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EFFECT OF COLCHICINE TREATMENT ON SEED GERMINATION, PLANT GROWTH AND YIELD TRAITS OF COWPEA (*VIGNA UNGUICULATA* (L.) Walp)

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ABSTRACT

The study investigated the effect of colchicine on germination of cowpea seeds, as well as morphological and selected growth traits of cowpea plant (*Vigna unguiculata* (L.) Walp). Dry cowpea seeds were treated with colchicine at concentrations of 0.05g/dl, 0.10g/dl, 0.15g/dl and 0.20g/dl. Quantitative characters such as percentage germination, plant height, number of leaves, length of longest branches, number of primary branches, pod and seed yield were measured. Percentage germination was high in the control (89.3) and0.05g/dl (90.2) of first generation of colchicine treatment (C_1). Significant differences ($P<0.05$) were recorded in the mean of most of the quantitative characters such as number of leaves, branches and seeds per plant in some of the colchicine treatments in the study.

Keywords: Colchicine, seed germination, plant growth, yield traits, cowpea.

INTRODUCTION

Cowpea is an important legume and among the crops considered for national crop improvement program in Ghana (Oppong-Konadu *et al*., 2005). It serves as a source of food for many people in Ghana. Cowpea contains between 22 and 32% of protein on a dry weight basis (Fatokun, 2002) and 50-67% carbohydrate and starch (Pavadai *et al*., 2009). The legume can also serve as potential food source that can be used to manage food shortage in developing countries (Fatokun, 2002).

Mutation techniques have been used to derive many varieties of food crops including cowpea. These methods have proved useful in obtaining new traits, creating genetic variability and supplementing conventional breeding (Sangsiri *et al.*, 2005; Anbarasan*et al*., 2013). This genetic variability is what is required for crop improvement (Novak and Burnner, 1992; Aliero, 2006; Bolbhat*et al*., 2012) as variability existing in all organisms including our crop plants has been generated by mutation and subsequent recombination.

Colchicine is known to inhibit mitosis in a wide variety of plant and animal cells by interfering with the orientation and structure of the mitotic fibres and spindle (Finnie and Staden, 1994). Since chromosome segregation is driven by microtubules, colchicine is therefore applied to interfere with mitosis to induce polyploidy and mutations in plant cells (Kleinhofs *et al.*, 1978).While polyploidy is fatal in animal cells, it is usually well tolerated in plant cells and mostly results in fruits and seeds that are larger, hardier and faster growing and more desirable (Finnie and Staden, 1994).

For the above mentioned reasons, this type of genetic manipulation is frequently used in breeding plants to create genetic variability. Several high yielding variants of crop plants have been developed using colchicine (Bragal, 1955). The objective of the present study was to induce mutations in cowpea using colchicine treatment.

MATERIALS AND METHODS

Experimental material
Cowpea accession was obtained from the Plant Genetic Resources Research Institute in Bunso, Ghana. The seeds were planted and self pollinated to multiply them. They were then stored in a freezer until their use.

Chemical treatment of cowpea seeds
Dry and quiescent seeds of cowpea were pre-soaked in distilled water for six hours at room temperature. Varying concentrations of colchicine were prepared, that is, 0.05g/dl, 0.10g/dl, 0.15g/dl and 0.20g/dl. The pre-soaked seeds were again soaked in the different concentrations of the colchicine for three hours. The colchicine-treated seeds were thoroughly washed under running tap water and immediately planted in rows along side with controls (untreated) in ploughed field.

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Table 1. Effect of colchicine on percentage germination (cumulative) of C_1 and C_2 cowpea seeds (*Vigna unguiculata* L. Walp).

Generation	Treatment	Number of days after planting											
		4	5	6	7	8	9	10	11	12	13	15	18
C_1	Control	58.9	72.6	78.6	83	86.6	87.5	87.5	87.5	88.4	88.4	89.3	89.3
	0.05g/dl	60.2	70.8	83.9	86.6	88.4	88.4	88.4	90.2	90.2	90.2	90.2	90.2
	0.10g/dl	-	-	8.0	8.9	12.5	17.0	22.3	22.3	22.3	22.3	22.3	24.1
	0.15g/dl	-	-	4.5	7.1	8.9	8.9	9.8	9.8	9.8	10.7	10.7	10.7
	0.20g/dl	-	-	1.8	2.7	2.7	2.7	3.6	3.6	3.6	3.6	3.6	3.6
C_2	Control	59.5	73.8	79.8	83.3	86.9	86.9	86.9	86.9	86.9	86.9	86.9	86.9
	0.05g/dl	62.0	73.2	80.3	81.7	85.9	85.9	85.9	85.9	85.9	85.9	85.9	85.9
	0.10g/dl	44.9	63.2	67.7	70.6	70.6	70.6	70.6	70.6	70.6	70.6	70.6	70.6
	0.15g/dl	36.2	49.2	63.0	63.0	63.0	63.0	63.0	63.0	63.0	63.0	63.0	63.0
	0.20g/dl	33.1	62.2	73.2	73.2	73.2	73.2	73.2	73.2	73.2	73.2	73.2	73.2

Table 2. Effect of colchicine on plant morphology and growth traits of cowpea (*Vigna unguiculata* L. Walp).

Generation	Treatment concentration	Mean number of leaves/ plant	Mean plant height [cm]	Mean number of primary branches/plant	Mean length of longest branch/plant[cm]
C_1	Control	4.85 ± .26	11.88 ± .69	3.21 ± .14	43.46 ± 5.4
	0.05g/dl	3.88 ± .22	13.00 ± 1.2	3.38 ± .12	30.18 ± 4.9
	0.10g/dl	2.85 ± .22	8.50 ± .60	2.19 ± .27	23.17 ± 7.0
	0.15g/dl	2.80 ± .13	10.77 ± 1.5	2.55 ± .55	70.20 ± 14.4
	0.20g/dl	3.00 ± .58	7.75 ± 1.7	2.50 ± .87	19.67 ± 4.5
C_2	Control	7.91 ± 1.3	11.68 ± .65	3.32 ± .17	67.68 ± 14.8
	0.05g/dl	5.26 ± .67	12.48 ± .58	3.26 ± .30	34.56 ± 6.7
	0.10g/dl	6.14 ± .77	12.68 ±.80	3.26 ± .22	107.66 ± 19.1
	0.15g/dl	6.47 ± .77	12.33 ± .76	4.06 ± .19	110.47 ± 14.2
	0.20g/dl	6.17 ± .65	13.11 ± .75	3.82 ± .19	71.62 ± 14.8

Table 3. Effect of colchicine on reproductive and yield traits of cowpea plants (*Vigna unguiculata* L. Walp).

Generation	Treatment [concentration]	Mean number of days to flowering	Mean number of pods /plant	Mean number of seeds per plant	Pollen grain viability[%]	Seed set [%]
	Control	48±2.1	11.92±.77	92.79±8.2	96.5	63.4
C_1	0.05g/dl	45±1.5	8.22±.67	72.16±10.1	92.6	61.9
	0.10g/dl	51±1.8	3.78±.66	22.16±5.7	81.1	79.4
	0.15g/dl	51±1.4	12.1±5.1	103.25±55.2	93.6	69.4
	0.20g/dl	54±2.4	6.16±1.1	46.0±12.5	92.2	41.6
C_2	Control	47±2.3				
	0.05g/dl	46±2.3				
	0.10g/dl	46±2.3				
	0.15g/dl	48±2.6				
	0.20g/dl	50±2.8				

C_2 data on Pollen grain viability, Seed set and Mean number of pods /plant not available.

Plants raised from the initially treated seeds were designated C_1 (first generation of colchicine treatment) while plants raised from the C_1 seeds were designated C_2 (second generation of colchicine treatment).

Quantitative measurements and Data Analysis

Data were collected on the following plant characters in the study: number of days to germination of seeds, mean number of leaves per plant, mean plant height [cm], mean number of primary branches per plant, mean length of longest branch per plant [cm], mean number of days to flowering, pollen grain viability [%] by IKI (iodine + potassium iodide) test (Baker and Baker, 1979), seed set [%], mean number of pods per plant, and mean seed weight [g].

The means obtained were compared for differences within and between treatments using one-way ANOVA test and Dunnett Post-Hoc analysis respectively.

RESULTS

The data gathered in the study are analysed and summarised into the tables 1, 2, and 3. Percentage germination (cumulative) after planting and the overall percentage recorded are presented in table 1, with the highest of 90.2% recorded in treatment 0.05g/dl of C_1. Reduced germination percentages were observed with increasing concentration of the mutagen especially in the C_1 plants which were from the initially treated seeds, whereas in the C_2 generation, the germination rate ranged from 63.0% in Treatment 0.15g/dl to 86.5% in the control. Table 2 shows data on mean number of leaves, primary branches per plant and mean height and length of longest branch per plant which were all significant (P< 0.05) within treatments but not between treatments in comparison to the control in C_1. However, these same parameters did not record differences within or between treatments in their means in C_2 generation plants, except for length of longest branch per plant (within treatment). Mean number of days to flowering, mean number of pods per plant, mean number of seeds per plant, mean seed weight and percentage pollen grain viability and Seed set are also presented in table 3. Significant difference (P< 0.05) within treatment was recorded in the mean number of seeds and pods per plant in the C_1 generation. Treatments 0.05g/dl and 0.10g/dl in C_1 generation recorded significant differences in comparison with the control for mean number of pods per plant and also 0.10g/dl for number of seeds per plant. Effect of colchicine on percentage germination (cumulative) of C_1 and C_2 cowpea seeds (*Vignaunguiculata* L. Walp).

DISCUSSION

Chemical mutagens have been reported to have inhibitory effects on seeds leading to low percentage germination (Dhakhanamoorthy *et al.*, 2010; Pande and Khetmalas, 2012). Reduction in percentage germination with increasing concentration has also been reported in Sesame with the effects more pronounced in C_1 generation than C_2 generation (Mensah *et al.*, 2005; Mensah and Akomeah, 1997). Treatments 0.10g/dl, 0.15g/dl and 0.20g/dl in the present study also recorded reduced percentage germination probably due to the high concentration of colchicine as reported by the earlier researchers. However, studies by Udensi and Ontui (2013) on pigeon peas recorded a contrary result even though they recorded a prolonged period for the emergence of seedlings.

Results reported in Sesame (Mensah *et al*., 2007) and *Trigonella foenumfraeum* (Datta and Biswa, 1988) show significant difference in the number of leaves per plant. Treatments in the C_1 generation in our study recorded similar results as reported, inferring an effect as a result of the treatment. In the C_1 generation in this study, the effect of the colchicine may have resulted in reduced or increased plant height in that generation leading to significant differences (P<0.05) observed in various treatments. The effect may have probably normalized in the C_2 generation hence the normal growth of plants which occurred thereafter. The same research on Sesame (Mensah *et al*., 2007) and *Trigonella foenumfraeum* (Datta and Biswa, 1988) treated with colchicine recorded significant differences in mean number of branches per plant. The present study also recorded significant differences (P< 0.05) in the mean number of primary and secondary branches in C_1 and C_2. Vegetative growth seems to have been enhanced in the colchicine treatment leading to development of more and longer branches.

Early flowering and fruit maturity may be due to the physiological changes caused by mutagen (Dhakhanamoorthy *et al.*, 2010). In the present study colchicine treatments in the C_1 generation with high concentration produced delay in flowering suggesting that the chemical may have interfered with maturity and early flowering. Comparing number of days to flowering in C_1 and C_2 it could be observed that the first generation showed a longer period to flowering while the second generation had a shorter period (in the difference of 1 to 3 days). This infers that the initial treatment interferes with early maturity and flowering in the cowpea. Hormones (e.g. florigen) may have also been induced or inhibited to initiate or impede flowering.

Due to the drastic effect of the initial treatment observed in the C_1 generation, it would have been expected that the highest seed sets would be recorded in the control; however the seed set records in the various treatments were higher than the control but not significantly different (P<0.05) from each other. It can therefore be inferred that the chemical mutagens applied may have enhanced or reduced the seed formation ability of plants. Mean number of pods per plant was also high in the treated rather than the untreated plants. This brings to light that yield per plant was enhanced in some of the colchicine treatments.

The number of seeds per plant was inconsistent among the treated and the control however, the differences observed were statistically significant (P<0.05). This result conforms to that of Nura *et al*. (2013) who studied the mutagenic effect of colchicine on pigeon peas (*Sesamum indicum* L.).

CONCLUSION

Colchicine induced marked vegetative growth leading to the formation of large plants and more number of leaves and branches per plant. High number of seeds per plant in some of the colchicine treatments in the study was also recorded. Further experiments should be carried out to select such plants for the production of cowpea leaves and seeds.

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