
RESEARCH ARTICLE

Evaluation of cowpea parents and hybrids through multivariate analysis under phosphorus limited soil

E. Chikalipa, M. Mwala, L. Tembo

The University of Zambia, School of Agricultural Sciences, Department of Plant Science, P.O BOX 32379, Lusaka, Zambia

Corresponding authors email Id: Chikalipawesu@gmail.com

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Abstract

Cowpea is an important cultivated grain legume pulse, vegetable and fodder crop of African origin. Twenty cowpea genotypes comprising of twelve crosses and eight progenitors were evaluated at the University of Zambia (15°23'S and 28°25'E, at 1250m above sea level)). The experiment was arranged as a two set experiment i.e. applied rate of i) 0kg P /ha and ii) 60kg P /ha P [a control]) and laid as a completely randomized design (CRD) with three replications. Comparisons using a paired t- test revealed a significant increase in performance in a control (60kg P /ha P) for all measured variables across genotypes when compared to a 0 Kg/ ha experimental set. In this study, genotype [LT 11-3-3-12] and a cross [LT 3-8-4-1 X LT 11-5-1-1] were identified as the highest performers exhibiting a mean yield of 1994.9 kg/ha and 1984.0 kg/ha respectively in a P-limiting soil. The variables plant biomass, root biomass, number of pods and pod length were identified as ideal at discriminating genotypic performance responses in P- limiting soil. This implies that the identified variable responses under cowpea genotypic selection in P-limiting soils cannot solely be used as indirect selection criteria for yield response but can be used as supplement to yield response.

Keywords: cowpea, genotypes, yield, principal component analysis, selection

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is a diploid ($2n = 2x = 22$) species with a genome size of 620 million base pairs and a self-pollinated species (Boukar *et al.*, 2018). It is cultivated for its fresh leaves, green pods, and grain which are rich sources of macro and micro-nutrients (i.e. protein, carbohydrates, vitamins, and minerals) (Bai *et al.*, 2020; ElMasry *et al.*, 2021; Silva *et al.*, 2021). Despite the role of cowpea as a food, nutrition and health security crop, its production is, however, hampered by both biotic and abiotic stress factors. Among the abiotic stresses, yield losses due to phosphorus (P) deficiency is an important factor. Cowpea like most other leguminous crops requires higher amounts of P (Singinga *et al.*, 2000). However, P is one of the least available plant nutrients and is deficient in many soils of the world (White and Brown, 2010). Presently, the use of P-formulated fertilizers appears to be a quick and easy fix for P deficiency soils, but that option has not been largely adopted by most smallholder cowpea growers because P-fertilisers are costly. This scenario is typical of the cowpea growing soils of Zambia where the smallholder farmers are resource-poor. Amending such P deficiency soil through increased application of inorganic fertilizer is neither sustainable, economically feasible nor environmentally safe.

Therefore, the most sustainable solution is to identify and select cowpea genotypes that have high P-use efficiency under low soil P. In Zambia, cowpea genotypes are limited and to resolve that, the University of Zambia developed gamma ray generated cowpea mutants with the hope of identifying mutant derived lines or crosses with favourable agronomic traits (Tembo et al., 2017). Multivariate analysis such as principle component analysis (PCA) has been used where factor assessment involves utilization of several associated measured variables. In this study, variables such as, plant height, shoot biomass, root biomass, plant biomass, hundred seeds weight, pod length, root length, number of pods per plant, number of seed per pod, yield/hectare and chlorophyll concentration index was used to evaluate performance of cowpea in P-limited soil. However, studies to determine the relevance of these variables in discriminating genotypes in P-limited soils are limited. This research serves as a foundation for breeding low P efficient cowpea varieties for farmer utilization in Zambia and similar

agro-ecologies. Hence, the objectives of this study, therefore, were to; (i) identify high yielding cowpea genotypes when exposed to P-limiting soil, and (ii) identify the variables with high discriminating capability among cowpea genotypes in P-limiting soil.

Materials and methods

Experimental site, material used and experimental details

The research was conducted at the University of Zambia, Green house in Lusaka (15°23'S and 28°25'E, at 1250m above sea level). Soil sample used in the study was collected from the top layer (0–30 cm) from the Liempe Farm (15°22'S and 28°26'E, at 1171m above sea level) in Chongwe District. Collected soil sample was sieved using 2mm sieve and analyzed for nutrient content, soil P using Bray II method. Twenty genotypes (Table 1) comprising of 12 F₁ Crosses and 8 progenitors were used for this study. Several crossing were done using same progenitors and successful crosses were recorded.

Table 1: Material used in the present study

Genotypes codes	Genotypes	Type
A	LT 3-8-4-6	Parent
B	LT 11-5-1-1	Parent
C	LT 11-5-2-2	Parent
D	LT 4-2-4-1	Parent
E	LT 3-8-4-1	Parent
F	LT 16-7-2-5	Parent
G	LT 10-7-1-12	Parent
H	LT 11-3-3-13	Parent
AXH	LT 3-8-4-6 X LT 11-3-3-13	Cross
BXG	LT 11-5-1-1 X LT 10-7-1-12	Cross
AXD	LT 3-8-4-6 X LT 4-2-4-1	Cross
CXE	LT 11-5-2-2 X LT 3-8-4-1	Cross
DXA	LT 4-2-4-1 X LT 3-8-4-6	Cross
EXB	LT 3-8-4-1 X LT 11-5-1-1	Cross
FXE	LT 16-7-2-5 X LT 3-8-4-1	Cross
AXE	LT 3-8-4-6 X LT 3-8-4-1	Cross
GXC	LT 10-7-1-12 X LT 11-5-2-2	Cross
CXG	LT 11-5-2-2 X LT 10-7-1-12	Cross
EXD	LT 3-8-4-1 X LT 4-2-4-1	Cross
HXA	LT 11-3-3-13 X LT 3-8-4-6	Cross

The experiments was arranged as a two set experiment and laid out in a completely Randomized Design (CRD), with three replications. The experimental plot was 15cm diameter polythene plastic bags filled with 3kg of soil and Single Super Phosphate (SSP) was applied three weeks after planting. The quantity of SSP added was calculated to achieve the soil P at two levels 60kg P/ha (optimal) was added to experimental set while 0kg P/ha (suboptimal) was added to control set. However, the recommended quantities of potassium (K) (30 kg K/ha) and nitrogen (N) (20 kg N/ha) was applied in form of potassium sulphate at the rates of 64 kg/ha as basal dressing. In each bag, three identical genotypes were planted which were later thinned 15 days after planting to two plants per bag. Other standard cowpea production agronomic practices were followed.

Data collection

The following variables were collected for each of the experiments; Plant height (cm); Shoot biomass (g); Root biomass (g); Plant biomass (g); 100 Seed weight (g) was calculated in grams from 100 randomly sampled seeds; Pod Length (cm); Root length (cm); Number of pods per plant were counted at physiological maturity; Number of seeds per pod were counted after harvest; yield/ha was recorded in grams and converted to kg/ha. Chlorophyll concentration index (CCI) was recorded during the vegetative growth stage from the adaxial surface of healthy leaf using a chlorophyll meter (model SPAD-502, Japan). The chlorophyll meter was calibrated to zero every time before taking on a new measurement.

Data analyses

A paired t-test was used to compare the performance of genotypic mean performance in two, experimental sets at added value of 0kg/ha P on one hand and a control experiment with added value of 60kg/ha P. Analysis of Variance (ANOVA) was performed assuming a fixed model to determine significant differences among measured genotypic variables in specific experimental sets. Means of measured variables was separated using

fisher protected least significant difference (LSD) method, at a significant level of $\alpha=0.05$. Further evaluation was computed by performing a multivariate approach tool, principle component analysis (PCA). It is well known fact that principal component analysis, or PCA, is a dimensionality-reduction method that is often used to reduce the dimensionality of large data sets, by transforming a large set of variables into a smaller one that still contains most of the information in the large set.

Principal component analysis was undertaken using genotypic means of measured variable across replications. Furthermore, discriminating capability of measured variables was computed using factor analysis and associated angle between a variable and each principal component was created on a two dimensional scatter plot. All data was performed using GenStat statistical software and XLSTAT in excel.

Results and discussion

Response of cowpea genotypes in phosphorus-limiting soil

The paired t-test showed that the mean performance of measured variables across genotypes was higher in a control experiment at an applied P rate of 60 kg/ha than at applied P rate of 0 Kg/ha (Table 2). Among the biotic stresses, yield losses due to phosphorus deficiency are an important factor. In this study, mean poor performance of genotypic response of measured variables in P-limiting medium compared to medium with optimum P attest to that fact.

The analysis of variance (ANOVA) in each experimental sets showed significant ($P < 0.01$) genotypic mean differences on all measured variables except CCI which was non-significant ($P=0.05$) in the 0 Kg P (Table 3). Mean squares agronomic response to 60 kg and 0 kg of phosphorus level showed highest significant differences under 60 kg phosphorus for seed yield.

Table 2: Comparisons of mean performance of measured variables at 0kg P and 60kg P using a paired t- test

Variables	Mean-0 ^x	Mean-60 ^y	Difference	P-value
Yield	2569.0	2644.5	75.5	<0.001
100 seed weight	67.2	76.0	8.7	<0.001
Number of seed per pod	16.1	17.5	1.3	<0.001
Number of pods	15.8	16.7	0.9	<0.01
Pod length	17.0	17.9	0.9	<0.001
Root biomass	28.0	29.6	1.7	<0.01
Plant height	52.7	59.1	6.4	<0.01

X-mean value of measured variables at fertilizer application rate of 0 Kg P across genotypes

Y-mean value of measured variables at fertilizer application rate of 60 Kg P across genotypes

Table 3: Mean squares for genotypic agronomic response at different phosphorus levels

Mean squares agronomic variables in response to 60 kg phosphorus level											
Source of variation	DF	100 seed weight	Chlorophyll concentration index	Number of pods per plant	Number of seed per pod	Plant biomass	Pod length	Plant height	Root biomass	Shoot biomass	Yield/ha
Genotype	19	450.6***	384.3***	30.36***	9.0***	104.7***	2.2***	162.8**	29.3***	60.8***	423772.0***
Error	40	12.2	118.3	5.8	1.2	1.4	0.3	58.8	1.1	0.3	277.3
Mean squares agronomic variables in response to 0 kg phosphorus level											
Genotype	19	486.1***	31.2***	9.9***	113.8***	4.3***	47.4**	21.4***	51.9***	451638.4***	80.4 ^{NS}
Error	40	8.9	5.4	2.1	1.4	0.2	30.9	0.9	0.4	954.0	2.5

***Significant at P= 0.001, **Significant at P= 0.01, NS=non-significant

Previous studies have also shown that genetic response variation in P-limited soils exists in cowpea genotypes and successful selection depends upon the information on the genetic variability and morpho-agronomic traits related to grain yield (Menssen *et al.*, 2017; Hassan *et al.*, 2005). It is known fact that phosphorus is important for cowpea production and is inherently low in many tropical soils. Selection of cowpea genotypes that produce good yield under low soil P or those with high P response efficiency can be a low input approach in solving this problem. In this study, genotype [LT 11-3-3-12] and a cross [LT 3-8-4-1 X LT 11-5-1-1] had the highest mean yield of 1994.9 kg/ha and 1984.0 kg/ha respectively in a P-limiting experimental set (Table 5).

Earlier studies agree that higher cowpea yields in P- limited soils can be obtained within the same range, 1900- 2000 kg/ha (Iseki *et al.*, 2021; Owusu *et al.*, 2021). On the other hand, genotypic cowpea yields as high as 3305.3 kg/ha were obtained in optimum P medium (60Kg/ha experimental set) (Table 4). This implies that the identified cowpea genotypes efficient at utilizing P in P- limiting soils can still be supplemented by additional dosages of fertilizer.

Table 4: Means of genotypes for agronomic variable responses evaluated in 60 kg phosphorus per hectare

Genotype	Plant height	Chlorophyll concentration index	Number of pods per plant	Pod length (cm)	Number of seed per pod	Hundred seed weight (g)	Root length (cm)	Root biomass (g)	Shoot biomass (g)	Plant biomass (g)	Yield (kg/ha)
AXH	50.7	32.0	11.0	16.8	14.6	70.0	36.6	23.2	42.4	65.6	2524.7
BXG	65.0	45.2	13.3	18.3	16.4	76.0	34.7	22.5	38.5	61.0	2698.2
AXD	67.0	54.4	13.7	17.8	15.9	76.0	31.4	24.9	29.4	54.3	2826.4
CXE	63.0	62.5	10.3	18.2	17.8	59.0	27.4	25.4	32.7	58.1	2754.7
DXA	49.0	34.1	15.7	17.9	16.7	84.0	23.9	20.6	36.5	57.0	2752.9
EXB	54.7	40.6	18.0	18.3	19.4	70.0	27.6	15.2	35.5	50.7	2909.4
FXE	65.3	48.5	14.7	16.4	15.2	61.0	24.5	19.5	36.2	55.8	2615.2
AXE	59.3	27.7	16.3	17.1	16.4	69.0	24.3	26.5	40.4	67.0	2349.4
GXC	73.7	35.4	21.0	18.2	16.0	58.7	22.6	26.8	43.1	69.8	2385.2
CXG	59.0	30.1	21.3	18.4	16.3	62.3	33.2	22.2	34.1	56.3	2751.0
EXD	68.3	33.1	22.3	18.4	19.0	89.0	32.7	25.1	39.9	65.0	3305.3
HXA	69.0	22.9	19.3	16.2	17.0	67.0	22.9	23.4	37.3	60.7	3194.1
A	59.7	61.8	16.7	19.3	22.3	88.0	39.6	16.5	34.9	51.4	2142.0
B	48.0	38.8	17.3	19.0	18.3	57.0	35.9	20.5	38.6	59.1	2334.3
C	56.7	32.0	15.7	18.1	18.3	68.0	33.8	23.7	32.2	55.9	2977.2
D	61.0	47.5	18.0	18.1	18.1	50.0	23.8	26.3	38.3	64.6	2177.8
E	51.7	31.3	17.3	17.3	18.2	69.3	25.5	20.6	35.5	56.1	2057.1
F	51.3	25.7	17.0	16.5	18.0	48.3	23.7	23.5	39.2	62.7	2724.6
G	55.0	36.5	19.3	18.3	16.7	76.0	33.5	22.6	23.6	46.2	3252.5
H	54.0	32.4	15.3	18.6	18.2	46.0	34.5	24.1	34.3	58.4	2158.9
Grand Mean	59.1	38.6	16.7	17.9	17.5	67.2	29.6	22.7	36.1	58.8	2644.5
LSD _{0.05}	12.7	18.0	4.0	1.0	1.8	5.8	2.8	1.8	0.9	1.9	27.5

A= LT 3-8-4-6, B= LT 11-5-1-1, C= LT 11-5-2-2, D= LT 4-2-4-1, E= LT 3-8-4-1, F= LT 16-7-2-5, G= LT 10-7-1-12, H= LT 11-3-3-13

Table 5: Means of genotypes for agronomic variable responses evaluated in 0 kg phosphorus per hectare

Genotype	Plant height	Chlorophyll concentration index	Number of pods per plant	Pod length (cm)	Number of seed per pod	Hundred seed weight, (g)	Root length (cm)	Root biomass (g)	Shoot biomass (g)	Plant biomass (g)	Yield (kg/ha)
AXH	59.0	29.6	12.0	15.4	12.6	62.0	32.6	21.6	35.2	56.8	1396.5
BXG	48.3	37.5	18.0	18.2	15.9	70.7	35.4	19.3	34.1	53.5	1607.7
AXD	52.0	38.5	13.0	14.9	15.2	91.0	34.2	28.0	34.9	62.9	1683.1
CXE	51.3	46.4	13.0	16.4	16.9	64.0	23.8	14.7	21.7	36.4	1620.9
DXA	49.7	32.9	14.7	16.7	16.3	99.0	22.9	21.5	34.4	55.9	1636.0
EXB	52.3	37.7	11.7	15.7	15.2	60.0	24.50	18.1	29.4	47.5	1984.8
FXE	55.7	27.3	15.0	14.8	11.9	92.0	23.5	20.6	35.3	55.9	1671.8
AXE	53.7	38.9	10.7	18.2	17.2	82.0	24.9	21.3	38.2	59.6	1355.0
GXC	56.7	35.1	16.3	17.8	14.9	67.0	23.7	17.7	30.1	47.8	1330.5
CXG	54.7	34.4	18.0	17.4	15.2	91.0	35.9	18.7	32.8	51.5	1530.4
EXD	58.0	50.1	24.0	16.9	17.9	77.0	39.5	21.0	38.1	59.1	1175.2
HXA	56.7	31.8	19.3	17.9	16.3	92.0	33.6	19.1	30.0	49.1	1737.8
A	46.0	50.4	16.0	18.5	16.4	67.0	23.6	18.6	30.1	48.6	1251.3
B	50.7	29.1	18.0	17.9	15.4	92.0	23.8	18.2	38.1	56.3	1370.1
C	55.3	39.5	18.3	18.1	19.2	71.0	26.6	16.6	28.7	45.3	1047.0
D	48.3	29.2	13.7	15.6	14.9	79.0	25.6	19.0	30.3	49.2	1055.2
E	55.3	41.5	13.3	17.8	18.2	62.0	25.6	19.7	29.8	49.4	1859.1
F	48.7	30.7	17.7	17.6	18.2	63.0	25.9	21.5	37.3	58.8	1703.8
G	55.7	22.4	14.3	15.7	17.0	70.7	27.5	20.6	29.2	49.8	1369.4
H	45.7	28.0	18.0	17.5	17.4	67.0	24.9	17.7	31.2	49.0	1994.9
Grand Mean	52.7	35.6	15.8	17.0	16.2	76.0	27.9	19.7	32.4	52.1	1551.8
LSD _{0.05}	9.2		3.8	0.8	2.4	4.9	2.6	1.6	1.1	2.0	51.0

A= LT 3-8-4-6, B= LT 11-5-1-1, C= LT 11-5-2-2, D= LT 4-2-4-1, E= LT 3-8-4-1, F= LT 16-7-2-5, G= LT 10-7-1-12, H= LT 11-3-3-13

Multivariate evaluation of measured agronomic variables

Principal component analysis revealed three PCs contributing a sum total of 63.4% and 60.6% total phenotypic variation explained respectively for treatment application of 0Kg /ha P and control (Table 6). In the applied rate of 0 kg/ ha P, variables, plant biomass (PBM), shoot biomass (SBM) and root biomass (RBM) were identified as important at differentiating genotypes with regards to principal component (PC1), attaining factor

loading values 0.9, 0.8 and 0.8 respectively. On the other hand, in the control experiment PBM and yield were identified as important with regards to PC1 and PC2 respectively, each attaining factor loading value of 0.8 and 0.9 respectively. Generally genotypic screening is costly and identification of important traits/variables with ideal discriminating capability helps to narrow down to only few essential variables to utilize and this ultimately save cost (Walubita *et al.*, 2022; Simasiku *et al.*, 2021).

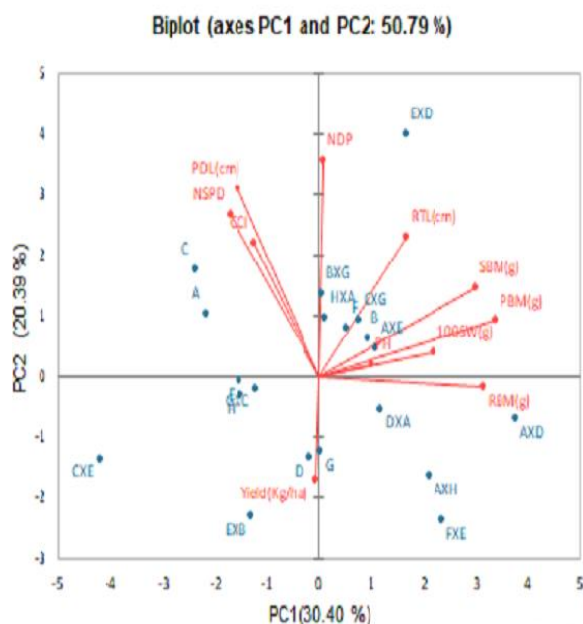
Table 6: Factor loadings of measured variables

Variables	0 kg P			60 kg P		
	1(30.4%)	2(20.4%)	3(12.6%)	1(30.6%)	2(16.2%)	3(13.8%)
Plant height	0.3	0.1	0.7	0.3	0.4	0.4
Chlorophyll concentration index	-0.3	0.5	0.1	-0.5	-0.3	-0.1
Number of pods per plant	0.1	0.8	0.1	0.1	0.4	0.7
Pod length, (cm)	-0.4	0.7	-0.3	-0.6	-0.3	0.5
Number of seed per pod	-0.5	0.6	-0.3	-0.6	-0.3	0.5
Hundred seed weight (g)	0.6	0.1	-0.1	-0.5	0.4	0.2
Root length (cm)	0.4	0.5	0.5	-0.7	-0.2	0.1
Root biomass (g)	0.8	0.1	-0.1	0.7	0.1	0.1
Shoot biomass (g)	0.8	0.3	-0.3	0.6	-0.4	0.4
Plant biomass (g)	0.9	0.2	-0.3	0.8	-0.3	0.3
Yield (kg/ha)	0.1	-0.4	-0.4	-0.1	0.9	0.1

Further analysis in the applied rate 0 kg/ha P indicated RBM and NDP as the leading associated linked variables to PC1 and PC2 as evident by the smallest acute angles on the respective axis respectively (Figure 1). In the control experiment yield was identified as the leading associated linked variables to PC1 and PC2 as evident by the smallest acute angles on the respective axis respectively (Fig. 2). Interestingly, yield response in P- limiting medium didn't positively correlate with any other measured variables in the experimental

set as observed by the created obtuse angles (Fig.1). This implies that under cowpea genotypic selection in P-limiting soils variable responses PBM, RBM, NDP and PDL cannot solely be used as indirect selection criteria for yield response but can be used as supplement to yield response. However, in the control set 60 kg per hectare yield was positively correlated with 100 seed weight, number of pods, plant height and root biomass exhibiting an acute angle with yield response.

Fig1: Principal component score plot of PC1 and PC2 describing the variation among cowpea genotype estimated using the data set of measured traits for 0kg P. The first two PCs explained 50.79% of the total variation. Blue dots=genotype; red dots= measured variables

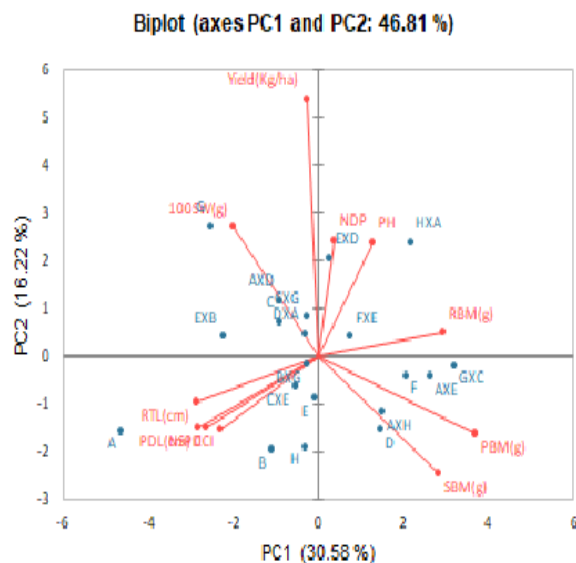


Hence, it is concluded that in this study, genotype [LT 11-3-3-12] and a cross [LT 3-8-4-1 X LT 11-5-1-1] were identified as the highest performers exhibiting a mean yield of 1994.9 kg/ ha and 1984.0 kg/ ha respectively

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Fig 2: Principal component score plot of PC1 and PC2 describing the variation among cowpea genotype estimated using the data set of measured traits for 60kg P. The first two PCs explained 46.81% of the total variation. Blue dots=genotype; red dots= measured variables



in a P-limiting soil. Variables PBM, RBM, NDP and PDL were identified as ideal at discriminating genotypic performance response in P- limiting soil.

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