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Mutation Studies in Black Turmeric *(Curcuma caesia Roxb.)*

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Abstract

Black turmeric is an underground rhizomatous medicinal crop with bluish-black rhizomes. The rhizomes are employed in treating piles, bronchitis, asthma, impotency, cancer, epilepsy, fever and other health conditions especially by the tribes in North East India which is the home for this species. Due to over exploitation, it is listed under "Endangered species" category necessitating its conservation and cultivation. No named varieties are available as of now in black turmeric. Hence the present experiment was planned with the objective of locating useful genotypes by inducing variability through mutation treatments. The rhizomes were exposed to physical and chemical mutagens *viz.* gamma rays (10, 15, 20, 25, 30, 35 Gy), EMS (1, 1.25, 1.5 %) and colchicine (0.1, 0.2, 0.3 %). The experiment was laid out in RCBD design with thirteen treatments and two replications. The mutant populations were evaluated for growth, yield and quality parameters and were compared to untreated control. Among the different treatments, T13 (control) recorded maximum plant height (71.80cm), number of leaves (24.20), rhizome yield/plant (130.03g), number of primary (4.59) and secondary rhizome (11.64), oleoresin (6.8 %), crude fibre (4.69 %), curcumin (0.008 %) and essential oil (0.36 %) content. The mutagenic treatment resulted in reduction of growth and yield and altered the biochemical composition in the treated rhizomes.

Key words: *Curcuma caesia, black, turmeric, mutation,*

Introduction

Black turmeric (*Curcuma caesia* Roxb.) is a medicinal plant belonging to family Zingiberaceae comprising of more than 1300 species of flowering plants. Ginger and turmeric are the most commercially exploited plants of in the family along with many other economically important species. Black turmeric is one such economically important species which is enlisted as endangered species by the central forest department of India due to overexploitation from the wild (Venugopal *et al*., 2017). The plant of black turmeric resembles that of turmeric but the leaves have purple streaks running along the midrib to the length of the lamina. Unlike the bright yellow rhizomes of turmeric the rhizomes are bluish-black inside and have a bitter taste (Plate 1). The rhizomes are credited with high medicinal value and are used in the treatment of piles, bronchitis, asthma, impotency, cancer, epilepsy and fever *etc*.

Due to the lack of seed set in the species, genetic improvement by other approaches such as hybridization is challenging. Mutation breeding has been proven to be an effective tool in the improvement of vegetatively propagated crops by generating variability in the existing population. So, inducing mutation and isolating desirable mutants are the means of crop improvement. Since no systematic attempts in crop improvement are done in black turmeric, an attempt was done to induce desirable mutants in the species.

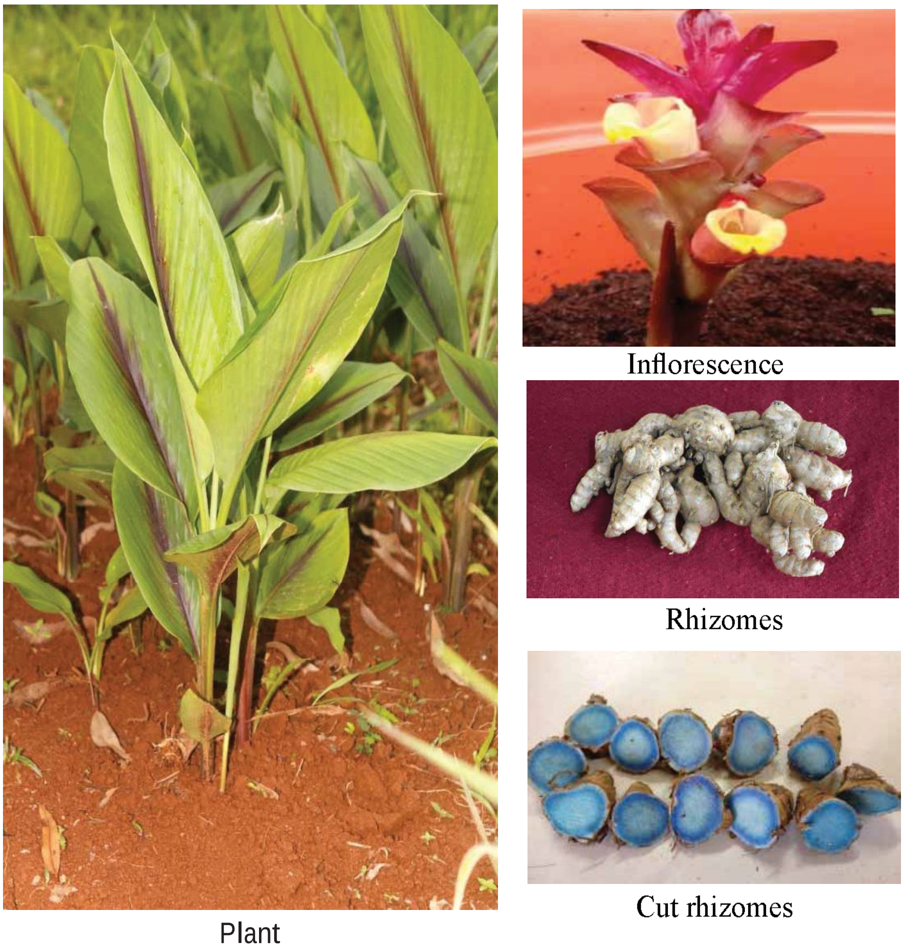

Plate 1. Plant morphology of black turmeric

Material and Methods

Physical mutagen gamma (10, 15, 20, 25, 30, 35 Gy) and chemical mutagens, EMS (1, 1.25, 1.5%) and colchicine (0.1, 0.2, 0.3%) were employed in the study. The rhizomes were cut into pieces of 15 to 20g having an active eye bud and were subjected to mutagenic treatments. Gamma irradiation was carried out using 60Co (Cobalt 60) as irradiation source in a Gamma Chamber. Rhizomes were dipped in EMS and colchicine solutions in a glass beaker according to treatments. Treated rhizomes were field planted at a spacing of 30 x 30 cm after having sprouted in a nursery protray. The experimental design was RCBD with two replications comprising of fifty plants each. Standard cultural practices were practiced as recommended for turmeric.

The observations were recorded for various morphological parameters such as plant height, number of tillers per clump, number of leaves per clump, chlorophyll mutants and morphological mutants. Chlorophyll mutants were observed 2 months after planting and it was classified according to Gustafsson (1940) as Xantha and Striata. Yield parameters like yield per plant, number of primary rhizomes, number of secondary rhizomes were recorded immediately after harvest. Quality parameters *viz*., curcumin content, oleoresin content, crude fibre and essential oil were also assesses from rhizome samples of each treatment.

The curcumin content was calculated using the method described by Manjunath *et al.* (1991). Soxhlet apparatus was used for estimating oleoresin content as per the AOAC (1980) method. Crude fibre content was estimated as per the procedure suggested by Chopra and Kanwar (1976). Essential oil was extracted by hydro-distillation using Clevenger apparatus.

The recorded data was statistically analyzed to compare and find out the significant differences among the various growth, yield and quality parameters among different treatment group.

Results and discussions

The data pertaining to plant height, number of tillers per clump and number of leaves per clump are presented in table 1. Maximum plant height of 71.80 cm was observed in control (T13) and the minimum plant height (43.35 cm) was noticed in T12 (colchicine @ 0.3 %). In the present study, plant height recorded a gradual increase and then decreased with increase in the dose of gamma irradiation up to 35 Gy. In case of EMS and colchicine treatment, increase in the concentration resulted in reduction in plant height. Gamma rays might have negatively affected the apical meristems or caused the partial failure of the internodes to elongate resulting in reduction in the number of proliferating cells (Khalil *et al.*, 1986).

Table 1. Effect of different mutagens on growth parameters of black turmeric

Treatment	Plant height (cm)	No. of tillers/ clump	No. of leaves/ clump
T1 (Gamma @ 10 Gy)	60.98^{de}	4.60^{bcdef}	19.90^{bcde}
T2 (Gamma @15 Gy)	61.84^{de}	5.20^{abcd}	20.85^{bcde}
T3 (Gamma @ 20 Gy)	66.20^{b}	5.50^{ab}	22.65^{ab}
T4 (Gamma @ 25 Gy)	64.48^{bc}	5.40^{abc}	21.10^{abcd}
T5 (Gamma @ 30Gy)	59.93^{ef}	4.30^{cdefg}	18.90^{cdef}
T6 (Gamma @ 35 Gy)	58.20^{f}	4.10^{defg}	18.60^{def}
T7 (EMS @ 1%)	64.65^{bc}	5.30^{abc}	22.00^{abc}
T8 (EMS @ 1.25 %)	63.08^{cd}	5.00^{abcde}	19.75^{bcde}
T9 (EMS @ 1.5 %)	60.81^{def}	4.30^{cdefg}	17.70^{ef}
T10 (Colchicine @ 0.1%)	47.30^{g}	3.90^{efg}	16.25^{fg}
T11 (Colchicine @ 0.2%)	45.78^{g}	3.55^{fg}	14.15^{gh}
T12 (Colchicine @ 0.3%)	43.13^{h}	3.30^{g}	12.55^{h}
T13 (Untreated control)	71.80^{a}	5.85^{a}	24.20^{a}

Note: DMRT at 5% of level of significance to compare the pair of treatment means. Any two means having common letter, are not significantly different at 5% level of significance.

Similar results have been obtained with gamma treatments by Giridharan (1984) in ginger, Norfadzrin *et al.* (2007) in tomato and okra. Misra and Bajai (1983) reported decrease in plant height in gladiolus with EMS treatment and opined it might be due to physiological disturbance and reduction in cell division by suppressing the mitotic division and negative effect on auxin. Damon (1958) reported that colchicine treatment arrests metaphase cells in the shoot tip.

Significant differences were noted for tiller and leaf production. Maximum number of tillers per clump (5.85) and number of leaves per clump (25.30) were observed in control (T_{13}). The lowest value was noticed in T_{12} (colchicine @ 0.3 %). Mutagens at higher doses having lethal effect might have caused inactivation or killing of growing points, thus, resulted in the reduction of tiller and leaf production.

Among different treatments, highest chlorophyll mutation frequency (13.30 %) was observed in T_4 (Gamma @ 25 Gy). Both xantha and striata were observed. Chlorophyll mutants were not recorded in T_7 (EMS @1%), T_8 (EMS @1.25%), T_{11} (Colchicine @ 0.2%) and T_{13} (untreated control). Dwarf mutants were observed in EMS @ 1.5% and Colchicine @ 0.2% (Table 2).

Table 2. Effect of mutagens on induction of chlorophyll and morphological mutants

Treatments	Xantha(%)	Striata(%)	Total chlorophyll mutation (%)	Split leaf type (%)	Dwarf plant type (%)
T_1 (Gamma @ 10 Gy)	4.00 (2.12)	2.67(1.78)	6.67(2.68)	0.00(0.71)	0.00(0.71)
T_2 (Gamma @15 Gy)	6.94 (2.73)	1.39(1.38)	8.33(2.97)	5.00(2.35)	0.00(0.71)
T_3 (Gamma @ 20 Gy)	6.67 (2.68)	0.00(0.71)	6.67(2.68)	3.33(1.96)	0.00(0.71)
T_4 (Gamma @ 25 Gy)	11.64(3.48)	1.67(1.47)	13.30(3.71)	6.67(2.68)	0.00(0.71)
T_5 (Gamma @ 30Gy)	10.00(3.24)	0.00(0.71)	10.00(3.24)	0.00(0.71)	0.00(0.71)
T_6 (Gamma @ 35 Gy)	8.33(2.97)	1.67(1.47)	10.00(3.24)	1.67(1.47)	0.00(0.71)
T_7 (EMS @ 1%)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)
T_8 (EMS @ 1.25 %)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)
T_9 (EMS @ 1.5 %)	3.33(1.96)	0.00(0.71)	3.33(1.96)	5.00(2.35)	1.67(1.47)
T_{10} (Colchicine @ 0.1%)	1.67(1.47)	0.00(0.71)	1.66(1.47)	0.00(0.71)	0.00(0.71)
T_{11} (Colchicine @ 0.2%)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	1.67(1.47)
T_{12} (Colchicine @ 0.3%)	0.00(0.71)	1.67(1.47)	1.66(1.47)	0.00(0.71)	0.00(0.71)
T_{13} (Untreated control)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)

Note: Figures in parenthesis are the values of square root transformation ($\sqrt{x}+0.5$) for zero observation

Significant effects of mutagenic treatment on black turmeric were observed on various yield parameters as presented in table 3. Maximum fresh rhizome yield (130.03 g/clump), number of primary rhizomes (4.59), number of secondary rhizomes (11.64/ clump) were observed in control (T_{13}). The lowest values were noticed in T_{12} (colchicine @ 0.3 %).

There was a reduction in yield with increasing concentration of EMS and colchicine. This could be related to the fact that mutagenic treatment at higher dose damaged or disrupted the physiology of the plant, affecting photosynthesis and respiration, resulting in poor plant growth in terms of plant height, number of leaves, leaf area and number of tillers. Similar line of work had been reported by Raju *et al.* (1980) in ginger, Gupta *et al.* (1982) in costus, Madhuri (2017) in ginger and Laxmi *et al.* (2019) in turmeric.

The maximum curcumin (0.008 %), oleoresin (6.80 %), crude fibre (4.69 %) and essential oil (0.36 %) contents were observed in control (T_{13}). The lowest value was noticed in colchicine @ 0.3% (Figures 1, 2 and 3).

Changes in the production of essential oil, oleoresin and crude fibre could be attributed to the promotive or inhibitory influence of mutagens at various dosages. These findings are in accordance with the reports of Gupta *et al.* (1982) in costus. The change in chemical constituents in response to mutagenic treatment is most likely owing to mutagenic sensitivity of these components at various doses.

Table 3. Effect of different mutagens on yield parameters of black turmeric

Treatments	Yield/ plant (g)	Number of primary Rhizomes/ clump	Number of secondary Rhizomes/clump
T1(Gamma@10Gy)	115.13[cd]	3.59[de]	9.10[cd]
T2(Gamma@15Gy)	119.99[bc]	3.91[cd]	9.24[c]
T3(Gamma@20Gy)	123.61[b]	4.31[ab]	10.84[ab]
T4(Gamma@25Gy)	121.87[b]	3.99[bc]	9.44[c]
T5(Gamma@30Gy)	110.96[de]	3.59[de]	8.44[cde]
T6(Gamma@35Gy)	106.88[e]	3.10[fg]	7.44[ef]
T7(EMS@ 1%)	122.09[b]	3.90[cde]	9.74[bc]
T8(EMS @1.25 %)	114.87[cd]	3.68[cde]	9.14[cd]
T9(EMS @1.5 %)	98.29[f]	3.61[cde]	7.84[de]
T10(Colchicine@0.1%)	50.93[g]	3.50[ef]	6.48[fg]
T11(Colchicine@0.2%)	47.05[gh]	2.90[g]	6.24[fg]
T12(Colchicine@0.3%)	42.67[h]	2.71[g]	5.84[g]
T13(Untreated control)	130.03[a]	4.59[a]	11.64[a]

Note: DMRT at 5% of level of significance to compare the pair of treatment means. Any two means having common letter, are not significantly different at 5% level of significance.

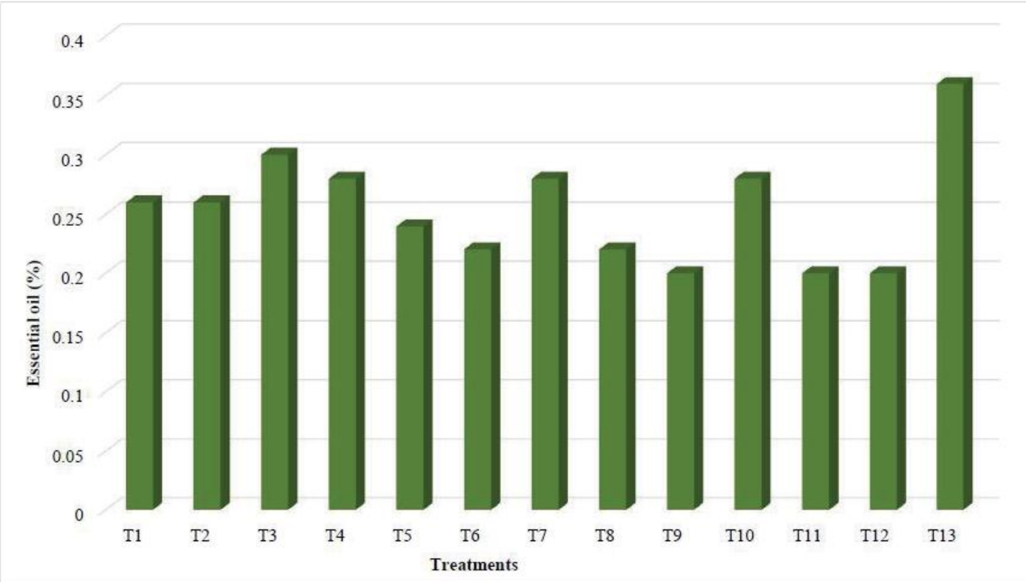

Figure1. Effect of different mutagenic treatments on curcumin content (%) in black turmeric

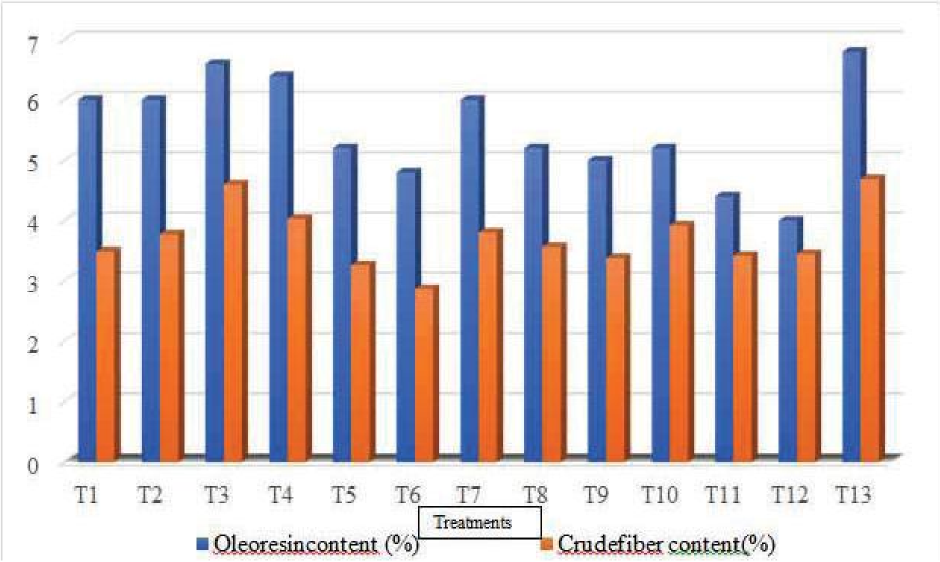

Figure 2. Effect of different mutagenic treatments on oleoresin (%) and crude fibre content

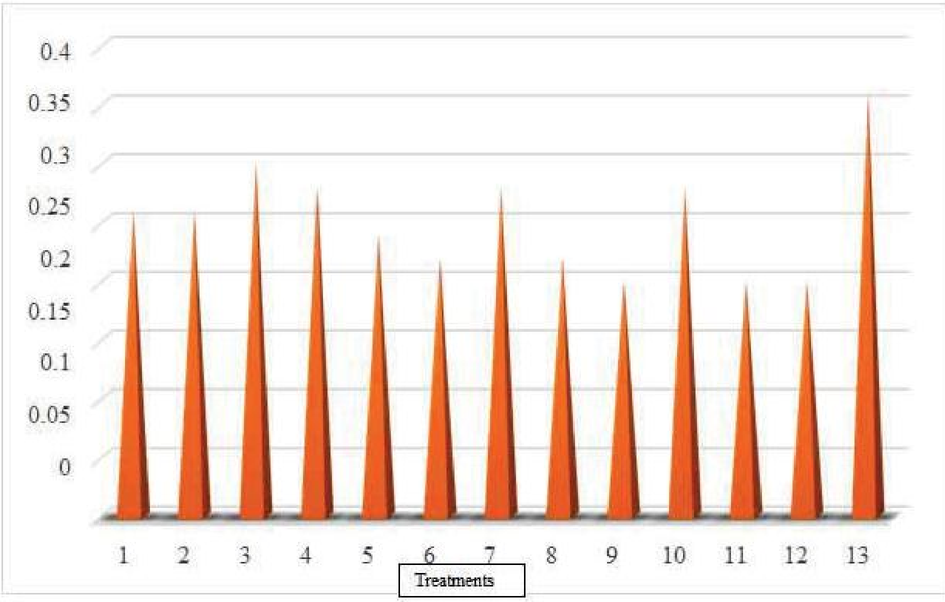

Figure 3. Effect of different mutagenic treatments on essential oil content (%)

Conclusion

It was evident from the study that mutagenic treatment resulted in creation of considerable degree of variability in the existing black turmeric population for plant growth, yield and secondary metabolite contents. The populations need to be further evaluated in ensuing generations for their consistency in performance for yield and quality attributes.

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