



Regeneration of Stem Cuttings of Pomegranate cv. Bhagwa as Influenced by PGR's and Planting Time

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ABSTRACT

Background: Pomegranate is being cultivated in the tropical and sub-tropical parts of the world for its delicious fruits. It can be propagated from seeds as well as from softwood, semi-hardwood and hardwood cuttings. The application of auxins encourages rooting in stem cutting owing to their ability to active cambium regeneration, cell division and cell multiplication. To meet the demand of pomegranate in the current situation and owing to its importance, the present study was undertaken.

Methods: For regeneration of stem cuttings of pomegranate cv. Bhagwa as influenced by PGR.s and planting time a study was conducted at the Department of Horticulture, Khalsa College, Amritsar during 2019-2020. The trial was undertaken with ten treatments comprising of IBA, PHB and NAA (500, 750 and 1000 ppm) each by quick dip method along with control planted in the first week of August and January.

Result: The results of the study revealed that IBA 1000 ppm (T_3) proved to be the best in terms of minimum days to first sprouting (11.06), maximum sprouting (91.10%), rooting (83.31%), number of roots per cutting (60.03), root length (11.55 cm), fresh weight of roots (1.08 g) and dry weight of roots (0.55 g). Among planting time the cuttings planted on first week of January proved to be effective in terms of sprouting (87.83%), survival (84.70%), rooting (78.81%) and number of roots (52.06) respectively.

Key words: Cuttings, Planting time, Pomegranate, Quick dip, Rooting, Sprouting, Survival.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient fruit crop, belonging to the family Punicaceae and genus *Punica*. It has been derived from the latin words *Pomum* (apple) and *granatus* (grainy or seeded). It is cultivated in the tropical and sub-tropical parts of the world for its delicious fruits. Commercial orchards of pomegranate tress are grown in Mediterranean basin and in Asia. Iran, China, India, USA and Turkey are the five countries which are major producers of pomegranate. It is also extensively cultivated in India, Spain, Morocco, Egypt, Iran, Afghanistan, Arabia and Baluchistan province of Pakistan (Seiar 2017). Pomegranate is a shrub or small tree with 6 to 10 m height. Fruit is a large globose berry called 'balusta' which comprises of juicy arils with seeds confined in seed coat known as sarcotesta. It is a rich source of minerals, vitamins and tannins while its juice is an excellent source of vitamins (B and C), sugars, minerals (K, Fe) and an antioxidant polyphenols (ellagic acid and punicalagin) which not only lower cholesterol but is also helpful in lowering blood pressure and prevent both heart attack and strokes (Karimi and Mirdehgan 2013). Some parts of the pomegranate tree (leaves, immature fruits, fruit rind and flower buds) have been used traditionally for their medicinal properties and also for tanning of leather. The use of the pomegranate juice, peel and oil has been indicated that pomegranate have anticancer activities, including interference with tumour cell proliferation, cell cycle, invasion and angiogenesis. Pomegranate can be propagated from seeds as well as softwood, semi-hardwood and hardwood cuttings. External auxin application encourages rooting in stem cutting owing to their ability to active cambium

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regeneration, cell division and cell multiplication (Rymbai and Reddy 2010). PHB a non-auxinic compound also has the capacity to synergize the root promoting action of auxins (Kumar *et al* 1995). Phenoxy compounds also enhances the formation of root co-factor rhizocaline. It has been reported that IBA enhances the rooting by increase of internal free IBA or modifying the action of IAA (Krieken *et al.*, 1990). It has been found that season of planting and application of growth regulators largely influence the rooting. The cuttings taken 45 days before sprouting in trees gives better success percentage (Kahramanoglu and Umar 2018) whereas cuttings taken in January gave better sprouting percentage (Singh *et al.*, 2011). Hence, to meet the demand of pomegranate in the current situation and owing to its importance, the present study was undertaken.

MATERIALS AND METHODS

The present study was carried out at the nursery of Department of Horticulture, Khalsa College, Amritsar during 2019-20.

The cuttings were taken from healthy uniform pomegranate trees of cultivar Bhagwa from pomegranate block of college orchard. The shoots selected for preparation of cuttings were healthy and disease free. The cuttings of 20 cm length having 3-6 buds were taken with preferably pencil thickness. A round cut was given at the upper side and a slanting cut was given at the lower end of the cuttings on the beds prepared for the purpose by mixing sand, soil and farmyard manure in equal proportions. While planting about 2/3rd length of cutting was buried in the soil, leaving 1/3rd part exposed to the environment. The cuttings were planted 10 cm apart with a row to row distance of 30 cm in the first week of August and January. They were dipped for 2 minutes in different solutions containing IBA, PHB and NAA each @ (500, 750 and 1000 ppm) along with control comprising of total 10 treatments. For treatment soaking method of cuttings was used. 1000 ml growth regulator solution of appropriate concentration was taken in beaker and a unit of 20 cuttings was placed in each approximately 3-4 cm of the basal ends of cuttings were dipped in solution for 2 minutes in quick dip treatment. In case of control, the cuttings were immersed in distilled water for the same period of time. Various observations on sprouting (%), rooting (%), number of roots, length of roots (cm), fresh weight of roots (g), dry weight of roots (g) and survival (%) in the nursery were recorded after 120 days of planting. Field observations were statistically analysed by using randomized block design with factorial arrangement and the data was analysed through EDA software (Elementary Designs Application).

RESULTS AND DISCUSSION

Days to first sprouting

According to the results as shown in Table 1, minimum days taken to sprouting (11.06) were noticed with the application of IBA 1000 ppm (T_3) while the untreated cuttings (T_{10}) took maximum (15.50) days. Lesser days taken for sprouting might be due to the better utilization of stored carbohydrates, nitrogen and other factors with the application of auxins (Chandramouli 2001). Enhanced IBA concentration in the cell might have increased the cell division resulting in quick callus formation in the hardwood grape cuttings as reported by Patil *et al.* (2000). Also the external application of auxins led to the promoted growth and produced more favourable conditions for sprouting of dormant buds on the cuttings (Hartmann *et al.*, 2002). The present results are in corroborates with the research findings of Thota (2012) in fig cv. Poona. Rafael (2005) and Adelson (2009) in olive also reported the same. Regarding the planting time, the pomegranate cuttings planted in January took lesser days (11.63) as compared to the planting in August which took 12.80 days. This might be attributed to the reason that the environmental conditions relative to temperature, humidity and light prevailing at that time, might have reflected the detrimental effects on the sprouting of cuttings (Singh *et al.*, 2011).

The interaction of planting time and treatments were found to be significant statistically. Minimum days taken to first sprouting (10.33) were recorded under IBA 1000 ppm (T_3) during January while maximum days to sprouting (15.80) were found in T_{10} (control) in the month of August.

Sprouting percentage

It was noted and depicted in Table 1 that maximum sprouting (91.10%) was recorded with the application of IBA 1000 ppm (T_3). Minimum sprouting (73.83%) was observed in cuttings under control (T_{10}). This might be attributed to cell division stimulated by the auxin at sprout-union initiation (Seiar 2017). Hydrolysis and translocation of carbohydrates and nitrogenous substances results in the accelerated cell growth and division which might be triggered by use of auxins. It also tend to promote the histological features like formation of callus and tissues and then further differentiation of vascular tissues (Singh 2017). The possible explanation to this lies in better development of root system with good quality root and shoot parameters enabling the rooted cuttings to make better growth under field conditions after plantation and there by accounted the highest field survivability (Sharma *et al.*, 2009). These findings are in agreement with the research work of Ram *et al.* (2005) in pomegranate cv. Ganesh and Kandhari and Shukla *et al.* (2010) in peach. Planting time also tend to have significant influence on the sprouting percentage of planted cuttings. Cuttings planted in the month of January tend to have maximum sprouting (87.83%), while minimum sprouting percentage was recorded in the cuttings of August plantation accounting to be 76.84 per cent. This is often a refraction on the response of cuttings to environmental conditions such as low relative humidity and high temperature (Yagoub 1998). The availability of reserved food substrates in cuttings also tend to help them in more sprouting. This data is in accordance with Singh *et al.* (2011) and Singh (2013) in pomegranate. Interaction of PGR's and planting time was found to be non significant statistically.

Survival percentage

Maximum survival (88.31%) was noted in the cuttings treated with IBA 1000 ppm (T_3) (Table 1). The untreated cuttings (T_{10}) were recorded minimum (66.09%) survival percentage. This can be attributed to better sprouting and root growth due to the superiority of treated cuttings with the auxins. The facilitated absorption of nutrients and moisture from soil and better growth might have developed capacity to withstand for a longer period (Ram *et al.*, 2005). The research findings of Iqbal *et al.* (1999), Kaur and Kaur (2016) in pomegranate cv. Ganesh, Thota (2012), Kishorbhai (2014) and Kaur *et al.* (2018) in fig support the present results. According to the results cuttings planted in the month of January resulted in maximum survival (84.70%), whereas minimum survival percentage (70.70) was noted in August plantation. More survivability in cuttings might be due to catalyzing of stored food resources until better root and shoot

development. The present findings corroborate the research findings of Singh *et al.* (2003) in long pepper (*Piper longum* L.) cuttings and Mehta *et al.* (2018) in pomegranate. Interaction between PGR's and planting time were found to be in non-significant statistically.

Rooting percentage

According to the results shown in Table 2, IBA 1000 ppm (T_3) tends to have resulted in maximum rooting percentage (83.31). Minimum rooting percentage (59.15) was noted in

the cuttings which were under control (T_{10}). Formation of roots is likely to be favoured by high carbohydrates and low nitrogen. Polat and Caliskan (2009) reported that IBA 1000 ppm improved root characteristics. The better rooting in cuttings treated with auxin might be due to the enhancement in hydrolysis activity which favours the formation of high carbohydrates and low nitrogen and leads to the increment in root formation (Narula 2018). The findings are in line with Kumar *et al.* (2004) in lime and Tripathi and Shukla (2004) in pomegranate and Husen (2008) in *Dalbergia sissoo*.

Table 1: Days to first sprouting, sprouting percentage (%) and survival percentage (%) in pomegranate cuttings cv. Bhagwa as influenced by PGR's and planting time.

Observations → Treatment ↓	Days to first sprouting			Sprouting percentage (%)			Survival percentage (%)		
	August	January	Mean	August	January	Mean	August	January	Mean
T_1 : IBA 500 ppm	12.40	10.80	11.60	76.66	88.33	82.49	69.98	85.53	77.75
T_2 : IBA 750 ppm	11.60	11.20	11.40	79.44	91.66	85.55	73.88	88.86	81.37
T_3 : IBA 1000 ppm	11.80	10.33	11.06	84.99	97.22	91.10	82.20	94.43	88.31
T_4 : PHB 500 ppm	13.33	11.40	12.36	73.88	86.10	79.99	65.54	82.75	74.14
T_5 : PHB 750 ppm	11.73	11.33	11.53	76.66	88.33	82.49	72.76	85.53	79.15
T_6 : PHB 1000 ppm	13.00	10.86	11.93	81.22	90.55	85.88	77.21	88.33	82.77
T_7 : NAA 500 ppm	12.46	11.93	12.20	71.66	83.88	77.77	64.41	79.98	72.20
T_8 : NAA 750 ppm	13.33	11.26	12.30	75.55	85.00	80.27	68.86	81.65	75.25
T_9 : NAA 1000 ppm	13.26	12.00	12.63	78.88	88.88	83.88	73.31	86.65	79.98
T_{10} : Control (Distilled water)	15.80	15.20	15.50	69.44	78.33	73.88	58.86	73.31	66.09
Mean	12.87	11.63		76.84	87.83		70.70	84.70	
	Days to first sprouting C.D. ($p=0.05$), Treatment = 1.82 Time of planting = 0.81 Interaction (Treatment × Time of planting) = NS			Sprouting percentage (%) C.D. ($p=0.05$), Treatment = 0.48 Time of planting = 0.22 Interaction (Treatment × Time of planting) = 0.68			Survival percentage (%) C.D. ($p=0.05$), Treatment = 3.40 Time of planting = 1.52 Interaction (Treatment × Time of planting) = NS		

Table 2: Rooting percentage (%), number of roots per cutting and average root length (cm) in pomegranate cuttings cv. Bhagwa as influenced by PGR's and planting time.

Observations → Treatment ↓	Rooting percentage (%)			Number of roots per cutting			Average root length (cm)		
	August	January	Mean	August	January	Mean	August	January	Mean
T_1 : IBA 500 ppm	62.76	79.98	71.37	38.73	52.00	45.36	7.06	9.12	8.09
T_2 : IBA 750 ppm	68.31	82.21	75.26	46.73	57.86	52.30	7.48	9.90	8.69
T_3 : IBA 1000 ppm	77.20	89.43	83.31	55.20	64.86	60.03	10.38	12.73	11.55
T_4 : PHB 500 ppm	58.31	75.53	66.92	33.00	49.73	41.36	5.30	8.16	6.73
T_5 : PHB 750 ppm	65.55	78.86	72.20	41.40	54.20	47.80	6.96	9.33	8.14
T_6 : PHB 1000 ppm	71.65	82.75	77.20	46.20	60.73	53.46	7.69	10.76	9.22
T_7 : NAA 500 ppm	57.20	74.43	65.81	30.26	46.53	38.40	5.52	8.49	7.01
T_8 : NAA 750 ppm	62.21	77.76	69.99	37.00	50.33	43.66	5.66	8.12	6.89
T_9 : NAA 1000 ppm	65.53	80.55	73.04	40.20	54.46	47.33	6.66	9.00	7.83
T_{10} : Control (Distilled water)	51.65	66.65	59.15	25.06	29.86	27.46	4.50	6.76	5.63
Mean	64.04	78.81		39.38	52.06		6.72	9.23	
	Rooting percentage (%) C.D. ($p=0.05$) Treatment = 2.40 Time of planting = 1.07 Interaction (Treatment × Time of planting) = NS			Number of roots per cutting C.D. ($p=0.05$) Treatment = 1.40 Time of planting = 0.62 Interaction (Treatment × Time of planting) = 1.98			Average root length (cm) C.D. ($p=0.05$) Treatment = 0.86 Time of planting = 0.38 Interaction (Treatment × Time of planting) = NS		

Maximum rooting percentage was recorded in cuttings planted in January depicting 78.81 per cent while minimum rooting (64.04%) was observed in August. The findings are in correlation with the results reported by Kumar *et al.* (2016). The enhanced hydrolytic activity in presence of applied IBA coupled with appropriate planting time might be responsible for the increased percentage of rooted cuttings (Mehta *et al.* 2018). The findings of present study are supported by Pirlak (2000) in Cornelian cherry (*Cornus mas* L.) and Sharma *et al.* (2009) in pomegranate cv. Ganesh. Interaction between PGR's and planting time were found to be non-significant statistically.

Number of roots per cutting

Maximum number of roots (60.03) per cutting were found in the cuttings treated with IBA (1000 ppm) (Table 2). Least number of roots *i.e.* 27.46 per cutting were counted in untreated cuttings under control. The production of a good amount of roots in auxin treated cuttings pertains to the fact that the auxins promoted cell division and their elongation which led to differentiation of cambial initials into root primordia and in the mobilization of reserve food material to sites of root initiation there by giving higher number of roots per cutting (Sharma 1999). These findings are in agreement with the research work of Tripathi and Shukla (2004) in pomegranate, Kaur *et al.* (2018) in fig, Diwaker and Katiyar (2013) in kagzi lime, Shukla *et al.* (2010) in peach, Kumar *et al.* (2004) in lime, Ram *et al.* (2005) in pomegranate cvs. Ganesh and Kandhari, Rafael (2005) and Adelson (2009) in olive and Kaur and Kaur (2016) in pomegranate cv. Ganesh. Season of planting exhibited significant influence on the number of roots developed per cutting. Maximum roots (52.06) were explored in the cuttings planted during January while the number of roots (39.38) appeared to be lesser in August plantation. The variation in root formation

in cuttings in both the planting times might be due to the indulgence of environmental factors relative to fluctuations in temperature, humidity and light. Also, auxins might have triggered the hydrolysis and translocation of carbohydrates and nitrogenous substances at the base of cuttings responsible for cell elongation and cell division in suitable environment (Hartmann *et al.*, 2007). The present findings are in line with the research findings of Kumar *et al.* (2016) and Mehta *et al.* (2018) in pomegranate who advocated the results of present study. Interaction was found to be significant between PGR's and planting time, maximum number of roots were found in IBA 1000 ppm (T_3) in the month of January which registered (64.86) roots while minimum number of roots (25.06) were recorded in Control (T_{10}) during August. The given results are in agreement with the findings of Mehta *et al.* (2018) in pomegranate.

Average root length (cm)

Maximum average root length (11.55 cm) was measured in the cuttings treated with IBA 1000 ppm (T_3) while minimum average root length (5.63 cm) was measured in cuttings which were under control (T_{10}) (Table 2). Auxins might have helped in amplifying rooting and boosted length of roots as the root elongation stage is very responsive to auxin concentrations (Hartmann *et al.*, 2002). The availability of carbohydrates is often considered exclusively as an energetic requirement and carbon skeleton source to drive root development (Correa *et al.*, 2005). Similar observation have also been reported by Upadhyay and Badyal (2007) in pomegranate, Kumar *et al.* (2004) in sweet lime, Reddy *et al.* (2008), Thota *et al.* (2012) and Kaur *et al.* (2018) in fig, Rafael (2005) in olive respectively.

Maximum length of roots (9.23 cm) was recorded in the cuttings which were planted in January where as the minimum average root length (6.72 cm) was found in

Table 3: Fresh weight of roots (g) and dry weight of roots (g) in pomegranate cuttings cv. Bhagwa as influenced by PGR's and planting time.

Observations →	Fresh weight of roots (g)			Dry weight of roots (g)		
Treatment ↓	August	January	Mean	August	January	Mean
T_1 : IBA 500 ppm	0.41	1.03	0.72	0.24	0.53	0.38
T_2 : IBA 750 ppm	0.50	1.20	0.85	0.28	0.62	0.45
T_3 : IBA 1000 ppm	0.68	1.47	1.08	0.36	0.74	0.55
T_4 : PHB 500 ppm	0.31	0.78	0.55	0.17	0.41	0.29
T_5 : PHB 750 ppm	0.35	0.89	0.62	0.20	0.48	0.34
T_6 : PHB 1000 ppm	0.43	1.07	0.75	0.25	0.56	0.40
T_7 : NAA 500 ppm	0.30	0.74	0.52	0.16	0.40	0.28
T_8 : NAA 750 ppm	0.34	0.85	0.59	0.18	0.45	0.32
T_9 : NAA 1000 ppm	0.38	0.97	0.68	0.22	0.52	0.37
T_{10} : Control (Distilled water)	0.22	0.66	0.44	0.11	0.34	0.22
Mean	0.39	0.96		0.22	0.50	
	Fresh weight of roots (g)			Dry weight of roots (g)		
	C.D. ($p=0.05$)			C.D. ($p=0.05$)		
	Treatment = 0.037			Treatment = 0.02		
	Time of planting = 0.02			Time of planting = 0.01		
	Interaction (Treatment ×			Interaction (Treatment ×		
	Time of planting) = 0.05			Time of planting) = 0.03		

untreated cuttings planted during August (Table 2). It might be due to the favourable climatic conditions pertaining to root length. The present findings are similar to the findings of Panwar *et al.* (2001) in pomegranate cuttings.

Fresh weight of roots (g)

It is evident from the data depicted in Table 3 that maximum fresh weight of roots (1.08 g) was there in the treatment. The roots of the cuttings devoid of any treatment (T_{10}) had the least weight of roots (0.44 g). The increase in root weight might be due to the reserved food materials in the cuttings, the translocation of reserved carbohydrates may have also helped in better root growth and weight (Singh 2013). The findings of the present study are in line with the findings of Chalfun *et al.* (2003) and Kaur *et al.* (2018) in fig, Kaur and Kaur (2016) in pomegranate cv. Ganesh and Deb *et al.* (2009) in lemon cuttings.

It is apparent from the data in Table 3 that the planting time had a considerable effect on the fresh weight of roots. January planted cuttings had maximum root weight (0.96 g) whereas the cuttings planted during August had minimum fresh root weight (0.39 g). The empowering fact that the prevailing conditions at the time of planting was responsible for better root development justifies the present results. Also the transfer of stored food and nutrients from cuttings to rooting part might have helped for its better growth. The present research studies are in agreement with the findings of Panwar *et al.* (2001) in pomegranate cuttings. Interaction between PGR's and planting time were found to be significant statistically. The maximum fresh weight of roots (1.47 g) was found in T_3 (IBA 1000 ppm) treated cuttings planted during the month of January as compared to the untreated cuttings with fresh root weight (0.22 g) planted during August. This might be due to the seasonal variations in humidity, light and temperature parameters. The above findings are in accordance with the research findings of Singh *et al.* (2011), Mehta *et al.* (2018) and Polat and Caliskan (2009) in pomegranate respectively.

Dry weight of roots (g)

From the data in Table 3, it is clear that maximum dry weight (0.55 g) was found under IBA 1000 ppm (T_3) treated cuttings while the cuttings under control were found to have the least dry weight (0.22 g). Increase in dry weight of roots might be attributed to an increase in the root number and length of roots resulted in higher accumulation of dry matter. The present investigation is in positive correlation with the findings of Galavi *et al.* (2013) in grapes and Kaur *et al.* (2018) in fig. According to the results maximum dry weight of root (0.50 g) was found in January planted cuttings with minimum (0.22 g) being observed in the cuttings planted in August. The differences were found to be significant statistically. The increase might be attributed to the higher metabolic reserves for root initiation and its growth as well as higher rooting potential of such cuttings, that led to an increase in fresh and dry weight of root (Singh, 2013). The present results are in positive correlation with Narula and

Kaur (2018) in plum, Singh *et al.* (2011) and Kaur and Kaur (2016) in pomegranate. The interaction between PGR's and planting time were found to be significant statistically with the maximum dry weight of roots (0.74 g) found in T_3 (IBA 1000 ppm) in January planted cuttings whereas minimum dry weight of roots (0.11 g) being noticed in untreated cuttings of August. The prevailing climatic conditions at planting time might have led to the advanced root growth with good dry weight. The findings are in accordance to Polat and Caliskan (2009), Singh *et al.* (2011) and Mehta *et al.* (2018) in pomegranate.

CONCLUSION

As a result of the present study it was observed that among PGR's, the application of IBA 1000 ppm proved to be promising in the regeneration of the stem cuttings of pomegranate cv. Bhagwa. With respect to the planting time, the cuttings of January plantation performed much better in terms of sprouting, survival and root parameters. Hence, it can be concluded that the pomegranate cuttings of cv. Bhagwa propagated with the application of IBA 1000 ppm and planted during the first week of January can help the growers to raise the superior planting material which can improve the yield and fruit quality enabling them to fetch good price in the market and will help to boost the pomegranate cultivation.

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