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SHORT COMMUNICATION

Nodule Function in Symbiotic Bambara Groundnut (*Vigna subterranea* L.) and Kersting's Bean (*Macrotyloma geocarpum* L.) is Tolerant of Nitrate in the Root Medium

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Nitrogen-fixing activity in two nodulated African legumes, Bambara groundnut (*Vigna subterranea* L.) and Kersting's bean (*Macrotyloma geocarpum* L.), was assessed in the presence of nitrate (NO_3^-) ions in the rooting medium. Nitrogenase activity was unimpaired by the supply of 5 mol m^{-3} NO_3 to both species. Also, large concentrations of ureides dominated the transpiration stream of NO_3-fed plants. Compared to other symbiotic legumes cultured with similar NO_3 concentrations, nodule functioning in the tested landraces of Bambara groundnut and Kersting's bean is tolerant of NO_3 ions in the rhizosphere. The potential benefits of such naturally occurring NO_3-tolerant symbioses are substantial, as they would permit inorganic N fertilizer application in intercropping systems without inhibiting N_2 fixation in the associated legumes.

Key words: NO_3 tolerance, Bambara groundnut, Kersting's bean, nitrogenase activity, xylem ureides.

INTRODUCTION

Inhibition, by nitrate (NO_3^-) ions, of nodule development and nitrogenase activity in symbioses of rhizobial bacteria with legumes has been well documented (Streeter, 1988). Although the mechanisms underlying the inhibitory effects of NO_3 are still not fully understood, most nodulated species grown in the presence of large concentrations of this solute tend to depend more on it for growth than on forms of nitrogen acquired symbiotically. Consequently, the continued uptake of soil N under such conditions could lead to a decline in soil N fertility and a concomitant decrease in yields of subsequent crops. According to Layzell and Moloney (1994), increases in N_2 fixation of up to 300% could be achieved in soils with large concentrations of NO_3 if highly effective NO_3-tolerant symbioses were identified. In pursuit of that goal, various workers have isolated NO_3-tolerant supernodulating (*nts*) mutants of *Pisum sativum* (Jacobsen and Feenstra, 1984), *Glycine max* (Carroll *et al*., 1987; Gremaud and Harper, 1989) and *Phaseolus vulgaris* (Buttery and Park, 1989). These mutants have greater ability to nodulate in the presence of large concentrations of NO_3 than the parent cultivars (Hansen *et al*., 1989; Wu and Harper, 1990). However, the amounts of N fixed are either the same as the parents, or even smaller (Hansen *et al*., 1989) apparently due to reduced accumulation of total biomass (Day *et al*., 1986). Consequently, field yields are also not significantly different between mutants and their parents (Song *et al*., 1995). Thus, while development of *nts* mutants from mutagenesis represents a useful tool for understanding NO_3 tolerance in N_2-fixing legumes, their actual contribution to increased agronomic yields cannot be demonstrated in the field (Song *et al*., 1995).

The development of legume cultivars which can fix large amounts of N_2 in the presence of high NO_3 concentrations in soil is currently a top priority. So far, however, the *nts* phenotype has not been observed in nature outside mutagenized plant material apart from a limited nodulation tolerance of soil NO_3 seen when some soybean varieties are inoculated with high rhizobial numbers (Betts and Herridge, 1987; Herridge and Betts, 1987; Lawson, Carroll and Gresshoff, 1988). Unfortunately, in these studies, no data were collected on nodule functioning which may have been adversely affected.

Bambara groundnut and Kersting's bean are two geocarpic legumes indigenous to Africa. These plants grow in differing regimes of soil N fertility, ranging from the N-poor soils of the Sahel savanna to the N-rich soils of the transitional forest zones. Landraces and ecotypes able to nodulate and fix N_2 optimally in soils containing large concentrations of NO_3, and with some ability to overcome NO_3 inhibition of nodule development and nitrogenase activity may exist.

In a previous study (Dakora, Atkins and Pate, 1992), increasing the concentration of NO_3 to Bambara groundnut and Kersting's bean inhibited nodule growth and N_2 fixation in both species and decreased dependence on symbiotically-fixed N as a consequence of a progressively reduced nodule specific activity. Although those integrated values of nodule function suggest that the symbiosis of the two species is sensitive to NO_3, the scale of intolerance remains unknown relative to other N_2-fixing legumes. In order to compare the NO_3 response of these species with other symbioses, as well as to understand the components of that response, new

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experiments to monitor nitrogenase activity using the acetylene reduction assay (the technique popularly used for assessing instantaneous response of a legume's symbiosis to NO_3 or other external factors) are required.

This study examines mineral N nutrition in nodulated plants of Bambara groundnut and Kersting's bean with special reference to NO_3 tolerance.

MATERIALS AND METHODS

Plant culture

Seeds of Bambara groundnut (*Vigna subterranea* L.) and Kersting's bean (*Macrotyloma geocarpum* L.) were sown in sand in a glasshouse and inoculated with a peat suspension of *Bradyrhizobium* strain CB 756. Following germination, five seedlings were transferred to liquid culture (3·5 l) and grown hydroponically with their root systems bathed in well aerated N-free nutrient solution under glasshouse conditions. Later, they were thinned to four plants per pot. At 40 d after planting (DAP) the nutrient solution was altered to contain different concentrations (0, 2 and 5 mol m^{-3}) of NO_3, NH_4 and NH_4NO_3. Four replicate pots were used for each treatment. Nutrient solutions were replaced four times a day to avoid changes in pH, which might adversely affect symbiotic performance.

Nitrogenase activity

Nitrogenase activity in both Bambara groundnut and Kersting's bean was measured using the flow-through system of the acetylene reduction assay (ARA) as described by Dakora and Atkins (1990). In each case, ethylene formation was assessed for periods up to 40 min following exposure to acetylene, and the averages of constant maximum rates recorded.

Xylem analysis

At harvest, plants were decapitated and xylem sap collected from all four plants per pot. Equal volumes of sap from the four replicate pots were pooled, and samples analysed for ureides (allantoin and allantoic acid), the products of N fixation which are transported (Dakora *et al.*, 1992). HPLC separation of xylem sap into amides and amino acids was carried out as described by Atkins, Dakora and Storer (1990).

Statistical analysis

Acetylene reduction values were analysed statistically by one-way ANOVA using STATISTICA software.

RESULTS

Nitrogenase activity of Bambara groundnut and Kersting's bean plants supplied with NO_3, NH_4 and NH_4NO_3

Providing NH_4 and NH_4NO_3 to nodulated plants of Bambara groundnut and Kersting's bean resulted in a 39 to 97 % decrease in nitrogenase activity within 3 to 15 d (data not shown). With a 5 mol m^{-3} NO_3 supply however, both Bambara groundnut and Kersting's bean showed only a small decline in nitrogenase activity relative to controls (Fig. 1). When expressed as a percentage of the control, nitrogenase activity in the two species declined by about 33 % over an 8 d period, and dropped by 76 % after repeated acetylene reduction assays for 15 d.

Xylem sap composition of plants supplied with NO_3, NH_4 and NH_4NO_3

Amino acids, as opposed to ureides, dominated the xylem sap of Kersting's bean fed with NH_4 or HN_4NO_3, and their

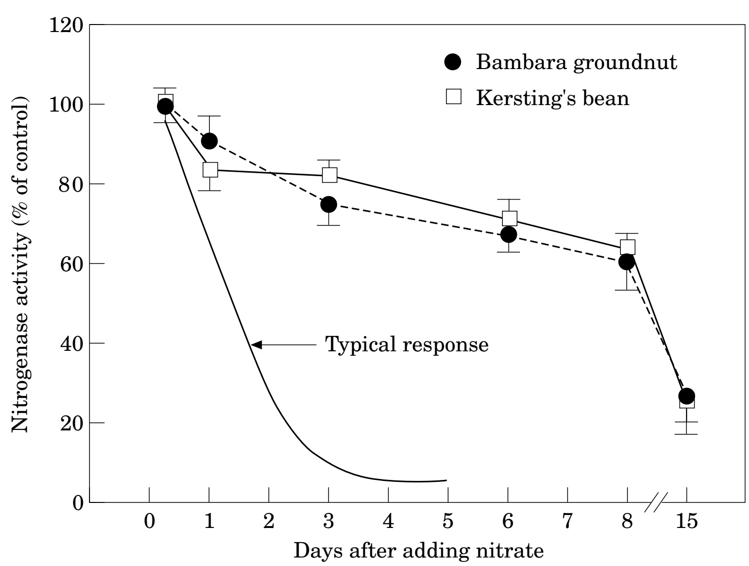

FIG. 1. Nitrogenase (acetylene reduction) activity of Bambara groundnut (*Vigna subterranea*) and Kersting's bean (*Macrotyloma geocarpum*) exposed to 5 mol m^{-3} NO_3. A typical response to NO_3 by other nodulated legumes is also indicated (based on data from Schuller *et al.*, 1986; Hansen *et al.*, 1989; Wu and Harper, 1990). Bars are ±s.e.m.

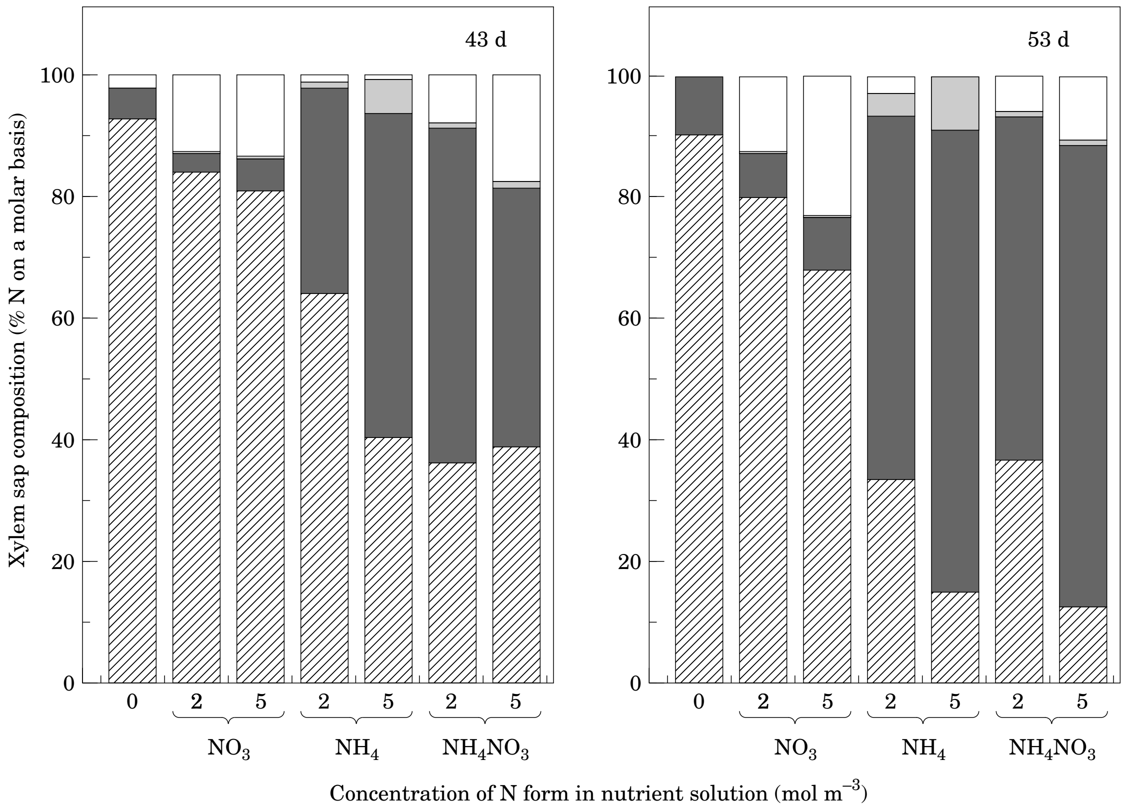

FIG. 2. Proportional distribution of ureides (▨), amino acids (■), ammonium (▩) and nitrate (□) in xylem exudate collected from *Macrotyloma geocarpum* L. plants fed different concentrations of three forms of mineral N. Each component is the average of a duplicate run of a pooled xylem sap sample from four replicate pots.

concentrations increased with prolonged exposure of the plant to the two nitrogen sources (Fig. 2). However, ureides constituted the major proportion of organic N in xylem sap in purely symbiotic and NO_3-fed plants (Fig. 2). The concentration of ureides in xylem increased with increasing plant dependence on symbiotic N in Kersting's bean. The proportion of components in the xylem sap in response to feeding with NO_3 followed a similar pattern (Fig. 2). The plants which showed greater concentrations of ureides in their xylem stream with NO_3 supply (Fig. 2) also exhibited higher nitrogenase activities (Fig. 1). The xylem sap composition of Bambara groundnut supplied with the different forms of N was similar to that of Kersting's bean (data not shown).

DISCUSSION

Nitrate and ammonium ions both affect symbiotic activity in nodulating legumes (Streeter, 1988), often leading to decreased nitrogenase activity at higher concentrations. In this study, nodule function in Bambara groundnut and Kersting's bean was not markedly altered when supplied with 5 mol m^{-3} NO_3 (Fig. 1). There was, however, some decline in nitrogenase activity following repeated acetylene reduction assays and/or prolonged exposure of the two species to 5 mol m^{-3} NO_3. However, the decrease in nodule function with NO_3 supply was so gradual and unusually slow that it suggests some protection of nitrogenase from NO_3 inhibition. This argument is supported by the high concentrations of ureides in the xylem stream of NO_3-fed plants (Fig. 2), which is consistent with the acetylene reduction values obtained (Fig. 1).

Bambara groundnut and Kersting's bean showed a dramatic decline in nitrogenase activity after 15 d exposure to 5 mol m^{-3} NH_4 (data not shown). This inhibition of nitrogenase by NH_4 nutrition has been noted before in symbiotic legumes (Parsons and Baker, 1996), and is apparently due to increased accumulation of the amide glutamine in phloem sap which restricts O_2 diffusion to bacteroids (Neo and Layzell, 1997). Although phloem components were not analysed in this study, the xylem fluids of NH_4-fed plants were dominated by a large pool of amino acids (Fig. 2).

The observed slow response of nitrogenase to NO_3 in the two species was in sharp contrast to other legume symbioses supplied with similar concentrations of NO_3 (Fig. 1). Nitrogenase activities were generally greater in this study than those reported for other symbiotic systems under similar conditions (Oghoghorie and Pate, 1971; Pate *et al.*, 1980; Minchin *et al.*, 1986; Schuller *et al.*, 1986; Carroll *et al.*, 1987; Wu and Harper, 1990). This clearly indicates that although integrated values of N fixed from a previous study (Dakora *et al.*, 1992) revealed sensitivity of the two species to NO_3, nitrogenase activity was still nevertheless more tolerant of free NO_3 than other symbioses. As indicated in Fig. 1, Bambara groundnut and Kersting's bean did not show the typical NO_3 response exhibited by other nodulated legumes when supplied with NO_3 (Oghoghorie and Pate, 1971; Pate *et al*., 1980; Minchin *et al*., 1986; Carroll *et al*., 1987; Wu and Harper, 1990). In fact, nitrogenase activity in soybean can decrease to 40% of control after only 6 d of exposure to 5 mol m^{-3} NO_3 (Schuller *et al*., 1986). Compared with the so-called *nts* mutants of soybean (Hansen *et al*., 1989; Wu and Harper, 1990), nodule function in Bambara groundnut and Kersting's bean still appears to be relatively more tolerant of NO_3. On this basis, both species are considered to be NO_3-tolerant.

The mechanisms involved in NO_3 inhibition of nitrogenase activity have not been clearly defined for many symbiotic legumes. Most hypotheses are based either on the direct toxicity of NO_2 from nitrate reductase activity on nitrogenase and leghaemoglobin (Rigaud and Puppo, 1977), NO_2 conversion of leghaemoglobin to nitrosylleghaemoglobin (Kanayama, Watanabe and Yamamoto, 1990), NO_3 alteration of leghaemoglobin components (Becana and Sprent, 1989), or on an indirect effect of NO_3 assimilation elsewhere in the plant, which results in reduced photosynthate supply to nodules (Oghoghorie and Pate, 1971; Harper and Gibson, 1984). The data from this study are insufficient to establish which of these mechanisms operates to produce the low NO_3 sensitivity in the two geocarpic legumes.

Traditional farming in Africa and elsewhere in the tropical world is based largely on mixed cropping, a system which does not easily permit N fertilizer application to associated, N-starved cereal and vegetable crops. The identification of naturally-occurring NO_3-tolerant legume symbioses in Bambara groundnut and Kersting's bean may allow N fertilization in intercropping systems without inhibiting N_2 fixation, and thus provide an added advantage to that cultural practice in addition to sparing soil N during monocropping in high NO_3 environments.

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