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Effect of Colchicine on Andrographis Variety Phichit 4-4 for Plant Breeding in Stem and Leaf Characteristics

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ABSTRACT

Background: *Andrographis paniculata* (Burm.f.) Nees is a plant native to Thailand. Thais use andrographis as an herb for treating the common cold. During pandemic the Andrographis leaves and stem has been used to treat COVID-19. In Thailand, there is a demand for andrographis, leading to a shortage of andrographis. Breeding andrographis by inducing polyploid plants is one way to increase yields in stem and leaf sections.

Methods: The study was conducted in May-September 2022 at Kasetsart University, Chalermprakiat Sakon Nakhon Campus, Thailand, by inducing andrographis of Phichit 4-4 variety into polyploids with colchicine concentrations of 0.0, 0.1, 0.2, 0.3 and 0.4%. After that, study of agricultural characteristics and identification of polyploids with flow cytometry analysis were carried out.

Result: A study on the characteristics of andrographis Phichit 4-4 after inducing colchicine in various treatments at 2 months of age showed that andrographis received with colchicine had a higher stem height and number of node than those without colchicine. Comparison of characteristics of andrographis Phichit 4-4 at 4 months of age. The number of leaves per plant and leaf weight are greater than those treated without colchicine. When examining the polyploids of andrographis plants using flow cytometry analysis, it was found that the plants with abnormal characteristics with tall stems, large leaves, thick leaves, dark green leaves are mixoploids. Studies have found that there are many abnormal plants in colchicine-treated treatments. 0.1% for 24 and 48 hours.

Key words: Andrographis, Colchicine, Mixoploid, Polyploidy

INTRODUCTION

Fa Thalai Chon, literally :Heaven Breaks Down the Bandit or *Andrographis paniculata* (Burm.f.) Nees is also known as Fa Thalai, Fa Satan, Khun Chon Haroi, Ya Kan Ngu, Namlai Pangpon, Mek Thalai, Chuang Chim Noi, Jek kiang Hee, Kwo Chao, Chuan Si Lian. The plant has been used as medicine for more than 2,000 years. It is deemed a regional herb of many countries in East Asia, India, China, Sri Lanka and Thailand. Indeed, the medicinal use of the plant has been widely practiced in the Asian continent Department of Agriculture, 2021. Its leaves and underground stems are mostly utilized as herbal remedy particularly for influenza. Research on *A. paniculata* has been conducted to find its extensive effects on antiviral activities. The plant is included in the World Health Organization's medicinal list for curing upper respiratory tract infections, bronchitis and tonsillitis. The properties of *A. paniculata* have been signified these days as many studies have reported its effective actions on healing the cold, flue, inhibiting and killing viruses (anti-virus). According to the National List of Essential Medicine 2021 (No. 2) issued by the Dispensary System Committee of Thailand, the extract from *A. paniculata* has been included in the Herbs section as the medicine for alleviating the influenza and non-severe Covid-19 (Technology Transfer and Development Bureau, 2021). Since then, the importance of *A. paniculata* has become better recognized. In Thailand, *A. paniculata* varieties which are mostly recommended for planting are Phitsanulok 5-4 and Phichit 4-4. The yield of Phitsanulok 5-4 is 11,087-26,168.75 kg ha-1 with the amount of 10.74-11.79% lactone of the dry weight. As for Phichit 4-4 variety, its yield is 10,787.5-24,250 kg ha-1 and the amount of 10.59-12.00% lactone of the dry weight (Department of Agriculture, 2021). At present, the demand for *A. paniculata* to treat diseases has been increased. So, *A. paniculata* breeding to gain better and more yield of this crop has been vigorously attempted to adequately fulfill the demand. As a matter of fact, *A. paniculata* has small flowers and the species is self-pollination. For these reasons, it is quite difficult to successfully improve *A. paniculata* through breeding. Unlike other cultivated plants, *A. paniculata* variation in the nature will become less in the future.

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Therefore, we should seek for other techniques to create more *A. paniculata* variation. One effective way which can help increase *A. paniculata* variation with better yield is by applying polyploidy induction from *A. paniculata* seeds. As reported in several studies, polyploidy plants provide good agricultural characteristics (Chen *et al.,* 2021; Dzimega *et al.,* 2024) whereas the polyploids also contain more essential compounds (Chen *et al.,* 2021; Talei *et al.,* 2020; Sadat Noori *et al.,* 2017). Furthermore, the polyploidy plants can tolerate the unfavorable environment like inappropriate temperature or drought better (Zhang *et al.,* 2015; Saravanan, 2019). They can also resist or adapt well to the salty soil or other poor soil conditions (Tu *et al.,* 2014; Luo *et al.,* 2017; Meng *et al.,* 2016). Polyploidy induction from the plant seeds is a strategy for chromosome duplication providing various genotypical autopolyploids of heterozygosity or gene diversity (Wang *et al.,* 2020; Dzimega *et al.,* 2024). Polyploidization may occur through cytological mechanism or from applying antimitotic chemicals such as colchicine, oryzalin, trifluralin (Talei *et al.,* 2020). Using colchicine to induce polyploidy, the plants' morphology, physiology, cytology or even at the gene expression level will be altered (Valnona, 2000; Talei *et al.,* 2020). However, the efficiency of polyploidy induction depends on many factors: the organs of the plants, types of chemicals and their concentration, and treatment durations (Wang *et al.,* 2020; Baby *et al.,* 2023). It was indicated in some studies that using colchicine to induce polyploidy from the cotton seeds worked more successfully than from the cotton seedlings and cotton branches (Dhamayanthi and Gotmare, 2010). In this study, polyploidy induction was conducted with Phichit 4-4 *A. paniculata*. It was expected that the study would offer guidelines for inducing the polyploid Phichit 4-4 *A. paniculata*. In addition, the effects of colchicine concentrations and colchicine treatment durations to induce polyploidy on the morphological characteristics and the ploidy level of the plant were investigated.

MATERIALS AND METHODS

The experiment took place from 2022 to 2023 at the Faculty of Agricultural Technology at Sakon Nakhon Rajabhat University and the Faculty of Natural Resources and Agro-Industry at Kasetsart University Chalermphrakiat Sakon Nakhon Province, Sakon Nakhon Province, Thailand.

The experimented *A. paniculata*. variety

In this experiment, the Phichit 4-4 *A. paniculata* was used.

Polyploidy induction in *A. paniculata*

The seeds of Phichit 4-4 *A. paniculata* were washed through the running tap water for 5 minutes. After that, the seeds were washed again with the solution of Sunlight dishwashing liquid for 3 minutes. The seeds had to be washed one more time with the running tap water for 5 minutes. Next, use 10% Clorox solution to wash the seeds for 5 minutes. Afterwards, the seeds were washed 3 times, 5 minutes a time, using Singha water bottles. Soak the seeds in colchicine of different concentrations (0.0%, 0.1%, 0.2%, 0.3% and 0.4%) for a period of 24 and 48 hours, respectively. The seeds were then grown in the planting trays with 60 holes filled with compressed peat moss. There were 10 treatments in the study and each treatment was conducted 4 times. Completely Randomized Design (CRD) was adopted for the experiment. All means were compared using DMRT and SPSS version 16. After the seeds had been planted for a period of 1 month, their germination, seedlings' abnormalities and the polyploidy of the abnormal seedlings were investigated.

Investigation of the seedlings' morphological traits

After the seeds had been planted for a period of 2 months, the shoots of the seedlings were seen to germinate above the compressed peat moss. The seedlings were observed and compared between the normal (from the control trays which were not given colchicine treatment) and abnormal seedlings. Transplant and grow the seedlings in the 7×15 inches black plastic bags which were filled with the mixture of the soil, raw rice husk and farmyard manure. When the seedlings were in their two and four months, their growth traits were recorded: stem, height, number of nodes/plant, number of branches/plant, number of leaves/plant, leaf width and leaf length. Finally, the collected data were compared using DMRT and SPSS version 16.

Examination of abnormal plants using flow cytometry analysis

After the Phichit 4-4 *A. Paniculata* seeds had been grown for 5 months, the plants would be categorized as the normal or abnormal plants and they were checked through flow cytometry analysis. The abnormal plants' polyploidy were examined and they were compared with the controlled normal plants. The amount of 0.1-0.5 gram (1-2 centimeters) of leaves picked from each plant were chopped on the plastic Petri dish before adding 500 microliter of Quantum Stain NA UV 2 (A). After that, add 0.05 gram of Polyvinyl-Pyrrolidone (PVP) in order to remove the waste particles. Then, the mixture was filtered through 30 micron strainer so that any nucleuses of suspended solids could be got rid and soon afterwards more 500 microgram of Quantum Stain NA UV 2 (B) was added in the tube. Shake the tube well to blend the substances before checking the polyploidy of the plants through flow cytometer.

RESULTS AND DISCUSSION

Traits of one-month-old *A. paniculata*

Germination of one-month-old *A. paniculata*

It was found that the germinations of Phichit 4-4 *A. paniculata* seeds in each treatment significantly differed. In this study, colchicine treatment had increased the germination of *A. paniculata*. In contrast, other studies demonstrated that colchicine treatment decreased the germination of other plants (Nura *et al.,* 2013; Surson *et al.,* 2021; Yan *et al.,* 2022). Nevertheless, the results gained from this study agreed with the experiment which was conducted with the indigenous *A. Paniculata*-the indigenous *A. paniculata* seeds which were treated by 0.1% and 0.2% colchicine concentrations germinated better than *A. paniculata* seeds which were not treated by colchicine (Table 1). Furthermore, the longer colchicine treatments of 24 and 48 hours also brought about higher germination of Phichit 4-4 *A. paniculata*.Contemplating each treatment, 0.2% colchicine treatment yielded the highest percentage of germination (Table 1).

Abnormal traits of one-month-old seedling

The abnormalities in seedlings were categorized into two groups: the normal and abnormal. The abnormal seedlings had two shoots with large, distorted, dark green foliage leaves and swollen stems. The treatment which received 0.1% colchicine for 48 hours had most abnormal seedlings (24.58%). At the same time, the treatment which received 0.4% colchicine for 24 hours had the second most abnormal seedlings of all treatments, the seeds of Phichit 4-4 *A. paniculata* plants which received 0.1% colchicine for both 24 and 48 hours provided high percentage of abnormal seedlings (Table 1). Such results agreed with the study of polyploidy induction of *Indigo suffruticosa* previously conducted (Surson *et al.,* 2018). Moreover, no abnormal seedlings were found in treatments 1 and 2. Similar results were reported in the study of using 0.2%colchicine to induce from *Citrus reticulata* Blanco in which the highest percentage of tetraploids were gained (Surson *et al.,* 2015; Surson *et al.,* 2018).

Traits of two-month-old *A. paniculata*

Height of two-month-old *A. paniculata*

The colchicine treated two-month-old Phichit 4-4 *A. paniculata* seedlings' heights significantly varied at different concentration. The two-month-old Phichit 4-4 *A. paniculata* seedlings whose

Table 1: The effect of concentration and duration of colchicine treatment on germination and abnormalities of andrographis Phichit 4-4 at the age of 2 months.

Treatment (%, h)	Germination (%)	Abnormalities (%)
T1 (0.0, 24)	24.17±3.97[a]	0.00 ±0.00[a]
T2 (0.0,48)	37.08±8.54[a-b]	0.00±0.00[a]
T3 (0.1,24)	51.67±15.57[c-d]	17.08±10.13[b-c]
T4 (0.1,48)	42.08±10.66[b-c]	24.58±9.85[c]
T5 (0.2,24)	74.17±12.36[e]	14.17±5.18[b]
T6 (0.2,48)	100.00±0.00[f]	10.00±6.53[a-b]
T7 (0.3,24)	34.58±2.84[a-b]	10.00±3.0 4[a-b]
T8 (0.3,48)	56.25±4.38[d]	13.75±9.27[b]
T9 (0.4,24)	72.09±8.21[e]	19.59±4.59[b-c]
T10 (0.4,48)	88.08±12.05[f]	12.50±6.16[b]
F-test	**	**
C.V.(%)	15.76	53.45

Letter (s) in each column indicated least significant differences at probability (p) <0.05, ns = non significant, ** Represent significant at the P = 0.01 level and * Represent significant at the P = 0.05 level.

Table 2: The effect of concentration and duration of colchicine treatment on plant height number of node and number of leaves of andrographis Phichit 4-4 at the age of 2 months.

Treatment(%, h)	Plant height (cm)	Number of node	Number of leaves
T1 (0.0, 24)	6.05±1.87[a]	2.53±0.40[a]	10.05±4.89
T2 (0.0,48)	9.87±2.52[a-c]	2.96±0.37[a-b]	11.75±2.20
T3 (0.1,24)	13.11±4.54[c-d]	3.23±1.15[a-b]	11.34±2.84
T4 (0.1,48)	8.77±3.70[a-b]	2.54±0.64[a]	10.28±3.94
T5 (0.2,24)	9.64±0.90[a-c]	2.78±0.25[a-b]	8.56±1.71
T6 (0.2,48)	11.11±1.22[b-d]	3.19±0.13[a-b]	7.93±0.88
T7 (0.3,24)	8.77±2.27[a-b]	3.59±0.63[b]	9.59±2.58
T8 (0.3,48)	9.16±0.98[a-b]	3.10±0.41[a-b]	8.26±1.43
T9 (0.4,24)	14.33±2.43[d]	4.37±0.37[c]	10.49±0.77
T10 (0.4,48)	8.19±0.98[a-b]	3.26±0.36[a-b]	7.85±0.66
F-test	**	**	ns
C.V.(%)	24.62	17.21	26.67

Letter (s) in each column indicated least significant differences at probability (p) <0.05, ns = Non significant, ** Represent significant at the P = 0.01 level and * Represent significant at the P = 0.05 level.

seeds were treated by 0.4% colchicine for a period of 24 hours were the tallest. At the same time, the two-month-old Phichit 4-4 *A. paniculata* seedlings whose seeds were treated by 0.2% colchicine for a period of 48 hours were the second tallest (Table 2). The results coincided with the studies of polyploidy induction from the seeds conducted by Nura *et al.* (2011) and Nura *et al.* (2013); according to these referred research works, the sesame seeds which received colchicine treatments had taller stems. On the contrary, in other studies which polyploidy induction had been conducted in other plants, the higher colchicine concentrations and the longer treatment periods the seeds received, the shorter their seedlings would become (Surson *et al.,* 2015; Surson *et al.,* 2021).

Number of nodes of two-month-old *A. paniculata*

The investigation demonstrated that the number of nodes of the two-month-old Phichit 4-4 *A. paniculata* seedlings whose seeds received different colchicine concentrations and different treatment durations significantly varied at statistical level. *A. paniculata* seedlings whose seeds were treated by 0.3% colchicine for 24 hours had the most nodes (3.59 nodes/plant) of all treatments, the two-month-old seedlings treated by colchicine had more nodes than those without colchicine treatment (Table 2). The results agreed with the effects of colchicine on *A. paniculata* stems; the two-month-old Phichit 4-4 *A. paniculata* seedlings whose seeds were treated by colchicine were taller than those whose seeds were not treated by colchicine. The experiment's results agreed with those results indicated in the black sesame polyploidy induction in the seeds which showed that the abnormal sesame seedlings had more nodes than the normal seedlings in the last week of the experiment (Surson *et al.,* 2021). Similar results were reported in another experiment. According to the polyploidy induction in *Citrus reticulata* Blanco, the tetraploid seedlings had more nodes than those of the diploids (Surson, 2017).

Number of leaves of two-month-old *A. paniculata* seedlings

The study showed that the two-month-old seedlings whose seeds received different colchicine concentrations and different treatment durations did not statistically have different number of leaves (Table 2). However, in the past experiment, the number of leaves of the five-week-old *Indigoflera tinctoria* L. seedlings were checked and it was found that their diploids and tetraploids' number of leaves statistically differed; the diploids had more leaves than the tetraploids. Despite the different numbers of leaves at this stage, when the *Indigoflera tinctoria* L. plants fully grew, the tetraploid *Indigoflera tinctoria* L. plants' leaves did not statistically differ from those of the *Indigoflera tinctoria* L. plants whose seeds did not receive colchicine treatment (Surson, 2018b). In case of the black sesame, the leaf number of the normal black sesame plants significantly and statistically varied from that of the abnormal black sesame plants; the abnormal black sesame plants whose seeds received colchicine treatments had more leaves than the normal black sesame plants whose seeds did not receive colchicine treatment (Surson *et al.,* 2021).

Traits of four-month-old *A. paniculata* plants

Height of four-month-old *A. paniculata* plants

It was revealed that the plant heights of different treatments were not statistically different from each other in different concentration of colchicine (Table 3). The results agreed with those gained from the black sesame experiment (Surson *et al.,* 2021) and from the *Indigoflera tinctoria* L. study (Surson, 2018a). Although the heights of the diploid and tetraploid the *Indigoflera tinctoria* L. plants did not vary, their chromosome numbers differed. As for Phichit 4-4 *A. paniculata* plants, in spite of the fact that both their chromosome numbers and heights did not statistically differ, it is possible that their chromosome structure could differ due

Table 3: The effect of concentration and duration of colchicine treatment on plant height, number of node and number of branches of andrographis Phichit 4-4 at the age of 4 months.

Treatment (%, h)	Plant height (cm)	Number of node	Number of branches
T1 (0.0, 24)	38.49±8.80	9.63±2.04	15.1±3.46
T2 (0.0,48)	36.07±9.07	9.18±0.42	16.3±3.15
T3 (0.1,24)	36.55±6.98	9.37±1.52	13.62±3.84
T4 (0.1,48)	30.03±4.67	8.16±1.39	12.43±2.38
T5 (0.2,24)	53.27±33.02	10.48±1.08	15.63±1.44
T6 (0.2,48)	34.63±6.00	9.59±0.98	13.30±2.21
T7 (0.3,24)	34.02±5.91	10.32±1.52	13.71±1.90
T8 (0.3,48)	36.32±7.72	9.48±2.08	16.43±3.79
T9 (0.4,24)	44.88±4.49	11.51±0.73	18.88±2.19
T10 (0.4,48)	36.34±4.70	8.99±1.35	14.63±4.61
F-test	ns	ns	ns
C.V.(%)	32.11	14.51	20.36

Letter (s) in each column indicated least significant differences at probability (p) <0.05, ns = Non significant, ** Represent significant at the P = 0.01 level and * Represent significant at the P = 0.05 level.

to other characteristics of variations. Such assumption was supported by the case of the cotton experiment; it was unveiled that colchicine had altered genetic characteristics of the quantitative yield and the mutated cotton characteristics could be inherited to the next generation (Luckett, 1989). In similar vein, as reported by Samadi *et al.,* (2022), colchicine 0.025% would double the gene expression of *ALDH*, *BGL* and *CCD2.*

Node number of four-month-old *A. paniculata* plants

The node number per plants of different treatments did not differ statistically (Table 3). The results agreed with the previous study which colchicine treatments made the black sesame plants to have more nodes (Surson *et al.* (2021). However, in other studies, the effects of colchicine treatments on the seeds of *Citrus reticulata* Blanco (Surson, 2017) and *Indigofera tinctoria* L. (Surson, 2018b) were conducted. It was reported in these studies that the tetraploids of both plants had fewer nodes than the diploids.

Number of branches of four-month-old *A. paniculata* plants

It was found that the branches of all treatments were not statistically different. The results disagreed with the study conducted by Talei *et al.* (2020) whose experiment showed that the branches of stevia whose seeds were soaked in 0.05%, 0.1% and 0.2% colchicine had more branches than the treatment of 0.0% colchicine (Table 3). The results in this experiment also differed from the past experiment conducted by Surson *et al.* (2021). According to Surson *et al.* (2021), the abnormal black sesame plants whose seeds received colchicine treatments had fewer branches than the normal black sesame plants whose seeds were not treated by colchicine. The black sesame polyploidy induction results (Surson *et al.,* 2021) agreed with the results of an experiment performed by Liu *et al.,*(2007). Based on this study, the tetraploids induced by colchicine had fewer branches than the diploids (Liu *et al.,* 2007).

Leaf number of four-month-old *A. paniculata* plants

It was revealed that the leaf number of four-month-old *A. paniculata* plants whose seeds were soaked in colchicine of different concentrations did not significantly vary at statistical level. Comparing the plants whose seeds were soaked with those whose seeds were not soaked in colchicine concentrations for the same duration, the results tended to show that those whose seeds received colchicine treatments had more leaves than the ones whose seeds were not soaked in colchicine. The experiment revealed that the plants of treatment 5, T5 (0.2, 24); treatment 9, T9 (0.4, 24) and treatment 10, T10 (0.4, 24), had the most leaves but without statistical difference (Table 4). These results agreed with the study conducted by Talei *et al.,* (2020) who experimented on stevia. According to Talei *et al.,* (2020), the plants whose seeds were soaked in colchicine 0.05%, 0.1% and 0.2% had more leaves than those whose seeds were soaked in 0.0% colchicine. Similar results happened to the study conducted on sesame (Nura *et al.,* 2011; Nura *et al.,* 2013).

Leaf width of four-month- old *A. paniculata* plants

The leaf width of the four-month-old *A. paniculata* plants grown from the seeds soaked in colchicine of different concentrations (0.0%, 0.1%, 0.2%, 0.3% and 0.4%) were investigated. It was revealed that the leaf widths of all treatments were not statistically different (Table 4). The results disagreed with the previous study conducted by Surson *et al.* (2021). In her aforementioned study, it was indicated that the abnormal plants whose seeds were soaked in colchicine had wider leaves than the leaves of the normal plants whose seeds were not soaked in colchicine (Surson *et al.,* 2021).

Table 4: The effect of concentration and duration of colchicine treatment on number of leaves, leaf width, leaf length and leaf weight of andrographis Phichit 4-4 at the age of 4 months.

Treatment (%, h)	Number of leaves	Leaf width (cm)	Leaf length (cm)	Leaf weight (g)
T1 (0.0, 24)	104.52±55.00^{b-c}	2.38±0.17	7.51±1.49	0.29±0.07^{a-b}
T2 (0.0,48)	45.47±4.75^{a}	2.48±0.39	8.32±1.11	0.27±0.04^{a}
T3 (0.1,24)	108.26±32.36^{c}	2.76±0.19	9.82±1.92	0.38±0.0 4^{b-c}
T4 (0.1,48)	50.16±19.23^{a}	2.38±0.22	8.15±1.48	0.35±0.09^{b-c}
T5 (0.2,24)	129.80±11.92^{c}	2.58±0.41	7.79±0.73	0.40±0.03^{c}
T6 (0.2,48)	108.77±22.47^{c}	2.56±0.44	7.90±0.84	0.38±0.06^{b-c}
T7 (0.3,24)	70.20±6.79^{a-b}	2.41±0.47	7.41±0.93	0.32±0.05^{b-c}
T8 (0.3,48)	110.79±20.49^{c}	3.23±1.56	8.24±1.12	0.41±0.04^{c}
T9 (0.4,24)	124.72±14.85^{c}	2.95±0.29	9.35±1.45	0.52±0.07^{d}
T10 (0.4,48)	122.17±11.11^{c}	2.76±0.60	8.55±1.59	0.53±0.07^{d}
F-test	**	ns	ns	**
C.V.(%)	24.96	19.37	15.86	16.47

Letter (s) in each column indicated least significant differences at probability (p) <0.05, ns = Non significant, ** Represent significant at the P = 0.01 level and * Represent significant at the P = 0.05 level.

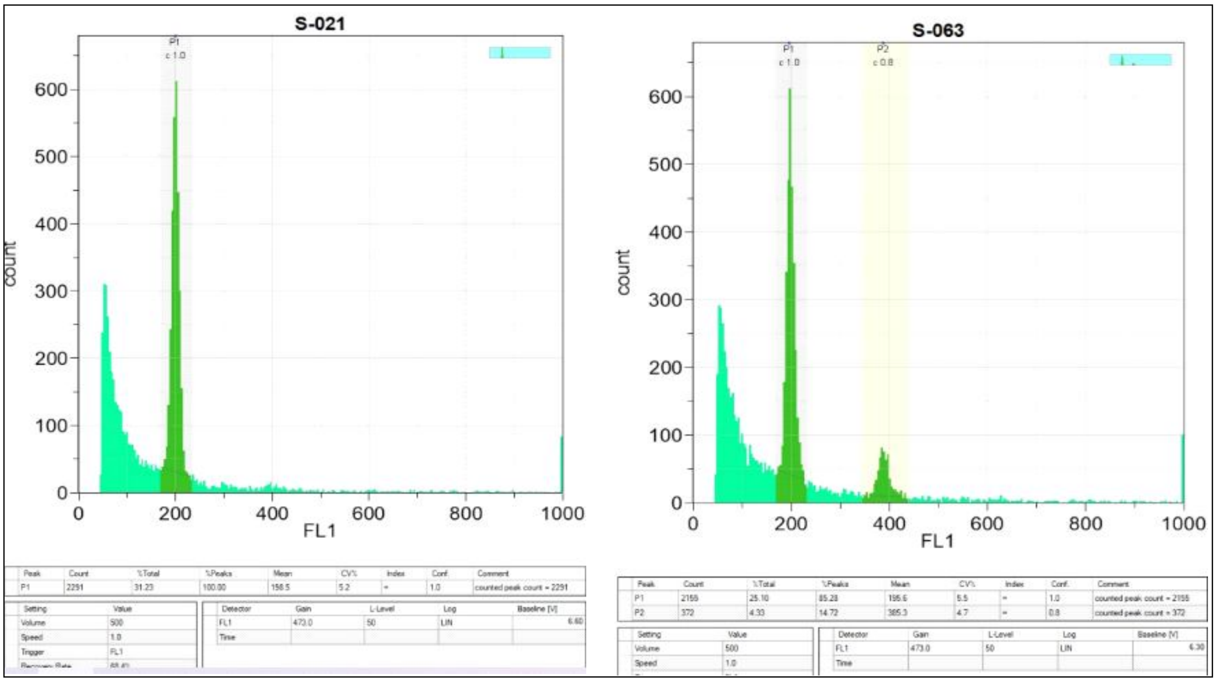

Fig 1: Flow cytometric analysis on DNA content of Andrographis Phichit 4-4 diploid (left) and mixoploid (right).

Fig 2: Andrographis Phichit 4-4 diploid (left) and andrographis mixoploid (right).

Leaf length of four-month-old plants

The investigation showed that the leaf length of every treatment whose seeds were soaked in colchicine did not vary at statistical level (Table 4). Such results contradicted the study conducted by Talei *et al.,* (2020). According to Talei *et al.,* (2020), the leaves of stevia plants whose seeds were soaked in colchicines of 0.05%, 0.1% and 0.2% were longer than those whose seeds were treated by 0.0% colchicine.

Leaf weight of four-month-old plants

The study revealed that the leaf weight of the plants treated in different treatments of colchicines were statistically differed. The leaves of the plants which received colchicine treatments were heavier than those without colchicine treatment. Nonetheless, neither the leaf width nor the leaf length of all treatments differed (leaf size was not different). The investigation indicated also that the leaves of the plants whose seeds received colchicine treatments were denser than those of the plants whose seeds were not soaked in colchicine (Table 4). The results went in line with the study of black sesame which showed that the leaves of the abnormal sesame plants weighed more than the leaves of normal sesame plants whose seeds were not soaked in colchicine (Surson *et al.,* 2021).

Flow cytometry analysis

All the abnormal seedlings were indicated their abnormalities through flow cytometry analysis. It showed that most of the abnormal seedlings whose shoots had large, distorted, dark green foliage leaves and swollen stems were diploids, except for the seedlings whose seeds were soaked in colchicine 0.2% for 24 hours. Some abnormal seedlings were mixoploids (Fig 1), whose stems were big with large, dark green leaves that spread out. These mixoploids had their flowers later than most of the diploids. Most of the diploids flowered and abundantly developed their pods (Fig 2). The polyploidy of the next generation of these mixoploids will be planted and investigated in the next experiment. Although there are not many polyploids in this study, it was reported in other experiments (Dermen *et al.,* 1943; Samadi *et al.,* 2022) that colchicine could alter the plant varieties by deleting or inserting one or some chromosomes in addition to increasing the number of chromosomes. The changes induced by the mutagens will depend on the plant species, concentrations of mutagens/chemicals, and treatment period.

CONCLUSION

After the seeds of Phichit 4-4 *A. paniculata* had been soaked in 0.0%, 0.1%, 0.2%, 0.3% and 0.4% for 24 and 48 hours, it was found that the two-months old *A. paniculata* plants which received colchicine treatments had higher percentage of germination than the plants which did not receive colchicine treatment. There were more abnormal plants in 0.1% colchicine treatments of both 24 and 48 hours of exposure times. Moreover, it was noticeable that many abnormal *A. paniculata* plants were gained from 0.4% colchicine treatment of 48 hours. As for their morphological traits, the *A. paniculata* plants in all 24 hours colchicine treatments were taller and had more number of nodes than the control plants which were not given colchicine treatment. Regarding *A. paniculata* plants in all 48 hours colchicine treatments, these plants tended to be tall with fewer number of nodes than the control plants which did not receive colchicine treatment. Nevertheless, in terms of the number of leaves, the number of leaves in all treatments did not statistically differ. The study revealed also that the four-month-old Phichit 4-4 *A. paniculata* did not have statistically different height, number of nodes, number of branches, leaf width and leaf length. However, the four-month-old Phichit 4-4 *A. paniculata* plants which received colchicine treatments had higher number of leaves and leaf weight. Applying flow cytometry analysis, it was unveiled that the abnormal plants could be classified as the diploids and the mixoploids. In addition, the mixoploids were bigger/taller and had more number of leaves. Their dark green leaves were also larger and thicker containing good agricultural traits.

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Conflict of intrest

The authors declare that there are no conflicts of interest**.**

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