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Rootstock potential of auto and Allotetraploid Citron [*Citrullus lanatus* var. *citroides* (L. H. Bailey) Mansf.] for Watermelon [*Citrullus lanatus* var *lanatus* (Thunb.) Matsum. & Nakai] under hydroponic conditions: plant growth and some physiological characteristics

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Abstract

The emergence of some physiological and fruit quality problems due to the common squash rootstocks used in watermelon has led researchers to search for alternative rootstocks sources. Exploitation of novel Citrullus germplasm such as citronmelon (*Citrullus lanatus* var. *citroides*) is an alternative to avoid these problems. In this study, rootstocks potential of auto and allotetraploid citrullus genotypes for watermelon were investigated as regard to plant growth and some physiological parameters under hyroponic conditions. Plant length was significantly affected by rootstock genotype and the longest plant stem was measured in watermelon plants grafted on N7-4T tetraploid rootstock (62.67 cm) while the shortest stem was measured in grafted plants onto autotetraploid Calhounn Gray with 14.33 cm. Among the graft combinations, N7-4T/CT (93.33 g) and CN7-5T/CT 95.00 g) graft combination produced the highest shoot fresh and dry weight. As in shoot fresh weight, the exploitationted on to tetraploid rootstock produced higher root fresh and dry weight than the plants grafted on diploid rootstocks and commercial rootstock. The highest root fresh and dry weight were determined in the plants grafted on to autotetraploid N5-4T and allotetraploid CN7-5T. Root characteristics were significantly affected by rootstock genotypes. The N, P, K and Ca contents of the leaves of the CT watermelon cultivar grafted on different rootstocks were significantly affected by the rootstocks. This study showed that citrullus tetraploid genotypes (auto and allo) to be produced by polyploidy method can be an important alternative rootstock source for watermelon.

Keywords: Hydroponic culture, Rootstock, Scion, Tetraploid, Citron 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 101620420 tons in an area of 3053258 ha in 2020. Watermelon, grown in many parts of the world, covers 7% of the fruit and vegetable production areas. However, watermelon production is concentrated in China, Middle East countries, India, USA, Africa, Japan and some European countri (Faostat, 2022). As in other vegetable species, watermelon is also susceptible to many abiotic and biotic stress factors. The primary factor in coping with stress factors is genotypic resistance/tolerance. The development of cultivars with multiple resistance to both biotic and abiotic stress factors takes a very long time or is sometimes not possible due to genetic barriers between species. For this reason, one of the approaches used to overcome with stress conditions and to support plant growth is to graft sensitive commercial genotypes onto the stress-resistant genotypes (rootstocks) (Amaro et al., 2014; Rouphael, Cardarelli, Rea, & Colla, 2012; Zhong & Bie, 2007) . The primary starting point for grafting in vegetable crops is to prevent damage caused by soil-borne pests and pathogens (Oda, 2002; H Yetisir & Sari, 2003). However, in the last few decades, it has also been reported that grafting of vegetable crops onto suitable rootstock improves tolerance to abiotic stresses such as drought, low soil temperature and salinity, to increase water and nutrient use efficiency, and fruit yield and quality (Edelstein, Plaut, & Ben-Hur, 2011; Lee & Oda, 2010; Nisini et al., 2002; Oda, 2002; Rivero, Ruiz, & Romero, 2003; Romero, Belakbir, Ragala, & Ruiz, 1997; Shimada & Nakamura, 1977). Although utilization rates varies, the most common commercial rootstocks for watermelons are Cucurbita interspecific hybrids (*C. moschata* Duch. × *C. maxima* Duch.) and bottle gourd (*Lagenaria siceraria* Standl.) hybrids. Grafting can affect the plant performance positively or negatively depending on the rootstock/scion interaction. There is consensus among researchers that grafting watermelon on Cucurbita or Lagenaria rootstocks tends to increase plant vigor, fruit weight, and yield (Alexopoulos, Kondylis, & Passam, 2007; Cushman & Huan, 2008; Yetişir, Kurt, Sari, & Tok, 2007). Rootstock/scion incompatibility is the most obvious cause of the adverse effect on the grafted watermelons. However, different effects on pant growth, yield and fruit quality were commonly reported even in compatible graft combinations. These effects are due to rootstock/scion interaction, which can alter nutrient and water uptake, hormone synthesis, photosynthesis, and other metabolic processes (Aloni, Cohen, Karni, Aktas, & Edelstein, 2010). Grafted plants onto vigorous roostocks absorb more water and ions than ungrafted plants and carry these water and ions to the above ground graft. Ion uptake is regulated by a complex communication mechanism between the scion and rootstock and vigorous rootstocks with higher root volume increase plant nutrient uptake (Albacete et al., 2009; Gregory et al., 2013; Huang et al., 2013; Nawaz et al., 2016; Schwarz, Öztekin, Tüzel, Brückner, & Krumbein, 2013; Uygur & Yetisir, 2009).

It has been recently reported that grafting on different rootstocks can play an important role in the differentiation of physiological processes of watermelons and induce different gene expressions in the scion (Aslam et al., 2020; Liu et al., 2016). Therefore, there are conflicting reports in the literature about the effect of grafting on pumpkin rootstocks on the fruit quality of watermelon. One way to solve the quality problems in grafted watermelon production mentioned above may be to use rootstocks developed from the Citrullus genus (Edelstein et al., 2014). The most important disadvantage of the genotypes that can be used as rootstock for watermelon in the Citrullus germplasm is that their plant vigor is weaker than the existing commercial gourd rootstocks. With polyploidy breeding method, it is possible to develop polyploid genotypes with higher plant vigor. An important promising alternative rootstock candidate for watermelon is Citrullus lantus var. citroides (LH Bailey) Mansf. ex Greb., also known as citron melon (Fredes et al., 2016). Levi et al., (2014), reported that seedless (triploid) watermelon cultivars produced higher yield when grafted onto autotetraploid *Citrullus lanatus* var. *citroides* rootstocks compared with those grafted onto commercial Cucurbita or Lagenaria rootstocks. According to available literature, studies on the rootstock potential of autotetraploid genotypes developed from the Citrullus genera are quite limited and there are no studies on the rootstock potentials of allotetraploid Citrullus genotypes.

Therefore, in this the rootstock potential of auto and allotetraploids of *Citrullus lanatus* var. *citroides* for watermelon was investigated under hydroponic conditions and it has been determined that there are promising tetraploids producing vegetative growth results close to commercial rootstocks.

MATERIALS AND METHODS

Plant materials

Plant materials used in hydroponic testing are auto and allotetraploids, original diploid parent lines, commercial rootstocks (RS841 and Argentario), and the Crimson Tide watermelon variety. In 2019 growing season, hybrids were produced between Calhoun Gray watermelon cultivar and N3, N5 and N7 citron genotypes. Auto and allotetraploid genotypes were produced at Erciyes University, Faculty of Agriculture, Department of Horticulture within the framework of a Ph.D. study. Chromosome number was duplicated according to (Ra et al., 1995) by %0.5 colchicine application. Argentario Commercial *Cucurbita* and *Lagenaria* rootstocks and Crimson Tide watermelon cultivar were used for comparison. The list of plant materials used in the study is given in Table 1.

Seedling Production and Grafting

One hundred seeds from each rootstock and a sufficient number of seeds from Crimson Tide (scion) were sown in multipots filled witha mixture of peat (pH: 6.0–6.5) and perlite in a 2:1 ratio and then the appropriate seedlings were selected for the grafting process at the first true leaf stage. The seedlings were grafted "Splice Grafting" (single cotyledon) following the procedure described by Lee & Oda (2010), while non-grafted Crimson Tide seedlings were used as control plants Thirty seedlings from each rootstock were grafted. Grafted seedlings were kept in a post-graft care unit in 90-95% humidity, 22-25 °C, and semi-shaded for one week. The graft success rate was evaluated 14 days after grafting and the survival rate was expressed as a percentage of the total number of the grafted plants. Plants with severely wilted scion and root-

Table 1. Rootstocks code, name and ploidy level

Rootstock Code	Rootstock Ploidy Level
Calhoun GrayT (*Citrullus lanatus*)	Autotetraploid
N3T (*Citrullus lanatus* var. *citroides*)	Autotetraploid
N5-3T (*Citrullus lanatus* var. *citroides*)	Autotetraploid
N5-4T (*Citrullus lanatus* var. *citroides*)	Autotetraploid
N7-3T (*Citrullus lanatus* var. *citroides*)	Autotetraploid
N7-4T (*Citrullus lanatus* var. *citroides*)	Autotetraploid
CN3-2T (*Citrullus lanatus* var. *citroides)*	Allotetraploid
CN3-3T (*Citrullus lanatus x C. lanatus* var. *citroides*)	Allotetraploid
CN5T (*Citrullus lanatus x C. lanatus* var. *citroides*)	Allotetraploid
CN7-4T (*Citrullus lanatus x C. lanatus* var. *citroides*)	Allotetraploid
CN7-5T (*Citrullus lanatus x C. lanatus* var. *citroides*)	AlloTetraploid
Calhoun GrayD (*Citrullus lanatus)*	Diploid
N3D (*Citrullus lanatus* var. *citroides*)	Diploid
N5D (*Citrullus lanatus* var. *citroides*)	Diploid
N7D (*Citrullus lanatus* var. *citroides*)	Diploid
CN3D (*Citrullus lanatus x C. lanatus* var. *citroides*)	Diploid
CN5D (*Citrullus lanatus x C. lanatus* var. *citroides*)	Diploid
CN7D (*Citrullus lanatus x C. lanatus* var. *citroides*)	Diploid
RS841 (*C. maxima* x *C. moschata*)	Diploid
Argentario (*Lagenaria siceraria*)	Diploid
Crimson Tide (Scion) (*Citrullus lanatus*)	Diploid

stock were considered dead. The graft succes rate was calculated with the formula = (Number of live plants/total grafted plants) × 100%.

Establishment of Experiment in Hydroponic Culture

A hydroponic culture test was carried out in the fully automated venlo type glass R&D greenhouse of Kırşehir Ahi Evran University. The grafted plants acclimated to greenhouse conditions were transferred to 136 L plastic containers after roots were washed from growth media, each container was filled with nutrient solution and continuously aerated by an air pump. The upper surface of the containers is covered with styrofoam and the plants are placed in the holes. The nutrient solution contained 1.5 mM calcium nitrate ($Ca(NO3)_2$ 250 µM monopotassium phosphate (KH_2PO_4), 500 µM potassium sulfate (K_2SO_4), 325 µM magnesium sulfate ($MgSO_4.7H_2O$), 50 µM sodium chloride (NaCl). Micronutrients were 80 µM iron (Fe) (III) ethylenediaminetetraacetic acid (EDTA) sodium (Na), 0.4µM manganese sulfate ($MnSO_4$), 0.4µM zinc sulfate ($ZnSO_4$), 0.4µM copper sulfate ($CuSO_4$), 8µM boric acid (H_3BO_3), 0.4µM sodium molybdate (Na_2MoO_4). The electrical conductivity of the growing solution was maintained at 1.50 dS/m and the pH was between 6.5-7 (Hoagland & Arnon, 1950). The study was carried out under controlled greenhouse conditions (22-24 °C day / 16-18C night and 60% relative humidity) for 21 days. The experiment was set up according to a completely randomized block design with three replications and six plants in each replication.

Plant Growth Measurements

After three weeks of growing, plants were harvested and separated into shoot and roots. Stem length (cm), diameter (mm) and number of leaf per plant were determined. The fresh and dry weights (g plant^{-1}) of stem and root were determined. In order to determine shoot and root dry weight, plant materials were dried in a air-forced oven for 48 h at 70 °C. The root length (cm plant^{-1}), volume (cm^3) and diameter (mm) of the plants was determined by using the special software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada) (Ulas, Doganci, Ulas, & Yetisir, 2019).

Chlorophyll Meter Measurements

Leaf chlorophyll index was determined with the CM 1000 Chlorophyll Meter. During the growth period, leaf chlorophyll index in fully developed leaves in all graft combinations was determined by two measurements with the CM 1000 chlorophyll meter.

Plant Nutrient Analysis

After harvest fresh plant material was divided into two parts. One part was frozen in liquid nitrogen and storedfor later use. The remaining fresh plant material was dried at 70°C for 24 hours. For the determination of N, Ca and K concentrations, 100 mg dried plant material was extracted by boiling in 5 ml MilliQ for one hour. The solution was filtered through 0.2 mm filters (Whatman, England) and N, Ca, P and K contents in the filtrate were analyzed using high-performance liquid chromatography (HPLC, Shimadzu Japan). The HPLC system was equipped with a ø 4.6 mm 6125 mm Shodex IC YS-50 column (Showa Denko). As an eluent, 4.0 mM methane sulfonic acid was used in HPLC graded H_2O (J.T. Baker, The Netherlands) with a flow rate of 1 ml min^{-1}. Final ion concentrations in the filtrate were calculated according to a calibration curve.

Statistical Analysis

Data from the hydroponic culture study was sunjected to one-way analysis of variance (ANOVA) at 5% significance level (IBM, Chicago, IL, USA) using SPSS version 18.0 and means were compared using the Duncan test. Correlation analysis was performed root characteristics and other measured parameters in grafted watermelon onto different rootstock grown under hydroponic conditions using SPSS software (version 22.0, Chicago, IL, USA).

RESULTS AND DISCUSSION

Grafting success in Citrullus rootstocks was 8% to 17% higher than in commercial rootstocks. While the graft success rate was 98% in grafted plants onto Citrullus rootstocks, it was 83.33% in grafted plants onto Argentrio (*Lagenaria siceraria)* rootstock and 90.00% in grafted plants onto RS841 (*C.maxima* x *C. moschata*) rootstock (Table 2). Since the cost of grafted watermelon seedlings is higher than that of ungrafted seedlings, the survival rate after grafting should be high. The grafting techniques used, rootstock/scion genotype, pre- and post-grafting care conditions affect the success of grafting in watermelon. The single cotyledon grafting method is preferred in cases where the scion has a thin as in watermelon, cucumber and melon (Sakata, Ohara, & Sugiyama, 2007). This method is done when the rootstock and scion are of similar size and the first true leaf of the rootstock begins to develop (approximately 7 to 10 days after planting) (Oda, 2002). In our study, the high success rate of grafting on Citrullus rootstocks may be due to the fact that the rootstock and the scion belong to the same genera, or the diameter of the Citrullus rootstocks is proportional to the diameter of the scion. Similarly, a higher graft success in Citrullus rootstocks was reported in previous studies (Aydın et al., 2022; Kaseb et al., 2020; Levi et al., 2014).

The results of stem diameter, length and number of leaves per plant are given in Table 3. Plants grafted on tetraploid (auto-allo) Citrullus rootstocks had thicker stems than those grafted on diploid rootstocks. The highest stem diameter was measured in N7-3T/CT (6.45 mm), CN3-3T/CT (6.44 mm) and CN7-5T/CT (6.18 mm) respecti-

Table 2. Number of grafted plants, number of grafted plants, and graft success (%)

Rootstock Ploidy Level	Graft Combinations (Rootstock/Scion)	Number of Grafted Plants	Number of Surviving Plants	Graft Success Rate (%)
Autotetraploid	Calhon GrayT/Crimson Tide	30	30	100.00
Autotetraploid	N3T/CT	30	29	96.67
Autotetraploid	N5-3T/CT	30	30	100.00
Autotetraploid	N5-4T/CT	30	30	100.00
Autotetraploid	N7-3T/CT	30	30	100.00
Autotetraploid	N7-4T/CT	30	29	96.67
Allotetraploid	CN3-2T/CT	30	30	100.00
Allotetraploid	CN3-3T/CT	30	29	96.67
Allotetraploid	CN5T/CT	30	29	96,67
Allotetraploid	CN7-4T/CT	30	29	96.67
Allotetraploid	CN7-5T/CT	30	29	96.67
Average				98.18
Diploid	Calhon GrayD/CT	30	30	100.00
Diploid	N3D/CT	30	29	96.67
Diploid	N5D/CT	30	30	100.00
Diploid	N7D/CT	30	29	96.67
Diploid	CN3D/CT	30	30	100.00
Diploid	CN5D/CT	30	29	96.67
Diploid	CN7D/CT	30	29	96.67
Average		30		98.10
Diploid	RS841/CT	30	27	90.00
Diploid	Argentario/CT	30	25	83.33

vely. The lowest stem diameter was measured in ungrafted Crimson Tide plants with 3.94 mm. Plant main stem length length was significantly affected by rootstock genotypes. The longest main stem length was 62.67 cm in plants grafted on tetraploid rootstock N7-4T, while the shortest main stem length was determined in Calhoun Gray T/ CT (14.33 cm), CN7D/CT (19.33 cm) and N7D/CT (20.33 cm) graft combination. The number of leaves per plant in plants grafted on tetraploid rootstocks ($\bar{x}$=10.75) is higher than the number of leaves in diploid plants grafted onto diploid rootstocks ($\bar{x}$= 9.22). In the study, the highest number of leaves per plant was recorded in the CN3-3T/CT (15.33 leaves plant-1) graft combination, while the lowest number of leaves was determined in the ungrafted Crimson Tide (5.33 leaves plant^{-1}) (Table 3). It was reported that 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, and an increase in DNA content usually results in increased cell and organ size in plants (Corneillie et al., 2019).

The shoot and root fresh and dry weights of grafted plants onto rootstocks with diploid and tetraploid ploidy levels were presented in Table 4. The plants grafted on diploid rootstocks had less biomass than plants grafted on tetraploid rootstocks. Plants grafted on tetraploid plants produced more shoot fresh weight than plants grafted on diploid rootstocks, except plants grafted on auto-tetraploid Calhoun Gray. At the same time, the plants grafted on tetraploid rootstocks produced higher shoot fresh weight than ungrafted control plants (CT) and grafted plants on commercial rootstock RS841.The highest shoot fresh and dry weights were obtained in N7-4T/CT graft combination with 93.33 g plant^{-1}, and 10.25 g plant^{-1}, respectively while the lowest shoot fresh and dry weights were determined in the plants grafted onto diploid Calhoun with 11.00 g plant^{-1} and 1.38 g plant^{-1}, respectively. Plants grafted on both diploid and tetraploid Calhoun Gary had the lowest shoot fresh and dry weights. As in shoot biomass, the root biomass was also significantly affected by rootstock genotype and ploidy level. Auto and allotetraploid produced higher root fresh weight than diploid corresponding rootstocks, ungrafted control plants and commercial rootstocks. The average root fresh weight of plants grafted on tetraploid rootstocks was 30.45 g plant^{-1}, while the average root fresh weight of plants grafted on diploid rootstocks was 11.46 g plant^{-1}. The graft combination with the highest root fresh weight was N5-4T/CT with 61.67 g plant^{-1} while the lowest root fresh weight was measured in the Calhon Gray D/CT graft combination with 5.20 g plant^{-1}. Similar to root fresh weight, tertaploid rootstocks had higher root dry weight. The highest root dry weight was measured in the CN7-5T/CT (1.43 g plant^{-1}) and N7-4T/CT (1.40 g plant^{-1}) graft combinations, while the lowest root dry weight was measured in the Calhoun Gray D/CT (0.23 g plant^{-1}) and N3D/CT (0.24 g plant^{-1}) graft combinations

Table 3. Plant stem diameter, plant lenghtht and number of leaves per plant in watermelon grafted on to different rootstocks grown under hydroponic conditions

Rootstock Ploidy Level	Graft Combinations (Rootstock/ Scion)	Plant Stem Diameter (mm)	Plant Height (cm plant^{-1})	Number of Leaves per Plant
Autotetraploid	Calhon GrayT/CT	4.83c-f*	14.33i	7.67gh
Autotetraploid	N3T/CT	5.53a-e	30.67d-h	9.33e-h
Autotetraploid	N5-3T/CT	5.14b-f	34.67cg	10.00c-f
Autotetraploid	N5-4T/CT	5.85ab	39.33b-e	12.00bc
Autotetraploid	N7-3T/CT	6.45a	27.67e-h	9.40e-h
Autotetraploid	N7-4T/CT	5.36a-e	62.67a	9.67d-g
Allotetraploid	CN3-2T/CT	5.73a-d	41.33b-d	9.70e-g
Allotetraploid	CN3-3T/CT	6.44a	37.67b-f	15.33a
Allotetraploid	CN5T/CT	5.58a-d	45.33bc	13.67ab
Allotetraploid	CN7-4T/CT	5.40a-e	26.67f-i	11.67b-d
AlloTetraploid	CN7-5T/CT	6.18ab	48.00b	9.77d-g
Average		5.68	37.12	10.75
Diploid	Calhon GrayD/CT	4.24d-f	18.67 hi	9.33ce
Diploid	N3D/CT	5.62a-d	23.33g-i	9.00e-h
Diploid	N5D/CT	3.98f	30.00d-h	9.57c-g
Diploid	N7D/CT	4.26d-e	20.33hi	8.00f-h
Diploid	CN3D/CT	3.89f	26.67f-i	13.33b
Diploid	CN5D/CT	3.96f	20.33 hi	7.33hi
Diploid	CN7D/CT	4.51c-e	19.33 hi	8.00f-h
Average		4.24	22.67	9.22
Diploid	RS841/CT	4.63c-f	20.33 hi	7.35hi
Diploid	Argentario/ CT	5.50a-e	29.33d-h	7.43hi
Diploid	Crimson Tide (ungrafted)	3.94f	30.67d-h	5.33i

*Values denoted by different letters are significantly different between genotypes within columns at $p< 0.05$

(Table 4). In agreement with the current study, a significant increase in shoot dry weight in tetraploid and hexaploid compared to diploids was reported in Arabidopsis (Zhang et al., 2019). The general belief of many researchers (del Pozo & Ramirez-Parra, 2014; Dudits et al., 2016; Głowacka, Jeżowski, & Kaczmarek, 2010; Li et al., 2012) that ploiploidization increases biomass production is consistent with our results.

The genotypic difference in leaf chlorophyll index was significant. The leaf chlorophyll index varied from 325 (CT) to 510 (CN3-3T/CT). The highest chlorophyll index was recorded in CN3-3T/CT, N7-4T/CT and CN3-2T/CT graft combinations, while ungrafted control plants and the grafted plants onto Argentario and tetraploid Calhoun Gray had the lowest chlorophyll index (Table 5). When shoot/root ratio were compared, it was determined that there was a significant difference between rootstock/scion combinations and the ratios of diploid rootstocks were higher than tetraploids. This shows that since tetraploid plants promote shoot growth, the shoot/root ratio of tetraploid plants is less than that of plants grafted on diploid plants. In a way that confirms our findings. Fredes et al., (2016) and Levi et al., (2014), reported that the use of tetraploid (allo and auto) watermelon rootstocks with a strong root system can provide high graft compatibility and a high survival rate, while the increased chlorophyll content and high antioxidant activities of tetraploid watermelon rootstocks can promote plant growth and stress tolerance without negatively af-

Table 4. Shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of watermelon grafted on different rootstocks grown under hydroponic conditions

Rootstock Ploidy Level	Graft Combinations (Rootstock/Scion)	Shoot Fresh Weight (g plant^{-1})	Shoot Dry Weight (g plant^{-1})	Root Fresh Weight (g plant^{-1})	Root Dry Weight (g plant^{-1})
Autotetraploid	Calhon GrayT/CT	16.67jk*	2.34h-j	6.63i	0.35gh
Autotetraploid	N3T/CT	33.33f-h	4.09e-g	20.00ef	0.69c-g
Autotetraploid	N5-3T/CT	40.00f-h	4.71c-f	18.33e-g	0.77c-f
Autotetraploid	N5-4T/CT	75.00b	8.16b	61.67a	1.28ab
Autotetraploid	N7-3T/CT	50.00ce	5.58c-e	15.00e-i	0.55d-h
Autotetraploid	N7-4T/CT	93.33a	10.25a	43.33c	1.40a
Allotetraploid	CN3-2T/CT	56.67c	6.05c	30.00d	0.87cd
Allotetraploid	CN3-3T/CT	60.00c	5.93cd	31.67d	0.80c-e
Allotetraploid	CN5T/CT	35.00f-h	3.97e-h	21.67e	0.52d-h
Allotetraploid	CN7-4T/CT	55.00cd	6.12c	33.33d	0.96bc
AlloTetraploid	CN7-5T/CT	95.00a	9.50ab	53.33b	1.43a
Average		55.45	6.06	30.45	0.88
Diploid	Calhon GrayD/CT	11.00k	1.38j	5.20i	0.23h
Diploid	N3D/CT	20.00i-k	2.38h-j	6.70i	0.24h
Diploid	N5D/CT	33.33f-h	4.34d-g	18.33e-g	0.64c-h
Diploid	N7D/CT	35.00f-h	3.35f-I	11.67f-i	0.37f-h
Diploid	CN3D/CT	43.33e-g	4.32d-g	10.00g-i	0.35gh
Diploid	CN5D/CT	18.33i-k	1.92ij	11.67f-i	0.39f-h
Diploid	CN7D/CT	30.00g-i	2.87g-i	16.67e-h	0.57d-h
Average		27.29	2.94	11.46	0.44
Diploid	RS841/CT	26.67h-j	2.67g-j	8.93hi	0.28h
Diploid	Argentario/CT	40.00e-g	3.67f-h	15.00e-i	0.39f-h
Diploid	Crimson Tide (ungrafted)	25.00h-j	2.96g-i	15.00e-i	0.46e-h

*Values denoted by different letters are significantly different between genotypes within columns at $p<0.05$

Table 5. Leaf chlorophyll content and shoot/root ratio of grafted watermelon grown under hydroponic conditions

Rootstock Ploidy Level	Graft Combinations (Rootstock/ Scion)	Leaf Chlorophyll Content (CM 1000 Chlorophyll Meter)	Shoot/Root Ratio
Autotetraploid	Calhon GrayT/CT	335.00gh	1.13f
Autotetraploid	N3T/CT	413.67ef	1.67c-f
Autotetraploid	N5-3T/CT	396.67f	2.22b-d
Autotetraploid	N5-4T/CT	442.00cd	1.22ef
Autotetraploid	N7-3T/CT	510.00a	3.33a
Autotetraploid	N7-4T/CT	506.67a	2.22b-d
Allotetraploid	CN3-2T/CT	500.00ab	1.94e-f
Allotetraploid	CN3-3T/CT	521.00a	1.94e-f
Allotetraploid	CN5T/CT	483.33b	1.67c-f
Allotetraploid	CN7-4T/CT	348.33cd	1.65c-f
AlloTetraploid	CN7-5T/CT	430.00de	1.77c-f
Average		444.24	1.89
Diploid	Calhon GrayD/CT	436.67df	2.17c-e
Diploid	N3D/CT	430.00de	3.05ab
Diploid	N5D/CT	426.67de	1.83e-f
Diploid	N7D/CT	431.67de	3.00ab
Diploid	CN3D/CT	420.00de	3.50a
Diploid	CN5D/CT	455.00b	1.67c-f
Diploid	CN7D/CT	440.00cd	2.00e-f
Average		434.29	2.46
Diploid	RS841/CT	355.00g	1.59c-f
Diploid	Argentario/CT	340.00gh	2.67a-c
Diploid	Crimson Tide (ungrafted)	325.00h	1.22ef

*Values denoted by different letters are significantly different between genotypes within columns at $p<0.05$

fecting fruit quality in watermelon.

The average root length of the grafted plants onto tetraploid rootstocks grown under hydroponic conditions is approximately 57% longer than the average root length of the plants grafted onto diploid rootstocks. In general, all grafted plants produced longer roots than the non-grafted control plants. The graft combination with the highest root length was CN5T/CT 7906.86 cm and the while the lowest root length was measured in plants grafted on diploid Calhoun Gray rootstock (272.69 cm) and non-grafted Crimson Tide (187.13 cm) respectively. In similar manner, the CN5T/CT graft combination producing the highest root length also had the highest root volume with 11.48 cm^3 $plant^{-1}$. The lowest root volume was measured in the CN3D/CT graft combination (0.16 cm^3) and non-grafted Crimson Tide plants (0.41 cm^3). The mean root diameter affected by rootstock genotype ranged from 0.34 mm to 0.58 mm. Plants grafted on tetraploid rootstocks ($\bar{x}$=0.45) had thicker roots than those grafted on diploid rootstocks ($\bar{x}$=0.41). CN5T/CT (0.58 mm) and CN3-3T/CT (0.52 mm) graft combinations had the highest root diameter, while the lowest root diameter was measured in plants grafted on Argentario (0.34 mm) rootstock. The mean root diameter of commercial rootstocks was calculated as 0.37 mm. (Table 6). Similarly, increases in plant biomass due to polyploidization 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, root length and leaf width, compared to diploid orchids (Chung, Kuo, & Wu, 2017). Polyploid plants showed higher leaf and root growth compared to diploid plants in *Artemisia cina* (Kasmiyati, Kristiani, & Herawati, 2020), Kim et al., (2004), reported that the number of adventitious roots in polyploid ginseng plants is higher than in diploid plants. Similar to the results of the present study the tetraploid watermelon line USVL-360 (citron) showed vegetative growth as much as commercially available cucurbit rootstocks and provided resistance against root-knot nematode (Levi et al., 2014).

Grafted plants onto different rootstocks grown under hydroponic conditions were compared in terms of leaf N, P, K, and Ca content (Table 7). All plant nutrients showed significant differences based on rootstocks. Plants grafted on tetraploid rootstocks had higher leaf nitrogen content than those grafted on diploid rootstocks, and the leaf nitrogen content of watermelons grafted onto tetraploid and diploid rootstocks was 2% and 1.7% respectively. The highest N content was determined in the CN3-3T/CT (2.57%) and N7-4T/CT graft combination, respectively, while the lowest N content was measured in the CN7D/CT (1.19%) graft combination. Leaf P content showed variation from 0.10% to 0.27% between graft combinations. In general, higher content of P was recorded in the plants grafted onto tetraploid rootstocks compared to control and diploid rootstocks. While the plants grafted onto tetraploid Calhoun Gray, N3T, N5-3T, N5-4T, N7-3T, and N7-4T had the highest leaf P content,

Table 6. Root length, root volume, and root diameter of grafted watermelon grown under hydroponic conditions

Rootstock Ploidy Level	Graft Combinations (Rootstock/ Scion)	Root Length (cm plant^{-1})	Root Volume (cm^3/plant^{-1})	Root Diameter (mm plant^{-1})
Autotetraploid	Calhoun Gray T/ CT	1199.55ef	2.98df	0.43e
Autotetraploid	N3T/CT	1756.36e	1.88f	0.37gh
Autotetraploid	N5-3T/CT	5646.29b	6.49c	0.39fg
Autotetraploid	N5-4T/CT	4086.59cd	6.18c	0.44de
Autotetraploid	N7-3T/CT	3870.89d	5.56c	0.44de
Autotetraploid	N7-4T/CT	5277.32	2.46ef	0.37gh
Allotetraploid	CN3-2T/CT	3972.10cd	6.07c	0.45de
Allotetraploid	CN3-3T/CT	3333.54d	6.54c	0.52b
Allotetraploid	CN5T/CT	7906.86a	11.48a	0.58a
Allotetraploid	CN7-4T/CT	4847.44bc	8.81b	0.48c
AlloTetraploid	CN7-5T/CT	5871.39b	8.36b	0.43e
Average		4342.58	6.07	0.45
Diploid	Calhoun Gray D/CT	272.69fg	0.61gh	0.36hi
Diploid	N3D/CT	1506.58e	1.88f	0.40f
Diploid	N5D/CT	3147.09d	3.78de	0.40f
Diploid	N7D/CT	1532.92d	2.17f	0.43e
Diploid	CN3D/CT	1506.86e	0.16h	0.46d
Diploid	CN5D/CT	3370.60d	3.94d	0.39fg
Diploid	CN7D/CT	3067.14e	5.35c	0.40f
Average		1857.75	2.56	0.41f
Diploid	RS841/CT	3333.54d	5.67c	0.39fg
Diploid	Argentario/CT	1506.58d	5.33c	0.34i
Diploid	Crimson Tide (non-grafted)	187.13g	0.41h	0.40f

Values denoted by different letters are significantly different between genotypes within columns at $p<0.05$

non-grafted control plants had the lowest P content with 0.01% (Table 6). Leaf potassium content was significantly affected by rootstocks and an increase in leaf K content was observed with polyploidization. The highest K content was determined in the CN3-3T/CT (4.59%) graft combination, while the lowest K content was obtained in the plants grafted onto commercial rootstocks (Argentario and RS841) and non-grafted control plants. (Table 7). When the graft combinations were evaluated in terms of leaf Ca content, the average leaf Ca content of the plants grafted on tetraploid rootstocks was 1.34%, while the average leaf Ca content of the plants grafted on diploid rootstocks was 1.02%. The appropriate rootstock/scion combination in vegetable grafting increased the nutrient uptake and use efficiency in many vegetable species (Nawaz et al., 2016; Schwarz, Rouphael, Colla, & Venema, 2010). In agreement with current study, it has been reported in previous studies that grafting onto rootstocks with strong root system increases the plant nutrient uptake and use efficiency (Schwarz et al., 2013; Uygur & Yetisir, 2009). Yetisir et al., (2013), that the N, Ca and K contents of watermelon leaves grafted on different gourd rootstocks (Cucurbita and Lagenaria) with strog root system were higher than the non-grafted plant. Huang et al., (2016), reported that grafting watermelon on squash rootstocks with a strong root system increased plant growth and grafted plants had twice the P content than non-grafted watermelon plants. Similarly, previous studies have reported improved water and nutrient uptake, nutrient utilization, and plant biomass growth by selecting appropriate rootstocks for spesific scion cultivars (Albacete et al., 2009; Gregory et al., 2013;

Table 7. N, P, K and Ca content of watermelon leaves grafted on different rootstocks grown under hydroponic conditions

Rootstock Ploidy Level	Graft Combinations (Rootstock/ Scion)	N%	P%	K%	Ca%
Autotetraploid	Calhoun Gray T/ CT	2.23bc*	0.26ab	2.16b	1.46a
Autotetraploid	N3T/CT	2.28bc	0.27a	2.09c	1.45a
Autotetraploid	N5-3T/CT	2.13ce	0.25a-d	2.12bc	1.45a
Autotetraploid	N5-4T/CT	2.16cd	0.25a-c	2.16b	1.48a
Autotetraploid	N7-3T/CT	1.93d-h	0.25a-d	1.77d	1.41ab
Autotetraploid	N7-4T/CT	2.57a	0.25a-d	1.77d	1.45ab
Allotetraploid	CN3-2T/CT	2.07c-f	0.24b-d	1.92d	1.33bc
Allotetraploid	CN3-3T/CT	2.57a	0.18hi	4.59a	1.19d
Allotetraploid	CN5T/CT	1.91d-h	0.22d-g	1.91d	1.30c
Allotetraploid	CN7-4T/CT	1.75g-j	0.20f-h	1.61d	1.29c
AlloTetraploid	CN7-5T/CT	1.78g-i	0.23c-e	1.65d	1.32c
Average		2.00	0.23	2.16	1.34
Diploid	Calhon Gray D/CT	1.84f-i	0.18hi	1.09de	0.82i
Diploid	N3D/CT	1.85e-i	0.22d-g	1.72d	0.81i
Diploid	N5D/CT	1.65h-j	0.20e-h	1.65d	1.12de
Diploid	N7D/CT	1.50j	0.18hi	1.38d	1.10ef
Diploid	CN3D/CT	1.61i-j	0.20f-h	1.50d	1.04ef
Diploid	CN5D/CT	1.48j	0.17hi	1.48d	1.01
Diploid	CN7D/CT	1.19k	0.15i	1.10d	0.87hi
Average		1.70	0.19	1.42	1.02
Diploid	RS841/CT	1.86d-i	0.19gh	1.02de	0.87hi
Diploid	Argentario/CT	2.46ab	0.16i	0.78e	0.94gh
Diploid	Crimson Tide (non-grafted)	1.94d-h	0.10j	1.06de	0.85hi

Values denoted by different letters are significantly different between genotypes within columns at $p<0.05$

Yetisir et al., 2013).

Correlation Between Root Characteristics and Plant Growth

The correlation between root growth parameters and shoot growth parameters, chlorophyll index, leaf N %, % P, % K and % Ca parameters are presented in Table 8. A significant positive correlation was found between root fresh weight and stem diameter (0.476), plant height (0.699), number of leaves per plant (329), shoot fresh weight (873), shoot dry weight (841), and N% (413) at $p<0.01$ level and a significant positive correlation was found at $p<0.05$ level with chlorophyll index (292). There was a positive correlation at $p<0.01$ level between root dry weight and stem diameter (0.366), plant height (0.591), shoot fresh weight (0.764), shoot dry weight (0.728), and leaf N content (0.436) and there was a positive correlation between chlorophyll index (0.314) and P% (0.305) at $p<0.05$ level. Significant positive correlations between root length and stem diameter (0.364), plant height (0.561), shoot fresh weight (0.515), shoot dry weight (0.522), chlorophyll index (0.353) and leaf N (0.436) at $p<0.001$ level was determined and correlation between root length and number of leaves per plant was significant (0.271) at $p<0.05$. The root volume was positively correlated with stem diameter (0.415; p<0,01), plant length (0.319; $p<0.01$) shoot fresh weight (0.369; $p<0.01$), shoot dry weight (0.330; $p<0.01$) and leaf nitrogen content (0.248; $p<0.01$). A positive correlation was determined between root diameter and number of leaves per plant (0.382) and %K (0.347) at $p<0.01$ significance level. Significant positive correlations between root characteristics of rootstocks and vegetative growth parameters of scions have also been reported in several previous studies (Albacete et al., 2009; Gregory et al., 2013; Schwarz et al., 2010; Uygur & Yetisir, 2009; Yetisir et al., 2013)

Tablo 8. Pearson's correlation coefficients (r values) between root characteristics and other measured parameters in grafted watermelon onto different rootstock grown under hydroponic conditions

Parametetrs	Stem Diameter	Plant Length	Leaves Number Per Plant	Shoot Fresh Weight	Shoot Dry Weight	Chlorophyll Content	N	P	K	Ca
Root Fresh Weight	0.476**	0.699**	0.329**	0.873**	0.841**	0.292*	0.413**	0.195	0.147^{ns}	-0.031^{ns}
Root Dry Weight	0.366**	0.591**	0.244^{ns}	0.764**	0.728**	0.314*	0.436**	0.305*	0.190^{ns}	0.025^{ns}
Root Length	0.364**	0.561**	0.271*	0.515**	0.522**	0.353**	0.462**	0.240^{ns}	0.151^{ns}	-0.229^{ns}
Root Volume	0.415**	0.319*	0.243^{ns}	0.369**	0.330**	0.140^{ns}	0.248*	0.014^{ns}	0.156^{ns}	-0.188^{ns}
Root Diameter	0.165^{ns}	-0.121	0.382**	0.094^{ns}	0.079^{ns}	0.021^{ns}	0.217^{ns}	0.029^{ns}	0.347**	-0.136^{ns}

* and ** denote P ≤ 0.05 and 0.01, respectively, and ns as not significant.

CONCLUSION

In this study, it has been shown that tetraploid (auto and allo) genotypes of the Citrullus genus can increase plant growth and plant nutrient uptake when used as rootstock. According to the results of our study, plant biomass development and leaf nutrient content of plants grafted on tetraploid rootstocks are higher than the plants grafted on diploid rootstocks. Some auto (N7-3T and N7-4T) and allo (CN3-2T and CN3-3T) tetraploid genotypes that we developed in our previous studies and used as rootstock in this study presented superior performance to commercial rootstocks (RS841 and Argentaio) in terms of plant growth parameters. Therefore, in terms of vegetative growth and nutrient uptake, tetraploid (allo and auto) watermelon rootstocks developed from Citrullus genus are expected to become commercially available for different horticultural crops in the near future. More detailed studies are needed to determine the effect of tetraploid rootstocks on the fruit yield and quality characteristics, plant nutrient metabolism and some stress tolerance of the scion when they are used as rootstocks for cultivated watermelons.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Ethics committee approval is not required.

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Data availability

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