STENTING (CUTTING AND GRAFTING) – A TECHNIQUE FOR PROPAGATING POMEGRANATE (*Punica granatum* L.)

Hamid Reza Karimi

Department of Horticultural Sciences, Faculty of Agriculture
University of Vali-e-Asr, Rafsanjan, IRAN
e-mail: h_karimi1019@yahoo.com

(Received September 7, 2010/Accepted July 27, 2011)

A B S T R A C T

There is inadequate information on the application of rootstocks in the propagation of pomegranate. The aim of this research was to study propagation of pomegranate using the stenting method. The experiment was conducted on the basis of a completely randomised design (CRD) with two factors: rootstock (R_1, R_2 and R_3) and the concentration of indole-3-butyric acid (IBA) (0 and 500 mg/l), in three replications. The rootstocks were 'Gorj-e-Dadashi' (R_1), 'Gorj-e-Shahvar' (R_2) and 'Gool Safid-e-Ashk-e-Zar' (R_3), and the scions were of the cultivar Malas-e-Yazdi. The results indicated that rootstock type and IBA treatment influenced bud-take and root formation. The highest bud-take percentage was obtained with R_1 in combination with 500 mg/l IBA.

Key words: IBA, rootstock, scion, stenting method

INTRODUCTION

The pomegranate (*Punica granatum* L.) is one of the most important horticultural crops in Iran. Many pomegranate orchards are located in arid and semi-arid areas with low quality soil and water. Therefore, grafting selected scions onto suitable rootstocks is important for increasing yield efficiency of this crop. Furthermore, there are evidences that the rootstock influences the tolerance to soil-borne diseases (Ashworth, 1985), resistance to chlorosis induced by calcareous soils (Bavaresco et al., 1991; Romera et al., 1991; Shi et al., 1993), tolerance to soil salinity (Behboudian et al., 1986; Walker et al., 1987; Picchioni et al., 1990) and also affects leaf nutrient content (Pool and Nyirenda, 1981; Ruiz et al., 1996; Vazifeshenas et al., 2009).

The pomegranate is traditionally propagated by seed or stem cuttings (Olmez et al., 2007). The most important problem with reproductive propagation is genetic variation. Therefore, stem cutting is the best way to retain the properties of the original cultivars. Most of the propagation studies on pomegranate have focused on the rooting capacity of pomegranate varieties in response to rooting hormones (Ghosh et al., 1988; Sandhu et al., 1991; Singh, 1994; Melgarejo et al., 1998; Owis, 2010). Singh (1994) reported that the application of IBA on stem cuttings of pomegranate increased rooting percentage.

Stenting is a method for quick propagation of plants. Cutting and grafting is performed simultaneously. The scion is grafted onto a non-rooted rootstock. The formation of the union and adventitious roots on the rootstock occurs simultaneously. Stenting is now being used worldwide by rose growers (Nazari et al., 2009) and is also a valuable technique in propagating species of conifers and also rhododendron, apple, plum and pear (Hartman et al., 2002). Currently, about 760 genotypes, specimens and cultivars of pomegranate from different provinces of Iran have been gathered in the Yazd Pomegranate collection (Zamani et al., 2006). In this rich collection, it is likely that some genotypes are tolerant to adverse environmental conditions and disease, but have been neglected because of the low quality of the fruit. These genotypes could be evaluated and used as potential rootstocks. In the last decades, there has been a tremendous tendency towards using grafted plants in fruit orchards (Rivero et al., 2003). Furthermore, pomegranate cultivation could be improved by using cultivars grafted on tolerant rootstocks; thus, evaluation of pomegranate propagation through grafting and budding techniques seems necessary. The present study aimed to evaluate the vegetative propagation of pomegranate by the stenting method (simultaneous cutting and grafting).

MATERIAL AND METHODS

This study was conducted in a greenhouse of the Department of Horticulture, College of Agriculture, Vali-e-Asr University of Rafsanjan, over 2009-2010. Stem cuttings of three pomegranate genotypes, namely 'Gorj-e-Dadashi' (R_1), 'Gorj-e-Shahvar' (R_2) and 'Gool Safid-e-Ashk-e-Zar' (R_3), were used as rootstocks. The scion was 'Malas-e-Yazdi' obtained from the University's Pomegranate Collection. The experiment was conducted as a factorial experiment based on a completely randomised design with two factors: rootstock (R_1, R_2 and R_3) and the concentration of indole-3-butyric acid (IBA) (0 and 500 mg/l), in three replications. Each replication consisted of 20 samples. The stem cuttings were taken from ten-year-old trees and cut into 20-cm lengths. The scion was a piece of stem with two buds. The rootstocks (stem cuttings) and scions were transferred to a greenhouse and stenting was carried out. The base end of the grafted cuttings was dipped in distilled water and 500 mg/l IBA potassium salt (K-IBA) for 30 min. The grafted plants were then placed in a substrate of moist sawdust. They were kept at a temperature of 18 °C (±2 °C) for six weeks until the formation of callus and then planted in a nursery bed. Successful callus formation at the graft union, bud-take percentage, scion shoot length, internode length, the chlorophyll index, leaf surface area, the number of leaves and shoot fresh and dry weight were measured at the end of the experiment. Data analysis was performed using MSTAT-C software and the means were compared with DNMRT at 5%.

RESULTS

A comparison of mean rootstock and IBA effects (data not shown) on successful callus formation at the graft union indicated that rootstock type and IBA concentration had no significant effect on this process. However, the results also indicated that the effect of the interaction between the rootstock and IBA concentration on successful callus formation at the graft union was significant. The R_1 rootstock (‘Gorj-e-Dadashi’) treated with 500 mg/l IBA produced more successful callus formation at the graft union.

Bud-take (BT) was significantly affected by rootstock type. The highest BT percentage was observed in R_1 (‘Gorj-e-Dadashi’). BT was increased by using IBA, so that the highest BT percentage was obtained with 500 mg/l IBA. The interaction between the rootstock and IBA concentration was such that the highest BT percentage was obtained in the R_1C_2 treatment combination, although there was no significant difference between this treatment combination and R_2C_2. The lowest BT percentage was obtained in the R_3C_1 and R_2C_1 treatment combinations (Tab. 1).

The analysis of variance indicated that the effect of the interaction between the rootstock and IBA concentration on root fresh and dry weight was significant and the cutting-grafting combinations treated with IBA had a higher root fresh weight than the control. The highest root fresh and dry weight was found in R_1C_2 and R_2C_1 treatment combinations, respectively (Tab. 1).

Shoot fresh and dry weight was also significantly affected by the rootstock and IBA concentration. The highest and lowest shoot fresh and dry weight was observed in R_3 and R_1, respectively. Shoot fresh and dry weight was decreased by applying the IBA treatment (Tab. 2).

A comparison between the IBA concentrations indicated that shoot length was not affected by IBA concentration, but this characteristic was influenced by the rootstock. The highest and lowest shoot length was observed in the R_3 and R_1 rootstocks, respectively (Tab. 2).

Leaf chlorophyll index was affected by the rootstocks and IBA concentration. The highest chlorophyll index was obtained with the R_3 rootstock.

Table 1. The effect of the interaction of IBA concentration and rootstock on bud-take and root fresh and dry weight

IBA concentration [mg/l]	Rootstock			Mean
	R_1	R_2	R_3	
Bud take [%]				
0 (C_1)	70.00 bc*	65.00 c	65.00 c	66.66 B
500 (C_2)	86.67 a	85.00 a	76.66 b	82.77 A
Mean	78.33 A	75.00 AB	70 83 B	
Root fresh weight [g]				
0 (C_1)	0.28 c	1.70 ab	1.55 ab	1.17 B
500 (C_2)	2.38 a	1.14 bc	1.91 ab	1.81 A
Mean	1.33 A	1.42 A	1.73 A	
Root dry weight [g]				
0 (C_1)	0.10d	0.71 a	0.57 ab	0.46 A
500 (C_2)	0.54 b	0.29 c	0.51 b	0.45 A
Mean	0.32 B	0.50 A	0.54 A	

*Means followed by the same letter in each column are not significantly different (Duncan test, $p = 0.05$)

Table 2. The effect of the interaction of IBA concentration and rootstock on shoot fresh and dry weight, shoot length and chlorophyll index

IBA concentration [mg/l]	Rootstock			Mean
	R1	R2	R3	
Shoot fresh weight [g]				
0 (C_1)	5.80 b*	12.67 a	11.03 a	9.83 A
500 (C_2)	2.77 c	4.55 bc	11.23 a	6.18 B
Mean	4.90 C	8.61 B	11.13 A	
	Shoot dry weight [g]			
0 (C_1)	2.72 b	5.50 a	4.46 a	4.23 A
500 (C_2)	1.08 c	1.58 bc	4 62 a	2.43 A
Mean	1.09 C	3.54 B	4.54 A	
Shoot length [cm]				
0 (C_1)	16.33 c	28.00 b	33.33 ab	25.88 A
500 (C_2)	17.50 c	31.66 ab	37.33 a	28.83 A
Mean	16.10 C	29.83 B	35.33 A	
Chlorophyll index				
0 (C_1)	42.7 bc	45.4 bc	63.6 a	50.5 A
500 (C_2)	34.6 cd	30.2 d	53.4 ab	39.4 B
Mean	38.6 B	37.8 B	58.5 A	

*Explanation: see Table 1

DISCUSSION

At present, the pomegranate is propagated by stem cuttings in all producer countries. Low quality of irrigation water, soil salinity, nutrient imbalance and soil-borne diseases are the most limiting factors in the production areas. Propagation of pomegranate using the stenting (cutting-grafting) method will permit grafting of superior genotypes onto tolerant rootstocks. Application of rootstocks in the propagation of many horticultural crops is a common practice. Although the cultivation of pomegranate, revered as a holy fruit in the Iranian culture, has a rich history, no research has been reported on the subject of grafting and budding of this fruit tree. Most of the studies have focused on the rooting of pomegranate cuttings.

According to the present findings, the IBA treatment increased the BT percentage compared to the control. This may be associated with cell division stimulated by the auxin at the graft union.

Based on this study, BT was affected by the rootstock, which corresponds to the results reported by Orlova (2007), Hamdi et al. (2007), and Sadowski and Gorski (2003) for plum, kiwifruit and apple.

In stenting, the graft union must be formed before root initiation. After leaf formation on the scion, carbohydrates and natural hormones are produced and translocated from the leaves to the rootstock for growing.

The effect of the rootstock on BT may be related to the formation of the callus bridge at the graft union. An unexpected result was that the IBA treatment decreased shoot fresh and dry weight. This may be associated with the competition between newly developing roots and shoots.

Shoot length was reduced by the rootstocks, indicating a possible dwarfing effect of some pomegranate genotypes. The results presented here are in agreement with those of Agbaria et al. (1996) for rose.

In conclusion, the present findings indicated that stenting could be practiced for pomegranate propagation. To our knowledge, this is the first report on pomegranate propagation by the stenting method. On the other hand, high variability exists among pomegranate genotypes; consequently, further research with other cultivars is suggested.

Acknowledgment: The authors would like to thank the research office of Vali-e- Asr University of Rafsanjan for financial support of this study (Grant Number: P/2753/2010).

REFERENCES

Agbaria H., Heuer B., Zieslin N. 1996. Shoot-root interaction effects on nitrate reductase and glutamine synthetase activities in rose (*Rosa hybrida* cvs. IIesta and Mercedes) graftings. J. PLANT PHYSIOL. 149: 559-563.

Ashworth J. 1985. *Verticillium* resistant rootstock research. Annual Report California Pistachio Industry, pp. 54-56.

Bavaresco M., Fregoni M., Fraschini P. 1991. Investigations on iron uptake and reduction by excised roots of different grapevine rootstocks and a *V. dnifera* cultivar. PLANT SOIL. 130: 109-l13.

Behboudian M.H., Walker R.R., Torokfalvy E. 1986. Effects of water stress and salinity on photosynthesis of Pistachio. SCI. HORT. 29: 251-261.

Ghosh D., Bamdyopadhyay A., Sen S.K. 1988. Effect of NAA and IBA on adventitious root formation in stem cuttings of Pomegranate (*Punica granatum* L.) under intermittent mist. INDIAN AGRI. 32: 239-243.

Hamdi Z.M., Ozcan M., Haznedar A., Demir T. 2007. Comparisons of methods and time of budding in kiwifruit (*Actinida deliciosa* A. Chev.). INTER. J. NAT. ENG. SCI. 1: 23-28.

Hartman H.T., Kester D.E., Davies Jr. F.T., Geneve R.L. 2002. Plant Propagation: Principles and Practices. 5^{th} Ed, Prentice-Hall Inc. Englewood, Cliffs, NJ., USA, 647 p.

Melgarejo P., Martinez J., Martinez J.J., Martinez-Valereo R., Amoros A. 1998. Study of the rooting capacity of eleven Pomegranates (*Punica granatum* L.) clones using plastic to cover the soil. CIHEAM - Options Mediterraneennes, pp.169-179.

Nazari F., Khosh-Khui M., Salehi H. 2009. Growth and flower quality of four *Rosa hybrida* L. cultivars in response to propagation by stenting or cutting in soilless culture. SCI. HORT. 119: 302-305.

Olmez Z., Temel F., Gokturk A., Yahyaoglu Z. 2007. Effect of sulphuric acid and cold stratification pretreatments on germination of pomegranate (*Punica granatum* L.) seeds. A. J. PLANT. SCI. 427-430.

Orlova A.A. 2007. Effect of type on rootstock on development of plum seedlings. RUS. AGRI. SCI. 23: 373-374.

Owis S.J. 2010. Rooting response of five Pomegranate varieties to Indole Butyric Acid (IBA). PAK. J. BIOT. SCI. 13: 51-58.

Picchioni G.A., Miyamoto S., Storey J.B. 1990. Salt effects on growth and ion uptake of Pistachio rootstock seedlings. J. AM. SOT. HORT. SCI. 115: 647-653.

Pool P., Nyirenda H.E. 1981. Effects of rootstocks on the components of yield in tea (*Camellia sinensis* L.). J. HORT. SCI. 56: 121-123.

Rivero R.M., Ruiz J.M., Romero L. 2003. Role of grafting in horticultural plants under stress conditions. FOOD. AGR. ENV. 1: 70-74.

Romera F.J., Alcantara E., De La Guardia M.D. 1991. Characterization of the tolerance to iron chlorosis in different peach rootstocks grown in nutrient solution: I. Effect of bicarbonate and phosphate. PLANT SOIL 130: 115-119.

Ruiz J.M., Belakbir A., Romero L. 1996. Foliar level of phosphorus and its bioindicators in *Cucumis melo* grafted plants. A possible effect of rootstocks. J. PLANT PHYSIOL. 149: 400-404.

Sadowski A., Gorski M. 2003. Quality of apple planting stock depending on its methods of production. PLANT SCI. 11: 394-398.

Sandhu A.S., Minhas P.P.S., Singh S.N., Kambhoj J.S. 1991. Studies on *Rhizogenesis* in hard wood cuttings of Pomegranate. INDIAN J. HORT. 48: 302-304.

Shi Y., Byrne D.H., Reed D.W., Loeppert R.H. 1993. Iron chlorosis development and growth response of peach rootstocks to bicarbonate. J. PLANT NUT. 16: 1039-1046.

Singh R.S. 1994. Effect of growth substances on rooting of Pomegranate cutting. CUR. AGRI. 18: 87-89.

Walker R.R., Torokfavy E., Behboudian M.N. 1987. Uptake and distribution

of chloride, sodium and potassium ions and growth of salt-treated Pistachio plant. AUST. J. AGRI. RES. 38: 383-394.
Vazifeshenas M., Khayyat M., Jamalian S. 2009. Effect of different scion-rootstocks combinations on vigor, tree size, yield and fruit quality on three Iranian cultivars of Pomegranate. ACTA HORT. 463: 143-152.
Zamani Z., Sarkhosh A., Fatahi R., Ebadi A. 2006. Genetic relationships among pomegranate genotypes studied by fruit characteristics and RAPD markers. J. HORT. SCI. BIOT. 82: 11-18.

STENTOWANIE (CIĘCIE I SZCZEPIENIE) – METODA ROZMNAŻANIA GRANATU (*Punica granatum* L.)

Hamid Reza Karimi

STRESZCZENIE

Jest mało informacji na temat stosowania podkładek w rozmnażaniu granatu. Celem niniejszej pracy było zbadanie rozmnażania granatu metodą stentowania. Doświadczenie przeprowadzono jako czynnikowe na podstawie układu kompletnie zrandomizowanym z dwoma czynnikami: podkładkami (R_1, R_2 i R_3) oraz kwasem indolilo-3-masłowego (IBA) (0 i 500 mg/l), w trzech powtórzeniach. Podkładkami były ‘Gorj-e-Dadashi’ (R_1), ‘Gorj-e-Shahvar’ (R_2) i ‘Gool Safid-e-Ashk-e-Zar’ (R_3), a zrazami odmiana Malas-e-Yazdi. Badania wykazały, że rodzaj podkładki i traktowanie kwasem IBA mają wpływ na przyjmowanie się oczek i formowanie się korzeni. Najwyższy procent przyjętych oczek uzyskano z podkładką R_1 w połączeniu z 500 mg/l IBA.

Słowa kluczowe: IBA, podkładka, zraz, metoda stentowania