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Colchicine-induced polyploidy and morphological changes in wild *Silene compacta* Fischer: Potential as an ornamental plant in Türkiye

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Abstract

This study aimed to induce polyploidy in *Silene compacta* Fischer by applying varying colchicine doses and soaking durations while evaluating resultant changes in plant growth and morphology. *Silene compacta* seeds growing naturally in Erzurum and its surroundings were treated with different colchicine doses (0.01%, 0.05%, 0.1%, 0.2% and 0.4%) and soaking durations (6, 12, 24, 48 and 72 hours). In addition, two different application methods (dripping on the shoot tip of the seedlings and application to the root tip meristem regions) were also tested. The control group received only pure water. As a result of our study, the toxic effect of colchicine at low doses (0.01% and 0.05%) in root application was not lethal and the plants survived 100%. However, higher doses and prolonged applications (e.g. 0.2% and 0.4%) led to plant death. Application to the shoot tip was more toxic, especially high doses (e.g. 0.2% and 0.4%) caused plant death. Colchicine treatments affected stomatal number and stomatal size. In root applications, stomatal width increased at 0.01%, 0.05% and 0.1% doses, but decreased at higher doses. In shoot tip treatments, the highest stomatal number was determined at 0.01% dose. Stomatal length decreased with increasing dose and this decrease was significant compared to the control group. The optimal soaking duration for the increasing stomatal length was determined as 12 hours for both root and shoot tip applications. As a result, it was found that the *S. compacta* plants kept at 0.1% colchicine dose for 12 hours in both treatment methods were likely to be tetraploid. Also, it was predicted that various *S. compacta* cultivation materials with improved properties that can be used as main materials in future breeding programs can be developed.

Keywords: *Silene compacta*, Colchicine, Polyploidy

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1. Introduction

The native flora of Türkiye is relatively rich in the genus Silene. *Silene compacta* Fischer, widely distributed in Türkiye, shows significant potential as an ornamental plant, due to its striking pink flowers. In this context, it is important to improve the characteristics of local plant species without disturbing the adaptation of traditional varieties to the growing area. For the cultivation of ornamental plants, especially species for which little breeding has been done, various biotechnological interventions such as polyploidization, haploids, mutation breeding and in vitro soma clonal variations can accelerate reproduction and selection of new mutants. Among all these methods, mutation breeding and polyploidy are used to develop new varieties and obtain different genetic variations in plants.

To accelerate the development of new varieties and achieve agronomic traits unattainable through conventional breeding, non-traditional approaches such as mutation and polyploidy are increasingly emphasized.

Polyploidization, chromosome folding, can be achieved using various chemicals. Colchicine is the most widely used chemical to induce polyploidy. The effects of colchicine have been tested in many studies to obtain tetraploid plants. It has been found that the stems of polyploid plants are thicker, the leaves are large and dark colored, the roots are strong and spread wider than diploids, and the flowers, pollen and seeds are larger than diploids (Motosugi et al., 2002; Sattler et al., 2016).

The mutation effect can be easily seen in ornamental plants in terms of changes in flower color, shape and size (Ari et al., 2015). Polyploids have been successfully bred in many ornamental plants in the last few decades (Sajjad et al., 2013). Polyploidy breeding is an effective method compared to mutation breeding and conventional hybridization, which is easy to use in a short time and increases germplasm availability (Niu et al., 2016). Polyploidy leads to intensification of flower color, increase in flower size and change in plant shape (Sajjad et al., 2013). Besides increasing the size of various vegetative parts in tetraploid plants, it can alter growth habits, sterility and sometimes increase cold hardiness (Dibyendu, 2010).

Colchicine not only helps to double chromosomes, but also causes mutation in plants. Colchicine is usually applied as an aqueous solution. It is recommended to make a fresh aqueous solution before application (Kumar & Rani, 2013). Colchicine concentrations for seed treatment usually range from 0.1% to 0.8%, but high doses cause malformation and reduce the production of tetraploid plants. Therefore, it is recommended to use colchicine in concentrations as low as possible (Pirkoohi et al., 2011). Colchicine is highly toxic to plants. Therefore, low doses with long exposure time are considered safe to reduce its toxic effect and increase the rate of polyploid production (Sajjad et al., 2013).

One of the simplest, easiest and most effective methods is to use a large number of seedlings with small and actively growing meristematic tissues, depending on the plant species. Seedlings can be dipped/soaked or apical meristems can be immersed in different concentrations of anti-mitotic agent solution at different exposure times or frequencies. Furthermore, a specific protocol is required for each plant species. Seneviratne et al. (2002) in African violet (*Saintpaulia ionantha*), leaf bases (stem part) were immersed in colchicine solution at concentrations of 0.025, 0.04, 0.04, 0.05, 0.05, 0.06, 0.1% and for 18, 23.5, 27, 43, 47 and 117 hours to obtain plants with different flowers. Morphologically different plant size, leaf shape and size, flower color, shape and size, and mutations attributed to polyploidy were reported in the new plants obtained at different doses and times. These polyploid plants can also be taken as a new variation or a genotype that can be used in future breeding programs for crop improvement. Due to the enormous importance of polyploidy, many economically important crops have been artificially induced, with the highest success reported in the ornamental plants industry. Chromosome doubling through colchicine has been achieved in many ornamental plants such as lily, salvia, phlox, gladiolus, petunia and marigold using different application methods (Manzoor et al., 2019). These studies, in which colchicine was applied on a large number of ornamental plants and successful results were obtained, were generally carried out in in-vitro environments. The research was carried out under greenhouse conditions. In addition, the method applied in the studies is generally applied to the seed and shoot tip meristem region. In this study, colchicine was applied to the shoot tip meristem region and root tip meristem region and the effects of these two application regions were also revealed. In the literature review, no study on the use of colchicine to obtain polyploid plants of our local plant species *S. compacta* was found.

This study was carried out with the aim of obtaining polyploid plants and selecting the best quality plants with the most suitable characteristics and realizing their propagation in order to bring this species, which has a very high potential to be an ornamental plant, into the sector and to be more demanded in terms of ornamental plant properties. Determination of the most appropriate dose and soaking duartion for this species is the most important goal of the research.

2. Material and Methods

The research was carried out in the research greenhouse, in the Atatürk University Faculty of Agriculture (in Türkiye). The latitude of the research site is 39°53′57,4″N and longitude is 41°14′14,8″ E. The height of the research area is 1890 m above sea level.

In the study, the seeds of *Silene compacta* Fischer grown naturally in Erzurum (Türkiye) and its surroundings were collected from the determined locations during the seed ripening period and were used as plant material (Figure 1).

The peat used as growing medium in the experiment was obtained with a grain diameter of 0-40 mm, pH value (CaCl2) approximately 5.2-6.0, porosity weight 95-99%, volume weight (dry state) <55-90 g/l and organic matter content (dry state) 95-99%. A mixture of garden soil, sand and peat was prepared and filled into pots. The seeds were cold-dressed until the seedling stage to stimulate germination and grown in vials. Colchicine treatments were applied to the plants and was transplanted into pots after the seedling stage. Plants in the control group were treated with pure water.

The stratification treatments were carried out by placing the seeds on moistened blotting papers (towel napkins) in the refrigerator conditions set to +4 °C for 64 days. The moisture content of the blotters and the germination of the seeds were checked at regular intervals. In cases where humidification was necessary, the lost moisture was added by spraying water (Figure 2).

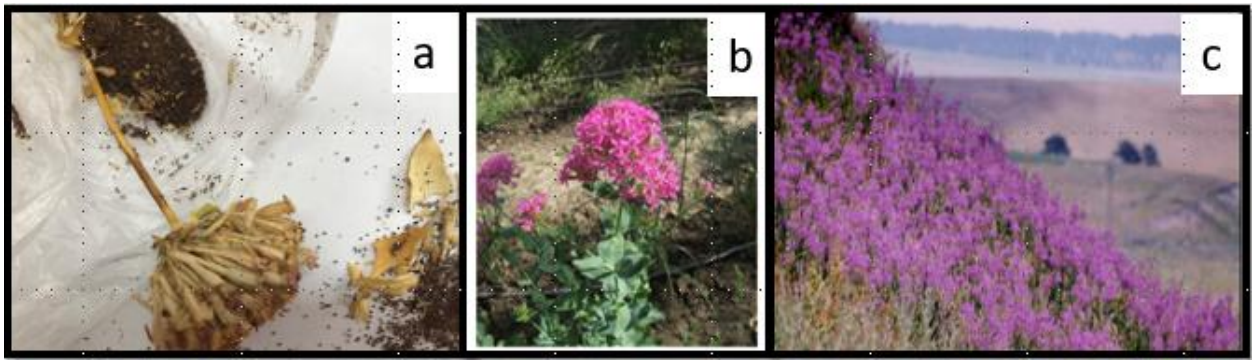

Figure 1. (a) View of *S. compacta* Fischer seeds (August) at the mature stage and seeds (Original); (b) Natural appearance of *S. compacta* Fischer (Draghia et al., 2013); (c) (Özer et al., 2009)

Figure 2. Seeds placed on moistened blotting papers (towel napkins) (a,b), rolled (c), placed in transparent bags, left open for ventilation (d) and folded

The doubled seeds were removed and cleaned in tap water and then transplanted in greenhouse conditions into peat-containing viols (70 wells). By following irrigation procedures regularly, these germinated plantlets were grown in viols (seedling growing container) and brought to seedling size (Figure 3).

Colchicine was dissolved in 1% Dimethyl sulfoxide (DMSO) (Yang et al., 2006). Five different (0.01%, 0.05%, 0.05%, 0.1%, 0.1%, 0.2% and 0.4%) colchicine doses were applied to the shoot tip meristem region of the seedlings by placing cotton wool impregnated with colchicine for five different times (6, 12, 24, 48 and 72 hours) and covered with aluminum foil. In these treatments, the cotton impregnated with colchicine was changed daily. The root tip meristem region treatment was done by immersing in solutions prepared with the same dose and soaking durations. Control plants were treated with pure water only (Ma et al., 2014; Sattler et al., 2016). After 6, 12, 24, 48 and 72 hours, the plants of the treatment groups were transplanted into pots filled with growing medium prepared with a mixture of garden soil, sand and peat in equal proportions. The treatments were arranged according to the randomized plots experimental plan with 3 replicates and 10 seedlings in each replicate (Figure 4).

The experiment was completed 100 days after the application of colchicine and the following observations and measurements were made:

Plant height (cm): Plant height of all plants grown after colchicine applications to the growing tip and root tip of the seedlings from the soil to the longest shoot tip of the plant was determined in cm with the help of a ruler.

Plant stem diameter (mm): The stem diameters of all plants grown after colchicine applications to the growing tip and root tip of the seedlings, 5-6 cm above the root collar, were determined in mm with the help of a digital caliper.

Number of leaves (number/plant): The number of leaves per plant was determined by counting the number of leaves of all plants grown after the application of colchicine to the growing tip and root tip of the seedlings.

Number of branches (number/plant): The number of branches per plant was determined by counting the number of branches of all plants grown after colchicine applications to the growth tip and root tip of the seedlings.

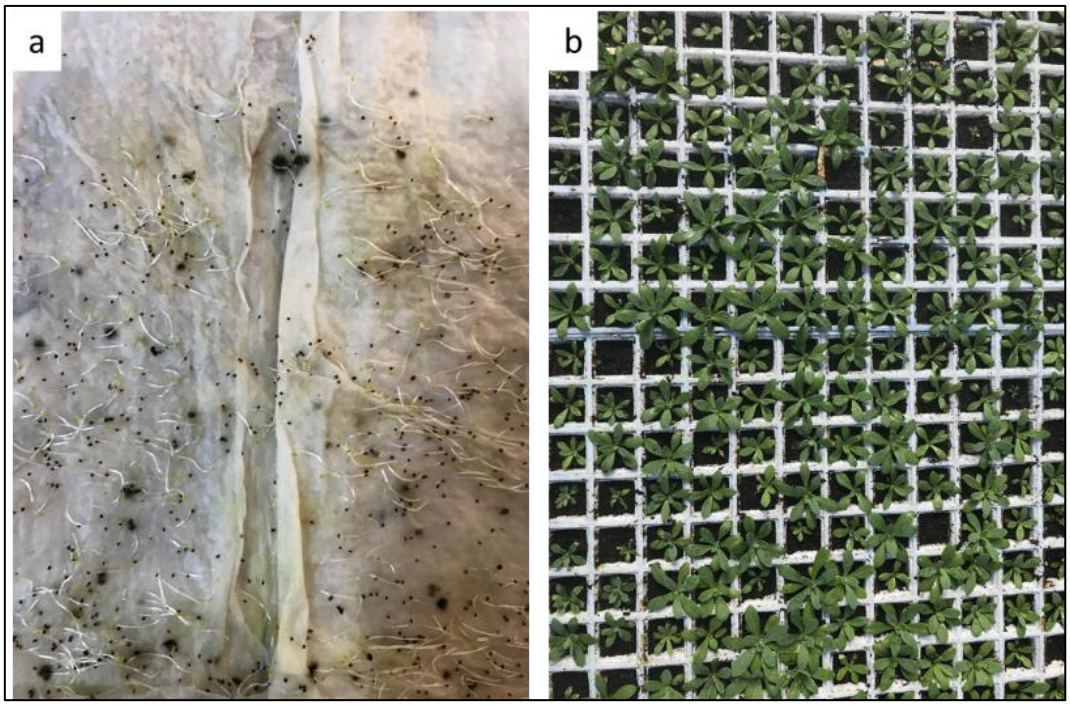

Figure 3. Germination and emergence of seeds after stratification (a); view of seedlings after germination (b)

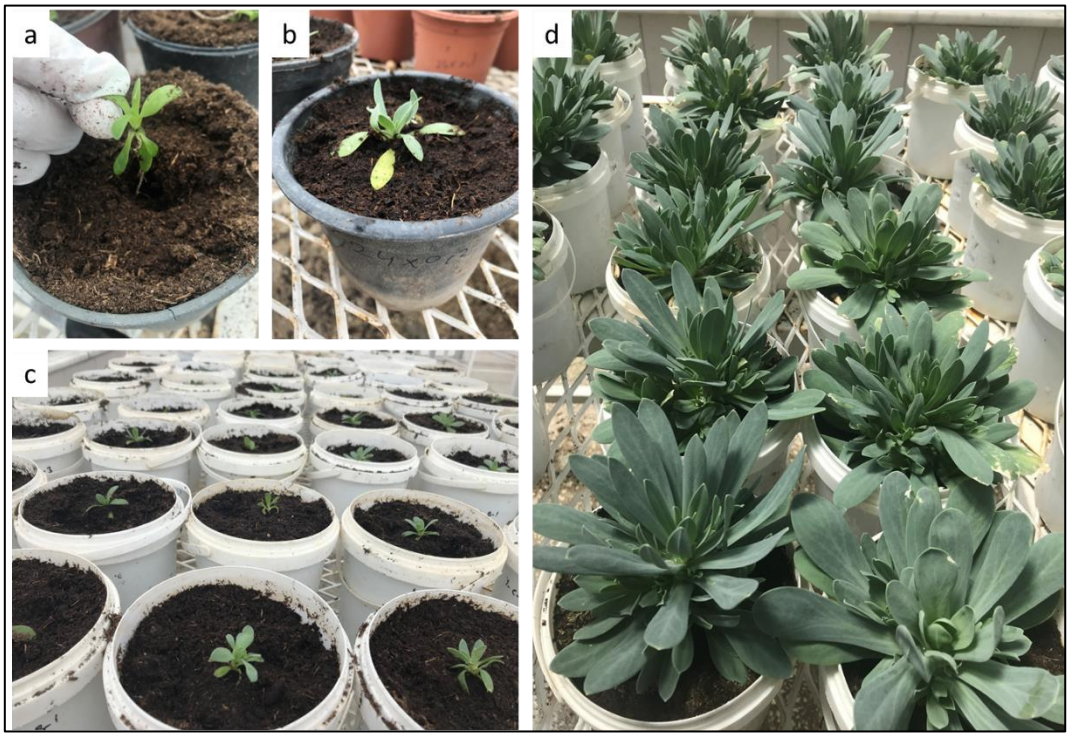

Figure 4. Stages of plant growth after colchicine application in the research greenhouse

Leaf length (cm): The lengths of the leaves taken from the leaves growing in the middle part of the seedlings after the application of colchicine to the growing tip and root tip of the seedlings were determined in cm with the help of a ruler.

Leaf width (cm): The width of the leaves taken from the leaves growing in the middle part of the seedlings after the application of colchicine to the growing tip and root tip of the seedlings was determined in cm with the help of a ruler. The measurement of plant height, plant stem diameter, number of leaves, number of branches, leaf length and leaf width parameters is described above. Also, these measurements were made according to the methods described by Błażewicz-Woźniak et al. (2021) and Dikbas et al. (2023).

Stomatal number (pcs mm^{-2}): Stomata were measured on the leaves of chemically mutated *S. compacta* plants. In order to determine the number of stomata, 3 leaves were selected from each plant. Nail polish method (Akal, 2001) was used for stomatal counts. According to the method, mono nitro cellulose was applied on the underside of the leaves and kept until it dried thoroughly. Then the lower epidermis was peeled off with this material and placed on a coverslip and the number of stomata in an area of 1 mm2 was counted and recorded for three different parts of the leaf using a 20-magnification objective and a 10-magnification ocular micrometer using a 'Net Micrometer' (Figure 5).

Stomatal width and length (µm): The width and length of the stomata of the specimens, whose lower epidermis was stripped and placed on coverslips, were measured with a 100-magnification objective and a 10-magnification ocular micrometer by taking 3 readings for three different parts of each leaf (Figure 6).

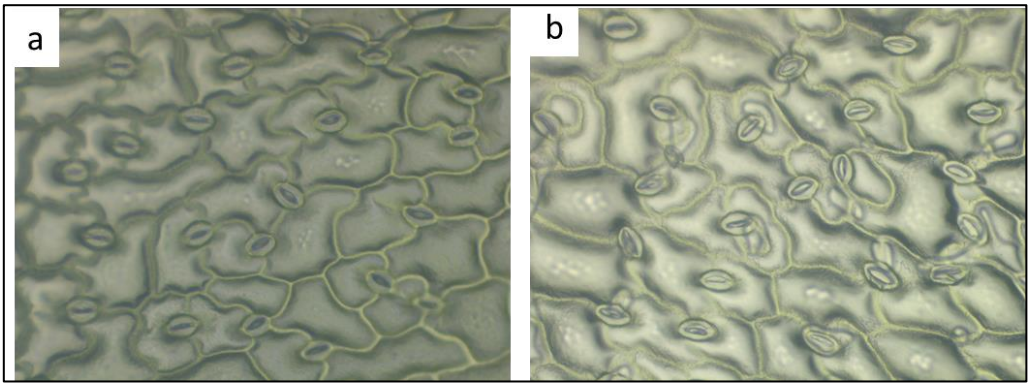

Figure 5. Examples of images used to determine the number of stomata in an area of 1 mm^2 using a 2- magnification objective and a 10-magnification ocular micrometer on the leaf

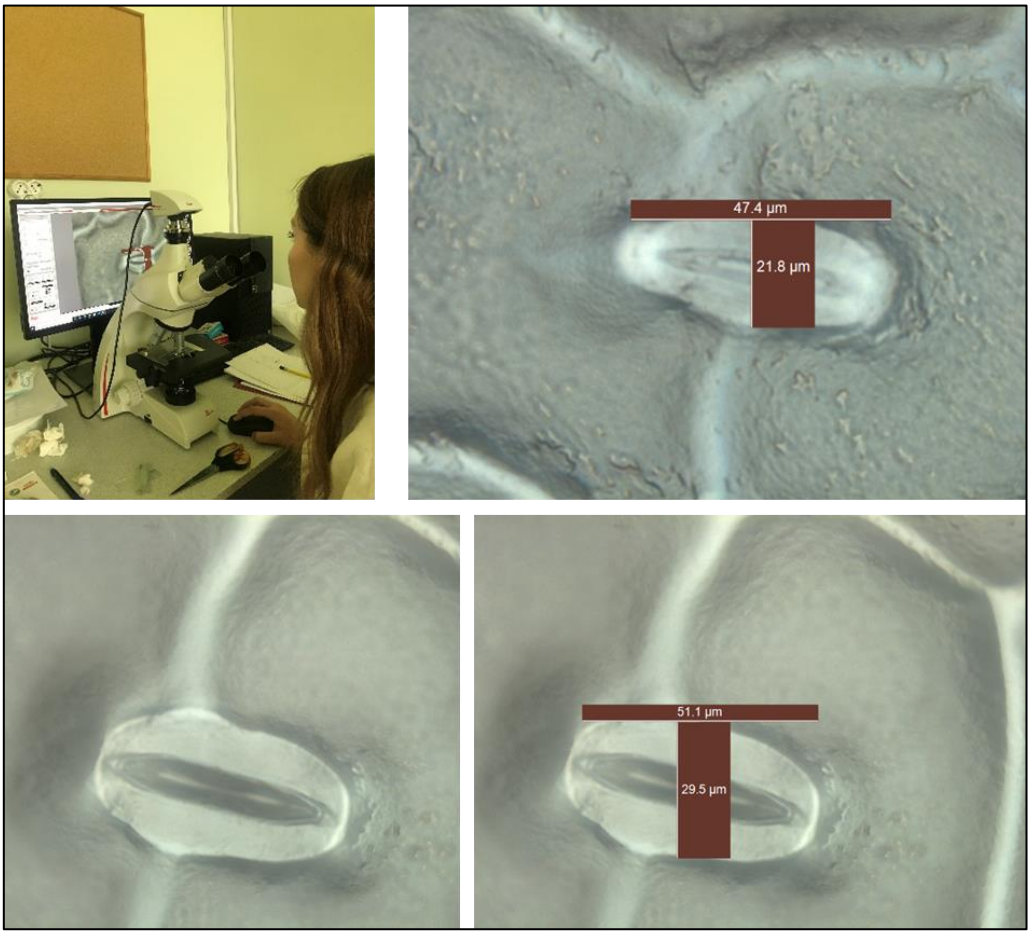

Figure 6. Determination of stomatal width and length using a 100-magnification objective and a 10-magnification ocular micrometer

The data obtained as a result of colchicine applications were compared with Duncan multiple comparison test in SPSS 20.0 statistical program (SPSS Inc, Chicago, IL, USA) at $p<0.05$ significance level.

3. Results and Discussion

Silene compacta Fischer plants were treated with colchicine and plant height was measured after 100 days. For rooting tip meristem application, the mean plant height was lowest at 4.92 cm with 0.4% colchicine dose, while the highest plant length was 9.94 cm with 0.05% colchicine dose. The application of 0.05% colchicine dose, in which the highest plant height was obtained, was in the same statistical group as the control application at $p<0.05$ significance level (Table 1). For the rooting tip meristem application, the lowest plant stem diameter was 3.80 mm at 0.4% colchicine dose and the highest plant stem diameter was 8.20 cm at 0.0% colchicine dose (control treatment). This application, in which the highest plant stem diameter was obtained, was in the same statistical group as the 0.01% and 0.05% colchicine doses applications. In the root tip meristem application, the number of leaves decreased from 32.40 leaves per/plant in the control treatment to 12.57 leaves per/plant at 0.4% colchicine dose (Table 1). The number of branches varied between 0.60 and 3.83 number plant-1 branches per plant according to the treatments; the highest number of branches was obtained from 0.05% colchicine dose in root tip meristem application. In the general evaluation of the treatment doses, all treatment doses except 0.05% colchicine dose were in the same statistical group and there was no significant difference between them in terms of the number of branches. The effect of root tip meristem application and different doses on leaf length was not statistically significant. As a result of the evaluation made in the experimental group applied from the root, it was determined that the leaf width varied between 0.74 and 1.91 cm according to the applications. The highest leaf width was obtained from the 0.05% dose application. In the general evaluation of the application doses, the 0.05% colchicine dose was the control, 0.01%, 0.05% and 0.1% doses were in the same statistical group and no significant difference was determined between them in terms of leaf width (Table 1).

The lowest plant height was 5.26 cm at 0.4% colchicine dose and the highest plant height was 11.07 cm at 0.05% colchicine dose in the shoot tip meristem application method. It was determined that plant height decreased as the doses and soaking durations of application increased regardless of the type of application (Table 2). In a study conducted by Gupta and Koak (1976), tetraploid plants obtained after 0.01% colchicine applied to *Zinnia elegans* plants were compared with diploid zinnia plants. According to this comparison, tetraploid zinnias were shorter than diploids. The results reported by these researchers were in parallel with the results of our study.

The lowest plant stem diameter was 3.57 mm at 0.4% colchicine dose and the highest plant stem diameter was 7.93 mm at 0.0% colchicine dose (control treatment) in the shoot tip meristem application method. It was determined that the plant stem diameter decreased as the application doses and times increased regardless of the application type (Table 2).

In a study conducted by Gupta and Koak (1976), tetraploid plants obtained after colchicine applied to Zinnia elegans plants had thinner main stem diameter than diploid zinnia plants. The results reported by these researchers are compatible with the results of our study. The different doses of colchicine applied from the shoot tip became shorter with the increase in the soaking duration. In general, the morphology of the plants decreased with increasing soaking duration and dose. In this case, more than 0.2% of colchicine was toxic to the plants. This toxic effect of the dose increased with the soaking duration.

Plant growth and development depends on the amount of assimilate produced by photosynthesis. The amount of assimilate is mainly undertaken by the leaves, which is the main site of photosynthesis in plants. In other words, plant growth and development are related to the number of leaves. In the study, the number of leaves decreased with the increase in colchicine doses as a result of the treatments. While the highest number of leaves was obtained from the control treatment, the lowest number was determined in the highest colchicine dose treatment. This decrease in the number of leaves also increased with the increase in colchicine doses and soaking durations. In both root tip (Table 1) and shoot tip application method (Table 2), the number of leaves decreased with increasing doses and soaking durations. At the highest soaking durations in the study, 48 and 72 hours (and at the highest colchicine doses) plants died. 48 and more than 48 hours of soaking durations not only caused a decrease in the number of leaves but also slowed down the growth of the plants. In the shoot tip meristem application, the highest number of leaves was 29.97 leaves per plant and the lowest number of leaves was 13.00 leaves per plant (Table 2). The explanation for this situation is that overdose of colchicine can be fatal by toxic effect on plants (Table 1, Table 2). As a result of lateral branching, plants can fill the pot volume and create a more compact texture.

This situation can be successfully created by increasing the leaf area and with the lateral branch. In this study, the leaves of plants with a high number of lateral branches had narrow and thin leaves. Therefore, less lateral branching may positively affect the leaf area of the plant. The lowest number of lateral branches was determined at 0.4% colchicine dose and the highest number of lateral branches was determined at 0.05% dose. Based on this result, colchicine applied at low levels was effective in increasing the number of lateral branches, and as the dose increased, both the number of lateral branches decreased and dwarf appearance occurred in the plant. In general, plants died at

Table 1. The effects of root tip meristem application method, different doses (%) and soaking durations of colchicine

			Soaking durations in colchicine					
		Doses	*6 hours*	*12 hours*	*24 hours*	*48 hours*	*72 hours*	Mean
Root tip meristem application method	Plant height (cm)	Control	9.57 c**	9.63 ns	9.94 a*	9.59 a***	8.82 a***	9.51 AB***
		0.01	11.97 a	9.48	10.60 a	8.00 b	7.53 ab	9.51 AB
		0.05	12.87 a	10.37	9.80 a	8.01 b	8.67 a	9.94 A
		0.1	12.83 a	10.47	9.03 a	6.83 c	6.75 b	9.18 B
		0.2	11.10 ab	10.17	9.25 a	0.00 d	0.00 c	6.10 C
		0.4	9.37 c	8.75	6.50 b	0.00 d	0.00 c	4.92 D
		Mean	11.28 A***	9.81 B	9.19 C	5.41 D	5.30 D	
	Plant stem diameter (mm)	Control	8.20 ns	7.87 ns	8.87 ns	8.54 a***	7.54 b***	8.20 A***
		0.01	8.27	7.99	7.44	5.50 c	9.29 a	7.70 A
		0.05	8.63	9.03	5.77	6.53 b	8.68 ab	7.73 A
		0.1	7.44	6.83	7.82	4.56 d	4.86 c	6.30 B
		0.2	7.65	8.22	5.66	0.00 e	0.00 d	4.31 C
		0.4	8.83	5.61	4.56	0.00 e	0.00 d	3.80 C
		Mean	8.17 A***	7.59 A	6.69 B	4.19 B	5.06 B	
	Number of leaves (number plant^{-1})	Control	31.33 ab*	33.67 a**	34.67 a**	32.33 a***	30.00 a***	32.40 A***
		0.01	34.67 a	33.67 a	24.00 b	19.00 bc	26.00 ab	27.47 B
		0.05	32.67 ab	32.00 a	21.33 b	18.67 c	27.67 a	26.47 B
		0.1	30.67 ab	29.00 a	25.67 b	20.33 b	21.00 b	25.33 B
		0.2	29.00 bc	27.33 a	20.00 b	0.00 d	0.00 c	15.27 C
		0.4	25.33 c	18.50 b	19.00 b	0.00 d	0.00 c	12.57 D
		Mean	30.61 A***	29.03 A	24.11 B	15.06 D	17.44 C	
	Number of branches (number plant^{-1})	Control	2.33 b**	0.67 ns	1.33 ns	1.33 a*	0.67 ns	1.28 B***
		0.01	1.67 b	2.33	1.33	0.00 b	0.00	1.07 B
		0.05	7.33 a	3.33	2.67	0.67 ab	0.33	2.87 A
		0.1	1.00 b	1.00	0.67	0.33 b	0.00	0.60 C
		0.2	1.00 b	2.00	2.00	0.00 b	0.00	1.00 B
		0.4	1.67 b	3.00	1.00	0.00 b	0.00	1.13 B
		Mean	2.50 A***	2.06 A	1.50 B	0.39 D	0.17 C	
	Leaf length (cm)	Control	8.33 ns	8.00 ns	8.67 ns	9.03 a***	8.03 a***	8.41 ns
		0.01	10.92	7.73	7.97	6.50 b	7.10 ab	8.04
		0.05	10.33	8.33	7.50	6.90 b	7.67 a	8.15
		0.1	9.00	8.50	6.83	6.77 b	5.85 b	7.39
		0.2	8.67	7.33	6.40	0.00 c	0.00 c	4.48
		0.4	7.27	6.75	5.33	0.00 c	0.00 c	3.87
		Mean	9.09 ns	7.78	7.12	4.87	4.78	
	Leaf width (cm)	Control	1.47 ns	1.33 ns	1.30 ns	1.40 a***	1.17 a***	1.33 AB*
		0.01	1.37	1.63	1.43	1.10 b	1.10 a	1.33 AB
		0.05	1.37	4.73	1.13	1.17 b	1.17 a	1.91 A
		0.1	1.33	1.30	1.13	1.10 b	1.00 a	1.17 AB
		0.2	1.70	1.23	0.80	0.00 c	0.00 b	0.75 B
		0.4	1.30	1.25	1.13	0.00 c	0.00 b	0.74 B
		Mean	1.42 AB**	1.91 A	1.16 B	0.79 B	0.74 B	

ns: not significant at $p>0.05$, *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ are statistically significant at the probability level. There is no difference at the 5% significance level between means indicated with the same letter.

Table 2. The effects of shoot tip meristem application method, different doses (%) and soaking durations of colchicine

			Soaking durations in colchicine					
		Doses	*6 hours*	*12 hours*	*24 hours*	*48 hours*	*72 hours*	Mean
Shoot tip meristem application method	Plant height (cm)	Control	9.57 ^{ns}	9.53 b***	8.89 b**	9.00 c***	6.00 c***	8.60 C***
		0.01	9.28	12.55 a	13.00 a	9.95 a	10.07 a	10.97 A
		0.05	10.40	12.03 a	12.75 a	10.50 a	9.67 ab	11.07 A
		0.1	11.57	11.87 a	10.40 b	7.95 b	8.67 b	10.07 B
		0.2	11.17	11.83 a	8.78 b	0.00 d	0.00 d	6.36 D
		0.4	9.65	7.67 c	9.00 b	0.00 d	0.00 d	5.26 E
		Mean	10.27 B***	10.91 A	10.47 AB	6.23 C	5.74 C	
	Plant stem diameter (mm)	Control	8.87 b**	7.87 ^{ns}	8.87 ^{ns}	7.90 a***	6.13 a***	7.93 A***
		0.01	9.25 b	7.27	8.64	6.64 b	4.31 b	7.22 A
		0.05	9.78 ab	7.92	8.69	6.22 b	4.43 b	7.41 A
		0.1	10.03 ab	8.65	7.98	6.13 b	5.43 a	7.62 A
		0.2	10.62 a	7.77	8.53	0.00 c	0.00 c	5.38 B
		0.4	7.18 c	4.78	5.90	0.00 c	0.00 c	3.57 C
		Mean	9.29 A***	7.38 C	8.11 B	4.48 D	3.38 E	
	Number of leaves (number plant^{-1})	Control	31.33 a**	33.33 a***	31.33 ^{ns}	31.33 a***	22.50 a***	29.97 A***
		0.01	34.67 a	30.33 a	25.33	21.67 b	20.67 a	26.53 B
		0.05	34.33 a	31.33 a	30.00	22.00 c	13.00 b	26.13 BC
		0.1	32.67 a	29.67 a	26.50	22.50 c	9.67 c	24.04 C
		0.2	33.33 a	21.00 b	24.33	0.00 d	0.00 d	15.73 D
		0.4	21.00 b	19.33 b	24.67	0.00 d	0.00 d	13.00 E
		Mean	31.22 A***	27.50 B	27.06 B	16.25 C	10.97 D	
	Number of branches (number plant^{-1})	Control	1.00 b*	1.67 b*	1.00 b*	2.67 a**	2.00 ^{ns}	1.67 C***
		0.01	2.00 b	5.00 a	3.00 b	2.00 a	2.33	2.87 AB
		0.05	2.67 b	5.00 a	6.00 a	2.50 a	3.00	3.83 A
		0.1	0.33 b	5.00 a	2.00 b	0.00 ab	3.00	2.07 BC
		0.2	5.33 a	1.67 b	1.33 b	0.00 b	0.00	1.67 C
		0.4	1.50 b	2.00 ab	2.67 b	0.00 b	0.00	1.23 C
		Mean	2.14 BC***	3.39 A	2.71 AB	1.19 C	1.72 BC	
	Leaf length (cm)	Control	8.33 ^{ns}	9.00 ab***	9.37 ab*	7.20 ab***	5.53 a ***	7.89 A***
		0.01	8.62	10.33 a	9.50 a	7.00 a	6.33 a	8.36 A
		0.05	8.57	10.33 a	9.75 a	8.00 b	3.50 b	8.03 A
		0.1	9.17	9.33 ab	7.95 abc	5.85 c	3.67 b	7.14 B
		0.2	9.33	8.60 b	7.58 bc	0.00 d	0.00 c	5.10 C
		0.4	7.90	5.80 c	6.57 c	0.00 d	0.00 c	4.05 D
		Mean	8.65 A***	8.90 A	8.48 A	4.68 B	3.17 C	
	Leaf width (cm)	Control	1.47 ^{ns}	1.63 ^{ns}	1.57 ^{ns}	0.97 a***	1.45 a***	1.42 A***
		0.01	1.60	1.43	1.20	1.33 a	1.07 b	1.33 AB
		0.05	1.50	1.53	1.25	1.40 b	0.55 c	1.25 AB
		0.1	1.40	1.40	1.25	1.45 b	0.63 c	1.23 B
		0.2	1.63	1.33	1.50	0.00 c	0.00 d	0.89 C
		0.4	1.40	1.17	0.97	0.00 c	0.00 d	0.71 D
		Mean	1.50 A***	1.42 AB	1.29 B	0.86 C	0.62 D	

^{ns}: not significant at $p>0.05$, *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ are statistically significant at the probability level. There is no difference at the 5% significance level between means indicated with the same letter.

the highest doses of 48 and 72 hours (0.2% and 0.4%). In terms of the number of lateral branches, this formation decreased significantly as the soaking duration increased (Table 1, Table 2). The application of 0.01% colchicine solution to *Impatiens balsamina* L. seedlings was able to induce polyploidy in a study conducted by Wiendra et al. (2011). Observation on morphological traits showed that colchicine treatment increased plant height, stem diameter, leaf length and number of branches (Wiendra et al., 2011). The results of Wiendra et al. (2011) and the increase in the number of branches at 0.05% colchicine dose obtained in our study can be explained. Treatments from the shoot tip of colchicine significantly ($p \leq 0.001$) shortened the leaf length in terms of both dose and soaking duration (Table 2). It was reported by Compton et al. (1996) that chloroplast density, ovary diameter, petal-anther diameter of male flowers and leaf length/width ratio are good indicators of plant ploidy. The evaluation of the leaf length parameter was also carried out for this purpose. According to the results obtained, it was determined that 0.05% and 0.01% doses of colchicine application from the shoot tip increased the leaf length (Table 2). In applications made from the shoot tip, the lowest leaf width was determined at 0.4% colchicine dose; the highest leaf width was determined at 0.0% dose. Based on this result, high-level colchicine application reduced leaf width; as the dose increased, both leaf width decreased and dwarf appearance occurred in the plant. In general, plants died at the highest doses (0.2% and 0.4%) of 48 and 72 h soaking durations. Leaf width increased as the waiting period increased at the colchicine dose, and the decrease in leaf width increased (Table 2). It was reported by Compton et al. (1996) that chloroplast density, ovary diameter, petal-anther diameter of male flowers and leaf length/width ratios are good indicators in determining plant ploidy.

Polyploid plants have a special place in the breeding of ornamental plants. The typical characteristics of polyploidy, such as dark green coloration and large, showy flowers, are of significant importance in the field of ornamental plants. In root tip meristem application, the treatments that resulted in the lowest number of stomata compared to the control group plants were those with 0.2% colchicine for 72 hours and 48 hours, as well as 0.4% colchicine for 48 and 72 hours. While an average of 18.73 stomata were counted on the leaves of the control group plants, the application of 0.2% colchicine resulted in 9 stomata mm^{-2}, and the application of 0.4% colchicine resulted in 9.67 stomata/mm^2. The evaluation of the treatments applied to the shoot tip showed that, in terms of the overall application doses, the highest number of stomata per unit area was 20.20 stomata, observed with a 0.01% colchicine dose, while the lowest number was determined with the highest colchicine doses (Table 3). As it is known, the number of stomata of mutated plants is less compared to the control group plants. Xing et al. (2011) applied colchicine to the seeds of Catharanthus roseus (L.) G. Don and in their stomatal studies, they found that the stomatal size and density of stomata were higher in tetraploid lines than in the control group, and the total stomatal area in tetraploid plants was 1.76% ± 0.01%, while it was 1.24% ± 0.02% in control group plants. Tepe et al. (2002) found that a relationship between stomatal number and chromosome number can be established. The stomatal numbers obtained from this study can be used as a preliminary key to identify folded plants.

Stomatal density per leaf unit area, length and width of stomatal guard cells are used as morphological markers to determine ploidy level in many plant species. In general, the size of leaf stomatal guard cells increases with increasing ploidy level, while stomatal density per leaf unit area decreases (Yen etal., 2010). As a result of flow cytometry analysis, it was determined by Doğan (2017) that the plantlets determined to be polyploid also showed differences in the number, diameter and length of stomata in the 4-hour application of 0.1% colchicine and confirmed polyploidy. Çimen et al. (2016) reported that the number of stomata per mm2 was denser in diploid plants than in tetraploid plants when the leaf stomatal characteristics of clementine 22D mandarin plants at diploid ploidy level and tetraploid ploidy level obtained by colchicine application were examined. The number of stomata obtained as a result of the present research decreased according to the increasing colchicine dose, while the soaking durations did not show a significant difference compared to the control.

As it is known, the stomatal diameters of the mutated plants are higher compared to the control group plant. Xing et al. (2011) applied colchicine to the seeds of *Catharanthus roseus* (L.) G. Don in their study. In their stomatal studies, they found that the stomatal size and density in tetraploid lines were higher than those in the control group. Padoan et al. (2013) reported that triploid *Clementine mandarin* plants had larger stomatal sizes, both in length and width, compared to diploid plants. Similarly, Sharif et al. (2013) noted that the stomatal sizes of tetraploid individuals obtained from certain citrus rootstocks were larger than those of the diploid ones. In root applications, when compared to the control group in terms of stomatal width, the treatments with 0.01%, 0.05%, and 0.1% colchicine doses showed the highest stomatal width (with average values of 33.36, 33.39, and 35.61 µm, respectively). In shoot tip applications, the highest values of stomatal width compared to the control group were observed with the 0.01%, 0.05%, and 0.1% colchicine doses, while a decrease was noted with the highest colchicine dose (Table 3). This decrease can be explained by the toxic effect of the high dose. The optimal soaking duration for increasing stomatal width was found to be 24 hours, as shown in Table 3.

In the present study, as the dose amount increased, stomatal length decreased and this decrease was statistically significant compared to the control. It was determined that 12 hours was the most appropriate soaking duration for the increase in stomatal length in root applications; 6 and 12 hours were determined as the most appropriate soaking duration in shoot tip applications. As it is known, the stomatal length of the mutated plants is higher compared to

the control group plants. Maghbel (2015) observed that stomatal length was 128.01 nm and 181.86 nm, respectively, in 24-hour application of 0.05% and 0.1% colchicine for *Glycyrrhiza glabra* L. var. glandulifera plant, while it was determined to be 84.7 nm in the control group. For *Carthamus tinctorius*, the stomatal length was 132.67 nm and 123.83 nm in 0.05% and 0.1% colchicine 24 h treatment, respectively, while the control group was 99.44 nm (Maghbel 2015).

Table 3. The effects of different application methods, different doses (%) and soaking durations of colchicine on stomatal number, stomatal width and stomatal length

			Soaking durations in colchicine					
		Doses	*6 hours*	*12 hours*	*6 hours*	*48 hours*	*6 hours*	Mean
Root tip meristem application method	Stomatal number (pcs mm^{-2})	Control	16.67 ^{ns}	17.67 ^{ns}	18.33 bc**	20.00 c***	21.00 a***	18.73 B***
		0.01	23.00	27.00	24.33 a	24.67 b	21.00 a	24.00 A
		0.05	25.50	22.00	24.00 a	24.00 b	18.67 a	22.83 A
		0.1	18.33	15.00	23.00 ab	36.33 a	21.00 a	22.73 A
		0.2	11.33	16.67	17.00 c	0.00 d	0.00 b	9.00 C
		0.4	18.33	15.50	14.50 c	0.00 d	0.00 b	9.67 C
		Mean	18.86 A***	18.97 A	20.19 A	17.50 A	13.61 B	
	Stomatal width (µm)	Control	22.23 ^{ns}	22.23 b***	22.23 ***c	22.57 d***	21.90 c***	22.23 B***
		0.01	22.83	23.20 b	34.38 b	45.38 a	41.02 a	33.36 A
		0.05	27.23	24.20 b	29.60 bc	43.43 b	42.50 a	33.39 A
		0.1	25.43	30.67 a	55.27 a	31.83 c	34.85 b	35.61 A
		0.2	27.2	21.73 b	45.00 a	0.00 e	0.00 d	18.79 C
		0.4	30.67	33.80 a	47.25 b	0.00 e	0.00 d	22.34 B
		Mean	25.93 B***	25.97 B	38.96 A	23.87 BC	23.38 C	
	Stomatal length (µm)	Control	37.23 c**	34.57 ^{ns}	39.23 b***	37.48 a***	34.82 ab***	36.67 A***
		0.01	36.99 c	42.73	47.57 a	25.51 c	23.95 b	35.35 A
		0.05	41.83 bc	50.27	46.73 a	27.33 b	25.73 b	38.38 A
		0.1	43.40 bc	56.73	24.67 d	26.56 bc	39.50 a	38.17 A
		0.2	55.30 a	52.3	30.15 c	0.00 d	0.00 c	27.55 B
		0.4	47.23 ab	54	29.15 c	0.00 d	0.00 c	26.08 B
		Mean	43.66 B***	48.43 A	36.25 C	19.48 D	20.67 D	
Shoot tip meristem application method	Stomatal number (pcs mm^{-2})	Control	16.67 ab**	19.67 ab*	21.33 a**	16.33 b***	23.67 a***	19.53 A***
		0.01	19.67 a	23.00 a	15.00 bc	19.00 a	24.33 a	20.20 A
		0.05	14.00 bc	20.67 ab	14.50 bc	10.00 c	23.00 a	16.43 B
		0.1	18.67 a	19.33 ab	12.50 c	22.00 ab	19.33 b	18.79 A
		0.2	12.33 c	15.33 c	18.00 b	0.00 d	0.00 c	9.13 C
		0.4	17.50 ab	12.00 bc	16.33 b	0.00 d	0.00 c	9.17 C
		Mean	16.47 B***	18.33 A	16.50 B	11.22 D	15.06 C	
	Stomatal width (µm)	Control	22.23 b*	23.23 c**	23.60 b***	53.73 b***	34.39 c***	31.44 B***
		0.01	25.87 ab	28.99 bc	46.98 a	52.34 a	44.70 b	39.77 A
		0.05	28.07 a	26.93 bc	47.25 a	51.25 a	45.43 ab	39.79 A
		0.1	30.57 a	26.27 bc	53.10 a	34.39 c	48.70 a	37.57 A
		0.2	25.67 ab	33.67 b	52.85 a	0.00 d	0.00 d	22.44 C
		0.4	30.05 a	43.73 a	50.40 a	0.00 d	0.00 d	24.84 C
		Mean	27.08 D***	30.47 BC	45.26 A	31.95 B	28.87 CD	
	Stomatal length (µm)	Control	37.23 c**	35.57 c*	36.48 a**	25.93 b***	25.04 b***	32.05 C***
		0.01	48.53 ab	46.20 abc	29.16 b	34.37 a	34.07 a	38.47 AB
		0.05	56.43 a	49.17 abc	29.15 b	35.15 a	34.14 a	40.81 A
		0.1	53.60 ab	41.77 bc	31.15 b	25.04 b	28.25 b	36.30 B
		0.2	44.97 bc	54.33 ab	27.75 b	0.00 c	0.00 c	25.41 D
		0.4	57.55 a	58.30 a	27.03 b	0.00 c	0.00 c	28.58 D
		Mean	49.72 A***	47.56 A	30.06 B	20.08 C	20.25 C	

^{ns}: not significant at $p>0.05$, *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ are statistically significant at the probability level. There is no difference at the 5% significance level between means indicated with the same letter.

In addition, autotetraploid plants could be obtained from the application of 0.5% colchicine to the growth tip of basil (*Ocimum basilicum*) seedlings at the cotyledon development stage. Tetraploid plants showed larger stomata, pollen, increased number of chloroplasts in guard cells and decreased stomatal density compared to diploids (Omidbaigi et al., 2010). In the present study, it was concluded that the plants kept in 0.1% dose of colchicine for 12 hours in both treatments could be tetaploid plants in terms of stomatal length, stomatal number and stomatal length parameters (Table 3). However, it was stated that stomatal examinations before determining the ploidy level were beneficial in reducing the cost of flow cytometry (Aydın et al., 2021).

4. Conclusion

In this study, due to the increasing interest in the use of native plant species in planting designs and landscaping arrangements, it was tried to obtain tetraploid individuals of *Silene compacta* Fischer, which is rich in the native flora of Erzurum (Türkiye), by applying colchicine to the root tip and shoot tip of young seedlings at different soaking durations and doses.

The highest doses of colchicine applied both from the root and shoot tip were found to have a lethal effect on the plants at the highest soaking durations. In general, high doses and prolonged application of colchicine caused a decrease in the viability rate. In conclusion, as a result of this study, which aimed to obtain larger leaves, flowers and plants, it was determined that the most effective concentration of colchicine for polyploid induction of *S. compacta* was 0.1%, the application time was 12 and 24 hours and there was no statistically significant difference between the application methods and success could be achieved with the use of both application methods. With this study, it is also suggested that a variety of *S. compacta* breeding material with improved characteristics can be developed that can be used as parent material in future breeding programs.

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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