PRELIMINARY STUDIES ON THE CREATION OF ANATOMICAL CHANGES BY COLCHICINE TREATMENT IN CHINESE KALE (*Brassica alboglabra* L.H. Bailey)

M. Teerarak[1]*, S. Thitavasanta[1] and S. Khamchutr[2]

[1] Department of Horticulture, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.
[2] Applied Biology Program, Faculty of Science and Technology, Rambhai Barni Rajabhat University, Chantaburi 22000, Thailand.

ABTRACT

Seeds of chinese kale were treated with 0, 0.125, 0.25, 0.50, 1 and 2% colchicine for 6, 12 and 24 hours in order to form polyploid plants and were sown in glass Petri dishes. The results showed that treatments of seeds with all colchicine concentrations had no effect on seed germination and colchicine did induce abnormality in the seedlings. In the continuous experiment, seeds of chinese kale were soaked in 0, 0.1, 0.2, and 0.4% colchicine solutions for 12 hours and were grown in a greenhouse. The results indicated that the stomatal sizes both on the upper and lower epidermis of colchicine treated plant were significantly larger than those of the normal plants while the frequencies of the stomata were reduced significantly.

KEYWORDS: colchicine, chinese kale, anatomical variants, polyploidization

1. INTRODUCTION

Foods that are sources of lutein and vitamin C are dark green leafy vegetables. Lutein and vitamin C are antioxidant substances that occur naturally in the diet. Several important and very popular vegetables especially leafy vegetable are belonged to the Genus Brasscica. Chinese kale (*Brassica alboglabra* L.H. Bailey), a number of which, is an important leafy vegetable in the tropical region in Asia. It is highly valuable for its deliciousness. In addition, chinese kales are the sources of lutein and vitamin C. Chinese kales are major components of daily meal in many countries in Asia, it is becoming increasingly important to be able to assess their nutritional value in terms of antioxidant content.

Polyploidy has been used as a breeding tool in horticulture for obtaining new characteristics. Polyploidization usually leads to thicker leaves, stem and root, deeper green color, increased width-to-length ratio of leaves, larger and more texture flower and a more compact growth habit [1]. Colchicine has been effective for chromosome doubling in many species. Colchicine has been *in situ* or *in vitro* at various dose and duration depending upon the species used.

Studies on the effects of colchicine on other characteristics are underway for plant improvement. Therefore, the objective of the current study was to analyze the effects of colchicine application on anatomical variants. In a large number of crops, leaf color increases in accordance with increasing of ploidy level. Thus, the ultimate goal is to improve the characteristics of chinese kale by increasing nutritional value in terms of antioxidant content through polyploidy induction.

* Corresponding author. Tel: 662-3264318 Fax: 662-3264318
E-mail: noomonmon@hotmail.com

2. MATERIALS AND METHODS

2.1 Morphological development of seedlings

For the observation of morphological development, fifty seeds were treated by soaking in aqueous colchicine solution at 0.125, 0.25, 0.5, 1 and 2% for 6, 12 and 24 hours. The seeds were then rinsed three times with distilled water and laid on paper towel to remove excess water. The seeds treated with colchicine were transferred into 9 cm diameter of glass Petri dishes containing 5 ml of sterile distilled water and laid on a piece of germination paper. Simultaneously, fifty seeds without colchicine treatment were sown into a glass Petri dish as controls. The seedlings developed roots and shoots from these seeds, and morphological characteristics such as root length, stem length, diameter of stem, percentages of seed germination and normal seedlings were examined.

In the experiment, replications were not included hence statistical analysis was not carried out and only standard deviations were calculated.

2.2 Colchicine application method

Twenty germinable seeds of chinese kale were soaked in 0.1, 0.2 and 0.4% colchicine solution for 12 hours followed by being washed three times with water. Each seed separated was planted in a pot and maintained in a greenhouse. Simultaneously twenty seeds of normal plants were sown into pots as controls. The anatomical characteristics were examined. The experimental design was completely randomized design. Each treatment was replicated five times with four plants in each replicate.

2.3 Size and density of stomata

From the bottom of both normal and colchicine treated plants, fully expanded third leaf was cut and taken into the laboratory for the observation of anatomical changes. A certain amount of upper and lower epidermal cells were obtained from both sides by tearing with a nail and were then rubbed on to a microscope slide by a razor blade. In order to measure stomatal diameter and length, the under surface of a leaf was placed onto a microscopic slide after the addition of one drop of distilled water, the cover glass was then put on the slide. The density of stomata was determined as number of stomata /mm^2.

Under a light microscope, the diameter and length of three stomata per leaf were measured with magnification of 400x. For those three parameters, statistical analysis was carried out; the completely randomized experimental design was applied, and the average values were then compared by least significant difference.

3. RESULTS AND DISCUSSION

Treatment of seeds with all colchicine concentrations had no effect on seed germination (data not shown). Moreover, the percentage of normal developing seedling decreased after colchicine application with increased concentrations and longer soaking periods (data not shown). The results of the morphological developing seedlings are summarized in Table 1. The shoot growth of all colchicine treated seedlings ranged from 0.38 to 2.01 centimeters. The root length of all colchicine treatment seedlings ranged from 0.61 to 4.01 centimeters. In colchicine treated plants, the stem diameter reached 1.13 centimeters while in controls it was about 1.11 centimeters. The abnormal developing seedling rate with colchicine treatment was higher than those of the controls, especially at higher concentrations and longer durations. Similarly, this rate of abnormal developing seedling was obtained in a previous study [2]. Obviously, abnormal seedling occurred because there was sufficient penetration of colchicine into tissue which affected the division of cells.

Subsequent analyses of stomata size and density in colchicine treated plants are presented in Table 2. In controls and colchicine treated chinese kale plants, the stomata diameter and stomata density were found to be significant different. In colchicine treated plants, the stomata diameter reached 24.42 μm while in the controls it was about 16.71 μm. No significant difference in stomata length both of the upper and lower epidermal cells of the controls and the colchicine treated plants were found. In 0.4% colchicine treated plant, the stomata density of upper epidermal and lower epidermal cells was about 170.56 and 196.03 number/mm^2, respectively while those in the controls were about 210.94 and 281.34 number/mm^2, respectively. Stomata of epidermal cells of normal and colchicine treated plants are shown in Fig. 1. This study indicated that stomata sizes of colchicine treated chinese kale increased the stomata diameter and lowered the stomata frequency as in muskmelon [3] and watermelon [4].

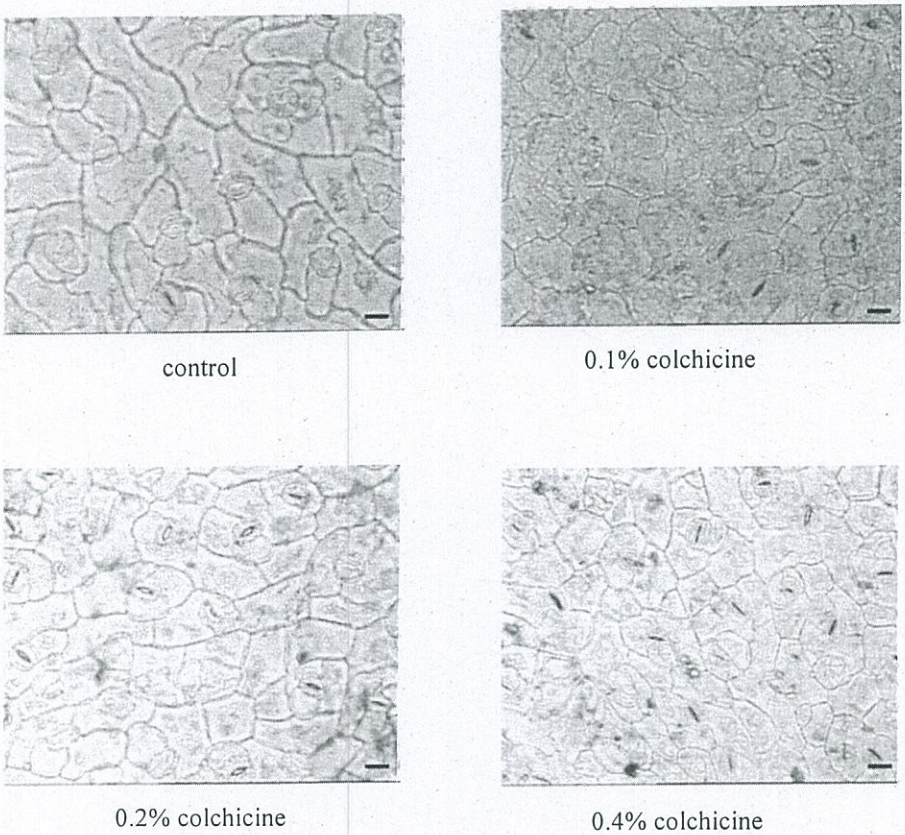
Table 1. Development of chinese kale seedlings after *in vitro* colchicine application.

Plant development	control			0.125% colchicine			0.25% colchicine		
	6 h	12 h	24 h	6 h	12 h	24 h	6 h	12 h	24 h
Stem length (cm)	1.75±0.49	1.71±0.47	1.91±0.83	2.01±0.41	1.70±0.67	0.71±0.23	1.20±0.52	1.56±0.68	1.53±0.49
Diameter of stem (cm)	0.12±0.05	1.11±0.63	0.07±0.02	0.16±0.03	1.13±0.02	0.12±0.03	0.17±0.04	0.16±0.03	0.11±0.02
Root length (cm)	2.40±0.79	1.35±0.51	1.36±0.35	2.76±1.38	1.65±0.57	2.55±1.26	2.45±0.46	2.75±1.71	2.18±0.45
Plant development	0.5% colchicine			1% colchicine			2 % colchicine		
	6 h	12 h	24 h	6 h	12 h	24 h	6 h	12 h	24 h
Stem length (cm)	1.06±0.53	1.05±0.55	0.88±0.71	0.98±0.26	0.65±0.31	0.38±0.12	0.70±0.22	0.68±0.10	0.53±0.10
Diameter of stem (cm)	0.13±0.03	0.15±0.04	0.16±0.02	0.17±0.01	0.17±0.03	0.12±0.02	0.20±0.01	0.19±0.01	0.15±0.01
Root length (cm)	4.01±1.31	3.33±0.98	1.95±1.92	2.46±1.91	2.48±1.51	0.80±0.75	2.20±1.53	0.61±0.18	1.30±0.43

Table 2. Stomata size and density of chinese kale after colchicine application.

Treatment	Stomata diameter (μm)	Stomata length (μm)	Stomata density (number/mm^2)
Upper epidermal cell			
control	18.15 c*	28.10 a	210.94 b
0.1% colchicine	20.99 b	27.59 a	274.30 a
0.2% colchicine	20.95 b	27.17 a	188.61 a
0.4% colchicine	24.42 a	28.86 a	170.56 c
Lower epidermal cell			
control	16.71 c	27.59 a	281.34 a
0.1% colchicine	19.59 b	27.13 a	325.06 a
0.2% colchicine	20.61 b	26.24 a	273.86 a
0.4% colchicine	23.44 a	28.62 a	196.03 b

*Data within columns followed by the same letters do not differ significantly at the 5% level according to LSD test.



control 0.1% colchicine

0.2% colchicine 0.4% colchicine

Figure 1 Stomata of lower epidermal cells of chinese kale plants after colchicine application (400x). Bar (—) = 20 μm.

In the future, different methods such as chromosome counting and flow cytometry will be studied in order to confirm the polyploid levels.

4. CONCLUSIONS

Treatment of seeds with all levels of colchicine concentration had no effect on seed germination. The percentage of normal developing seedlings decreased after colchicines concentration and soaking time were increased. Most colchicine treated plants showed increased values for stomata diameter, but lower values for stomata frequency.

5. ACKNOWLEDGEMENTS

The authors thank Dr. Chamroon Loasinwattana for valuable assistance during the experiments.

REFERENCES

[1] Gao, S.T., Zhu, D.N., Cai, Z.H. and Xu, D.R. **1996** Autotetraploid Plants from Colchicine Treated Bud Culture of *Salvia miltiorrhiza* Bge. *Plant Cell, Tissue and Organ Culture, 47*, 73-77.

[2] Caglar, G. **1997** In Vitro Colchicine Application of Haploid Cucumber Plants. *Cucurbit Genetics Cooperative Report, 20*, 21-23.

[3] Yetisir, H. and Sari, N. **2003** A New Method for Haploid Muskmelon (*Cucumis melo* L.) Dihaploidization. *Scientia Horticulturae, 98*, 277-283.

[4] Sari, N., Abak, K. and Pitrat, M. **1999** Comparison of Ploidy Level Screening Methods in Watermelon: *Citrullus lanatus* (Thunb.) Matsum and Nakai. *Scientia Horticulturae, 82*, 265-277.