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Assessment of Rooting Capability and Rootstock Potentials of Some Turkish Bottle Gourd (*Lagenaria siceraria*) Accessions Used as Rootstocks for Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Grafting practice has been common agricultural application in fruit-bearing vegetables in several countries. Since scion can be grafted on rootstock seedling with or without root in Cucurbitaceous crops, rooting capabilities of rootstocks during healing processes has critical importance. Rooting characteristics of some commercial hybrid rootstocks and eight Turkish bottle gourd accessions (*Lagenaria siceraria*) was studied when they were used as rootstocks for watermelon in splice grafting method. The study was carried out at three steps. At the first step, gourd seedling cuttings were prepared by cutting one cotyledon and growing point and roots then they were transplanted into irrigated rooting media consist of peat and perlite (2:1 v:v) under high air humidity and low light

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intensity conditions in a plastic tunnel covered with shading material (post graft care unit). Root formation capability of the cuttings was evaluated three weeks after transplanting. Crimson Tide watermelon cv. was grafted onto gourd genotypes without root by splice grafting method, and they were transplanted in growth media (2:1 v:v) in post graft care unit at the second stage of the experiment. At the third stage, rootstock effect of the bottle gourd accessions on plant growth parameters was investigated. Rooting performance (1-5 scale), root characteristics (length, thickness, fresh and dry weight) and graft compatibility rate (%) were determined three weeks after grafting. Rootstocks effect of different local bottle gourd accessions on leaf number and area, plant length, and fresh and dry weight of shoots and roots were investigated at the last stage of the study. While bottle gourd accessions showed significant variation in rooting capabilities after grafting, root characteristics, graft compatibility and plant growth (leaf number, leaf area and biomass accumulation), it was found out that local calabash accessions presented similar rootstock performance with commercial hybrid rootstocks. The survival rate of grafted plants ranged from 84% to 100%. All grafted combinations produced significantly higher biomass than ungrafted control plants. A number of local bottle gourd accessions produced higher leaf number, leaf area, dry root weight and shoot dry weight than commercial rootstocks were three, five, six and six, respectively.

Keywords: Cucurbita maxima; Cucurbita moschata; Lagenaria siceraria; Citrullus lanatus; grafting; rooting; survival rate; plant growth.

1. INTRODUCTION

Grafting in fruit-bearing vegetable species is a unique horticultural method performed for a long time in East Asian countries such as Japan and Korea to solve problems caused by intensive vegetable production in the limited agricultural area. Grafting fruit-bearing vegetables onto resistant rootstocks to control soil-borne diseases and pests has been practiced for many years in some Asian and European countries [1]. According to Lee and Oda [2] a self-grafting method to increase fruit size in bottle gourd by increasing root volume to promote water and plant nutrient uptake through bunch grafting was reported in a historical book written in China in the 5th century and in Korea in the 17th century. The first interspecific grafting in fruit-bearing vegetables as a pest/disease management and yield increase method was reported in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], by grafting on a squash (*Cucurbita moschata* Duch.) rootstock in Japan [3] and bottle gourd (*Lagenaria siceraria*) rootstock in Korea [4]. The success of these early trials led to increase the number of grafted vegetables species and cultivation areas. Research on cucumber grafting (*C. sativus* L.) also were introduced at the same time with watermelon grafting in the late 1920s, but widely agricultural applications occurred after 1960 [5]. In the Solanaceaous species, the earliest grafting practice was in eggplant (*Solanum melongena* L.) by grafting on *S. integrifolium* Poir (scarlet eggplant) in the 1950s [6]. Tomato (*S. lycopersicum* L.) has been commercially grafted since 1960s [2]. At present, grafted plants are used for mostly watermelon, tomato, eggplant, cucumber, melon and pepper in both open field and protected cultivation in Japan and Korea as well as other Asian and European countries such as China, Spain, Italy, Greece, Turkey and Israel, where fruit-bearing vegetables are being cultivated intensively [7,8,9]. Commercial grafting in vegetable crops was originated from Japan and Korea and was commercially used for more than 50 years. Some European countries introduced it in the early 1990s and it is currently common horticultural application using local/introduced scion varieties and imported rootstocks in Cucurbitaceous and Solanaceous crops [1].

The ban of methyl bromide (MB) fumigation has led to research on alternative control methods for the soil-borne pathogen in vegetable production, particularly in protected cultivation. Although alternative chemical applications and other cultural practices have been tested and developed, one of the best strategies to control soil-borne diseases is grafting susceptible cultivars onto resistant rootstocks. Furthermore, grafting onto suitable rootstocks can influence plant growth parameters, flowering, fruit ripening date, yield, and quality. After MB fumigation was phased out in Turkey, researchers and producers began to use grafting as an alternative method to avoid soil-borne pests and diseases [10].

Watermelon is one of the most grafted crop species. Because of the limited availability of plant rotation, use of grafted transplants has provided an effective alternative way for producing watermelon on land which would require soil fumigation or to be abandoned. Watermelon was mainly grafted to manage the soil-borne disease such as *Fusarium* wilt [11,12] and to enhance the plant nutrient absorption [13]; however, the purpose of the vegetable grafting have varied and increased significantly over the time. Common objectives of the grafting in vegetables are tolerance to high [14] and low [15] temperatures, to iron deficiency in high pH soil conditions [16] to saline soil conditions [17], to promote plant nutrition absorption [18] and to increase water uptake and use efficiency [19].

Interspecific squash hybrids (*Cucurbita maxima* Duch. × *C. moschata* Duch.), calabash (*L. siceraria* L.), squash (*C. moschata* Duch.), pumpkin (*C. pepo* L.), wax gourd (*Benincasa hispida* Thunb.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai), African horned cucumber (*Cucumis metuliferus* E. Mey. Ex Naud) (Lee et al., 2010), sponge gourds (*Luffa* spp.) [8,20] and wild watermelon (*Citrullus lanatus* var. *citroides*) [21] can be used as rootstocks for watermelon for different objectives. Commercial grafted watermelon production is widely practiced by grafting onto hybrid squash and bottle gourd rootstocks [1].

There are different grafting methods applicable for watermelon grafting. These are cleft, tongue approaching, hole insertion and splice (slant cut) grafting methods [1]. The grafting techniques used change according to scion and rootstock characteristics, conditions of grafting units and experience of researchers or farmers. Rootstocks/scion selection has a significant effect on survival rate of grafted seedling, fruit yield and quality characteristics [13,10,22].

Turkey is the second important watermelon producing country after China, with 3.9×10^6 t per year [23]. Watermelon has been produced intensively for many years in some areas of the country. Soil-borne vascular bundle diseases, especially *Fusarium*, and successive watermelon cultivation on the same field are among the main reasons for yield reduction in watermelon production areas of Turkey. One of the most effective and well known agricultural practices to control *Fusarium* wilt of watermelon is integrated crop management, suggesting that watermelon should be grown with a rotation of the suitable crops such as cereals at least for five years in the same field contaminated with the *Fusarium* wilt agent [24]. On the other hand, grafting disease sensitive cultivars onto resistant/tolerant rootstocks is an effective alternative way in controlling of some soil-borne diseases and has favorable effects on vegetative growth, fruit yield and quality [8,25,2].

Grafting in herbaceous plants is not new agricultural practice in some part of the world, and it has been practiced for about more than one hundred years [2]. But grafting in watermelons in Turkey has not been previously practiced due to available arable land for plant rotation, availability of methyl bromide for soil sterilization and high cost of grafted seedlings. However, producers have experienced in recent years constraints of new field for rotation, ban of methyl bromide and increased soil-borne disease problem [11]. The first experiment on production of grafted watermelon in Turkey was carried out in 1998-2000 [20]. After this successful study, commercial seedling companies has introduced and adapted the grafting technologies to their conditions. Number of grafted watermelon seedling increased more than 15 fold increased last decade [26]. Interspecific squash hybrid rootstocks (*C. maxima* x *C. moschata*) are widely used rootstocks for watermelon in Turkey. Seedling companies commonly used splice grafting technique in watermelon grafting. Rooting capacity of the rootstocks after grafting is important characteristics because previously formed roots are cut off during grafting. The complaint that rooting capacity of *Lagenaria* rootstocks after grafting was weaker than squash hybrids was received from seedling producer (personal communication). Previous literature was reviewed but sufficient information could not found about rooting capacity of rootstocks. Therefore, this experiment aimed to investigate rooting capacity, morphological root characteristics and effects of local bottle gourd genotypes as rootstocks on plant growth parameters of grafted watermelon at early stage of plant development.

2. MATERIALS AND METHODS

The study was conducted at three stages in Department of Horticulture, Faculty of Agriculture, University of Mustafa Kemal in Hatay-Turkey located at 36°19'32.62'' N latitude, 36°11'40.54'' E longitude and elevation above sea level is 130 m. At the first stage, rooting capacity and morphological root characteristics of the different rootstock cuttings were determined. At the second stage, watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] cv.

Crimson Tide was grafted onto rootstocks by splice grafting technique then survival rate of grafted seedling were determined. At the final stage of the experiment, effects of bottle gourd [*Lagenaria siceraria* (Molina) Standl.] accessions as a rootstock on plant growth parameters of watermelon (Crimson Tide cv.) were investigated.

2.1 Materials

Plant materials: Eight bottle gourd landraces (01-17, 07-06, 09-01, 31-43, 33-35, 35-01, 48-01, 47745), collected from Mediterranean region of Turkey, two commercial *Lagenaria* hybrids ((Macis (Nunhems) and Argentario (Syngenta)) and four commercial squash hybrid (*Cucurbita maxima* Duch. x *C. moschata* Duchesne ex Poir.) rootstocks ((Kublai (Syngenta), Kazako (Syngenta), Ferro (Rijk Zwaan), RS841 (Seminis)) were used as rootstocks. Crimson Tide (Syngenta) watermelon cultivar was used as scion. At the third stage of experiment, eight local bottle gourd genotypes and two commercial *Lagenaria* hybrid rootstocks, Macis and Argentario were used as rootstock. Peat:Perlite (2:1 v:v) mixture was used as seed sowing, rooting and growth media. The study was carried out in an unheated greenhouse.

2.2 Methods

Rootstock seeds were sown in mixture of peat and perlite (2:1 v:v). At the first true leaf stage (30 days after seed sowing), roots, growing point and one cotyledon of the rootstock seedlings were cut off and they were replanted in the mixture of peat and perlite and placed in postgraft care units for three weeks. After three weeks, rooting level (1-5 scale) (Fig. 1), root length (cm), root diameter (mm), root fresh and dry weight (g) were recorded. At the second stage, Crimson Tide watermelon cultivar was grafted according to the procedure described by Lee and Oda [2] at the first true leaf stage (30 days old rootstock and 40 days old scion) by splice grafting method (Fig. 2) and grafted plants were placed in postgraft care unit for one week. Survival rate of the grafted seedling were recorded as percentage four weeks after grafting (15.05.2009) and plants were taken from postgraft care unit to greenhouse. The rooting and graft compatibility experiments were designed as a randomized complete block design and each graft combination was replicated three times with 15 plants.

Plant growth experiment: Twelve grafted seedlings from each rootstock genotype were transplanted to 8 liter pots filled with a peat and perlite mixture (1:1 v:v) in an unheated greenhouse. Ungrafted Crimson Tide watermelon cv. was used as the control. The experiments were designed as a randomized complete block design with three replication and four plants (pots) for each replication. Plants were irrigated by two days interval. Plants were watered once a week with a nutrient solution

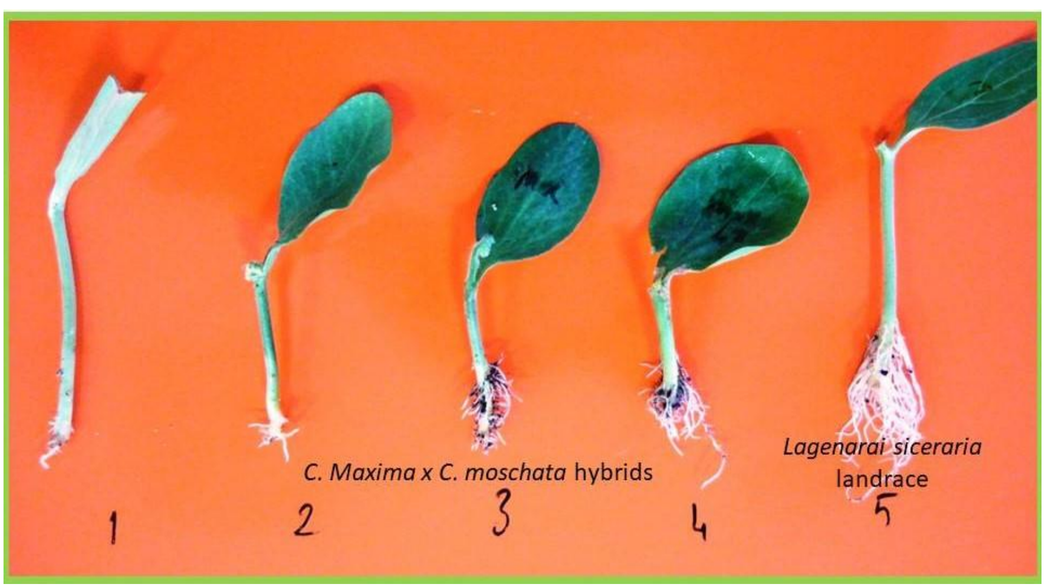

Fig. 1. 1-5 rooting scale the rootstocks

prepared according to Yetişir et al. [27]. Experiment was terminated four weeks after transplanting (15.06.2009) and plants were harvested. Plant length (main shoot length) (cm), leaf number (leaf/plant) and total leaf area (cm^2) per plant (LI-COR 3100 Area Meter, LI-COR, Lincoln, NE, USA), fresh and dry weight of plant organs (root and shoot) were determined. After the roots were washed and cleaned of growth media, the roots and shoots were dried in an oven with circulating air at 70°C for 48 h and dry weight of the plant organs were determined.

Statistical analysis: Data were subjected to an analysis of variance using SAS software [28] SAS statistical program and mean separation was performed with Tukey's test at P <0.01 and P<0.05 levels and LSD values are also presented.

3. RESULTS AND DISCUSSION

Rooting level and root characteristics showed significant difference based on rootstock genotypes (Table 1). All *Lagenaria* rootstocks showed better rooting than *Cucurbita* rootstocks except Kublai and Ferro. The highest rooting was observed in 07-06 while lowest rooting was observed in Kublai and RS841. Root length was significantly affected by rootstocks genotype and RS41, Kublai, 47745, 31-43 had longer root than other rootstocks had.

Root thickness significantly varied based on genotype. Commercial rootstocks and *Lagenaria* landraces 48-07, 47745, and 35-01 had thicker roots than other *Lagenaria* landraces. The thickest root was recorded in Macis (1.07 mm), Ferro (1.08 mm) and Argentario (1.02 mm) while thinnest root was determined in 01-17, 09-01, 31-43 and 33-35. Root fresh and dry weight was significantly affected by rootstocks. The highest root fresh weight was produced by 33-35 while lowest root fresh weight was recorded in 01-17 and commercial rootstocks Argentario. Other rootstocks produced similar result regarding to root fresh weight. *Lagenaria* rootstocks produced significantly higher root dry weight than *Cucurbita* rootstocks except 01-17 and Argentario. The highest root dry weight was recorded in 35-01 while the lowest root dry weight was determined in Kublai. When rootstocks grouped (local bottle gourd landraces, commercial *Lagenaria* and *C. maxima* x *C. moschata* hybrids), there was no significant differences between the groups as regarded to rooting level and root characteristics (Table 2).

Fig. 2. Splice grafting technique in watermelon. a: Scion at the first true leaf stage, b: Rootstocks at cotyledon leaf stage, c: Removing of growing point and one cotyledon of the rootstock, d: Grafted and replanted plants at postgraft care unit, d: Grafted seedling in greenhouse ready for transplanting

Table 1. Rooting level and root characteristics of different rootstocks

Rootstocks/genotypes	Rooting level (1-4)	Root			
		Length (cm)	Thickness (mm)	Fresh weight (g)	Dry weight (g)
01-17	3.26	10.52	0.68	1.89	0.10
07-06	4.15	11.41	0.78	2.43	0.24
09-01	3.41	11.19	0.68	2.71	0.23
31-43	3.41	12.70	0.69	2.54	0.24
33-35	3.37	10.60	0.69	3.49	0.21
35-01	3.59	10.74	0.88	2.84	0.28
48-07	3.11	10.61	0.99	2.71	0.26
47745	3.00	12.80	0.90	2.46	0.25
Macis	3.80	11.57	1.07	2.27	0.22
Argentario	3.80	11.67	1.02	1.97	0.07
Kublai	3.00	13.63	0.96	2.19	0.06
Ferro	3.47	12.47	1.08	2.47	0.15
Kazako	2.60	10.07	0.96	2.07	0.11
RS841	2.93	14.43	0.98	2.18	0.15
LSD	0.80*	1.87**	0.23**	0.44**	0.04**

**, ** significant at 0.05 and 0.01 respectively*

Table 2. Rooting capability and some root characteristics of the rootstock groups

Rootstock group	Rooting level (1-5)	Root			
		Length (cm)	Thickness (mm)	Fresh weight (g)	Dry weight (g)
Local bottle gourds	3.41	11.32	0.79	2.63	0.23
C. max. x *C. mosc.* hybrids	3.00	12.65	0.97	2.23	0.12
Bottle gourd hybrids	3.80	11.62	1.05	2.12	0.15
LSD	ns	ns	ns	ns	ns

ns: non-significant at 0.05 significance level

In this study, significant differences were determined between rootstocks regarding rooting properties and root characteristics (Table 1). These differences may be attributed to genotypes, interior characteristics, hypocotyls diameter and size of cotyledon. Cuttings with thicker diameter and rich in plant nutrition and hormones may have better rooting capacities. Tripathy et al. [29] stated that factors affecting rooting are physiological status of cutting, circumstances under which cuttings are grown and rooting media. It was previously reported that *Lagenaria* and *Cucurbita* species had different hypocotyl diameters and various number of vascular bundle in hypocotyls [25,30].

A survival rate of grafted plants was presented in Fig. 3. Survival rate was significantly affected by rootstock genotypes. The highest survival rate was recorded in RS841 and Argentario while the lowest survival rate was determined in Kazako. When rootstock groups was compared, the highest survival rate was ascertained from commercial bottle gourd hybrids (99 %) while the lowest rate was recorded in squash hybrids 86%. Mean survival rate of local bottle gourds was 91%. Watermelon can be successfully grafted onto bottle gourd rootstocks and high level of graft compatibility was reported [8,2,31]. Results of the study are agreement with previous experiments that economically sufficient survival rate and grafted seedling with high quality in *Lagenaria* rootstocks were gained [31,31]. Economically sufficient survival rate was obtained in local bottle gourd landraces by grafting slant cut grafting technique currently used in commercial seedling companies. As it was also pointed out in previous studies [25,30] reduction in diameter difference of scion and rootstocks hypocotyls and corresponding of vascular bundle of the rootstock and scion were effective in increasing the survival rate and promoting the growth of grafted seedling. Growth rate and emergence period of the rootstocks and scion should be known and the seeds should be sown based on this information in slant cut and approaching grafting methods in cucurbits.

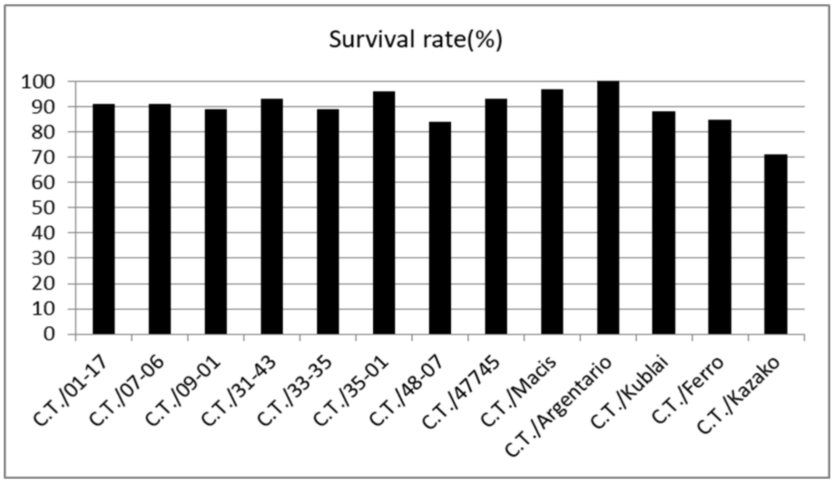

Fig. 3. Survival rate of graft combinations four weeks after grafting

Table 3. Leaf number, leaf area, plant length, fresh and dry weight of Crimson Tide watermelon cultivar grafted onto different local bottle gourd (*Lagenaria siceraria*) accessions four weeks after grafting

Graft combinations	Leaf number per plant	Total leaf area (cm^2)	Plant length (cm)	Root weight (g)		Shoot weight (g)	
				Fresh	Dry	Fresh	Dry
C.Tide	10.8	998	98	11.4	0.6	56.7	5.9
C.T./01-17	22.2	2536	118	51.3	2.7	157.4	17.8
C.T./07-06	19.7	2140	127	43.4	2.3	133.1	15.6
C.T./09-01	18.7	1907	123	47.7	2.3	112.7	12.4
C.T./31-43	19.3	2317	127	54.2	2.6	133.3	14.9
C.T./33-35	20.3	2383	116	57.4	2.8	146.6	16.0
C.T./35-01	18.5	2115	132	46.9	2.2	126.5	13.7
C.T./48-07	15.5	1635	118	39.9	1.8	99.8	10.1
C.T./47745	18.8	2235	122	41.4	1.9	130.3	14.6
C.T./Macis	18.7	1997	122	41.7	2.1	114.4	12.4
C.T./Argentario	14.7	1497	109	39.0	2.0.	88.4	9.4
LSD	5.4**	637**	10**	17.3**	1.1*	38.5**	4.4**

*C.T.: Crimson Tide; *, ** : Significant at 0.05 and 0.01 respectively*

Plant length was significantly affected by rootstocks (Table 3). All grafted plants had significantly longer plant length than the ungrafted control plants. The graft combinations C.T./07-06, C.T /31-43 and C.T./35-01 had longer plant than both commercial rootstocks. Other grafted plants showed similar plant length to that of the commercial rootstocks. Leaf number also showed significant differences based on rootstocks, and all of the grafted plants had a higher leaf number per plant than the ungrafted control plants. The lowest leaf number was recorded in ungrafted control plants with 10.8 leaves/plant while the highest leaf number was determined in C.T./01-17 graft combination with 22.2 leaves/plant. Four graft combinations (C.T./01-17, C.T./07-06, C.T./31-43 and C.T./33-35) produced higher leaf number than commercial rootstocks graft combinations. In comparison to the ungrafted control plants, increases in leaf number varied from 36% to 100% based on the rootstocks. The leaf area also showed significant variation based on rootstocks. All grafted plants had larger leaf area than ungrafted control plants. Plants grafted onto 01-17, 33-35, 47745, 31- 43, 07-06 and 35-01 produced significantly higher leaf area than that of the commercial rootstocks (Table 3). Shoot and root growth of the grafted plants were significantly promoted by different rootstocks. While the lowest root fresh weight was recorded in ungrafted control plants, the highest root fresh weight was determined in C.T./33-35 graft combination. The plants grafted onto 33-35, 31-43, 01-17 09-01 and 35-01 had higher root fresh weight than the plants grafted onto commercial rootstocks. Root dry weight results showed similar manner with root fresh weight. Shoot growth was significantly affected by rootstock and all of the grafted plants produced more shoot fresh and dry weight than the ungrafted control plants. The lowest shoot fresh weight was produced by ungrafted control plants with 56.7 g/plant whereas the highest shoot fresh weight was produced by the grafted plants onto 01-17 with 157.4 g/plant. The number of graft combination produced higher shoot fresh and dry weight was six. Root dry weight was significantly increased by different rootstocks and the increase rate in the shoot dry weight in comparison to control plants varied from 50% to 200% among the graft combinations (Table 3).

Bottle gourd accessions used in this study showed that all rootstocks promoted shoot biomass of scion. These results are agreement with previous studies in watermelon [10,32] and melon [16]. Plant growth promoting by rootstocks were attributed to root physical characteristics [33,34,35,10,36], high level of phytohormone production capacity of roots [37,34] and tolerance to biotic [11,38] and abiotic [18,13, 19,14] stress conditions.

4. CONCLUSION

Bottle gourd rootstocks showed rooting capability as high as commercial squash hybrids had and when splice grafting technique was used they had over 90% survival rate. It was also found that local bottle gourd accessions had sufficient rooting capacity, root quality and survival rate as compared to commercial *Lagenaraia* and *Cucurbita* type rootstocks. Rooting capacity and producing sufficient amount of roots after grafting in splice grafting method in cucurbitaceous rootstocks is one of the key concerns in rootstock breeding program. Because most of the commercial grafted seedling producers used splice grafting and root removal methods. The current study on subsample of Turkish bottle gourd germplasm pointed that Turkish *Lagenaria siceraria* germplasms has powerful rootstock potential for watermelon but local bottle gourds should be bred for agronomical traits such as seedling quality (hypocotyl diameter and length), effects on yield, quality, against biotic and abiotic stress factors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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