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Improvement of *Dendranthemum grandiflora* cv. canter with colchicine *in vitro*

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Abstract The mutation of chrysanthemum with ray florets was induced by colchicine. The explants were cultured on Murashige and Skoog (MS) medium supplemented with 2 mg/l NAA and 4 mg/l Kinetin for inducing calli for 12 weeks. The shoots were regenerated from callus when cultured on MS medium for 8 weeks. The plant growth, size of stomata and number of chloroplasts per stomata were not statistically different in each treatment. However, the ray florets were received high levels of colchicine is less to survivor rate. The plantlet of chrysanthemum was transfered to pot plant containing soil and chopped coconut. Five of ten plant showed the flowers color changed from purple into a pinkish-orange at the 0.10% colchicine for 12 hours. The explant obtained from soaked of 0.15 % colchicine for 12 hours with the highest number of flowers per plant, 72 flowers per plant.

Keywords: Chrysanthemum, Mutation, Colchicine, Tissue culture

Introduction

Mutation breeding is an establishment method for crop improvement in ornamental plants (Broertjets and Harten, 1988). Physical mutagens such as X-ray, gamma-rays, ion beam (Matsumura *et al*., 2010) and chemical mutagens sush as ethyl-methane-sulphonate and colchicine has been applied to expand genetic resourse in plants. Polyploidization can be induced faster and more reliably with mutagen such as colchicine, oryzaline and trifluralin. Polyploidization using colchicine in horticultural plants has been practised for more than 80 years. Colchicine still remain as the prominent antimiotic agent in polyploid production (Eng and Ho, 2019). Recently, colchicine has been found to induce mutation of ornamental plant such as anthurium (Chen *et al*., 2011),

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gerbera (Gantiat *et al*., 2011), petunia (Regarado *et al*., 2017), tolonia (Ragasova *et al*., 2016) and vanda (Tuwo and Indrianto, 2016).

Chrysanthemum (*Dendranthemum grandiflora*) is in the compositae family. Chrysanthemums are one of the most important cut flowers and pot plants in the world. The native is in China and Japan. Chrysanthemum is a popular flower for cultivation and is important for trade in both domestic and international markets. The improvement is interesting in improving the character of flower like flower color, the character of petal, size involves resistance to various environments. In the breeding process, colchicine is used to induce morphological changes such as study of the effect of colchicine on polyploid induction in many plant (Eng and Ho, 2019). The aim of this study was to induce color and size mutations by using colchicine in chrysanthemum cv. Canter.

Materials and Methods

Plant material

The ray floret of chrysanthemum (*Dendranthemum grandiflora*) cv. canter was used as explants. The ray florets were washed by running through the water for 30 minutes, 1 minutes of 70% ethanal and surface sterilized with 1.8% sodium hypochlorite and tween-20 add 1-2 drop for 30 minutes. The explants were washed 3 times with sterilizing distilled water for 5 minutes.

Colchicine induce mutation

The sterilized ray floret was soaked in colchicine concentration 0, 0.5, 0.1, 0.15 and 0.2 % for 12 and 24 hours on the 80 rmp shaker. The experiment was 5×2 factorial in randomized complete design with ten treatments three replications and ten explants per replication was used. The percentage of servival rate, callus size and percentage of shoot regeneration was recored. The ray floret segments were transferred onto sterilized papers, and cutted into the size of 0.5x0.5 cm., then culture on MS medium (Murashige and Skoog, 1962) supplemented with 2 mg/l NAA and 4 mg/l kinetin for 12 weeks under light for 16 hr. photoperiod at temperature 25±2 ℃ for callus induction. The calli were transfered onto regeneration shoot medium (hormone free MS medium).

Morphological observation

The rooted plantlets were transferred to plastic pots in 6 inchs pots by using soil and chopped coconut material. A set of morphological characteristics were measured size of flowers, number of lateral bud and number of flowers were measured.

Statistic analysis

The experiment was repeated three times. All data were analyzed by analysis of varience (ANOVA) and mean were compared using the least significant difference (LSD) at 5% probability level and Duncan's multiple range tests.

Results

The ray florets were treated with 0.2% colchicine highest concentration found 50% survival rate. Callus formation began during the second weeks of culture the explant. Callus size and percentage of shoot regeneration and number of shoots per callus decrease when using higher colchicine concentration (Table 1). All of the shoots regenerated from colchicine treatd explants were morphologically shownheight of shoot, number of leaves, stomata size and number of chloroplast which similar to the control during in tissue culture stage (Table 2). After growth pantlet from each treatment in green hosue for 16 weeks, it was found that there are not different survival percentage, height of stem and number of lateral buds, at 0.15% colchicine for 12 hours showed the most of flower number(Table 3). Orange ray florets mutants emerged from chrysantimum cv. canter original color was found in 0.1% colchicine for 12 and 24 hours and 0.05% colchicine for 24 hours treated (Fig. 1A and 1C, Fig. 2D and 2C). The ray florets petal shape mutated pink double bloomed mutant was found in 0.15% colchicine for 12 hours (Fig. 1D). After selection, we found 5 types of flower such as original flower (Fig. 2A), light orange mutant (Fig. 2B), dark orange mutant (Fig. 2C), pinkish-orange mutant (Fig. 2D) and double bloomed mutant (Fig. 2E). From transplanting to greenhouse ten plants per treatment found at 0.15% colchicine for 12 hours the petal shape changes to double bloomed accounted for 60 percent, at 0.1% colchicine for 12 hours that flower color change into a pinkish-orange accounted for 50 percent, at 0.05% colchicine for 24 hours change into a light orange accounted for 5 percent and at 0.1% colchicine for 24 hours change into a dark orange accounted for 5 percent.

Table 1. The effect of colchicine concentration and duration of soak on callus induction from ray floret after 12 weeks culture

colchicine (%)	Duration (hours)	Survival rate (%)	Callus width (cm.)	Callus length (cm.)	Shoot regeneration (%)	Number of shoot/callus
colchicine (%)	**0**	100.00 ±0.00a	1.58 ±0.08a	1.61 ±0.15a	76.67 ±10.32a	2.11 ±0.07a
	0.05	85.00 ±13.78b	1.21 ±0.09b	1.30 ±0.13b	60.00 ±6.32b	1.61 ±0.07b
	0.1	70.00 ±0.00c	1.20 ±0.08b	1.30 ±0.16b	51.67 ±4.08c	1.45 ±0.05c
	0.15	63.33 ±5.16d	1.09 ±0.04c	1.12 ±0.02c	45.00 ±5.47c	1.30 ±0.08d
	0.2	51.67 ±4.08e	1.11 ±0.01c	1.09 ±0.03c	45.00 ±5.47c	1.21 ±0.07d
F-test		**	**	**	**	**
Duration (hours)	**12**	77.33 ±19.07a	1.29 ±0.20a	1.37 ±0.24a	58.67 ±15.05a	1.55 ±0.35
	24	70.67 ±17.51b	1.19 ±0.17b	1.20 ±0.13b	52.670 ±11.62b	1.52 ±0.31
F-test		**	**	**	**	ns
colchicine (%)	**Duration (hours)**					
0	**12**	100.00 ±0.00a	1.65 ±0.05a	1.74 ±0.04a	83.33 ±5.77a	2.16 ±0.05a
	24	100.00 ±0.00a	1.51 ±0.01b	1.48 ±0.05b	70.00 ±10.00b	2.06 ±0.05a
0.05	**12**	96.67 ±5.77a	1.28 ±0.01c	1.41 ±0.01b	63.33 ±5.77bc	1.63 ±0.05b
	24	73.33 ±5.77b	1.14 ±0.08d	1.19 ±0.09c	56.67 ±5.77cd	1.60 ±0.10b
0.1	**12**	70.00 ±0.00b	1.28 ±0.02c	1.44 ±0.08c	53.33 ±5.77cde	1.46 ±0.05c
	24	70.00 ±0.00b	1.12 ±0.02d	1.16 ±0.01c	50.00 ±0.00de	1.43 ±0.05cd
0.15	**12**	66.67 ±5.77b	1.16 ±0.01d	1.13 ±0.03cd	46.67 ±5.77de	1.30 ±0.10de
	24	60.00 ±0.00c	1.11 ±0.01d	1.11 ±0.01cd	43.33 ±5.77e	1.30 ±0.10de
0.2	**12**	53.33 ±5.77d	1.12 ±0.01d	1.11 ±0.01cd	46.67 ±5.77de	1.23 ±0.05e
	24	50.00 ±0.00d	1.07 ±0.06e	1.06 ±0.01d	40.33 ±5.77e	1.20 ±0.10e
F-test		**	**	**	**	**
%CV		**4.93**	**3.25**	**3.8**	**10.87**	**5.02**

Values within a column followed by the same letter are not significantly different by Duncan's multiple range test at P<0.01; ** Significant different.

Table 2. The effect of colchicine concentration and duration of soak on shoots from callus after 8 weeks culture

colchicine (%)	Duration (hours)	Shoot height (cm.)	Leaf number	Stomata size		Chloroplast number/ stomata
				width (µM)	length (µM)	
colchicine (%)	**0**	4.08 ±0.01	7.83 ±0.15	22.89 ±1.91	53.57 ±5.95	32.50 ±2.86
	0.05	4.09 ±0.02	7.85 ±0.20	24.56 ±1.62	55.40 ±5.15	33.08 ±3.44
	0.1	4.09 ±0.02	7.81 ±0.21	24.25 ±1.72	50.40 ±3.81	32.15 ±2.20
	0.15	4.09 ±0.03	7.85 ±0.23	24.12 ±1.23	56.41 ±6.23	34.17 ±3.31
	0.2	4.09 ±0.02	7.80 ±0.12	24.58 ±1.68	58.33 ±3.82	34.78 ±2.12
F-test		ns	ns	ns	ns	ns
Duration (hours)	**12**	4.09 ±0.02	7.83 ±0.19	24.12 ±1.25	53.81 ±5.15	32.99 ±3.07
	24	4.09 ±0.02	7.82 ±0.17	24.04 ±2.04	55.84 ±5.76	33.66 ±2.61
F-test		ns	ns	ns	ns	ns
colchicine (%)	**Duration (hours)**					
0	**12**	4.09 ±0.01	7.86 ±0.20	22.89 ±0.75	49.51 ±4.52	30.23 ±2.00
	24	4.08 ±0.02	7.80 ±0.10	22.90 ±2.93	51.30 ±3.67	34.76 ±1.04
0.05	**12**	4.09 ±0.02	7.86 ±0.25	23.98 ±1.00	55.43 ±4.66	31.23 ±2.23
	24	4.08 ±0.03	7.83 ±0.20	25.14 ±2.14	55.38 ±6.68	34.93 ±3.78
0.1	**12**	4.09 ±0.02	7.80 ±0.26	24.86 ±0.64	50.51 ±7.71	32.36 ±2.20
	24	4.08 ±0.02	7.83 ±0.20	23.64 ±2.33	56.64 ±0.93	31.93 ±3.45
0.15	**12**	4.09 ±0.03	7.86 ±0.20	25.19 ±1.28	53.84 ±3.77	35.23 ±3.85
	24	4.09 ±0.03	7.83 ±0.30	23.04 ±0.70	58.70 ±3.89	33.00 ±2.98
0.2	**12**	4.09 ±0.03	7.76 ±0.15	23.68 ±1.31	57.97 ±4.58	35.90 ±2.29
	24	4.09 ±0.02	7.83 ±0.11	25.48 ±1.72	58.99 ±7.93	33.67 ±1.50
F-test		**ns**	**ns**	**ns**	**ns**	**ns**
%CV		**0.66**	**2.69**	**6.99**	**9.55**	**7.8**

Values within a column followed by the same letter are not significantly different by Duncan's multiple range test at P<0.05; ns: non-significant.

Table 3. Survival percentage and plant growth rate of chrysanthemum (cv. canter) after transfer to soil 16 weeks

colchicine (%)	Duration (hours)	Survival percentage (%)	Shoot height (cm.)	Lateral bud number	Flower number
Colchicine (%)	**0**	16.90±8.33	34.80±9.13	12.90±6.55	39.70±21.42
	0.05	16.50±8.75	35.40±7.67	14.80±2.84	57.10±23.30
	0.1	15.60±8.49	34.70±23.09	13.50±7.69	54.70±29.63
	0.15	15.20±8.43	30.80±5.41	13.40±3.47	55.70±17.29
	0.2	15.70±6.74	33.50±5.78	12.70±5.07	53.40±20.07
F-test		ns	ns	ns	ns
Duration (hour)	**12**	17.36±8.90	36.48±8.35	14.84±4.81	56.36±23.32
	24	14.66±9.53	31.20±16.67	12.08±7.61	47.88±27.76
F-test		ns	ns	ns	ns
Colchicine(%)	**Duration (hour)**				
0	**12**	18.40±10.00	36.40±4.03	13.00±3.78	31.40±27.32b
	24	15.40±10.00	33.20±9.78	12.80±7.82	48.00±16.00ab
0.05	**12**	19.00±10.00	38.00±4.50	15.00±3.03	61.40±31.48a
	24	14.00±8.36	32.80±0.00	14.60±0.00	52.80±0.00ab
0.1	**12**	17.40±10.00	38.40±5.76	15.40±3.76	58.60±16.52ab
	24	13.80±0.00	31.00±0.00	11.60±0.00	50.80±0.00ab
0.15	**12**	15.00±8.94	32.60±7.41	15.60±3.80	72.00±23.57a
	24	15.40±8.94	29.00±1.94	11.20±2.77	39.40±10.18b
0.2	**12**	17.00±4.47	37.00±7.08	15.20±3.64	58.40±19.05ab
	24	14.40±8.94	30.00±3.74	10.20±3.96	48.40±16.36ab
F-test		**ns**	**ns**	**ns**	*****
%CV		**41.56**	**42.25**	**50.79**	**49.41295**

Values within a column followed by the same letter are not significantly different by Duncan's multiple range test at P<0.05; ns: non-significant, * Significant different.

Figure 1. The chrysanthemum mutation by colchicine: (A) control 0%, (B) 0.05%, (C) 0.10%, (D) 0.15%, (E) 0.20 %, colchicines for 12 hours and (F) 0%, (G) 0.05 %, (H) 0.10%, (I) 0.15 %, (J) 0.20%, colchicines for 24 hours after transfer to soil for 12 weeks

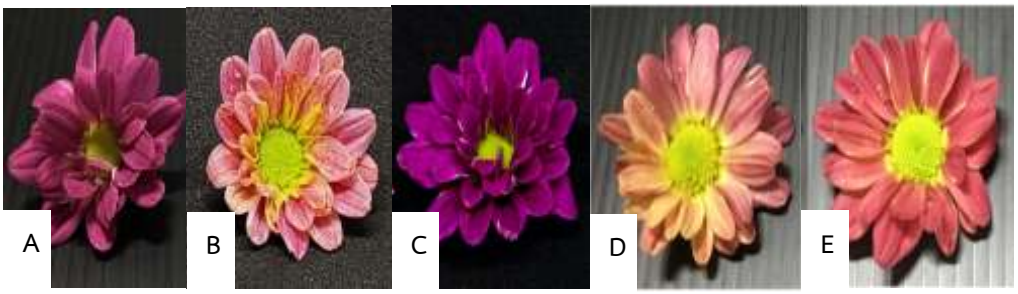

Figure 2. The Chrysanthemum flower type after transfer to soil 12 weeks (A) control 0% (B) 0.1% (C) 0.15% colchicines for 12 hours. (D) 0.05% (E) 0.1% colchicines for 24 hours

Discussion

Our results suggested that tissue culture mutations with colchicine would contribute to breeding mutant varieties in a relatively short time compared with the conventional method. Matsumura *et al*. (2010) reported that various color mutants emerged from *Chrysanthemum moriflorum* 'H13' and Shiroyamate after ion beam irradiation and tissue culture of ray floret. In this study, the concentration of 0.2% colchicine for 12 hours was soaked the samples was the lethal dose of 50% (LD50) which LD50 is the effective doses that can cause the highest variant frequency for plants. Petbanna *et al.* (2009) studied mutation in *Spathoglottis plicata* Blume by seeding in aseptic conditions soaked in colchicine solution. It was found that the survival rate decreased. Kerdsuwan and Te-chato (2012) studied on the effects of colchicine on survival rate, morphological, physiological and cytological characters of chang daeng orchid (*Rhynchostylis gigantean* var. *rubrum* Sagarik) that induced the highest percentage of tetraploid (60%) by using 0.2% colchicine for 72 hours. The ability to transport mutants into plant tissues and the genetic characteristics of plants are factors that affect the survival percentage and increase chromosome number sets of plant parts (Allum *et al*., 2007). The concentration and duration affected to induce the change of characteristic in different plants. The mutagen various concentrations at 0.1%, 0.15% colchicine for 12 hours and 0.05%, 0.1% colchicine for 24 hours can change some of the morphological characteristics and flower color mutants from purple into a pinkish-orange, double layer petal, light orange and solid orange respectively.

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