = 4$). In this and all subsequent tables, exudate collection time was 2 h. The complete nutrient solution (pH 5.5) contained either ammonium (*Am*; for ammonium plants) or nitrate (*Nit*; for nitrate plants). The glycine-betaine solution (pH 5.5) contained 59 and 63 mM betaine for ammonium and nitrate plants, respectively. The initial pH (5.5) decreased to 3–4.2 with ammonium plants and increased to 6.4–7.4 with nitrate plants. The root sugar concentrations for ammonium and nitrate plants were 3.62 ± 0.74 and 3.06 ± 0.64 μmol (g FW)$^{-1}$, respectively

Rooting medium	Sugars exuded [nmol (g FW^{-1}) h^{-1}]	
	Am plants	Nit plants
Nutrient solution	28.49 ± 10.26	0.74 ± 0.24
Glycine-betaine	37.06 ± 32.06	0.82 ± 0.55

($P < 0.01$). Concentrations of exuded sugars in the collection medium increased during the first 2 h, and remained constant thereafter for periods of up to 10 h, at values between 1 and 10 μM (sum of hexoses plus sucrose, not shown). Occasionally, concentrations of exuded sugars had already decreased after 4 h of incubation, which was probably due to microbial contamination and/or resorption by roots. The major sugars exuded were glucose, fructose and sucrose. When related to the internal hexose or sucrose content, hexose exudation, under all conditions, was about 4-fold higher than that of sucrose, and for both sugars, ammonium plants exuded 20-times (mean value from all experiments) more than nitrate plants. In another experiment using glycine-betaine (without nutrients) as the exudate-collection medium, exudation of sugars, amino acids and inorganic ions was measured (Table 2). Here, ammonium plants exuded 33-times more sugars ($P < 0.001$) and 3-times more amino acids ($P < 0.01$) than nitrate plants. In this experiment, ammonium plants showed 2- and 6-fold higher root concentrations of sugars and amino acids, respectively. However, with exudation expressed as a percentage of the respective concentration in the roots, ammonium plants exuded 20-times more sugars than nitrate plants, whereas amino acid exudation was similar.

Exudation of major cations was 1.3- to 6-fold higher for ammonium than nitrate plants, though the differences were statistically significant only for Ca^{2+} ($P < 0.01$), Mg^{2+} ($P < 0.05$), and NH_4^+ ($P < 0.001$). Exudation of major anions was up to 3-times higher from ammonium than from nitrate plants ($P < 0.01$), except nitrate exudation which, as expected, was observed only from nitrate-grown plants. Exudation of inorganic ions, expressed as a percentage of the respective root concentration, was usually equal or only slightly higher for ammonium than nitrate plants. It should be noted that exudation of inorganic ions was linear with time during the 2-h exudation period used here as a standard condition (not shown). We also tried to measure exudation of organic acids by direct CE (capillary electrophoresis) determination. However, exudation was usually too low to give reliable results, due to the much lower sensitivity of CE with indirect UV detection as compared with pulsed amperometric detection of sugars.

Table 2 Exudation of sugars, amino acids and inorganic ions from roots of kallar grass grown under ammonium (*Am*) or nitrate (*Nit*) as N source (mean ± SD, $n = 4$). Glycine-betaine solution (initial pH 5.5) was used for collection of root exudates. The final pH of the rooting medium was 4.71 ± 0.01 for ammonium plants and 7.52 ± 0.17 for nitrate plants. Figures in parentheses represent the initial root concentration [μmol $gFW)^{-1}$]; *n.d.* not detected

Compound exuded	Nitrogen source	
	Am	Nit
Sugars [nmol (g FW^{-1}) h^{-1}]		
Glucose	22.6 ± 14.2 (4.4 ± 1.3)	1.1 ± 0.4 (1.7 ± 0.2)
Fructose	61.6 ± 45.2 (4.0 ± 1.7)	1.1 ± 0.9 (1.6 ± 0.3)
Sucrose	8.8 ± 7.1 (6.8 ± 1.4)	0.7 ± 0.9 (5.1 ± 0.5)
Total	93.0 ± 66.4 (15.2 ± 3.8)	2.8 ± 2.2 (8.4 ± 0.2)
Amino acids [nmol (g FW^{-1}) h^{-1}]		
All	0.30 ± 0.11 (14.80)	0.10 ± 0.02 (2.50)
Cations [μmol (g FW^{-1}) h^{-1}]		
Na^+	1.61 ± 0.81 (12.10)	1.22 ± 0.57 (36.70)
K^+	2.39 ± 1.62 (86.10)	1.78 ± 0.24 (67.50)
Ca^{2+}	1.26 ± 0.56 (4.70)	0.53 ± 0.05 (7.60)
Mg^{2+}	0.54 ± 0.17 (6.80)	0.24 ± 0.11 (5.40)
NH_4^+	0.12 ± 0.04 (1.85)	0.02 ± 0.00 (0.91)
Anions [μmol (g FW^{-1}) h^{-1}]		
Cl^-	7.01 ± 1.79 (63.9)	2.14 ± 1.48 (32.0)
PO_4^{2-}	0.83 ± 0.20 (30.0)	0.30 ± 0.05 (2.5)
NO_3^-	n.d.	0.10 ± 0.03 (68.1)
SO_4^{2-}	1.06 ± 0.27 (2.3)	0.33 ± 0.11 (2.4)
Malate	n.d.	n.d.

Effect of external Ca^{2+} concentration on sugar exudation

As recorded in Table 2, the root concentration of Ca^{2+} for ammonium plants was almost one-half that of the nitrate plants. In order to investigate whether increased external Ca^{2+} concentration could reduce sugar exudation from ammonium plants, the latter were grown for 15 days in nutrient solution with different levels of Ca^{2+} supplied as $CaSO_4$. Exudation of sugars was similar from ammonium plants grown under 1 mM Ca^{2+} [14.9 nmol (g FW)$^{-1}$ h^{-1}], 4 mM Ca^{2+} [15.1 nmol (g FW)$^{-1}$ h^{-1}] and 8 mM Ca^{2+} [12.2 nmol (g FW)$^{-1}$ h^{-1}], but was 4-times higher than that [3.2 nmol (g FW)$^{-1}$ h^{-1}] recorded for nitrate plants grown under 4 mM $CaSO_4$ ($P < 0.01$).

Role of root sugar concentration in sugar exudation

Since N starvation may lead to accumulation of sugars in the shoot, roots of N-starved plants may be expected to possess a higher sugar concentration compared with N-sufficient plants. If the exudation process were only concentration dependent, roots of N-starved plants might exude higher amounts of sugars. To elucidate the effect of root sugar concentrations on exudation of sugars, kallar grass plants previously grown for 10 days under either ammonium or nitrate were subjected to N starvation for 5 days. Exudation was studied in a complete nutrient solution (pH 5.5) with composition similar to that from which plants were harvested, i.e. with and without ammonium or nitrate. Although the root sugar concentration of ammonium plants showed a 48% increase due to N-starvation ($P < 0.05$), exudation was 4-fold lower from N-starved ammonium-grown plants than in ammonium control plants ($P < 0.001$, Table 3). For nitrate plants, although a 31% increase in the root sugar concentration ($P < 0.01$) and a 39% increase in the sugar exudation ($P < 0.05$) were recorded due to N-starvation, the amount of sugar exuded was severalfold lower than for ammonium-sufficient plants ($P < 0.001$). Moreover, sugar exudation expressed as percent of the

root concentration in ammonium-sufficient plants was 6-times that of the ammonium-starved plants, and 12-times that of the nitrate plants. These results indicate that it was not the higher root sugar concentration but the presence of ammonium that was responsible for higher sugar exudation from ammonium plants.

To further establish the stimulatory role of ammonium in root exudation, an experiment was conducted in which plants grown for 4 weeks under ammonium were transferred into nutrient solution containing nitrate as the sole N source. Twelve or 24 h after transfer into nitrate nutrition, exudation of sugars was studied in nutrient solution containing nitrate. Twenty-four hours after shifting to nitrate, ammonium plants accumulated a considerable quantity of nitrate, whereas a 4-fold reduction was observed in the ammonium content of roots ($P < 0.001$, Table 4). The root sugar concentration of ammonium plants did not change during the 24 h growth under nitrate, and was almost 2-times that of the plants grown continuously under nitrate nutrition ($P < 0.01$). However, shifting from ammonium to nitrate nutrition reduced sugar exudation to the level recorded for plants originally grown under nitrate ($P < 0.01$). In contrast, for ammonium plants that continued their growth on ammonium, sugar exudation was, respectively, 34- and 79-fold higher than from ammonium plants shifted to nitrate nutrition or continuously grown under nitrate ($P < 0.001$). Similar trends were recorded 12 h after shifting ammonium plants to nitrate nutrition (data not shown), confirming that it was the ammonium concentration and not the sugar concentration of the roots that was responsible for higher sugar exudation under ammonium compared with nitrate nutrition.

Table 3 Effect of N starvation on root sugar content and exudation from kallar grass grown on ammonium (*Am*) or nitrate (*Nit*) (mean ± SD, $n = 4$)

Treatment	Root sugar content [μmol (g FW)$^{-1}$]	Sugar exudation [nmol (g FW^{-1}) h^{-1}]
Am plants, control		
Glucose	0.9 ± 0.2	7.6 ± 1.1
Fructose	0.8 ± 0.2	6.1 ± 0.9
Sucrose	1.6 ± 0.4	1.4 ± 0.1
Total	3.3 ± 0.6	15.1 ± 2.0
Am plants, starved		
Glucose	0.8 ± 0.1	1.1 ± 0.3
Fructose	0.8 ± 0.1	1.7 ± 1.7
Sucrose	3.3 ± 0.9	1.3 ± 0.5
Total	4.9 ± 0.8	4.1 ± 1.8
Nit plants, control		
Glucose	1.1 ± 0.1	0.6 ± 0.2
Fructose	0.6 ± 0.1	0.8 ± 0.5
Sucrose	2.6 ± 0.3	0.4 ± 0.3
Total	4.3 ± 0.1	1.8 ± 0.4
Nit plants, starved		
Glucose	0.7 ± 0.1	0.7 ± 0.2
Fructose	0.8 ± 0.1	1.6 ± 0.2
Sucrose	4.1 ± 0.4	0.2 ± 0.0
Total	5.6 ± 0.5	2.5 ± 0.2

Effect of external pH

When exudation into betaine solution was studied (Table 2), the initial pH (5.5) of the exudate collection-medium decreased to 4.7 or even lower for ammonium plants, in contrast to nitrate plants where it rose to 7.4. Therefore, increased exudation by the ammonium plants might be traced back at least partly to increased membrane permeability through acidification and eventually to a replacement of membrane-bound Ca^{2+} during ammonium assimilation. Nevertheless, such an effect of ammonium was reversible and was offset within 24 h after shifting onto nitrate nutrition (Table 4). To further elucidate a potential effect of external acidification, sugar exudation from nitrate plants was compared at pH 4.5 and pH 6.5 (data not shown separately). With an initial pH of 6.5, sugar exudation from nitrate plants in nitrate nutrient solution was 1.0 ± 0.2 nmol (g FW)$^{-1}$ h^{-1}, compared with 1.6 ± 0.4 nmol (g FW)$^{-1}$ h^{-1} exuded at pH 4.5. Thus, while an acidic pH indeed increased sugar exudation slightly, at pH 4.5, exudation from nitrate plants remained severalfold lower than the amount exuded in the presence of ammonium at similar pH.

Effect of N source on glucose uptake

To investigate if the higher sugar exudation in the presence of ammonium was due to lower sugar resorption, uptake of [^{14}C]glucose from a 10 μM solution was studied. The kinetics and magnitude of glucose uptake were very similar for ammonium and nitrate plants (Fig. 1).

Root architecture

As exudation may be affected by the number of root tips/side roots, the root surface area, or the root surface/volume ratio, we also analysed these root archi-

Table 4 Effect of a 24-h shift of kallar grass plants from ammonium (*Am*) to nitrate (*Nit*) nutrition on sugar exudation and root concentration of sugars, ammonium and nitrate (mean ± SD, $n = 4$; *n.d.* not detected)

Treatment	Sugar exudation [nmol (g FW)$^{-1}$ h^{-1}]	Sugar content [μmol (g FW)$^{-1}$]	Am content [μmol (g FW)$^{-1}$]	Nit content [μmol (g FW)$^{-1}$]
Am, control	94.6 ± 33.9	19.0 ± 3.6	0.8 ± 0.2	n.d.
Am → Nit	2.8 ± 0.9	19.7 ± 5.6	0.2 ± 0.0	18.9 ± 4.3
Nit, control	1.2 ± 0.1	10.2 ± 2.8	0.2 ± 0.0	80.3 ± 21.5

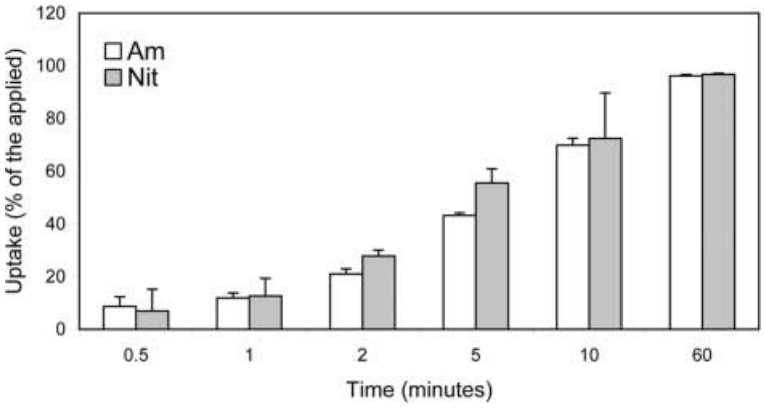

Fig. 1 Uptake of [^{14}C]glucose by roots of kallar grass (*Leptochloa fusca*) in the presence of ammonium (*Am*) or nitrate (*Nit*) (mean ± SD, $n = 3$). The initial glucose concentration in the rooting medium was 10 μM. Uptake was followed by counting the remaining radioactivity in aliquots (100 μl) of the root incubation medium

tectural parameters in kallar grass grown under ammonium or nitrate nutrition. Plants grown under the two N sources did not differ in the average root diameter, and root length and root surface area g^{-1} root FW were also similar (Table 5). Although the number of root tips/side roots was 1.4-times higher in ammonium than nitrate plants, statistically the difference was only marginally significant ($P < 0.1$). Stratifying the data into different root-diameter classes revealed that the majority of the root length and surface could be attributed to the finest root populations, having diameters in the range of 0–0.4 mm (Fig. 2). A considerable proportion of the root volume (20–30%) also belonged to that group, but the thicker roots contributed more to the total root volume than to the total root length or surface. Roots belonging to the diameter class 0–0.4 mm comprised about 85% of the total length, and slightly more than 60% of the total surface. Neither the length, nor the surface area of that root population was significantly different in ammonium or nitrate plants. Moreover, the observed stimulatory effect of ammonium nutrition on root exudation was almost similar in magnitude, whether exudation was expressed as cm^{-2} root surface area, or as g^{-1} root FW.

Table 5 Root architecture of kallar grass grown under ammonium (*Am*) or nitrate (*Nit*) nutrition (mean ± SD, $n = 4$). Total root fresh weights under ammonium and nitrate nutrition were 1.46 ± 0.38 and 3.03 ± 0.25 g plant^{-1}, respectively. Note that the "apparent volume" derived from the projection area and the length, is considerably larger than expected from the fresh weight. This is at least partly dependent on the scanning resolution, but may also indicate a deviation from perfectly circular cross-sections

Root parameter	Am	Nit
Length [cm (g FW)$^{-1}$]	5,612 ± 1,983	5,153 ± 0,785
Mean diameter [mm]	0.29 ± 0.06	0.33 ± 0.07
Surface area [cm^2 (g FW)$^{-1}$]	483 ± 198	523 ± 131
Apparent volume [cm^3 (g FW)$^{-1}$]	3.46 ± 1.90	4.14 ± 1.51
Tips/side roots [(g FW)$^{-1}$]	10,130 ± 2,466	7,210 ± 1,776

Discussion

Kallar grass grown hydroponically developed a root system with little or no root hairs, yet numerous very thin side roots. Mechanical stress during growth (no aeration) and handling was kept as low as possible, and during the short exudation period release of solutes by cell death was probably negligible. Under these gentle conditions, most exuded compounds should have been released from intact cells. Accordingly, total exudation of organic compounds (mainly sugars, but also amino acids and organic acids) was very low. Maximum exudation rates (on a molar carbon ratio) were roughly 0.02% of the photosynthetic carbon assimilation rate, which was estimated to be 50 μmol C (g FW)$^{-1}$ h^{-1} (whole-plant FW).

In the present study, shoot biomasses of ammonium and nitrate plants were similar, whereas the root growth under ammonium was reduced to about 50%. Therefore, the somewhat higher root sugar concentrations of ammonium plants may be traced back to a lower consumption of sugars.

Although root biomass yield was less under ammonium than nitrate nutrition, the root surface of the majority of the fine roots (0–0.4 mm diameter, which comprised more than 80% of the total root surface) was not significantly different. Root tips or points of lateral root emergence are often considered as major sites of exudation. Ammonium plants indeed possessed a higher number of root tips/side roots than nitrate plants. However, that difference was much smaller than the difference in the sugar exudation between ammonium and nitrate plants. Therefore, in our conditions, root architecture appeared to be irrelevant for the increased sugar exudation under ammonium nutrition. This conclusion is further supported by the fact that sugar exudation from ammonium plants was drastically reduced within 12–24 h upon shifting into nitrate medium (Table 4) and during this short time period significant alterations in the root architecture were not detectable.

If root tips are considered as the major site of exudation, the amount and composition of the root exudates should be better correlated with the content and composition of the compounds in the root tips than in the bulk root. Although the evidence is not well documented, the results of Ayers and Thornton (1968) for exudation of amino acids from wheat and pea roots

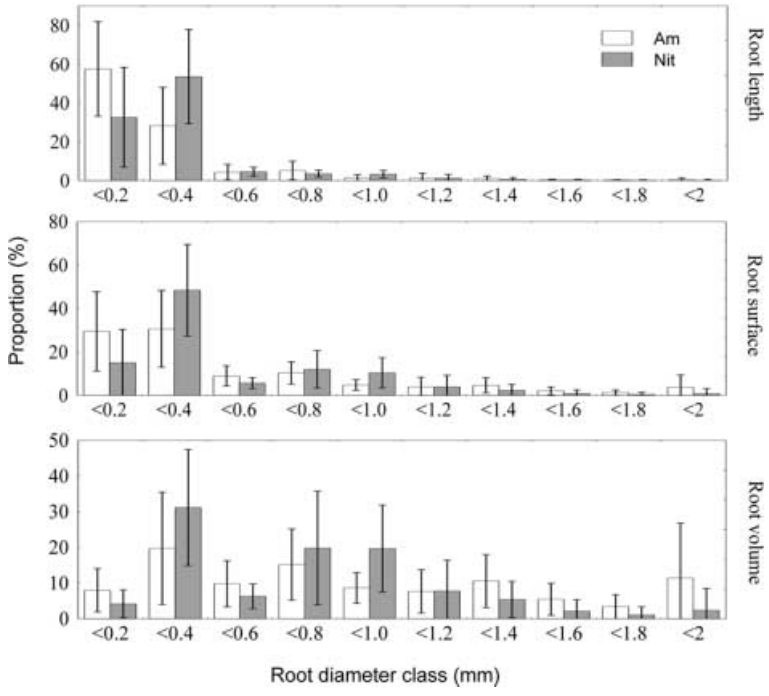

Fig. 2 Proportion (%) of kaller grass root length, surface and volume in different root-diameter classes under ammonium (*Am*) and nitrate (*Nit*). Vertical bars indicate ± SD ($n=4$)

support this hypothesis. In the present study, only the overall root sugar content was measured, which was only occasionally higher in ammonium roots (compare Tables 1, 2 and 3). Shifting ammonium plants to nitrate nutrition did not change the root sugar concentration but drastically reduced the exudation (Table 4). Similarly, N-starved ammonium plants showed higher sugar content, but exudation was reduced when ammonium was absent from the rooting medium. Obviously, sugar exudation was not determined by the total sugar content of roots.

Compared with nitrate as N source for wheat, ammonium has been reported to impair the survival of root cortical cells, thus increasing amino acid exudation (Brown and Hornby 1987). We cannot exclude the possibility that during the 2-h exudation period more cortical cells died in ammonium versus nitrate conditions. However, if sugar exudation were preferentially due to cell death, other compounds should be unspecifically released to the same extent as sugars. However, the relative release (related to internal contents) of inorganic cations and anions and of amino acids from roots, which was roughly one order of magnitude higher than the release of sugars, was not much different for ammonium and nitrate plants. Thus, cell death was not a major source for exudates.

The relatively higher hexose exudation would require that, for example, cortical cells or root tips contained more hexoses than sucrose. The latter may be preferentially located in the central cylinder, therefore contributing only little to total sugar exudation. On the other hand, the higher hexose over sucrose exudation might also indicate a specific release of hexoses over sucrose, or a different compartmentation of hexoses and sucrose between cytosol and vacuole within one and the same cell type.

Increased external Ca^{2+} supply did not reduce the exudation, nor was the exudation significantly affected by external pH within the range observed during the above experiments, indicating that altered membrane permeability or altered H^+-gradients due to external acidification were hardly responsible for the large stimulation of sugar exudation under ammonium nutrition.

The results of the glucose-uptake experiment indicate that the type of N source did not influence the effective retrieval of sugars. Therefore, the much higher net sugar exudation by ammonium plants was not attributable to lower resorption.

The results of the present study show that ammonium specifically stimulates, by as yet unknown mechanisms, sugar exudation. This may represent an important mechanism for kallar grass to feed associative diazotrophs, which provide ammonium-N for the plant.

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