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Differential Responses of Planting Time and Auxin on Rooting and Growth Behaviour of Hardwood Cuttings of Wild Pomegranate (*Punica Granatum* L.)

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Abstract: An investigation was carried out to elucidate the influence of planting time and auxin treatments on the rooting and growth of hardwood cuttings of wild pomegranate. The experiment was laid out in Randomized Block Design (Factorial) with 28 treatment combinations comprising 7 auxin levels (IBA 2000, 2500 & 3000 ppm, NAA 2000, 2500 & 3000 ppm and control without IBA/NAA) and 4 planting times (15th & 30th December and 15th & 30th January). 2500 ppm of IBA (auxin) was found to be the optimum concentration used and 30th January was the most suitable time for planting hardwood cuttings of wild pomegranate under subtropical conditions to produce quality plants with a good root and shoot system.

Keywords: Hardwood cuttings, Indole-Butyric Acid, N-Acetic Acid, Wild pomegranate

Wild pomegranate belongs to the family Punicaceae and is one of the important fruit scattered in wild form along the roadsides and upper extremities of sub-tropical forests in Western Himalayan regions of India including Himachal Pradesh, Jammu & Kashmir, Uttarakhand, and Punjab (Rana et al., 2022). *Punica granatum* L. originated from Iran and is found in almost all tropical and subtropical regions of the world and is an important fruit tree growing wild in hilly tracts and forests of Jammu and Kashmir state between 1000-2500 m above mean sea level (Ge et al., 2021). These fruits of wild pomegranate are collected by local people for self-use or petty scale to generate a part of their annual income.

The flowering season of wild pomegranate in the North western Himalayan region is observed to be from March to the last week of May and the collection of ripe fruits usually starts during August and continues upto October (Dhandar and Singh 2002). The Wild pomegranate being resistant to drought and diseases requires less after-care and therefore plays an important role in pushing up the economy of farmers of the areas lacking good cash crops. The rooting ability of cuttings is influenced by several factors, such as the genetic background of the rootstock, their horticultural management, and nutritional status, the age of the stock plant, the cutting collection season, the endogenous content of photoassimilates and hormones, the type of cutting, the environmental manipulation of cuttings, the rooting media, and the hormonal treatment of the tissues (Tewfic 2002). The propagation through cutting with the application of plant growth regulators (PGRs) is one of the most common practices. Auxin is one of the most important PGRs regulating the speed of rooting. Plants produce natural auxin in their branches and young leaves, but synthetic auxin should be applied for better rooting. Natural auxins are more sensitive to catabolism enzymes than synthetic auxins. In a study on the effect of different levels of IBA on the rooting of semi-hardwood cuttings like kiwifruit, the best results have been obtained with the treatment of 4000 ppm IBA. There are no systematic plantations of this crop and plants are available mostly in marginally and neglected areas along roadsides due to a lack of vegetative propagation means. Keeping in view these facts, the present study was conducted with objectives to standardize the doses for auxin and planting time for survival and growth of shoots of rooted hardwood cuttings of wild pomegranate.

MATERIAL AND METHODS

The study was carried out at Research Farm, Division of Fruit Science, SKUAST-Jammu, Chatha from 2020 to 2021, situated at an elevation of 300 m above mean sea level and lies at 32˚43" North latitude and 74˚54" East longitude. The climatic is sub-tropical with hot and dry summer, hot and humid, rainy season and cold winter months. The maximum temperature rises to 45˚C during summer and the minimum temperature falls to 10˚C during winter. The mean annual rainfall is about 1000-1200 mm. The meteorological data regarding the climatic conditions that prevailed during the period of experimentation. The soil of the experimental field is sandy loam with pH of 7.5. The available soil nitrogen, phosphorus and potassium were 237.21, 16.23 and 153.25 kg ha^{-1}, respectively.

Treatment details: The experiment was laid out according to randomized block design (factorial) with seven harmonal treatment combinations with dates of planting replicated thrice. Uniform, healthy, disease-free semi-hardwood cuttings of pencil-size thickness were selected from elite mother plants producing good quality anardana with the help of officials of the state horticulture and forest department in Chennai, Jammu. From the selected branches, 20 cm long cuttings having 4 to 5 nodes were taken from the hardwood portion of the branches. IBA and NAA solutions of different concentrations viz., 2000, 2500 and 3000 ppm were prepared by dissolving 2.0 g, 2.5.0 g and 3.0 g of IBA and NAA in distilled water and the volume in each concentration were made up to 1000 ml by adding distilled water**.** The basal 1.5-2.0 cm portion of the cuttings were dipped in IBA and NAA for 5-10 sec before planting. Cuttings were planted on the beds. The beds contained Soil: Sand: FYM. Weeding and watering were done at regular intervals when needed.

Survival percentage: The number of days taken for first sprouting is calculated observing the sprouting of cuttings daily and data was recorded for initiation of sprouting of each treatment**.** The number of shoots per cutting that emerged was calculated after 90 days of planting. The shoot diameter of the longest shoot was recorded using a vernier caliper and was expressed in millimeters. The length of the longest shoot was recorded from each cutting with the help of scale and measured in centimeters, length of the shoot was taken from point of its emergence to the tip of the fully opened leaf.

Leaf characteristics: The number of leaves per cutting that emerged was calculated after 90 days of planting. For measuring leaf area, five leaves were randomly sampled and their cumulative area was recorded with the help of a leaf area meter, Systronics -211 model at 90 days and expressed as average leaf area in square centimeters (cm^2).

Root characteristics: The mean root length of five roots per cutting was calculated by adding the length of each root of the same cutting and dividing it by the number of roots in that cutting. The diameter of the longest root was calculated using the vernier caliper and was expressed in millimeters. The roots detached from the plant and the average fresh weight of leaves and roots was also calculated. This sample taken for calculating the fresh weight was subjected to a temperature of 60˚C for 12 hours in an oven for drying. Electronic weighing balance was used to calculate the dry weight of leaves and roots only after complete drying of leaves and roots has taken place.

Chlorophyll content: Chlorophyll content in leaves of wild pomegranate was determined by the use of chlorophyll meter SPAD-502 manufactured by Konica Minalta Sensing, Inc. Japan. The data was expressed in percentage.

Partitioning coefficient: The partitioning coefficient of root and shoot of wild pomegranate cuttings was determined to know the distribution of photoassimilates in different parts of pomegranate cuttings (shoot and root) following equation was used to determine the partitioning coefficient of specific plant parts. The partitioning coefficient [(dry weight of plant part/total dry weight) x 100] was expressed in percentage.

Statistical analysis: The data analyses were performed using SPSS-21 software (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

The effect of plant growth regulators and planting dates on the survival rate of wild pomegranate indicated that there were no statistically significant differences between treatments (Table 1). Among different planting dates, 30th January planted cuttings showed maximum mean survival percentage followed by 30th December and minimum mean survival percentage was observed on 15th December. Similarly, wild pomegranate cuttings treated with IBA 2500 ppm resulted in the highest survival percentage and the lowest mean survival of cuttings was observed under control. The interaction effect of plant growth regulators and planting dates on survival percentage in wild pomegranate also exhibited positive effects. The planting dates and doses of plant growth regulator exhibited a positive impact on wild pomegranate. The delayed planting reduced root growth and also restricted soil volume exploited by roots and hence nutrient uptake, hormone synthesis and metabolism in the root system resulting in less survival of cuttings. The better development of the root system with good quality root and shoot parameters enables the rooted cuttings to make better use of nutrients resulting in luxuriant growth.

The minimum number of days for supporting when wild pomegranate cuttings were planted on 30th January and treated with IBA 2500 ppm. Cuttings took maximum number of days for sprouting in untreated cuttings planted on 15th December. Treating the cuttings with growth regulators such as IBA results in earliness in sprouting because of better utilization of stored carbohydrates, nitrogen and other factors. Earliness in sprouting, due to timely planting, the increase in the number of sprouts and sprout length may be due to greater utilization of stored carbohydrates, nitrogen and other factors with the help of growth regulators (Chandramouli 2001).

The maximum number of shoots per cutting is observed with IBA 2500 ppm planted on 30th January whereas, the minimum number of shoots per cutting (2.49) was in control planted on 15th December. Treatment of cuttings with IBA causes an increase in the number of roots which in turn results in an increased number of shoots because the increase in the number of roots enhances the nutrient uptake, which affects the cell division and cell expansion in the cambium resulting in an increased number of shoots (Kamboj et al., 2017). Maximum mean shoot diameter was in cuttings treated with IBA 2500 ppm planted on 30th January, while minimum mean shoot diameter was recorded in untreated cuttings on 15th December planting. This may be due to the maximum number of roots, which helped in nutrition and water absorption. The timely planting of cuttings might have resulted in a better-rooting establishment leading to a higher number of leaves, which in turn led to an increase in shoot diameter, gathering more biomass in the shoot.

The length of the longest sprout per cutting was markedly influenced by plant growth regulators and planting dates. The differences in shoot length may be due to better growth of cuttings during January. Poor growth and success of cuttings during December may be attributed to the reduced rate of division of cambial cells, their differentiation and consequent development in the healing of cuttings. This in turn may be due to decreased synthesis of endogenous auxin and mobilization of reserved food material caused can be correlated to higher cell activity and early sprouting which are responsible for a higher number of leaves and shoot length, thus synthesizing more food material and reduced activity of hydrolysis enzymes. The maximum mean the number of leaves per cutting was in wild pomegranate cuttings treated with IBA 2500 ppm planted on 30th January, which was closely followed by cuttings treated with IBA 2000 ppm planted on 15th January while the minimum mean number of leaves was recorded under control planted on 15th December (Table 2). The increased leaves per shoot might be the result of rapid cell division and cell elongation due to the treatment of cuttings with plant growth regulators. Kepinski and Leyser (2005) also observed that increase in the number of leaves was due to the auxin treatment, which increased the development of primary shoots and their number.

The influence on leaf area was observed during the present investigation with pre-treatment of pomegranate cuttings with rooting hormones and different times of planting. Maximum leaf area was in cuttings treated with IBA 2500 ppm planted on 30th January and minimum leaf area was under control when planted on 15th December. Since, the number of green leaves was significantly influenced by variation in dosages of plant growth regulators and consequently the leaf area also showed variation. The increase in leaf area can be attributed to the joint effect of timely planting and conducive soil and climatic conditions having a positive effect on water holding capacity, porosity, soil aeration and supplying a substantial amount of nutrients for good root and shoot growth over control. The maximum mean fresh and dry weight of stem was recorded when cuttings were treated with IBA 2500 ppm, whereas, untreated cuttings recorded minimum mean dry weight of stem (Table 2). The auxins activated shoot growth, which might have resulted in elongation of stems and leaves through cell division accounting for higher dry weight of shoot (Abraham

Table 1. Effect of hormones on survival and shoor parameters at different treatment dates

Treatment details	Survival percent (Number of days taken for first sprouting)					Number of shoots per cutting (Shoot diameter)				
	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean
T_1- IBA 2000 ppm	75.00 (15.60)	74.95 (15.35)	74.60 (15.20)	76.32 (15.08)	75.22 (15.31)	3.91 (2.48)	4.13 (2.51)	4.22 (2.97)	4.45 (3.01)	4.18 (2.74)
T_2- IBA 2500 ppm	75.82 (12.90)	76.09 (12.75)	76.68 (12.42)	76.73 (12.32)	76.33 (12.60)	4.31 (2.52)	4.18 (2.56)	4.56 (3.06)	4.85 (3.11)	4.48 (2.81)
T_3- IBA 3000 ppm	72.51 (17.00)	74.91 (16.85)	74.37 (16.70)	74.19 (16.45)	74.00 (16.75)	3.71 (2.41)	3.97 (2.45)	4.16 (2.74)	4.35 (2.88)	4.05 (2.62)
T_4- NAA 2000 ppm	73.22 (15.56)	75.16 (15.30)	74.31 (15.04)	73.78 (14.92)	74.12 (15.21)	3.31 (2.38)	3.53 (2.42)	4.13 (2.51)	3.92 (2.59)	3.72 (2.48)
T_5- NAA 2500 ppm	74.29 (13.75)	74.49 (13.55)	75.43 (13.30)	75.15 (13.16)	75.24 (13.44)	4.05 (2.49)	4.09 (2.52)	4.20 (2.61)	4.60 (2.65)	4.23 (2.57)
T_6– NAA 3000 ppm	73.06 (17.85)	73.77 (17.50)	72.58 (17.45)	73.71 (17.26)	73.28 (17.52)	3.27 (2.32)	3.75 (2.36)	3.95 (2.51)	3.76 (2.54)	3.68 (2.43)
T_7- Control	50.72 (19.91)	51.59 (19.75)	51.64 (19.43)	53.79 (19.28)	52.24 (19.59)	2.49 (1.08)	2.59 (1.10)	2.91 (1.14)	2.71 (1.19)	2.68 (1.13)
Mean	70.96 (16.08)	71.53 (15.86)	71.37 (15.65)	72.09 (15.50)		3.58 (2.24)	3.75 (2.27)	4.02 (2.51)	4.09 (2.57)	
	D	T	D x T				D	T	D x T	
CD (p=0.05)	1.27 (0.44)	1.67 (0.58)	3.35(1.17)				0.22 (0.22)	0.28 (0.28)	0.39 (0.39)	

Table 2. Effect of hormones on shoot, leaves and biomass at different treatment dates

Treatment	Length of longest shoots					Number of leaves (Leaf area, cm^2)					Fresh weight of shoots (dry weight of shoot, g)				
	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean
T_1-IBA 2000 ppm	22.69	23.72	24.11	24.56	23.77	206.00 (28.57)	208.67 (28.75)	209.00 (29.45)	210.67 (32.96)	208.59 (29.93)	17.89 (10.68)	17.77 (11.11)	17.90 (11.47)	18.58 (11.45)	18.04 (11.18)
T_2-IBA 2500 ppm	25.51	24.69	25.05	26.38	25.41	210.67 (28.71)	209.00 (30.12)	211.33 (31.56)	214.00 (33.45)	211.25 (30.96)	18.26 (11.26)	18.26 (11.30)	18.48 (11..44)	18.87 (12.09)	18.47 (11.52)
T_3- IBA 3000 ppm	22.45	22.93	23.88	23.72	23.25	208.00 (27.42)	207.00 (28.89)	210.33 (28.53)	210.33 (30.45)	208.92 (28.82)	17.82 (10.49)	17.97 (11.16)	18.05 (11.15)	18.54 (11.41)	18.18 (11.05)
T_4- NAA 2000 ppm	21.34	21.35	21.67	23.08	21.86	205.33 (27.98)	208.33 (27.42)	208.33 (29.77)	208.67 (31.52)	207.67 (39.17)	17.56 (10.79)	17.61 (10.88)	18.00 (11.12)	18.58 (11.42)	18.01 (11.05)
T_5-NAA 2500 ppm	23.08	22.97	24.85	24.99	23.97	208.00 (27.45)	208.67 (29.68)	209.00 (29.56)	211.00 (32.29)	209.17 (29.75)	17.03 (11.08)	17.54 (11.51)	18.00 (11.75)	18.64 (12.03)	17.78 (11.59)
T_6– NAA 3000 ppm	21.52	20.08	20.89	21.58	21.02	203.67 (26.98)	206.67 (27.26)	207.33 (27.88)	208.00 (29.85)	206.42 (27.99)	16.78 (10.38)	17.03 (10.64)	17.93 (11.13)	17.90 (11.15)	17.41 (10.82)
T_7- Control	12.65	12.72	13.75	13.55	13.17	169.00 (16.11)	174.33 (16.88)	173.33 (17.52)	172.00 (18.88)	172.17 (17.35)	13.26 (8.21)	13.50 (8.83)	13.90 (9.31)	14.50 (10.02)	15.65 (9.09)
Mean	21.32	21.21	22.03	22.55	21.78	201.52 (26.17)	203.24 (27.00)	204.09 (27.75)	204.95 (29.91)		16.94 (10.41)	17.10 (10.77)	18.04 (11.05)	18.51 (11.37)	
	D	T	D x T				D	T	D x T			D	T	D x T	
CD (p=0.05)	1.70	2.25	4.50			CD (p=0.05)	1.86 (0.08)	2.45 (0.11)	4.91 (0.22)		CD (p=0.05)	0.38 (0.26)	0.51 (0.35)	1.01 (0.70)	

1996). The total chlorophyll content was appreciably influenced by pre-treatment of rooting hormones and time of planting of wild pomegranate found in cuttings treated with IBA 2500 ppm and planted on 15^{th} January after treatment with NAA. However, the lowest chlorophyll content was observed in untreated cutting planted on 15^{th} December (Table 3). This was due to the the abundant supply of nutrients in balanced quantity and more particularly in addition the optimum time of planting cuttings. Wong et al. (1995) reported chlorophyll content increase in hormones treated plots in comparison to control.

A marked influence on the partitioning coefficient was observed during the present investigation with pre-treatment of pomegranate cuttings with rooting hormones and different times of planting. The maximum partitioning coefficient was recorded in cuttings treated with IBA 2500 ppm planted on 30^{th} January whereas, the minimum partitioning coefficient was recorded in untreated cuttings planted on 15^{th} January. Our result is supported by Law and Davis (1990), who reported that a close correlation was observed between the level of tree auxin and the rate of stem growth in a range of genetic lines of peas differing in height. For maintaining the height of the plant, the highest source-sink relationship shows a better partitioning coefficient of the photosynthates towards the stem. However, the present investigation revealed that wild pomegranate cuttings treated with IBA 2500 ppm and planted on 30^{th} January resulted in maximum length of the longest root. However, the minimum length of the longest root was observed in cuttings planted on 15^{th} December under control. Treatment of cuttings with auxins enhances the hydrolysis of carbohydrates, accumulation of metabolites at the site of application, synthesis of new protein, cell enlargement and cell division, which results in increased length of roots in cuttings. Treatment of cuttings with IBA promotes the translocation of metabolites and carbohydrate metabolism, which ultimately affects the beginning of rooting and increases the length of roots and number of roots. Auxins cause hydrolysis and translocation of carbohydrates and nitrogenous substances at the base of cuttings, which results in accelerated cell elongation and cell division in a suitable environment.

The diameter of the longest root was significantly influenced by both rooting hormones and the time of planting of cuttings (Table 4). The maximum diameter of the longest root (1.59 mm) was observed in cutting treated with IBA 2500 ppm and planted on 30^{th} January whereas, a minimum diameter of the longest root was found under control and cuttings were planted on 15^{th} December. Gulifoyle and Hagen (2007) opinioned that auxins would bring about various physiological changes, but the mechanism by which these changes are brought about is not fully understood except for the effect on cell elongation and differentiation. This mechanism of cell differentiation was quite evident in terms of variability in root diameter. Similar results have also been recorded earlier by Bemkaireima et al. (2012) in passion fruit, Seiar (2017) in pomegranate and Rolaniya et al., (2018) in

Table 3. Effect of hormones on chlorophyll and root parameters at different treatment dates

Treatment details	Chlorophyll content (partitioning coefficient of dry shoots)					Average root length, cm (Diameter of longest root, mm)				
	15^{th} December (D_1)	30^{th} December (D_2)	15^{th} January (D_3)	30^{th} January (D_4)	Mean	15^{th} December (D_1)	30^{th} December (D_2)	15^{th} January (D_3)	30^{th} January (D_4)	Mean
T_1- IBA 2000 ppm	45.79 (66.92)	46.54 (67.66)	48.89 (68.79)	51.35 (69.64)	48.14 (68.25)	22.61 (1.40)	23.59 (1.41)	22.85 (1.44)	24.42 (1.48)	23.37 (1.43)
T_2- IBA 2500 ppm	52.79 (70.51)	54.41 (71.45)	55.33 (72.03)	51.78 (75.14)	53.58 (72.8)	25.45 (1.43)	24.68 (1.47)	24.82 (1.52)	26.16 (1.59)	25.28 (1.50)
T_3- IBA 3000 ppm	46.45 (63.32)	48.05 (68.41)	49.31 (69.31)	50.30 (70.34)	48.53 (68.85)	21.44 (1.36)	22.83 (1.38)	23.81 (1.41)	23.66 (1.44)	22.94 (1.40)
T_4- NAA 2000 ppm	41.44 (67.90)	43.67 (66.38)	44.00 (67.60)	45.28 (68.33)	43.60 (66.55)	21.27 (1.28)	21.24 (1.31)	21.58 (1.36)	22.94 (1.39)	21.76 (1.34)
T_5- NAA 2500 ppm	45.93 (67.25)	47.47 (68.32)	51.38 (70.71)	55.22 (70.99)	50.00 (69.32)	22.72 (1.40)	22.89 (1.41)	24.14 (1.43)	24.87 (1.46)	23.66 (1.43)
T_6– NAA 3000 ppm	42.41 (63.96)	41.72 (64.46)	44.99 (67.54)	46.11 (70.80)	43.81 (66.69)	20.74 (1.29)	19.70 (1.31)	20.58 (1.35)	21.38 (1.38)	20.60 (1.33)
T_7- Control	26.45 (56.23)	29.64 (57.12)	29.53 (56.32)	30.59 (56.64)	29.05 (56.58)	12.67 (0.89)	12.72 (0.91)	13.21 (0.94)	13.14 (1.38)	12.93 (0.93)
Mean	43.04 (65.16)	44.50 (66.26)	46.20 (67.47)	47.23 (68.84)		20.99 (1.29)	21.09 (1.31)	21.57 (1.35)	22.37 (1.40)	
		D	T	D x T			D	T	D x T	
CD (p=0.05)		1.94 (1.08)	2.56 (1.43)	5.13 (2.87)		CD (p=0.05)	1.07	1.42	2.85	

Table 4. Effect of hormones on root biomass at different treatment dates

Treatment details	Fresh weight of roots (dry weight of roots. g)				
	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean
T_1- IBA 2000 ppm	0.99 (0.83)	0.98 (0.90)	1.00 (1.00)	0.97 (0.99)	0.99 (0.91)
T_2- IBA 2500 ppm	1.04 (0.93)	1.12 (0.91)	1.02 (1.02)	1.11 (1.07)	1.07 (0.97)
T_3- IBA 3000 ppm	0.90 (0.78)	0.93 (0.89)	0.94 (0.94)	0.96 (0.93)	0.93 (0.86)
T_4- NAA 2000 ppm	0.92 (0.77)	0.97 (0.86)	0.93 (0.93)	0.91 (0.93)	0.93 (0.87)
T_5- NAA 2500 ppm	1.05 (0.89)	1.03 (0.94)	0.99 (0.99)	1.06 (1.03)	1.03 (0.95)
T_6– NAA 3000 ppm	0.89 (0.73)	0.93 (0.89)	0.93 (0.93)	0.95 (0.92)	0.92 (0.85)
T_7- Control	0.71 (0.60)	0.72 (0.63)	0.72 (0.72)	0.74 (0.72)	0.72 (0.65)
Mean	0.93 (0.79)	0.95 (0.86)	0.94 (0.87)	0.96 (0.89)	
		D	T	D x T	
CD (p=0.05)		0.05 (0.04)	0.06 (0.05)	0.12 (0.10)	

grapes. Both rooting hormones and the time of planting of cuttings significantly influenced the fresh weight and dry weight of roots. Maximum fresh and dry weight of root were recorded in cuttings treated with IBA 2500 ppm when planted on 30th January and minimum were observed in cuttings planted on 15th January under control. This may be due to the maximum number of primary and secondary roots, higher length, thickness and perhaps the ability to regenerate further new fibrous roots from main roots, which probably absorb more nutrients and water from the soil and resulted in an increment in weight of roots either fresh or dry (Kathrotia and Singh 1995).

CONCLUSION

The cuttings treated with IBA 2500 ppm and planted on 30th January was the most suitable concentration of IBA and time for planting hardwood cuttings, respectively of wild pomegranate under subtropical conditions to produce quality plants.

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