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# Utilization of phosphorus from different sources by genotypes of promiscuous soybean and cowpea in a low-phosphorus savanna soil

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The differential ability of genotypes of soybean (*Glycine max*) and cowpea (*Vigna unguiculata*) to thrive under low-phosphorus (P) conditions by utilising P from sources with low solubility was assessed in a greenhouse study with a low-P savanna soil collected from a research field in Fashola, south-western Nigeria. The P sources added (21 mg P kg<sup>-1</sup> soil) were calcium phosphate (Ca-P), iron phosphate (Fe-P), aluminium phosphate (Al-P), and triple superphosphate (TSP). Soil without P addition served as a control. The soybean genotypes were TGm 1039, TGm 1196, TGm 1293, TGm 1360, TGm 1420, TGm 1511, and TGm 1540. The cowpea genotypes were Dan-ila, IT89KD-349, IT89KD-391, IT90K-59, and IT82D-716. Nearly all the soybean genotypes significantly increased their shoot dry matter yield (DMY) and accumulation of P from the various sources when compared with the control; the ranking for P acquisition was control<Al-P<Fe-P<Ca-P=TSP. The shoot DMY and shoot P accumulation of most of the cowpea genotypes were also significantly increased by the addition of Ca-P, Fe-P, and TSP; the addition of Al-P had no significant effect. The cowpea genotypes varied widely in acquiring P from the P sources. However, the general ranking was control=Al-P<Fe-P<Ca-P=TSP. For both crop species, the shoot DM yields under Ca-P and TSP treatments were not significantly different. From the analysis of shoot P accumulation with the Additive Main Effects and Multiplicative Interaction (AMMI) model, the cowpea genotype IT89KD-391 was better than other genotypes with Ca-P as P source; genotype IT90K-59 was better when Fe-P was the P source. In contrast, most of the soybean genotypes appeared to have access to the P sources in a similar manner

**Key words:** Soybean, cowpea, genotypes, savanna soil, sparingly soluble phosphorus.

## INTRODUCTION

Overcoming nutrient deficiencies, particularly of nitrogen (N) and phosphorus (P), associated with some soils in the moist savanna zone of West Africa is one of the major tasks the smallholder farmers face in their attempts to increase crop production. Therefore, N and P fertilizers are necessary inputs if optimal crop yields are to be realized as agricultural production intensifies. However,

inorganic fertilizers are not readily available and/or the resource-poor farmers cannot afford them. As a result, most farmers apply often inadequate amounts to their food crops. Tremendous variability exists in the types of soil that occur in the area. Lixisols (FAO World Soil Legend, 1988) are the most common (Kang and Juo, 1979; Mokwunye, 1979), and most are characterized by low activity clays containing crystalline, low specific surface minerals (e.g., kaoline) as well as oxides and hydroxides of Al and Fe (Mokwunye, 1979). These oxides contribute to the removal of P from the soil solution and thereby render a large proportion of soil and applied P

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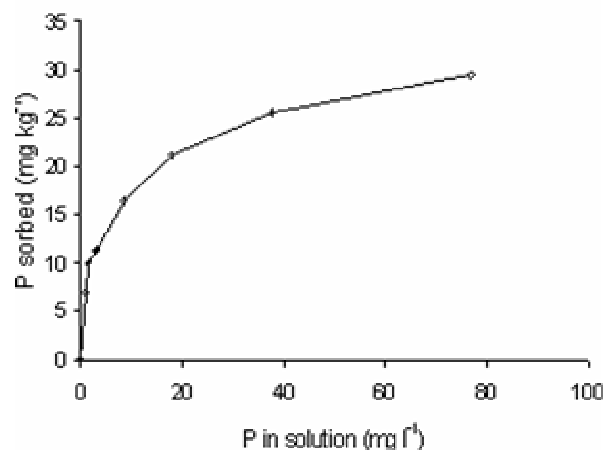
unavailable for plant uptake. The NaOH extractable P pool in the sequential soil P fractionation scheme (Hedley et al., 1982) is thought to be characterized by P associated with Fe and Al. Recent studies (Kolawole et al., 2003; Nwoke et al., 2004) indicate that these P pools occur in relatively large amounts in some West African savanna soils, compared to other biologically meaningful pools. Therefore, crops that can access these P pools can be chosen for such soils.

Cropping systems that are common in the region incorporate high-N-fixing grain legumes, such as promiscuous soybean and cowpea, intercropped or grown in rotation with a cereal crop. Promiscuous soybean and cowpea form nodules freely with indigenous rhizobia (and thus reduce the need for inoculation) to effect N fixation. These legumes not only provide food and fodder but also contribute N to the soil through biological N fixation (BNF) resulting from their association with effective rhizobium strains. It is estimated that some cowpea genotypes can fix as much as 70% of their total N content (Sanginga et al., 2000). However, the potential for BNF cannot be fully exploited under P limiting conditions. The development or selection of genotypes that can grow under low P-availability by obtaining P from sparingly soluble soil P pools or sources is, therefore, crucial.

Differences exist among plant species or even among genotypes in the strategies employed for enhanced P acquisition under limiting conditions. Root characteristics, capability for mycorrhizal fungi infection, and rhizosphere modification through exudates are considered important traits for this process. Genotypic differences have been reported in the growth of promiscuous cowpea (Sanginga et al., 2000) and soybean (Abdelgadir, 1998) under low-P conditions. Krasilnikoff et al. (2003) found large differences in the ability of cowpea genotypes to utilize soil P from strongly bound P pools. These pools may include sparingly soluble P associated with aluminium (Al), iron (Fe), and calcium (Ca). The ability of soybean genotypes grown in the same environment as cowpea to utilize these forms of P has not been assessed. This study was conducted to evaluate the differential ability of some promiscuous soybean and cowpea genotypes to utilize P from sparingly soluble sources in savanna soils.

## MATERIALS AND METHODS

Topsoil (0 - 10 cm) of low P-availability was collected from a research field in Fashola (7° 50'N, 3° 55'E), Nigeria. It was air-dried and sieved (4 mm) to remove large plant debris, and a subsample was taken for laboratory analyses. The pH (H<sub>2</sub>O, 1:2.5) of the soil was 6.0 and available P content was 4.47 mg kg<sup>-1</sup> (Bray-1), typical of savanna soils. Other characteristics were (g kg<sup>-1</sup>): organic C 4.8, total N 0.4, sand 800, silt 120, and clay 80, and a low P sorption capacity (Figure 1). The soil was weighed (3 kg) into plastic pots and amended with one P source or another. The P sources differed in solubility and were iron (III) phosphate, FePO<sub>4</sub>·4H<sub>2</sub>O (Fe-P) and aluminium phosphate, AlPO<sub>4</sub> (Al-P). Both are sparingly soluble compounds obtained from Sigma-Aldrich Laborchemikalien GMBH; calcium phosphate CaHPO<sub>4</sub> (Ca-P), obtained from BDH Laboratory



**Figure 1.** The phosphorus sorption capacity of the soil determined after 2 h of equilibration with series of phosphate standard solutions; determination of orthophosphate remaining in solution was by the method of Murphy and Riley (1962). The amount of P sorbed was estimated by subtracting the amount in solution from the amount added.

Supplies, England, and triple superphosphate (TSP), a commercially available P fertilizer. Although both TSP and Ca-P are primarily calcium phosphate compounds, the latter is a sparingly soluble compound (Sample et al., 1980) whereas the former is readily soluble. Each P source was in a powdered form and was mixed thoroughly with whole soil at the rate of 21 mg P kg<sup>-1</sup> soil. Distilled water was added to the soil to bring the moisture level to about 60% of the water holding capacity.

The soil was sampled 24 h after P addition for the sequential determination of P fractions, as described by Tiessen and Moir (1993). The P fractions measured were (a) the biologically important pools which comprised of resin-P (anion exchange resin extractable inorganic P regarded as freely exchangeable), the labile pool extractable with 0.5 M NaHCO<sub>3</sub>, the moderately labile pool extractable with 1 M NaOH, and (b) the relatively stable pools (which may contribute to P availability in the long term) extractable with dilute and concentrated HCl, and the residual fraction estimated after digestion with H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>. A control treatment (that is, without P addition) was included, and all treatments (that is, genotype × P source) were replicated four times. The pots were arranged on greenhouse benches in a randomized complete block design. The promiscuous soybean genotypes (TGm 1039, TGm 1196, TGm 1293, TGm 1360, TGm 1420, TGm 1511, and TGm 1540) and cowpea genotypes (Dan-ila, IT89KD-349, IT89KD-391, IT90K-59, and IT82D-716) from the collection of IITA, Nigeria, were selected, based on their good growth under low-P conditions (Abdelgadir, 1998; Sanginga et al., 2000). These were planted (4 seeds per pot; thinned to 2 one week after emergence) and allowed to grow for 7 weeks. Each pot received at planting 83 ml of a nutrient solution containing (litre): 2.307 g K<sub>2</sub>SO<sub>4</sub>, 0.124 g Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O, 0.144 g ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.144 g CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.288 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.144 g MnSO<sub>4</sub>·4H<sub>2</sub>O, 4.19 mg CoCl<sub>2</sub>·6H<sub>2</sub>O, 4.95 mg Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O and 15.728 g KNO<sub>3</sub>. The plants were watered daily with distilled water and sprayed with an appropriate insecticide, whenever necessary, to control pests.

The plants were harvested by cutting the shoot at the soil surface. The roots were separated by sieving the soil with a 4 mm sieve and later washed with water. Nodules were detached from the roots, counted, and weighed. Subsamples of the roots were taken for the estimation of arbuscular mycorrhizal fungi (AMF) infection.

**Table 1.** The inorganic P pools classified as labile (resin-P and  $\text{NaHCO}_3\text{-P}$ ), moderately labile ( $\text{NaOH-P}$ ), stable (dilute  $\text{HCl-P}$ ), and recalcitrant (concentrated  $\text{HCl-P}$  and residual-P) fractions extracted from the soil, and the soil pH 24 h after P addition. Values are means of four determinations (standard deviation in parentheses). The anion exchange resin was used in the chloride form.

Fraction	Type of P added				
	0-P	Ca-P	Fe-P	Al-P	TSP
	$\text{mg kg}^{-1}$				
Resin-P	4.5 (1.3)	14.9 (0.6)	5.1 (0.8)	5.3 (0.4)	14.8 (1.2)
$\text{NaHCO}_3\text{-P}$	4.2 (0.9)	6.8 (1.5)	19.0 (0.8)	5.7 (1.0)	6.5 (0.8)
$\text{NaOH-P}$	8.1 (0.8)	11.6 (1.6)	12.8 (0.9)	20.0 (0.8)	9.9 (1.3)
Dilute $\text{HCl-P}$	5.1 (0.3)	6.8 (0.9)	5.5 (0.5)	5.7 (0.4)	5.8 (0.4)
Concentrated $\text{HCl-P}$	14.8 (2.3)	15.2 (3.5)	12.2 (1.3)	10.8 (1.1)	13.1 (3.4)
Residual-P	25.7 (2.8)	30.0 (4.3)	29.1 (2.8)	28.3 (3.3)	32.6 (4.9)
Total	62.4	85.3	83.8	75.8	82.7
pH ( $\text{H}_2\text{O}$ , 1:2.5)	5.98 (0.05)	5.85 (0.06)	5.88 (0.05)	5.88 (0.05)	5.80 (0.08)

This was done by the method of Giovanetti and Mosse (1980), following clearing and staining as described by Phillips and Hayman (1970). The roots were also subsampled and scanned with a flatbed scanner (HP *ScanJet 4C*) using DeskScan II software. The scanned roots were analyzed for root length and average diameter, using the image analysis software *DT-SCAN* (Delta-T Devices, Cambridge, UK). The shoot and root samples were dried at  $65^\circ\text{C}$  and weighed for the determination of dry matter yield (DMY), then ground separately for the measurement of total P content (IITA, 1982).

#### Calculations and statistical analyses

The GLM procedure of the SAS software (SAS, 1999) was used to analyze treatment and interaction effects; mean separation was conducted with the PDIFF option. Correlation coefficients were also computed to assess the relationships among selected parameters. The ANOVA indicated a significant genotype  $\times$  P source interaction for shoot P accumulation. Therefore, the Additive Main Effects and Multiplicative Interaction (AMMI) statistical model was applied to assess the interaction patterns. It combines the usual analysis of variance (ANOVA) for additive effects with Principal Components Analysis (PCA) for the multiplicative effects of a 2-way data structure. The model separates the additive variance from the multiplicative variance, and then uses PCA to decompose the interactions (residual portion of ANOVA) into interaction principal components axes that can diagnose the patterns of the interaction more effectively. The AMMI analysis (including fitting the multiplicative variance by PCA) was done with the microcomputer software MATMODEL Version 2.0 (Gauch, 1990). The AMMI model is an effective tool for diagnosing patterns of the type genotype  $\times$  treatment or environment (Shafii and Price, 1998).

## RESULTS AND DISCUSSION

### Soil P fractions following the addition of different P forms

The effects of the P sources, 24 h after addition, were observed only on the labile and moderately labile inorganic P pools (Table 1). While the addition of Ca-P and

TSP increased mainly resin-P (the readily exchangeable fraction), addition of Fe-P increased both  $\text{NaHCO}_3\text{-P}$  and  $\text{NaOH-P}$  fractions, and Al-P increased only the  $\text{NaOH-P}$  fraction. Resin-P and  $\text{NaHCO}_3\text{-P}$  are labile pools that are more available to plants than  $\text{NaOH-P}$ , the moderately labile pool. Since the P sorption capacity of the soil is low (Figure 1), the rapid disappearance of P from soil solution due to sorption is not anticipated.

### Plant growth parameters

The shoot DMY of all the soybean genotypes significantly increased under the various forms of P relative to the control, indicating that these genotypes were able to utilize P from the sparingly soluble sources. The exception was TGM 1360 that was not increased by addition of Al-P (Figure 2). However, no significant ( $P > 0.05$ ) interaction was observed between soybean genotype and P source. On average, the increases were 40% (Al-P), 54% (Fe-P), and 79% (Ca-P and TSP). Abdelgadir (1998) attributed differences observed in the growth of soybean genotypes on a low-P soil under field conditions to their differential ability to acquire P under limiting conditions. In our study, differences in shoot DMY were not large among the genotypes within and across the P sources, even though significantly different amounts of P were accumulated in their shoot biomass. This might be attributed to the influence of factors other than P availability on DMY. Consequently, the effects of the P sources on the growth of the studied genotypes were better detected by looking at shoot P acquisition than at shoot DMY. For cowpea, the addition of Ca-P and TSP increased the shoot DMY of all genotypes significantly when compared with the control (Figure 2).

The addition of Fe-P resulted in a significant increase in shoot DMY of only some genotypes, whereas addition of Al-P did not lead to a significant increase of shoot DMY in

any of the cowpea genotypes. As in soybean, there was no significant genotype  $\times$  P source interaction ( $P > 0.05$ ). The increases were, on average, 33% (Al-P), 57% (Fe-P), 72% (TSP) and 84% (Ca-P).

The root DMY of soybean was higher with the addition of Ca-P and TSP than with Al-P and Fe-P, and varied little among genotypes within each P source (Table 2). Only small increases in root DMY due to P addition was observed in some cowpea genotypes and there was no significant genotype  $\times$  P source interaction. On average, the growth of the soybean and cowpea genotypes under Ca-P was comparable to their growth under TSP. The shoot/root ratio differed significantly ( $P < 0.001$ ) among the soybean genotypes but the effect of the P sources was not significantly ( $P > 0.05$ ) different (data not shown). For cowpea, this parameter did not differ significantly ( $P > 0.05$ ) among the cowpea genotypes, both within and across P sources. Although some cowpea genotypes (e.g., IT82D-716) exhibit the strategy of root interception (development of root hairs and the exploration of a large volume of soil) to access sparingly soluble P (Krasilnikoff et al., 2003), the results of this present study showed no evidence of root development at the expense of shoot biomass formation as there was no difference in the shoot/root ratio of the cowpea genotypes grown with sparingly soluble and readily soluble P.

The length and average diameter of the roots differed among the genotypes of both legumes and also among the P sources. As there was no significant genotype  $\times$  P source interaction (except for the root length of soybean), only the average values across genotypes and across P sources are presented (Table 3). The root length of the soybean genotypes varied from 76 m (TGm 1360) to 132 m  $\text{pot}^{-1}$  (TGm 1039), and the average diameter ranged between 0.49 mm (TGm 1420) and 0.59 mm  $\text{pot}^{-1}$  (TGm 1039). Soybean has been reported to access P by exploring a large volume of soil through root proliferation (Watt and Evans, 2003). It is unclear whether the morphology of the roots of the genotypes used in the present study played an important role. Nevertheless, the soybean roots were, on average, twice as long as those of cowpea. The root length of the cowpea genotypes varied from 39 m (IT89KD-349) to 77 m  $\text{pot}^{-1}$  (IT90K-59), and the average root diameter from 0.45 mm (IT89KD-349) to 0.53 mm  $\text{pot}^{-1}$  (IT90K-59).

### Phosphorus in the shoot biomass

Accumulation of P in the shoot biomass of the soybean genotypes was significantly higher with P addition than without (Figure 2). There was a significant genotype  $\times$  P source interaction ( $P < 0.05$ ), but differences within each P source were not large. However, most of the genotypes accumulated more P when the P source was Ca-P than when it was either Al-P or Fe-P. For cowpea, a significant genotype  $\times$  P source interaction ( $P < 0.01$ ) was also observed in the accumulation of P. The addition of Ca-P, Fe-P,

and TSP increased P accumulation in all the genotypes significantly but the addition of Al-P did not, except for IT82D-716.

Analyzing the shoot P accumulation with the AMMI model revealed the patterns of interaction between the soybean and cowpea genotypes and the P sources. The first two PCA axes were significant at the 5% probability level. PCA axis 1 accounted for 55.46% of the variability (interaction sum of squares) while PCA axis 2 accounted for 29.33%. The two together recovered 85% of the interaction sum of squares with about half of the interaction degrees of freedom and were therefore retained (Table 4). The biplot of PCA 1 and PCA 2 revealed the differential ability of the genotypes to acquire P from the different P sources (Figure 3a). The biplot showed a close association between cowpea genotype IT89KD-391 and Ca-P. This implies that this genotype acquired more P from this P source than the other genotypes. Similarly, genotype Dan-ila was closely associated with TSP. For the soybean genotypes, the patterns of the interaction with P sources were not very distinct. However, some genotypes (e.g., TGm 1360) were located close to the center of the PCA axes. This shows that these genotypes had small interactions with the P sources (Shaffi and Price, 1998) which signifies they acquired P from each of the P sources in a somewhat similar manner. Perhaps these varieties are genetically close to each other. In contrast, the cowpea genotypes varied widely in their acquisition of P from the various sources. The biplot of PCA axis 1 against mean shoot P accumulation for both genotypes and P sources (Figure 3b) showed that cowpea genotype IT89KD-391 took up more P than the other genotypes, and that the mean P accumulation from Ca-P and TSP was similar.

When the two species are compared, the degree of genotype  $\times$  P source interaction for shoot P accumulation showed that variability was larger among the cowpea genotypes than among the soybean. The data on shoot P indicate that some of the cowpea genotypes (e.g., IT90K-59) could utilize P from Fe-P nearly as much as from Ca-P or TSP, but could take very little from Al-P. This implies that such genotypes may be unable to acquire P sufficiently from aluminium-associated soil P pools, even though they may utilize P from other strongly bound pools of soil P. In a pot experiment, Krasilnikoff et al. (2003) observed that IT90K-59 and IT89KD-391 were able to acquire P from the sparingly soluble P pools. They noted that these genotypes invest less in root development but depend on root-induced processes dissolving soil P to acquire P from strongly bound P pools. These pools invariably contain some amounts of P associated with Al, Fe, and Ca. In the present study, IT90K-59 and IT89KD-391 were poor in utilizing Al-P, even though they were relatively good in utilizing Fe-P and Ca-P. This could be attributed to differences in rhizosphere chemistry. Moreover, the intensity (and nature) of such root-induced processes could be a critical factor. In contrast, nearly all the

**Table 2.** The effect of various P sources on root dry matter yield (DMY), arbuscular mycorrhizal fungi (AMF) infection rate, and number of nodules formed by the roots of soybean and cowpea genotypes under greenhouse conditions. *Con* control, *Al-P* aluminium phosphate, *Ca-P* calcium phosphate, *Fe-P* iron phosphate, *TSP* triple superphosphate, *SEM* standard error of means.

Genotypes	Root DMY (g pot <sup>-1</sup> )					AMF (%)					Nodule (number pot <sup>-1</sup> )				
	Con	Al-P	Ca-P	Fe-P	TSP	Con	Al-P	Ca-P	Fe-P	TSP	Con	Al-P	Ca-P	Fe-P	TSP
<b>Soybean</b>															
<b>TGm 1039</b>	2.61	2.77	4.40	3.20	4.50	35.04	18.50	7.66	14.68	0.00	0.3	1.0	2.8	1.0	15.0
<b>TGm 1196</b>	2.39	2.76	3.70	2.16	3.19	40.10	15.19	25.06	14.17	0.00	0.5	0.8	1.5	0.3	0.5
<b>TGm 1293</b>	1.67	2.79	3.18	2.51	3.58	37.22	27.62	14.86	12.57	0.00	20.0	60.8	95.8	31.3	107.8
<b>TGm 1360</b>	1.85	2.14	2.83	2.08	2.67	32.32	5.87	9.30	17.59	0.00	0.5	0.0	6.3	1.5	3.0
<b>TGm 1420</b>	1.78	2.60	3.72	2.86	3.84	21.36	28.13	19.60	25.42	0.00	1.0	0.9	3.5	1.8	9.8
<b>TGm 1511</b>	1.89	3.07	3.22	3.33	4.03	41.49	14.52	19.59	18.00	0.00	8.8	12.3	46.3	18.0	28.5
<b>TGm 1540</b>	1.75	2.28	3.29	2.89	2.40	38.43	2.66	16.38	17.69	0.00	7.3	35.8	71.8	35.6	46.0
<b>SEM</b>	0.276					2.028					9.32				
<b>Cowpea</b>															
<b>Dan-ila</b>	0.97	1.74	2.39	1.40	1.49	41.62	14.92	28.62	22.14	10.90	73	102	167	160	182
<b>IT89KD-349</b>	0.62	1.62	1.62	2.63	1.64	40.16	18.59	20.34	26.82	0.00	41	79	80	69	98
<b>IT89KD-391</b>	1.13	2.02	1.75	2.33	2.40	44.71	12.83	30.63	24.64	24.16	34	84	112	64	114
<b>IT90K-59</b>	1.16	2.53	2.75	2.54	2.85	39.84	16.83	22.27	31.50	8.29	34	60	104	69	93
<b>IT82D-716</b>	1.25	1.37	2.55	1.90	2.36	46.19	15.31	26.25	24.00	4.91	129	96	129	134	111
<b>SEM</b>	0.366					2.648					16.85				

**Table 3.** The average diameter and length (averaged across genotypes and P sources) of roots of promiscuous soybean and cowpea genotypes grown in soil amended with different forms of P. *SEM* standard error of means.

	Average diameter (mm)	Length (m pot <sup>-1</sup> )		Average diameter (mm)	Length (m pot <sup>-1</sup> )
<b>Soybean</b>			<b>Cowpea</b>		
TGm 1039	0.58	132	<b>Dan-ila</b>	0.49	57
TGm 1196	0.55	99	<b>IT89KD-349</b>	0.45	39
TGm 1293	0.55	116	<b>IT89KD-391</b>	0.49	51
TGm 1360	0.57	77	<b>IT90K-59</b>	0.53	77
TGm 1420	0.48	107	<b>IT82D-716</b>	0.48	57
TGm 1511	0.52	109			
TGm 1540	0.54	99			
<b>SEM</b>	0.018	6.8	<b>SEM</b>	0.016	6.0
<b>P source</b>					
<b>Al-P</b>	0.53	112		0.46	65
<b>Ca-P</b>	0.55	118		0.53	57
<b>Fe-P</b>	0.52	106		0.47	63
<b>TSP</b>	0.55	107		0.52	57
<b>Control</b>	0.54	84		0.46	38
<b>SEM</b>	0.017	6.0		0.015	5.7
<b>P &gt; F</b>					
<b>Genotype(G)</b>	0.0039	<0.0001		0.0071	0.0006
<b>P source(P)</b>	0.7174	0.0003		0.0009	0.0037
<b>G × P</b>	0.0649	0.0270		0.2162	0.6398

**Table 4.** Analysis of variance of the additive main effects and multiplicative interaction (AMMI) model for shoot P accumulation of soybean and cowpea genotypes grown with four P sources (and a control). *PCA* principal components analysis.

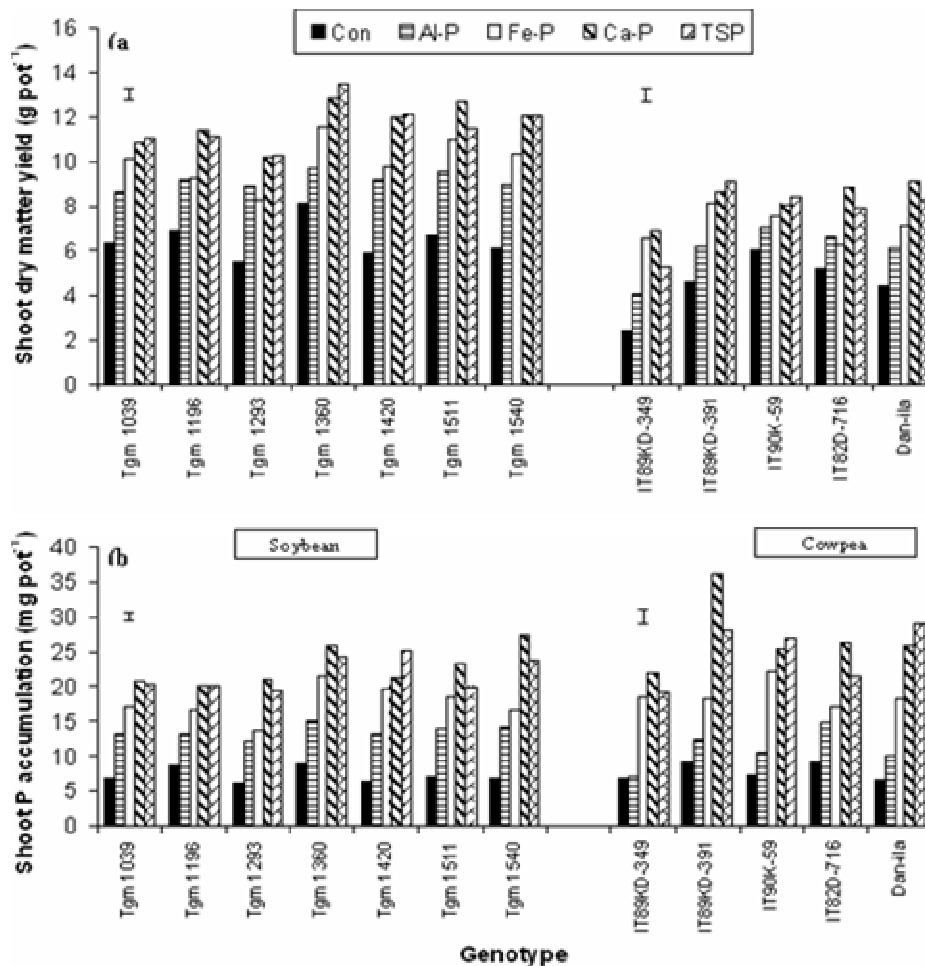
Source of variation	Degrees of freedom	Sum of squares	Proportion of G × P	Mean square	P > F
<b>Model</b>	59	11772.07	-	199.53	< 0.0001
<b>Genotype (G)</b>	11	742.05	-	67.46	< 0.0001
<b>P source (P)</b>	4	9876.46	-	2469.11	< 0.0001
<b>G × P</b>	44	1153.56	-	26.22	< 0.0001
<b>PCA axis 1</b>	14	639.77	55.5	45.70	< 0.0001
<b>PCA axis 2</b>	12	338.33	29.3	28.19	0.0005
<b>Residual</b>	18	175.46	-	9.75	0.3843
<b>Error</b>	179	1628.32	-	9.10	
<b>Corrected total</b>	238	13400.38		56.30	

soybean genotypes increased shoot P accumulation significantly with Al-P addition. The roots of the soybean genotypes might have induced changes in the rhizosphere that promoted P acquisition from Al-P since Ae et al. (1993) reported that only small amounts of P ( $\leq 5 \text{ mg kg}^{-1}$ ) were solubilized from  $\text{AlPO}_4$  added to sand-vermiculite (at pH ca. 6.0) without plants.

#### **Nodule formation and arbuscular mycorrhizal fungi (AMF) infection**

Addition of the various forms of P significantly enhanced

the number of nodules formed by the promiscuous soybean and cowpea genotypes (Table 2). Soybean genotype TGm 1293 produced more nodules than the others with and without P addition. No significant effect of P addition was observed among the soybean genotypes (e.g., TGm 1196) that nodulated poorly under the control treatment. The number of nodules produced by cowpea increased with P addition but differences within genotypes were not statistically significant. In general, the large number of nodules formed by cowpea compared to soybean suggests apparent BNF that was enhanced by incr-



**Figure 2.** The effect of various P sources on (a) the shoot dry matter yield (DMY) and (b) the shoot P accumulation of promiscuous soybean and cowpea genotypes grown under greenhouse conditions. Bars indicate standard error of means (SEM). *Con* control, *Al-P* aluminium phosphate, *Fe-P* iron phosphate, *Ca-P* calcium phosphate, *TSP* triple superphosphate.

eased P acquisition as the number and fresh weight of the nodules correlated significantly with shoot P accumulation (Table 5).

The rate of AMF infection of the root of both legumes depended on the P status of the soil (Table 2). Generally, P addition reduced the rate of infection significantly and differences occurred among the genotypes both within and across the various P sources. On average, the AMF infection rate of the cowpea genotypes dropped from 43% (control) to about 25% under Ca-P and Fe-P, to 15% with Al-P, and to 9% with TSP. The AMF infection rate of the soybean genotypes decreased from 35% (control) to about 15%, following the addition of the Al-P, Fe-P, and Ca-P. No evidence of infection was observed with the addition of TSP and the reasons are unclear. However, the level of soil solution P influences mycorrhizal infection of roots, and high levels (beyond the optimum) can be inhibitory (Aziz and Habte, 1987). The relatively high level of AMF infection of the roots of cowpea

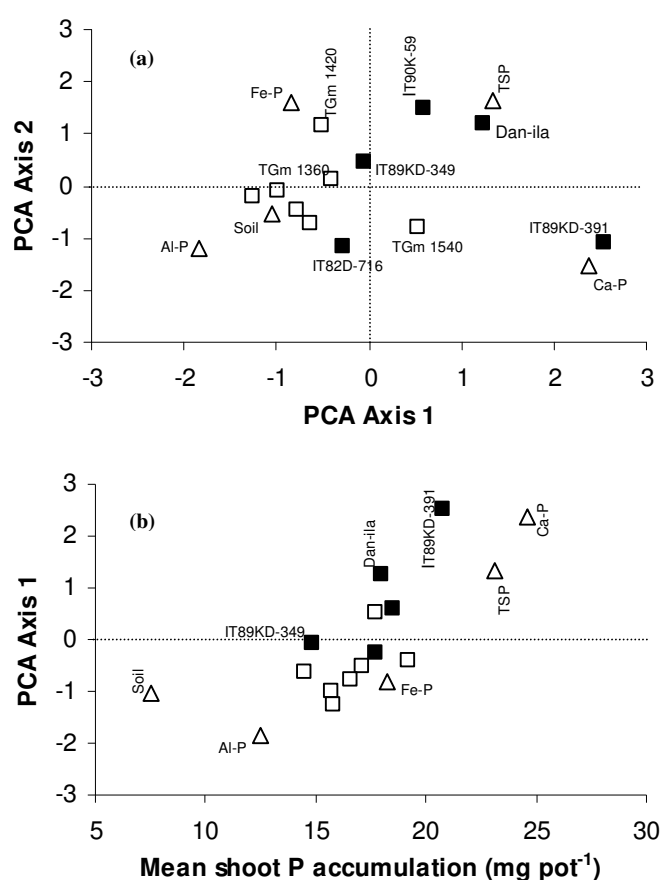
genotype IT89KD-391 in all the treatments may signify dependency on AMF for P acquisition, and needs to be evaluated further.

### Relationships between selected plant growth parameters

The shoot DMY of the cowpea genotypes correlated positively and significantly with the root parameters (average diameter and length), and with the number and fresh weight of nodules formed (Table 5). A lower but significant correlation was observed between the root parameters and P accumulation in the shoot biomass. In contrast to cowpea, the shoot DMY and shoot P accumulation of the soybean genotypes did not correlate with the length and average diameter of the roots (Table 5). A negative correlation (stronger for soybean than for cowpea) was found between P accumulation and AMF infection rate. However, when analyzed separately for each of

**Table 5.** Pearson correlation coefficients between shoot DMY and P accumulation from different sources and root length, root diameter, nodule parameters, and arbuscular mycorrhizal infection (AMF) rate of soybean ( $n = 140$ ) and cowpea ( $n = 100$ ) genotypes. \* Indicate significance at 5% level, \*\* Indicate significance at 1% level, and \*\*\* Indicate significance at 0.1% level.

Parameter	Soybean		Cowpea	
	Shoot DMY (g pot <sup>-1</sup> )	P accumulation (mg pot <sup>-1</sup> )	Shoot DMY (g pot <sup>-1</sup> )	P accumulation (mg pot <sup>-1</sup> )
Root diameter (mm pot <sup>-1</sup> )	0.050	0.068	0.448***	0.361***
Root length (m pot <sup>-1</sup> )	0.213*	0.167	0.419***	0.202
Nodule fresh weight (mg pot <sup>-1</sup> )	0.221**	0.275**	0.586***	0.582***
Number of nodules (pot <sup>-1</sup> )	0.185*	0.255**	0.374***	0.462***
AMF (%)	-0.588***	-0.612***	-0.269**	-0.305**



**Figure 3.** The biplots of (a) interaction principal components analysis (PCA) axis 2 against PCA axis 1, and (b) PCA axis 1 against mean shoot P (grand mean = 17.2 mg pot<sup>-1</sup>) for promiscuous soybean (open block) and cowpea (solid block) genotypes grown in soil amended with four P sources (triangle). Only distinct genotypes are labelled. The label 'soil' refers to the treatment that did not receive P (control).

the P sources, no significant relationship was observed between these parameters for soybean but the relationship between P accumulation and AMF became positive

for cowpea under Fe-P ( $r=0.46, P < 0.05$ ) and Ca-P ( $r=0.49, P < 0.05$ ).

In conclusion, the results of this study emphasize the variability among promiscuous cowpea genotypes in accessing various forms of sparingly soluble P. For example, IT89KD-391 acquired more P from Ca-P than the other genotypes and IT90K-59 had more from Fe-P than the other genotypes. Also, all the genotypes used acquired small amounts of P from Al-P. In contrast, most of the soybean genotypes significantly increased P acquisition with the addition of the various forms of P. Although the soybean genotypes acquired different amounts of P from these sources, the data presented indicate that the variability was small among genotypes within a P source. Nevertheless, further studies are warranted to relate these P forms to sparingly soluble soil P pools, and to test the ability of these legume genotypes to utilize such P pools under field conditions.

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## REFERENCES

- Abdelgadir AH (1998). The role of mycorrhizae in soybean growth in P-deficient soil in the humid tropics. PhD thesis. Cornell University, Ithaca, NY, USA. p. 255.
- Ae N, Arihara J, Okada K, Yoshihara T, Otani T, Johansen C (1993). The role of pisdic acid secreted by pigeonpea roots grown in an Alfisol with low-P fertility. In: Randall PJ, Delhaize E, Richards RA, Munns R (eds) Genetic aspects of mineral nutrition. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 279-288.
- Aziz T, Habte M (1987). Determining vesicular-arbuscular mycorrhizal



- effectiveness by monitoring P status of leaf disks. *Can. J. Microbiol.* 33: 1097-1101.
- FAO (Food and Agriculture Organization of the United Nations) (1988). Soil map of the world. Revised legend. FAO World Soil Resources Reports 60, FAO, Rome, Italy. Reprinted as Technical Paper 20, ISRIC, Wageningen, The Netherlands. 1989, p.138.
- Gauch HG JR (1990). MATMODEL version 2.0. AMMI and Related Analyses for Two-way Data Matrices. Microcomputer Power, Ithaca, NY, USA.
- Giovanetti M, Mosse B (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* 84: 489-500.
- Hedley MJ, Stewart JWB, Chauhan BS (1982). Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Sci. Soc. Am. J.* 46: 970-976.
- IITA (International Institute of Tropical Agriculture) (1982). Automated and semi-automated methods for soil and plant analysis. Manual series no. 7. IITA, Ibadan, Nigeria.
- Kang BT, Juo ASR (1979). Balanced phosphate fertilization in Humid West Africa. *Phosphorus Agric.* 76: 75-85.
- Kolawole GO, Tijani-Eniola H, Tian G (2003). Dynamics of phosphorus fractions during fallow with natural vegetation and planted *Pueraria phaseoloides* in south-western Nigeria. *Plant Soil* 257: 63-70.
- Krasilnikoff G, Gahoonia T, Nielsen NE (2003). Variation in phosphorus uptake efficiency by genotypes of cowpea (*Vigna unguiculata*) due to differences in root hair length and induced rhizosphere processes. *Plant Soil* 251: 83-91.
- Mokwunye U (1979). Phosphorus needs of soils and crops of the savanna zones of Nigeria. *Phosphorus Agric.* 76: 87-95.
- Murphy J, Riley JP (1962). A modified single solution for the determination of phosphorus in natural waters. *Analytica Chemica Acta* 27: 31-36.
- Nwoke OC, Vanlauwe B, Diels J, Sanginga N, Osonubi O (2004). The distribution of phosphorus fractions and desorption characteristics of some soils in the moist savanna zone of West Africa. *Nutr. Cycl. Agroecosystems* 69: 17-141.
- Phillips JM, Hayman DS (1970). Improved procedure for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.* 55: 158-161.
- Sample EC, Soper RJ, Racz GJ (1980). Reactions of phosphate fertilizers in soil. In: Kwasanwneh FE, Sample EC, Kamprath EJ (eds) The role of phosphorus in agriculture. American Society of Agronomy-Crop Science Society of America-Soil Science Society of America, Madison, WI, USA, pp. 263-310.
- Sanginga N, Lyasse O, Singh BB (2000). Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in a low P soil of the derived savanna zone in West Africa. *Plant Soil* 220: 119-128.
- SAS (1999). Statistical analyses software. SAS Version 8.2. SAS Institute Inc, Cary, NC, USA.
- Shafii B, Price WJ (1998). Analysis of genotype-by-environment interaction using the additive main effects and multiplicative interaction model and stability estimates. *J. Agric. Biol. Environ. Stat.* 3: 335-345.
- Tiessen H, Moir JO (1993). Characterization of available P by sequential extraction. In: Carter MR (ed) Soil Sampling and Methods of Analysis. Canadian Society of Soil Science (Lewis Publishers), Boca Raton, FL, USA, pp. 75-86.
- Watt M, Evans JR (2003). Phosphorus acquisition from soil by white lupin (*Lupinus albus* L.) and soybean (*Glycine max* L.) species with contrasting root development. *Plant Soil.* 248: 271-283.