

Genetic Evaluation of Phosphorus Utilization in Tropical Cowpea (*Vigna unguiculata* (L) WALP)

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ABSTRACT: Genetics evaluation of phosphorus utilization in cowpea (*Vigna unguiculata* (L) Walp) was studied in a cross involving a tropically adapted genotype (IT90K-277-2) with a reported positive response to rock phosphate (RP) and a second genotype (IT89KD-288) that responds negatively to RP, using generation mean analysis of the parents, their F₁, F₂ and the two backcross generations. Highly significant differences among the six generations with respect to seed phosphorus (P) concentration and grain yield per plant were observed. The F₁ progenies produced seeds with higher seed P concentration (0.31 mg/g) than the mid-parent value (0.27 mg/g). Mean yield per plant in the F₁ (48.03g) that was significantly greater than the mid-parent value (28.85g) was also observed suggesting positive heterosis for the two traits. Backcrossing to both parents showed good convergence of genes on the recurrent parents. Possibility of transgressive segregation for seed P concentration and grain yield per plant in the F₁ and F₂ populations suggested lack of maternal and cytoplasmic effects. Non-additive gene action (Dominance and epistasis) made significant contributions to total genetic variations in both traits within the cross. Whereas seed P concentration was observed to be under polygenic control, number of genes for seed yield per plant was biased downward by epistasis. Observed narrow-sense heritability estimate (H_N) for seed P concentration of 50.15% and very low (0.040) expected genetic gain in the F₃ over the F₂ means suggested that although expected progress from selection could be lowered by non-additive gene effects, this trait could be transmitted to the offsprings of hybridization program with huge success, through backcrossing. Although, available soil P (6.03 – 8.09 mg/kg) was observed to be below the critical level, phosphorus uptake in the F₁ and the segregating generations was observed to be higher than what obtained in the parents to suggest that the progenies of a P-efficient x P-inefficient cross would be efficient in P utilization. [The Journal of American Science. 2007;3(1):77-83].

Keywords: Generation mean analysis, Phosphorus uptake, Polygenes, Transgressive segregants, Tropical vigna.

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INTRODUCTION

Cowpea has been identified as one of the keys to crop-livestock integration in the Sahelian region of Nigeria because of its drought tolerance, growth habit in sandy soils, its contribution to soil fertility improvement and its usefulness as food and fodder (Singh, 1999). Generally, soils in the West African semi-arid tropics are low in available and total phosphorus (P) and the main limiting nutrient for legume production in the tropics are nitrogen and phosphorus (Fox and Kang, 1977). Uptake of soil P varies between cowpea genotypes for nodulation (Aukomah *et al.*, 2000). Efficient P utilization and tolerance to moisture stress and micronutrient toxicity will increase adaptation and yield potential of cowpea in marginal lands. However, inorganic fertilizers are not readily available to the resource poor African farmers. Thus, direct application of indigenous rock phosphate (RP) remains an economic alternative to the use of water-soluble fertilizer (Singh, 1999).

Frageria *et al.* (1988) have observed that cowpea cultivars that can perform well under a low level of soil

P-tolerant cowpea lines that could assess a greater proportion of total soil P pool and at the same time efficiently associate with soil mycorrhizal for better utilization of added P from applied RP.

Genetic analysis of the progenies of an iron-efficient by iron-inefficient cross in soybeans has established that efficiency was due to a single, major dominant gene that controlled the reducing power of the root surface (Brown *et al.*, 1958). Epstein, (1972) also reported a single, major gene inheritance for the recurring feature of micronutrient efficiency characters in crop plants. In tomatoes, iron efficiency has been shown to be based on a major gene coding of nictoneamide and string of minor genes (Brown and Wann, 1982; Coyne *et al.*, 1982) also observed that dominant alleles at two loci were required for full expression of iron efficiency in dry beans (*Phaseolus vulgaris* L.). Hoan *et al.* (1992) observed that interaction between the two genes at the loci stated was more additive than complementary. However, Majumder *et al.* (1990) reported that additive, and to a lesser extent, dominance gene effects, were responsible

for zinc efficiency traits in rice. Bolland and Baker, (1988) observed an increased shoot yield and seedling vigour with progressive increases in phosphorus concentration in wheat seeds sown in pots which, in effect, extended to an increase in grain yield (Bolland and Baker, 1988).

A cowpea breeding line (IT90K-277-2) with reported positive response to RP in terms of increased yield has been identified (Singh, 1999). Another line (IT89KD-288) that responded negatively to P treatment by giving high yields without P and low yield with P was also identified. Singh *et al.* (2002) also reported significant positive correlations between high yield and improved nutritional and cooking qualities among the identified cowpea genotypes. There exists, therefore, sufficient genetic variability needed in the improvement and production of cowpea lines that could combine high grain yield and high seed protein with acceptable nutritional qualities even when sown in low P West African semi-arid tropical (WASAT) soils.

However, studies have shown that some legumes are capable of releasing large amount of organic acids into the rhizosphere in response to P deficiency (Mugwira *et al.*, 1997; Otani and Ae, 1997). It has been hypothesized (Belanger *et al.*, 2002) that crop genotypes that are exhibit contrasting P concentration because of a strong relationship that exist between N and P concentrations in plants.

Although, critical level of available soil P for grain legumes (10.8 mg.kg) has been documented (Adeoye and Agboola, 1985; Anne and Lai, 1995), rate of depletion of soil P in African soils was 10 kg ha⁻¹ year⁻¹ (Stoorvogel and Smalling, 1998). Low P levels in tropical soils, diminishing P reserves and the ability of some legumes to perform optimally under low P levels are a signal to the need to develop crop varieties that are efficient users of available soil P or at least, tolerant to P deficiency.

In comparison with iron (Fe⁺⁺) and other micronutrient efficient studies in Soybean (Beeghly and Fehr, 1989), dry beans (Coyle *et al.*, 1982; Hoan *et al.*, 1992) and rice (Majunder *et al.*, 1990), there is virtually no definitive information with respect to the genetics of P utilization in tropical Vigna.

There is a possibility that one or more of the lines identified by Singh (1999) is significantly more efficient in P utilization compared with others, in which case the P utilization characteristic may be controlled by a single dominant gene. Transgressive segregation for P utilization may result in the F₂ generation from a cross involving P-efficient x P-inefficient parents or its reciprocals due to the presence of many genes, with small but significant effects (Fehr, 1982; Borojevic, 1990). It is not equally certain whether there is an interaction of the P utilization

characteristic with desirable traits in the cowpea cultivars.

The aim of this study, therefore, was to determine the genetic basis of phosphorus utilization in tropical cowpeas.

MATERIAL AND METHODS

Seeds of cowpea line (IT90K-277-2) that has been reported to show positive response as well as the line (IT89KD-288) that responded negatively to rock phosphate treatment in terms of grain yield (Singh, 1999) were obtained from IITA, Ibadan for this study. Line IT90K-277-2, in particular, is photo-insensitive, has white-rough seed coat texture with faster cooking time and acceptable taste. Line IT89KD-288 on the other hand is late-maturing with high seed protein (26%), higher fodder and seed yield without P and low productivity with P (Singh *et al.*, 2002).

Genetic evaluation of phosphorus utilization was investigated by artificial crossing between IT90K-277-2 and IT89KD-288 in the screen house at IITA, Ibadan, Nigerian, to obtain the F₁ progenies. The F₁ generation was selfed to obtain the F₂ progenies. Two backcrosses were obtained by making crosses between the F₁s and each of the parents to ensure effective gene transfer. Reciprocal crosses were also made to determine possible maternal and cytoplasmic effects.

The soil available P was determined before and after the planting of seeds from the six generations using Bray No. 1 methods (Bray and Kurtz, 1945). Fifteen plants were selected at random from each of the parents, 25 plants from the F₁ and the two backcrosses whereas 75 plants were selected at random from the F₂ generation at harvest maturity. Total nitrogen (N) concentration in soil samples was determined using kjeldahl digestion and colorimetric technicon auto-analyser (TNC, 1971). Particle sizes were determined by the pipetal method (Gee and Bauder, 1986) cation determination with the flame photometry and atomic absorption spectrophotometry, whereas soil acidity was determined by the chromic acid digestion procedure (Heanes, 1984).

Subsequently, 5g seed samples of selected plants from the six generations were tested for P concentration first by perchloric acid digestion (Novosky *et al.*, 1983), followed by simultaneous colorimetric P determination using an auto analyzer (IITA, 1982).

Phosphorus uptake in seed samples of selected plants in the six generations was determined as the product of P concentration (%) in seed and weight of seed per plant (Ahmed *et al.*, 2001).

Observed means were subjected to weighted generation means analysis according to Mather and Jinks (1982) to estimate the genetic parameters such as the mean [m], additive [d], dominance [h] and to detect

the presence or absence of additive x additive [i], additive x dominance [j] and dominance x dominance [l] effects in a generalized inverse equation matrix.

The data collected were also analyzed of broad-sense and narrow-sense heritability estimates following the methods of Mahmud and Kramer (1951) and Warner (1952), respectively. Expected genetic gain at 5% selection was estimated following (Allard, 1969). The numbers of genes controlling both traits were also estimated following (Mather, 1949; Lande, 1981).

RESULTS AND DISCUSSION

The physico-chemical properties of soil samples used for the experiment (Table 1) showed that the soil was sandy, low in total acidity with total N (0.05%) and available P (7.62 mg/kg) that were below the critical levels (Aune and Lai, 1995).

Means and variances of cowpea seed phosphorus (P) concentration and yield per plant in the six generations are presented in Table 2. The results showed that cultivar IT89KD-288 had significantly higher seed P concentration than IT90K-277-2. Genotypic differences in P utilization were, probably, not only related to P participation in metabolic process (Fageria and Baligar, 1999) they were also due to

absorption rate as a result of large root systems, particularly, in legumes as reported by Sanginga *et al.* (2000). However, mean seed yield of the two parents (27.96g and 29.73g) were statistically similar. The F₁ values for each character were significantly different from those of the parents on one hand and the mid-parent values on the other hand suggesting positive heterosis for the two characters. Partial dominance of alleles for high seed P concentration and overdominance of alleles with higher seed yield per plant was also observed. The above observations suggest that cultivar IT89KD-288 depends upon available soil P rather than efficiently utilizing the large P concentration in its seed for agronomic productivity.

Backcrossing to the two parents resulted in convergence of gene for higher yield and higher P concentration in the recurrent parents. Although, the two backcrosses were not statistically different, the backcross breeding method could provide a profitable means of improving seed P concentration in tropical cowpea. Transgressive segregation for increased seed P concentration was also observed in the F₂ generation because nearly half of the segregants (47%) in the F₂ generation performed better than their better parent.

Table 1. Physico-chemical properties of the top soil samples used for the Experiment

Soil characteristics	
Sand (%)	
Silt (%)	16.00
Clay (%)	2.00
pH (H ₂ O)	6.00
Organic C (%)	0.35
Total N (%)	0.05
Bray 1 P (mg/kg)	7.62
Ca	4.35
Mg	0.35
Mn	0.04
K	0.13
Total acidity (cm01/kg)	0.01

Table 2. Means and variances of seed P concentration (mg/g) and yield per plant (g) in six generations of a cowpea cross

Generation	N	Seed P concentration		Yield/plant	
		Mean ^t	Variance (δ^2) x 10 ⁻³	Mean ^t	Variance (δ^2)
IT89KD-288 (P ₁)	15	0.43b	0.63	27.96d	18.72
IT90K-277-2 (P ₂)	15	0.11d	1.68	29.73d	16.33
Mid-parent		0.27		28.85	
P ₁ x P ₂ (F ₁)	15	0.31c	7.74	48.03	26.46
P ₂ x P ₁ (RF ₁)		0.35c	7.91	46.24c	27.85
F ₁ x P ₁ (B ₁)	25	0.82a	8.58	43.51b	45.49
F ₁ x P ₂ (B ₂)	25	0.79a	7.57	42.23b	38.82
F ₁ x F ₁ (F ₂)	75	0.49b	2.81	35.89c	49.13
RF ₁ x RF ₁ (RF ₂)	75	0.50b	3.12	34.33c	43.85

The joint scaling tests for seed P and yield per plant as shown in Table 3 were considered because the simple additive-dominance model was not adequate in explaining the total genetic variability observed for the two characters. Large and significant chi-square values in the joint scaling tests also confirmed the inadequacy of the additive-dominance model to suggest presence of dominance or digenic epistasis. The m, d and h components of the joint scaling test for P concentration were significantly different from zero. For this trait, the dominant component [h] was larger and positive (0.657) compared with the additive [d] component (0.136). Low additive [d] compared with large dominance gene effect [h] indicated the preponderance of dominance gene effect in the inheritance of seed P concentration in the cowpea cross. Positive sign of dominance effects also indicated that dominance was in the direction of parent with larger concentration of seed phosphorus. Negative dominance component [h] of -11.07 for yield per plant indicated that dominance was in eh direction of IT89KD-288, the parent with smaller yield per plant to suggest a net dominance for genes that controlled low seed yield per plant. The various observations tend to suggest that the more the seed P content of IT98D-288, the lower was seed yield per plant. These observations are in conformity with a similar observation (Singh, 1999) that addition of rock phosphate to IT89KD-288 resulted in reduced yield.

The six parameter model in the estimation of various genetic components for seed P concentration and yield per plant is shown in Table 4. For seed P-concentration only the additive component [d] of the main effects was not significant. The digenic epistatic terms [i], [j] and [l] were statistically significant (t-test, $P > 0.05$). Significant digenic interaction terms indicated that epistasis was responsible for the departure from simple additive-dominance model with respect to seed P concentration in the cross. For seed yield per plant, however, both the additive[d] of the main effects and the epistatic term [j] sign of additive x dominance [j] interaction also suggested dispersion of genes in the parents. According to Mather and Jinks (1977), positive sign of dominance [h] and negative sign of dominance x dominance [l] suggest duplicate gene action.

Estimates of variance components as shown in Table 5 revealed that the additive variance (V_A) was smaller than dominance variance (V_D) for yield but larger than V_D for seed P concentration. Narrow-sense heritability estimate was relatively high (50.57%) for seed phosphorus concentration but was low (28.39%) for seed yield per plant suggesting large effect of the environment on the latter. Genetic gain was low (0.04) for seed P concentration compared with 4.12 for seed yield per plant. Number of effective factor for P

concentration was between 0.168 and 12.09 and between 0.03 and 0.89 for yield per plant. With the assumption of no dominance, no linkage and no epistasis (Kumar and Sign, 1995) the estimates (0.03 – 0.89) obtained for seed yield per plant were particularly most likely biased downward by epistatic effects. The first estimate (Lande, 1981) was assumed to be less affected by the presence of dominance and would provide a more reliable estimate of the minimum number of effective factors for this trait. In the estimation of effective factors, it is assumed that the segregating factors are iso-directionally distributed between the two parents and they have equal additive [d] effects. According to Aryeetey and Laing (1973), non-validity of either or both assumptions would result in an underestimation of the true value.

John and Thanyavelu (1997) have reported that heritability estimates could not solely provide sufficient information for the genetic improvement that would result from selection of best plant genotypes. However, heritability estimates is more important than just knowing the number of genes involved because heritability provides a realistic means of measuring progress in plant improvement by indicating how easy or difficult it would be to provide changes in a given trait by applying selection (Graham and Wetch, 1996). According to the authors, the closer heritability estimates is to 100%, the less environmental influence a trait is subjected to and the easier it is to make progress through selection. Thus, predominance of dominance effects coupled with relatively high heritability estimates and low genetic advance obtained for seed phosphorus in this study tends to suggest that phosphorus utilization in tropical cowpea could lower the expected progress from selection during early segregating generating and thus, lower the gain anticipated from continued inbreeding.

Soil available P before and after plating and estimates of seed P uptake from the six generations is presented in Table 6. Uptake of P in IT89KD-288 (P_1) was higher than in IT90K-277-2 (P_2) suggesting that P_1 was more efficient in P utilization than P_2 because it could absorb more P and, thus, yield more without P. also, P uptake in the F_1 and the segregating generations was higher (14.89 – 35.68 mg/plant) relative to the parental (3.27 – 12.02 mg/plant) values, to suggest that the progenies of a P efficient x P-inefficient cross would be efficient in P utilization because of transgressive segregation for higher seed P in cowpea. The implication is that the progenies of this cross would not require addition of synthetic Phosphorus of RP for agronomic productivity and this would be in the interest of the resource-poor West African farmers.

Using the current study as a yardstick, selection for phosphorus utilization may not be profitable and

feasible in the early segregating populations, the relatively high heritability estimate notwithstanding. The backcross breeding method would be an ineffective means of transferring the genes for seed P concentration among tropical cowpea genotypes. Graham and Welch (1996) had earlier reported that the

presence of many genes even with small effects could offer a possibility of obtaining transgressive segregants in the F₂ generation as observed in the current study to further suggest that P utilization trait is continuous and quantitative and further improvement by recurrent selection could also be employed.

Table 3. Joint scaling tests for seed P concentration and yield per plant

Parameter	Seed P concentration	Yield/plant
Mean [m]	0.3085 ± 0.0059*	41.44 ± 0.81*
Additive [d]	0.1356 ± 0.0660*	0.01 ± 0.72 ^{ns}
Dominance [h]	0.6574 ± 0.016*	-11.07 ± 1.58*
Chi-square [X ²]	189.58	599.37

Table 4. Six parameter model for estimation of various genetic components (Jinks and Jones, 1958) for seed P concentration and yield per plant

Parameter	Seed P concentration	Yield/plant
Mean [m]	0.49 ± 0.00790*	35.891 ± 1.044*
Additive [d]	0.03 ± 0.0328*	1.28 ± 2.37 ^{ns}
Dominance [h]	2.77 ± 0.0752*	47.10 ± 6.45*
Additive x Additive [i]	1.26 ± 0.0728*	27.92 ± 6.32 ^{ns}
Additive x Dominance [j]	-0.13 ± 0.0338*	2.17 ± 2.49 ^{ns}
Dominance x Dominance [l]	-3.32 ± 0.0400*	-45.65 10.67*

Table 5. Estimates of variance components, heritability and minimum number of effective factors for seed phosphorus concentration and yield per plant in a cross of cowpea

Estimates	Seed P Concentration	Yield/plant
Genetic variance [V _G]	1.636 x 10 ³	27.14
Additive variance [V _A]	1.053 x 10 ³	13.95
Dominance variance [V _D]	0.583 x 10 ³	13.19
Environmental variance [V _E]	0.446 x 10 ³	21.99
Heritability		
Broad sense [H _B] %	78.58	55.24
Narrow sense [H _N] %	50.57	28.39
Genetic gain	0.04	4.12
Minimum no. of genes		
Lande (1981)	12.09	0.03
Mather (1949)	0.168	0.89

Table 6. Phosphorus concentration before and after planting and estimates of seed phosphorus uptake (mg/plant) in the six generations

Generation	Before planting	After planting	P uptake (mg/plant)
P ₁	8.09	7.64	12.02
P ₂	7.65	7.56	3.27
F ₁	7.97	7.62	14.89
B ₁	7.88	7.09	35.68
B ₂	6.86	6.03	33.36
F ₂	7.48	6.98	17.57

Phosphorus uptake (mg/plant) = phosphorus concentration in seed x weight of seed (g) per plant

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