The Pharma Innovation Journal 2023; 12(9): 1152-1159

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 1152-1159

www.thepharmajournal.com
Received: 14-07-2023
Accepted: 18-08-2023

Poonam Nawalkar
Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Sunil Kumar Verma
Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Corresponding Author:
Poonam Nawalkar
Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Impact of colchicine on cowpea plant growth and yield characteristics (*Vigna unguiculata* L. Walp)

Poonam Nawalkar and Sunil Kumar Verma

Abstract
In Africa's tropical and subtropical climes, the cowpea is especially important since it can fix atmospheric nitrogen in addition to giving residents a source of protein. While many crop species have exploited forced mutation successfully, legumes like cowpeas have had the least success. The effects of colchicine on cowpeas were investigated. The study investigated the effects of colchicine treatment on the germination of cowpea seeds as well as the morphology and a few particular growth traits of cowpea plants (*Vigna unguiculata* L. Walp). Dry cowpea seeds were treated with colchicine in concentrations of 0.02, 0.04, and 0.05 percent. Quantitative parameters such the percentage of germination, plant height, number of leaves, length of longest branches, main branch count, pod and seed production. The percentage of germination was high in the control (89.3) and the colchicine-treated group (0.04%, 90.2). In some of the colchicine treatments in the study, significant variations ($p<0.05$) were noted in the mean of the majority of the quantitative features, including the number of leaves, branches, and seeds per plant.

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

Introduction

One of the crops Ghana is considering for its national agricultural enhancement effort is the cowpea, a vital legume (Oppong-Konadu *et al*., 2005) [15]. It offers food for a big number of people in India. Cowpeas have between 22 and 32% of protein by dry weight and 50-67% of carbohydrates and carbs, according to Fatokun (2002) [8]. (Pavadai *et al*., 2009) [17]. The possibility of using the bean as a food source to reduce food shortages in developing countries is also present (Fatokun, 2002) [8]. In terms of global production, Nigeria produces the most cowpeas, followed by Niger, Mali, Burkina Faso, and Senegal. Hybridization and selection have been the main sources of cowpea improvements over time (Traditional breeding methods).

Mutation techniques have been used to create a wide variety of food crop variants, most notably the cowpea. These techniques have been effective in increasing genetic variety, obtaining distinctive traits, and traditional breeding (Sangsiri *et al*., 2005; Anbarasan *et al*., 2013) [18, 2]. It is crucial for crop development since mutation and subsequent recombination are the sources of genetic variety in all species, including our agricultural plants (Novak and Burnner, 1992; Aliero, 2006; Bolbhat *et al*., 2012) [14, 1, 3].

After the discovery of colchicine in 1940, work on induced mutagenesis to improve plants by chromosomal duplication started. In an effort to enhance plant quality, favourable mutations generated through colchicine-induced mutagenesis led to the establishment of numerous novel crop kinds with enhanced phenotypic features.

Colchicine inhibits the orientation and structure of the mitotic fibres and spindle, which stops mitosis in a number of plant and animal cells (Finnie and Stadan, 1994) [9]. Because microtubules are essential for chromosome segregation, colchicine is used to interrupt mitosis in order to create polyploidy and mutations in plant cells (Kleinhofs *et al*., 1978) [10]. Although polyploidy is lethal to animal cells, it is frequently tolerated by plant cells and predominantly produces fruits and seeds that are bigger, tougher, grow faster, and seem more appealing (Finnie and Stadan, 1994) [9].

For the aforementioned reasons, this form of genetic modification is frequently utilised in plant breeding to boost genetic variety. Many crop plant types with excellent yields have been developed using colchicine. Using colchicine, a number of crop plant varieties with high yields have been created (Bragal, 1955) [4]. The goal of the current investigation was to use colchicine to treat cowpea in order to cause mutations.

Materials and Methods

Experimental material

The experiment was carried out in the Department of Plant Molecular Biology and Biotechnology's several laboratories and the Instructional Cum Research Farm at Raipur's Indira Gandhi Krishi Vishwavidyalaya for Agriculture. Colchicine treatment was applied to Cowpea variety CG Chara Barbatti-1 (RCC-48) seeds in order to produce polyploidy.

Methodological Description

As a control, cowpea seeds were pre-soaked in distilled water for 2.5 hours at room temperature. Whereas colchicine was made in concentrations of 0, 0, 0, and 0.05 percent. Again, the seeds were incubated for 2 hours and 30 minutes at varying colchicine doses. Before being sown right away, the seeds that had been exposed to colchicine were thoroughly cleansed under running water. Place the treatment group adjacent to the untreated group in the tilled ground after 14 days.

List of Observations

Colchicine's impact on cowpeas was evaluated using the following observations: seed germination (%), number of leaves per plant, number of branches per plant, etc. Height, number of leaves per plant, size of leaves in square centimetres (m2), number of flowers per plant, size of seeds per pod, number of seeds per pod, and number of pods per plant; Pod length and 10 seed weight (g) were measured four times, at intervals of 30 days each: at 30, 60, 90, and 120 days after sowing. When the plants were fully grown, measurements were made of the number of branches per plant, the quantity of seeds per pod, the length of the pods, and the weight of the seeds on the control and colchicine-treated plants. We took mean values, and Mean values were taken and the percentage difference was calculated by taking the means of the data recorded for colchicine treated and control plants.

Procedure of Cytological study/ stomatal study & count

Data analysis

The plants displaying the characteristics of polyploid plants were subjected to the cytological criteria after the analysis of morphological attributes. The stomata of the diploid control plants and the likely colchicine-treated plants were measured for length, width, and density. The diameters of the cell and nucleus in respect to the stomata were also assessed to confirm polyploidy in the suspicious plants.

Stomata size (µm)

The treated plants showed a little increase in stomatal size (length & width). Changes in stomatal size are frequently associated with plant development. The best outcomes for all treatments were seen at 0.04% CT for 2.5 hours, indicating 19.68 m and 19.4 m, respectively. The mean stomatal width observed in the plants treated with colchicine 0.02% & 0.04% was found to be 13.52 mm and 13.25 mm, respectively, with a range of 11.26-13.52 m. In contrast, the mean stomata length in the diploid control plants was found to be 12.9 mm with a range of 11.02-12.9 mm.

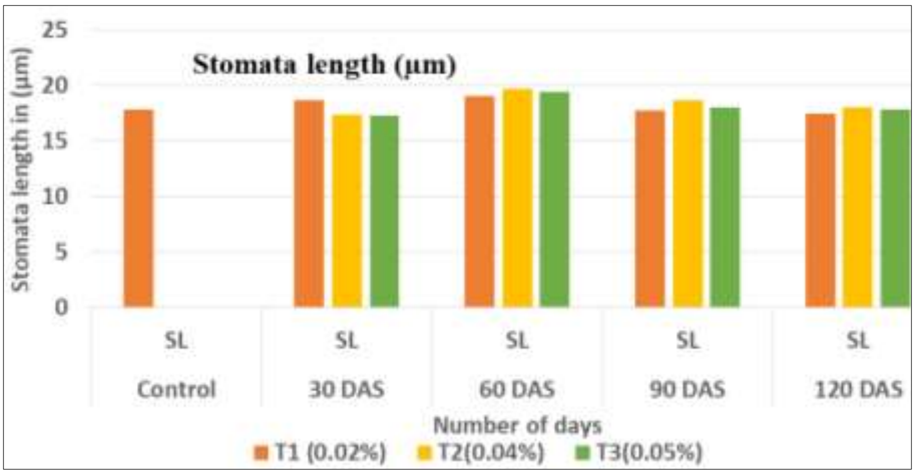
When compared to the untreated control, the one treatment, 0.04 (12.77%) delivered for 2.5 hours, demonstrated the biggest percentage increase in stomata length. The aforementioned treatment also resulted in a 0.04% (4.88%) increase in stomata width (Graph 4.24). Similar results have also been discovered for the plant species *Carthamus tinctorius* L., Trachyspermum ammi, and *Guizotia abyssinica* (Moghbel *et al*., 2015; Noori *et al*., 2017) [20, 21]. (Chambhare *et al*., 2021) [22]. Citrus species Dendrobium cariniferum (Cimen. 2020) [23]. Bedhar noted in 1961 in Lycopersicum esculentum that stomata length increased, their width did as well in tetraploids, and the length of the stomatal opening was also noted to increase. Bertioli *et al*. reached the same verdict (2012).

Table 1: Mean length and width of stomata of control and CT plant

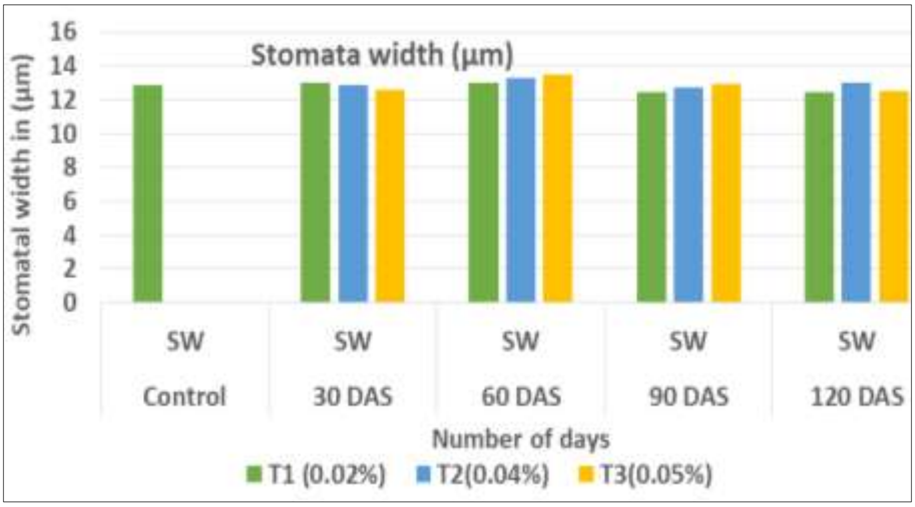
Number of days	Stomata length	Colchicine treatment		
		T_1 (0.02%)	T_2 (0.04%)	T_3 (0.05%)
Control	SL	17.85	0	0
30 DAS	SL	18.71	17.39	17.26
60 DAS	SL	19.02	19.68	19.40
90 DAS	SL	17.72	18.65	18.07
120DAS	SL	17.49	18.00	17.89

Table 2: Mean width of stomata of control and CT plant

Number of days	Stomata length	Colchicine treatment		
		T_1 (0.02%)	T_2 (0.04%)	T_3 (0.05%)
Control	SW	12.90	0	0
30 DAS	SW	13.00	12.84	12.58
60 DAS	SW	13.04	13.25	13.52
90 DAS	SW	12.46	12.70	12.91
120 DAS	SW	12.46	13.00	12.50



Graph 1: Mean stomatal length of control and treated plants



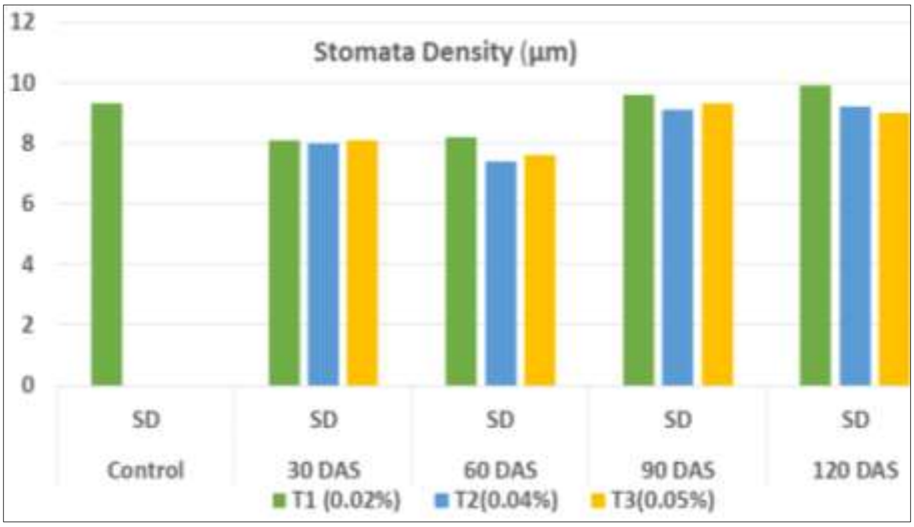
Graph 2: Mean stomatal width of control and treated plants

Stomatal density (per microscopic area)

The size of stomata grew but their density reduced in plants treated with colchicine as a result of a rise in chromosomal numbers. The results show that treated Cowpea plants have a significant number of larger stomata. When stomata density per microscopic area was calculated, plants treated with colchicine were found to have less stomata overall. The number of stomata per microscopic area was 9.3, which was somewhat greater than the 9.3 stomata seen in other CT plants. All treatment plants treated for 2.5 hours with 0.05% and 0.1%, or 7.4 and 7.6, respectively, showed lower stomatal densities. Compared to the control, there was a percent decrease of 20.43% and 19.35% in the plants treated with 0.05 and 0.1%, respectively. The same results in Arachis paraguariensis were reported (Aina *et al.* al., 2012) [24], *Helianthus annuus* L.

Table 3: Mean stomatal density per microscopic area of control & treated plants

Sr. No.	Exposure time (hrs.)	Colchicine concentration (%)	Stomata density per microscopic area
1	Control	0	9.3
2	30 DAS	T_1- 0.02	8.1
		T_2- 0.04	8.0
		T_3- 0.05	8.1
3	60 DAS	T_1- 0.02	8.2
		T_2- 0.04	7.4
		T_3- 0.05	7.6
4	90 DAS	T_1- 0.02	9.6
		T_2- 0.04	9.1
		T_3- 0.05	9.3
5	120 DAS	T_1- 0.02	9.9
		T_2- 0.04	9.2
		T_3- 0.05	9.0



Graph 3: Mean stomatal density in control and colchicine treated plants

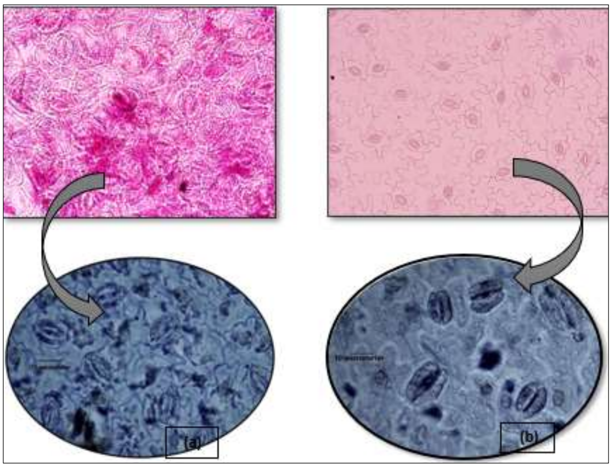


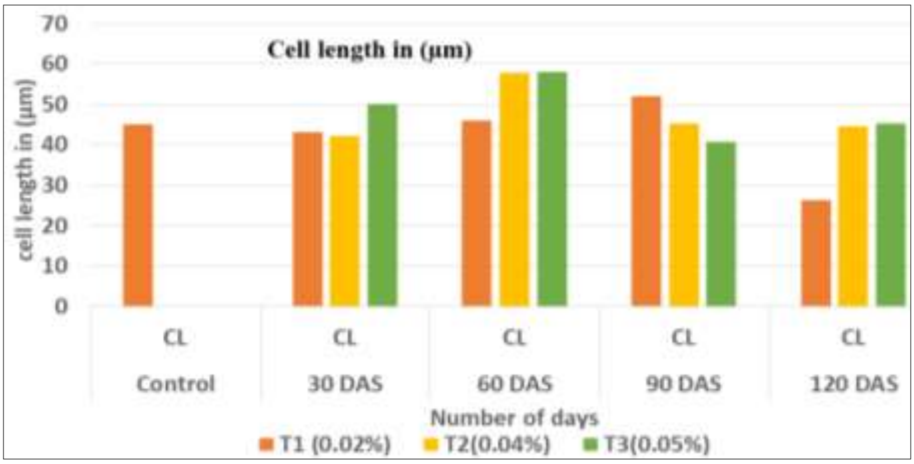
Fig 1: Stomatal density of control and colchicine treated for 0.04% for 2.5 hrs.

a) Stomatal density from the leaf of control plant
b) Stomatal density of CT plant at 0.04% for 2.5 hrs.

Cell size per microscopic area

As ploidy levels change, cell size typically changes as well (Sourour *et al*., 2014) [25]. When chromosomes double, a cell's size immediately increases as a result of an increase in nuclear material, which prevents a cell from splitting as it grows and develops, according to Manzoor *et al*. in 2019. In the current study, the cell length and width of the treated and control groups were measured from the root tips at the commencement of growth. The plants that were given colchicine treatments of 0.05% and 0.1%, or 57.68 mm and 58.01 mm, respectively, displayed the mean cell length. The measured length of the control cell was 44.98 m.

The mean cell width in the diploid control was 17.89 m, whereas the mean cell width in the cells treated for 16 hours with 0.05 and 0.1% of colchicine was 18.63 m and 18.92 m, respectively. Comparing the treated plants to the control, the treated plants' cell widths increased by 5.75% and 4.10%, respectively. Greater cell size is often associated with polyploidization, which modifies the morphology and anatomy of plants, according to Sattler *et al*. (2016) [26].



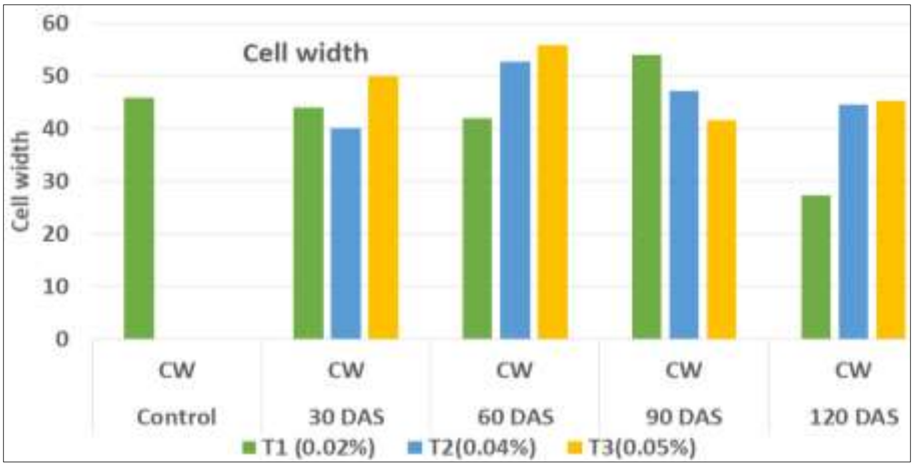
Graph 4: Mean cell length of control and colchicine treated plants

Table 4: Mean cell length of control and colchicine treated plants

Number of Days		T_1 (0.02%)	T_2 (0.04%)	T_3 (0.05%)
Control	CL	44.98	0	0
30 DAS	CL	43.10	42.10	50.07
60 DAS	CL	45.96	57.68	58.01
90 DAS	CL	52.10	45.20	40.63
120 DAS	CL	26.25	44.51	45.39

Table 5: Mean cell width of control and colchicine treated plants

Number of Days		T_1 (0.02%)	T_2 (0.04%)	T_3 (0.05%)
Control	CW	45.98	0	0
30 DAS	CW	44.1	40.1	50.07
60 DAS	CW	41.96	52.68	56.01
90 DAS	CW	54.1	47.2	41.61
120 DAS	CW	27.25	44.41	45.29



Graph 5: Mean cell width of control and colchicine treated plants

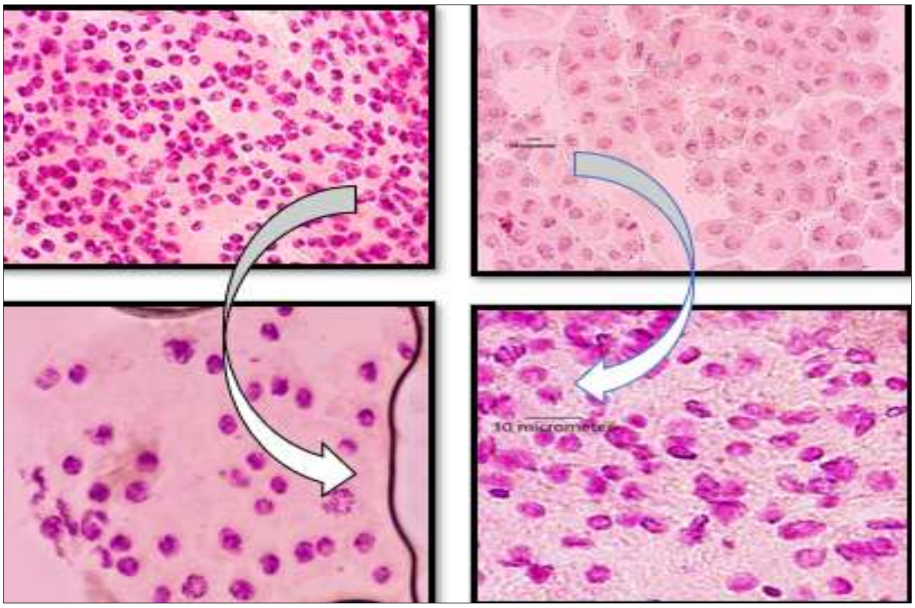


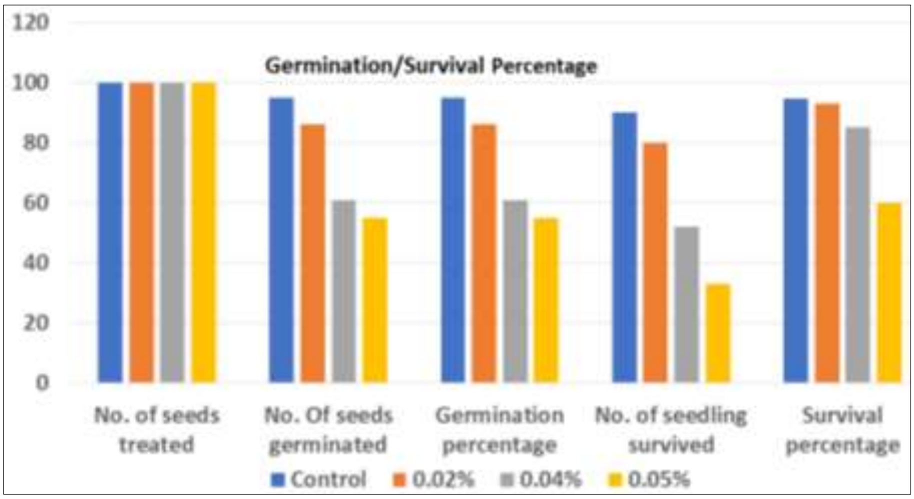
Fig 2: Cell size of control and colchicine treated for 0.05%
a) Cell length from the root tip of the control plant.
b) Cell length from the CT at 0.04% for 2.5hrs.

Table 6: Effect of colchicine on germination percentage of cowpea seeds

Treatment	Exposure time(in hrs)	Colchicine concentration %	No. of seed treated	No. of seeds germinated	Germination Percentage	No. of Seedlings survived	Survival Percentage
Colchicne	2.5	0.00	100	95	95	90	94.73
		0.02	100	86	86	80	93.02
		0.04	100	61	61	52	85.24
		0.05	100	55	55	33	60.00

The effects of colchicine treatment (CT) on the seeds were measured by determining the germination percentage and death rate after 7 days. The estimated percentages of germination and mortality are shown in the table below. The plantlets were then transferred to the field for more investigation.

According to the findings, whereas the rate in the control group was high, at 95%, the proportion of RCC-48 seeds that germinated after being treated with colchicine ranged from 55% to 86%. 0.04% of colchicine concentration treated for 2.5 hours produced the lowest germination percent, or 61%, whereas other treatments showed less germination than control. Of the treated seeds, 0.02% of the concentration treated for 2.5 hours had a germination rate that was 86% similar to the control. It was demonstrated that when the concentration of colchicine rose, the rate of germination reduced. The results of Javadian *et al* 2017 [27] study are also comparable to This might be because colchicine binds to the protein molecules of living things, interfering with their ability to function (Deysson 1968) [28]. Similar results i.e., decrease in germination percent with increase in colchicine concentration was also reported by Noori *et al*., 2017 [21].



Graph 6: Germination percentage after colchicine treatment

Fig 3: Germination percentage of control and treated seeds

Table 7: Effect of colchicine on plant height (cm)

Variety	Colchicine Concentration	30 DAS	60 DAS	90 DAS	120 DAS
CG Chara Barbatti-1 (RCC-48)	0.02%	29.42	40.13	43.42	54.58
	0.04%	30.21	44.01	43.54	55.13
	0.05%	28.98	40.86	35.93	51.03
	Control	29.31	32.99	34.73	50.89

The treated plants first developed normally when compared to the untreated control plants, but with time they showed robust growth. The administration of colchicine at varied concentrations at each growth stage was contrasted with the RCC-48 control. After 30 days, researchers found that the highest mean value for plant height in control plants was 55.13. The plants that were given different doses of colchicine had different average heights after 2.5 hours of treatment. The RCC-48 variety showed variations in plant height when compared to the population of control plants.

The maximum mean plant height recorded in control plants was 55.13, as shown in the table. The average height of the plants treated for 2.5 hours varies between the plants treated with the various concentrations of colchicine.

Fig 4: Plant height observation of control vs treated after 20 days

Effect of colchicine on number of branches

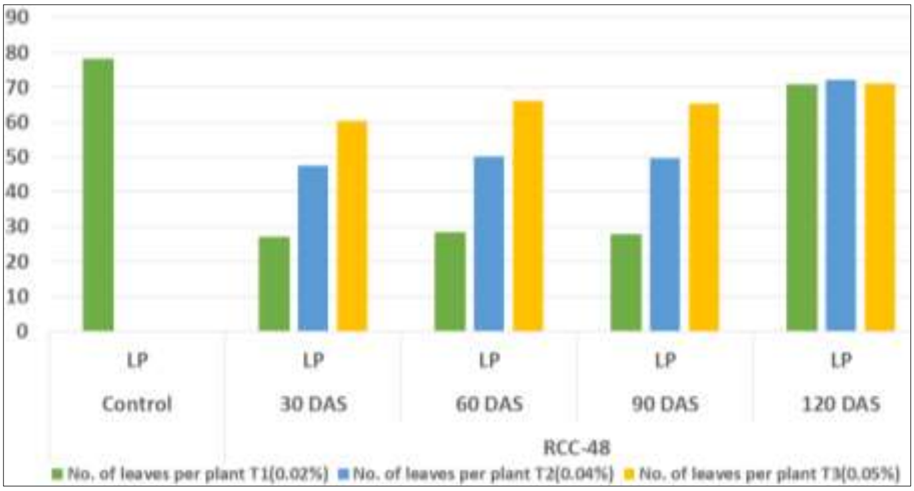
For plants given colchicine, the mean number of branches varied from 6 to 9.6, whereas for plants given control, it varied from 5.7 to 8.3. The treatment at two concentrations, 0.04% and 0.05%, produced better results as compared to the majority of the colchicine-treated plants, with 9.6 and 9 branches per plant. Table no 8: Effect of colchicine on number of branches

Number of Days		Colchicine treatment		
	Control	T_1 (0.02%)	T_2 (0.04%)	T_3 (0.05%)
30 DAS	8.0	7.0	6.0	7.6
60 DAS	8.3	8.3	9.6	9.0
90 DAS	8.1	7.3	7.1	8.5
120 DAS	8.3	7.6	7.0	8.4

After 2.5 hours of therapy, improvements of 15.66% and 8.44% were found as compared to the control group, which had improvements of 0.04% and 0.05%, respectively. For Trigonella foenum fraeum (Datta and Biswa, 1988) [5], sesame (Mensah *et al*., 2007) [13], and cowpea (Essel *et al*., 2015), similar studies with colchicine treatment were carried out that revealed significant changes in the mean number of branches per plant.

Table 9: Colchicine's impact on the quantity of leaves per plant of cowpea seeds

Variety	Colchicine Concentration	No. of leaves per plant			
		30 DAS	60 DAS	90 DAS	120 DAS
CG-Chara Barbatti-1(RCC48)	Control	24.83	35.8	50.13	60.93
	0.02%	27.26	47.53	60.40	70.93
	0.04%	28.43	50.26	66.06	72.16
	0.05%	28.03	49.66	65.26	71.03



Graph 7: Average number of leaves per plant

At intervals of 30, 60, 90, and 120 days after seeding, the number of leaves per plant was counted. As with the number of branches in the previous section, the average and improvement% were computed. The table shows that the mean number of leaves per plant for check plants was 24.83, whereas the mean number of leaves per plant for verified plants was 28.43, and the mean number of leaves per plant for confirmed plants was 72.16.

Effect of colchicine on Seed weight (g)

In particular, diploid seeds were lighter than tetraploid seeds. According to Stevens *et al*. in 2020, tetraploid plants produced heavier, more viable seeds than diploid plants. Ten seeds from each condition were collected, and they were all individually weighed. The care given to ten seeds from diploid plants was uniform. The table demonstrates that colchiploid seed weights were higher than diploid seed weights in each case. Haploids normally weigh between 0.50 g and 1.06 g, while diploids typically weigh between 0.50 g and 1.06 g. In contrast, 0.02 percent of haploids weigh between 1.06 g and 1.17 g, 0.04 percent between 0.99 g and 1.05 g, and 0.05 percent between 1.16 g and 1.21 g.

Table 10: Effect of colchicine on Seed weight of cowpea

Sr.no.	Colchicine conc. (%)	Ten seed weight (g)	
		Diploids	Tetraploids
1	0.02	1.01	1.05
2		0.68	1.22
3		0.74	1.15
4		1.06	1.05
5	0.04	0.99	1.06
6		0.50	1.06
7		0.48	1.21
8	0.05	0.51	1.17
9		0.53	1.09
10		0.50	1.10
Average		0.7	1.1

Discussion

The characteristics of polyploidy, according to Eigsti and Dustin (1955) [29], include delayed plant growth, thicker, darker-green leaves, larger stomata and pollen grains, high pollen sterility, larger flowers and seeds, and slower plant growth. By seed treatment, phenotypic variations between diploid and colchiploid cowpea plants were found in this study. Plant height, branch and leaf number and size, days till first blooming, flower number and size, and number of pods per plant were among these variations. Cytological tests revealed that tetraploids had the largest nuclei and the smallest stomata, despite the fact that their seed weight (g) was higher than that of diploids'. as well as the size of the individual cells.

The seedling treatment, on the other hand, was found to have the lowest survival rate and the highest mortality rate. The plants in the seedling treatment exhibited dwarf-like morphology, small, thick, dark green leaves, no discernible branches, and short survival times before passing away. Gunckel (1957) [30] argued cytological characteristics were connected to physiological changes and identified physiological causes for plant dwarfing. Plants are getting smaller and having more branches, something Bose and Hati have also seen (1966).

Conclusion

Colchicine significantly increased vegetative growth, resulting in the development of bigger plants with more leaves and branches per plant. To choose these plants for the production of cowpea leaves and seeds, more research should be done.

References

1. Aliero AA. Effect of hydroxylamine on the germination of and growth of Sesame (*Sesamum indicum*). Journal of Plant Science. 2006; 1(4):356-361.
2. Anbarasan K, Sivalingam D, Rajendran R, Anbazhagan M, Chidambaram AA. Studies on the mutagenic effect of EMS on seed germination and seedling characters of Sesame (*Sesamum indicum* L.) Var. T MV3. International Journal of Research in Biological Sciences. 2013;3(1):68-70.
3. Bolbhat SN, Bhoge VD, Dhumal KN. Effect of mutagens on seed germination, plant survival and quantitative characters of horse gram (*Macrotyloma uniflorum* (LAM.) VERDC). International Journal of Life Science and Pharma Research. 2012;2(4):130-136.
4. Bragal M. Production of polyploids by colchicine. Euphytica. 1955;4:76-82.
5. Datta AK, Biswa AK. Induced mutation in two *Trigonella* species. Banglad J Bot. 1988;17:211-214.
6. Dhakhanamoorthy D, Selvaraj R, Chidambaram A. Physical and chemical mutagenesis in *Jatropha curcas* L. to induce variability in seed germination, growth and yield traits. Rom. J Plant Biol. 2010;55(2):113-125.
7. Essel E, Asante IK, Laing E. Effect of colchicine treatment on seed germination, plant growth and yield traits of cowpea (*Vigna unguiculata* (L.) Walp). Canadian Journal of Pure and Applied Sciences. 2015;9(3):3573-3576.
8. Fatokun CA. International institute of tropical agriculture; c2002 p. 52-61.
9. Finnie JF, Stadan VJ. *Gloriosa superba* L. (Flame lily): Micropropagation and *in vitro* production of colchine. Medicinal and Aromatic Plants. 1994;6:146-166.
10. Kleinhofs AW, Owais, M. and Nilan RA. Azide. Mutal. Res. 1978;55:165-195.
11. Mensah JK, Akomeah PA, Ekpekurede EO. Gamma radiation induced variation of yield parameters in cowpea (*Vigna unguiculata* (L) Walp). Global J Pure Appl. Sci. 2005;11(3):327-330.
12. Mensah JK, Akomeah PA. Mutagenic effect of hydroxylamine and streptomycin on the growth and seed yield of cowpea, *Vigna unguiculata* (L) Walp. Legume Res. 1997;15:39-44.
13. Mensah JK, Obadoni BO, Akomeah PA, Ikhajabe B, Ajibolu J. The effect of sodium azid and colchicine treatment on morphological and yield traits of sesame seeds (*Sesame indicum* L.). African Journal of Biotechnology. 2007;6(5):534-538.
14. Novak FJ, Burnner H. Plant breeding: Induced mutation technology for crop improvement. IAEA Bulletin. 1992, 25-31.
15. Oppong-Konadu EYR, Akromah HK, Adu-Dapaah, Okai E. Genetic diversity within Ghanaian cowpea germplasm based on SDS-PAGE of seed proteins. African Crop Science Journal. 2005;13(2):117-123.
16. Pande S, Khetmalas M. Biological Effect of Sodium

Azide and Colchicine on Seed Germination and Callus Induction in *Stevia Rebaudiana*. Asian J Exp. Biol. Sci. 2012;3(1):93-98.
17. Pavadai P, Girija M, Dhanavel D. Effectiveness, efficiency and biochemical content of physical and chemical mutagens in soybeans (*Glycine Max* (L.) *Merr*.). Journal of Phytology. 2009;1(6):444-447.
18. Sangsiri C, Sorajjapinun W, Srinives P. Gamma radiation induced mutations in mungbean. Science Asia. 2005;31:251-255.
19. Udensi OU, Ontui V. Determination by Flow Cytometry Polyploidy Inducing-capacity of Colchicine in *Cajanus cajan* (L.) Mill sp. Pakistan Journal of Biological Sciences. 2013;16:630-635.
20. Moghbel N, Borujeni MK, Bernard F. Colchicine effect on the DNA content and stomata size of Glycyrrhiza glabra var. glandulifera and Carthamus tinctorius L. cultured *in vitro*. Journal of Genetic engineering and Biotechnology. 2015 Jun 1;13(1):1-6.
21. Noori A, Ashrafi SJ, Vaez-Ghaemi R, Hatamian-Zaremi A, Webster TJ. A review of fibrin and fibrin composites for bone tissue engineering. International journal of nanomedicine. 2017 Jul 12:4937-61.
22. Chambhare MR, Nikam TD. *In vitro* induction and assessment of tetraploid plants from shoot cultures of diploid Niger (*Guizotia abyssinica* (Lf) Cass.): a multipurpose oilseed crop. Plant Cell, Tissue and Organ Culture (PCTOC). 2021 Jun 23:1-3.
23. Çimen H, Çetinkaya N, Vasquez JC, Guerrero JM. A microgrid energy management system based on non-intrusive load monitoring via multitask learning. IEEE Transactions on Smart Grid. 2020 Sep 29;12(2):977-87.
24. Aina AJ, Falade KO, Akingbala JO, Titus P. Physicochemical properties of Caribbean sweet potato (Ipomoea batatas (L) Lam) starches. Food and Bioprocess Technology. 2012 Feb;5:576-83.
25. Sourour A, Ameni B, Mejda C. Efficient production of tetraploid barley (*Hordeum vulgare* L.) by colchicine treatment of diploid barley. J Exp. Biol. 2014 Mar;2(1S):113-9.
26. Sattler MC, Carvalho CR, Clarindo WR. The polyploidy and its key role in plant breeding. Planta. 2016 Feb;243:281-96.
27. Javadian S, Darbasizadeh B, Yousefi A, Ektefa F, Dalir N, Kakemam J. Dye-surfactant aggregates as corrosion inhibitor for mild steel in NaCl medium: Experimental and theoretical studies. Journal of the Taiwan Institute of Chemical Engineers. 2017 Feb 1;71:344-54.
28. Deysson G. Antimitotic substances. International review of cytology. 1968 Jan 1;24:99-148.
29. Eigsti OJ, Dustin Jr P. Colchicine in Agriculture. Medicine, Biology, and Chemistry, Ames, Iowa. 1955.
30. Gunckel JE. IV. The effects of ionizing radiation on plants: morphological effects. The Quarterly Review of Biology. 1957 Mar 1;32(1):46-56.