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Standardization of Propagation Techniques in Annual Moringa (*Moringa oleifera* Lam.) for Enhancing Crop Uniformity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

An investigation on annual Moringa, *Moringa oleifera* Lam., var., (PKM 1 and PKM2) with the objective of standardizing the method of vegetative propagation was carried out at Horticultural College and Research Institute, Periyakulam. the treatment consisted of three factors viz., cultivars (Factor 1: Moringa variety PKM 1 and PKM2), propagation methods (Factor 2: hardwood cuttings, semi hardwood cuttings and air layering) and application of different concentration of growth regulator IBA (Factor 3:0, 1500, 2500 and 4000 ppm) and the trial was laid out with twenty four

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treatments using Factorial Randomized Block Design with three replications. The overall result revealed that the treatment combinations of PKM 1 air layering treated with IBA 4000 ppm registered the highest dry matter content of root (70.00%) and PKM 1 hardwood cuttings treated with IBA 4000 ppm recorded the highest dry shoot to root ratio (9.19). In PKM 2 semi hardwood cuttings treated with IBA 1500 ppm registered the highest root length (16.30cm) and dry matter content of shoot (61.99%). PKM 2 air layers treated with IBA 4000 ppm took minimum number of days to root (17.99 days), days to sprout (9.85 days) had the highest root fresh weight (2.70g), root dry weight (1.80g), shoot fresh weight (12.52g) and shoot dry weight (6.90g). This study concluded that air layering with 2500 to 4000 ppm IBA treatment is best pro pragation method for PKM1 and PKM 2 annual moringa.

Keywords: Moringa; vegetative propagation; growth regulator; IBA.

1. INTRODUCTION

Moringa oleifera, a significant vegetable crop in the family Moringaceae, is known by numerous names including the Spinach Tree, Mother's Best Friend, Miracle Tree, Horse Radish Tree, Drumstick Tree, West Indian Ben, and Murungai in Tamil [1]. This species is native to North West India and Africa but is now cultivated worldwide in tropical and subtropical regions [2,3]. It can be either a deciduous or evergreen shrub or small tree typically grows between 5 to 10 meters tall. It is known for being a fast-growing tropical perennial with soft wood [4]. *Moringa oleifera* is highly valued for its nutritional content. The leaves, flowers, and pods are especially noted for their high levels of vitamins A, B, and C, as well as riboflavin, nicotinic acid, folic acid, pyridoxine, starch, calcium, iron, and alpha-tocopherol [5]. Its leaves, fruits, flowers, and immature pods are used as vegetables in many countries, including India, Pakistan, the Philippines, Hawaii, and parts of Africa. The plant provides gum (from tree trunks), green manure (from leaves), biogas (from leaves), cosmetic oil (from seeds), wood pulp, rope (from bark), forage for livestock, and powdered seeds for water purification [6,7,8,9,10].

India is the leading producer of Moringa, with an annual production of 2.2 million tonnes of tender fruits, translating to a productivity of 51 tonnes per hectare. Tamil Nadu is the largest producer within India, with an annual yield of 671,000 tonnes from an area of 13,042 hectares [11]. The introduction of annual Moringa cultivars such as 'PKM 1' and 'PKM 2' has increased their popularity due to ease of propagation, convenience in transport, and high yield potential. Though widely used seed propagation leads to variability forced the necessary for producing true-to-type plants with consistent yield and quality. With the rising importance of Moringa in agriculture, studies aim to standardize vegetative propagation techniques for annual varieties like PKM1 and PKM2 was conducted at Horticultural College and Research Institute, Periyakulam.

2. MATERIALS AND METHODS

The experimental materials for the study were comprised of three type of vegetative propagule *viz.,* hard wood cuttings, semi hardwood cuttings and air layering of annual Moringa (*Moringa oleifera* Lam.) var. PKM 1and var. PKM 2 and four IBA concentrations of 1500, 2500 and 4000 ppm. Thus altogether, there were twenty four treatments that were replicated thrice in Factorial Randomized Block Design. The prepared cuttings were kept under mist chamber for hardening.

2.1 Selection of Healthy Branches for Vegetative Propagation

Healthy branch cuttings of both Annual Moringa varieties were collected from the selected mother plants grown in the western block of Horticultural College and Research Institute, Periyakulam. Hardwood cuttings (2-3 cm thickness) and semi hardwood cuttings (1-2 cm thickness) of 25-30 cm long with at least 2 to 3 nodes in the stem were selected and separated. In the basal end slanting cut was given just below the basal node while the top end cut was made straight and 2 to 3 cm above the node. For air layering, juvenile branches were selected with 1 to 2 cm diameter.

2.2 Preparation of Growth Regulator Solution

A stock solution of 10,000 ppm was prepared by dissolving 5 g of IBA in ethanol and making final volume to 500 ml with 50 per cent ethanol (v/v). From the stock solution each of different IBA concentrations viz., 1500, 2500 and 4000 ppm were prepared.

List 1. Treatment combinations

Treatment	Notation	Details
T_1	$V_1P_1G_1$	PKM 1- Hard wood cuttings (Control)
T_2	$V_1P_1G_2$	PKM 1-Hardwood cuttings along with 1500 ppm of IBA
T_3	$V_1P_1G_3$	PKM 1-Hardwood cuttings along with 2500 ppm of IBA
T_4	$V_1P_1G_4$	PKM 1-Hardwood cuttings along with 4000 ppm of IBA
T_5	$V_1P_2G_1$	PKM 1- Semi hard wood cuttings (control)
T_6	$V_1P_2G_2$	PKM 1- Semi hard wood cuttings with 1500 ppm of IBA
T_7	$V_1P_2G_3$	PKM 1- Semi hard wood cuttings with 2500 ppm of IBA
T_8	$V_1P_2G_4$	PKM 1- Semi hard wood cuttings with 4000 ppm of IBA
T_9	$V_1P_3G_1$	PKM 1- Air layering (Control)
T_{10}	$V_1P_3G_2$	PKM 1-Air layering along with 1500 ppm of IBA
T_{11}	$V_1P_3G_3$	PKM 1-Air layering along with 2500 ppm of IBA
T_{12}	$V_1P_3G_4$	PKM 1-Air layering along with 4000 ppm of IBA
T_{13}	$V_2P_1G_1$	PKM 2- Hard wood cuttings (Control)
T_{14}	$V_2P_1G_2$	PKM 2-Hardwood cuttings along with 1500 ppm of IBA
T_{15}	$V_2P_1G_3$	PKM 2-Hardwood cuttings along with 2500 ppm of IBA
T_{16}	$V_2P_1G_4$	PKM 2-Hardwood cuttings along with 4000 ppm of IBA
T_{17}	$V_2P_2G_1$	PKM 2- Semi hard wood cuttings (control)
T_{18}	$V_2P_2G_2$	PKM 2- Semi hard wood cuttings with 1500 ppm of IBA
T_{19}	$V_2P_2G_3$	PKM 2- Semi hard wood cuttings with 2500 ppm of IBA
T_{20}	$V_2P_2G_4$	PKM 2- Semi hard wood cuttings with 4000 ppm of IBA
T_{21}	$V_2P_3G_1$	PKM 2- Air layering (Control)
T_{22}	$V_2P_3G_2$	PKM 2-Air layering along with 1500 ppm of IBA
T_{23}	$V_2P_3G_3$	PKM 2-Air layering along with 2500 ppm of IBA
T_{24}	$V_2P_3G_4$	PKM 2-Air layering along with 4000 ppm of IBA

2.3 Treatments Given to Cuttings and Air Layering Shoots

The basal one third portions of hardwood and semi hardwood cuttings were dipped in 0, 1500, 2500 and 4000 ppm solution of IBA for 5 minutes. In air layering, the bark of the twig (approximately 1 inch wide ring) was removed with the help of knife and 1500, 2500 and 4000 ppm IBA was applied to the wounded surface. Untreated layers served as control. Sphagnum moss about two handfuls moistened with water and thereafter squeezed to remove excess water was placed around the treated area and wrapped with a polyethylene sheet and tied at both ends with thread. After giving above mentioned treatments, the cuttings were planted in poly bags containing the mixtures of 2:1:1 sand: vermicompost: soil. In air layering, when the stem produced root system, a cut was given just below the point of rooting. The separated portion was then transplanted in poly bags kept in mist chamber for hardening.

2.4 Observations Recorded

Five plants per replication were randomly selected for recording observations in each treatment and mean was worked out. By emptying the poly bag and washing the roots with running water on 45th day, length of the primary roots was measured using meter scale and expressed in centimeters. Then the roots were separated and weighed as fresh weight using an electronic balance and expressed in grams. After weighing the fresh weight of root, the samples were oven dried at 105°C for 30 minutes and dried samples were again weighed and expressed in grams. Using the fresh and dry weight of roots dry matter content of root was calculated and expressed in percentage. The success percentage is ratio between the cuttings with roots and without roots. It was calculated and expressed in percentage. Regarding shoot characters, the number of days to sprouting was measured after transplanting the cuttings and air layered stem to polybag and expressed in days. Fresh and dry weight of shoots was measured from the newly developed shoots of 3 leaf stages after detachment from the mother plants was weighed as fresh weight and dried at 105˚C for 30 minutes and expressed in grams. After the secondary nursery, before transplanting the plants were sampled and calculated using the below formula:

$$\text{Dry shoot to root ratio} = \frac{\text{Dry weight of shoot (g)}}{\text{Dry weight of shoot (g)}}$$

3. RESULTS AND DISCUSSION

In the present study, root characters of annual Moringa (PKM 1 and PKM 2) were observed under different concentrations of growth regulators. Among the varieties, PKM 2 exhibited better root characters. This result was confirmed

Table 1. Effect of propagation methods and growth regulators in annual Moringa Var. PKM 1 and PKM 2 for root characters

Treatments	Days to rooting (days)				Root length (cm)				Root fresh weight (g)				Root dry weight (g)				Root dry matter content (%)			
	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4
V_1P_1	30.00	21.70	23.38	25.49	5.30	9.00	7.50	6.00	0.40	1.20	0.80	0.60	0.18	0.80	0.45	0.31	45.00	66.66	50.00	46.66
V_1P_2	27.75	21.56	24.73	26.89	7.00	12.30	10.56	8.15	0.50	1.30	0.70	0.45	0.15	0.70	0.35	0.20	30.00	53.84	50.00	44.44
V_1P_3	25.00	24.10	23.75	21.00	4.60	5.15	7.00	8.25	0.35	0.90	1.40	2.05	0.22	0.45	0.70	1.40	62.85	50.00	50.55	70.00
V_2P_1	28.19	19.00	22.09	26.85	6.87	10.35	9.00	7.57	0.90	1.60	1.35	1.00	0.35	0.90	0.70	0.55	38.89	56.25	51.85	48.00
V_2P_2	25.55	19.10	22.75	23.84	8.55	16.30	14.52	10.25	0.60	1.80	1.20	0.90	0.25	1.00	0.70	0.50	41.66	55.55	58.33	55.60
V_2P_3	23.00	20.15	19.25	17.99	5.00	6.52	8.22	10.00	1.00	1.60	2.00	2.70	0.40	0.85	1.25	1.80	40.00	53.12	62.50	68.00
SE (d)	0.503				0.473				0.048				0.033				1.467			
CD (0.05%)	1.014				0.952				0.097				0.068				2.954			

Table 2. Effect of propagation methods and growth regulators in annual Moringa Var. PKM 1 and PKM 2 for shoot characters

Treatments	Days to sprouting (days)				Shoot fresh weight (g)				Shoot dry weight (g)				Shoot dry matter content (%)				dry shoot to root ratio			
	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4
V_1P_1	30.88	18.40	23.80	27.34	4.81	9.15	7.51	5.78	1.00	5.50	4.00	2.85	20.00	57.89	51.28	47.50	5.40	6.87	8.89	9.19
V_1P_2	23.16	15.76	18.98	21.33	2.89	6.84	5.78	4.57	0.90	4.20	2.90	1.30	30.00	59.15	48.33	27.37	6.00	6.10	8.28	6.50
V_1P_3	18.72	16.53	14.73	12.86	4.19	5.49	7.85	9.49	0.90	2.70	4.10	5.50	20.69	47.37	50.31	55.83	4.09	6.00	5.85	3.93
V_2P_1	26.37	16.72	20.39	23.76	5.87	9.63	8.38	6.93	2.50	6.00	5.00	3.45	40.98	60.00	57.47	47.92	6.54	6.66	7.14	6.27
V_2P_2	21.28	13.57	16.44	18.34	4.52	8.23	6.84	5.78	1.85	5.30	4.10	2.80	39.36	61.99	57.74	46.66	6.90	5.30	5.85	5.60
V_2P_3	13.12	12.16	10.69	9.85	5.20	7.22	10.50	12.52	2.20	3.80	5.50	6.90	40.74	50.66	50.45	53.07	5.50	4.47	4.40	3.83
SE (d)	0.355				0.154				0.014				0.980				0.118			
CD (0.05%)	0.715				0.311				0.284				1.965				0.237			

V_1- PKM 1, V_2- PKM 2; P_1- Hardwood cutting, P_2- Semi hardwood cutting, P_3- Air layering; G_1- Control, G_2- IBA 1500 ppm, G_3- IBA 2500 ppm, G_4- IBA 4000 ppm.

by [12] which stated that easy to root woody plant species develop adventitious roots earlier from phloem ray parenchyma cells. The PKM 2 air layering treated with IBA 4000 ppm recorded early days to rooting, the highest root fresh weight and root dry weight. This might be due to high carbohydrate and low nitrogen to favour the root formation and also the highest roots per layering, increased fresh weight and dry weight of the roots. Air layering technique is successful in propagating plants because, the layered branch is not separated from the mother plant and therefore, receives continuous supply of water and mineral nutrients through the xylem and remain alive [12] and intact shoots (with leaves) possibly synthesize some unknown auxiliary substances which helps in induction of adventitious roots [13]. The root length was found to highest in PKM 2 semi hardwood cuttings treated with 1500 ppm IBA. The PKM 1 air layering treated with 4000 ppm IBA recorded highest dry matter content of root. Probably mature stem portion taken for cuttings and layering had more reserves of carbohydrates, other food materials and had higher C: N ratio that resulted in morenumber of roots. There was a positive correlation between rooting behavior of plum species and C: N ratio. This might be due to increase in the dry mass of layers has been correlated with the rooting ability of layers and rooting may also be related to photosynthesis of layers [14]. The results showed both of dry mass increment and photosynthetic rate leads to increase the dry matter content of root.

3.1 Shoot Characters

Among the varieties, PKM 2 recorded the best performance for all shoot characters except dry shoot to root ratio. It might due to extremely fast-growing genetic character of Moringa tree [15].Among the propagule, air layering recorded early days to sprouting, shoot fresh weight and shoot dry weight invariably in both the varieties growth regulators concentrations. The dry matter content of shoot and dry shoot to root ratio was found to be highest in hardwood cuttings. These results are in line with the earlier finding which reported that increase in production of leaves and leaf area ultimately increased the photosynthesis, relative growth rate and growth of lateral branches of shoots which increased fresh and dry biomass of shoots [16].

With respect to growth regulators, IBA 1500 ppm recorded superior performances for all shoot characters except dry shoot to root ratio. IBA 2500 ppm also recorded higher shoot fresh weight. Exogenous application of IBA had significant influence on shoot parameters, because it enhances hydrolysis of nutritional reserves under the exogenous auxin [17]. PKM 1 Hardwood cuttings treated with IBA 4000 ppm recorded the highest dry shoot to root ratio. It might be due to favourable water relations and high shoot nutrient levels allowed the layers to convert most of their assimilates into new biomass. The PKM 2 air layering treated with IBA 4000 ppm recorded the earliness to sprouting, shoot fresh weight and shoot dry weight. The PKM 2 semi hardwood cuttings treated with IBA 1500 ppm recorded the highest dry matter content of shoot. Removal of endogenous auxin source, leads to a large increase in the cytokinin content of xylem exudates [18]. The effect of auxin on cytokinin concentrations in the xylem suggests that auxin can influence apical dominance *via,* inhibition of cytokinin synthesis or export from the roots [19].

4. CONCLUSION

The experimental results concluded that, for vegetative propagation of annual Moringa air layering the best method for both the varieties PKM1 and PKM2 followed by semi hardwood cuttings. For the rooting and shoot growth IBA could be used at a concentration of 2500 ppm to 4000 ppm.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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