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Original Article

Polyploidy induction of black sesame (*Sesamum indicum* L.) for yield component improvement

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Abstract

This study was conducted with the aim of gaining the outcomes of polyploidy induction by soaking black sesame seeds, *Sesamum indicum* Linn, in 0.0%, 0.1%, 0.2%, 0.3% and 0.4% colchicine for 24 hours. When checking the characteristics of polyploidy using the fifteen days old seedlings' abnormalities as indicators. The abnormal seedlings grew very slowly. The stems were short containing only cotyledon leaves and the first pair of unifoliate leaves. Their hypocotyls were swollen, fat and short while epicotyls did not extended but stunted. However, these hypocotyls could be extended or grew longer but they would take more time. The examination revealed that the sesame seedlings which were not treated by using colchicine could grow normally. In contrast, those, which were treated by using colchicine, yielded abnormal seedlings. The higher percentage of the concentrations of colchicine, the more abnormal seedlings were obtained as the following.

Keywords: black sesame, *Sesamum indicum* L., polyploid, colchicine

1. Introduction

Sesame is an annual crop. The capsule contains white, black or red seeds which can be extracted for oil or taken either as food or used as a spice. Sesame seeds have been specifically eaten a great deal in the Middle East and Asia. Originally, sesame was probably an indigenous plant found in Asia or the east of Africa. At present, sesame is found to be grown in the tropics, subtropics, and all areas of the southern tropics. Black sesame seeds are also a rich source of vitamin E which is good to moisturize and protect the person's complexion. Besides, the sesamin and sesamolin, the fibers called lignans found in these seeds can lessen the level of cholesterols in human blood. In addition to the quality of lowering cholesterols, it is believed also that the phytosterols gained from taking black sesame seeds will help prevent people from having cancer and heart disease.

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One way to make the component of the black sesame seeds better, particularly in terms of bigger seeds which contain more sesamin, is by inducing polyploidy like tetraploid. Tetraploid components of sesame seeds can yield more antioxidants of sesamin, sesamolin and sesamol. As reported in many studies, more metabolite production of important chemicals had been attained from polyploidy induction. More octoploid, the secondary metabolite, was found in tetraploid *Panax ginseng* C.A. Meyer (Kim, Hahn, Murthy, & Paek, 2004). The tetraploid roots of *Artemisia annua* L. (clone YUT16) on the 14th day also contained more artemisinin (according to Chinese medicine, the substance is used to cure malaria) than those of the diploid roots (De Jesus-Gonzalez & Weathers, 2003); likewise the tetraploid and mixoploid *Centella asiatica* (L.) Urban provided increasing biomass and triterpenoid (the substance used for healing wound, thinking and emotional disorders in the seniors). It was also found that tetraploid *Hyoscyamus muticus* L. could produce 200% higher scopolamine than in their diploid *Hyoscyamus muticus* L. (Dehghan, Häkken, & Oksman-

Caldentey, & Ahmadi, 2012). Although the amount of metabolites in diploid and tetraploid *Echinacea purpurea* (L.) Moench were similar, the fresh and dry roots of tetraploid *Echinacea purpurea* (L.) Moench were heavier at 39.32% and 40.48%, respectively. In addition, it was confirmed by many research works that polyploid plants could tolerate unsuitable environments better, especially drought and salt. According to Wang, Wang, Liu, & Meng (2003), tetraploid *Robinia pseudoacacia* L. could resist the salt stress better than diploid *Robinia pseudoacacia* L. Tetraploid *Coccinia palmate* and tetraploid *Lagenaria sphaerica* had more chlorophylls and could tolerate drought better, resisted stress from dehydration better than their diploid counterparts (Ntuli & Zobolo, 2008). Moreover, tetraploid *Coccinia palmate* and *Lagenaria sphaerica* could resist pests better and their growth rates were higher (Otto & Whitton, 2000).

The experiment aimed to find the appropriate approach for creating tetraploid black sesame plants. The investigation intended to gain suitable methodology, colchicine concentrations, periods of treatment, the comparison between black sesame diploids and tetraploids to find good black sesame plants. In the future, these appropriate techniques will be employed for inducing polyploidy of several good black sesame plants which are adjustable to survive and grow in the changing environment of the earth.

2. Materials and Methods

2.1 Black sesame varieties

The variety of black sesame "Ubon Ratchathani 3" used in the study was obtained from Ubon Ratchathani Field Crops Research Center

2.2 Polyploidy induction

The black sesame seeds were washed through the running tap water for 5 minutes. Then, the seeds were washed again using the solution of Dishwashing Liquid (Sunlight) for 3 minutes. In order to kill germs, the seeds were washed again with the running tap water for 5 minutes before they were soaked in 7% Clorox solution for 5 minutes. Afterwards, the seeds were washed 3 times, 5 minutes per time using filtered water. Treat the seeds by soaking them in colchicine of different concentrations: 0.0%, 0.1%, 0.2%, 0.3% and 0.4% for a period of 24 hours. After the seeds were grown in the germination trays for 15 days, the seedlings were examined to find their growth rates, abnormalities, height, and number of leaves. The data was analyzed by SAS program (version 9.1). In the succeeding step, all treatments were compared by adopting Duncan's Multiple Range Test (DMRT). When the seeds had been grown for 25 days, the growing seedlings were tested to find their polyploidy through flow cytometry analysis technique.

2.3 Morphological examination of seedling abnormalities

After the black sesame seeds had been planted for 15 days, the seedlings were up and sprouted from the peat moss sheets stuck to the bottom of the germination trays. The normal and abnormal seedlings were observed and discriminated (from control seedlings which were not treated by colchicine (Surson, 2018a).

2.4 Comparison of morphological normalities and abnormalities of sesame plants

After the black sesame seeds were treated by colchicine of different concentrations: 0.0%, 0.1%, 0.2%, 0.3% and 0.4% for a period of 24 hours and the seeds had been grown for 15 days, normal and abnormal seedlings were classified.

2.4.1 Stem characteristics

These stem characteristics were examined: height, circumference, number of nodes per plant, and number of branches per plant.

1) Stem height was measured 1 centimeter above from the planting mixes to the tip of the plant stem
2) Stem circumference was measured around the stem about 1 centimeter above the peat moss.
3) Number of nodes were counted from the main stems to the stem tips
4) Number of branches were counted from the main stems to the stem tips

2.4.2 Leaf characteristics

These characteristics of the black sesame leaves were studied: number of leaves per plant, the fresh weight of the leaves, leaf length, leaf width, leaf index, fresh weight per leaf area.

1) Number of leaves per plant could be gained by counting all leaves on the plant

2) Leaf length was obtained from measuring the base to the apex of all fourth leaves and calculating the average length

3) Leaf width was obtained from measuring the widest part of all fourth leaves and calculating the average width

4) Leaf index could be obtained by measuring the width and length of all fourth leaves**.** Then, based on the formula given by Liu, Li, & Bao (2007), the leaf index was calculated by dividing the leaf length with the leaf width.

5) Fresh weight was available by weighing fresh the fourth leaves from the top

2.4.3 Growth rate

The growth rate of black sesame plants was examined by measuring their height, circumference, number of nodes, number of branches, and number of compound leaves of the 5 to 13 weeks old black sesame plants for a period of 60 days. Then, by adopting the formula set by He *et al.* (2012), the growth rate was calculated as below:

$$\text{Growth rate (\%)} = \frac{\text{The figures gained on the last day} - \text{The figures gained on the first days}}{\text{Number of days x The figures gained on the first day}} \times 100$$

2.5 Analysis of the results

Twenty diploids and twenty tetraploids of the black sesame plants were examined. Then, the information was analyzed by using SAS version 9.1 (1998). The population characteristics of these groups of black sesame plants which received different treatments were compared by employing a t-test.

3. Results and Discussion

3.1 Black sesame polyploidy induction by using colchicine solutions of different concentrations

3.1.1 Characteristics of 15 days old black sesame seedlings

Soaking the black sesame seeds in 0.0%, 0.1%, 0.2%, 0.3%and 0.4% colchicine for 24 hours, these black sesame seeds were grown on half peat moss cushioned germination trays of 35x45x10 centimeters. After having planted these seeds for 15 days, it was found that these black sesame seedlings could be categorized into the normal and abnormal seedlings. The normal seedlings had normal epicotyls, hypocotyls, cotyledons, and foliage leaves. At the same time, the abnormal seedlings had abnormal epicotyls and hypocotyls; their first two foliage leaves were also unusual with swollen hypocotyls. Some seedlings even had very short, abnormal roots which were incapable of growing; their epicotyls grew slower than those of the normal seedlings. Regarding foliage leaves, the first two foliages of normal seedlings had similar characteristics to those of the original species whereas the foliages of the abnormal seedlings were curled and deformed owing to errors of cell division (Table 1). In this study, polyploidy examination of both, normal and abnormal seedlings were tested by using flow cytometry technique. Unfortunately, the extract from the leaves of the seedlings which had germinated for a month had too much mucus making the polyploidy testing impossible. However, based on polyploid induction in **'***Kram Phak Troung***'** (*Indigofera tinctoria* L.) conducted by Surson (2018a), it was unveiled that more than 83% of the abnormal *Indigofera tinctoria* L. plants which were treated by colchicines were polyploids. These polyploidy abnormal *Indigofera tinctoria* L. plants consisted of both the mixoploids and tetraploids. In addition, the mixoploid *Indigofera tinctoria* L. plants were similar to the tetraploid *Indigofera tinctoria* L. plants (Surson, 2018a, 2018b).

3.1.2 Germination and abnormality percentage of black sesame seedlings

Examining the 15 days old black sesame seedlings after they had been treated by 5 different concentrations of colchicine (0.0%, 0.1%, 0.2%, 0.3% and 0.4%) for a period of 24 hours, it was revealed that varied colchicine concentrations did not statistically differed from the germination of these black sesame seedlings. However, there was a tendency that the higher percentage of colchicine solutions, the lower germination percentage of the black sesame seedlings. It can be said that all black sesame seeds which received colchicine treatments germinated or grew less. At the same time, it was noticeable from the experiments that the higher colchicine concentrations the black sesame seeds were treated, the less germination or less growth percentage could be seen as shown in Table 2. These results corresponded to those obtained from the study conducted by Liu *et al.* (2007); the higher percentage of colchicine solutions, the less germination rate of *Platanusa cerifolia*. In addition, the investigations performed with *Indigofera suffruticosa* (Surson, Sitthapanit, & Wongma, 2018), *Indigofera tinctoria* L (Surson, 2018a) *Citrus reticulata* Blanco (Surson, Sitthapanit, & Wongma, 2015) and *Eribotrya japonica* (Thumb.) Lindl. (Blasco, Badenes, & Naval, 2015) also yielded similar results; the higher colchicine concentrations to treat the seeds, the lower germination rates were obtained.

Counting the number of normal and abnormal plants, it was found that different colchicine concentrations significantly induced normal and abnormal plants at statistical levels (Table 2). In another research experimented by Surson *et al.* (2018), it was found that the germinated seeds of *Indigofera suffruticosa* which were treated by 0.1%, 0.2% and 0.4% colchicine for the periods of 6 and 12 hours yielded 20-67.67% abnormal indigo plants. At the same time, the

Table 1. Characteristics of black seedlings on the fifteenth day after they had been soaked with colchicine for 24 hours.

Types of seedlings	Characteristics
Normal seedlings	Having normal epicotyls and hypocotyls. Epicotyls and hypocotyls grew and extended. Their stems were long and thin. They had a pair of cotyledons, and one or two pairs of foliage leaves. The first pair of foliages (unifoliolate) was simple leaves. The second pair of foliages was green compound leaves.
Abnormal seedlings	The seedlings were short and they grew slower. Most of them had only cotyledons and the first pair of foliage leaves (unifoliolate). The hypocotyls swelled and were short while their epicotyls did not extend. It took longer time for the epicotyls to extend. Unlike the normal seedlings, the first pair of foliages did not spread out but were curled and distorted.

Table 2. Germination and abnormality percentage of black sesame seedlings on the fifteenth days after they had been treated by 0.0%, 0.1%, 0.2%, 0.3%, and 0.4% for a period of 24 hours.

Treatment	Germinated seedlings (%)	Ungerminated seedlings (%)	Normal seedlings (%)	Abnormal seedlings (%)
A1 (0.0%)	80.63	19.38	80.63a	0.00d
A2 (0.1%)	78.44	21.56	67.54ab	10.90cd
A3 (0.2%)	71.88	28.13	52.50bc	19.38bc
A4 (0.3%)	65.94	34.06	40.63cd	25.31ab
A5 (0.4%)	62.81	37.19	28.75c	34.06a
F-test	ns	-	**	**
cv.(%)	18.19	-	22.12	41.80

Letter (s) in each column indicated least significant differences at probability (p) < 0.05, ns = non-significant, ** Represents significant at the P = 0.01 level, and * Represents significant at the P = 0.05 level germinated seeds of *Indigofera tinctoria* L (Surson, 2018a) which were treated by 0.1%, 0.2%, and 0.4% colchicine gave 100% abnormal indigo plants. Nevertheless, according to this investigation, the ungerminated black sesame seeds treated by 5 different concentrations of colchicine (0.0-0.4%) for 24 hours yielded only 10.90% - 34.06% abnormal black sesame plants. The studies indicated that treating the ungerminated seeds were less effective for polyploidy induction than treating the germinated seeds.

3.1.3 Height and leaf number of black sesame seedlings on the fifteenth day

Measuring the height of 15 day-old black sesame seedlings after they have been treated with five different colchicine solutions, 0.0%, 0.1%, 0.2%, 0.3% and 0.4% for 24 hours, it was found that these different concentrations of colchicine significantly influenced the height of the black sesame seedlings at statistical levels. Seedlings which were not treated by colchicine grew the highest. On the contrary, when the seedlings were treated with increasing colchicine concentrations, the height of the seedlings tended to decrease (Table 3).

When examining the number of leaves of 15 days old black sesame seedlings after they had been treated by colchicine solutions 0.0%, 0.1%, 0.2%, 0.3% and 0.4% for 24 hours, it was found that different concentrations of colchicine significantly differed from the leaf numbers of the black sesame seedlings at statistical levels (Table 3). The results gained from this investigation agreed with those obtained in the two studies previously conducted with straight pod indigo plants, *Indigofera tinctoria* L. (Surson, 2018a), and the *Citrus reticulate* Blanco (Surson *et al.*, 2015).

Table 3. Height and number of leaf of black sesame seedlings on the fifteenth days after they were treated by 0.0%, 0.1%, 0.2%, 0.3%, and 0.4%, colchicine concentrations for 24 hours.

Types of treatments	Height (cm)	Number of leaves
A1 (0.0%)	4.18ab	5.05a
A2 (0.1%)	4.63a	4.09b
A3 (0.2%)	3.36bc	4.00b
A4 (0.3%)	3.21bc	3.74b
A5 (0.4%)	2.91c	4.29b
F-test	*	**
cv.(%)	19.23	9.89

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

3.2 Comparison of morphological characteristics of the black sesame plants during the tenth to fourteenth week

3.2.1 Plants height of black sesame plants

The heights of normal and abnormal black sesame plants were compared using SAS (version 9.1) and t-test. Comparing the normal and abnormal black sesame plants during the tenth to the fourteenth weeks, for five weeks, it was found that their heights did not show significant differences at statistical level. Nevertheless, the normal black sesame plants tended to be a little taller than the abnormal black sesame plants. The results agreed with the study conducted by Blasco *et al.* (2015) which demonstrated that the tetraploids were shorter than the diploids. However, the former studies experimented with *Citrus reticulate* Blanco and *Indigofera tinctoria* L. by Surson (2018b) and Surson *et al.* (2015) indicated that the heights of the tetraploids and diploids of both species did not differed. It can be said that more studies should be conducted and the heights of the black sesame tetraploids and diploids which were planted at the same time without colchicine treatment should be investigated and compared further.

3.2.2 Circumference

The information about the circumferences of normal and abnormal black sesame plants had been collected for five weeks starting from the tenth week and every two weeks afterwards. It was found that in the tenth to twelfth weeks the normal and abnormal black sesame plants' circumferences did not significantly differed at statistical level. However, in the fourteenth week, the circumferences of the normal and abnormal black sesame plants were significantly varied at statistical level (Table 5). The results corresponded to those results obtained in the previous studies which were experimented with *Indigofera suffruticosa* (Surson *et al.*, 2018) and *Citrus reticulate* Blanco (Surson, 2017). In these research works, the tetraploids and diploids' circumferences of both plants *Indigofera suffruticosa* and *Citrus reticulate* Blanco did not significantly differed at statistical levels.

Table 4. Heights of the normal and abnormal black sesame plants during the tenth to the fourteenth weeks

Age of black sesame plants (weeks)	Height (cm)		t-test
	Normal	Abnormal	
10	26.05±2.00	21.90±1.17	ns
12	31.45±2.13	26.05±1.56	ns
14	60.10±2.97	40.85±2.02	ns

** Represents significant at the P = 0.01 level, * Represents significant at the P = 0.05 level and ns not significant

Table 5. Circumferences of normal and abnormal black sesame plants during the tenth to the fourteenth weeks

Age of the black sesame plant (weeks)	Circumference (cm)		t-test
	Normal	Abnormal	
10	1.98±0.13	2.33±0.17	ns
12	2.48±0.13	2.85±0.17	ns
14	3.30±0.15	4.63±0.25	*

** Represents significant at the P = 0.01 level, * Represents significant at the P = 0.05 level and ns not significant

3.2.3 Number of nodes/plant

To study a number of nodes/plant, the normal and abnormal black sesame plants had been examined for five weeks (week 10th-14th). It was discovered that in week ten, the normal and abnormal black sesame plants did not significantly have different number of nodes at statistical level. In contrast, the study showed that the normal and abnormal black sesame plants significantly had different number of nodes at statistical level in the fourteenth week (Table 6). These results did not go in alignment with the results found in previous studies. In the experiments formerly conducted with *Indigofera suffruticosa* M. (Surson *et al.*, 2018) and *Citrus reticulate* Blanco (Surson, 2017). According to these research studies, the tetraploids and diploids' node numbers did not differ statistically. Moreover, there was a tendency that the tetraploids seemed to have fewer nodes than those of the diploids.

3.2.4 Number of branches

The information about the number of branches/plant would be collected every two weeks afterwards. It was revealed that the normal and abnormal black sesame plants, as shown in Table 7, did not significantly have different number of branches/plant at statistical level. Results complied with those results gained from the studies conducted by Grouh, Meftahizade, Lotfi, Rahimi, and Baniasadi (2011) in *Salvia hains* and Liu *et al.* (2007) which experimented with *Platanusa cerifolia* and a study by Surson *et al.* (2018) which experimented with *Indigofera suffruticosa*.

3.2.5 Number of leaves/plant

The information about the number of leaves/plant was collected since the tenth week and then the same information was recorded every two weeks afterwards. It was discovered that in week ten, the normal and abnormal black sesame plants did not significantly have different number of leaves at statistical level. In contrast, when the number of leaves of the normal and abnormal black sesame plants was collected in the twelfth week, it was found that the normal and abnormal black sesame plants significantly had different number of leaves at statistical level (Table 8). The results did not correspond to those results obtained from the previous studies which reported that the tetraploids seemed to have fewer leaves than those of the diploids (Surson, 2017, 2018b; Surson *et al.*, 2018).

3.2.6 Leaf length

The information about the leaf length was collected since the tenth week and then the same information was recorded every two weeks afterwards. As reported in Table 9, the normal and abnormal black sesame plants did not significantly have different leaf length at statistical level. However, the leaves of the abnormal black sesame plants tended to be longer than those of the normal black sesame plants. The results corresponded to those gained in the previous research works experimented with *Indigofera tinctoria* L. (Surson, 2018b) with *Indigofera suffruticosa* (Surson *et al.*, 2018), and with *Citrus reticulata* Blanco (Surson, 2017). Still, it was reported that the leaves of tetraploid *Platanusa cerifolia* were longer than their diploid counterparts (Liu *et al.*, 2007).

Table 6. Number of nodes of normal and abnormal black sesame plants during the tenth to the fourteenth weeks

Age of the black sesame plant (weeks)	Number of nodes		t-test
	Normal	Abnormal	
10	21.05±1.17	19.95±1.74	ns
12	24.10±1.25	23.80±2.09	*
14	21.30±1.18	30.70±2.24	**

** Represents significant at the P = 0.01 level, * Represents significant at the P = 0.05 level and ns not significant

Table 7. Number of branches of normal and abnormal black sesame plants during the tenth to fourteenth weeks

Age of the black sesame plant (weeks)	Number of branches		t-test
	Normal	Abnormal	
10	3.10±0.35	2.10±0.24	ns
12	4.10±0.35	2.95±0.38	ns
14	5.10±0.42	3.80±0.49	ns

** Represents significant at the P = 0.01 level, * Represents significant at the P = 0.05 level and ns not significant

Table 8. Number of leaves of normal and abnormal black sesame plants during the tenth to fourteenth weeks

Age of black sesame plants (weeks)	Number of leaves		t-test
	Normal	Abnormal	
10	37.20±2.73	29.15±3.65	ns
12	40.45±2.73	35.70±4.57	*
14	44.00±3.02	66.55±7.11	**

** Represents significant at the P = 0.01 level, * Represents significant at the P = 0.05 level and ns not significant

Table 9. Leaf lengths of normal and abnormal black sesame plants during the tenth to fourteenth weeks

Age of black sesame plants (weeks)	Leaf length (cm)		t-test
	Normal	Abnormal	
10	4.30±0.35	3.58±0.42	ns
12	6.53±0.44	5.40±0.51	ns
14	8.48±0.62	10.25±0.79	ns

** Represents significant at the P = 0.01 level, * Represents significant at the P = 0.05 level and ns not significant

3.2.7 Leaf width

The leaf width information of these black sesame plants had been collected since the tenth week and then the information was collected every two weeks afterwards. It was found that in the fourteenth week, the study revealed that the leaves of the normal black sesame plants were significantly differed from those of the abnormal black sesame plants. It was found that the leaves of the abnormal black sesame plants were wider than those of the normal black sesame plants. Results agreed with the research projects conducted earlier with *Indigofera tinctoria* L. by Surson (2018), *Salvia hains* by Grouh *et al.* (2011), *Citrus reticulata* Blanco by Surson (2017) and *Indigofera suffruticosa* by Surson *et al.* (2018).

3.3 Growth rate of black sesame plant

The study investigated the growth rate of the normal and abnormal black sesame plants It was revealed that the growth rate of the abnormal black sesame plant in terms of circumference, number of nodes, number of leaves, and number of branches was significantly higher than that of the normal black sesame plant at a statistical level. However, regarding number of nodes, it was unveiled that the normal black sesame plant had significantly higher number of nodes than the abnormal black sesame plant at a statistical level while the growth rate of the abnormal black sesame plant was higher than the normal sesame plant. Concerning their height, the study revealed that the normal and abnormal black sesame plants were not differently taller than each other at statistical level (Table 11). Contemplating all traits of their growth rate, it was found that the growth rate of the abnormal black sesame plants was higher than that of the normal black sesame plants in every aspect except for their height. Similarly, as reported in other studies, the growth rate of the tetraploids was mostly higher than the growth rate of the diploids (Surson, 2017, 2018b).

3.4 Characteristics of 14 weeks old normal and abnormal black sesame plants

In the fourteenth week, the normal and abnormal black sesame plants had been compared. It was found that their height, number of branches and leaf length were not different at statistical levels. However, the abnormal black sesame plants had longer circumference, more nodes, more leaves, wider leaves, and heavier leaves than those of the normal black sesame plants even though the leaf index of the normal black sesame plant was higher than that of the abnormal black sesame plant. In light of productivity, it was found that the normal and abnormal black sesame plants did not yield the number of pods differently. Nevertheless, the abnormal black sesame plant tended to yield more pods than the normal sesame plants (Table 12).

Table 10. Leaf width of normal and abnormal black sesame plant during the tenth to fourteenth weeks

Age of plants (weeks)	Leaf width (cm)		t-test
	Normal	Abnormal	
10	1.95±0.22	2.30±0.45	**
12	2.43±0.22	1.78±0.20	ns
14	2.79±0.19	4.30±0.37	**

** Represents significant at the P = 0.01 level, * Represents significant at the P = 0.05 level and ns not significant

Table 11. Growth rate of the normal and abnormal black sesame plants in the fourteenth weeks

Organs	Growth rate (percentage)		t-test
	Normal plant	Abnormal plant	
Height	3.58±0.38	2.64±0.58	ns
Circumference	1.79±0.21	2.77±0.47	**
Node	0.12±0.59	1.54±0.29	*
Number of leaves	0.67±0.27	4.16±0.77	**
Number of branches	2.23±0.46	2.71±1.01	**

** Represents significant at the P = 0.01 level, * Represents significant at the P = 0.05 level and ns not significant

Table 12. Characteristics of normal and abnormal black sesame plants in the fourteenth weeks

Characteristics of sesame	Abnormalities of black sesame		t-test
	Normal	Abnormal	
Height (cm)	60.10±2.97	40.85±2.02	ns
Circumference (cm)	3.30±0.15	4.63±0.25	*
Number of Nodes/plant	21.30±1.18	30.70±2.24	**
Number of branches	5.10±0.42	3.80±0.49	ns
Number of leaves	44.00±3.02	66.55±7.11	**
Leaf width (cm)	2.79±0.19	4.30±0.37	**
Leaf length (cm)	8.48±0.62	10.25±0.79	ns
Leaf index	3.34±0.33	2.54±0.20	*
Leaf weight (g)	0.29±0.08	0.41±0.14	*
Number of capsules	16.85±2.83	21.90±3.53	ns

** Represents significant at the P = 0.01 level, * Represents significant at the P = 0.05 level and ns not significant

4. Conclusions

In order to gain the appropriate method to induce polyploid black sesame plants by treating black sesame seeds with colchicines of different concentrations: 0.0%, 0.1%, 0.2%, 0.3% and 0.4% for 24 hours, it was found that a number of abnormal sesame plants were obtained from all colchicine treatments. It could be concluded that the most appropriate polyploid induction of the black sesame seeds was using 0.4% colchicine treatment for 24 hours. Regarding the morphological study of the black sesame seedlings in the fourteenth weeks, it was found that there was no statistically significant difference between abnormal and normal black sesame seedlings in terms of their height, number of branches, and leaf length. However, the abnormal black sesame plants had longer stem circumference, more nodes, more leaves, wider leaves, and heavier leaf weight than those of the normal black sesame plants. In light of the productivity, it was revealed that the normal and abnormal black sesame plants did not have different number of capsules. Nonetheless, the abnormal black sesame plants tended to have more capsules than the normal ones. The study will be investigated further by planting them in either the planting pots or in the field.

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References

Blasco, M., Badenes, M. L., & Naval, M. M. (2015). Colchicine-induced polyploidy in loqouat (*Eriobotrya japonica* (Thunb.) Lindl.). *Plant Cell, Tissue and Organ Culture, 120,* 453-461.

De Jesus-Gonzalez, L., & Weathers, P. J. (2003). Tetraploid *Artemisia annua* hairy roots produce more artemisinin than diploids. *Plant Cell Reports*, *21*, 809-813.

Dehghan E., Häkkinen, S. T., Oksman-Caldentey, K., & Ahmadi, F.S. (2012). Production of tropane alkaloids in diploid and tetraploid plants and in vitro hairy root cultures of Egyptian henbane (*Hyoscyamus muticus* L.). *Plant Cell, Tissue and Organ Culture*, *110,* 35–44

Grouh, M. S. H., Meftahizade, H., Lotfi, N., Rahimi, V., & Baniasadi, B. (2011). Doubling the chromosome number of *Salvia hains* using colchicines: Evaluation of morphological traits of recovered plants. *Journal of Medicinal Plant Research*, *5*, 4892−4898.

He, L. Y., Ding, Z., Jiang, F., Jin, B., Li, W., Ding, . . . Li, G. (2012). Induction and identification of hexadecaploid of Pinellia ternate. *Euphytica*, *186*, 479–488.

Kim, Y., Hahn, E., Murthy, H. N., & Paek, K. (2004). Effect of polyploidy induction on biomass and ginsenoside accumulations in adventitious roots of ginseng. *Journal of Plant Biology*, *47,* 356-360.

Liu, G., Li, Z., & Bao, M. (2007). Colchicine induced chromosome doubling in *Platanusa cerifolia* and its effect on plant morphology. *Euphytica*, *157*, 145–154.

Ntuli, N. R., & Zobolo, A. M. (2008). Effect of water stress on growth of colchicine induced polyploid *Coccinia palmata* and *Lagenaria sphaerica* plants. *African Journal of Biotechnology*, *7,* 3648-3652.

Otto, S. P., & Whitton, J. (2000). Polyploid induce and evolution. *Annual Review of Genetics*, *34*, 401–437.

SAS. (1998). SAS/STAT User's guide, Version 6. Cary, NC: Author.

Surson, S. (2017). The morphological characterization of diploid and tetraploid tangerine (*Citrus reticulata* Blanco). *Agricultural Science Journal*, *48,* 100-107.

Surson, S. (2018a). Polyploid Induction in 'kram phak troung' indigo (*Indigofera tinctoria* L.). *Khon Kaen Agriculture Journal*, *46*, 439-448.

Surson, S. (2018b). Comparative of growth rate and morphology of 'Kram phak troung' (*Indigofera tentoria* L.), between diploid and tetraploid plant. *Khon Kaen Agriculture Journal*, *46,* 559-570.

Surson, S., Sitthapanit, S., & Wongma, N. (2015). In vivo induction of tetraploid in tangerine citrus plants (*Cirus reticulata* Blanco) with the use of colchicine. *Pakistan Journal of Biological Sciences*, *18,* 37-41.

Surson, S., Sitthapanit, S., & Wongma, N. (2018). An investigation on polyploidy induction and verification of Kram Ngo plant (*Indigofera suffruticosa*) for biomass production in Northeast Thailand. *Thai Journal Agricultural Science*, *51*, 32-42.

Wang, Z., Wang, M., Liu, L., & Meng, F. (2013). Physiological and proteomic responses of diploid and tetraploid black locust (*Robinia pseudoacacia* L.) subjected to salt stress. *International Journal of Molecular Sciences*, *14*, 20299-20325.