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Effect of different concentrations of IBA and Saccharides on growth parameters of semi hard wood cuttings of pomegranate (*Punica granatum* L.) cv. Bhagwa

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Abstract
The present experiment entitled "Effect of IBA and Saccharides on rooting and growth in stem cuttings of pomegranate (*Punica granatum* L.) cv. Bhagwa" was carried out during 2019-2020 at Fruit Research Station, Imalia, Department of Horticulture, College of Agriculture, J.N.K.V.V., Jabalpur (M.P.). The present experiment was laid out with seventeen treatments and three replications in Completely Randomized Design (CRD) under polyhouse condition to study the effect of IBA and Saccharides on root and shoot growth parameters and establishment of rooted cuttings. There results revealed that different growth parameters were significantly varied among different treatments. Among the seventeen treatments sucrose @ 5000 ppm (T_4) performed superior in shoot parameters and IBA @ 5000 ppm (T_{16}) performed superior in root parameters. Besides, sucrose @ 5000 ppm recorded maximum sprouting percentage (86.58%), shoot parameters *viz*., number of shoots (6.33, 6.90, 7.46 and 8.83), number of leaves (26.33, 37.67, 48.67 and 57.33) at 30, 60, 90 and 120 days and length of new shoots (30.60 cm, 37.10 cm and 39.83 cm) at 60, 90 and 120 days. However, IBA @ 5000 ppm recorded maximum number of primary and secondary roots (34.33, 168.33), length of primary and secondary roots (29.50 cm and 9.40 cm), longest root length of primary roots (31.33 cm), success and survival percentage of rooted cutting (76.67% and 70.97%), respectively. Therefore, from the present investigation results found that the cuttings treated with sucrose @ 5000 ppm and IBA @ 5000 ppm gave pronounced effect on shoot and root formation indicating its effectiveness for propagation in pomegranate under polyhouse conditions.

Keywords: Indole-3-butyric acid, Saccharide, Semi hard wood cuttings

Introduction
Pomegranate (*Punica granatum* L.) belongs to the dicotyledonous family Punicaceae. Its scientific title derives from the Latin words 'pomum' (apple) and granatus (grainy), meaning seeded apple (Dhillon, 2013) [8]. Pomegranate is native to Central Asia, notably Iran. The pomegranate is wide spread throughout the world including the Mediterranean, Asia, and California due to its highly adaptive to a wide range of soils and climatic conditions including arid regions, particularly the mediterranean regions, which are the leading producers (Hussein and Gouda, 2018) [12].

In India, the pomegranate is one of the most suitable fruit crops for arid and semi-arid regions and gaining popularity in the Deccan plateau of India. For the last decade, its area, production and export have increased greatly in India and the country has occupied the prime position globally (Chandra *et al.*, 2006; Jadhav and Sharma, 2007) [2, 13]. India is the only country in the world where pomegranate is available throughout the year (January- December) in three seasons (*Ambe, mrig and hasthbahar*) in the Deccan plateau of India. During 2018-19, the pomegranate was cultivated over an area of 0.24 million ha with an annual production of 2.86 million tones and productivity of 10.3 MT/ha. The production share (%) of pomegranate was 0.9% among the total fruit production of the country during 2018-2019. At present, Maharashtra is the leading state under pomegranate cultivation concerning area and production, while Tamil Nadu leads in pomegranate productivity. The other important states with successful cultivation of pomegranate are Karnataka, Gujarat, Madhya Pradesh, Andhra Pradesh, Rajasthan, Tamil Nadu, Telangana, etc. in the country (Anonymous, 2018-19) [1].

Pomegranate is commercially propagated vegetatively using stem cuttings, layering, and grafting is rarely done because many different types of grafts have not been successful enough for use in commercial production (Hartmann *et al*., 1997) [11]. Propagation by stem cuttings is an easy, fast, and economic technique of plant multiplication. Generally, pomegranate is propagated by cuttings with 15-20 cm in length and pencil size in diameter and use of hardwood or semi-hardwood (Melgarejo *et al*., 2008; Saroj *et al*., 2008; Polat and Caliskan, 2009) [18, 27]. Rajkumar *et al*., (2016; 2017) [22-23] reported that the rooting capability of cuttings varies from cultivar to cultivar, location to location, season to season, and age of branch. Although, propagation of pomegranate through cuttings is not a simple task without the use of rooting substrate under arid conditions, although the use of perlite and vermiculite in combination as rooting substrates lead to high rates of rooting and better root and shoot development. The success percent of pomegranate cutting depends upon many factors such as the condition of the mother plant, part of the tree from where the cutting is made, time of operation, rainfall, temperature fluctuation and aftercare etc. Besides different environmental conditions growth regulators also play a major role in rooting, sprouting and growth of successful cuttings.

The exogenous application of auxins favours more success of rooting in cuttings. Auxins are the only group of chemicals (synthetic and natural) that consistently improve root formation in cuttings. Auxin is thought two influence the downward movement of carbohydrates. Indole-3-Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) are usually more effective than Indole Acetic Acid (IAA). IBA is also much more stable than IAA. Multiplication of the pomegranate cuttings depend on concentrations of IBA, type of cuttings, and even the media used for rooting of cuttings (Singh, 2017; Kumari, 2014) [15]. IBA application is increase number of roots per cutting and promotes root elongation due to their ability to achieve to the active cambium, regeneration, cell division, and cell multiplication. while, GA_3 inhibited rooting by Paul and Yang (2006) [20], Rymbai and Reddy (2010) [26] and Mani *et al.*, (2018) [16].

The rooting in woody cuttings is chiefly influenced by the concentration of carbohydrates. High carbohydrate levels in the shoot are thought to be conducive to root formation (Hartmann and Kester, 1983) [9]. Sucrose is commonly known as “table sugar” or “cane sugar” is a carbohydrate formed from the combination of glucose and fructose. Glucose is a simple carbohydrate formed as a result of photosynthesis. Fructose is nearly identical except for the location of double-bonded oxygen. They are both six-carbon molecules, but fructose has a slightly different configuration. When the glucose and fructose combine it become sucrose. Sucrose is a good source of carbohydrate which gives direct energy to the cuttings. High sugar level affects rooting by reducing the level of nitrogen which is essential for the rooting process (Yeboah *et al.*, 2009) [33]. This may be the reason due to which sucrose produces higher rooting percentage compare to control. The better response of sucrose might be due to the reason that carbohydrates are known as building blocks which act as necessary energy source for plant tissues. The availability of carbohydrates is often considered exclusively as an energetic requirement and a carbon skeleton source to drive root development (Correa *et al.*, 2005) [3]. Sucrose play a significant role for vegetative propagation by cutting which is reported in Karonda (*Carissa carandas* L.) (Deepika *et al*., 2015) [4] and pear cv. Patharnakh (Dhand *et al.*, 2019) [7]. Therefore, keeping in view the growing demand of uniform plants, there is a need to obtain large number of identical plants from a single mother plant through reliable propagation protocols by using different concentrations of IBA and Saccharides on root and shoot growth parameters of pomegranate semi hard wood cuttings.

Materials and Methods

The present investigation entitled “Effect of IBA and Saccharides on Rooting and Growth in Stem Cuttings of Pomegranate *(Punica granatum L.)* cv. Bhagwa.” was carried out during the year 2019-2020 at Fruit Research Station, Imalia, Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.). The present experiment was conducted to study the effect with seventeen treatments and three replications in Completely Randomized Design (CRD) under polyhouse condition on root and shoot parameters and establishment of rooted cuttings. The type of cuttings was semi hard wood, length of cuttings was 15-20 cm and size of polybags was 30 x 15 cm^2. The concentration of IBA and Saccharides for treating the semi hard wood pomegranate cuttings were used for getting better growth parameters. The cuttings were treated with different concentration of IBA and Saccharides before the planting of cuttings and observation were recorded afterwards. All five cuttings were tagged for recording the observations and average value for each treatment was computed and recorded.

Results and Discussion

Sprouting percentage (%)

The data recorded on various aspects were tabulated and subjected to statistical analysis in comparison with treatments showed significant sprouting percentage which has been presented in Table 1. The maximum sprouting percentage (86.58%) was recorded by treating the cuttings with Sucrose @ 5000 ppm (T_4), which was significantly superior and differ significantly to the rest of the treatments. The better performance with the use of Sucrose could be explained by the larger carbon skeleton provided by this carbohydrate, resulting in higher availability of biosynthetic blocks (Correa *et al.*, 2005) [3].

Shoot parameters

Shoot parameters differs significantly among the treatments as shown in Table 1. The maximum number of shoots per cutting at 30, 60, 90 and 120 days after planting (6.33, 6.90, 7.46 and 8.83, respectively), highest shoot length at 60, 90 and 120 DAP (30.60 cm, 37.10 cm and 39.83 cm, respectively), maximum leaves per cutting at 30, 60, 90 and 120 days after planting (26.33, 37.67, 48.67 and 57.33, respectively) were recorded in cuttings treated with Sucrose @ 5000 ppm (T_4).

New callus tissue arising from the cambial region is composed of thin walled, turgid cells which are boost up by the accumulation of sucrose as reported by Hartmann *et al.*, (2007) [10] and Singh (1994).

The more number of shoot formation might be due to the vigorous root system which increase the uptake of nutrients and they are actively participated in the cell division of the vascular cambium, cell expansion and control of differentiation into different types of cambial (Devi *et al.*, 2016). The better performance with the use of sucrose was explained by the larger carbon skeleton provided by the carbohydrate present within it, resulting in higher availability of biosynthetic building blocks (Correa *et al.*, 2005) [3]. These results are in line with the finding of Dey *et al*., (2017) in Karonda. The difference in carbohydrate content in the cuttings was considered as an important factor that determines the variability in rooting capacity among genotypes (Hartmann *et al.*, 2007) [10]. Length and number of shoots increase might be due to nutritional status of plant; it play vital role in protein synthesis cell reproduction and expansion (Devi *et al.*, 2016). The better performance with the use of sucrose could be explained by the larger carbon skeleton provided by the carbohydrate present within it, resulting in higher availability of biosynthetic building blocks (Correa *et al.*, 2005) [3]. Our results are collaborated with findings in karonda and pear cv. Patharnakh by Dey *et al.*, (2017) and in (Dhand *et al.*, 2019) [7], respectively.

The development of more number of leaves might be due to activation of physiological process at meristematic tissue regions activated physiological process and it results enhanced the vegetative growth of the cuttings. The more number of leaves with sucrose application might be more number of sprouts and leaves in the plants by more produced and photosynthates, which may contribute to initiation of rooting. Rapidly developing buds sometimes tend to promote root formation, whereas buds in the rest period may inhibit root development (Deepika *et al.*, 2015) [4]. These findings are in agreement with the findings of Dey *et al.*, (2017) [6] also reported that sucrose at 4% gave maximum leaves in hard wood cuttings of karonda.

Root parameters

The results depicted in Table 2 showed that maximum number of primary and secondary roots per cutting (34.33 and 168.33), the longest primary and secondary root length (29.50and 9.40 cm), Longest root length of primary root (31.33 cm), the highest percentage of rooted cuttings (76.67%) and highest survival percentage of rooted cuttings (70.97%) was observed in cuttings treated with IBA @ 5000 ppm (T_{16}).

Various rooting substrates can be used for pomegranate cuttings, the use of vermiculite and IBA @ 2500 ppm appears to be the most appropriate rooting substrates under arid conditions of Rajasthan, India (Rajkumar et al., 2016) [22]. The increase in number of roots was probably due to hormonal effect and accumulation of other internal substances and their downward movement as reported in citrus species (Panday *et al.*, 2003). The application of IBA may have an indirect influence by enhancing the speed of transformation and movement of sugar to the base of cuttings and consequently rooting. These results were also in conformity with (Ribeiro *et al*., 2010) [25] that found highest number of roots with IBA 7500 ppm in *Prunus species* and reported that auxin application could provide an earlier of faster root growth which is important for quality and quantity. Root primordial requires auxins, which are elicited by externally applied auxins and also known to allocate carbohydrates to the base of the cuttings. Endogenous auxin level might have been supplemented by exogenously applied auxin bringing about certain anatomical and physiological changes that resulted in more number and length of primary roots. Singh, (1994) has reported that application of IBA at different concentrations was the best for rooting in pomegranate and other fruits crops. Along with, the effect of sucrose on rooting could be elucidated that commonly carbohydrates supply the energy and carbon skeleton for the synthesis of organic compounds in the plant metabolism which are activated the root formation (Deepika *et al*., 2015) [4].

Maximum length of primary and secondary roots might be due to auxin application which had been found to enhanced the histological features like formation of callus and tissues differentiation of vascular tissue. Indole-3- Butyric Acid (IBA) is a leading plant growth hormone used to generate new roots in the cloning of plants through cuttings which is active in inhibiting axillary bud break on developing shoots and stimulates the root initiation. It also promotes cell elongation which promoted the root length. Results findings of Randhawa and Nito (1980) [24] and they stated that higher percentage of rooting, increase in length of roots and average number of roots per cutting in *Malus sp*. with increasing concentration of IBA. Similarly reported by Hartmann *et al*. (1997) [11] that auxin might have increased rooting and ensured root length.

Longest root length of primary root might be due to hydrolysis and translocation of carbohydrates and nitrogenous substances at the base of cuttings, and resulted in accelerating cell elongation and cell division (Singh *et al.*, 2003).The rooting in woody cuttings is chiefly influenced by the concentration of carbohydrate. Sucrose is good source of carbohydrate which gives direct energy to the cuttings. Higher level of sugar affects rooting of plants by reducing the level of nitrogen (Yeboah *et al.*, 2009) [33]. This might be the reason higher rooting percentage in sucrose treated cuttings as compared to control.

The response of IBA could be that it is slowly degraded by the auxin degrading enzyme linked system (Sharma *et al*., 2009) [28]. Deepika *et al*. (2015) [4] suggested that, since IBA translocate poorly, it is retained near the site of application and very effective. The application of IBA might have an indirect influence by enhancing the speed of transformation and movement of sugar to the base of cuttings and consequently rooting as mentioned by Torkashv and and Shadparvar (2012) [32] in hibiscus. Krieken *et al*. (1993) [14] reported that IBA might enhanced the rooting by increase of internal free IBA, or synergistically modify the action of IAA or due to synthesis of endogenous IAA. Melgarejo *et al.* (2000) [17] opined that treatment of cuttings with increasing concentrations of IBA could combined with endogenous auxins already present in the cuttings leads to optimization of auxin levels and consequently improved the percentage of rooting in cuttings. Application of IBA @ 2500ppm in the combination with vermiculite as rooting media resulted in the highest rooting percentage, root numbers, root length, fresh and dry weight (g) of the roots, numbers of shoots per cutting and survival percentage in the nursery cuttings of ‘Phule Arakta’ cultivar of pomegranate (Rajkumar *et al*., 2016) [22].

Table 1: Effect of IBA and Saccharides on sprouting percentage of cuttings at 15 days and number of shoots per cutting at 30, 60, 90 and 120 days:

Treatments	Sprouting% at 15 days	No. of shoots per cutting			
		30 days	60 days	90 days	120 days
Sucrose @1000ppm (T_1)	61.17	2.33	4.20	5.23	6.67
Sucrose @2000ppm(T_2)	73.53	4	4.70	6	7.03
Sucrose @3000ppm (T_3)	82.19	5.66	5.63	6.36	7.77
Sucrose @ 5000ppm(T_4)	86.58	6.33	6.90	7.46	8.83
Glucose @1000ppm(T_5)	52.45	1.66	3.93	5.23	5.60
Glucose @ 2000ppm(T_6)	67.58	3.33	4.03	5.66	5.87
Glucose @3000ppm(T_7)	76.89	4.33	4.13	5.93	6.40
Glucose @5000ppm(T_8)	80.56	4.33	5.80	6.5	7.77
Honey @1000ppm (T_9)	53.92	2	4.30	6.06	6.10
Honey @ 2000ppm(T_{10})	71.87	3.66	4.70	6.43	6.53
Honey @3000ppm(T_{11})	78.54	4.66	5.07	6.83	6.83
Honey @5000ppm(T_{12})	83.04	4.90	5.82	6.96	8.10
IBA @1000ppm (T_{13})	57.23	1.33	4.43	5.4	6.90
IBA @2000ppm(T_{14})	64.22	2.66	4.97	6	7.17
IBA @3000ppm(T_{15})	73.38	3	5.47	6.46	7.73
IBA @5000ppm(T_{16})	84.45	5.68	6.57	7.4	8.63
Control(T_{17})	44.78	1	3.87	4.56	5.43
S.Em±	1.17	0.49	0.37	0.16	0.16
CD at 5%	3.36	1.41	1.07	0.48	0.47

Table 2: Effect of IBA and Saccharides on length (cm) of new shoots of cutting at 60, 90 and 120 days:

Treatments	Length of new shoot (cm)		
	60 days	90 days	120 days
Sucrose @1000ppm (T_1)	16.17	21.33	24.37
Sucrose @2000ppm(T_2)	19.0	26.63	29.47
Sucrose @3000ppm (T_3)	28.33	36.70	39.40
Sucrose @ 5000ppm(T_4)	30.60	37.10	39.83
Glucose @1000ppm(T_5)	15.30	20.77	23.73
Glucose @ 2000ppm(T_6)	17.83	25.77	28.67
Glucose @3000ppm(T_7)	22.73	27.90	30.90
Glucose @5000ppm(T_8)	24.33	31.80	34.07
Honey @1000ppm (T_9)	15.67	20.93	23.93
Honey @ 2000ppm(T_{10})	18.67	26.27	29.37
Honey @3000ppm(T_{11})	23.67	30.07	32.17
Honey @5000ppm(T_{12})	26.33	33.63	35.70
IBA @1000ppm (T_{13})	15.13	20.80	23.67
IBA @2000ppm(T_{14})	16.67	23.27	25.93
IBA @3000ppm(T_{15})	23.27	25.67	29.37
IBA @5000ppm(T_{16})	27.0	35.20	36.83
Control(T_{17})	12.60	15.93	18.93
S.Em±	2.84	2.38	2.20
CD at 5%	8.17	6.85	6.33

Table 3: Effect of IBA and Saccharides on total number of leaves at 30, 60, 90 and 120 days

Treatment detail	Total no. of leaves at			
	30 days	60 days	90 days	120 days
Sucrose @1000ppm (T_1)	14.33	19.67	23.0	31.67
Sucrose @2000ppm(T_2)	17.67	24.33	32.0	41.0
Sucrose @3000ppm (T_3)	24.0	34.33	44.33	53.67
Sucrose @ 5000ppm(T_4)	26.33	37.67	48.67	57.33
Glucose @1000ppm(T_5)	14.0	19.0	22.0	29.0
Glucose @ 2000ppm(T_6)	16.67	23.0	28.0	37.0
Glucose @3000ppm(T_7)	20.0	27.33	36.0	44.0
Glucose @5000ppm(T_8)	20.33	29.0	38.67	47.33
Honey @1000ppm (T_9)	14.33	19.33	22.33	30.67
Honey @ 2000ppm(T_{10})	17.0	23.33	29.33	39.33
Honey @3000ppm(T_{11})	20.33	28.33	39.0	46.0
Honey @5000ppm(T_{12})	23.67	33.33	43.67	52.33
IBA @1000ppm (T_{13})	13.33	18.67	21.67	28.67
IBA @2000ppm(T_{14})	15.0	20.0	24.33	34.33
IBA @3000ppm(T_{15})	15.33	20.33	26.67	36.0

IBA @5000ppm(T_{16})	19.67	27.0	35.33	43.67
Control(T_{17})	11.0	17.0	19.0	24.33
S.Em±	1.94	1.67	1.89	1.86
CD at 5%	5.60	4.81	5.45	5.34

Table 4: Effect of IBA and Saccharides on primary and secondary root numbers, root length (cm) and longest root length (cm) of primary root at 120 days

Treatments	Primary roots at 120 days		Secondary roots at 120 days		Longest root length of primary root (cm)
	Numbers	Length (cm)	Numbers	Length (cm)	
Sucrose @1000ppm (T_1)	17.33	16.0	102.33	6.33	18.33
Sucrose @2000ppm(T_2)	19.0	18.67	123.67	7.17	20.33
Sucrose @3000ppm (T_3)	21.67	19.20	131.33	7.60	21.33
Sucrose @ 5000ppm(T_4)	27.33	24.47	138.67	8.40	27.0
Glucose @1000ppm(T_5)	16.0	15.67	85.67	5.37	17.0
Glucose @ 2000ppm(T_6)	17.67	17.33	113.67	6.67	18.67
Glucose @3000ppm(T_7)	20.0	19.0	126.0	7.23	21.0
Glucose @5000ppm(T_8)	23.0	23.67	134.67	8.27	26.0
Honey @1000ppm (T_9)	16.33	16.0	98.33	5.93	17.33
Honey @ 2000ppm(T_{10})	18.33	18.57	122.67	7.0	20.0
Honey @3000ppm(T_{11})	21.0	19.17	128.0	7.33	21.27
Honey @5000ppm(T_{12})	24.67	24.0	136.67	8.33	26.33
IBA @1000ppm (T_{13})	21.67	19.67	133.33	7.67	21.67
IBA @2000ppm(T_{14})	28.33	24.99	143.0	8.57	27.33
IBA @3000ppm(T_{15})	30.67	25.10	160.67	9.20	27.67
IBA @5000ppm(T_{16})	34.33	29.50	168.33	9.40	31.33
Control(T_{17})	17.33	14.67	78.0	4.0	14.67
SEm±	1.77	1.57	6.64	0.72	1.83
CD at 5%	5.11	4.52	19.12	2.09	5.27

Table 5: Effect of IBA and Saccharides on success and survival percentage of rooted cuttings at 120 days

Treatments	Success percentage	survival percentage
Sucrose @1000ppm (T_1)	46.67	38.87
Sucrose @2000ppm(T_2)	50.0	44.97
Sucrose @3000ppm (T_3)	64.0	56.43
Sucrose @ 5000ppm(T_4)	70.67	63.63
Glucose @1000ppm(T_5)	39.33	35.57
Glucose@ 2000ppm(T_6)	43.33	40.33
Glucose @3000ppm(T_7)	49.67	46.20
Glucose @5000ppm(T_8)	66.67	55.07
Honey @1000ppm (T_9)	40.33	38.13
Honey@ 2000ppm(T_{10})	45.33	41.80
Honey @3000ppm(T_{11})	48.0	45.47
Honey @5000ppm(T_{12})	68.0	60.20
IBA @1000ppm (T_{13})	53.33	52.43
IBA @2000ppm(T_{14})	61.67	60.27
IBA @3000ppm(T_{15})	70.0	66.70
IBA @5000ppm(T_{16})	76.67	70.97
Control(T_{17})	31.0	27.87
S.Em±	2.47	1.91
CD at 5%	7.11	5.51

Conclusion

Based on the results of present investigation, result revealed that Sucrose @ 5000 ppm (T_4) had significantly effective and promote the maximum sprouting, gave more leaves, shoots and length of shoots. IBA @ 5000 ppm (T_{16}) gave more number of primary and secondary roots, highest length of primary and secondary roots and the longest root length of primary roots. Similarly, the concentration IBA @ 5000 ppm (T_{16}) had significant effect and improved success and survival percentage of rooted cutting significantly.

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