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EFFECT OF COLCHICINE ON MORPHOLOGICAL AND BIOMETRICAL TRAITS IN AFRICAN MARIGOLD

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ABSTRACT

An experiment on effect of colchicine on polyploidy induction in white marigold was conducted in CRD with six treatments and five replications during year 2016-2017. The seeds of white marigold were treated with 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 % colchicine at room temperature for 12 hrs. and germinated in protray. Thirty days old seedlings were transplanted in the field with spacing of 45 cm × 30 cm in ridges and furrow. Observations on morphological traits and biometrical traits were recorded on each and every plant in each treatment and the data were subjected to statistically analysed. Reduced stem elongation, slow growth, slower node development as compared to control seedlings, abnormal first 1-2 true leaves were major morphological and growth characteristics observed due to colchicine treatments. These effects on seedling growth were most evident at the higher colchicine concentrations (1.5 to 2.5 %). Maximum number of 12 variant types were observed in 0.5 % colchicine followed by 1 % colchicine which recorded 8 variant types. Effect of colchicine treatments on 12 biometrical traits showed significant variation among the different colchicine treatments as observed from F test. Increase in the concentration of colchicine reduced germination percentage, survival rate, height of plant, number of branches plant^{-1}, days required for flowering, disc diameter. While increase in the concentration of colchicine increased stem diameter, number of flowers plant^{-1}, weight of flower, diameter of fully open flower, length of flower along with pedicel. This study lead to the conclusion that seeds treated with 0.5 % colchicine for 2 hrs. was optimum for inducing variation in marigold .

(Key words : Marigold, colchicine, variation)

INTRODUCTION

Marigold is grown for loose flowers, making garlands, decoration during puja and several religious functions, besides its use in landscape gardening. Apart from its significance in ornamental horticulture, it has been valued for other purposes too. The aromatic oil extracted from marigold, is called as “Tagetes oil”. It is used in preparation of high grade perfumes and also as an insect fly repellent. Recently dried flower petals of marigold are used as poultry feed in order to improve the colour of egg yolk as well as broiler’s skin. Flowers of African marigold can be used for extraction of L-limonene, ocimene, L-linalylacetate, L-linalool. Marigold petals are used for extraction of xanthophylls. Lutein which is the major constituent of xanthophylls is used for colouring food stuffs. Purified extract of marigold petals containing lutein dipalmitate is marketed as an ophthalmologic agent under the name adaptinol.

Marigold is widely cultivated as bedding plant in landscape design. Beside the pristine uses as loose flower and the bedding plants, marigold occupies anthelmintic, analgesic, anti-inflammatory, aromatic, bronchodilatory, digestive, diuretic, emmenagogue, sedative and stomachic properties. It is also widely used as perfumes, herbal, gulal, organic manure, anticarcinogenic agent, antioxidant in retinotherapy and for oil extraction. The floral extract of marigold is used for treating eye diseases and ulcers. Flowers are important for their economic use as well as aesthetic value. Among the flowers grown by farmers, marigold has its own importance. It has gained popularity among flower growers because of its easy cultivation and wide adaptability. The growers are attracted towards marigold flower as it has a habit of free flowering, short duration to produce marketable flowers of attractive colours having good keeping quality.

Total area under marigold crop in India during the year 2014-2015 was 56.04 thousand ha with the production of 497.59 thousand metric tonnes of loose flowers and 4.28 lakhs number of cut flowers (Anonnymous, 2015). Flower

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characters of marigold are generally one of the important aspects that acquire a tremendous attention from morphological breeders and growers. Those of undesired flower characters could possibly be improved by polyploidization. According to Azmi *et al*. (2016) perfection of flower in the floriculture plants have probably been achieved by polyploidy. In recent times, polyploidy program had been used to bring about variation in chromosome number, in order to fill up the needs of the industry. Polyploidy can increase genetic variability and improve flower characteristics, therefore, floriculture is probably the most benefited from this techniques. Those of several previous studies have been showed valuable role of polyploidy in marigold and various crops improvement.

Keeping in mind the above views, this study was planned in white marigold (*Tagetu serecta*, 2n = 2x = 24) using colchicines with the objective of creating more genetic variability, high yield and novel flower characters.

MATERIALS AND METHODS

The seeds of diploid white marigold were collected from Horticulture section of College of Agriculture, Nagpur. Before sowing the seeds were soaked in water for 12 hr and after that water soaked seeds were treated with different concentration of colchicine for 12 hr. The seeds at the rate of 50 per treatment were soaked in aqueous solution of colchicine with a concentration of 0.00 (control), 0.5, 1.0, 1.5, 2.0 and 2.5 per cent w/v at room temperature (25° C) for 12 hrs. Five replications of 10 seeds for each treatment were planted in the holes of protray filled with potting mixture of coco pit and vermicompost which was then gently covered with the soil. Trays were watered lightly with the help of hands. After about 3 to 4 days the seeds started germinating and potential germination was completed within ten days. Thirty days old uniform well developed and healthy seedlings of 10-15 cm length were transplanted in the field with 45×30 cm^2 spacing in ridges and furrow. The experiment was conducted in CRD design with five replications. The data were subjected to statistical analysis as per the method given by Pansen and Sukhatme (1954). Per cent data were subjected to arcsine transformation before analysing the data.

Observations on morphological variations and biometrical traits viz., germination percentage, survival rate (%), plant height (cm), stem diameter (cm), number of branches plant^{-1}, days to 1st flower initiation, leaf dimension, number of flowers plant^{-1}, weight of flower (g), diameter of fully opened flower (cm), length of flower along with pedicel (cm) and disc diameter (cm) were recorded on each and every plant in each treatment in each replication. The data recorded were analysed statistically by following CRD design as described by Panse and Sukhatme (1954). Per cent data were subjected to arcsine transformation before analysing the data.

RESULTS AND DISCUSSION

Morphological observations

The treated plants were regularly observed from germination to harvesting for recording morphological variations. Major morphological and growth habit characteristics observed in seedlings or plants treated with colchicine showed reduced stem elongation, slow growth and slower node development relative to control seedlings, and in some cases, the first 1-2 true leaves were morphologically abnormal eg. wrinkled, subsequent leaves appeared normal. These effects on seedling growth were most evident at the higher colchicine concentrations (1.5 to 2.5%). In consistent with this results Liu (2007) also reported morphological differences of the colchiploid plants which included a more compact growth habit, broader and thicker leaves in marigold.

The data recorded on the type of variants along with their frequency are depicted in table 1 and plate 2, 3. Maximum number of variants were observed in the treatment of 0.5 % colchicine followed by treatment 1 % colchicine. Least number of variants were recorded in 2.0 % colchicine followed by 2.5 % colchicine. All the variants identified were labelled and harvested separately. The results on morphological observations revealed that 0.5 % colchicine for 12hrs. was more effective in inducing variation followed by 1 % colchicine for 12 hrs.

In consistent to this result Hanzelka and Kobza (2001) reported 1- 1.5 % colchicine for 5 days to be the best treatment for variant induction in Aster. Kazi (2013) observed maximum variation with 0.2 % and 0.3 % colchicine treatment for 12 hrs in Marigold seeds. In *Torenia fournieri*, the most effective treatments were 5 ppm colchicine for 1 day and 15 ppm for 3 days which yielded maximum variants as reported by Jiranapapan *et al*. (2011).

Biometrical observations

The data recorded on biometrical observations are presented in table 2. The F-test was found to be significant for all the 12 traits studied. This indicates the prevalence of significant variation among the different colchicine treatments. Similar to this result significant variation among the different colchicine treatments were also reported by Hanzelka and Kobza (2001) in Aster, Kazi (2013) in Aster and Kazi *et al.* (2015) in French marigold.

Germination percentage

Germination was found to be maximum in control (66 %) and was significantly superior over all other treatments. This was followed by 0.5 % colchicine (45.9 %) and 1 % colchicine (42.3 %). These two treatments were at par with each other and significantly superior over the other treatments. The least germination per cent was observed in 2.5 % colchicine (26.7 %). It was further observed that colchicine treated seeds germinated slowly and took more time as compared to control. The results revealed that increase in the concentration of colchicine reduced germination percentage. This results are in agreement with the observations in *Salvia hains* as reported by Mohammad *et al*. (2011). They reported 72% germination in control and 66.8 % to 21.6 % in different colchicine treatments. Liu (2007) also reported high germination frequency of 68 % in control and 62.2 % to 32.1 % with different colchicine treatments in *Platanus acerifolia*.

Survival rate (%)

Highest survival rate was observed in control (57.5 %) and was significantly superior over other treatments. This was followed by 0.5 % colchicine treatment (35.3 %) and by 1.0 % colchicine (28.8 %) which were at par with each other and significantly superior over other treatments. The least survival % was observed in 2.5 % colchicine (6 %). The results indicated that increase in the concentration lead to decrease in survival rate. These findings were in consistent with the finding of Jiranapapan *et al.* (2011) in *Torenia fournieri*, Mohammad *et al*. (2011) in *Salvia hains* and Liu (2007) in *Platanus acerifolia*, who reported that the survival rate of colchicine treated shoots were lower, with the extent of the reduction depending on the colchicine concentration. Decrease in survival rate with increase in concentration of colchicine might be due to the cause of tissue necrosis when soaked in different concentrations of colchicine solution. This is because colchicine does not only have an effect on cell division but spreads through the cell, interfering with cellular mechanism and causing toxicity at high concentration as reported by Sasiree *et al*. (2013). Colchicine apparently affects the viscosity of cytoplasm so the cell can not function normally.

Plant height (cm)

Maximum height of 112.6 cm was exhibited in control, followed by 0.5 % colchicine treatment (107.5 cm) and at 1 % colchicine treatment (100.16 cm). The least height of 72.0 cm was exhibited by 2.5 % colchicine treatment. The data revealed that height of the plant was reduced with the increase in the concentration of colchicine. Though some of the treatments were at par with each other, numerical reduction in height was observed due to colchicine treatment. The high coefficient of variation observed for this trait revealed that within treatment variation was very high. The reduction in height due to colchicine treatment is caused due to shorter intermodal distance. In accordance to this results Mohammad *et al.* (2011) in *Salvia hains*, Liu (2007) in *Platanus acerifolia*, Sasiree *et al*. (2013) in *Torenia fournieri* reported lower plant height in tetraploid plants than the diploids. Kazi *et al.* (2015) reported 50 % reduction in the height of the plants of tetraploid as compared to diploid in French marigold.

Stem diameter (cm)

Significant variation was observed at all stages of observations which indicated that stem diameter was significantly influenced by different colchicine treatments. Maximum stem diameter (3.76 cm) was noticed in 2.5 % colchicine followed by 2 % colchicine (3.62 cm) and were at par with each other and significantly superior over other treatments. The least stem diameter was observed in control (2.28 cm). The data on stem diameter revealed that increase in the concentration of colchicine simultaneously increased the diameter of stem. The differences in stem diameter between control and different colchicine treatments were numerical rather than statistical significance as many of the treatment were at par with one another. Within treatment variation for this trait was also observed to be very less. Among the different treatments only few number of the plants recorded stem diameter of 4 cm and other were between the range of 2 to 3.6 cm. Similar to this result Sasiree *et al*. (2013) observed that there was no statistically significant difference in stem thickness between the diploid and tetraploid plants of *Torenia fournieri*.

Number of branches plant^{-1}

Significant variation was observed at harvest stage for this trait. Significantly maximum number of branches plant^{-1} was recorded by 2.5 % colchicine treatment (18.6) and was significantly superior over all other treatments. This was followed by control (13.2), 0.5 % colchicine (10.6), 1 % colchicines treatment (10.2) which were at par with each other. The least number of branches were produced in 2 % colchicine (8.4). The results on number of branches plant^{-1} indicated that increase in the concentration of colchicine decreased the number of branches except for 2.5 % colchicine treatment. Even though maximum number of branches was produced in 2.5 % colchicine treatment, the number of reproductive branches yielding flowers were very few. Similar to this result Mohammad *et al.* (2011) in *Salvia hains* reported that the number of branches reduced in tetraploid plants and the length of branches was shorter than diploid plants. Liu (2007) also observed reduced number of branches in tetraploid than diploid in *Platanus acerifolia*.

Days to first flower initiation

Significantly maximum days to first flower initiation (80.20 days) was noted in control when compared with rest of the treatments under study. This was followed by 0.5 % colchicine (69.2 days), 1 % colchicine (67.80 days), 1.5 % colchicine (63.6 days) and 2 % colchicine (61.8 days) which were all at par with each other. The results on days to first flower initiation revealed that time taken for flowering was more in control as compared to colchicine treatments. Further with the increase in the concentration of colchicine days required for flowering was reduced. Early flowering in treated plants may be due to the reason that plants might have received shock due to colchicine, reduction in height of plants and vigorous growth. In contrary to this finding Kazi (2013) reported 0.1 % colchicine for 9 hrs, 0.2 % colchicine for 1 day and control produced flowering in 32 days. He also reported that there was no significant difference in control and treated plants for days to first flower bud initiation and days required for flowering.

Leaf dimension (cm²)

The range of leaf length recorded was 5.90 cm (2.5 % colchicine) to 7.82 cm (0.5 % colchicine). It is observed that 0.5 % colchicine treatment exhibited maximum leaf length followed by 1 % colchicine. But these two treatments were at par with control. Higher concentration of colchicine 1.5 %, 2 % and 2.5 % resulted in reduction in leaf length. Leaf breadth ranged 1.94 cm (2.5 % colchicine) to 2.26 cm (0.5 % colchicine). Maximum leaf breadth was recorded in 0.5 % colchicine treatment followed by 1 % colchicine and were at par with control and was significantly superior over all other treatments. The results on leaf dimension revealed that colchicine treatment at lower concentration had increased length and breadth of leaf and at higher concentration had reduced length and breadth of leaf. In addition to increase and decrease of leaf length and breadth, treated plant showed thicker leaves as compared to untreated control. In accordance to this result Ravandi *et al*. (2013) in Chicori, Dario and Paul (2009) in *Vacinium* and Liu (2007) in *Platanus acerifolia* reported significant difference in leaf width and length in colchiploid plant as compared to untreated plants. Raghunath *et al*. (2014) observed that the colchiploid plants were having larger and thicker leaves with deep green pigment as compared to control in African marigold. Mohammad *et al*. (2011) also reported thicker and broader leaves in colchiploid plants of *Salvia hains*.

Traits related to flower

In the present study data on effect of colchicine on number of flowers plant^{-1}, weight of flower (g), diameter of fully open flower (cm), length of flower along with pedicel (cm), disc diameter (cm) were studied. All the traits related to flower showed significant variation. Significantly highest number of flowers plant^{-1} was recorded by 0.5 % colchicine (33 flowers) followed by 1 % colchicine (32.4 flowers) and control (28.60 flowers). All these treatments were at par with each other. The treatment 2.0 % colchicine recorded minimum number of flowers plant^{-1} (3.80). The mean number of flowers plant^{-1} in treated plants though found to be at par with control, lot of variation within treatments were observed. Weight of the flower was significantly highest in 0.5 % colchicine (13.40 g) followed by 1 % colchicine (13 g). These two treatments were significantly superior when compared with control and rest of the treatments. Significantly minimum weight of flower was recorded in 2.5 % colchicine (5.04 g). From the data on diameter of fully opened flower, it is observed that treatments 0.5 % (4.80 cm), 1 % (4.70 cm), control (4.52 cm) and 1.5 % (4.38 cm) in a descending manner were found at par with each other and superior over other treatments. The treatments 2.5 % colchicine (3.42 cm) and 2 % colchicine (3.80 cm) produced least mean diameter of fully opened flower. Length of flower along with pedicel was significantly longest in 0.5 % colchicine (13.92 cm) followed by 1 % colchicine (12.42 cm) and 1.5 % colchicine (12.04 cm) when compared with control and rest of the treatments. The 2.5 % colchicine produced significantly least length of flower (7.44 cm). The range of disc diameter obtained was 1.56 cm in 2 % colchicine to 2.24 cm in 2.5 % colchicine. The treatment 2.5 % colchicine (2.24 cm) followed by 1.5 % colchicine (2.10 cm) showed increased disc diameter, when compared with control and rest of the treatments.

The data on characters related to flower revealed that 0.5 % and 1 % colchicine resulted in increased number of flowers plant^{-1}, weight of flower, diameter of fully opened flower, length of flower along with pedicel and decrease in disc diameter. Colchicine treatment has thus increased as well as decreased the value of traits related to flower depending on the concentration of colchicine. Further, it was observed that the traits like number of flowers plant^{-1}, diameter of fully opened flower and disc diameter exhibited lot of variation within the treatment and between the treatment. In consistent to this result, Sasiree *et al*. (2013) in *Torenia fournieri* observed that the flowers on tetraploids were wider than the flowers on diploids to a statistically significant degree and no statistical significant difference in mean flower length or petal thickness between the diploid and tetraploid plants. Ravandi *et al*. (2013) in *Cichorium intybus* reported increase in flower diameter in cholchiploid plants when compared to untreated plants.

The inferences drawn from the data related to morphological, biometrical and cytological traits revealed that seeds treated with 0.5 % colchicine for 12 hrs. was more effective as this treatment gave maximum number of variants. This treatment also showed favourable influence for the characters studied. Increase in the concentration of colchicine was observed to adversely affect the plants in respect to all the traits studied. At higher concentration of any chemical/ nutrients outside the cell more than that of cytoplasmic concentration the exo-osmosis takes place and cell losses its turgidity and electron also losses out. This might be the reason for adverse effect in all the traits due to higher concentration of colchicine in the present study. Hence, 0.5 % colchicine was considered as an optimum concentration in marigold.

Table 1. Morphological variations induced by various colchicine concentrations

Treatment	Total No. of variants	Type of variant characters	No. of plants showing variant characters
T_0	-	-	-
T_1 (0.5 %)	12	Tall	11
		Compact growth	9
		Drooping	2
		Large leaf	19
		Small leaf	3
		Dark green leaf color	19
		Thick leaved plant	3
		Small compact flower	5
		Large flower	17
		Thick and hard stem plant	15
		Thin stem	7
		Profuse branching	10
T_2 (1.0 %)	8	Plant with single stem	2
		Dense growth	13
		Large leaf	11
		Small leaf	4
		Dark green leaf	9
		Flower with few petals	2
		Medium size flower	13
		Thick stem plant	9
T_3 (1.5 %)	7	Plant with single branch	3
		Dwarf and stunted	6
		Larger leaf	2
		Small leaf	7
		Compact small flower	3
		Medium size flower	6
		Thick stem	4
T_4 (2.0 %)	2	Tall erect with thick and rigid growth habitat	2
		Dwarf plant habitat	2
T_5 (2.5 %)	4	Medium height with dense growth	All plants
		Small flower	2
		Large flower	1
		Flower with few petals	1

Table 2. Effect of colchicine treatments on different biometrical traits

Tr. No	Colchicine concentrations	Germination (%)	Survival rate (%)	Plant height (cm)	Stem diameter (cm)	No. of branches $plant^{-1}$	Days to first flower initiation	Leaf dimension (cm)		Traits related to flower				
								Length	Breadth	No. of flowers $plant^{-1}$	Wt. of flower (g)	Diameter of fully open flower (cm)	Length of flower along with pedicel (cm)	Disc diameter (cm)
T_0	0.0 % (Control)	66.0 (54.31)	57.5 (49.31)	112.60	2.28	13.20	80.20	6.88	2.18	28.60	11.30	4.52	10.94	1.92
T_1	0.5 %	45.9 (42.64)	35.3 (36.48)	107.05	2.42	10.60	69.20	7.82	2.26	33.00	13.40	4.80	13.92	1.82
T_2	1.0 %	42.3 (40.59)	28.8 (32.43)	100.16	2.50	10.20	67.80	7.76	2.20	32.40	13.00	4.70	12.42	1.78
T_3	1.5 %	32.0 (34.42)	11.8 (20.08)	93.30	3.20	9.40	63.60	6.66	2.08	22.00	7.20	4.38	12.04	2.10
T_4	2.0 %	29.3 (32.79)	8.2 (16.64)	77.90	3.62	8.40	61.80	6.52	2.06	3.80	6.60	3.80	9.28	1.56
T_5	2.5 %	26.7 (31.11)	6.0 (14.17)	71.00	3.76	18.60	59.00	5.90	1.94	17.20	5.04	3.42	7.44	2.24
	SE (m) ±	1.91	2.04	6.88	0.15	1.32	3.40	0.42	0.04	3.99	0.71	0.20	0.75	0.10
	CD (5%)	5.52	5.90	19.92	0.43	3.82	9.85	1.24	0.13	11.54	2.06	0.58	2.19	0.29
	CV (5%)	10.86	16.20	16.44	11.47	25.23	11.37	13.86	4.83	39.09	16.95	10.63	15.41	11.88

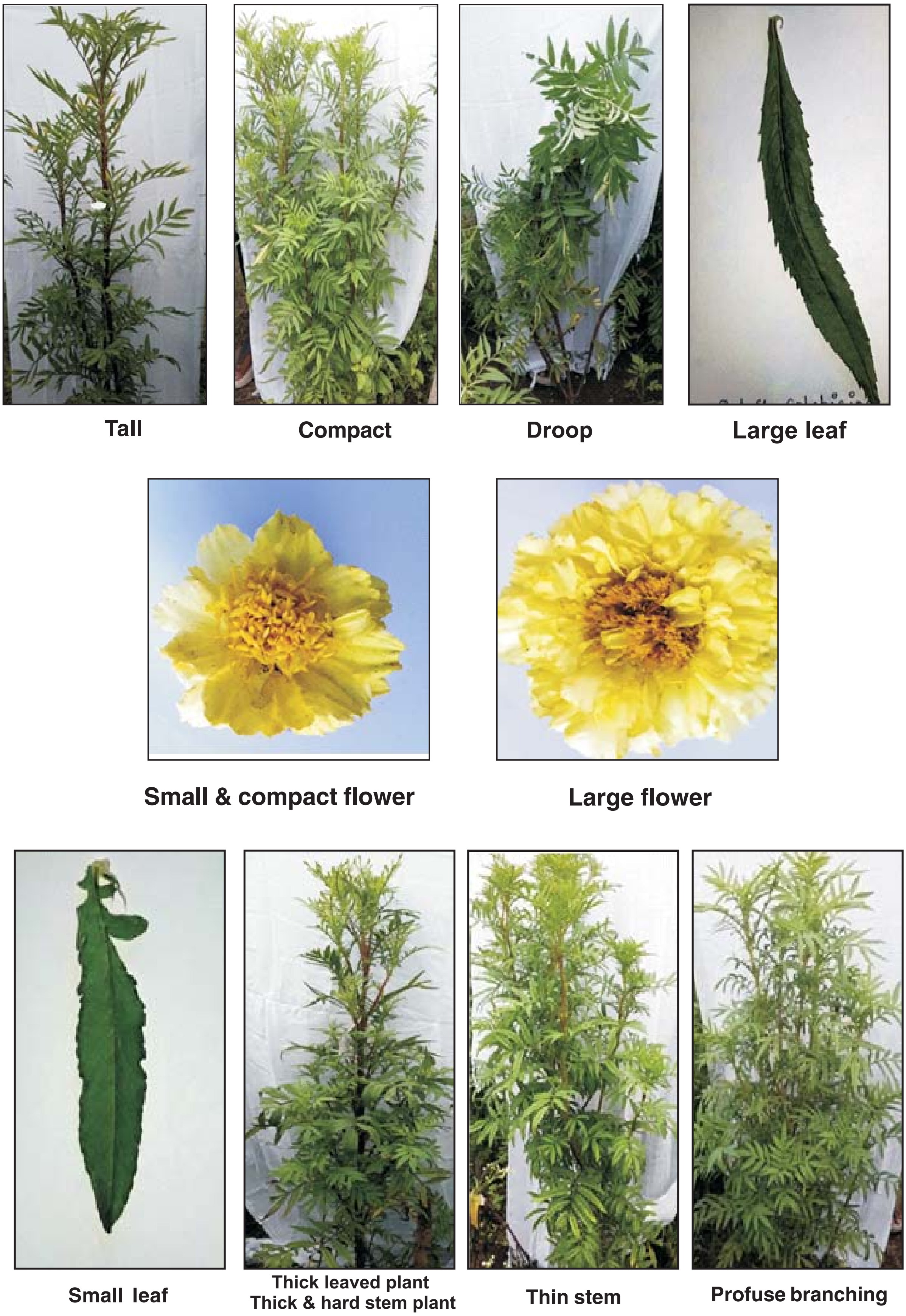
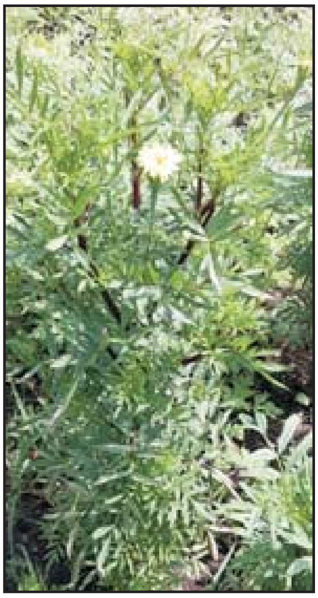


Plate 1. Morphological variations induced by 0.5 % colchicine treatment

Plant with single stem

Flower with few petals

Large leaf



Dense growth

Small leaf

Medium size flower

Thick stem plant

Plate 2. Morphological variations induced by 1.0 % colchicine treatment

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