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Genotypic variation for phosphorus uptake and dinitrogen fixation in cowpea on low-phosphorus soils of southern Cameroon

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Summary

In cowpea, efficient N_2-fixing genotypes are being selected to promote sustainable cropping systems in southern Cameroon (SC). However, N_2 fixation and growth of these genotypes are largely hampered by low levels of soil plant-available P. To evaluate the genotypic variation in N_2 fixation and P uptake among cowpea (*Vigna unguiculata* L.) genotypes, field experiments were conducted over two years on two acid soils low in available P. The experiments were laid out in a split-block design with four replications on typic (TK) and rhodic (RK) Kandiudult soils with seven cowpea genotypes. Phosphorus (P) fertilizers were applied on the main plots with 0 kg P, 30 kg P ha^{-1} as triple superphosphate (TSP) and 90 kg P ha^{-1} as Togo phosphate rock (PR). Nodule dry matter (DM), shoot DM, grain yield, and P uptake of cowpea significantly varied with site, P application, and genotype ($p < 0.05$). The N_2 fixation of the cowpea genotypes ranged from 29 to 51 kg N ha^{-1} on both TK and RK soils and was significantly increased with P application. Significant genotypic variations in N_2 fixation were observed with superior ability of the genotypes IT89KD-391 and IT90K-59 to fix N_2. The harvest index (HI) did not significantly differ between soils and P application levels ($p > 0.05$). Four genotypes were selected to investigate root mechanisms responsible for efficient P acquisition in pot experiments. The results suggest that a better root infection by arbuscular mycorrhizal fungi (AMF) in genotype IT90K-59 and root morphological and physiological characteristics in IT89KD-391 were the most important factors for increasing P uptake.

Key words: arbuscular mycorrhizal fungi (AMF) / nodulation / P availability

1 Introduction

Cowpea (*Vigna unguiculata* L. Walp) is one of the most important food, fodder, and cover crops in the semi- and tropical regions of Africa (*Padulosi* and *Ng*, 1990; *Jackai* and *Adalla*, 1997). The most important beneficial attribute of this legume is its contribution to the soil N status through symbiotic N_2 fixation, thereby enhancing soil fertility and reducing the need for N-fertilizer application (*Martins* et al., 2003). In addition, cowpea is considered to be less prone to drought damage and has a high yield potential especially when P fertilizers are applied. Its grain has relatively high protein content in the range of 20% to 28% (*Giami* et al., 2001; *Mortimore* et al., 1997). The crop's grain-yield potential has been reported to reach 3000 kg ha^{-1}, however, under farmers' conditions, actual grain yields average only 200–400 kg ha^{-1} in Uganda (*Sabiti* et al., 1994), 200–300 kg ha^{-1} in Nigeria (*Alghali*, 1992), and 400–800 kg ha^{-1} in the dry area of Cameroon (*Langyintuo* et al., 2003).

In the humid forest zone (HFZ) of southern Cameroon (SC), cowpea is increasingly used to improve the productivity of existing cropping systems (*Wendt* and *Atemkeng*, 2004). However, among the factors curtailing growth and yield are soil acidity and low P availability (*Menzies* and *Gillman*, 1997; *Eswaran* et al., 1997; *Vitousek* and *Farrington*, 1997). Soil acidity and low soil-P availability are especially problematic for growth and N_2 fixation, since nodules have a high P requirement and their growth is limited by P deficiency and low pH (*Vance* et al., 2001; *Giller* and *Wilson*, 1991).

Correcting P deficiency of cowpea in acid soils of SC through application of P fertilizers is not affordable for resource-poor farmers, because of the cost of P fertilizer (*Sample* et al., 1980). Also, applied P is rapidly converted into P forms of low plant availability in these soils (*Selles* et al., 1995). The desirable development of P-efficient genotypes requires a better understanding of the plant mechanisms controlling P efficiency and efficient field evaluation techniques to differentiate between efficient and inefficient genotypes (*Araújo* et al., 1998).

Cowpea genotypes exhibit a high degree of variation in N_2 fixation and P uptake under low-P conditions (*Ankomah* et al., 1995; *Krasilnikoff* et al., 2003; *Sanginga* et al., 2000). To our knowledge, no attempt has so far been made to identify cowpea genotypes that grow and fix N_2 well in the low–available P and acidic soils of SC.

Phosphorus efficiency can be achieved through P-acquisition efficiency (PAE), defined as the ability of the plant or genotype to acquire P from soils that are low in available P, or by P-utilization efficiency (PUE), the ability of a genotype or species to convert P into growth or yield once it is acquired from the soil (*Blair*, 1993; *Sattelmacher* et al., 1994). Plant-root characteristics that can increase PAE include rhizosphere acidification (*Marschner* et al., 1987), exudation of organic acid anions (*Neumann* and *Römheld*, 1999), production of phosphatase enzymes (*Li* et al., 1997; *Kamh* et al., 1999), uptake kinetics (*Nielsen* and *Barber*, 1978), association with arbuscular mycorrhizal (*Smith* and

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Read, 1997), and root morphology (*Lynch* and *Brown*, 2001; *Gahoonia* and *Nielsen*, 2004).

The objectives of the present study were: (1) to study intraspecific differences in N_2 fixation and P-uptake efficiency among cowpea genotypes on the low–available P soils of SC and (2) to investigate root traits that may account for the variability in P efficiency observed under the field conditions.

2 Materials and methods

2.1 Field experiments

2.1.1 Site description

Field trials were conducted in two P-deficient soils in farmers' fields at Abang village and at the research station of the Institut de la Recherche Agricole pour le Développement (IRAD) at Minkoameyos in 2001 and 2002. Abang (3°24' N, 11°47' E; 336 m a.s.l.) and Minkoameyos (3°51' N, 11°25' E; 727 m a.s.l.) are both located in the forest margins of SC; Abang is situated 10 km south of Mbalmayo and Minkoameyos 7 km north of Yaoundé. Following the USDA classification, the soils are classified as a Typic (TK) and a Rhodic (RK) Kandiudult at Abang and Minkoameyos, respectively. The study sites were selected on the basis of their low P availability (< 3–5 µg Bray1-P g^{-1}) and soil acidity (pH < 5.50). The annual rainfall was 1643 and 1513 mm at Abang and Minkoameyos sites, respectively, with a bimodal distribution. Selected physico-chemical properties of the topsoil (0–10 cm depth) are presented in Tab. 1.

2.1.2 Experimental setup

Experiments were laid out in split-block design with four replications. Phosphorus fertilizers were applied on the main plots at 0 kg P, 30 kg P ha^{-1} as triple superphosphate (TSP) and 90 kg P ha^{-1} as Togo phosphate rock (PR). Subplots, measuring 4 m × 4 m, comprised seven cowpea genotypes of different morphological and growth characteristics (Tab. 2). No N fertilizer or bradyrhizobia inoculation was applied. Cowpea was sown on September 24, 2001 with two seeds per hill at 75 cm × 25 cm, and the seedlings were thinned to one plant, 1 week after emergence. The experiments were repeated in September 2002. All cowpea plants were sprayed with the insecticide Thiodan® (endosulfuran organochlorine) at the rate of 0.33 mg L^{-1} at 2 and 4 weeks after planting (WAP) and hand-weeded at 2, 4, and 6 WAP. Cowpea genotypes were provided by the grain legume program of IITA, Ibadan, Nigeria. Morphological and growth characteristics of the genotypes are presented in Tab. 2.

2.1.3 Plant sampling and analysis

Plants were sampled for shoot growth, nodule formation, and N_2 fixation at mid-pod fill, *i.e.*, 56 d after planting (DAP). Six plants were chosen in the three middle rows, their shoots were cut at 5 cm aboveground level, and the fresh weight was recorded. A representative subsample of 500 g of fresh shoots was retained, transferred to the laboratory, and oven-dried at 70°C for 72 h. Shoot biomass at mid-pod fill was calculated. To minimize damage to the root system, the soil around the plant was loosened using forks, and the roots were carefully removed from the soil. Fresh nodules were cautiously removed from the roots, their number determined, oven-dried for 72 h, and their dry matter recorded. At grain maturity (84 DAP), a subplot of 1.50 m × 1.50 m was harvested, the fresh weights of the shoot biomass and grain yield were determined in the field. Subsamples of 500 g of fresh shoot biomass and 100 g of fresh grain were oven-dried at 70°C for 72 h, and the dry weight was measured.

Analysis of P in the shoots was done using the vanadomolybdate-yellow method (*Motomizu* et al., 1983), and total N was

Table 1: Physical and chemical properties of the topsoil (0–10 cm) in the experimental field sites in southern Cameroon. (Mean SE, n =36).

Soil properties	Sand	Silt	Clay	pH[1]	OC	TN	Ca	Mg	K	Al
	[%]				[g kg^{-1}]		[cmol (+) kg^{-1}]			
Rhodic Kandiudult	40.8	10.2	48.9	4.5	22.5	2.1	1.1	0.33	0.08	0.65
	(5.3)	(1.8)	(7.1)	(0.1)	(1.9)	(0.1)	(0.1)	(0.01)	(0.01)	(0.13)
Typic Kandiudult	50.6	9.7	39.7	5.4	16.0	1.6	1.35	0.64	0.06	0.12
	(1.58)	(0.85)	(1.2)	(0.1)	(1.1)	(0.05)	(0.17)	(0.06)	(0.04)	(0.02)

	P sorption[2]	FeO_x[3]	P (Bray 1)	$NaHCO_3$-P_i[4]	NaOH-P_i[5]	HCl-P[6]	H_2SO_4-P[7]
	[%]		[mg kg^{-1}]				
Rhodic Kandiudult	88.3	0.69	2.50	1.7	25.4	1	154.1
	(3.2)	(0.04)	(0.95)	(0.29)	(3.2)	(0.5)	(2.3)
Typic Kandiudult	77.5	2.3	5.0	3.2	29.2	0.86	190.9
	(0.3)	(0.07)	(1.20)	(0.5)	(1.2)	(0.38)	(12)

Figures in parentheses represent standard error of the means.
[1] pH = measured in water, [2] P sorption = percentage of P sorbed into Fe and Al ions, [3] FeO_x = oxalate-extractable Fe, [4] $NaHCO_3$-P_i = $NaHCO_3$-extractable inorganic P, [5] NaOH-P_i = NaOH-extractable inorganic P, [6] HCl-P_i = concentrated HCl–extractable inorganic P, [7] H_2SO_4-P_i = H_2SO_4-extractable inorganic P.

Table 2: Characteristics of the cowpea genotypes included into the study (Singh, pers. comm.).

Genotype	Maturity type	Growth type	Resistance to biotic and abiotic stresses stresses	*Photosensitivity	Origin of germplasm	Seed type
IT82D-849	early (70 d)	erect broad	major diseases, striga	PIS	Nigeria	brown, smooth
IT 89KD-349	early (70 d)	semispreading	aphids, thrips, bruchids, disease	PS	Nigeria, Kanananndo, Tanzania	white, rough
IT 82D-716	medium (75 d)	very erect	multiple diseases	PIS	Nigeria, Tanzania	white, brown eye
IT 81D-715	medium (75 d)	long peduncles, over canopy	multiple diseases	PIS	Nigeria, Tanzania, USA	white, rough
IT 90K-59	medium (75 d)	semi-erect	major diseases, striga, electra	PIS	Nigeria, Tanzania	brown, rough
IT 89KD-391	late (80–85 d)	semi-erect	aphids, thripsdisease	PIS	Nigeria, Tanzania	brown, rough
Dan'ila	late (80–85 d)	spreading	drought, diseases	PIS	Nigeria	white, rough

* PIS: photoperiod-insensitive; PS: photoperiod-sensitive

determined following *Powers* et al. (1981) after ashing of the samples in concentrated sulfuric acid.

2.1.4 N_2 fixation and harvest index (HI)

The xylem ureide–assay method (*Peoples* et al., 1989; *Herridge* and *Peoples*, 1990) was used to compare/assess N_2 fixation in all cowpea genotypes. Three plants per replicate were harvested at pod filling. The stems and petioles of the dried plants were finely ground, and a subsample of 0.5 g was used to extract the xylem solutes in boiling water. The ureide and nitrate concentrations in the extract were measured according to *Young* and *Conway* (1942) and *Cataldo* et al. (1975), respectively. The relative ureide-N abundance (RUA) of the sample was calculated based on the molar concentrations of ureides and nitrate with the assumption of four N atoms per ureide molecule, using the following equation:

$$RUA = 4\,N_1 / (4\,N_2 + N_1) \times 100, \quad (1)$$

where N_1 is the concentration of ureide and N_2 the concentration of nitrate in the stem and petiole extracts in nmol.

The harvest index (HI) of the cowpea genotypes was calculated with the following formula:

$$HI = (\text{grain DM}) / (\text{total shoot-biomass DM}) \quad (2)$$

2.2 Greenhouse experiment

A pot experiment was conducted in a greenhouse at IITA in Nkolbisson (Cameroon). The soil used was the RK, sampled at 0–10 cm depth at the Minkoameyos site in 2001. The soil was air-dried and sieved through a 4 mm sieve before filling 2.5 kg in 2.5 L pots. Seeds of cowpea genotypes were surface-sterilized and pregerminated for 3 d after which four seedlings were transplanted into each pot. One week after planting, the seedlings were thinned to two per pot. The experiment was laid out in a factorial randomized complete-block design with two factors. The first factor was P application with three levels equivalent to 0 kg P ha^{-1} (0P), 90 kg P ha^{-1} as phosphate rock (PR), or 30 kg P ha^{-1} as triple superphosphate (TSP). The second factor comprised four cowpea genotypes selected from the field experiment: two that had the highest N_2 fixation and P uptake and two that were amongst the lowest yielding under low-P (0P) conditions. Plants were daily watered with deionized water to 60% water-holding capacity (WHC) and harvested 8 weeks after planting.

2.2.1 Sampling

Harvested plant samples were assessed for dry matter, nodule formation, arbuscular mycorrhizal–fungi (AMF) colonization of roots, and root growth. Total P and N concentrations were measured in shoots and roots as described above.

2.2.2 Arbuscular mycorrhizal–fungi colonization

Subsamples of 1 g of fresh roots were washed free of soil, preserved in 50% alcohol and stored at 4°C prior to assessment of AMF infection. Roots were later allowed to attain room temperature and cut into 1 cm-length pieces and cleared in KOH (10%) solution. The cut roots were stained using acid fuchsin in lacto-glycerin at room temperature according to *Phillips* and *Hayman* (1970) and *Merryweather* and *Fitter* (1991). Roots were then examined for colonization by AMF using the grid-line intersection method of *Giovannetti* and *Mosse* (1980) under a stereomicroscope at 40× magnification.

2.2.3 Root-length and root-weight determination

The entire root systems of the two cowpea plants per pot were harvested and separated from soil by washing gently under slowly running tap water. Subsamples were spread out on a shallow plastic tray and scanned using ScanJet IIcx software (Hewlett-Packard ScanJet IIcx, England). Dt-Scan software from Delta-T Devices, Cambridge, England (*Webb* et al., 1993) was used to measure total length and diameter of roots. The scanned samples were subsequently oven-dried at 70°C for 72 h and their dry weights determined.

2.2.4 Statistical analysis

The growth parameters of the cowpea genotypes did not differ significantly between the two experimental years 2001 and 2002 on both soil types. Therefore, mean data over the two cropping years per site are presented. Statistical analyses of the data were carried out using the Statistical Analysis System (SAS) software version 8.2 (2001). Analysis of variance (ANOVA) was performed using the General Linear Model Procedure. Levels of significance in tables and graphs are given by ns, *, **, *** for not significant, significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Values in columns followed by the same letter are not significantly different at $p < 0.05$ (LSMEANS/PDIFF option). Regression analyses were used to establish relationships between pairs of variables.

3 Results

3.1 Field experiments

3.1.1 Nodulation and N_2 fixation

The nodule DM of the seven cowpea genotypes evaluated in our study was significantly higher on the TK than the RK soils (Tab. 3). Significant genotypic differences among the cowpea genotypes were observed on both soils for nodulation and N_2 fixation ($p < 0.05$). The cowpea genotypes IT90K-59, Dan'ila, IT81D-715, IT82D-716, and IT89KD-391 had significantly higher nodule DM than IT89D-349 and IT82D-849 on both soils (Tab. 4). The level of N_2 fixation was not significantly different between the two soils ($p > 0.05$, Tab. 3). Cowpea genotypes with higher nodule DM, (except IT82D-716, on the RK soil type) also had significantly higher N_2 fixation than IT89D-349 and IT82D-849 (Tab. 4).

Table 3: Nodule DM, N_2 fixation, and P uptake at mid-pod filling (56 DAP) of seven cowpea genotypes grown on Typic (TK) and Rhodic (RK) Kandiudult soils of southern Cameroon. Means across genotypes, and 2001 and 2002 are presented, n = 24.

P application	Nodule dry matter [mg plant^{-1}]		N_2 fixation [kg ha^{-1}]		P uptake [kg P ha^{-1}]	
	TK	RK	TK	RK	TK	RK
0P	290 c	295 b	35 b	37 b	3.7 c	3.5 c
PR	402 b	365 b	36 b	39 b	4.3 b	4.1 b
TSP	730 a	472 a	55 a	48 a	5.1 a	5.1 a
Source of variation		*F value*				
Soil (S)	9.0 ***		ns		4.9 *	
P application (P)	25.6 ***	3.5 *	6.2 **		11.9 ***	10.4 ***
Genotype (G)	3.3 **	2.3 *	2.9 *		3.4 *	4.2 *
S × P	7.6 ***		2.4 *		ns	
S × G	ns		2.4 *		ns	
P × G	ns	ns	ns		ns	ns
S × P × G	ns		ns		ns	

Numbers followed by the same letter within a column are not significantly different at $p > 0.05$ (LSMEANS/PDIFF test).
ns: not significant; *, **, ***, significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Table 4: Nodule dry matter, N_2 fixation, and P uptake of seven cowpea genotypes grown on Typic (TK) and Rhodic (RK) Kandiudult soils of southern Cameroon. Means across soils, P rates, and 2001 and 2002. n = 24.

Genotype	Nodule dry matter [mg plant^{-1}]		N_2 fixation [kg N ha^{-1}]		P uptake [kg P ha^{-1}]	
	TK	RK	TK	RK	TK	RK
IT 89KD-349	277 c	280 b	36.3 b	29.0 c	3.3 c	3.0 b
IT 82D-849	234 c	192 c	34.9 b	35.4 b	2.8 c	3.6 b
IT 82D-716	441 b	347 a	31.5 b	28.7 c	4.4 ab	4.0 ab
IT 81D-715	412 b	344 ab	47.0 a	50.4 a	4.6 ab	3.9 ab
IT 90K-59	633 a	490 a	42.5 ab	49.9 a	5.4 a	5.1 a
Dan'ila	676 a	366 ab	49.7 a	45.2 ab	4.5 ab	4.6 a
IT 89KD-391	443 b	485 ab	49.8 a	51.8 a	5.5 a	5.2 a

Numbers followed by the same letter within a column are not significantly different at $p > 0.05$ (LSMEANS/PDIFF test).

Phosphorus application significantly increased nodule DM and N_2 fixation ($p < 0.05$) on both soils whereby TSP was in general more effective than PR (Tab. 3). The S × P interaction was significant for both nodule formation and N_2 fixation. The interaction S × G was significant only for N_2 fixation ($p < 0.05$). However, the interactions P × G and S × G × P were not significant for both nodulation and N_2 fixation.

The individually calculated regressions between nodule DM and P content (Fig. 1a) and between N_2 fixation and P uptake (Fig. 1b) were significant over all P treatments for both soils, with no soil-related differences in the slopes of the regressions.

3.1.2 Shoot dry matter production and P uptake

Effects of soil types, cowpea genotypes, and P application on P uptake and cowpea biomass at the first harvest were similar to those on nodule DM ($p < 0.05$). The genotypes IT90K-59, Dan'ila, and IT89KD-391 generally had a higher P uptake than IT89KD-349 and IT82D-849 on both soil types (Tab. 4). The shoot DM of the cowpea genotypes IT90K-59 and IT89KD-391 were significantly higher than of the genotypes IT89KD-349 and IT82D-849, but not compared to the other genotypes on the TK soil (Tab. 5 and 6). On the RK soil, Dan'ila and IT89KD-391 produced higher shoot DM than the cowpea genotypes IT89KD-349 and IT82D-849.

3.1.3 Grain yield and harvest index

Significant genotypic differences in grain yield were observed among the cowpea genotypes on both soils ($p < 0.05$, Tab. 5). The genotypes Dan'ila and IT89KD-391 were among the highest yielding on both soils across P rates. Genotypic differences in the harvest index (HI) were similar to those in grain yield on the TK soil. In contrast, lower genotypic variation was observed on the RK soil.

Phosphorus application significantly increased the grain yield of the cowpea genotypes on the TK, but not on the RK soil (Tab. 6). The harvest index of the cowpea genotypes remained unaffected by P application on both soils.

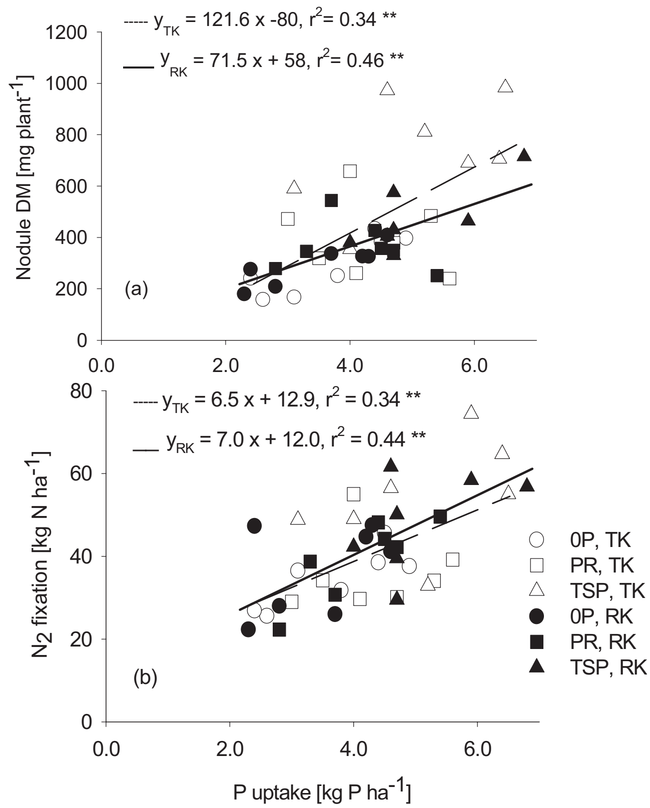

Figure 1: Relationship between nodule DM (a), N_2 fixation (b), and the P uptake of seven cowpea genotypes at mid-pod filling (56 DAP) grown on a typic (TK) and rhodic (RK) Kandiudult soil, in southern Cameroon. Open and filled symbols represent data points for the TK and RK soils, respectively.

Grain yields of the genotypes were significantly correlated with the nodule DM per plant (Fig. 2a), to the N_2 fixation (Fig. 2b), but not to shoot DM (Fig. 2c).

3.2 Greenhouse experiment

Based on the field results obtained, four genotypes, two evaluated as good performers at 0P (IT89KD-391 and IT90K-59)

Table 5: Shoot dry matter, grain yield, and harvest index of seven cowpea genotypes grown on Typic (TK) and Rhodic (RK) Kandiudult soils of southern Cameroon. Means across soils, P rates, and 2001 and 2002, n = 24.

Genotype	Shoot dry matter [kg ha⁻¹]		Grain yield [kg ha⁻¹]		Harvest index (HI) [%]	
	TK	RK	TK	RK	TK	RK
IT 89KD-349	1682 b	1065 b	833 c	608 ab	33 b	36 a
IT 82D-849	1697 b	1263 b	791 c	491 b	32 b	28 b
IT 82D-716	1531 b	1451ab	836 c	631 ab	35 b	30 b
IT 81D-715	1448b	1302 ab	1136 bc	817 ab	44 a	39 a
IT 90K-59	1842 a	1246 b	1244 b	840 ab	40 a	40 a
Dan'ila	1682 b	1541a	1235 b	917 ab	42 a	37 a
IT 89KD-391	2016 a	1505 a	1596 a	963 a	44 a	39 a

Numbers followed by the same letter within a column are not significantly different at $p > 0.05$ (LSMEANS/PDIFF test).

Table 6: Shoot dry matter, grain yield, and harvest index of seven cowpea genotypes grown on Typic (TK) and Rhodic (RK) Kandiudult soils of southern Cameroon as affected by P application. (0P = 0 kg P ha^{-1}, PR = 90 kg P ha^{-1} phosphate rock, TSP = 30 kg P ha^{-1} triple superphosphate P). Means across genotypes, and 2001 and 2002, n = 28.

P application	**Shoot dry matter** [kg ha^{-1}]		**Grain yield** [kg ha^{-1}]		**Harvest index (HI)** [%]	
	TK	RK	TK	RK	TK	RK
0P	1420 b	1080 b	883 c	650 a	38 a	37 a
PR	1640 b	1341 ab	1109 b	731 a	40 a	35 a
TSP	1989 a	1596 a	1295 a	877 a	39 a	35 a
Source of variation		*F value*				
Soil (S)	21.8 ***		83.5 ***		ns	
P application (P)	14.7 ***	16.5 **	17.3 ***	ns	ns	ns
Genotype (G)	3.0 **	2.9 *	11.0 ***	6.4 ***	2.4 *	ns
S × P	ns		18.2 ***		2.69 *	
S × G	ns		3.8 **		ns	
P × G	ns	ns	ns	2.3 *	ns	ns
S × P × G	ns		ns		ns	

Numbers followed by the same letter within a column are not significantly different at $p > 0.05$ (LSMEANS/PDIFF test).
ns: not significant; *, **, ***: significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively

and, therefore, classified as P-efficient genotypes (EG), and two with poor performance at low-P (IT82D-849 and IT89KD-349), classified as inefficient genotypes (ING), were selected to investigate probable root mechanisms responsible for efficient P acquisition in a greenhouse pot experiment. At the low-P level, cowpea shoot DM, P uptake, AMF root-colonization rates of the EG were generally significantly higher than of the ING, whereas P-uptake rate, root length, and root-to-shoot ratio did not differ between genotypes (Tab. 7). Generally, the EG responded positively to PR and TSP application by increasing all parameters except root length, which was negatively affected. In contrast, the ING barely responded to P application, generally. Only the P uptake of the IT89KD-349 increased with P application. Arbuscular mycorrhizal–fungi colonization was overall significantly increased with P application in both EG and ING.

In order to assess which plant parameters might have been responsible for P uptake, linear regressions were calculated between these parameters and P uptake for each of the genotypes separately (Tab. 8). Since P uptake and growth (see Tab. 7) of ING IT82D-849 did not respond to P application, it is not surprising that hardly any of the parameters (only P-uptake rate for genotype IT82D-849) was related to P uptake. The positive response of IT82KD-349 to P application can be related exclusively to a higher P-uptake rate. The comparison of the two EG revealed differences between the genotypes. In IT90K-59, AMF, root-infection rate, and shoot growth, but not root characteristics, were positively related to P uptake. In contrast, in IT89KD-391 shoot and root characteristics, but not AMF colonization were related to P uptake.

4 Discussion

Low levels of plant-available soil P induce P-deficiency symptoms in plants, which are generally accompanied by a decrease in leaf expansion, leaf surface area, the number of leaves, and consequently reduction in shoot biomass (*Marschner,* 1995). The results from our investigation showed an increase in shoot dry matter of the cowpea genotypes used with P application (Tab. 5), and significant genotypic differences in shoot dry matter were observed. Increases in shoot dry weight of cowpea following P application have been also shown by other authors (*Othman* et al., 1991; *Sanginga* et al., 2000), with bean (*Araújo* et al., 1998; *Christiansen* and *Graham,* 2002). In contrast, *Buerkert* et al. (2001) did not observe significant variation in shoot dry matter of cowpea and groundnut legumes after N and P application on a nutrient-depleted sandy soil of Sub-Saharan West Africa low in P-sorption capacity. The significant genotypic variation in shoot dry weight in cowpea and the superior ability of the genotypes IT90K-59, Dan'ila, and IT89KD-391 to produce higher biomass suggest the possibility to identify P-efficient cowpea genotypes adapted to the low–available P soils of SC. When cowpea is being used for the replenishing of soil fertility, these genotypes may be of great interest to farmers as they will supply available nutrients to the soil after decomposition and mineralization of the crop residues (*Martins* et al., 2003).

Significant genotypic variation for grain-yield production was also observed among the cowpea genotypes tested (Tab. 5). The increase in grain yield following P application on the TK soil was two times higher than on RK soil. Although significant differences in grain yield between the two soils can be attributed to the variation in soil chemical composition (Tab. 1), such as high P fixation, Al toxicity (*Aune* and *Lal*,

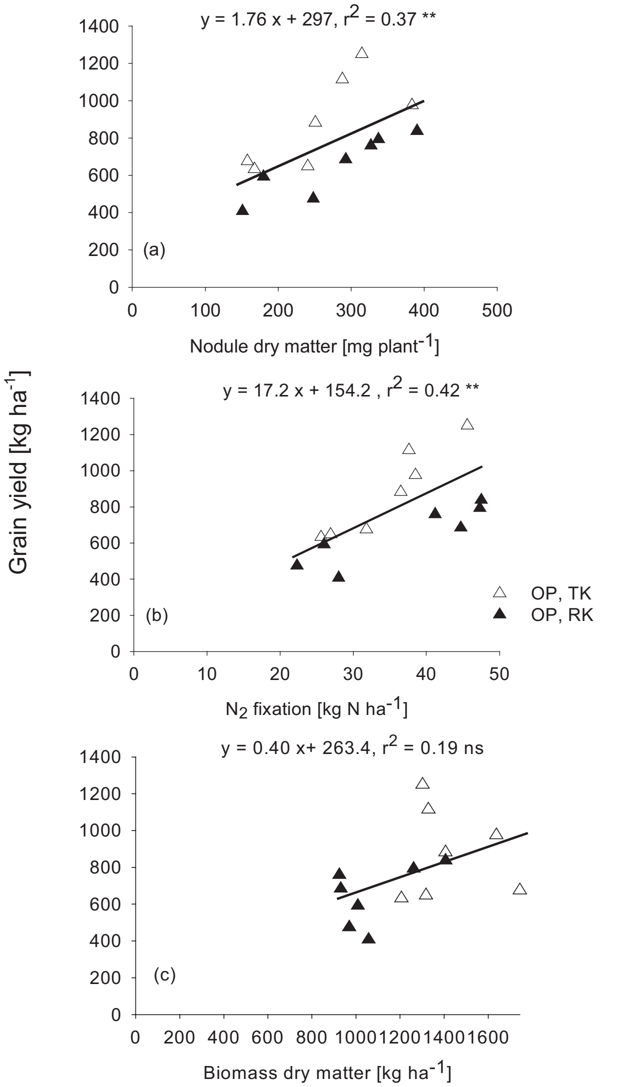

Figure 2: Relationship between nodule dry matter (a), N_2 fixation (b), and shoot biomass (c) at mid-pod filling (56 DAP) and grain yield of seven cowpea genotypes on low–available P soils of southern Cameroon. Means at OP and across soils.

1997), and cropping history, the significant genotypic variation observed on both soils is indicative of genetic control of cowpea grain yield. In the present study, yields of the genotypes IT90K-59, Dan'ila, and IT89KD-391 were higher compared to other cowpea genotypes previously used on these soils (*Wendt* and *Atemkeng,* 2004) which produced 981 kg ha^{-1}, at moderately available–soil P levels (8.8 μg (g soil)$^{-1}$). Such findings provide useful information on the possibility to improve cowpea grain yield on soils low in available P in SC. The harvest index (HI) ranged from 0.33 to 0.44 on the TK, with significant genotypic variation, and from 28% to 40% on the RK soil. Although comparable data for acid and P-deficient soils of SC are lacking, the range of variation presented here is in the range (26%–42%) obtained by other authors for cowpea (*Thiawa* and *Hall*, 2004).

The present study showed a significant increase in nodule dry matter and N_2 fixation with the application of P (Tab. 3) and a significant difference among the cowpea genotypes tested (Tab. 4), for both nodulation and N_2 fixation (Tab. 3). The ureide method has not yet been widely used for the estimation of the N_2 fixation by legumes in the field and challenged by *Unkovich* and *Pate* (2000). However, comparisons with the established natural ^{15}N abundance and ^{15}N isotope–dilution methods indicate the suitability of this method not requiring non-nodulating controls, particularly for the comparison of the N_2 fixation of legume species and genotypes (*Alves* et al., 2000; *Herridge* and *Peoples*, 1990, 2002; *Osunde* et al., 2003). Enhanced nodulation and N_2 fixation after adequate levels of P application have been reported for other legume species (*Dhingra* et al., 1988; *Hart,* 1989), but only a few authors (*Adu-Gyamfi* et al., 1989) reported positive plant responses to applied P in P-deficient soils to be associated with increased N_2 fixation. The significant relationships between N_2 fixed and total P uptake on both soils suggests that the genetic differences for N_2 fixation under P deficiency were due to differences in P-uptake efficiency. This is in agreement with reports that genotypic differences in N_2 fixation under P-deficiency conditions in common bean could be attributed to differences in P-uptake efficiency (*Pereira* and *Bliss*, 1989, 1987). The variability of N_2 fixation of the genotypes at low P suggests the potential for identifying genotypes with high N_2-fixation potential under low soil-P conditions (*Vadez* et al., 1999).

The superior N_2-fixation ability of the genotypes IT89KD-391, IT90K-59, and Dan'ila at 0P could be due to different genotypic strategies to acquire P from sparingly available P sources necessary for plant growth and N_2 fixation, a process which requires large amounts of P for each atom of N_2 fixed (*Vance,* 2001). In many tropical soils, where the transport of P to the root is the main limiting factor for P acquisition rather than root uptake of P (*Barber*, 1995; *Jungk* and *Claassen*, 1997), the efficient establishment of a symbiosis with arbuscular mycorrhizal fungi will allow the plants to access soil P up to several centimeters away from the root (*George* et al., 1995) thus enhancing P acquisition. The significant relationships between AMF-infection rate together with a higher uptake rate and P uptake in EG IT90K-59 (Tab. 8) suggest that this is a strategy of P acquisition in this genotype. For the EG IT89KD-391, the lack of a correlation between P uptake and AMF-infection rate, but significant positive correlations with root morphological characteristics in particular, and P-uptake rate indicate that an enhancement of P uptake through a more extensive root system (*Lynch* et al., 1997; *Manske* et al., 2000) and mobilization of soil and fertilizer P through root exudates (*Kirk* et al., 1999; *Gerke* et al., 2000) are more important for this genotype. The P uptake of the P-efficient cowpea genotypes was also significantly related to the shoot-to-root ratio (Tab. 8). The results may imply that a better translocation of P from root to shoot may also account for higher P efficiency in cowpea. Similar findings were made by *Araújo* et al. (1998) and *Caradus* and *Snaydon* (1986) in studies on common bean and white clover genotypes, respectively. The results are also in agreement with those obtained by *Christiansen* and *Graham* (2002) for bean genotypes.

Table 7: Shoot dry matter (DM), P uptake, root length, P-uptake rate, shoot-to-root ratio, and arbuscular mycorrhizal–fungi (AMF) colonization of each two P-efficient and P-inefficient cowpea genotypes grown for 8 weeks in a pot experiment as affected by P application (0P = 0 kg P ha^{-1}, PR = 90 kg P ha^{-1} phosphate rock, TSP = 30 kg P ha^{-1} triple superphosphate), n = 4.

Genotype	**Shoot dry matter** [g $plant^{-1}$]			**P uptake** [mg $plant^{-1}$]			**P-uptake rate** [µg (cm $root)^{-1}$]			**Root length** [m $plant^{-1}$]			**Shoot-to-root ratio** [mg dwt (m root $length)^{-1}$]			**AMF** [%]		
	0P	PR	TSP	0P	PR	TSP	0P	PR	TSP	0P	PR	TSP	0P	PR	TSP	0P	PR	TSP
Efficient genotypes (EG)																		
IT 90K-59	7.7 aB	8.8 aA	8.6 aA	15.4 aB	19.8aA	18.8 bA	2.1 aB	3.1 aA	2.5 abAB	73 bA	64 abA	77 aA	1.1 aB	1.4aA	1.1 aB	24 aB	30 b A	28 aAB
IT 89KD-391	7.7 aB	8.7 aA	8.7 aA	16.0 aB	19.6 aA	19.9 aA	1.9 aB	3.3 aA	2.7 aA	87 aA	61 bB	75 aAB	0.9 aB	1.4 aA	1.2 aA	25 aB	34 aA	24 bB
Inefficient genotypes (ING)																		
IT 89KD-349	7.0 bA	7.9 bA	7.8 bA	14.2 bB	16.9 bA	17.8 cA	2.1 aA	2.4 bA	2.7 aA	73 bA	69 aA	66 bA	1.0 aA	1.1 bA	1.2 aA	16 cB	29 bA	21 bA
IT 82D-849	7.1 bA	7.6 bA	7.8 bA	15.6 aA	15.8 cA	16.9 dA	1.9 aA	2.3 bA	2.2bA	81aA	68aA	77 aA	0.9 aB	1.1 bA	1.0 aAB	22 abB	30 b A	28 aA

Numbers followed by the same lower- or upper-case letter between genotypes within P treatments or between P treatments within genotypes, respectively, are not significantly different at $p >$ 0.05 (LSMEANS/PDIFF test).

Table 8: Coefficients of determination of the linear relationships between P uptake and selected plant characteristics for two P-efficient and two P-inefficient cowpea genotypes grown for 8 weeks in a pot experiment. Analysis across P rates, n = 24.

P uptake [mg $plant^{-1}$]						
Genotype	Shoot DM*	Root DM	Shoot-to-root	P-uptake rate	Root length	AMF
Efficient genotypes (EG)						
IT90K-59	0.63 *	ns	0.49 *	0.48 *	ns	0.31 *
IT89KD-391	0.57 **	0.46 *	0.53 *	0.54 **	0.39 *	ns
Inefficient genotypes (ING)						
IT82D-849	ns	ns	ns	0.57 **	ns	ns
IT82KD-349	ns	ns	ns	ns	ns	ns

For units see Tab. 7.
ns: not significant; *, **, ***: significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively

5 Conclusions

Differences among the cowpea genotypes were observed in growth, N_2 fixation, and P acquisition under low soil-P condition of SC. Genotypes IT90K-59 and IT89KD-391 were identified as efficient in N_2 fixation and P uptake. The results of the pot experiment suggest that in the genotype IT90K-59, the higher P uptake was primarily due to an effective symbiosis with arbuscular mycorrhizae, while in genotype IT89KD-391, root morphological and physiological characteristics increasing the P-uptake rate were more important. Both genotypes with high P-uptake efficiency and high N_2-fixation ability can be recommended for incorporation into cropping systems on the acid and low-P soils of SC.

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