**Evaluation of Cowpea (*Vigna unguiculata* L) Mutant Genotypes under Hydroponic Conditions using Different Levels of Phosphorus Concentration**

Abstract

Cowpea yields in the sub-Sahara African region are low primarily due to low available soil health, poor varieties and farming management. The present study aims to assess the performance of mutant genotypes of cowpea (*Vigna unguiculata* L) under hydroponic conditions, specifically focusing on different levels of phosphorus concentration. This study was conducted at the University of Zambia, School of Agricultural Sciences, plant physiology laboratory. The experiment was set up as a 5 x 8 factorial completely randomized design replicated three times with varying P amounts of 0mg/L, 4mg/L, 8mg/L, 12mg/L, and 16mg/L in a nutrient media and eight genotypes, giving a total of 120 experimental units assigned randomly to each plot (black plastic container). Genotypes showed significant differences (p<0.001) in response to all measured parameters (shoot length, root length, lateral roots, shoot biomass, and root biomass) across phosphorus concentrations. Similarly, the responses of all measured parameters in different P concentrations across genotypes were significantly different (P < 0.001). The current study identified the mutant line LT-3-8-4-1 as a better performer in the variables under consideration across different P-concentrations. However, mutant lines LT-3-8-4-1, LT-11-5-1-1, and LT-4-2-4-1 were identified as the best performers in the P-limiting solution (with P amount at 4mg/L). Furthermore, mutant line LT-3-8-4-1 exhibited a better adaptive flexibility performance than the parent (Lutembwe) in media with a P concentration ranging from 0 to 16 mg/L while the three genotypes, LT-3-8-4-1, LT-11-5-1-1, and LT-4-2-4-1 were considered as genotypes of choice in areas where there are p-limiting soils. In addition, the phenotypic variation explained showed that only 9.79% of the change in shoot length is explained by the change in root length. This implies that shoot length can only be used to estimate the root length in selecting for genotypic efficiency at utilizing P in the hydroponic medium. Based on the current study's findings, it is recommended that mutant line LT-3-8-4-1 replace the parental line regarding phosphorus use efficiency. Additionally, shoot length can be used to determine what is occurring in the root length as the two parameters are correlated.

**Keywords:** Cowpea, Hydroponic, Mutant lines, Phosphorus use efficiency.

1.0 Introduction

Cowpea (*Vigna unguiculata* L.) is an annual legume that is considered to be a vital cash and nutritional security grain legume in the semi-arid regions of sub-Saharan Africa (Siyunda et al., 2022a). In sub-Saharan Africa, the demand for cowpeas is at 20,000 metric tons per year and is projected to grow by more than 40,000 metric tons over the next ten years (Mwila et al., 2022a). In Zambia, production of cowpeas on average increased from 2722 metric tons to 10603 metric tons, which represents a 74.3% annual increase, and the area planted also increased on average from 11189ha to 20866 ha, representing a 46% annual increase from 2009/2010 to 2015/2016 agricultural farming seasons (Mwila et al., 2022b). The increase in cowpea production might indicate the importance of cowpea among subsistence farmers who are major producers of cowpea overall. Regardless of the recorded increase in cowpea production, grain yield in cowpea production remains low, rarely exceeding 0.5 t/ha in the traditional production system (Siyunda et al., 2022b) due to the low level of available P in most soil in the region. Despite the legumes' advantage of fixing N in the soils, legumes also need a good amount of phosphorus to grow and develop enough nodules to carry out N fixation effectively and efficiently.

Phosphorus deficiency is widely considered the primary biophysical constraint to food production in large areas of farmland in Africa (van Kessel and Hartley, 2000). Phosphorus dynamics in soils are complex because they involve chemical and biological processes and the long-term effects of sorption (fixation) and desorption (release) processes. Phosphorus (P) is one of the significant essential plant nutrients that is required in relatively considerable amounts since it contributes to the growth and uptake of water and other plant nutrients and is involved in the maturity phase of most crops. The low concentration and low solubility of P in soils frequently make P a limiting factor. However, smallholder farmers face low crop yields, income, and food scarcity due to low available P in most Sub-Saharan African (SSA) soils. This is due to diverse causes, including inherent poor soil fertility, soil acidity, over-cultivation with no adoption of good management measures, and soil erosion, among others (Buresh and Smithson, 1997). These factors affect soil available P in different manners, including absorption of P by Al, Fe oxides, and clay materials (Buresh and Smithson, 1997), physical loss through erosion, and removal by crop through over-cultivation (Jama et al., 2000). Cowpea yields in the sub-Sahara African region are low primarily due to low available soil phosphorus (P) status. Phosphorus-efficient cowpea genotypes with a remarkable ability to utilize P from P-deficient soils are essential in soils with low nutrient availability. Thus, this study was conducted to evaluate different mutant-derived genotypes for phosphorus use efficiency, as this has the potential to increase yield and reduce the cost of cowpea production.

2.0 Materials and Method

2.1 Location and Site Description

This study was conducted at the University of Zambia, School of Agricultural Sciences, plant physiology lab (15o23'41.3' ’S; 28o20'11.8''E). The experimental units were further moved to the greenhouse. The temperature range of 24-28 degrees Celsius and the humidity percent of 85-91% was maintained in the greenhouse. The parental cowpea line Lutembwe and its gamma ray-derived mutants used in the study are listed in Table 1 below.

Table 1: Germplasm used in the study for a hydroponic experiment at the University of Zambia, plant physiology laboratory.

|  |  |  |
| --- | --- | --- |
| No. | Genotype | Description |
| 1 | Lutembwe | Parent |
| 2 | Lt 16-7-2-5 | Lt mutant |
| 3 | Lt 4-2-4-1 | Lt mutant |
| 4 | Lt 3-8-4-1 | Lt mutant |
| 5 | Lt 11-3-3-12 | Lt mutant |
| 6 | Lt 11-5-2-2 | Lt mutant |
| 7 | Lt 3-8-4-6 | Lt mutant |
| 8 | Lt 11-5-1-1 | Lt mutant |

*Lt; Lutembwe (cowpea parental genotype)*

The mutants that were used in this study were obtained from the Cowpea Mutation Breeding Project at the University of Zambia under the School of Agricultural Sciences, Plant Science Department. At the time of the study, the 7th mutation generation seed was used for the experimental study. Undamaged seed from the parent and mutant lines was carefully selected before planting to ensure an increased germination rate.

Table 2: Nutrient solution used in the hydroponics study at the University of Zambia, School of Agricultural Sciences.

|  |  |  |  |
| --- | --- | --- | --- |
| Nutrient | Con(mg/L) | Chemical formula | Compound name |
| N | 60.90 | NH4NO3 | Ammonium nitrate |
| P | Varied\* | K2HPO4.3H2O | Potassium hydrogen phosphate trihydrate |
| B | 0.92 | H3BO3 | Boric acid |
| Zn | 0.32 | ZnSO4.7H2O | Zinc sulfate heptahydrate |
| Mg | 74.03 | MgSO4.7H2O | Magnesium sulfate heptahydrate |
| Cu | 0.12 | CuSO4 | Copper II sulfate |
| Fe | 3.98 | FeSO4.7H2O | Iron II sulfate |
| Ca | 88.20 | CaCl2.2H2O | Calcium chloride dehydrate |
| Mo | 0.04 | NaMoO4.2H2O | Sodium molybdate |
| Mn | 0.05 | MnSO4.H2O | Manganese II sulfate |

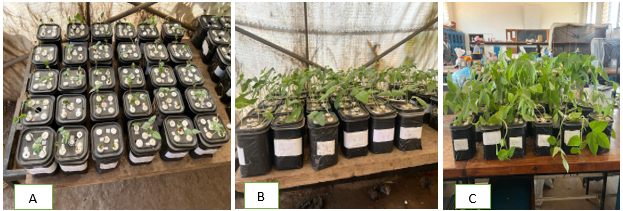
*N: Nitrogen; K; Potassium; Zn: Zinc; Mg: Magnesium; Cu: Copper; Fe: Iron; Ca: Calcium; Mo: Molybdenum; Mn: Manganese; B: Boron; Al: Aluminum; P: Phosphorous hydroponic combinations with either 0mg/L, 4mg/L, 8mg/L, 12mg/L, and 16mg/L.*

2.2 Experimental design and medium preparation

The experiment was set up as a 5 x 8 factorial completely randomized design (CRD), replicated 3 times with eight (8) genotypes (Table 1) and varying P amounts of 0mg/L, 4mg/L, 8mg/L, 12mg/L, and 16mg/L in a nutrient media (Table 2). The total number of experimental units was 120 and assigned randomly to each plot (black plastic container). Each container had a size of 850ml. The limiting concentration of P was 4mg/L, and 0mg/L as the control, with the excess concentration being 8mg/L, 12mg/L, and 16mg/L. Kerridge and Kronstad (1968) reported the optimal concentration as 4.65mg/L. The pH of the solutions was adjusted to 6.5 using HCL and NaOH buffer solutions as phosphorus is made readily to plants at the said pH.

2.3 Placement of cowpea seedlings

Each genotype was germinated on separate petri dishes lined with filter paper and soaked in distilled water. The seeds were disinfected using sodium hypochlorite for 1 minute, rinsed with sterile water, and placed in the germination chamber for 3 days at 25oC. After 3 days, the germinated seeds with uniform root lengths were selected and placed in the containers covered with black plastic to avoid any algae growth. Aeration was done twice daily, in the morning and late afternoon, using an aquarium air pump. This was done to provide oxygen to the roots of the plant. The 8 treatments were assigned randomly to each experimental unit, resulting in 4 plants per unit, and the other 4 to another unit, resulting in 8 total per replication, as shown in Figures 1A and 1B below. Harvesting was done on the 21st DAP (Day after Planting) (Figure 1C).



*Figure 1: A; Seedling at 5 DAP, B; Cowpea plants at 13 DAP, C; Harvesting at 21st DAP*

2.4 Data Collection and Analysis

Data was collected on the experimental material concerning shoot length, root length, lateral roots, shoot biomass, and root biomass (Tembo, 2018). The lateral roots were counted using a magnifying glass and a pair of tweezers, and the roots were then cut from the shoot, placed in different khaki envelopes, and oven-dried for 24 hours at 70 oC. After drying, the shoot biomass and root biomass were weighed using an electronic weight balance. The Analysis of variance (ANOVA) for all measured parameters was performed. The means for the main effects were separated using Fisher-protected least significant difference (LSD) at a significant level of = α 0.05. The dissection of the interaction effect was only computed for shoot length. The correlation of shoot and root length was determined using linear regression, taking root length and shoot length as explanatory. All data was analyzed using Genstat statistical package 18th edition.

3.0 Results

3.1 Evaluation of genotypic and phosphorous concentration main effects

The result from the analysis of variance is presented in table 3. The genotypes, phosphorus concentration and genotypes by phosphorus concentration were highly significant for root biomass. The genotypes were also highly significant for all the traits measured except for shoot biomass which was significant. Phosphorus concentration for the different levels was significant for shoot length, root length and shoot biomass except for number of lateral roots and root biomass while genotype by phosphorus concentration for all the measured traits was highly significant except for lateral root which was significant.

Table 3: Analysis of variance for mean squares of shoot length, root length, lateral roots, shoot biomass, and root biomass evaluated under hydroponics conditions.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Source of variation** | **DF** | **SL** | **RL** | **LR** | **SB** | **RB** |
| Genotypes(G) | 7 | 22.71\*\*\* | 58.49\*\*\* | 217.87\*\*\* | 0.049\* | 7.45x10-4\*\*\* |
| Phosphorus(P) Conc. | 4 | 182.67\* | 30.03\*\* | 219.87\*\*\* | 0.033\*\* | 3.16x10-4\*\*\* |
| G x P Conc. | 28 | 18.89\*\*\* | 93.96\*\*\* | 131.74\* | 0.022\*\*\* | 5.97x10-4\*\*\* |
| Error | 80 | 3.18 | 9.89 | 13.63 | 0.005 | 6.94x10-4 |
| Total | 119 | 227.46 | 192.37 | 569.37 | 0.11 | 0.00172 |

*\*\*\* Significant at P<0.001: \*\* Significant at P<0.01: \* Significant at P<0.05: respectively, DF: Degrees of freedom, SL: Shoot length, RL: Root Length, LR: No. of lateral roots**, SB: Shoot Biomass, RB: Root Biomass*

Some of the mutant-derived genotypes outperformed the parental line Lutembwe, for all measured parameters across P concentration (Table 4). Similarly, the responses of all measured parameters in different P concentrations across genotypes were significantly different (P < 0.001).

Regarding the shoot length, only the LT-16-7-2-5 (15.01 cm) genotype performed below the parental line, though it was still statistically at par with the parental line. The other genotypes outperformed the parent line. The best-performing genotype was LT-11-5-2-2 (18.56 cm), which was statistically at par with LT-3-8-4-1 (17.42 cm) and LT-3-8-4-6 (18.33 cm). In terms of the root length, three genotypes, namely, LT-11-3-3-12 (18.51cm), LT-11-5-1-1 (20.48 cm), and LT-4-2-4-1 (21.36cm), performed below the parental line. However, of the three genotypes, only LT-11-3-3-12 (18.51cm) was statistically below par, while the other two were statistically at par with the parental line. The best-performing mutant line regarding root length was LT-3-8-4-6, which recorded 24.67cm. However, LT-3-8-4-6 was statistically at par with three mutant lines namely, LT-11-5-2-2 (23.83cm), LT-16-7-2-5 (23.37cm), and LT-3-8-4-1 (22.27 cm). The rest of the genotypes were statistically at par with the parent line.

LT-3-8-4-6 recorded the highest shoot biomass of 0.32g. LT-3-8-4-6 was statistically at par with LT-3-8-4-1 (0.30g), LT-4-2-4-1 (0.27g), and parental line (0.27g). Mutant lines LT-11-3-3-12 (0.21 g) and LT-11-5-1-1 (0.14 g) recorded significantly lower shoot biomass than the parental line. Mutant line LT-3-8-4-1 recorded the highest root biomass of 0.05g, which was statistically at par with the parental line (0.05g). All the other genotypes were statistically below par, with mutant line LT-11-3-3-12 recording the least root biomass of <0.01g. Furthermore, LT-3-8-4-1 showed the best performance regarding lateral roots, having a record number of 32 lateral roots on average, which was statistically different from all the genotypes under consideration in the current study. Four genotypes performed statistically below the parental: LT-11-3-3-12, LT-11-5-2-2, LT-3-8-4-6, and LT-16-7-2-5, they recorded 22, 22, 22, and 19 respectively. The genotype that performed well across all selected levels of phosphorus was LT-3-8-4-1. On the other hand, genotypes LT-3-8-4-1, LT-11-5-1-1, and LT-4-2-4-1 performed well in the p-limiting medium. (Table 4).

Table 4: Genotypic means of measured parameters across genotypes in phosphorus concentration evaluated at University of Zambia (UNZA).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| GENOTYPE | SL (cm) | RL (cm) | LR | SB (g) | RB (g) |
| PARENT | 15.07 | 21.92 | 26 | 0.27 | 0.05 |
| LT-11-3-3-12 | 16.89 | 18.51 | 22 | 0.21 | 0.00 |
| LT-11-5-1-1 | 17.16 | 20.48 | 25 | 0.14 | 0.02 |
| LT-11-5-2-2 | 18.56 | 23.82 | 22 | 0.22 | 0.03 |
| LT-16-7-2-5 | 15.01 | 23.37 | 19 | 0.24 | 0.03 |
| LT-3-8-4-1 | 17.42 | 22.27 | 32 | 0.30 | 0.05 |
| LT-3-8-4-6 | 18.33 | 24.67 | 22 | 0.32 | 0.04 |
| LT-4-2-4-1 | 16.46 | 21.36 | 24 | 0.27 | 0.04 |
| Means | 16.93 | 22.05 | 23.99 | 0.24 | 0.04 |
| LSD (α 0.05) | 1.29 | 2.29 | 2.69 | 0.05 | 0.006 |

*SL: Shoot length; RL: Root Length; SB: Shoot Biomass; RB: Root Biomass; LR: No of Lateral Roots; ns: not significant; LSD: Least Significant Difference.*

3.2 Means of variables by phosphorus concentration across Genotypes

Significant differences (p<0.001) were observed in shoot length, shoot biomass, lateral roots, root length, and root biomass across various levels of phosphorus concentration (Table 5). Shoot length increased as the phosphorus concentration level increased while root length ranged between 20.78cm (16mg/L) to 23.46cm (12mg/L). Shoot biomass had a mean value of 0.25g with the lowest value at 0mg/L (0.19g) and highest value at 12mg/L (0.29g) phosphorus concentration level. Root biomass values was between 0.03g and 0.04g for the five different concentration levels. Also, the number of lateral roots decreased as the concentration levels increased.

Table 5: Means of measured parameters across genotypes in varying phosphorus concentrations.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| P Con. | SL (cm) | RL (cm) | SB (g) | RB (g) | LR |
| 0 | 12.22 | 22.79 | 0.19 | 0.04 | 28.92 |
| 4 | 17.56 | 21.13 | 0.25 | 0.04 | 23.92 |
| 8 | 17.03 | 22.08 | 0.24 | 0.03 | 23.81 |
| 12 | 18.68 | 23.46 | 0.29 | 0.03 | 22.49 |
| 16 | 19.09 | 20.78 | 0.26 | 0.04 | 20.83 |
| Means | 16.97 | 22.21 | 0.25 | 0.04 | 20.83 |
| LSD (α 0.05) | 1.03 | 1.81 | 0.04 | 0.01 | 2.12 |

*SL: Shoot length; RL: Root Length; SB: Shoot Biomass; RB: Root Biomass; LR: No. of Lateral Roots. LSD: Least Significant Difference.*

3.3 Linear regression of root and shoot length.

The model given in Figure 2 indicates the y-intercept of 12.284. This shows that at 0 cm development of the shoot system, the root length would be at 12.284 cm on average. Furthermore, the relationship between the root length and shoot length indicates that every 1 cm growth in the shoot length would be accompanied by 0.53 cm growth in the root length. However, the relationship in the developed model can only explain 9.79% of what is happening between the shoot length and root length.

*Figure 2: Linear regression of shoot and root length*

4.0 Discussion

The availability of phosphorus in the soil heavily influences the development of legumes. This has been confirmed by previous researchers such as Nachilima and Tembo (2021) and many others. In the Zambian context, many soils have low levels of phosphorus; as a result, P must be supplemented most of the time if legumes are to yield optimally. However, identifying genotypes that can utilize the low level of phosphorus available in the soil and yield optimally gives hope to a breeder and becomes a suitable alternative for farmers. In this hydroponic study, the source of P (Potassium hydrogen phosphate trihydrate) equally supplied another major element, Potassium (K). However, in plants and cowpea development inclusive, potassium plays a vital role in the opening and closing of stomata and regulates CO2 uptake in photosynthesis (Trankner et al., 2018). In that regard, genotypic parameter responses, particularly shoot length, were directly associated with the utilization of P.

In mutation breeding, the performance of mutant-derived lines is compared to their parental genotype. Desirable derived mutants outperform the parental line and can be identified as candidates for variety release or selected for further breeding (Tembo and Munyinda 2015; Tembo et al. 2017). The current study identified the best-performing genotype across all phosphorus levels as LT3-8-4-1. On the other hand, genotypes LT-3-8-4-1, LT-11-5-1-1, and LT-4-2-4-1 performed well in the p-limiting medium. These genotypes outperformed the parental line (Lutembwe). This might result from genetic divergence created by gamma rays between the parental line and the said mutants regarding phosphorus use efficiency. Furthermore, LT-3-8-4-1, LT-11-5-1-1, and LT-4-2-4-1 might have genes that could utilize phosphorus effectively and efficiently compared to the parental genotype and other mutant lines. The results of the current study are like the findings reported by Nachilima and Tembo (2021), in which the researchers conducted a similar study on the common bean crop. In their study, the mutant line SK 46-17-1 had a higher mean performance concerning the parental line for all measured parameters across P concentration.

The model developed in the current study had a phenotypic value explained (R2) of 0.0979. This implies that the change in the root length variable explains only 9.79% of the phenotypic variation in shoot length. This implies that shoot length can only be used to estimate the root length in selecting for genotypic efficiency at utilizing P in the hydroponic medium. The findings of the current research buttressed findings from work done in the past that showed indirect selection using agronomic traits can only supplement the direct selection of a trait, especially when the trait under consideration is quantitatively inherited (Tembo et al., 2016). The low value for phenotypic value explained (PVE) could be due to the interaction between the seed size and seed quality used for this research as both traits were found to be responsible for early seedling development during germination, as found in the research done by Lima et al. (2005) and Gupta (2008).

5.0 Conclusion and recommendation

In the current study, mutant line LT-3-8-4-1 was identified to perform better among variables under consideration and across different P-concentration levels. However, mutant lines LT-3-8-4-1, LT-11-5-1-1, and LT-4-2-4-1 were identified as the best performers in the P-limiting solution. Furthermore, mutant line LT-3-8-4-1 exhibited a better adaptive flexibility performance than the parent (Lutembwe) in media with a P concentration ranging from 0 to 16 mg/L, while the three genotypes, LT-3-8-4-1, LT-11-5-1-1, and LT-4-2-4-1 were considered as genotypes of choice in areas where there are p-limiting soils. In addition, the phenotypic variation explained (PVE) showed that only 9.79% of the change in shoot length is explained by the change in root length. This implies that shoot length can be used indirectly to determine the root length in selecting for genotypic efficiency at utilizing P in the hydroponic medium. Based on the findings of the current study, it is recommended that mutant line LT-3-8-4-1 can be used to replace the parental line with regard to phosphorus use efficiency. It is further recommended that shoot length can be used to determine what is happening in the root length due to the positive correlation between the two parameters, although with slightly weaker strength. In addition, it is also recommended that other researchers conduct similar research to validate the current result from our findings.

7.0 References

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