Microbiology

Nitrogen Transport Compounds as a Potential Measure of Nitrogen-Fixing Activity of Pigeonpea

The acetylene-reduction technique provides a useful index of N_2-fixation, and has been used to determine the nitrogenase activity of field-grown legumes. Unfortunately, there are particular problems with the use of this assay for pigeonpea. From 30 days after planting most of the nodules occur on secondary roots and their attachment to these roots is fragile. The roots can grow to a depth of up to 2 m and nodules form deep in the soil in later stages of plant growth. Since the crop growth and maturity extends into the dry season, it becomes extremely difficult and time-consuming to quantitatively recover pigeonpea nodules. For this reason, alternative methods are particularly desirable with this species.

The possibility of using the compounds present in the xylem flow, exuded after decapitation of the plant, as a measure of nitrogen fixation was stimulated by the studies of Matsumoto et al. (1975, 1976). They showed that 90% of the nitrogen exported from soybean nodules was in the form of ureides-a group of compounds including allantoin and allantoic acid. Ureide levels were minimal in the xylem sap of a nonnodulated soybean isoline. These studies suggested a close link between the forms of nitrogenous compounds transported in the xylem sap and the nodulation status of the plant. Some plants such as chickpea (*Cicer arietinum*) and groundnut (*Arachis hypogaea*) contain negligible amounts of ureides in their xylem sap.

When the soil is adequately moist, xylem sap exudes from the stem of pigeonpea for at least 1 hour after the plant has been cut off in the hypocotyl region. In the sap of 50-day-old plants of cv ICP-1 growing in an Alfisol at ICRISAT, ureides were the main nitrogenous compounds, amounting to 57% of the total soluble Kjeldahl-N. Amino acids and amides amounted to 37%, the remainder being predominantly nitrate.

We examined the effect of increasing N levels in the growth medium on the composition of the xylem exudate in two pot experiments, one conducted at ICRISAT with ICP-1 (Table 13) and the other at the University of the West

Table 13. Effect of nitrate on nodulation, nitrogenase activity, and reduced nitrogen compounds in the xylem sap of 80-day-old pigeonpea[a].

Nitrate N (ppm)	Nodule number/ plant	Nodule weight/ plant (mg)	N_2-ase activity (µmol C_2H_4/ plant/hr)	Ureides in xylem sap (µgN/plant/hr)	Ureides as % total soluble Kjeldahl-N	Amino acids + amides in xylem sap (µgN/plant/hr)
0	104	203	5.3	312	54	88
25	111	256	6.5	265	50	98
50	57	117	2.8	215	42	101
75	63	64	1.9	80	16	55
100	62	65	1.2	74	16	59

a. Pigeonpea cultivar ICP-1 was grown in sand culture and watered daily to excess with nutrient solution containing nitrate at the levels indicated

Indies with UWI-17 (Fig.8). In both studies the amount of ureide in the xylem exudate decreased as the nitrogen supplied to the growth medium increased to 100 ppm N. There was a parallel reduction in nodulation. At ICRISAT for cv ICP-1 with no added nitrate, ureide accounted for 54% of the total soluble Kjeldahl-N at 90 days after planting. With 100 ppm N in the applied nutrient solution the ureide level was reduced to 16%. For cv UWI-17 (Fig.8) the concentration of nitrate present in the xylem exudate increased with increasing levels of nitrate in the growth medium, but the concentration remained relatively constant with time. The concentration of amides and amino acids in the xylem exudate increased with increasing nitrate level in the growth medium, and there was a

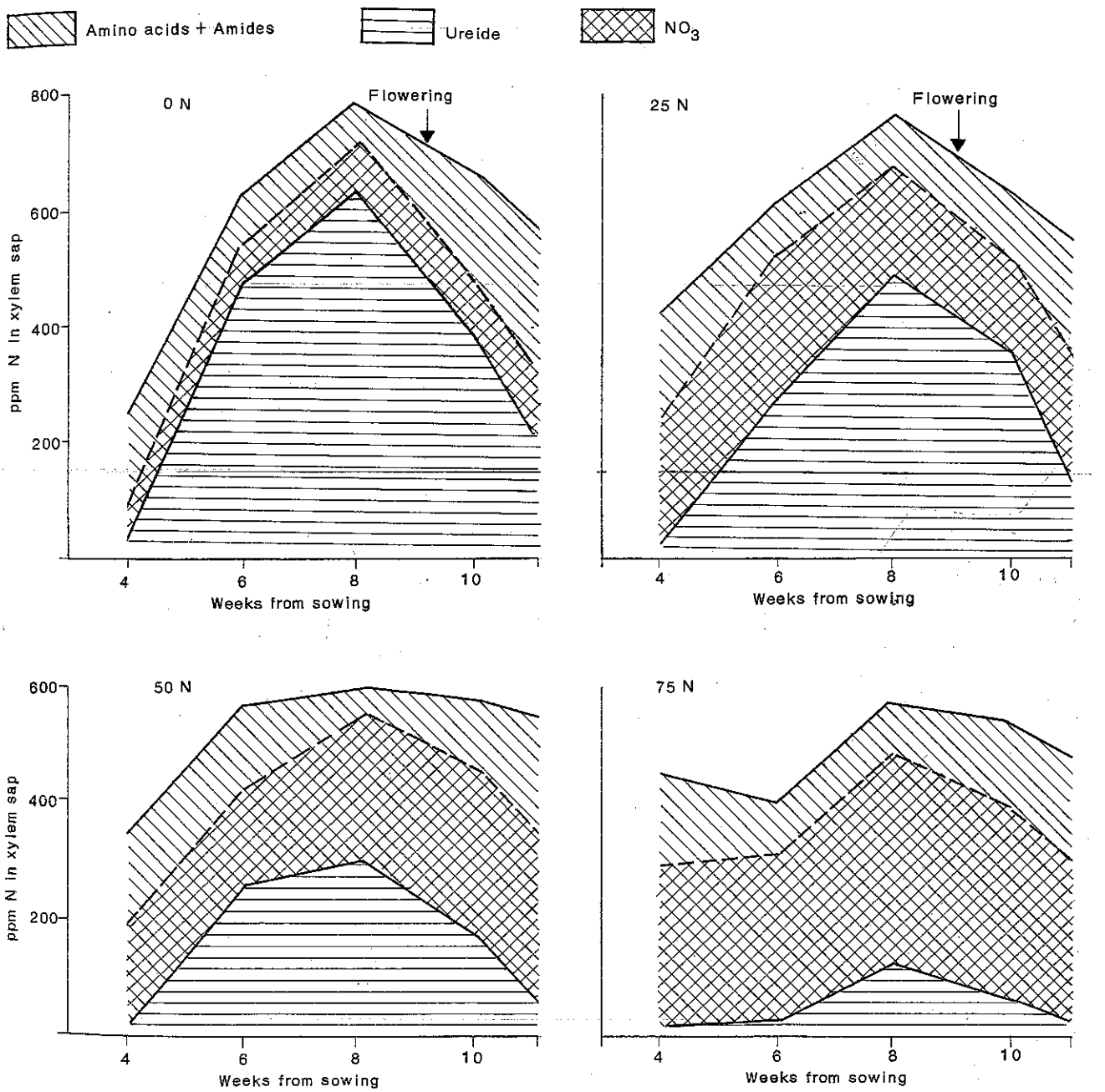

Fig. 8. Concentration of ureide (allantoin and allantoic acid), amino acids plus amides and nitrate in the xylem sap of pigeonpea cv UWI-17, grown with nutrient solutions containing 0, 25, 50, and 75 ppm nitrate N.

pronounced increase after 8 weeks when the concentration of ureide was declining.

In another field experiment at ICRISAT, five pigeonpea cultivars were grown in an Alfisol, and the ureide and amino acid + amide nitrogen were determined. The ureide flux from roots increased up to the last harvest at 90 days, and followed reasonably well the nitrogenase activity. The ureides, allantoin, and allantoic acid were the major nitrogenous compounds in the sap. There appear to be differences between cultivars in the amount of ureides and amino + amide N in the sap. There was a close relationship between the ureide content, particularly of the xylem exudate, and the nodulation status of the plant. However, there is always an appreciable amount of amide and amino acid N present in the xylem exudate and it is the source of this N that will determine the usefulness of xylem exudate analysis as a measure of N_2-fixation. The amide and amino N could come either from fixation or via nitrate reductase activity in the roots. If nitrate is not reduced in the roots to any significant degree, then the total reduced N, i.e., ureide plus amino and amide N, would be derived from nitrogen fixation, and the amount of nitrate would reflect the uptake from soil N. If, however, appreciable NO_3-reductase activity occurs in the roots of pigeonpeas, the relationship between fixation and allantoin in the sap is less reliable.

References

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Grain Quality/Biochemistry

Pigeonpea Grain Quality Investigated at ICRISAT

From the utilization point of view, the grain quality of pigeonpea is important. It has several components, including nutritional quality, antinutritional factors, digestibility and bioavailability of nutrients, cooking quality, consumer acceptability, and storage stability.

We have standardized a method for the determination of protein and have analysed over 20,000 samples consisting of germplasm accessions and breeding material. The range in the protein content in dhal (decorticated split seed) was from 15.1 to 31.5% with a mean protein content of 23.2%. Some of the species of *Atylosia*, a related genus, were found to have higher protein levels, and intergeneric lines from crosses of T-21 and *Atylosia* species showed that a few lines had more than 30% protein.

Methionine, cystine, and tryptophan are the important amino acids that are deficient in grain legumes. Accurate analysis of these amino acids requires tedious, careful, and expensive procedures, and analyses of large numbers of samples involve a great deal of time and effort. Attempts are therefore being made to check the suitability of rapid colorimetric procedures for the estimation of these amino acids.

Although pigeonpea has lower levels of trypsin and chymotrypsin inhibitor activities, as compared with soybeans, some of the wild relatives of pigeonpea have been found to contain higher concentrations of these inhibitors. Some of the antinutritional constituents are reported to be destroyed on cooking. However, these factors may have a role in insect- or disease-resistance characteristics. The presence of polyphenolic compounds (loosely termed tannins) and their role in the utilization of nutrients has been receiving some attention. Analysis of four pigeonpea cultivars with different seed coat colors showed that the seed coat contained the highest proportion of polyphenols and red seed appears to have a higher concentration of polyphenols than white seed.

Attempts are being made to find out the factors that influence the cooking time of