ISSN: 2287-688X

Annals of
Plant Sciences

Volume 14, Issue 04 (2025) pp. 6775-6784

Review Article

Resistance to *Ascochyta phaseolorum* and Multivariate Analysis of Mutational Derived Cowpea Lines

Judith Malumo, Paul Kachapulula and Langa Tembo

Department of Plant science, School of Agricultural Sciences, University of Zambia

Abstract

Cowpea plays a vital role in diets of many households, primarily as a source of protein. Among the biotic stresses Ascochyta blight caused by *Ascochyta phaseolorum* reduces cowpea yields among the small-scale farmers. The University of Zambia (UNZA), developed cowpea derived mutational lines as a way of increasing genetic diversity among cowpea germplasm in the country. The objective of this study, was therefore i) to identify resistant cowpea mutational derived genotypes to *Ascochyta phaseolorum*, ii) to cluster cowpea genotypes into distinct groups and iii) identify the best parental set to utilize as a cross in a further breeding program. The experiment was laid at the University of Zambia and Mt Makulu as a randomized complete block design (RCBD) with three replications It was artificially inoculated with *Ascochyta phaseolorum*. Significant differences (P = 0.001) were obtained among genotypes with regards to disease severity score. The genotypes, BB 8 –1 - 5- 2, LT 4 -2- 4- 1, LT 11- 3 -3- 12 and MS 1- 1 -8 – 4 were identified as resistant genotypes to *Ascochyta phaseolorum* and also obtained higher yield than the genotypic mean value. In this study, principal component analysis revealed six distinct groups of cowpea genotypes. Detailed analysis revealed that a genotypic cross, LT 11-3-3-12 (From group A) x BB PRT (from group E) were most dissimilar genotypes with a similarity score level of 30.3 %. Thus, this cross (LT 11-3-3-12 x BB PRT) is expected to segregate and exhibit highest phenotypic variation in advanced generations.

Keywords: *Vigna unguiculata, Aschochyta phaseolorum, Principal component analysis, Similarity matrix.*

Introduction

Cowpea [*Vigna unguiculata* (L) Walp] is an important annual leguminous crop in Africa that belongs to Fabacea family (Langyintuo *et al.*,., 2003; Moalafi *et al.*, 2010). It grows well in a wide range of soils with preference to sandy soils which has less restriction to root growth (DAFF 2011; Sankie *et al.*, 2012). It develops a long tap root which makes it adapt better in light and sandy soils than other legumes and has many spreading lateral roots on the soil surface (DAFF 2011). Cowpea is heat and drought tolerant crop (Lucas *et al.*, 2013). In addition, it tolerates water lodging and utilizes soil moisture efficiently. Cowpea can be produced with annual rainfall ranging between 400 mm and 700 mm even though adequate rainfall is essential during flowering/ podding stage. It is extensively grown for its nutritious source of protein for both humans and livestock (Agbogidi 2010a, Tembo *et al.*, 2017). All the plant parts of cowpea such as leaves, fresh and dry pods can be prepared differently and consumed as food (Ajeigbe *et al.*, 2012; Dube and Fanadzo 2013). Cowpea grains contain an average 38.1mg/kg of zinc. (Boukar *et al.*, 2011). Daily consumption of 100 – 135g of dry beans reduces the serum cholesterol level by 20%, in turn reducing the risk of developing heart disease by 40% (Adaji *et al.*, 2007).

The production constraints of cowpea production range from abiotic to biotic factors. The biotic stress factors are caused primarily by predators, parasites and parasitoids. Among the parasites, a fungus *Ascochyta phaseolorum* causes Ascochyta blight disease which leads to yield losses of about 50 – 75% (Salam *et al.*, 2011). Cultural control measures such as crop rotation with non-host

***Corresponding Author:**
Langa Tembo;

DOI: https://doi.org/ 10.5281/zenodo.15288374

crops of the disease, field sanitation and mixed intercropping can be employed to manage the diseases (Than *et al.,* 2008). In addition, chemical control method, by use of fungicides to eradicate the disease pressure can be employed, but they are not an economical and environmentally friendly approach. The use of resistant genotypes to *Ascochyta phaseolorum* is not only the cheaper option but also a feasible one for small scale farmers.

In this study, selected mutational derived lines generated by the University of Zambia, School of Agriculture Sciences, in corroboration with National Institute for Scientific and Industrial Research (NISIR) were evaluated for resistance to *Ascochyta phaseolorum*. Induced mutations generate new alleles and produce variants that are different from the parent, of which when advanced beyond M5 generation produce non-segregating, distinct lines (Tembo *et al.,* 2017).

The benefits of host plant resistance, especially as a component of integrated pest management make it a worthwhile investment of time, effort, and resources. A resistant genotype can be released as a variety or used as a parental genotype in a breeding program. It must be noted that in cowpea, the more genetically distinct the parental genotypes are, the more diverse the phenotypic variations in the segregating population (Tembo *et al.,* 2015). Multivariate approach techniques such as cluster and principal component analysis (PCA) have been previously employed to categorize genotypes into distinct groups (Simasiku *et al.,* 2015).

The objective of the study was therefore to i) to identify resistant cowpea mutational derived genotypes to *Ascochyta phaseolorum*, ii) to cluster cowpea genotypes into distinct groups and iii) identify the best parental set to utilize as a cross in a further breeding program.

Materials and Methods

Location of Study

The experiment was conducted during the 2018/ 2019 cropping season at two locations: The School of Agricultural Science field station at the University of Zambia in Lusaka, (15°23'S, 28°20'E) and at Zambia Agricultural Research Institute field in Chilanga (15°55'S, 28°25'E).

Germplasm used and conduct of experiment

Sixteen (16) genotypes (Table 1) that included: thirteen (13) mutational derived cowpea lines and their three (3) respective parental genotypes were obtained from University of Zambia, Department of Plant Science. The mutational derived cowpea lines were developed as explained by Tembo *et al.,*. 2017. Approximately 3000 seeds per parental line were irradiated.

Table 1: List of cowpea genotypes used for the experiment

No	Genotype	Description
1	BB 10 – 2 -2 – 3	Bubebe derived mutational derived line
2	BB 3 – 9 – 7 - 5	Bubebe derived mutational derived line
3	BB 8 – 1 – 5 -2	Bubebe derived mutational derived line
4	BB 14 – 16 – 2 – 2	Bubebe derived mutational derived line
5	BB PRT	Bubebe parent – released variety
6	LT 3 – 8 – 4 - 6	Lutembwa derived mutational line
7	LT 16 – 7 – 2 - 5	Lutembwe derived mutational line
8	LT 11 – 5 – 2 – 2	Lutembwa derived mutational line
9	LT 11 – 3 – 3 – 12	Lutembwa derived mutational line
10	LT 11 – 3 – 3 – 13	Lutembwa derived mutational line
11	LT 3 – 8 – 4 – 1	Lutembwa derived mutational line
12	LT PRT	Lutembwe parent – released variety

13	LT 4 – 2 – 4 – 1	Lutembwa derived mutational line
14	MS 10 – 11 – 1 – 1	Musandile derived mutational line
15	MS 1 – 1 – 8 – 4	Musandile derived mutational line
16	MS PRT	Musandile parent – released variety

BB- Bubebe, LT- Lutembwe, MS- Musandile

Conduct and Experimental Design

The experiment was laid as a randomized complete block design (RCBD) with three replications. Each replication consisted of 16 plots (with 3 rows each), giving 48 plots for the whole experiment. Land preparation was done with a hand hoe where planting furrows were made and seed planted at 2 cm depth. The plants were established using intra-row and inter-row spacing of 15 cm and 60 cm respectively. After two (2) weeks from planting, seedlings were thinned to 10 plants per row. Weed control was done manually using a hand hoe. Each block consisted of 48 lines. Plot area was $2.7m^2$ that accommodated 3 rows of 1.5m long with the total experimental area being $137.6m^2$. The genotype was indirectly inoculated at 14 days after planting by placing diseased cowpea leaves and pods between the rows in each plot. The diseased cowpea leaves and pods, used as a source of inoculum was obtained from UNZA field station.

Verification of the pathogen

Samples of suspected infected leaves and pods used in the experiment were assessed to verify the existence of *Ascochyta phaseolorum* as done by Wamali (1984) (Plate 1 and Plate 2).

Plate 1. Fungal growth (whitish stuff) of *Ascochyta phaseolorum* on PDA at 9^{th} day after inoculation.

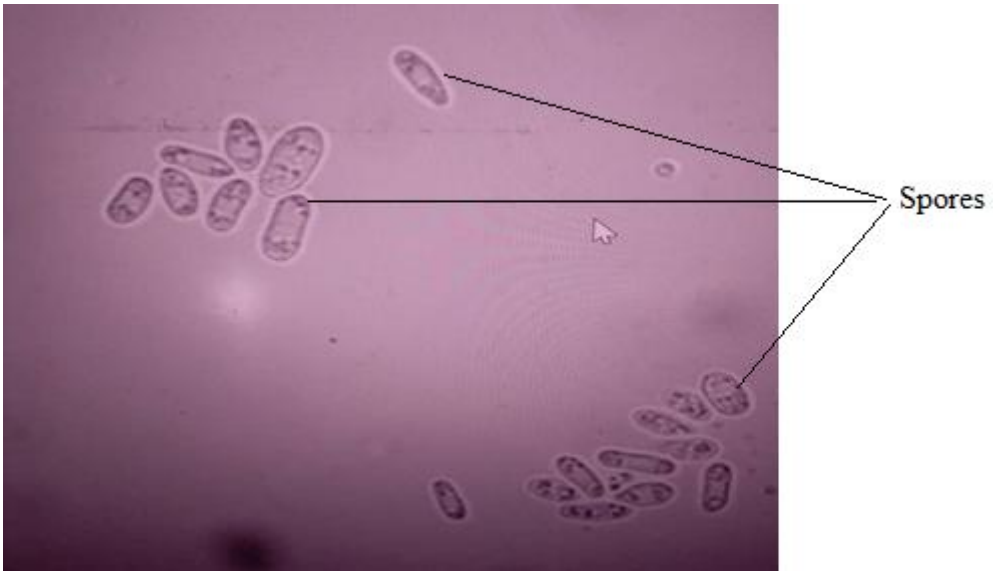

Plate 2. Spores of *Aschochyta phaseolorum* as visualized on magnification X40 magnifying microscope on 18th day after inoculation

Data Collection and Analysis

Scoring for disease severity response among genotypes was done at 10, 20 and 30 days after emergence and the results recorded as D10, D20 and D30 respectively. The severity rating was scored on a scale of 1 to 9 scale according to Schoohonven and Corrales (1987). However, the genotypic characteristics associated with response to resistance was undertaken at D30.

Data was taken from the middle row where the disease severity score per replicate was determined as a genotypic mean of plants and the crop was later harvested after 3 months**.** At harvest the above ground biomass was weighed and recorded and thereafter pods were then shelled and the grain was weighed separately and recorded accordingly. In this study, the scoring of genotypes were scored as done by Schoonhoven and Corrales (1987) where 1-3 Resistant, 6-9 Susceptible.

Clustering of the genotypes was achieved using a multivariate analysis approach particularly using principle component analysis (PCA). A similarity matrix was generated using cluster analysis. Differences among genotypes with regards to disease, yield and biomass were computed using analysis of variance (ANOVA) assuming a mixed model with genotypes fixed and location random. The mean genotypic differences were separated using the Fisher protected Lsd (α= 0.05). All data was performed in GenStat 18 the edition (Payne *et al.,*. 2007). Harvest index (HI) for each genotype was computed as a percentage grain weight divided by total biomass.

Results

Evaluation of Germplasm

Significant differences were obtained on genotypic responses on all measured parameters (P< 0.001) (Table 2). The interaction effect (Genotype [G] x Location [L]) was not significant for all measured parameters except at D10 and D20. With locational main effect, no significant differences among measured parameters were revealed except for yield (P < 0.001).

Table 2: Mean square for measured parameters on genotypic evaluation in two (2) locations during 2018/2019 planting season

SOV	df	D10	D20	D30	BMS	HI	Yld
L	1	11.8	4.94	0.13	11221842	0.78	6793688***
Rep/ L	4	1.7	0.9	1.6	507659	0.03	548
Genotype	15	15.3***	26.2***	36.6***	1831685***	0.19***	66139***
L x G	15	1.2***	0.4***	0.5	407798	0.04	40078
Error	60	0.17	0.10	0.31	354158	0.04	14685

*** Data significant at P= 0.001. df- degrees of freedom. D10, D20, D30- Disease score after 10, 20 and 30 days emergency respectively. HI- Harvest index, BMS- Biomass. L- Location, G- Genotype, Rep - Replication

Further analysis on genotypic response in Mt Makulu revealed that B 8-1-5-2, LT 11-3-3-12, LT 4-2-4-2, LT 3-8-4-1 and MS 1-1-8-4 as the most resistant genotypes exhibiting a score of 1, significantly lower than their respective parental genotypes at all inoculation levels (D10 to D30) (Table 3). With the exception LT 4-2-4-1, all the resistant genotypes exhibited higher yield than the overall genotypic mean value.

Table 3: Genotypic mean response to infection *Ascochyta phaseolorum* at Mount Makulu

Genotype	D10	D20	D30	BMS	HI	Yld
BB 10 - 4 - 2 – 3	3.50	4.83	6.46	1414	0.257	321
BB 14 - 16 - 2 - 2	4.07	5.33	6.73	1444	0.213	292
BB 3 - 9 - 7 - 5	2.33	4.67	5.80	1025	0.470	453
BB 8 - 1 - 5 – 2	1.00	1.00	1.00	2000	0.197	395
BB PRT	4.70	5.80	6.33	548	0.743	336
LT 11 - 3 - 3 - 12	1.00	1.00	1.00	1636	0.307	489
LT 11 - 3 - 3 - 13	1.00	4.00	5.20	2210	0.083	215
LT 11 - 5 - 2 – 2	3.33	4.67	5.30	1667	0.18	245
LT 16 -7 - 2 - 5	4.63	5.00	6.17	444	0.64	312
LT 3 - 8 - 4 - 1	1.00	1.00	1.01	1593	0.37	500
LT 3 - 8 - 4 – 6	3.50	4.67	5.17	1595	0.27	409
LT 4 - 2 - 4 – 1	1.00	1.00	1.04	1722	0.25	357
LT PRT	4.33	5.40	7.07	1673	0.16	273
MS 1 - 1 - 8 – 4	1.00	1.00	1.00	1160	0.43	444
MS 10 - 11 -1 - 1	3.67	4.67	5.00	1778	0.24	417
MS PRT	4.23	5.50	6.50	879	0.59	394
Overall Mean	2.77	3.72	4.42	1424.2	0.34	365.8
LSD (=0.05)	0.81	0.53	0.73	937	0.33	251.1

df- degrees of freedom. D10, D20, D30- Disease score after 10, 20 and 30 days of inoculation respectively. HI- Harvest index, BMS- Biomass (g/ plot), YLD- Yield (Kg/ ha), LSD – Least Significant difference.

Table 4 below exhibit further analysis on genotypic response at UNZA and interestingly, results were similar to the genotypic response at Mt Makulu location (Table 3). Bubebe 8-1-5-2, LT 11-3-3-12, LT 4-2-4-2, LT 3-8-4-1 and MS 1-1-8-4 were the most resistant genotypes exhibiting a score of 1, significantly lower than their respective parental genotypes at all inoculation levels (D10 to D30).

Table 4: Genotypic response to infection *Ascochyta phaseolorum* at University of Zambia

Genotype	D10	D20	D30	Bms	HI	Yld
BB 10 - 4 - 2 – 3	4.33	5.50	6.70	1552	0.46	664
BB 14 - 16 - 2 - 2	4.62	5.67	6.50	1772	0.33	621
BB 3 - 9 - 7 - 5	3.60	5.33	6.20	1574	0.50	731
BB 8 - 1 - 5 – 2	1.00	1.00	1.00	1981	0.42	775
BB PRT	4.77	5.73	6.38	780	0.75	543
LT 11 - 3 - 3 - 12	1.00	1.00	1.00	2664	0.25	600
LT 11 - 3 - 3 - 13	2.33	4.25	5.20	2463	0.22	476
LT 11 - 5 - 2 – 2	3.60	4.67	5.18	2074	0.27	560
LT 16 -7 - 2 - 5	4.82	5.60	6.50	660	0.81	570
LT 3 - 8 - 4 - 1	1.00	1.00	1.01	2086	0.41	796
LT 3 - 8 - 4 – 6	3.92	5.00	5.58	1717	0.35	562
LT 4 - 2 - 4 – 1	1.00	1.00	1.04	2225	0.34	562
LT PRT	4.83	5.90	7.30	2231	0.19	439
MS 1 - 1 - 8 – 4	1.00	1.00	1.00	1481	0.54	634
MS 10 - 11 -1 - 1	3.75	4.70	5.23	1735	0.43	739
MS PRT	4.37	5.67	5.70	1260	0.59	672
Overall Mean	3.12	3.94	4.47	1766	0.43	621.5
Lsd (α=0.05)	0.47	0.35	0.64	687.3	0.23	140.0

Lsd- Least significant difference, BB- Bubebe; MS -Musandile, YLD –Yield (kg/ ha), D10, D20, D30- Disease score after 10, 20 and 30 days emergency respectively. HI- Harvest index, BMS- Biomass (g/ plot).

Application of multivariate analysis

Five distinct cluster groups A, B, C, D and E were formulated (Figure 1). Principle component (PC) 1 and PC2 explained 60.2 % and 30.0 % of the percentage variation respectively giving a total of 90.20 %. The similarity matrix revealed that genotype LT-3-3-4 from group A and BB PRT from group E were the most distinct genotypes exhibiting a similarity level of 30.30 % (Table 5)

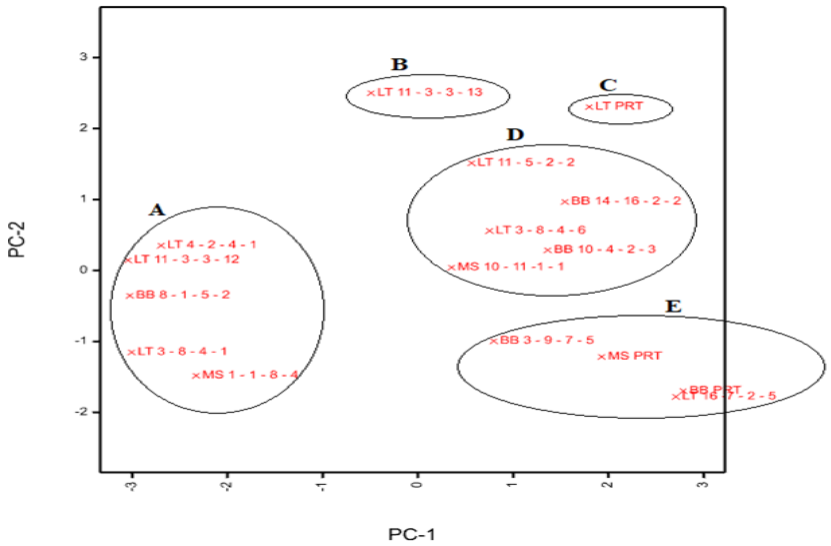

Figure 2. Scatter plot for Principal Component analysis with a percentage variation explained by PC 1 and PC2 of 60.2 and 30.0 respectively. Three cluster groups A, D and E with singletons B and C were generated. BB- Bubebe; MS –Musandile, LT- Lutembwe,

Table 5: Similarity matrix among germplasm derived from evaluated variables

Genotype	No.	Similarity Level (%)								
BB 10 - 4 - 2 - 3	1	----								
BB 14 - 16 - 2 - 2	2	99.0	----							
BB 3 - 9 - 7 - 5	3	96.0	91.4	----						
BB 8 - 1 - 5 - 2	4	60.5	54.2	68.8	----					
BB PRT	5	87.8	84.4	86.2	32.1	----				
LT 11 - 3 - 3 - 12	6	60.4	55.1	66.5	99.5	30.3	----			
LT 11 - 3 - 3 - 13	7	82.6	83.5	75.2	72.4	53.3	76.5	----		
LT 11 - 5 - 2 - 2	8	95.7	96.8	87.9	68.6	76.0	70.8	93.9	----	
LT 16 -7 - 2 - 5	9	87.8	84.2	86.4	34.7	99.8	32.7	53.4	76.2	----
LT 3 - 8 - 4 - 1	10	58.5	50.5	70.3	98.8	33.1	97.0	64.0	62.8	35.7
LT 3 - 8 - 4 - 6	11	99.0	98.3	95.0	69.7	83.2	70.1	87.4	98.1	83.4
LT 4 - 2 - 4 - 1	12	62.1	57.5	65.6	97.1	35.7	98.5	80.7	74.3	38.3
LT PRT	13	93.0	96.9	79.1	41.8	74.3	45.0	85.6	95.6	73.8
MS 1 - 1 - 8 - 4	14	62.3	54.2	72.2	95.8	46.9	94.4	67.2	66.9	49.9
MS 10 - 11 -1 - 1	15	97.1	95.1	96.7	73.6	79.5	72.7	81.6	93.7	79.7
MS PRT	16	95.9	92.5	96.2	51.6	96.1	49.5	65.9	85.5	95.9
		1	2	3	4	5	6	7	8	9
LT 3 - 8 - 4 - 1	10	----								
LT 3 - 8 - 4 - 6	11	66.8	----							
LT 4 - 2 - 4 - 1	12	93.0	71.6	----						
LT PRT	13	34.4	92.8	49.7	----					
MS 1 - 1 - 8 - 4	14	96.0	69.5	94.9	39.4	----				
MS 10 - 11 -1 - 1	15	72.8	98.3	71.1	86.2	71.5	----			
MS PRT	16	52.9	93.0	51.0	81.5	59.4	92.7	----		
		10	11	12	13	14	15	16		

BB- Bubebe, **LT**- Lutembwe, **MS**- Musandile. Highlighted in yellow- are genotypic pairs with low similarity levels

Discussion

Screening genotypes for response to plant disease require an understanding of the associated symptoms. The more consistent and accurate the scoring procedure is, the more reliable the phenotypic evaluation of the genotypes under investigation (Bock *et al.*,., 2016). In this study the screened genotypes were classified as resistant, moderate resistant and susceptible as by Schoohonvan and Corrales (1987).

Evaluation of Germplasm for Resistance to Ascochyta phaseolorum

Mutational derived cowpea genotype were evaluated for resistance to *Ascochyta phaseolorum* under field condition. The analysis of variance revealed significant differences ($p< 0.001$) among responses of mutant derived genotypes evaluated at all three stages after germination to disease severity score. In this study identification of resistant genotypes was undertaken at the final (D3) stage. Mutational derived genotypes, BB 8 –1 - 5- 2, LT 4 -2- 4- 1, LT 11- 3 -3- 12, LT 3-8-4-1 and MS 1- 1 -8 – 4 were found to be resistant to *Aschochyta Phaseolorum* in both locations. They exhibited a significant lower mean score values than their parental genotypes. This entails that induced mutation may have created new alleles not present in the parental genotype but in the mutational derived genotypes. Previous reports also suggested that induced

mutation widens the genetic base by increasing the genetic variability that may help solve agricultural challenges (FAO/IAEA 2004: Singh 2007, Tembo and Munyinda, 2015, Olasupo *et al.,*. 2016). Interestingly, a susceptible mutational derived genotype LT 11 – 3 - 3 -13 to *Ascochyta phaseolorum* performed better than the parent (LT PRT) in both grain yield and biomass production (Table 4). This entails that the creation of an allele associated with one desirable trait is independently associated with other alleles. Implying that induced mutational approach in crop improvement is a random effect which requires a substantial number of offspring in earlier generation selection (M2) to increase chances of identifying mutational derived lines with desirable agronomic trait (Tembo *et al.,*. 2017).

Multi-variate evaluation of genotypes

Identified mutational derived resistant genotypes can be evaluated and released as varieties or employed as parents in cowpea breeding to generate genotypes that will be tolerant to *Ascochyta phaseolorum*. Multivariate analysis such as principal component analysis and cluster enables identification of closely related genotypes by grouping them into distinct groups and computes the level of similarity among genotypes respectively (Simasiku *et al.,*. 2021).

In this study, PCA reaffirmed that mutational breeding may have created new alleles as evident by mutational derived lines, which fell in completely different clusters compared to their parental genotypes (Fig. 2). The use of molecular markers in genetic diversity studies has been advocated for (Edema *et al.,*. 2023; Tembo and Munyinda, 2015) as an accurate method of generating genetic diversity among genotypes. However, application of multivariate analysis utilizing phenotypic traits produce more reliable information especially where genotype by environmental effect is at play (Tembo *et al.,*. 2017, Simasiku *et al.,*. 2021). In this study PC1 and PC2 contributed 60.2 and 30.0 % giving a total of 90.2 % of variation indicating a higher reliable information with only 10 % unexplained.

To wrap it up, parental genotype for a further breeding approach can be obtained from any of the distinct groups labelled as A to E but not from the same group. In this study one parent should be from group A as all resistant genotypes clustered together in that group. The parental cross with the least similarity percentage between them is expected to exhibit highest phenotypic variation among genotypes (Tembo and Munyinda 2015; Simasiku *et al.,*. 2021). Detailed analysis using the similarity matrix (Table 5) revealed that genotypic pair LT 11-3-3-12 and BB PRT were most dissimilar genotypes with a similarity score of 30.3 %**.** Further on, low similarity levels computed between a parental genotype and some of their derived mutational lines (e.g LT PRT and LT 3-8-4-1; p and BB PRT and BB 8-1-5-2) reinforces the concept that induced mutation can create a new genotype completely differently from the parent.

Conclusion

Five (5) cowpea mutational derived genotypes were identified as resistant to *Ascochyta phaseolorum*, Thus BB 8 –1 - 5- 2, LT 4 -2- 4- 1, LT 11- 3 -3- 12, LT 3-8-4-1 and MS 1- 1 -8 – 4. These genotypes can further be evaluated for varietal release and or used as parents in the cowpea breeding program for tolerant to *Ascochyta phaseolorum* and other desirable traits. In this study, Principal component analysis revealed six distinct groups, stated as A to E. Parental genotype can be obtained from any of the distinct groups labelled as A to E but not both parents from the same group. However, in this study one parent should be from group A as all resistant genotypes clustered in one group (A). Detailed analysis using the similarity matrix (Table 5) revealed that genotypic cross pair LT 11-3-3-12 (From group A) and BB PRT (from group E) were most dissimilar genotypes with a similarity score level of 30.3 %. Thus, the cross (LT 11-3-3-12 x BB PRT) is expected to segregate and exhibit highest phenotypic variation in advanced generations.

Acknowledgements

The authors would like to acknowledge the Agriculture Productivity Programme for

Southern Africa(APPSA)for providing funding for this research

Competing Interests

Authors have declared that no competing interests exist

References

1. Adaji, M. J., Olufala, O. O., and Aliyu, L. "Effect of Intra-Row Spacing and Stand Density on the Growth and Yield of Cowpea (*Vigna unguiculata* (L.) Walp)." *Proceedings of the 41st Annual Conference of the Agricultural Society of Nigeria (ASN)*, edited by Olulaja O. O., Omokore D. F., Akpa G. N., and Sanni S. A., 22–26 Oct. 2007, pp. 153–157.
2. Agbogidi, O. M. "Response of Six Cultivars of Cowpea (*Vigna unguiculata* (L.) Walp.) to Spent Engine Oil." *African Journal of Food Science and Technology*, vol. 1, 2010b, pp. 139–142.
3. Agbogidi, O. M. "Screening Six Cultivars of Cowpea (*Vigna unguiculata* (L.) Walp) for Adaptation to Soil Contaminated with Spent Engine Oil." *Journal of Environmental Chemistry and Ecotoxicology*, vol. 7, 2010a, pp. 103–109.
4. Ajeigbe, H. A., *et al.*,. "Potentials for Cowpea (*Vigna unguiculata*) for Dry Season Grain and Fodder Production in the Sudan and Sahel Zones of West Africa." *Innovative Research Along the Cowpea Value Chain*, edited by O. Boukar *et al.*,., International Institute of Tropical Agriculture (IITA), 2012, pp. 189–202.
5. Bock, C. H., Hotchkiss, M. W., and Wood, B. W. "Assessing Disease Severity: Accuracy and Reliability of Rater Estimates in Relation to Number of Diagrams in a Standard Area Diagram Set." *Plant Pathology*, vol. 65, 2016, pp. 261–272. https://doi.org/10.1111/ppa.12403
6. Boukar, O., Massawe, F., and Muranaka, S. "Evaluation of Cowpea Germplasm Lines for Protein and Mineral Concentrations in Grains." *Plant Genetic Resources: Characterization and Utilization*, vol. 9, 2011, pp. 515–522.
7. DAFF. *Cowpea Production Guidelines*. Directorate Agricultural Information Services, 2011, www.daff.gov.za. Accessed 6 May 2019.
8. Dube, E., and Fanadzo, M. "Maximizing Yield Benefits from Dual-Purpose Cowpea." *Food Security*, vol. 5, 2013, pp. 769–779.
9. Edema, R., Adjei, E. A., and Ozimati, A. A. "Genetic Diversity of Cowpea Parental Lines Assembled for Breeding in Uganda." *Plant Molecular Biology*, vol. 41, 2023, pp. 713–725. https://doi.org/10.1007/s11105-023-01394-6
10. FAO/IAEA. *Mutation Breeding Review*. IAEA, 2004, pp. 1–42.
11. FAOSTAT. *Food and Agriculture Organization of the United Nations Statistics Division*. 2016.
12. FAOSTAT. *Food and Agriculture Organization Statistics*. 2000, www.fao.org/statistics.
13. Fatokun, C. A., *et al.*,. *Challenges and Opportunities for Enhancing Sustainable Cowpea Production*. International Institute for Tropical Agriculture, 2012, ISBN 578-131-190-8.
14. Langyintuo, A. J., *et al.*,. "Cowpea Supply and Demand in West and Central Africa." *Field Crops Research*, vol. 82, 2003, pp. 215–231.
15. Lucas, M. R., *et al.*,. "Markers for Breeding Heat-Tolerant Cowpea." *Molecular Breeding*, vol. 31, 2013, pp. 529–536.
16. Moalafi, A. I., Asiwe, J. A. N., and Funnah, S. M. "Germplasm Evaluation and Enhancement for Development of Cowpea (*Vigna unguiculata* (L.) Walp) Dual Purpose F2 Genotypes." *African Journal of Agricultural Research*, vol. 5, 2010, pp. 573–579.
17. Olasupo, F. O., *et al.*,. "Mutagenic Effects of Gamma Radiation on Eight Accessions of Cowpea (*Vigna unguiculata* [L.] Walp.)." *American Journal of Plant Sciences*, vol. 7, 2016, pp. 339–351.
18. Ragagnin, V., *et al.*,. "A New Inoculation Procedure to Evaluate Angular Leaf Spot Disease in Bean Plants (*Phaseolus vulgaris* L.) for Breeding Purposes." *Annual Report of the Bean Improvement Cooperative*, 2005, pp. 90–91.

19. Salam, M. V., *et al.*,. "A Meta–Analysis of Severity and Yield Loss from Ascochyta Blight on Field Peas in Western Australia." *Australasian Plant Pathology*, vol. 40, 2011, pp. 591–600.
20. Sankie, L., Addo-Bediako, K. O., and Ayodele, V. "Susceptibility of Seven Cowpea (*Vigna unguiculata* L. Walp) Cultivars to Cowpea Beetle (*Callosobruchus maculatus*)." *Agricultural Science Research Journals*, vol. 2, 2012, pp. 65–69.
21. Schoonhoven, A., and Corrales, P. *Standard System for the Evaluation of Bean Germplasm*. Centro Internacional de Agricultura Tropical (CIAT), 1987, p. 53.
22. Shiringani, R. P., and Shimeles, H. A. "Yield Response and Stability Among Cowpea Genotypes at Three Planting Dates and Test Environments." *African Journal of Agricultural Resources*, vol. 6, 2011, pp. 3259–3263.
23. Singh, B. B., *et al.*,. "Improving the Production and Utilization of Cowpea as Food and Fodder." *Field Crops Research*, vol. 84, 2003, pp. 169–177.
24. Singh, B. B., and Ajeigbe, H. A. "Improved Cowpea-Cereals-Based Cropping Systems for Household Food Security and Poverty Reduction in West Africa." *Journal of Crop Improvement*, vol. 19, 2007, pp. 157–172.
25. Simasiku, M., Wasige, E. J., and Tembo, L. "Multivariate Analysis of Cotton Genotypes Based on Agronomic Traits." *Journal of Genetics, Genomics and Plant Breeding*, vol. 5, 2021, pp. 86–94.
26. Tembo, L., and Munyinda, K. "Clustering Bean Mutants Based on Heterotic Grouping." *African Crop Science Journal*, vol. 1, 2015, pp. 1–7.
27. Tembo, L., *et al.*,. "Resistance to *Callosobruchus maculatus* Developed via Gamma Radiation in Cowpea." *Journal of Agriculture and Crops*, vol. 3, 2017, pp. 65–71.
28. Than, P. P., *et al.*,. "Characterization and Pathogenicity of *Colletotrichum* Species Associated with Anthracnose Disease of Chilli (*Capsicum* spp.) in Thailand." *Plant Pathology*, vol. 57, 2008, pp. 562–572.
29. Türnnkkan, M., and Dolar, F. S. "Determination of Pathogenic Variability of *Didymella rabiei*, the Agent of Ascochyta Blight of Chickpea in Turkey." *Turkish Journal of Agriculture Forum*, vol. 33, 2009, pp. 585–591.
30. Wamali, P. A. *Studies on Ascochyta Blight on Cowpea (Vigna unguiculata L. Walp) and Characterization of Isolates of Its Causal Organism, Ascochyta phaseolorum Sacc.* MSc Thesis, University of Nairobi, 1984.

Source of support: Nil;
Conflict of interest: The authors declare no conflict of interests.

Cite this article as:
Malumo, J., Kachapulula, P. and Tembo, L. "Resistance to *Ascochyta phaseolorum* and Multivariate Analysis of Mutational Derived Cowpea Lines.' *Annals of Plant Sciences.* 14.04 (2025): pp. 6775-6784.