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A comparative study of morphological characteristics in diploid and tetraploid (auto and allotetraploids) *Citrullus* genotypes

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ABSTRACT

In this study, ploidy levels were determined by stomatal observations and flow cytometry analysis of plants polyploidised by the application of 0.05% colchicine to seedlings at the first true leaf stage. In the study of developing polyploid watermelon rootstocks, the survival rate of the plants was 77%, and the polyploidisation rates were 11% and 3% according to stomatal observations and flow cytometry analysis, respectively. According to the results of flow cytometry, 22 polyploid genotypes were determined. Auto- (12) and allotetraploids (10) of *Citrullus* genotypes were developed, and their plant growth performance was determined in hydroponic culture in comparison with diploids, commercial rootstocks (RS841, 'Argentario') and watermelon cultivar ('Crimson Tide'). Putative tetraploids and their diploid controls were grown in hydroponic culture for 21 days, and their vegetative growth performances were determined. The results showed that the increases in plant biomass depending on polyploidisation were 100% in autotetraploids and 156% in allotetraploids as compared to diploid controls.

Keywords: hydroponic culture, ploidy levels, rootstock, tetraploid and watermelon

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], a member of the Cucurbitaceae family and a fruit-bearing vegetable, was the second most produced vegetable after tomato worldwide, with a production of 101,620,420 tons in an area of 3,053,258 ha in 2020 (FAOSTAT, 2022). Watermelon fruit is a rich source of water and health-beneficial nutrients for humans (sugars, minerals, amino acid, organic acids, carotenoids, lycopene, etc.) (Akashi et al., 2017; Guo et al., 2019). In general, the phytochemical content of 100 g of fresh watermelon juice is energy (30–46.2 Kcal), carbohydrates (7.6–11.6 g), protein (0.6–0.9 g), total fat (0–0.15 g), vitamin A (569–864.88 IU), vitamin C (8.1–12.31 mg), lycopene (3.38–11.34 mg), ash (5.2–5.4%), water (93.12–95.2%), calcium (7 mg), iron (0.24 mg), magnesium (10 mg), potassium (112 mg) and phosphorus (11 mg) (Fila et al., 2013; Oberoi and Sogi, 2017; USDA, 2022).

The first principle of producing high-yielding and quality watermelon is to grow healthy seedlings and plants. In the production of vigorous and healthy seedlings, biotic and abiotic factors in the growing environment, genotype, fertilisation, growing conditions of the mother plant of seed, harvest maturity stage of the seed, seed processing techniques, seed moisture content during storage and seed quality are effective factors.

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In addition, plant growth and vigour can be increased by using varieties or rootstocks with strong roots that provide more water and plant nutrients, synthesise hormones and similar substances that increase plant strength and health (Kovalev and Lisovskaya, 1989; Kovalev, 1990; Ra et al., 1995; Fernández-García and Martínez-Arbelaiz, 2002; Yarşi and Sari, 2006). This can only be achieved either with genotypes with strong roots or by grafting onto suitable rootstocks with a vigorous root system (Yetisir and Sari, 2003; Lee and Oda, 2010).

The grafting in fruit-bearing vegetables is becoming a well-developed practice with many agricultural advantages. The primary starting point for grafting vegetable crops is to prevent damage caused by soil-borne pests and pathogens (Oda, 2002; Yetisir and Sari, 2003). However, in the last few decades, it has also been reported that grafting of vegetable crops improves tolerance to abiotic stresses such as drought, low soil temperature and salinity, the efficiency of water and nutrient use, and fruit yield and quality (Shimada and Nakamura, 1977; Romero et al., 1997; Nisini et al., 2002; Oda, 2002; Rivero et al., 2003; Lee and Oda, 2010; Edelstein et al., 2011). Grafting onto suitable rootstocks can contribute to global food security by enabling the cultivation of watermelon and other fruiting vegetables under stressful environmental and soil conditions.

Although utilisation rates vary, the most common commercial rootstocks for watermelons are *Cucurbita* interspecies hybrids (*C. moschata* Duch. × *C. maxima* Duch.) and bottle gourd (*Lagenaria siceraria* Standl) hybrids. It should be noted that the decrease in fruit quality does not represent a general trend but depends on the specific rootstock–scion interaction under specific growing conditions (Yetisir and Sari, 2003). Therefore, it has been recommended that appropriate rootstock–scion combinations and rootstock–scion–growing condition interactions should be determined to prevent these quality deteriorations in the cultivation of watermelon grafted onto *Cucurbita* and *Lagenaria* rootstocks (Davis et al., 2008; Karaca et al., 2012).

One way to solve the quality problems in grafted watermelon production mentioned earlier could be to use rootstocks developed from the *Citrullus* genus (Edelstein et al., 2014). An important promising alternative rootstock candidate for watermelon is *Citrullus lanatus* var. *citroides* (LH Bailey) Mansf. ex Greb., which is also known citron melon (Fredes et al., 2016). The *citroides* group is an ancient cultivar group originating from southern Africa that can be found globally today and is generally considered the ancestor of cultivated sweet watermelons (Paris, 2015). Citron melons are grown worldwide mainly for animal food and fruit preserves. Resistance to nematodes, expressed as less galling than that of *Cucurbita* hybrids and bottle gourd rootstocks (Thies et al., 2016), and *Fusarium* wilting (Patrick Wechter et al., 2012) has been reported in some citron melon accessions, suggesting that this group is a promising alternative source of rootstock for managing root-knot nematodes (RKNs) and fusarium wilt in watermelon. It has been reported by different researchers that the use of citron rootstock in the production of grafted watermelon can be an interesting alternative to the currently used commercial rootstock (Edelstein et al., 2014; Levi et al., 2014; Fredes et al., 2016). The use of citron rootstocks slightly reduced the total yield but improved the aroma compounds, in particular dry matter content, soluble solids and acidity, and has no serious negative effects on other morphometric and textural characteristics of watermelon fruit (Edelstein et al., 2014; Levi et al., 2014; Fredes et al., 2016; Bigdelo et al., 2017).

The most important disadvantage of potential citron genotypes as rootstocks for watermelon is that they have slightly lower plant vigour than *Cucurbita* and *Lagenaria* rootstocks. The vigour of citron plants can be improved by using approaches such as crossing citron genotypes with a high special combining ability (heterosis) or the generation of auto- or allopolyploids using the polyploidy breeding method. Artificially produced polyploids exhibit new characteristics such as larger tissues and organs; higher secondary metabolite activity; increased yield; higher chlorophyll, lycopene, fructose and glucose contents; and higher tolerance to abiotic and biotic stress conditions than diploid plants in some species (Jaskani et al., 2005; Zhang et al., 2010; Sattler et al., 2016; Soltis et al., 2016; Doyle and Coate, 2018; Zhu et al., 2018). Levi et al. (2014) reported that seedless (triploid) watermelon cultivars produced higher yield when grafted onto autotetraploid *Citrullus lanatus* var. *citroides* rootstocks than those grafted onto commercial *Cucurbita* or *Lagenaria* rootstocks. According to the extant literature, studies on autotetraploid watermelon rootstocks are very limited, and there are no studies on allotetraploid rootstocks. Therefore, in this study, it was aimed to produce allotetraploids of cultivated watermelon and *Citrullus lanatus* var. *citroides* and to investigate their rootstock potential for watermelon.

MATERIALS AND METHODS

Production of tetraploids

The study was carried out in Kırşehir Ahi Evran University, Agricultural Research and Application Greenhouse. The list of plant materials applied with colchicine is presented in Table 1. Cultivated watermelon ('Calhoun Gray') and *Citrullus lanatus* var. *citroides* (W1482, W1832 and W2001) and their hybrids were produced in 2020 by selfing and crossing under Kırşehir conditions. The seeds of the genotypes (Table 1) to be applied with colchicine were sown in multiple pots filled with a mixture of peat and perlite at a ratio of 2:1 (v/v). The seedlings were grown until

Table 1. List, genotype codes and numbers of plant materials applied with colchicine.

Genotype	Genotype code	Plants used in colchicine application (number)
Calhoun Gray (*Citrullus lanatus*)	C	180
W1482 (*Citrullus lanatus* var. *citroides*)	N3	210
W1832 (*Citrullus lanatus* var. *citroides*)	N5	210
W2001 (*Citrullus lanatus* var. *citroides*)	N7	180
CxW1482	CxN3	210
CxW1832	CxN5	180
CxW2001	CxN7	210
Total		1,380

they reached the first true leaf stage under controlled greenhouse conditions. The roots of the seedlings at the first true leaf stage were washed and cleaned from the growing medium, and the seedlings were dipped in a 0.05% colchicine solution in the dark at 24 °C for 16 hr. After the plants were removed from the colchicine solution, they were washed with sterile distilled water three times to remove colchicine from the surface of the tissues, and the seedlings were replanted in multiple pots filled with the same medium used in seedling production. Replanted seedlings were kept in clear plastic containers at a relative humidity of 80%–90% and a temperature of 22–25 °C under 50% shade conditions for 5–7 days for re-establishment of the seedlings. The number of seedlings treated with colchicine from each genotype is given in Table 1. The numbers of seedlings treated with colchicine were 180 in C, N7 and CxN5 and 210 in N3, N5, CxN3 and CxN7. A total of 30 days after colchicine application, the stomatal diameter (µm), stomatal length (µm), stomatal density (number · mm^{-2}) and chloroplast number in guard cells were determined in diploid and possible polyploid plants, and flow cytometry analysis was performed in plants with stomatal differentiation.

Ploidy-level determination

Stomatal measurements

The stoma dimension (length and diameter) and density and the number of chloroplasts in guard cells were determined in the lower epidermis of colchicine-treated plant leaves. For this purpose, the fifth and sixth fully developed leaves developed after colchicine application were used. Under a light microscope, the diameter and length of four stomata per leaf were measured magnifying by 40 × 10 objective and oculars, respectively. Obtained values were multiplied by 2.439, a coefficient obtained by adjusting the ocular micrometre, so as to obtain the true length of the stomata. The chloroplast number in each of the two guard cells of stomata was scored under a light microscope. Measurements and counts were performed in two leaves from each genotype and in 10 stomata in two samples from each leaf. The density of stomata was determined as a number of stomata · mm^{-2}. Stomatal measurement and chloroplast count were performed following the procedure described by Sari et al. (1999) and Yetisir and Sari (2003).

Flow cytometry analysis

Flow cytometry analysis was performed to determine the ploidy levels of the plants found to be different as a result of stomatal observations. Flow cytometry analysis was carried out in the Department of Field Crops, Faculty of Agriculture, University of Tekirdağ. Samples were obtained from plant leaves for flow cytometry analysis, as reported by Tuna et al. (2016). The ploidy levels were examined by using a Partec CyFlow Space (Partec GmbH, Münster, Germany) flow cytometer. Nuclei were isolated from each plants leaves following the protocol described by Tuna et al. (2016). Flow cytometry analyses were performed using a Partec kit as per the manufacturers' instructions (Aleza et al., 2009; Tuna et al., 2016). Diploid control samples (cell nucleus isolates) were prepared from leaves of original diploid watermelon plants.

Seed production, seed and seedling properties

Colchicine-applied plants, which were determined to be tetraploid by stomatal measurements and flow cytometry analysis, were planted in Kırşehir Ahi Evran University, Agricultural Research and Application area. Seeds were produced by selfing in each plant grown with regular cultural practices for watermelon. Plants obtained as a result of colchicine application have monoecious flower types, and the fruit set rate of self-fertile flowers is high. Thousand seed weight is the weight of 1,000 seeds in grams.

The seeds used in the germination test were harvested from the fruit produced by selfing in diploids and tetraploids. For each genotypes, 30 uniform seeds were used in the germination test, and the germination rate was determined on the 12^{th} day of incubation. Seeds were placed between two layers of wet filter paper in 11-cm petri dishes and incubated at 24 °C in the dark. The germination response was scored visually as radicle emergence. In 25-day-old seedlings, the cotyledon width (mm), cotyledon length (mm) and hypocotyl diameter (mm) were measured using a digital calliper.

Comparison of vegetative development of auto- and allopolyploid genotypes in hydroponic culture

Plant materials used in hydroponic experiments are auto- and tetraploids, original diploid parental lines, commercial rootstocks (RS841 and Argentaio) and watermelon cultivar (Crimson Tide). Commercial *Cucurbita* and *Lagenaria* rootstocks and watermelon cultivars were used for comparison. A list of plant materials used in this experiment is given in Table 2. Hydroponic culture tests were carried out in Kırşehir Ahi Evran University's fully automated (fan-pad cooling, high-pressure fogging, heat screen, circulation fan and geothermal heating) Venlo-type glass R&D greenhouse. The seeds of the genotypes to be used in the hydroponic culture experiment were sown in multiple pots filled with a peat–perlite (2:1) mixture with an electrical conductivity of 0.4 ds · m^{-1} and a pH of 5.8. After germination, seedlings were grown in a greenhouse at 24/18 (day/night), a temperature of 25 °C and a relative humidity of 60%. The seedlings were irrigated daily and fertilised with Hoagland solution (Hoagland and Arnon, 1950), with an electrical conductivity of 1.5 dS · m^{-1} every 3 days.

Establishment of hydroponic culture

The seedlings with 2–3 true leaves were transplanted to 136-L plastic pots after cleaning from the growth substrate by washing with tap water on 3rd of March 2021. The upper surface of the pots was covered with Styrofoam, and the plants were placed in the holes drilled on the Styrofoam plate. The cultivation solution was constantly aerated with a pump. A total of 14 plants were grown in each pot. The nutrient solution contained 1,125 µM $Ca(NO_3)_2$, 375 µM $(NH_4)_2SO_4$ 750 µM K_2SO_4, 650 µM $MgSO_4$, 500 µM KH_2PO_4, 10 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.4 µM $CuSO_4$, 0.4 µM $MoNa_2O_4$ and 80 µM Fe EDDHA. The electrical conductivity of the cultivation solution was maintained at 1.50 dS · m^{-1} and the pH between 6.5 and 7 (Hoagland and Arnon, 1950). The experiment was designed according to the randomised plot design with three replications and three plants in each replication. The study was continued for 21 days under controlled greenhouse conditions (22–24 °C day/16–18 °C night and 60% relative humidity). The experiment was terminated on the 24th of March 2021.

Parameters measured in hydroponic culture

The plant stem diameter (PSD) (mm), plant stem length (cm), and shoot and root fresh and dry weights (g · $plant^{-1}$) were determined after harvest. The PSD was measured using a digital calliper 1–2 cm below the cotyledons and plant stem length was measured using a tape measure. After harvest, the fresh weights of roots and shoots were determined using a digital balance, with an accuracy of 0.01 (g · $plant^{-1}$). Their tissues were dried in a forced-air oven at 80 °C for 72 hr for dry biomass determination until a stable weight was achieved. Then, they were weighed on an electronic digital scale (g × $plant^{-1}$).

Determination of root length (RL) (cm · $plant^{-1}$), root diameter (RD) (cm^2 · $plant^{-1}$) and root volume (RV) (cm^3 · $plant^{-1}$)

The plant RL, RD and RV were measured by using special image analysis software Program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc., Québec, QC G1V 1V4, Canada) in combination with an Epson Expression 11000XL scanner. The root samples

Table 2. List of genotypes used in hydroponic culture.

Genotype code	Ploidy level	Genotype code	Ploidy level
M_1Calhon Gray	Tetraploid	M_1CXN5-1	Tetraploid
M_1N3	Tetraploid	M_1CXN7-1	Tetraploid
M_1N5-1	Tetraploid	M_1CXN7-2	Tetraploid
M_1N5-2	Tetraploid	M_1CXN7-3	Tetraploid
M_1N5-3	Tetraploid	M_1CXN7-4	Tetraploid
M_1N5-4	Tetraploid	M_1CXN7-5	Tetraploid
M_1N5-5	Tetraploid	Calhoun Gray	Diploid
M_1N5-6	Tetraploid	CXN3	Diploid
M_1N7-1	Tetraploid	CXN5	Diploid
M_1N7-2	Tetraploid	CXN7	Diploid
M_1N7-3	Tetraploid	N3	Diploid
M_1N7-4	Tetraploid	N5	Diploid
M_1CXN3-1	Tetraploid	N7	Diploid
M_1CXN3-2	Tetraploid	Crimson Tide	Diploid
M_1CXN3-3	Tetraploid	RS841	Diploid
M_1CXN3-4	Tetraploid	Argentario	Diploid

(~0.5 g) from each plant were placed in the scanner tray. Water was added using a plastic wash bottle, the roots were homogeneously spread across the tray, and the scanning and analysis were carried out using the WinRHIZO system's interface on a computer connected to the scanner. The total plant RL, RD and volume were then determined as the ratio of sampled root fresh weight (RFW) to the total RFW (Ulas et al., 2019).

Statistical analysis

Data from the hydroponic experiment were analysed by one-way analysis of variance (ANOVA) using SPSS version 18.0 at a 5% significance level (IBM, Chicago, IL, USA), and the means were separated using the Duncan test. Classification of genotypes was achieved by principal component analysis (PCA) using XLSTAT software (XLSTAT, New York, USA). Correlation analysis was performed between hydroponic culture parameters and DNA content and stomatal parameters, using SPSS software (IBM SPSS Statistics version 22.0, Chicago, IL, USA).

RESULTS AND DISCUSSION

Survival rate

The survival rate of the seedlings after the application of colchicine was calculated as a percentage and is given in Table 3. In the study, colchicine was applied to a total of 1,380 plants in seven genotypes; 858 of these plants survived, and the average survival rate was 62.17%. Depending on the application of colchicine, the survival rates of the seedlings varied from 52.38% to 87%. The N7 genotype was the least (87.78%) damaged from the negative effects of colchicine application, and the genotype most adversely affected by colchicine application was N5 (52.38%). The low survival rate of the colchicine-treated seedlings can be explained as a negative effect of colchicine. It was reported that colchicine is a toxic chemical, and exposure of young plant cells to high doses of colchicine can be fatal (Oh et al., 2015b). In a study, conducted *in vitro* to duplicate chromosome numbers of the haploid watermelon plants, it was reported that 64% of the plants treated with a 0.05% concentration of colchicine survived, and 56% of these developed and transformed into intact plants. In the same study, it was stated that the survival and development rates were 40% at a dose of 1% colchicine, and the plants remained haploid at low doses and for short periods of application time (Abak et al., 1998). A decrease in the survival rate after colchicine treatment has been reported in many species. The difference between reported results can be explained by the genotypic difference, colchicine concentration and duration of application, and the conditions of application (*in vivo*/*in vitro*) (Do Valle and Penteado, 2000; Kerdsuwan and Te-chato, 2012; Kwon et al., 2015).

Stomatal analysis

The width and length of guard cells, stomatal density and chloroplast number in guard cells are presented in Table 4. While the stomatal length, stomatal width and number of chloroplasts in guard cells increased in putative tetraploid plants, the number of stomata $\times$ mm^{-2} decreased (Table 4 and Figure 1). Stoma widths, which ranged from 16.09 µm to 18.90 µm in diploids, varied from 20.56 µm to 23.87 µm in putative tetraploids.

Table 3. Number of plants applied, the number of surviving plants and their ratio (%).

Genotype	Plants applied (number)	Surviving plants (number)	Surviving plants (%)
C	180	120	66.67
N3	210	119	56.67
N5	210	110	52.38
N7	180	158	87.78
CXN3	210	116	55.24
CXN5	180	119	66.11
CXN7	210	116	55.24
Total/mean	1,380	858	62.17

Table 4. Mean stomatal diameter, stomatal length, stomatal density and chloroplast number in presumed polyploid and diploid plants.

	Stomatal diameter (µm)		Stomatal length (µm)		Stomatal density (number · mm^{-2})		Chloroplast number (number)	
Ploidy level	Diploid	Tetraploid	Diploid	Tetraploid	Diploid	Tetraploid	Diploid	Tetraploid
Maximum	16.09	20.56	23.30	31.42	627.30	341.75	10.18	16.41
Minimum	18.90	23.87	26.04	34.01	927.77	470.34	12.03	19.37
Mean	17.50	22.22	24.67	32.72	777.54	406.05	11.11	17.89

As the stomatal width, the stoma length increased in putative tetraploid plants. While the minimum and maximum stomatal lengths in diploid plants were 23.3 µm and 26.04 µm, respectively, the minimum and maximum stomatal lengths in tetraploids were 31.42 µm and 34.01 µm, respectively. The stoma density per unit area decreased in putative tetraploids. Minimum and maximum stomatal densities in diploid plants were 627.30 and 927.77 number · mm^{-2}, respectively, while the minimum and maximum stomatal densities in tetraploids were 341.75 and 470.35 number · mm^{-2}, respectively. The number of chloroplasts in stomatal guard cells was significantly affected by colchicine application and increased in putative tetraploid plants. The chloroplast number in guard cells of the diploid plants ranged from 10.18 to 12.03 chloroplasts/stoma, and the chloroplast number in putative tetraploid plants' guard cells varied from 16.41 to 19.37 chloroplast/stoma.

In parallel with our results, it was reported that the average number of stomata per unit area in the leaves of diploid plants is higher than the number of stomata per unit area of leaves of polyploid plants (Chen, 2010; Bae et al., 2020). It was also reported that the stomatal length and width increased in polyploid plants after colchicine application (Yang et al., 2006; Kwon et al., 2015). Consistent with our results, an increase in the number of chloroplasts per guard cell has been reported in tetraploid watermelons (Şimşek et al., 2013; Zhang et al., 2014). A similar increase in the chloroplast number in guard cells from 7.6–8.1 chloroplast/stoma to 17.7–18.8 chloroplast/stoma was also reported by Oh et al. (2015a) in watermelon. It is also known that the number of chloroplasts of guard cells in various plant species is a good characteristic in estimating ploidy levels (Koh, 2002; Oh et al., 2015a).

As a result of stomatal and chloroplast measurements, it was estimated that a total of 84 plants from seven genotypes could be polyploid plants. Morphological parameters, and stomatal and chloroplast measurements are often used as the first primary selection criteria for polyploids, but are often not entirely reliable (Zhang et al., 2010; Guo et al., 2019). It was confirmed that 22 of 84 plants, which were analysed by flow cytometry, were tetraploids. According to the results of flow cytometry, the doubling rate of the chromosome number with colchicine application showed significant variations between genotypes. The highest number of tetraploid plants (6) was obtained in the N5 genotype, while the least number of tetraploid plants (1) was obtained in

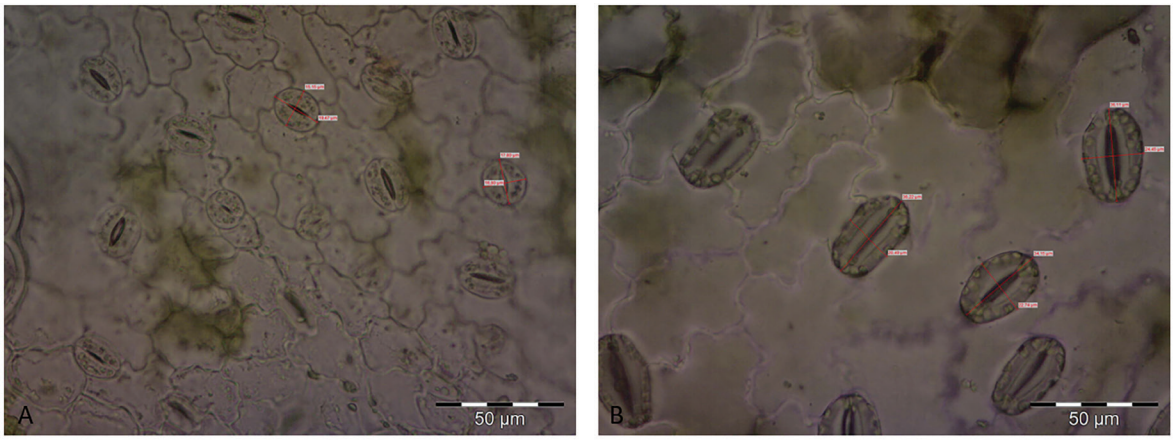


Figure 1. Stomal diameter and length in diploid (A) and tetraploid (B) plant leaves (magnified 400×).

Table 5. The number and percentage of tetraploid plants obtained as a result of microscope and flow cytometry analyses.

Genotype	Number of tetraploid plants		Flow cytometry analysis percentage of tetraploid plants (%)
	Stomatal examinations (number)	Flow cytometry analysis	
C	8	1	12.50
N3	15	1	6.67
N5	11	6	54.55
N7	12	4	33.33
CXN3	14	4	28.57
CXN5	11	1	9.09
CXN7	13	5	38.46
Total/mean	84	22	26.19

'Calhoun Gray', N3 and CXN5 genotypes. The success rate of chromosome duplication was 26.19% (Table 5). Flow cytometry analysis, chloroplast number and DNA content are correlated in tetraploid genotypes. It has been reported that the DNA content analysis method with flow cytometry is more reliable, especially in watermelon genome analyses (Jaskani et al., 2005; Oh et al., 2015a). Flow cytometry is an effective method that saves space and time by quickly determining the ploidy level of many plants at the early development stage (Väinölä, 2000; Leus et al., 2009). In a study conducted on the same plant material in *Acacia mearnsii*, stomatal measurements, chloroplast number and flow cytometry analysis results were found to be 0.64 correlative (Beck et al., 2005). The correlation reported between methods is higher than that obtained in our study. This difference can be explained by the large variation in materials used in the present study and possible chimeric structures.

While the average nuclear DNA content of 22 tetraploid genotypes after colchicine applications was 1.90 pg/2C, the average DNA content of original diploid plants used as a control was determined as 0.92 pg/2C. The nuclear DNA content of tetraploid plants was approximately twice that of diploid plants (Figure 2). Flow cytometry allows polyploid plants to be analysed at an early stage, saving space and time (Väinölä, 2000). Zhang et al. (2014, 2019) reported that the fluorescence intensity of the main peak of the diploid E46 watermelon genotype is 203.46 pg/2C, and the fluorescence intensity of the main peak of the tetraploid E46 watermelon genotype is about 371.07 pg/2C, which means that the DNA content of cells of tetraploid plants is about twice that of diploid plants. This result is in consensus with our fluorescence intensity results.

Seed and seedling characteristics

Plants estimated to be tetraploid were planted in the field and evaluated in terms of seed weight after seed production by selfing and seed extraction. While the average of 1,000 seed weight of tetraploid plants was 162.67 g, the average of 1,000 seed weight of diploid plants was 144.12 g. When the germination rates of tetraploid and diploid plants were compared, the germination rate of tetraploid plants decreased by 30.59% compared to that of diploid plants. While the cotyledon width of tetraploid seedlings increased by 7.76% compared to that of diploid seedlings, the cotyledon length and stem diameter increased by 12.58% and 20.62% (Table 6 and Figure 3), respectively. Similarly, previous studies have reported significantly increased seed length, width and thickness of tetraploid plants compared to those of diploid plant seeds in watermelon (Oh et al., 2015a; Zhang et al., 2019). The increase in seed size was also reflected in 1,000 seed weights, and the seed weight of tetraploid genotypes was found to be higher than that of diploids. Garber (1972) and Bakulin (1980) reported that auto- or allotetraploid plants generally

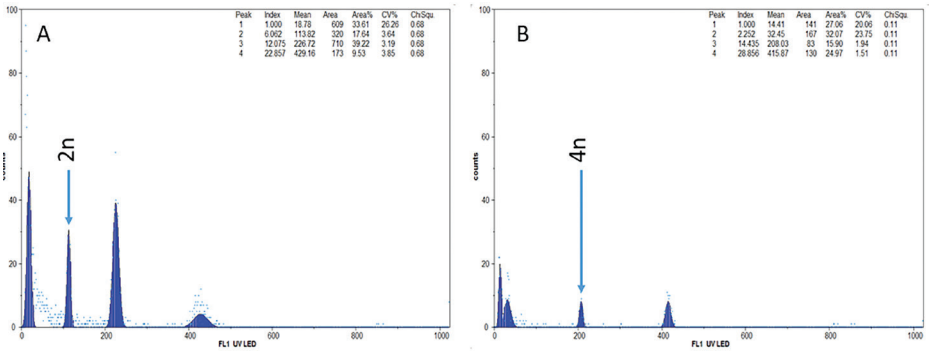


Figure 2. Ploidy level of diploid (A) and tetraploid watermelon (B).

Table 6. Thousand seed weights, germination rate, cotyledon width, cotyledon length, stem diameter and % change in genome size and 1,000 seed weights.

	Ploidy level		
	Tetraploid	Diploid	% Change
1,000 seed weight (g)	162.67	144.12	11.40
Germination rate (%)	65.8	94.8	–30.59
Cotyledon width (mm)	27.92	25.89	7.76
Cotyledon length (mm)	39.99	35.53	12.58
Hypocotyl diameter (mm)	3.14	2.57	20.62

have larger nuclei and cell size than corresponding diploids. This remarkable increase in the cell volume of polyploids is reflected in the dimensions or organs of their phenotype.

Vegetative growth

Since cell structures of tetraploid plants are larger than those of diploid plants, it has been reported that some vegetative organs of tetraploid plants are quite different from those of diploid plants (Väinölä, 2000; Hiaba and Mohamed, 2009). In this study, diploid and tetraploid M_1 plants (the first generation of colchicine-treated plants) were morphologically compared under hydroponic culture. The highest PSD was recorded in RS841 and 'Argentario' commercial rootstocks with 9.73 mm and 9.62 mm, respectively. The stem diameter ranged from 2.79 mm to 6.13 mm and did not show a significant variation among watermelon genotypes (diploids and tetraploids). A significant variation (11–115.83 cm) was found among genotypes as regarded to plant length (PL) (Figure 4). The longest plant was observed in 'Argentario' (115.83 cm) and RS841 (95.83 cm) commercial rootstocks, followed by M_1N7-4 (75.65 cm), M_1N5-3 (73.67 cm) and M_1N5-4 (70.33 cm) genotypes,

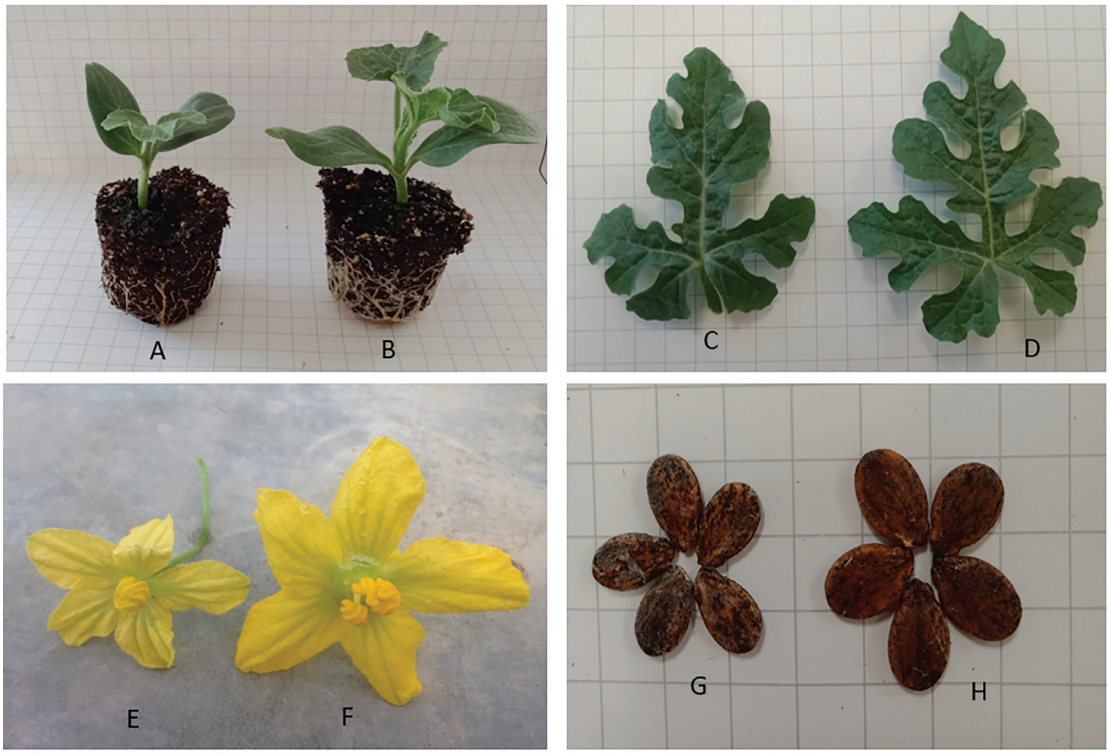


Figure 3. Seedling of diploid (A) CXN5 and tetraploid (B) M_1CXN5-1 genotypes, leaf of diploid (C) CXN5 and tetraploid (D) M_1CXN5-1 genotypes, male flower of diploid (E) N5 and tetraploid (F) M_1N5-1 genotypes and seeds of diploid (G) CXN3 and tetraploid (H) M_1CXN3-1 genotypes.



Figure 4. Diploid N7 (A) and tetraploid M_1N7-1 (B) genotypes and diploid CXN3 (C) and tetraploid M_1-CXN3-1 (D) genotype plants grown in hydroponic culture for 21 days after transplanting.

while the shortest plant was recorded in 'Crimson Tide' (11.00 cm), CXN3 (15.55 cm) and 'Calhoun Gray' (17.83 cm) genotypes. As in PL, genotypes showed significant differences in shoot fresh weight (SFW). The highest SFW was recorded in RS841 (84.00 g) and 'Argentario' (73.33 g) commercial rootstocks, followed by M_1CXN3-2 (58.33 g) and M_1CXN7-4 (55.00 g) genotypes, while the lowest SFW was found in commercial watermelon cultivars 'Crimson Tide' (2.72 g), CxN3 (3.60 g) and 'Calhoun Gray' (5.08 g). Shoot dry weight (SDW) results showed parallelism with SFW results. Similarly, the highest SDW was obtained in RS841 (8.58 g), 'Argentario' (7.43 g), M_1CXN3-2 (5.93 g) and M_1CXN7-4 (5.65 g) genotypes, while the lowest SFW was recorded in Crimson tide (0.39 g), CxN3 (0.41 g) and 'Calhon Gray' (0.67 g) genotypes (Table 7). In general, our data showed that auto- and allopolyploid plants produced significantly higher shoot biomass than their corresponding diploids. Polyploid plants have notable differences from diploids in their external morphological characteristics, mainly the shape and size of roots, stems, leaves, flowers and fruits due to chromosome duplication (Zhang et al., 2019), and an increase in DNA content mostly results in increased cell and organ size (Corneillie et al., 2019). Tetra- and hexaploid Arabidopsis plants have a significant increase

Table 7. PSD, plant height, SFW and SDW of the plants grown in hydroponic culture.

Genotype code	Ploidy level	PSD (mm)	PL (cm)	SFW (g · plant^{-1})	SDW (g · plant^{-1})
M_1Calhon Gray	Tetraploid	5.82 b–d	30.83 mn	12.58 no	1.42 op
M_1N3	Tetraploid	5.61 b–d	35.53 k–m	21.37 k–m	2.30 l–n
M_1N5-1	Tetraploid	5.53 b–d	67.40 c–f	49.14 de	4.96 d–f
M_1N5-2	Tetraploid	5.57 b–d	67.67 c–f	37.50 f–i	3.92 g–j
M_1N5-3	Tetraploid	5.17 b–d	73.67 cd	39.17 f–h	4.08 g–i
M_1N5-4	Tetraploid	5.23 b–d	70.33 cd	39.17 f–h	3.97 g–j
M_1N5-5	Tetraploid	5.26 b–d	54.00 e–j	30.33 h–j	3.21 i–k
M_1N5-6	Tetraploid	4.94 b–d	51.12 f–k	30.83 h–j	3.16 jl
M_1N7-1	Tetraploid	5.32 b–d	49.70 g–l	23.33 j–m	2.45 k–n
M_1N7-2	Tetraploid	4.88 cd	65.05 c–g	25.00 j–m	2.68 k–m
M_1N7-3	Tetraploid	5.19 b–d	64.25 c–h	40.83 e–g	4.21 f–h
M_1N7-4	Tetraploid	5.38 b–d	75.65 c	43.33 e–g	4.51 e–h
M_1CXN3-1	Tetraploid	6.05 b–d	46.67 i–m	30.83 h–j	3.19 jk
M_1CXN3-2	Tetraploid	5.90 b–d	48.18 h–l	58.33 c	5.93 c
M_1CXN3-3	Tetraploid	5.77 b–d	67.67 c–f	45.00 e–g	4.68 e–h
M_1CXN3-4	Tetraploid	6.13 b–d	52.02 f–k	25.83 j–l	2.69 k–m
M_1CXN5-1	Tetraploid	6.48 b–d	45.47 j–m	46.33 ef	4.75 e–g
M_1CXN7-1	Tetraploid	6.12 b–d	64.00 c–h	44.67 e–g	4.62 e–h
M_1CXN7-2	Tetraploid	5.93 b–d	34.55 l–n	40.17 fg	4.07 g–i
M_1CXN7-3	Tetraploid	5.82 b–d	27.67 no	36.35 g–i	3.81 h–j
M_1CXN7-4	Tetraploid	6.05 b–d	60.62 c–j	55.00 cd	5.65 cd
M_1CXN7-5	Tetraploid	6.53 b–d	62.17 c–i	49.33 de	5.10 d–f
Calhoun Gray	Diploid	6.07 b–d	17.83 o–r	5.08 op	0.67 pr
CXN3	Diploid	3.86 d	15.55 o–r	3.60 p	0.41 r
CXN5	Diploid	5.48 b–d	44.67 j–m	30.83 h–j	3.25 i–k
CXN7	Diploid	5.07 b–d	58.52 d–j	29.67 k	3.13 jl
N3	Diploid	4.76 d	26.22 n–p	16.52 mn	1.70 no
N5	Diploid	4.96 b-d	37.23 k–n	20.82 mn	2.26 mn
N7	Diploid	4.11 d	30.83 mn	17.33 l–n	1.81 no
C. tide	*Citrullus lanatus*	2.79 d	11.00 p–r	2.72 p	0.39 r
RS841	*C.maxima* × *C. moschata*	9.73 a	95.83 b	84.00 a	8.58 a
Argentario	*Lagenaria siceraria*	9.62 ab	115.83 a	73.33 b	7.43 b

Values denoted by different letters are significantly different between genotypes within columns at $p < 0.05$.
PL, plant length; PSD, plant stem diameter; SDW, shoot dry weight; SFW, shoot fresh weight.

in stem dry weight compared to diploids (Zhang et al., 2019). Researchers' general belief that polyploidy increases biomass production is agreement with our results (Głowacka et al., 2010; Li et al., 2012; del Pozo & Ramirez-Parra, 2014; Dudits et al., 2016). When the average of the original diploid genotypes and tetraploids was compared, a 100% increase in autotetraploids and a 156% increase in allotetraploids were determined in shoot biomass production (Table 7)

Significant variations were found in root biomass production and root morphological characteristics among genotypes grown in hydroponic culture for 21 days after transplanting (Table 8). The highest RFW was measured in RS841 (38.17 g), 'Argentario' (34.00 g), M_1CXN5-1 (33.02 g) and M_1CXN3-2 (28.37 g) genotypes, respectively (Figure 1). The lowest RFW was measured in 'Crimson Tide' (1.19 g), CXN3 (1.70 g) and 'Calhoun Gray' (3.16 g) genotypes. Similar to RFW, RS841 (4.00 g), 'Argentario' (3.50 g), M_1CXN5-1 (3.41 g) and M_1CXN7-4 (3.04 g) produced the highest root dry weights (RDWs), while CXN3 (0.22 g), 'Crimson Tide' (0.24 g) and 'Calhoun Gray' (0.48 g) produced

Table 8. RFW, RDW, RL, RV and RD of the plants grown in hydroponic culture for 21 days after transplanting.

Genotype code	Ploidy level	RFW (g · plant^{-1})	RDW (g · plant^{-1})	RL (cm)	RV (cm^3 · plant^{-1})	RD (mm)
M_1Calhon Gray	Tetraploid	9.81 mn	1.15 pr	1,319.16 m	3.31 l–n	0.40 g
M_1N3	Tetraploid	10.50 l–n	1.22 o–r	1,702.86 m	1.82 o	0.37 i
M_1N5-1	Tetraploid	22.13 fg	2.26 h–k	4,818.78 ef	3.52 k–m	0.44 f
M_1N5-2	Tetraploid	22.94 d–f	2.46 f–i	3,570.95 g–k	3.85 kl	0.38 i
M_1N5-3	Tetraploid	23.61 d–f	2.53 e–h	5,175.32 de	5.92 gh	0.53 c
M_1N5-4	Tetraploid	26.78 c–e	2.73 d–g	3,470.66 h–l	5.25 h–j	0.44 f
M_1N5-5	Tetraploid	20.26 f–h	2.20 h–k	3,514.55 h–k	4.32 l	0.40 g
M_1N5-6	Tetraploid	23.48 d–f	2.43 f–i	3,660.05 g–j	4.48 i–k	0.58 a
M_1N7-1	Tetraploid	13.97 k–m	1.52 n–p	4,046.80 f–i	4.44 i–k	0.37 i
M_1N7-2	Tetraploid	14.51 j–l	1.63 m–o	2,764.20 kl	3.95 kl	0.55 b
M_1N7-3	Tetraploid	20.65 f–h	2.19 h–k	3,722.38 g–j	5.38 h–j	0.44 f
M_1N7-4	Tetraploid	21.48 fg	2.33 g–j	6,083.45 c	2.81 mn	0.37 i
M_1CXN3-1	Tetraploid	15.73 i–k	1.68 l–n	2,658.33 l	4.35 j–l	0.46 e
M_1CXN3-2	Tetraploid	28.37 c	2.93 de	4,223.48 f–h	6.53 fg	0.46 e
M_1CXN3-3	Tetraploid	22.50 fg	2.43 f–i	3,333.78 i–l	6.67 fg	0.51 d
M_1CXN3-4	Tetraploid	18.24 g–j	1.93 j–n	2,905.59 j–l	3.79 k–m	0.40 g
M_1CXN5-1	Tetraploid	33.02 b	3.41 bc	7,678.59 b	11.09 b	0.44 f
M_1CXN7-1	Tetraploid	27.09 cd	2.86 d–f	6,001.24 c	7.40 d–f	0.40 g
M_1CXN7-2	Tetraploid	19.91 f–i	2.05 i–m	4,416.07 e–g	4.50 i–k	0.35 j
M_1CXN7-3	Tetraploid	20.60 f–h	2.23 h–k	3,658.58 g–j	7.28 ef	0.51 d
M_1CXN7-4	Tetraploid	28.94 c	3.04 c–e	4,104.75 f–i	8.21 de	0.51 d
M_1CXN7-5	Tetraploid	22.82 d–f	2.45 f–i	5,825.66 cd	8.29 d	0.44 f
Calhoun Gray	Diploid	3.16 o	0.48 s	344.85 n	0.78 p	0.44 f
CXN3	Diploid	1.70 o	0.22 s	173.10 n	0.27 p	0.46 e
CXN5	Diploid	19.29 f–i	2.09 h–l	3,645.02 g–j	4.22 l	0.40 g
CXN7	Diploid	16.70 h–k	1.83 k–n	4,428.47 e–g	5.51 hi	0.40 g
N3	Diploid	8.37 n	0.89 r	1,454.43 m	1.82 o	0.40 g
N5	Diploid	13.90 k–n	1.57 n–p	3,591.94 g–k	4.31 l	0.40 g
N7	Diploid	10.77 n	1.16 pr	1,719.21 m	2.44 no	0.44 f
C. tide	*Citrullus lanatus*	1.19 o	0.24 s	148.66 n	0.32 p	0.37 i
RS841	*C. maxima* × *C. moschata*	38.17 a	4.00 a	11,098.84 a	13.04 a	0.39 h
Argentario	*Lagenaria siceraria*	34.00 b	3.50 b	11,000.44 a	9.70 c	0.34 k

Values denoted by different letters are significantly different between genotypes within columns at $p < 0.05$.
RD, root diameter; RDW, root dry weight; RFW, root fresh weight; RL, root length; RV, root volume.

the lowest root weights. The genotypic variations in RL, volume and diameter were found significant. The genotypes with the highest RL in hydroponic culture were RS841 (11,098.84 cm), 'Argentario' (11,000.44 cm), M_1CXN5-1 (7,678.59 cm) and M_1N7-4 (6,083.45 cm), while genotypes with the shortest RL was commercial 'Crimson Tide' watermelon cultivar (148.66 cm). The highest RV was recorded in RS841 (13.04 cm^3), followed by M_1CXN5-1 (11.09 cm^3), while the lowest RV was observed in CxN3 and 'Crimson Tide' had (0.27 cm^3 and 0.32 cm^3), respectively. Root thickness, which showed significant variation among genotypes, ranged from 0.58 mm to 0.34 mm. The highest and lowest RDs were recorded in M_1N5-6 and 'Argentario' with 0.58 mm and 0.34 mm, respectively. In general, tetraploid genotypes produced more root biomass than diploids. When diploid and tetraploid genotypes were compared in terms of root fresh biomass, an 126% increase in all tetraploids, a 100% increase in autotetraploids and a 180% increase in allotetraploids were found (Table 8 and Figure 4). Similarly, increases in plant biomass due to polyploidisation have been reported in different species in previous studies. It has been reported that polyploid orchids significantly increased in various growth parameters, including fresh weight, dry weight, shoot length, RL and leaf width, compared to diploid orchids (Chung et al., 2017). Polyploid plants showed higher leaf and root growth than diploid plants in *Artemisia cina* (Kasmiyati et al., 2020). Kim et al. (2004) reported that the number of adventitious roots in polyploid ginseng plants is higher than that in diploid plants. The tetraploid watermelon line USVL-360 showed vegetative growth at the same level as commercially available cucurbit rootstocks and provided resistance against root-knot nematode (Levi et al., 2014). In the current study, tetraploid genotypes (auto and allotetraploids) as strong as commercial rootstocks RS841 and 'Argentario' were not determined. However, it was determined that an average of 100%–180% biomass increase was achieved compared to the corresponding diploid genotypes (open-pollinated and hybrid). Polyploid plants, when used as rootstock, can provide desirable traits such as biotic and abiotic stress tolerance. The use of tetraploid (allo- and autotetraploids) watermelon rootstocks with a strong root system can provide high graft compatibility and a high survival rate, while the increased hormone content and high antioxidant activities of tetraploid watermelon rootstocks can promote plant growth and stress tolerance

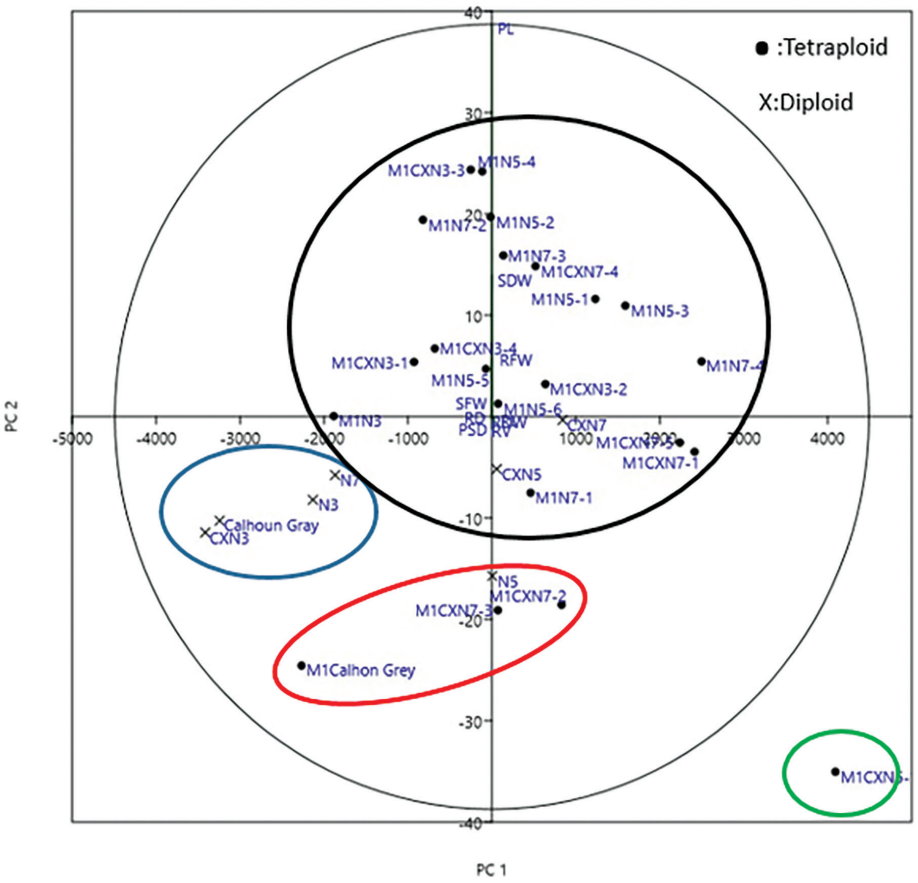


Figure 5. PCA of diploid (D) and tetraploid (T) watermelon genotypes based on hydroponic culture morphological features. PCA, principal component analysis; PL, plant length; PSD, plant stem diameter; RD, root diameter; RDW, root dry weight; RFW, root fresh weight; RL, root length; RV, root volume; SDW, shoot dry weight; SFW, shoot fresh weight.

without negatively affecting fruit quality in watermelon (Levi et al., 2014; Kaseb et al., 2020).

Principal component analysis

PCA was used for classifying of diploid (D) and tetraploid (T) genotypes based on plant growth parameters in hydroponic culture. According to the analysis, two principle components described 99% of total variation (98% by PC1 and 1% by PC2). When PCA charts were examined, it could be seen that genotypes were separated into four different groups based on measured one feature (Figure 5). The first group is the group containing four of the five diploid genotypes in region III of the graph and is indicated by the blue circle. There is a negative correlation between diploids and biomass parameters. Diploid N5, autotetraploid 'Calhoun Gray', and allotetraploids CxN7 and CxN7 formed the second group and are indicated by the red circle between regions II and IV of the graph. This group also showed less vegetative development than the main group. CxN5 is scattered in the far corner of the III region of the graph and is considered group III (green circle). Group IV, with 18 (auto- and allo-) tetraploid and two diploid genotypes, is located in the upper part of the graph (black circle). When group IV is analysed within itself, it was seen that the two diploid genotypes have lower vegetative growth performance than tetraploids. Although there are deviations, in general, tetraploids formed the large main group with higher values, while diploids formed the other group with low vegetative growth performance (Figure 5).

Correlation analysis

DNA content was positively correlated with SFW, SDW, RFW, RDW ($p < 0.01$), PSD, PL and RV ($p < 0.05$). As the DNA content increased, notable increases were observed in plant biomass, where significant genetic variation was determined. The PSD and RD did not show any positive or negative correlation with any stomatal characteristics. While the stomatal diameter and density were positively correlated with plant biomass, the stomatal diameter was negatively correlated with plant biomass. There was no significant correlation between plant growth parameters and chloroplast number of gourd cells ($p < 0.01$ or 0.05, Table 9).

Correlation analysis between the DNA content and stomatal measurement indicated that stomal dimensions and chloroplast number of guard cells were positively correlated with DNA content. By contrast, a significant negative correlation was found between stomatal density and DNA content in diploid and tetraploid plants ($p < 0.01$ or 0.05, Table 10). As stated earlier, polyploidisation often causes an enlargement in the habitus and organs of plants. The enlargement in cell volume was accepted as the reason for this increase in plant and organ sizes. Similarly, it has been reported that the stomatal length and stomatal diameter increase as the ploidy level increases (Yetisir and Sari, 2003). Autopolyploids mostly have larger nuclei and cell sizes than corresponding diploids. This significant increase in the cell volume of polyploids is reflected in the dimensions or organs of their phenotype (Garber, 1972;

Table 9. Pearson's correlation coefficients (*r* values) between plant growth parameters and direct (DNA content) and indirect (stomatal measurement) ploidy determination methods in 22 tetraploid and seven diploid watermelon genotypes.

	PSD	PL	SFW	SDW	RFW	RDW	RL	RV	RD
DNA content	0.33*	0.48*	0.60**	0.60**	0.60**	0.61**	0.45*	0.47*	–0.02^{ns}
Stomatal diameter	–0.15^{ns}	–0.49*	–0.56**	–0.56**	–0.58**	–0.58**	–0.53**	–0.44*	0.26^{ns}
Stomatal length	0.26^{ns}	0.50**	0.49*	0.49*	0.52*	0.53*	0.38*	0.32*	–0.15^{ns}
Stomatal density	0.30^{ns}	0.50**	0.55**	0.55**	0.59**	0.59**	0.43*	0.40*	–0.12^{ns}
Chloroplast number	–0.19^{ns}	0.19^{ns}	–0.10^{ns}	–0.10^{ns}	0.02^{ns}	0.01^{ns}	–0.05^{ns}	–0.24^{ns}	–0.26^{ns}

**$p \leq 0.05$; **$p \leq 0.01$, respectively; ns, not significant; PL, plant length; PSD, plant stem diameter; RD, root diameter; RDW, root dry weight; RFW, root fresh weight; RL, root length; RV, root volume; SDW, shoot dry weight; SFW, shoot fresh weight.

Table 10. Pearson's correlation coefficients (*r* values) between DNA content and stomatal measurement in 22 tetraploid and 62 diploid watermelon genotypes.

	Stomatal diameter	Stomatal length	Stomatal density	Chloroplast number
DNA content	0.96**	0.90**	–0.79**	0.88**

Variable of significance **$p \leq 0.01$; ns, not significant.

Beck et al., 2005). However, in polyploid genotypes, the number of stomata per unit area decreased (Yetisir and Sari, 2003; Beck et al., 2005). The present study also confirmed the results of previous studies by producing a strong positive correlation between stoma size and DNA content and a negative correlation between DNA content and stoma density.

CONCLUSIONS

Polyploidy is a notable approach in the development of high-yielding crops as about half of the crop species are polyploid. While many polyploid plants show higher vigour than their diploid ancestors, they show a feature similar to the heterosis effect by showing a high rate of heterozygosity. Therefore, polyploids show new and useful phenotypic variations. One of these approaches is the generation of auto- or allopolyploids using the polyploidy breeding method. Auto- and allotetraploid *Citrullus lanatus* var. *citroides* genotypes developed in our study showed promising results in improving plant vigour in hydroponic conditions. According to the results of this study, the effects of selected tetraploid genotypes on plant growth, yield and quality in watermelon are being studied in ongoing projects. The results will be shared with the public in the near future. More detailed studies are needed to determine the effect of polyploids on the fruit quality characteristics, plant nutrient metabolism and some stress tolerance of scions when they are used as rootstocks for cultivated watermelons.

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AUTHOR CONTRIBUTIONS

The contributions of all authors are as follows: study concept and design: A. A and H.Y; data collection: A.A; analysis and interpretation of results: A.A and H.Y; and preparing a draft text: A.A and H.Y. All authors reviewed the results and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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