

Effects of Cucurbita, Lagenaria and Citrullus rootstocks on pollen and fruit characters, seed yield and quality of F₁ hybrid watermelon

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Abstract

In the study, two commercial *Cucurbita maxima*×*Cucurbita moschata* hybrid (Nun9075 and TZ148), *Lagenaria* spp. hybrid (Argentario) and one local *Lagenaria* spp. genotype (3335) and one *Citrullus amarus* genotype (PI 296341) were used as rootstocks. The male and female parents of two watermelon hybrids (187×125 and 11×162) were used as scions. Ungrafted plants formed the control group and also each parent was grafted on itself. Male parents were used for pollen measurements. Fruit weight, fruit height, fruit diameter, fruit rind thickness, total soluble solid content, total number of seeds, total seed weight and 1000 seeds' weight and seed vigor tests were carried out in fruits of female parents crossed with male parents grafted onto the same rootstock. According to results of this study, the highest pollen production was obtained from Argentario while in terms of pollen germination and pollen viability there was no difference between applications in general. It was determined that grafting on Argentario and 3335 rootstocks increased fruit height, fruit diameter, fruit rind thickness, and the amount of total soluble solid, and the best performing rootstocks in terms of fruit weight were found to be Argentario, 3335, TZ148 and NUN9075. The highest seed number, seed weight and 1000 seeds weight were obtained from those grafted on TZ148 and NUN9075 rootstocks. Although variable results were obtained in terms of early germination, there was no rootstock that came to the fore in general. There was no difference between the rootstocks regarding accelerated aging test and controlled deterioration test.

Keywords: Watermelon, Grafting, Pollen Tests, Seed Yield And Quality

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is one of the most economically important species of the Cucurbitaceae family. The world watermelon production is about 101.620.420 million tonnes and Turkey ranks as 2nd most producer country after China (Faostat 2020). Grafting has been used on fruit trees and vines for thousands of years (Kombo & Sari 2019). Grafting in vegetables began in the late 1920s, first by grafting watermelon on pumpkin (*Cucurbita moschata*) and later on bottle gourd (*Lagenaria siceraria*) in Korea and Japan (Lee 1994). Grafting was originally adopted in China, Japan and Korea, where land use was intensive and crop rotation was difficult. Subsequently, grafting became widespread in countries such as Australia (Tran-Nguyen et al. 2012). It became widespread in western countries in the early 1990s and is now widely used around the world. The usage of grafted seedlings in watermelon has expand-

ed fast due to their resistance to Fusarium wilt, earliness, yield, fruit quality, and their tolerance to biotic and abiotic stress conditions. *C. maxima* x *C. moschata* interspecific hybrids and *Lagenaria siceraria* hybrids are the most extensively employed commercial rootstocks for watermelon (Qin et al. 2014; Edelstein et al. 2017; Fallik et al. 2019). Splice grafting of watermelon, in which both cotyledons are excised from the rootstock, has recently advanced to achieve all these goals (Devi et al. 2020).

Grafting is a process that first includes the selection of the appropriate rootstock and scion, then combining the rootstock and scion with the appropriate grafting method, and acclimatizing the grafted plant until it heals. Grafting watermelon (*Citrullus lanatus*) onto resistant rootstocks is an efficient biotic and abiotic stress control approach (Devi et al. 2021). Since the first report of watermelon grafting for resistance to disease in 1927, adoption has progressively increased in Greece, Japan, Israel, Korea, and Turkey reaching 95% (Devi et al. 2020).

The use of rootstocks in watermelon can rapidly transfer nutrients from the soil to the pen, avoid soil diseases and increase plant tolerance in soil salinity, low soil temperatures and moist soil conditions (Salam et al. 2002; Claudio et al. 2019; Tripodi et al. 2019). The type of rootstock affects plant growth, yield and fruit quality (Yetişir et al. 2003; El-Kersh et al. 2016; Fallik et al. 2016; Fredes et al. 2016; Huang et al. 2016; Maršić et al. 2016; Özdemir et al. 2016; Zaaroor et al. 2016; Liu et al. 2017; Ceylan et al. 2018; Gaion et al. 2018; Zhong et al. 2018; Meng et al. 2019; Siamak & Paolo 2019; Zhang et al. 2019) and also seed yield and qualities (Kombo & Sari 2019; Hussein & Sari 2020).

According to our knowledge so far, no study has been found on the effect of rootstocks on hybrid watermelon seed production. In this study, the effects of Cucurbita, Lagenaria and Citrullus rootstocks on the seed yield and quality of two hybrid watermelons were tried to be determined. In addition, the effects of grafting on pollen productivity in male parents and fruit characters in female parents were also investigated.

MATERIALS AND METHODS

This study was carried out at the greenhouses and laboratories of the Alata Horticultural Research Institute, Mersin-Turkey during the spring/summer seasons of 2019 and 2020.

Plant Materials

Nun9075 (Nunhems Seed Company, Holland) and TZ148 (Syngenta seed Company, Holland) from commercial rootstocks of *C. maxima* x *C. moschata* group; Argentario (HM Clause Seed Company, France) from commercial rootstock and 3335 from Turkish landraces of *Lagenaria* spp. group; PI 296341 from *Citrullus amarus* were used as rootstocks. Nun9075 and TZ148 are hybrid rootstocks widely grown in Türkiye. Gourd group rootstocks are

not widely used in Türkiye lately. The hybrid Argentario, which was used in Turkey for a period, was selected from the gourd group. In addition, from the gourd group, Yetişir et al. (2007) and Karaca et al. (2012) developed 3335 lines were used. PI296341 is from the *Citrullus amarus* group and is a genotype resistant to races 0, 1, 2 of fusarium in watermelon. Each parent was also self-grafted. 187x125 and 11x162 hybrids developed by Alata Horticultural Research Institute, Türkiye were used as scions. The 187x125 hybrid is a hybrid with a striped rind pattern and a fruit weight of 8-9 kg. The 11x162 hybrid is a hybrid with a dark green rind pattern and a weight of 9-10 kg. Both watermelon hybrids were transferred to a private sector company. Two hybrids' parents were grafted onto each rootstock. Non-grafted watermelon plants of parents were considered as controls. The experimental design was the factorial experiment in randomized complete block design with three replicates with thirty plants in each replicate.

Seed sowing, grafting, and transplanting

In this study, seed sowing for all scions and rootstocks was started on January, 3rd, 2019 for the first year of the experiment and on December, 30th 2019 for the second year (2020). The grafting was performed with the splice/one cotyledon grafting method in Antalya Seedling Company.

Inoculation is done when the rootstock, cotyledons and the first true leaf begin to develop. A cotyledon and growth tip is cut. The seedling is cut obliquely from the base of one cotyledon to 0.8-1.0 cm below the other cotyledon; one cotyledon and its growth tip are removed. The length of the cut on the hypocotyl of the pen should be the same as that of the rootstock and should be at an angle of 35° to 45° (Davis et al., 2008; Bie et al., 2017). The pen is attached to the rootstock and fixed with the grafting clip. The grafted plants were kept in a dark environment at 25 °C and 100% humidity for three days before being moved to a greenhouse maintained at 21 °C to 30 °C or until the joint had healed. All seedlings were planted as follow: for 2019, seedlings were planted on 22 March 2019 at 36°37'47.91"N latitude, 34°20'38"E longitude, and 4 m above sea level; for the year 2020, on 06 March 2020, at 36°37'50.73"N latitude, 34°20'43.68"E longitude and 4 m above sea level, different E.P.E. covered greenhouses in Alata Horticultural Research Institute with a 1.5 m between and 0.4 m within plant distances. Plants were grown in a single stem by hanging them on a rope and removing their secondary axes.

Climatic data were taken in both years by hobo devices placed in greenhouses. Monthly minimum, maximum and average temperature and relative humidity values are given in Table 1. In 2019, the highest temperatures were seen in April (51.19 °C) and May (50.01 °C), after which shading dust was applied on the plastic greenhouse. In terms of relative humidity, the highest value was taken in June (100.00%) and the lowest value was

taken in March (91.36%). In 2020, the highest (46.4 °C) and the lowest (4.2 °C) temperatures were seen in March. In terms of relative humidity, the highest (97.5%) relative humidity was recorded in March, and the lowest (23.1%) relative humidity was recorded in May (Table 1).

The irrigation and fertilization were performed with a drip irrigation system. The irrigation system started with the planting seedlings of all accessions used in this study and were given when necessary depending on the climate observed in the greenhouse. The analysis of soil of used greenhouses was carried out during the experiment. The first year of the study (2019) was sandy, calcareous, slightly salty, weak inorganic substances, slightly alkali, and sufficient in phosphorus and potassi-

stage is the period from when the fruits reach the size of an apple until harvest. Pesticides were applied for observed diseases and pests regularly, and weeding and pruning were mechanically performed.

Crossing started on 15 April, in 2019 and on 06 April in 2020, and was completed on 17 May in 2019 and on 15 May in 2020. The hybridizations were carried out by closing the male and female flowers with clips one day before the anthesis and crossing female flowers by the males, in the next morning. 187 female parent with 125 male parent and 11 female parent with 162 male parent were pollinated. Three male flowers were used in each pollination. Female parents and male parents grafted on the same rootstocks were crossed. One fruit was set on

Table 1. Climate values in greenhouses during the trial months in 2019 and 2020

Year	Climate Factor	March	April	May	June	July	
2019	Temperature (°C)	Minimum	10.42	7.40	10.94	15.11	16.63
		Maximum	45.17	51.19	50.01	47.07	48.82
		Average	22.91	23.01	27.00	29.23	32.39
	Relative humidity (%)	Minimum	33.74	25.86	22.71	34.21	29.45
		Maximum	91.36	95.87	96.81	100.00	92.60
		Average	70.87	72.35	72.21	76.83	63.57
2020	Temperature (°C)	Minimum	4.2	7.5	10.5	12.9	22.3
		Maximum	46.4	33.4	44.6	41.2	40.5
		Average	19.9	19.4	23.9	26.4	29.9
	Relative humidity (%)	Minimum	25.5	24.0	23.1	33.3	41.9
		Maximum	97.5	72.4	97.2	97.0	89.6
		Average	71.4	72.4	67.0	71.0	70.8

Table 2. Soil analysis of used greenhouses

Analyzes	Limit Values	Analysis Results (0-30 cm)	
		2019	2020
Texture (100 g/ml)	30-50	28.00 (sandy)	34.00 (loamy)
Total Calcitic (CaCO ₃ %)	5-15	24.00 (calcareous)	27.20 (high calcareou)
Salinity E.C. ds/m (25 °C)	0-0.8	1.55 (slightly salty)	0.85 (optimum)
Organic matter (%)	3-4	2.76 (defficient)	2.20 (defficient)
pH 1: 2,5	6.0-7.0	7.72 (slightly alkaline)	7.66 (alkaline)
Available potassium (mg/kg)	244-300	250.60 (sufficient)	54.70 (very low)
Receivable phosphorus (mg/kg)	20-40	25.10 (sufficient)	34.10 (optimum)

um; and in another hand for the second year (2020) was loamy, very calcareous, normal in salinity, weak inorganic substances, alkali, poor in potassium sufficient for phosphorus quantity (Table 2). According to the results of soil analysis, fertilizers were given as pure substance as 140-160 kg N/ha, 80-100 kg P₂O₅/ha, 60-80 kg K₂O/ha (Güçdemir 2012). Fertilizers were given by drip irrigation. Nitrogen, phosphorus and potassium are given by dividing the watermelon into three according to the three growing stages of the watermelon. The first stage is the period until the first female flower. The second period is the period from the stage when the first female flower is seen until the fruits reach the size of an apple. The third

each plant.

To determine the effect of grafting on pollen viability, germination and amount, male flowers at anthesis stage were picked from each replication and brought to the laboratory. Nine male flowers were used for each replication. For pollen viability TTC test (Eti 1991; Gök et al. 2007; Kombo & Sari 2019), for pollen germination agar in petri dishes test (Gök et al. 2007), and for the amount of pollen per flower hemacytometer test (Eti 1990) were performed.

Fruit harvest was carried out on 18 July in 2019 and on 08 July in 2020. Then, fruit weight, fruit height, fruit diame-

ter, fruit rind thickness, and total soluble solid content of grafted and ungrafted female parents (187 and 11) were determined. Fruit measurements were made on 5 fruits for each application. Seeds were manually extracted, and thoroughly washed and put on fine wire mesh to dry.

Total number of seeds, total seed weight and 1000 seeds weight, germination at 52nd, 60th and 68th hours after sowing on germination paper, total germination rate in accelerated aging test and total germination rate in controlled deterioration test were determined for grafted and ungrafted female parental lines (187 and 11) crossed by male parents (125 and 162). The standard germination test (International Seed Testing Association, 2018) was conducted using 400 seeds (100 seeds per replication) for each treatment for germination at 52nd, 60th and 68th hour after seed sowing, which were incubated at 25°C for 14 days on moisturized paper towels. Hundred seeds (25 seeds per replication) for each treatment were used for germination, accelerated aging test and controlled deterioration test. For the early germination (early count) test, the seeds of the controls and all hybrids were germinated in the dark using the paper method. Germinated seeds at the 52nd, 60th and 68th hours were counted and rates were determined (Mavi and Demir, 2010; Ermis et al. 2022). Accelerated aging test, aging pots consisting of two parts were used. The aging pot consists of an outer box of 15 × 9 × 6.5 cm and an inner chamber made of 15 × 7 × 7 cm sieve wire placed inside it. In order to provide a high relative humidity, 40 mL of pure water was placed in the outer box. The seeds were laid on the sieve wire in a single layer and placed inside the sieve wire outer box. Before all hybrid seeds were placed on the sieves. The seeds of each hybrid placed on the sieves in the aging boxes were kept at 45 °C for 144 hours (“Mettler 854 Schwabach W-Germany” trademark incubator) (Bhering et al. 2004). Then standard germination trials were established. Seed moisture was initially increased to 24% for controlled deterioration test. In order to ensure the moisture balance of the seeds whose seed moisture was increased, the seeds were kept at 5 °C for 3 days. Then it was packed hermetically by putting it in airtight packages with aluminum mixture. For the controlled deterioration test of seeds, 45 °C temperature and 48 hours aging times were used. At the end of the specified period, the seeds were taken out of their packages and standard germination tests were established (Bhering et al. 2004).

Statistical analysis

Statistical analyses were performed using the Tukey Test in the JMP 7.0 statistics software (v7.00, SAS Institute Inc., NC 27513-2414, USA) at a significance level of $P \leq 0.05$. Statistical analyses were performed after applying angle transformation to percentage values.

RESULTS AND DISCUSSION

Pollen development

Pollen viability, pollen germination, and pollen production per male flower in male parents were examined (Figure 1). The results showed that there were no statistically significant differences in male line 125 combinations in 2019, while the highest values were obtained in Argentario and 3335 rootstocks in 2020. TZ148 had the highest value in male line 162 in 2019, while Argentario gave the highest value in 2020. In terms of pollen germination, there was no statistically significant difference between 125 and 162 male lines combinations in 2019. In 2020, the highest results were obtained from the 125/125 combination for the 125 male line and the Argentario/162 combination for the 162 male line. In terms of pollen viability, there was no statistically significant difference between the combinations in 125 male line in both years and in 162 male line in 2020. However, in 2019, the highest values in 162 male lines were obtained in combinations of 162 (control) and Nun9075/162. The reason for the high difference between the data of the two years is that it was made in different greenhouses and different soil conditions every year. The results obtained in terms of the number of pollen per flower were found to be higher than the results obtained by Stanghellini & Schultheis (2005), their average is 32 000. Regarding pollen germination results, Gök et al. (2007) had similar results, their results being 70% on average. Pollen viability results per flower are higher than those of McGregor & Waters (2013), their average being 80%, but Gök et al. (2007) and Freeman et al. (2008) got similar results comparing our study (average 90% and 97%).

Fruit development

Fruit yield and quality results are presented in Figure 2 for the 2019 and 2020 growing seasons. Regarding fruit weight, Argentario and Nun9075 rootstocks came to the fore in both hybrid female parents and in both years. 3335 and Argentario rootstock came to the fore in terms of fruit height, fruit diameter, fruit rind thickness and total soluble solid content. Since the roots of *Cucurbita maxima* × *Cucurbita moschata* and gourd (*Lagenaria* spp.) rootstocks are much stronger than ungrafted watermelons, more brix accumulates and watermelon fruit weights and fruit shell thicknesses increase. The reason for the high difference between the data of the two years is that it was made in different greenhouses and different soil conditions every year. The difference in fruit weight between the data of the two years is high, because the research was carried out in different greenhouses and different soil conditions every year. Compatible with our study, Turhan et al. (2012), Abd Alla et al. (2012), Öztekin et al. (2012), Petropoulos et al. (2012), Özmen et al. (2015), El-Kersh et al. (2016), Maršić et al. (2016), Hussein & Sari (2020), Ozbahce et al. (2021a), Ozbahce et al. (2021b), Ulas (2021) and Suárez-Hernández et al. (2022) reported

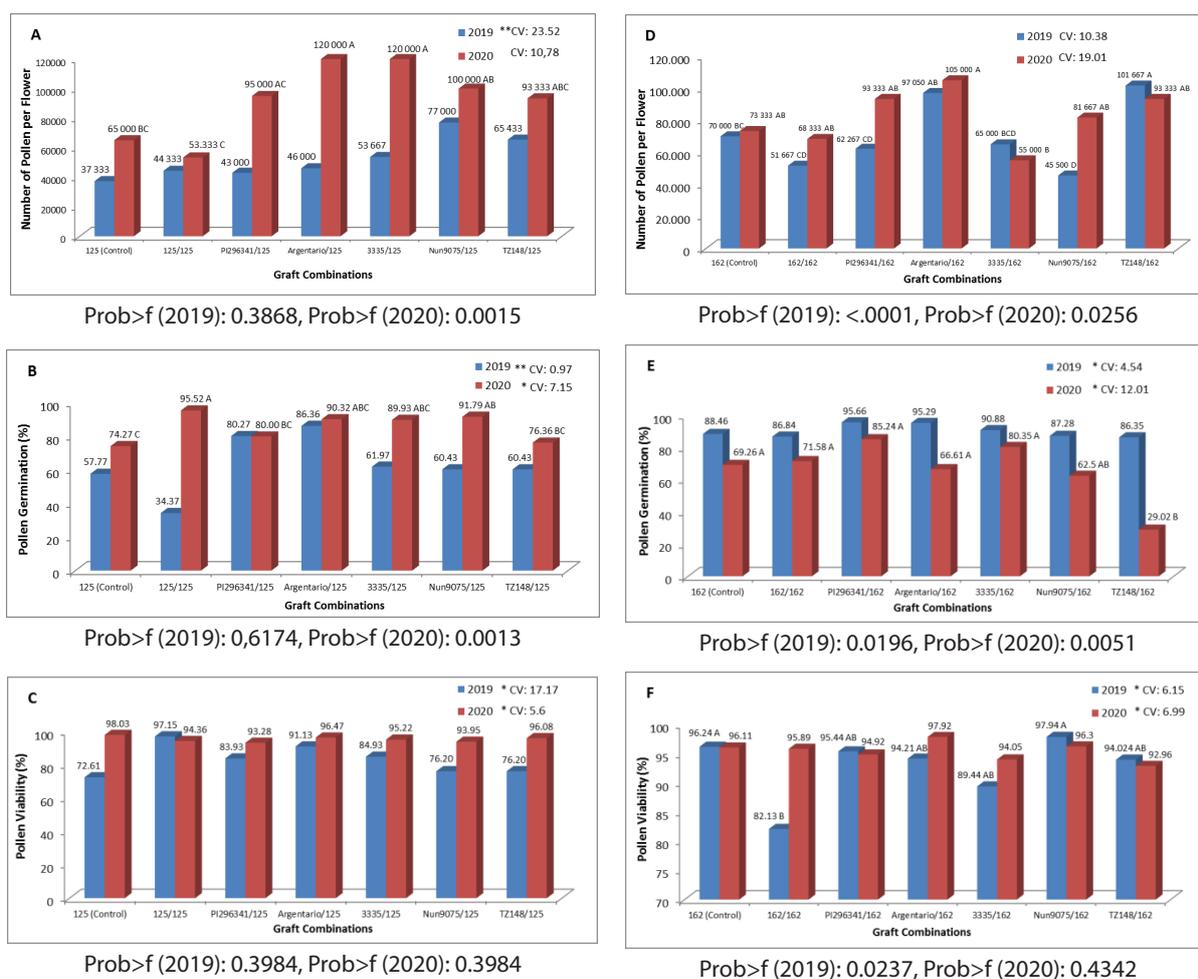


Figure 1. Effect of rootstocks on (A) number of pollen per flower of 125, (B) pollen germination of 125, (C) pollen viability of 125, (D) number of pollen per male flower of 162, (E) pollen germination of 162, (F) pollen viability of male parent 162 in watermelon hybrids. *Angle transformation was applied to the percent values, **Square root transformation was applied. No letters on bars means that there is no significant difference between graft combinations

that grafted watermelons result in higher yield and quality. Eventough some studies (El-Sayed et al. 2015; Zhong et al. 2018) revealed that grafting had a negative impact on the TTS content it was also, reported that there was no difference in the content of TTS by Mohamed et al. (2012), Liu et al. (2017), Meng et al. (2019).

Seed yield

There was no statistically significant difference between the combinations in terms of total number of seeds, total seed weight and 1000 seeds weight of female line 11 in 2019. However, TZ148 and Nun9075 rootstocks stand out among the combinations in terms of total number of seed, total seed weight and 1000 seeds weight of female line 187 in both years and female line 11 in 2019 (Figure 3). TZ148 and Nun9075 rootstocks are very strong rootstocks, their roots can go very deep and the root grafted on them is minimally affected by abiotic conditions. As a result, the scion is well fed and produces healthy male and female flowers. More pollen is produced in male flowers formed by male parents grafted on these rootstocks than in ungrafted ones. As a result of these, it is

not a coincidence that the seed yields of the scions grafted on TZ148 and Nun9075 rootstocks are high seed yield. Grafting was found to be important regarding to seed yield, and Abd Alla et al. (2012), Öztekin et al., (2012), Yetişir & Sari (2018), Kombo & Sari (2019), Hussein & Sari (2020) and stated that grafting increased seed yield, but Claudio et al. (2019) reported that grafting did not make any difference on seed yield. Ulas (2021), in her study on eggplant, reported that Pala grafted on Köksal F₁ rootstock had a 72.03% increase in total seed yield compared to ungrafted plants.

Seed quality

There was no statistically significant difference between the combinations in both years and in both lines for total germination at 52nd hour. Regarding total germination at the 60th hour, there was no difference in the combinations of 187 lines for 2019, while 3335 (11.3%) rootstocks stood out in 2020; while TZ148 (14.1%) stood out in the combinations of 11 lines in 2019, no statistical difference was found between the combinations in 2020. In terms of total germination at the 68th hour, there was no differ-

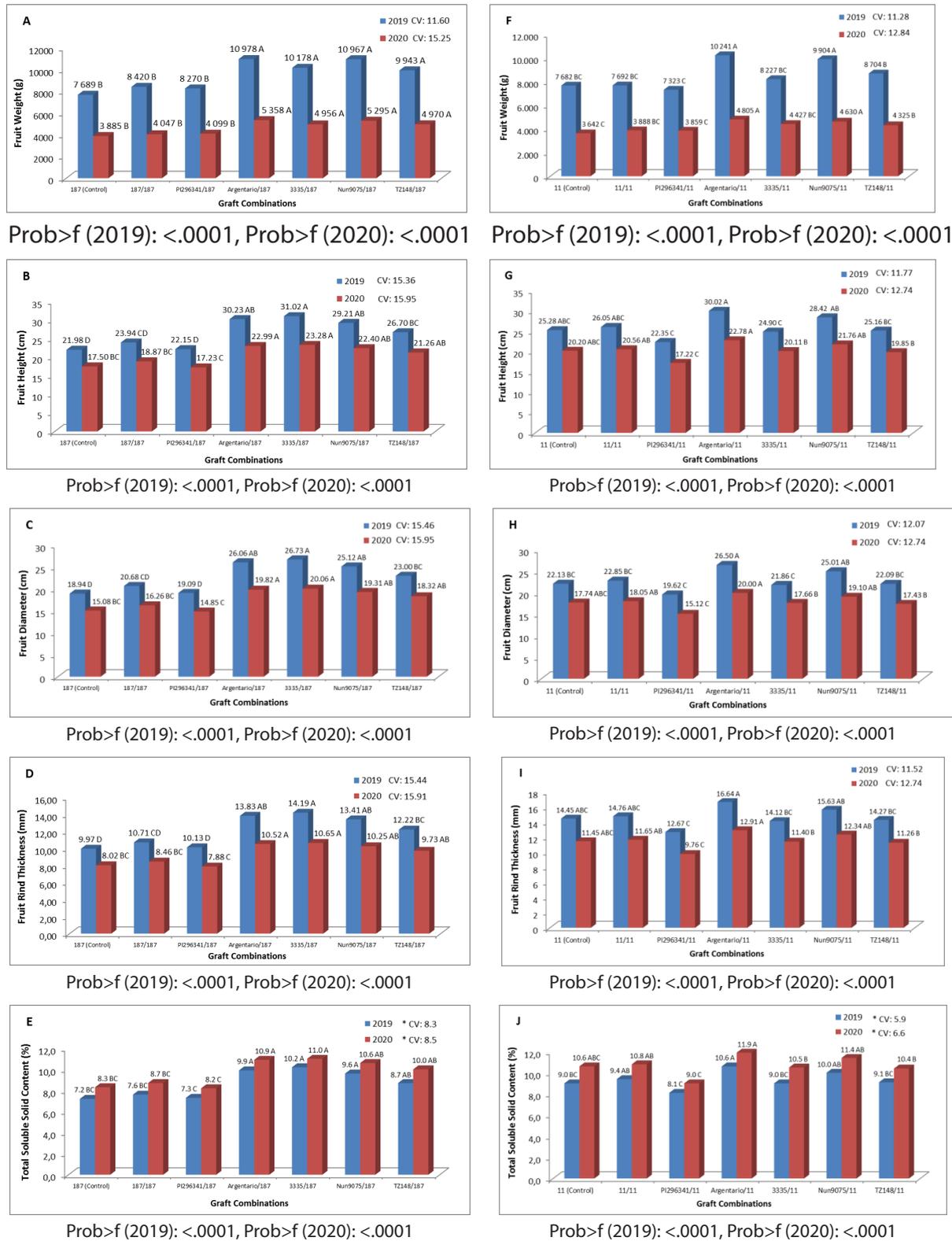


Figure 2. Effect of rootstocks on (A) fruit weight of 187, (B) fruit height of 187, (C) fruit diameter of 187, (D) fruit rind thickness of 187, (E) total soluble solid content of 187, (F) fruit weight of 11, (G) fruit height of 11, (H) fruit diameter of 11, (I) fruit rind thickness of 11, (J) total soluble solid content of 11 female parents in watermelon hybrids. *Angle transformation was applied to the percent values. No letters on bars means that there is no significant difference between graft combinations

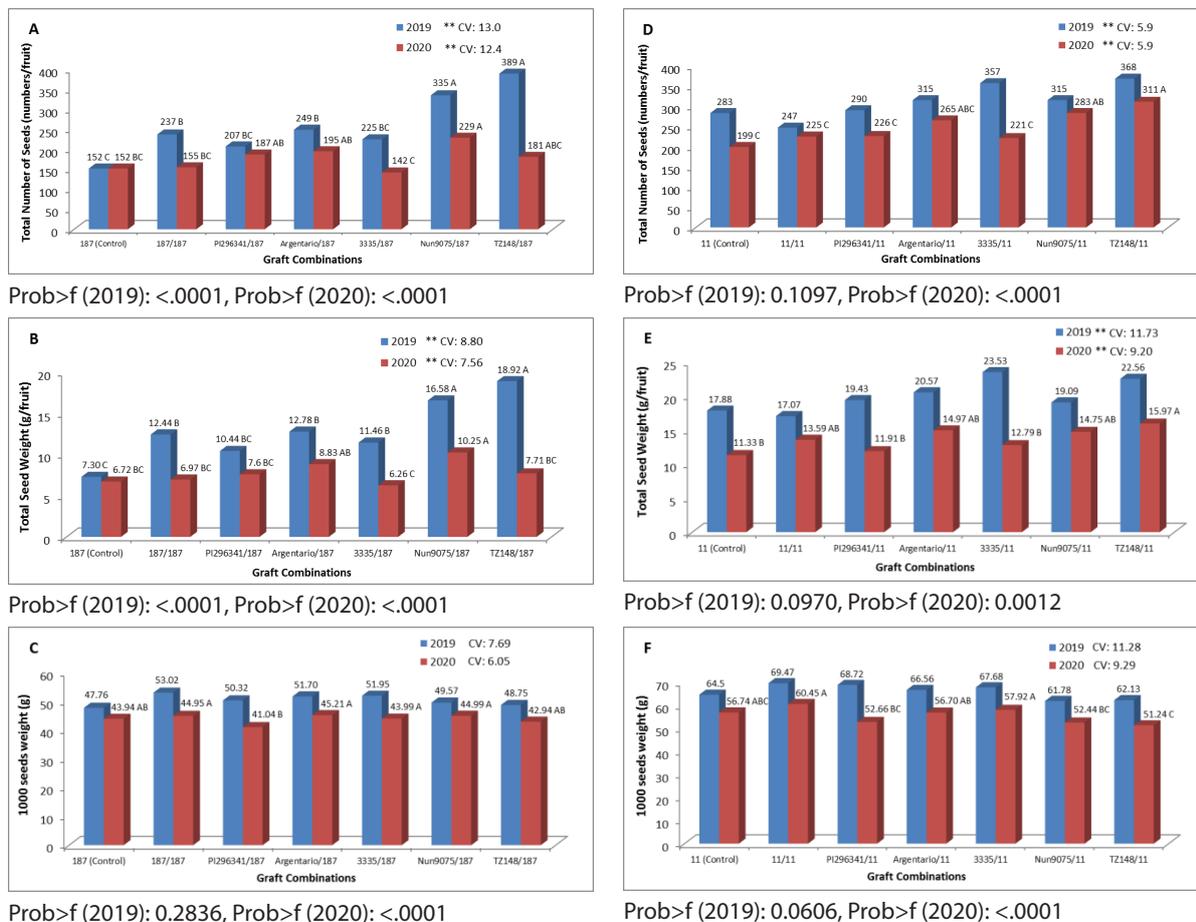


Figure 3. Effect of rootstocks on (A) total number of seeds of 187, (B) total seeds weight of 187, (C) 1000 seeds weight of 187, (D) total number of seeds of 11, (E) total seeds weight of 11, (F) 1000 seeds weight of female parent 11 in watermelon hybrids. *Angle transformation was applied to the percent values, **Square root transformation was applied. No letters on bars means that there is no significant difference between graft combinations

ence between the applications in both lines in 2019, but TZ148 and PI296341 rootstocks stand out in general in 2020 (Figure 4). Seed viability and quality of watermelon seeds obtained from ungrafted plants are very high in the first years. For this reason, the results obtained from both grafted and ungrafted watermelons were almost close to each other. The grafted plants feed the fruits better and as a result, better quality seeds will be obtained. In such studies to be carried out in the future, the difference between the preservation of seeds in cold storage and the quality of seeds obtained from ungrafted and grafted plants will be revealed more clearly.

There was no significant difference between grafted combinations for total germinated at accelerated aging test and total germinated at controlled deterioration test in both years and in both lines overall (Figure 5).

The rapid aging test shows that it is suitable for assessing the physiological quality of watermelon seeds and thus becomes a promising test for the evaluation of vigor expression (Mavi & Demir 2010; Mavi, 2011; Radke et al. 2017). Bhering et al. (2003) and Mavi (2011) found differ-

ences between applications in accelerated aging tests in their studies on watermelon.

CONCLUSION

According to the results of this study, the best results in terms of pollen production was obtained from Argentario rootstock, however regarding pollen germination and pollen viability, there was no difference between rootstocks in general. It was determined that grafting on Argentario and 3335 rootstocks increased fruit height, fruit diameter, fruit rind thickness, and the amount of total soluble solid, and the best performing rootstocks in terms of fruit weight were Argentario, 3335, TZ148 and NUN9075. The highest values for seed number, seed weight and 1000 seeds weight were obtained from those grafted onto TZ148 and NUN9075 rootstocks. Although different results were obtained in terms of early germination, there was no rootstock that came to the fore in general. No difference was found between the applications in terms of accelerated aging test and controlled deterioration test. According to the results of this study, Argentario, NUN-9075 and TZ148 rootstocks performed

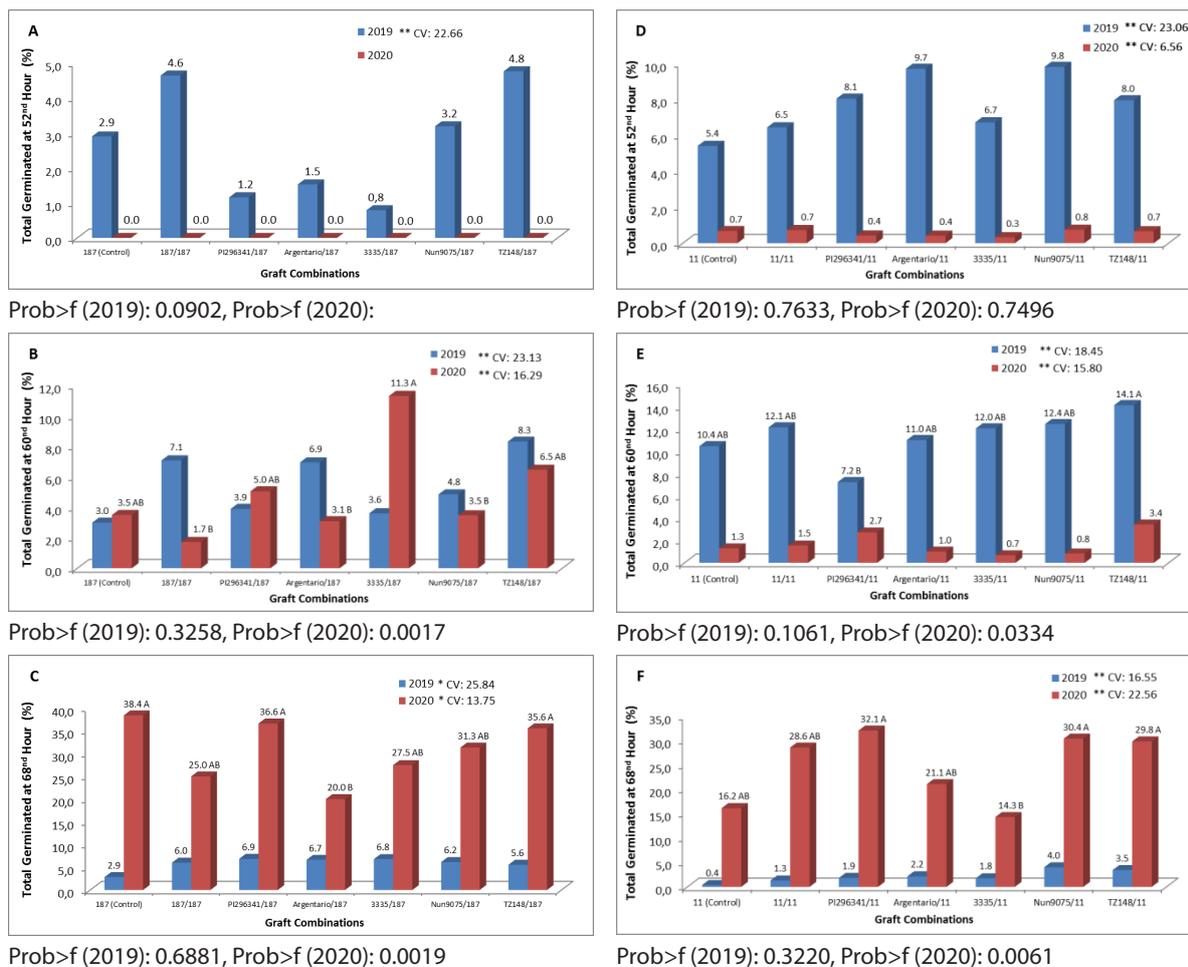


Figure 4. Effect of rootstocks on (A) total germinated at 52nd hour of 187, (B) total germinated at 60th hour of 187, (C) total germinated at 68th hour of 187, (D) total germinated at 52nd hour of 11, (E) total germinated at 60th hour of 11, (F) total germinated at 68th hour of 11 female parents in watermelon hybrids. *Angle transformation was applied to the percent values, **Square root transformation was applied. No letters on bars means that there is no significant difference between graft combinations

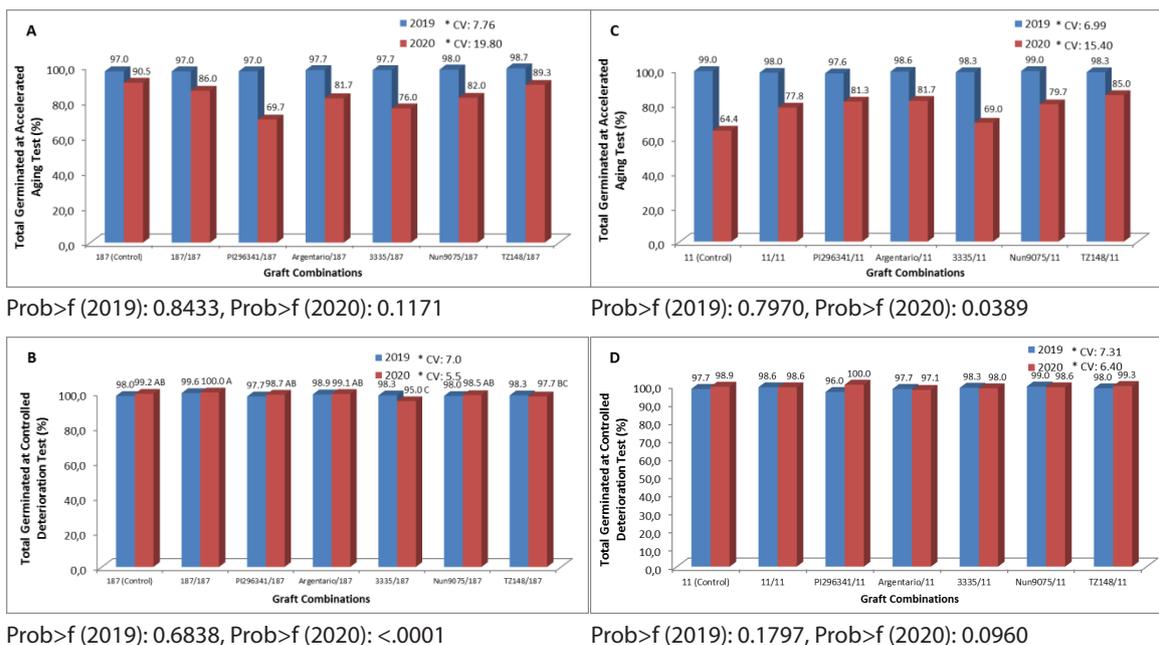


Figure 5. Effect of rootstocks on (A) total germinated at accelerated aging test of 187, (B) total germinated at controlled deterioration test of 187, (C) total germinated at accelerated aging test of 11, (D) total germinated at controlled deterioration test of 11 female parents in watermelon hybrids. *Angle transformation was applied to the percent values. No letters on bars means that there is no significant difference between graft combinations

better than the other rootstocks and are therefore recommended as the best rootstock. In addition to the results we have obtained, we recommend the use of rootstock in F_1 hybrid watermelon seed production to companies due to the biotic and abiotic resistance characteristics of rootstocks.

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.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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