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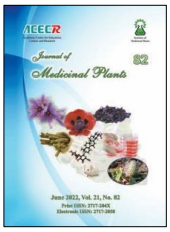

Research Article

Variations in morphological, biochemical and phytochemical traits of diploid and induced tetraploid plants of downy thorn-apple (*Datura innoxia* Mill.)

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ABSTRACT

Background: *Datura* species produce a wide range of tropane alkaloids with medicinal values. *D. innoxia* is a species of the *Datura* genus which is poisonous and has an unpleasant odor due to the presence of tropane alkaloids. **Objective**: The study aimed to induction of polyploidy by colchicine treatment on **downy thorn-apple** and the effect of it on appearance and hyoscyamine content. **Methods:** In order to induce tetraploidy in downy thorn-apple, a factorial experiment was carried out with two factors: colchicine concentrations (0.05, 0.1, and 0.2 %) and exposure time (48 and 72 h) with 3 plants for each treatment with using a cotton ball included colchicine on shoot tip. Morphological changes were observed and the plants were analyzed by flow cytometry in near to the flowering time. **Results:** The results showed that the concentration and exposure time of colchicine, and their interaction affected the percentage of tetraploidy. The maximum percent of tetraploidy was observed in concentration of 0.2 % and 72 h. On the other hand, the application of different concentrations of colchicine and exposure time didn't have a significant effect on plant survival. The number of stomatal guard cells were decreased but no significant differences were observed in the content of total chlorophyll and carotenoids. Also, the hyoscyamine content in tetraploid plants was decreased to half in comparison to diploid ones. **Conclusion**: In general, induction of tetraploidy in this plant could change some phenotypic traits but has a negative effect on hyoscyamine content.

1. Introduction

Secondary metabolites play an important role in the adaptation of plants to the environment and on the other hand, are an important source of active drugs such as alkaloids. Plant alkaloids are one of the largest groups of natural compounds from which various medicinal products are produced. Although research on alkaloids has a long history, humans are still at the beginning of biotechnological use of these compounds. Therefore, any research and studies on understanding the mechanism of their biosynthesis and identifying plant species that produce these substances are very important [1, 2].

Abbreviations: HPLC, High Performance Liquid Chromatography

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Tropane alkaloids, the most important of which are hyoscyamine and scopolamine, are among the most important compounds in many plants of the Solanaceae family. The anticholinergic effects of these compounds have made their common use in medicine. *D. innoxia* is a species of the *Datura* genus which is poisonous and has an unpleasant odor due to the presence of tropane alkaloids. The average content of alkaloids in the vegetative parts (stem and leaves) is 0.2 to 0.25 %. Scopolamine is 70 to 80 % of alkaloids in this plant and the rest, are other alkaloids such as hyoscyamine, tiglodin, atropine and tropine [2].

Manipulation of the ploidy level is a powerful tool in the genetic modification of many plants. Induction of polyploidy in plants often leads to the production of new variants with distinct quality. [3]. In an experiment conducted by Berkov and Philipov [4] on the diploid form and the fourth generation of tetraploid *D. stramonium*, the results showed that increasing the biosynthesis capacity of tetraploid roots increased the alkaloids stored in the leaves of tetraploid plants.

According to Dehghan and Shahriari [5], tetraploidy also affected growth rate and the content of tropane alkaloids in Egyptian henbane plants. Studies have shown that the use of colchicine in vitro conditions has increased and in rare cases has reduced or no significant effect on metabolites [6]. The application of colchicine with a concentration of 0.05 % has increased the production of biomass in *Bacopa monnieri* and increased the production of Bacoside content in this plant [7].

The diploid form of *Datura* contains more alkaloids than the haploid one and its tetraploid form is expected to have more alkaloids than its diploid form [8]. This study aimed to investigate the effect of different treatments for polyploidy induction and the influence of this change on the content of hyoscyamine alkaloid produced in *D. innoxia*.

2. Materials and Methods

2.1. Polyploidy induction

The seeds were collected from the plant grown at Gonabad region located in South Khorasan Province. Then the seeds were confirmed as *innoxia* species in the Research Institute of Plant Sciences of the Ferdowsi University of Mashhad with herbarium code 11257 (FUMH).

Initial germination of seeds was done in seedling trays. After the plants reached to the 3-4 leaf stage, colchicine treatments with concentrations of 0.05, 0.1, and 0.2 % were applied by placing cotton balls on the apical meristem. To prevent the evaporation, the pots were covered with clear plastic. During 48 and 72 hours add of the treatments, colchicine was applied twice and three times on a cotton ball, respectively. After the treatment, the cotton was removed and the plants were placed in the greenhouse. All the plants near to flowering stage (4 months) were studied for their appearance, measurement of total chlorophyll and carotenoids, flow cytometry analysis and alkaloid content.

2.2. Appearance comparison and measurement of total chlorophyll and carotenoids

The appearance of diploid and tetraploid plants just compared without data recording. So analysis of variance was not done on morphological traits. Also, samples were taken from the upper epidermis of treated and control plant leaves separately to observe the stomata cells with an X40 objective lens of a light microscope.

Lichtenthaler's [9] method was used to measure the content of chlorophylls and carotenoids. First, 0.1 g of fresh leaves were ground with 4 ml of 80 % acetone in a porcelain

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mortar, and then the resulting solution was centrifuged for 5 minutes at 3000 rpm, and then the supernatant was used to determine the content of chlorophylls and carotenoids in wavelengths of 647, 664 and 470 nm by spectrophotometer (80 % acetone was used to blank the spectrophotometer). Total chlorophyll, chlorophyll a and b, chlorophyll a to b ratio, and carotenoids (micrograms per ml) were calculated using the following equations.

Equation 1 $Chl_b = 21.21A_{647} - 5.1A_{664}$

Equation 2 $Chl_a = 12.25A_{664} - 2.79A_{647}$

Equation 3 $Chl_T = Chl_a + Chl_b$

Equation 4

$$carotenoid = \frac{1000A_{470} - 1.8chla - 85.02chlb}{198}$$

In which A_{647}: Light absorption at 647 nm, A_{664}: Light absorption at 664 nm, and A_{470}: Light absorption at 470 nm. (A is the output data of spectrophotometer instrument)

2.3. Flowcytometry analysis

Ploidy analysis of treated plants was performed by flowcytometer (Partec pA, Germany). Control plants were also used for comparison and the peak obtained from them was used as the basis for nucleus volume comparisons.

The procedure was as follows: 0.5 cm square pieces were prepared from fully grown leaves, 400 µl of nucleus extraction buffer (Partec device solution A) was poured on them and the leaf section was finely chopped with a sharp razor to prevent tissue crushing. The resulting solution was then passed through filters for the device and 1600 µl of 4 and 6-diamidino-2-phenyl indole (DAPI) nucleus staining solution was added to it and after 30-60 seconds it was given to the device for counting. Normally, for each sample, the volume of at least 5,000 nuclei was interpreted by the measuring device, and the peaks obtained were interpreted by Mode Fit LT 3.1 software.

2.4. Extraction and measurement of hyoscyamine content

Different samples were freeze-dried and 300 mg of each powder was weighed and added into 50 ml Falcon tubes. 10 ml of 96 ° ethanol was added to each of them and placed on a shaker for 48 hours with a gentle motion at 25 °C. After, the ethanol was evaporated by vacuum evaporation and 5 ml of 5 % sulfuric acid was added to each and placed in a gentle rotation for 16 hours at 25 °C. Then, in 3 steps, 3 ml of chloroform was added to the mixture each time, and the chloroform precipitate containing colorants was removed [4].

The precipitated acidic solution was filtered and adjusted to pH = 10 using 10 N NaOH. Then, in 3 steps, each time 3 ml of chloroform was added to the remaining solution, and the chloroform precipitate containing the alkaloids was removed. The evaporated solvent was dissolved in the mobile phase and after passing through a syringe filter, 20 µl of each was injected into the device. The system used in this study included an HPLC device from the German Company Zorbox, with a two-pump system, equipped with a photodiode detector model S2600 and a loop volume of 20 microliters, and the EZ Chrom Elite software program with integration capabilities.

The used constant phase was the reverse phase C_{18} column with an inner diameter of 4.6 mm and a length of 250 mm, and water: methanol: acetonitrile with a ratio of 80:10:10 was used as the mobile phase. The used wavelength was also 254 nm. The mobile flow rate in this experiment was considered to be 1 ml/min. Dissolution of pure hyoscyamine powder in methanol was used to prepare 2500 ppm of hyoscyamine as standard.

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2.5. Data analysis

The data for chlorophyll and carotenoid measurement and flow cytometry were recorded after 40 days of treatments. Analysis was done based on factorial experiment in completely randomized design with two factors of treatment time (48 and 72 hrs of colchicine treatment) and its concentration include 0.05, 0.1, and 0.2 % of colchicine. Control treatment was no application of colchicine. For each treatment, 3 replications were considered. All data were analyzed with JMP4 software.

3. Results

3.1. Survival of treated plants

The effect of colchicine on seedling viability was determined about one month after treatment. Application of different concentrations of colchicine and treatment time did not have a significant effect on plant survival and 100% survival was observed in treated plants, so no statistical analysis was done in this section. Although some plants initially showed pallor. But, their recovery power was very good (Fig. 1). Seedling growth retardation was the first visible effect in colchicine treatment. The treated seedlings showed a growth delay of about 10 days compared to the control plants and the phenotype of pallor and deformation and callus-like texture was common in the treated samples. About 40 days after the beginning of growth, the treated samples had shorter stems than the control (Fig. 1).

3.2. Differences in appearance of diploid and polyploid plants

Differences in the appearance of treated plants compared to diploid ones, were mentioned but no data were recorded and analyzed. Maybe the appearance could be a parameter in the prediction of putative tetraploids. In most tetraploid and mixoploid plants, the early leaves had an abnormal appearance but the later leaves created at the treatment site had a normal appearance. Apparently leaf size and plant height were larger in polyploid plants, which was visually observable (Fig. 2). Morphological characteristics such as flower size, stem length and diameter, flowers, seeds, etc. have been mentioned as an indirect identification method, an easy, fast and reliable method, but it takes time for the plant to grow, but less certainty than by direct methods (chromosome counting and flow cytometry) [10].

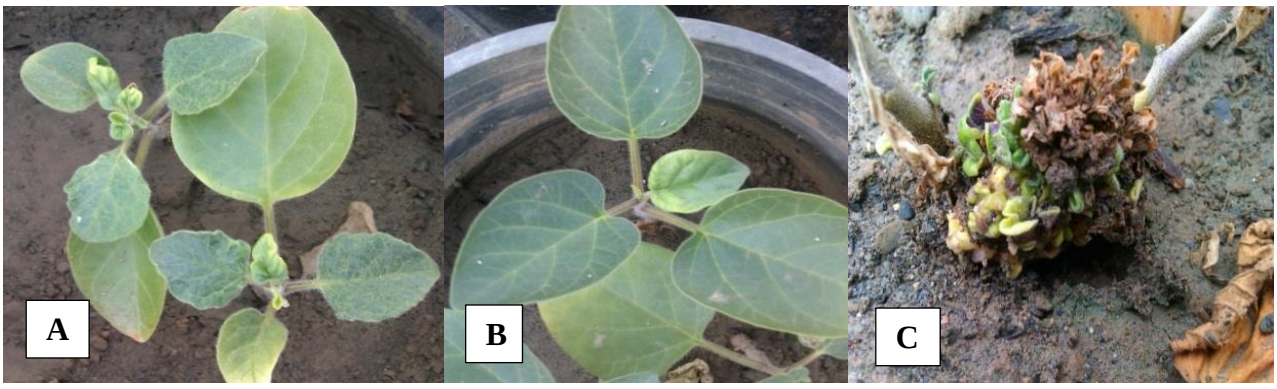

Fig. 1. Deformation of treated plants. Treated plant with 0.2 % colchicine and a duration of 72 hours (A) Diploid plant (B), Creating callus-like texture in the treatment site (0.2 % colchicine and a duration of 72 hours (C).

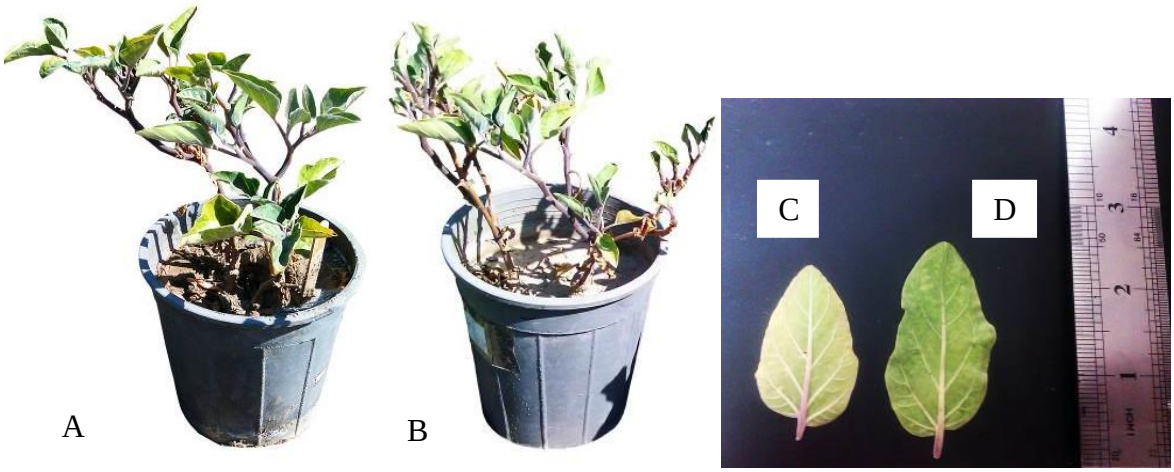

Fig. 2. Morphological changes in treated (0.2 % colchicine and a duration of 72 hours) (A) and control plants (B) and leaves of control (C) and treated plants (0.2 % colchicine and a duration of 72 hours) (D).

3.3. Difference in Stomata cells

The epidermal cells of tetraploid plants were larger than those of diploid plants. As can be seen in Fig. 3, the larger epidermal and stomata cells than diploid plants are quite evident, which can be considered a strong criterion in the diagnosis and isolation of *D. innoxia* plants with different ploidy levels.

In fact, it can be stated that increasing cell size is one of the general effects of polyploidy induction in *D. innoxia*. It appears to be a genetic effect and causes an increase in the size of organs such as leaves and stems. Increased cell size is one of the fastest and most widespread consequences of polyploidy [11].

In general, the results of morphological comparisons were according to other observations such as flow cytometry experiments, so they can be used as appropriate and efficient parameters to determine possible tetraploids.

3.4. Determination of chlorophyll and carotenoid content

According to the results, no significant difference was observed in the comparison of chlorophyll a, chlorophyll b and carotenoids in tetraploid and diploid plants (Table 1).

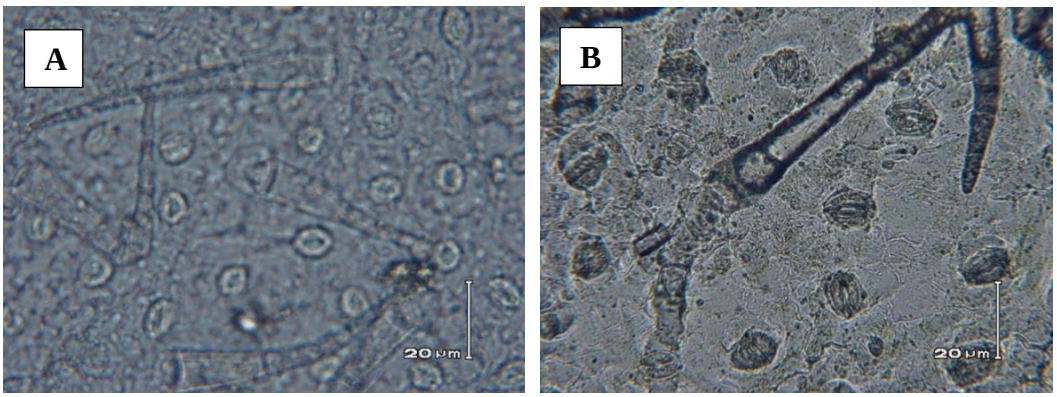

Fig. 3. Stomata cell size changes in diploid plant (A) and tetraploid (B). The same magnification is used in both images (40X)

Table 1. Levels of chlorophyll a, chlorophyll b and carotenoids in diploid and tetraploid plants of *Datura innoxia*

Ploidy level	Average of chlorophyll a (µg/ml)	Average of chlorophyll b (µg/ml)	Average of carotenoids (µg/ml)
Diploid	9.54a	3.67a	760.76a
Tetraploid	9.89a	3.92a	839.62a

3.5. Flow cytometry analysis

Flow cytometry analysis was carried out for all plants treated with colchicine and some diploid plants as control. Young leaf samples were used to increase the accuracy of flow cytometry analysis. The results showed the presence of plants with diploid, mixoploid and tetraploid levels (Fig. 4). The peaks in step G1 of cell division show the content of nucleus material which can be predicted the ploidy level. Fig. 4A is for diploid plants, Fig. 4B is for tetraploid plants, and Fig. 4C is for mixoploid plants. As can be seen in the Fig. 4, the G1 peak of tetraploid plants is located on channel 90, while the G1 peak of control sample shows the channel 40. The presence of G1 peak of tetraploid plants on channel 90 indicates that their DNA content is twice of diploid samples. On the other hand, mixoploid plants have shown peaks related to diploid and tetraploid plants at the same time, which indicates the presence of diploid and tetraploid cells in them.

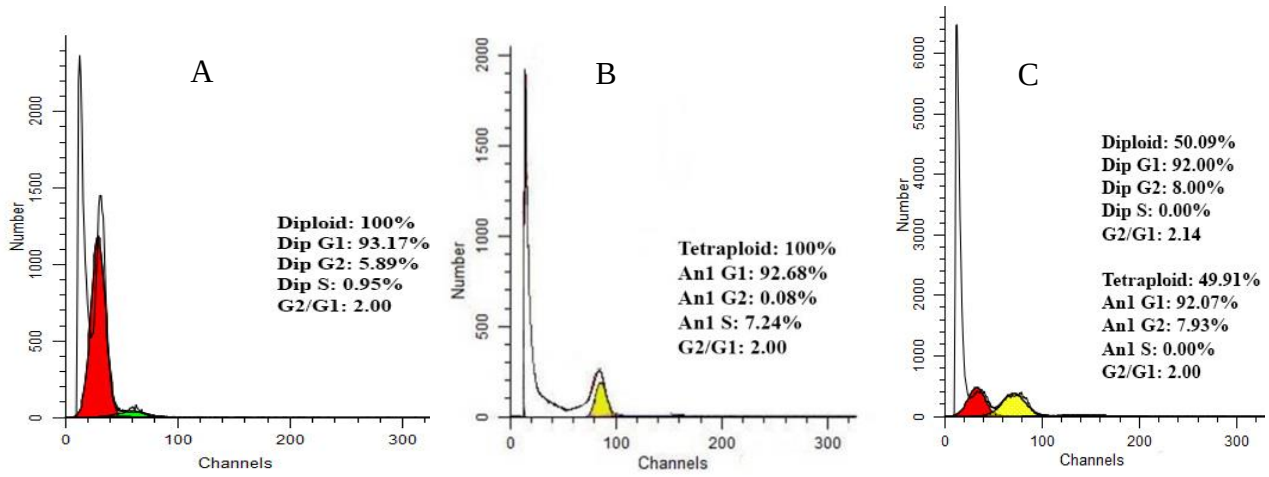

Fig. 4. Flow cytometry profiles of diploid (A), tetraploid (B) and mixoploid (C) plants

The results of flow cytometry analysis showed the presence of plants with diploid, mixoploid and tetraploid levels. According to the obtained curves for all samples analyzed by flow cytometry, the highest mixoploidy rate with 80 % was in plants treated with 0.2 % colchicine concentration in 48 hours. Tetraploid plants were observed only at a concentration of 0.2 % colchicine and a duration of 72 hours at 13.33 %. The results of data analysis showed that different concentrations of colchicine and treatment time had a significant effect on the percentage of tetraploid induction, so that the highest probability of tetraploidy (13.3 %) at a concentration of 0.2 % and during a time of 72 h is observed (Fig. 5, 6, 7).

Eleven months later, the control plants entered the reproductive phase, but in the treated plants,

even after a month of waiting, no signs of entering the flowering phase were observed, so a suitable fertilizer program was applied for inducing of flowering phase, but no change was observed in the treated plants. Finally, it was concluded that colchicine treatment may have led to sterility of plants. Due to the fact that in most medicinal plants only leaves, stems and roots are used, sterility and non-production of seeds due to polyploidy induction are not as important as crops [12].

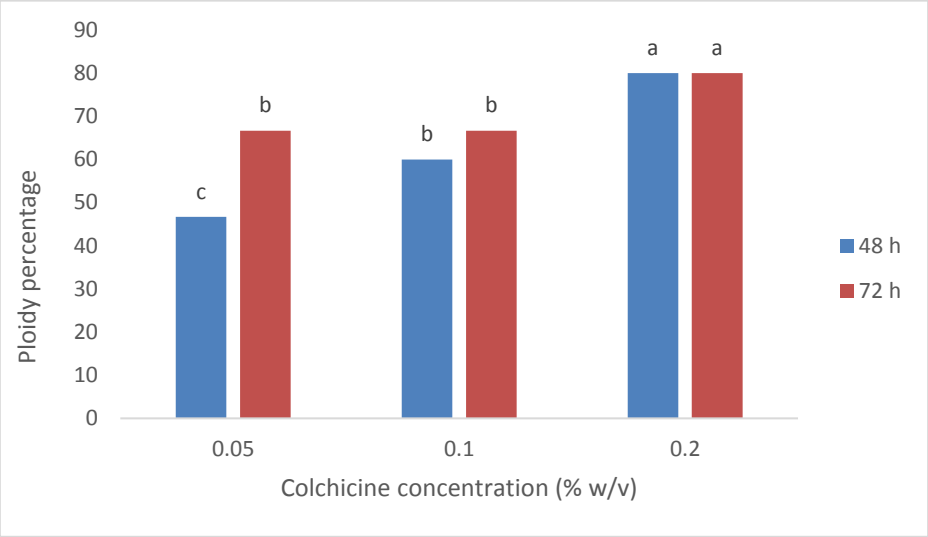

Fig. 5. The effect of time and different concentrations of colchicine treatment on polyploidy percentage of *Datura innoxia* plant

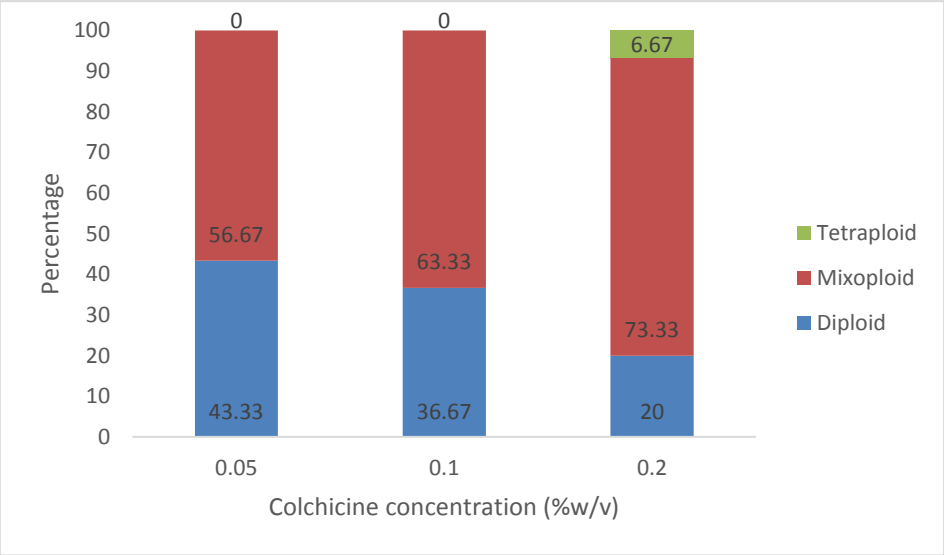

Fig. 6. The effect of different concentrations (% w/v) of colchicine treatment on the percentage of different ploidy levels (diploid, mixoploid, tetraploid) in *Datura innoxia* plant

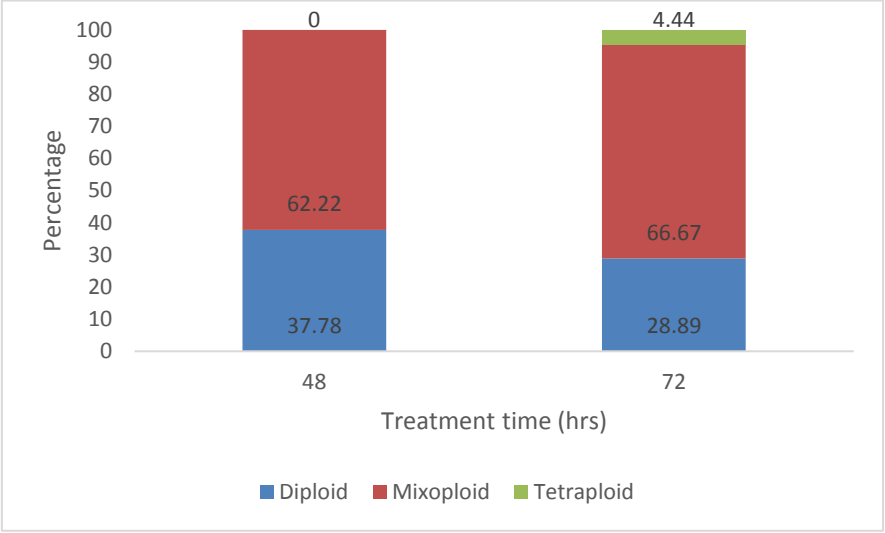

Fig. 7. The effect of time treatment on the percentage of different ploidy levels (diploid, mixoploid, tetraploid) in *Datura innoxia* plant.

In general, the results of this study showed that a concentration of 0.2 % and a duration of 72 hours is the best treatment for ploidy induction in *Datura innoxia* plants. However, in this experiment, treatment with colchicine resulted in sterility of the treated plants and none of these plants entered the reproductive phase (Fig. 8).

3.6. Hyoscyamine content

First, 20 µl of standard hyoscyamine methanolic solution with a concentration of 2500 ppm was injected into the device and a retention time of 4.8 minutes was obtained (Fig. 10A). Based on this time, the diagrams of other samples were interpreted. Then 20µl of the samples extracted from the leaves of diploid and tetraploid plants dissolved in methanol, were injected into the column and the area under the curve was compared to the peaks obtained in 4.8 minutes.

As can be seen, the area under the curve in the leaves of diploid plants was 4.09 mg / g (mg of hyoscyamine per gram of dry weight), and in the leaves of tetraploid plants showed 2.16 mg / g which indicates a 47 % decrease in hyoscyamine of tetraploid ones (Fig. 9 B, C).

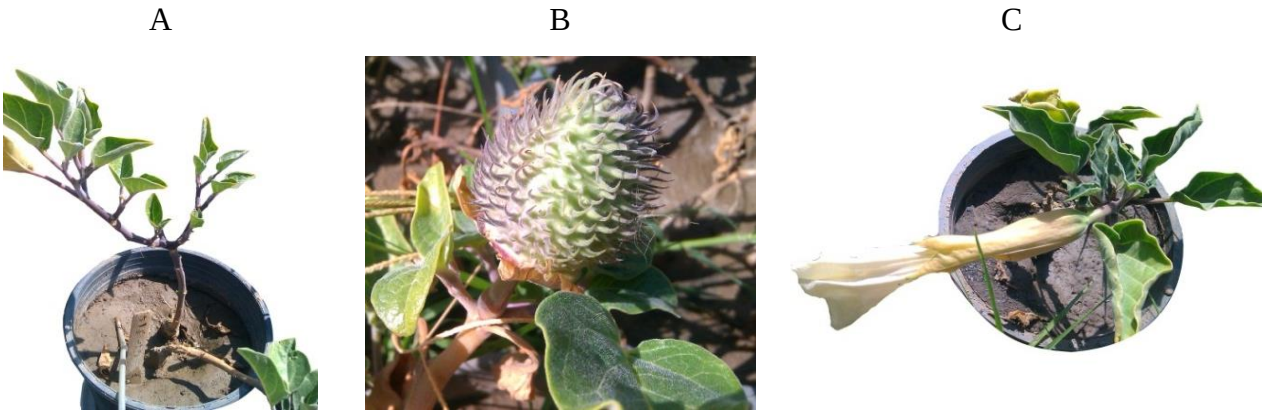

Fig. 8. Lack of flowers in tetraploid plant (A), formation of capsules containing seeds in control plant (B) and Flowering in control plant (C).

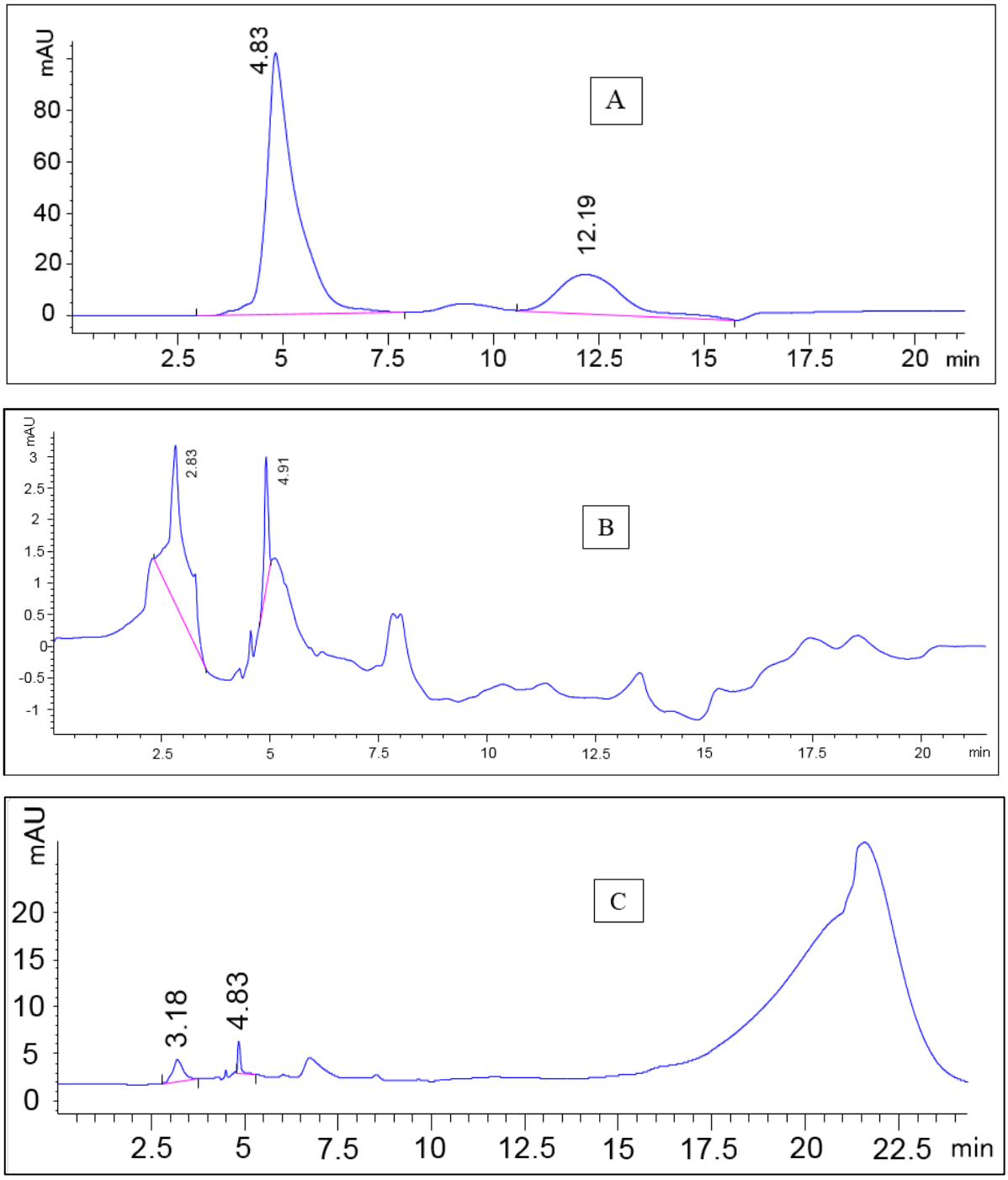

Fig. 9. Curve related to the hyoscyamine standard (A), HPLC analysis of tetraploid plant (B) and HPLC analysis of the diploid plants (C)

4. Discussion

All concentrations of colchicine used, had no effect on seedling viability and the best one for tetrapolidy induction was 0.2 % of colchicine and exposure time 72h. The effective concentration for ploidy induction in different plants has been reported between 0.0006 to 3 % and the effective concentration varies depending on the type of plant, treatment method and duration of treatment [13, 14].

It has been reported that high concentrations of colchicine, especially above 0.2 % in many plants, cause the death of all or a large number of samples, and this inverse relationship between colchicine concentrations and the survival of plants was observed in other plants [15, 16].

The low growth of plants may be due to physiological interference caused by colchicine, which leads to a decrease in cell division rate [17].

Tetraploid induction in *Eclipta alba* at in vitro condition with different concentrations and times of colchicine treatment showed that concentration of 0.1 % and duration of 24 hours of treatment on the shoot tip had the highest rate of tetraploid induction of 30 % and the composition of wedelolactone has increased about 2.3 times compared to diploid plants [18].

The size of tetraploid cells is usually about twice and their membrane area is about 1.5 times that of diploid cells, which causes the plants to grow larger [17]. The results of several reports have shown that the characteristics of the stomatal structure (abundance and length of stomatal guard cells) can be easily used to determine the ploidy level in plants. The use of stomatal characteristics is recommended as a cheap, easy and fast method to determine ploidy levels in different plant species. In all studied species, with increasing ploidy levels, the frequency of stomata (number per square millimeter) decreased, but the length of stomatal guard cells (microns) increased.

Stomatal length in safflower seedlings treated with colchicine was significantly larger than the stomatal length in control seedlings [19]. Also, tetraploid cells of Egyptian seedlings were larger than diploid samples. This was clearly evident in the stomatal guard cells [5]. In general, the size of stomatal guard cells is one of the most suitable factors in identifying tetraploid plants from diploids, but the factor is not 100% reliable, especially in identifying chimer samples (mixture of diploid and tetraploid) from pure tetraploid [11].

Colchicine treatment on seeds of *Stevia rebaudiana* between 0.05 to 0.2 % showed that different concentrations of colchicine had a significant effect on morphological traits such as plant height, number of leaves, number of shoots and stomatal density and increasing colchicine concentration often caused Increased traits [20]. Lavania [11] also reported sterility increase as one of the observed effects of tetraploid induction. This effect was also observed in tetraploid samples of Egyptian hyoscyamus [5].

Determination of chlorophyll content as a tool to detect ploidy levels in different species has been associated with different results [21, 22, 23].Chlorophyll content in autotetraploid plants of acacia has been reported to be 40 % higher than that of their diploid ones [22]. Omidbeigi et al. [24] reported that with increasing ploidy levels in basil, total chloroplast per guard cell also increased significantly. Therefore, it is inferred that the type of species and cultivar studied can also affect chlorophyll content at different ploidy levels [25].

As seen in this study, the use of new techniques such as flow cytometry can have many advantages. Manzoor et al. [12] reported that colchicine may not only induce tetraploidy, but also there is the possibility of mixoploids (chimers with tetraploid and diploid tissue) production. Their study also showed that the type of tissue used for flow cytometry analysis can affect the results and leaf tissue can be the best source of analysis when there is a need to determine the ploidy level accurately [12]. Therefore, it can be concluded that flow cytometry technique can clearly be used as a reliable tool to determine commercial genotypes

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and seedlings. In addition, it is able to quickly detect haploid, mixoploid, and polyploid cells after routine ploidy tests. Therefore, it saves money, energy and especially time.

In our experiment, the highest percent of tetraploid plants were observed only at a concentration of 0.2 % colchicine and a duration of 72 hours at 13.33 %. Effective concentrations for polyploidy induction vary in different plants. For example, in the chamomile plant, different concentrations of 0.1, 0.2, 0.5 and 1 % and for a period of 48 hours were used in the two-leaf stage and the results showed that 0.2 % colchicine solution is the best concentration for producing tetraploid plant [26]. Malekzadeh et al. [16] in the treatment of basil with concentrations of 0, 0.05, 0.1 and 0.2% and the duration of treatment in three levels of 6, 12 and 24 hours on the 6- to 8-leaf stage, showed the highest percentage of tetraploid plants (3.63%) was obtained in the concentration of 0.05%. Also, in the hyoscyamus plant using 0.1% of colchicine resulted in the highest percentage of tetraploid plants (7.2%) in the two-leaf stage [26].

Decreased hyoscyamine content in tetraploid plants may be due to increased h6h gene activity. In other words, tetraploidy may have accelerated and increased the conversion of hyoscyamine to scopolamine in tetraploid plants. The change in the metabolic profile of autoploids can be explained by the disruption of the metabolic mechanisms that regulate the biosynthesis of individual compounds. Autotetraploidy has increased enzyme activity in cells per milligram of protein in various systems. In *Todea barbara*, there is a direct relationship between gene dose and peroxidation activity . Similarly, this relationship has been reported for alcohol dehydrogenase and esterase in a number of plants [11]. The biosynthetic pathway of hyoscyamine is such that first ornithine is converted to tropin and phenylalanine is converted to tropic acid, and then from the esterification of tropin to tropic acid, hyoscyamine is produced, which is eventually converted to scopolamine after epoxidation [5]. Polyploidy increases the gene dose and activity of enzymes per cell [11]. Therefore, the change in the production of tropane alkaloids, especially hyoscyamine, may be due to the increased epoxidation of hyoscyamine to scopolamine.

Similar results have been reported by other researchers in other plants. In Egyptian hyoscyamus tetraploid seed plants, although the concentration of tropine, as a precursor of hyoscyamine, did not change compared to the diploid sample, but the production of hyoscyamine decreased and more scopolamine was produced, and the percentage of scopolamine was reduced to hippocampus. It has increased to about 6% to 38% [5]. Lavania reported a 30% decrease in hyoscyamine and an increase in the ratio of scopolamine to hyoscyamine due to tetraploid induction in *Datura stramonium* [11].

Application of Orizalin on *Thymus vulgaris* plant by 80 μM and 24-hour treatment increased the content of essential oil especially the content of thymol and carvacol increased by 18 and 0.5%, respectively [27]. In some cases, no effect on some compounds has been reported. For example, the use of colchicine at in vitro condition in *Centella asiatica* by concentration of 0.05 to 0.2% was effective in inducing tetraploidy, but no significant increase was observed in triterpene production [28]. In *Solanum bulbocastanum*, tetraploid induction with a combination of orizalin at a dose of 10 mg / l for 24 hours caused the content of metabolites and phenylpropanoid to have the same content or even less than the usual plant [29].

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5. Conclusion

The maximum percentage of tetraploidy according to flow cytometry analysis was obtained in concentrations of 0.2 % colchicine and 72 h treatment time but the survival percentage was not affected by different concentrations of colchicine. Apparently, increasing the stem diameter, size, thickness and number of leaves in tetraploid plants were observed but no significant differences were observed in the content of total chlorophyll and carotenoids. Contrary to expectations, a decrease in the content of hyoscyamine was observed in tetraploid plants compared to diploid plants. Therefore, although increasing ploidy level causes changes in vegetative traits, but did not have a positive effect on hyoscyamine production.

Author contributions

NM: Study supervision and data interpretation; PN: Experimental work and analysis; AB and SM: study supervision.

Conflict of interest

The authors declare that there is no conflict of interest.

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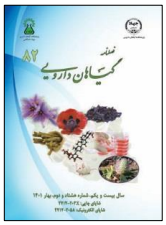

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مقاله تحقیقاتی

تغییرات در صفات مورفولوژیکی، بیوشیمیایی و فیتوشیمیایی گیاهان دیپلوئید و تتراپلوئید القایی داتوره تماشایی

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اطلاعات مقاله

گل‌واژگان:
کلروفیل
کروماتوگرافی مایع با کارایی بالا
هیوسیامین
متابولیت
پلی‌پلوئیدی

چکیده

مقدمه: گیاهان جنس داتوره طیف وسیعی از آلکالوئیدهای تروپانی با ارزش دارویی را تولید می‌کنند. گیاه داتوره تماشایی یکی از گونه‌های سمی جنس داتوره است و به دلیل داشتن آلکالوئیدهای مختلف دارای بوی نامطبوعی می‌باشد. هدف: این مطالعه با هدف القای پلی‌پلوئیدی با تیمار کلشی‌سین بر روی داتوره تماشایی و تأثیر آن بر ظاهر گیاه و محتوای هیوسیامین انجام شد. **روش بررسی:** به منظور القای تتراپلوئیدی در داتوره تماشایی، آزمایشی به صورت فاکتوریل با دو عامل غلظت کلشی‌سین (۰/۰۵، ۰/۱ و ۰/۲ درصد) و زمان تیمار (۴۸ و ۷۲ ساعت) با ۳ بوته برای هر تیمار با استفاده از گلوله پنبه‌ای آغشته به کلشی‌سین در نوک مریستم انتهایی گیاهان انجام گرفت. تغییرات مورفولوژیکی مشاهده شد و نزدیک به زمان گلدهی، گیاهان با فلوسایتومتری آنالیز شدند. **نتایج:** نتایج نشان داد که غلظت و زمان قرار گرفتن در معرض کلشی‌سین و اثر متقابل آنها بر درصد تتراپلوئیدی تأثیر می‌گذارد. بیشترین درصد تتراپلوئیدی در غلظت ۰/۲ درصد و ۷۲ ساعت مشاهده شد. از سوی دیگر، استفاده از غلظت‌های مختلف کلشی‌سین و زمان، تأثیر معنی‌داری بر بقای گیاه نداشت. تعداد سلول‌های محافظ روزنه کاهش یافت، اما تفاوت معنی‌داری در محتوای کلروفیل کل و کاروتنوئیدها مشاهده نشد. همچنین میزان هیوسیامین در گیاهان تتراپلوئید نسبت به دیپلوئید به نصف کاهش یافت. **نتیجه‌گیری:** به طور کلی، القای تتراپلوئیدی در این گیاه می‌تواند برخی از صفات فنوتیپی را تغییر دهد اما بر محتوای هیوسیامین تأثیر منفی دارد.

مخففها: HPLC، کروماتوگرافی مایع

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