JPMB | Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan
2023 | Volume 11 | Issue 2

Effects of polyploidy induction on the performance of anise (*Pimpinella anisum* L.)

Haniyeh Ahmadinia and Parviz Heidari *

Faculty of Agriculture, Shahrood University of Technology, Shahrood 3619995161, Iran.

Original paper

OPEN ACCESS

Edited by
Prof. Ahmad Arzani,
Isfahan University of Technology, Iran

Date
Received: 15 January 2024
Accepted: 03 February 2024
Published: 17 February 2024

Correspondence
Dr. Parviz Heidari
heidarip@shahroodut.ac.ir

Citation
Ahmadinia, H., and Heidari, P. (2024). Effects of polyploidy induction on the performance of anise (*Pimpinella Anisum* L.). *J Plant Mol Breed 11* (2): 17-30.
doi: 10.22058/jpmb.2024.2020503.1289.

Abstract: Plant chromosome manipulation is a powerful tool in plant breeding due to its significant impact on various genetic traits and diversity. To investigate the effect of polyploidy induction in anise (*Pimpinella anisum* L.), three different concentrations (0.01%, 0.5%, and 5%) of colchicine were tested. In this study, the seeds and terminal buds of five-week-old plants were treated with colchicine, and the process was repeated for three consecutive days. Subsequently, molecular, physiological, and morphological traits of both control (diploid) and induced (autotetraploid) plants were investigated. The results revealed that 0.01% colchicine had no significant effect on ploidy induction, while significant effects were observed at 0.5% and 5%. Seedlings treated with concentrations higher than 0.5% colchicine exhibited larger stomatal size, lower stomatal density, and darker leaf color. In addition, the contents of DNA, RNA, and total protein increased in seedlings treated with concentrations of 0.5% and 5%. Karyotype observation confirmed polyploidy induction in plants treated with colchicine concentrations above 0.5%. Overall, this study shows that colchicine can alter anise plants' ploidy by 0.5% and 5% and boost leaf size and pigments associated with photosynthesis, resulting in stronger plants.

Keywords: medical plants; pigment content; nucleic acid content; ploidy level; plant vigor.

Introduction

Anise (*Pimpinella anisum* L.), 2n = 2x = 18, is a medicinal and aromatic plant belonging to the Apiaceae family that is used in the pharmaceutical, perfume production, and food industries (Gülçın et al., 2003). Additionally, the fruits and essential oils of this plant have antispasmodic, antioxidant, antimicrobial, insecticidal and antifungal effects (Özcan and Chalchat, 2006; Tepe et al., 2006). The seeds of this plant contain 5 to 5.5% essential oil, which is mainly composed of volatile phenylpropanoids such as transanthol (Tabanca et al., 2006). In addition, anise seed essential oil contains a small amount of estragole, anisaldehyde and cis-anthole (Omidbaigi et al., 2003; Tabanca et al., 2006). Anise seed and its essential oil are used both in ancient and modern times in salty foods, baked goods and various drinks. Anise seeds are a good source of many essential B complex vitamins, such as pyridoxine, niacin, riboflavin and thiamine. Considering all the positive features, including anti-diabetes, blood fat reduction, antioxidant activities, anticancer and antimicrobial properties, anise seeds and essential oils are recommended for safe use as dietary supplements (Sun et al., 2019).

Today, various methods, such as metabolite engineering, cell culture and the use of elicitors, are used to increase the production of secondary metabolites in medicinal plants. Increasing the ploidy level (polyploidy) is also known as an effective method for producing new genotypes and increasing the yield of plants (Chung et al., 2017; Liu et al., 2023). Polyploidy can also affect secondary metabolites in terms of quantity and chemical diversity. These changes are due to structural and functional modifications caused by an increase in the allelic level (Zahumenická et al., 2018). Polyploid plants are larger than diploids in terms of morphological characteristics such as leaf, stem, and root size, which can be beneficial for yield and crop production (Dhooghe et al., 2011), especially for fodder, vegetables, and medicinal plants. Polyploidy occurs spontaneously in plants but can be induced in a short period of time by using antimitotic substances such as colchicine, which interferes with spindle formation (Eng and Ho, 2019). Polyploidized plants are more tolerant to adverse environmental conditions due to their relatively stronger foundation (Fang et al., 2019). Additionally, polyploidy affects the increase in photosynthesis by increasing the amount of chlorophyll, and as a result, polyploid plants have a stronger foundation than their ancestors (Zahumenická et al., 2018).

Polyploidization is often associated with morphological and physiological changes that can increase plant growth rate and yield other commercially beneficial traits (Cheng et al., 2015). It was previously reported that polyploid plants have advantages in terms of creating tissues and organs; in general, compared with those of diploid species, the size of vegetative tissues, the shape of large flowers and resistance to environmental stresses are improved (Liu et al., 2011; Meng et al., 2011). For example, autotetraploid lavenders (*Lavandula angustifolia*) had larger flowers and leaves, thicker peduncles and larger shield hairs on their leaves than did their diploid genotypes (Urwin et al., 2007). Treating the terminal bud of watercress plants (*Nasturtium officinale*) with 0.5% colchicine was able to induce tetraploidy. Similarly, compared with diploid plants, tetraploid plants exhibit significant differences in leaf dimensions, stomatal number and chlorophyll content (Aqafarini et al., 2019). Investigation of different methods of polyploidy induction in Ispaghul (*Plantago ovata* Forsk.) showed that the use of 0.3% colchicine treatment for 24 hours or 22.5% trifloralin treatment for 72 hours on seeds results in the most tetraploid plants (Sabzehzari et al., 2020). Induction of polyploidy with different concentrations of colchicine was successfully performed in Salvia species such as *Salvia bowleyana* (Duan et al., 2006) and *S. hains* (Grouh et al., 2011). In both species, compared with diploid plants, chromosomally doubled plants had thicker and wider leaves, darker colors, thicker roots and larger stomata. It has been reported that in some cases, increased resistance to pathogens can be associated with polyploidy. For example, the tetraploid *Trifolium pratense* is more resistant to clover rot than is its diploid ancestor (Vestad, 1960; Vleugels et al., 2013), and the tetraploid *Glycine tabacina* is more resistant to leaf rust disease (*Phakopsora pachyrhizi*) than is its diploid ancestor (Burdon and Marshall, 1981). Recent hypotheses show that ploidy can affect plant defense by increasing genetic adaptation to

pathogens through replacement or through new functions of resistance genes and genes related to plant defense (Innes et al., 2008; King et al., 2012). In the present study, different concentrations of colchicine were applied to induce polyploidy in anise. We found that terminal bud treatment with 0.5% and 5% colchicine could increase the polyploidy rate in anise plants, as evidenced by changes in the morphological and physiological traits along with alterations in nucleic acid content.

Materials and Methods

Plant materials and Seed treatment

In the first experiment, anise seeds, landraces prepared from Shahrood region, were transferred to 3% sodium hypochlorite solution for 15 minutes for sterilization, after which the seeds were washed three times with distilled water to remove the residues of the solution. Then, the anise seeds were placed between two layers of filter paper and irrigated with distilled water. After one week, the water in the Petri dishes was removed, and the samples (200 seeds) were treated with different concentrations of colchicine (0.01% and 0.5%) for different periods of time (6, 24 and 48 hours). Ten microlitres of colchicine solution was added to each petri dish. Finally, after seven days, the number of plants was counted, and the results were used to measure the survival rate.

Terminal bud treatment

In the second experiment, sterilized anise seeds were grown in pots. First, perlite and peat moss were prepared at a ratio of 2:1, mixed together and transferred to the pots. Seven seeds were subsequently planted in each pot (finally, four plants remained in each pot for treatment). Pots were maintained under a 17-hour photoperiod (6000 lux) and a 25 ± 3°C temperature. After five weeks, the terminal buds of the plants were treated with different concentrations of colchicine (0 (as a control), 0.01%, 0.5%, or 5%). The selection of colchicine concentrations was determined based on the review of previous studies. Between 10 and 11 am (when the mitotic divisions were most common), the terminal bud was treated with 5 µl of colchicine. This work was repeated for three consecutive days.

Measurement of morphological traits

After applying the terminal bud treatment, new leaves were considered to confirm polyploidy. Determination of ploidy level in treated plants was first based on morphological changes in different stages of growth and development by observing plants suspected of polyploidy in comparison with control plants. Morphological traits such as the length and width of new leaves, length of petioles, distance between nodes, and density and size of stomata were measured. A digital caliper was used to measure seedling length, leaf length, the distance between the second leaflet, the length and width of the terminal leaflet and the diameter of the petiole.

Measurement of plant stomata

To determine the number of stomata on the surface of the new leaves, the upper layer of the leaf was covered with a thin layer of colorless varnish. After drying, the above layer was placed on a clean glass slide using adhesive tape, and the number and size of the stomata were counted with an Olympus CH2 optical microscope.

Leaf pigments

The method outlined by Lichtenthaler and Wellburn (1983) was employed to measure leaf chlorophyll pigments. In brief, the chlorophyll and carotenoid contents were measured without crushing. For this purpose, we mixed 50 mg of leaf tissue with 5 ml of dimethyl sulfoxide and put it in an oven at 65°C for 4 hours. After that, using a spectrophotometer, the absorbance was recorded at 470, 645 and 665 nm. Finally, the contents of chlorophyll a and b and carotenoids were obtained using the following relationships:

Chla = (12.19 A665) - (3.45 A645)

Chlb = (21.99 A645 - 5.32 A645)

Cartenoeid = (1000 A470 - 2.14Chla - 70.16Chlb)/200

Protein content

The Bradford method was used to measure the amount of protein (Bradford, 1976). The Bradford method is a fast, simple, accurate and sensitive colorimetric method used to measure the total protein concentration in biological samples. The basis of this method is the binding of protein molecules to Coomassie dye under acidic conditions, which leads to a change in color from brown to blue, and as a result, a change in light

absorption occurs. In fact, Coomassie blue dye binds to proteins and produces a blue color that can be measured at a wavelength of 595 nm.

DNA and RNA extraction

DNA extraction was performed using the CTAB method (Porebski et al., 1997). To ensure comparability of DNA quantity, 100 mg of young leaves from each treatment was used. RNA extraction was performed using a TRIzol reagent kit (Kiagene Fanavar, Iran) according to the manufacturer's instructions. Likewise, to extract RNA, the same amount of plant tissue was used for all samples. Finally, the quantity of the extracted DNA and RNA samples was measured by a Nano Photometer (Implen N50).

Survival rate and seed germination

The number of surviving plants after terminal bud treatment with colchicine was evaluated as the survival percentage. Additionally, the seedlings that were treated with different concentrations were allowed to produce seeds. The seeds were subsequently distributed in Petri dishes, after which the germination rate was calculated.

Preparation of karyotypes and chromosome counts

Karyotyping is the process of depicting the set of chromosomes in the cells of a living organism to check the chromosomal content. To perform anise karyotyping, first, the plants that were treated with different concentrations of terminal buds were allowed to produce seeds, after which the seeds were germinated. The root tips of the germinated seeds were used to prepare the karyotype. After cutting the young roots, karyotype preparation was performed in five steps, namely, root pretreatment (0.01% colchicine), fixation with Carnoy 1 solution (one part of absolute acetic acid and three parts of absolute ethanol), hydrolysis (normal hydrochloric acid at a temperature of 60°C for 8 minutes), root staining with 0.1% Aceto Carmen for 45 minutes, and squashing the roots with 45% acetic acid. Chromosomes were observed using an OLYMPUS CH2 light microscope.

Data analysis

The data were analyzed via Minitab 17 software (version 17) via ANOVA, and the means were compared using Tukey's test at the 5% probability level. Graphs were drawn based on the mean and standard deviation for each treatment by GraphPad Prism 5.0 software.

Results

Toxic effects of colchicine on embryos

The results showed that seed treatment with colchicine caused nongermination and nongrowth of a large number of seeds (Figure 1). The highest percentage of plant survival was obtained in the control treatment (no use of colchicine).

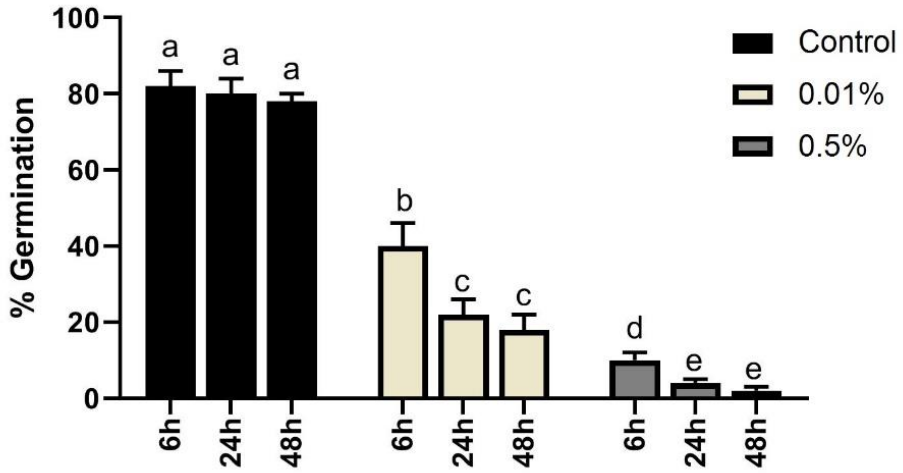

Figure 1. Germination rate of anise seeds treated with colchicine. Different letters indicate significant differences (p value < 0.05) between experimental treatments

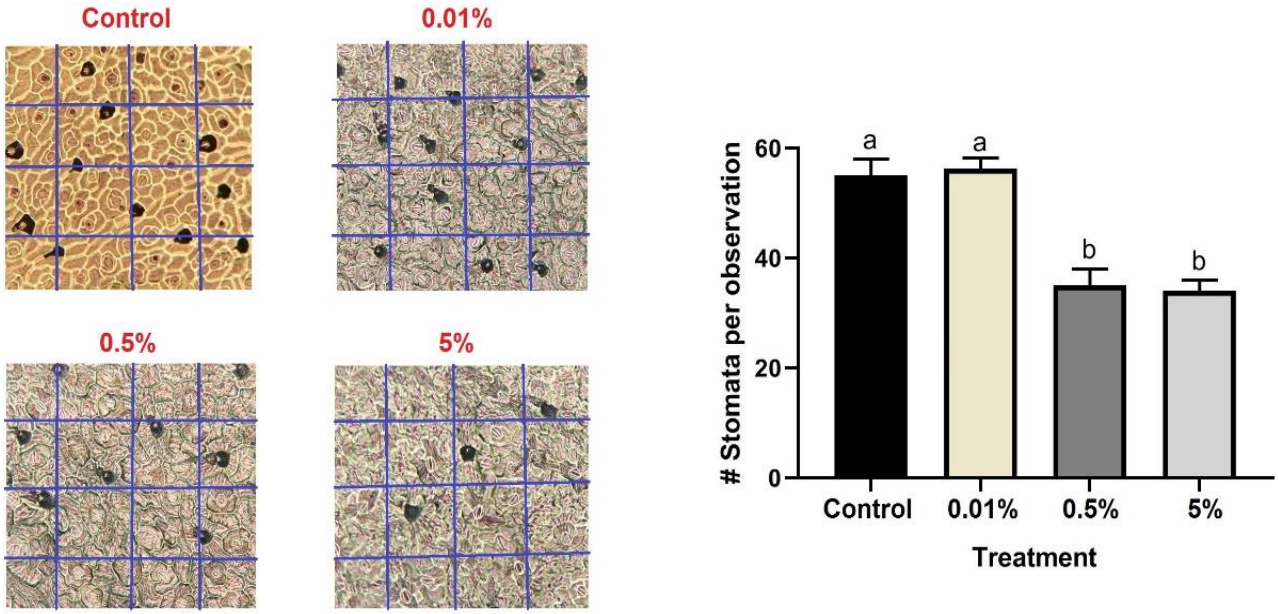

Figure 2. Stomatal density of young leaves after treatment of top buds with different concentrations of colchicine.

The survival percentage of the seedlings (germination) decreased with increasing treatment duration; the highest survival percentage was related to the duration of 6 hours at a concentration of 0.01%, and the lowest survival percentage was related to the duration of 48 hours at a concentration of 0.5%. These results indicate that colchicine has toxic effects on embryos and that seed treatment is not recommended for inducing polyploidy in anise plants.

Stomatal density

Based on microscopic observations of leaf stomatal characteristics in plants treated with colchicine compared to diploids, the density and size of stomata on new leaves were affected by polyploidy induction (Figure 2). A decrease in stomatal density and an increase in stomatal size were observed in seedlings treated with 0.5% and 5% colchicine. The size and density of stomata are among the important traits related to the ploidy level and can be used as biomarkers.

Morphological traits

The effects of polyploidy induction on morphological traits were investigated. A comparison of the means of plants treated with different concentrations of colchicine revealed that the length of the seedlings and leaves and the petiole diameter were not affected by treatment with different concentrations of colchicine (Figure 3). However, treatment with a 5% concentration of colchicine somewhat increased the length of the leaf and petiole diameter. The dimensions (length and width) of the terminal leaflets and the length between the leaflets were significantly greater for the plants treated with high concentrations (0.5% and 5%) of colchicine. In general, the induction of polyploidy did not significantly alter the height of the plant, but it caused changes in the dimensions of the new leaflets and caused compact seedlings with broad leaves (Figure 4a and 4b). In this study, compared with those of the control plants, the shapes of the new leaves on the plants treated with 5% and 0.5% colchicine were wrinkled, uneven, wide and thick (Figure 4b).

Leaf pigment content

A comparison of the mean chlorophyll a content revealed that there was a significant difference between the 5% colchicine treatment and the control treatment. However, the 5% treatment did not significantly differ from the 0.5% treatment (Figure 5).

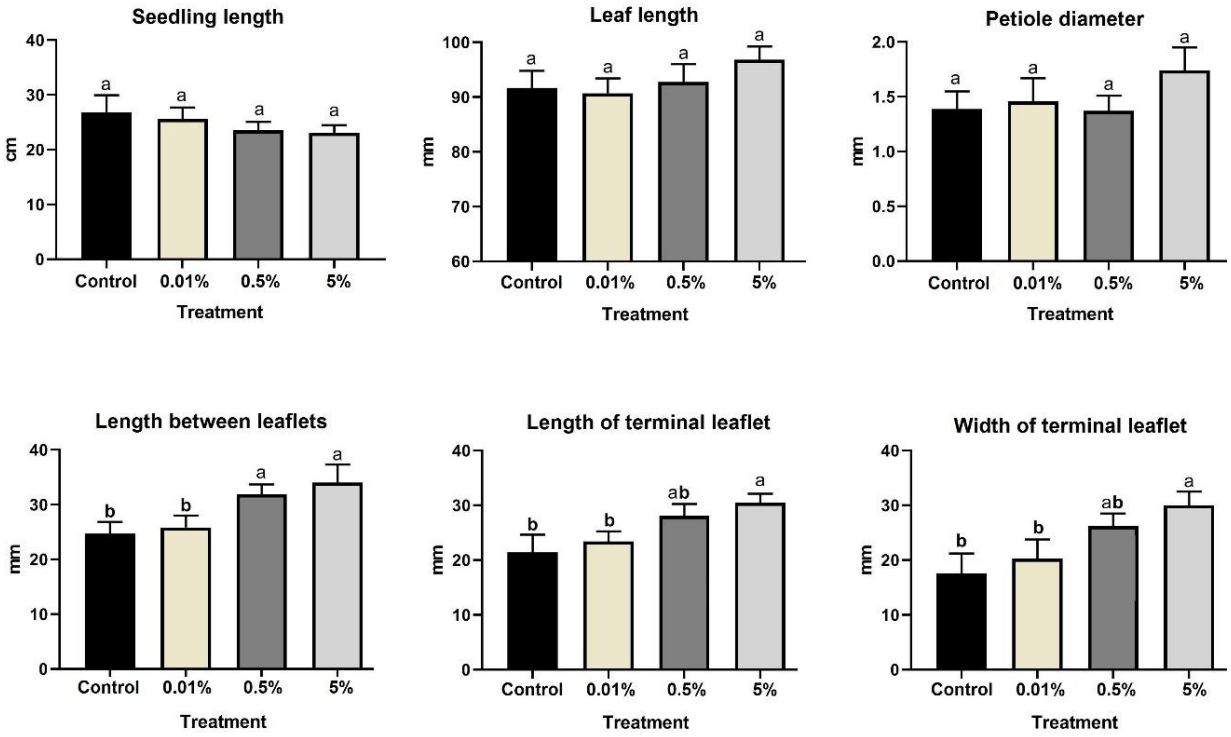

Figure 3. Comparison of the means for morphological traits, including seedling length, leaf length, petiole diameter, length between leaflets, length and width of terminal leaflet, between the control treatment and treatment with different colchicine concentrations. Different letters indicate significant differences ($p < 0.05$) between experimental treatments

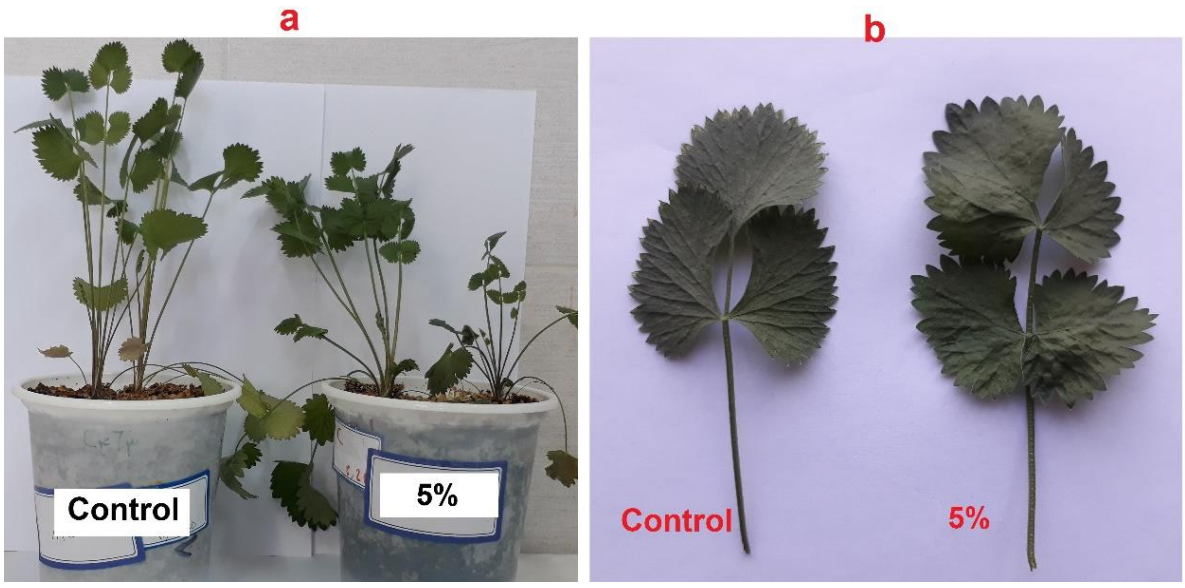

Figure 4. Morphological modifications between the control and 5% colchicine treatment groups in terms of seedling height (a) and leaf length and width (b).

It seems that the content of chlorophyll a increases in induced tetraploid plants. No significant differences were detected in the contents of chlorophyll b and carotenoids between the colchicine treatments and the control, although the amounts of chlorophyll b and carotenoids were somewhat greater in the induced plants and in the 0.5% and 5% treatment groups.

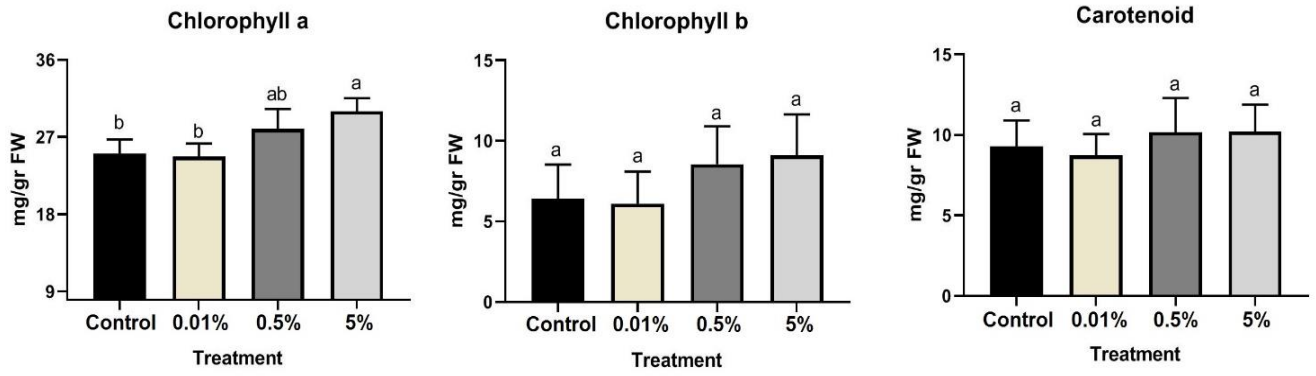

Figure 5. Mean comparison of chlorophyll a and b and carotenoid content under the colchicine treatments. Different letters indicate significant differences (p value < 0.05) between experimental treatments.

Nucleic acids and total protein content

In the present study, the levels of three macromolecules, protein, DNA, and RNA, were measured in seedlings treated with colchicine (Figure 6). Significant increases in the concentrations of protein, DNA, and RNA were observed in the new leaves treated with 0.5% and 5% colchicine. Interestingly, the total RNA content sharply increased in the 5% treatment group compared with the control sample. An increase in the amount of DNA causes an increase in the number of genes and, as a result, the content of RNA and protein is increased. As a result, the effect intensity of each gene increases in related cellular pathways.

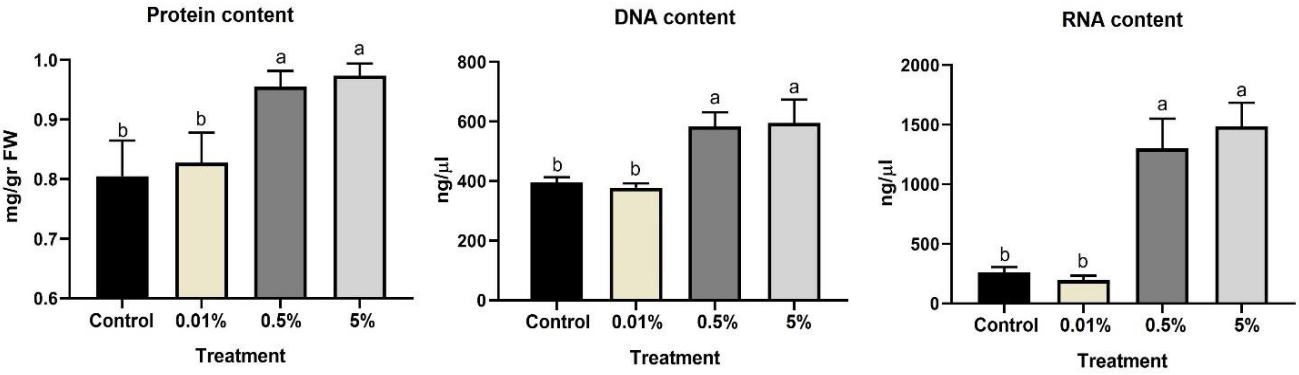

Figure 6. Mean comparison of protein, DNA, and RNA content under the colchicine treatments. Different letters indicate significant differences (p value < 0.05) between experimental treatments.

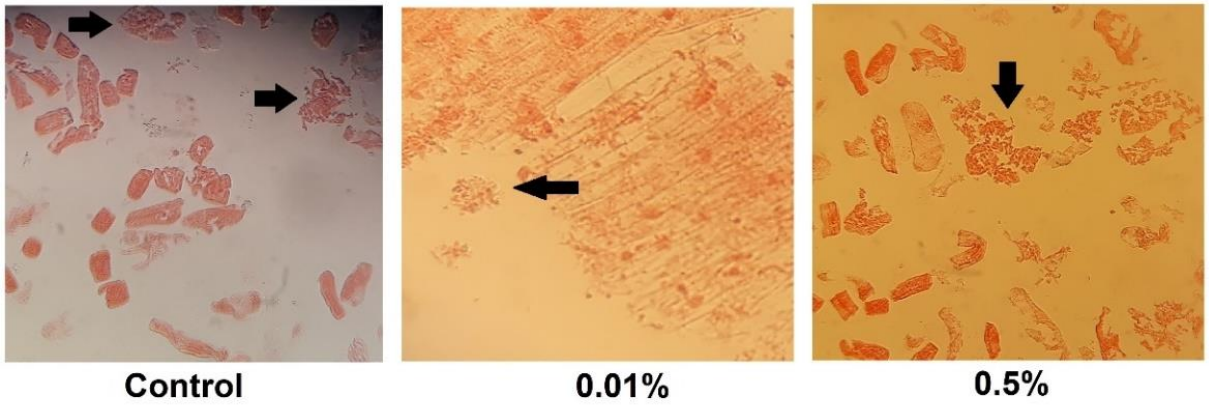

Figure 7. Karyotype analysis of the roots of plants treated with 0.01% or 0.5% colchicine.

Chromosome observation

Chromosome counting is a direct, unambiguous and common way to determine the number of chromosomes in plant cells (Figure 7). This method is vital in plant systematic studies, classification and breeding. To investigate the effects of colchicine treatments on the polyploidy of treated plants, we examined and analyzed the root tip cells. The results revealed that the chromosome number of the plants treated with a concentration of 0.5% colchicine was duplicated comparing to those in the control and 0.01% colchicine samples. The limited seed production in the first generation prevented the preparation of a suitable karyotype for the 5% treatment. However, based on changes at the 0.5% level, the occurrence of polyploidy in this treatment can also be confirmed.

Effects of colchicine on survival and induction rate

The results revealed that tetraploid induction was increased at two high colchicine concentrations, 0.5% and 5%, based on morphological traits and nucleic acid content (Figure 8a). However, fewer than 40% of the seedlings treated with 5% colchicine survived. In addition, the germination rate of seeds from plants treated with 0.5% or 5% colchicine was sharply reduced (Figure 8b). Based on the percentage of tetraploid induction, seedling survival and seed germination, 0.5% colchicine was introduced as an effective dose for inducing tetraploid anise seedlings with high fertility ability.

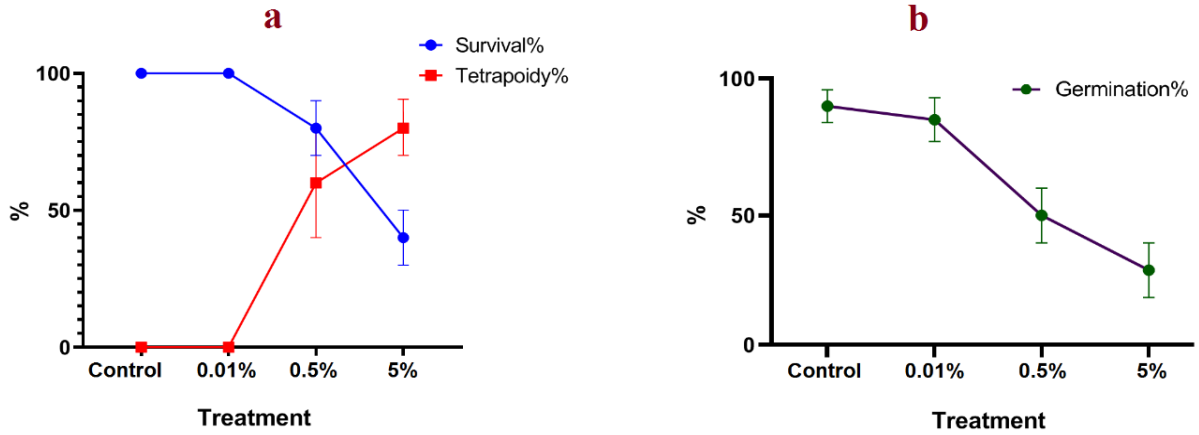

Figure 8. Effects of colchicine on the percentage of surviving plants and tetraploid induction (a) and on the percentage of generated seeds from seedlings (b).

Discussion

Elevating the ploidy level (polyploidy) is recognized as an effective approach for generating novel genotypes with enhancing the biomass, consequently augmenting the bioactive compounds of medicinal plants (Nilanthi et al., 2009; Chung et al., 2017). Polyploidized plants exhibit enhanced tolerance to negative conditions due to their relatively stronger organs (Fang et al., 2019). Additionally, polyploidy affects photosynthesis by increasing the amount of chlorophyll, and as a result, polypoid plants have a stronger foundation than their ancestors (Azoush et al., 2014; Zahumenická et al., 2018). In this research, the induction of polyploidy in anise was investigated. The results showed that applying colchicine to anise seeds is not a suitable method for increasing ploidy. Colchicine likely has toxic effects on embryos and young plants and can cause their death or severe weakening. On the other hand, terminal bud treatment had significant effects on morphological characteristics and other traits related to genome content. In general, according to the results obtained from the morphological studies, it can be said that the treatment of the terminal bud of anise seedlings with concentrations of 5% and 0.5% colchicine has been able to have changes in the distance between the leaflets and the dimensions of the leaves. These results indicate that changes likely occurred at the genomic level, which led to morphological changes in new leaves after treatment. Autopolyploid plants and their organs are often larger than their diploid parents are, which makes them attractive to plant breeders (Dai et al., 2015). Considering that vegetative organs are the main source of active compounds in most medicinal plants, increasing the chromosomal level can be considered a valuable and fast way to increase drug production (Dhawan and Lavania, 1996). However, in many cases, it has been reported that tetraploid plants are smaller than or equal in size to diploid plants (Lavania, 2005).

The usual result of polyploidy in plants is reduced growth due to decreased cell division, which occurs as a result of disturbance of the auxin content in cells. In this regard, the rate of respiration and the activity of a large number of enzymes also decrease (Stebbins, 1971). In our study, a decrease in seedling height and length was observed with increasing colchicine concentration, which was consistent with the results observed for the height of anthurium plants, which showed a decrease in the height of plants treated with colchicine compared to diploids (Chen et al., 2011). Additionally, the induction of polyploidy in chamomile (Saharkhiz, 2007) and *Platanus acerifolia* (Liu et al., 2007) was associated with reduced growth. The decreased growth of induced polypoid plants is due to a decrease in the rate of cell division (Eigsti, 1947), a decrease in growth hormones and decreased activities of metabolites in plants (Larsen and Tung, 1950). The decrease in height of tetraploid plants is probably due to the high toxicity of colchicine and its negative effects on physiological activities, including the production and activity of plant growth hormones. Moreover, differences in leaf margins and leaf shapes were observed between induced plants treated with colchicine (5% and 0.5%) and diploid plants. Some of the observed irregularities in the leaf size, shape, texture and color of the seedlings may be due to initial disturbances caused by colchicine. The leaves of tetraploid plants are significantly thicker than the leaves of diploid plants because of the presence of thick parenchyma, spongy parenchyma and epidermal cells and large intercellular spaces in the spongy parenchyma (Allario et al., 2011).

A decrease in stomatal density and an increase in stomatal size were observed in seedlings treated with 0.5% and 5% colchicine. Increasing the ploidy level of the nucleus often causes structural changes such as stomatal density, increasing the size of stomatal cells and the number of chloroplasts in the cell. Research has shown that the size of stomatal guard cells is more affected by genetic factors than is that of other plant cells and is less influenced by environmental conditions (Watrous and Wimber, 1989). With increasing ploidy level, the length and width of the stomata increase, and as a result, the stomatal density decreases. Increasing ploidy also increases the number of chloroplasts in stomatal guard cells. Studies on chicory and Egyptian bean plants have shown that the number of chloroplasts in the stomatal guard cells of tetraploid plants is greater than that in diploid plants (Ghotbi Ravandi et al., 2013). On the other hand, in tetraploid hop plants, despite the increase in stomatal size, no

significant difference in stomatal density per unit area was observed between diploid and tetraploid plants (Roy et al., 2001). Therefore, the type of species under study plays an important role in determining stomatal indices at different ploidy levels. In most cases, polyploid leaves exhibit a greater volume of mesophyll cells with more chloroplasts and chlorophyll than diploids, resulting in a greater rate of photosynthesis per cell (Coate et al., 2012). Previous research has shown that polyploidization increases the number of chloroplasts and photosynthesis in each cell, which may be due to the increase in genome content and cell size (Warner and Edwards, 1993). The results of the present study showed that colchicine treatment had a significant effect only on the content of chlorophyll a. In the research conducted by Liao et al. (2016)Liao et al. (2016)Liao et al. (2016) on Populus trees, the photosynthetic rate and chlorophyll content were significantly greater in tetraploids than in diploids. In addition, Abdoli et al. (2013)Abdoli et al. (2013)Abdoli et al. (2013) reported that an increase in ploidy causes an increase in the number of chloroplasts in the leaf and thus increases photosynthesis. An increase in the number of gene copies causes an increase in the number of transcripts and the amount of protein, which in turn affects the cell volume and plant stem (Tsukaya, 2008). The results of this research confirmed that the content of genomic material and the amount of total RNA increased in plants treated with 5% colchicine, and as a result, the total protein content also increased in the cells. Additionally, Yan et al. (2014)Yan et al. (2014)Yan et al. (2014) reported an almost twofold increase in total protein in radish plants. Although there is sometimes no direct relationship between RNA and protein levels, different factors affect the translation and stability of proteins. Increasing the genomic level can be considered as a strategy in the breeding of medicinal plants to increase biomass and effective substance

Conclusion

Based on the results obtained from this research, it can be concluded that colchicine, as a disruptor of mitotic spindles, can induce polyploidy in anise plants. Terminal bud treatment was found to be more effective than seed treatment. It seems that colchicine concentrations higher than 0.5% are suitable for increasing ploidy, although the toxicity associated with higher concentrations reduces the survival rates of seedlings and their fertility. Based on four important traits related to polyploidy- namely, DNA content, total RNA content, protein content, and stomatal size and density- it is concluded that it is possible to produce autotetraploid plants using colchicine. An increase in the amount of genomic content caused a change in the appearance of anise plants, as these plants had larger leaves. Additionally, compared with that in the control plants, the amount of chlorophyll in the polyploidized plants increased. An increase in leaf size and chlorophyll content can increase the photosynthesis rate and water consumption efficiency in plants, thereby potentially increasing production rates. In the end, the success of autoploid plant production can be influenced by factors such as the concentration of the inducing substance, the chosen growth stage for treatment and the method of applying the treatment.

Supplementary Materials:

No supplementary material is available for this article.

Author contributions

Conceptualization, P.H.; methodology, P.H. and H.A.; validation, P.H.; formal analysis, P.H. and H.A.; writing—original draft preparation, P.H.; writing—review and editing, P.H.; project administration, P.H.; supervision, P.H. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Acknowledgments

We thank the Shahrood University of Technology for financially supporting and providing the needed facilities.

Conflict of interest statement

The authors declare no conflict of interest.

References

Abdoli, M., Moieni, A., and Naghdi Badi, H. (2013). Morphological, physiological, cytological and phytochemical studies in diploid and colchicine-induced tetraploid plants of *Echinacea purpurea* (L.). *Acta Physiol Plant* 35(7): 2075-2083.

Allario, T., Brumos, J., Colmenero-Flores, J.M., Tadeo, F., Froelicher, Y., Talon, M., Navarro, L., Ollitrault, P., and Morillon, R. (2011). Large changes in anatomy and physiology between diploid Rangpur lime (*Citrus limonia*) and its autotetraploid are not associated with large changes in leaf gene expression. *J Exp Bot* 62(8): 2507-2519.

Aqafarini, A., Lotfi, M., Norouzi, M., and Karimzadeh, G. (2019). Induction of tetraploidy in garden cress: morphological and cytological changes. *Plant Cell Tissue Organ Cult* 137: 627-635.

Azoush, S., Kazemitabar, S.K., and Heidari, P. (2014). Polyploidy induction in Iranian Borage (*Echium amoenum* L.) by colchicine treatment. *Biharian Biol* 8(2): 87-89.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72(1-2): 248-254.

Burdon, J., and Marshall, D. (1981). Inter-and intra-specific diversity in the disease-response of *Glycine* species to the leaf-rust fungus *Phakopsora pachyrhizi*. *J Ecol*: 381-390.

Chen, C., Hou, X., Zhang, H., Wang, G., and Tian, L. (2011). Induction of *Anthurium andraeanum* "Arizona" tetraploid by colchicine in vitro. *Euphytica* 181: 137-145.

Cheng, S., Zhu, X., Liao, T., Li, Y., Yao, P., Suo, Y., Zhang, P., Wang, J., and Kang, X. (2015). Gene expression differences between high-growth *Populus allotriploids* and their diploid parents. *Forests* 6(3): 839-857.

Chung, H.-H., Shi, S.-K., Huang, B., and Chen, J.-T. (2017). Enhanced agronomic traits and medicinal constituents of autotetraploids in *Anoectochilus formosanus* Hayata, a top-grade medicinal orchid. *Molecules* 22(11): 1907.

Coate, J.E., Luciano, A.K., Seralathan, V., Minchew, K.J., Owens, T.G., and Doyle, J.J. (2012). Anatomical, biochemical, and photosynthetic responses to recent allopolyploidy in *Glycine dolichocarpa* (Fabaceae). *Am J Bot* 99(1): 55-67.

Dai, F., Wang, Z., Luo, G., and Tang, C. (2015). Phenotypic and transcriptomic analyses of autotetraploid and diploid mulberry (*Morus alba* L.). *Int J Mol Sci* 16(9): 22938-22956.

Dhawan, O., and Lavania, U. (1996). Enhancing the productivity of secondary metabolites via induced polyploidy: a review. *Euphytica* 87: 81-89.

Dhooghe, E., Van Laere, K., Eeckhaut, T., Leus, L., and Van Huylenbroeck, J. (2011). Mitotic chromosome doubling of plant tissues in vitro. *Plant Cell Tissue Organ Cult* 104: 359-373.

Duan, Y.-Z., Ke, S.-Y., Cao, J., Niu, Y.-Z., and Peng, C.-Z. (2006). Study on induction of polyploidy in *Salvia bowleyana* by colchicine treatment. *Zhongguo Zhong Yao Za Zhi* 31(6): 445-448.

Eigsti, O. (1947). The pollen tube method for making comparisons of differences in mitotic rates between diploids and tetraploids. *Genetics* 32: 85.

Eng, W.-H., and Ho, W.-S. (2019). Polyploidization using colchicine in horticultural plants: A review. *Sci Hortic* 246: 604-617.

Fang, C., Fernie, A.R., and Luo, J. (2019). Exploring the diversity of plant metabolism. *Trends Plant Sci* 24(1): 83-98.

Ghotbi Ravandi, E., Rezanejad, F., Zolala, J., and Dehghan, E. (2013). The effects of chromosome-doubling on selected morphological and phytochemical characteristics of *Cichorium intybus* L. *J Hortic Sci Biotechnol* 88(6): 701-709.

Grouh, M.S.H., Meftahizade, H., Lotfi, N., Rahimi, V., and Baniasadi, B. (2011). Doubling the chromosome number of *Salvia hains* using colchicine: Evaluation of morphological traits of recovered plants. *J Med Plant Res* 5(19): 4892-4898.

Gülçın, İ., Oktay, M., Kıreççı, E., and Küfrevıoğlu, Ö.İ. (2003). Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chem* 83(3): 371-382.

Innes, R.W., Ameline-Torregrosa, C., Ashfield, T., Cannon, E., Cannon, S.B., Chacko, B., Chen, N.W., Couloux, A., Dalwani, A., and Denny, R. (2008). Differential accumulation of retroelements and diversification of NB-LRR disease resistance genes in duplicated regions following polyploidy in the ancestor of soybean. *Plant Physiol* 148(4): 1740-1759.

King, K., Seppälä, O., and Neiman, M. (2012). Is more better? Polyploidy and parasite resistance. *Biol Lett* 8(4): 598-600.

Larsen, P., and Tung, S.M. (1950). Growth-promoting and growth-retarding substances in pollen from diploid and triploid apple varieties. *Botanical Gazette* 111(4): 436-447.

Lavania, U. (2005). Genomic and ploidy manipulation for enhanced production of phyto-pharmaceuticals. *Plant Genet Resour* 3(2): 170-177.

Liao, T., Cheng, S., Zhu, X., Min, Y., and Kang, X. (2016). Effects of triploid status on growth, photosynthesis, and leaf area in *Populus*. *Trees* 30: 1137-1147.

Lichtenthaler, H.K., and Wellburn, A.R. (1983). "Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents", in: *Biochem Soc Trans.* Portland Press Ltd.).

Liu, G., Li, Z., and Bao, M. (2007). Colchicine-induced chromosome doubling in *Platanus acerifolia* and its effect on plant morphology. *Euphytica* 157: 145-154.

Liu, S., Chen, S., Chen, Y., Guan, Z., Yin, D., and Chen, F. (2011). In vitro induced tetraploid of Dendranthema nankingense (Nakai) Tzvel. shows an improved level of abiotic stress tolerance. *Sci Hortic* 127(3): 411-419.

Liu, Y., Duan, S.-D., Jia, Y., Hao, L.-H., Xiang, D.-Y., Chen, D.-F., and Niu, S.-C. (2023). Polyploid induction and karyotype analysis of dendrobium officinale. *Horticulturae* 9(3): 329.

Meng, H.-b., Jiang, S.-s., Hua, S.-j., Lin, X.-y., Li, Y.-l., Guo, W.-l., and Jiang, L.-x. (2011). Comparison between a tetraploid turnip and its diploid progenitor (*Brassica rapa* L.): the adaptation to salinity stress. *Agric Sci China* 10(3): 363-375.

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.

Omidbaigi, R., Hadjiakhoondi, A., and Saharkhiz, M. (2003). Changes in content and chemical composition of *Pimpinella anisum* oil at various harvest time. *J Essent Oil Bear Plant* 6(1): 46-50.

Özcan, M.M., and Chalchat, J.C. (2006). Chemical composition and antifungal effect of anise (*Pimpinella anisum* L.) fruit oil at ripening stage. *Ann Microbiol* 56: 353-358.

Porebski, S., Bailey, L.G., and Baum, B.R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol Biol Rep* 15: 8-15.

Roy, A., Leggett, G., and Koutoulis, A. (2001). In vitro tetraploid induction and generation of tetraploids from mixoploids in hop (*Humulus lupulus* L.). *Plant Cell Rep* 20: 489-495.

Sabzehzari, M., Hoveidamanesh, S., Modarresi, M., and Mohammadi, V. (2020). Morphological, anatomical, physiological, and cytological studies in diploid and tetraploid plants of Ispaghul (*Plantago ovata* Forsk.). *Genet Resour Crop Evol* 67: 129-137.

Saharkhiz, M. (2007). The effects of some environmental factors and ploidy level on morphological and physiological characteristics of feverfew (*Tanacetum parthenium* L.) medicinal ornamental plant. *Tarbiat Modares University, Tehran.[In Persian]*.

Stebbins, G.L. (1971). Chromosomal evolution in higher plants. *Q Rev Biol* 48(1).

Sun, W., Shahrajabian, M.H., and Cheng, Q. (2019). Anise (*Pimpinella anisum* L.), a dominant spice and traditional medicinal herb for both food and medicinal purposes. *Cogent Biol* 5(1): 1673688.

Tabanca, N., Demirci, B., Ozek, T., Kirimer, N., Baser, K.H.C., Bedir, E., Khan, I.A., and Wedge, D.E. (2006). Gas chromatographic–mass spectrometric analysis of essential oils from *Pimpinella* species gathered from Central and Northern Turkey. *J Chromatogr A* 1117(2): 194-205.

Tepe, B., Akpulat, H.A., Sokmen, M., Daferera, D., Yumrutas, O., Aydin, E., Polissiou, M., and Sokmen, A. (2006). Screening of the antioxidative and antimicrobial properties of the essential oils of *Pimpinella anisetum* and *Pimpinella flabellifolia* from Turkey. *Food Chem* 97(4): 719-724.

Tsukaya, H. (2008). Controlling size in multicellular organs: focus on the leaf. *PLoS Biol* 6(7): e174.

Urwin, N.A., Horsnell, J., and Moon, T. (2007). Generation and characterisation of colchicine-induced autotetraploid *Lavandula angustifolia*. *Sci Hortic* 156: 257-266.

Vestad, R. (1960). The effect of induced autotetraploidy on resistance to clover rot (*Sclerotinia trifoliorum* Erikss.) in red clover. *Euphytica* 9(1): 35-38.

Vleugels, T., Cnops, G., and Van Bockstaele, E. (2013). Screening for resistance to clover rot (*Sclerotinia* spp.) among a diverse collection of red clover populations (*Trifolium pratense* L.). *Euphytica* 194: 371-382.

Warner, D.A., and Edwards, G.E. (1993). Effects of polyploidy on photosynthesis. *Photosynth Res* 35: 135-147.

Watrous, S.B., and Wimber, D. (1989). Artificial induction of polyploidy in *Paphiopedilum*. *Lindleyana* 3(4): 177-183.

Yan, P., Xu, Y.-y., Zhu, X.-w., Zhe, L., Gong, Y.-q., Liang, X., Gong, M.-y., and Liu, L.-w. (2014). Molecular characterization and expression profiles of myrosinase gene (*RsMyr2*) in radish (*Raphanus sativus* L.). *J Integr Agric* 13(9): 1877-1888.

Zahumenická, P., Fernández, E., Šedivá, J., Žiarovská, J., Ros-Santaella, J.L., Martínez-Fernández, D., Russo, D., and Milella, L. (2018). Morphological, physiological and genomic comparisons between diploids and induced tetraploids in *Anemone sylvestris* L. *Plant Cell Tiss Org Cult* 132: 317-327.

JPMB
Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan
2023 | Volume 11 | Issue 2

مقاله پژوهشی

اثرات القای پلی‌پلوئیدی بر عملکرد انیسون (*Pimpinella Anisum* L.)

هانیه احمدی‌نیا و پرویز حیدری *

دانشکده کشاورزی، دانشگاه صنعتی شاهرود، شاهرود ۳۶۱۹۹۹۵۱۶۱، ایران

چکیده: دستکاری کروموزوم‌های گیاهی به دلیل تأثیر آن بر چندین صفت مهم و تنوع ژنتیکی، روشی قدرتمند برای اصلاح نباتات است. برای بررسی اثر القاء پلی‌پلوئیدی در انیسون (*Pimpinella anisum* L.)، سه غلظت مختلف (۰/۰۱٪، ۰/۵٪ و ۵٪) کلشی‌سین مورد آزمایش قرار گرفت. صفات مولکولی، فیزیولوژیکی و مورفولوژیکی گیاهان شاهد و القاء شده بررسی گردید. نتایج نشان داد که کلشی‌سین در غلظت ۰/۰۱ درصد تأثیر معنی‌داری بر سطح پلوئیدی ندارد، در حالیکه تأثیر معنی‌داری در غلظت‌های ۰/۵ و ۵ درصد کلشی‌سین مشاهده شد. اندازه روزنه بیشتر، تراکم روزنه کمتر و رنگ برگ تیره‌تر در گیاهچه‌های تیمار شده با غلظت‌های بالاتر از ۰.۵ درصد کلشی‌سین مشاهده شد. علاوه بر این، محتوای DNA، RNA و پروتئین کل در گیاهچه‌های تیمار شده با غلظت‌های ۰/۵ و ۵ درصد افزایش یافت. مشاهدات کاریوتیپ همچنین القاء پلی‌پلوئیدی را در گیاهان انیسون تیمار شده با غلظت‌های بالاتر از ۰/۵ درصد کلشی‌سین تایید کرد. یافته‌های این مطالعه نشان داد که کلشی‌سین می‌تواند سطح پلوئیدی گیاهان انیسون را در غلظت ۰/۵ و ۵ درصد تغییر دهد و اندازه برگ و رنگدانه‌ها را افزایش دهد.

کلمات کلیدی: گیاهان دارویی، محتوی رنگدانه، محتوی اسید نوکلئیک، سطح پلوئیدی، بنیه گیاه.

ویراستار علمی
دکتر احمد ارزانی،
دانشگاه صنعتی اصفهان، ایران

تاریخ
دریافت: ۲۵ دی ۱۴۰۲
پذیرش: ۱۴ بهمن ۱۴۰۲
چاپ: ۲۸ بهمن ۱۴۰۲

نویسنده مسئول
دکتر پرویز حیدری
heidarip@shahroodut.ac.ir

ارجاع به این مقاله
Ahmadinia, H., and Heidari, P. (2024). Effects of polyploidy induction on the performance of anise (*Pimpinella Anisum* L.). *J Plant Mol Breed 11* (2): 17-30.
doi: 10.22058/jpmb.2024.2020503.1289.