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Original Article

EFFECTS OF COLCHICINE ON SOME AGRO-MORPHOLOGICAL TRAITS OF *PHASEOLUS LUNATUS* (L.) AT M_1 AND M_2 GENERATION

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ABSTRACT

The mutagenic effects of colchicine on the agro-morphological traits at M_1 and M_2 generations of *P. lunatus* were studied. Seeds of the *P. lunatus* obtained from local farmers within Zaria, was treated with colchicine at various concentrations of 0.2mM, 0.4mM, 0.6mM and 0.8mM before they were sown with their respective control (0.00mM), to assess the effects of the different concentration of the mutagen on the plant at M_1 and M_2 generation. The result revealed that the effects of the chemical mutagen, varied with the concentration. However, lower concentration (0.2mM) of colchicine favored percentage germination, days to first and 50% flowering while higher concentration (>0.2mM) enhanced some yield traits such as pod length, pod diameter, number of pods per plant, number of seed per pod, pod weight and 100 seeds weight. Agro-morphological data obtained in this study were significant at $p \leq 0.05$.

Keywords: Colchicine, Mutagenic effect, Agro-morphological, *P. lunatus,* M_1 and M_2 generation.

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INTRODUCTION

The use of mutagens in crop improvement helps to understand the mechanism of mutation induction and to quantify the frequency as well as the pattern of changes in different selected plants by mutagens; mutation breeding generates a knowledge base that guides future users of mutation technology for crop improvement (Mensah *et a*l., 2007). Colchicine is both a polyploidising and mutagenic agent (Bragal, 1955). This chemical has been used for a long time to produce polyploid plants. The mutagenic effects on plant morphology, chlorophyll, sterility and yield have earlier been confirmed by Ahoowalia (1967), Mensah (1977) and Castro *et al*. (2003). Lima bean (*Phaseolus lunatus* L.) is a tropical and subtropical legume cultivated for its edible seeds, its sprouts, leaves, young pods and green seeds (immature or dry) are edible and eaten as vegetables. The dry seeds are

eaten boiled, fried, ground into powder and baked, and used in so-ups and stews (Baudoin, 2006). The vines, leaves and empty pods left after the harvest can serve as fodder, and can be made into hay or silage. Lima bean may be used for green manure or as a cover crop. Lima bean might be valuable in intercropping systems, though only few cultivars are suitable for this (Baudoin, 2006). However, there is paucity of information on the locally cultivated landraces, which lack variability because of lack of improved technology targeted towards the production of the crop. Therefore, this research was focused at determining the effects of colchicine on some agro-morphological traits of *P. lunatus* at M_1 and M_2 generation.

MATERIALS AND METHODS

Seed Source and Mutagenic Treatment

Healthy landraces of *P. lunatus* were obtained from local farmers in Zaria, Kaduna State, Nigeria and landrace identity was confirmed at International Centre for Tropical Agriculture (CIAT), Cali, Colombia. About 0.25kg of healthy dry seeds of *P. lunatus* was presoaked in distilled water for 6 hours and afterwards, soaked in freshly prepared concentrations of Colchicine [0.0mM (control), 0.2mM, 0.4mM, 0.6mM, 0.8mM] for 6 hours. After treatment with the mutagen, the seed were washed thoroughly to remove the residual effects of mutagens, if any.

Experimental design and sowing of seeds

Top soil was collected from uncultivated land within the botanical garden in Ahmadu Bello University, Zaria and after the soil was treated by heating, it was filled into one hundred and fifty (150) polythene bags arranged in a randomized complete block design (RCBD) with three replications for each treatment to raise M_1 and M_2 generation. The polythene bags (51.5 x 38.3cm) were half filled each weighing 8.5kg and placed at a spacing of 10 x 90 cm. A total of 1.35×10^{-3} hectares (13.5msq) of land was used for this research. Three seeds were sown in each bag which was later thinned to one per bag.

Sample collection

Agro-morphological data such as Percentage germination, plant height at 4 weeks after sowing, 8weeks after sowing,12 weeks after sowing, number of days to first flowering, number of days to 50% flowering, number of pods per plant, pod length, pod weight, pod diameter, number of seeds per pod and 100 seeds weight.

Data analyses

Agro-morphological data obtained was subjected to one way analysis of variance (ANOVA) using SAS (2002) version 9.1 to determine the significant effect of different concentrations of the mutagens ($p \leq 0.05$) and Duncan's Multiple Range test (DMRT) was used to separate the means obtained, where significant.

RESULTS

Effects of Colchicine on Agronomic Traits of *P. lunatus* at M_1 Generation

Percentage germination, days to first flowering, number of pods per plant, pod length, pod diameter, pod weight and 100 seed weight were significantly different ($P \leq 0.05$) as shown in Table 1. The control treatment recorded the highest percentage germination (100.00%) while the least germination percentage (70.00%) was observed at 0.8mM. the highest (76.33) number of days to first flowering was recorded at 0.8mM and this was closely (75.00) followed by 0.4mM while the fewest

(71.33) was recorded at 0.2mM. The number of pods per plant increased with an increase in concentration of colchicine, 0.8mM produced the highest (23.40) number of pods while 0.2mM produced the least (14.07). Pod length was highest (6.20cm) at 0.4mM and least (5.53cm) at 0.8mM while pod diameter was highest (1.67cm) at 0.2mM and least (1.33cm) at the control. Pod weight also varied with concentration as 0.4mM recorded the highest (7.83g) and the least (5.50g) was recorded by the control. Weight of a 100 seeds was highest (34.17g) at 0.2mM and least (29.33g) at 0.6mM of colchicine which was closely followed by the control (29.63g). All the traits mentioned above varied significantly at $P \leq 0.05$.

Effects of Colchicine on Agronomic Traits of *P. lunatus* at M_2 Generation

The result of different concentrations of colchicine in the M_2 generation of *P. lunatus* recorded significant difference in days to first flowering, days to 50% flowering, pod length, pod diameter, pod weight and 100 seed weight as shown in Table 2. The highest (58.00) number of days to first flowering was observed at 0.8mM while the fewest (51.33) was recorded at 0.6mM which was closely followed by 0.2mM with 51.67 days. days to 50% flowering were differed slightly with 0.8mM fewest (61.67) number of days to 50% flowering while the control and 0.2mM recorded the highest (64.33) number of days. Pod length and pod diameter were observed to increase with an increase in concentration, the highest pod length and diameter (5.88cm and 1.64cm) were recorded at 0.8mM while the control recorded the least (4.81cm) pod length and 0.2mM recorded the least (1.48cm) pod diameter which was closely followed by the control with a pod diameter of 1.50cm. Similarly, the pod weight increased with an increase in concentration of the mutagen where 0.8mM produced the highest (6.43g) while the control had the least (4.33g). On the other hand 100 seed weight had the significantly highest mean value (33.83g) at 0.6mM and the least mean value (28.50g) at 0.4mM.

Table 1: Effects Colchicine on Agronomic Traits of *P. lunatus* at M_1 Generation

TRT	PGERM (%)	PH4 (cm)	PH8 (cm)	PH12 (cm)	DFF	D50%F	NPPP	PDLT (cm)	PDDIA (cm)	PDWT (g)	NSPP	100SWT (g)
0.0Mm	100.00^{a}	33.17^{a}	96.83^{a}	148.40^{a}	74.33^{ab}	83.00^{a}	17.53^{b}	5.67^{b}	1.33^{c}	5.50^{b}	2.80^{a}	29.63^{b}
0.2Mm	83.33^{b}	35.17^{a}	121.17^{a}	161.17^{a}	71.33^{b}	80.33^{a}	14.07^{d}	5.85^{ab}	1.67^{a}	6.70^{ab}	2.73^{a}	34.17^{a}
0.4Mm	80.00^{bc}	31.58^{a}	150.50^{a}	210.07^{a}	75.00^{a}	83.00^{a}	15.27^{c}	6.20^{a}	1.60^{ab}	7.83^{a}	2.67^{a}	32.17^{ab}
0.6Mm	73.33^{bc}	36.83^{a}	124.42^{a}	140.20^{a}	73.33^{ab}	81.33^{a}	16.60^{b}	5.58^{b}	1.51^{abc}	6.47^{ab}	2.83^{a}	29.33^{b}
0.8mM	70.00^{c}	42.18^{a}	132.25^{a}	159.97^{a}	76.33^{a}	84.33^{a}	23.40^{a}	5.53^{b}	1.45^{bc}	6.00^{b}	2.47^{a}	32.17^{ab}
SEM±	3.42	3.44	18.51	28.37	0.88	1.45	0.33	0.13	0.06	0.5	0.19	0.98

NOTE: Means with the same letter within a column are not significantly different at $P \leq 0.05$

TRT- treatment, PGERM- percentage germination, PH4-plant height at 4 weeks, PH8-plant height at 8 weeks, PH12-plant height at 12weeks, DFF-days to first flowering, D50%F-days to 50% flowering, NPPP-number of pods per plant ,PDLGTH-pod length, PDDIA- pod diameter, PDWT-pod weight, NSPP-number of seeds per pod 100SWT-one hundred seeds weight

Table 2: Effects Colchicine on Agronomic Traits of *P. lunatus* at M_2 Generation

TRT	PGERM (%)	PH4 (cm)	PH8 (cm)	PH12 (cm)	DFF	D50%F	NPPP	PDLT (cm)	PDDIA (cm)	PDWT (g)	NSPP	100SWT (g)
0.0mM	90.00^{a}	44.17^{a}	97.76^{a}	114.19^{a}	54.33^{b}	64.33^{a}	7.29^{a}	4.81^{c}	1.50^{b}	4.33^{b}	2.27^{a}	30.00^{b}
0.2mM	96.67^{a}	49.95^{a}	104.05^{a}	95.49^{a}	51.67^{c}	64.33^{a}	5.87^{a}	5.25^{b}	1.48^{b}	5.27^{ab}	2.13^{a}	27.00^{b}
0.4mM	96.67^{a}	40.04^{a}	81.58^{a}	92.99^{a}	52.33^{c}	63.33^{a}	6.60^{a}	5.67^{ab}	1.54^{ab}	5.27^{ab}	2.00^{a}	28.50^{b}
0.6mM	93.33^{a}	41.57^{a}	98.12^{a}	96.29^{a}	51.33^{c}	63.57^{a}	7.00^{a}	5.65^{ab}	1.58^{ab}	6.17^{a}	2.07^{a}	33.83^{a}
0.8mM	90.00^{a}	52.76^{a}	109.95^{a}	113.65^{a}	58.00^{a}	61.67^{b}	8.33^{a}	5.88^{a}	1.64^{a}	6.43^{a}	2.40^{a}	31.00^{ab}
SEM±	5.73	7.11	8.23	7.69	0.41	0.45	0.7	0.13	0.03	0.37	0.18	0.98

NOTE: Means with the same letter within a column are not significantly different at $P \leq 0.05$
TRT- treatment, PGERM- percentage germination, PH4-plant height at 4 weeks, PH8-plant height at 8 weeks, PH12-plant height at 12weeks, DFF-days to first flowering, D50%F-days to 50% flowering, NPPP-number of pods per plant ,PDLGTH-pod length, PDDIA- pod diameter, PDWT-pod weight, NSPP-number of seeds per pod 100SWT-one hundred seeds weight

DISCUSSION

Percentage germination in the M_1 generation decreased continuously with an increase in concentration of the mutagen and may be as a result of the inhibitory effect of chemical mutagens on seeds leading to a reduced germination percentage (Dhakhanamoorthy *et al.*, 2010; Pande and Khetmalas, 2012). At M_2 generation, although percentage germination was not significantly different from the control, the effect of the chemical mutagen was not as pronounced at M_2 compared to the M_1 generation. This result conforms to that of Mensah *et al.* (2005) and Essel *et al.* (2015) when they reported reduction in percentage germination due to increasing concentration in sesame and cowpea respectively with the effects more pronounced in C_1 generation than C_2 generation. However, studies by Udensi and Ontui (2013) on pigeon peas recorded a contrary result.

The number of days to first flowering in both generations and 50% in the first mutant generation differed from the control with the highest number of day to first flowering and 50% flowering observed at the highest concentrations of treatment with colchicine, which may be due to the physiological changes caused by mutagen (Dhakhanamoorthy *et al.*, 2010). This conforms to the findings of Essel *et al.* (2015) when they reported that higher concentrations of colchicine treatments in the C_1 generation led to delay in flowering suggesting that the chemical may have interfered with maturity and early flowering.

Pod length, pod diameter, pod weight and 100 seeds weight in both generations differed significantly from the control and this may be as a result of the mechanism of action of colchicine. This result conforms to the findings of Sadiq (2015) who treated Soybean with Colchicine, Mathew (2014) who treated pigeon pea with sodium azide as well as Wani and Khan (2006) who reported similar increase in number of pod, 100 seed weight, pod dry weight and also decrease in pod dry weight in *Vigna radiata* (L).

The absence of significant difference in the number of pods at the M_2 generation as compared to the M_1 generation may be due to the fact that the change induced in the genome at M_1 is not heritable or was due to the influence of the environment and therefore cannot be passed on to the subsequent (M_2) generation.

Plant height 4WAS, 8WAS, 12WAS and Number of seeds per pod were not significantly different from their control in both generations, this maybe as a

result of the fact that the gene responsible for the expression of these traits were not affected by treatment with the chemical mutagen, thus showing no significant agronomic variation from the control.

CONCLUSION

This study generally revealed that lower concentration (0.2mM) of colchicine favored percentage germination, days to first and 50% flowering while higher concentration (>0.2mM) enhanced some yield traits such as pod length, pod diameter, number of pods per plant, number of seed per pod, pod weight and 100 seeds weight. These concentrations depending on the objective of the plant breeder can be adopted for further improvement of *Phaseolus lunatus*.

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