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Induced Mutation of Chrysanthemum by Colchicine

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Chrysanthemum (*Dendranthema grandiflora*) cv.crystal white is a potted plant with a shrub and many buds. The bush is about 20-30 cm height. The study on colchicine induced mutation with chrysanthemum. The rooting plants from tissue culture were transferred to soil pot plant for 14 days were used plant material. The shoots were dropped in 0.1 ml colchicine solution with 6 concentration levels (0, 0.20, 0.40, 0.60, 0.80 and 1.00%) on apical bud, three times a day, 9.00, 13.00 and 17.00 the for 6 days to induce mutation. The colchicine has efficiency to induce mutation. One of the shoot that was dropped with 0.80% colchicine has a height of 28.70 cm in which it is higher than the one with control (19.06 cm). There are only 2 branches of lateral bud, while the other plants are splitted with 7-9 branches. It has 6.09 mm stem diameter which were significantly higher than the other plants. The stomata sizes were 56.84 µm larger than the control. The chlorophyll content of 120.2 µg/gFW is higher than the control. However, the results showed no difference in DNA content.

Keywords: Dendranthema, colchicine, mutation

Introduction

Chrysanthemum (*Dendranthema grandiflora*) cv. crystal white is one of the most popular ornamental plants in Thailand. However, they produces only white flowers. In Thailand, chrysanthemum flowers are grown in Chiang Mai, Chiang Rai, Nonthaburi, Surat Thani, Songkhla, Yala, Ubon Ratchathani, Udon Thani, Khon Kaen, Nong Khai and Nakhon Ratchasima. Some varieties can grow in specific area. Chrysanthemum is extensively grown as a pot plant as well as a cut flower worldwide. Breeding programs have focused on improving various characteristics to enhance ornamental values, including flower colors, sizes forms and production quality. Breeding of chrysanthemum can be made

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through two ways i.e. cross between two cultivars and by artificial induced mutation. Mutation cultivar derived vegetatively from successful cultivars that differ from the original cultivars in some traits.

A common technique used to induce plant mutation for many years is the induction of chromosome doubling to generate polyploids by treatment of plants with mitotic spindle poisons such as colchicine (Dhooghe *et al*., 2011). The polyploid plants can have larger flowers, fruits and seeds than their diploid plant (Hancock, 1997; Dhooghe *et al*., 2011).

Colchicine is widely used in the induction of plant mutations. Because it is not harmful to the plant, even if it is used as a plant extracts, it can be transported to different parts of the plant, maintaining the same shape and inducing mutation for a long time. It also does not change the structure of the gene. Various types of colchicine treatments can be applied to the plants. Whether it is a mix in a culture medium (Miyoshi *et al.*,1996), seeds soaking in a colchicine solution (Yetisir and Sari 2003, Ye *et al.*, 2009) or dropping the solution onto the plant's meristems (Luckett, 1989, Blassco *et al.*, 2014). Each method can induce chromosomal change in the plant, the concentration of the solution will vary according to the plants.

Colchicine is also used in the chrysanthemum mutations, most commonly using colchicine in combination with tissue culture, either by mix in medium or immersing seeds, explants before planting in medium culture or planted in the pot, both of these can mutant chrysanthemum as well (Liu and Gao, 2007, Urwin, 2014, Gao *et al.*, 2016).

This experiment was objective to investigate of colchicine concentration solution for the induced mutation of chrysanthemum. The experiment was conducted outside the laboratory. It is inexpensive technique and requires no special equipment or expertise**.**

Materials and methods

Plant material

Petal of chrysanthemum from flower was used the explants. The ray floret washed thoroughly under running water for 30 min. They were surface sterilized with 20% Clorox (30% sodium hypochlorite) for 30 min and washed 3 times with sterilized distilled water for 5 min. The callus was initialed by culturing ray floret 0.5×0.5 cm on MS medium supplemented with 2 mg/l NAA (α-Napthalene acetic acid) and 4 mg/l Kinetin (N6-furfuryladenine). The explants were cultured under cool fluorescent lamps at the light intensity 40 $\mu Molm^{-2}$ with a 16 h/day light at 25±2 ℃. The cultures were transferred to the

fresh medium at 30 days. After the shoot formation, they were cultured on MS medium free hormone for root development.

Colchicine treatment

The rooting plants from tissue culture were transferred to soil pot plant for 14 days. The shoots that were covered with cotton, were dropped in 0.1 ml colchicine solution with 6 concentration levels (0, 0.20, 0.40, 0.60, 0.80 and 1.00%) on apical bud, three times a day, at 9.00, 13.00 and 17.00 for 6 days to induce mutation**.** The treatment has 4 replications, 5 pots per a replication and experimental design with completely randomized design.

Morphological observation and Chlorophyll content

After 6 weeks of culture, the treatment and control plants were compared for morphological traits including plant height (cm), measured from basal to highest shoot. Stem diameter (mm) measured at the basal of the plant material above 1 cm, and number of lateral bud, leaves characteristic. The size of stomata and chlorophyll content were detected.

Flow cytometry analysis

Mutant traits were selected for comparation DNA content with control plants to test with the flow cytometry analysis. Extraction of DNA from the leaf components by chopped the leaves in a Petri dish with 500 ml of Pacrtec CyStain (a one-step exraction and DAPI stain solution) and filtered through a 30 µm before being analyzed in a Partec PAII flow cytometer.

Statistic analysis

All data were analized using ANOVA and Duncan's multiple range tests at $p \leq 0.05$ by SAS programme.

Results

Effects of colchicine concentrations on growth of chrysanthemum

Chrysanthemum cv. crystal white is a potted plant with a shrub and many buds. The bush is about 20-30 cm height. For study colchicine induces mutation with chrysanthemum by shoot dropped technique. In 0.8% colchicine concentration, the average of height growth has a shortest (14.63 cm), while the

size of the stem and shoots did not differ with the control. We found only one the hightest shoot was obtained from the 0.8% colchicine treatment with 28.70 cm high, which have more than the average height of the control (19.06 cm) and average in treatment (14.63 cm). There have only 2 branches of lateral bud, while the other plants have splitted with 7-9 branches. It has 6.09 mm stem diameter were significantly higher than other plants (Table 1 and Figure 1).

Table 1. The effects of colchicine concentrations on growth of chrysanthemum

Colchicine concentration (%)	**Plant height(cm.)[a]**	**Stem width(mm.)**	**No. of lateral bud**	**Suvival rate (%)**
0.00	19.06 ±1.68 ^{A}	3.75 ±0.57	7.90 ±1.45	100
0.20	18.03 ±5.89 ^{AB}	3.61 ±0.96	7.80 ±1.11	95
0.40	17.15 ±6.73 ^{AB}	3.43 ±1.47	8.25 ±1.45	90
0.60	17.23 ± 4.91^{AB}	4.08 ±1.18	8.30 ±1.49	95
0.80	14.63 ±5.26 ^{B}	3.33 ±1.43	7.75 ±2.07	90
1.00	16.27 ±5.85 ^{AB}	3.82 ±0.80	7.55±1.54	95
F – test	*	ns	ns	
CV (%)	31.09	30.35	19.47	

[a] The values within a column followed by the same leter are not significantly different, as determind by Duncan's multiple range test.
ns, non-significant, * significant (P < 0.05)

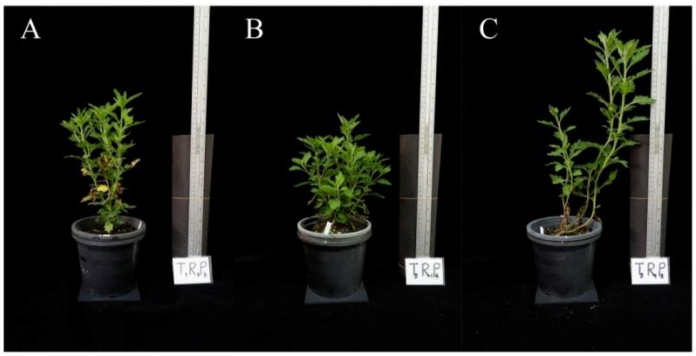

Figure 1. Height of mutant plant with colchicines after 6 weeks. (A) control (B,C) 0.8% colchicine

Chlorophyll content and Stomata size

Chlorophyll a, chlorophyll b content and total chlorophyll in the treatment highter concentration of colchicine decreased and was significantly lower than the control treatment (Table 2). The 0.8 % colchicine treatment was showed lowest chlorophyll content. However, one plant in this treatment showed the highest total chlorophyll content (120.2 mg/gFW) and longest stomata 56.84 µm. The result has shown that all treatments had no different of average stomata length (Table 2, Figure 2).

Table 2. Effect of colchicine concentration on chlorophyll content and stomata length

Colchicine concentration (%)	Chlorophyll A (µg/gFW)[a]	Chlorophyll B (µg/gFW)	Total Chlorophyll (µg/gFW)	Stomata length (µm.)
0.00	46.4 ±23.5 ^{ABC}	42.8 ±11.1 ^{A}	89.2 ±27.7 ^{A}	50.16 ±4.33
0.20	59.4 ±19.2 ^{A}	35.3 ±10.9 ^{AB}	94.8 ±35.9 ^{A}	50.14 ±3.77
0.40	51.4 ±19.8 ^{ABC}	29.6 ±13.6 ^{BC}	81.1 ±42.0 ^{AB}	49.76 ±5.20
0.60	54.0 ±16.2 ^{ABC}	33.1 ±08.2 ^{B}	87.9 ±29.1 ^{A}	47.32 ±3.61
0.80	36.0 ±20.0 ^{C}	21.1 ±10.5 ^{D}	57.8 ±34.8 ^{C}	47.11 ±6.54
1.00	40.9 ±13.9 ^{BC}	22.7 ±07.1 ^{CD}	63.3 ±25.3 ^{BC}	47.11 ±4.58
F – test	**	**	**	ns
CV (%)	46.811	39.89	41.62	9.82

[a] The values within a column followed by the same leter are not significantly different, as determind by Duncan's multiple range test.
ns, non-significant, ** significant ($P < 0.01$)

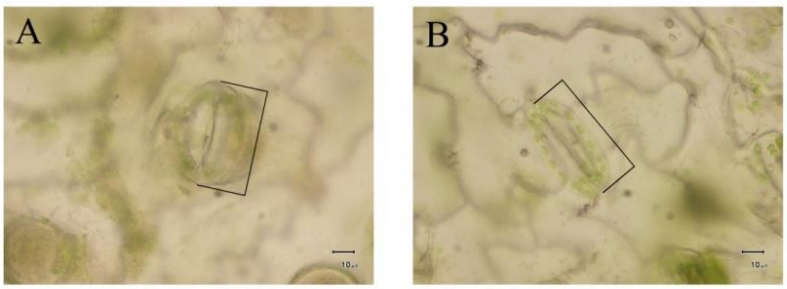

Figure 2. Stomata size in abaxial leaf epidermis of chrysanthemum (A) control (B) mutated plant in 0.8% colchicine treated.

Flow cytometric analysis

Flow cytometry that performed on the regenerated plant was employed to give an accurate estimation of nuclear DNA content. Figure 3 showed the flow cytometric results of leaf examination for chrysanthemum. The results showed no difference in nuclei. This means that the DNA content of mutated plant in 0.8% colchicine treated is unchanged.

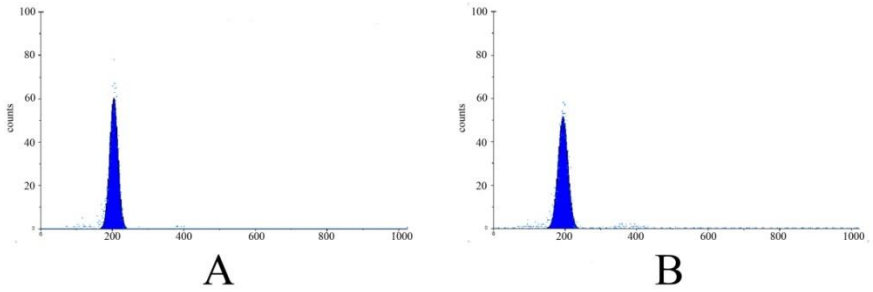

Figure 3. Flow cytometric analysis of chrysanthemum. (A) control plant; (B) mutnat plant from 0.8% colchicine treated

Discussion

Colchicine, acts as an antimitotic agent which has been widely used to induce polyploidy in plant breeding (Ye *et al*. 2009.) In this study, the colchicine induced mutation with chrysanthemum. The shoots were dropped in 0.1 ml colchicine solution with 6 concentration levels 0, 0.20, 0.40, 0.60, 0.80 and 1.00%. Morphology identification, determination of stomata and flow cytometry were used for polyploid identification. There was one putative mutation plant occurred in the 0.8% clochicine treatment when used morphology observable. There is a 28.70 cm height, is more than the average height of the control (19.06 cm). There are only 2 branches of lateral bud, while the other plants are splitted with 7-9 branches. It has 6.09 mm stem diameter were significantly higher than other plants. The stomata sizes were 56.84 μm larger than the control. The chlorophyll content of 120.2 μg/gFW is higher than the control.Chlorophyll content increasing in plant may be the effective way to increase product and yield (Berg *et al.*, 2006; Dewick, 2009; Ge *et al*., 2012 ;Guo *et al*., 2005 and Jiang *et al*., 2007).

The concentration and period time of colchicines also influenced the mutation rate of plant. Although the plant can survived heighest rate of survive when treated in the low concentration of colchicines. However, the variation effert was hightest when treated in high concentration of colchicines (Dhooghe

et al., 2011 and Cui *et al*., 2017). The putative mutation plant was observed by stomatal size and chlorophyll content. It showed the longest stomata size and highest chlorophyll content. The utility of stomatal size in distinguishing plants with different ploidy levels was used in other plant types (Chen and Gao, 2007; Mishra, 1997; Aryavand *et al*., 2003; Khazaei *et al*., 2010 and Gao *et al*., 2016). The efficient colchicines mediated technique by dropping chlchicines on shoot induction mutation of chrysanthemum showed low mutation. Each of the treatment showed high survival rate. We suggest to use the higher concentration with this technique. The putative mutation was determined their ploidy by flow cytometry. The result showed ploidy of putative mutation has no difference when comparing to the control. Althought there are differences between the putative mutation and the control, with regard to plant height, branches number, chlorophyll content and stomata size index. They are no difference in the ploidy. It was maybe chimera plant (Roy *et al*., 2001 and Zhang *et al*., 2010).

Acknowledgement

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