Does ammonium uptake influence xylem sap composition in *Phaseolus vulgaris* L. and *Glycine max* (L.) Merill?

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Received 3 February 1995; accepted 28 March 1995

Abstract. The question of whether ammonium uptake influences the occurrence of ureides in legumes has been addressed in this study by investigating three *P. vulgaris* genotypes as well as one cultivar of *Glycine max*. All plants were raised in sand culture during the dry season in northern Thailand and irrigated daily with nitrogen-free nutrient solution, or the same solution containing 12 mol m^{-3} nitrogen in the form of $(NH_4)_2SO_4$ or KNO_3, each treatment consisting of different proportions of either compound. Regression analyses of xylem sap composition relative to ammonium vs. nitrate supply of plants harvested at V4, R1 and R6 indicated close positive correlations of xylem amino nitrogen content and negative correlations with xylem nitrate content and ammonium supply. Statistically significant correlations between relative xylem ureide content and ammonium availability could be established for the *P. vulgaris* cultivar Brilliant up to stage R1, but not for the other plants investigated. It was concluded that at least for some genotypes of common bean a relationship exists between ureide production and ammonium uptake by the root system. Since the extent to which ureide production is stimulated remains quite small, its relevance to the xylem solute technique for measurement of N_2 fixation may be limited. Nevertheless, due to the possibility of large genotypic differences in the impact of ammonium on ureide production, this factor must be considered in calculations if N_2 fixation is to be determined in soils containing significant amounts of ammonium, e.g. in paddy fields.
Key words. Allantoic acid; allantoin; ammonium; nitrate; common bean; soybean; xylem ureide assay.

Quantification of N_2 fixation under field conditions remains an important tool for legume research, ultimately aiming to enhance symbiotic activity of legume crops. The choice of method depends largely on the specific objective of the experiment, the conditions and species to be evaluated, and the analytical means available to the researcher. While ^{15}N techniques are direct measures of symbiotic activity and may have certain advantages, indirect assessment procedures are considerably cheaper and can be conducted by laboratories with a less sophisticated technical set-up. Apart from the controversial acetylene reduction assay, the xylem solute technique is a potentially reliable indirect method to quantify symbiotic activity in the field. However, such indirect procedures are only as reliable as the calibration used to convert collected data to estimates of N_2 fixation. Conventional calibrations rest upon assessments of changes in xylem sap composition in a series of plants treated with different concentrations of ^{15}N-labelled nitrate. Since such long-term nitrate applications at various concentrations induce plants to develop with different dependencies on N_2 fixation, one can easily establish a correlation between the plants' proportional utilisation of dinitrogen and respective changes in xylem sap composition. The method has proven most reliable in ureide (e.g. allantoin and allantoic acid)-producing plants (e.g. soybean), consistently reflecting a positive correlation between N_2 fixation and the relative quantity of nitrogen present in the xylem sap in form of ureides[1,2]. Intensive studies focusing on the optimisation of the method resulted in a range of recommendations for routine applications. If properly followed, reliable results can be achieved without excessive expense and technology[3,4].

However, ureide production in plants can also occur irrespective of N_2 fixation. Ureides are produced via purine degradation in enlarged peroxisomes of interstitial cells in the infected tissue of nodules[5]. The same process may take place in non-symbiotic plant tissue, where glutamine may serve as an efficient precursor for purine formation[6–8]. Glutamine may arise from N_2 fixation as well as ammonium or nitrate uptake, in the later case following nitrate reduction. Hence, a certain quantity of ureides in N_2-fixing legumes is not produced in the wake of nitrogenase activity. Since this ureide background varies with species, genotype and quantity as well as quality of combined nitrogen, it is essential to accurately assess the proportion of ureides not of symbiotic origin. Therefore, in addition to other precautions which need to be taken[9], the effect of urea or ammonium in the rooting medium and the question of whether their up-

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take influences xylem sap composition remains to be some cause for concern[6]. Some studies have suggested that the nitrogen source may have no effect on the relative ureide index, or only if ammonium becomes the major source of nitrogen taken up by the legume[9]. Since the latter conditions only rarely apply, any effect of ammonium was not considered to be very significant, at least for soybean and pigeonpea. However, studies on *Phaseolus vulgaris* have indicated that the situation may be somewhat different in this species[10,11], suggesting that calibrations based exclusively on plants' exposure to a series of different nitrate concentrations may not be as reliable in situations where ammonium and nitrate constitute mineral nitrogen sources.

The objective of this present study was to evaluate more closely the effect of ammonium nutrition on the relative abundance of ureides in the xylem sap of several genotypes of *P. vulgaris* in comparison to one soybean cultivar. For that purpose plants were raised under non-symbiotic conditions and exposed to high mineral nitrogen concentrations, which were supplied as different proportional amounts of ammonium or nitrate. One fully symbiotic set of plants was included as a control. Data are discussed in relation to their significance for the application of the xylem solute technique.

Materials and methods

Plant material and culture. Seeds of three common bean (*Phaseolus vulgaris* L.) cultivars (i.e. Mokcham, Brilliant and Rico) as well as one soybean (*Glycine max* (L.) Merill) cultivar (i.e. NW 1) were sown in pots filled with 10 kg of nutrient-free, washed river sand, and inoculated with 1 cm^3/seed of a suspension containing either 10^8 cells/cm^3 of *Rhizobium leguminosarum* bv. *phaseoli* strain UMR 1899 or *Bradyrhizobium japonicum* strain USDA 110 for common bean and soybean respectively. For the first 12 days after sowing pots were irrigated daily with water. Thereafter, seedlings were culled to 10 per pot and subjected to different nutrient solutions depending on the respective treatment (see below). Plants were raised during the dry season 1992/93 (December–March) in an open plant culture area at the Multiple Cropping Center of Chiang Mai University in northern Thailand. Mean monthly maximal and minimal temperatures experienced by the plants during the experiment ranged between 28.7–33.7 and 12.6–18.0 °C respectively, with an average relative humidity between 60.7–71.6%. Except for some heavy rain on two days (10–11 days after sowing) prior to the beginning of any experimental treatment, no rainfall was recorded at the research station during the remainder of the experiment. Plants were harvested during vegetative development at stage V4 (between 31 and 39 days after sowing, depending on developmental stage of the different genotypes) and subsequently during reproductive growth at the stages R1 (between 42 and 56 days after sowing) and R6 (between 70 and 91 days after sowing) following the physiological age classifications of Nuland and Schwarz[12] and Fehr et al.[13]. At each harvest, four replicate pots from each treatment were sampled for root bleeding sap (see ref. 5). To avoid any artefacts resulting from diurnal fluctuations in the relative abundance of ureides, sap samples were only collected between 11.00 h and 14.00 h[14]. Post-collection metabolism of nitrogen compounds in the sap was avoided by rapid stabilisation of samples at -20 °C. Analyses of sap for ureides, amino-nitrogen and nitrate as well as computation of data followed the methods described by Peoples et al.[9].

Nutrient solutions. The experiment involved either plants treated with a minus-nitrogen nutrient solution[15] or five equally large groups of plants exposed to the same solution supplemented with 12 mol m^{-3} nitrogen applied in different proportions of $(NH_4)_2SO_4$ or KNO_3 to yield the following combinations: 0/100, 20/80, 50/50, 80/20, 100/0 and 0/0 (minus-nitrogen treatment). The figures refer to the percentage of nitrogen supplied to the plants as ammonium or nitrate respectively (i.e. NH_4NO_3). Plant cultures were irrigated daily with 0.5 liter and later (past the 27th day after sowing) with 1.0 liter of quarter strength nutrient solution. All pots were flushed weekly with water to avoid accumulation of unabsorbed salts in the rooting medium of the plants.

Statistics. Statistical analyses were carried out on a Macintosh computer using Statview for calculations of regressions and correlation coefficients together with generally associated statistical evaluations. Spreadsheet calculations relied upon the program Excel.

Results

Nodulation and nitrogen accumulation. Plants not exposed to mineral nitrogen were well-nodulated with maximal nodule weights ranging between 0.5 and 1.2 g for the different genotypes of common bean and reaching 2.0 g for soybean. Nitrogen-treated plants on the other hand were essentially devoid of nodules, except for soybean which consistently maintained up to 20% of the nodule weight carried by the fully symbiotic plants. Hence, nitrogen-treated soybeans may have maintained some symbiotic activity, while the three *P. vulgaris* genotypes exposed to the same treatment were incapable of any N_2 fixation due to a lack of nodules.

Figure 1 depicts the nitrogen content of the experimental plants close to maturity (R6). Data for earlier harvest are not shown since they reflected similar general patterns. Nitrogen accumulation of symbiotically active plants was lowest, but almost identical to plants receiving ammonium as the sole source of mineral nitrogen. Under these conditions soybean reached the highest values followed by the common bean genotypes

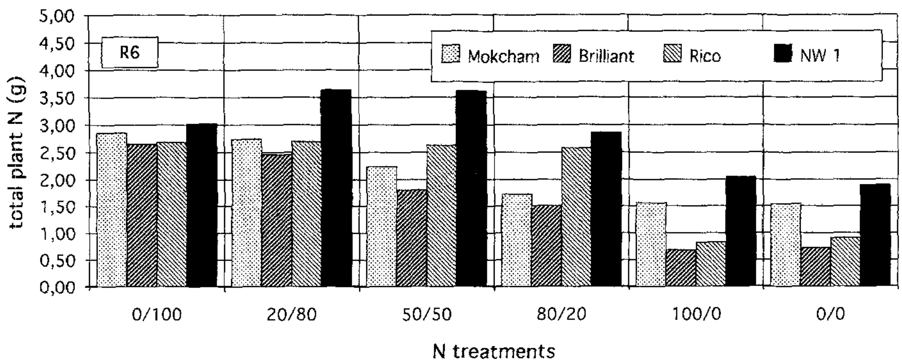

Figure 1. Total plant nitrogen content at stage R6 of three cultivars of *Phaseolus vulgaris* cv. Mokcham, Brilliant and Rico as well as the soybean (*Glycine max*) cultivar NW 1. Plants were raised in sand culture during the dry season in northern Thailand and regularly irrigated with either nitrogen-free nutrient solution (0/0), or the same solution supplemented with 12 mol m^{-3} nitrogen supplied in the form of $(NH_4)_2SO_4$ or KNO_3, with each solution containing a different proportion of nitrogen in either form. Percentages of nitrogen are indicated by the numbers separated by a slash (e.g. 20/80) with the first number representing the value for $(NH_4)_2SO_4$ and the second the value for KNO_3. Each value represents a mean of plants grown in four pots, each containing ten plants (SE < 8%).

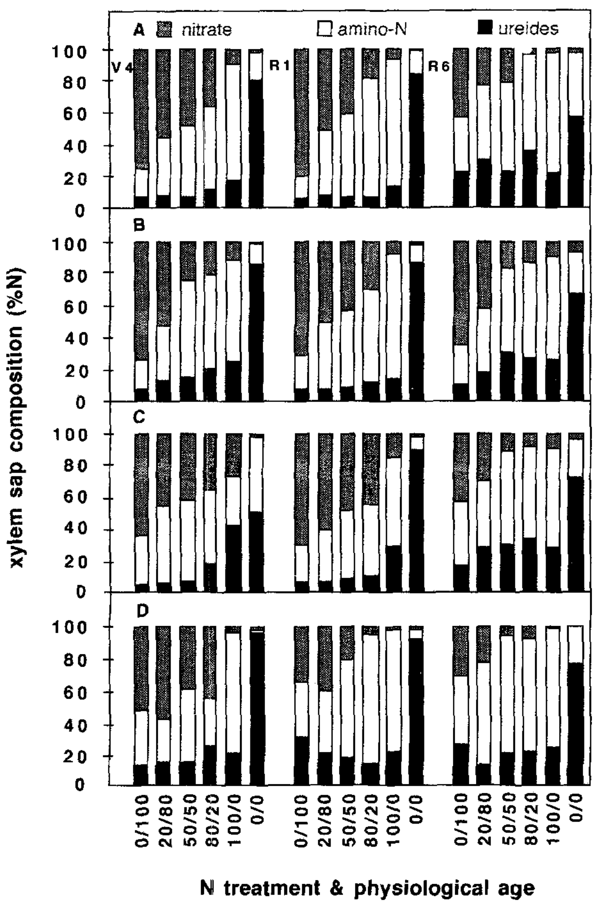

Figure 2. Changes in nitrogen-solute composition of xylem sap collected as root bleeding sap from *Phaseolus vulgaris* cv. Mokcham (*A*), Brilliant (*B*) and Rico (*C*) as well as the soybean (*Glycine max*) cultivar NW 1 (*D*), dependent on mineral-nitrogen source supplied in the rooting medium and physiological age (i.e. V4, R1 and R6). Nitrogen was supplied in the form of $(NH_4)_2SO_4$ or KNO_3 (12 mol m^{-3}) in different proportions. The relative supply of nitrogen in either form is indicated on the X axes by the figures separated by a slash (e.g. 20/80) with the first number representing the value for $(NH_4)_2SO_4$ and the second the value for KNO_3.

Mokcham, Rico and Brilliant. This ranking was consistently maintained throughout all treatments but with variable magnitudes of differences. Judging from figure 1 it is obvious that the presence of up to 50% ammonium in the rooting medium was beneficial for plant nitrogen accumulation in soybean and Rico, while both remaining common bean cultivars reflected a gradual, but continuous decline in nitrogen acquisition in the presence of any ammonium. Rico appeared to be most tolerant to ammonium since it remained essentially unaffected even if 80% of the mineral nitrogen in the rooting medium was in the form of ammonium sulphate (80/20).

Patterns of xylem sap composition. Partitioning of nitrogen transported in the xylem into ureides, amino acids and nitrate was subjected to alterations induced by plant age, genotype, and to some extent the source of mineral nitrogen available to plants (see fig. 2). While all genotypes of *P. vulgaris* showed rather accentuated patterns of increased proportional representation of ureides with increasing access to ammonium up to the R1 stage, such patterns were not maintained to the late reproductive period (R6). The cultivars Brilliant and to a lesser extent Rico reflected the most pronounced trends for the first two harvests, while data for Mokcham were clearly less conclusive. Regression analyses confirmed the existence of statistically significant linear relationships ($y = 0.16x + 7.224$; $r^2 = 0.979$ and $y = 0.072x + 5.18$; $r^2 = 0.876$ for the stages V4 and R1 respectively) between ammonium availability in the rooting medium of the plants and relative ureide abundance in the xylem sap of Brilliant ($p < 0.05$) during early development, while values for Rico remained just outside the range of statistical significance on a 95% level ($y = 0.33x + 1.624$; $r^2 = 0.726$ and $y = 0.182x + 2775$; $r^2 = 0.624$ for the stages V4 and R1 respectively). No such pattern regarding ureides could be established for soybean at any stage of development, pointing to an absence of any relationship between ammonium uptake and ureide production in the investigated genotype of this species.

In contrast, every investigated common bean cultivar at each developmental stage reflected significant correlations ($p < 0.05$) between nitrate supply to the plant and its recovery in the xylem sap. However, this result was less pronounced in soybean, suggesting more active nitrate reduction in the roots of the latter species. All studied specimens at the latest developmental stage showed reduced quantities of nitrogen in form of nitrate in their xylem sap; this is most likely due to reduced nitrate uptake at R6 and enhanced redistribution of N from other plant organs (i.e. leaves).

Amino-nitrogen was in most cases significantly ($p < 0.05$) correlated with ammonium supply. The notable exceptions were only Rico at the V4 stage and soybean at the stages V4 and R6. Hence, in this case again, the relationship proved to be less well established for soybean than for the common bean genotypes.

Fully symbiotic plants (0/0) generally reflected progressively increasing amino-nitrogen abundance in xylem sap with plant age at the expense of ureides. This may again be a consequence of retranslocation of nitrogen during this late growth phase and reduced symbiotic activity.

Discussion

The interaction between technological advancement and ever increasing scientific progress enhances experimental scrutiny, leading to a greater awareness of the methodological limitations of techniques used for the evaluation of N_2 fixation. Consequently, the caution first expressed in the literature regarding acetylene reduction assays has spilled over to other procedures including ^{15}N techniques and the xylem solute analyses[5,16–22]. In this context ureide formation by legumes in response to uptake of combined nitrogen in the form of ammonium (see ref. 6) is potentially very significant for measurements of symbiotic activity by xylem solute analyses. This specifically applies under conditions facilitating ammonium uptake by plants, since most calibrations of the method are based on nitrate as the sole source of mineral nitrogen. As high ammonium concentrations are not very common in agricultural soils and effects on ureides so far have only been reported if ammonium was the major form of mineral nitrogen available to plants, examinations of the problem have been limited. Evidence for soybean and pigeonpea suggested only insignificant effects[9]. However, other reports point to a correlation between ureide production and ammonium uptake[11,23] as well as an effect on amino nitrogen production. Such an influence on the presence of ureides, not associated to N_2 fixation, has also been reported when urea served as a source of combined nitrogen[6]. The results of this present study confirm a clear effect of ammonium uptake on amino-nitrogen production. For ureide-producing legumes this result is of no great importance since N_2 fixation determinations rely only on the relative ureide content. However, for amide-producing species this effect may have to be taken into account if xylem solute-based determinations of N_2 fixation are undertaken.

The presented data for soybean do not suggest any specific effect of the mineral nitrogen source, despite some variation in the relative ureide content of the xylem sap of plants exposed to different treatments[4,9]. Since nodulation was not completely suppressed by the different N treatments, some symbiotic activity may have occurred. Such activity could have had some influence on xylem sap composition and might have camouflaged potential changes due to ammonium uptake by the soybean genotype investigated in this experiment. However, considering the mostly very small amount of nodules retained by the soybeans exposed to mineral nitrogen, symbiotic activity is unlikely to have been very substantial. Hence, a significant response due to ammonium probably did not occur.

The situation, however, is less clear for at least some cultivars of common bean. Although a statistically confirmed correlation between ammonium uptake and ureide production could only be established for Brilliant up to stage R1, Rico also showed a very similar pattern. These results are consistent with other reports documenting such a relationship for some species including *P. vulgaris*[11,23,24]. In contrast, data recorded for the genotype Mokcham fail to confirm conclusively any substantial influence of ammonium on ureide synthesis. Consequently, one can assume that there may be not only species-, but also genotype-dependent differences in this matter. If one takes into account the potential errors which can occur in the calculation of nitrogen fixation as a consequence of ammonium-induced ureide production, it becomes clear that they may be considerable (up to 30%) in the most extreme situation, i.e. when plants are relying exclusively on ammonium for mineral nitrogen uptake. Under more common conditions where ammonium would only account for a minor fraction of the assimilated mineral nitrogen, an error close to $\pm 5\%$ is realistic, which may be viewed as acceptable considering other sources of inaccuracy in the technique. The reason for this rather minor effect can be found in the small relative increase of ureides in the xylem sap in response to ammonium in the rooting medium (see fig. 2). Despite the statistical significance of the correlation for the genotype Brilliant, the slope of the linear regression is too small to suggest a greater impact. Nevertheless, since apparently an interaction between ammonium uptake and ureide production exists and some genotypes show a clear response to ammonium, calculations should take this factor into account if the plants which are subsequently due to be investigated are cultivated in areas where ammonium is a considerable source of mineral nitrogen for plants (e.g. some forest soils, paddy fields, or in areas of heavy ammonium application). This could be facilitated during calibrations by cultivation of plants with access to varying quantities of nitrogen in form of ammonium and nitrate, rather than using nitrate alone as a mineral nitrogen source to achieve an estimate of dependencies on N_2 fixation and respective ureide contents in xylem sap. In this way potential errors can be reduced and reliability of results will be increased.

Acknowledgments. The authors are thankful to Prof. Tadakatsu Yoneyama for the critical evaluation of the manuscript. The financial support of the Deutsche Forschungsgemeinschaft (DFG), the Bundesministerium für wirtschaftliche Zusammenarbeit (BMZ) and the National Research Council of Thailand (NRC) was greatly appreciated.

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