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Preliminary Test of Polyploidy Induction in Cotton (*Gossypium arboreum*) Using Colchicine Treatment

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ABSTRACT

Two local varieties of *Gossypium arboreum*, PM_2 and PM_3 were treated with colchicine solution for polyploidy induction. Two colchicine solutions, colchicine solution # 1 (0.1% colchicine) was derived from powder sold by Sigma and colchicine solution # 2 (0.5% colchicine) from drug tablets for gout treatment. Three experiments were undertaken which were, Experiment 1: apical meristem dropping with colchicine solution # 1; Experiment 2: seed treatment with colchicine solution # 2. Experiment 3 : apical meristem dropping with colchicine # 2. All were compared with the untreated (water treatment) controls. The results showed % germination of PM_2 and PM_3 after seed treatment to be lower than those of the controls. The same were found with their heights. Eleven, nine and three presumably polyploidy plants of PM_2 in Experiment 1, PM_3 in Experiment 1 and PM_3 in Experiment 2 respectively were found to have stomata sizes of 24.9%, 34.9% and 31.4% increased and stomata frequencies of 24.2%, 46.5% and 45.9% decreased compared to those of the controls respectively.

Key words: cotton, *Gossypium arboreum*, colchicine, polyploidy

INTRODUCTION

Cotton originally cultivated in Thailand were native cotton, *Gossypium arboreum* origin and the so-called 'Indian cotton'. Earlier attempt to improve cotton production was by introducing the 'Cambodian cotton' from Cambodia to Thailand in the early 1950's. The so-called 'Cambodian cotton' was in fact the American Upland cotton, *Gossypium hirsutum* introduced to Cambodia earlier (Na Pompeth, 1994). *G. arboreum* consists of both white and brown cotton which produces short, sparse seed hairs that are not spinnable. It is diploid and possess 13 pairs of chromosome (2n=26) while *G. hirsutum* is allotetraploids with 26 pairs of chromosome (4n=52) (Fryxell, 1969)

American cotton, *G. hirsutum* is very likely an alloploid derived from hybridization between *Gossypium thuberi* and *G. arboreum* (http://www. biology. ualberta.ca/courses hp/gen275/problem-set-key-2-0.1 htm) followed by chromosome doubling resulting in well-developed seeds. However, hybridization between different species may not be so successful due to different chromosome numbers or sizes. Polyploidy, therefore, could be artificially induced by some treatments, such as colchicine. In the late 1930's, it was discovered that colchicine inhibited the formation of spindle fibers and effectively arrested mitosis at the anaphase stage. At this point, the chromosomes have multiplied but cell division have not yet been taken place resulting in polyploidy

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cells. In later year, a number of other mitotic inhibitors including oryzalin, trifluralin, amiprophos-methyl and N_2O gas have been identified and used as doubling agents (Bouvier *et al*., 1994; van Tuyl *et al*, 1992; Taylor *et al*, 1976).

Colchicine treatment is the classical method to induce doubling of chromosome number. The techniques used for chromosome doubling of barley haploid with colchicine have been reported by Jensen (1974), Subrahmanyam and Kasha (1975) and Thiebaut and Kasha (1977). Yan (2001) demonstrated that ploidy of waxflowers could be doubled using colchicine. The tetraploid plantlets were found to have large leaves with fewer large stomata than the diploid, an indicator of increased ploidy level. Most species of Fuchsia are either diploid or tetraploid. Addink (2002) reported that a crossing between a diploid and tetraploid of Fushia often resulted in a triploid which was mostly sterile and could not be used for further breeding. Doubling the chromosome number with colchicine treatment from the diploid parent and the triploid could solve breeding problem in Fushia. Hybrid HAR was obtained from crossing of *G. hirsutum* and *G. arboreum* and *G. raimondie* using colchicine (DOA, 1984). Stephens (1947) also reported the morphology of cotton hybrids through colchicine treatment.

Chromosome counting which is time consuming and laborous is not suitable for detection mixoploidy in tissues with lower proportion of dividing cells such as leaves (Uhlik, 1981) Recently stomata size, leaf index value, stomata frequency, pollen grain diameter and other changes in plant morphology were found to be useful indicators in the primary screening for new ploidy levels. Evan(1955) and Speckman *et al* (1965) stated that stomata length was the accurate indicator of polyploidy level in many plants. Wright (1976) also showed that stomatal measurement was a quick way to determine whether or most of the leaves on a branch were polyploidy. The purpose of this study is to induce polyploidization in *G. arboreum* by colchicine treatment using plant attributes as primary indicators.

MATERIALS AND METHODS

Two local varieties of *G. arboreum*, PM_2 (light brown cotton) and PM_3 (white cotton) were used for polyploid induction through colchicine treatment. PM_2 and PM_3 were grown in pots, 30 pots/variety/treatment. Three seeds per pots were grown to obtain three plants. Twenty pot plants were treated with colchicine while 10 pot plants with just water.

Colchicine solutions were derived from two sources

1) Sigma sold colchicine powder was diluted in water into 0.1 % colchicine solution (colchicine solution # 1)

2) Colchicine tablets (drug for gout treatment) were dissolved in water to obtain 0.5% colchicine solution (colchicine solution # 2)

Three experiments were undertaken as followed

Experiment 1

A few drops of colchicine solution # 1 were applied on the apical meristem of seedlings between the first pair of true leaves. To aid in absorption, cotton balls were placed on the treated spots. The treatments were repeated during 8:00-9:00 am for 3 consecutive days. The controls were treated with water.

Experiment 2

This experiment was achieved by soaking seeds in colchicine solution # 2. The bottles with seed treatment were periodically shaken. After 24 hours, the treated seeds were washed in the flowing water for 3 hours, and then planted in the pots. The controls were treated with water.

Experiment 3

The similar procedure was followed as in

Experiment 1 except colchicine solution # 2 (with no cotton balls covered the apex) was used instead of colchicine solution # 1.

All plants, both treated and untreated (the controls) in all experiments were allowed to grow normally. Seed germination, plant height, stomata size, and stomata frequency per 1 mm of leaf area were checked.

Stomata measurement

Similar sizes of treated and untreated leaves from 3-4 months old plants were sampled, 5 leaves/plant. For stomata sizes, lower epidermis from both left and right sides of each leaf sample were peeled off, placed on glass slides covered with cover glasses and measured under 40X stereomicroscope. Stomata lengths were measured employing ocular micrometer, 10 measurements/leaf (5 from left and 5 from right sides of the mid vein). It was replicated 5 times. The values obtained were computed into micrometer (mm) using stage micrometer.

To obtain stomata frequency, the measurements were undertaken under 40X stereomicroscope through TV monitor. Surface cells of 5 leaves/plant, treated and the control, were used, 10 measurements/plant The measurements were undertaken from both left and right sides of each leaf.

RESULTS AND DISCUSSION

Changes in the morphological characteristics such as plant height, stomata size and stomata frequency were important indicators for the detection of ploidy levels in M_1 generation of cotton varieties. Table 1 presents % germination of PM_2 and PM_3 after seed treatment (Experiment 2) with colchicine solution # 2 to be lower than those of the controls. The results half agreed with explanation from Addink (2002) who stated that colchicine with too high concentration could inhibit the development of living part resulting in mortality of organism. Although most treated seeds survived to produce healthy plants, only a few could become polyploidy. This might be due to the fact that even though colchicine solution # 2 had high contration of colchicine (0.5%), the starch suspension from tablets could interfere with the absorbtion of colchicine through the seed coats. However, the

Table 1 Averaging percent germinations and height of *G. arboreum* the local varieties, PM_2 and PM_3, after colchicine treatments compared to the (untreated) control.

Experiment	Treatment	Germination (%)		Plant Height (cm)			
				1st measurement/1		2nd measurement/2	
		PM_2	PM_3	PM_2	PM_3	PM_2	PM_3
1	Control	-	-	12.50	13.15	62.40	66.90
	Treated	-	-	9.70	11.15	60.50	68.70
2	Control	95	100	14.40	13.05	71.10	89.60
	Treated	78	72	11.90	11.95	66.40	94.80
3	Control	-	-	11.75	10.60	60.40	62.90
	Treated	-	-	10.10	11.50	60.50	60.90

1 Averaged heights of PM_2 and PM_3 plants from the first measurement on Days 75, 60 and 45 after planting for Experiment 1, 2 and 3 respectively.

2 Averaged heights of PM_2 and PM_3 plants from the second measurement on Days 130, 120 and 100 after planting for Experiment 1, 2 and 3 respectively.

24 – hour period of seed soaking still allowed sufficient time for some seeds to receive high doses resulting in no germination while some obtained low or no dose resulting in polyploidization or normal germination respectively.

The averaging heights of treated plants of PM_2 and PM_3 in all experiments were also found to be lower than those of the untreated except that of PM_3 treated plant in Experiment 3 on the first measurement. This exception might be similarly explained to the result of % germination that the starch suspension from tablets in colchicine solution could prevent absorbtion of colchicine doses through meristem droppings, hence, no apparent height different from the control to be seen. For seedling treatment, colchicine solution # 1 revealed more effectively than 0.5 % from drug tablets with different effects. Stebbin (1950) stated that the decrease growth rate of polyploids was caused by the reduced ratio of cell division. The supply of the cells with auxin, a phyto-hormone was interrupted, the respiratory intensity was reduced and the activity of many enzymes was disminished. The results on plant height on first measurement also agreed with Kerr (2001) and Wright (1976) who stated that the induced 4n seemed to grow more slowly and growth abnormality were the first indication of successful colchicine treatment.

However, the inhibition continued for 5-6 weeks after which the surviving plants of both varieties in all experiments resumed normal growth and development as appeared on the second measurements. This was confirmed by Yan (2001) who treated shoot tips of waxflower by immersion in 0.05 % colchicine and found them to exhibit a deformation of the new growth but it was only transient, later growth returned back to normal.

In Table 2 the stomata size of selected plants are found to range from 26.25-34.65, 32.35-37.40 and 30.15-33.85 mm while those of the control are 22.50-27.25, 24.65-27.75 and 22.05-26.65 mm for PM_2 in Experiment 1, PM_3 in Experiment 1 and PM_3 in Experiment 2 respectively.

Compared to the control plants, the treated plants of both cotton varieties had epidermis with

Table 2 Anatomical differences in leaves between the controls and the treated cotton plants of the two cotton varieties, PM_2 and PM_3.

Experiment	Variety	Treatment	Ranges of Stomata Length (mm)	Stomata	
				Length (±S.D) (mm)	Frequency (±S.D) [1]
1	PM_2	Control	22.50-27.25	25.20±1.25	427.3±19.30
		Treated	26.25-34.65	30.40±3.01	324.0±89.60
		Difference	-	20.90% increase	24.20% decrease
1	PM_3	Control	24.65-27.75	26.00±1.00	451.50±33.02
		Treated	32.35-37.40	35.00±1.54	241.70±15.47
		Difference	-	34.90% increase	46.50% decrease
2	PM_3	Control	22.05-26.65	24.70±1.00	451.50±33.02
		Treated	30.15-33.85	32.40±1.95	244.44±43.00
		Difference	-	31.40% increase	45.90% decrease

1 = number of stomata per 1 mm^2

larger stomata sizes but lower stomata frequency (Table 2). It was found that the leaves of plants of PM_2 in Experiment1 and PM_3 in Experiment 1 and 2 had 24.2, 46.5 and 45.9 % fewer stomata that were 20.9, 34.9 and 31.4 % longer in length than those of the untreated plants respectively. The differences were observed by microscopy as described in the procedure.

The results agreed with those of Uhlik (1981) who reported that the polyploid plants usually had gigantic characteristics such as thicker, wider and greener leaves with greater stomata size and larger flowers. Stomata size, frequency of stomata including pollen grain diameter were favorable used as preliminary indicator of plants with polyploidy levels. Tan and Dunn (1973) also studied the correlation of the above characters with ploidy levels of *Bromus inermis* and found the possitive correlations of stomata length and pollen grain diameter while negative correlation of stomata frequency with ploidy levels. That meant stomata length and pollen grain diameter increased with the increasing of polyploidy levels. The opposite was noticed in stomata frequency. The results agreed with Yan (2001) who reported that the leaves of the tetraploid plantlets of waxflower had 54% fewer stomata that were 15% shorter in length than those of the diploid plantlets. Collins (1933, 1960) and Kerns and Collins (1947) studied the diploid, triploid hybrids and diploid and autotetraploid plants of pineapple variety 'Smooth Cayenne' and found that the sizes of cell, trichomes and stomata also increased with the ploidy levels while the stomata frequency was reduced. Sax and Sax (1973) also found greater somata frequency in diploid than tetraploid leaves of equal area of *Tradescantia camaliculata*.

In Experiment 1, chimera was observed from 5 out of 11 PM_2 polyploidy plants which indicated that as colchicine rarely acted on cells in a growing point, artificial induction usually resulted in a mixture called mixoploid or chimera (Wright, 1976). Similarly reported by Addink (2000) and Kehr (2001), they explained colchicine treatments to plant constituting of many cells to have chimera appearances. The results showed that the induced polyploidy plants still composed of plants with differing number of chromosomes.

Primary screening of morphological characteristics revealed 22 plants [11 plants of PM_2 (Experiment 1), 8 plants of PM_3 (Experiment 1) and 3 plants of PM_3 (Experiment 2)] to be presumably polyploids with higher ploidy levels. The 0.1% solution of colchicine derived from colchicine powder sold by Sigma seemed to give better results than the 0.5 % solution from the drug tablets. The rate of polyploidy occurrence from seedling in Experiment 1 appeared to be higher than seed treatment in Experiment 2. To confirm polyploidization, the selected plants will be furtherly analyzed and chromosome number will be determined.

CONCLUSION

The preliminary investigation had demonstrated that

1. 0.1% colchicine solution derived from powder sold by Sigma gave better results than 0.5% colchicine solution derived from drug tablets.
2. The presumably polyploidy plants of PM_2 and PM_3 had slower growth at the beginning and later resumed normal growth, larger stomata and less stomata frequency compared to those of the controls.

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