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RESEARCH ARTICLE

# Performance of cowpea progenitor and hybrids in varying concentration levels of phosphorus in hydroponic setup

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## Abstract

Among abiotic factors limiting cowpea productivity, phosphorus (P) is an important element. However, in Zambia, no study has determined optimal soil P concentrations for the available cowpea germplasm. The objectives of this study were (i) to identify the cowpea genotype that performs better in different concentration levels of phosphorus in hydroponic setups and ii) to determine the phenotypic response of agronomic variables in different concentration levels of phosphorus. The experiment was set up as a 4 x 10 completely randomized design (CRD) replicated 3 times, with 4 doses of P. Thus, 0mg/L, 4.7mg/L, 9.3mg/L and 13.95mg/L in a nutrient medium and ten genotypes (G). Genotype LT BT1h was identified as a better performer across P concentration. Further exploration of G x P interaction main effects revealed that genotype LT BT1 h performed better than the progenitor in all doses of P concentration except in a medium with 9.3mg/L and 13.95mg/L. Overall, the results showed that applying a concentration above 9.3mg/L P impeded seedling productivity. Significant better performances were obtained with shoot biomass, plant biomass and shoot length of measured variables, implying that the identified variables in varying levels of P concentration can initially be used to aid in selecting for high performing genotypes. The identified novel and best-performing cowpea hybrids for economic traits be recommended for further evaluation.

**Keywords:** *Vigna unguiculata*, Hydroponic, Phosphorus, Progenitor, Hybrids

## 1.0 Introduction

Cowpea (*Vigna unguiculata* [L.] Walper) is an annual edible grain legume that belongs to the family Fabaceae tribe Phaseolea and the genus Vigna (Chikalipa, 2023; Chikalipa and Tembo, 2023; Ehlers and Hall, 1997; Singh *et al.*, 2002). It is a self-pollinating diploid (2n = 2x = 22) species with a genome size of 620 million base pairs (Chikalipa *et al.*, 2022; Boukar *et al.*, 2018). Cowpea is a staple seed-pod crop in contributing to food security among small-scale farmers in sub-Saharan Africa (SSA) (Tarawali *et al.*, 2002). Additionally, it is a valuable source of cheaper dietary protein for poor livelihoods and sources of income.

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In Zambia, the mean grain yields of cowpeas are between 100 to 599 kg/ha, which is far below the potential yield of 3000 kg/ha (Chikalipa, 2023; Horn *et al*., 2015, Gerrano *et al*., 2017). Cowpea production is hampered by both biotic and abiotic factors, which severely affect productivity. Among the abiotic factors, phosphorus (P) deficiency leads to significant yield losses. Phosphorus is one of the most important plant nutrients, required in large quantities, comprising 0.2% of the plant's dry weight (Lambers and Shane, 2007; Schachtman *et al*., 1998) and ranging from 0.05% to 0.5% (Vance *et al*., 2003). However, P is one of the least available plant nutrients and is deficient in many soils of the world (White, 2010). Presently, the use of P-formulated fertilizers by some farmers appears to be a quick and easy fix for P-deficient soils (Chikalipa and Tembo, 2023). However, the current option has not been widely adopted by most smallholder cowpea growers because it is not financially and ecologically sustainable. In this study, the researcher investigated the use of morphological variables such shoot biomass, root and shoot length as an indirect selection criterion for screening genotypic variability in the response to P in the hydroponic medium. The objectives of the study were i) to identify a genotype that performs better in varying concentration levels of P in hydroponic setups and ii) to determine the phenotypic response of agronomic variables in different concentration levels of P.

## 2.0 Materials and Methods

### 2.1 Experimental site and germplasm used

Ten genotypes, including Parental Lutembwe and nine Lutembwe mutants, were used in the study (Table 1). The study was conducted at the University of Zambia in the Plant Science Department Laboratory, School of Agricultural Sciences in Lusaka (15°23’S and 28°25’E, at 1250 m above sea level).

**Table 1: Genotype used in the study**

| Genotype | Type |
|---|---|
| Lutembwe | Parent |
| LT 3-8-4-1J | Mutant |
| LT BT1h | Mutant |
| LT 4-2-4-1 | Mutant |
| LT 11-5-2-2d | Mutant |
| LT 16-7-2-5b | Mutant |
| LT 4-2-4-1a | Mutant |
| LT 16-7-2-5c | Mutant |
| LT 11-5-2-2k | Mutant |
| LT 11-5-2-2T | Mutant |

LT=Lutembwe

### 2.2 Design of the experiment and medium preparation

The study was set up as a 4x10 factorial design in a completely randomized design (CRD) replicated 3 times, with 4 doses of P; thus, 0mg/L, 4.65mg/L, 9.30mg/L and 13.95mg/L nutrient medium (Table 2), ten genotypes giving a total of 118 experimental units randomly assigned to each plot (Test tube). Each test tube had a diameter of 2.3cm and a height of 14.5cm. 0mg/L P was used as a control, while 4.56mg/L P was taken as the optimal concentration, as determined by Kerridge and Kronstad (1968). The sub-optimal concentration used was 4.65mg/L, and the above optimal concentrations were provided as two doses of 9.30mg/L and 13.95 mg/L. The pH was adjusted to pH 6 using HCl and NaOH buffer solutions.

**2.3 Placement of cowpea seedlings**

Forty-five (45) seeds per genotype were germinated on separate petri dishes lined with filter paper soaked in distilled water and placed in the germination chamber at 25oC for 5 days. After 5 days, 30 seedlings of each genotype having uniform root length were selected and then transferred into 50ml test tubes covered with black plastic having nutrient solution of varying P concentration and supported over the solution with the stopper. Covering test tubes with black plastic was done to prevent any possible algae growth in the solution. The nutrient solution was aerated twice daily, morning and evening, using an aquarium air pump (Sonic 9905) to provide oxygen and allow refilling of the solution.

**2.4 Data collection**

On the 15th day after placement, the seedlings were harvested. Data was collected on all experimental units with respect to Shoot biomass(g); Root biomass(g); Number of root hairs; Shoot length (cm), and Root length (cm). Measurements of root length, shoot length and the number of root hair count on all experimental units were conducted based on the methods by by Tembo (2018). This was followed by cutting the roots from the shoots using a surgical blade for each experimental unit. The shoots were separately placed per experimental unit in clearly labeled khaki envelopes, and then oven dried for 24 hours at $70^0$C. After drying, the shoot biomass was weighed using the electronic weight balance.

**2.5 Statistical Data Analysis**

The analysis of variance (ANOVA) for all measured variables was performed assuming a Fixed model. The means were separated using Fisher-protected least significant difference (LSD), at a significant level of $\alpha$ =0.05. A correlation between shoot and root length was determined using linear regression with root and shoot lengths as explanatory variables.

**Table 2: Nutrient solution used in the study**

| Chemical Formula | Nutrient | Conc (mg/L) | Compound name |
|---|---|---|---|
| $K_2HPO_43H_{2O}$ | P | Varied* | Potassium hydrogen phosphate trihydrate |
| $H_3BO_3$ | B | 0.32 | Boric acid |
| $ZnSO_47H_20$ | Zn | 0.16 | Zinc sulfate heptahydrate |
| $MgSO_47H_2O$ | Mg | 14.60 | Magnesium sulfate heptahydrate |
| $CuSO_45H_20$ | Cu | 0.06 | Copper sulphate pentahydrate |
| $FeSO_47H_20$ | Fe | 1.60 | Iron sulfate heptahydrate |
| $CaCl_22H_2O$ | Ca | 48.10 | Calcium chloride dehydrate |
| $NaMoO_42H_2O$ | Mo | 0.03 | Sodium molybdate dehydrate |
| $NH_4NO_3$ | N | 42.61 | Ammonium Nitrate |
| $MnSO_4H_2O$ | Mn | 0.03 | Manganese sulfate monohydrate |

**K: Potassium; B: Boron; Al: Aluminium; N: Nitrogen; Zn: Zinc; Mg: Magnesium; Cu: Copper; Fe: Iron; Ca: Calcium; Mo: Molybdenum; Mn: Manganese; *Phosphorus combinations with either 0, 4.56, 9.30 and 13.95mg/L; Conc: Concentration. Source: Kerridge et al., 1968.**

## 3.0 RESULTS

### 3.1 Evaluation of measured variables in varying concentrations of P nutrient medium

Genotypes exhibited significant differences in all measured variables (root length, shoot length, shoot biomass and number of root hairs) across P concentrations (Table 3).

**Table 3: Analysis of variance for mean squares for measured variables evaluated in the study**

| Source of Variation | DF | Shoot biomass | Root biomass | Shoot length | Root length | Number of root hairs |
|---|---|---|---|---|---|---|
| Genotype (G) | 9 | 0.00859* | 0.0009428*** | 31.805*** | 35.672*** | 567.95*** |
| Phosphorus Conc (PC) | 3 | 0.048356 | 0.005726*** | 250.79** | 311.683*** | 1266.31*** |
| GXPC | 27 | 0.001959ns | 0.000358* | 5.871ns | 33.523*** | 331.86*** |
| Error | 78 | 0.001974 | 0.000134 | 9.047 | 2.904 | 32.7 |
| Total | 117 | 0.428331 | 0.428331 | 1898.757 | 2371.154 | 20356.81 |

***- Significant (p<0.01); ** - very significant (p<0.05); ***- highly significant (p<0.001); ns- Not significant; DF: Degrees of Freedom; Conc: Concentration.**

Similarly, the responses of all measured variables to different P concentrations across genotypes were significant (P<0.001) except for shoot biomass and shoot length, which were non-significant (P>0.05). Genotype LT BT1h had a higher mean performance for all measured variables (Table 4; Figure 1). Furthermore, there was an increase in measured variables across genotypes as P concentration increased at 6.97mg/L and dropped drastically at the 9.30mg/L and 13.95mg/L of P concentration, respectively. Further analysis revealed that genotype LT BT1h performed better than the Lutembwe parent (LT PRT) in all doses of P concentration except in a medium with 9.30 mg/L and 13.95 mg/L. Significant differences were exhibited between the interactions of the main effects (Genotype X Phosphorus concentration) for all measured variables, except for shoot biomass and shoot length, which were non-significant (Table 3).

**Table 4: Genotypic means of measured variables across genotypes in varying phosphorus concentration evaluated.**

| Genotype | SB | RB | SL | RL | NRH |
|---|---|---|---|---|---|
| Lutembwe PRT | 0.26 | 5.60 | 7.24 | 6.08 | 23.17 |
| LT 3-8-4-1J | 0.32 | 6.07 | 8.27 | 5.78 | 30.53 |
| LT Bt1h | 0.39 | 7.67 | 10.17 | 6.56 | 37.73 |
| LT 4-2-4-1 | 0.28 | 5.49 | 9.12 | 5.19 | 23.13 |
| LT 11-5-2-2d | 0.31 | 6.43 | 8.75 | 5.49 | 26.33 |
| LT 16-7-2-5b | 0.33 | 5.79 | 6.99 | 6.16 | 25.00 |
| LT 4-2-4-1a | 0.28 | 6.21 | 6.46 | 6.00 | 22.78 |
| LT 16-7-2-5c | 0.30 | 6.64 | 8.34 | 5.83 | 31.87 |
| LT 11-5-2-2k | 0.34 | 5.85 | 8.03 | 6.12 | 26.35 |
| LT 11-5-2-2T | 0.27 | 6.21 | 8.81 | 5.17 | 31.13 |
| Mean | 0.32 | 6.20 | 8.22 | 5.84 | 27.80 |

**SB: Shoot biomass; RB: Root biomass; SL: Shoot Length, RL: Root Length, NRH: Number of root hairs; PRT: Parent.**

**3.2 Correlation of root and shoot length**

Root and shoot lengths were significantly (P<0.01) correlated (r = 0.64). This gave the phenotypic evaluation (R2) explained of 34.6%.

## 4.0 Discussion

Cowpea growth and yield are largely dependent on the ability of a crop to explore soil, and absorb water and nutrients. Phosphorus (P) is one of the major nutrients influencing cowpea productivity and production. Genotypic variations in response to available P exist among Zambian cowpea germplasm (Chikalipa, 2023; Mourice and Tryphone, 2012). In this hydroponic study, the source of P, thus Potassium hydrogen phosphate trihydrate, equally supplied another major element, Potassium (K). However, in plants and cowpea development inclusive, potassium plays a key role in opening and closing of stomata and regulates CO2 uptake in photosynthesis (Trankner *et al*., 2018). The relationship between genotypic variable responses, notably root length, and P was evident.

In mutation breeding, derived mutants are those which outperform the parental and can be identified as candidates for variety release or selected for further breeding (Tembo *et al*. 2017; Tembo and Munyinda, 2015). Genotype LT BT1h was identified as a better adaptive flexibility performer than the progenitor (LT PRT) in media with P concentration doses, ranging from 0mg/L to 6.97 mg/L, implying that it can be the genotype of choice for several environments.

The results show that root elongation is a direct function of P concentration within a range of 0 mg/L to 6.97 mg/L. Additionally, it was noted that seedling productivity was significantly hindered by concentrations of 9.30mg/L and 13.95 mg/L, as evidenced by inadequate genotypic responses for all assessed variables. This may be the result of an excess of P in the solution, which prevents the absorption and translocation of Zinc and Magnesium by cowpea seedlings, ultimately resulting in a metabolic malfunction. Symptoms associated with Zinc and Magnesium deficiencies in cowpeas include the appearance of yellow veins on new leaves s and a reduction in the size of plant parts, respectively (Hosier and Brandley, 1999). The symptom of reduced plant size is similar to what was observed in this study, at an application rate of 9.3mg/L and 13.95mg/L. Consequently, in light of the findings and consideration of soil conditions during cowpea production, it is critical to perform soil tests in order to verify the addition of the recommended amount of P. It is worth noting, nevertheless, that the essential quantity of Soil-Specific P (SSP) varies in accordance with factors such as crop rooting depth, soil type, and the availability of supplementary nutrients (Macintosh *et al*., 2019; Manschadi *et al*., 2014). In the event of soil with excess P, Snyder et al. (2019) recommend minimizing the application of manure and alternatively applying inorganic fertilizers that contain less P.

The phenotypic variation explained (PVE) entails that only 34.6% of the change in shoot length is explained by the change in root length variable. This implies that shoot length can only be used as a supplement to root length when selecting genotypic efficiency at utilising P in the hydroponic medium. These results agree with previous work by Tembo et al. (2016), which showed that indirect selection using agronomic traits can only supplement the direct selection of a trait, especially when the trait under consideration is quantitatively inherited. The low value for PVE could be due to the interaction with seed size and quality used. It was found that during germination, early seedling development is a function of seed size and quality (Mandal *etal*., 2008; Lima *et al*., 2005).

## Conclusion

In this study, genotype LT BT1h was identified as a better performer across P-concentration, implying that it can be recommended across several environments. It exhibited a better adaptive flexibility performance than the parent (LT PRT) in media with P concentration ranging from 0mg/L to 6.97mg/L. Overall, the results showed that applying a concentration of above 6.97mg/L impedes seedling productivity in cowpeas. Finally, the phenotypic variation explained (PVE), showed that only 34.6% of the change in shoot length is explained by the change in root length. This implies that shoot length can only be used as a supplement to root length in selecting for genotypic efficiency at utilising P in the hydroponic medium. Additionally, it is recommended that molecular mapping studies be conducted in order to identify quantitative trait loci that are linked to phosphorus use efficiency in cowpeas.

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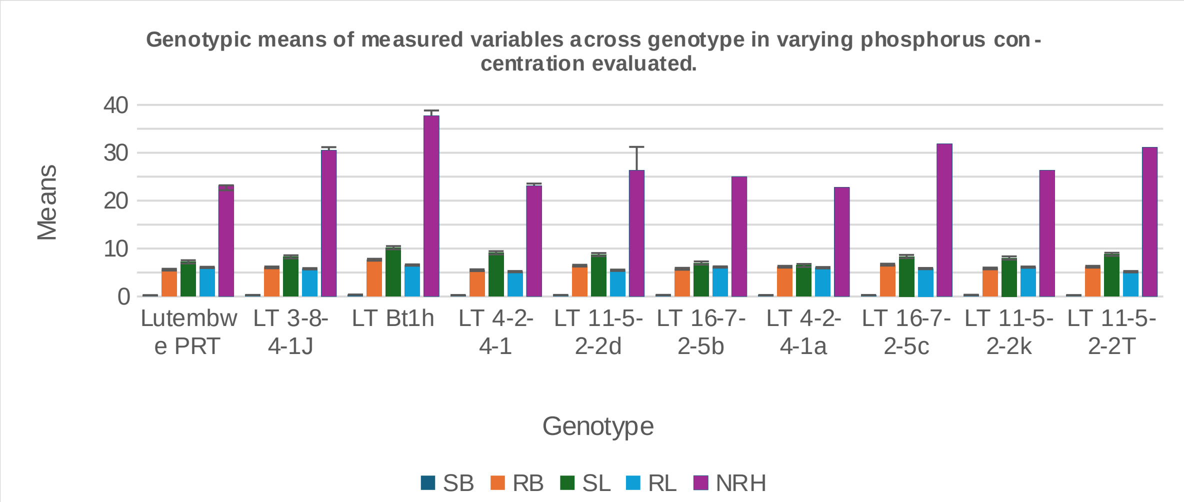
## APPENDICES

### APPENDIX A: FIGURES




**SB: Shoot biomass; RB: Root biomass; SL: Shoot Length, RL: Root Length, NRH: Number of root hairs.**

**Figure 1. Mean performance of genotypes of measured variables.**