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Horticultural Science

Elixir Horti. Sci. 58 (2013) 14886-14890

ISSN: 2229-712X

Effect of Single-node Cutting Method on Rooting of Pomegranate (Punica granatum)

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ARTICLE INFO

Article history:

Received: 3 May 2013;

Received in revised form:

7 May 2013;

Accepted: 13 May 2013;

Keywords

Pomegranate,
Rooting,
Single-nude cuttings,
IBA hormone,
Sulfuric acid.

ABSTRACT

Using hardwood cuttings is the most common method for propagation of pomegranate. Due to the high demand for raw materials in pomegranate production nurseries, and to reduce raw material consumption, comparison of different cutting methods as single-node, two-nodes and four-nodes with smaller and larger than 8 mm diameters, was investigated in greenhouse condition, and on a mixture of sand and perlite bed in the ratio of 2 to 1. Also effects of two levels of IBA hormone treatment with concentrations of 1500 ppm and 3000 ppm and 2N sulfuric acid on increasing of pomegranate single-node cuttings rooting were tested. Cultivars used in this study were Shirin-e-Saveh and Malas-e-Torsh planted on March 2010 and January 2011, and statistically analyzed as factorial in a completely randomized design. According to the obtained results single-node cuttings with diameters smaller than 8 mm had better rooting. Shirin-e-Saveh cultivar showed better rooting compared to Malas-e-Torsh cultivar and also IBA hormone treatment with concentrations of 1500 ppm and 3000 ppm did not show significant effect on increasing of single-node cuttings rooting. Whereas acid treatment in Shirin-e-Saveh cultivar caused facilitation in rooting,best average for some of roots (11 roots) were found in single-node cuttings of Shirin-e-Saveh cultivar treated with sulfuric acid.

Introduction

Pomegranate with *punica granatum* scientific name is one of the oldest fruits in Iran, long history of cultivation and wide genetic diversity in iran tell us that this fruit is native to iran (Sarkhosh et al., 2006).Common propagation method for this plant is woody stem cuttings (Hartman et al., 2007). One of the ways that makes raw materials were saved in production nurseries is single-nude cutting method (Hartman et al., 2007). In the single node cuttings in propagation mint plant *Mentha sp.* Along with the auxin hormone treatment, positive effect were seen (Croteau, 1986 & El-Keltavi), and in the single-node cuttings of rose plants *Rosa sp.* ,The auxin treatment has shown positive effects (Devries & Dubois, 1988). IBA is one of the important plant hormone, that will facilitate the rooting of plants (Arteca, 1995). pea cuttings required At least one bud for rooting, When all the buds were removed on the cuttings and pea cuttings treated with auxin, cuttings had no roots, which defines that certain factors except auxin involved in rooting (Arteca, 1995). Usually a hormone like substance has made in the leaves and buds move down base of stems and facilitates rooting of cuttings (Vanderlek, 1930). Sulfuric acid (H_2SO_4) with destruction of tissue that prevents rooting in plants facilitates rooting here and destroys tissue that promotes cell division and increased uptake of rooting hormone in plants facilitates rooting and has similar effect on cuttings that were treated with wounds (Kosh-Khui & Tafazoli, 1979).

Sharma and colleagues reviewed the effect of IBA hormone on rooting of pomegranate cuttings and between the 12 treatments with different concentrations of hormones and borax, hormonal treatment with IBA 500 ppm + borax 1% has shown highest rooting percent and average rooting per cuttings, and it was in the hardwood cuttings and semi hardwood cuttings 78% and 65% and in average 16 cuttings per cutting were rooted out, boron has shown a synergistic effect on rooting of pomegranate cuttings (Sharma et al., 2009).

Karimi et al investigated effect of IBA hormone and salicylic acid on rooting of two cultivars of pomegranate cuttings and observed that differences between cultivars have shown significant differences on rooting of pomegranate cuttings. results showed that Gorj-e-Shahvar had 85% and Gorj-e-Dadashi 60 % of rooting. Different results were observed when cuttings were treated with salicylic acid and rooting percentage increased in the Gorj-e-Shahvar while it was reducedin the other cultivar rooting (karimi et al., 2012). In another study by Karimi grafted cuttings from Pomegranate were treated with IBA hormone and positive effect of hormon on rooting and sprouting was observed (karimi, 2011).

Time of the year that cuttings were taken has a great effect on the results obtained in cuttings rooting. Time to take cuttings of plants is related to physiological conditions of plants and not related to a specific time (Hartman & Books, 1985). Why rootings of many plants are hard to do, or not do at all, depends on genetic of plants that effects the anatomy and physiology. Determine the exact time of taking cuttings is not easy. accurate detection time to take cuttings depends on a person's intelligence and experience (MacDonald, 2000).

In this study, in order to conserve the raw material in the nursery production, potential of pomegranate single-nude

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cuttings were examined for rooting And due to possible reduction of rooting in cuttings because of the smaller size of the cuttings, treatment with IBA hormone and sulfuric acid to increase rooting was used. Also two different times of the year to determine the appropriate time for taking cuttings and two commercial cultivar of pomegranate Cultivars due to compares cultivars rooting potential were considered.

Materials and Methods:

This research has been done at Horticulture Departments greenhouse of the College of Agriculture and Natural Resources of University of Tehran, and took two years and two examination were defined, First,a factorial experiment in a completely randomized design with four factors, the number of nude, cuttings diameter, cultivar and date of cutting in two different years was designed.

In the first experiment, the number of nudes was: 4 nodes, 2 nodes and single node, cuttings diameter including: diameter larger than 8mm and a diameter smaller than 8mm, two cultivar include: Shirin-e-Saveh and Malas-e-Torsh, on making cuttings during two time March 2010 and January 2011 respectively. beside of this, In the second experiment the effect of two levels of IBA hormone concentration and one level sulfuric acid on rooting of single-node cuttings was defined. two cultivars in a compelete randomized design with the control sample cuttings, was studied.

In the second experiment, the levels of IBA hormone include: 3000 PPM and 1500 PPM, one level of sulfuric acid at 2N concentration and cultivars include: Shirin-e-Saveh and Malas-e-Torsh, as well a control sample (no hormones and no acid) for comparing treatments were considered.

Pomegranate cuttings were of the Research Center of Horticultural Science department and of selected Diverse of commercial varieties, from both Shirin-e-Saveh and Malas-e-Torsh. Bed of planting was chosen from a mixture of sand and perlite in a ratio of 2 to 1 and poured into pots. Then it was placed on platforms that have a height of about 1.5 meters.

Hormone levels were made in two levels of concentratin; 1500 PMM and 3000 PMM and used with the method of 'Quick deep method' for 5 seconds. Acid, used in this study was sulfuric acid with 2N concentration. To make this solution with the supposed concentration the $N_1.V_1 = N_2.V_2$ formula should be used. in this experiment the cuttings were immersed in acid solution from the bottom side and immediately planted in the bed, with 'Quick deep method' for 15seconds.

Taking cuttings and planting, for the first time were done on early March 2010 and the obtained cuttings were statically analyzed after two months and then transferred to pots. The pots were taken out and transferred to outside of greenhouse for adaptability.

The Second Date of planting was on January 2011. harvesting and transplanting of planted cuttings took place after two months. 10 cuttings were planted in each pot and from the obtained data, the rooting average, and rooting percentage were calculated in each repeat.

The experiment data were analyzed and compared statically by SAS software and by Duncan's multiple range test and Excel software was used to plot graphs.

Results:

As observed in table (1), the impact of a single-node cutting on rooting was very significant in compare with two nodes and four nodes, so that it is significant at the 1% level. Beside of this, rooting of both cultivars was ideal by single-node cuttings. But between the two cultivars that were studied, Shirin-e-Saveh, showed better rooting percentage result than Malas-e-Torsh and The difference between them is significant at the 1% level. As well, The effect of nodes number in diameter was significant at 5% level and single-node cuttings of smaller than 8mm diameter, showed better rottings than cuttings with diameter larger than 8 mm.

According to table (1) the Average of rooting numbers in rooted cuttings have shown significant difference at 1% among rooted in the single-node, two-node and four-node. As well the effect of cuttings diameter on the average root number was significant at 5% level. between the interaction of treatments, interaction between diameter and the time on the root numbers in cuttings were significant at the 5% level. Other interactions between treatments showed no significant difference.

According to table (2) the effect of different hormonal treatments and acid on percentage of cuttings rooting of a single -node wase valuated and the effect of treatments on rooting percentage indicated a significant difference. As well the interaction of treatments and cultivar on rooting percentage was significant at the 5% level.

The effect of IBA hormone treatments and sulfuric acid, on the average roots number of single-node cuttings were evaluated, and was shown in table (2), and significant differences were observed at the 1% level. As well the interaction between the hormone,acid and cultivars was significant at 1%.

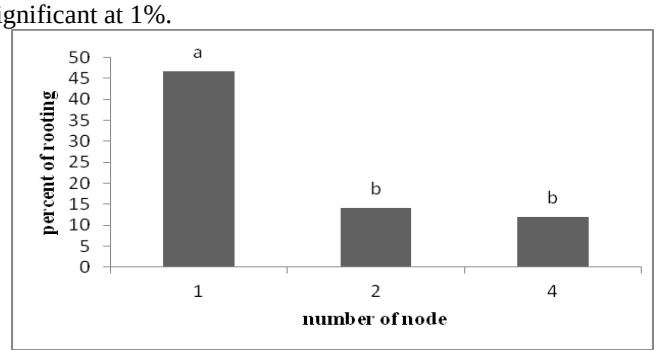

Figure1-Effect of number of node on rooting percent of two cultivars of pomegranate

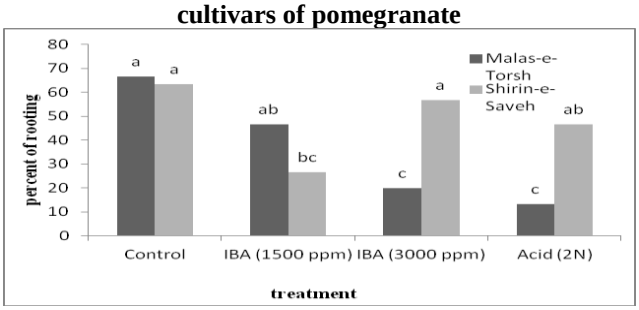

Figure2- Effect of acid and IBA on rooting percent of two cultivar

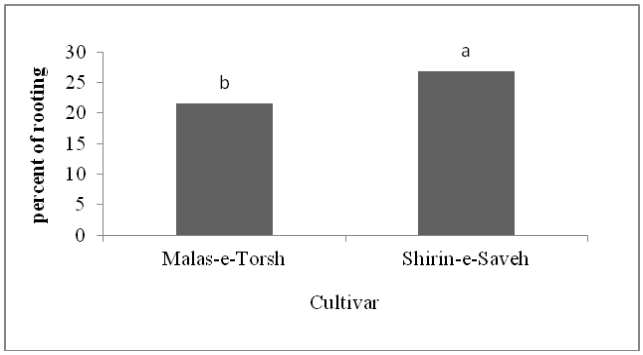

Figure3-Effect of Cultivar on rooting percent

Table1- Variance analysis table of percent of rooting and average of rooting

		Means of square	
s.o.v	♀Df	Percent of Rooting	Average of Rooting
node	2	3984.143 **	4.247**
diameter	1	9.256ns	0.475*
cultivar	1	217.197**	0.296ns
time	1	0.22ns	0.055ns
node*diameter	1	60.551*	0.038ns
node*cultivar	1	41.718ns	0.145ns
node*time	2	9.759ns	0.007ns
diameter* cultivar	1	7.513ns	0.061ns
diameter*time	1	75.456ns	0.997*
diameter*time	1	2.189ns	0.037ns
node*diameter* cultivar	1	45.323ns	0.05ns
node*diameter*time	2	45.557ns	0.34ns
node*cultivar*time	2	13.034ns	0.115ns
diameter*cultivar*time	1	1.539ns	0.084ns
node*diameter*cultivar *time	2	20.278 ns	0.134ns
Error	48	21.188	0.097ns
C.V %		16.302	17.236

*,** significant at 5% and 1% levels respectively; ns: not significant.

Table 2-- Variance analysis table of acid and IBA treatment on percent of rooting and average of rooting

S.O.V	Df	Means of square Percent of Rooting	Average of Rooting
treatments	3	549.850**	16.295**
cultivar	1	337.842 ns	2.178 ns
treatments* cultivar	3	*447.615	14.141**
Error	16	89.979	1.331
C.V %		23.6913	27.9303

*,** significant at 5% and 1% levels respectively; ns: not significant.

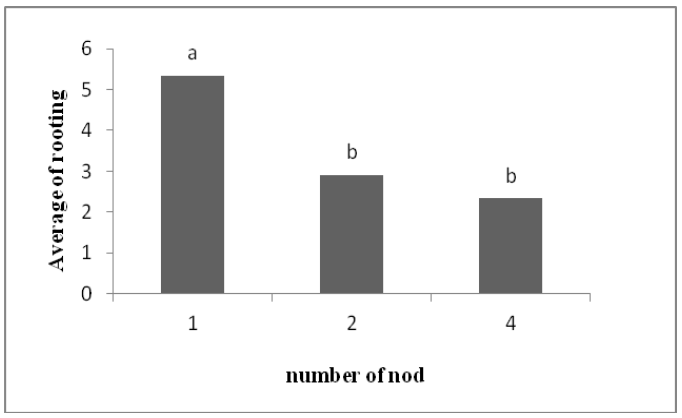

Figure4- Effect of number of nod on average of cuttings rooting

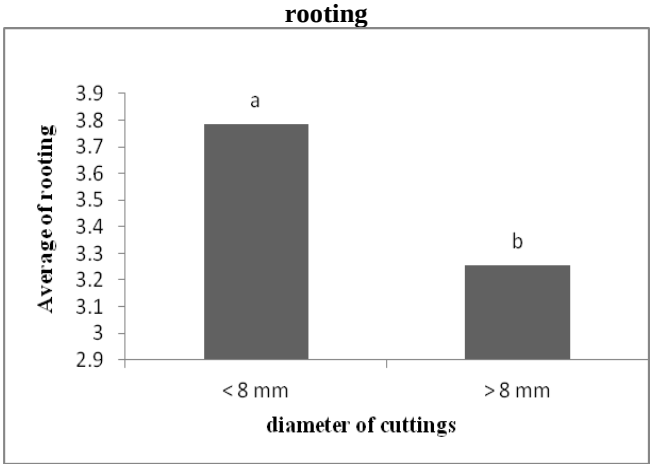

Figure5- Effect of cuttings diameter on average of rooting

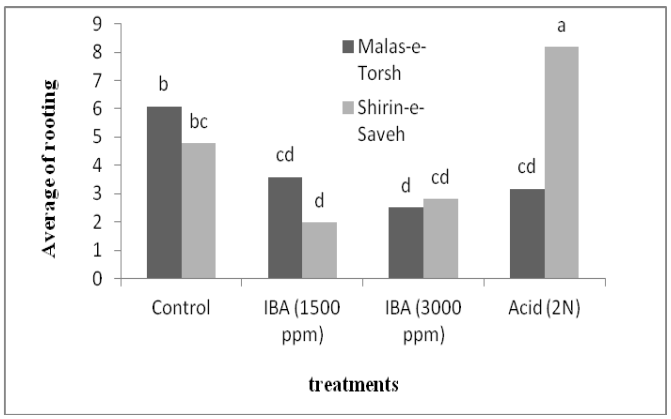

Figure6- Effect of Acid and IBA on average of rooting in two cultivar

Discussion:

Single-node cuttings had higher rooting percentage and average compared to two and four-node cuttings, this is clear in figure (1) and figure (4). According to table (1) interactions between the number of nodes and diameter on rooting percentage showed that single-node cuttings with diameters smaller than 8 mm had better rooting than single-node cuttings with diameters larger than 8 mm. It can be seen in figure (3) that cuttings with diameters smaller than 8 mm have higher average of number of roots than cuttings with diameters larger than 8 mm. Thus it can be said that in general rooting of cuttings, cutings with diameters smaller than 8 mm are better than cuttings with diameters larger than 8 mm.

In figure (2) you can see that control cuttings had the highest percentage of rooting and hormone and acid treatments did not have significant effect on increasing the percentage of

rooting in single-node cuttings and even reduced them, this is probably related to the high levels of endogenous auxin in pomegranate cuttings. Because addition of external hormone to cuttings containing sufficient endogenous hormone, not only will not increase the rooting but if the limit is exceeded plays an inhibitory role in the successful rooting (Arteca, 1955). This conclusion is contrary to a study on rooting of cuttings of ten pomegranate cultivars conducted by Melgarejo and colleagues, in this study, 30 cm pomegranate cuttings with at least two buds and five months time for rooting were investigated and ultimately positive effect of IBA hormone treatment and wounding on rooting were observed (Melgarejo et al., 1997). Whereas in a study on rooting of pomegranate cuttings conducted by Hamooh, positive effect of IBA hormone was only observed in not wounded cuttings and in wounded cuttings IBA hormone in high concentrations had a negative impact, the reason has been reported to be toxicity of auxin in high concentrations on cells and fresh tissues, which show similarity to the results of this experiment (Hamooh, 2005).

In figure (2) acid treatment effect on increase the percentage of single-node cuttings rooting of Shirin-e-Saveh cultivar is significant. Figure (6) shows that acid treatment had significant effect on average number of roots in rooted cuttings. While acid treatment has an average slightly higher than the control, but the difference is not significant. Acid by destruction of cells in the rooting zone stimulate cell division to repair damaged tissue and formation of young cells, this young cells differentiate into young roots while placed in rooting appropriate conditions of temperature, bed and hormones differentiate into young roots (Arteca, 1955), also acid destroys hard and wooden layers which prevent emergence of adventitious roots from germinal layer of root and in fact has a function similar to scarification treatment (Khosh-khui & Tafazoli, 1979).

Considering figure (2) and figure (3) Shirin-e-Saveh cultivar had higher rooting percentage compared to Malas-e-Torsh cultivar and the difference between them is significant at 1% level, which shows that rooting is different in various cultivars, this difference in rooting between cultivars is also seen in cuttings of pomegranate cultivars in Owais study in 2010, that the highest rooting percentage was 70% and the lowest rooting percentage was about 40% (Owais, 2010). Also in comparison of rooting of pomegranate cuttings in the study conducted by Karimi and colleagues on two pomegranate cultivars, it became clear that different cultivars had different rooting potentials (Karimi et al., 2012).

Figure (6) illustrates effects of hormonal treatments, acid and cultivar on average of number of roots in single-node cuttings which acid treatment in Shirin-e-Saveh cultivar has the highest rooting rate and in acid treatment the mean difference of roots in cuttings between Shirin-e-Saveh and Malas-e-Torsh cultivars is quite significant. However, in control treatment there is no significant difference between Shirin-e-Saveh and cultivars. So we can say that acid treatment in Shirin-e-Saveh cultivar was effective for increasing the average of number of roots in single-node cuttings.

In this study, single-node cuttings without hormone treatment showed better rooting, otherwise when the number of nodes in cuttings increases, rooting is reduced. this result lies in the research of Kracke and colleagues which investigated changes in endogenous auxin in cuttings of two hard rooting and easy rooting grape species. Based on this study level of endogenous auxin is high in easy rooting cuttings whereas gibberellic acid and abscisic acid levels which are rooting inhibitors are low in these cuttings. In hard rooting cuttings level of endogenous auxin is low and levels of gibberellic acid and abscisic acid are high. During rooting endogenous auxin levels rise in cuttings of both easy rooting and hard rooting species until cutting buds open, and opened buds produce abscisic acid and gibberellic acid which have an inhibitory role in rooting (Kracke et al., 1981). Also according to the study of hormones conducted by Kawai between single-bud cuttings and cuttings without bud of grapes, levels of endogenous IAA hormone in single-bud cuttings have been reported higher than cuttings without bud so far as causing significant differences in rooting of single-bud cuttings compared to cuttings without bud (Kawai, 1996). Although buds are involved in auxin production, but after opening, buds prevent rooting by producing inhibiting substances like abscisic acid and gibberellic acid (Arteca, 1955).

Conclusion:

High number of buds does not always lead to better rooting in cuttings, but in order to have higher and better rooting there should be hormone balance in the plant and in fact an optimum level of stimulating and inhibiting hormones in cuttings is considered for higher and better rooting and since single-node cuttings had better hormonal balance than two-buds and four-buds cuttings or cuttings under external hormone treatment, these cuttings showed better rooting in this study.

Different cultivars have different potential of rooting and sulfuric acid has a positive effect on rooting of woody cuttings.

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