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Effect of indole-3-butyric acid (IBA) on vegetative propagation of a rare medicinal plant *Moringa concanensis* Nimmo ex Dalz & Gibson using stem cuttings

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ABSTRACT

A method for vegetative propagation of *Moringa concanensis* was developed in the present study. Stem cuttings were collected from wild populations in Tamil Nadu. The cuttings with 2-4 nodes were treated with various concentrations of Indole Butyric acid (IBA), Indole Acetic Acid (IAA) and α-Naphthalene Acetic Acid (NAA) for 5 min. The effectiveness of auxins on shoot bud induction and rooting response of stem cuttings was in the order of IBA>NAA>IAA. The auxin treated cuttings were planted on planting media and placed in the greenhouse. The survival rate and relative growth rate of stem cuttings were analyzed at every 4 week interval for 24 week. Stem cuttings treated with 250 mg/L IBA was responded better and survived for two years. Indole-3 acetic acid treated stem cuttings initiated branches within 7 weeks but very few branches arose. The cuttings treated with NAA responded with less relative growth than IBA. This method proves the rooting ability of IBA and growth performance of stem cuttings, which can be used for the mass propagation and conservation of this rare medicinal plant to prevent the exploitation from the wild.

***Keywords*: *Moringa concanensis*; vegetative propagation; auxins; conservation**

1. INTRODUCTION

Moringa concanensis Nimmo ex Dalz & Gibson belongs to the family Moringaceae, the genus *Moringa* consists of about 13 species but only two species recorded in India. It is a glabrous deciduous tree, commonly known as Kattumurungai, Avacunari, Kacappumurunkai, Kanalmurunkai, Kanamurunkai, Karikamuli, Karimurungai etc. in Tamil (Bhamadevi, 2015). The leaves are 10-20 cm long, distant and mostly bipinnate but very rarely tripinnate with 5-6 pairs. Leaflets are elliptic, blunt at the ends, notched at tip and arranged in 4-6 pairs with an odd one at the apex. Inflorescence measures approximately 45 cm long and the white colored flowers are arranged in pubescent panicles. Fruits are straight capsules, triquetrous, and constricted between the seeds. Seeds are white colored with three wings and three angles (Kirtikar and Basu, 1985). Flowering starts in the months of February-April and fruiting from April months onwards (Dey *et al.,* 2014).

M. concanensis is extremely revered for the existence of valuable secondary metabolites. Alkaloids, flavonoids, volatile oils, tannins, saponins, steroids, anthraceneglycosides, cardiac glycosides, xanthoproteins, gums and mucilage are reported from the leaves (Arora *et al.,* 2013; Balamurugan and Balakrishnan, 2013; Bhamadevi, 2015). The seeds and fruits are reported to possess ascorbic acid myristic acid, linoleic acid, oleic acid, palmitic acid, stearic acid and arachidic acid (Dogra *et al.,* 1975; Verma *et al.,* 1976).

The plant has been used as antifertility agent for decades by tribals of Western Ghats. Traditionally the plant have been used to treat inflammation, thyroid complaints, diabetes, fever, sore eyes, jaundice, high blood pressure and skin tumour (Adesokan *et al.,* 2007). It possess noticeable bioactive properties such as analgesic, anti-inflammatory, anticancer, anti-pyretic, antimicrobial, anti-convulsant and antifungal activities (Rao *et al.,* 2008; Jayabharathi and Chitra, 2011; Joy *et al.,* 2015).

Though the plant provides enormous goods for the human welfare, its population is gradually declines. The plant is reported as vulnerable (Soosairaj *et al.,* 2007; Gritto *et al.,* 2012) and rare species (Bhamadevi, 2015) in India. The plant is found in tropical dry forest from south-eastern Pakistan and southern tip of India only (Edeoga *et al.,* 2005).

Vegetative propagation of rare plants through stem cuttings could overcome the limitations of propagation through seeds, such as altered phenotype, seed dormancy and poor viability of the seeds with prolonged time for maturation (Somashekar and Sharma, 2002). But the plants which are raised through vegetative stem cuttings resemble the mother plant and help in maintaining the essential qualities (Henrique *et al.,* 2006). Recently a number of plant species under the threat were conserved through this technique like *Olea europaea* (Negash, 2003), *Cedrus deodara* (Tamta *et al.,* 2007), *Gingko biloba* (Pandey *et al.,* 2011), *Hildegardia populifolia* (Saradha and Paulsamy, 2012), *Paris polyphylla* (Danu *et al.,* 2015) and *Salacia oblonga* (Deepak *et al.,* 2016).

Therefore, the present investigation aims to develop methods for the conservation of *M. concanensis* through vegetative propagation using stem cuttings of a mature tree.

2. MATERIALS AND METHODS

2. 1. Plant material and preparation of cuttings

Field surveys were conducted for selection of mature and healthy plants of *M. concanensis* throughout the coromandel coast of the south India. The plants were identified with the help of "An excursion flora of Central Tamil Nadu and Carnatic" (Matthew, 1982). The juvenile stem cuttings were harvested from the garden maintained healthy trees in the Thiruvannamalai District (Tamil Nadu, India). The stem cuttings with 2-4 nodes were harvested regularly with the help of sterilized wood cutter. Average length of the cuttings used for the study was 55 cm. All the branches were removed, and the cut ends were dipped in 0.1 % bavistin (fungicide, BASF Ltd, Mumbai) solution (w/v) for 5 min subsequently washed with distilled water and treated with various concentrations of different auxins.

2. 2. Preparation of planting medium (soil mixture)

Soil mixture plays important role in induction of roots from the cuttings. The planting medium is composed of a mixture of garden soil, red soil, vermi-compost and farm yard manure in equal ratio (1:1:1:1). All the four components of soil mixture were mixed properly and finally filled in the earthen pots to distribute nutrients uniformly to the growth of the cuttings.

2. 3. Auxins used in roots induction

Three types of auxins [Indole-3-Butyric acid (IBA), Indole-3-Acetic Acid (IAA) and α-Naphthalene Acetic Acid (NAA)] were used to induce roots. The basal end (5.0 cm) of the cuttings were immersed in solutions of different concentrations (50, 100, 150, 200, 250, 300, 350 and 400 mg/L) of auxins i.e. IAA, IBA and NAA for 5-10 min. The pulse treated cuttings were transferred to earthen pots containing soil mixture. Homogenous nodal stem cuttings of comparable size were used for all the experiments. Each pot comprised of a single stem cutting.

2. 4. Greenhouse conditions

Greenhouse plant growth unit lined with netted polysheet was used for adventitious shoot induction in all the stem cuttings procured in the present study. Field soil was used as potting mixture to compare response of stem cuttings in soil as well as soil mixture in greenhouse. The cuttings were maintained in the greenhouse at 28-30 °C temperature with approximately 80-90% relative humidity. The cuttings were immersed for the same time in distilled water as control experiments.

2. 5. Culture Methods

First irrigation to the pots containing stem cuttings was flooded and thereafter, the cuttings were watered with fine jet sprayer once every day, until completion of the experiment. Cuttings were defined as "dead" when severely rotted accompanied by discoloring, drooping or bleaching. The propagation period for the experiment was 28 week.

2. 6. Statistical analysis

The experiments were carried out inside the greenhouse and open fields. The experiments were conducted in randomized block design method with three replicates. The phenotypic observations were taken periodically according to the time period of shoot induction for all the three treatments in greenhouse and in soil (control). The cuttings having at least one shoot were considered for various parameters such as rooting and shooting percentage, and number and length of shoots. The data for shoots length were observed after 30 and 60 days of insertion of stem cuttings in the soil mixture. The data were analyzed statistically using SPSS v.16 (SPSS, Chicago, USA). The significance of differences among mean values was carried out using Duncan's multiple range test or paired sample T test at $P<0.05$. The results are expressed as mean ±SE of three experiments.

3. RESULTS AND DISCUSSION

The vegetative propagation method aids in the conservation and restoration of numerous rare, endangered and threatened taxa. The stimulation of roots and shoots formation in stem cuttings with the aid of exogenous auxins is effective in species which are difficult to propagate (Purohit *et al.*, 2009). The vegetative propagation approach greatly minimizes the exploitation of wild stocks, and this is the first reports on the vegetative propagation of *M. concanensis* through stem cuttings using exogenous auxins. Vegetative propagation of tree species provides the best planting material with highest genetic uniformity, and it can be achieved within short duration than the raising of plants through seeds (Kesari *et al.,* 2009).

3. 1. Effects of auxins on induction of shoots

Auxins untreated (control) stem cuttings did not survive more than two week. All the stem cuttings with 2-4 nodes treated with different types of auxins were survived. Higher survival rates (100%) were observed on IBA treated stem cuttings than the other auxins. At the end of 8 week the induced shoots (4.0) attained maximum of 7.4 cm length on this concentration. Stem cuttings treated with 300 mg/L NAA showed 80% survival after 8 week (3.6 shoots with 5.5 cm length). Only 76% of IAA treated shoots were survived at the 250 mg/L concentration. Maximum 3.2 shoots with 5.3 cm length were observed on IAA. None of the cuttings were survived on lower concentrations of IAA. Induction of shoot buds in most of the stem cuttings was observed after 6 week (Fig. 1A). The response of shoot bud induction on stem cuttings through the treatment of auxins was statistically analyzed after 8 week and presented in Fig. 1B and Table 1.

Table 1. The effect of auxins on shoot bud induction from stem cuttings of *M. concanensis* after 8 week of treatment under greenhouse condition.

Concentration of auxins (mg/L)			Shoot bud induction response (%)	Average no. of shoots per shoot cutting (mean ±SE)	Average length of shoots (mean ±SE)
IAA	IBA	NAA			
50	-	-	44	$1.5±0.16^{a}$	$3.4±0.21^{b}$
100	-	-	51	$1.9±0.13^{b}$	$3.9±0.19^{b}$
150	-	-	59	$2.4±0.25^{c}$	$4.4±0.11^{c}$
200	-	-	64	$2.7±0.11^{d}$	$4.8±0.15^{d}$

250	-	-	76	3.2±0.21e	5.3±0.26[f]
300	-	-	70	2.9±0.16[d]	5.0±0.17[e]
350	-	-	67	2.3±0.10[c]	4.2±0.22[c]
400	-	-	60	2.0±0.00[c]	3.6±0.10[b]
-	50	-	73	2.4±0.12[c]	4.0±0.23[c]
-	100	-	85	2.9±0.17[d]	4.7±0.20[d]
-	150	-	91	3.1±0.29[e]	5.8±0.13[f]
-	200	-	97	3.5±0.10[f]	7.0±0.14[h]
-	250	-	100	4.0±0.32[g]	7.4±0.20[i]
-	300	-	94	3.6±0.11[f]	6.9±0.10[h]
-	350	-	81	3.0±0.20[e]	6.0±0.19[g]
-	400	-	77	2.2±0.13[d]	4.3±0.25[c]
-	-	50	58	1.4±0.00[a]	3.0±0.17[a]
-	-	100	63	1.9±0.22[b]	3.7±0.20[b]
-	-	150	69	2.5±0.19[cd]	4.0±0.15[c]
-	-	200	74	2.7±0.17[d]	4.4±0.10[c]
-	-	250	78	3.1±0.29[e]	4.7±0.22[d]
-	-	300	80	3.6±0.14[f]	5.5±0.19[f]
-	-	350	72	3.0±0.22[e]	5.1±0.10[e]
-	-	400	60	2.5±0.10[cd]	4.3±0.21[c]

Note: Stem cuttings with at least one shoot bud were considered for calculating percentage of shoot bud induction, SE – Standard Error, Level of significance $P<0.05$. All values are an average of 10 replicates.

All cuttings were checked periodically for emergence of shoots or shoot primordial. Though the shoot buds were induced on all the treatments except control, the percentage of response varied with the type and concentration of auxins used. Maximum shoot bud induction and rooting response was observed on stem cuttings pulsed with IBA at 250 mg L^{-1}. The higher concentration of IBA (400 mg L^{-1}) was found to be less effective than the lower concentrations in this study.

Vegetative propagation of economically important, rare and endangered plants using auxins were achieved in *Cedrus deodara* (Nandi *et al.*, 2002), *Robinia pseudoacacia* and *Grewia optiva* (Swamy *et al.*, 2002), *Prunus africana* (Tchoundjeu *et al.*, 2002), *Pongamia* *pinnata* (Kesari *et al.*, 2009), *Onobrychis viciifolia* (Avci *et al.*, 2010), *Gingko biloba* (Pandey *et al.*, 2011), *Salacia oblonga* (Deepak *et al.*, 2016) etc. revealed that the IBA was more effective for shoots and roots induction in stem cuttings.

The development of adventitious roots and shoots from the cuttings were influenced by various internal and external factors (Stefancic et al., 2005). Auxins were used to stimulate adventitious rooting stem cuttings from last three decades (Tchoundjeu *et al.*, 2004; Overvoorde *et al.*, 2010). Role of exogenous auxins for induction of roots of stem cuttings were reported in *Jatropha curcas* (Adekola *et al.*, 2012), *Melissa officinalis* (Sevik and Guney, 2013), *Dillenia suffruticosa* (Abidin and Metali, 2015).

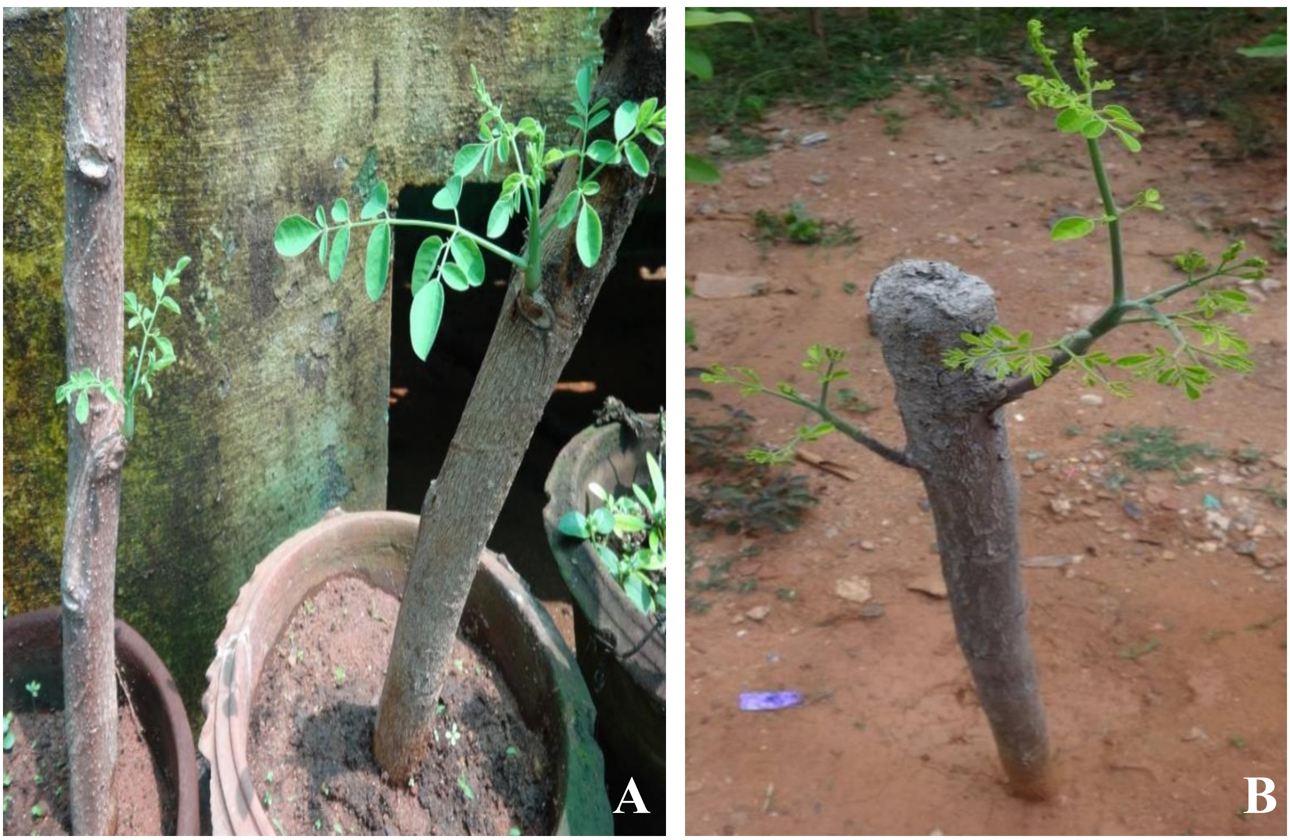

Fig. 1A. Stem cuttings treated with auxins in greenhouse after 6 week.
Fig. 1B. Shoots from the stem cuttings after 8 week (IBA treatment).

3. 2. Effect of auxins on rooting from the stem cuttings

Ninety percentage of stem cuttings on NAA responded with 3.0 roots with the average length of 5.0 cm. Maximum 85% of rooting with 4.5 roots (4.7 cm length) per cuttings was achieved with 250 mg/L IAA. The gradual increase of rooting response was observed till 250 mg/L. Among the different concentrations of IBA used, the highest percentage (cent percentage) of rooting was observed on IBA treated stem cuttings (5.8 roots with 6.2 cm length). Further increase in the concentration of auxins from the optimized concentrations showed a noteworthy decline in the number of roots (Table 2). According to Abidin and

Metali (2015) NAA was the most effective auxin to induce rooting on stem cuttings of *Dillenia suffrutiosa*.

Table 2. The effect of auxins on rooting of stem cuttings.

Concentration of auxins (mg L^{-1})			Rooting response (%)	Average no. of roots per shoot cutting (mean ±SE)	Average length of roots (mean ±SE)
IAA	IBA	NAA			
50	-	-	54	$2.0±0.19^{a}$	$2.7±0.11^{a}$
100	-	-	66	$2.6±0.24^{b}$	$3.2±0.00^{b}$
150	-	-	71	$2.8±0.15^{b}$	$3.7±0.19^{b}$
200	-	-	76	$3.3±0.17^{c}$	$4.1±0.31^{c}$
250	-	-	85	$4.5±0.29^{d}$	$4.7±0.20^{c}$
300	-	-	80	$3.9±0.11^{d}$	$4.0±0.33^{c}$
350	-	-	71	$3.0±0.25^{c}$	$3.7±0.15^{b}$
400	-	-	65	$2.7±0.22^{b}$	$3.1±0.21^{b}$
-	50	-	44	$3.8±0.17^{d}$	$4.3±0.18^{c}$
-	100	-	59	$4.3±0.13^{d}$	$5.6±0.11^{d}$
-	150	-	71	$4.9±0.19^{de}$	$5.9±0.16^{e}$
-	200	-	88	$5.1±0.10^{e}$	$6.0±0.15^{e}$
-	250	-	100	$5.8±0.16^{f}$	$6.2±0.25^{e}$
-	300	-	92	$4.4±0.20^{d}$	$5.9±0.20^{e}$
-	350	-	84	$4.0±0.12^{d}$	$4.8±0.10^{c}$
-	400	-	77	$3.5±0.16^{c}$	$4.1±0.16^{c}$
-	-	50	64	$1.9±0.00^{a}$	$3.0±0.19^{b}$
-	-	100	71	$2.3±0.19^{b}$	$3.6±0.11^{b}$
-	-	150	75	$2.8±0.21^{c}$	$4.1±0.23^{c}$
-	-	200	79	$3.3±0.00^{c}$	$4.5±0.19^{c}$
-	-	250	82	$3.5±0.25^{c}$	$4.9±0.33^{d}$
-	-	300	90	$3.8±0.19^{c}$	$5.0±0.21^{c}$

-	-	350	83	2.7 ± 0.11^{b}	4.0 ± 0.14^{c}
-	-	400	72	2.1 ± 0.16^{b}	3.2 ± 0.20^{b}

Note: Stem cuttings with at least one root were considered for calculating percentage of rooting, SE – Standard Error, Level of significance $P<0.05$. All values are an average of 10 replicates.

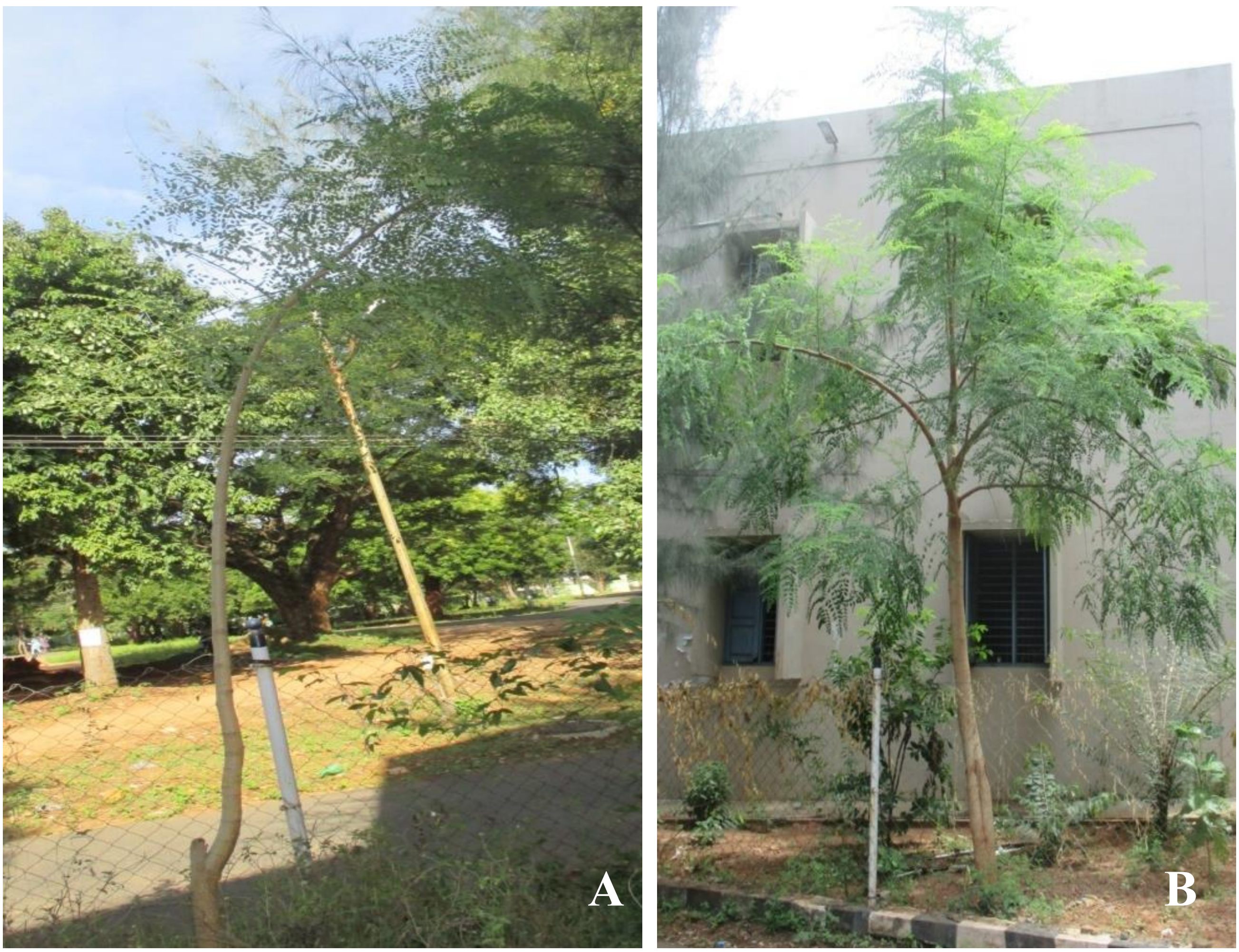

Fig. 2A. Elongated shoot after 4 months.
Fig. 2B. Small tree formed after one year.

Developments of lateral roots are highly dependent on the transport of exogenous auxins (Chhun *et al.*, 2003). Both IAA and IBA were used for the induction of roots in *Malus pumila* (Alvarez et al., 1989), *Prunus species* (Stefancic et al., 2005). Hossain *et al.* (2004) and Martin (2002) reported superior effect of IBA in root induction from the cuttings of *Swietenia macrophylla*, *Chukrasia velutina* and *Holostemma ada-kodien*. IAA and NAA were induced roots on soft wood cuttings of *Centaurea tchihatcheffii* (Ozel *et al.*, 2006).

In the present study it was observed that longer cuttings and low concentrations of auxins promoted better growth as compared to the shorter cuttings and higher concentrations of auxins. Similar reports were made by Kathiravan *et al.* (2009) and Aminul-Islam *et al.* (2010) in *Jatropha curcas*. This is in contrast with the findings of Raha and Roy (2001) and Shrivastava and Barnejee (2008). They reported that the shorter stem cuttings with higher concentration of auxins produced better results in shoot and root formation.

In the present study, stem cuttings sprouted sooner when treated with IBA than the other auxins. After 8 weeks, the stem cuttings with shoots and roots were cautiously removed from the pots and planted to the field conditions (Fig. 1C and 1D).

The maturation of rooting was achieved through the potting media and the conditions of greenhouse. The soil mixture also played a significant role in vegetative propagation of *M. concanensis* through stem cuttings. In contrary, the stem cuttings on soil alone exhibited less sprouting and rooting. Maximum 90% of rooted stem cuttings were successfully survived in the field.

4. CONCLUSION

The vegetative propagation methods for candidate plus trees of M. concanensis were evaluated using different auxins, which ensure conservation through its propagation. Standardization of the vegetative propagation techniques for this rare species using exogenous auxins could prevent over exploitation of this species in nature. Our results on rooting and sprouting response could assure a large scale production of genetically uniform plantlets throughout the year. It offers an alternative conservation tool for the ecorestoration of this valuable genotype.

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