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Impact of IBA concentrations and growing media on growth and rooting performance of apple rootstock Bud 9

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Abstract

The research experiment was executed to assess the impact of Auxin (IBA) in different concentrations and rooting/growing media on the growth and rooting performance of Bud 9 apple rootstock. The experiment was laid out in a randomized complete block design (factorial) with twelve treatment combinations replicated thrice. It comprised three IBA levels (0, 2000 and 2500 ppm) and four growing media (soil, sawdust, crop residue and FYM). Different levels of IBA, growing media and their interaction significantly affected shoot and root parameters of Bud 9 rootstock *i.e.* length of daughter shoots, the diameter of shoots, number of leaves per shoot, leaf area, leaf chlorophyll content, fresh weight of shoots, dry weight of shoots, percent of rooted shoots, the average number of roots, longest root, shortest root, average root length, root diameter, fresh weight of roots, dry weight of roots and root: shoot (on dry weight basis). The investigation concluded that treatment combination IBA @ 2500 ppm with sawdust as growing media was found to be superior over all other treatment combinations.

Keywords: IBA, growing media, sawdust, apple-rootstock, BUD 9, mound layering

1. Introduction

Apple (*Malus x domestica* Borkh.) is the world's most popular, significant and dominant temperate fruit crop in the North-Western Himalayan region of India. It belongs to the Rosaceae family.

In India, important apple hub states are Himachal Pradesh, Jammu and Kashmir, and Uttarakhand, on a smaller scale in Arunachal Pradesh, Sikkim, Nagaland and Meghalaya. It is cultivated on over 3,13,000 hectares in India, with an output of 24,37,000 MT^[1]. Seedlings as well as clonal rootstocks are utilized for apple production around the world but it is mainly propagated through clonal rootstock. Clonal rootstocks are usually propagated using vegetative methods such as mound layering, cuttings and micro-propagation. Mound layering is a traditional propagation method for mass multiplication of true-to-type planting material. In this methodology, the standing shoots are given cut near the ground level and covered by rooting medium or normal soil to stimulate the development of roots at their bases^[6]. The rooting capacity of clonal rootstocks can be enhanced by using growth regulators like Auxins such as IAA, IBA and NAA as well as a variety of growing conditions. Synthetic auxins are either of the most widely utilized growth regulators for promoting roots. IBA, a synthetic auxin, is frequently utilized because it promotes rooting and generates a fibrous root structure. Rooting can also be increased by the utilization of organic as well as inorganic substrates as the growing medium. Rooting media such as FYM, sawdust and crop residue, etc. allows plants to achieve optimal nutrient intake, growth and development as well as maximize water and oxygen retention. It also improves the physical and biological qualities of the soil and increases the rooting percentage of apple rootstocks. The objective of the research was to assess the influence of IBA and growing/rooting media on the growth and rooting of rootstocks in mound layers.

2. Materials and Method

Mother stools of Bud 9 apple clonal rootstock were imported for research trial from the USA, by the College of Horticulture in February 2020 and planted for multiplication at Experimental Farm of the Department of Seed and Science and Technology, Dr. YSP UHF Nauni, Solan, HP 173230. in 2021.

After establishment in the field, the mother stools of Bud 9 rootstock were cut back to 2 inches above ground level in February 2021. In the spring season, the new stool shoots emerged near the cut portion the of stub and were named daughter shoots. The prepared concentrations of IBA solution were applied to the basal portion of shoots in the month of May (2021) when shoots attained 10 to 15 cm height and mounded up with growing media as per the below treatments. The experiment was designed in RBD factorial to study the impact of different concentrations of IBA and rooting/growing media on rooting in mound layers. Three concentrations of IBA (0, 2000 and 2500 ppm) and four growing media (Soil, Sawdust, Crop residue and FYM) were employed as treatments.

Treatment details

Number of treatment combinations	=	12
IBA levels (A)	=	3 (0, 2000, 2500 ppm)
Growing media (B)	=	4 (Soil, Sawdust, Crop residue, FYM)
Number of replications	=	3
Number of Plots	=	36
Crop	=	Apple rootstock Bud 9
Spacing	=	0.25 m x 1 m
Design of Experiment	=	Randomized Complete Block Design (Factorial)

The observations recorded were the length of daughter shoots, the diameter of shoots, the number of leaves per shoot, leaf area, leaf chlorophyll content, fresh weight of shoots, dry weight of shoots, percent of rooted shoots, the average number of roots, longest root, shortest root, average root length, root diameter, fresh weight of roots, dry weight of roots and root: shoot ratio.

Leaf area was calculated by randomly taking five fully expanded leaves from different shoots per replication. The leaves were taken from the middle portion of the shoot during the month of October. Leaf area was measured with the help of the 'LI-COR' leaf area meter (Model LI - 3000C) and was expressed in square centimetres (cm²). For the estimation of leaf chlorophyll content, five leaves were collected from the field per sample then leaves from each sample were thoroughly washed and chopped into fine pieces under

subdued light and 100 mg of chopped material was placed in a vial containing 7 ml of dimethyl sulphoxide (DMSO). The contents of the vials were incubated at 65 °C for 30 minutes and then the extract was transferred to the graduated test tube and the final volume was made to 10 ml with dimethyl sulphoxide. The optical density (OD) of the above extract was recorded on Spectronic 20D at 645 nm and 663 nm wavelength against a DMSO blank and total chlorophyll content was calculated by using the following formula:

$$\text{Total chlorophyll (mg g}^{-1}\text{)} = \frac{20.2 A_{645} + 8.02 A_{663}}{A \times 1000 \times W} \times V$$

Where

V=Volume of extract used

A =Length of the light path in cell (1 cm)

W =Weight of the sample(g)

A₆₄₅=Absorbanceat645nm wavelength

A₆₆₃=Absorbance at663 nm wavelength

3. Results and Discussion

It is evident from the data given in Tables 1 to 8 that the different levels of IBA, growing media and their interaction exhibited significant effects on all the shoot and root parameters of Bud 9 rootstock.

In an interaction between levels of IBA and growing media, the maximum length of daughter shoot (143.73 cm), the diameter of shoots (12.12 mm) and the number of leaves per daughter shoot (79.16) was observed in treatment T₁₀ i.e. IBA @ 2500 ppm + sawdust while, minimum no of leaves per daughter shoot (63.10), length (85.60) and diameter of daughter shoots (6.24 mm) were recorded in treatment combination of IBA @ 0 ppm and soil as growing medium i.e. T₁. Our findings corroborate with [9], who reported that the application of IBA at 2500 ppm and 3000 ppm increased stool shoots' growth due to cell division and cell elongation. The present results also got support from the findings of [3], who recorded the highest average linear growth in M7 rootstocks of apples treated with the highest concentration of IBA (2500 ppm). Our findings of the length of daughter shoots, the diameter of daughter shoots and the number of leaves per daughter shoots corroborate the findings of [8], [2] and [17].

Table 1: Impact of IBA and growing media on length of daughter shoot and diameter of daughter shoots in Bud 9 rootstock

	Length of daughter shoot (cm)				Mean (A)	Diameter of daughter shoots (mm)				Mean (A)
	Soil	Sawdust	Crop residue	FYM		Soil	Sawdust	Crop residue	FYM	
0 ppm	85.60	96.40	93.10	88.20	90.82	6.24	8.50	8.41	7.66	7.70
2000 ppm	105.10	114.23	111.10	107.73	109.54	9.15	10.56	9.88	9.22	9.70
2500 ppm	130.13	143.73	140.10	135.10	137.26	9.26	12.12	10.44	9.82	10.41
Mean (B)	106.94	118.12	114.76	110.34		8.21	10.39	9.58	8.90	
CD _{0.05}										
IBA (A)				0.08		0.43				
Growing media (B)				0.09		0.49				
IBA X Growing media (A x B)				0.17		0.86				

Table 2: Impact of IBA and growing media on the number of leaves per daughter shoots and leaf area in Bud 9 rootstock

	Number of leaves per daughter shoot				Mean (A)	Leaf area (cm ²)				Mean (A)
	Soil	Sawdust	Crop residue	FYM		Soil	Sawdust	Crop residue	FYM	
0 ppm	63.10	67.03	66.10	64.11	65.08	25.33	31.03	29.93	30.80	29.27
2000 ppm	68.10	76.11	74.11	71.18	72.37	33.28	39.56	37.32	39.26	37.35
2500 ppm	72.40	79.16	78.99	75.07	76.40	38.80	48.18	44.97	48.03	44.99
Mean (B)	67.86	74.10	73.06	70.12		32.47	39.59	37.41	39.36	
CD _{0.05}										
IBA (A)					0.08	0.27				
Growing media (B)					0.09	0.31				
IBA X Growing media (A x B)					0.17	0.54				

Among interaction, the maximum leaf area (48.18 cm²), fresh weight (143.50 g) and dry weight (74.50 g) of daughter shoot were observed in treatment combination of IBA @ 2500 ppm and sawdust *i.e.* T₁₀ however, higher leaf chlorophyll content (2.35 mg/g) in IBA @ 2500 ppm and FYM *i.e.* T₁₂. Minimum leaf area (25.33 cm²), leaf chlorophyll content (1.43 mg/g), and fresh and dry weight (69.00, 33.00 g) of daughter shoot were recorded in treatment combination of IBA @ 0 ppm and soil *i.e.* T₁. Chlorophyll is responsible for photosynthetic activities. Treatment of IBA and growing medium resulted in initiation, growth and further proliferation of adventitious roots, the more the root growth, the more will be the uptake of minerals, nutrients and water absorption to the shoot growth. Thus, the active shoot growth enriched with more nutrition and more photosynthetic activities enhanced the metabolic

processes in the plant system which ultimately resulted in increased leaf chlorophyll content as well as leaf area. Similar results of increased leaf chlorophyll content aligned with [18], and increased leaf area content with [15]. The maximum fresh and dry weight of daughter shoots is because, IBA 2500 ppm along with growing media sawdust resulted in the development of a healthy root system which promoted a greater number of leaves, greater leaf area and more photosynthetic favoured into the higher accumulation of carbohydrates. A higher rate of photosynthesis favoured more synthesis and assimilation of metabolites in the vegetative part resulting in increased fresh and dry weight of shoots. Our findings of the increased fresh and dry weight of daughter shoots are in line with the findings of [8], [21] and [22].

Table 3: Impact of IBA and growing media on leaf chlorophyll content and fresh weight of daughter shoots in Bud 9 rootstock

	Leaf chlorophyll content (mg g ⁻¹)				Mean (A)	Fresh weight of daughter shoot (g)				Mean (A)
	Soil	Sawdust	Crop residue	FYM		Soil	Sawdust	Crop residue	FYM	
0 ppm	1.43	1.57	1.59	1.62	1.55	69.00	86.33	81.98	79.33	79.16
2000 ppm	1.67	1.71	1.94	2.32	1.91	94.99	121.66	110.66	98.33	106.41
2500 ppm	1.86	1.75	2.09	2.35	2.01	96.83	143.50	126.00	108.29	118.65
Mean (B)	1.65	1.67	1.87	2.10		86.94	117.16	106.21	95.32	
CD _{0.05}										
IBA (A)					0.01	1.10				
Growing media (B)					0.02	1.27				
IBA X Growing media (A x B)					0.03	2.20				

Table 4: Impact of IBA and growing media on the dry weight of daughter shoot and percent rooted shoot in Bud 9 rootstock

	Dry weight of the daughter shoot (g)				Mean (A)	Percent rooted shoot (%)*				Mean (A)
	Soil	Sawdust	Crop residue	FYM		Soil	Sawdust	Crop residue	FYM	
0 ppm	33.00	44.08	42.00	41.66	40.18	43.13 (41.03)	52.30 (46.30)	50.40 (45.21)	47.70 (43.66)	48.38
2000 ppm	49.16	68.66	61.00	53.66	58.12	68.43 (55.79)	74.66 (59.75)	72.30 (58.22)	70.26 (56.93)	71.41
2500 ppm	51.33	74.50	63.97	56.83	61.66	71.13 (57.47)	76.23 (60.79)	75.16 (60.08)	73.40 (58.92)	73.98
Mean (B)	44.50	62.41	55.65	50.72		60.90	67.73	65.95	63.78	
CD _{0.05}										
IBA (A)					0.81	0.14				
Growing media (B)					0.94	0.16				
IBA X Growing media (A x B)					1.63	0.29				

In the interaction between both factors (IBA and growing media), the maximum percent of rooted shoots (76.23%), the average number of roots (13.16), the longest root (32.11 cm) and the shortest root (9.11 cm) were found in treatment T₁₀ *i.e.* IBA @ 2500 ppm and sawdust. However, the minimum percent of rooted shoots (43.13%), the average number of roots (4.84), the longest root (15.63 cm) and the shortest root (2.56 cm) were found in treatment T₁ *i.e.* IBA @ 0 ppm and soil. Exogenous application of auxins in layering leads to the breakdown of starch into simple sugars, which results in increased respiratory activity in regenerating tissues at the

time of initiation of new root primordia. It also leads to increased cell wall elasticity, speed-up cell elongation and cell division resulting in more roots ([4], [11]). IBA favours the conjugation between endogenous IAA and amino acids, which leads to the synthesis of specific proteins necessary for the formation of root initials [13]. This might be the reason for the increase in the number of roots. The above results of an increase in the average percent number of roots including longer and shorter roots have also been found in findings of [5], [12], [14], [20] and [7] in different fruit crops.

Table 5: Impact of IBA and growing media on the average number of roots and longest in Bud 9 rootstock

	Average number of roots				Mean (A)	Longest root (cm)				Mean (A)
	Soil	Sawdust	Crop residue	FYM		Soil	Sawdust	Crop residue	FYM	
0 ppm	4.84	7.08	6.84	5.09	5.96	15.63	20.30	20.15	18.07	18.54
2000 ppm	7.23	10.18	9.02	8.07	8.62	21.32	30.13	26.96	24.14	25.64
2500 ppm	9.04	13.16	11.09	10.10	10.85	23.07	32.11	29.26	27.37	27.95
Mean (B)	7.03	10.14	8.98	7.75		20.01	27.51	25.46	23.19	
CD _{0.05}										
IBA (A)				0.05		0.36				
Growing media (B)				0.06		0.42				
IBA X Growing media (A x B)				0.10		0.73				

Table 6: Impact of IBA and growing media on shortest root and average root length in Bud 9 rootstock

	Shortest Root (cm)				Mean (A)	Average root length (cm)				Mean (A)
	Soil	Sawdust	Crop residue	FYM		Soil	Sawdust	Crop residue	FYM	
0 ppm	2.56	3.21	3.11	2.85	2.93	10.23	19.10	18.23	15.15	15.68
2000 ppm	4.06	7.33	6.74	5.37	5.88	19.82	30.66	28.10	22.26	25.21
2500 ppm	4.93	9.11	7.17	6.18	6.85	20.03	32.16	29.16	24.16	26.38
Mean (B)	3.85	6.55	5.67	4.80		16.69	27.31	25.16	20.53	
CD _{0.05}										
IBA (A)				0.10		0.13				
Growing media (B)				0.11		0.15				
IBA X Growing media (A x B)				0.20		0.26				

Among interaction, maximum average root length (32.16 cm), root diameter (2.34 mm), fresh weight of root (86.16 g) and dry weight of root (50.50 g) were recorded in treatment T₁₀ i.e. IBA @ 2500 ppm + sawdust while, minimum average root length (10.23 cm), root diameter (0.75 mm), fresh weight (28.00 g) and dry weight (13.33 g) of roots was recorded in treatment T₁ i.e. IBA @ 0 ppm + soil. Interaction between IBA concentrations and growing media also exerted a significant effect on the root: shoot ratio. The significantly higher root: shoot ratio (0.73) was observed in IBA @ 2500 ppm and crop residue as a growing medium treatment combination. The minimum root: shoot ratio (0.39) was observed in the treatment combination of IBA @ 0 ppm and soil as the growing medium. The reason for the higher root

length and diameter is the physical properties of rooting media. Growing media not only provides proper aeration, porosity, water retention capacity and ideal conditions for the growth of roots but also hormones and other metabolites improved the length of roots. External application of auxin which stimulated the root growth and further movement of natural auxin and other metabolites in downward directions from the leaves and root tips favourably increased the number of roots and more dry matter accumulation in roots ultimately resulting in higher fresh and dry weight of roots. Similar results of higher root length, diameter, and fresh and dry weight of root have also been reported in findings of [19], [10], [21] and [16] in different fruit crops.

Table 7: Impact of IBA and growing media on root diameter and fresh weight of roots in Bud 9 rootstock

	Root diameter				Mean (A)	Fresh weight of roots (g)				Mean (A)
	Soil	Sawdust	Crop residue	FYM		Soil	Sawdust	Crop residue	FYM	
0 ppm	0.75	0.93	0.81	0.76	0.81	28.00	40.07	38.33	36.33	35.68
2000 ppm	1.07	2.30	1.49	1.14	1.50	46.16	73.00	61.66	49.33	57.54
2500 ppm	1.21	2.34	1.74	1.34	1.66	56.00	86.16	76.33	57.66	69.04
Mean (B)	1.01	1.86	1.34	1.08		43.38	66.41	58.77	47.77	
CD _{0.05}										
IBA (A)				0.01		0.76				
Growing media (B)				0.01		0.87				
IBA X Growing media (A x B)				0.03		1.52				

Table 8: Impact of IBA and growing media on the dry weight of roots and root: shoot ratio in Bud 9 rootstock

	Dry weight of roots (g)				Mean (A)	Root: shoot (on a dry weight basis)				Mean (A)
	Soil	Sawdust	Crop residue	FYM		Soil	Sawdust	Crop residue	FYM	
0 ppm	13.33	21.00	20.07	18.50	18.22	0.39	0.47	0.47	0.44	0.44
2000 ppm	22.00	36.33	30.33	24.33	28.25	0.44	0.52	0.49	0.45	0.48
2500 ppm	26.66	50.50	47.00	31.66	38.95	0.51	0.67	0.73	0.55	0.61
Mean (B)	20.66	35.94	32.47	24.83		0.45	0.55	0.56	0.48	
CD _{0.05}										
IBA (A)				0.48		0.01				
Growing media (B)				0.55		0.01				
IBA X Growing media (A x B)				0.96		0.02				

4. Conclusion

On basis of present investigations, it is concluded that the applications of IBA (2500 ppm) along with growing medium sawdust *i.e.* T₁₀ resulted in better rooting and growth of apple clonal rootstock BUD 9 under mound layering while, treatment while T₁ *i.e.* IBA @ 0 ppm + soil recorded minimum values.

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