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Cytological and Morphological Responses of Strawberry (*Fragaria spp.*) to Polyploidization

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ABSTRACT

Strawberry (*Fragaria spp.*) is broadly planted for its fruit, fragrance, color, taste, and sweetness. Some people are concerned about transgenic plants and their products. Furthermore, genetic diversity is one of the basics of plant breeding programs. Polyploidy induction is one of the solutions to create genetic diversity and to reduce the concerns about transgenic plants. This experiment was conducted in a randomized complete block design to induce polyploidy in strawberry (*Fragaria spp*. cv. Kurdistan) using different concentrations of colchicine. We aimed to examine the possibility of polyploidy induction, to determine the best concentration, and to evaluate strawberry responses to polyploidy. The tetraploidy percentage in the plants treated by 0.7% colchicine was more than 80%. Tetraploid plants had a greater fruit length and diameter, fresh fruit weight, and larger fruit volume than the controls. Such plants had a lower stomata number with a larger size and a larger chloroplast number than the diploids. Ultimately, considering the flow cytometry results, and the number of modified plants, the best concentration was 0.7% colchicine treatment, which could be used as a suitable concentration for ploidy induction in strawberries.

Keywords: Polyploidy induction, Tetraploidy, Flow cytometry, Genetic diversity, Colchicine

INTRODUCTION

Strawberry (*Fragaria spp.*) is broadly planted for its fruit, trademark fragrance, color, taste, and sweetness (Jamali *et al*., 2011; Sharma *et al*., 2014), and it is consumed in different forms (El-Denary *et al*., 2016). The most common cultivated variety in Kurdistan Province, Iran, is known as the Kurdistan (Mohammadi and Hanafi, 2014); a highly reproductive and short-day strawberry plant, resistant to pests and diseases, and appropriate as perennial crops. This variety has small fruits and low permanency, but high quality in taste and aroma (Mohammadi and Hanafi, 2014).

Strawberry plants are usually propagated by runners (asexual reproduction), and it requires new replanting every year to enhance yields and allow normal density plantings (El-Denary *et al*., 2016). The vegetative or asexual reproduction of the plants is not an evolutionary advantage, and it limits the genetic changes. Such a situation could lead plants to accumulate deleterious mutations (Scarcelli *et al*., 2006) and finally might lead to a reduction in crop production (Crutsinger *et al*., 2008).

Whether or not these concerns are justified, some people around the world today are concerned about transgenic plants and their products. In addition, genetic diversity in plants is one of the basics of plant breeding programs. Using mutations

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and polyploidy induction to create the required genetic diversity and to reduce the concerns of the people about transgenic plants is one of the suggested solutions. High rates of chromosome mutations provide new sources of variation, which could be desirable in modern breeding techniques (Comai, 2005). Polyploidy offers a mechanism for adaptation and enables plants to be improved genetically (Lavania, 2005, Chen *et al*., 2007). About 50-70% of angiosperms have undergone polyploidy during their evolutionary process (Comai, 2005, Chen *et al*., 2007). Artificial polyploidy inducement is routine in plant breeding, for which colchicine is used for crop plants, frequently (Compton *et al*., 1996). Recent reports indicate the importance of mutation in plants and its superiority as an alternative to transgenic plant techniques (Razmi *et al*., 2019). Artificial polyploidy is accompanied by an increase in the size of different organs of plants, mostly due to increased cell size (Lavania, 2005). Specific changes, such as genetic composition, physiological mechanisms, structural composition, and vigor, might arise in the polyploidy process (Dhawan and Lavania, 1996, Acquaah, 2007). Hybrid vigor is one of the exploited advantages of polyploidy in plant breeding (Dhawan and Lavania, 1996). Seedless fruits, bridge crossing (Comai, 2005), ornamental and forage breeding (Levi *et al*., 2002, Acquaah, 2007), production of apomictic crops (Dhawan and Lavania, 1996, Levi *et al*., 2002), disease resistance (Acquaah, 2007, Zahirnejad *et al*., 2018), drought resistance (Ghaderi *et al*., 2015), industrial applications and increase in primary and secondary metabolites (Dhawan and Lavania, 1996, Levi *et al*., 2002), and the production of bio-insecticides (Liu and Gao, 2007) are the most common applications of polyploidy in plants.

This study was performed to investigate the possibility of tetraploid induction in the strawberry plants using different concentrations of colchicine, and to determine the best concentration for this purpose. Another aim of this experiment was to evaluate the morphological and the genetic material response of the strawberry plants to polyploidy.

MATERIALS AND METHODS

Plant Materials and Experimental Design

The experiment was conducted to induce polyploidy in strawberry (*Fragaria spp.*) Kurdistan variety; which is a diploid variety (2n= 2x= 14). Treatments included the check (zero colchicine), 0.7, 0.9, and 1.1% colchicine concentrations with five replications in a Randomized Complete Block Design (RCBD). Transplants; reproduced from a single clone, were cultured in pots filled with a 1:1 ratio of cocopeat: perlite medium. After plant establishment (ten days after transplanting) and confidence in the detection of central buds, chemical treatments were injected at the tips of plants. The apical meristems were also inoculated with cotton pellet dipped by colchicine treatments. The treatments were repeated every 24 hours for 72 hours (Nilanthi *et al*., 2009; Omidbaigi *et al*., 2010).

Morphological Measurements

Before the first flowering, the number of full leaves, the leaf area of each plant (using the AM200 Leaf Area Meter), and the leaf chlorophyll index [using the chlorophyll meter (SPAD-502 Readings Minolta, Japan] on young plant leaves were measured. The length and diameter of the fruit were measured using a digital calliper, and the shape of the fruit was calculated as the ratio of length to diameter of the fruits. The Brix (grams of sugar per 100 grams of fruit extract), or the TSS (total soluble solids) was determined utilizing the ATAGO (E1 model, Japan) refractometer instrument.

The titration method was used to measure the Total Acid (TA) of the fruits. Fruit juice samples were extracted and titrated using phenolphthalein as an indicator and 0.1N NaOH, till the colour changed to pink. The total acid content in terms of citric acid was obtained through the following formula:

A= (SNFE)/C

Where, A is the quantity of Acid in fruit extract (g mL^{-1}), S the quantity of the consumed NaOH (mL), N= 0.1 (Normality of NaOH), F is NaOH Factor, E is Equivalent of the preferred acid (citric acid), and C is quantity of fruit extract (mL).

Anthocyanin concentration was determined by Wagner (1979) method. A sample of 0.1 g of fresh fruit tissue was thoroughly ground in a mortar containing five mL of acidic methanol. The resulting extract was transferred to the test tube, with tightly closed lid, and was placed in the dark at room temperature for 24 hours. Thereafter, the resulting extract was centrifuged at 4000g for 10 minutes. The supernatant was poured into the cuvette and its adsorption intensity was read at 550 nm using a spectrophotometer. To calculate the concentration of anthocyanin, an extinction coefficient of 133,000 $Mm^{-1}cm^{-1}$ was used. The equation A= εbc was used to calculate the anthocyanin concentration, where, A is the Absorption rate at 550 nm, ε is the extinction coefficient, b is the width of the cuvette (cm), and c is the concentration of anthocyanin (mol per gram of sample used).

Chloroplasts Analysis

Farmer's fixative solution (3:1 95% ethanol: glacial acetic acid) (Owen and Miller, 1993) was applied to view the chloroplast of stomata guard cells. After five days, when the leaves discoloured and were fully transparent, they were washed several times. Subsequently, they were put in distilled water for 30 minutes, and the leaves were dried with paper towels. For chloroplast staining, the leaves were placed in Lugol's solution for 30 minutes. Small pieces of the treated leaves were excised and washed with water to remove any additional colour. After that, the chloroplast of stomata guard cells was counted under a 40X magnification of a light microscope (Ghanavati and Eskandari, 2011).

Flow Cytometry Analysis

The number and size of stomata and chloroplast variations were also investigated. By viewing the visual changes, some samples from each treatment were evaluated using the flow cytometer instrument (Germany Partec Pa) equipped with an arc-UV lamp, using Gu *et al*. (2005) method. The young leaves were cut into squares of 5×5 mm, and stored in 1N HCl solution for 30 seconds to soften the leaf tissue partly. The leaf sections were crushed well, the suspension was poured in 1.5 mL microtubes, and was centrifuged for 10 minutes at 4°C in 10,000 rpm to sediment the nuclei. Afterward, the nucleus staining solution [DAPI (4′, 6-Diamidino-2-Phenylindole)] was added (1,200 µL) to the isolated nuclei in a new micro-centrifuge tube. After 30- 60 sec, they were used for evaluation by the flow cytometer instrument. For every sample, the device examined at least 5,000 nuclei, and the derived histogram peaks were analyzed using Mode Fit LT 3.1 software.

Analysis of Variance

The student t-test were used for data analysis of variance for the characteristics, and the differences of the individual plants were assessed using SPSS v.19.

RESULTS

Morphological Characteristics

The evaluated individual plants were significantly different in leaf area,

chlorophyll, stomata number, fruit length, fruit diameter, TSS (total soluble sugar), and anthocyanin based on the student t-test (Table 1). Out of 25 plants, 5 plants receiving 0.7% colchicine treatment were different ($P< 0.01$) from the control in fruit volume and fresh weight (Table 2). Four plants represented larger fruit diameter and length, and five plants showed a lower stomata number than the control treatment. Fifty-one of the plants treated by the 0.7% colchicine concentration were modified (Table 2).

According to the t-test, the mutants derived from the treatment with 0.9% colchicine were different in leaf area, fruit length and diameter ($P< 0.05$), number of stomata, TSS, and anthocyanin ($P< 0.01$), compared with the control treatment (Table 1). Individual plants receiving 0.9% colchicine treatment showed a significant difference in the leaf area, stomata number, and different characteristics of fruit (Table 2). Thirty-three modified plants were derived from the plants treated with 0.9% colchicine concentration (Table 2), which was lower than the 51 plants treated with 0.7% colchicine concentration.

Table 1 demonstrates the t-test results between the control and the 1.1% concentration colchicine treatments. The mutants and the control plants were different ($P< 0.05$) in leaf area, the stomata number, TSS, acidity, and anthocyanin. In such a situation, 16 modified plants were derived in comparison with the control treatment.

The frequency of modified plants (mutants) due to 1.1% colchicine concentration was lower than that of the 0.7 and 0.9% colchicine concentration treatments (Table 2).

Ploidy Level Confirmation by Flow Cytometry Analysis, Stomata Guard Cell Size, and Chloroplasts Number

As shown in Figure 1, the peaks of histogram resulting from the assessment and validation of ploidy occurrence using flow cytometry in the G1 stage of the cell division cycle show the amount of hereditary material, which is relative to the number of investigated cells, and their relative position confirms the ploidy level. Changes in stomata size are also represented in Figures 1, 2, 3, and 4.

Figure 1 is related to the control plants with 100% diploidy. The G1 peak displays channel 75.48, and the G2 peak was placed at channel 150.59, which is twice the G1, representing the nuclei with synthesized DNA. The stomata size in diploid leaves and the density of the chloroplasts in the diploid guard cells could be seen in Figure 1.

Figure 2 shows the ploidy situation of the plants treated with 0.7% colchicine concentrations. The percentage of tetraploidy in these plants was more than

Table 1. The results of t-test between the control and other the concentration treatments for the evaluated traits.

	Mean±Standard deviation			
Characters	Control	0.07 (%)	0.9 (%)	1.1 (%)
Leaf area (mm^2)	5582 ± 25.05	9803 ± 53*	5198 ± 31.38*	3163 ± 10.15**
Chlorophyll	55.13 ± 2.1	56.90 ± 1.9*	55.37 ± 1.92	54 ± 1.7
Stomata number	23.46 ± 1.66	20.23 ± 1.73**	20.7 ± 2.30**	20.6 ± 2.1**
Fruit length (cm)	2.23 ± 0.45	2.91 ± 0.94*	2.97 ± 0.93*	2.15 ± 0.44
Fruit diameter (cm)	1.66 ± 0.38	2.17 ± 0.66*	2.19 ± 0.60*	1.66 ± 0.29
Fruit fresh weight (g)	6.16 ± 1.76	9.93 ± 6.89	9.62 ± 7.17	4.82 ± 2.19
Dry weight (g)	0.625 ± 0.126	0.989 ± 0.719	1.063 ± 0.873	0.557 ± 0.268
Fruit volume (mL)	6.27 ± 1.75	10.04 ± 6.75	10.10 ± 7.42	4.88 ± 2.24
Special weight	0.981 ± 0.030	0.975 ± 0.053	0.948 ± 0.051	1.01 ± 0.065
TSS	6.96 ± 2.47	9.59 ± 2.32*	10 ± 2.02**	8.97 ± 1.07*
Acidity	3.45 ± 0.84	3.92 ± 1.11	4.68 ± 2.61	5.0 ± 1.97*
Anthocyanin (mol g^{-1})	0.056 ± 0.023	0.091 ± 0.026	0.08 ± 0.01**	0.092 ± 0.021**

* and **: Mean significantly difference at 5, and 1% probability levels, respectively.

Table 2. Number of different plants from the controls based on the t-test between the control and individual plants receiving colchicine treatment.

Colchicine (%)	Number of modified plants for each characteristic														
	Leaf area	Chlorophyll	Stomata number	Fruit length	Fruit diameter	Fruit fresh weight	Fruit dry weight	Fruit volume	Special weight	Juice	Acidity	Anthocyanin	TSS	Taste	Total number
0.7	8	2	5	4	4	5	8	5	1	4	2	3	-	-	51
0.9	1	-	2	3	3	3	8	3	2	3	3	-	1	1	33
1.1	-	-	3	-	-	-	5	-	2	-	5	-	-	1	16

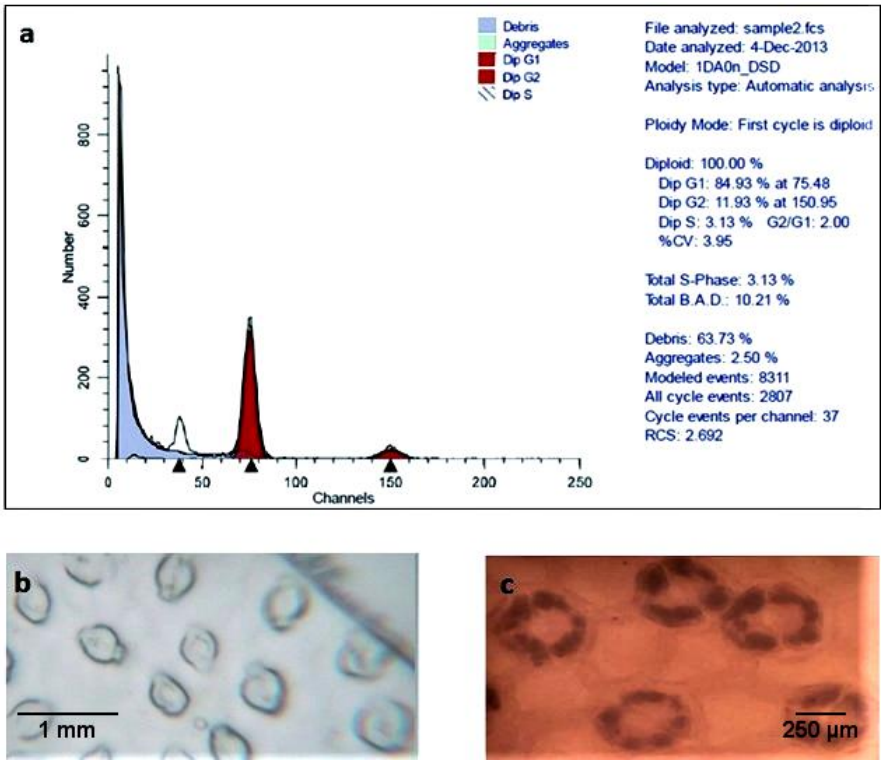

Figure 1. Diploid plants (control treatment) properties: (a) Flow cytometry histogram, (b) The stomata size in a microscope view field with 20X magnification, and (c) The chloroplast situation (number or density) with a 40X magnification.

80%, according to the flow cytometry analysis. The G1 peak in tetraploid cells showed channel 66.48, which was almost twice the G1 peak in diploid cells with channel 33.39. The aneuploidy rate in this plant was 0.01%, a trivial rate. These plants had a greater extent of fruit length and diameter, fresh fruit weight, and fruit volume than the control. These plants had a lower number of stomata, but the guard cell size and the number of chloroplasts remarkably increased compared to the diploid plants (Figure 2).

Figure 3 is related to the plants with 0.9% colchicine concentration treatment, which showed an approximately 46% tetraploidy level. Regarding the plants with 0.9% treatment, the level of tetraploidy was up to about 64%. The G1 Peak associated with the diploidy state represented channel 38.39, and the G1 peak related to the tetraploidy level showed channel 73.87. Aneuploidy in

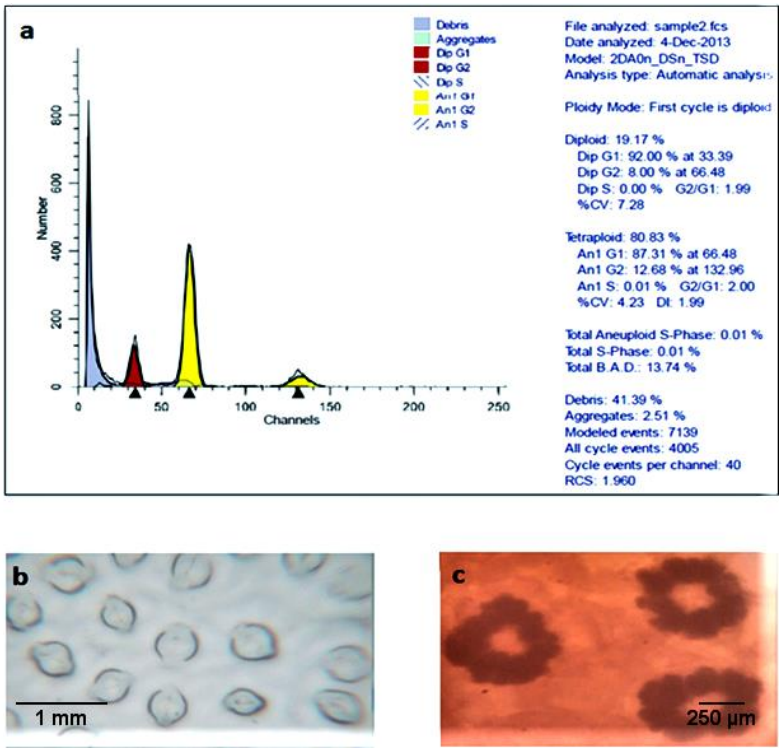

Figure 2. Plants properties treated with 0.7% colchicine concentration: (a) Flow cytometry histogram, (b) Stomata size in a microscope view field with 20X magnification, and (c) Chloroplast situation (number or density) with a 40X magnification.

these plants was about 5%. These plants had a significantly higher fruit length and diameter, fruit fresh-weight, and volume than the control. Still, the number of stomata decreased considerably in the plants receiving 0.9% treatment compared to the control. Furthermore, it could be seen that the plants treated with the 0.9% colchicine concentration showed an increased stomata size and higher density of the chloroplasts of guard cells compared to the control treatment.

The plants of 1.1% colchicine treatment approximately showed half-and-half diploid and tetraploid cells (Figure 4). The G1 peak of the diploid and tetraploid cells was on channels 41.3 and 80.3, respectively, and the aneuploidy rate was about 1- 5%. These plants had statistically no difference with the control plants, though they showed a slight increase compared to the control in terms of the stomata size and the chloroplast number of the guard cells.

DISCUSSION

The polyploid plants are usually detected based on the increase in morphological characteristics such as leaf, flower, fruit size, plant height, fruit fresh and dry weight, and yield. An increase in fruit size has been reported in tetraploid tomato and red pepper (Ogawa *et al*., 2012). Todorov and Dimitrov (1974) produced greater stomata cells and more chloroplast in stomata guard cells by tetraploidy induction. Tetraploid cells were larger in size than the diploid ones, and the increase in cell size was one of the most rapid effects of polyploidy. Given an increase in ploidy levels, a reduction in the stomata cell number and an increase in their cell size at the same time has been reported (Omidbaigi *et al*., 2010). In general, the

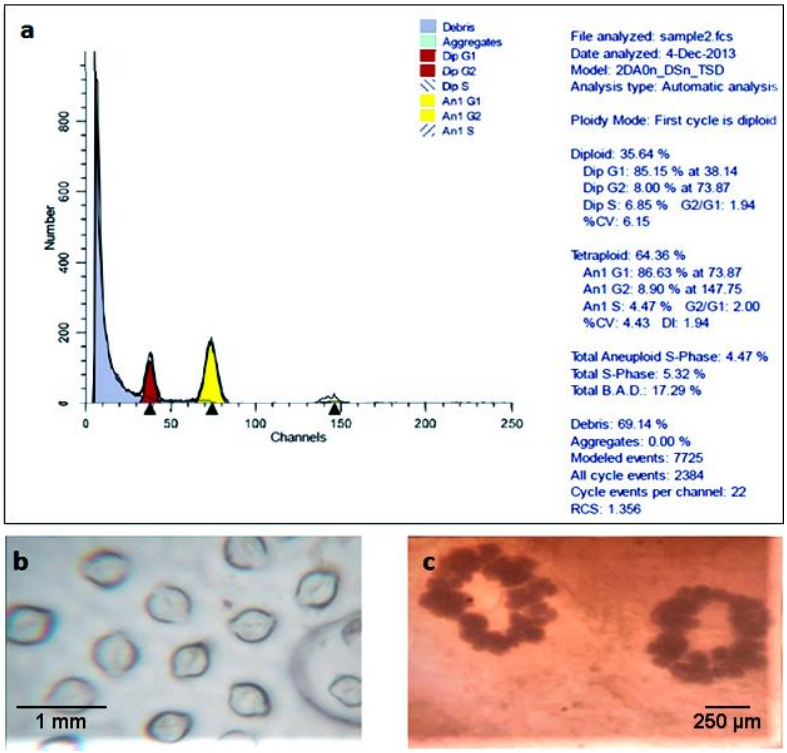

Figure 3. Plants properties treated with 0.9% colchicine concentration: (a) Flow cytometry histogram, (b) Stomata size in a microscope view field with 20X magnification, and (c) Chloroplast situation (number or density) with a 40X magnification.

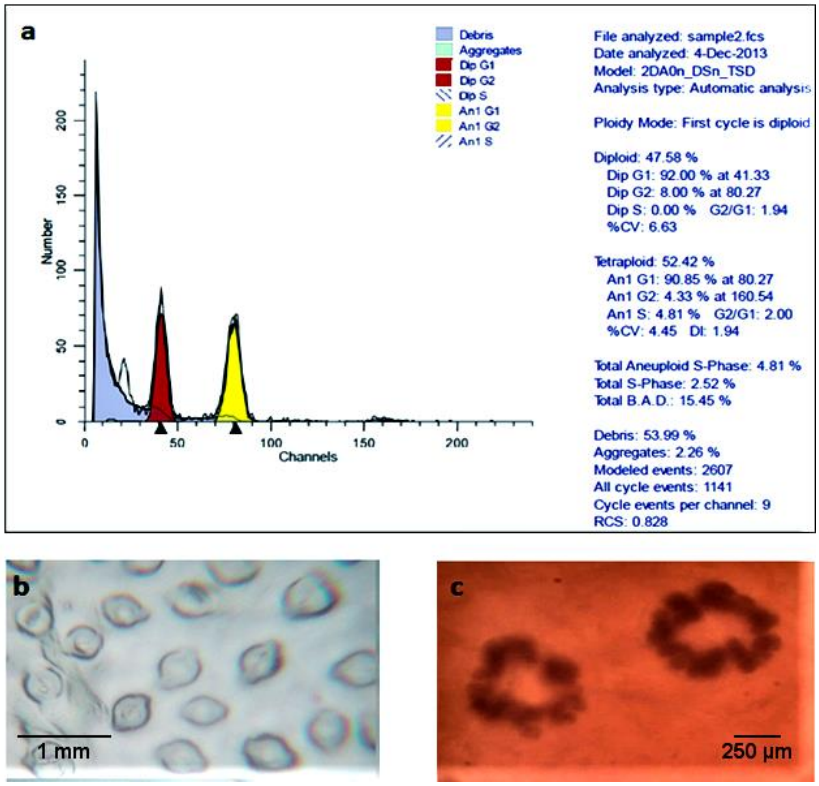

Figure 4. Plants properties treated with 1.1% colchicine concentration: (a) Flow cytometry histogram, (b) Stomata size in a microscope view field with 20X magnification, and (c) Chloroplast situation (number or density) with a 40X magnification.

stomata guard cell size is a highly suitable factor for distinguishing the tetraploid plants from diploids (Gao *et al*., 2002). Sun *et al*. (2009) created seedlings with higher ploidy levels, more weight, and larger stomata than diploid plants, using a 0.4% colchicine solution on "Fertility" pear variety. Tulay and Unal (2010) located seeds in a 0.005% solution of colchicine to induce polyploidy and achieved 12% tetraploid mungbean with lower flowers but significantly larger seeds than the diploid plants. Iyer and Randhawa (1965) used autotetraploidy in grapevine breeding for the first time, using 0.25 to 0.5% concentrations of colchicine. Kuliev (1991) created tetraploid grapevine using colchicine with 0.1 to 0.5% concentrations on the "Malakhati" variety, in which tetraploid grapes produced larger berries compared to the diploids.

However, it is not a highly confident factor, particularly in identifying chimera samples from pure tetraploids. In our study, the assessment of the stomata cell size confirmed the changes in the size of some stomata cells. It was similar to the results reported by Thao *et al*. (2003) on ornamental Alocasia (*Arctium* spp.), Omidbaigi *et al*. (2010) on dragonhead (*Dracocephalum moldavica* L.) plant, and Gao *et al*. (2002) on peppermint.

The number of chloroplasts of the stomata guard cells increased, which was in agreement with the results reported by Cardi *et al*. (1992) on potato and Fassuliotis and Nelson (1992) on melon. The central region of the meristem includes shoot dividing cells, and it is comprised of several layers of tissue. Since the central section is responsible for the production of terminal cells of the tissue, duplication of the chromosomes in this region leads to the production of terminal polyploid tissues (Borgheei *et al*., 2010).

The tetraploid cells created due to colchicine absorption by different cells (Tambong *et al*., 1998) prevail in mixoploid plants. All the results derived from the flow cytometry in this study revealed the existence of mixoploidy in the plants, similar to the results reported by Tambong *et al*. (1998), Ascough *et al*. (2007), Nilanthi *et al*. (2009), and Borgheei *et al*. (2010).

CONCLUSIONS

In general, the size of stomata guard cells is a highly appropriate factor for distinguishing the tetraploid plants from diploids. Still, it is not a highly confident factor, particularly in identifying chimera samples from the pure tetraploids. The percentage of tetraploidy in the plants treated with 0.7% colchicine concentration was more than 80%, which was more than the tetraploidy (about 46%) in the plants treated with 0.9% colchicine concentration. Alternatively, the plants′ cells that received 1.1% colchicine treatment had statistically no difference with the control plants. Tetraploid plants had a greater extent of fruit length and diameter, fresh fruit weight, and fruit volume compared to the control, and these are the most critical objectives for plant breeders. Such plants had a lower stomata number, but the guard cell size and their chloroplast number increased remarkably compared to the diploid ones. One of the critical points in using chemical compounds to induce mutations in plants is to determine the concentration of the chemical compound used in such a way that with the highest percentage of modifications, it also has the lowest mortality in the plant. Ultimately, based on the flow cytometry results and the number of modified plants, the best concentration of colchicine for ploidy induction in strawberry was the 0.7% treatment among the plants treated with 0.7, 0.9, and 1.1% concentrations. Thus, it could be used as a suitable concentration for ploidy induction in strawberries.

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REFERENCES

1. Acquaah, G. 2007. *Principles of Plant Genetics and Breeding*. Wiley-Blackwell, Malden, UK.
2. Ascough, G., Erwin, J. and van Staden, J. 2007. *In Vitro* Propagation of Four Watsonia Species. *Plant Cell Tiss. Org.*, **88**: 135-145.
3. Borgheei, S. F., Sarikhani, H., Chaichi, M. and Kashi, A. 2010. *In Vitro* Induction of Polyploidy in Lemon Balm (*melissa officinalis* l.). *Iran. J. Med. Aromat. Plants*, **26(3)**: 283-295.
4. Cardi, T., Carputo, D. and Frusciante, L. 1992. *In Vitro* Shoot Regeneration and Chromosome Doubling in 2x and 3x Potato Clones. *Am. Potato J.*, **69**: 1-12.
5. Chen, L., Lou, Q., Zhuang, Y., Chen, J., Zhang, X. and Wolukau, J. N. 2007. Cytological Diploidization and Rapid Genome Changes of the Newly Synthesized Allotetraploids Cucumis× Hytivus. *Planta*, **225**: 603-614.
6. Comai, L. 2005. The Advantages and Disadvantages of Being Polyploid. *Nat. Rev. Genet.*, **6**: 836.
7. Compton, M. E., Gray, D. and Elmstrom, G. 1996. Identification of Tetraploid Regenerants from Cotyledons of Diploid Watermelon Cultured in Vitro. *Euphytica*, **87**: 165-172.
8. Crutsinger, G. M., Reynolds, W. N., Classen, A. T. and Sanders, N. J. 2008. Disparate Effects of Plant Genotypic Diversity on Foliage and Litter Arthropod Communities. *Oecologia*, **158**: 65-75.
9. Dhawan, O. and Lavania, U. 1996. Enhancing the Productivity of Secondary Metabolites via Induced Polyploidy: A Review. *Euphytica*, **87**: 81-89.
10. El-Denary, M., Ali, R. A. M. and Gomaa, S. 2016. Morphological Markers for Early Selection in Strawberry Breeding Programs. *Alex. Sci. Exch. J.*, **37(2)**: 102-115.
11. Fassuliotis, G. and Nelson, B. 1992. Regeneration of Tetraploid Muskmelons from Cotyledons and Their Morphological Differences from Two Diploid Muskmelon Genotypes. *J. Am. Soc. Hortic. Sci.*, **117**: 863-866.
12. Gao, S., Chen, B. and Zhu, D. 2002. *In Vitro* Production and Identification of Autotetraploids of *Scutellaria baicalensis*. *Plant Cell Tiss. Org.*, **70**: 289-293.
13. Ghaderi, N., Normohammadi, S. and Javadi, T. 2015. Morpho-Physiological Responses of Strawberry (Fragaria×ananassa) to Exogenous Salicylic Acid Application under Drought Stress. *J. Agr. Sci. Tech.*, **17**: 167-178.
14. Ghanavati, F. and Eskandari, H. 2011. Relationship between the Chloroplast Number in Stomatal Guard Cells, Flow Cytometry and Ploidy Level in Onobrychis spp. *Seed Plant Improv. J.*, **27-1(3)**: 427-439.
15. Gu, X., Yang, A., Meng, H. and Zhang, J. 2005. *In Vitro* Induction of Tetraploid Plants from Diploid *Zizyphus jujuba* Mill. cv. Zhanhua. *Plant Cell Rep.*, **24**: 671-676.
16. Iyer, C. and Randhawa, G. 1965. Increasing Colchicine Effectiveness in Woody Plants with Special Reference to Fruit Crops. *Euphytica*, **14**: 293-295.
17. Jamali, B., Eshghi, S. and Tafazoli, E. 2011. Vegetative and Reproductive Growth of Strawberry Plants cv. 'Pajaro' Affected by Salicylic Acid and Nickel *J. Agr. Sci. Tech.*, **13**: 895-904.
18. Kuliev, V. 1991. The Use of Induced Tetraploid Forms in Grape Breeding. *THIK*, **2: 150-151**.
19. Lavania, U. 2005. Genomic and Ploidy Manipulation for Enhanced Production of Phyto-Pharmaceuticals. *Plant Genet. Resour.*, **3**: 170-177.
20. Levi, A., Thomas, C., Joobeur, T., Zhang, X. and Davis, A. 2002. A Genetic Linkage Map for Watermelon Derived from a Testcross Population: (*Citrullus lanatus* var. citroides×*C. lanatus* var. lanatus)×*Citrullus colocynthis*. *Theor. Appl. Genet.*, **105**: 555-563.
21. Liu, Z. and Gao, S. 2007. Micropropagation and Induction of Autotetraploid Plants of *Chrysanthemum cinerariifolium* (Trev.) Vis. *In Vitro Cell. Dev. Biol. Plant*, **43**: 404-408.
22. Mohammadi, H. and Hanafi, Q. 2014. Effect of Different Atmospheres on Quality Changes of Kurdistan Strawberry. *J. Food Chem. Nut.*, **2**: 61-69.
23. Nilanthi, D., Chen, X. -L., Zhao, F. -C., Yang, Y. -S. and Wu, H. 2009. Induction of Tetraploids from Petiole Explants through Colchicine Treatments in *Echinacea purpurea* L. *Biomed. Res. Int.*, 2009.
24. Ogawa, D., Ishikawa, K. and Mii, M. 2012. Difference in the Polysomaty Degree during Fruit Development among Plants with Different Ploidy Levels Produced by

Artificial Chromosome Doubling of a Pepper (*Capsicum annuum*) Cultivar 'Shishitou No. 562'. *Sci. Hortic.*, **134**: 121-126.
25. Omidbaigi, R., Yavari, S., Hassani, M. E. and Yavari, S. 2010. Induction of Autotetraploidy in Dragonhead (*Dracocephalum moldavica* L.) by Colchicine Treatment. *J. Fruit Ornam. Plant Res.*, **18**: 23-35.
26. Owen, H. R. and Miller, A. R. 1993. A Comparison of Staining Techniques for Somatic Chromosomes of Strawberry. *Hortsci.*, **28**: 155-156.
27. Razmi, H., Amiri Fahliani, R., kavoosi, B. and Masoumi Asl, A. 2019. Mutation Induction and Creation of Variation in Strawberry, Kurdistan Cultivar, by Gamma Irradiation and Determination of Suitable Irradiation Dosage. *J. Sci. Techn. Greenhouse Cult.*, **10**: 25-40.
28. Scarcelli, N., Tostain, S., Vigouroux, Y., Agbangla, C., Daïnou, O. and Pham, J. L. 2006. Farmers' Use of Wild Relative and Sexual Reproduction in a Vegetatively Propagated Crop. The Case of Yam in Benin. *Mol. Ecol.*, **15**: 2421-2431.
29. Sharma, G., Yadav, A. and Thakur, M. 2014. Studies on Growth and Flowering Attributes of Different Strawberry Cultivars (*Fragaria x ananassa* Duch.) in Himachal Pradesh. *Asian J. Adv. Basic Sci.*, **3**: 1-4.
30. Sun, Q., Sun, H., Li, L. and Bell, R. L. 2009. *In Vitro* Colchicine-Induced Polyploid Plantlet Production and Regeneration from Leaf Explants of the Diploid Pear (*Pyrus communis* L.) Cultivar,'Fertility'. *J. Hortic. Sci. Biotechnol.*, **84**: 548-552.
31. Tambong, J., Sapra, V. and Garton, S. 1998. *In Vitro* Induction of Tetraploids in Colchicine-Treated Cocoyam Plantlets. *Euphytica*, **104**: 191-197.
32. Thao, N. T. P., Ureshino, K., Miyajima, I., Ozaki, Y. and Okubo, H. 2003. Induction of Tetraploids in Ornamental Alocasia through Colchicine and Oryzalin Treatments. *Plant Cell Tiss. Org.*, **72**: 19-25.
33. Todorov, I. and Dimitrov, B. 1974. Study on Some Indirect Methods for Determination of Ploidy Level in the Grape Variety Bolgar (*Vitis vinifera* L.). *Genet. Plant Breed*., 7(3): 218-225.
34. Tulay, E. and Unal, M. 2010. Production of Colchicine Induced Tetraploids in *Vicia villosa* Roth. *Caryologia*, **63**: 292-303.
35. Wagner, G. J. 1979. Content and Vacuole/Extravacuole Distribution of Neutral Sugars, Free Amino Acids, and Anthocyanin in Protoplasts. *Plant Physiol.*, **64**: 88-93.
36. Zahirnejad, B., Bahramnejad, B. and Rostamzadeh, J. 2018. Isolation and Expression Analysis of a Defensin Gene from Strawberry (Fragaria×ananassa cv. Paros). *J. Agr. Sci. Tech.*, **20**: 1243-1257.

پاسخ‌های سیتولوژیکی و ریخت شناسی توت فرنگی (*Fragaria* spp.) به پلی‌پلوئیدی

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چکیده

توت فرنگی (*Fragaria* spp.) به خاطر میوه، عطر، رنگ، طعم و شیرینی آن به طور گسترده کاشته می‌شود. برخی از مردم نگران گیاهان تراریخته و محصولات آنها هستند. بعلاوه، تنوع ژنتیکی یکی از اصول برنامه‌های به‌نژادی گیاهی است. القاء پلی‌پلوئیدی یکی از راه حل‌های ایجاد تنوع ژنتیکی و کاهش نگرانی‌ها در مورد گیاهان تراریخته است. این آزمایش برای ایجاد پلی‌پلوئیدی در توت‌فرنگی

(*Fragaria spp*. cv. Kurdistan) رقم کردستان با استفاده از غلظت‌های مختلف کلشی‌سین در قالب طرح بلوک‌های کامل تصادفی انجام شد. این تحقیق در گیاهان توت‌فرنگی برای بررسی امکان ایجاد پلی‌پلوییدی، ارزیابی پاسخ آنها به پلی‌پلوئیدی، و تعیین بهترین غلظت کلشی‌سین انجام شد. درصد تتراپلوئیدی در گیاهان تیمار شده با 0/7٪ کلشی‌سین بیش از 80٪ بود. طول و قطر میوه، وزن میوه تازه و حجم میوه گیاهان تتراپلوئید بیشتر از گروه شاهد (بدون کلشی‌سین) بود. چنین گیاهانی تعداد روزنه‌های کمتر، اما اندازه بزرگتر و تعداد کلروپلاست بیشتری نسبت به دیپلوئیدها داشتند. در نهایت، با توجه به نتایج فلوسیتومتری و تعداد گیاهان تغییریافته، بهترین تیمار کلشی‌سین غلظت 0/7٪ بود. می‌توان از آن به عنوان غلظت مناسب برای القای پلوئیدی در توت‌فرنگی استفاده کرد.