Available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Sciences (December, 2023), 31(2):66-72
DOI: http://dx.doi.org/10.4314/njbas.v31i2.10
ISSN 0794-5698

Full Length Research Article

Variability in Tolerance to Low Soil Phosphorus among Cowpea (*Vigna unguiculata* L. Walp) Landraces from Northern Nigeria

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ABSTRACT
Phosphorus (P) is an important precursor for nitrogen fixation as it initiates nodule formation and enhances rhizobium-legume symbiosis. This study was conducted to (i) evaluate the tolerance of cowpea landraces to low soil P and (ii) assess the correlation among agronomic traits under limited soil P conditions. A total of 420 cowpea genotypes were evaluated using a 20 × 21 alpha lattice design with two replications under high and low soil P conditions. Significant variations ($p<0.05$) were observed among the landraces under low P for all the measured traits indicating the presence of genetic variability and potential for crop improvement. Grain yield ranged from 58.7 kg/ha to 1488.0 kg/ha under low soil P. Low soil P significantly reduced grain and biomass yields of cowpea landraces compared to optimum conditions. Reduction in grain yield between the low and high P treatments ranged between 24% and 97%. 'Red beans medium' 'Dan Misra_Zamfara' and 'Dan Zaria' with the least yield reduction could serve as potential resources for the development of varieties tolerant to low soil P. Grain yield was significantly correlated with weight of 100 seeds ($r= 0.25$), the number of branches ($r=0.14$) and biomass ($r=0.58$), indicating that selection for these traits could be used as indirect selection metrics to increase grain yield under low soil P. The wide variability observed among the landraces for the measured traits suggests the availability of sufficient variability for genetic improvement for tolerance to low soil P.
KEYWORDS: cowpea, yield, landraces, Nigeria, phosphorus, tolerance

INTRODUCTION
Cowpea is an annual grain legume predominantly grown in the northern parts of Nigeria and widely consumed across the country, especially for its high protein (over 30%) content (Boukar *et al*., 2016). Nigeria is the largest producer and consumer of cowpea grain with an annual yield of over 3.4 million metric tons, which accounts for over 45% of global production (FAOSTAT, 2016). The young leaves and immature pods are consumed in some parts of the country while its fodder serves as animal feed (Iseghohi *et al*., 2019). The crop's yield on farmers' fields has been reported to be 863 kg ha^{-1} (Akah *et al*., 2021) compared to a potential of 1500–2500 kg ha^{-1} (Boukar *et al*., 2018; ICRISAT 2017) due to biotic and abiotic stresses (Aliyu *et al.*, 2023; Indabo *et al*., 2023; Sakariyahu *et al*., 2023). Low soil fertility especially low soil nitrogen (N), organic matter, and phosphorus (P) in the savannah soils is a major abiotic constraint for cowpea production (Boukar *et al*., 2018). Cowpea's productivity is highly associated with the amount of P fertilization, as several reports have shown increased yield and performance of the crop as P input increases (Adusei *et al.,* 2016; Kyei-Boahen *et al.,* 2017). Although empirical evidence has shown a strong cowpea response to P fertilization, the use of synthetic P fertilizer is not well adopted among smallholder farmers due to poor availability, especially in rural areas, low awareness of the need for P fertilizer, and high cost (Mohammed *et al*., 2020; Adusei *et al.,* 2015). P performs a critical role in cowpea growth and development as it is reported to stimulate growth, initiate nodule formation as well and influence the efficiency of the rhizobium-legume symbiosis (Haruna, 2011, Namakka *et al*., 2017). P is also involved in several key functions including energy metabolism, N fixation, synthesis of nucleic acids and membranes, photosynthesis, respiration, and enzyme regulation (Karikari and Arkorful, 2015). It is required in large quantities in young cells such as shoot and root tips with high metabolism rates and rapid cell division. It also aids flower initiation, seed, and fruit development (Nkaa *et al.*, 2014). Earlier reports have shown that legumes require more P for N fixation since P is needed in energy transformation in nodules (Hussain, 2017).

Conventional breeding programmes accompanied by modern agricultural practices gradually resulted in the loss of genetic diversity for many cultivated plant species (Bozokalfa *et al*., 2017). Consequently, vulnerability to biotic and abiotic stress of the elite germplasm is increased due to a narrowing genetic base of some cultivated crops. Nigerian cowpea landraces show high variability in seed shapes, sizes, colours, texture, pigmentation, and growth habits (Iseghohi *et al*., 2019). They represent valuable resources that can be explored for the introgression of new genes in varietal improvement (Hegde and Mishra, 2009; Xu *et al*., 2010; Iseghohi *et al*., 2019). The increasing population of Nigeria without a commensurate increase in cowpea production is likely to further widen the demand-supply gap, thus resulting in a persistent increase in the price of this commodity among other staple crops (Saka *et al*., 2018). The high cost of fertilizers and environmental concerns necessitate the development of cowpea genotypes with better nutrient use efficiency to ensure higher yields. Therefore, the development and utilization of varieties that are efficient in uptake and use of P will improve the productivity of the crop, and ensure a cleaner environment that would come from reduced P application and increased yield in low P soils. Hence, the need to explore landraces; a potential powerhouse of novel genes for improved cowpea performance under a sub-optimal P environment. This study was conducted to (i) phenotype cowpea landraces for their tolerance to low soil P, and (ii) assess the correlation among agronomic traits under limited soil P conditions.

MATERIALS AND METHODS

Experimental Materials

Plant materials used in the experiment consisted of 420 cowpea landraces comprising 311 landraces collected from the 19 states of northern Nigeria and 109 sourced from the International Institute for Tropical Agriculture (IITA).

Soil Analysis

The field used for the low P treatment has been left uncultivated for several years and had low P (4.2 mg/kg, Bray-1 method), N (0.13 g/kg), and K (0.68 cmol/kg) and organic carbon (0.79 g/kg of soil content), as determined by the Department of Soil Science, Ahmadu Bello University, Nigeria. The Bray 1 test is a widely used method for determining available phosphorus in soils. It involves the extraction of phosphorus using a 0.03 M calcium chloride ($CaCl_2$) solution. Soil samples were collected, air-dried, and sieved by passing them through a sieve with a mesh size of 2 mm to remove any physical impurities. 10 grams of the sieved soil was measured and placed in a 100 mL volumetric flask, and 50 mL of the 0.03 M Calcium Chloride ($CaCl_2$) solution was added to the flask. The soil and $CaCl_2$ solution were thoroughly stirred to ensure proper contact and extraction of available P from the soil. The mixture of soil-$CaCl_2$ was briefly incubated for 30 mins to enhance soil P extraction. After the incubation, the soil-$CaCl_2$ mixture was filtered using filter paper, and the filtrate was collected in a clean beaker.

Thereafter, 5 mL of the extract for each sample was pipetted into a 25 mL volumetric flask and 5 mL of a colour-developing agent made up of ammonium molybdate solution was prepared, ascorbic acid solution and sulfuric acid solution was prepared following the specified concentrations as per Bray I method. Then, 10 mL of the filtrate was taken and transferred into a new beaker. As per the test protocol, a measure of the ammonium molybdate solution was added to the filtrate. The solution was thoroughly mixed and then allowed to stand for 5-10 minutes to develop the blue colour. This was then followed by colorimetric determination, where the developed blue colour's intensity was measured using a colourimeter at a specific wavelength (460 nm). A calibration curve was prepared with known P concentrations to relate the colour intensity to the P concentration of the soil samples. The concentration of available P in the soil was calculated based on the absorbance and the calibration curve.

Methodology

The experiment was laid out using a 20 x 21 alpha lattice design with two replications under two levels of soil P; low phosphorus (LP) and high phosphorus (HP). The low P experiment was conducted in a low P field at the Institute for Agricultural Research farm (11°10'31.7"N 7°36'43.9"E), Samaru. P was applied to the high P treatment using a single super phosphate fertilizer (SSP 18% P_2O_5, 11% Sulphur, 18% Ca, and 4% moisture) at the rate of 60 kg P_20_5 ha^{-1} based on previous phosphate studies on cowpea (Mohammed *et al*., 2021). Plots were one row of 1.5 m long, with a 0.75 m unplanted walkway between plots, giving a pot size of 1.1 m^2 with an intra- and inter-row spacing of 0.20 and 0.75 m, respectively. All the plots received a plot equivalent of 30 kg/ha N (46% N, Urea) and K (30% K_2O, Muriate of Potash) to avoid any confounding effects of N and K deficiency on the plants. The fertilizers were applied by placing them below the soil surface five days after sowing. Plants were protected against insect pests by spraying insecticide (Karate 50 g/L lambda-cyhalothrin, Syngenta Crop Protection AG, Basel, Switzerland).

Data Collection

Data collected from the experiment include plant height (cm) measured as the height of the main axis from ground level to the apical leaflet of plants at maturity, number of branches (count) taken as the number of branches borne on the main axis of plants at maturity, dry fodder weight (g) was measured as the dry weight of above ground biomass obtained after harvest, days to maturity (days) taken as the number of days from planting to physiological maturity per plot, the weight of seeds (g/100 seeds) was measured as the weight of 100 seeds collected from well dried and threshed pods per plot, and grain yield (kg/ha) was estimated from the weight of grains obtained per plot.

Statistical Analysis

Data were analyzed using the general linear model procedure of the statistical analysis system (SAS) (SAS, 2002) and mean comparison was undertaken using the least significant difference (LSD) test at a 5% level of significance. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) as well as broad sense heritability were also estimated according to Singh and Chaudhary (1985).

Genotypic coefficient of variation (G.C.V) = $\dfrac{\sqrt{\sigma_g^2}}{\bar{x}} x\,100$

Phenotypic coefficient of variance (P.C.V) =

$$\frac{\sqrt{\sigma^2_{ph}}}{\bar{x}} x\,100$$

Where;

σ^2_g = genotypic variance; σ^2_{ph} = phenotypic variance

$\bar{x}$ = mean of character

Broad sense heritability, $h_b = \frac{\sigma^2_g}{\sigma^2_{ph}}\ \mathrm{X}\,100$

GCV and PCV estimates were considered as high (> 20), medium (between 10 and 20) or low (< 10) according to Deshmukh *et al*. (1986). Heritability estimates were classified according to Pramoda and Gangaprasad (2007); ≥ 80 % as very high, 60 to 79 % as moderately high, 40 to 59 % as medium and < 40 as low. The relationship among agronomic traits was investigated with the Pearson-product moment correlation.

RESULTS AND DISCUSSION

Performance of Cowpea Landraces Under Low and High Soil P Conditions

Significant differences (p<0.05) were observed among the landraces for all the measured traits under low and high P, except dry fodder yield under high P treatment. This indicates the presence of substantial variability among the cowpea landraces to enable the selection of superior genotypes for further genetic improvement. Similarly, Olayiwola and Soremi (2014) reported significant mean squares for dry fodder weight, seed yield, and 100-seed weight in cowpea. The grain yield varied significantly between the soil P treatments, with high phosphorus (HP) plants having a higher yield over the low phosphorus (LP). There was a wide range in the grain yield measured in the HP compared to LP. Some lines in the LP had grain yield values close to the first quartile of the HP plants. The mean grain yield of landraces under low and high P was 345.0 kg/ha and 1817.0 kg/ha, respectively (Figure 1A). Grain yield ranged from 58.7 kg/ha for 'Dan Misra_Sokoto' to 1488.0 kg/ha for 'Red Beans-medium' under low P and from 194.8 kg/ha to 3735.0 kg/ha under high P. Biomass varied between 91.0 kg/ha for 'Dan Misra_Sokoto' and 2418.0 kg/ha for 'Yambari' under low P. It ranged from 282.0 kg/ha for 'Mada' to 7959.0 kg/ha for 'Olotu' under high P (Table 1). Significant genetic variability has been reported for traits under low P (Gerrano *et al*. 2015).

Low soil phosphorus significantly reduced 100-seed weight, grain and biomass yields of cowpea landraces compared to optimum conditions (Figure 1). 'Red beans medium', 'Dan Misra_Zamfara' and 'Dan Zaria' had the highest grain yields of 1488, 1190 and 1094 kg/ha, respectively, under low P (Table 1). This indicates their tolerance to low soil P. The performance of these landraces may be attributed to their capacity to explore larger volumes of the soil due to their root architecture and ability to dissolve strongly bound soil phosphorus (Adusei *et al*., 2021). 'Pea beans' (68%), and 'Dan Misra_Sokoto' (59%) had the highest percentage reduction in grain yield under low soil P compared to optimum conditions indicating their susceptibility to low soil P. Superior cowpea landraces with better performance under low soil P should be exploited for cowpea improvement considering the significant yield losses attributable to phosphorus deficiency. Similarly, Mohammed *et al*. (2021) reported a positive association between P fertilization with increased growth and performance of cowpea plants

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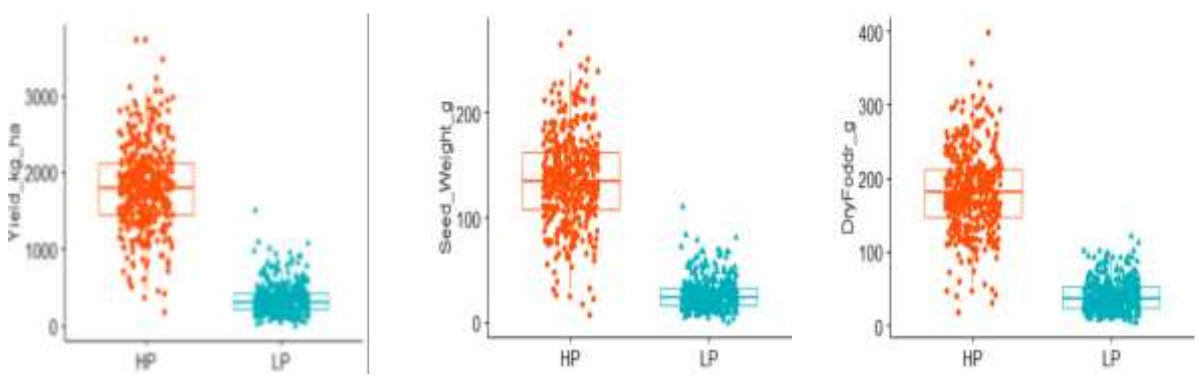

(A) (B) (C)

Figure 1. Phenotypic distribution of yield (grain and biomass) measured under varying soil phosphorus levels in the field environment. Boxplot displays the median of each group bounded by the first and third quartile, where the x-axis is soil phosphorus concentrations, with HP = 60 kg P2O5/ha and LP = no-P application. (A) Grain yield in kilogram per hectare (B) Grain weight in gram per plot (C) Dry fodder weight in gram per plot.

Table 1. Performance of cowpea landraces under low and high P conditions evaluated at Samaru in 2020

Yielding ability	Landrace	GRAIN YIELD (kg/ha)			100-SEED WEIGHT (g)			BIOMASS (kg/ha)		
		Low P	High P	Reduction %	Low P	High P	Reduction %	Low P	High P	Reduction %
High	Red beans Medium	1488	1096	36	19	21	10	778	2228	65
	Dan Misra_Zamfara	1190	2473	52	23	29	19	1162	2535	54
	Dan Zaria	1094	1676	35	27	25	-8	1414	2765	49
	Iron Beans_Kadu-	1008	2895	65	19	32	42	942	3663	74
	Tvu-17038	976	1703	43	27	23	-17	1558	1602	3
	Tvu-16946	949	2067	54	19	16	-23	1603	1846	13
	Komfita	899	1379	35	22	23	6	1253	2084	40
	Bosop_Gombe	894	1177	24	25	29	14	589	1987	70
	Gwalam	894	1200	26	30	29	-2	842	2782	70
	Brown Beans_Abuja	892	1932	54	15	36	58	320	3388	91
Low	Waken Rumfa	78	1191	93	14	17	18	148	1996	93
	Iron Beans_Big_Sokoto	73	2073	96	14	34	59	161	2473	93
	Pea Beans	68	1836	96	12	25	53	123	2389	95
	Lagos	59	1550	96	13	12	-17	537	2106	75
	Dan Misira_Sokoto	59	2265	97	13	21	36	92	3427	97
	Tvu-17045		823	100		9	100		987	100
Mean		346	1817		20	22		603	2588	
CV		73	33		40	21		78	51	
LSD		350.7*	843.8*		10.9*	6.3*		647.7*	1839.3	

*Significant $p<0.05$

Variance Components and Heritability of Traits Measured for Cowpea Landraces

The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) for yield and yield-related traits are presented in Table 2. The PCV was generally higher in magnitude than the GCV for all measured traits indicating the high influence of environment on the expression of the traits. Bationo and Anan Kumar (2002) reported that environmental factors affect the response of cowpea genotypes to P levels. The existence of genetic variability among the landraces for the measured traits under low P suggests the possibility of selection for crop improvement under low soil P. Phenotypic and genotypic coefficients of variation for traits indicate the presence of variation and provide the basis for crop genetic improvement (Omoigui *et al*., 2006; Olayiwola and Soremi, 2014). The high magnitude of difference between phenotypic and genotypic coefficients of variation conforms with the study of Olayiwola and Soremi (2014) in cowpea. The GCV was low for days to 95% maturity (2.9), moderate for the number of branches (18.5) and 100-seed weight (12.8) and high for plant height (33), grain (25.4) and biomass yield (30.9) (Table 2). The PCV ranged from low (8.9) for days to 95% maturity to high for biomass (82.4). Broad sense heritability was low for all the measured traits except plant height (43.7%) which had moderate heritability. Heritability estimates for a trait determine the extent to which it can be transmitted from parents to offspring. The moderate heritability for plant height indicates a moderate genetic influence on the trait. On the other hand, the low heritability for days to 50% flowering, number of branches, 100-seed weight, grain, and dry fodder yield indicate low genetic control and imply that direct selection in early generations for these traits may not be effective for crop improvement. Recurrent selection has been reported to be a powerful means of accumulating favourable alleles in a population and often is the method of choice for improving traits with low heritability (Fribourg and Hannaway, 2009). The results obtained in this study contradict the findings of Olayiwola and Soremi (2014) who reported high heritability for 100-seed weight as well as fodder yield and moderate heritability for days to 50% flowering and seed yield.

Table 2: Estimates of variance components and heritability for landraces evaluated under low soil P conditions at Samaru in 2020

TRAITS	$\sigma^2 e$	$\sigma^2 g$	$\sigma^2 p$	GCV	PCV	H^2
Days to 95% Maturity	59.8	7.1	66.9	2.9	8.9	10.6
Number of Branches	0.5	0.1	0.6	18.5	40.6	20.6
Plant Height	46.4	36.0	82.4	33.0	49.9	43.7
Grain Yield	59396.6	7730.3	67126.9	25.4	75.0	11.5
100-Seed Weight	63.0	6.2	69.2	12.8	42.6	9.0
Biomass	212044.7	34683.5	246728.2	30.9	82.4	14.1

Relationships among Yield and Yield-Related Traits Under Low P

Grain yield was significantly correlated with 100-seed weight (r= 0.25), the number of branches (r=0.14), and biomass (r=0.58) under low P. This indicates that selection for these traits under low P will improve grain yield. The correlation between traits is highly important in crop improvement. Improvement in one trait as a result of the selection of another depends not only upon the correlation between them but also on the genotypic and phenotypic variance association with them.

CONCLUSIONS

Phosphorus availability is crucial for cowpea growth and development. Cowpea landraces used in this study possess variability for grain yield and yield-related traits under low soil P. This is an essential requirement for the improvement of tolerance to low P. Low soil phosphorus significantly reduced grain and biomass yields of cowpea landraces compared to optimum conditions. 'Red beans medium', 'Dan Misra_Zamfara' and 'Dan Zaria' which had the highest grain yields under low soil P could serve as potential resources for the development of varieties tolerant to low soil P. Moderate to high genotypic variability observed for the measured traits under low soil P suggested the feasibility of selections for crop improvement. In addition, significant associations observed among the measured traits will enhance the selection of tolerant landraces.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

ACKNOWLEDGMENTS

This research was funded by TETFUND with grant number TETFund/DR&D/CE/NRF/STI/04/VOL1. We also wish to acknowledge undergraduate students of the Departments of Botany and Biology, Ahmadu Bello University, Zaria, for their support during data collection.

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