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1 author:

Saidou Addam Kiari
National Institute of Agricultural Research, INRAN Niger

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Full Length Research Paper

Response of cowpea lines to low Phosphorus tolerance and response to external application of P

A. K. Saidou[1]*, B. B. Singh[1], R. C. Abaidoo[1], E. N. O. Iwuafor[2] and N. Sanginga[3]

[1]International Institute of Tropical Agriculture (IITA), Oyo Road, Ibadan, Nigeria.
[2]Institute of Agricultural Research (IAR) Ahmadu Bello University, Samaru, Zaria, Nigeria.
[3]Tropical Soil Biology and Fertility (TSBF), Nairobi, Kenya.

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Low availability of phosphorus (P) is a major constraint to crop production in Sahel and Sudan Savanna of West Africa, and efforts are being made to identify cowpea genotypes with tolerance to low P and greater P use efficiency. Arbuscular mycorrhizal fungi (AMF) enhance P uptake in low P soil. Cowpea breeding lines (200) were examined at 3 P levels (0P, 90 kg P ha^{-1} as Rock Phosphate and 30 kg P ha^{-1} as Simple Super Phosphate (SSP) in 2002 at Minjibir, Nigeria and Toumnia, Niger. Wide apparent variation in grain and fodder yields was found among the 200 genotypes. Grain yield response to RP and SSP ranged from 1 to 160%. Based on the genotypes performance at low and high (SSP), genotypes were classified. Fifteen genotypes were selected from the different P use and response groups and further examined in the greenhouse and field studies for growth characteristics related to tolerance to low applied P. Phosphorus application significantly ($P \leq 0.05$) increased the grain and fodder yield at both locations., shoot-root ratio but decreased AMF colonization of cowpea roots. There were large differences in the relative P used in efficiency and the values ranged from –11 to 38 kg grain (kg P)$^{-1}$ applied. AMF infection was reduced by at least 50%, while shoot-to-root ratio was significantly increased with P application. Variation between genotypes was significant for certain paired means but not consistent for all parameters measured, and the locations. The cowpea genotypes differ in AMF colonization, shoot growth relative root development, and relative P use efficiency for tolerance to low P soils and response to external application.

Key words: Arbuscular mycorrhizal fungi (AMF), cowpea genotypes, P-tolerance/response, relative P-use efficiency, shoot, root ratio.

INTRODUCTION

Low availability of phosphorus (P) is a major constraint to cowpea production in the low P environment of the Sudano-Sahelian region of West Africa, (Danso, 1992). Legumes like alfalfa (Deng et al., 1998), clover, common bean, cowpea (Cassman et al., 1981) and Pigeon pea Itoh, (1987) showed a high positive response to P supplementation. However, farmers' inability to purchase chemical fertilizer is another crucial problem especially in land locked country like Niger Republic where the P fertilizer is scarce. There is, therefore, need for screening cowpea for low P tolerance and response to added external P to obtain suitable genotypes for these predominantly low P soil environments. The identification/establishment of criteria for useful P efficiency for cowpea would be needed for a successful selection and breeding program. The P use efficiency (grain yield per unit of nutrient supplied) Moll et al. (1982) has been established to a certain extent (Sanguinga et al., 2000; Bationo, 2002). The AMF root symbiosis has been shown to enhance P absorption by increasing the effective root area (Hayman and Mosse, 1971). A strong correlation between the P use efficiency and the root fungus AMF was pointed out in moist savanna of West Africa (Sanguinga et al., 2000). Shoot-root ratio could

*Corresponding author. E-mail: kaddam2001@yahoo.fr.

Table 1. Physico-chemical properties of Minjibir(M), Toumnia(T) and River Sand (RS) 0-10 cm.

Soil-sand characteristics	M	T	RS
pH (H_2O)	6.5	7.3	6.3
pH (KCL)	5.4	6.5	5.8
Organic Carbon (g kg^{-1})	0.25	0.24	0.036
Total N (g kg^{-1})	0.013	0.01	ND
Olsen P (mg kg^{-1})	2.3	0.7	8
Bray I P (mg kg^{-1})	7.6	2.7	ND
NH_4-Acetate-extractable Cations (cmol kg^{-1})			
Ca	0.9	0.8	1
Mg	0.2	0.3	0.13
K	0.1	0.1	0.06
Na	0.4	0.4	0.23
Sand%	88	91	92
Silt%	5	2	2
Clay%	7	7	6
Textural class		Sandy soil	

also be an indicator of the response of cowpea lines to low P soils. Evidence available so far indicates that genes controlling tolerance to P deficiency appear to be independently inherited (heritable) and recombinable (Polle and Konzack, 1990). The implication of this to the cowpea breeder is that any identified tolerant cultivar can be crossed with another to transfer the tolerance trait. The objectives of this paper were: (1) to identify cowpea genotypes that maintain high yields under low P condition and respond to P application and (2) to identify criteria or mechanisms to explain the efficient use of low P and response to applied P.

MATERIALS AND METHODS

A total of 200 cowpea (*Vignaunguiculata* (L) Walp.) genotypes comprising plant types, duration of growth, yield and origins were obtained from International Institute for Tropical Agriculture (IITA), Institute of Agricultural Research (IAR) Samaru, Zaria and National Institute for Agronomic Research of Niger Republic (INRAN). Field experiments were conducted at Minjibir (12°8.73'N, 8°39.97'E) on an Alfisol (Olsen P, 2.1 mg kg^{-1}), located in Sudansavanna of Nigeria and at Toumnia, Zinder in NigerRepublic (13°28.76'N, 9°.79'E) located in Sahel zone of Niger on soils with an Olsen P level of 0.7 mg kg^{-1}. The mean rainfall for 2002 was 841.4 mm at Minjibir, and 372.8 mm at Toumnia. The field experiments were set up after P response curves in potted soils from the two locations had been determined. The land was cleared of trees, ploughed and then disc harrowed. Cowpea genotypes were planted at an inter-row distance of 1 m and 0.25 cm within row. Single super phosphate (SSP, 30 kg P ha^{-1}) or Rock P from Niger (90 kg P ha^{-1}) were the sources of P applied by banding before planting. The rock P has the following characteristics: 36% P_2O_5, 52%Ca, 0.5% H_2O, 0.1% S and 3.7% F (Bationo et al., 1991). Each genotype was grown in a single row 4 m long with 42 plants in each row. The experimental design was a strip-plot design with 4 replications. The cowpea fields were sprayed with Delphos (Monocrotophos AC-E2) for aphids and Upper Cott for Maruca damages and weeded according to standard agricultural practice. Plants were harvested at maturity and grain and fodder yields measured.

In 2003 cropping season, the fifteen-cowpea lines selected from the 2002 field screening experiments were planted on newly selected low available P fields at Toumnia and Minjibir (430.3 and 1031.4 mm rainfall respectively). Cowpea genotypes were planted at a spacing of 25 cm within rows and 75 cm between rows at five seeds sown $hill^{-1}$ and the seedlings thinned to two, a week after emergence. Phosphorus was applied as SSP at 30 kg P ha^{-1} and as RP at 90 kg P ha^{-1} and each cowpea cultivar in a plot of 4 × 3 m received a basal application of 30 kg K ha^{-1} as KCl. No N fertilizer, mycorrhizal fungi or –*Bradyrhizobia* inocula were applied. The treatments of cowpea genotypes as vertical factor and P sources as horizontal factor were arranged in a strip plot design with 3 replications. Fresh roots were collected in sampling vials at early podding for AMF colonization rate by the method of Giovanetti and Mosse (1980). The clearing and staining procedure described by Phillips and Hayman (1970) was used prior to detection of AMF colonization of cowpea roots.

Statistical analysis

Analysis of variance (ANOVA) was done using the SAS 8e program (SAS, 2001) to determine treatment and interaction effects. Least significant differences (LSD) were also calculated to assess treatment differences.

RESULTS

The soils used in the experiments were sandy, slightly acidic at Minjibir and slightly alkaline at Toumnia. The organic C and total N as well as the available P were very low (Table 1). Phosphorus application significantly($P \leq 0.05$) increased the grain and fodder yield at both locations. The increase was in the order SSP > RP >

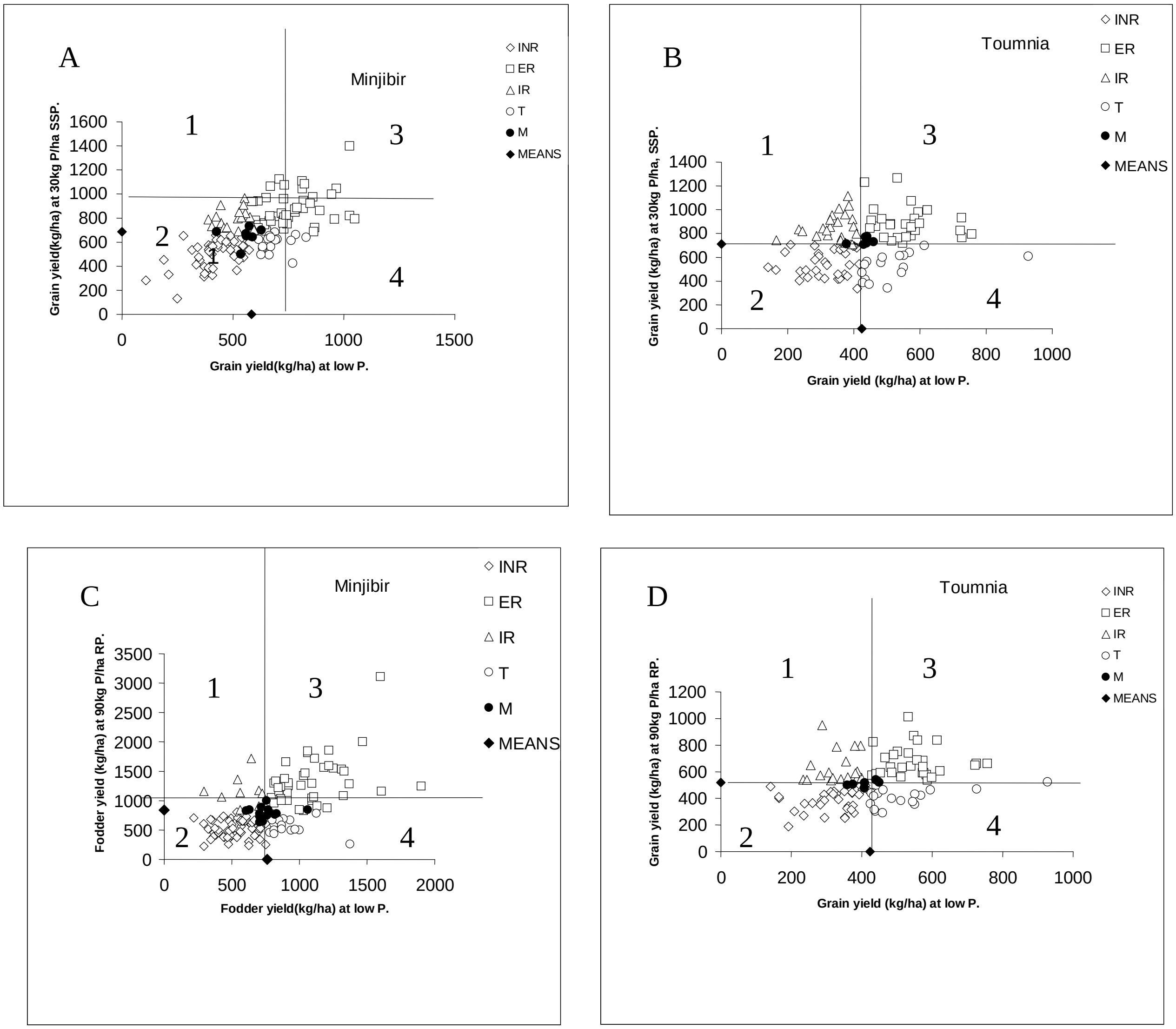

Figure 1. Grain yield variability of 200 cowpea genotypes to low P (no P applied) and high P (30 kg P ha-1 as SSP or 90 kg P ha-1 as RP). Graphs A and B (above), 0P and SSP application; C and D (below), 0P and RP application. Legend: 1, inefficient; 2, efficient response; 3, inefficient response; and 4, efficient no response.

Control without P. Five major P-response groups were identified at both Toumnia and Minjibir fields based on grain (Figure 1) and stover (Figure 2) yields as follows: (1) No P response, (2) Efficient response to high P, (3) response to low P, (4) Response or P-tolerant group, For example, the lines IT99K-213-21, TN256-80, IT98K-476-8 and IT98D-1399 were recorded as efficient in low as well as in high P while IT97K-813-21 maintained high yields under low P (0P) conditions at Minjibir, Kano, Nigeria. This was found to be slightly different at ToumniaIT97K-340-1, IT98D-1399, IT97K-819-170, TN256-80 and IT99K-826-119 were recorded as responding to fertilizer P addition and TN28-87 was recorded as Efficient in uptake of added P, the line IT97K-819-154 was recorded as tolerant to low P at Toumnia. Significant ($P < 0.01$) differences in shoot: root ratio of cowpea genotypes were observed in the fields at both Toumnia and Minjibir (Table 2).

At Minjibir, the cultivar TN256-80 had the highest shoot: root ratio (14) both with and without P application suggesting that the line had efficiently responded to SSP/RP and tolerant at Minjibir. The genotype IAR was recorded as having the lowest shoot: root ratio (4) in the field at Minjibir. On the other hand the cultivars TN256-80, IT98K-813-21, IT98D-1399 and DANILLA had shoot: root ratios of 14, 13, 13, and 12, respectively when no P was applied.This suggests that these genotypes/varieties inefficient in P uptake to get P from the P-deficient soil have to be in relation with AMF, hence the increase in shoot: root ratio (Table 2).

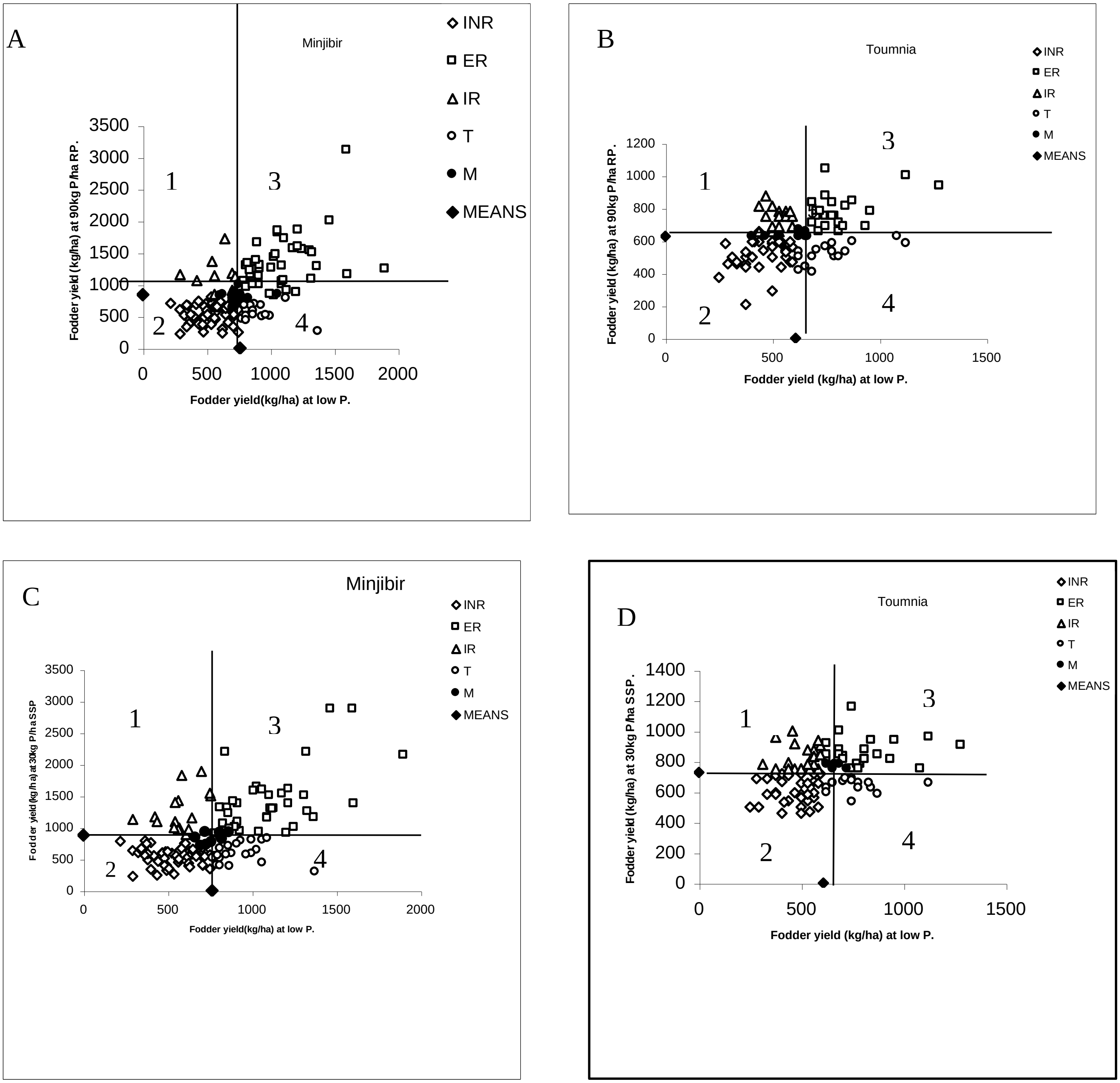

Figure 2. Stover yield variability of 200 cowpea genotypes to low P (no P applied) and high P (30 kg P ha-1 as SSP or 90 kg P ha-1 as RP). Graphs A and B (above), 0P and RP application; C and D, (below) 0P and SSP application. Legend: 1, inefficient; 2, efficient response; 3, inefficient response; and 4, efficient no response.

Phosphorus application suppressed AMF colonization in more than 53% of field grown-cowpea genotypes at both Minjibir and Toumnia (Table 3).The line IT98K-476-8 was observed to have the highest AMF colonization rate (21%)at Minjibir, while IT97K-813-21 had the highest AMF rate (24%) at Toumnia (Table 3).The relative P use efficiency (RPUE) of the cowpea genotypes also varied significantly at both Minjibir and Toumnia.The local variety Danila had the highest RPUE (38 kg grain (kg P)$^{-1}$ at Minjibir and the line IT98D-1399 was observed to be the most efficient P user (13 kg grain (kg P)$^{-1}$ at Toumnia (Table 4).

Table 2. Variability of cowpea lines shoot-to-root ratio cowpea lines grown in P-deficient soils at Toumnia and Minjibir.

	TRTS							
Varieties	**C**	**RP Minjibir**	**SSP**	**RMQ[1]**	**C**	**RP Toumnia**	**SSP**	**RMQ[1]**
90K-277-2	6	7	8	INR	8	5	8	IR
97K-340-1	-	*	*		12	8	14	ER
97K-813-21	13	8	11	T	11	15	11	ER
97K-819-154	8	9	10	INR	22	13	17	IR
97K-819-170	10	10	12	INR	16	19	10	IR
98D-1399	13	8	11	IR	12	11	7	T
98K-476-8	10	9	8	ER	9	8	8	ER
99K-826-119	6	7	6	INR	10	11	8	T
ALOKA	9	12	11	INR	19	24	10	ER
DANILA	12	12	11	IR	13	13	11	INR
IAR-48	5	4	6	IR	8	13	11	ER
00K-1148	12	12	12	INR	12	13	13	ER
TN256-80	14	14	14	ER	16	14	15	ER
TN28-87	12	14	11	T	13	10	9	ER
								ER
MEANS	10	10	11		13	13	11	ER
Cv%	33				30			
	T	V	T×V		T	V	T×V	
SE	1	2	3		1	3	5	
LSD	3	3	5		4	6	10	
S	NS	**	NS		NS	**	NS	

1=IR, Inefficient Response; INR, Inefficient no response; ER; Efficient response; and T, Tolerant; NS, not significant**, very significant.

DISCUSSION

Differences in P use efficiency among the cowpea genotypes were observed in 2002 field screening experiment and different P response groups were established depending on location and whether grain yield or stover yields were used as basis of the evaluation. The study supported observations made by Sanguinga et al., (2000); Bationo and Anan Kumar (2002) that environmental factors affect response of cowpea genotypes to P levels. However, some lines like IT98K-476-8, IT97K-826-119 and IT97K-813-21 were consistent in response at both locations indicating heritable traits of cowpea lines under existing/inherent soil P conditions.

On the other hand a local variety Danilla was efficient in utilizing inherent soil P. This suggests that such a variety will usually rely on the external application of P for optimum growth. It is inefficient in utilizing inherent soil P, is not adapted to existing soil conditions. P requirement for cowpea varies widely within the germplasm. The response of the sister lines IT97K-819-154 and IT97K-819-170, which are not far from each other indicate the influence of the same gene expression within the cowpea lines.

The increase in shoot: root ratio upon P application supported observations made by Anghioni and Barber (1980) on the effect of P application on shoot: root ratio.

The increase in the shoot: root ratio in the different lines has been explained as a mechanism used by some crops to efficiently use P added to soils for dry matter production. The reduction in AMF colonization rate in the fields indicates the role played by the fungus in absence of the fertilizer P in these dry areas of West Africa (Bagayoko et al., 2000). This also explains how AMF colonization enhances host-plant uptake of relatively immobile nutrient like P (Thompson, 1987). The relative P use efficiency increased from 0 to 38 kg grain (kg P^{-1}) for the local variety Danilla and this indicated that the variety is an inefficient at low P levels but responding to external application of SSP.

However, certain improved varieties (e.g., 97K-813-21, 97K-826-119) have shown that they are non-responsive to P application by giving almost the same relative P use efficiency (RPUE) values with and without SSP. The results clearly indicate that the P use efficiency by the

Table 3. Variability of the selected lines to P for AMF infection in P-deficient soil at Toumnia and Minjibir.

Varieties	TRTS					
	TOUMNIA			MINJIBIR		
	C	RP	SSP	C	RP	SSP
	--------------------------AMFIR (%) -------------------------					
90K-277-2	15	11	8	19	12	5
97K-340-1	8	29	9			
97K-813-21	24	16	10	17	13	3
97K-819-154	17	11	7	2	1	2
97K-819-170	6	7	10	7	1	3
98D-1399	14	23	9	20	10	6
98K-476-8	13	11	9	21	20	6
99K-826-119	21	28	12	8	5	8
ALOKA	16	15	11	4	8	3
DANILA	11	15	4	11	13	3
IAR-48	20	13	12	14	8	5
00K-1148	19	10	6	16	12	6
TN256-80	19	15	12	15	8	6
TN28-87	15	6	10	8	7	5
Means	16	15	9	12	9	5
CV(%)	47			46		
	T	V	TxV	T	V	TxV
SE	1	3	5	1	2	3
LSD	4	6	10	3	4	7
S	*	**	*	**	***	**

NS: not significant; *: significant; **: very significant; ***: highly significant.

Table 4. Relative phosphorus use efficiency of cowpea varieties in the P deficient soil of Toumnia and Minjibir.

Varieties	RPUE (kg grain (kg P)$^{-1}$)					
	TOUMNIA			MINJIBIR		
	RP	SSP	RMQ	RP	SSP	RMQ[1]
90K-277-2	1.17	8.44	IR	0.10	2.00	INR
97K-340-1	3.52	8.2	ER	ND	ND	
97K-813-21	3.17	0.00	ER	3.50	18.70	T
97K-819-154	1.04	7.26	IR	0.60	2.80	INR
97K-819-170	2.32	2.53	IR	-2.60	7.60	INR
98D-1399	7.92	13.13	T	-4.60	21.50	IR
98K-476-8	-5.62	-1.00	ER	7.10	0.30	ER
99K-826-119	ND	ND	T	-2.50	-2.50	INR
ALOKA	1.31	4.69	ER	2.10	3.20	INR
DANILA	1.15	0.75	INR	2.40	38.10	IR
IAR-48	0.00	9.93	ER	9.70	30.00	IR
00K-1148	-11.55	1.00	ER	6.40	14.20	INR
TN256-80	2.82	0.00	ER	-0.70	3.20	ER
TN28-87	-3.23	3.61	ER	-1.70	1.10	T
MEANS	0	5		2	11	
	TRT	VAR	TRT×VAR	TRT	VAR	TRT×VAR
SE	2.218	2.645	4.929	5	8	14
LSD	6.159	5.278	9.895	13	16	29

ND: Not defined.

breeding lines is a good mechanism differentiating the lines and selecting lines for specific sites.

Conclusion

This study observed wide differences between cowpea cultivars in grain and fodder yield, P use efficiency, AMF colonization rate and shoot: root ratio parameters. We have found out that the genotypes IT97K-476-8, IT98D-1399 and IT97K-813-21 with high RPUE and high shoot: root ratio under low P field's conditions will be suitable for cropping systems of dry savannas. We have also identified Danilla, IAR-48, and TN256-80 as good performers under no or minimal external P application.

The potential to use Rock P in combination with selected improved P-efficient lines exists from this study. The cowpea genotype IT97K-340-1, which exhibited some response to Rock P application, needs further testing to confirm this attribute.

There is a crucial role of AMF in these soils and this may eventually compel consideration of AMF when breeding new cowpea lines. The results also, affirm the importance of considering P requirements of these cowpea lines when introducing them in Sudan and Sahel savanna zones.

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