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In-vitro Induction of Polyploidy in Niger (*Guizotia abyssinica* L.f. Cass) through Colchicine Treatment

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ABSTRACT

The present study was conducted to induce polyploidy through colchicine treatment to obtain better yield by enhancing the vigour, improving morphological and yield attributing traits. The Niger seeds were exposed to four different colchicine concentrations (0.025,0.05,0.1 and 0.2%) at different time duration (6, 16, 24 and 48 hrs.). The experiment was carried out in two different conditions i.e., *ex-vitro* and *in-vitro.* The survival percentage of treated plants was found better under *in-vitro* conditions while the mortality rate was found higher in *ex-vitro* condition. The germination percentage was found higher in control as compared to colchicine treated except the 16 hrs treatment at 0.05% and 0.1% which showed similar germination as control. On assessing the morphological parameters including plant height, number of branches per plant, capitulum size, number of capitulum per plant and seed yield per plant, the treated plantlets with 0.05% and 0.1% colchicine at 16 hrs. showed increment compared to control. Stomatal length and width were found increased whereas stomatal density per microscopic area was found higher in control plants. The size of the cell and nucleus was also found increased in the above treatment as compared to control. Comparing the morphological and cytological results it can be anticipated that the plants treated for 16 hrs at 0.05% and 0.1% colchicine concentration may show the putative polyploidy.

Keywords Colchicine, Cytology, Putative polyploidy, Oilseed, Yield.

INTRODUCTION

Polyploidy is widely acknowledged as a major mechanism of adaptation and speciation in plants. The improvement of plant material through induced polyploidy has been one of the major targets of plant breeding programs in the past century. The main advantage of induced polyploidy is that the plants achieved usually have improved morphological and yield characteristics than their intact diploids (Chambhare and Nikam 2021). Polyploidy can be induced through the application of mitotic spindle poisons, such as colchicine and oryzalin. Colchicine is an alkaloid functions as anti-mitotic metabolite. It is commonly used for chromosome doubling and induction of polyploidy in plant cells (Gantait and Mukherjee 2021). The technique of subjecting plant cells and tissue to colchicine, oryzalin, and trifluralin

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in vitro has been described in multiple examples over the past few decades, among the many known induction methods (Podwyszynska *et al.* 2017, Zhang and Gao 2021).

India accounts about 20 % of the global area and 10% of the global production of the oilseeds through which India stands as a fourth largest producer of oilseeds, despite of that imbalance between supply and demand for edible oils has grown over time. The average yield gap in edible oilseeds is about 60% in the year 2021. The target of reducing the yield gap to 20% with this increasing population in the next five years is not possible if, we only concentrate on the major oilseed crops. It has previously been reported that in comparison with major oilseed crops there are some minor oilseed crops having similar properties but there is a very little efforts have been made for the improvement in minor oilseed crops. The present investigation was carried out in Niger crop to mitigate with the above-mentioned problem.

Niger, *Guizotia abyssinica* (L.f.) Cass is generally regarded as a minor oilseed crop. Seed oil contains protein (20–30%), oleic acid (25–38%), and linoleic acid (30-50%) which shows the promising role in human nutrition and health (Geleta *et al.* 2011). The productivity of Niger in India (321 kg/ha) is low and it contributes only 2% of total vegetable oil (Solvent Extraction Association of India). So, the study was aimed to induce polyploidy through colchicine treatment to obtain better yield by enhancing the vigour, improving morphological and yield attributing traits.

MATERIALS AND METHODS

Plant material

The material used in the study was seeds of JNS 9 variety of Niger, *Guizotia abyssinica* (L.f.) Cass developed in JNKVV Jabalpur M.P., JNS 9 variety is high yielding variety of Niger with chromosome number 2n=30 having average yield of 650–700 kg/ha. For induction of polyploidy seeds of JNS-9 variety were collected from field at Indira Gandhi Krishi Vishwavidyalaya, Raipur (CG) India.

Colchicine treatment

Seeds were surface sterilize with tween 20 followed by 1% Bavistin and 0.1% (w/v) $HgCl_2$ for 5 minutes and rinsed with sterile distilled water for 2 to 3 times at each step to remove the traces. To induce polyploidy in Niger two different approaches were carried out i.e., *ex-vitro* and *in-vitro* process. The seeds of JNS 9 variety were surface sterilized and treated with the colchicine on petri plates at *ex-vitro* condition and at *in-vitro* conditions seeds were cultured on full strength MS (Murashige and Skoog 1962) medium, supplemented with different concentrations of colchicine, including 0.025, 0.05, 0.1, and 0.2% (w/v). Before autoclaving, the medium's pH was adjusted to 5.8±0.2 by using hydrochloric acid and sodium hydroxide. Stock solutions of colchicine were prepared by dissolving the aforementioned concentration of colchicine in sterilized distilled water. In final, filter-sterilized colchicine was added to media after autoclaving. All cultures were maintained at 25±2°C under a 16/8 h (light/dark) photoperiod with 60–65% relative humidity for different exposure times, i.e., 6, 16, 24, and 48 h. Sixty seeds were cultured in each treatment concentration. Following treatment, seeds were subculture in colchicine free full strength MS medium. All the cultures were then incubated in growth room under the different conditions. After 7 days, the seedlings that survived were first adopted and then transferred to pots. After 20 days plantlets were then transplanted to the field. Morphological analysis was conducted after at the maturity stage.

Growth parameters

Germination percentage, shoot length and root length were taken to examined the effect of control and colchicine treated plants in controlled condition.

Cytological observations

The cytological features of treated plants were evaluated to screen the effect of colchicine on treated plants. Stomatal size and density play a key role in identification of polyploids. Stomata characteristics like stomata length, width and density were measured by applying the thin layer of clear nail polish on the epidermal layer (abaxial side) and removed after

drying the layer of expanded leaves. Increasing in the ploidy often results in increased cell size (Sourour *et al.* 2014). In the present study, the cell and nucleus size of colchicine treated as well as control were taken from the root tips at the initial stages of growth. For this study the 0.5 cm of root tip was excised and dip into the 1% acetocarmine solution for 24 hrs. Then slide was prepared by squash method and visualize under microscope. In the present study trinocular microscope with digital display system optima camera type- DG1510CCD were used and high-quality images were taken through Dewinter software.

Morphological parameters

For studying the effect of different concentration of colchicine on morphological traits of Niger, viz., plant height (cm), number of branches, leaf length and width (cm), number of capitulum, capitulum size (mm), seed length, seed width and seed yield per plant were observed in colchicine treated and control plants.

RESULTS AND DISCUSSION

Effect of colchicine on germination percentage, shoot and root length

The germination percentage of cultured seeds treated with different concentration of colchicine was taken after 7 days of treatment. The germination percentage in the control was found 85% which is higher than any other colchicine treatment. Among all treatments 0.1% of colchicine treated for 16 hrs showed the better germination percentage as compared to the other treatments. The lowest germination percentage was observed at 24 hrs treatment (Fig. 1). Earlier it was reported that, as the concentration of colchicine increased the rate of germination decreased (Javadian *et al.* 2017). Decrease in germination percent with increase in colchicine concentration was also reported by Noori *et al.* (2017).

The slight variation in shoot and root length of control and treated plants were also observed. Among treated plants 0.1% for 16 hrs attained the mean shoot length of 7.22 cm followed by 6.9 cm in 0.05% and control showed the shoot length of 6.3 cm (Fig. 2). The mean root length recorded in control was 1.9 cm, whereas the plants treated with 0.05% and 0.1% of colchicine treatment for 16 hrs showed 2.4 cm and 2.2 cm respectively (Fig. 3) which shows an increase in shoot and root length may be because of colchicine at this stage.

Cytological observations

A comparison of stomata size and density in control and treated plants showed the increased length and width of stomata in colchicine treated plants of Niger. Microscopic observations also proved the decrease in stomatal density in the colchicine treated plants as compared to the untreated control plants (Fig. 4). The results of a paired T-test analysis showed differences between control and treated plants for stomata length, width, and density at 5% probability level (Table 1). One of the most appropriate features that can be used as a strong indicator of the ploidy level in plants is stomatal density (Baydar and Tuglu 2022). Ploidy induction usually leads to increase in stomata length and width, stomata density, most likely due to the larger stomata and epidermal cells (Gantait and Mukharjee 2021). An increase in stomata length and width and

Table 1. Statistical analysis of cytological parameters. Significant at *p<0.05.

Sl. No.	Characters	Control		Putative polyploids	
		Min-max	Mean±SEm	Min-max	Mean±SEm
1	Cell length (µm)	43.21-46.21	44.98±0.32	54-62.55	57.68±0.89*
2	Cell width (µm)	16.21-21.11	17.89±0.56	17.25-21.21	19.05±0.40
3	Nucleus length (µm)	6.6-8.2	7.66±0.16	6.5-8.9	8.25±0.24*
4	Nucleus width (µm)	4-5.7	4.78±0.18	4.2-5.8	4.97±0.13
5	Stomata length (µm)	15.37-19.61	17.43±0.39	17.19-20.63	19.54±0.35*
6	Stomata width (µm)	12.12-14.23	12.99±0.19	12.21-15.26	13.7±0.38*
7	Stomata density per microscopic area	8-13	10.2±0.46	7-11	8.6±0.45*

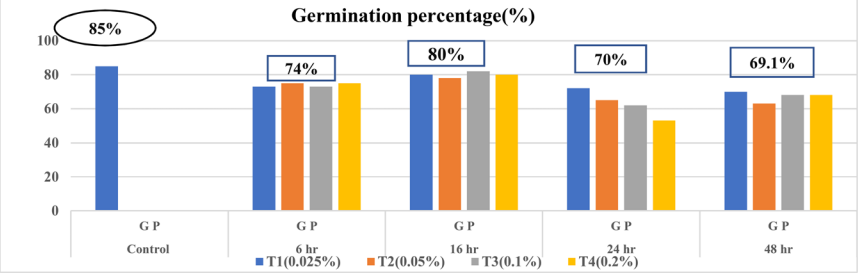

Fig 1. Germination percentage of control and colchicine treated seeds at different time duration.

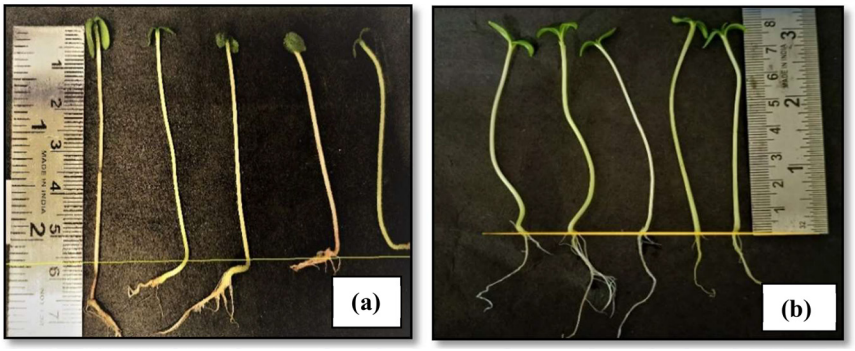

Fig 2. a) Shoot length of control; b) Shoot length of colchicine treated plants at 0.05% for 16 hrs.

reduction in the stomata density of colchicine induced tetraploid plants have also been reported in other ploidy induction experiments (Moghbel *et al.* 2015, Chambhare and Nikam 2021).

Increasing in the ploidy often results in increased cell size (Sourour *et al.* 2014). Manzoor *et al.* 2019 have reported that when there is chromosome doubling, the immediate effect is an increase in cell size due to increase in nuclear content. In the present study, plants treated with 0.05 and 0.1% for 16 hrs of exposure showed increase in the cell length and width as compared to the untreated control (Table 1, Fig. 5). The polyploidy indicates the doubling in the chromosomes which will directly shows the increment in nuclear content and size of the nucleus. The nucleus length (µm) and width (µm) from root tip cells of control and colchicine treated plants was taken to assess the nucleus size of the cell. In the present study it was observed that the length of nucleus in

Table 2. Statistical analysis of morphological parameters. Significant at *p<0.05.

Sl. No.	Characters	Control		Putative polyploids	
		Min-max	Mean±SEm	Min-max	Mean±SEm
1	Plant height (cm)	49 - 56	52.7±0.77	46.3-58.2	60±1.25*
2	Number of branches	6-10	8.6±0.44	8-11	9.6±0.33*
3	Leaf length (cm)	10-12	10.9±0.31	10.3-13	11.6±0.24
4	Leaf width (cm)	2.8-3.6	3.11±0.08	2.5-4.5	3.19±0.18
5	Capitula size (mm)	6-7.1	6.4±0.13	6-7.4	6.7±0.17*
6	Number of capitulum per plant	8-12	10.3±0.39	10-13	11.5±0.34*
7	Seed width (cm)	0.14-0.17	0.15±0.03	0.14-0.18	0.15±0.04
8	Seed length (cm)	0.48-0.54	0.51±0.05	0.42-0.54	0.49±0.01
9	Seed yield/plant	2.5-3.2	2.93±0.05	2.8-3.6	3.16±.0.08*

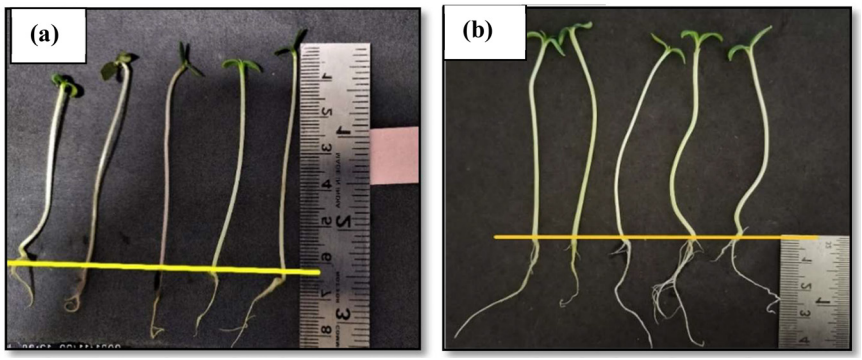

Fig 3. a) Root length of control; b) Root length of colchicine treated plants at 0.05% for 16 hrs.

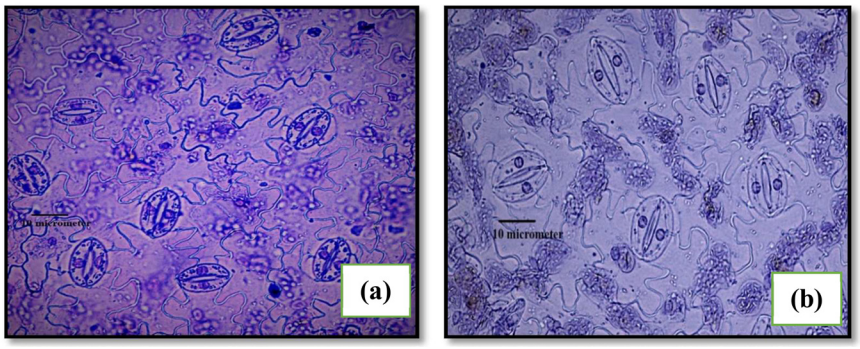

Fig 4. a) Stomatal size of control b) Stomatal size of the colchicine treated plants at 0.05% for 16 hrs.

control varied from 6.9 to 7.66 µm whereas, treated ranged from 7.1 to 8.25 µm (Table 1, Fig. 6).

Statistical analysis

The best result obtained for every parameter was analyzed using paired t-test: Two sample assuming Unequal Variances to check the significant variations for different parameters (Tables 1 and 2).

Comparison based on morphological observations

In the present study, observation was assessed on morphological characters of colchicine treated vs control plants that are usually affected by ploidy levels. The statistical analysis showed the effect of colchicine

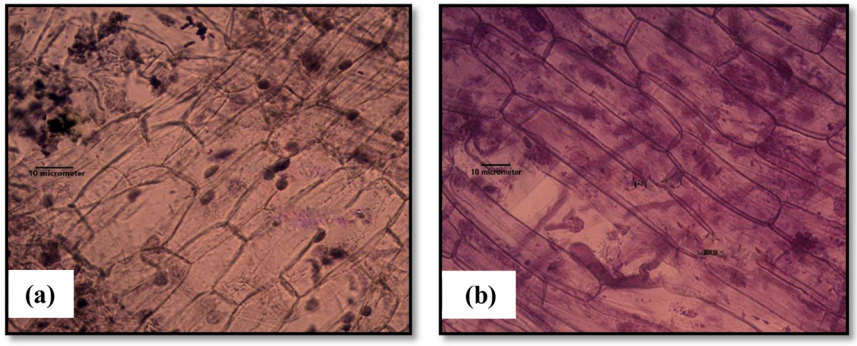

Fig 5. a) Cell size of control b) Cell size of the colchicine treated plants at 0.05% for 16 hrs.

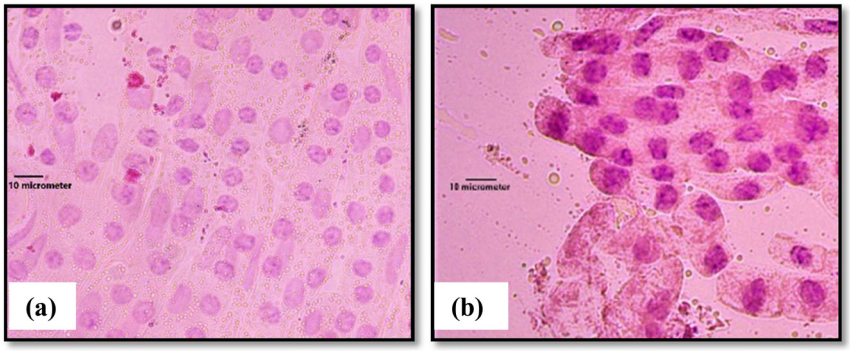

Fig 6. a) Nucleus size of control b) Nucleus size of the colchicine treated plants at 0.05% for 16 hrs.

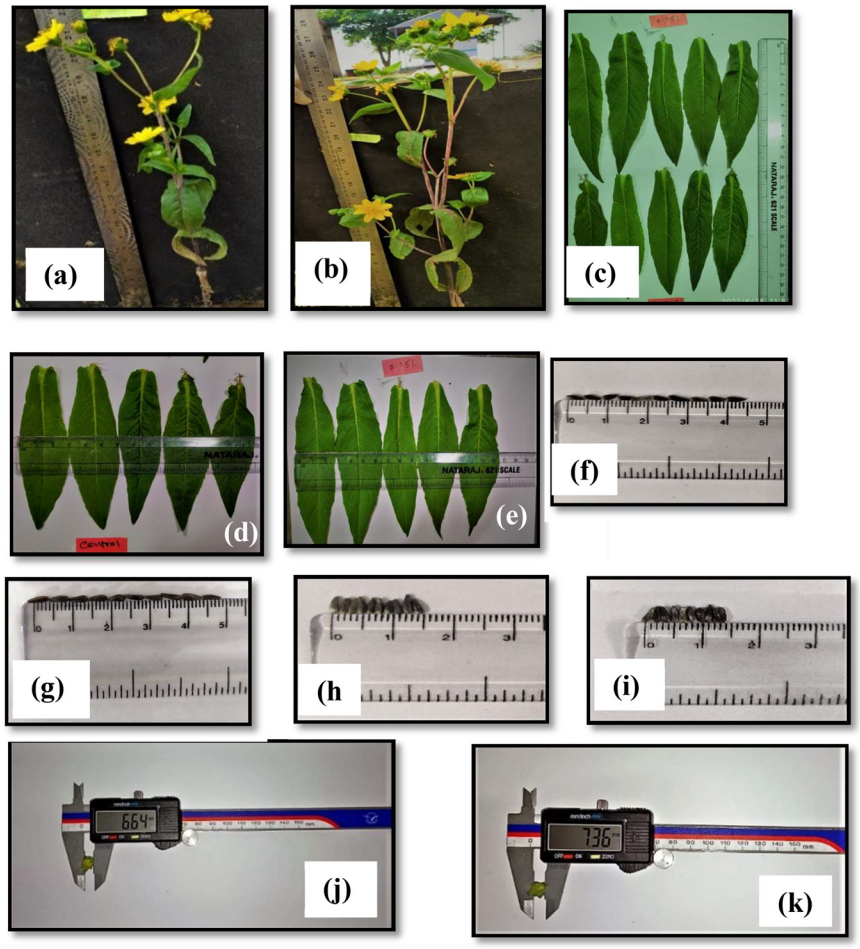

Fig 7. a) Plant height of control at maturity stage b) Plant height of colchicine treated (CT) at 0.05% for 16 hrs. c) Leaf length of control and CT at 0.1% for 16 hrs. d, e) Leaf width of control and CT at 0.1% for 16hrs. f, g) Capitulum size (mm) of control and colchicine treated at 0.05% for 16hrs. h, i) Seed length of control and CT at 0.05% for 16 hrs. j, k) Seed width of control and CT at 0.05% for 16 hrs.

concentration on some investigated morphological traits at 5% probability level (Table 2). Mean comparison showed the variation in plant height (cm) by applying the 0.1% colchicine for 16 hrs (Table 2, Figs. 7 a-b). Application of 0.05% colchicine for 16 hrs. led to increase in the number of branches per plant as compared to the control (Table 2). Very little changes were also observed in the leaf length and width of the treated and control plants (Table 2, Figs. 7 c–e). The mean in number of capitulum per plant corresponds to using 0.05% of colchicine for 16 hrs showed the better performance (Table 2). The capitulum size (mm) also increased in the plants treated for 16 hrs. at 0.05% concentration (Table 2, Figs. 7. f-g). After harvesting, seed size i.e., seed length (cm) and seed width (cm) and seed yield per plant (g) was taken and it was observed that the seed length and width was slightly lower than the treated plants but the seed yield per plant showed increase in the treated plants (Table 2, Figs. 7 h-k).

It was reported that the plants with increased ploidy level are sometimes apparent by their distinct morphology (Sourour *et al.* 2014). The colchicine treated plants showed the reduce plant height due to the increase colchicine concentration which directly affects the cell division was observed in *Guizotia abyssinica* (Chambhare *et al.* 2021). Essel *et al.* 2015 also observed the significant difference in number of branches of the treated and control plants. The increase in leaf size and thickness was also observed by Zhang and Gao (2021) in *Dendrobium carinferum*. After analyzing morphological parameters, result reveals that colchicine treatment for different time intervals has shown some effect on the treated plants. Most of the treated plant showed results similar or little better than control while reduction was also observed in some treatments. Overall, the 16 hrs at 0.05% and 0.1% have shown the better result in plant height, number of branches, capitulum size, number of capitulum than any other treatment when compared to the control.

CONCLUSION

In the present study, the *in-vitro* treatment was found most appropriate for induction of polyploidy in Niger as compared to the *ex-vitro* process. From all the given treatments the prominent result was obtained with 0.05% and 0.1% of colchicine concentration, treated for 16 hrs of time duration and cytological analysis also showed overall increment in stomatal size and density along with increase in cell and nucleus length. The morphological and cytological study for the polyploidy induction reveals that, the colchicine concentration of 0.05% and 0.1% at 16 hrs was found effective in altering the traits which may lead to increase in yield and attributing traits in Niger.

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