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Effect of colchicine on *Vigna radiata* l

Ankit Yadav, Shaun Rodrigues, Ryan Sequeira, Samruddi palambe
Department of Botany, Chiki tsak Samuha's SS and LS Patkar-Varde College, Goregaon (West), Mumbai, India

Abstract
The study investigated the effect of colchicine on germination of mungbean seeds (*Vigna radiata L.*). Dry seeds were treated with colchicine of different concentration 0.1%, 0.2%, 0.3%, 0.4% for 6 hours. Quantitative characters such as percentage seed germination, stem height, radicle length, leaf length was calculated. In cytological parameter mitosis was performed along with estimation of DNA and protein. For all criteria, the 0.1 percent treatment seeds performed admirably following increase in concentrations significant difference was observed.

Keywords: colchicine, seed germination, mitosis, DNA, protein

Introduction
Many food crops, like mungbean, have been developed using mutation techniques. These methods have proved useful in obtaining new traits, creating genetic variability and supplementing conventional breeding (Sangsiri *et al*, 2005 [18]; Anbarasan *et al*, 2013) [2]. This genetic variability is what is required for crop improvement (Novak and Burnner, 1992 [13]; Aliero, 2006 [1]; Bolbhat *et al*, 2012) [3] 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) [8]. 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) [9]. 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) [8].This form of genetic modification is widely used in plant breeding to establish genetic diversity for the reasons mentioned above. Colchicine has been used to grow a number of high-yielding crop plant varieties (Bragal, 1955) [4]. The aim of this study was to use colchicine treatment to induce mutations in mung bean seeds.

Material and Method
Material
Dry and healthy seeds of experimental plant material i.e., *Vigna radiata L.* were obtained from, Ratanshi's Agro, Byculla, Mumbai. They were washed with running tap water soaked and stored for further use.

Chemical treatment of seeds
Seeds were pre-soaked in distilled water for 1 hour after being washed and dried. The seeds were then soaked in the appropriate concentration for 6 hours (0.1 %, 0.2 %, 0.3 %, 0.4%).

After 6 hours, the seeds were washed under running tap water and placed in a dry petri dish for 24 hours. After 24 hours, the percentage of seeds that germinated and the length of the radicle were determined. The colchicine treated seeds were thoroughly washed under running tap water and immediately planted in pots under laboratory condition.

Quantitative measurement and Data analysis
Germinating index
For each concentration, 50 seeds were used to determine the germinating index. 0.1% and 0.2 % of seeds germinated 100 %, while 0.3% and 0.4 % germinated 98 % and 92 %, respectively**.**

Table 1: Effect of colchicine on germinating index of mung bean seeds (*Vigna radiata L.*)

Sr.no.	Treatment (Colchicine)	No. of seeds total	No. of seeds germinated	GI
1.	Control	50	50	100%
2.	0.1%	50	50	100%
3	0.2%	50	50	100%
4.	0.3%	50	49	98%
5.	0.4%	50	46	92%

Radical length
At 0.1% concentration, the mean radical length of treated seeds was 1.32cm, followed by 1.1cm for 0.2%, 0.92cm for 0.3%, and 0.9cm for 0.4%, respectively. (Fig 1)

Table 2: Effect of colchicine on radical length of mung bean seeds (*Vigna radiata L.*)

Treatment	Radical length (cm)					Mean length (cm)
Control	2	2	2.1	2.5	2	2.12
0.1%	1.8	1.3	1.2	1.2	1.1	1.32
0.2%	1.1	1.1	0.8	1.1	1.4	1.1
0.3%	0.9	0.8	0.9	1	1	0.92
0.4%	0.9	0.8	1	0.9	0.9	0.9

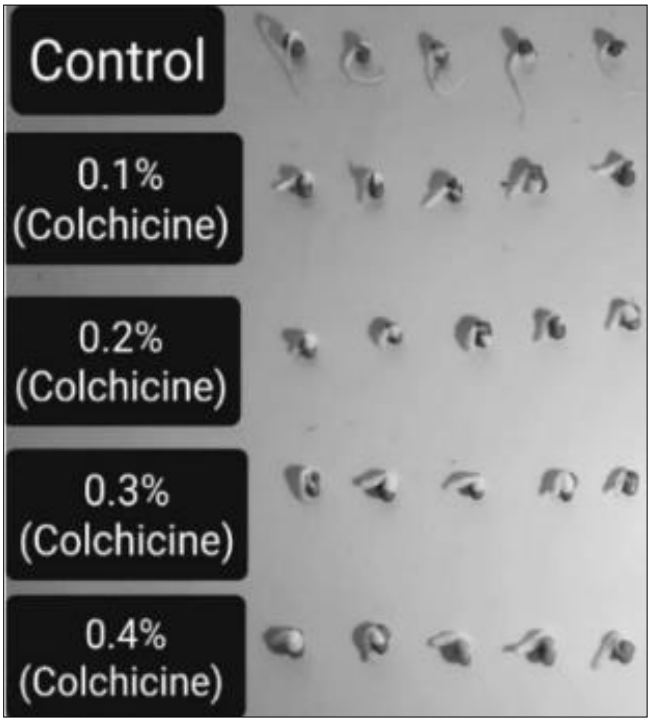

Fig 1: Shows the length of the radical in mung bean seeds (*Vigna radiata L.*) that have been treated with colchicine.

Leaf length

Table 3 shows the average percentages observed, with the highest concentration being 0.1%. Increased concentration, on the other hand, resulted in shorter leaf length. (Fig 2)

Table 3: Effect of colchicine on leaf length of mung bean seeds (*Vigna radiata L.*)

Control	0.1%	0.2%	0.3%	0.4%
3cm	4.2cm	2.7cm	2.8cm	1.8cm

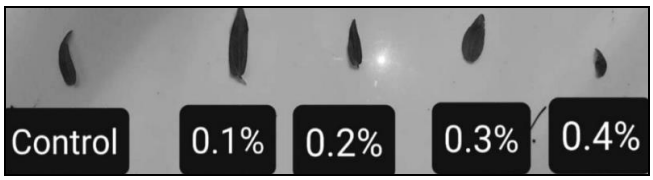

Fig 2: The effect of colchicine on the mean leaf length of mung bean seeds (*Vigna radiata* L.). The effect was positive at 0.1 %, but as the concentration increased, leaf length decreased while leaf thickness increased.

Mean height of stem

Table 4 shows data on mean stem height, which was notable at 0.1 %concentration and 0.2% concentration. With increasing concentration, the length shrank. However, at 0.3% and 0.4%, a significant observation was made, namely, the growth of leaves per node.

Table 4: Effect of colchicine on mean height of stem of mung bean seeds (*Vigna radiata L.*)

Control	0.1%	0.2%	0.3%	0.4%
20.5cm	21.5cm	14cm	11cm	4cm

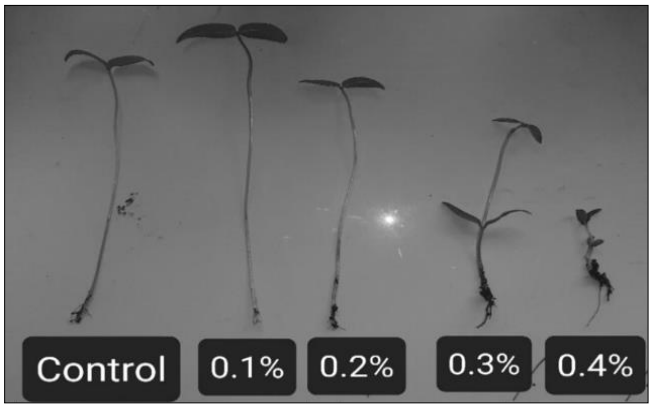

Fig 3: The average stem height was measured, and a significant effect of colchicine was found in 0.1 % of the sample. It was observed that as concentration length increases, the number of leaves per node decreased, but at 0.3 and 0.4 %, the number of leaves per node increased substantially.

Cytological parameter

As compared to the control, the volume of DNA calculated with a significant increase of 0.1%. With increasing concentration, the sum decreased. The amount of protein measured using Lowry's method was 490mg/ml, which was important when compared to the control. Mitosis was also conducted, and it was found that as the concentration of colchicine increased, the size cell appears to be larger as compared to control.

Table 5: Effect of colchicine on DNA content of mung bean seeds (*Vigna radiata L.*)

Control	0.1%	0.2%	0.3%	0.4%
0.21 mg of DNA	0.35 mg of DNA	0.32 mg of DNA	0.30 mg of DNA	0.25 mg of DNA

Table 6: Effect of colchicine on protein content of mung bean seeds (*Vigna radiata L.*)

Control	0.1%	0.2%	0.3%	0.4%
400 mg of Protein	490 mg of Protein	484 mg of Protein	480 mg of Protein	456 mg of Protein

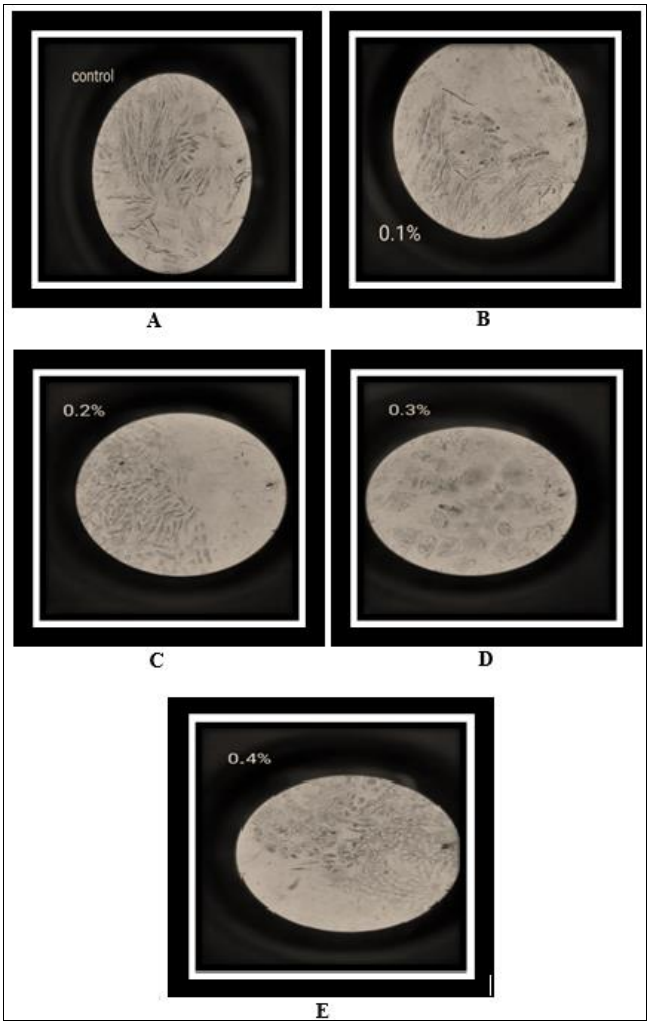

Fig 4: A- Mitosis of control, B- 0.1% cells appear thin, C- 0.2% Thickness of the cell increased, D- 0.3% Maximum thickness of cell, E- Minimum thickness of cells

Result

From the present research work, it has been found that colchicine acts as a good chemical mutagen.

1. The radical length decreased with an increase in concentration of colchicine.
2. The germination index decreased with increase in concentration of colchicine.
3. The mean leaf length was found to be maximum at 0.1% concentration (4.2cm), as compared to control (3cm).
4. The mean stem height was found to be maximum at 0.1% concentration (21.5cm) as compared to control (20.5).
5. The DNA content was maximum in 0.1% concentration and decreased with increase in concentration. Minimum DNA content was observed in control.
6. The protein content was maximum in 0.1% concentration and decreased with increase in concentration. Minimum protein content was observed in control.
7. The cells appeared larger than those in control in the order 0.1% greater than 0.2% greater than 0.3% greater than 0.4%.

Conclusion

- In the present work, the treatment of colchicine showed an increase in the stem height and leaf length at 0.1%.
- The germination index at 0.1% and 0.2% was found to be 100% which is equal to that of control.
- The amount of DNA estimated was found to be maximum at 0.1% i.e., 0.35 mg/gm.
- The amount of protein estimated was maximum at 0.1% i.e., 490 mg/gm.
- Colchicine treatment might be inducing poly ploidy which is the probable cause for increase in stem height, leaf length, protein content, DNA amounts and other parameters in 0.1% treated plantlets.
- Further research studies need to be conducted to analyze the increase in the overall yield in the plants treated with 0.1% colchicine.

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