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Najibullah Mujadadi
College of Horticulture, UHS Campus, GKVK Post, Bengaluru, Karnataka, India

Naqibullah Mujadidi
Department of Horticulture, Afghanistan National Agricultural Sciences and Technology University (ANASTU), Kandahar Afghanistan

Fakrudin B
College of Horticulture, UHS Campus, GKVK Post, Bengaluru, Karnataka, India

Jagannath S
College of Horticulture, UHS Campus, GKVK Post, Bengaluru, Karnataka, India

Zmarialay Tani
Department of Horticulture, Afghanistan National Agricultural Sciences and Technology University (ANASTU), Kandahar Afghanistan

Abdul Wasi Amiri
Herat University, Department of Horticulture, Herat Afghanistan

Corresponding Author:
Jagannath S
College of Horticulture, UHS Campus, GKVK Post, Bengaluru, Karnataka, India

Microbial inoculants enhance the rooting and establishment of stem cuttings of cvs. Ruby and Mrudula of pomegranate (*Punica granatum* L.) for propagation

Najibullah Mujadadi, Naqibullah Mujadidi, Fakrudin B, Jagannath S, Zmarialay Tani and Abdul Wasi Amiri

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Abstract
The influence of different microbial inoculants and their combination for inducing rooting in hardwood cuttings of pomegranate cv. Ruby and Mrudula in greenhouse and open condition was studied. Shoot and root parameter such as days taken for first sprouting, length of sprouts, plant height number and length of roots, dry weight of shoot and root, percentage of rooting were analysed. Among various microbial cultures used *Trichoderma* + *Azotobacter* each at 2.5 ml/kg of potting mixture resulted in maximum number of branches, diameter of shoot and plant height, followed by IBA 2000 ppm + *Azotobacter* at 5 ml / kg of potting mixture and IBA 2000 ppm + *Trichoderma*at 5 ml / kg of potting mixture. Maximum number of sprouts per cutting recorded at weekly interval and length of shoot recorded on 30th day after planting till 90th day after planting were observed in cv. Ruby, when treated with microbial inoculants vi iz., *Azotobacter* + *Trichoderma* each at 2.5 ml /kg of potting mixture. High number of adventitious roots, secondary roots, length of longest adventitious root, length of longest secondary root and establishment after transplanting were also in cv. Ruby compared to cv. Mrudula. Cuttings under high cost greenhouse performed better with respect to both vegetative parameters and root parameters than those kept in open condition. The interaction effect of varieties, microbial inoculants and growing condition also influence various shoot and root parameters of hardwood cuttings. The combination of IBA @ 2000 ppm +*Azotobacter*at 5 ml /kg of potting mixture performed better in greenhouse condition. Therefore, it is advisable to use microbial consortia in place of IBA, since it is cheaper and provides other benefits apart from producing quality, planting material which will be cost effective. Further, treating the cutting with IBA @ 2000 ppm + *Trichoderma*@ 5 ml/ kg of potting mixture (T_6) under greenhouse gave better results while under open condition the treatment with *Azotobacter* + *Trichoderma* each @ 2.5 ml/kg of potting mixture (T_4) was economically feasible for propagation of pomegranate.

Keywords: Microbial inoculants enhance, rooting, establishment, stem cuttings, Ruby and Mrudula

Introduction
Pomegranate (*Punica granatum* L.) is an important fruit crop of the tropical and subtropical countries of the world and it belongs to the family Punicaceae. It is rich in beneficial antioxidants, polyphenols, tannins and anthocyanins, and regular consumption of pomegranate juice can help fight osteoarthritis and reduce down the corrosion of the cartilage. Further, this fruit juice is believed to increase blood flow to the heart, and is beneficial for people with ischemic heart disease and some other diseases. Pomegranate has anti-viral and anti-tumor properties and is said to be a good source of vitamins, especially vitamin A, vitamin C, and vitamin E, as well as folic acid.

In India, although the pomegranate crop is grown all over on a small scale to meet requirements of local markers, it is commercially grown in Maharashtra, Madhya Pradesh, Gujarat, Odisha, West Bengal, Tamil Nadu, Andhra Pradesh, Telangana and Karnataka staes, and the produce is exported to the Middle East and European countries. Being a drought enduring crop, the area under this crop is increasing and many non-traditional areas are coming under the cultivation of pomegranate in recent years. In Karnataka, pomegranate crops growing districts are Bijapur, Bagalkot, Belgaum, Bellary, Chikkaballapur, Kolar, Mysuru, Gadag, Bidar, Dharwad, Haveri, Chamarajanagar and Chitradurga.

There is a huge demand for the quality planting marterial specially coinciding to the kharif season. Vegetative propagation through stem cutting is the cost effective and quicker method to produce genetically pure planting material in relatively larger proportion. Planting material produced through this approach will have seldom genetic purity issues compared to the tissue culture-based approach of producing planting material. Further, propagation through conventional cutting / air layering is simpler and many nurserymen with training have been able to adopt the technique and produce the planting material. A few growth substances applied exogenously to cuttings are found to advance root formation and elongation which is crucial for the establishment of seedling both in nursery and later in the field. Use of microbial consortia is found to have multiple benefits in this endevour.

Beneficial microorganisms are those that can stimulate plant growth by fixing atmospheric nitrogen, decomposing organic wastes and residues, enhance nutrient recycling, detoxifying pesticides, suppressing plant diseases, pests and soil borne pathogens by producing bioactive compounds such as vitamins, hormones and enzymes. Using some of these beneficial microorganisms, various microbial inoculants or combination has been prepared for use in plant propagation and production to reduce the cost on synthetic chemicals and minimize environmental pollution. Since, the microbial inoculants are useful in reducing the problems associated with the use of synthetic chemicals and pesticides; they are now widely applied in eco-technology. Microorganisms such as *Trichoderma* spp. *Glomus* spp. and some bacteria are reported to produce phytohormones which induce rooting of different plant species (Constracurta and Vandeleyden, 1995) [3]. These microorganisms can be employed in propagation of many fruit crops of horticulture impotence (Amy *et al*., 1995) [1].

There has been advancement in the use of beneficial microbial inoculants to reduce chemical treatments, to control soil borne pathogens (Linderman, 1993) [4] and to enhance rooting and survival of rooted cuttings (McLean *et al*., 1994) [5]. Microorganisms are known to produce survival growth regulating substances, having beneficial effects on plant growth and developmental processes, including cell division, cell elongation and root proliferation. Riker *et al*., (1930) [7] was the first to report the action of *Agrobaterium rhizogenes* on the induction and proliferation of secondary roots at the wounded sites on a wide range of host plants. Similarly, *Trichderma harzianum*, a fungus, apart from controlling soil borne pathogens, enhanced root and shoot growth of chrysanthemum cuttings during propagation possibly by the production of growth regulating substances or by chemically antagonizing or competing with pathogens (Amy *et al*., 1995) [1]. We report the beneficial effects of some of the microbial inoculants together with IBA in enhancing the parameters related to rooting and establishment of cuttings during the process of root initiation in pomegranate.

Material and Methods

The study was carried out during 2007-08 at the Deportment of Horticulture, University of Agricultural Science, Bangalore. The suitability of the microbial inoculants for multiplication of pomegranate through hardwood cuttings was conducted in a greenhouse and under open (shade net) conditions. A green house with cool cell pad and fan system, measuring 300 sq.m area (30 m L x 10 m W x 4 m H) was utilized. Two cultivars were included in the present experiment: the details of the cultivars is as follows.

Ruby: This variety is developed at IIHR, Bangalore. The mature fruits resemble cv. 'Ganesh' with respect to shape and size. The rind of this variety is reddish brown with green streaks containing red blood arils. The fruit weighs 270g with an average yield of 16-18 tones/h.

Mrudula: This variety has all the characters of the Ganesh variety except the arils are dark red in colour. The colour of the arils in 'Ambe' and 'Mrig' bahars is dark red in colour, while it is pink during the 'Hasta' bahar. The average fruit weight is 250-300 g. Following treatments were included in the experiment:

T_1: IBA @ 2000 ppm (control).
T_2: Azotobacter @ 5 ml/kg of potting mixture.
T_3: Trichoderma @ 5ml/kg of potting mixture.
T_4: Azotobacter + Trichoderma each @ each 2.5 ml/kg of potting mixture.
T_5: IBA @ 2000 ppm + Azotobacter @ 5 ml/kg of potting mixture.
T_6: IBA @ 2000 ppm + Trichoderma@ 5 ml/kg of potting mixture

Results and Discussion

Growth parameters

Higher concentration of IBA (2000 ppm) as quick dip treatment induced the bud sprouts as quickly as other treatments in combination with the microbial agents tested: no significant differences with respect to the number of days taken to the induction of first srout in the cutting. However, numerical merit of microbial agents was recorded (Table 1). Similar results were reported in pomegaranate (Ram, *et al*. 2005). The hardwood cuttings, raised under high cost greenhouse showed sprouting in lesser number of days (4.69), compared to open condition (4.81), which is statistically non-significant. Among the two cultivars, Mrudula took a minimal number of days for sprouting (4.64) as compared to Ruby (4.86) (Table 1). The maximum (2.43 cm) shoot diameter was recorded in T_4 : *Azotobacter* and *Trichoderma* each at @ of 2.5 ml/kg of potting mixture under polyhouse conditions whereas, it was low in T_2 : *Azotobacter* at 5 ml/kg of potting mixture (Table 2).

The significant difference was recorded between conditions with maximum plant height (20.21 cm) observed on75th day after planting under high cost: under open condition compared to high cost greenhouse (17.26 cm and 17.67 cm).

Root parameters

The data pertaining to the influence of microbial inoculants on percentage of rooting showed a significant difference between conditions (Table 1). Cuttings under high cost greenhouse recorded maximum rooting percentage (58.61 %) as compared to open condition (27.22 %) with respect to percentage of rooting. The data on the number of adventitious roots per cutting as influence by microbial inoculants and growing conditions on different cultivars are presented in (Table 2). No significant differences were observed among growing conditions, and treatments and interactions with respect to number of adventitious roots.

Among the cultivars, significantly higher number of adventitious roots were recorded in cv. Ruby (7.21) and it was least in cv. Mrudula (5.56). Interaction effect was also found to be non-significant among the growing conditions, cultivars and treatments with respect to number of adventitious roots per cuttings. The data on the number of secondary roots per

cutting showed a significant difference between the growing conditions and cultivars (Table 4).

Cuttings under high cost greenhouse recorded significantly higher number of secondary roots per cutting (9.57) as compare to open condition (7.05). While among the cultivars, Ruby recorded maximum number of secondary roots per cuttings (8.08) and was significant as compared to Mrudula (5.59).

Propagation is an important aspects as source of planting material in commercial cultivation of horticultural crops. Vegetative propagation achieved by cuttings is widely employed method, which is cost effective and quickest method to produce large number of true-to-type planting material of variety of horticultural crops. Application of auxins to stimulate rooting of cutting and application of chemicals to control pathogen and pest during propagation is in commercial usage by nurseryman, but utilizing beneficial microbial inoculants for induction of rooting is a novel approach to reduce the inorganic chemical with the recent trend of organic cultivation. Many microorganisms are known to control soil borne pathogens, besides their ability to produce auxin which in turn help in induction of rooting (McLean *et al.* 1994 and Linderman 1993) [5, 4]. Our results demonstrated the beneficial effects of microbial inoculants in enhancing the rooting and sprouting aspects of propagation through cutting in two cultivars of pomegranate.

Shoot and root parameter recorded the influence of conventional growth regulator and the microbial consortia used. A combination of *Trichoderma* + *Azotobacter* each at 2.5 ml/kg of potting mixture resulted in maximum number of branches, diameter of shoot and plant height. *Azotobacter* + *Trichoderma* each at 2.5 ml /kg of potting mixture recorded maximum number of sprouts per cutting recorded in both cultivars. High number of adventitious roots, secondary roots, length of longest adventitious root, length of longest secondary root and establishment after transplanting were also in cv. Ruby compared to cv. Mrudula. Cuttings under high cost greenhouse performed better with respect to both vegetative parameters and root parameters than those kept in open condition. Taken together, it is advisable to use microbial consortia in place of IBA, since it is cheaper and provides other benefits apart from producing quality, planting material which will be cost effective. Further, treating the cutting with IBA @ 2000 ppm + *Trichoderma*@ 5 ml/ kg of potting mixture (T_6) under greenhouse gave better results while under open condition the treatment with *Azotobacter* + *Trichoderma* each @ 2.5 ml/kg of potting mixture (T_4) was economically feasible for propagation of pomegranate. Testing different combination microbial consortia of known potential ones can lead to pinning down the right combination and levels to achieving maximum benefit in propagation of horticultural crops through semi hardwood cutting

Table 1: Effect of microbial inoculation on days taken for first sprouting in pomegranate cvs. Ruby and Mrudula

Treatment	Ruby			Mrudula			Average	Condition	
	Polyhouse condition	Open condition	Average	Polyhouse condition	Open condition	Average		Polyhouse	Open
T1	4.00	5.33	4.67	4.00	4.33	4.17	4.42	4.00	4.83
T2	5.00	6.00	5.50	5.00	4.67	4.83	5.17	5.00	5.33
T3	4.33	4.67	4.50	4.67	5.00	4.83	4.67	4.50	4.83
T4	4.67	5.33	5.00	5.33	4.00	4.67	4.83	5.00	4.67
T5	5.33	4.67	5.00	5.00	4.00	4.50	4.75	5.17	4.33
T6	5.00	4.00	4.50	4.00	5.67	4.83	4.67	4.50	4.83
Average	4.72	5.00	-	4.67	4.61	-	-	4.69	4.811
Average	Ruby (4.86)			Mrudula (4.64)					
	A	B	C	A x B	A x C	B x C	A x B x C		
F. value	1.07	0.27	0.88	0.60	0.71	1.27	2.40		
S.Em±	0.152	0.152	0.264	0.215	0.373	0.373	0.527		
CD at 5%	NS	NS	NS	NS	NS	NS	NS		

A – Variety; B – Conditions; C - Treatments

Table 2: Effect of microbial inoculation on plant height (cm) pomegranate cvs. Ruby and Mrudula (75th days after planting).

Treatment	Ruby			Mrudula			Average	Condition	
	Polyhouse condition	Open condition	Average	Polyhouse condition	Open condition	Average		Polyhouse	Open
T_1	20.23	19.33	19.78	19.43	19.70	19.57	19.68	19.83	19.52
T_2	20.23	18.80	19.52	20.17	19.30	19.73	19.63	20.20	19.05
T_3	19.83	19.47	19.65	22.13	19.63	20.88	20.27	20.98	19.55
T_4	21.20	18.53	19.87	19.27	19.83	19.55	19.71	20.23	19.18
T_5	19.87	20.53	20.20	19.90	19.43	19.67	19.93	19.88	19.98
T_6	19.83	20.00	19.92	20.43	20.70	20.57	20.24	20.13	20.35
Average	20.20	19.44	-	20.22	19.77	-	-	20.21	19.61
Average	Ruby (19.82)			Mrudula(19.99)					
	A	B	C	A x B	A x C	B x C	A x B x C		
F. Value	0.48	5.92	0.89	0.36	1.21	1.31	2.35		
S.Em±	0.176	0.176	0.305	0.249	0.431	0.431	0.609		
CD at 5%	NS	0.501	NS	NS	NS	NS	NS		

A – Variety; B – Conditions; C - Treatments

Table 3: Effect of microbial inoculation on number of adventitious roots in pomegranate cvs. Ruby and Mrudula

Treatment	Ruby			Mrudula			Average	Condition	
	Polyhouse condition	Open condition	Average	Polyhouse condition	Open condition	Average		Polyhouse	Open
T_1	9.10	7.67	8.38	5.72	6.57	6.14	7.26	7.41	7.12
T_2	8.40	7.00	7.70	5.95	4.17	5.06	6.38	7.18	5.58
T_3	6.45	7.30	6.88	4.95	4.27	4.61	5.74	5.70	5.78
T_4	7.30	7.17	7.23	6.79	5.90	6.35	6.79	7.05	6.53
T_5	5.96	5.87	5.91	5.00	5.33	5.17	5.54	5.48	5.60
T_6	7.80	6.50	7.15	7.33	4.73	6.03	6.59	7.57	5.62
Average	7.50	6.92	-	5.96	5.16	-	-	6.73	6.04
Average	Ruby (7.21)			Mrudula(5.56)					

	A	B	C	A x B	A x C	B x C	A x B x C
F. value	16.57	2.90	1.71	0.07	0.69	0.78	0.50
S.Em±	0.287	0.287	0.496	0.405	0.702	0.702	0.992
CD at 5%	0.815	NS	NS	NS	NS	NS	NS

A – Variety B – Conditions C - Treatments

Table 4: Effect of microbial inoculation on number of secondary roots in pomegranate cvs. Ruby and Mrudula

Treatment	Ruby			Mrudula			Average	Condition	
	Polyhouse condition	Open condition	Average	Polyhouse condition	Open condition	Average		Polyhouse	Open
T_1	9.87	7.67	8.78	7.78	5.00	6.39	7.58	9.42	7.57
T_2	6.73	7.00	6.87	7.23	3.13	5.18	6.03	9.04	8.19
T_3	8.44	6.80	7.62	6.39	4.77	5.58	6.60	9.49	6.67
T_4	9.67	8.17	8.92	8.17	3.07	5.62	7.27	9.84	7.84
T_5	9.15	7.10	8.12	6.67	3.23	4.95	6.54	10.29	6.70
T_6	8.64	7.77	8.20	6.33	5.33	5.83	7.02	9.33	6.12
Average	8.75	7.42	-	7.09	4.09	-	-	9.57	7.05
Average	Ruby (8.08)			Mrudula (5.59)					

	A	B	C	A x B	A x C	B x C	A x B x C
F. value	30.28	22.95	1.03	3.40	0.32	0.58	0.71
S.Em±	0.320	0.320	0.555	0.453	0.785	0.785	1.109
CD at 5%	0.911	0.911	NS	NS	NS	NS	NS

A – Variety; B – Conditions; C – Treatments

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