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Determination of the effect of cucumber grafting on some morphological and physiological characteristics in hydroponic conditions

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Abstract

Due to the benefits and importance of the use of grafted seedlings, the demand for high quality grafted seedlings is increasing. In this study, it was aimed to determine the effect of rootstocks on some morphological and physiological parameters of cucumber (*Cucumis sativus* L.) plant under hydroponic growing conditions. In this study, grafted on seven different rootstocks and non-grafted plant characteristics were compared. Non-grafted seedlings were used as control plants. The values measured in at least one grafting application in 9 different morphological and/or physiological parameters among the 12 parameters measured were higher than the non-grafted plants. In leaf chlorophyll content (SPAD), photosynthetic active radiation (PAR) and root dry weight/fresh weight rate measurements, the values measured in non-grafted plants were not lower than grafted plants. It was determined that Cremna and Devrim rootstocks were effective in terms of shoot length and biomass values, but rootstock use did not have a significant effect in terms of photosynthetic activity. It was concluded that grafting of cucumber plants on different rootstocks may cause significant advantages in terms of some parameters, but the effects largely depend on the rootstock selection.

Keywords: *Cucumis sativus*, Hydroponic, Photosynthetic activity, Grafting, Rootstock

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INTRODUCTION

Environmental pollutants, which are one of the most important global problems today and increasing as a result of human activities, also threaten the existence of biota (Adrees et al., 2015). Some pollutants can affect plant growth, photosynthesis mechanism and biochemical activities to a degree that may decrease (Ali et al., 2013; Keller et al., 2015; Rizwan et al., 2015). The deterioration of the soil ecosystem as a result of environmental pollutants causes loss of agricultural land, yield and quality decreases in agricultural products. Due to intensive production and monoculture cultivation in greenhouses, soil-related problems can cause yield and quality losses in aquaculture (Vural et al., 2000). For this reason, ways to reduce or eliminate yield and quality losses caused by pollutants are investigated.

One of the most important methods to reduce the negative effects of environmental conditions is grafting. Due to its advantages, the use of grafted plants has become a common agricultural method in many parts of the world (Turhan et al., 2011). In order to reduce the negative effects of soil-based stress factors, use of resistant/tolerant rootstocks is preferred. Grafting applications in vegetables can be evaluated to improve fruit quality under biotic or abiotic stress conditions (Sabatino et al., 2019; Singh et al., 2020; Ulas et al., 2020; Ulas, 2021; Ulas et al., 2021). The advantages of grafting in vegetables include providing tolerance to low soil and air temperatures, prolonging the economic harvest period and increasing yield accordingly, preventing damage to the environment as a result of better uptake of plant nutrients in the soil (Yetisir et al., 2004). With grafting, nutrient intake and use efficiency can also be increased (Colla et al., 2010; Ulas et al., 2021). Producer demand for grafted seedlings has increased in vegetable growing, especially in *Solanaceae* and *Cucurbitaceae* family vegetable species (Güngör and Balkaya, 2016; Karaağaç et al., 2018). Grafted seedling production in Turkey is carried out commercially mostly in tomato, watermelon, eggplant, cucumber and melon species.

Cucumber (*Cucumis sativus* L.) is one of the most economically valuable species of the *Cucurbitaceae* family. Cucumber production in the world is 91.258.272 tons, and in Turkey it is 1.926.883 tons (FAO, 2020). It is an environmentally friendly application that provides an important advantage to solve the problems encountered in cucumber growing is grafted seedlings. In some studies on cucumber, it has been determined that grafting increases plant growth, yield and water use efficiency (Günay, 2011). One of the most important factors determining the effectiveness of grafting is the determination of suitable rootstocks in grafted seedling production (Colla et al., 2012; Balkaya, 2014; Coşkun et al., 2022). Rootstock-scion interaction affects many features such as mineral uptake, fruit growth, fruit quality (Martínez-Ballesta et al., 2010). In this study, it was aimed to determine some morphological and physiological parameters of cucumbers grafted on different rootstocks in hydroponic culture medium.

MATERIALS AND METHODS

Plant material

In the study, cucumber was grafted different rootstocks. Minimix F1 was used as scion and TZ148, Devrim, Cremna, Kubai, Strong, RS841 and Maximus were used as rootstocks. The rootstocks used are *Cucurbita maxima* x *Cucurbita moschata* hybrid rootstocks and non-grafted plants were used as control.

Method

The study was carried out in the greenhouse and laboratory of Hatay Mustafa Kemal University, Agriculture Faculty, Horticulture Department. The seeds were sown in mixture of peat:perlite (3:1) with an EC of 0.4 and the seedlings were grown until they reached the first true leaf stage. Cucumber variety used as a scion was planted four days before the rootstocks and grafting was done when the first true leaves started to form. As the grafting method, single cotyledon grafting method was used. The disinfection of the equipment to be used in grafting was provided by using 75% ethyl alcohol. Grafted plants were stored for 7 days in grafting care unit with a relative humidity of 95% and a temperature of 27 °C. After the grafting had taken place, the plants were gradually acclimated to the normal growing medium, then the roots were washed and transferred to aerated water culture in 8 liter pots. The experiment was carried out according to the randomized trial plots design with 3 replications. Hydrophonic medium was changed every 7 days and 4 plants were used in each replication. The plants are grown in a nutrient solution containing 1125 µM $Ca(N0_3)_2$, 375 µM $(NH_4)_2SO_4$, 750 µM K_2SO_4, 650 µM $MgSO_4$, 500 µM KH_2PO_4, 10 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.4 µM $CuSO_4$, 0.4 µM $MoNa_2O$ and 80 µM Fe. The electrical conductivity (EC) of the growing solution was kept at 1.50 dS/m and the pH between 6.5-7. On the 15th day, shoot length, stem diameter, leaf number, leaf proportional water content, SPAD (leaf chlorophyll content), PAR (photosynthetic active radiation), shoot weights and root weights were measured in non-grafted and grafted plants. In order to determine the relative water content of the leaves, the fresh weight, turgor weight and dry weights of the samples taken from the 3rd and 4th leaves of the plants were calculated. For turgor weight, leaf samples were kept in water for 4 hours and weighed. For dry weight was determined after 48 hours at 65 °C. Leaf relative water content values were calculated with the formula "RWC=(Fresh Weight-Dry Weight)/(Turgor Weight-Dry Weight)x100". One-way ANOVA test was used to determine the statistical significance of the difference between the groups. The post-hoc Duncan test was used to compare the mean values of each group. All statistical analyzes were performed using the SPSS 16.0 package program.

RESULTS AND DISCUSSION

Shoot length (cm)

In grafted plants, mean shoot length values varied between 101.33±2.91 cm $plant^{-1}$ and 174.53±1.18 cm $plant^{-1}$. Shoot length values in non-grafted plants were determined as 113.00±9.45 cm. It was concluded that some rootstock-scion grafting may be more effective in increasing shoot length. The significantly highest shoot length values were measured in Cremna-Minimix grafting. The significantly lowest shoot length values were measured in non-grafted plants and when Strong rootstock was used. In the case of Cremna, Devrim, Kubai and RS841 rootstocks, it was determined that the shoot length increased significantly compared to the non-grafted plants. The average shoot length values were measured as 136.45 cm $plant^{-1}$ in all grafted plants, and 133.52 cm $plant^{-1}$ in all applications with non-grafted plants (Table 1).

Shoot length is one of the most important parameters affecting yield. In optimum growing conditions, some rootstocks have advantages in terms of shoot length. In this study, shoot length values measured in four different rootstock applications (Cremna, Devrim, Kubai

Table 1. Shoot length values in different grafting combinations

	Mean±S.E.	Minimum	Maximum
Non-grafted	113.00±9.45 ef	99	131
TZ148-Minimix	119.33±1.07 e	117.6	121.3
Devrim-Minimix	156.33±5.04 b	149	166
Cremna-Minimix	174.53±1.18 a	172.5	176.6
Kubai-Minimix	146.37±1.27 bc	144.2	148.6
Strong-Minimix	101.33±2.91 f	96	106
RS841-Minimix	135.93±6.14 cd	124.6	145.7
Maximus-Minimix	121.33±6.39 de	111	133
Mean	133.52±5.00	96	176.6

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

Table 2. Stem diameter values in different grafting combinations

	Mean±S.E.	Minimum	Maximum
Non-grafted	5.14±0.01 d	5.12	5.17
TZ148-Minimix	3.66±0.01 f	3.64	3.67
Devrim-Minimix	5.72±0.01 a	5.68	5.74
Cremna-Minimix	5.48±0.02 b	5.45	5.51
Kubai-Minimix	5.70±0.06 a	5.61	5.86
Strong-Minimix	5.37±0.01 c	5.32	5.39
RS841-Minimix	3.63±0.01 f	3.58	3.64
Maximus-Minimix	4.94±0.01 e	4.89	4.96
Mean	4.96±0.17	3.58	5.86

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

and RS841) were higher than the non-grafted plants. In some studies, it has been determined that grafting is effective on the vegetative development of plants (Salehi-Mohammedi et al., 2009; Ulas, 2021). Similarly, in this study, shoot length values of some grafting applications were determined higher in the control application. Aktaş and Üre (2019) determined grafted on TZ148 plants produced significantly higher shoot lenght than non-grafted plants. amar and Solmaz (2020) determined in their study that non-grafted plants are in the shortest group. Similarly, in this study, non-grafted plants had lower shoot length values compared to some grafting combinations. The findings of this study are different from the findings of the study of Kacjan Maršić and Jakše (2010), who reported that the stem length of the cucumber was not affected by grafting in the hydroponic system. In this study, which was carried out in a hydroponic environment, a significant variation in shoot length was detected between grafted and non-grafted plants. Similar to the results of the study carried out by Heidari et al (2010) and Ban et al (2014), it was determined that the stem length of the stem increased in cucumbers grafted on some interspecies hybrid rootstocks. It is evaluated that different rootstock-scion combinations may produce different responses in terms of shoot length.

Stem diameter (mm)

Stem diameter values varied between 5.12 mm plant^{-1} and 5.17 mm plant^{-1} in non-grafted plants. The average of the stem diameter was 5.14 mm in non-grafted plants. Stem diameter values in grafted plants ranged from 3.58 mm plant^{-1} to 5.86 mm plant^{-1}, with an average of 4.93 mm plant^{-1}. An average of 4.96 mm plant^{-1} stem diameter values were obtained in all grafted and non-grafted plants. It was determined that some rootstock applications were higher than the non-grafted plants in terms of stem diameter values, while some were lower. The stem diameter values obtained in the combinations using Devrim and Kubai rootstocks are significantly higher than the non-grafted plants and the plants using other rootstocks (Table 2).

In grafted plants, the stem diameter depends on the rootstock variety (Kurum, 2010; Aktaş and Topçu, 2020). In this study, the mean stem diameter values were determined as 4.96 mm. The minimum and maximum values ranged from 3.58 mm to 5.86 mm, resulting in a wide variation. In this study, stem diameter increased significantly in Devrim and Kubai rootstocks. Damar and Solmaz (2020) found the stem diameter to be higher in the TZ148-Solo grafting combination than the non-grafted plants. In this study, the stem diameter values obtained using TZ148 and Maximus rootstocks were found to be lower than the non-grafted plants. It can be evaluated that the reason for the differences depends on the rootstock types used, and rootstock-scion interaction may cause significant differences.

Leaf Number

The number of leaves in non-grafted plants ranged from 8 to 12 LN plant^{-1}, with an average of 10.0 LN plant^{-1}. The

Table 3. Leaf number values in different grafting combinations

	Mean±S.E.	Minimum	Maximum
Non-grafted	10.0±0.58 c	8	12
TZ148-Minimix	14.2±0.57 b	13	15
Devrim-Minimix	20.3±0.88 a	19	22
Cremna-Minimix	15.6±1.12 b	13	17
Kubai-Minimix	15.1±1.11 b	13	17
Strong-Minimix	13.3±1.09 b	11	15
RS841-Minimix	15.0±1.15 b	13	17
Maximus-Minimix	12.7±0.58 bc	10	14
Mean	14.52±0.65	8	22

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

Table 4. Leaf relative water content values in different grafting combinations

	Mean±S.E.	Minimum	Maximum
Non-grafted	68.06±0.04 d	67.99	68.11
TZ148-Minimix	77.25±0.24 c	76.85	77.69
Devrim-Minimix	82.67±4.43 ab	75.18	90.51
Cremna-Minimix	76.23±0.01 c	76.23	76.23
Kubai-Minimix	86.77±0.28 a	86.23	87.18
Strong-Minimix	82.97±0.07 ab	82.86	83.11
RS841-Minimix	78.79±0.16 bc	78.48	79.01
Maximus-Minimix	83.29±0.19 ab	82.95	83.64
Mean	79.51±1.22	67.99	90.51

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

number of leaves in grafted plants varied between 10-22 LN plant^{-1} and the average was determined as 15.17 LN plant^{-1}. The number of leaves of all grafted and non-grafted plants was found to be between 8-22 LN plant^{-1} and an average of 14.52 LN plant^{-1}. The highest leaf number values (20.3±0.88) were obtained from Devrim-Minimix rootstock-scion combination. The leaf number values obtained in plants using Devrim rootstock were higher than both non-grafted plants and other rootstock applications. The leaf number values measured in non-grafted plants were lower than all graft combinations except the plants in which Maximus used as rootstock (Table 3).

Variation in the number of leaves is high in non-grafted plants, grafted plants and between different rootstock uses. The number of leaves detected in all rootstocks except the plants in which Maximus rootstock is used is higher than the non-grafted plants. Heidari et al. (2010) and Ban et al. (2014) determined that the use of hybrid rootstocks between species increased the leaf number values. Similar results were also found in this study. The increase in the number of leaves is directly proportional to the increase in the vegetative part and may cause high photosynthetic activity. In this case, it can related to yield and is advantageous in terms of hydrophonic culture.

Relative Water Content (RWC) (%)

The relative water content values of leaves in non-grafted plants were determined between 67.99% and 68.11% and an average of 68.06%. In the grafted plants, the relative water content of the leaves increased. The leaf relative water content values obtained as a result of all rootstock applications were statistically significantly higher than the non-grafted plants. Significant differences were also detected between different rootstock-scion combinations. Leaf relative water content values obtained in plants using Kubai rootstock are higher than TZ148, Cremna and RS841 rootstock applications (Table 4).

Leaf relative water content, which was determined as 79.51% on average, was measured at the lowest values in non-grafted plants. Although there were different results between rootstocks, the highest values were obtained in the use of Kubai rootstock.

Leaf chlorophyll content (SPAD) and Photosynthetic Active Radiation (PAR) Values (µmol/m²/s)

The SPAD values were found to be between 26.4-34.2 in non-grafted cucumber plants, with an average of 29.72±1.44. In grafted plants, these values showed a wider variation (14.5-37.7) and averaged 28.92. When all plants were examined, the SPAD averages were 28.77. Statistically, no significant differences were detected between grafted and non-grafted plants, but differences were detected between some rootstock applications. SPAD values obtained in plants applied Maximus, Strong and Kubai rootstocks were statistically significantly higher than TZ148 and Cremna rootstock applications

(Table 5).

The PAR values in grafted and non-grafted plants varied between 18.12-74.33 and were calculated as 46.33 on average. Average PAR values are 50.67 for non-grafted plants and 45.49 for grafted plants. The PAR values obtained in the Kubai-Minimix rootstock combinations among the graft combinations were found to be lower than the non-grafted plants. At the same time, the PAR values obtained from Devrim and Cremna rootstock applications are higher than the Kubai-Minimix combination. No statistically significant difference was found between the other groups (Table 5).

and non-grafted cucumber plants and found that the difference between grafted and non-grafted plants was insignificant in terms of chlorophyll a, chlorophyll b and total chlorophyll. Similarly, in this study, the difference in SPAD values between grafted and non-grafted plants is insignificant. Differently, in this study, it was determined that SPAD values varied between different rootstocks. In this study, it is evaluated that the low variation between grafted and non-grafted plants in terms of photosynthetic parameters may be due to the absence of a stress cause. The difference between rootstocks is an indication that different rootstock-scion interactions can produce different responses under the same conditions.

Table 5. SPAD and PAR values in different grafting combinations

	SPAD	PAR
Non-grafted	29.72±1.44 a-c	50.67±8.99 a
TZ148-Minimix	24.67±2.077 c	49.75±4.38 a
Devrim-Minimix	26.07±2.32 bc	48.83±5.09 a
Cremna-Minimix	24.6±1.6 c	49.67±3.63 a
Kubai-Minimix	30.6±2.02 ab	30.2±5.87 b
Strong-Minimix	32.23±1.12 a	46.5±3.30 ab
RS841-Minimix	30.02±2.41 a-c	46.3±6.77 ab
Maximus-Minimix	34.28±0.75 a	47.17±4.61 ab
Mean	28.77±0.79	46.33±2.06

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (*p*<0.01).

Depending on the rootstock variety, the parameters affecting the photosynthesis metabolism, which have an important effect on plant growth and development, may change (Öztekin, 2009). In this study, SPAD and PAR analyzes were performed to determine photosynthetic activity. SPAD values have very close values in grafted and non-grafted plants. Although there are no statistically significant differences between grafted and non-grafted plants, it has been determined that some rootstocks have higher values than other rootstocks. It was determined that the PAR values determined in the non-grafted plants were not lower than the grafted plants, and even higher than the Kubai-Minimix grafting combinations. Uysal (2010) determined leaf chlorophyll contents in grafted

Shoot Fresh Weight and Dry Weight (g plant^{-1})

The average of shoot fresh weight values of all plants were 43.81±2.37 g plant^{-1}, shoot dry weight values were 4.16±0.32 g plant^{-1}, and shoot dry weight/shoot fresh weight ratio was determined as 0.093±0.003 g plant^{-1}. Significantly higher results were obtained when rootstock was used in terms of shoot fresh weight values. A significant variation was also obtained among rootstocks, the highest shoot fresh weight values were obtained in Cremna and Devrim rootstock usage cases. Significantly higher results were obtained when rootstock was used in terms of shoot dry weight values. A significant variation was also obtained between rootstocks, the highest shoot dry weight values were obtained in the use cases of

Table 6. Shoot fresh-dry weight values in different grafting combinations

	Shoot Fresh Weight	Shoot Dry Weight	Dry W./ Fresh W.
Non-grafted	29.41±0.27 g	2.84±0.01 f	0.097±0.001 c
TZ148-Minimix	36.60±0.12 e	2.62±0.01 h	0.072±0.001 f
Devrim-Minimix	54.94±0.27 b	6.82±0.03 a	0.124±0.001 a
Cremna-Minimix	66.80±0.05 a	6.38±0.03 b	0.096±0.001 c
Kubai-Minimix	43.75±0.16 c	3.56±0.01 e	0.082±0.001 e
Strong-Minimix	44.12±0.17 c	4.34±0.02 c	0.098±0.001 b
RS841-Minimix	41.79±0.59 d	3.98±0.02 d	0.095±0.001 c
Maximus-Minimix	33.08±0.16 f	2.75±0.04 g	0.083±0.001 d
Mean	43.81±2.37	4.16±0.32	0.093±0.003

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (*p*<0.01).

Devrim and Cremna rootstock. When the ratios of shoot dry weight to shoot fresh weight were examined, it was determined that non-grafted plants had higher values than some rootstock plants. The highest values were determined for Devrim rootstocks, the lowest values for TZ148 rootstocks (Table 6).

Table 7. Root fresh-dry weight values in different grafting combinations

	Root Fresh Weight	Root Dry Weight	Dry W./ Fresh W.
Non-grafted	7.98±0.01 h	0.92±0.01 b	0.115±0.001 a
TZ148-Minimix	8.92±0.01 g	0.72±0.01 c	0.081±0.002 b
Devrim-Minimix	14.58±0.12 d	1.23±0.01 a	0.084±0.001 b
Cremna-Minimix	16.85±0.02 c	1.20±0.06 a	0.071±0.003 c
Kubai-Minimix	20.47±0.08 a	1.20±0.03 a	0.059±0.001 d
Strong-Minimix	9.64±0.06 f	0.65±0.01 c	0.067±0.001 c
RS841-Minimix	18.00±0.27 b	0.99±0.02 b	0.055±0.001 d
Maximus-Minimix	10.61±0.16 e	0.50±0.02 d	0.047±0.001 e
Mean	13.38±0.92	0.93±0.05	0.073±0.004

*Results are given as mean±standard error. Different letters indicate statistical differences between rows (p<0.01).

Root Fresh Weight and Dry Weight (g)

Average root fresh weight values were 13.38±0.92 g plant^{-1}, root dry weight values were 0.93±0.05 g plant^{-1} and root dry weight/shoot fresh weight ratio was determined as 0.073±0.004 g plant^{-1} in all plants. Significantly higher results were obtained when rootstock was used in terms of root fresh weight values. A significant variation was also obtained between rootstocks, with the highest root fresh weight values obtained in the Kubai and RS841 rootstock use cases. In terms of root dry weight values, the values obtained in non-grafted plants were found to be higher than some rootstocks used. The lowest root dry weight values belong to Maximus-Minimix grafting combination. When the ratios of root dry weight to root fresh weight were examined, it was determined that non-grafted plants had higher values than all rootstocks used (Table 7).

Biomass values vary according to rootstocks. In terms of shoot weight measurements, all rootstock applications have higher values than non-grafted plants in terms of both fresh and dry weight values. In this study, shoot fresh and dry weights were determined by Yarşi et al. (2008) was found to be higher in grafted plants, similar to the our findings. In the study of Uysal (2010), while the highest stem fresh and dry weights were obtained in Maximus rootstock grafted plants, the highest shoot fresh and dry weights were obtained from other rootstocks in this study. The reason for the difference may be the difference in the type of scion used and the cultivation in the hydroponic environment. It has been determined in other studies that the biomass values increase with the use of rootstock, and plant growth is encouraged depending on the rootstock (Khah, 2005). Although biomass production is determined higher in grafted plants (Ulas et al., 2020; Ulas, 2021), the rate of change varies according to rootstocks (Öztekin, 2009). Similarly, in this study, it was concluded that rootstock-scion combinations were effective in determining biomass.

CONCLUSION

The values measured in at least one grafting application in 9 different morphological and/or physiological parameters among the 12 parameters measured were higher than the non-grafted plants. In SPAD, PAR and root dry weight/fresh weight ratio measurements, the values measured in non-grafted plants were not lower than grafted plants. In root dry weight/fresh weight measurements, the values measured in non-grafted plants are significantly higher than all grafted plant groups. This shows that grafting in cucumber has no significant effect on photosynthetic activity under optimum conditions, but it has an effect on other parameters. While no significant differences were detected between rootstocks in some parameters, significant differences were detected in some parameters. TZ148 was in the group with the highest measurements in one parameter (PAR), RS841 in two parameters (SPAD and PAR), Maximus in three parameters (RWC, SPAD, PAR), Strong in three parameters (RWC, SPAD, PAR). Cremna rootstock in four parameters (shoot length, PAR, shoot fresh weight and root dry weight), Kubai in five parameters (stem diameter, RWC, SPAD, root fresh weight and root dry weight), and Devrim rootstock in seven parameters (stem diameter, number of leaves, RWC, PAR, shoot dry weight, shoot dry weight/fresh weight and root dry weight) was in the group with the highest measurements. It was concluded that Cremna and Devrim rootstocks stand out in terms of shoot length and biomass values, and rootstock use did not have a significant effect in terms of photosynthetic activity.

It is important to take measures to minimize the risks of factors that adversely affect soil, vegetation, water and atmosphere. Grafting and hydroponic media culture in vegetables are environmentally friendly applications that can be used for this purpose. Due to the benefits of using grafted seedlings, the demand for high quality grafted seedlings is increasing. In grafting technique, it is important to select the appropriate rootstock and scion for superior yield and quality. In addition, rootstock-scion interactions and growing environment factors affect the yield and quality of grafted vegetables. In this study, it was determined that grafting of cucumber plants on different rootstocks had a positive effect on some morphological and physiological parameters in hydroponic medium cultivation, but the effects could vary greatly with rootstock selection. In order to better understand the efficacy of grafted species of the *Cucurbitaceae* family, a scientific approach to genomics, proteomics and/or metabolomics can be performed in addition to the current study.

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 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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