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ISSN: 0975-928X

Volume: 10

Number:2

EJPB (2019) 10(2):922-929

DOI:10.5958/0975-928X.2019.00118.2

<https://ejplantbreeding.org>

Research Article

Assessment of colchicine sensitivity in African marigold (*Tagetes erecta*) var. Pusa Narangi Gaiinda

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(Received:15 Apr 2019; Revised:06 Jun 2019; Accepted:08 Jun 2019)

Abstract

The present experiment was carried out to determine colchicine sensitivity in African marigold (*Tagetes erecta*) var. Pusa Narangi Gaiinda to various levels of colchicine (0.00, 0.02, 0.04, 0.06, 0.08 and 0.1%) at different duration of soaking (0, 1, 2 and 3 hours). The experimental results indicated that the effect of colchicine concentration and duration of treatment has a significant effect on morphological parameters of African marigold. Result showed gradual and significant reduction in germination percentage (40%), survival of seedlings (9%), shoot length (6.1cm), root length (3.2cm), leaf length (3.25cm) and leaf width (1.35cm) with the increase in concentration of colchicine at 0.1% for 3 hours of soaking. The probit curve analysis based on mortality percentage of treated seeds revealed, the LD₅₀ dosage of colchicine to be 0.11% with a soaking duration of one hour for African marigold var. Pusa Narangi Gaiinda.

Key words

African marigold, Polyploidy breeding, colchicine, probit analysis, LD₅₀

Introduction

Marigold belonging to Asteraceae is one of the most popular loose flower and is commercially cultivated in different parts of our country. Among different marigold species, African marigold (*Tagetes erecta* L.) is grown in tropical conditions and subtropical conditions. It is hardy herbaceous annual plant with erect growing habit along with lanceolate and serrated leaves. Flower colour varies from lemon yellow to yellow, golden yellow or orange. Flowers are widely used as loose flower, possess great demand for garland making, floral arrangements and landscaping as herbaceous border, bedding and potted plants. Apart from ornamental purpose, it is considered as source of natural colour for dye industry. Marigold can be used as trap crop for nematodes due to presence of thiophenes (alpha-terthienyl) and also used as repellent against flies. Flower petals contain lutein, a xanthophyll pigment which is potentially utilized as food colorant. Dried petal concentrates are added to poultry feed to intensify the yellow colour of egg's yolk.

Polyploidy breeding is an effective method for doubling the chromosome number in most of the ornamental crops. Among various anti-mitotic agents utilized for polyploid induction, colchicine is extensively used for chromosome doubling. Affected cells may increase in size due to

the increased number of chromosomes and also has various changes in their functions. On comparing with the normal plants, the plants developed by colchicine treatment shows change in height, thickness of stem and branches; in size, shape and texture of leaves and flowers. Tetraploids are more vigorous and larger in size with thick and dark green leaves.

Flower form is an important character for African marigold which is one of the prime breeding objective. As colchicine increases the chromosomal count, colchiploidy can be used as a potential breeding method for altering the flower form in African marigold. Considering the above points, to increase the potentiality of marigold flowers in terms of qualitative traits through polyploidy breeding, the present study was planned. In order to avoid excessive loss of actual experimental materials, sensitivity tests must be conducted to determine LD₅₀(the safe dose at which half population of the planting material survive) doses before massive exposure of similar materials. LD₅₀ dose is considered as the dose at which highest frequency of chromosome doubling occurs. With this background, the present investigation was undertaken aiming to determine the optimum lethal dose (LD₅₀) for colchicine in marigold (*Tagetes erecta* L.) var. Pusa Narangi Gaiinda.

Materials and Methods

Seeds of Pusa Narangi Gaiinda (African marigold) collected from ICAR-IARI, New Delhi were used for the experimental study.

Factorial completely Randomized Design with–2 factors (24 treatments and 2 replications).

Treatment details	Concentration(%) – Duration (hours)
1	0.00 % - 0 hour
2	0.00 % - 1 hour
3	0.00 % - 2 hours
4	0.00 % - 3 hours
5	0.02 % - 0 hour
6	0.02 % - 1 hour
7	0.02 % - 2 hours
8	0.02 % - 3 hours
9	0.04 % - 0 hour
10	0.04 % - 1 hour
11	0.04 % - 2 hours
12	0.04 % - 3 hours
13	0.06 % - 0 hour
14	0.06 % - 1 hour
15	0.06 % - 2 hours
16	0.06 % - 3 hours
17	0.08 % - 0 hour
18	0.08 % - 1 hour
19	0.08 % - 2 hours
20	0.08 % - 3 hours
21	0.10 % - 0 hour
22	0.10 % - 1 hour
23	0.10 % - 2 hours
24	0.10 % - 3 hours

Seeds of African marigold var. Pusa Narangi Gaiinda were immersed in water for 4-5 hours at room temperature. Seeds that are visibly swollen and ready to germinate were selected. Colchicine at various concentration were prepared and seeds were treated with various concentration of colchicine as described in treatment details. After treatment, seeds were washed with sterile distilled water 10 times to wash out excess colchicine. In control, seeds were sown directly. After 2-3 days, the treated seeds were sown in portrays, filled with mixture of sand, vermicompost and coir pith @ ratio of 1: 0.5: 1. The morphological parameters such as germination percentage, survival percentage, shoot length, root length, leaf length and leaf width were measured on 15th day after sowing. The LD₅₀ value was calculated based on probit analysis (Finney, 1978) using the mortality percentage of seedlings compared with control.

Result and Discussion

Lethal dose for 50 percent mortality (LD₅₀) for colchicine treatment was fixed based on germination percentage. Probit analysis was carried

out based on mortality percentage of seeds after treatment compared with untreated seeds. In this study, LD₅₀ value for colchicine treatment was assessed from probit curve analysis and results were depicted. It was found that LD₅₀ value of colchicine for African marigold var. Pusa Narangi Gaiinda was 0.11% at a duration of one hour (Table1).

In this experiment, among the treated seeds the maximum seed germination percentage was observed in seeds treated with 0.02% colchicine for 1 hour (80%) followed by 0.04% for 1 hour which recorded a germination percentage of 76% and lower seed germination percentage was observed in 0.1% for 3 hours (40%) treated seeds. 94% of seed germination was observed in control (T₁). The maximum survival percentage was found in seeds treated with 0.04% colchicine for 1 hour (51%) and lowest survival percentage was found in 0.1% for 3 hours (9%). The survival percentage in control was estimated as 78.5% (T₁) (Table2). Reduced survival percentage at higher doses of colchicine mainly may be due to killing of cells or chromosomal aberration.

Similar result was reported by Sajjad *et al.*, 2013 in *Tagetes erecta*, wherein the highest mortality rate (72.4%) was observed in 0.05% of colchicine treated plants. Increased mortality and decreased survival percentage was found with increased colchicine concentration in *Lilium* (0.02% for 9 hours) (Heo *et al.* 2016), in *Toreniafournieri* (Saisree *et al.*, 2013), in *Dendrobium* (Atichart and Bunnag, 2007) and *Jatropha* (0.4% for 4 and 10 days) (Niu *et al.* 2016). The higher level of dosages might have caused inhibition in mitosis on cell division and elongation due to inactivation and/or decrease in auxin concentration resulting in poor establishment and survival as reported earlier (Mahure *et al.*, 2010).

Morphological characters viz., shoot length, leaf length, leaf width and root length was observed in this study. The highest shoot length was found in 0.06% of 1 hour (8.45 cm) treated seeds followed by seeds treated with 0.02% colchicine for 1 hour (8.10 cm) and lowest shoot length was recorded in 0.1% for 3 hours (6.10 cm). In control it was observed as 8.55 cm (T₁) (Table3). The reduction in shoot length can be due to delay in sprouting and slow growth rate.

Root length was observed to be highest in seeds treated with 0.02% colchicine for 1 hour (4.10 cm) followed by seeds treated with 0.08% colchicine for 1 hour (4.05 cm). Lowest root length was observed in 0.1% for 3 hours (3.20 cm). In control, it was recorded as 5.40 cm (T₁) (Table3). Tuwo *et*

al., 2016 reported that minimum number of roots and root length was noted in colchicine treated plants of orchid (*Vanda* hybrids).

In this present study, the maximum leaf length was found in seeds treated with 0.02% colchicine for 1 hour (4.55 cm) and minimum was found in 0.04% for 1 hour (4.1 cm) which is on par with seeds treated with 0.08% for 1 hour and in control it was observed as 5.25 cm (Table4). The higher leaf width was reported in 0.02% for 1 hour (2.25 cm) which is on par with seeds treated with 0.04% for 1 hour followed by 0.06% for 1 hour (2.15 cm) treated seeds and lower leaf width was found in 0.1% for 3 hours (1.35 cm). The leaf width of 2.30 cm was observed in control (T_1) (Table4).

Similar result with minimum length, width and number of leaves was found in plants treated with colchicine in orchid (*Vanda* hybrid) (Tuwo *et al.*, 2016) and in chrysanthemum (Kushwah *et al.*, 2018). Leaf shape was found to be different from treated and untreated seedlings, wherein the leaves of treated seedling were linear shaped while the leaves of control were elongated leaves. Thi *et al.*, (2003) reported in *Alocasia*, variation in leaf shape was found among the multiplied plants under same growing condition. While the leaves of diploid plants has elongated heart shaped leaves, the leaves of tetraploids and chimera tends to be round in shape.

Reduced morphological characteristics were observed in this present study, this is because colchicine does not only have an effect on cell division but spreads throughout the cell, interfering with cellular mechanisms and causing toxicity at high concentrations (Dermen, 1940). Colchicine apparently affects the viscosity of the cytoplasm so the cell cannot function normally (Cook and Loudon, 1952).

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Table 1. Probit analysis for calculating LD₅₀ in African marigold (*Tagetes erecta*) var. Pusa Narangi Gainda

Treatments	Concentration (%)	Duration (hours)	Log ₁₀ of doses	Observed mortality percentage	Corrected mortality percentage	Emprical probit unit	LD ₅₀ value
1	0.00	0	-	6	-	-	0.1%
2	0.00	1	-	14	-	-	
3	0.00	2	-	12	-	-	
4	0.00	3	-	14	-	-	
5	0.02	0	1.7	20	14.9	3.96	
6	0.02	1	1.7	20	7.0	3.52	
7	0.02	2	1.7	28	18.2	4.09	
8	0.02	3	1.7	36	25.6	4.34	
9	0.04	0	1.4	22	17.0	4.05	
10	0.04	1	1.4	24	11.6	3.81	
11	0.04	2	1.4	28	18.2	4.09	
12	0.04	3	1.4	38	27.9	4.41	
13	0.06	0	1.2	26	21.3	4.20	
14	0.06	1	1.2	36	25.6	4.34	
15	0.06	2	1.2	42	34.1	4.59	
16	0.06	3	1.2	46	37.2	4.67	
17	0.08	0	1.1	26	21.3	4.20	
18	0.08	1	1.1	46	37.2	4.67	
19	0.08	2	1.1	46	38.6	4.71	
20	0.08	3	1.1	54	46.5	4.91	
21	0.1	0	1.0	28	23.4	4.27	
22	0.1	1	1.0	52	44.2	4.85	
23	0.1	2	1.0	58	52.3	5.06	
24	0.1	3	1.0	60	53.5	5.09	

Table 2. Effect of colchicine on Survival Percentage (%) of African marigold(*Tagetes erecta*) var. Pusa Narangi Gaiinda

Treatments	Concentration (%)	Duration (hours)	Actual	Percent over control	Percent reduction over control
1	0.00	0	79.00	100.00	0.00
2	0.00	1	75.00	94.94	5.06
3	0.00	2	77.00	97.47	2.53
4	0.00	3	75.00	94.94	5.06
5	0.02	0	48.00	60.76	39.24
6	0.02	1	47.50	60.13	39.87
7	0.02	2	42.00	53.16	46.84
8	0.02	3	30.00	37.97	62.03
9	0.04	0	58.00	73.42	26.58
10	0.04	1	51.00	64.56	35.44
11	0.04	2	33.00	41.77	58.23
12	0.04	3	30.00	37.97	62.03
13	0.06	0	48.00	60.76	39.24
14	0.06	1	39.00	49.37	50.63
15	0.06	2	29.00	36.71	63.29
16	0.06	3	27.00	34.18	65.82
17	0.08	0	56.00	70.89	29.11
18	0.08	1	26.00	32.91	67.09
19	0.08	2	22.00	27.85	72.15
20	0.08	3	18.00	22.78	77.22
21	0.1	0	53.00	67.09	32.91
22	0.1	1	19.00	24.05	75.95
23	0.1	2	16.00	20.25	79.75
24	0.1	3	9.00	11.39	88.61
Mean			41.98		
SE(d)			8.46		
CD(5%)			17.45		



Table 3. Effect of colchicine on shoot length and root length (cm) African marigold (*Tagetes erecta*) var. Pusa Narangi Gaiinda

Treatments	Concentration (%)	Duration (hours)	Shoot length (cm)			Root length (cm)		
			Actual	Percent over control	Percent reduction over control	Actual	Percent over control	Percent reduction over control
1	0.00	0	8.55	100	0	5.40	100	
2	0.00	1	8.50	99.42	0.58	5.15	95.37	4.63
3	0.00	2	8.35	97.66	2.34	5.25	97.22	2.78
4	0.00	3	8.10	94.74	5.26	5.25	97.22	2.78
5	0.02	0	8.15	95.32	4.68	4.30	79.63	20.37
6	0.02	1	8.10	94.74	5.26	4.10	75.93	24.07
7	0.02	2	7.90	92.40	7.60	3.95	73.15	26.85
8	0.02	3	7.80	91.23	8.77	3.95	73.15	26.85
9	0.04	0	8.10	94.74	5.26	4.05	75.00	25.00
10	0.04	1	8.05	94.15	5.85	3.75	69.44	30.56
11	0.04	2	7.40	86.55	13.45	3.95	73.15	26.85
12	0.04	3	7.20	84.21	15.79	3.65	67.59	32.41
13	0.06	0	8.50	99.42	0.58	4.15	76.85	23.15
14	0.06	1	8.45	98.83	1.17	3.95	73.15	26.85
15	0.06	2	7.55	88.30	11.70	3.65	67.59	32.41
16	0.06	3	7.05	82.46	17.54	3.55	65.74	34.26
17	0.08	0	8.50	99.42	0.58	4.20	77.78	22.22
18	0.08	1	7.65	89.47	10.53	4.05	75.00	25.00
19	0.08	2	7.15	83.63	16.37	3.95	73.15	26.85
20	0.08	3	6.90	80.70	19.30	3.90	72.22	27.78
21	0.1	0	8.30	97.08	2.92	4.05	75.00	25.00
22	0.1	1	7.95	92.98	7.02	3.75	69.44	30.56
23	0.1	2	7.00	81.87	18.13	3.55	65.74	34.26
24	0.1	3	6.10	71.35	28.65	3.20	59.26	40.74
Mean			7.80			4.11		
SE(d)			0.52			0.94		
CD (5%)			1.08			1.94		



Table 4. Effect of colchicine on leaf length and leaf width (cm) African marigold (*Tagetes erecta*) var. Pusa Narangi Gaiinda

Treatments	Concentration (%)	Duration (hours)	Leaf length (cm)			Leaf width (cm)		
			Actual	Percent over control	Percent reduction over control	Actual	Percent over control	Percent reduction over control
1	0.00	0	5.25	100.00	0.00	2.30	100.00	0.00
2	0.00	1	4.35	82.86	17.14	2.23	96.74	3.26
3	0.00	2	4.30	81.90	18.10	2.01	87.39	12.61
4	0.00	3	5.00	95.24	4.76	2.05	89.13	10.87
5	0.02	0	4.75	90.48	9.52	2.09	90.65	9.35
6	0.02	1	4.45	84.76	15.24	2.25	97.83	2.17
7	0.02	2	4.05	77.14	22.86	2.00	86.96	13.04
8	0.02	3	3.65	69.52	30.48	1.75	76.09	23.91
9	0.04	0	5.20	99.05	0.95	2.15	93.48	6.52
10	0.04	1	4.10	78.10	21.90	2.25	97.83	2.17
11	0.04	2	3.95	75.24	24.76	2.00	86.96	13.04
12	0.04	3	3.45	65.71	34.29	1.35	58.70	41.30
13	0.06	0	4.95	94.29	5.71	2.18	94.78	5.22
14	0.06	1	3.95	75.24	24.76	2.15	93.48	6.52
15	0.06	2	3.65	69.52	30.48	1.85	80.43	19.57
16	0.06	3	3.50	66.67	33.33	1.55	67.39	32.61
17	0.08	0	5.10	97.14	2.86	2.28	99.13	0.87
18	0.08	1	4.10	78.10	21.90	1.90	82.61	17.39
19	0.08	2	3.95	75.24	24.76	1.80	78.26	21.74
20	0.08	3	3.45	65.71	34.29	1.55	67.39	32.61
21	0.1	0	5.05	96.19	3.81	2.22	96.52	3.48
22	0.1	1	3.75	71.43	28.57	1.75	76.09	23.91
23	0.1	2	3.40	64.76	35.24	1.55	67.39	32.61
24	0.1	3	3.25	61.90	38.10	1.35	58.70	41.30
Mean			4.19			1.94		
SE(d)			0.66			0.54		
CD (5%)			1.37			1.17		

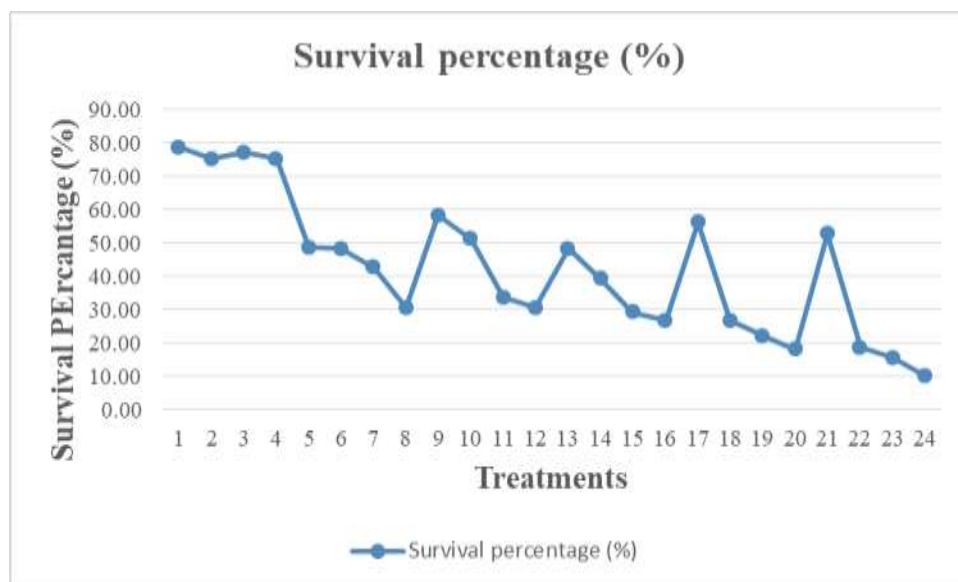


Fig. 1. Colchicine sensitivity on survival percentage (%) of African marigold var. Pusa Narangi Gaiinda

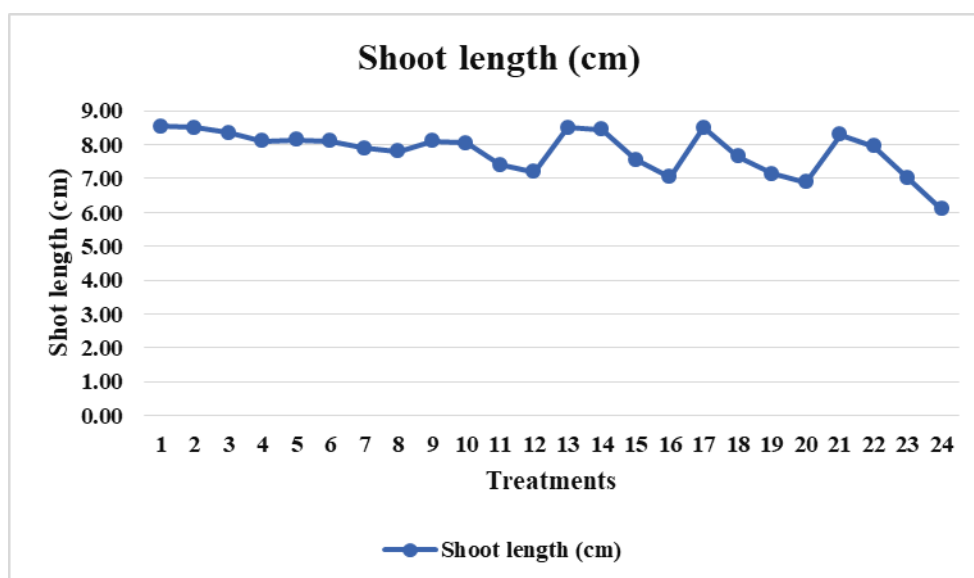


Fig. 2. Colchicine sensitivity on shoot length (cm) of African marigold var. Pusa Narangi Gaiinda

