International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.4.2

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(4):211-216 (2019)

Contribution of roots to growth and physiology of watermelon grafted onto rooted and unrooted seedlings of various bottle gourd rootstocks

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Abstract

A hydroponic experiment was conducted between April and May in 2018 by using an aerated Deep Water Culture (DWC) technique in a controlled growth chamber of Erciyes University, Agricultural Faculty in Kayseri, Turkey. To evaluate contribution of roots for growth and physiology a commercial watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] cultivar (Crimson Tide F_1) was grafted onto two different bottle gourd (*Lageneria siceraria*) genotypes (39-01 and 47-02) and one commercial rootstock genotype (Argenterio) by using two propagation techniques (unrooted or rooted seedlings). Plants were grown in 8 L pots filled continuously aerated nutrient solution, in Randomized Block Design with 4 replications for 6 weeks. Results indicated that shoot and root fresh (FW) and dry (DW) weights, main stem length, total leaf area, leaf chlorophyll index (SPAD), photosynthetic activity of leaves of watermelon were significantly ($P<0.001$) affected by rooting type, genotype and genotype x rooting type interaction. Irrespective of rooting type, the grafted genotypes usually showed significantly higher performance in growth and physiological development than ungrafted control plants. Among graft combinations, the highest growth performance was shown by C.Tide/Argenterio while the lowest was shown by C.Tide/39-01. In terms of rooting type, watermelon plants usually showed a better performance in growth and physiological development when they were used as rooted seedlings compared to unrooted ones. Grafting watermelon onto unrooted seedlings caused a significant reduction in shoot FW by 21.6%, in shoot DW by 12.8%, in root FW by 29.5%, in root DW by 33.7%, in stem length by 11.5%, in total leaf area by 26.3%, in SPAD by 11.2% and in photosynthesis by 18.2%. All these clearly indicate that roots are playing very essential role in contribution to growth and development of plants, particularly at the beginning of growth stage. Therefore, our study suggested that grafting with unrooted seedlings is not a useful application strategy for watermelon plants grown under hydroponic conditions, even when they are grafted onto vigorous rootstocks.

Keywords: Grafting, Genotype, Rootless cuttings, DWC, Hydroponic

Introduction

Grafting is an important and widely applied practice for the production of cucurbit and solanaceous vegetable crops which are usually propagated by using grafted seedlings (Alan et al., 2017). The first grafted vegetable was achieved in Korea and Japan in the late 1920s by grafting watermelon onto gourd rootstocks to manage the soilborne Fusarium wilt (*Fusarium* spp.) diseases (Sibomana et al., 2013). Later on, several studies were carried out on grafting which represents a feasible alternative propagation technique in fruit bearing vegetables such as in tomato, watermelon, cucumber and eggplant to solve issues related to biotic and/or abiotic stress factors that affecting the fruit yield and quality (Lee, 1994; Davis et al., 2008; Schwarz et al., 2010; Savvas et al., 2010). Depending on the scion cultivars, the effects of the rootstocks on plant growth, fruit yield and quality can be resulted diversely either

Cite this article as:
Ulas, A., Aydın, A., Ulas, F., Yetisir, H. (2019). Contribution of roots to growth and physiology of watermelon grafted onto rooted and unrooted seedlings of various bottle gourd rootstocks. Int. J. Agric. Environ. Food Sci., 3(4), 211-216
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.2
Received: 08 September 2019 **Accepted:** 09 November 2019 **Published:** 23 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 211-216
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

positively in enhancement (Ozdemir et al., 2016) or negatively in decline (Edelstein, 1999; Lee and Oda, 2003). Since, not all the rootstock species are appropriate and useful for the all scion cultivars.

Generally, watermelon cultivars are grafted onto *Cucurbita moschata, C. maxima, Benincasa hispida* and *Lagenaria siceraria* rootstocks which are the widely used rootstock species for watermelon (Lee, 1994). In Turkey, grafting studies on watermelon plants were started in 2000s by testing 10 rootstocks consisting of Lagenaria, landrace and cucurbit hybrids (Yetisir, 2001). Ozdemir et al. (2016) reported that, watermelon varieties Crimson Tide and Crisby were grafted onto hybrid rootstocks of RS 841, Ferro, Argentario and Macis rootstocks and observed that more yield, fruit size, plant development and fruit quality were produced by the grafted plants.

Cucurbits grafting can be done by applying different propagation techniques, such as using by unrooted cuttings or rooted seedlings as rootstocks. However, there are several advantages and disadvantages of using some of these propagation techniques (unrooted cuttings or rooted seedlings) in rootstock grafting (Lee and Oda, 2003). The advantages of unrooted grafting are; quick and easy method, for some vegetables, very well seedling homogeneity, regulates able to stem length regulation, more hygienic. On the other hand, disadvantages of this method are; delay in root formation during healing of graft part, slow growth, and infection risk. The aim of this work was to evaluate the significance of roots for growth and physiology of watermelon grafted onto rooted and unrooted cuttings of various bottle gourd (*Lagenaria sicerari*) rootstock genotypes under hydroponic condition.

Materials and Methods

Plant Material

In this study a commercial watermelon cultivar (Crimson Tide F1) was used as scion and two different bottle gourd (*Lageneria siceraria*) landrace genotypes (39-01 and 47-02) and one commercial bottle gourd rootstock (Argenterio) genotype were used as rootstock materials (Table 1).

Experimental Site and Plant Growth Conditions

An experiment was conducted between April and May in 2018 by using an aerated Deep Water Culture (DWC) technique in a controlled growth chamber situated in the Plant Physiology Laboratory of Erciyes University, Faculty of Agriculture, central Anatolia in Turkey. For the vegetation period, the average day/night temperatures were 25/22 °C, the relative humidity was 65-70% and about 350 µmol m^{-2} S^{-1} photon flux was supplied in a photoperiod of 16/8 h of light/dark regimes in the controlled growth chamber. To produce homogenous seedling for hydroponic growth medium, seeds of watermelon were sown one week earlier than quickly germinating bottle gourd's seeds in a multi-pots contained a mixture of peat (pH: 6.0-6.5) and perlite in a 2:1 (v:v) ratio for 2 weeks. When the seedlings developed two or three true leaves, scions were grafted onto rootstocks. Some of the ungrafted watermelon (Crimson Tide) plants were used as scion control plants while some of them were grafted onto different rootstocks.

After grafting process, plants were healed and acclimatized in the tunnel covered with double-layered plastic film and shade cloth in the climate chamber for one week (Leoni et al., 1990). In order to prevent grafted plants from wilting by the excessive transpiration and to enhance healing, the tunnel was closed for the first three or four days of healing and acclimatization period. For the next three or four days, the opening and closing of the tunnel were done depending on the conditions of grafted plants and growth room. This was done for the acclimatization of grafted plants to environmental conditions outside tunnel. After the end of healing and acclimatization period, the grafted and ungrafted control plants were carefully freed from the growth medium with no root damage and then transferred into 8 L plastic pots filled with nutrient solution in growth chamber. Each pot was filled with 8 L nutrient solution that was aerated by an air pump to supply sufficient oxygen. The experiment was arranged in a completely randomized block design with four replications and three plants in each pot (replication). In the hydroponic experiment the total vegetation period from transplanting into 8 L plastic pots up to final harvest was almost six weeks.

The nutrient solution was prepared by using distilled water contained analytical grade (99% pure) chemicals according to modified Hoagland and Arnon formulation. In hydroponic experiment, 2000 µM nitrogen was supplied by using two different proportional N sources (75% $Ca(NO_3)_2$ and 25% $(NH_4)_2SO_4$). Furthermore, basic nutrient solution had the following composition (µM): K_2SO_4 (500); KH_2PO_4 (250); $CaSO_4$ (1000); $MgSO_4$ (325); NaCl (50); H_3BO_3 (8.0); $MnSO_4$ (0.4); $ZnSO_4$ (0.4); $CuSO_4$ (0.4); $MoNa_2O_4$ (0.4); Fe-EDDHA (80). All nutrients were replaced when the N concentration of the nutrient solution in the 2.0 mM N rate pots fell below 0.3 mM, as measured daily with nitrate test strips (Merck, Darmstadt, Germany) by using a NitracheckTM reflectometer. Distilled water was added every two days to replenish the water lost to evaporation, and the solution was changed weekly.

Harvest, Shoot- Root Fresh and Dry Weight, Root: Shoot Ratio Measurements

At the end of the experiment plants were harvested by separating them into shoot and roots. For the fresh weight determination plant organs were fractioned into the leaf, stem and roots and then weighted. After measuring the fresh weights of each shoot and root fraction, samples were stored separately in paper bags and dried in a ventilated oven at 70 °C for 72 hours. Root: shoot ratio was calculated from the dry weight.

Main Stem Length and Leaf Physiological Measurements

At the end of the experiment the main stem length and leaf physiological measurements of plants were determined destructively. Main stem length (cm) was measured by using a ruler. Total leaf area (cm^2) of harvested plants was measured destructively with a leaf area measuring device (LI-COR LI-3100C, Inc., Lincoln, NE, USA).

On the other hand, the leaf chlorophyll index (SPAD) was determined non-destructively by using a portable chlorophyll (SPAD) meter (Minolta SPAD-502). During the growth period, SPAD readings were performed on 3^{th} and 4^{th} week of the vegetation period at the center of the leaves on the fully expanded youngest leaf of whole plants for each treatment.

The leaf-level CO_2 gas exchange (μmol CO_2 m^{-2} s^{-1}) measurements were done in controlled growth chamber by using a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). The leaf photosynthesis measurement was performed on the most recent fully expanded leaves, using four replicate leaves per treatment on 3th and 4th week of the vegetation period.

Statistical Analysis

Statistical analysis of the nutrient solution experiment data was performed using SAS Statistical Software (SAS 9.0, SAS Institute Inc., Cary, NC, USA). A two-factorial analysis of variance was performed to study the effects of graft combination (genotype) and rooting type and genotype x rooting type interactions on the plants. Levels of significance are represented by *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ns means not significant. Differences between the treatments were analyzed using Duncan's Multiple Test.

Table 1. The scion, rootstock and their graft combinations under two propagation techniques

Genotypes	Scion/Rootstock (S/R)	Propagation (Root Type)
C.Tide	Ungrafted Control (Crimson Tide)	Rooted - Unrooted
C.Tide/39-01	Crimson Tide/Landrace bottle gourd (*L. siceraria*)	Rooted - Unrooted
C.Tide/47-02	Crimson Tide/ Landrace bottle gourd (*L. siceraria*)	Rooted - Unrooted
C.Tide/Argenterio	Crimson Tide/Commercial bottle gourd (*L. siceraria)*	Rooted - Unrooted

Results and Discussion

Shoot and Root Fresh Biomass Production and Main Stem Length

Results obtained from hydroponic experiment indicated that shoot and root fresh (FW) and main stem length of watermelon were significantly (P<0.001) affected by rooting type, genotype and genotype x rooting type interaction (Table 2). Irrespective of rooting type, the grafted genotypes usually showed significantly higher shoot (85% increase in shoot FW and 41% increase in stem length) and root growth (170% increase in FW) performance than ungrafted control plants. Among graft combinations, the highest shoot growth was shown by unrooted C.Tide/Argenterio while the lowest was shown by unrooted C.Tide/39-01. On the other hand, the highest root growth was shown by rooted C.Tide/39-01 graft combinations while the lowest was shown by rooted C.Tide/47-02. The lowest fresh biomass and shortest stem length of both rooted and unrooted C.Tide/39-01 graft combinations, might be due to differences in partitioning of dry matter between scion (watermelon) and rootstock (39-01). The vigor of the rootstock is important in conferring scion vigor (Gisbert et al., 2011), but its effect on scion may depend also on watermelon variety (Ozdemir et al., 2016). Our results clearly indicated that grafting with vigor rootstocks either with rooted seedlings or unrooted cuttings have pronounced positive effect on shoot and root growth.

A higher performance in shoot and root growth of grafted watermelon plants might be results of vigorous and active root system of bottle gourd rootstocks that contributed to water and mineral nutrient uptake (Rivero et al., 2003) which led to increase in leaf area formation and photosynthetic activity of leaves. Since, the leaf area formation plays an important role for the light interception and carbon assimilation by crops (Grosse, 1989). Consequently, biomass production and yield of a crop is strongly dependent on its leaf area as well as the rate of leaf photosynthesis (Hirasawa and Hsiao, 1999).

In collaboration with our study, Wei et al., (2009) reported similar results and stated that plants grafted onto *Lageneria siceraria* rootstock genotypes have significantly higher fresh matter in shoots and roots than those of ungrafted plants. Also, other authors stated similar reports about the grafting effects on plant growth and yield (Chouka and Jebari, 1999; Yetisir and Sarı, 2004; Yetisir et al., 2006). Irrespective of grafting process, watermelon plants usually showed a better performance in growth and physiological development when they were used as rooted seedlings compared to unrooted cuttings. Because, unrooting treatment caused a significant reduction in shoot FW by 21.6%, in root FW by 29.5%, and in stem length by 11.5% of watermelon plants. This might be due to lower water transport and mineral uptake (Rivero et al., 2003) from roots to shoots that caused a decline in leaf area formation and thus a low photosynthetic activity of scion leaves.

Shoot and Root Dry Matter Accumulation and Partitioning

The accumulation of shoot and root dry matter and its partitioning of watermelon were significantly (P<0.001) affected by rooting type, genotype and genotype x rooting type interaction (Table 3). Irrespective of rooting type, the grafted genotypes usually produced significantly higher shoot (96% increase), and root (119% increase) dry matter than ungrafted control plants. Graft combinations differed significantly and thus the highest shoot dry matter accumulation was shown by C.Tide/Argenterio while the lowest was shown by C.Tide/39-01. On the other hand, the highest root growth was shown by C.Tide/Argenterio and C.Tide/39-01 graft combinations while the lowest was shown by C.Tide/47-02. This is the similar variation existed also in shoot and root fresh matter production among the same graft combinations (Table 2).

Our results clearly indicated that grafting with vigor rootstocks either with rooted seedlings or unrooted cuttings have pronounced positive effect on shoot and root growth and hence on dry matter accumulations (Table 3). This might be results of stronger root growth of the rootstock (Yetisir and Sari, 2004; Khah, 2011) that contributed to water and mineral nutrient uptake (Rivero et al., 2003) and to augmented endogenous hormone production (Zijlstra et al., 1994) which led to increase in leaf area formation and photosynthetic activity of scion leaves. However there was no significant difference between ungrafted watermelon control plants and grafted C.Tide/Argenterio and C.Tide/47-02 graft combinations in dry matter partitioning (root:shoot ratio), while significantly highest root:shoot ratio was demonstrated only by C.Tide/39-01 (Table. 3).

It has also been reported that grafting promotes vegetative growth at different levels depending on rootstock characteristics. Many studies reported that an interaction between rootstocks and scions exists resulting in high vigor of the root system and greater water and mineral uptake leading to an increased yield and to fruit growth enhancement (Besri, 2002; Kacjan Marsic and Osvald, 2004). Irrespective of grafting process, watermelon plants usually showed a better performance in growth and physiological development when they were used as rooted seedlings compared to unrooted cuttings. Because, unrooting treatment caused a significant reduction in shoot DW by 12.8%, in root DW by 33.7%, and in root:shoot ratio by 40.6% of watermelon plants. This might be due to lower water transport and mineral uptake (Rivero et al., 2003) from roots to shoots that caused a decline in leaf area formation and thus a low photosynthetic activity of scion leaves.

Physiological Leaf Development and Photosytetic Activity of Leaves

The total leaf area, leaf chlorophyll index (SPAD) and photosynthesis of watermelon were significantly ($P<0.001$) affected by rooting type, genotype and genotype x rooting type interaction in hydroponic experiment (Table 4). Irrespective of rooting type, the grafted genotypes significantly increased the total leaf area almost by 124%, the leaf SPAD value by 19% and the photosynthetic activity by 44% as compared to ungrafted control plants.

Among graft combinations, highly significant differences were found in physiological leaf development and photosynthetic activity of leaves. Significantly highest total leaf area, SPAD and photosynthesis were demonstrated consistently by the graft combination of C.Tide/Argenterio while the lowest was shown by C.Tide/39-01. The leaf area formation is evidently affected by the scion, but the rootstock may also have significant effects on plant growth (Davis et al., 2008).

Many researchers found that grafting on hybrid rootstocks promoted plant yield increase (Yetisir et al., 2006; Alan et al., 2007; Alexopoulos et al., 2007). In this study, an increase in leaf area formation was determined to be consistent with previous studies.

Since the interspecific hybrid rootstocks with vigorous root system are able to absorb water and nutrient elements more efficiently in addition to disease resistance, they are superior to ungrafted plants in terms of plant yield (Huitron et al., 2009).

Furthermore, concerning leaf chlorophyll index (SPAD) and photosynthesis, our results agreed with the finding of other researchers (Lee, 1994; Besri, 2008). The increased yield of grafted plants is also believed to be due to enhanced water and mineral uptake (Rivero et al., 2003). Pulgar et al. (2000) found that grafting influences absorption and translocation of phosphorus, nitrogen, magnesium, and calcium. Therefore, improving nutrient uptake increases photosynthesis, these conditions allow grafted plants to produce higher yields (Hu et al., 2006). Regardless of grafting process, watermelon plants usually showed a better performance in physiological leaf development and photosytetic activity when they were used as rooted seedlings compared to unrooted cuttings. Since, unrooting treatment caused a significant reduction in total leaf area by 26.3%, in SPAD by 11.2% and in photosynthesis by 18.2% of watermelon plants. This might be due to lower water transport and mineral uptake (Rivero et al., 2003) from roots to shoots that caused a decline in leaf area formation and thus a low photosynthetic activity of scion leaves.

Table 2. Shoot and root fresh matter and main stem length of rooted and unrooted control and grafted watermelon genotypes

Genotypes	Shoot Fresh Weight (g plant^{-1})		Root Fresh Weight (g plant^{-1})		Main Stem Length (cm plant^{-1})	
(Scion/ Rootstock)	Rooted	Unrooted	Rooted	Unrooted	Rooted	Unrooted
C.Tide	19.95 B	17.95 d	4.35 C	2.69 b	9.28 D	7.31 c
C.Tide/39-01	36.23 A	22.87 c	11.75 A	7.23 a	9.79 C	11.34 a
C.Tide/47-02	37.48 A	27.51 b	9.92 B	7.40 a	11.79 A	10.19 b
C.Tide/Argenterio	36.46 A	33.70 a	10.20 B	8.18 a	10.61 B	10.68 b
Genotype	***		***		***	
Rooting type	***		***		***	
Genotype X Ro.type	***		***		***	

Values denoted by different letters (lower and upper case letters for rooted and unrooted, respectively) are significantly different between genotypes within columns at $P < 0.05$: ns, non-significant. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Table 3. Shoot and root dry matter, root: shoot ratio of rooted and unrooted control and grafted watermelon genotypes

Genotypes	Shoot Dry Matter ($g\ plant^{-1}$)		Root Dry Matter ($g\ plant^{-1}$)		Root:Shoot ($g\ g^{-1}$)	
(Scion/ Rootstock)	Rooted	Unrooted	Rooted	Unrooted	Rooted	Unrooted
C.Tide	1.62 C	1.16 d	0.62 C	0.24 b	0.38 B	0.21 c
C.T/39-01	2.38 B	1.45 c	1.17 A	0.72 a	0.49 A	0.50 a
C.T/47-02	2.57 A	2.34 b	0.99 B	0.74 a	0.39 B	0.32 b
C.T/Argenterio	2.50 A	2.97 a	1.02 B	0.82 a	0.41 B	0.28 b
Genotype	***		***		***	
Rooting type	***		***		***	
Genotype X Ro.type	***		***		***	

Values denoted by different letters (lower and upper case letters for rooted and unrooted, respectively) are significantly different between genotypes within columns at P < 0.05: ns, non-significant. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Table 4. Total leaf area, leaf chlorophyll index (SPAD) and photosynthetic activity of rooted and unrooted control and grafted watermelon genotypes

Genotypes	Leaf area ($cm^2\ plant^{-1}$)		Leaf chlorophyll Index (SPAD)		Photosynthesis ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	
(Scion/ Rootstock)	Rooted	Unrooted	Rooted	Unrooted	Rooted	Unrooted
C.Tide	300.79 C	240.52 d	36.77 D	32.88 d	6.47 D	5.83 d
C.T/39-01	596.50 B	340.10 c	40.22 C	35.78 c	8.59 C	6.40 c
C.T/47-02	605.37 B	440.19 b	42.46 B	37.26 b	9.48 B	7.59 b
C.T/Argenterio	647.71 A	565.02 a	43.91 A	39.24 a	9.63 A	8.12 a
Genotype	***		***		***	
Rooting type	***		***		***	
Genotype X Ro.type	***		***		***	

Values denoted by different letters (lower and upper case letters for rooted and unrooted, respectively) are significantly different between genotypes within columns at P < 0.05: ns, non-significant. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Conclusion

Grafted vegetable production has become a common practice in many parts of the world. It is an effective agricultural approach to improve plant growth, due to that the yield and quality of the shoot system, partially, depend on the root system. The results of the present experiments demonstrate that how the rootstocks improve plant vigor and productivity whether grafted on to rooted seedlings or unrooted cutting plants. The grafted plants were more robust in terms of main stem length, leaf area, leaf chlorophyll index (SPAD), photosynthesis, fresh and dry weights than those of the ungrafted control plants. This effect, which is present only in some grafting combinations, therefore the scion x rootstock combination is of major importance in terms of growth and development, whereas the choice of the right combination could be a useful means in grafting vegetable production. Watermelon scion variety interacts significantly different when they are grafted onto rooted seedlings or unrooted cutting as a scion-rootstock combination under hydroponic conditions. Unrooting process on the cuttings caused a significant reduction in shoot and root growth, total leaf area, leaf SPAD value and in photosynthesis. All these clearly indicate that roots are playing very essential role in contribution to growth and development of watermelon plants, particularly at the beginning of growth stage. Therefore, our study suggested that grafting with unrooted cuttings is not a useful application strategy for growth and physiology of watermelon plants grown under hydroponic conditions, even when they are grafted onto vigorous rootstocks.

Acknowledgements: We thank to all staff members of the Plant Nutritional Physiology Laboratory of Erciyes University, Turkey for the technical supports and supplying all facilities during the experiments.

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