Turk J Agric For
28 (2004) 231-237
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Effect of Hypocotyl Morphology on Survival Rate and Growth of Watermelon Seedlings Grafted on Rootstocks with Different Emergence Performance at Various Temperatures

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Received: 01.09.2003

Abstract: The emergence performance of different rootstocks and watermelon cultivars at different temperatures, and the effect of hypocotyl structure on grafting success and survival rate were investigated. Evaluated rootstocks were *Cucurbita moschata* (landrace), *C. maxima* (Arıcan local cultivar), Strong Tosa (*C. maxima* x *C. moschata*), P360 (*C. maxima* x *C. moschata*), *Luffa cylindrica*, *Benincasa hispida*, *Lagenaria siceraria* (landrace), Skopje (Landrace), and 2 *Lagenaria* hybrids, 216 and FR Gold. The Crimson Tide watermelon cultivar was used as scion. Emergence tests at 14 °C, 18 °C and 25 °C were carried out for all rootstocks and scion seeds in a growth chamber. *Cucurbita* type rootstocks showed better emergence performance at low temperatures than did other rootstocks and scion. However, *B. hispida*, *L. cylindrica*, *Lagenaria* type rootstocks and scion did not emerge at 14 °C and they were rotten. At 18 °C, the earliest emergence was recorded in *Cucurbita* type rootstocks while the latest emergence was observed in *B. hispida* and *L. cylindrica*. Rootstocks and scion did not show any difference in emergence rate at 25 °C. *B. hispida* and *L. cylindrica* had the thinnest and shortest hypocotyls which made grafting difficult. The survival rate of grafted plants was inversely correlated with the difference in diameters of scion and rootstock. At the same time survival rate was significantly affected by the rootstocks genotype. Rootstocks had different numbers of vascular bundles but the survival rate was not affected by the number of vascular bundles whereas the plant growth rate was positively affected. In general, *Lagenaria* type rootstocks had a higher survival rate than did other rootstocks. The hole insertion grafting technique had a significantly lower survival rate than did the approaching technique in *Cucurbita* type rootstocks. All graft combinations produced higher plant biomass than did the control plant, except for the *L. cylindrica*. The study showed that rootstock influences survival rates and plant growth in the use of grafting applications in watermelon.

Key Words: *Citrullus lanatus*, emergence rate, grafting, rootstocks, scion, hypocotyl

Hipokotil Morfolojisinin Değişik Sıcaklıklarda Farklı Çıkış Performansı Gösteren Anaçlar Üzerine Aşılanmış Olan Karpuz Fidelerinde Aşı Tutum Oranına ve Bitki Büyümesine Etkisi

Özet: Bu çalışmada farklı anaçların ve karpuzun değişik sıcaklıklarda çıkış performansları ve hipokotil morfolojisinin aşı tutma oranına ve bitki gelişimine etkileri çalışılmıştır. Anaç olarak; *C. moschata*, *C. maxima* (Arıcan), Strong Tosa (*C. maxima* x *C. moschata*), P360 (*C. maxima* x *C. moschata*), Luffa. cylindrica, *Benincasa hispida*, *Lagenaria siceraria*, Skopje ve 2 adet *Lagenaria* melezi kullanılırken, kalem olarak Crimson Tide karpuz çeşidi kullanılmıştır. Çıkış testleri 14 °C, 18 °C ve 25 °C'de bütün anaçlar ve kalem için yapılmıştır. *Cucurbita* grubuna giren anaçlar düşük sıcaklıklarda diğer anaçlardan ve kalemden daha iyi çıkış performansı göstermiştir. *B. hispida*, *L. cylindrica*, diğer su kabağı anaçları ve Crimson Tide karpuz çeşidi çıkış göstermemiş ve yapılan incelemede tohumların çürümüş olduğu görülmüştür. *Cucurbita* grubu anaçlar, 18 °C'de de en erken çıkan anaçlar olurken, *B. hispida* ve *L. cylindrica*, en geç çıkan anaçlar olmuşlardır. 25 °C'de anaçlar ve kalem arasında çıkış bakımından önemli bir fark bulunmamıştır. *B. hispida* ve *L. cylindrica* en ince ve en kısa hipokotillere sahip olmuşlar ve bu özellikleri aşı yapımını güçleştirmiştir. Anaç ve kalemin arasındaki çap farkı büyüdükçe aşı tutma başarısı düşmektedir. Aynı zamanda aşı tutma oranı anaçlardan da önemli oranda etkilenmiştir. Hipokotillerdeki iletim demeti sayısı aşı tutma oranı üzerine etkili olmazken, bitki gelişimi üzerinde etkili olmuştur. Genel olarak bakıldığında *Lagenaria* cinsine giren anaçlar daha yüksek aşı tutma oranı göstermiştir. *Cucurbita* grubu anaçlarda kakma (koltuk) aşıda aşı tutma oranı İngiliz dilcikli aşıdan daha düşük bulunmuştur. Bütün aşı kombinasyonları CT/LCY hariç kontrol bitkilerinden daha yüksek biyomas değerlerine sahip olmuştur. Çalışma sonuçları göstermiştir ki karpuzda aşılama uygulamalarında anaçların özellikleri aşı tutumunu ve bitki gelişmesini etkilemektedir.

Anahtar Sözcükler: *Citrullus lanatus*, çıkış oranı, aşılama, anaç, kalem, hipokotil

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Introduction

In Turkey, 24.7 million t of vegetables is produced in about 785,000 ha per year, and 31% of this production belongs to Cucurbitaceous species. Watermelon is one of the most important crops in this group with 16% production. Turkey is the second largest watermelon producing country, after China (FAO, 2002) with about 4 million t. Watermelon cultivation is performed mostly under low tunnels for early production in the Çukurova region, located in southern Turkey. Watermelon has been cultivated intensively for many years in this region. Problems caused by soil borne diseases, in particular *Fusarium* (Yücel et al., 1989) and continuous cropping (Messiaen, 1974) in watermelon have been reported. One of the classical solutions is crop rotation, with at least 5 year intervals for watermelon in the same field due to *Fusarium* damage. Producers have to pay at least twice as much in wages as in field rent for watermelon production. A combined breeding program might be applied to control soil-borne diseases (McCreight et al., 1993). However, developing new cultivars resistant to diseases is time-consuming and enhances the danger of resistant cultivars becoming susceptible to new races of pathogens. Grafting onto resistant rootstocks may be a solution to these problems (Balaz, 1982; Lee, 1994; Oda, 1995).

Grafting is widely used for the production of fruit bearing vegetables in Japan, Korea and some other Asian and European countries where intensive and continuous cropping is performed. Grafting in vegetables was first performed in Korea and Japan in the late 1920s by grafting watermelons onto gourd rootstocks (Ashita, 1927; Yamakawa, 1983). With the promising results from the first study, the cultivated area and species of grafted vegetables have been consistently increased. Currently, watermelon is one of the vegetables species in which grafting is performed intensively in those countries.

Other than to control soil-borne diseases, watermelons are grafted to increase low temperature tolerance and yield by increasing water and plant nutrients uptake (Masuda et al., 1981; Heo, 1991; Jang, 1992; Oda, 1995; Bletsos et al., 2003; Yetisir et al., 2003,). For these purposes, watermelons are grafted onto *C. moschata, C. maxima, Benincasa hispida* and *Lagenaria siceraria* species as rootstock (Lee, 1994). However, grafting needs time, space, materials and experience. Graft incompatibility and a decrease in fruit quality may appear depending on the combination of scion and rootstocks. Furthermore, grafted plants require improved cultivation methods and intensive post-graft care (Lee, 1994; Papodopoulos, 1994; Oda, 1995; Edelstein et al., 1999, Lee and Oda., 2003).

Vegetable grafting is a new topic in Turkey. There is a limited number of studies on grafted vegetable production. However, attention to grafting by researchers and industry has recently increased, especially with the banning of methylbromide. Suitable rootstocks should be identified and characterized for the effective utilization of grafting. Vegetables of the family *Cucurbitaceae* are grown in all regions of Turkey and there is great genetic diversity. There are many landraces of squash, pumpkin, bottle gourd, wax gourd and *Luffa cylindrica* that can be used as rootstocks for watermelon. However, to our knowledge there has been only 1 study conducted on the subject (Yetisir, 2001). Thus, more information on the rootstock characteristics of candidates in germplasm will be of great value. The objectives of the present study were to collect data on the rootstock performance of some candidates and in particular to determine the effect of hypocotyl morphology on the survival rate and growth of watermelon seedlings grafted on different rootstock, and to ascertain emergence performance of different rootstocks at different temperatures.

Materials and Methods

Plant material and experimental design

The watermelon [C*itrullus lanatus* (Thunb.) Matsum. and Nakai] cultivar Crimson Tide (CT) was grafted onto 10 different rootstocks. The names, definitions and sources of rootstocks are given in Table 1. Ungrafted Crimson Tide was used as the control. The hole insertion grafting technique (HIGT) (peg), and tongue approaching grafting technique (TAGT) were used and plants were grafted by following the procedure described by Lee (1994).

For emergence performance, each genotype was replicated 3 times with 30 seeds. Seeds were sown in a peat perlite mixture (1:1) in plug trays (cell volume 50 ml) and emergence testing was carried out at 3 different temperatures (14 ± 1, 18 ± 1 and 25 ± 1 ^{o}C) in a growth chamber. Daily observation was carried out and the

Table 1. Name, definition and source of rootstocks and scion.

Rootstock	Definition	Source
Cucurbita moschata (CMO)	Landrace	Mersin-Turkey
Cucurbita maxima (Arıcan)(CMA)	Landrace	Adapazarı-Turkey
Strong Tosa (ST)	*Cucurbita* hybrid	Korea
P360	*Cucurbita* hybrid	France
Luffa cylindrica (LCY)	Landrace	Adana-Turkey
Benincasa hispida (BH)	Landrace	Ankara- Turkey
FR Gold (FRG)	*Lagenaria* hybrid	Korea
Lagenaria siceraria (LSC)	Landrace	Urfa-Turkey
216	*Lagenaria* hybrid	Korea
Skopje (SK)	Landrace	Adana-Turkey
Watermelon cv. Crimson Tide (CT)	Hybrid	Syngenta seed company

emergence of 50% of seeds was taken as the emergence time. One hundred seeds were planted from each rootstock genotype for hypocotyl measurement and grafting in an unheated greenhouse under a low plastic tunnel. Seeds of the scion were sown 7 days earlier for grafting.

Six grafted plants from each rootstock genotype were transplanted to 8 l puts filled with topsoil, under low tunnels and the tunnels were removed when the air temperature was suitable (20-25 °C) for watermelon. The experimental design was a randomized complete block design. Each treatment was replicated 3 times with 4 plants in each replicate. The study was carried out in the spring of 2000. The following measurement and observations were done in both 2000-2001 studies;

Measurements

Hypocotyl length and diameter were measured at the first true leaf stage. Distance between soil surface and cotyledon leaves was measured as hypocotyl length (cm). Hypocotyl diameter was measured by digital compases (mm). The number of vascular bundles was counted in 50 hypocotyl sections using a 100x light microscope at the first true leaf stage. One hundred plants were grafted, and surviving grafted plants were counted 15 days after grafting. The survival rate was determined as a percentage. Two plants representing each replicate were rooted for 20 days after planting (DAP). Root length (cm), main stem length (cm), number of leaves per plant, total fresh weight (g) and total dry weight (g) were determined. Rooted plants were weighed for fresh weight and were placed in oven with circulating air at 70 °C for 48 h then weighed for dry weight.

Statistical Analysis

Data were subjected to analysis of variance by the COSTAT statistical program and means were compared by Tukey's test at the 0.01 or 0.05 significance levels. Correlation coefficients were calculated between hypocotyl diameter, hypocotyl length, vascular bundle and survival rates with the Microsoft Excel program.

Results and Discussion

Emergence rates of rootstocks at different temperatures were significantly affected by rootstock genotype. *C. moschata* (CMO), *C. maxima* (CMA) and P360 emerged 24 days after sowing (DAS) but other rootstocks and scion (Crimson Tide) did not emerge at 14 ± 1 °C. When examined, the seeds were found to be rotten. It was apparent the seeds had absorbed water but germination had not taken place (Table 2).

At 18 ± 1 °C, the earliest emergence was observed in CMA and Strong Tosa (ST) with 11 DAS. C*ucurbita* type rootstocks emerged earlier than *L. cylindrica* (LCY), *B. hispida* (BH) and *Lagenaria* type rootstocks. LCY and BH were the latest emerging rootstock at 22 DAS. On the other hand, rootstocks and scion did not show significant differences in emergence rate at 25 ± 1 °C (Table 2). *Lagenaria* type rootstocks need higher temperatures to emerge or emergence takes longer at lower temperature compared to *Cucurbita* type rootstocks. BH and LCY did

Table 2. Emergence performance of rootstocks and scion at different temperatures. CT: Crimson Tide

Rootstocks and CT	Emergence performance (Days After Sowing)		
	Temperatures (°C)		
	14 ± 1	18 ± 1	25 ± 1
C. moschata	24	13.0 de[c]	6.0
C. maxima	24	11.0 e	6.3
Strong Tosa	24	11.0 e	6.0
P360	24	12.0 e	6.3
L .cylindrica	NE[a]	22.0 a	7.3
B. hispida	NE	22.0 a	7.3
L. siceraria	NE	17.0 bc	6.0
216	NE	18.0 b	6.0
FR Gold	NE	15.0 bcd	6.3
Skopje	NE	16.0 bc	6.0
Crimson Tide	NE	15.0 cd	6.0
LSD	[b]	(1%) 1.91	(5%) NS

[a] No emergence,
[b] Since some of the rootstocks did not emerge, statistical analysis could not be performed,
[c] Same letter indicates the absence of any significant difference (P 0.01 or 0.05)

not emerge at 14 ± 1 °C and the latest emergence was determined in BH and LCY at 18 ±1 °C whereas they emerged at about the same time as other rootstocks. It might be concluded that BH and LCY need higher temperatures to show normal growth habit. Similar results were reported by Yetisir (2001) and Yetisir and Sari (2003). Some *Cucurbita* species are tolerant of low air and soil temperatures in extracting nutrients when soil temperature is low (Tachibana, 1987). Although BH and LCY showed high compatibility with watermelon (cv. Crimson Tide), they were not suitable rootstocks for early watermelon production, but they can be considered for late watermelon production because they were resistant to 3 known races of *Fusarium oxysporum f.* sp. *niveum* (Yetisir et al., 2003).

Hypocotyl length and diameter were measured and the results are presented in Table 3. The highest diameter was recorded in CMA (4.3 mm) while Crimson Tide (CT) had the smallest (2.8 mm) diameter. All rootstocks had higher diameters than did CT, except for CMO. There was a significant difference between rootstocks and scion with regard to hypocotyl length. The longest hypocotyl was determined in Skopje (SK) at 6.9 cm, followed by *L.siceraraia* (LSC) at 4.9 cm. LCY and BH had the shortest hypocotyls at 2 cm. The greatest difference between the diameters of rootstocks and scion in grafting was that between CMA and CT, at 1.5 mm. This was followed by SK, FRG and LSC (Table 3). Correlation coefficients were separately calculated between morphological characteristics of hypocotyls and survival rates for grafting techniques. The correlation between hypocotyl length and survival rate was insignificant (r = 0.19), and hypocotyl diameter and vascular bundle numbers were negatively correlated with survival rates (r = -0.38 and -0.65) in TAGT. The correlation between hypocotyl length, hypocotyl diameter, and number of vascular bundles with survival rate was also low (r = 0.30, r = 0.16, r = -0.14 respectively) in HIGT. The correlation between the morphological characteristics of hypocotyls and the survival rate of grafted plants ranged from very weak (r = 0.14) to modest (r = -0.65) significance levels. Oda et al. (1993) reported that a smaller difference in hypocotyl diameter between rootstocks and scion may increase the survival rate. However, in this study, it is difficult to make the assumption that survival rate could be explained by hypocotyls' morphological characteristics. Therefore, the affinity status of rootstocks and scion should also be accounted for. Incompatibility between watermelon and some *Cucurbita* rootstocks was reported by Yetisir and Sarı (2003). The length of rootstock hypocotyl is also an important characteristic in grafting because grafting is performed easily and efficiently in rootstocks with longer hypocotyls specifically in TAGT. With regard to this parameter, *Lagenaria* type rootstocks showed a better performance than did the others. BH and LCY, with the shortest and thinnest hypocotyls, showed the poorest performance in hypocotyl length. Hence, grafting needed more time and attention in these 2 rootstocks.

Rootstocks and CT had different numbers of vascular bundles in the hypocotyls. The highest number of vascular bundles was recorded in CMA with 10.44 vascular bundles per hypocotyls, while BH had the lowest number of vascular bundles with 4.54 bundles. *Lagenaria* type rootstocks and CT had similar numbers of vascular bundles (about 7 vascular bundles/hypocotyl) in their hypocotyls while ST, P360 and LCY had about 6 vascular bundles in theirs (Table 3).

Table 3. Morphological characteristics of rootstocks and scion hypocotyls and survival rate of graft combinations.

Rootstocks	Hypocotyl			Number of vascular bundles per hypocotyl	Survival rate (%)	
	Diameter (mm)	Length (cm)	RHD-SHD[d] (mm)		TAGT[e]	HIGT
CMO	2.7 e	2.9 de	-0.1	8.10 ± 1.10	85.0	63.0 d
CMA	4.3 a	3.3 c	+1.5	10.44 ± 1.00	85.0	82.0 bc
ST	3.1 d	3.2 cd	+0.4	6.40 ± 0.79	90 0	74.0 c
P360	3.1 d	3.1 cd	+0.3	6.36 ± 0.84	90.0	75.0 c
LCY	2.5 f	2.0 e	-0.3	6.00 ± 0.35	95.0	90.0 ab
BH	2.6 ef	2.0 e	-0.2	4.54 ± 0.82	95.0	90.0 ab
LSC	3.4 c	4.9 b	+0.7	7.97 ± 0.56	95.0	94.0 a
216	2.8 ef	3.0 cd	-0.1	7.20 ± 0.87	91.0	95.3 a
FRG	3.5 bc	3.2 cd	+0.7	7.7 ± 1.00	84.0	95.0 a
SK	3.7 b	6.9 a	+0.9	6.92 ± 0.86	94.0	95.0 a
CT	2.8 e	3.4 c	0.0	7.00 ± 0.89	----	---
LSD	(1%) 0.14	(1%) 0.2			(5%) NS	(1%) 6.29

[d]RHD: rootstock hypocotyls diameter, SHD: scion hypocotyls diameter
[e]TAGT: tongue approaching grafting technique, HIGT: hole insertion grafting technique

The survival rates of plants grafted onto different rootstocks by 2 different grafting methods are presented in Table 3. Although the difference in survival rates was not significant in TAGT, the lowest survival rate was 84% and the highest rate was 95%. There was a 10% difference between rootstocks, and this difference in survival rate was practically significant, in particular when hybrid seed was used in both rootstocks and scion. On the other hand, rootstocks showed significant differences with regard to survival rate in HIGT. The highest survival rate was found in LSC, 216, FRG, and SK at 95%, while CMO showed the lowest survival rate at 63%. In general, *Cucurbita* type rootstocks had a lower survival rate than did LCY, BH and *Lagenaria* type rootstocks in HIGT. Survival rate was not affected by the number of vascular bundles in hypocotyls of rootstocks. Oda (1993) reported a survival rate increase in *C. maxima* in which the larger number of vascular bundles was assumed to increase the chance of contact between the vascular bundles at the cut surface of hypocotyls. In this study, *Cucurbita* type rootstocks had a lower survival rate than did BH, LCY and *Lagenaria* type rootstocks. It seems that the difference between hypocotyl diameters may not be the only reason for the lower survival rate since the difference between hypocotyl diameters in CT and SK was 0.9 mm, but the survival rate was higher than that in other combinations with smaller differences in hypocotyl diameters. The survival rate in CMO, CMA, ST and P360 decreased in HIGT. This decrease can be explained by the larger pith in these rootstocks. Scion may be inserted in pith without contacting any cut surface, and then the scion will die (Yetisir, 2001, Yetisir and Sarı, 2003).

The growth performances of plants grafted onto different rootstocks were compared with CT and each other. During early spring, BH did not emerge and plants grafted for growth performance did not produce. As presented in the emergence test, LCY and BH were susceptible to low temperatures. Grafted plants had longer roots than those of CT, except for LCY. CMA and SK had the longest roots at 71.50 cm and 72.50 cm, respectively (Table 4). Main stem length was also significantly affected by rootstocks. *Lagenaria* type rootstocks produced longer main stems than did the control and other rootstocks. P360 and LCY produced the shortest main stems with 7.33 cm and 6.00 cm, respectively (Table 4). FRG, CMA and SK had the highest numbers of leaves at 22.17, 21.67, and 21.00 leaves/plant, respectively, whereas LCY at 10.17 produced the lowest number of leaves per plant (Table 4).

Table 4. Biomass measurements of grafted watermelon plants on different rootstocks at 20 days after planting.

Treatments	Root length (cm)	Main stem length (cm)	Number of leaves (number/plant)	Total fresh weight (g)	Total dry weight (g)
CT/CMO	53.17 ab	19.33 b	18.83 ab	43.33 bc	4.1 bc
CT/CMA	71.50 a	18.67 b	21.67 a	65.00 a	5.25 ab
CT/ST	55.67 ab	9.33 cd	17.00 abc	28.33 cd	2.41 def
CT/P360	64.00 ab	7.33 d	18.00 ab	33.66 cd	2.26 def
CT/LCY	42.67 b	6.00 d	10.17 d	12.5 e	1.38 f
CT/BH	[f]	[f]	[f]	[f]	[f]
CT/LSC	62.50 ab	16.50 bc	14.50 bcd	58.00 ab	3.56 cd
CT/216	65.00 ab	15.33 bc	12.67 bcd	30.33 cd	3.16 cde
CT/FRG	59.83 ab	26.27 a	22.17 a	67.66 a	5.13 ab
CT/SK	72.50 a	22.33 ab	21.00 a	69.67 a	5.50 a
CT	42.33 b	15.83 bc	12.33 cd	24.00 de	2.21 ef
LSD 1%	24.43	9.68	6.34	10.32	0.87

[f] There were not enough plants for field experiments

Total plant fresh and dry weights are presented in Table 4. As with leaf number, FRG, SK, and CMA produced the highest fresh weights while LCY had the lowest fresh weight. Plant dry weight was also significantly affected by rootstocks. Similar results were obtained to those for total fresh plant weight: CMA, SK and FRG produced the highest dry weight whereas LCY had the lowest dry weight.

It seems, therefore, that *Cucurbita* type rootstocks were more tolerant of low soil temperatures than were the other rootstocks and scion used in this study. This resistance may be attributed to ability in ion absorption, in utilizing the ATP and to the higher activity at lower reaction temperatures (Choi et al., 1995). Many authors stated that rootstocks affected yield and growth in grafted plants (Nielsen and Kappel, 1996; Chouka and Jebari, 1999; Ruiz and Romero, 1999; Traka-Mavrona et al., 2000; Bletos et al., 2003). This may be explained by the interaction of some or all of the following phonomena: increased water and plant nutrient uptake (Kato and Lou, 1989), augmented endogenous hormone production (Zijlstra et al., 1994), tolerance of low soil temperatures (Den Nijs and Smeets, 1987; Tachibana, 1989) and salinity tolerance (Zerki and Parsons, 1992; Santa-Cruz et al., 2002). In this study, plant fresh and dry weights were significantly affected by rootstocks and plant vigor was closely related to root length and vascular bundles in the hypocotyls, which supply excess water and plant nutrients to the scion.

Conclusion

On the basis of these results, *Cucurbita* species may be used as rootstocks for early watermelon production areas where soil temperatures are low in early spring, although the survival rate of plants grafted onto *Cucurbita* type rootstocks was lower than those of other rootstocks. Therefore, economic analysis should be conducted before the use of these rootstocks with lower survival rates. BH, LCY and *Lagenaria* used in this study were not suitable rootstocks for watermelon in early spring since they showed a similar emergence performance to that of watermelon in low soil temperatures. Other disadvantages of BH and LCY are their short and thin hypocotyl that causes difficulties in grafting. It may also be concluded that the reduction in the difference in hypocotyl diameters of rootstocks and scion increase the survival rate: however, this is not enough to explain the difference in survival rates of plants grafted onto different rootstocks. It is possible that the genotype of rootstocks was more fluential than the differences in diameter of rootstocks and scion. The *Lagenaria* type rootstocks, BH and LCY were in close histological structure and showed higher survival rates, and *Lagenaria* species have been used as main rootstocks for watermelon worldwide (Lee, 1994). Grafted plants produced more fresh and dry matter than did control plants, except for the CT/LCY graft combination. LSC and SKP, open pollinated landraces, may be utilized for hybrid rootstock development for watermelon cultivars.

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