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Poonam

Research Scholar, Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Suman Bodh

Assistant Professor, Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Praveen Verma

Assistant Professor, Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Influence of different growing media and IBA concentration on rooting of cutting in mulberry (*Morus alba* L.)

Poonam, Suman Bodh and Praveen Verma

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Abstract

Among the various methods of vegetative multiplication, the use of hardwood cuttings is considered one of the simplest and most cost-effective. Several factors can influence the rooting potential of stem cuttings, including the specific requirements of the species and cultivar, the source and type of cutting, the presence of wounds or removal of leaves, etiolation of the stock plant, planting time and growing conditions such as the growth medium, use of hormones, and fertilizer. The experimental objectives were to study the effect of different concentration of IBA on rooting of cuttings in mulberry as well as to find out the influence of different soilless growing media on rooting of cuttings and shoot growth in mulberry. The experiment was laid out in a Completely Randomized Design (CRD) with three replications comprising of seven treatment combinations were prepared viz., T₁, T₂, T₃, T₄, T₅ and T₆. To record the data on various growth parameters like shoot and root parameters were measured. Among all the treatment T₄ showed the best results.

Keywords: Mulberry, coco peat, perlite, vermiculite, FYM, IBA.

Introduction

Over thousands of years, the domestication of Mulberry (*Morus alba*) has occurred, resulting in its adaptation to various tropical, subtropical and temperate zones across Asia, m Europe, North and South America and Africa (Ozgen *et al.*, 2009) ^[20]. There are around 68 species of mulberry (*Morus*) worldwide, with *Morus alba* and *Morus indica* being the most prevalent types (Datta, 2000) ^[5]. The primary use of mulberry globally is for its leaves in the cultivation of silkworms, with China and India being the most significant countries in this regard. The Moraceae family encompasses *Morus alba*, and the majority of the native variants in India belong to the *Morus indica* genus. To reproduce fruit, it is recommended to utilize cultivated mulberry clones. Unlike tissue culture propagation and layering systems commonly used in plant production, grafting and cutting propagation techniques are typically employed (Guo *et al.*, 2007) ^[7]. Among the various methods of vegetative multiplication, the use of hardwood cuttings is considered one of the simplest and most cost-effective. Recently, auxin treatments have been employed to stimulate the development of roots in cuttings (Fourrier, 1984) ^[6]. Several factors can influence the rooting potential of stem cuttings, including the specific requirements of the species and cultivar, the source and type of cutting, the presence of wounds or removal of leaves, etiolation of the stock plant, planting time and growing conditions such as the growth medium, misting, bottom heat, use of hormones, fertilizer and supplemental lighting (Hartmann *et al.*, 2002) ^[11]. In comparison to studies on softwood cuttings, a considerable number of studies on mulberry have predominantly focused on hardwood cuttings and have been conducted on a large scale. In addition, the selection of a suitable rooting medium plays a crucial role in the growth and advancement of plants obtained from stem cuttings (Bakhsh *et al.*, 2000; Yıldız and Koyuncu 2000; Koyuncu *et al.*, 2004) ^[3, 18]. The propagation process necessitates the inclusion of rooting media as a vital component. A recommended combination of peat, perlite, and coco peat in a ratio of 2:2:1 has been found to promote taller plant growth and enhance leaf production in citrus cuttings.

Corresponding Author:

Suman Bodh

Assistant Professor, Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Furthermore, successful results have been achieved by utilizing mixtures comprising vermiculite, peat, and perlite Albouyeh (2007) ^[1]. The auxin hormone, which is wellknown for its role in stimulating plant growth and the development of leaves, fruits, shoots, and lateral roots, is employed in stem cutting techniques. In a study conducted by Sokhuma *et al.*, (2018) ^[28], IBA and NAA, two specific types of auxin, were utilized as tools for stem cuttings. The researchers focused on the rooting and axillary branch outgrowth of mulberry stem cuttings. Interestingly, they discovered that the application of 1,000 mg of IBA and NAA did not have any noticeable impact on the percentage of stem cuttings that successfully rooted (Sokhuma *et al.*, 2018) ^[28]. Mulberry is commonly cultivated through the use of hard wood cuttings due to its various advantages, such as the rapid multiplication of parent material and the ability to maintain desirable plant properties. The timing of cutting preparation plays a crucial role in determining the quantity and success of root development in mulberry. Factors such as the physiological state of the plant and its surrounding environment influence the optimal time for cutting preparation and planting. Additionally, the current weather conditions and outside temperature impact the collection of cuttings and their subsequent rooting success. Other factors that can affect the likelihood of stem cuttings rooting include the plant's juvenility and overall condition, as well as the source, location, and type of cutting taken. Furthermore, any injury or leaf removal can also influence the rooting process. It is important to consider these factors in order to maximize the success of mulberry propagation through stem cuttings (Hartmann *et al.*, 2002) ^[1]. The rooting ability of cuttings is influenced by various internal and external factors, including the type and concentration of hormones present in the cuttings, as well as the variety and planting season. In the context of large-scale mulberry plant reproduction, the application of growth regulators to cuttings has proven to be more advantageous and cost-effective compared to the grafting method (Rao and Khan, 1963) ^[24]. So considering all these, this study aims to evaluated the influence of different growing media and different concentration growth regulator on cutting of mulberry.

Materials and Methods

The study "Influence of different growing media and IBA on rooting of cutting in mulberry (*Morus alba* L)" was conducted in the experiment farm of school of agriculture, department of Horticulture, Lovely professional University under shade net house during the academic year (2023-2024). The variety used for this trial was collected from the field of agriculture farm lovely professional university, Punjab. The main objectives of study the effect of growth regulator on rooting of cutting and influence of different growing media on root and shoot growth of mulberry. The study was conducted using a completely randomized design, consisting of three replications and seven treatments. Each treatment included ten cuttings. One-year-old cuttings of the fruitless cultivar of white mulberry (*Morus alba* L) were acquired from mature and healthy trees in the gardens of the main campus of the University, using a sterilized wood cutter. Cuttings were made using partially mature branches with a thickness ranging from 0.75 to 1.00 cm. These cuttings, measuring 15-20 cm in length and containing 5-6 buds, were prepared for planting by completely removing the leaves. Various growth substrates such as soil, farmyard manure (FYM), cocopeat, vermiculite and perlite were prepared. Subsequently, different concentrations of IBA solution (1000 ppm, 2000 ppm, 3000 ppm) were prepared from the stock

solution in the laboratory. The prepared substrates are then placed into polybags and transferred to the shade net house. The prepared cuttings were immersed in IBA solutions of varying concentrations before being placed into the polybags. The study focused on evaluating the effect of different growing media and IBA concentration on mulberry hardwood cuttings. A total of seven treatment combinations were prepared viz., T₁ (control), T₂ (Soil + FYM + IBA@1000ppm), T₃ (Soil + FYM + IBA@2000ppm), T₄ (Soil + FYM + IBA@3000 ppm), T₅ (Cocopeat + Vermiculite + Perlite + IBA@1000 ppm), T₆ (Cocopeat + Vermiculite + Perlite + IBA@2000 ppm), T₇(Cocopeat + Vermiculite + Perlite + IBA@3000ppm). Various vegetative and root growth parameters were evaluated. After mulberry cuttings, were subjected in poly house, they were investigated in the way maximum number of days required for sprouting per cutting, the number of sprouts per cutting, the percentage of sprouted cuttings, the survival percentage of sprouted cuttings, the number of leaves, the length and width of the leaves in centimeters, the fresh weight and dry weight of the shoots in grams, the number of roots generated per cutting, the number of primary roots, the number of axillary roots, the length of the longest root in centimeters, as well as the fresh and dry weight of the roots in grams.

Results and Discussion

Goal of the experiment was to determine the effect of growth regulator on rooting of cutting and influence of different growing media on root and shoot growth of mulberry (*Morus alba* L). In addition to organic amendments like soil, vermiculite, perlite and cocopeat, the treatments included changes in the concentration of indole-3-butyric acid (IBA).

As per table 1 treatment T₄ (Soil + FYM + IBA 3000 ppm) showed the shortest time, with cuttings sprouting (6.33) as compare to the other treatments followed by T₃, T₂, and T₇. T₁ showed a more days-to-sprouting with a value of (15.33). After conducting a thorough analysis of various parameters, it was determined that the application of IBA at a concentration of 100 ppm is the most effective plant growth regulator for the vegetative propagation of *Morus alba* under the current environmental conditions (Rafeeq *et al.*, 2020). The similar findings have also been reported by Khan *et al.*, (2007) investigated the best Sprouting percentage and number of leaves was found maximum in T₄.

The data pertaining number of sprouts per cutting in T₄ having the maximum number of sprouts (30.33) as per table 1, followed by T₃ and T₂. The minimum number of sprouts shown (14.33) in T₁. Among all the treatments, numbers of sprouted cuttings, length of the roots per cutting, percentage of rooted cutting, lengths of longest root were higher in IBA 2000 mg /L investigated by (Singh *et al.*, 2014) ^[26]. The similar findings have also been reported by Pallavi *et al.*, (2018) ^[22] studied the effect of IBA and NAA on rooting and growth of mulberry cuttings. They found that maximum number of shoots (2.67) and number of leaves (7.31) was observed when treated with 2000 ppm IBA. The minimum value was observed when treated with control. The variation in dose response to the number of roots might be attributed to the varietal and climatic differences in the location.

The sprouting percentage varied significantly between treatments, showing the varying impacts of soil amendments and hormone combinations on the rooting performance of mulberry cuttings. As per table 1 T₄ exhibited the highest sprouting rate (91.11%) than other treatments and T₁ showed the minimum sprouting percentage (35.55%). In case of rooting media, the

maximum number of sprouted cutting, diameter of thickest sprout, number of leaves on new shoots, survival percentage, rooting percentage, number of primary root, length of longest root was observed under vermicompost rooting media by (Singh *et al.*, 2018) [27]. The similar findings reported by Kalyoncu *et al.*, (2009) [15].

Among the treatments, T₄ exhibited the highest survival percentage of sprouted cuttings, with a rate of (95.55%). In T₃ and T₂ survival percentages of were (77.78%) and (66.67%) respectively. On the other hand, the treatment T₁ exhibited lower survival percentage of (40.00%) compared to the other treatments. In terms of the survival percentage of sprouted cuttings in T₇, T₆, and T₅ showed moderate rates with IBA concentrations of 1000, 2000, and 3000 ppm. The role of indole-3-butyric acid (IBA) for rapid propagation of mulberry for higher biomass and large-scale production; and examines the associated biochemical changes during rooting. The non-treated (control) and treated (1000, 2000 and 3000 IBA mg L⁻¹) soft stem cuttings were cultured in mist chamber; IBA increased rooting phenomenon and activated carbohydrate metabolism (Husen *et al.*, 2015) [12]. similar finding also have been reported by Hawramee *et al.*, (2019) [9] cuttings treated with different concentrations of IBA significantly increased success

percentage. the highest values of success rate of rooting 1st and immersed in 1500ppm of IBA solution (T₇), while success rate of rooting (58.33%) and all other qualitative parameters of shoots height were obtained from cuttings taken on the same time but treated with 2000 ppm.

The number of leaves per cutting shown highest in T₄ with the value (25.33) consistently produced competitive or higher leaf counts (25.33) at 90 days after planting, indicating that enhance leaf growth as shown in table 2. On the other hand, T₁ showed less number of leaves (11.33) under controlled conditions (Table 2). The study shows that the effect of IBA concentrations on rooting of mulberry cuttings with varying number of buds, the data of two budded cuttings treated with 3000 ppm of IBA showed at par results with three budded cuttings treated with 3000 ppm of IBA on rooting parameters and also proved that IBA play a significant role in rooting of mulberry saplings (Pooja *et al.*, 2022) [8]. The similar findings have also been reported by Karabulut and Saracoglu (2022) [16], investigated the impact of IBA and cinnamic acid treatments on the rooting of black mulberry cuttings. The results revealed that among the different treatments and periods, the combination of 6000 ppm IBA + 100 ppm CA applied during July and January exhibited superior performance compared to the others.

Table 1: Effect of different treatments on sprouting of cutting

Treatment	Number of days taken for sprouting	Number of sprouts per cutting 30 days after planting	Sprouted cutting percentage (%)	Survival percentage (%) of sprouted cutting
T ₁	15.33 ^a	14.33 ^f	35.55 ^f	40.00 ^f
T ₂	9.33 ^{de}	24.33 ^c	68.88 ^c	66.66 ^c
T ₃	8.33 ^e	26.33 ^b	80.00 ^b	77.77 ^b
T ₄	6.33 ^f	30.33 ^a	91.11 ^a	95.55 ^a
T ₅	13.33 ^b	18.33 ^e	44.44 ^e	46.66 ^{ef}
T ₆	11.67 ^c	20.00 ^e	48.88 ^e	51.11 ^{de}
T ₇	10.33 ^d	22.00 ^d	55.55 ^d	57.77 ^d
CD _{0.05}	1.021	1.85	6.301	8.134

Treatment combination: T₁ (control), T₂ (Soil + FYM + IBA@1000), T₃ (Soil + FYM + IBA@2000), T₄ (Soil + FYM + IBA@3000), T₅ (Cocopeat + Vermiculite + Perlite + IBA@1000), T₆ (Cocopeat + Vermiculite + Perlite + IBA@2000), T₇ (Cocopeat + Vermiculite + Perlite + IBA@3000)

Table 2: Effect of different treatments on shoot characteristics of cuttings

Treatment	Number of leaves			Leaf length(cm)	Leaf width (cm)	Fresh shoot weight(g)	Dry shoot weight(g)
	30 DAP	60 DAP	90 DAP				
T ₁	10.00 ^d	10.66 ^d	11.33 ^d	8.66 ^d	3.06 ^c	29.26 ^a	2.54 ^d
T ₂	16.00 ^{bc}	16.00 ^{bc}	17.33 ^c	18.00 ^{bc}	5.33 ^b	35.35 ^a	6.25 ^{bc}
T ₃	18.00 ^b	18.66 ^b	20.66 ^b	21.33 ^{ab}	6.00 ^{ab}	34.24 ^a	7.26 ^b
T ₄	23.33 ^a	24.00 ^a	25.33 ^a	24.00 ^a	7.00 ^a	37.03 ^a	9.44 ^a
T ₅	11.33 ^d	11.33 ^d	12.00 ^d	11.33 ^d	4.5 ^{bc}	30.50 ^a	3.17 ^d
T ₆	12.66 ^{cd}	13.33 ^{cd}	14.00 ^{cd}	16.66 ^c	4.66 ^b	32.17 ^a	3.50 ^d
T ₇	13.33 ^{cd}	15.33 ^c	16.00 ^c	17.00 ^{bc}	5.00 ^b	34.79 ^a	4.60 ^{cd}
CD _{0.05}	3.364	3.18	3.36	4.314	1.41	N/A	2.122

Treatment combination: T₁ (control), T₂ (Soil + FYM + IBA@1000), T₃ (Soil + FYM + IBA@2000), T₄ (Soil + FYM + IBA@3000), T₅ (Cocopeat + Vermiculite + Perlite + IBA@1000), T₆ (Cocopeat + Vermiculite + Perlite + IBA@2000), T₇ (Cocopeat + Vermiculite + Perlite + IBA@3000)

The length of the leaves per cutting was significantly affected by the concentrations of IBA and the various soilless media used. The longest leaf length of (24.00 cm) was observed in T₄ as shown in table 2. It was statistically at par with treatments T₃ and T₂ (Soil +FYM) with different concentration of IBA (1000, 2000ppm) having value (21.33 cm), (18.00 cm). On the other hand, the smallest leaf length of (8.66 cm) was observed in T₁. The study's findings demonstrate that the amount length of leaves was significantly increased by applying 3000 ppm IBA. A similar result was found by Polat, (2008) [21] the effect of Indole butyric acid on rooting of mulberry Cuttings four local cultivars of mulberry were assessed for IBA rooting. One-year-old shoots were cut into 20-25 cm lengths throughout the

dormant stage. Before planting, basal sections (1-2 cm) of the cutting were treated with IBA solution at different doses for 10 seconds. After 93-118 days of cutting, rooting proportion, root number per cutting, and average root length (cm) were measured. Cuttings treated with 5000 ppm IBA provided the best rooting performance. The data clearly indicates that both the IBA concentrations and various soilless media have a significant impact on the width of the leaves per cutting T₄ having the maximum width of the leaf, measuring (7.00cm) as shown in table 2. Conversely, the T₃ and T₂ treatments had narrower leaf widths compared to T₄, with T₃ measuring (6.00cm) and T₂ measuring (5.22 cm). The control T₁, displayed the minimum leaf width of (3.07cm) than all other treatments.

Among the treatments, treatment T₄ exhibited the highest fresh shoot weight, measuring (37.03 g) T₄ treatment having the width of the leaf, measuring (7.00 cm) as shown in table 2. The control group (T₁) showed lower shoot weights compared to the all treatments. The treatment with the highest dry weight of shoot

was observed in T₄ (9.44 g) followed by T₃ a dry shoot weight of (7.26g), T₂ produced a dry shoot weight of (6.25 g). Control (T₁) showed the lowest dry weight of shoot, measuring (2.54 g) then all other treatments as shown in table 2.

Table 3: Effects of different treatments on root characteristics of cutting

Treatment	Number of roots generated per cutting	Number of Primary Roots	Numbers of axillary roots	Length of the longest root (cm)	Fresh Root weight(g)	Dry Root weight(g)
T ₁	4.00 ^d	4.06 ^d	4.13 ^d	11.00 ^d	2.70 ^d	1.13 ^c
T ₂	7.33 ^{abc}	6.19 ^{bc}	6.23 ^{bc}	18.53 ^b	5.83 ^{bc}	1.70 ^b
T ₃	8.00 ^{ab}	7.83 ^{ab}	7.60 ^b	19.50 ^b	6.85 ^{ab}	2.16 ^a
T ₄	9.00 ^a	9.60 ^a	9.40 ^a	25.06 ^a	7.41 ^a	2.46 ^a
T ₅	4.66 ^d	3.96 ^d	3.96 ^d	14.00 ^{cd}	2.26 ^d	1.26 ^{bc}
T ₆	5.66 ^{cd}	5.19 ^{cd}	4.93 ^{cd}	15.90 ^{bc}	2.90 ^d	1.40 ^{bc}
T ₇	7.00 ^{bc}	5.39 ^{cd}	5.40 ^{cd}	17.56 ^{bc}	5.16 ^c	1.60 ^{bc}
CD _{0.05}	1.76	1.86	1.53	3.88	1.20	0.45

Treatment combination: T₁ (control), T₂ (Soil + FYM + IBA@1000), T₃ (Soil + FYM + IBA@2000), T₄ (Soil + FYM + IBA@3000), T₅ (Cocopeat + Vermiculite + Perlite + IBA@1000), T₆ (Cocopeat + Vermiculite + Perlite + IBA@2000), T₇ (Cocopeat + Vermiculite + Perlite + IBA@3000)

As per table 3, T₄ exhibited the number of roots generated was the highest, with a value of (9.00) then all other treatments. T₁ had the lowest value (4) among all treatments. It is evident that the primary roots were most abundant in T₄ with value (9.60). Following closely behind were T₃ and T₂ respectively, with values of (7.83) and (6.19). On the other hand, the control group (T₁) had the lowest number of primary roots, measuring at (4.06). The number of roots generated was the highest in T₄, with a value of (9.40) then all the other treatment. Whereas, T₅ with a concentration of IBA (1000 ppm) showed a value of (3.97), T₆ and T₇ values of (4.93) and (5.40) respectively. T₁ had the lowest value among all treatments (4.13). The data conferred table 3 revealed that the length of longest root was significantly influenced by the use of IBA concentrations and different soilless media. Maximum length of longest root (25.07 cm) was found in treatment T₄. Treatment T₁ (control) recorded minimum average length of longest root (11.00 cm). T₃ and T₂ having the value (19.50 cm) and (18.00 cm). As per table 3, T₄ exhibited a significant rise in fresh root weight (7.42). Similarly, Treatment T₃ showed an elevated fresh root weight, reaching (6.85 g). This highlights the positive impact of using FYM organic amendments on enhancing root biomass accumulation, thereby promoting the robust growth of mulberry cuttings. On the other hand, Treatments T₅, T₆, and T₇ with varying IBA concentrations resulted in fresh root weights of (2.27g), (2.90 g), and (5.17g) respectively. In contrast, Treatment T₁ (control) exhibited the lowest fresh weight (2.70 g) compared to all other treatments. Treatment T₄, resulted in a substantial enhancement in dry root weight, measuring (2.467g). Table 3 highlight that T₃, which involved soil, FYM, and IBA at 3000 ppm, also contributed to an increase in dry root weight. T₅, root weight (1.27 g), T₆, and T₇, resulted in fresh root weight (1.40 g), and (1.60 g), respectively. In contrast, T₁ (control) exhibited the lowest dry weight of (1.13 g) compared to all the other treatments.

The effects of (IBA) dosages and relative humidity on softwood top cuttings of two varieties of black mulberry were examined. Cuttings were applied to the various IBA doses. The maximum root branching value was discovered from Type 3 in 3000 ppm IBA dose application (16.20 number per cutting), and the longest root was determined from 3000 ppm IBA dose of Type 1 (11.23 cm). In a study investigation of IBA at different dose on mulberry cutting (*Morus alba*) was conducted. Which showed that stem cuttings of the plant were subjected to different

treatments; IBA concentration, IBA was found to be most effective at 4000 ppm for best rooting and good root growth, and at 2000 ppm for healthy leaf growth and to enhance the number of primary and secondary roots in Mulberry (Regmi *et al.*, 2022) [25]. The highest root numbers were obtained when 6000 ppm IBA and 2000 ppm Pacroburazol applications. Cekic *et al.*, (2013) [4].

Similar studies also found by Singh *et al.*, (2018) [27], the effect of auxins and rooting media on rooting in stem cutting of mulberry (*Morus nigra* L.). They used the different treatments IBA concentration and rooting media (Sand+ FYM, Vermicompost and Coco peat). In case of IBA concentration, the maximum number of sprouted cutting, average length of longest sprout, length of longest root was recorded under 2000 ppm concentration of IBA. In case of rooting media, the maximum number of sprouted cutting, diameter of thickest sprout, number of leaves on new shoots, survival percentage, rooting percentage, number of primary root, length of longest root was observed under vermicompost rooting media. The basal portions of mulberry hardwood stem cuttings were subjected to different concentrations of IBA using the slow dip.

The similar findings have also been reported by Aziz *et al.*, (2020) [2] the effects of different concentrations of IBA and soaking in water for 24 hours on propagation black mulberry (*Morus nigra* L.) by hardwood cuttings. Effect of individual factors showed that the highest rooting percentage (15%) was achieved in cuttings soaked in water for 24 hours, as well as improving other traits. Also, the best (23.33%) rooting was found in cuttings dipped in 4000 ppm IBA. The findings revealed that the concentration of 3000 ppm of IBA yielded the most favorable results (Sokhuma *et al.*, 2018) [28].

Conclusion

Based on the findings of the current study, it can be concluded that the utilization of various treatments resulted in significant variations in several parameters. These parameters include the maximum number of days required for sprouting per cutting, the number of sprouts per cutting, the percentage of sprouted cuttings, the survival percentage of sprouted cuttings, the number of leaves, the length and width of the leaves in centimeters, the fresh weight and dry weight of the shoots in grams, the number of roots generated per cutting, the number of primary roots, the number of axillary roots, the length of the longest root in centimeters, as well as the fresh and dry weight

of the roots in grams. Notably, treatment T₄ (Soil + FYM + IBA 3000 ppm) exhibited the highest values for all these parameters.

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