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Nodulation, nitrogen fixation and nitrogen uptake in pigeonpea (*Cajanus cajan* (L.) Millsp) of different maturity groups*

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Summary The seasonal patterns of nodulation, acetylene reduction, nitrogen uptake and nitrogen fixation were studied for 11 pigeonpea cultivars belonging to different maturity groups grown on an Alfisol at ICRISAT Center, Patancheru, India. In all cultivars the nodule number and mass increased to a maximum around 60–80 days after sowing and then declined. The nodule number and mass of medium- and late-maturing cultivars was greater than that of early-maturing cultivars. The nitrogenase activity per plant increased to 60 days after sowing and declined thereafter, with little activity at 100 days when the crop was flowering. At later stages of plant growth nodules formed down to 90 cm below the soil surface but those at greater depth appeared less active than those near the surface.

All the 11 cultivars continued to accumulate dry matter until 140 days, with most biomass production by the late-maturing cultivars (up to 11 t ha^{-1}) and least by the early-maturing determinate cultivars (4 t ha^{-1}). Total nitrogen uptake ranged from 69 to 134 kg ha^{-1}. Nitrogen fixation by pigeonpea was estimated as the difference in total nitrogen uptake between pigeonpea and sorghum and could amount to 69 kg N ha^{-1} per season, or half the total nitrogen uptake. Fixation by pigeonpea increased with crop duration, but there were differences within each maturity group. The limitations of the methods used for estimating N_2 fixation by pigeonpea are discussed.

Introduction

Pigeonpea is an important grain legume of the semi-arid tropics, with about 90 per cent of the total world production in India[8] where it is generally grown as an annual crop, though it is intrinsically perennial[1]. A wide range of maturity groups exists in pigeonpea, an important factor in the adaptation of the crop to diverse agroclimatic areas and agronomic systems. Based on days to 50% flowering at ICRISAT, Patancheru, India, ten maturity groups ranging from 60 days to more than 160 days have been identified[3]. The crop is usually sown at the onset of the monsoon in June or July. In peninsular India, the early-maturing cultivars are harvested after 4–5 months, medium-maturing cultivars after

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Table 1. Plant habit, maturity group and days to flowering of the pigeonpea cultivars used for experimentation

Cultivar	Plant habit[a]	Days to 50%[b] flowering	Maturity group[b]
Pant A-3	D	60	0
Prabhat	D	61–70	I
UPAS 120	I	71–80	II
T 21	I	81–90	III
BDN 1	I	101–110	V
No.148	I	101–110	V
Bhedaghat	I	131–140	VII
JA 275	I	141–160	VIII
Bhandra	I	141–160	VIII
T 7	I	141–160	VIII
NP(WR) 15	I	Above 160	IX

[a] D – Determinate, I — Indeterminate.
[b] see ICRISAT, 1978

5–6 months and late-maturing cultivars after 6–9 months. The first 3–4 months of growth takes place during the monsoon, and subsequent growth is mainly dependent on moisture stored in the soil.

It is particularly important to know the ability of pigeonpeas to nodulate and fix nitrogen as they are generally grown on marginal soils with little input. Although Kumar Rao and Dart[4] discussed the biology of nodulation of a medium-maturing pigeonpea cultivar, little information is available on the comparative nodulation and nitrogen-fixing ability of cultivars from different maturity groups. We have therefore studied the seasonal pattern of nodulation, nitrogen fixation, and nitrogen uptake of 11 pigeonpea cultivars of different maturity groups.

Materials and methods

The experiment was conducted at ICRISAT Center, Patancheru, India, on an Alfisol (fine loamy, Udic Rhodustalfs) fertilized with single superphosphate at a rate of 17 kg P ha^{-1}. No nitrogenous fertilizer was applied. At sowing, the pigeonpea seed was inoculated with an effective rhizobium strain, IHP 114. The trial was sown on 8 July 1977. The pigeonpea cultivars used were selected from those most commonly grown by farmers and plant breeders in India (Table 1). The cultivars are grouped into three major maturity groups as follows: early (maturity groups 0 to III), medium (group V) and late (groups VII to IX). A long-duration and photosensitive sorghum, cv IS 11758, that matures in about eight months at Hyderabad, was grown as one of the treatments to serve as an indicator of soil N available for plant growth, providing a non-fixing control for estimating N_2 fixed by different cultivars of pigeonpea.

The experimental design was a randomized complete block with three replicates. Each plot measured 10 m × 6 m, with eight rows 75 cm apart. For pigeonpea, two seeds were sown per hill that were 20 cm apart; sorghum seeds were sown at a spacing of 10 cm. The seedlings were thinned to one per hill. The plots were weeded by hand as and when required. Insect pests of aerial plant parts, particularly *Heliothis armigera*, were controlled by spraying the crop with endosulfan at 0.7 kg a.i. ha^{-1}. The crop was grown under rainfed conditions and the monthly rainfall and mean maximum and minimum temperatures during the growing period are given in Table 2.

Table 2. Monthly rainfall and mean maximum and minimum temperatures at ICRISAT Center, Patancheru, India, during the pigeonpea growing season, 1977–78

Month	Rainfall (mm)	Temperature (°C)	
		Mean maximum	Mean minimum
1977			
July	184	30.9	22.5
August	194	29.1	22.3
September	40	30.8	21.7
October	59	30.6	20.0
November	28	29.1	20.4
December	2	27.7	13.0
1978			
January	17	28.0	15.8
February	26	29.6	18.1
March	4	34.4	19.8

Each pigeonpea cultivar was sampled 20, 40, 60, 80, 100, and 140 days after sowing. Samples were collected from near the end of a sample row after removing the first few plants to eliminate any border effects. Because of the extensive root system and labour involved in digging out the roots the sample size varied depending on the plant age so that ten plants plot^{-1} were taken up to 40 days, five plants plot^{-1} up to 80 days and three plants plot^{-1} up to 140 days after sowing. At each sampling the shoot was cut at ground level before the roots with nodules were carefully dug out with a crowbar, the depth of soil sampled increased with plant age. Nodulated roots were assayed for nitrogenase activity by acetylene reduction. Roots and nodules were placed in a glass container of suitable size and with a rubber septum fitted in the lid. After a 30 min incubation in a 10% atmosphere of C_2H_2 at ambient air temperature in the field, a 3.0 ml gas sample was removed and stored in a pre-evacuated venoject tube. The sample was analyzed for C_2H_4 on a Pye Unicam 104 gas chromatograph fitted with a flame ionization detector and a 150 cm-long glass column of 0.6 cm O.D., packed with Porapak N. The oven temperature of the gas chromatograph was 100°C and the carrier gas (N_2) flow rate 45 ml min^{-1}.

After the acetylene reduction assay, roots and nodules were cleaned of soil by washing in water and the nodules separated and counted. Plant tops, roots and nodules were dried at 70°C for 48 h, weighed and ground for N determination. Fallen plant parts were collected from 3 m^2 subplots at a fixed place within each plot at regular intervals, starting around 80 days after sowing. Their oven-dry weights and N contents were determined. At maturity, pods were harvested by hand, air-dried, and threshed. Grain, husk, and plant top dry weight and N content were determined. The N contents of plant material were determined by block digestion method and a Technicon Autoanalyzer (Industrial method No. 218-72A).

Results

Nodulation and nitrogenase activity

The results of nodulation and nitrogenase activity of a six pigeonpea cultivars selected to represent the three maturity groups are presented in Figures 1 and 2 respectively. The pattern shown by these cultivars is fairly representative of other cultivars of similar maturity.

Pigeonpea is a deep-rooted legume and in this experiment it formed nodules to a depth of at least 90 cm in the soil. The nodules formed as

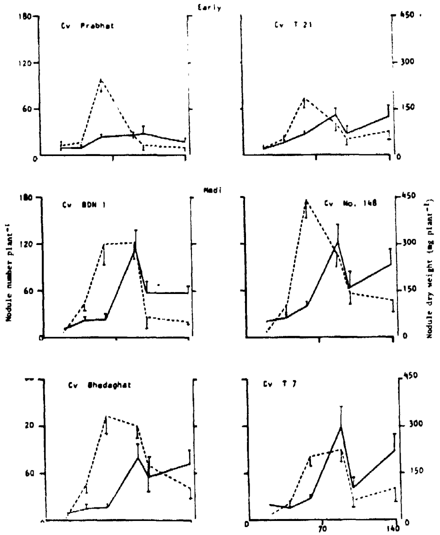

Fig. 1. Nodule number ( ) and dry weight (- - -) over time of pigeonpea cultivars of different maturities. *Bars* indicate standard error of mean.

early as 20 days after sowing were mostly on the primary root. These early-formed nodules senesced rapidly and nodules formed after 20 days were mostly found on secondary roots. In all the cultivars tested, nodule number and nodule mass increased with plant age and reached a maximum around 60–80 days after sowing, and declined thereafter (Figure 1). The nodulating ability of medium- and late-maturing pigeonpea cultivars was greater than that of early-maturing cultivars. Though the nodule number continued to increase up to 80 days after sowing, nodule weight did not show a corresponding increase. This was mainly because the nodules were destroyed by the larvae of the insect *Rivellia angulata*[7].

In Figure 2, active N_2 fixation was measurable by ARA within 20 days after sowing in all cultivars. The nitrogenase activity per plant increased

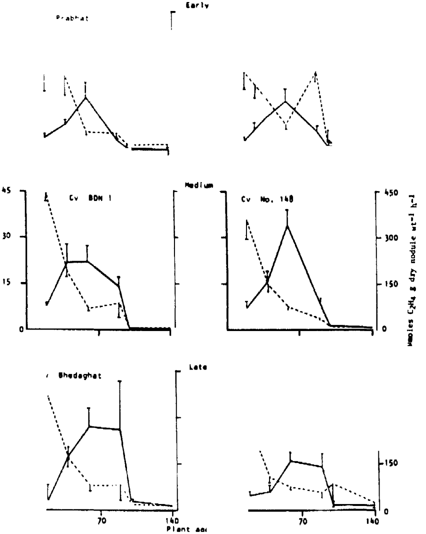

Fig. 2. Nitrogenase activity plant^{-1} (——) and g dry weight nodule^{-1} ( ) hour^{-1} over time of pigeonpea cultivars of different maturities. *Bars* indicate standard error of mean.

with plant age to a maximum at about 60 days after sowing. Thereafter activity declined and was minimal by 100 days, coinciding with the beginning of the postrainy season and the drying of the soil. The pattern of nitrogenase activity over the season in cultivars of different maturity groups closely followed that of nodule weight. The specific nitrogenase activity (μM C_2H_4 g nodule dry wt^{-1}h^{-1}) was greatest 20 days after sowing and then declined with time (Figure 2). A similar trend was seen in all cultivars and maturity groups except in cv T 21 when a rise in specific nitrogenase activity was observed at 80 days after sowing. The reasons for this are not known. The deep-rooted nature of pigeonpea enables the crop to explore a large volume of soil for moisture and nutrients, particularly in the postrainy season when the medium- and late-maturing cultivars flower and mature. The nodules formed at deeper layers might not be so active as the nodules formed at a shallow depth (0–30 cm). At the 80-, 100-, and 140-day harvests the color of cut nodule tissue of nodules from below 50 cm in the soil was usually white or brown, compared with a pink color for active nodules in the surface layers of soil. The decline in nitrogenase activity after 60 days could be due to several factors. Firstly, increased insect damage of nodules could have reduced the amount of active nodule tissue. Secondly, the recovery of nodules that were formed deep in the soil particularly in later harvests was probably incomplete. Thirdly, the cessation of nitrogenase activity after 100 days may well result from soil moisture deficit, even though the plants were apparently not stressed. Modelling studies suggest that for soybean the reduction in nitrogenase activity during pod fill is related to the decline in soil water below 50% of the plant available soil water holding capacity (T.R. Sinclair, Personal communication).

Dry matter production

Accumulation of dry matter in shoots and roots up to 140 days after sowing in pigeonpea plants of different maturity groups is presented in Figure 3. It increased similarly for the different cultivars. Final dry matter accumulated by cultivars increased with their maturity, but not grain yields which were as follows: early, 1.0–1.3 t ha^{-1}; medium. 1.3–1.4 t ha^{-1}; late, 0.7–1.0 t ha^{-1}. There was a wide range in harvest index, from 7 to 28% tending to decrease as plant biomass production and duration increased (Table 3). The cultivars also differed in the quantity of plant parts shed during the life cycle. It ranged from 0.5–1.0 t ha^{-1} in early-maturing cultivars, from 1.3–1.6 t ha^{-1} in medium-maturing cultivars and from 1.2–1.9 t ha^{-1} in late-maturing cultivars.

Nitrogen uptake and fixation

The changes in nitrogen concentration with growth up to 140 days after sowing in plant tops, roots and nodules of pigeonpea cultivars of different maturity groups are presented in Figure 4. As expected, young plants had the highest nitrogen concentration. The decline in nodule tissue nitrogen concentration paralleled that for nitrogenase activity per plant, with both measurements being at a maximum around 60 days after sowing. Though new nodule formation continued beyond 60 days after sowing, the presence of senescent tissue in the older nodules reduced the overall nodule nitrogen content. In plant tops, the nitrogen concentration declined after 40 days after sowing. The root nitrogen content declined rapidly after 20 days, but was quite stable after 60 days.

In the present study we attempted to estimate available soil nitrogen

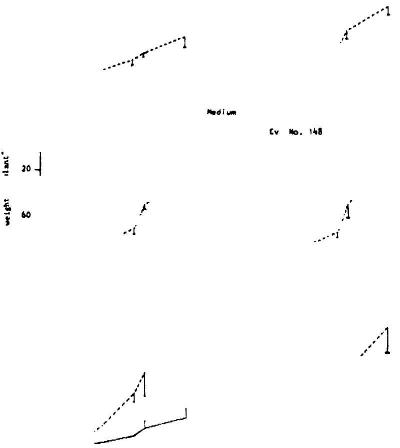

Fig. 3. Dry matter produced in shoot (---) and root without nodules (——) by pigeonpea cultivars of different maturities. *Bars* indicate standard error of mean.

by growing a long-duration sorghum cultivar. If we make the assumption that the roots of sorghum explore a similar volume of soil to that explored by pigeonpea over both time and space, and that both species take up all available soil nitrogen, then nitrogen fixation by the legume can be calculated as total legume nitrogen uptake minus total nonlegume nitrogen uptake. Tables 3 and 4 show the results of dry matter produced and total nitrogen uptake by pigeonpea and sorghum and estimates of N fixed by the 11 pigeonpea cultivars.

Total nitrogen uptake and net nitrogen fixation by pigeonpea generally increased with crop duration, but there were substantial differences between cultivars within a maturity group. Early-maturing determinate cultivars apparently fixed little nitrogen (maximum $7\,kg\,N\,ha^{-1}$). Indeterminate early- and medium-maturing cultivars fixed more nitrogen,

Table 3. Grain, dry matter production (kg ha^{-1}) and harvest index at maturity of some pigeonpea cultivars and sorghum grown on an Alfisol at ICRISAT Center, Patancheru, India, 1977-78.

Cultivar	Harvest time (days)	Grain	Pod wall	Plant top	Roots	Nodules	Fallen plant parts	Total dry matter	Harvest index (%)
Pant A-3	140	1050	560	1390	440	0.8	540	3980	26
Prabhat	140	1140	330	1560	330	2.0	680	4050	28
UPAS 120	140	1270	270	2120	710	1.9	900	5270	24
T 21	140	1350	740	2460	620	3.5	1060	6240	22
BDN 1	140	1300	660	2630	480	3.8	1570	6640	20
No 148	150	1370	550	3290	990	7.1	1260	7480	18
Bhedaghat	172	720	670	3440	1590	4.6	1310	7730	9
JA 275	172	830	460	2470	1160	1.8	1160	6082	14
Bhandra	230	1040	880	3100	1010	4.3	1910	7940	13
T 7	220	780	530	6240	2010	6.8	1370	10940	7
NP (WR) 15	241	700	760	5210	1280	5.4	1200	9150	8
Sorghum	175	150	560	8280	470		0	9460	
S.E.		± 118	± 1·15	± 371	± 241	± 1.23	± 149	± 534	

the amount increasing with days to 50% flowering rather than to final harvest date (140 days). These cultivars ranged from 27 to 55 kg N ha^{-1} based on the most conservative estimate. In late-maturing pigeonpea cultivars, the estimate of nitrogen fixed ranged from 13 to 69 kg N ha^{-1}. Even for the best-fixing cultivar, T 7, N_2 fixation represented only 52% of the total nitrogen uptake. The harvest index for nitrogen, that is the amount of N in the grain as a proportion of total plant nitrogen uptake was also small, ranging from 21% to 57% (mean of 38%) and decreased with crop duration; this was about double the harvest index for dry matter (range 7 to 28%, mean 17%).

The amount of nodule tissue at the end of the season is very low and accounts for less than 0.2 kg N ha^{-1}. However, this is an underestimate of the total amount of nitrogen returned to the soil as nodules senesce, since this is a continual process starting about 30 days after sowing. Even so, such nodules contain on an average only 3.7% N, much less than that found in active nodules (about 5.5% N). Maximum nodule weight per plant was 300 mg for T 7 with a nitrogen content of 15 mg, extrapolating to 1.0 kg N ha^{-1}.This is still an underestimate of the return of N to the soil via nodule decay.

Discussion

The nitrogenase activity profiles differed relatively little between genotypes despite the large differences in net nitrogen fixation estimated by the difference method. Seasonal estimates of nitrogen fixation, derived by integrating the area under the curves for acetylene reduction

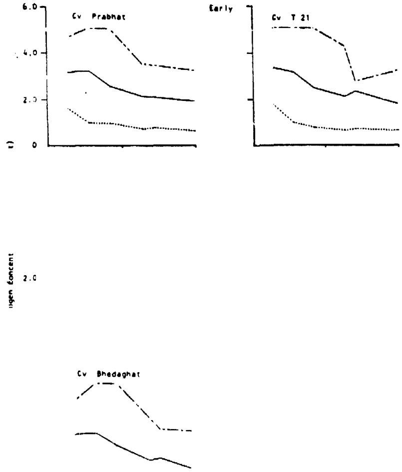

Fig. 4. Nitrogen concentration in tops (—), roots (· · ·) and nodules (· · ·) of pigeonpea cultivars of different maturities.

activity per plant over the season (Figure 2), and multiplying this by the plant population, using a conversion factor of 3 (1 mole of N_2 fixed for 3 moles of C_2H_2 reduced to C_2H_4) were much lower than that estimated by the difference method (3–5 kg N ha^{-1} cf 43–13 kg N ha^{-1} for cultivars T 21 and JA 275[9]). This may be partly due to a large variation in the conversion factor during the life cycle. The attachment of pigeonpea nodules to the root system is also very fragile, and invariably nodules become detached from the roots during preparation for the assay. In other species such as soybean, nitrogenase activity is much reduced in detached nodules compared with nodules still attached to the root (J F Witty, personal communication).

It is difficult to measure net nitrogen fixation by pigeonpea grown in the field, because of the problem of estimating nitrate uptake by such a

Table 4. N content ($kg\,ha^{-1}$) in fractions of pigeonpea cultivars and a sorghum grown on an Alfisol at final harvest

Cultivar	Grain	Pod wall	Plant top	Roots	Nodules	Fallen plant parts	Resid.[a] N	Total N up-take	Estim.[b] N_2-fix.	Harvest index for N (%)
Pant A-3	38.8	5.5	14.9	3.9	0.03	8.5	16	72	7	54
Prabhat	39.4	4.0	11.6	2.6	0.07	11.4	16	69	4	57
UPAS 120	43.4	2.7	24.9	5.2	0.06	15.5	26	92	27	47
T 21	49.1	6.6	31.1	4.5	0.11	16.5	26	108	43	45
BDN 1	45.7	6.6	37.2	4.0	0.11	24.6	33	118	53	39
No. 148	48.8	4.4	40.9	7.9	0.22	17.6	33	120	55	41
Bhedaghat	25.5	7.9	34.7	11.8	0.15	21.0	45	101	36	25
JA 275	28.7	5.0	19.0	7.4	0.06	17.7	32	78	13	37
Bhandra	38.9	10.1	23.0	7.5	0.14	28.1	43	108	43	36
T 7	27.7	5.6	64.2	15.1	0.22	21.3	51	134	69	21
NP (WR) 15	25.3	8.7	54.1	11.3	0.16	14.7	37	114	50	22
Sorghum	4.3	5.2	51.6	3.6	-	0	-	65		
S.E.	±4.42	±1.23	±6.22	±1.87	±0.039	±2.07		±9.3		

[a] Calculated as root N × 2 + N in fallen plant parts (see text).

[b] N_2-fixation calculated as total N uptake of pigeonpea minus that for sorghum.

long-duration and deep-rooted crop. In the present study a long-duration sorghum was grown and harvested only once, 175 days after sowing, to measure available soil nitrogen. Thus the value of the nitrogen fixed by pigeonpea cultivars of early maturity (particularly Pant A-3 and Prabhat) is likely to be an underestimate. In addition the sorghum may have gained a small amount (up to 15 kg ha^{-1}) of nitrogen from nitrogen fixation by bacteria associated with its roots[2], resulting in an underestimate for all values for pigeonpea nitrogen fixation.

Nitrogen fixation by pigeonpea generally increased with crop duration, but there were substantial differences between cultivars within a maturity group. Nevertheless, the present results suggest that the early-maturing cultivars Pant A-3 and Prabhat fixed little nitrogen. They were also efficient in partitioning nitrogen to the grain, as evidenced by the harvest index for nitrogen. It may be that this efficient partitioning of both carbohydrate and nitrogen resulted in an early diversion of photosynthate from the root system, particularly the nodules, so that nitrogen fixation was reduced.

A considerable amount of nitrogen (9 to 28 kg ha^{-1}) was lost as fallen plant parts (mostly leaves), with large differences between cultivars, but with a surprisingly similar and high percentage of nitrogen (mean 1.54%, range from 1.23 to 1.68%). The fallen plant parts represented 12 to 26% of the total N taken up by the plant. Assuming, with Sheldrake and Narayanan[6], that we recover only 50% of the roots, then for cv BDN 1, fallen plant parts and roots plus nodules potentially return 33 kg N ha^{-1} to the soil, a figure very similar to the 40 kg N ha^{-1} estimated by Sheldrake and Narayanan[6] for ICP 1, a variety with a similar maturity to BDN 1. The residual N ranged from 16 kg N ha^{-1} for the shortest-duration cultivars to 51 kg N for T 7, the longest-duration cultivar, the amount correlating well with plant duration. This could account for the large residual effect of pigeonpea reported on a succeeding cereal crop[5].

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