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Yield, nodulation, and N_2 fixation by cowpea cultivars at different phosphorus levels

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Abstract A greenhouse experiment was conducted to investigate the effect of a P application (0 vs. 50 mg P kg^{-1}) on yield, nodulation, and N_2 fixation by three cowpea cultivars (Soronko, Amantin, and IT81D-1137) using the ^{15}N isotope-dilution method. When P was not applied the inoculated cowpea genotypes showed significant differences (Soronko>Amantin> IT81D-1137) in N accumulation, in contrast to the uninoculated cowpea cultivars, which accumulated similar amounts of N. The differences in shoot N in inoculated plants were thus caused by differences in N_2 fixation. The average values of N fixed (for both P levels) were 74% in Soronko, 59% in Amantin, and 42% in IT81D-1137, corresponding to 80, 51, and 24 mg N $plant^{-1}$, respectively. Inoculation increased the total shoot-N accumulation in cv. Soronko by 270% without P and by 204% with P, cv. Amantin by 152 and 104%, and cv. IT81D-1137 by 74 and 58%, respectively. With P, the % N derived from atmosphere (%Ndfa) was 42% for IT81D-1137, 62% for Amantin, and 76% for Soronko. The high value for Soronko indicates that in a soil of medium fertility, certain cowpea cultivars are capable of satisfying their total N requirement through N_2 fixation. The P effect on N_2 fixation was mainly in the total amount of N fixed rather than on the percentage derived from the atmosphere.

Key words Biological nitrogen fixation · Cowpea · N assimilation · ^{15}N isotope dilution · P assimilation

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Introduction

The cowpea [*Vigna unguiculata* (L) Walp.] is an important grain legume crop in the lowland tropics, with west Africa producing well over 60% of the world's output (Rachie and Roberts 1974). Despite the importance of the cowpea as a dominant dietary protein source, as an organic fertilizer (Eaglesham et al. 1981), and as a cash crop, seed yields are low and inconsistent (Summerfield et al. 1974).

Deficiencies in N and P are among the major constraints on higher crop productivity in most tropical soils. Although these soils may be fertilized to increase cowpea yields, a better option may be to identify, select, or breed cowpea genotypes for increased N_2 fixation, and thereby increase the efficient use of soil or fertilizer P.

The identification and selection of genotypes for increased N_2 fixation requires accurate methods of estimating this process. The use of the stable isotope ^{15}N offers a reliable means of measuring N_2 fixation in legumes (Danso et al. 1993; Fried and Broeshart 1975). However, there are only few reports on ^{15}N-based estimates of N_2 fixation in the cowpea (Eaglesham et al. 1977, 1981; Ofori et al. 1987; Awonaike et al. 1991).

There is also a lack of information on the influence of P fertilizer application on N_2 fixation in the cowpea. Studies on the performance of this crop in relation to P have concentrated on the P effects on yield and nodulation (Mason and Leihner 1988; Seripong 1988; Daramola and Afolabi 1989).

The present experiment was carried out to study the P nutrition of three cowpea cultivars in order to determine how differences in the P level affect yield, nodulation, and N_2-fixation, using the ^{15}N isotope-dilution technique.

Materials and methods

A pot experiment was conducted in the greenhouse of the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf, Austria, from June 1 to September 5, 1989. The greenhouse was naturally lit with interior mean day/night temperatures of 35/16°C and a relative humidity of 50/70%.

The experiment consisted of 12 treatments, three determinate cowpea cultivars grown at two P levels (0 and 50 mg P kg^{-1}) and two inoculation treatments (inoculated vs. uninoculated). Each treatment was replicated three times, with the pots arranged in a completely randomized design. The reference plants for calculating N_2 fixation by ^{15}N methodology comprised a non-nodulating soybean isoline (cv. 129) and uninoculated cowpea plants.

The cowpea cultivars were (1) Amantin, with medium-sized, mottled dark red seeds, maturity 65–70 days; (2) IT81D-1137, with large, white seeds, maturity 70–80 days; and (3) Soronko, with small, brown seeds and maturity in 70–80 days. The cultivars were among those recommended in Ghana and seeds were supplied by the Crops Research Institute of Ghana.

An Austrian Waldviertel soil was used. It had a pH(H_2O) of 6.1, 14.2 g organic C kg^{-1}, 0.77 g total N kg^{-1}, 8.5 mg (NH_4Ac-ethylenediaminetetraacetic acid-extractable) P kg^{-1}, and 72, 155, and 773 g kg^{-1} clay, silt, and sand, respectively.

Plastic pots (18 cm deep, 17 cm top diameter) were each filled with 4.5 kg of a 1:1 soil:sand mixture as a growth medium. Ten milligrams of N per kilogram of soil and 10% ^{15}N atom excess ^{15}N-labelled $(NH_4)_2SO_4$ was applied in solution to each pot sown to cowpeas, while the non-nodulating soybeans were supplied with 20 mg N kg^{-1} of 10% ^{15}N atom excess as $(NH_4)_2SO_4$. Half the pots sown to cowpea were supplied with 50 mg P kg^{-1} soil, in the form of triple superphosphate (19.6% P) mixed into the soil, while the other half had no P fertilizer. Four seeds of each cowpea cultivar and of the non-nodulating soybean were sown per pot. Half the pots with and half those without P fertilizer were then inoculated with cowpea rhizobial strain NUM 716, applied in suspension onto the seeds. Three days after emergence, the plants were thinned to two per pot. Every week 50 ml of N- and P-free Hoagland solution (full strength) was applied to each pot and the soil was maintained at 75% field capacity by watering.

The plants were harvested 96 days after planting, when Amantin had reached full physiological maturity and 1T81D-1137 and Soronko were at the pod-filling stage. All plants were separated into shoots and roots, and dried at 70°C for 72 h for dry weight determinations. Total N was determined by Kjeldahl analysis (Eastin 1978) on ground (40-mesh) plant material, and %^{15}N atom excess by a VG 602 mass spectrometer (Fiedler and Proksch 1975).

The proportion (%) and amount (mg $plant^{-1}$) of cowpea N derived from atmospheric N_2 were estimated by three different approaches: (1) ^{15}N isotope dilution (Fried and Middelboe 1977), using uninoculated cowpea plants as a reference; (2) the *A* value method (Fried and Broeshart 1975), using non-nodulating soybean plants as a reference; and (3) the total N difference method (Talbott et al. 1982; Rennie 1984), using the uninoculated cowpea plants as a reference.

An analysis of variance was performed to assess the effects of cultivar (C), P level, inoculation (I), and their interactions (C×P, C×I, P×I) on yield, nodulation, and N_2 fixation. Comparisons between treatment means for various measured parameters were made by the Duncan multiple range test. The relationship between pairs of selected parameters was assessed by simple linear regression analysis.

Total P in plant samples was determined by the colorimetric (phosphovanadomolybdate, yellow complex) method of Barton (1948).

Results and discussion

Nodulation

Without P fertilizer, Soronko formed the largest number of nodules, about 41% more than Amantin, and almost three times as many as IT8lD-1137 (Fig. 1). Nodule mass followed the same trend as nodule numbers, with Soronko having the largest nodule mass, about twice that of

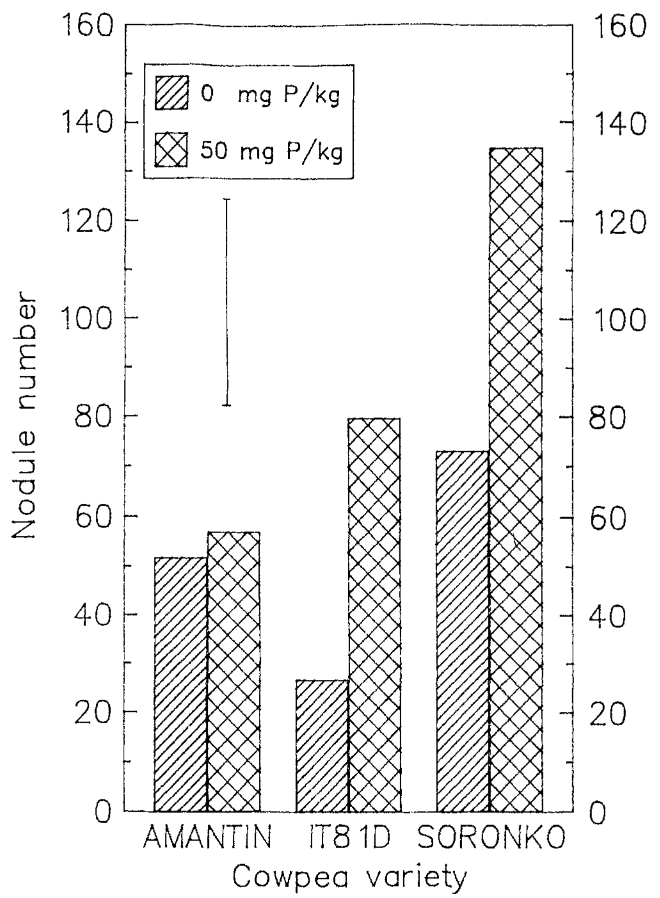

Fig. 1 Number of nodules on three inoculated cowpea cultivars at two P levels

IT81D-1137 (Fig. 2). These results show that there were large genotypic differences in the nodulation capacities of the cultivars under low-P conditions.

The application of P resulted in a significant increase in the number of nodules, especially on Soronko and IT81D-1137, with a much lower increase in Amantin (Fig. 1). Although a positive effect of P on cowpea nodulation has also been reported by Andrew (1977), Seripong (1988), and Daramola and Afolabi (1989), it is interesting that one cultivar, Amantin, did not show a significant response, thus indicating genotypic differences in the effect of P on nodulation. Also, although nodule numbers on IT8lD-1137 were increased by as much as 200% with P fertilization, these nodules were comparatively small and consequently the nodule mass was increased by only 70%. In contrast, there was a close match between the corresponding increase in nodule numbers and mass of Soronko.

There were no nodules on the roots of the uninoculated treatments and these plants were therefore valid as reference plants for measuring N_2 fixation by the ^{15}N methodology.

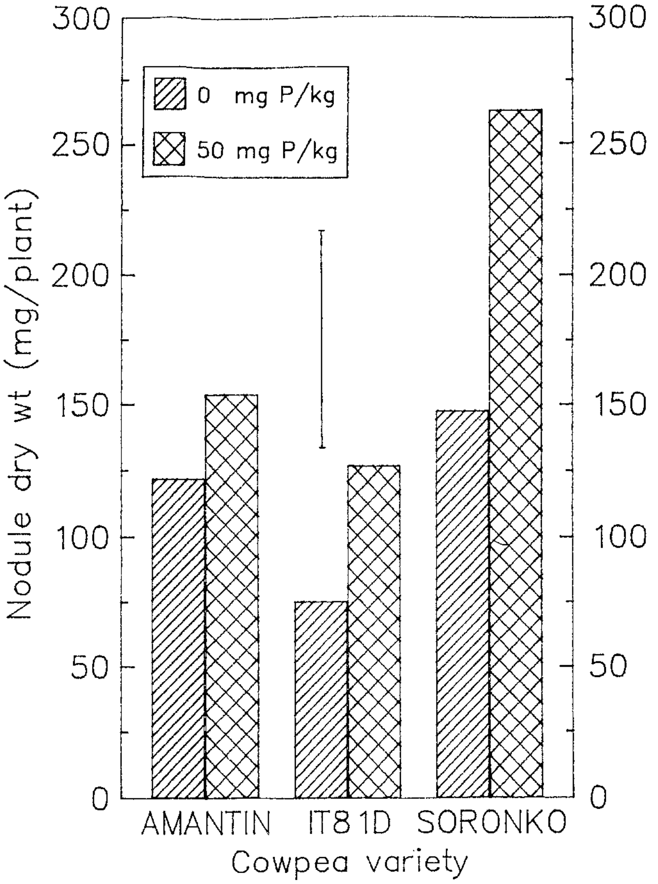

Fig. 2 Nodule dry weight (*wt*) of three inoculated cowpea cultivars at two P levels

N_2 fixation

Without P fertilizer, the percentage of N derived from the atmosphere as estimated by the isotope-dilution method was 42% for IT8lD-1137, 57% for Amantin, and 72% for Soronko; these values were significantly different (P<0.05; Table 1). These data demonstrate the existence of a large genotypic difference in the N_2-fixing capabilities of cowpea cultivars under P-limiting conditions. The different cultivars were all harvested at the same time, when Amantin had reached physiological maturity with IT81D-1137 and Soronko at the pod-filling stage. In other studies in soybean (Zapata et al. 1987a) and fababean (Zapata et al. 1987b) the percentage of N derived from the atmosphere did not change much after the pod-filling stage. In the present experiments harvesting at slightly different growth stages is therefore unlikely to have affected this measurement although the total amount of N_2 fixed was probably slightly underestimated in cultivars IT81D-1137 and Soronko.

The percentage of N derived from the atmosphere was not significantly influenced by the P fertilizer application, with values of 42% for IT8lD-1137, 62% for Amantin, and 76% for Soronko. The high values for Soronko (72–76%) are very impressive and indicate that in soil of medium fertility, certain cowpea cultivars are capable of satisfying the total N requirement predominantly through N_2 fixation. Similar findings have been made in pot-grown cowpea cultivars K2809 from Nigeria, which obtained 80–90% of N from the atmosphere (Eaglesham et al. 1977; Minchin and Summerfield 1978), while Ofori et al. (1987) reported values of up to 66% for the pot-grown cowpea cultivar TVu 354. However, the lower value obtained in IT81D-1137 in the present study emphasizes the need for selection.

Also, there were significant genotypic differences in the amounts of N fixed (Fig. 3). With no P fertilizer and with 50 mg P kg^{-1}, IT81D-1137 derived 21 and 27 mg N $plant^{-1}$ from fixation, Amantin 41 and 60 mg N $plant^{-1}$, and Soronko 63 and 99 mg N $plant^{-1}$, respectively. Thus, although P had no significant effect on the percentage of N derived from the atmosphere, it increased the total N fixed, especially in Amantin and Soronko. As reported by Sanginga et al. (1991), the major effect of P on N_2 fixation is via the total N fixed rather than on the per cent derived from the atmosphere. There was a highly significant cultivar × P interaction on the amount of N fixed.

The amount of N fixed correlated well with nodule mass (r = 0.949; P<0.01) and also with total shoot N (r = 0.988; P<0.001) at both low and high P levels. This suggests that the nodule mass and total N in shoots of nodulated cowpea plants might be used to assess genotypic differences in N_2 fixation, especially during the screening stages, which would allow a substantial reduction in time, costs, and materials during the selection of improved genotypes. Nodule mass was a much better criterion than nodule number for predicting N_2 fixation, because the amount of N fixed was not as well correlated with nodule number (r = 0.818; P<0.05) as nodule mass was. A high

Table 1 Atom % ^{15}N excess and proportion of shoot N derived from air (*%Ndfa*), estimated by the isotope-dilution method, and from soil (*%Ndfs*) of three cowpea cultivars at two P levels, no P (P_0) and 50 mg P kg^{-1} (P_{50}). *LSD*: least significant difference

Inoculation status	Cultivar	Atom % ^{15}N excess		%Ndfa		%Ndfs	
		P_0	P_{50}	P_0	P_{50}	P_0	P_{50}
Uninoculated	Amantin	2.05	2.02	–	–	79.6	80.0
	IT81D-1137	2.18	1.90	–	..	78.4	81.2
	Soronko	2.30	2.13	–	–	77.2	78.9
Incoluated	Amantin	0.89	0.78	56.8	61.5	34.4	30.8
	IT81D-1137	1.25	1.11	42.4	41.6	45.2	47.4
	Soronko	0.65	0.50	71.6	76.4	22.0	18.7
LSD (0.05)		0.19		11.2		8.5	

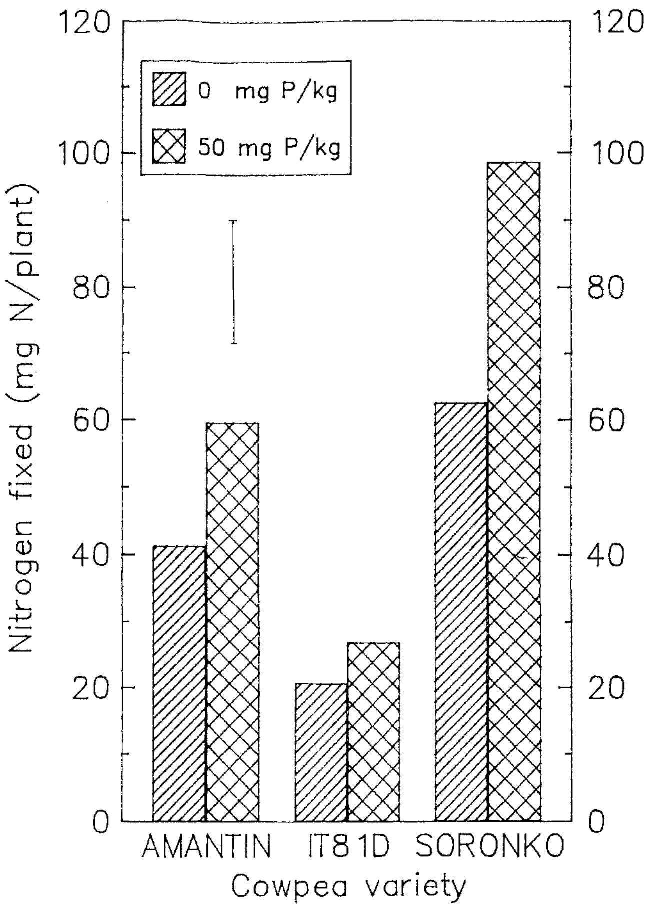

Fig. 3 Amount of N fixed by three inoculated cowpea cultivars at two P levels

correlation was also obtained between total dry matter production and N fixed ($r = 0.827$; $P<0.05$) and between shoot dry weight and N fixed ($r = 0.977$; $P<0.05$).

There were no significant differences in the percentage of N derived from the atmosphere as estimated by the isotope-dilution, *A* value, and total N difference methods (data not shown). However, the precision of the isotope methods was higher (CV 21–25%) than that of the total N difference method (CV 32%). There have been frequent reports that the total N difference method is less accurate than the isotope method (Hardarson et al. 1984; Henson and Heichel 1984; Rennie 1984; Talbott et al. 1982), and this has been ascribed to the more variable yield component in the total N difference estimate (Hardarson et al. 1984; Rennie 1984). However, good agreement between isotopic and difference methods has often been found (Martensson and Ljunggren 1984; Reichardt et al. 1987; Ndoye and Dreyfus 1988).

N assimilation

There were no significant differences in shoot N content among the three uninoculated cowpea cultivars (Table 2), in contrast to the significant genotypic differences that were observed in shoot N content of inoculated cowpea plants, at both P levels, with Soronko accumulating the largest amount of N followed by Amantin. These differences in shoot N content of the inoculated plants were therefore attributed to differences in N_2 fixation, as confirmed by Fig. 3. Compared to the corresponding uninoculated control, inoculation increased the total shoot N content of Soronko by 270% with no P fertilizer and 204% with 50 mg P kg^{-1} Amantin by 152 and 104% and IT81D-1137 by 74 and 58%, respectively.

Significant increases in the shoot N content of uninoculated plants with P fertilizer occurred in Amantin and Soronko (Table 2). The shoot N content of all inoculated cowpea plants increased with P application. For inoculated Soronko the increase was 48%, for Amantin 31%, and for IT81D-1137 28%. This ranking of the cultivars according to P effect on total shoot N was similar to that for the effect of P on amounts of N fixed, confirming the high correlation between amounts of N fixed and total shoot N content.

P assimilation

Without P fertilizer, there were no genotypic differences in P accumulated by the three cowpea varieties, and P contents were essentially similar in both inoculated and uninoculated plants (Table 2). These results suggest that at low P levels both the inoculated and uninoculated plants had the same ability to accumulate P.

Raising the P level to 50 mg kg^{-1} generally increased the P content in the shoots of all cultivars. Except for the uninoculated IT81D-1137, for which this increase was

Table 2 Content of P and N in shoots and P-use efficiency of three inoculated and uninoculated cowpea cultivars at two levels. For further explanations, see Table 1

Inoculation status	Cultivar	N in shoots (mg $plant^{-1}$)		P in shoots (mg $plant^{-1}$)		P-use efficiency (g dry matter mg^{-1}P)	
		P_0	P_{50}	P_0	P_{50}	P_0	P_{50}
Uninoculated	Amantin	28.8	46.7	4.12	7.81	0.51	0.39
	IT81D-1137	28.1	39.4	4.68	5.14	0.53	0.49
	Soronko	23.6	42.5	5.04	8.54	0.52	0.31
Inoculated	Amantin	72.6	95.4	5.06	8.26	0.67	0.51
	IT81D-1137	48.9	62.4	3.30	6.03	0.91	0.58
	Soronko	87.3	129.2	4.50	8.62	0.94	0.67
LSD (0.05)		12.22		2.02		0.13	

Table 3 Shoot, root, and total dry matter (*DM*) yields of three inoculated and uninoculated cowpea cultivars at two *P* levels. For further explanations, see Table 1

Inoculation status	Cultivar	Shoot DM (g plant^{-1})		Root DM (g plant^{-1})		Total DM (g plant^{-1})	
		P_0	P_{50}	P_0	P_{50}	P_0	P_{50}
Uninoculated	Amantin	2.01	3.05	0.38	0.29	2.39	3.34
	IT81D-1137	2.48	2.46	1.21	1.00	3.69	3.46
	Soronko	2.68	2.78	0.69	0.61	3.37	3.39
Inoculated	Amantin	3.34	4.22	0.35	0.38	3.69	4.60
	IT81D-1137	3.00	3.45	1.32	1.17	4.32	4.62
	Soronko	4.25	5.74	0.69	0.94	4.94	6.68
LSD (0.05)		1.14		0.46		1.40	

only about 10% (and not significant), the increases for the other inoculated and uninoculated treatments ranged from 63 to 92%. Overall, raising the P level from 0 to 50 mg kg^{-1} increased shoot P by almost 80%. Given the low P content of the soil used, the significant increase in the P content of the plants is not surprising. Both inoculated and uninoculated Soronko and Amantin had similar shoot P contents at 50 mg P kg^{-1} soil, and these were substantially greater than the amounts in the shoots of IT8lD-1137. The low ability of IT8lD-1137 to assimilate P might have accounted for its poor N_2-fixing performance.

Dry matter yield

Inoculated Soronko had the highest shoot dry matter yield at both P levels, but uninoculated plants showed no genotypic differences in shoot dry matter yields at any P level (Table 3). Significant genotypic differences in response to P were observed in inoculated plants. The average increase in the shoot biomass of inoculated plants at 50 mg P kg^{-1} soil was 35% and statistically significant for Soronko and for Amantin. Given the low available P status of the soil, though, it is surprising that greater responses were not obtained.

Without P fertilizer, inoculation increased the shoot yield by 66% in Amantin and 59% in Soronko, and with P fertilizer by 38% in Amantin and by 106% in Soronko. Thus, like N_2 fixation, when P was applied inoculation had a greater effect on the shoot yield of Soronko than the other two cultivars, more so than under low-P conditions. It is likely that P stimulation of N_2 fixation of Soronko was a major factor in the increased yield observed. Similarly, the lack of inoculation response in terms of N_2 fixation in IT81D-1137 may have been responsible for the small increase in dry matter yield from this cultivar when inoculated and supplied with P.

Overall, however, inoculation increased shoot dry matter yields more than P did; by inference, N must have limited growth more than P. High inoculation responses were obtained because there were no indigenous cowpea rhizobia, as shown by the absence of nodules on the roots of uninoculated plants. Some authors have reported a lack of inoculation response in cowpea plants grown in soil containing indigenous cowpea rhizobia (Ezedinma 1963; Doku 1969).

Neither inoculation nor the application of P had any effect on the cowpea root biomass (Table 3); this finding is contrary to the report by Seripong (1988) that P increases cowpea root yields. There were significant genotypic differences in root biomass, with IT8lD-1137 producing the greatest, and Amantin the least. Amantin had the greatest proportion of total biomass above ground, being significantly different from the other two cultivars. However, the greater root biomass of IT8lD-1137 did not confer any advantages on this cultivar over the others in absorption of N and P from the soil. A similar finding has been reported for soybean genotypes by Gunawardena et al (1993).

The vast differences in P-use efficiency, nodulation, N_2 fixation, and growth in cowpea genotypes without P fertilizer indicate that there is great potential for cowpea varietal identification and selection and for the use of these traits by breeders to create suitable cultivars with improved N_2-fixing abilities and growth in low-P soils. An initial identification and selection for improved N_2 fixation, especially from large collections, may be made on the basis of total shoot N and nodule dry weight. Accurate estimates of N_2 fixation may then be made by ^{15}N methodology.

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