Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2022) 35(3): 175-181
DOI: 10.29136/mediterranean.1067571

Morphological, physiological, cytological characteristics and agricultural potential of colchicine induced autotetraploid plants in safflower

Hasan BAYDAR, Ummu TUGLU

Department of Field Crops, Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Turkey

Corresponding author: H. Baydar, e-mail: hasanbaydar@isparta.edu.tr
Author(s) e-mail: ummutuglu123@gmail.com

ARTICLE INFO

Received: February 3, 2022
Received in revised form: July 22, 2022
Accepted: July 23, 2022

***Keywords*:**

Carthamus tinctorius
Autotetraploidy
Colchicine induction
Flow cytometer
Trait evolution

ABSTRACT

Safflower (*Carthamus tinctorius* L.) is one of the important oilseed and bio-energy crops. All of the safflower cultivars in the world have diploid genomes (2n= 2x= 24). In this research, autotetraploidy induction in safflower was performed by colchicine treatments to the emerging shoot tips at the cotyledonary stage. As a result of flow cytometric analyses performed in the C_2 progenies, autotetraploids (4x= 48) had DNA content of 4.88 pg $2C^{-1}$, while diploids (2x= 24) had 2.29 pg $2C^{-1}$. The autotetraploids in C2 generation exhibited bigger stomata size (33.40 µm to 46.90 µm in length) and a higher chloroplast number (9.5 to 17.2 in the guard cells), but less stomatal density (17.98% to 16.67% in index) compared to their diploid counterparts. However, autotetraploidy reduced the pollen viability from 80.24% to 16.20%, and seed set rate from 35.06% to 7.01% per capitula. As a result, autotetraploid plants were able to produce very few seeds despite the high unit seed size and weight in their heads. While oil content of the large-seeded autotetraploids was significantly lower, by two-fold, (26.37% to 13.23% in the whole seeds) than the small-seeded diploids, fatty acid composition was not significantly influenced by autopolyploidization.

1. Introduction

Safflower is one of the oldest cultivated annual crops in semi-arid and arid regions of the world for its seeds rich in omega fatty acids and flowers with high dyeing power. *Carthamus tinctorius* L., the only cultured species of safflower, carries the diploid genomes of its putative wild diploid progenitors, *C. oxyacanthus* Bieb (Knowles 1969) or *C. palestinus* Eig (Chapman and Burke 2007) without polyploidy. Hence all safflower varieties cultivated today have a diploid chromosome number (2n= 2x= 24) (Estilai 1971; Sheidai et al. 2009; Uysal et al. 2018). Although an *in vitro* study was carried out by Moghbel et al. (2015) to obtain artificial polyploid safflower, the results obtained from this research remained only at the experimental level.

Polyploidy refers to the presence of more than two complete sets of chromosomes per cell nucleus (Simmonds 1980). Polyploid plants carry multiple copies of alleles which can help increase allelic diversity and confer various evolutionary and adaptive advantages in comparison with diploid plants (Parisod et al. 2010). Based on numerous studies on artificially induced polyploidy by a potent mitotic inhibitor colchicine after the first treatment by Blakeslee and Avery (1937), it is most likely expected to be an enlargement or increment in both vegetative and generative cells, tissues and organs of the putative polyploids with a phenomenon described as gigas effect (Sattler et al. 2016).

However autoploidy induction can lead to high rates of multivalent pairing during meiosis and an associated reduction in fertility due to the production of aneuploid gametes (Acquaah 2012). Therefore autopolyploidy breeding is usually applied to the crops cultivated for their vegetative organs and those with vegetative propagation, due to the low rates of viable pollen and seed production (Levin 1983; Paterson 2005; Wang et al. 2016). The reduction in fertility is a hindrance to the use of induced autopolyploids, especially when the organs of interest are reproductive such as fruits or seeds (Dewey 1980). For example, incomplete or weak seed set remains the main constraint to the utilization of autotetraploid barley (Evans and Rahman 1990) and rye (Pfahler et al. 1987).

Carthamus tinctorius L. has less chromosome number (2n= 2x= 24) and haploid genome size (1.07 Gb) than many other plant species (Wu et al. 2021). The aim of the study was to establish an efficient system for generation of autotetraploid *Carthamus tinctorius* plants through colchicine induction and to identify their phenological, morphological, physiological, cytological and agricultural characteristics compared with its diploid parent. The findings from our study presented in this paper are also expected to be a fruitful resource for extensive investigation on evolutionary plant breeding.

2. Materials and Methods

2.1. Plant material

The seeds of a safflower (*Carthamus tinctorius* L.) cultivar "Olein" registered by the first author's breeding team in 2019 were used as genetic material. This diploid cultivar (2n= 2x= 24) was characterized with spiny capitulum, red flower color and oleic acid type.

2.2. Colchicine treatments and induced autopolyploidy

Safflower seeds were immersed in 70% ethanol for 1 min, followed by 10 mins of 10% NaOCl and then rinsed three times with sterile distilled water. The sterilized seeds were sown carefully in plastic seedling trays filled with sterile peat and grown in the growth chamber at 25±1°C until the emergence of cotyledeonal leaves. Small cotton swabs soaked in aquaepus colchicine solutions at the concentrations of 0% (used only pure water), 0.25%, and 0.5% (w/v) were placed on the emerging apical tip between two cotyledonary leaves, and the solution added in drops at regular intervals for 3 days, with 6 hours duration each day according to the cotton swab method explained in detail by Kushwah et al. (2018).

Colchicine treated and untreated (control) seedlings were transplanted in plastic pots with a 3:1 mixture of peat and perlite and cultured in a greenhouse under relatively consistent environmental conditions. From each colchicine treatments, 80 healty seedlings, at 4-6 true leaf stage, were selected and then transplanted to the experimental field on 22 March 2020. A total of 22 colchicine treated plants survived at only 0.25% concentration in C_1 generation, these were self-pollinated during the flowering period and then harvested at maturity stage for C_2 generation. The seeds of the individual C_1 plants were separately sown in 22 rows with 4 m length at 50x25 cm spacing on 29th April 2021. Thus diploid and putative autotetraploid plants were grown in the rows side by side based on the practices recommended for safflower cultivation. Each row was represented by 10 plants for identification and confirmation of autopolyploidy in C_2 generation.

2.3. Identification of autotetraploids by flow cytometric analysis

The ploidy levels and nuclear DNA contents of C_2 plants and their diploid parent "Olein" were detected by using a flow cytometry (CyFlow® Ploidy Analyser, Partec, Germany) equipped with an HBO lamp for UV. The fresh leaf tissues (0.5 cm^2) were evaluated for the flow cytometric analysis by using a commercial kit, the 'CyStain PI absolute P' nuclei extraction and staining kit (Partec GmbH, Munster) according to the manufacturer's instructions. The absolute core DNA content (pg) of the samples was calculated using the fluorescent intensities of the G1 peaks of the sample and the internal standard of common vetch (*Vicia sativa*). An ANOVA was performed on nuclear DNA content data by using the SPSS Ver. 15.0 software for Microsoft Windows (Nizam et al. 2020).

2.4. Confirmation of tetraploids by chromosome counting

Freshly grown root tips from the germinated seeds of diploid and autotetraploid plants, confirmed by flow cytometric analysis, were pretreated with 2 mmol L^{-1} of 8-hydroxyquinoline solution at 20°C for 2 h, and fixed in ethanol:acetic acid (3:1, v/v) at 4°C for 24 h. The fixed tips were then washed thoroughly in distilled water to remove the fixative solution, and hydrolyzed in 1 N HCl at 60°C for 5 min. After hydrolysis, root tips were stained with 2% aqueous aceto orcein (Sheidai et al. 2009; Uysal et al. 2018). The chromosomes at the metaphase plate were observed under an optical microscope (Zeiss Axiostar, Jena, Germany) with a 100x oil immersion objective.

2.5. Cytological observation of stomata, chloroplasts and pollens

The abaxial epidermis layers of diploid and autotetraploid leaves were observed for stomata measurements such as density, length and width (µm) through the light microscope (Nikon SE, Tokyo, Japan) at 400× magnification. The number of epidermis and stomata cells within a visual field of 0.196 mm^2 (objective 40×) were counted at the randomly selected 10 unit areas per leaf sample (Boso et al. 2016). Stoma index (SI) as a percent was calculated by the following formula: SI= [(Number of stomata per unit area) / (Number of stomata per unit area) + (Number of epidermal cells per unit area)] × 100 (Meidner and Mainsfield 1969). After the abaxial epidermal layers were placed on a microscope slide into a drop of 1% $AgNO_3$ with a cover glass, chloroplasts were counted in 10 pairs of stomatal guard cells at 400× magnification (Monakhos et al. 2014).

The length and width (µm) of pollen grains from the diploid and autotetraploid flowers were recorded on a microscope slide with the help of an ocular micrometer at 400x magnification (Pei et al. 2019). The Hemocytometric Thoma slide was used to estimate the pollen number per anther by way of a method explained in detail by Eti (1990). Pollen viability was carried out using 1% 2,3,5-triphenyltetrazolium chloride (TTC) stain. Among the pollen grains that were kept in a few drops of TTC on a slide for 3-4 hours, the reddish stained ones were recorded as viabile (Norton 1966).

2.6. Analysis of physiological, morphological and agricultural characteristics

In order to determine the leaf chlorophyll density at the rosette and anthesis stages, SPAD measurements with Minolta SPAD-502 (Chlorophyll Meter, Minolta Co. Ltd., Japan) were made on 10 randomly selected leaves from diploid and autotetraploid plants (Jiang et al. 2017). On the basis of changes in morphological and agricultural characteristics including plant height (cm) stem thickness (mm), leaf length and width (cm), corolla tube length (mm), flower weight per capitula (g), caputula number per plant, capitula size (cm), seed size (mm), seed weight per plant (g),1000 seed weight (g), seed hull thickness (mm) and seed hull content (%) were determined in both diploid and autotetraploid plants.

2.7. Determination of seed oil content and fatty acid composition

The seed oil content and fatty acid composition of diploid and autotetraploid plants were determined by Nuclear Magnetic Resonance (NMR, Bruker: mq one Total Fat Analyzer) and Gas Chromatography (GC-FID, Shimadzu GC-2025 with Teknokroma TR-CN100 capillary column) according to the methods described in detail by Erbas et al. (2016).

2.8. Statistical analysis

An analysis of Student's t-test (two sample t-test) was performed at the 5% significance ($P \leq 0.05$) level to compare diploid and autotetraploid plants (SAS 1998). Standard deviations (SD) of the means were calculated using Microsoft Excel 2010.

3. Results

As illustrated in Figure 1, two fold fluorescence intensity appeared in autotetraploid plants compared to that of G_1 nuclei of diploid plants. While diploid displayed a peak of DNA content at 518.0, the major peak representing the DNA content in autotetraploid was around 714.3. 2C nuclear DNA contents 2x and 4x plants were 2.29 and 4.89 pg $2C^{-1}$, respectively, suggesting that the induction of autotetraploidy was successful in *Carthamus tinctorius*. As a result of flow cytometric analyses performed in C_2 progenies of 22 putative C_1 plants, only one autotetraploid group, which was represented by 10 progenies, was detected in C_2 generation. So autopolyploidization efficiency in C_2 generation was 4.5% (1 of 22 C_1 plants from only 0.25% colchicine treatment).

Mitotic analysis confirmed that chromosome numbers in the somatic cells of diploids and autotetraploids were 2n= 2x= 24 and 2n= 4x= 48, respectively. However, counting 48 chromosomes in autotetraploids was very tedious due to the overlapping of chromosomes (Figure 1). Stomata, chloroplast and SPAD measurements were also performed for identification and confirmation of autopolyploidy as presented in Table 1. The autotetraploids in C_2 generation exhibited bigger stomata size (33.40 µm to 46.90 µm in length) and a higher chloroplast number (9.5 to 17.2 in the guard cells), but less stomatal density (17.98% to 16.67% in index) compared to their diploid counterparts ($P \leq 0.05$). Although autotetraploid plants had significantly higher leaf SPAD values at rosette leaf stage, the values at anthesis stage were not significantly different between both ploidy levels (Table 1).

When diploid and autotetraploid plants in C_2 generation were grown in the rows side by side in the same environmental conditions, autopolyploids exhibited thicker stems and leaves, longer and wider leaves, but produced a lower number of leaf per plant and the same height of the main stem compared to diploids (Table 2). Chromosome doubling caused 1.6 and 1.1 fold increase in leaf width and length, respectively as illustrated in Figure 2-C.

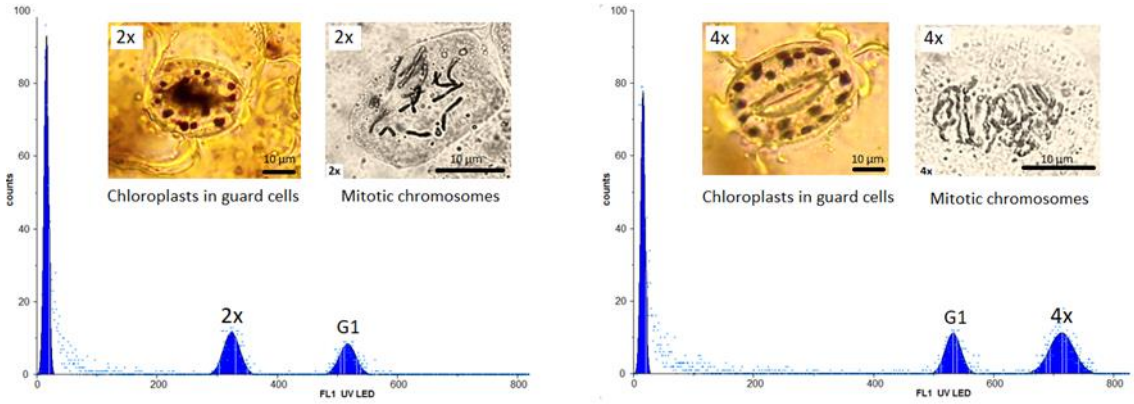

Figure 1. Flow cytometric histograms of the nuclear DNA contents, mitotic chromosomes in somatic cell and chloroplasts in guard cells in diploid (2n= 2x= 24) and autotetraploid (2n= 4x= 48) plants of *Carthamus tinctorius* L.

Table 1. Stoma and chloroplast characteristics, leaf SPAD values in diploid and autotetraploid plants

Characteristics	Ploidy levels		t- value
	Diploid (2x= 24)	Tetraploid (4x= 48)	
Stoma number in an unit area	23.30 ± 1.89§	10.20 ± 1.32	25.58*
Stoma index (%)	17.98 ± 0.89	16.67 ± 0.74	-4.73*
Stoma length (400x) (µm)	33.40 ± 2.91	46.90 ± 2.56	9.64*
Stoma width (400x) (µm)	24.80 ± 3.55	34.50 ± 2.07	10.69*
Chloroplast number in guard cells	9.50 ± 1.41	17.20 ± 1.40	13.13*
Leaf SPAD value at rosette stage	61.64 ± 4.54	83.51 ± 3.08	13.18*
Leaf SPAD value at anthesis stage	80.39 ± 4.63	87.34 ± 12.53	1.83

§ All values are means ± standard deviation (n= 10). * $P \leq 0.05$ according to Student's t-test.

Table 2. Certain plant and leaf characteristics of diploid and autotetraploid plants

Characteristics	Ploidy levels		t-value
	Diploid (2x= 24)	Tetraploid (4x= 48)	
Plant height (cm)	60.50 ± 3.02§	60.75 ± 6.41	0.09
Main stem thickness (mm)	7.66 ± 0.78	9.25 ± 1.04	2.91*
Leaf number per plant	24.63 ± 0.92	23.13 ±1.46	-5.85*
Leaf length (cm)	14.13 ± 1.43	15.58 ± 2.56	4.38*
Leaf width (cm)	4.51 ± 0.56	7.08 ± 0.38	10.12*
Leaf thickness (mm)	0.25 ± 0.06	0.35 ± 0.05	3.79*

§ All values are means ± standard deviation (n= 10). * $P \leq 0.05$ according to Student's t-test.

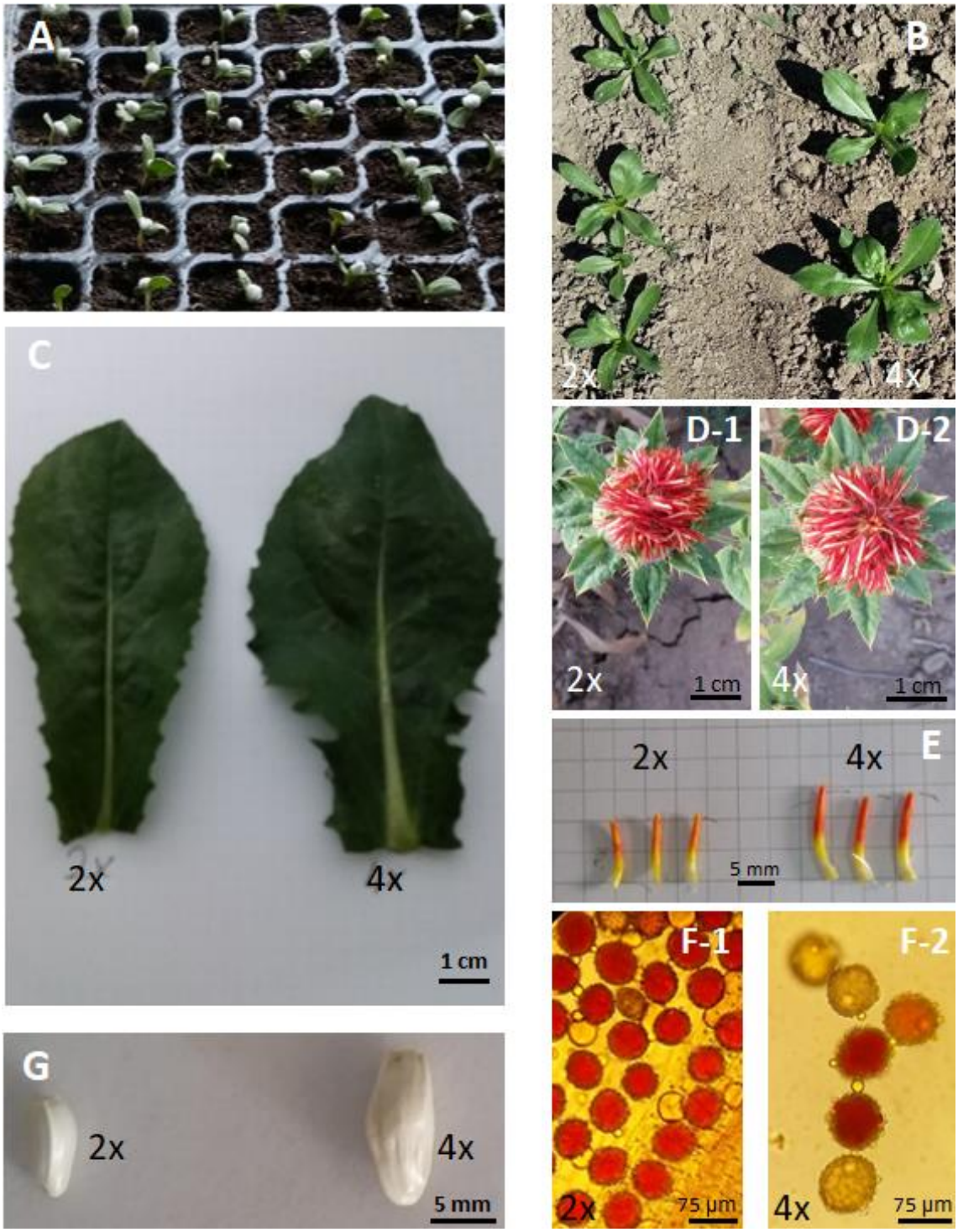

Figure 2. Cotton swap method (A), rosette growth stage (B), leaf size (C), capitulum size (D-1, D-2), corollo lenght (E), pollen size and viability in TTC test (F-1, F-2), and seed size (G) in diploid (2x) and tetraploid (4x) plants, respectively.

The autotetraploid plants remained longer in the rosette stage and started to bloom 2-3 days later than the diploids (Table 3). While there were not significant differences in capitulum number per plant (with an average of 5-6 heads) and flower number per capitulum (with an average of 100 flowers), the autotetraploid plants exhibited wider and longer flower parts. As a result, autotetraploid flowers had higher dry weight of flowers compared to diploids. For example, the length of corolla tube in the diploid and autotetraploid flowers was 12.43 mm and 16.80 mm, respectively (Table 3; Figure 2- E).

The pollen viability and productivity showed significant ($P \leq 0.05$) decreases with chromosome doubling in safflower (Table 3; F-1 and F-2 in Figure 2). The pollen viability as tested by 1% TTC was calculated as 80.24% for diploid and 16.20% for autotetraploid plants. In addition, the colchicine-induced autotetraploid plants showed significantly lower pollen grains per

anther than their diploids counterparts ($P \leq 0.05$). Another finding was that autoteraploid plants produced larger pollen than diploid plants (75.7 µm and 69.63 µm, respectively) (Table 3; F-1 and F-2 in Figure 2).

Although autotetraploid plants produced almost the same number but larger capitulum (D-1 and D-2 in Figure 2), diploid plants produced 79.04% more seeds and 93.15% more seed yield than autotetraploids (Table 4). Autotetraploid seeds were 2.7 times heavier, 1.5 times longer and 1.4 times wider than diploid seeds (Figure 2-G). In addition, higher hull (shell) thickness and lower seed germination rate of the autotetraploid safflower seeds were detected (Table 4).

The seed oil content of autotetraploid plants was significantly lower (26.37 to 13.23% in the whole seeds and 54.33 to 45.20% in the kernels) than the diploid ones ($P \leq 0.05$) (Table 5). The dramatic decrease in the oil content of large-seeded autotetraploids compared to small-seeded diploids was mainly due the the significant increase in the seed hull (achene shell) content (50.30 to 71.60%) (Table 4). However, there were not any significant differences among the fatty acid compositions of diploid and autotetraploid plants. The safflower oil contained two main unsaturated fatty acids, oleic and linoleic, which represent circa 90% of the total fatty acid content, while the remaining circa 10% corresponds to saturated fatty acids, palmitic and stearic. Oleic acid was the most abundant fatty acid which comprised 79.37% in the seed oil of diploid plants and 78.11% in the seed oil of autotetraploid plants. On the other hand, linoleic acid was found at the rates of 10.97% and 9.29% in the seed oils of diploid and autotetraploid plants, respectively (Table 5).

4. Discussion

Autopliploidization was successfully performed with colchicine treatment at 0.25% to the apical shoot tips between two cotyledonary leaves of the safflower seedlings. After confirmation of putative autotetraploids in C_2 generation, by way of flow ctytometric analysis and mitotic chromosome counting, polyploidization efficiency was only 4.5%. Autotetraploid genome (4x= 48) had about two fold higher DNA content than that of diploid genome (2x= 24). Autotetraploid safflower plants

Table 3. Certain floral characteristics of diploid and autotetraploid plants

Characteristics	Ploidy levels		t-value
	Diploid (2x= 24)	Tetraploid (4x= 48)	
First flowering days after sowing	59.6 ± 1.26[§]	62.8 ± 1.03	5.08*
Flower number per capitulum	97.01 ± 6.95	101.67 ± 7.64	0.64
Flower weight per capitula (g)	0.12 ± 0.02	0.18 ± 0.04	3.77*
Corolla tube length (mm)	12.43 ± 0.55	16.80 ± 0.10	7.74*
Pollen diameter (400x) (µm)	69.63 ± 3.93	75.7 ± 5.08	3.82*
Pollen viability (%)	80.24 ± 7.00	16.20 ± 6.30	-25.87*
Pollen number per anther	22000 ± 3771	9200 ± 2700	-7.56*

[§] All values are means ± standard deviation (n= 10). *$P \leq 0.05$ according to Student's t-test.

Table 4. Certain capitulum and seed characteristics of diploid and autotetraploid plants

Characteristics	Ploidy levels		t- value
	Diploid (2x= 24)	Tetraploid (4x= 48)	
Capitulum number per plant	5.13 ± 1.13[§]	5.63 ± 1.30	1.00
Capitulum diameter (cm)	2.53 ± 0.21	2.83 ± 0.10	3.38*
Seed number per capitula	34.01 ± 5.51	7.13 ± 4.02	-11.72*
Seed set rate (%)	35.06 ± 6.23	7.01 ± 5.49	-10.32*
Seed germination rate (%)	96.00 ± 3.27	66.67 ± 9.43	-3.19*
Seed yield per plant (g)	7.73 ± 2,63	0.53 ± 0.29	-7.10*
1000 seed weight (g)	27.75 ± 4.13	73.94 ± 24.64	5.19*
Seed length (mm)	7.10 ± 0.13	10.61 ± 0.50	20.51*
Seed width (mm)	3.94 ± 0.19	5.39 ± 0.48	7.89*
Seed hull content (%)	50.30 ± 4.04	71.60 ± 2.81	7.85*
Seed hull thickness (mm)	0.38 ± 0.02	0.75 ± 0.06	15.23*

[§] All values are means ± standard deviation (n= 10). *$P \leq 0.05$ according to Student's t-test.

Table 5. Seed oil and fatty acids content in diploid and autotetraploid plants

Characteristics	Ploidy levels		t- value
	Diploid (2x= 24)	Tetraploid (4x= 48)	
Oil content (%) in whole seed	26.37 ± 3.01[§]	13.23 ± 1.00	-13.03*
Oil content (%) in kernel	54.30 ± 2.56	45.20 ± 1.13	-7.81*
Palmitic acid (%)	7.27 ± 1.14	8.29 ± 0.37	1.37
Stearic acid (%)	1.56 ± 0.16	1.68 ± 0.35	0.49
Oleic acid (%)	79.37 ±3.10	78.11 ± 2.79	0.48
Linoleic acid (%)	10.97 ± 1.91	9.29 ± 1.59	-1.33
Linolenic acid (%)	0.81 ± 0.32	2.61 ± 1.42	2.25

[§] All values are means ± standard deviation (n= 10). *$P \leq 0.05$ according to Student's t-test.

had a smaller number of stomata per unit epidermal area and a greater number of chloroplasts in larger stomatal guard cells. These findings revealed that stoma size, stomata density and chloroplast number seem to be important morphological markers in order to distinguish ploidy levels. However, these morphological measurements may not always give accurate results to screen out pure tetraploids because of the chimeras (Jaskani et al. 2004). The SPAD value was not a reliable criterion to estimate the ploidy level in *Carthamus tinctorius*. To confirm this assumption, no significant differences were detected in the chlorophyll contents and net photosynthetic rates of diploid, autotetraploid and octoploid *Jatropha jurcas* plants (Niu et al. 2016).

Since autotetraploids produced similar main stem height and leaf number per plant, they exhibited more leaves, thicker stems and leaves plus longer and wider leaves. This result indicates that autopolyploidy promotes cell expansion rather than cell elongation in the leaves just as in the plant stem. The primary cell wall is largely composed of polysaccharides, such as cellulose, hemicellulose, lignin and pectin. A comparison of euploid series (2x, 4x, 6x, and 8x) in *Arabidopsis thaliana* showed that induced polyploidy had slower growth, enlarged cell size, and lower lignin and cellulose but higher pectin and hemicellulose in the stem (Corneillie et al. 2019). Autotetraploid safflower plants remained longer in the rosette stage, and started to bloom 2-3 days later than their diploid counterparts. Similar findings were obtained in the studies on colchicine-induced autotetraploid sunflower and barley (Evans and Rahman 1990, Srivastava and Srivastava 2002). It was expressed by Pei et al. (2019) that differences in endogenous phytohormone levels and flowering genes expression gave rise to delay flowering and bolting in polysomic tetraploids of radish (*Raphunus sativus*).

While there were not significant differences in capitulum number per plant and flower number per capitulum, autotetraploid flowers having larger and longer stamens and pistils had higher dry weight of flowers compared to diploids. Similarly, polyploid plants in *Arabidopsis thaliana* increased petal and sepal sizes in their flowers compared to diploids (Corneillie et al. 2019). Since safflower is a crop that is cultivated, not only for its seeds, but also for its flowers, autoteraploid varieties may be more advantageous than diploids in the safflower cultivation for only flower production.

Although colchicine-induced autotetraploid plants produced larger anthers and pollen grains, pollen numbers per anther were very low compared to diploids. On the other hand, the viability rates of the few pollens produced by tetraploids were also very low. Similarly, tetraploid plants of *Raphanus sativus* and *Helianthus annuus* had larger flowers and pollen grains, but lower pollen viability and germination rate than diploid plants (Limera et al. 2016; Srivastava and Srivastava 2002). The reduction in pollen fertility is a common consequence of autopolyploidy and may result from issues concerning the multivalent formation and meiotic irregularities (Acquaah 2012). Low pollen productivity and consequently low seed formation of autopolyploids can be eliminated by self-pollination and selection methods in advanced C generations (Baghyalakshmi et al. 2020; Singh 1992; Srivastava and Srivastava 2002).

As a result of the decrease in pollen number and viability by the autopolyploidy, it resulted in 80% lower seed set rate or seed forming efficiency. Similarly, the seed setting rate was reduced from 86% to 29% in sunflower autotetraploids due to the irregular pairings such univalents and multivalent of four homolog chromosomes during meiosis (Srivastava and Srivastava 2002). However, the seeds of autotetraploid safflower plants were heavier, longer and wider than the seeds of diploids. The seed weight of autotetraploids in *Ricunus communis* was also higher than their diploid counterparts (Baghyalakshmi et al. 2020).

In addition, the hull (shell) content and germination rate of achenes decreased with the chromosomal doubling in safflower. Perhaps the polysaccharides, such as cellulose, hemicellulose, lignin and pectin, that cause stem and leaf thickening caused similar thickening in the seed hull. Due to the high hull thickness and low seed germination rate of the large-seeded tetraploid plants, their seeds germinated on an average of 3 days later than the small-seeded diploids under the same field conditions. In fact, one reason for late flowering in the autotetraploid plants may be related to the thick-hulled large seeds which need more water absorption than the thin-hulled small seeds of diploid plants.

Safflower is primariliy a cultivated crop because of the high quality of edible oil in its seeds. Another area of interest of this research was to observe how the oil content and fatty acid composition would be in the seeds of the autotetraploids as compared to the diploid ones. Although autotetraploid plants produced a larger leaf area, higher chloroplast number and higher SPAD values, it was an unexpected result that their seed oil content was significantly lower by two fold in the whole seeds and 1.2 fold in the kernels than the diploid plants. This result is a considerable disadvantage for the industrial value of autotetraploid safflower. However, the fatty acid composition was not significantly affected by the autopolyploidization. Similarly, the autotetraploid plants of *Jatropha curcas* contained less oil in their seeds than diploids, mainly due to energy deficiency or unbalanced distribution (Niu et al. 2016). Since seed oil content has significant negative correlations with seed hull percentage (Rao et al. 1977) and seed size (Claassen et al. 1950), autotetraploid safflower seeds with higher hull contents had less crude oil than the diploid seeds.

5. Conclusion

After the identification and confirmation of autopolyploidy in C_2 generation by flow cytometric and cytological analysis, various phenological, morphological, physiological and agricultural characteristics of diploid and autotetraploid plants were comparatively evaluated during the growth and development stages. In brief, the results obtained from this research showed that the low seed yield and oil content of autotetraploids reduced their agricultural and industrial potential. These negative features can be eliminated by self-pollination and effective selection methods in advanced C generations. Also, further genetic studies, such as interspecific gene transfer and ploidy manipulation through genetic engineering, marker-assisted selection (MAS), and CRISPR/Cas9 genome editing, are needed to improve their agricultural prospects. Consequently, our research provides important findings in terms of revealing possible problems that may be encountered in the process of safflower breeding with chromosome doubling.

Acknowledgments

We would like to thank Prof. Dr. Metin Tuna from Tekirdağ Namık Kemal University for flow cytometry analysis.

References

Acquaah G (2012) Principles of Plant Genetics and Breeding (2nd Edition). Wiley-Blackwell, UK: John Wiley & Sons.

Baghyalakshmi K, Shaik M, Mohanrao M, Shaw R, Lavanya C (2020) Development and characterization of tetraploid castor plants. Characterization and Utilization 18: 98-104.

Blakeslee AF, Avery AG (1937) Methods of inducing doubling of chromosomes in plants by treatment with colchicine. The Journal of Heredity 28: 393-411.

Boso S, Gago P, Alonso-Villaverde V, Santiago JL, Martinez MC (2016) Density and size of stomata in the leaves of different *Vitis vinifera* varieties. Vitis 55: 17-22.

Chapman MA, Burke JM (2007) DNA sequence diversity and the origin of cultivated safflower (*Carthamus tinctorius* L.; Asteraceae). *BMC* Plant Biology 7: 60.

Claassen C, Ekdahl WG, Severson GM (1950) The estimation of oil percentage in safflower seed and the association of oil percentage with hull and nitrogen percentages, seed size, and degree of spineless of the plant. Agronomy Journal 42: 478-482.

Corneillie S, De Storme N, Van Acker R, Fangel JU, De Bruyne M (2019) Polyploidy affects plant growth and alters cell wall composition. Plant Physiology 179: 74-87.

Dewey DR (1980) Some applications and misapplications of induced polyploidy to plant breeding. Plenum Press, New York, pp. 445-470.

Erbas S, Tonguc M, Sanlı A (2016) Variations in the agronomic and quality characteristics of domestic and foreign safflower (*Carthamus tinctorius* L.) genotypes. Turkish Journal of Field Crops 20: 110-119.

Estilai A (1971) Cytogenetic studies of *Carthamus* species (Compositae) with eleven pairs of chromosomes. PhD, University of California, Davis, USA.

Eti S (1990) A practical method in used determination pollen production level. Journal of Çukurova University Agriculture Faculty 5: 49-58.

Evans GM, Rahman MM (1990) The basis of low grain yield and infertility in autotetraploid barley (*Hordeum vulgare*). Heredity 64: 305-313.

Jaskani MJ, Kwon SW, Koh GC, Huh YC, Ko BR (2004) Induction and characterization of tetraploid watermelon. Korean Society for Horticultural Science 45: 60-65.

Jiang C, Johkan M, Hohjo M, Tsukagoshi S, Maruo T (2017) A correlation analysis on chlorophyll content and SPAD value in tomato leaves. HortResearch 71: 37-42.

Knowles PF (1969) Centers of plant diversity and conservation of crop germplasm – Safflower. Economic Botany 23(4): 324-329.

Kushwah K S, Verma R C, Patel S, Jain NK (2018). Colchicine induced polyploidy in *Chrysanthemum carinatum* L. Journal of Phylogenetics & Evolutionary Biology 06. doi: 10.4172/2329-9002.1000193.

Levin DA (1983) Polyploidy and novelty in flowering plants. American Naturalist 122: 1-25.

Limera C, Wang K, Xu L, Wang Y, Zhu X (2016) Induction of autotetraploidy using colchicine and its identification in radish (*Raphanus sativus* L.). The Journal of Horticultural Science and Biotechnology 91: 63-70.

Meidner H, Mansfield TA (1969) Physiology of Stomata. Mc Graw-Hill, Newyork, USA.

Moghbel N, Borujeni MK, Bernard F (2015) Colchicine effect on the DNA content and stomata size of *Glycyrrhiza glabra* var. *glandulifera* and *Carthamus tinctorius* L. cultured *in vitro*. Journal of Genetic Engineering and Biotechnology 13: 1-6.

Monakhos SG, Nguen ML, Bezbozhnaya AV, Monakhos GF (2014) A relationship between ploidy level and the number of chloroplasts in stomatal guard cells in diploid and amphidiploid *Brassica* species. Sel'skokhozyaistvennaya Biologiya 5: 44-54.

Niu TY, Chena M, Fua Q, Donga Y, Hea H (2016) Identification and characterization of tetraploid and octoploid *Jatropha curcas* induced by colchicine. Caryologia 69: 58-66.

Nizam I, Gulcu R, Savas Tuna G & Tuna M (2020) Determination of nuclear DNA content and ploidy of some *Bromus* L. germplasm by flow cytometery. Pakistan Journal of Botany 52: 3. doi: 10.30848/PJB2020-3(12).

Norton JD (1966) Testing of plum pollen viability with tetrazolium salts. American Society of Horticultural Science 89: 132-134.

Parisod C, Holderegger R, Brochmann C (2010) Evolutionary consequences of autopolyploidy. The New Phytologist 186: 5-17.

Paterson AH (2005). Polyploidy, evolutionary opportunity, and crop adaptation. Genetica 123: 191-196.

Pei Y, He N YL, Deng D, Li W, Zhang W (2019) Comparative study of the morphological, physiological and molecular characteristics between diploid and tetraploid radish (*Raphunas sativus* L.). Scientia Horticulturae 257: 108739. doi: 10.1016/j.scienta.2019.108739.

Pfahler PL, Barnet RD, Luke HH (1987) Diploid/tetraploid comparisons in rye. Crop Science 27: 431-435.

Rao V, Ramachandram M, Arunachalam V (1977) An analysis of association of components of yield and oil in safflower (*Carthamus tinctorius* L.). Theoretical and Applied Genetics 50: 185-191.

SAS (1998) SAS Introductory Guide, 3rd Edition, NC, USA, pp. 99.

Sattler MC, Carvalho CR, Clarindo WR (2016) The polyploidy and its key role in plant breeding. Planta. doi: 10.1007/s00425-015-2450-x.

Sheidai M, Sotoode M, Nourmohammadi Z (2009) Chromosome pairing and cytomixis in safflower (*Carthamus tinctorius* L.) cultivars. Cytologia 74: 43-53.

Simmonds NW (1980) Polyploidy in plant breeding. SPAN 23: 73-75.

Singh RN (1992) Chromosomal abnormalities and fertility in induced autotetraploid *Helianthus annuus* in C_1 and C_2 generation. Cytologia 57: 277-281.

Srivastava R, Srivastava G (2002) Autopolyploids of *Helianthus annuus* L. var. morden. Cytologia 67: 213-220.

Uysal T, Tekkanat BS, Şimşek Sezer EN, Ada R, Bozkurt M (2018) Karyotype analysis of some lines and varieties belonging to *Carthamus tinctorius* L. species. Anatolian Journal of Botany 2: 1-9.

Wang X, Cheng ZM, Zhi S, Xu F (2016) Breeding triploid plants: a review. Czech Journal of Genetics and Plant Breeding 52: 41-54.

Wu Z, Liu H, Zhan W, Yu Z, Qin E (2021) The chromosome-scale reference genome of safflower (*Carthamus tinctorius*) provides insights into linoleic acid and flavonoid biosynthesis. Plant Biotechnology Journal 19: 1725-1742.