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Effect of IBA, boric acid and wounding treatments on rooting and growth of stem cuttings in pomegranate (*Punica granatum L.*)

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Abstract

The experiment was laid out using Factorial RBD with 10 chemical and mechanical treatments (i.e. control, IBA 1000 ppm, IBA 2000 ppm, IBA 1000 ppm + Boric Acid 1%, IBA 2000 ppm + Boric Acid 1%, Wounding, IBA 1000 ppm + Wounding, IBA 2000 ppm + Wounding, IBA 1000 ppm + Boric Acid 1% + Wounding, IBA 2000 ppm + Boric Acid 1% + Wounding). The stem cuttings when treated with IBA 2000 ppm + Boric acid 1% + Wounding had recorded significantly earliest sprouting and highest survival, shoot and root growth of stem cutting.

Keywords: Boric acid, IBA, hardwood stem cutting and wounding

Introduction

Pomegranate (*Punica granatum L.*) is a delicious and desert table fruit of tropical and subtropical arid and semi-arid regions of the world owing to its versatile adaptability, hardy nature, low maintenance cost, high yield and suitability to marginal lands. It is liked for its cool refreshing juice, nutritional and medicinal properties. It is a naturally dense, deciduous, busy and multistemmed shrub. Pomegranate is a highly remunerative crop for replacing subsistence farming and alleviating poverty.

Pomegranate belongs to the family Punicaceae. The genus 'Punica' has two species viz., *Punica granatum* (cultivated pomegranate) and *Punica protopunica* (wild pomegranate). Pomegranate is basically diploid in nature with $2n=2x=16$, 18 chromosomes (Smith, 1979, Darlington and Janakiammal, 1945) [4, 46]. The number of chromosome in the somatic complements of cvs., Dholka, Ganesh, Kandhari, Muscat White and Patiala were found to be $2n=16$, while the cv. Double Flower (ornamental type) had $2n=18$ (Nath and Randhawa, 1959a). The chromosome number in cvs. Vellodu and Kashmiri were found to be $2n=2x=18$ (Raman, *et al.*, 1963) [31]. Botanically the type of pomegranate fruit is berry balausta.

The pomegranate (*Punica granatum L.*) is believed to be originated from Iran (Primary centre of Origin). Besides, it is widely prevalent in Afghanistan, Pakistan and India, the Secondary Centers of Origin (De-Candolle, 1967) [5]. The usage of pomegranate is deeply embedded in human history with references in many ancient cultures about its use in food and medicine (Holland *et al.*, 2009) [11]. It is one of the oldest known edible fruits and is associated with ancient civilizations of the Middle East.

Pomegranate fruit has a good consumer preference for its attractive, juicy, sweet acidic and refreshing arils. Its fruit has great nutritional value due to which its demand in the local and international market is increasing day by day. It is known to have multiple health benefits and is therefore, consumed in fresh forms as well as processed products such as juices, jams, anardana etc. Of the several horticulture products in India, the global demand for pomegranate has been increasing at a much faster rate compared to others. The value of pomegranate is its large content in polyphenol compounds, which are present in the edible part as well as in the rest of the fruit. Pomegranate has a long history of nutritional (when consumed as raw fruit or juice) value. Apart from being eaten fresh, pomegranates are used to make juice, which is consumed around the world. About 100 g arils provides 72 kcal of energy, 1.0 g protein, 16.6 g carbohydrate, 1 mg sodium, 379 mg potassium, 13 mg calcium, 12 mg magnesium, 0.7 mg iron, 0.17 mg copper, 0.3 mg niacin and 7 mg vitamin C (Grove and Grove, 2008) [9]. Its fruit is good source of essential vitamins as foliate and vitamin. It is well known that pomegranate is a good source of anti-oxidants. Pomegranate juice and seeds are used to make toppings, sauces and dips for many types of food. There are about 153 phyto-chemicals, including their

derivatives, in pomegranate. Phyto-nutrients derived from pomegranate fruits offer the best protection against many diseases (Jyotsana and Maity, 2010) [14]. Pomegranate peel extract might be useful as multi-functional preservative in foods (Ibrahim, 2010) [12]. There is a growing demand for good quality fruits both for fresh use and processed products (Pruthi and Saxena, 1984) [28, 38, 39]. The ancient fruit has emerged as a commercially important fruit in the recent times due to its utility as processed products such as juice, syrup, squash, wine besides anardana, an acidulant (Saxena *et al.*, 1984) [28, 38, 39]. Fresh fruit is of exquisite quality while its processed products like juice, syrup, jelly etc. are highly appreciated.

Pomegranate fruit has excellent keeping quality, fine table and therapeutic values along with considerable pharmacological properties like antimicrobial, antiviral and anti-mutagenic effects (Negi *et al.*, 2003 and Seeram *et al.*, 2005) [2, 24, 40] which made this fruit more lucrative and remunerative in the market.

Nutritionally pomegranate is ranked better than grapes, mango, orange and papaya. Pomegranate is one of the richest sources of Riboflavin. Rind of the fruit, bark of stem and root of pomegranate contain more than 28% Gallo tannic acid and dye which is useful in tanning as natural bio-dye. Pomegranates are rich in polyphenols, specifically ellagic acid and punicalgins, which can act as potent antioxidants. Ellagic acid is found in the red arils of the pomegranate besides other red coloured berries. Punicalgins are found only in the outer skin of the pomegranate and are estimated to have twice the antioxidant capability of red wine and green tea (Sevda and Rodrigues, 2011) [41]. In India, there is a common adage '*Ek Anar Sau Bimar*' meaning "one fruit of pomegranate cures hundreds of diseases". The flower buds are very useful in Ayurveda for managing bronchitis. The bark of the stem, root and rind of the fruit are used for slimming treatments, control of gastro intestinal diseases and disorder like dysentery, diarrhoea and killing tape worms (Singh *et al.*, 1967) [30, 34, 35, 43-45]. The rind of the fruit has also some pharmaceutical values to prevent intestinal disorder (Patil and Karele, 1985) [27].

India ranks first in the world with respect to pomegranate area and production, but Iran is the largest exporter. During 2015, pomegranate in the country was cultivated over 0.19689 million ha with annual production of 2.30644 million tonnes (Saxena, 2017) [28, 38, 39]. The productivity of pomegranate in 2013-2014 was 11.7144 tonnes/ha. In India, pomegranate orchards are sprawling from Kashmir to Kanyakumari. It is commercially grown in Maharashtra, Karnataka, Andhra Pradesh, Gujarat, Tamil Nadu and Rajasthan. However, Maharashtra is the leading state in acreage covering about 68.7 per cent of the area under pomegranate. In Maharashtra, production of pomegranate is mainly concentrated in the western Maharashtra and Marathwada region. Pomegranates are commercially cultivated in Solapur, Sangli, Nashik, Ahmednagar, Pune, Dhule, Aurangabad, Satara, Osmanabad and Latur districts. The other important states next to Maharashtra with respect to pomegranate cultivation are Karnataka, Gujarat and Andhra Pradesh. There has been a steady increase in area and production of pomegranate in the country. By 2025, figures for the area, production and export of pomegranate are expected to reach 7.5 lakh ha, 114 lakh tonnes and 83,800 tonnes respectively. Of the different white and red aril cultivars, 'Ganesh' a promising selection from Maharashtra has gained popularity by virtue of its larger fruit, pink and sweet aril and soft seed character.

Quality of planting material is always prime need to the fruit growers. Therefore, it is necessary to produce quality planting material for distribution among the fruit growers. For the purpose, a rapid method for multiplication of planting material of pomegranate is needed to obtain good quality plants (Frey *et al.*, 2006) [6]. The pomegranate is propagated by seeds, cuttings, layering, off shoots and grafting. Of these methods of propagation, cutting is the most used form of multiplication in pomegranate. Propagation by stem cuttings are simple, easily to perform, rapid, economic and works well. New plants developed from stem cutting are true to type and uniform in growth. Such plants come into bearing earlier than the seedlings and do not require any special techniques necessary in grafting and layering (Saed Owais, 2010 and Saroj *et al.*, 2008) [25, 35].

Pomegranate can be propagated from softwood or hardwood cuttings. Hardwood cuttings are the preferred means of propagation, but softwood cuttings collected in early fall can be used with varying degrees of success. A tree from a hardwood cutting will bear fruit in a year or two after planting, while it will take at least three years from seed. Further propagation by seed leads to enormous variability in the progenies. Propagation by seed is unable to perpetuate characters of the parent tree. The seedling trees have long juvenile period and more vigorous growth habit which poses problem in performing various horticultural operations. Moreover, the fruits of seedling trees do not mature in one stroke and thereby affect their marketing. The other techniques of vegetative propagation of pomegranate, namely, layering, and grafting are cumbersome, laborious, time consuming and required skilled gardener. Not only this, these technique particularly layering is un-economical since only one plant is obtained from a long scion shoot. Because of development of more number of plants in short period and also because of development of plants more easily without requiring skilled gardener, stem cutting technique of vegetative propagation is better provided its success rate is improved.

In some fruit plants, propagation by stem cutting has become more feasible with the development of auxin treatment (Saroj *et al.*, 2008) [35]. For general use in rooting stem cuttings of the majority of plant species, NAA and IBA, particularly the latter is recommended, to determine the best materials and optimum concentration for rooting any particular species under a given set of conditions. (Hartmann *et al.*, 2007) [10]. Besides root promoting bio regulators, the application of boric acid at the basal portion of stem cutting has been found beneficial in stimulating adventitious root formation (Kumari *et al.*, 2013 and Sharma *et al.*, 2009) [16, 42].

The rooting of cutting of pomegranate varied according to different dates of stem collection. The cuttings collected at the end of February had higher rooting potential than those taken at the beginning of October. This result could be attributed to the different concentrations of auxins and rooting co factors. Cuttings should be maintained at a temperature between 21 - 27°C during the day and night time (Sandhu *et al.*, 1991) [34]. In addition to auxin and other chemical treatments, root production on stem cutting can be promoted by wounding the base of cutting in a number of plant species (Sarroj *et al.*, 2014 and Reddy, 2004) [33, 36]. Basal wounding is beneficial in rooting cutting of certain species especially cuttings with older wood at the base. Following wounding, callus production and root development frequently are heavier along the margins of the wound. Evidently wounded tissues are

stimulated in to cell division and production of root primordial (Hartmann *et al.*, 2007) [10].

The research findings on the above aspects of propagation in pomegranate are rather meagers and inadequate (Baloni *et al.*, 2017, Kumari *et al.*, 2013 [16] and Rajkumar *et al.*, 2016) [30]. Moreover, the success rate in case of stem cutting without auxin, nutrient and wounding treatments is not very encouraging (Manila *et al.*, 2017 and Panday and Bisen, 2010) [20, 26]. Therefore, it was realized to ascertain the performance of stem cutting under different conditions with auxin, nutrient and wounding treatments.

Materials and Methods

The present study was conducted at Horticultural Research Centre (HRC) and PG laboratory of department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (UP) during 2016 and 2017. The university is situated on Meerut- Roorkee road (Near Modipuram), about 11 km away from the Meerut city. Geographically, experimental field is located at 29.01° North latitude, 77.45° East longitude and at an altitude of 237.75 meter above the mean sea level. The climate of this region is sub-tropical with maximum temperature of about 42°C during summer (April to October) and a minimum temperature of about 7°C during winter (November to March). Frost occasionally occurs in this region during winter from December to February. The monsoon generally begins during the last week of June and ceases by the end of September. The average annual rainfall in the region is about 862.7 mm and the annual relative humidity varies from 67 to 83 percent. The stock solutions of 25000 ppm IBA (Indole Butyric Acid) and 10% boric acid were prepared as per the procedure described by Prakash (1984). The pH of the stock solution was adjusted at 7.00. Different concentrations of IBA and boric acid were prepared by dilution method. For dilution of stock solution, the distilled water was used. Wounding is done at the basal portion of stem cutting with the help of sharp knife. A vertical

cut with the tip of a sharp knife down each side of the cutting for 3.0 cm penetrating through the bark and into wood was given in treated and untreated cuttings. Wounding treatment was comprised of 3 vertical incisions at the base of each cutting. These incisions were equally spaced on the circumference of the base of the cuttings. Just after preparation of stem cuttings, the basal part (3 cm) of the cuttings were dipped in the solution of different concentrations of IBA and boric acid for 5 minutes, while for control treatment, stem cuttings were dipped in distilled water for 5 minutes. Subsequently the cuttings were air dried for 15 minutes and planted in poly bags containing respective rooting media. The rooting media around the base of the cutting was gently pressed to hold the cutting in right place, to eliminate air pockets and make sure that the base of the cutting was in good contact with the moist rooting media.

Results

Days taken for bud sprouting

The time of bud sprouting in stem cutting of pomegranate was significantly affected due to chemical treatments (IBA and boric acid), wounding, rooting media and growing conditions (Table 4.1.1(a) and Figure 4.1.1 (a, b)). Perusal of data recorded on days taken for bud sprouting revealed that stem cuttings treated with 2000 ppm IBA + 1% Boric acid + Wounding had earliest sprouting (10.86 and 11.08 days) during both the years of investigation, while control cuttings sprouted very late (17.39 and 17.56 days) during both the years of study. In comparison to control, bud sprouting was advanced by 6.50 days with 2000 ppm IBA + 1% Boric acid + Wounding.

The data also indicated that among mechanical and chemical treatments applied to stem cuttings, wounding alone was significantly least effective (15.82 and 16.23 days) in advancing bud sprouting of stem cuttings during 2016 and 2017.

Table 1: Effect of IBA and boric acid on shoot growth of stem cutting in pomegranate

S. No.	Treatments	IBA1000	IBA2000	IBA1000 + 1% Boric Acid	IBA2000 + 1% Boric Acid	Wounding (W)	IBA1000 + W	IBA2000 + W	IBA1000 + 1% Boric Acid + W	IBA2000 + 1% Boric Acid + W	Control	CD (5%)
1.	Days taken for bud sprouting	13.91	12.82	13.26	12.22	16.03	13.40	12.51	12.48	10.97	17.47	0.206
2.	Sprouting percentage (%)	95.56	98.89	96.11	98.89	96.67	97.78	97.78	98.89	100.00	90.83	2.941
3.	Survival percentage (%)	95.56	98.89	96.11	98.89	68.06	77.50	80.84	83.33	86.95	90.83	4.668
4.	Number of shoots	4.29	4.73	4.66	5.20	3.15	4.61	5.19	4.96	5.83	2.83	0.161
5.	Mean shoot length (cm)	46.98	50.44	49.42	53.60	26.03	50.24	53.60	53.12	56.54	22.84	1.244
6.	Length of longest shoot (cm)	77.10	80.22	80.39	85.68	42.30	83.21	87.36	86.74	91.53	37.99	1.605
7.	Diameter of longest shoot (mm)	3.62	4.09	4.04	4.67	2.35	3.82	4.43	3.99	5.06	1.91	0.094
8.	Total number of leaves	166.89	214.80	241.79	274.67	95.68	225.74	250.31	269.05	309.82	86.46	5.407
9.	Total leaf area (cm ²)	736.14	975.21	1121.87	1297.21	369.24	996.94	1134.79	1256.78	1482.96	313.10	39.562
10.	Fresh weight of shoot (g)	17.32	19.24	19.76	21.70	13.99	19.89	21.43	22.17	24.16	12.45	0.483
11.	Dry weight of shoot (g)	11.92	13.11	13.52	14.66	10.05	13.55	14.90	15.68	16.84	8.73	0.501

Table 2: Effect of IBA and boric acid on root growth of stem cutting in pomegranate

S. No.	Treatments	IBA1000	IBA2000	IBA1000 + 1% Boric Acid	IBA2000 + 1% Boric Acid	Wounding (W)	IBA1000 + W	IBA2000 + W	IBA1000 + 1% Boric Acid + W	IBA2000 + 1% Boric Acid + W	Control	CD (5%)
1.	Number of primary root	35.41	38.06	39.63	40.94	14.72	37.00	38.55	40.53	42.46	14.21	0.491
2.	Mean root length (cm)	23.56	25.24	26.43	27.79	13.87	24.84	26.36	27.50	29.54	11.90	0.462
3.	Length of longest root (cm)	31.83	34.24	37.59	39.19	19.09	33.04	35.55	38.71	43.13	17.39	0.605
4.	Mean root diameter (mm)	1.13	1.35	1.45	1.60	0.66	1.26	1.39	1.57	1.70	0.59	0.023
5.	Diameter of longest root (mm)	2.12	2.34	2.41	2.78	0.94	2.26	2.43	2.66	3.01	0.91	0.041
6.	Fresh weight of root (g)	7.03	7.52	7.76	8.30	4.96	7.48	8.05	8.17	8.87	4.57	0.193
7.	Dry weight of root (g)	4.72	5.16	5.42	6.02	3.00	5.24	5.79	5.96	6.64	2.63	0.174
8.	Per cent dry weight of root	66.19	67.93	68.92	71.70	59.78	69.12	71.22	72.07	73.80	56.28	1.420

Sprouting percentage

Hundred percent sprouting was recorded in cuttings treated with 2000 ppm IBA + 1% Boric acid + Wounding during 2016 and 2017. Other treatments i.e. 2000 ppm IBA + Wounding, 2000 ppm IBA + 1% Boric acid and 2000 ppm IBA had also recorded 100 percent sprouting but only in 2017. Among the treatments, significantly lowest percentage of sprouting (93.3% and 88.3%) in cuttings was recorded with control during 2016 and 2017. A maximum of 10.09% increase in sprouting percent over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Survival percentage

Perusal of data revealed significantly maximum percent survival in cuttings treated with 2000 ppm IBA + 1% Boric acid + Wounding (85.00% and 88.89%) followed by 2000 ppm IBA + 1% Boric acid (82.22% and 85.00%) and 1000 ppm IBA + 1% Boric acid + Wounding (80.55% and 86.11%), while control cuttings had recorded minimum survival (60.00% and 65.56%) during both the years of investigation. A maximum of 38.49% increase in survival percent over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Among the chemical and mechanical treatments applied to stem cutting, wounding alone was significantly least affective (66.11% and 70.00%) in increasing the survival percent of stem cutting preceded by 1000 ppm IBA (72.78% and 75.56%) and 2000 ppm IBA (75.00% and 78.33%) during both the years of investigations.

Number of shoots in stem cutting

All the treatments significantly increased the shoot production in stem cutting over control. Significantly maximum numbers of shoots per stem cutting were recorded with 2000 ppm IBA + 1% Boric acid + Wounding (5.77 and 5.88) followed by 2000 ppm IBA + 1% Boric acid (5.13 and 5.27) and 2000 ppm IBA + Wounding (5.09 and 5.28). However, control cuttings had minimum number of shoots (2.79 and 2.87) during both the years of investigation. A maximum of 106.00% increase in number of shoots over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Among the chemical and mechanical treatments applied to stem cuttings, significantly least number of shoots per cutting was recorded with wounding (3.10 and 3.20) preceded by 1000 ppm IBA (4.21 and 4.37) and 1000 ppm IBA + Wounding (4.56 and 4.66) during both the years of study.

Mean shoot length

Data revealed that maximum mean shoot length (56.18 cm and 56.90 cm) was recorded in cuttings treated with 2000 ppm IBA + 1% Boric acid + Wounding during both the years of investigation. Among the treatments applied to cuttings significantly shortest mean shoot length was recorded in control cuttings (22.32 cm and 23.37 cm) during 2016 and 2017. The effect of wounding alone on mean shoot length was found to be least significant (25.40 cm and 26.67 cm) preceded by 1000 ppm IBA (46.75 and 47.20), 1000 ppm IBA + 1% Boric acid (49.43 and 49.42) with 1000 ppm IBA + Wounding (50.05 and 50.44) during both the years of investigation. It is also evident from the data that rooting media significantly affected the mean shoot length of cutting during both the years. A maximum of 147.54% increase in mean shoot length over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Length of longest shoot

The length of longest shoot per cutting as affected by different treatments varied from 37.99 cm (control cuttings) to 91.53 cm (2000 ppm IBA + 1% Boric acid + Wounding) during both the years.

The stem cuttings treated with 2000 ppm IBA + 1% Boric acid + Wounding had significantly maximum length of longest shoot per cutting (91.0 cm and 92.0 cm) followed by 2000 ppm IBA + Wounding (87.1 cm and 87.6 cm) and 1000 ppm IBA + 1% Boric acid + Wounding (86.4 cm and 87.1 cm) during both the years of investigation. However, minimum length of longest shoot per cutting was recorded in control cuttings (37.7 cm and 38.3 cm) during both the years. A maximum of 140.93% increase in length of longest shoot over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Among the chemical and mechanical treatments applied to stem cuttings, wounding was found to be significantly least effective in increasing the length of longest shoot per cutting (42.5 cm and 42.1 cm) preceded by 1000 ppm IBA (76.7 cm and 77.5 cm), 2000 ppm IBA (80.0 cm and 80.5 cm) and 1000 ppm IBA + 1% Boric acid (80.0 cm and 80.8 cm) during both the years.

Diameter of longest shoot

Among the chemical and mechanical treatments applied to stem cutting of pomegranate, significantly maximum diameter (5.05 mm and 5.06 mm) of longest shoot was recorded in cuttings treated with 2000 ppm IBA + 1% Boric acid + Wounding, while the effect of wounding alone was found to be least significant (1.90 mm and 1.91 mm) than the combined effect of wounding, IBA and Boric acid during both the years of study. A maximum of 164.92% increase in diameter of longest shoot over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Total number of leaves

The total number of leaves in stem cutting as affected by treatments varied from 86.46 (control cuttings) to 309.82 (2000 ppm IBA + 1% Boric acid + Wounding) during both the years of investigation. Among the treatments, the total number of leaves per cutting was found to be significantly maximum with 2000 ppm IBA + 1% Boric acid + Wounding (308.8 and 310.9) followed by 2000 ppm IBA + 1% Boric acid (273.1 and 276.3) and 1000 ppm IBA + 1% Boric acid + Wounding (268.0 and 270.2) during both the years of investigation. However, control cutting untreated with pre-planting treatment had lowest number of leaves per cutting during both the years of study (84.8 and 88.2). A maximum of 258.33% increase in total number of leaves over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

While examining the individual effect of IBA, boric acid and wounding, it was found that wounding alone was significantly least effective in increasing the total number of leaves per cutting (94.4 and 97.0) preceded by 1000 ppm IBA (165.4 and 168.4) and 2000 ppm IBA (214.0 and 215.6) during both the years.

Total leaf area

The leaf area of stem cuttings as affected by different treatments varied from 313.10 cm² (control cuttings) to 1482.96 cm² (2000 ppm IBA + 1% Boric acid + Wounding) during both the years.

The data further indicated that 2000 ppm IBA + 1% Boric acid + Wounding treatment when given to stem cutting had

significantly increased the maximum leaf area of cutting (1471.9 cm² and 1494.1 cm²) followed by 2000 ppm IBA + 1% Boric acid (1283.8 cm² and 1310.7 cm²), 1000 ppm IBA + 1% Boric acid + Wounding (1244.5 cm² and 1269.1 cm²) and 2000 ppm IBA + Wounding (1122.8 cm² and 1146.8 cm²) during both the years of study. However, minimum leaf area was recorded in control cuttings (306.6 cm² and 319.6 cm²) during both the years. A maximum of 373.63% increase in total leaf area over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Fresh weight of shoot

Owing to the effect of different treatments, the fresh weight of shoot varied from 12.45 g (control) and 24.16 g (2000 ppm IBA + 1% Boric acid + Wounding) during both the years.

Of the treatments applied to the stem cuttings when treated with 2000 ppm IBA + 1% Boric acid + Wounding had significantly maximum fresh weight of shoot (23.92 g and 24.40 g) followed by 1000 ppm IBA + 1% Boric acid + Wounding (21.91 g and 22.44 g) and 2000 ppm IBA + 1% Boric acid (21.48 g and 21.92 g) during both the years of study. Untreated cuttings had recorded minimum fresh weight of shoot (12.18 g and 12.72 g) during both the years. A maximum of 94.05% increase in fresh weight of shoot over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Dry weight of shoot

The dry weight of shoot in stem cuttings as affected by treatments varied from 8.73 g (control) and 16.84 g (2000 ppm IBA + 1% Boric acid + Wounding) during both the years of study.

The stem cuttings treated with 2000 ppm IBA + 1% Boric acid + Wounding had significantly maximum dry weight of shoot per cutting (16.97 g and 16.71 g) followed by 1000 ppm IBA + 1% Boric acid + Wounding (15.64 g and 15.72 g) and 2000 ppm IBA + Wounding (14.83 g and 14.97 g) during both the years of investigation. However, minimum dry weight of shoot was recorded in control cuttings (8.87 g and 8.59 g) during both the years. A maximum of 92.89% increase in dry weight of shoot over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Number of primary root

Significance variation in number of roots developed in cuttings was recorded among the treatments. The number of roots per stem cutting recorded in different treatments varied from 14.21 (control) to 42.46 (2000 ppm IBA + 1% Boric acid + Wounding) during both the years.

The perusal of data further revealed that among the treatments, significantly maximum number of roots per cutting was recorded with 2000 ppm IBA + 1% Boric acid + Wounding (42.40 and 42.52) followed by 2000 ppm IBA + 1% Boric acid (40.89 and 41.00), 1000 ppm IBA + 1% Boric acid + Wounding (40.52 and 40.54) and 1000 ppm IBA + 1% Boric acid (39.55 and 39.71) during both the years. However, control cuttings had significantly least number of roots (14.36 and 14.07) during both the years. A maximum of 198.80% increase in number of primary over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Mean root length

The root length of cutting as affected by different treatments varied from 11.90 cm (control cuttings) to 29.54 cm (2000 ppm IBA + 1% Boric acid + Wounding) during both the years.

The stem cuttings treated with 2000 ppm IBA + 1% Boric acid + Wounding had significantly maximum mean root length (29.46 cm and 29.62 cm) followed by 2000 ppm IBA + 1% Boric acid (27.68 cm and 27.90 cm) and 1000 ppm IBA + 1% Boric acid + Wounding (27.42 cm and 27.58 cm) during both the years of investigation. However, minimum root length was recorded in control cuttings (11.83 cm and 11.97 cm) during both the years. A maximum of 148.23% increase in mean root length over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Among the chemical and mechanical treatments applied to stem cutting, wounding was found to be significantly less effective in increasing the root length of cutting (13.72 cm and 14.03 cm) preceded by 1000 ppm IBA (23.59 cm and 23.52 cm), 1000 ppm IBA + Wounding (24.69 cm and 24.99 cm) and 2000 ppm IBA (25.20 cm and 25.28 cm) during both the years.

Length of longest root

The length of longest root in stem cutting as affected by different treatments varied from 17.39 cm (control) to 43.13 cm (2000 ppm IBA + 1% Boric acid + Wounding) during both the years.

Data revealed that highest length of longest root (43.01 cm and 43.25 cm) was recorded in cuttings treated with 2000 ppm IBA + 1% Boric acid + Wounding during both the years of investigation. Among the treatments applied to cuttings, significantly minimum length of longest root was recorded in control cuttings (17.27 cm and 17.52 cm) during 2016 and 2017. The effect of wounding alone on length of longest root was found to be least significant (19.00 cm and 19.19 cm) preceded by 1000 ppm IBA (31.73 cm and 31.94 cm), 1000 ppm IBA + Wounding (33.03 cm and 33.04 cm) and 2000 ppm IBA (34.20 cm and 34.29 cm) during both the years of investigation. A maximum of 148.01% increase in length of longest root over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Mean root diameter

The mean root diameter of cuttings affected by different treatments ranged from 0.59 mm (control) to 1.70 mm (2000 ppm IBA + 1% Boric acid + Wounding) during both the years.

Among the chemical and mechanical treatments applied to stem cutting of pomegranate, significantly maximum mean diameter of root (1.68 mm and 1.72 mm) was recorded in cuttings treated with 2000 ppm IBA + 1% Boric acid + Wounding followed by 2000 ppm + 1% Boric acid (1.58 mm and 1.61 mm) and 1000 ppm IBA + 1% Boric acid + Wounding (1.55 mm and 1.59 mm) during both the years. However, control cuttings had recorded significantly minimum mean root diameter (0.58 mm and 0.61 mm) during both the years. A maximum of 188.13% increase in mean root diameter over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Diameter of longest root

The diameter of longest root of cuttings as affected by different treatments varied 0.91 (control) to 3.01 (2000 ppm IBA + 1% Boric acid + Wounding) during both the years.

All the treatments significantly increased the diameter of longest root in stem cutting over control. Significantly maximum diameter of longest root in stem cuttings were recorded with 2000 ppm IBA + 1% Boric acid + Wounding

(2.96 mm and 3.06 mm) followed by 2000 ppm IBA + 1% Boric acid (2.75 mm and 2.82 mm) and 1000 ppm IBA + 1% Boric acid + Wounding (2.61 and 2.70). However, control cuttings had minimum diameter of longest root (0.92 mm and 0.91 mm) during both the years of investigation. A maximum of 230.76% increase in diameter of longest root over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Of the chemical and mechanical treatments applied to stem cuttings, significantly minimum diameter of longest root was recorded with wounding (0.93 mm and 0.96 mm) preceded by 1000 ppm IBA (2.09 mm and 2.15 mm) and 1000 ppm IBA + Wounding (2.23 mm and 2.30 mm) during both the years of study.

Fresh weight of root

Significance variation in fresh weight of root per cutting was recorded among the treatments. The fresh weight of roots in stem cuttings affected by different treatments varied from 4.57 g (control) to 8.87 g (2000 ppm IBA + 1% Boric acid + Wounding) during both the years.

The perusal of data revealed that among the treatments, significantly maximum fresh weight of roots of cutting was recorded with 2000 ppm IBA + 1% Boric acid + Wounding (8.85 g and 8.90 g) followed by 2000 ppm IBA + 1% Boric acid (8.23 g and 8.37 g), 1000 ppm IBA + 1% Boric acid + Wounding (8.13 g and 8.22 g) and 1000 ppm IBA + 1% Boric acid (8.03 g and 8.08 g) during both the years. However, control cuttings had recorded significantly minimum fresh weight of root of cutting (4.55 g and 4.59 g) during both the years. A maximum of 94.09% increase in fresh weight of root over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Dry weight of root

The dry weight of root as affected by different treatments varied from 2.63 g (control cuttings) to 6.64 g (2000 ppm IBA + 1% Boric acid + Wounding) during both the years of study. Of the treatments applied to stem cuttings, 2000 ppm IBA + 1% Boric acid + Wounding had shown significantly maximum dry weight of root (6.59 g and 6.68 g) followed by 2000 ppm IBA + 1% Boric acid (5.94 g and 6.10 g) and 1000 ppm IBA + 1% Boric acid + Wounding (5.91 g and 6.01 g) during both the years of study. Untreated cuttings had recorded minimum dry weight of root (2.61 g and 2.66 g) among the treatments during both the years. A maximum of 152.47% increase in dry weight of root over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Percent dry weight of root

The percent dry weight of root in stem cuttings as influenced by different treatments varied from 56.28% (control) to 73.80% (2000 ppm IBA + 1% Boric acid + Wounding) during both the years.

Perusal of data revealed significantly maximum percent dry weight of root in cuttings treated with 2000 ppm IBA + 1% Boric acid + Wounding (73.49% and 74.11%) followed by 1000 ppm IBA + 1% Boric acid + Wounding (71.89% and 72.24%) and 2000 ppm IBA + 1% Boric acid (71.40% and 71.99%), while control cuttings had minimum percent dry weight of root (56.00% and 56.56%) during both the years of investigation. A maximum of 31.13% increase in percent dry weight of root over control was recorded with 2000 ppm IBA + 1% Boric acid + Wounding.

Among the chemical and mechanical treatments applied to stem cutting, wounding alone was significantly less affective (59.29% and 60.26%) in increasing the percent dry weight of root of stem cutting preceded by 1000 ppm IBA (65.98% and 66.41%) and 2000 ppm IBA (67.65% and 68.21%) during both the years of investigations.

Discussion

The promotive effect of chemical and mechanical treatments on bud sprouting as reported in the present study has also been observed by Panday and Bisen, (2010) [26] and Sarrou *et al.*, (2014) [36] who had reported that application of IBA and boric acid in stem cutting of pomegranate had significant effect on bud sprouting. The stimulatory effect of IBA + Boric acid + Wounding on sprouting in the present study has also been reported by Rajarama, 1997 and Sateesh, 1999 [29, 37].

In consistence effect of different treatments on percent bud sprouting was observed in the present study after 21 days of planting during 2016 and 2017. Luqman *et al.*, 2004 [18] also reported that auxin being root promoting growth regulator, had no direct impact on sprouting percentage of buds as bud sprouting is mainly attributed to the carbohydrate stored in the cuttings (Wahab *et al.*, 2001) [48]. In fact, sprouting is controlled by the epical dominance, physical status and food reserves of cuttings along with presence of appropriate environment i.e. temperature, moisture and oxygen. Cuttings utilize reserved food when temperature rises and start sprouting earlier than callus formation (Mabood *et al.*, 1996) [19].

The comparatively lesser time taken by cuttings to sprout as a result of treatment application in the present study might also be attributed to rapid sap flow in cuttings resulting in early callus formation and initiation of subsequent growth (Mabood *et al.*, 1996) [19].

Although initiation of bud development in stem cutting is a sign of sap flow in the treated cuttings but early callus formation determines subsequent survival of cutting prior to cambial and vascular connectivity. Therefore, the possible reason for maximum survival percentage of stem cuttings treated with IBA + wounding might be due to rapid sap flow in cuttings which could have favoured the healing process and established the continuity of cambial and vascular tissues for sprouting (Chandel *et al.*, 1998) [2]. The higher survival of cuttings treated with IBA + wounding may also be due to the fact that IBA and wounding treatments enhanced the absorption of nutrients, water and metabolites in cuttings. Following wounding, callus production and root development are increased along the margins of the wound. Wounding also increased the natural accumulation of auxins and carbohydrates in the wounded area. Besides this, ethylene production is also increased which promote adventitious root formation (Hartmann *et al.*, 2007) [10]. As a result, the survival rate of stem cuttings treated with IBA and wounding was significantly improved.

The significant improvement in sprouting and success rate in wounded cuttings over control in the present study might be attributed to the fact that wounded cuttings absorb more water from the medium than unwounded cutting. Further, wounding also permits greater absorption of applied growth regulators by the tissues at the base of the cuttings (Hartmann *et al.*, 2007) [10].

Advancement in bud in cuttings treated with 2000 ppm IBA + 1% Boric acid + wounding in the present study may be due to the fact that among the synthetic auxins, IBA is more

effective in inducing adventitious root formation in stem cutting (Hartmann *et al.*, 2007) ^[10]. It has been confirmed that auxin is a requirement for initiation of adventitious roots on stem cuttings (Hartmann *et al.*, 2007) ^[10] which has resulted in early sprouting in cuttings treated with IBA.

In the present study, wounding when applied along with other treatments also stimulated early bud sprouting in cutting. This may be because of the fact that following wounding, callus production and root development increases along the margins of the wound. Due to wounding, natural accumulation of auxins and carbohydrates in the wounded area is increased. Besides this, ethylene production is also increased which promote adventitious root formation in stem cutting resulting in early intake of buds in cuttings (Hartmann *et al.*, 2007) ^[10]. The promotive effect of wounding on bud sprouting as observed in the present study may also be attributed to the fact that wounded cuttings absorb more water from the medium than unwounded cuttings. Wounding also permits greater absorption of applied growth regulators by the tissues at the base of the cuttings (Hartmann *et al.*, 2007) ^[10].

The promotive effect of rooting hormone (IBA) containing treatment on survival of cutting is further supported by the fact that among the synthesis auxin, IBA is more effective in inducing adventitious root formation in stem cutting (Hartmann *et al.*, 2007) ^[10]. It has been confirmed that auxin is a requirement for initiation of adventitious roots on stem cuttings (Hartmann *et al.*, 2007) ^[10] which has resulted in early sprouting in cuttings treated with IBA.

The significant effect of 2000 ppm IBA + 1% Boric acid + Wounding on survival and growth of stem cutting has also been reported by Lata (2015) ^[17] and Upadhyay and Badyal (2007) ^[47], who has observed that stem cuttings of pomegranate when treated with IBA 2000 ppm + 1% Borax had highest success (85.55%) maximum number of shoots (6.74/cutting), shoot length (45.00 cm), number of leaves (203.57), number of roots (38.87/cutting) and root length (33.92 cm) in cuttings after three months of planting respectively, while control cuttings had poorest root growth among the treatments. Damar *et al.*, (2014) ^[3] also reported that stem cutting of pomegranate when treated with 2000 ppm IBA had maximum shoot growth in respect of number of shoots, plant height and total number of leaves. The significant response of IBA on growth of stem cutting as observed in the present study has also been reported by Raut *et al.*, (2015) ^[32] who have reported that IBA application at 2500 ppm in stem cutting of pomegranate gave maximum shoot growth. The findings of present study also supported the findings of Sharma *et al.*, (2009) ^[42] who have reported highest survival percentage of stem cuttings of pomegranate with IBA 500 ppm + Borax 1% /IBA 300 ppm + Borax 2% /IBA 5000 ppm.

However, Sharma *et al.*, (2009) ^[42] observed the significant effect of IBA on root growth of pomegranate cuttings at higher concentration (5000 ppm). In other crop (Fig) also, higher concentration (3000 ppm) of IBA was found effective in increasing the vegetative and root growth of cutting (Kaur and Kaur 2017) ^[15].

Wounding carried out at the base of the cutting increased the percentage of cuttings that rooted (Melgarejo *et al.*, 2008) ^[21, 22]. Melgarejo *et al.*, (2000) ^[21, 22] also observed that IBA (1200 ml) and wounding had the highest effects on rooting of cuttings.

The root elongation in stem cuttings may be due to suitable environmental conditions like soil and atmospheric temperature, humidity and internal condition of cuttings *viz.*,

endogenous auxin level, carbohydrate contents. The optimization of auxin level due to external application might have improved the root numbers, their length and diameter etc. (Singh 2014) ^[30, 34, 35, 43-45] has reported that auxin is required for initiation of adventitious roots on stems and indeed it has been considered that divisions of the first root initial cells are dependent upon either applied or endogenous auxin. The other possible reason for significant increase in root length of IBA and wounded treated cuttings may be due to the action of auxins in terms of hydrolysis and translocation of carbohydrate and nitrogen substances at the base of cutting which resulted in accelerating cell elongation and cell division in suitable environment (Singh *et al.*, (2003) ^[30, 34, 35, 43-45]. This might have caused early formation of root and more utilization of reserved food material of the treated cuttings.

The significant improvement in vegetative and root growth of stem cuttings due to wounding application in the present study might be because of the fact that wounded cuttings when treated with IBA + boric acid absorb greater amount of nutrients, hormones and metabolites (Hartmann *et al.*, 2007) ^[10]. Following wounding, callus production and root development increases along the margins of the wound. Due to wounding, natural accumulation of auxins and carbohydrates in the wounded area is also increased. As a result, endogenous level of nutrient and hormone is increased in the cuttings which might have increased the vegetative and root growth of stem cutting

The boric acid application in the present study has been found effective in stimulating the sprouting, survival, vegetative and root growth of cutting. This may be due to the involvement of boron in various processes such as cell division, nitrogen metabolism, carbohydrate metabolism, translocation and salt absorption. Further, starch is break down in simple sugar following the exogenous application of auxin and boron in cuttings (Ganie *et al.*, 2013 and Gauch and Dugger 1953) ^[7, 8]. Further, starch is break down in simple sugar following boron application, the mobilization of O₂ rich citric and iso-citric acids is enhanced which affects the acid metabolism of the cuttings (Lata 2015, Jain and Parmar 1993, and Sharma *et al.*, 2009) ^[17, 42]. As a result, sprouting, survival, vegetative and root growth of cuttings is stimulated in treated cuttings owing to rapid mobilization of sugar and better utilization of stored carbohydrates, nitrogen and other factors at the base of cuttings through photosynthesis following the application of growth regulator, boron and wounding in cuttings. Significant enhancement in bud in cuttings treated with boron containing treatment in the present study may be because of involvement of boron in various processes such as cell division, nitrogen metabolism, carbohydrate metabolism, translocation and salt absorption. Further, starch is break down in simple sugar following the exogenous application of auxin and boron in cuttings (Lata, 2015 and Ganie *et al.* 2013) ^[7, 17].

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