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Influence of Colchicine Treatments on Character Expression and Yield Traits in Cowpea (*Vigna Unguiculata* L. Walp)

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Abstract- Mutagenesis has been exploited to enhance genetic variability in cowpea (Vigna unquiculata L. Walp.); an important legume in the tropical and subtropical regions of the world. Mutation is regarded to be a shortcut breeding technique, which has produced new and high yielding varieties through heritable changes in genetic constitution of characters in some leguminous crops. Effects of 0.1% aqueous solution of colchicine for different periods of time, viz; - 0, 2, 4 and 6 hours were tested on the quantitative and yield characters of a cowpea variety popularly known as 'Oloyin' in M1 generation. Lethal dose value (LD40) of 59% was observed at 2 hours treatment. Treatment was significant (P = 0.05) for seedling emergence percentage (67 – 13%), plant height (21.52 – 15.63 cm), number of leaves (11.08 – 4.98), number of nodes on main stem (6.26 - 4.5), survival percentage (63.50 – 12.50%) and number of days to first flowering (55.52 – 47.30) while treatment was not significant for all other characters studied.

Keywords: *mutagenesis, genetic variability, induced variation.*

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Abstract- Mutagenesis has been exploited to enhance genetic variability in cowpea (Vigna unquiculata L. Walp.); an important legume in the tropical and subtropical regions of the world. Mutation is regarded to be a shortcut breeding technique, which has produced new and high yielding varieties through heritable changes in genetic constitution of characters in some leguminous crops. Effects of 0.1% aqueous solution of colchicine for different periods of time, viz; - 0, 2, 4 and 6 hours were tested on the quantitative and yield characters of a cowpea variety popularly known as 'Oloyin' in M1 generation. Lethal dose value (LD40) of 59% was observed at 2 hours treatment. Treatment was significant (P = 0.05) for seedling emergence percentage (67 – 13%), plant height (21.52 – 15.63 cm), number of leaves (11.08 – 4.98), number of nodes on main stem (6.26 - 4.5), survival percentage (63.50 – 12.50%) and number of days to first flowering (55.52 – 47.30) while treatment was not significant for all other characters studied. The results revealed that colchicine can be used to induce variations in cowpea which may be of agronomic importance in the production of this crop.

Keywords: mutagenesis, genetic variability, induced variation.

I. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is one of six major cultivated crop species of the family *Leguminosae* distributed throughout the tropics (Padulosi and Ng, 1997; Pasquet, 2001). It is the second most important grain legume crop after groundnut (Blade *et al*., 1997). Cowpea has been reported as an important food crop throughout Sub –Saharan Africa (SSA) (Kitch *et al*., 1998) and one of the major sources of plant protein in the developing countries including Nigeria (Adekola and Oluleye, 2007). Its grain and leaves have high quality protein and vitamins which serves as an excellent food supplement in developing countries (Kitch *et al*., 1998). Millions of relatively poor people in low income countries in the tropics rely on it for their livelihood and as protein supplement (Ajayi, 2014). Hence, it is a key staple food crop for ever-increasing population both in the rural and urban areas. Cowpea has a great ability to fix atmospheric nitrogen in the soil, thereby improving soil nutrients (Adetiloye *et al*., 2013). Ajayi and Adesoye (2013) reported that cowpea production has been consistently hindered by low grain yields and quality, and lack of improved cultivars. Dhanavel *et al* (2012) reported induced mutation as a valuable supplement to conventional breeding in crop improvement programs, but has been least applied in grain legumes like cowpea. Induced mutations have been used successfully to improve yield and yield components of many crops like *Oryza sativa, Hordeum vulgare, Triticum durum, Vicia faba, Cicer arietinum, Cajanus cajan,* in the world (Khan and Wani, 2006). Improvement of legumes such as cowpea through induced mutation could make it possible to identify new genes and thus broaden the spectrum of heritable changes and expand cowpea germplasms.

To enhance the limited genetic variability in cowpea, mutagenesis has been exploited and efforts made at identifying the proper mutagens in cowpea breeding which can produce mutants for future breeding programs (Achaya *et al*., 2007). Acceleration of frequency of mutation in cowpea has been accomplished by exposure of seeds to mutagenic agents like ionizing radiation and / or chemical mutagens (Natarajan, 2005). Colchicine treatment was reported as one of the best tools of inducing and enhancing genetic variability in some food crops within a very short period of time (Gnanamurthy *et al*., 2013). Hence, this study focused on the influence of colchicine treatments on characters expression and yield of cowpea.

II. Materials and Methods

This research was conducted at the Research Laboratory of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. Modified techniques of Khan and Wani (2006); Kumar and Verma (2011); Ajayi (2014) were employed for this study.

A cowpea variety popularly known as "Oloyin" was obtained from a local farmer in Akungba-Akoko, Ondo State, Nigeria. A total of 800 healthy seeds of uniform size were used for this study. Two hundred seeds per treatment were soaked in distilled water for 12 hours, after which the water was drained, and seeds spread and air dried on a filter paper before being treated with colchicine.

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Two hundred seeds per treatment were soaked in 0.1% (w/v) aqueous colchicine solution for 2, 4 and 6 hours at room temperature while the control was left untreated. Seeds were later transferred into a sterile cloth, tied and rinsed in running water for 20 min to terminate the residual effect of colchicine. The treated seeds were later air dried for 15 hours before sowing.

Treated seeds along with the control were sown in the field to generate M1 generation in a randomized complete block design with five replications, during the rainy season of 2013 at the Research Field of Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria. Two hundred seeds from each treatment and control were planted in the field adopting an intra-row spacing of 50cm and inter- row 30cm.

a) Data collection

Data on 15 quantitative traits were collected from 10 randomly selected plants per replicate for control and from all surviving plants per replicate for the treated plants. The quantitative traits included;- seedling emergence percentage (7- 20 days after sowing); plant height (cm) taken at 4weeks and 8 weeks after sowing; number of leaves; terminal leaflet length (cm); terminal leaflet width (cm); number of main branches; number of nodes on main stem taken at 7 weeks after sowing; survival percentage; days to first flowering; number of peduncles per plant; peduncle length (cm); number of pods per plant; pod length (cm); number of seeds per pod and 100-seed weight (g) were taken at maturity.

Data were analyzed using Statistical Package for Social Science (SPSS) version 20 (SPSS, Inc., Chicago IL). Analysis of variance (ANOVA) was performed, followed by least significant difference (LSD; P = 0.05% level of significance) computation for mean separation.

III. Results and Discussions

Chemical mutagenesis has been a beneficial technique in the improvement of yield characters in crop breeding. Variability of quantitative traits influencing yield have been greater in mutagenic progenies than in control. Ability of mutagens to enter the cell of the living organisms to interact with DNA produces the general toxic effects associated with their mutagenic properties (Mensah *et al*., 2007). It has been widely proved that chemical mutagens induce physiological damages (injury), gene mutations and chromosomal aberration in M1 individuals which can be detected and measured from seed germination or emergence of seedlings, survival reduction (lethality), plant height reduction (injury) and fertility reduction or sterility (reduction in pod and seed formation) (Kumar *et al*., 2009) which might not be restricted to M1 generation (Mak *et al*., 1986). Analysis of variance revealed that treatment effect was significant ($P \leq 0.05$) for emergence percentage, plant height, number of leaves, number of nodes on main stem, survival percentage, and number of days to flowering in the M1 generation whereas treatment effect was not significant for all other traits (Table 1). Means for emergence percentage from 7th day after sowing to 20th day after sowing established that colchicine treatment reduced emergence of seedlings with direct correlation with the duration of exposure. At 20days after sowing, emergence of seedlings ranged from 67% (control) to 25% (6 hours). Colchicine significantly decreased the emergence of seedlings compared with the control. About 40% reduction of emergence was found at 2 hours of exposure; therefore, LD_{40} value (59%) was fixed at 2 hours duration of treatment (Table 2). Reduction in emergence of seedlings and survival as a result of colchicine treatments agrees with the findings of many workers on cowpea (Kumar *et al*., 2009; Girija and Dhanavel, 2009; Gnanamurthy *et al*., 2013) and in many other crops like black gram (Deepalaskshmi, 2000; ThangaHamavathy, 2002). The damage to the biological materials according to these findings might be considered as an indication of the mutagenic effects.

Survival percentage at maturity ranged from 63.50% (control) to 12.5% (6 hours). Survival was significantly reduced with increased duration of colchicine treatment (Table 4). The linear relationship of treatment duration on survival has been observed by many workers. In most cases, the mortality may be due to poor seedling vigor resulting from inability to overcome the toxic effect of colchicine (Zlesak *et al*., 2005).

Plant height was significantly reduced by all treatments generally compared with the control, with the highest duration of time producing the shortest heights, 7.46cm (4 weeks) and 15.63cm (8 weeks); whereas the control had the highest heights of 15.57cm at week 4 and 21.52cm at week 8 (Table 3). The reduction of growth observed with increase in duration of treatment is very common in M1 generation of many mutated crops, which is actually as a result of reduction in the rate of cell division among the treated plants and also linked to chromosomal abnormality, reduction of auxin levels, inhibition of auxin synthesis and failure of assimilation mechanisms (Riley, 1954).

Number of leaves was more in the control than other treatments: the number of leaves being 11.08 (control), 8.86 (2 hours), 4.98 (4 hours) and 6.79 (6 hours). There was a significant difference in treatment effects for number of leaves. The treated plants were found to possess longer leaflets and wider leaflets compared to control as also confirmed by Ajayi *et al*. (2010). This may actually be a useful trait for breeding for its potential to increase the net photosynthetic area which may lead to increase in photosynthetic assimilates going into grains as a positive effect of seed yield (Priya, 2006). It has also been proved that

successful colchicine treatment has been found to result in plants that possess characters such as thicker-wider leaves with bigger and fewer stomata number (Uhlik, 1981).

Number of nodes on main stem showed a significant difference among the treatments, with the 2 hours having the highest number of nodes (6.26), followed by the control (6.22), and while the lowest number of leaves was for the 4hour treatment (4.50). Control flowered earlier (47.30 days) than all other treatments. Increase in duration of exposure delayed flowering. The mean number of days to flowering among the treated plants was 47.50 (2 hours), 54.97 (4 hours) and 55.12 (6 hours). The difference between the control and other treatments was significant. This delay in flowering with direct correlation with increased exposure period is not novel as it has been observed by many workers especially in soybean (Maheshwari *et al.*, 2003; Pavadai and Dhanavel, 2005), in mungbean (Khan and Wan, 2005).

The decrease in peduncle length, number of pods per plant, pod length and number of seeds per pod all contradict the results of Odeigah (1998) on M1 generation of cowpea but consistent with Kumar *et al.* (2009), who reported that reduction in pod number may be as a result of inhibiting action of enzymes, changes in enzymatic activities and toxicity of the mutagen on these traits, while reduced seed yield can be attributed to high seed sterility and reduced pod number, also as consequences of physiological and biochemical disturbances in the development of plants (Prabhakar, 1985) resulting from mutagenic treatment (Ajayi *et al.*, 2014). Seed weight was however enhanced by 2hour duration of exposure but was further reduced by treatment at higher exposure of time. Some of the characters studied decreased linearly as the duration of treatment increased why most of them showed irregular pattern of behaviour with increase in duration of treatment.

IV. Conclusion

From the results obtained, seed treatment has proven to be a viable method for inducing variability in cowpea through mutagenesis. The level of dissimilarity among the plants as a result of treatment was high and this indicates a possible improvement through this approach. Although colchicine reduced most of the morphological characters at M1, this is always expected as it is a usual phenomenon in M1 generation.

Table 1 : Mean square values of all traits for colchicine treatment

CHARACTER	DF	REPLICATION	TREATMENT	ERROR
EP (20 DAS)	3	452.66*	3753.75*	47.24
PH(8 WAS)	3	50.04*	36.04*	5.99
NL	3	2.45	34.64*	3.86
NN	3	1.34*	5.66*	0.78
TLL (cm)	3	1.21	1.78	2.31
TLW (cm)	3	0.84	0.56	0.82
NMB	3	0.63	0.83	1.19
SUP	3	358.91*	3176.98*	32.45
PPP	3	39.36	94.48	53.51
PEL (cm)	3	55.85*	50.19	14.42
NDF	3	45.07	97.51*	19.94
NPP	3	5.62	2.58	8.22
PL (cm)	3	2.88	1.48	1.35
SP	3	0.86	3.52	3.67
SW (g)	3	0.04	0.81	0.89

*: Significant

EP: Emergence percentage; PH: Plant height; NL: Number of leaves per plant; NN: Number of nodes on main stem; TLL: Terminal leaflet length; TLW: Terminal leaflet width; NMB: Number of main branches; SUP: Survival percentage; PPP: Peduncle per plant; PEL: Peduncle length; NDF: Number of days to flowering; NPP: Number of pods per plant; PL: Pod length; SP: Seeds per pod; SW: Seed weight; DAS: Days after sowing; WAS: Weeks after sowing

Table 2 : Emergence percentage of colchicine treated cowpea and control from 7 DAS to 20 DAS (Mean ±Standarderror)

Treatment	7DAS	8DAS	9DAS	13DAS	18DAS	20DAS
Control	55.00±7.70[a]	62.00±5.55[a]	63.50±5.45[a]	65.00±5.53[a]	67.00±5.26[a]	67.00±5.26[a]
2Hrs	28.50±4.07[b]	38.50±7.85[b]	44.00±7.05[b]	55.00±8.60[b]	55.50±8.11[b]	59.00±7.81[b]
4Hrs	11.00±2.44[c]	11.50±2.44[c]	12.00±3.00[c]	15.00±4.10[c]	16.80±4.58[c]	18.50±5.35[c]
6Hrs	11.50±2.31[c]	12.00±2.12[c]	12.50±2.50[c]	14.00±2.80[c]	13.00±2.42[c]	13.00±2.42[c]
LSD	12.27	13.48	11.46	11.76	9.66	9.47
CV (%)	33	32	25	23	18	18

Mean values followed by same letters within a column are not significantly different

DAS: Days after sowing; LSD: Least significant difference; CV: Coefficient of variation

Table 3 : Mean and Standard error values of plant height of colchicine treated and untreated cowpea

Week	Control	2Hrs	4Hrs	6Hrs	LSD	CV (%)
4WAS	15.57±0.6[a]	10.93±0.7[b]	6.69±0.50[c]	7.46±0.78[c]	1.93	13.9
8WAS	21.52±1.8[a]	17.53±1.4[b]	16.05±2.03[b]	15.63±2.04[b]	3.37	13.84

Mean values followed by same letters within the same row are not significantly different

LSD: Least significant difference; CV: Coefficient of variation; WAS: Weeks after sowing

Table 4 : Quantitative traits (Mean ± Standard error) of control and colchicine treated cowpea

Treatment	NL	NN	TLL	TLW	NMB	SUP	PPP	PEL	NDF	NPP	PL	NSP	SW
Control	11.08±1.29a	6.22±0.43b	11.73±0.07a	7.57±0.16a	7.30±0.41a	63.50±4.23a	27.56±3.29a	24.90±1.59a	47.30±1.43c	9.00±0.89a	14.65±0.54a	10.78±0.75a	12.92±0.51a
2hrs	8.06±0.42b	6.26±0.26a	15.04±0.70a	8.38±0.56a	7.84±0.42a	52.00±6.49b	28.52±4.27a	25.32±0.86a	47.50±0.83c	8.76±1.09a	14.62±0.36a	9.37±0.68a	12.50±0.32a
4hrs	4.98±0.35c	4.26±0.22c	14.36±0.81a	7.84±0.41a	6.40±0.60a	17.50±5.06	18.89±2.69a	24.85±2.36a	54.97±1.53b	8.58±1.65a	13.84±0.68a	11.06±0.73a	13.30±0.81a
6hrs	6.79±0.90c	4.50±0.36c	14.91±0.55a	7.96±0.36a	6.94±0.34a	12.50±2.37	24.25±1.91a	18.70±3.32a	55.52±3.99a	7.38±1.16a	13.58±0.71a	11.19±0.90a	12.44±0.38a
LSD	2.7	0.84	NS	NS	NS	7.85	NS	NS	6.15	NS	NS	NS	NS
CV (%)	24.78	11.52	10.43	11.41	15.34	15.66	29.49	16.19	8.72	34	8.21	18.06	7.37

Mean values followed by same letters within the column are not significantly different

NL: Number of leaves per plant; NN: Number of nodes on main stem; TLL: Terminal leaflet length; TLW: Terminal leaflet width; NMB: Number of main branches; SUP: branches; SUP: Survival percentage; PPP: Peduncle per plant; PEL: Peduncle length; NDF: Number of days to flowering; NPP: Number of pods per plant; PL: Pod length; SP: Seeds per pod; SW: Seed weight; LSD: Least significant difference; CV: Coefficient of variation.

References Références Referencias

1. Acharya, S.N., Thomas, J.E and Basu, S.K 2007. Improvement in medicinal and nutritional properties of fenugreek (*Trigonella foenum graecum* L). in: S.N. Acharya, J.E. Thomas (eds) Advances in medicinal plant research, Research Signpost, Trivandrum, Kerala, India.
2. Adekola, O.F and Oluleye, F. 2007: Induction of genetic variation in cowpea (*Vigna unguiculata* L.Walp) by Gamma Irradiation. Asian journal of plant sciences 6 (5): 869-873.
3. Adetiloye, I.S., Ariyo, O.J., Alake, C.O., Oduwaye, O.O and Osewa, S.O. 2013. Genetic diversity of some selected Nigeria cowpea using simple sequence repeats (SSR) marker, *Afr. J. Agric. Res*. 8 (7): 586 – 590.
4. Ajayi, A.T. 2014: Variations in germination, survival and yield characters of a colchicine-induced m1 generation of cowpea [*Vigna unguiculata* (L.) Walp]. Applied science research journal. 2014 vol 2 (1) 1 – 9.
5. Ajayi, A.T and Adesoye, A.I. 2013. Cluster analysis technique for assessing variability in cowpea *(Vigna unguiculata* L. Walp).
6. Blade S.F, Shetty S.V.R, Terao T, Singh B.B. 1997. Recent developments in cowpea cropping systems research In: Singh BB, Mohan Raj DR, Dashiell K.E, and Jackai L.E.N. (eds). Advances in Cowpea.
7. Deepalakshmi, A.J. 2000. Creation of variability in black gram (*Vigna mungo* L. Hepper) through induced mutagenesis. MSc. (Ag.) Thesis, Tamil Nadu Agric. Univ. Coimbatore, India.
8. Dhanavel, D., Gnanamurthy, S and Girija, M. 2012. Effect of gamma rays on induced chromosomal variation in cowpea (*Vigna unguiculata* L. Walp.). *Int. J. Curr.* Sci. 245 – 250.
9. Girija, M and Dhanavel, D . 2009. Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combined treatment in cowpea (*Vigna unguiculata* L. Walp.). Glo. *J. Mol. Sci.* 4 (2): 68 – 75.
10. Gnanamurthy, S., Dhanavel, D and Girija, M. 2013. Effect of gamma irradiation on the morphological characters of cowpea (*Vigna unguiculata* L. Walp.). *Int. J. Cur. Tr. Res.* 2 (1): 38 – 43. IITA-JIRCAS, Ibadan, pp 1–12.
11. Khan, S and Wani, M.R. 2005. Comparison on the effect of chemical Mutagens on mungbean. *Adv. Plant Science* 18 (11): 533-535.
12. Khan, S and Wani, M.R 2006. Estimate of genetic variability in mutated populations and scope of selection for yield attributes in *Vigna radiata* (L.) Wilczek. *Egyptian Journal of Biology,* 8 pp. 1 – 6.
13. Kitch, L.W., Boukar, O., Endondo, C. & Murdock, L.L., *Expl Agric.*, 1998, 34: 475–486.
14. Kumar, G and Verma, S. 2011. Induction of quantitative variability through EMS treatment in *Vigna unguiculata Rom. J. Biol* – Plant Biol. 56 (2): 91 – 97.
15. Kumar, V.A., Kumari, R.U., Amutha, R., Kumar, T.S., Hepziba, S.J and Kumar, C.R.A. 2009. Effect of chemical mutagen on expression of characters in arid legume pulse – cowpea (*Vigna unguiculata* L. Walp.). *Research Journal of Agriculture and Biological Sciences*, 5 (6): 1115 – 1120.
16. Maheshwari, J.J., Patil, S., Dhole, V.J and Rathod, D.R. 2003. Radiation induced variability for quantitative characters in soybean . *J. soils* and *crops* 13(12): 314-316.
17. Mak, C., Teoh, S.B., and Ratnam, A .1986. The influence of Gamma rays on the injury and chromosomal aberrations of long bean (*Vigna sesquipedalis Fruw*.). Journal of Petranika, ((1): 109-117.
18. Megloire, N. 2005. The genetic, morphological and physiological evaluation of African cowpea genotypes. University of Free State, South Africa.
19. Mensah, J.K., Obadoni, B.O., Akomeah, P.A., Ikhajiagbe, B and Ajibolu, J. 2006. The effects of sodium Azide and Colchicine treatments on morphological and yield traits of sesame. African Journal of Biotechnology 6(5), pp534-538.
20. Natarajan, A.T. 2005. Chemical mutagenesis: from plants to human. *Curr. Sci*. 89(2):312-316.
21. Padulosi S, Ng N.Q. 1997. Origin, taxonomy and morphology of *Vigna unguiculata* [L.] Walp). In: Singh B.B, Mohan Raji D.R, Dashiell K.E, Jackai L.E.N. eds. Advances in Cowpea Research 1997.
22. Pasquet R. *Vigna savi.* In: Mackinder B, Pasquet R, Polhill R, Verdcourt B eds. Flora zambesiaca, volume part *Phaseoleae.* 2001; Royal Botanic Gardens, Kew, pp 121–156.
23. Pavadai, P and Dhanavel, D. 2005. Effect of gamma rays on yield and its components in Soybean (Glycine max L.) Merrill. Var.Co. *Crop Research* 30 (3): 459-461.
24. Prabhakar, L.V .1985. Studies on induced mutagenesis in *Sesamum indicum* L. MSc. (Ag). Thesis, Tamil Nadu Agricultural University, Coimbatore, India.
25. Priya R.T. 2006. Induced macromutation in mungbean (*Vigna radiata* (L) Wilczek). *International Journal of Botany* 2(3): 219-228.
26. Riley, E.F .1954. The effect of X-rays upon growth of Avena seedlings. *Rad. Res.* 1: 227 – 228
27. ThangaHemavathy, A .2002. Creation of variation in black gram (*Vigna mungo* L. Hepper). M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ. Coimbatore.
28. Uno, G., Storey, R., and Moore, R. 2001. *Principles of Botany.* Mc Graw Hill New York 1-550pp.
29. Zlesak, D.C., Thill, C.A and Anderson, N.O. 2005. Trifluralin-medxiated polyploidization of *Rosa chinensis* minima (Sims) Voss seedlings. Euphytica 141: 281 – 290.