International Journal of Horticultural Science and Technology
Vol. 7, No. 1; March 2020, pp 69-79
Print ISSN: 2322-1461 Online ISSN: 2588-3143
DOI: 10.22059/ijhst.2020.284659.304
Web Page: https:// ijhst.ut.ac.ir, Email: ijhst@ut.ac.ir

First report: Grafting of Three Iranian Commercial Pomegranate Cultivars on Drought Tolerant Rootstocks

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(Received: 2 July 2019, Accepted: 15 October 2019)

Abstract
The objective of this study was to evaluate the stenting (grafted cutting) propagation of commercial pomegranate cultivars as scions on drought tolerant genotypes as rootstocks. The effect of drought stress on nine pomegranate rootstocks was analyzed. Cultivars including ‘Daneshgah 13’, ‘Daneshgah 32’ and ‘Daneshgah 8’ that were used as rootstocks showed the maximum drought tolerance among the studied cultivars; therefore, these three rootstocks were used to investigate the stenting propagation. Furthermore, three pomegranate cultivars including ‘Malas Saveh’, ‘Robab’ and ‘Bihasteh’ were used as scions. The highest percentage of graft success (58.88%) was obtained from grafting on ‘Daneshgah 13’. In addition, the highest percentage of graft success (84.22) was found in ‘Robab’. ‘Robab’ and ‘Malas Saveh’ had the longest shoot (11.50-11.93 cm) and highest shoot fresh weight (33.66-35.00 g) when grafted on ‘Daneshgah 13’. ‘Daneshgah 13’ had higher shoot dry weight (5.47 g) compared to the other rootstocks. Regarding the scion cultivars, ‘Robab’ and ‘Malas Saveh’ showed higher shoot dry weight (6.76-6.96 g) in comparison with ‘Bihasteh’. Using ‘Daneshgah 13’ as rootstock resulted in the highest content of chlorophyll a (18.11 mg/g), chlorophyll b (8.02 mg/g) and total chlorophyll (26.13 mg/g) in the scion leaves. ‘Robab’ and ‘Malas Saveh’ had highest content of chlorophyll a (18.11-18.33 mg/g), chlorophyll b (8.58-8.62 mg/g) and total chlorophyll (26.73-26.92 mg/g) among the scion cultivars. In all three rootstocks, a negative correlation was observed between the total phenolic content and the percentage of graft success. In addition, there was a negative correlation between the total phenolic content and the percentage of graft success in ‘Bihasteh’ scion.

Keywords: Chlorophyll, Grafting, Phenol, Scion, Stenting.

Introduction

Pomegranate (*Punica granatum* L.) is an important fruit tree of the tropical and subtropical areas of the world that is grown for its enjoyable fruits, medicinal properties and ornamental appearance (ValizadehKaji and Abbasifar, 2017). Pomegranate is considered native to Iran (Stover and Mercure, 2007) and it has been widely cultivated in arid and semiarid regions of this country. The total area under pomegranate cultivation in Iran is about 70,000 ha and Iran, with an annual pomegranate production of about 800,000 tonnes, ranks second in pomegranate production in the world (Kahramanoğlu and Usanmaz, 2016). However, drought in this area is a major limitation to pomegranate production resulting in

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serious adverse economic impacts to its growers. To meet the challenges of such extreme environmental conditions, different strategies were developed by the researchers (Dietmar et al., 2010). One of the methods is to enhance or improve the genetic tolerance to such stresses by developing tolerant varieties. However, due to less genetic variability in relation to tolerance, breeding remained a slow process in many horticultural crops. Recently, another significant method for adapting plants to counteract environmental stresses and improving the tolerance suggested by various researchers is grafting on selected tolerant rootstocks (Dietmar et al., 2010). Modern fruit growing makes extensive use of selected rootstocks for a variety of purposes, including vigor control (Basile et al., 2003; Tuwel et al., 2008), which enable high density planting, resistance to disease and pest (Hernández et al, 2010), tolerance to abiotic stresses like hypoxia (Dietmar et al., 2010), reduce the time to fruiting, increase profit returns (Tuwel et al., 2008), improve fruit quality and yield (Cantin et al., 2010), improve cold hardiness (Dietmar et al., 2010), decreasing the chilling requirement of the scion (Gainza et al., 2015), and alleviating certain syndromes like replant complex (Gainza et al., 2015). In addition, modern cultural methods are evolving towards the use of clonal rootstocks as seedling rootstocks that were commonly used in the past (Gainza et al., 2015).

Presently, there are about 760 genotypes and cultivars of pomegranate in the pomegranate collection of Yazd, a city located in the southeast of Iran. In this collection, some genotypes are tolerant to drought but they are neglected due to their low-quality fruits. These genotypes could be used as drought tolerant rootstocks. Grafting leading pomegranate cultivars on drought-tolerant rootstocks can be a promising tool for reducing drought stress under water deficient condition. Hence, developing an efficient grafting technique for the propagation of pomegranate is of great importance. In fruit trees, grafting was found to be effective for reducing the effect of water stress and it can considerably improve water use efficiency under drought conditions (Serra et al., 2014). A useful rootstock should be compatible with the scion cultivar, tolerant to pests and diseases, and adaptable to a wide range of soil types and climatic conditions (Hernández et al, 2010).

Pomegranate is conventionally propagated by hardwood and softwood cuttings. Pomegranate can also be propagated by stenting (simultaneous cutting and grafting). Stenting propagation of pomegranate has been previously reported by some researchers (Karimi and Nowrozy, 2017; Karimi, 2011). However, there is no report on grafting of commercial pomegranate cultivars on abiotic and biotic stresses tolerant rootstocks probably because this plant is established in orchards as an own-rooted plant. Nowadays, stenting is a valuable technique in propagating roses, conifers, rhododendrons as well as apple, plum and pear cultivars (Nazari et al, 2009; Hartman et al., 2002). Therefore, the objective of this study was to evaluate the stenting propagation of pomegranate using drought tolerant genotypes as rootstocks and commercial cultivars as scions. Here for the first time we report stenting of three Iranian leading pomegranate cultivars ‘Malas Saveh’, ‘Robab’ and ‘Bihasteh’ on the drought-tolerant rootstocks.

Material and methods

Plant materials

The experiment was carried out in a research greenhouse at the Department of Horticultural Sciences, Arak University (34°4′26″ N; 49°40′36″ E). In March 2017, hardwood cuttings with 20-30 cm long of three pomegranate cultivars ‘Malas Saveh’, ‘Robab’ and ‘Bihasteh’, and nine rootstocks (‘Daneshgah 5’, ‘Daneshgah 8’, 'Daneshgah 13', 'Daneshgah 19', 'Daneshgah 22', 'Daneshgah 25', 'Daneshgah 28', 'Daneshgah 32' and 'Daneshgah 40') were obtained from Pomegranate Research Station in Yazd (southeast Iran). In a preliminary study, more than 40 rootstocks were evaluated for drought tolerance under field conditions, and these nine rootstocks showed good drought tolerance and were transferred to the greenhouse for further evaluation to select the rootstocks with the highest drought tolerance.

Selection of drought-tolerant rootstocks

Six plants of uniform size were selected from each rootstock and subjected to two irrigation levels; control (T0) and 20-day drought stress (T1). Plants in control (T0) conditions were normally irrigated every three days. However, the plants in T1 were subjected to drought stress condition by stopping irrigation for 20 days. All the nine rootstocks were included in control treatment and their average was taken. The experiment was arranged according to completely randomized design. Plants were kept in the greenhouse to avoid the rain watering. Mean temperatures during day and night were 26 and 19°C, respectively, while the relative humidity varied from 65 to 85%. Plant height, number of shoots, plant fresh weight and plant dry weight were measured after 20 days both in control and stressed plants.

Stenting procedure

In late March, scions with two nodes and about 1.5 cm in diameter were grafted (splice grafting) onto rootstocks of 8 cm in length and of equal diameter. The base of scion and the top of rootstock were held together and were simultaneously cut at an angle of 45° for ease of grafting. The cut surfaces were treated with 1000 mg L^{-1} NAA, then the scions were placed on the rootstocks and the grafted parts were kept together with a parafilm tape. The bottoms of rootstocks were treated with 1000 mg L^{-1} NAA and were planted in two gallon plastic pots containing garden soil (soil:compost, 1:1). The pots were covered with polyethylene bags to maintain high humidity and were kept at 25±1°C for four weeks. Plants were then planted in the big pots. In a preliminary study, different concentration of NAA was used. It was found that 1000 mg L^{-1} NAA was the most effective and this concentration therefore was used in the following experiments.

Measurements

The percentages of graft success were determined two months after grafting based on the percentage of plump (swollen) buds and the suitable growth (shoots with appropriate appearance).

By the end of the study, grafted plants were taken out of culture media and some morphological traits such as root number, root length, root volume, shoot length, root and shoot fresh and dry weights were recorded. Root volume was determined by immersing root in a container of water placed on a balance. The displaced water (measured in grams) is equal to the volume (measured in cubic centimeters) of the root in a way that 1 g of water equals 1 cm^3 at room temperature (Burdett, 1979). To estimate the dry weight, samples were oven-dried at 75 °C for 48 hours until constant weight was reached.

The amount of photosynthetic pigments (chlorophyll a, b, and total) was determined according to the method of Lichtenthaler (1987). The pigment extract was measured against a blank of 80% (V/V) acetone at wavelengths of 646.8 nm and 663.2 nm for chlorophyll assays.

Total phenolic contents of scion and rootstock were determined spectrophotometrically (Cary Win UV 100; Varian, Sydney, Australia) according to the Folin-Ciocalteu method (Singleton et al., 1999). The total phenolic content was calculated from a calibration curve using gallic acid as a standard and the results were expressed as mg gallic acid equivalents per gram dry weight of extract (mg GAE g^{-1} DW) for the dried extract. Determination of total phenolic in this assay was based on their chemical reducing capacity relative to an equivalent reducing capacity of gallic acid (Katalinic et al., 2006).

Experimental design and statistical analysis

For evaluating the effect of rootstock and scion on the stenting propagation of pomegranate, the experiment was conducted as a factorial based on completely randomized design (CRD), where cultivars (‘Malas Saveh’, ‘Robab’ and ‘Bihasteh’) and rootstocks (‘Daneshgah 8’, ‘Daneshgah 13’, and ‘Daneshgah 32’) were the factors (Table 2). Each treatment consisted of three replicates with at least five grafted plants. The data were analyzed using SAS (Version 9.1) and significant differences were assessed using Duncan's multiple range test at $P \leq 0.05$. Data were expressed as percentages subjected to arcsine transformation before statistical analysis. All transformed data were presented in original values.

Results

Selection of drought-tolerant rootstocks

The effect of drought stress on morphological characteristics showed significant differences for plant height, number of shoots, plant fresh weight and plant dry weight among the rootstocks among nine pomegranate rootstocks (Table 1). Control plants had higher plant fresh and plant dry weights, plant height and number of shoots compared to their values in stressed plants. ‘Daneshgah 13’, ‘Daneshgah 32’ and ‘Daneshgah 8’ rootstocks showed the maximum plant height, number of shoots, plant fresh weight and plant dry weight than other rootstocks at 20th day of drought application (Table 1).

Therefore, these three rootstocks were utilized for the second experiment to investigate the stenting propagation of pomegranate using drought-tolerant genotypes as rootstocks and commercial cultivars as scions.

Effects of rootstock and scion on stenting propagation

Analysis of two-factorial experiment including main factors and interaction effects (Table 2 and 3) showed that rootstock, scion and their interactions had no significant effect on root number, root volume, root length and root fresh and dry weights (Table 3).

Table 1. Morphological characteristics of nine pomegranate rootstocks following 20 days of drought stress. The control represents the mean value for the nine rootstocks grown in well-watered condition.

Treatments	Plant height (cm)	Number of shoots	Plant fresh weight (g)	Plant dry weight (g)
Control	121.33± 7.09 a	8.41±0.57 a	196.00±5.29 a	101.53±1.42 a
‘Daneshgah 5’	96.66±2.08 d	3.13±0.44 c	128.43±7.63 dc	61.00±1.00 c
‘Daneshgah 8’	103.33±2.18 bc	5.33±0.47 b	167.00±3.60 b	74.16±1.89 b
‘Daneshgah 13’	109.33±2.01 b	5.56±0.52 b	169.36±4.72 b	72.43±2.08 b
‘Daneshgah 19’	96.33±3.21 de	3.36±0.53 c	133.19±10.40 dc	61.22±2.88 c
‘Daneshgah 22’	93.33±4.16 de	3.34±1.15 c	128.23±7.63 dc	60.71±1.52 c
‘Daneshgah 25’	92.00±2.00 de	3.62±0.57 c	121.36±2.88 d	58.66±2.18 c
‘Daneshgah 28’	90.33±2.08 e	3.55±0.55 c	135.00±8.66 c	59.24±2.30 c
‘Daneshgah 32’	104.33±1.15 bc	5.33±0.57 b	168.55±3.05 b	71.51±1.52 b
‘Daneshgah 40’	91.66±1.52 de	3.33±0.67 c	125.76±4.93 dc	58.33±1.52 c
Significance	**	**	**	**

Values represent the mean ± SD.

Means followed by similar letters under different treatments within the same column are not significantly different from each other at $P \leq 0.05$ (Duncan’s multiple range test).

**: significant at $P \leq 0.01$.

Table 2. Effect of three different rootstocks and scions on the percentage of graft success and some morphological and photosynthetic characteristics of pomegranate cultivars

Effects	Grafting success (%)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})
Rootstock							
‘Daneshgah 8’	55.22± 40.26 b	7.10±3.00	20.66±11.60	4.88±2.44 b	15.88±2.42 b	6.56±1.94 c	22.45±4.11 b
‘Daneshgah 13’	58.88± 42.25 a	9.16±3.84	25.11±13.88	5.47±2.76 a	18.11±2.66 a	8.02±2.33 a	26.13±4.93 a
‘Daneshgah 32’	54.77± 39.88 b	7.87±3.19	21.27±11.65	5.07±2.54 b	16.55±2.40 b	7.20±1.95 b	23.75±4.11 b
Scion							
‘Malas Saveh’	82.66±2.95a	10.11±1.24	29.66±3.39	6.76±0.34 a	18.11±2.02 a	8.62±0.99 a	26.73±2.86 a
‘Robab’	84.22±3.89 a	10.42±1.18	31.44±2.83	6.96±0.45 a	18.33±2.00 a	8.58±0.91 a	26.92±2.55 a
‘Bihasteh’	2.00±3.12 b	3.61±0.49	5.94±0.91	1.71±0.21 b	14.11±0.92 b	4.57±0.50 b	18.68±1.26 b
Rootstock×Scion							
‘Daneshgah 8’×‘Malas Saveh’	80.66±1.15	8.73±0.25 d	27.00±2.00 c	6.46±0.15	17.33±2.51	7.83±0.76	25.16±3.01
‘Daneshgah 8’×‘Robab’	83.33±4.16	9.43±0.40 c	29.66±0.57 b	6.56±0.11	17.00±1.00	7.76±0.92	24.76±0.25
‘Daneshgah 8’×‘Bihasteh’	1.66±2.88	3.13±0.23 f	5.33±0.57 d	1.63±0.15	13.33±1.15	4.10±0.17	17.43±1.25
‘Daneshgah 13’×‘Malas Saveh’	86.33±1.52	11.50±0.50 a	33.66±1.52 a	7.16±0.20	19.33±0.57	9.66±0.28	29.00±0.86
‘Daneshgah 13’×‘Robab’	87.66±2.51	11.93±0.11 a	35.00±1.00 a	7.46±0.25	20.33±0.57	9.43±0.51	29.76±0.68
‘Daneshgah 13’×‘Bihasteh’	2.66±4.61	4.06±0.40 e	6.66±0.76 d	1.80±0.26	14.66±0.57	4.96±0.56	19.63±0.90
‘Daneshgah 32’×‘Malas Saveh’	81.00±1.00	10.10±0.36 b	28.33±1.52 bc	6.66±0.15	17.66±2.51	8.36±0.77	26.03±3.28
‘Daneshgah 32’×‘Robab’	81.66±2.88	9.90±0.36 bc	29.66±1.52 b	6.86±0.32	17.66±2.30	8.56±0.40	26.23±2.40
‘Daneshgah 32’×‘Bihasteh’	1.66±2.88	3.63±0.32 ef	5.83±1.04 d	1.70±0.26	14.33±0.57	4.66±0.30	19.00±0.40
Significance							
Rootstock	*	**	**	**	*	**	**
Scion	**	**	**	**	**	**	**
Rootstock×Scion	ns	**	**	ns	ns	ns	ns

Values represent the mean ± SD.
Means followed by similar letters under different treatments within the same column are not significantly different from each other at $P \leq 0.05$ (Duncan’s multiple range test).
ns, * and **: not significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 3. Effect of three different rootstocks and scions on root number, root volume, root length and root fresh and dry weights of the pomegranate cultivars

Effects	Root number	Root volume	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Rootstock					
‘Daneshgah 8’	14.31± 1.31	4.08±0.90	13.22±2.20	16.25±2.33	5.23±0.62
‘Daneshgah 13’	15.12± 2.01	4.56±0.14	14.21±2.18	17.17±2.25	5.61±0.71
‘Daneshgah 32’	14.67± 1.38	4.77±0.19	31.17±1.85	16.41±2.33	5.44±0.45
Scion					
‘Malas Saveh’	13.97±2.15	4.11±0.34	13.96±2.21	16.67±2.44	5.51±0.32
‘Robab’	14.12±1.99	4.52±0.18	14.55±2.26	17.45±2.25	5.62±0.50
‘Bihasteh’	13.09±2.02	3.92±0.59	13.11±1.21	15.96±2.54	4.91±0.94
Rootstock×Scion					
‘Daneshgah 8’×‘Malas Saveh’	14.16±1.85	4.23±0.15	13.250±2.10	16.16±2.21	5.43±0.51
‘Daneshgah 8’×‘Robab’	14.23±1.76	4.13±0.30	13.56±1.97	16.65±2.06	5.50±0.43
‘Daneshgah 8’×‘Bihasteh’	14.56±1.98	4.21±0.19	13.13±2.07	16.51±2.25	5.23±0.44
‘Daneshgah 13’×‘Malas Saveh’	14.83±2.22	4.50±0.30	13.96±1.83	16.76±2.30	5.61±0.47
‘Daneshgah 13’×‘Robab’	15.16±2.31	4.83±0.31	14.21±2.12	17.39±3.31	5.86±0.39
‘Daneshgah 13’×‘Bihasteh’	14.26±2.11	4.56±0.20	13.59±1.77	16.10±2.36	5.26±0.31
‘Daneshgah 32’×‘Malas Saveh’	14.06±1.20	4.21±0.26	13.45±1.82	16.55±2.21	5.41±0.32
‘Daneshgah 32’×‘Robab’	14.56±1.25	4.27±0.17	13.43±1.84	16.45±3.22	5.29±0.40
‘Daneshgah 32’×‘Bihasteh’	14.41±1.35	4.38±0.42	13.33±1.54	16.36±2.16	5.43±0.37
Significance					
Rootstock	ns	ns	ns	ns	ns
Scion	ns	ns	ns	ns	ns
Rootstock×Scion	ns	ns	ns	ns	ns

Values represent the mean ± SD.
Means followed by similar letters under different treatments within the same column are not significantly different from each other at $P \leq 0.05$ (Duncan’s multiple range test).
ns: not significant

Effects of rootstock and scion on the percentage of graft success

Rootstock significantly ($P \leq 0.05$) affected the percentage of graft success (Table 2). Data in Table 2 show that the highest percentage of graft success (58.88%) was obtained from grafting on 'Daneshgah 13', followed by 'Daneshgah 8' then 'Daneshgah 32' in descending order.

The percentage of graft success was significantly influenced by the scion. The highest percentage of graft success (84.22%) was found in 'Robab', followed by 'Malas Saveh' (82.66%) then 'Bihasteh' (2.00%), respectively (Table 2).

Interaction of rootstock and scion on shoot growth

Multiple shoots were obtained from scions grafted on every three rootstocks. Interaction of rootstock and scion had a significant ($P \leq 0.01$) effect on shoot length (Table 2). 'Robab' and 'Malas Saveh' gave the longest shoot length when grafted on 'Daneshgah 13', whereas 'Bihasteh' scions had the shortest shoot length when grafted on each of the three rootstocks (Table 2).

The results of the variance analysis showed that the interaction of rootstock and scion had a significant effect on shoot fresh weight (Table 2). 'Robab' and 'Malas Saveh' scions, when grafted on 'Daneshgah 13' rootstock, gave the highest shoot fresh weight; whereas 'Bihasteh' scions when grafted on each of the three rootstocks showed the lowest shoot fresh weight (Table 2).

The results showed that rootstock and scion had significant effects ($P \leq 0.01$) on shoot dry weight (Table 2). 'Daneshgah 13' rootstock had higher shoot dry weight (5.47 g) compared to shoot dry weight of other rootstocks. No differences were found between 'Daneshgah 8' and 'Daneshgah 3' for shoot dry weight.

Regarding the scion cultivars, 'Robab' and 'Malas Saveh' had higher shoot dry weight compared to 'Bihasteh' (Table 2). Nevertheless, there was no significant difference among rootstocks and scions for shoot dry weight ($P \leq 0.01$).

Effects of rootstock and scion on leaf pigmentation

Rootstock significantly affected the content of chlorophylls a, b and total chlorophyll (Table 2). 'Daneshgah 13' rootstock resulted in the highest content of chlorophylls a, b and total chlorophyll in scion leaves. The scion leaves on 'Daneshgah 8' and 'Daneshgah 32' rootstock had the lowest content of chlorophyll, respectively.

Moreover, chlorophylls a, b and total chlorophyll contents were significantly affected by scion (Table 2). 'Robab' and 'Malas Saveh' showed higher content of chlorophylls a, b and total chlorophyll compared to chlorophyll content of 'Bihasteh'. Nevertheless, there was no significant interaction between rootstock and scion for their chlorophylls a ($P = 0.7200$), b ($P = 0.5364$) and total chlorophyll ($P = 0.6118$) contents.

Effects of rootstock and scion on total phenolic content

The results of the variance analysis showed that the interaction of rootstock and scion had a significant effect on total phenolic content (Fig. 1). 'Robab' and 'Malas Saveh' scions, when grafted on 'Daneshgah 13' rootstock had the lowest total phenolic content; whereas 'Malas Saveh' scions when grafted on 'Daneshgah 8' rootstock showed the highest total phenolic content, although no significant difference was found among most treatments (Fig. 1).

Correlation between phenolic content of rootstock and scion with percentage of graft success

Results indicated that in each of the three rootstocks, there was a negative correlation between total phenolic content and percentage of graft success (Table 4). In fact, due to high total phenolic content these rootstocks had low percentage of graft success (Table 2).

In addition, results showed that in ‘Malas Saveh’ and ‘Robab’ scions, the total phenolic content of wood had no impact on graft success percentage, while in ‘Bihasteh’, a negative correlation between the total phenolic content and the percentage of graft success was observed (Table 4).

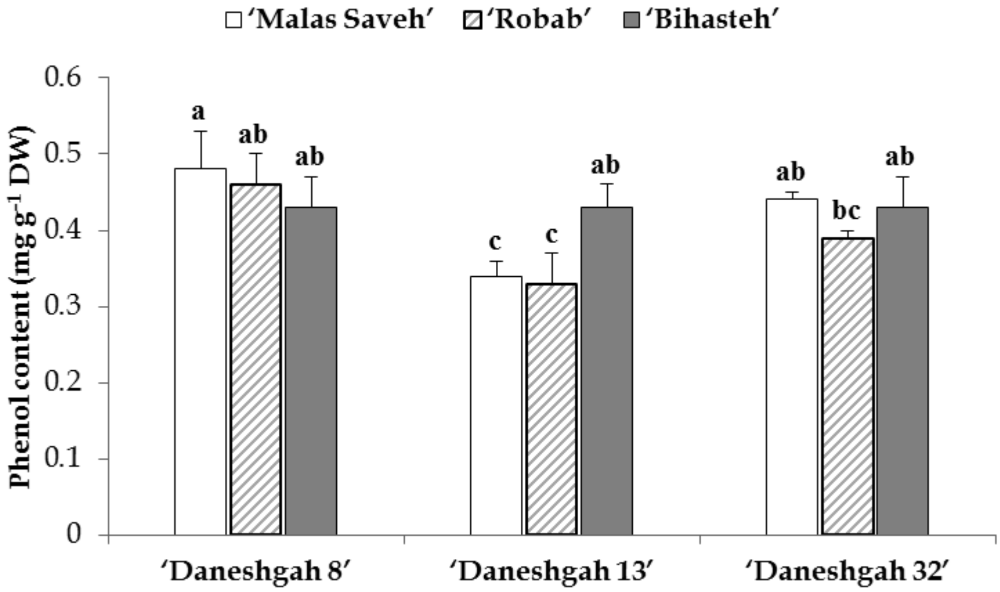

Fig. 1. Effects of rootstocks (‘Daneshgah 8’, ‘Daneshgah 13’ and ‘Daneshgah 32’) and scions (‘Malas Saveh’, ‘Robab’ and ‘Bihasteh’) on total phenolic content of pomegranate. Significant difference ($P \leq 0.05$) between data is expressed by different letters. Vertical bars indicate standard deviation.

Table 4. Pearson correlation coefficients between phenolic compounds of graft parts and percentage of graft success

Rootstock	Phenol (mg/g)	Scion	Phenol (mg/g)
‘Daneshgah 8’	-0.98410 ***	‘Malas Saveh’	-0.29321 ^{ns}
‘Daneshgah 13’	-0.99836 ***	‘Robab’	-0.34624 ^{ns}
‘Daneshgah 32’	-0.99812 ***	‘Bihasteh’	-0.63285 ^{ns}

ns: not significant; ***: Significant at 0.0001 probability.

Discussion

Drought stress is one of the major abiotic factors which decrease plant growth, development, and ultimately yield of plants. Our findings revealed that drought stress had negative effect on morphological characteristics of nine pomegranate rootstocks and ‘Daneshgah 13’, ‘Daneshgah 32’ and ‘Daneshgah 8’ rootstocks exhibited the maximum drought tolerance (Table 1). Differences in rootstock tolerance have been also reported for peach (Boonanunt et al., 2014) and apple trees (Fernandez et al., 1997) when subjected to drought stress. In apple, commercial rootstocks such as M26 and MM111 have been reported to be drought-tolerant rootstocks (Atkinson et al., 1999).

Results of present study suggest that the percentage of graft success was significantly influenced by the rootstock type (Table 2), which are in agreement with results reported for apple (Sadowski and Gorski, 2003), plum (Orlova, 2007) tea (Tuwel et al., 2008) and pomegranate (Karimi and Nowrozy, 2017). Fayek et al. (2004) reported that grafting success significantly varied according to rootstock and scion. Karimi (2011) also reported that the genotype of rootstock affected the percentage of graft success in pomegranate. This may be due to different levels of rootstocks endogenous compounds such as carbohydrates, auxins and phenolic contents. Some authors have reported that phenolic compounds and phytohormones interact with each other, for instance high concentration of phenol inhibits auxin transfer, which affects the differentiation of xylem and phloem in the graft site (Gainza et al., 2015).

In line with the findings obtained in the present study (Table 2), the significant effect of scion on the percentage of graft success among fruit tree cultivars has been previously reported (James, 2012). The significant variations in the percentage of graft success may be due to the different levels of compatibility of the scions with the rootstocks (Gainza et al., 2015). Graft incompatibility can be often determined at an early stage of graft development. For example, in a study of apricot grafts, accumulation of phenol compounds could serve as an indicator of incompatibility as early as the first week after grafting (Errea et al., 2001). Mng’omba et al. (2008) indicated that accumulation of phenol deposits at the place of graft union might inhibit graft compatibility. Another study also demonstrated that differences in phenol accumulation below and above the graft union might serve as an indicator of compatibility (Usenik et al., 2006). Additionally, a study on *Prunus persica*/*Prunus* spp. grafts indicated that a significant increase in peroxidase activity might be observed in incompatible unions (Zarrouk et al., 2010).

In the present research, shoot length and shoot fresh weight were significantly affected by the interaction of rootstock and scion (Table 2). Understanding mechanisms of the rootstock-scion interaction is yet under development. Several studies demonstrated mechanisms of scion-rootstock interaction on shoot growth. One of the hypotheses is that the growth regulator, auxin, which is transported at different rates between grafts on different rootstocks, affects cytokinin production in roots and causes differences in shoot growth (Webster, 1998). Another hypothesis assumes that phenols accumulating at the graft union reduce tissue viability and soluble transport across the graft union (Basile et al., 2003). Our results are in agreement with the findings of Karimi and Nowrozy (2017) on pomegranate and Fayek et al. (2004) on olive who reported that shoot fresh weight was affected by the interaction of rootstock and scion. On the contrary, results of present study are not in agreement with those reported by Karimi and Nowrozy (2017) and Fayek et al. (2004) who concluded that shoot dry weight was affected by the interaction of rootstock and scion.

In agreement with the results obtained in this study (Table 2), Misra et al. (1992) reported that rootstock had a significant effect on chlorophyll content of lemon scion leaves. Also, Morinaga and Ikeda (1990) reported that rootstocks had significant effects on photosynthesis of satsuma mandarin scion leaves. They reported that leaves of satsuma mandarin grafted on trifoliate orange strains, such as `Rubidoux', `Pomeroy', and `USDA' showed higher photosynthetic rates than those on common trifoliate orange (*Poncirus trifoliata* Raf.) rootstock. However, `Oba' (large leaf strain), `Barnes', and `sour orange' rootstocks had lower rates of photosynthesis. However, Barden and Ferree (1979) reported that rootstocks of apple trees did not influence photosynthesis of scion leaves. Trees on ‘Daneshgah 13’ rootstock had more vigorous shoots with higher shoot dry weight (Table 2). It can be suggested that this is due to more total photosynthetic product per tree than those on other rootstocks. Photosynthetic capacity was an excellent prediction characteristic for selection of superior rootstocks (Morinaga and Ikeda, 1990). While, results of present study are in contrast with findings of Karimi and Nowrozy (2017) on pomegranate who reported that chlorophyll fluorescence was affected by the interaction of rootstock and scion.

On the basis of phenol content in the tissue of three pomegranate cultivars grafted on the drought-tolerant rootstocks it can be deduce that the content of phenol compounds are related to specific cultivar/rootstock combination (Fig. 1), which is in agreement with several previous reports (Usenik and Sˇtampar, 2000; Usenik et al., 2006).

Results of the current study showed that there was a negative correlation between total phenolic content of rootstocks and percentage of graft success (Table 4), which is in accordance with the results of Karimi and Nowrozy (2017) on pomegranate who reported that phenolic compounds of rootstock had an effect on the percentage of graft success. In addition, there was a negative correlation between total phenolic content of ‘Bihasteh’ scion and percentage of graft success (Table 4). Similar results were also reported by Karadeniz et al. (1993) and Karadeniz and Kazankaya (1997) on chestnut and walnut confirming that the graft success can be affected by phenol contents of scion and there is a negative relation between the percentage of graft success and the phenolic contents of the scions. Phenolic compounds have important roles in woody plants, including the relationships between rootstock and scion (Usenik et al., 2006; Martínez-Ballesta et al., 2010). Phenols have been implicated in graft incompatibility (Pina and Errea, 2005) and differences in quantity or specific phenols above and below the union area play a role in reducing graft compatibility (Usenik et al., 2006). Phenols can adversely affect the movement of cytokinins and auxins which are important for cell differentiation and growth. Cytokinins and indole-3-acetic acid (IAA) are known to induce differentiation of sieve tubes and vessels (Usenik and Stampar, 2000). Furthermore, phenolic acids inhibit plant growth and increase oxidative decarboxylation of IAA (Mngomba et al., 2008). In addition, high content of phenolic compounds is associated with damage during graft formation, limiting the proliferation and differentiation of callus, as well as the formation of the new vascular tissues in incompatible grafts (Hartman et al., 2002).

Conclusion

Results of the current study showed that splice grafting technique can be used with success for stenting of pomegranate. Moreover, results indicated that rootstock and scion had significant effects on the percentage of graft success. ‘Daneshgah 13’ was introduced as the most effective rootstock for the stenting propagation of pomegranate cultivars ‘Robab’ and ‘Malas Saveh’. Also, ‘Bihasteh’ cultivar showed the lowest percentage of graft success when grafted on the three studied rootstocks.

Acknowledgments

This work was supported by Arak University under grant number of 94/11994. We would like to acknowledge the research team of Arak University. We thank Mr. Parastar (Pomegranate Research Station in Saveh, Iran) for supplying hardwood cuttings of scions and rootstocks.

Conflict of interest

The authors declare no conflict of interest.

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