RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 704-710
DOI: https://doi.org/10.31015/jaefs.2022.4.26

Comparison on flower, fruit and seed characteristics of tetraploid and diploid watermelons (*Citrullus lanatus* Thunb. Matsum. and Nakai)

Pınar Adıgüzel[1] • **İlknur Solmaz**[1] • **Şenay Karabıyık**[1] • **Nebahat Sarı**[2]

[1] Horticulture Department, Faculty of Agriculture, Cukurova University, Adana, Türkiye
[2] Department of Chemical and Biological Engineering, Princeton University, Princeton, New Jersey, USA

Abstract

In this study, the characteristics of tetraploid and diploid watermelon's flowers, fruits, and seeds were examined, and their differences were investigated. As plant material, tetraploid ST101 and ST82; diploid WL92, WL124, WL134, WL216, WL259-B and WL235 were used. Flower (pollen viability, pollen germination, number of anthers and number of pollen per anther and flower); fruit (weight, length, diameter, rind thickness and TSS) and seed (weight of 1000 seed, length, width, thickness, full seed number, seed yield, embryo/seed ratio, germination and emergence) parameters were investigated. In terms of pollen parameters, diploid watermelon has a higher value than tetraploid watermelon. According to research results, average pollen germination was found to be in diploid watermelons at 74.48% and tetraploid watermelons at 71.62%. Pollen viability was determined highest in WL 235 (95.53%). In fruit parameters, tetraploid watermelons had higher values, but fruit length decreased (diploid 19.32 cm; tetraploid 15.33 cm) significantly. Considering the average values of tetraploid watermelons, a significant decrease occurred in terms of seed germination, full seed number, seed yield and embryo/seed ratio (57.22%, 225.48 seed, 6.33 g/fruit, 57.51% respectively in diploids; 37.31%, 57.67 seed, 4.90 g/fruit, 46.42% in tetraploid), and no difference observed in other seed parameters.

Keywords: Tetraploid, Diploid, Pollen, Watermelon, Seed Germination

Citation: Adiguzel, P., Solmaz, I., Karabiyik, S., Sari, N. (2022). Comparison on flower, fruit and seed characteristics of tetraploid and diploid watermelons (*Citrullus lanatus* Thunb. Matsum. and Nakai). International Journal of Agriculture, Environment and Food Sciences, 6 (4), 704-710

Received: 01 November 2022
Revised: 12 December 2022
Accepted: 15 December 2022
Published Online: 24 December 2022

Correspondence: Pınar Adıgüzel
E-mail: pinaradiguzel63@gmail.com

INTRODUCTION

Watermelon (*Citrullus lanatus* L. Matsum. and Nakai) is an economically important vegetable in the *Cucurbitaceae* family (Zhang et al., 2019). Although watermelons are generally diploid (2n=2x=22), they can also be found in triploid (3n=3x=33) and tetraploid (4n=4x=44) structures as autopolyploids (Blakeslee and Avery, 1937). Autopolyploids differ from diploids in that they have different DNA content, contain high levels of secondary metabolite organisms, have large tissues and organs, have a high yield, and provide tolerance to biotic and abiotic stress factors (Soltis et al., 2016; Godfree et al., 2017). However, apart from these benefits, their low frequency and undetermined time of occurrence are also disadvantages (Zhang et al., 2019). To eliminate this situation, colchicine, which was applied first to the Datura plant by Blakeslee and Avery in 1937, has been the most successful method for obtaining tetraploids today (Blakeslee and Avery, 1937; Kihara, 1951; Andrus, 1971; Suying et al., 1993). Tetraploid lines are obtained by applying chemicals such as colchicine or oryzalin to diploid lines (Kihara, 1951; Lower and Johnson, 1969; Koh, 2002; Li et al., 2002; Jaskani et al.,

2004; Inan, 2007). As a result of this application, a limited number of tetraploid seedlings, a high rate of chimera seedlings, and diploid seedlings with unchanged structures have emerged (Compton and Gray, 1994; Jaskani et al., 2004). Inbreeding and tissue culture can be used to reproduce tetraploid lines (Compton and Gray, 1991; Krug et al., 2005; Zhang, 2010). Tetraploid lines can be identified through morphological observations of differences in leaf, ovary, and flower sizes as well as through chromosome counting (Sari et al., 1999; Jaskani and Khan, 2000) and flow cytometry (FCM) methods (Compton et al., 1996; Norrmann et al., 1997; Rhodes and Zhang, 2000). Finally, the qPCR method was found to be another method that allows for determining the plant ploidy level (Zhang et al., 2019). Because of the small and deeply embedded chromosomes in watermelon, the chromosome counting technique is not widely used (Fahleson et al., 1988; Şimşek et al., 2013). The flow cytometry (FCM) technique is the most preferred method because of its fast and reliable nature (Arumuganathan and Earle, 1991; Dolezel, 1998; Koh, 2002; Jaskani et al., 2004; Jaskani et al., 2005). Tetraploids are the primary parents in the triploid breeding program. The triploid plants are obtained by the cross-pollination of tetraploids and diploids (Kihara, 1951; Jaskani and Khan, 2000). Tetraploids are also valuable germplasm and cultivars (Jaskani et al., 2005). The objective of this study was to compare the flower, fruit, and seed properties of tetraploid and diploid watermelon lines.

MATERIALS AND METHODS

This study was conducted in 2018 growing seasons in the plastic greenhouse at the Research Application Area of Horticulture Department (latitude 37°1′48.63″N, longitude 35°22′3.74″E, altitude 56 m), Cukurova University, Adana, Turkey.

Plant Material

In this study two tetraploid (4n) lines (ST 101 and ST 82) were used as female parents, and six diploid (2n) lines (WL 124, WL 92, WL 235, WL 134, WL 259-B and WL 216) were used as male parents. Tetraploid female and diploid male parental lines were grafted onto Nun9075 interspecific hybrid (*C. maxima* x *C. moschata*) rootstock. The grafted combination seedlings were planted on 04.05.2018 in a plastic greenhouse of double row with (100-50) x 50 cm spacing distances within three repetitions. Twenty healthy plants of both tetraploid and diploid seedlings were planted. A total of 120 plants were used (20 plants x 3 replications, both tetraploid and diploid watermelon). Both female flowers and male flowers were isolated one day before anthesis. The following day morning selfing was performed. Fruit analysis was carried out on 4 plants from each plot (4 plants x 3 replicates). In the study, the number of anthers in a flower, pollen number in an anther, pollen viability rate (%) and germination rate (%); fruit characteristics [(fruit weight (g), length (cm), diameter (cm), rind thickness (mm), TSS (%)] and seed characteristics [yield (g/fruit), number of full seeds (number/fruit), 1000 seed weight (g), embryo/seed ratio (%), length (mm), width (mm) and thickness (mm), seed germination rate (%) time (day), emergence rate (%) and time (day)] parameters were examined. Seed length, width and thickness, were measured using a digital caliper (Mitutoyo), while seed weight was measured using a digital scale.

Flower Analysis

Pollen viability and germination tests were carried out with the pollen collected from the male flowers in the anthesis stage. The %1, 2,3,5 Triphenyltetrazolium Chloride (TTC) was used to determine pollen viability rates (Norton, 1966). The TTC indicator was used to assess seed viability. The red color variation, ranging from a light to a dark red hue, was shown to result from the reaction between the dehydrogenase enzyme and the reddish component known as formazan in this indicator. The viability of the seed was analyzed based on these colors (Shivanna and Rangaswamy, 1992; Sensoy et al., 2003). The empty pollen slides when it comes into contact with the fluid dye, so it is preferable to count the pollen in-between the preparations during counting (Elçi, 1982). The TTC test results demonstrated that viable pollen turned red, semi-viable pollen pink, and non-viable pollen was white (Eti, 1991). During the pollen counting stage, pollen in the center of the lamella should be counted because the pollen at the edge of the lamella contains more oxygen, this may cause a difference in the pollen staining rate (Shivanna and Rangaswamy, 1992). Pollen germination rates were identified by using the medium containing boric acid (250 ppm), agar (1%) and sucrose (10%) in petri dishes at 25°C (Eti,1991; Karabıyık et al., 2017).

Fruit and Seed Analysis

Fruits were weighed (g), cut in the middle and measured with a ruler to determine fruit length (cm) and diameter (cm). Rind thickness was determined using a digital caliper (Mitutoyo) total soluble solids (TSS, %) using a digital refractometer (Atago). Three fruits of each replication were used in fruit analysis. The seeds were fermented after being removed from the fruit for 3 days, then washed, and dried. Seed analyses were performed with 4 replications of 100 seeds in each repetition. Seed length, width, seed thickness, and fruit diameter were measured using a digital caliper (Mitutoyo), while seed weight was measured using a digital scale. Seeds have been germinated in an incubator (Memmert) at 25°C according to ISTA rules (ISTA, 2018) Seed germination is determined by dividing the germinated seeds by the total number of planted seeds.

The experiment was set up according to the randomized plot design. The data were analyzed using the JMP program (v8.00, SAS Institute Inc., NC 27513–2414, USA).

RESULTS AND DISCUSSION

In terms of the average number of anthers in male flowers, there was no statistical difference between used watermelon lines. The study resulted in 5.20 anthers per flower in tetraploids and 5.04 anthers per flower in diploids (Table 1). In previous reports, it was stated that the average number of anthers (3.00) in a flower of tetraploid-diploid gourd and diploid watermelon (Kombo, 2017; Hassan et al., 2020) and (3.40) of triploid watermelon (Hussein, 2017). Normally, the number of anthers in a watermelon flower is 5, however, most of the time 2 anthers appear to be attached, so they can be counted as a single anther.

The highest numbers of pollen in the anther (22112.82) and in the flower (112604.95) were obtained from WL 134 (Table 1). According to the mean values of evaluated parameters, the diploid watermelon lines had higher values (19.735.34; 99640.4, respectively) than tetraploid lines (17128.01; 89074.29, respectively). The highest pollen number in anther (124486.70) was found from the diploid Crimson Sweet grafted onto Argentario whereas the lowest pollen number (60917.76) was determined in ungrafted group (Kombo 2017).

In terms of pollen viability, the highest rate (95.53%) was found from WL 235 line while the lowest value (87.60%) was obtained from ST 82 line (Table 1). According to mean values of pollen viability rate among tetraploid and diploid, in diploids (92.23%) were higher than in tetraploids (87.76%) lines. There was no statistical difference between diploid and tetraploid lines in terms of pollen germination rates. The average values of pollen germination rates were 71.62% in tetraploids and 74.48% in diploids (Table 1). The pollen germination rate was affected by the pollen collection time, incubation conditions, environment, and pollen density (La Porta and Roselli, 1991). Moreover, various factors influence pollen viability and germination rates, such as environmental factors and the pollen viability period of each plant (Nepi and Pacini, 1993). In the study of Gok et al. (2005), 45 watermelon genotypes were evaluated and the highest pollen viability rates were determined as 97.40% and 97.36% while the lowest were 49.65% and 61.08% and between Furthermore, 89.43%–88.23%, pollen germination rates were found to be the highest, and the lowest were ranged between 19.62%–20.22%. Freeman et al. (2008), reported that pollen germination rates in diploid watermelons was in range of 97% and 99.2%.

Table 1. The number of anthers in a flower, number of pollen in an anther, number of pollen in a flower, pollen viability rate (%) and pollen germination rate (%) in tetraploid and diploid watermelon.

Genotype Name	The number of anthers in a flower	Number of pollen in an anther	Number of pollen in a flower	Pollen viability rate (%)	Pollen germination rate (%)
ST 82	5.20	14740.39 D	76650.02 D	87.60 D (70.01)	72.75 (64.31)
ST 101	5.20	19515.62 ABC	101498.57 ABC	87.92 CD (71.36)	70.50 (59.93)
Tetraploid Average	5.20	17128.01	89074.29	87.76	71.62
WL 134	5.08	22112.82 A	112604.95 A	92.11 BCD (73.15)	75.88 (63.36)
WL 216	5.00	16737.17 CD	83685.83 CD	93.59 AB (76.87)	74.00 (58.00)
WL 259-B	5.17	21395.42 AB	110718.46 AB	89.50 CD (71.26)	68.75 (54.71)
WL 92	5.00	19066.85 ABC	95334.25 A-D	93.16 ABC (74.45)	73.75 (59.48)
WL 124	5.00	18497.18 BC	92485.92 BCD	89.50 CD (69.01)	79.50 (61.23)
WL 235	5.00	20602.61 AB	103013.07 ABC	95.53 A (77.94)	75.00 (59.02)
Diploid Average	5.04	19735.34	99640.4	92.23	74.48

LSD (the number of anthers in a flower): NS; LSD (number of pollen in an anther): 3528.82***; LSD (number of pollen in a flower): 19460.16***; LSD (pollen viability, %): 4.40***; LSD (pollen germination, %): NS

NS: Not Significant; *: ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Transform values are given in parentheses.

Fruit weight (g), length (cm) diameter (cm), rind thickness (mm) and TSS (%) values are presented in Table 2. Fruit weight was found to be higher in WL92 (2846.67 g), followed by ST82 (2593.33 g) and WL 124 (2111.11 g). However, in terms of average weight, tetraploids' fruit (2193.33 g) was heavier than diploids' (2135.44 g). According to the fruit length (cm), the longest fruit (22.26 cm) was obtained from WL 134 line. The widest fruit (16.48 cm) was found from ST 82 line. Due to fruit rind thickness, the thickest fruit rind (14.61 mm) was in ST 82. The TSS was high (12.23%) in WL 124 line. Diploid watermelon lines showed the highest values in fruit length, while tetraploids were greater in terms of fruit diameter, rind thickness, and TSS.

Generally, tetraploid plants start to flower later than diploids. The diploid-triploid-tetraploid flowers sizes increase proportionally to the number of chromosomes

Table 2. Fruit weight (g), fruits length (cm), fruit diameter (cm), rind thickness (mm) and TSS (%) in tetraploid and diploid watermelon.

Genotype Name	Fruit Weight (g)	Fruit Length (cm)	Fruit Diameter (cm)	Rind Thickness (mm)	TSS (%)
ST 82	2593.33 AB	16.10 CD	16.48 A	14.61 A	9.66 C
ST 101	1793.33 C	14.57 DE	15.60 ABC	10.48 B	11.33 AB
Tetraploid Average	2193.33	15.33	16.04	12.55	10.50
WL 134	2104.00 BC	22.26 A	13.93 BCD	10.84 B	10.13 BC
WL 216	1780.00 C	13.92 E	14.89 A-D	9.45 BC	9.60 C
WL 259-B	2062.00 BC	21.59 A	13.85 CD	9.49 BC	9.43 C
WL 92	2846.67 A	21.97 A	16.03 A	13.22 A	11.43 AB
WL 124	2311.11 ABC	17.89 BC	15.70 AB	7.98 C	12.23 A
WL 235	1708.89 C	18.26 B	13.42 D	11.01 B	8.90 C
Diploid Average	2135.44	19.32	14.64	10.33	10.28

LSD (Fruit Weight): 691.14*; LSD (Fruits Length): 1.93***; LSD (Fruit Diameter): 1.80*; LSD (Rind Thickness): 1.75*** LSD (TSS): 1.40**

NS: Not Significant; *: ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Transform values are given in parentheses.

(Kihara, 1951). The rate of TSS was higher in tetraploid watermelons than in diploid watermelons, it was between 12% -14% in tetraploid watermelons (Zhang, 2010). Şimşek et al., (2013) reported the TSS rate ranged between 8% - 10% in diploid watermelons. The fruit weights of tetraploid and diploid watermelons are found to be similar (Kihara 1951; Jaskani et al., 2005), however, Henderson (1977) stated that tetraploids form smaller fruits. The fruit weight varied between 1.5 and 2 kg in tetraploid watermelons (Zhang, 2010). In our study, the average fruit weight of diploid was 2135.44 g and 2193.33 g in tetraploid watermelon lines which are found to be similar. Tetraploid watermelons had lower values than diploids in terms of fruit length and diameter. Zhang et al. (2019), evaluated the fruit length and diameter, and found higher values in diploids than in tetraploids. It has been determined by Jaskani et al. (2005) that the rind thickness was 12.7 mm in diploid and 17.2 mm in tetraploid watermelons similarly.

The seed analysis results were presented in Table 3. The highest values were obtained in seed yield from WL 134 (12.98 g/fruit); in full seed number from WL 92 line (451 seed/fruit); in 1000 seed weight from line ST 101 (165.1

Table 3. Seed yield (g/fruit), number of full seed (seed/fruit), 1000 seed weight (g), embryo/seed ratio (%), seed length (mm), seed width (mm) and seed thickness (mm) in teraploid and diploid watermelon

Genotype Name	Seed Yield (g/fruit)	Number of Full Seed (seed/fruit)	1000 Seed Weight (g)	Embryo/Seed Ratio (%)	Seed Lenght (mm)	Seed Width (mm)	Seed Thickness (mm)
ST 82	4.69 DE	70.67 EF	66.51 B	50.00 (45.02) CD	9.63 A	6.45 A	2.65 A
ST 101	5.11 CD	30.67 F	165.15 A	42.83 (40.89) D	9.82 A	6.06 B	2.38 B
Tetraploid Average	4.90	50.67	115.83	46.42	9.73	6.26	2.52
WL 134	12.98 A	339.27 B	38.25 B	62.14 (52.05) AB	8.81 B	5.55 C	1.68 D
WL 216	5.77 CD	166.44 D	34.63 B	57.07 (49.09) BC	8.33 C	5.06 E	1.95 C
WL 259-B	9.08 B	261.583 C	34.77 B	66.46 (54.70) A	8.64 B	5.28 D	1.54 E
WL 92	7.38 BC	451.00 A	16.37 B	54.29 (47.50) BC	6.46 D	3.91 F	1.59 DE
WL 124	0.59 F	47.89 EF	14.33 B	55.12 (47.99) BC	4.99 E	3.15 G	1.05 F
WL 235	2.16 EF	86.72 E	27.66 B	49.99 (45.02) CD	8.63 B	5.16 DE	1.49 E
Diploid Average	6.33	225.48	27.67 B	57.51	7.64	4.69	1.55

LSD (Seed Yield): 2.55***; LSD (Number of Full Seed): 48.93***; LSD (1000 Seed Weight): 56.69**; LSD (Embryo/Seed Ratio): 6.03*** LSD (Seed Lenght): 0.28*** LSD (Seed Width): 0.16***; LSD (Seed Thickness): 0.13***;

NS: Not Significant; *: ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Transform values are given in parentheses.

Table 4. Seed germination (%), germination time (day), seed emergence (%) and emergence time (day) in tetraploid and diploid watermelon

Genotype Name	Seed Germination Rate (%)	Germination Time (day)	Seed Emergence Rate (%)	Emergence Time (day)
ST 82	61.12 (51.48) A	2.70	100.00 (90.04)	1.80
ST 101	13.50 (21.56) B	1.50	60.00 (51.20)	2.00
Tetraploid Average	37.31	2.10	80.00	1.90
WL 134	65.00 (53.75) A	0.27	86.67 (72.32)	1.70
WL 216	65.00 (53.75) A	0.56	93.33 (81.21)	3.20
WL 259-B	63.33 (52.76) A	2.60	93.33 (81.20)	1.25
WL 92	55.00 (47.93) A	0.79	80.00 (68.10)	1.38
WL 124	44.44 (41.77) A	1.15	73.33 (63.88)	2.84
WL 235	50.56 (45.32) A	0.56	73.33 (59.24)	1.55
Diploid Average	57.22	0.98	83.33	1.99
LSD (Germination %): 22.11**; LSD (Germination Time): NS; LSD (Emergence %): NS; LSD (Emergence Time): NS				

NS: Not Significant; *: ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Transform values are given in parentheses

g). In seed length, tetraploid lines ST 101 (9.82 mm) and ST 82 (9.63 mm) were in the same statistical group and had superior values. The highest seed width and thickness were determined in tetraploid ST 82 (6.45 mm and 2.65 mm respectively). The embryo/seed ratio was highest in WL 259-B (66.46%) and WL 134 (62.14%), which are in the same statistical group. In terms of seed parameters on average; diploids had the highest values in full seed number, seed yield and embryo/seed ratio; Tetraploids were superior in 1000 seed weight, seed length, seed width and seed thickness.

The seeds of tetraploid plants are thicker and broader than diploid seeds (Kihara, 1951) and more oblong-round shaped than diploids (Jaskani et al., 2005). Also, as a single layer of enlarged palisade cells forms the epidermis, there is an indented seed coat (Chopra and Swaminathan, 1960) with cracking of the palisade layer (Kihara 1951; Jaskani et al., 2005). It has been determined that while the embryo fills the seed coat in diploids, it does not fill the seed coat due to the space on the chalazion side in the seeds of tetraploids (Jaskani et al., 2005). Seed length, width and thickness in tetraploid watermelons was found 9 mm; 6.3 mm, and 2.8 mm respectively, the shape of the seed was observed as rectangular round in tetraploids by Jaskani et al. (2005). Zhang et al. (2019) reported that the seeds of tetraploid watermelons are larger than diploid watermelons. Chopra and Swaminathan (1960) examined the number of seeds per fruit and found very few in selfed tetraploid watermelons compared to open-pollinated tetraploids and diploids. Due to the number of seeds, a total of 681 seed in diploids and 446 seed in tetraploids were obtained in the study of Compton et al. (1996), 20.6-75.3 in tetraploids and 182.8-733 in diploid watermelon; Jaskani et al. (2005) determined that there were 323.5 seeds in diploid and 37.9 seeds in tetraploid watermelons As stated in other studies, a decrease in the number of seeds was observed in tetraploids. This situation is thought to be caused by the embryos of tetraploids not filling the seed coat, thus affecting the number of filled seeds.

The seed germination rate was found to be high in all lines except ST 101 tetraploid line (Table 4). Seed germination time, seed emergence rate, and emergence time values were found to be non-significant. Due to the seed emergence time (day) and rate (%), the results indicated that the earliest seed emergence time (0.27 day) was found from WL 134 line. While the highest seed emergence rate (100%) was obtained from ST 82 line. In terms of averages of seed germination and emergence in tetraploids and diploids, diploids were found to have higher values than tetraploids. Seed germination was inhibited by the seed coat formation mechanism. Based on the hard seed coat of tetrapoid seed, the water and gases passage is limited. The germination rate (%) is limited due to the seed coat thickness and the excess of air space between the embryo and rind (Jaskani et al., 2006). The germination rate is reduced not only by the thickness of seed coat but also due to the high moisture content in seed. The moisture content increases in the immature embryo, and the germination rate decreases according to the large air gap between the embryo and the seed coat (Grange et al., 2000; Grange et al., 2003). In addition, Jaskani et al. (2006), reported that the germination rates in tetraploid watermelons were 76.6% and 93.3% in diploids. The researchers have also demonstrated that the germination rates increased in diploids (98%) and tetraploids (78%), after the seeds were cracked (Jaskani et al., 2005).

CONCLUSION

In this study, a comparison of the characteristics of tetraploid and diploid watermelons was performed. The results indicated that the increase in the number of chromosomes in watermelon plants affected the flower, fruit, and seed characteristics. Furthermore, seed yield, full seed weight, and embryo/seed ratio were decreased in tetraploid watermelon. On the other hand, the length,

width, thickness, and 1000 seed weight of the tetraploid seeds were increased. Tetraploid watermelons developed seeds larger with thicker rinds than diploid watermelons. This had a negative impact on seed germination rate/time, and emergence rate/time. The thickness of the fruit rind and seed coats in tetraploid plants has been proven to be a result of colchicine application which duplicates the chromosome number. Further studies are required to eliminate the effect of the colchicine chemical product on seed coat thickening in order to increase germination and emergence rates.

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

N. S: Conceptualization and design of the research; P. A and Ş. K: Study data analysis; P. A: Wrote the original draft; and İ. S: Editing and preparation of the manuscript. All authors have read and approved the manuscript after N. S and I. S revised. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

This study was funded by Cukurova University, Unit of Scientific Research Projects with project number FLY-2018-10957.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

The authors are thankful to Cukurova University, Unit of Scientific Research Projects. The authors also thank Antalya Tarım Productive, Consultant and Marketing Co., and Ismail SİMSEK for their collaboration and for providing grafted seedlings used in this work.

REFERENCES

Andrus, C. F. (1971). Production of Seedless Watermelons, 1424. US Department of Agriculture.

Arumuganathan, K., Earle, E. D. (1991). Estimation of Nuclear DNA Content of Plants by Flow Cytometry. Plant Molecular Biology Reporter, 9(3): 229-241. [CrossRef]

Blakeslee, A. F., Avery, A. G. (1937). Methods of Inducing Doubling of Chromosomes in Plants: by Treatment With Colchicine. Journal of Heredity, 28 (12): 393-411. [CrossRef]

Chopra, V. L., Swamınathan, M. S. (1960). Induction of Polyploidy in Watermelon. Proceedings of the Indian Academy of Sciences-Section B. Springer India, 51(2), p. 57-65. [CrossRef]

Compton, M. E., Gray, D. J. (1991). Shoot Organogenesis on Cotyledons of Watermelon. Horticultural Science, 26: 772.

Compton, M .E., Gray, D .J . (1994). Adventitious Shoot Organogenesis and Plant Regeneration from Cotyledons of Tetraploid Watermelon. HortScience, 29: 211-213. [CrossRef]

Compton, M. E., Gray, D. J., Elmstrom, G. W. (1996). Identification of Tetraploid Regenerants from Cotyledons of Diploid Watermelon Cultured *in vitro*. Euphytica, 87(3): 165-172. [CrossRef]

Dolezel, J. (1998). Flow Cytometry, its Application and Potential for Plant Breeding. Current Topics in Plant Cytogenetics Related to Plant Improvement (Ed.): T. Lelley. Universitätsverlag, Vienna, 80-90.

Elçi, Ş. (1982). Observations and Research Methods in Cytogenetics. Fırat University Faculty of Arts and Sciences Publications, Biology:3, Malatya. 159.

Eti, S. (1991). Determination of Pollen Viability and Germination Capability of Some Fruit Species and Cultivars by Different *in vitro* Tests. J. Agric. Fac. Cukurova. Univ. 6:69–80.

Fahleson, J., Dixelius, J., Sundberg, E., Glimelius, K. (1988). Correlation Between Flow Cytometric Determination of Nuclear DNA Content and Chromosome Number in Somatic Hybrids within *Brassicaceae*. Plant Cell Reports, 7(1): 74-77. [CrossRef]

Freeman, J. H., Olson, S. M. and Kabelka, E. A. (2008). Pollen Viability of Selected Diploid Watermelon Pollenizer Cultivars. HortScience, 43(1), 274-275.

Godfree, R. C., Marshall, D. J., Young, A. G., Miller, C. H., Mathews, S. (2017). Empirical Evidence of Fixed and Homeostatic Patterns of Polyploid Advantage in a Keystone Grass Exposed to Drought and Heat Stress. Royal Society Open Science, 4(11): 170934. [CrossRef]

Gok, P., Yetisir, H., Solmaz, I., Sari, N., Eti, S. (2005), September. Pollen Viability and Germination Rates of 45 Watermelon Genotypes. In III International Symposium on Cucurbits 731, pp. 99-102. [CrossRef]

Grange, S. L., Leskovar, D. I., Pike, L. M., Cobb, B. G. (2000). Excess Moisture and Seedcoat Nicking İnfluence Germination of Triploid Watermelon. HortScience, 35(7): 1355-1356. [CrossRef]

Grange, S., Leskovar, D. I., Pike, L. M., Cobb, B. G. (2003). Seedcoat Structure and Oxygen-Enhanced Environments Affect Germination of Triploid Watermelon. Journal of the American Society for Horticultural Science, 128(2): 253-259. [CrossRef]

Hassan, J., Miyajima, I., Ozaki, Y., Mizunoe, Y., Sakai, K., Zaland, W. (2020). Tetraploid Induction by Colchicine Treatment and Crossing with a Diploid Reveals Less-Seeded Fruit Production in Pointed Gourd (*Trichosanthes dioica* Roxb.). Plants, 9(3): 370. [CrossRef]

Henderson, W. R. (1977). Effect of Cultivar, Polyploidy and Reciprocal Hybridization on Characters İmportant in Breeding Triploid Seedless Watermelon Hybrids. Journal of The American Society For Horticultural Science, 102(3): 293-297.

Hussein, S. (2017). The Effects of Different Rootstocks on Seed Yield and Quality in Seedless Watermelon Seed Production in Greenhouse. Çukurova University Institute of Science, Department of Horticulture, Master Thesis, Adana,65 s.

Inan, S. (2007). Obtaining Tetraploid Plants from Watermelon (*Citrullus Lanatus* (Thunb.) Matsum and Nakai) by *in vivo* and *in vitro* Methods. Çukurova University, Institute of Science, Department of Horticulture, Master Thesis, Adana, 80 p.

ISTA (International Rules for Seed Testing), Full Issue. (2018). i–19-8 (298) https://doi.org/10.15258/istarules.

Jaskani, M.J., Khan, I.A. (2000). Characterization of Interploid Hybrids of Kinnow Mandarin. In Proceedings of International Society of Citriculture, 1: 165-166.

Jaskani, M. J., Kwon, S. W., Koh, G. C., Huh, Y. C., Ko, B. R. (2004). Induction and Characterization of Tetraploid Watermelon. Horticulture Envıronment and Bıotechnology, 45(2): 60-65.

Jaskani, M. J., Kwon, S. W., Kim, D. H. (2005). Comparative Study on Vegetative, Reproductive and Qualitative Traits of Seven Diploid and Tetraploid Watermelon Lines. Euphytica, 145(3): 259-268. [CrossRef]

Jaskani, M. J., Kwon, S. W., Kin, D. H. (2005). Flow Cytometry of DNA Contents of Colchicine Treated Watermelon as a Ploidy Screening Method at MI Stage. Pakistan Journal of Botany, 37(3): 685.

Jaskani, M. J., Kwon, S. W., Kim, D. H., Abbas, H. (2006). Seed Treatments and Orientation Affects Germination and Seedling Emergence in Tetraploid Watermelon. Pakistan Journal of Botany, 38(1): 89.

Karabıyık, Ş., M.A. Sarıdaş, S. Eti, and S. Paydaş Kargı. (2017). The Effects of Boron and Calcium Applications on Pollen Characteristics and Distorted Fruit Formation in Sweet ann Strawberry Varieties. Bahçe 46(1):271–279.

Kihara, H. (1951). Triploid Watermelons. Proc. Amer. Soc. Hort. Sci.,58: 217-230.

Koh, G. C. (2002). Tetraploid Production of Moodeungsan Watermelon. Journal-Korean Socıety for Hortıcultural Scıence, 43(6): 671-676.

Kombo, M. D., (2017). Rootstock Effects on Seed Yield and Quality in Watermelon. Cukurova University Institute of Natural and Applied Sciences Deparment of Horticulture PhD Thesis, 97 p.

Krug, M.G.Z., Stipp, L.C.L., Rodriguez, A. P. M., Mendes, B.M.J. (2005). *In vitro* Organogenesis in Watermelon Cotyledons. Pesquisa Agropecuaria Brasileira, 40(9): 861-865.

La Porta, N., Roselli, G. (1991). Relationship Between Pollen Germination *in vitro* and Fluorochromatic Reaction in Cherry Clone F12/1 (*Prunus avium* L.) and Some of its Mutants. Journal of horticultural science, 66(2): 171-175. [CrossRef]

Li, L., He, Y., Lu, K. (2002). Chemical Induction Mutation in Yellow Peel Watermelon (*Citrullus Lanatus*) and Its Application to Tetraploid Watermelon Breeding. Chine Vegetables, 3: 8-11.

Lower, R. L., Johnson, K. W. (1969). Observations on Sterility of İnduced Autotetraploid Watermelons. Proc Amer Soc Hort Sci., 94:367-369

Nepi, M., Pacini, E. (1993). Pollination, Pollen Viability and Pistil Receptivity in *Cucurbita pepo*. Annals of Botany, 72(6):527-536. [CrossRef]

Norrmann, G. A., Quarín, C. L., Keeler, K. H. (1997). Evolutionary İmplications of Meiotic Chromosome Behavior, Reproductive Biology, and Hybridization in 6x and 9x Cytotypes of Andropogon Gerardii (Poaceae). American Journal of Botany, 84(2): 201-207. [CrossRef]

Norton, J.D. (1966). Testing of Plum Pollen Viability with Tetrazolium Salts. Proc. J. Am. Soc. Hortic. 89:132–134.

Rhodes, B., Zhang, X. 2000. Hybrid Seed Production in Watermelon. Journal of new seeds, 1(3-4): 69-88. [CrossRef]

Sari, N., Abak, K., Pitrat, M. (1999). Comparison of Ploidy Level Screening Methods in Watermelon: *Citrullus Lanatus* (Thunb.) Matsum. and Nakai. Scientia Horticulturae, 82(3-4): 265-277. [CrossRef]

Shivanna, K.R. and Rangaswamy, N.S. (1992). Pollen Biology. Springer-Verlag Berlin Heidelberg, 119 sayfa.

Soltis, D. E., Misra, B. B., Shan, S., Chen, S., Soltis, P. S. (2016). Polyploidy and The Proteome. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1864 (8): 896-907. [CrossRef]

Suying, T., Xiuqiang, H., Jiwei, L., Wenge, L. (1993). Raising The Frequency of İnducing Tetraploid Watermelon by Treating of Colchicine. In International Symposium on Cultivar Improvement of Horticultural Crops. Part 1: Vegetable Crops 402: 18-22. Doi: 10.17660/ActaHortic.1995.402.2

Sensoy, A. S., Ercan, N., Ayar, F., Temirkaynak, M. (2003). Determination of Some Morphological Characteristics and Viability of Pollens in Some Vegetable Species in *Cucurbitaceae* Family. Journal of Akdeniz University Faculty of Agriculture, 16(1): 1-6.

Simşek, I., Göcmen, M., Sarı, N. (2013). Determination of Morphological and Cytological Differences in Diploid and Tetraploid Watermelon Plants., 30(1): 1-14. ISSN : 2149-2182

Zhang, X. (2010). Tetraploid Watermelon Producing Small Fruits. U.S. Patent No 7,652,193.

Zhang, N., Bao, Y., Xie, Z., Huang, X., Sun, Y., Feng, G., Chen, W. (2019). Efficient Characterization of Tetraploid Watermelon. Plants, 8(10): 419. [CrossRef]