

Effect of auxins on adventitious rooting from stem cuttings of candidate plus tree *Pongamia pinnata* (L.), a potential biodiesel plant

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Abstract *Pongamia pinnata*, commercially important tree species used to produce biofuels, is known for its multipurpose benefits and its role in agro-forestry. Present study examines the amenability of vegetative propagation and effect of maturation in candidate plus tree *P. pinnata* through rooting of stem cuttings treated with varying concentrations and combinations of auxins. The performance of the cuttings was evaluated using SAS GLM software and the data were analyzed as a one-way classified data with and without sub sampling for inferring auxin concentration that can be included in programmes aimed at genetic improvement of the tree species. All auxin treatments promoted sprouting and at lower concentrations triggered/enhanced rooting of cuttings. The effectiveness was in the order of IBA > NAA > IAA when applied singly. IBA at 4.92 mM was found to be most effective where rooting percentage and number of roots were significantly higher ($P < 0.01$) than in control. However higher concentrations of auxins above 7 mM in general inhibited the rooting of cuttings. The interaction among auxins was found to be effective in root induction and differentiation and the most stimulating effects were observed in three-component mixture. The effect of other

cutting characteristics such as juvenility and cutting position on rooting is also discussed.

Keywords Biodiesel · *Pongamia* · Plus tree · Stem cuttings · Vegetative propagation

Abbreviations

CPT	Candidate plus tree
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
NAA	Naphthalene acetic acid
NG	North Guwahati

Introduction

Pongamia pinnata popularly known as ‘Karanj’ or ‘Karanja’ is commercially important tree species used to produce biofuels that can grow on waste land or unproductive land. The tree is adaptable to wide agro climatic conditions. Besides the oil-yielding capacity, its multipurpose benefits as a provider of green manure and medicine and its role in agro-forestry make it a potential candidate for large-scale plantation on marginal lands. It is recommended as a shade tree for pastures and a windbreak for tea (Duke 1983). It can also play a role in rural economy by generating huge manpower employment during various stages of its cultivation as well as during downstream processing (Shrinivasa 2001). However, while utilizing these species as a source of biodiesel, there is a further need for research into various areas of production (Kesari et al. 2008). To increase the biodiesel production it’s important to have unlimited feed stocks of *Pongamia* bearing, high oil-yielding seeds. Thus large-scale plantation of clonal stocks

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of elite genotype needs to be done to encourage afforestation programme.

Tree breeding can be both by sexual and asexual means. Sexual method of propagation through seeds has the limitation that the seedlings raised, do not fully resemble the mother plant and often essential superior qualities or plus-traits of a mother plant fails to get transmitted to the young ones. Furthermore, there are chances that seeds exhibit dormancy or they have poor viability and long generation time before attaining maturity (Somashekar and Sharma 2002). On contrary, in asexual mode of propagation which uses the vegetative parts such as stem cuttings, the progeny always resembles the mother plant in all respects and, helps in maintaining the progeny of elite plants which exhibit special characters and plus qualities (Henrique et al. 2006).

Vegetative propagation is an integral part of tree improvement programmes, as it is needed for establishing clonal seed orchards. Also, mass vegetative multiplication of selected genotypes offers the promise of quick productive gains. In most tree species exogenous application of natural and synthetic auxins facilitates adventitious root production from branch cuttings (Kevers et al. 1997; Henrique et al. 2006). The current research explores the rooting potential in *Pongamia* species. Previous research demonstrates adventitious rooting in juvenile tissues of *Pongamia*; however success rates were correlated with seasons (Palanisamy et al. 1998).

Therefore, the current study was undertaken to examine the rooting ability of mature stem cuttings and regulation by different phytohormones from an elite genotype of *Pongamia*. Rooting was evaluated on stem cuttings treated with varying concentrations and combinations of natural and synthetic auxins, viz., indole-3-acetic acid-IAA, indole-3-butyric acid-IBA, and naphthalene acetic acid-NAA, respectively. Study was also performed to see the synergistic effects on two and three-component mixtures of auxins and effect of maturation in rooting. This is the first report on studying the synergistic effect of phytohormones for adventitious root production from mature stem cuttings of *P. pinnata*. All cuttings were collected from superior genotype of *P. pinnata*, earlier characterized, based on their phenology and reproductive characters from natural populations occurring in North Guwahati, Assam, India (Kesari et al. 2008). The aim was to develop a suitable technique for the large-scale production of superior clonal stock for testing and plantation establishment.

Materials and methods

Selection of planting material

Candidate plus trees of *Pongamia* occurring across ten locations in North Guwahati, were evaluated based on

morphological markers (vegetative and reproductive) to identify potential genotypes that can be included in programmes aimed at genetic improvement of the species. Individuals performing above average for 75% of traits were tagged as the candidate plus trees (Kesari et al. 2008).

Preparation of stem cuttings

Mature stem cuttings of characterized candidate plus tree of *Pongamia* were collected in the month of October from the study site near North Guwahati (Sila forest, Kamrup District, Assam, India). The leaves and shoot apices were excised and uniform leafless semi-hard wood cuttings (15–25 cm long and 0.5–1 cm diameter) comprising 3–4 nodes were prepared. The cuttings were dipped in 0.1% aqueous bavistin (fungicide, BASF India Ltd, Bombay) for 10 min, subsequently distilled water washed and treated with root promoting auxins at the basal end (2–3 cm basal portion). The cuttings received distilled water (control) or treatments of IBA, IAA and NAA individually at different concentration ranging from 1.23 to 7.38 mM, respectively for a maximum of 1 h duration to determine the rooting capacity. Different combinations of above rooting hormones were also tried to see any possible synergistic effects in promoting the rooting ability from the stem cuttings. Cuttings from 2-month-old seedlings of the same mother plant, raised in nursery bed were also used as source material for studying the effect of adventitious rooting and effect of juvenility treated with the best responding concentration. The top (apical) cut ends of the treated cuttings were sealed with paraffin wax to reduce the water loss. Subsequently the cuttings were planted in poly-bags containing sand and clay at the ratio of 1:4 and kept in the mist chamber at $28 \pm 2^\circ\text{C}$ and 70–80% relative humidity. Light intensity was reduced to 22% of ambient sunlight and day-length is 12 ± 1 h throughout. Intermittent mist was supplied for 30 s at 15 min intervals.

Data collection and statistical analyses

After 11 weeks the mature stem cuttings of CPT were stuck, data were recorded for shoot and root traits, viz. (1) number of cuttings that showed sprouting, (2) number of shoots per sprouted cutting, (3) maximum number of primary shoots, (4) shoot length, (5) maximum length of primary shoot, (6) number of cuttings that showed rooting, (7) number of primary roots per rooted cutting, (8) maximum number of primary roots, (9) root length, and (10) maximum length of primary root.

The data were analyzed as a one-way classified data with sub sampling for the traits, number of shoots (2), shoot length (4) number of primary roots (7) and root length (9). The treatments were the different concentrations

of the auxins. There were 26 treatments and 3 replications for each treatment. The sub samples correspond to the 15 cuttings within each replicate. The replications within the treatments do not have the same number of sub samples. ANOVA was performed on the weighted means to account for the variation in the relative contribution of the individual values to the treatment mean. The data was analyzed as a one-way classified data without sub sampling (the measurements are averaged across the 15 cuttings within each replicate) for the remaining traits. In both cases the following ANOVA model was used

$$y_{ij} = \mu + t_i + e_{ij}$$

where j_{ij} is the j th replication of the i th treatment, μ is the overall mean, t_i is the effect of the i th treatment and e_{ij} is the error. The SAS GLM (SAS Institute 1999) procedure was used for the analysis.

Results and discussion

Selection of material

Vegetative propagation forms an integral part of tree improvement programme that provides the best planting stock with highest genetic quality that is not always the case with sexually propagated progenies. The plants can moreover be raised throughout the year and the plantable stock for some species can be obtained in shorter time than those raised through seeds. With this objective in mind, selection of candidate plus trees was embarked.

A critical minimum value (mean performance) for various vegetative and reproductive traits from different populations of *Pongamia* tagged from the study site was done and the tree performing above this average value for 75% of the characteristics for two seasons were designated as candidate plus trees. Table 1 presents the traits that were scored for identification of candidate plus tree. The candidate plus tree is an individual tree of *P. pinnata* possessing superior morphological characters than other individuals of the same species. The data comprised of morphological (vegetative and reproductive) traits measured on 50 trees and each of the 50 trees is a different genotype (Kesari et al. 2008). Ten candidate plus trees were identified based on the morphological markers of which one elite genotype (henceforth referred with original tag number NGPP46) was progressed further for mass multiplication.

Tree breeding consists of selection of superior germ-plasm followed by large-scale propagation of true-to-type plus trees in the seed orchard for the production of improved seeds, which can be used for high quality plantations. The present study on genetic enhancement of potential biodiesel plant, *P. pinnata* is based on the above

Table 1 Plus tree identification characters in *P. pinnata*

Vegetative characters	Reproductive characters
Girth of the main stem at breast level (m)	Number of buds/inflorescence
Plant height (m)	Number of flowers/inflorescence
Number of leaves/g (wt)	Number of seeds/inflorescence
Canopy size (m)	Pod traits, viz., length (cm), breadth (cm) and 100 pod wt (g)
	Seed traits, viz., length (cm), breadth (cm) and 100 seed wt (g), pod to seed ratio

Scoring for traits was done for two seasons in the month of April–June (2006–2007) from naturally occurring populations in North Guwahati, each population consisting of 10 genotypes. Individuals performing above average for 75% of traits were tagged as the candidate plus trees and were subjected to further analysis

principle in which characteristics plus trees were identified based on phenotypic markers from natural populations. Selected elite genotype was used as a source material for large-scale vegetative propagation. Similar studies were performed by Rao et al (2001) for genetic enhancements from natural populations of mangrove trees growing in Pitchavaram, India for successful vegetative propagation.

Effects of a range of auxin type on sprouting and rooting

Auxins are well known to play a significant role in stimulating adventitious rooting from stem cuttings of tree species (Poupard et al. 1994; Tchoundjeu et al. 2004). It has been repeatedly confirmed that auxin is required for adventitious root formation on stems and that the divisions of the first root initials are dependent on exogenous and endogenous levels of auxins (Ludwig-Müller 2000; Kochhar et al. 2005). In addition to enhancing the rate of adventitious roots development, auxin application has been found to increase the number of roots initiated per rooted cutting in a variety of species (Mesen et al. 1997; Palanisamy et al. 1998).

In present study, cuttings from NGPP46 developed sprouts within 10 days and adventitious roots within 3 weeks after planting, although there were significant differences in rooting and sprouting percentage between auxin-treated cuttings and control. All three natural and synthetic auxins resulted in a significant induction and growth of adventitious roots in cuttings. By week 11, there were significant differences ($P < 0.05$) between the three auxin treatments (IBA > NAA > IAA) as the rate of rooting was strongly determined by the type of auxin. Among the treatments, 4.92 mM IBA induced maximum rooting (66.67%), followed by 1.34 mM NAA (40.0%) and 1.42 mM IAA (26.67%), respectively when applied singly (Table 2). IBA at 4.92 mM was also found to enhance the number of roots

Table 2 Effect of auxins on rooting of stem cuttings of *P. pinnata*

Treatments (mM)	No. of cuttings showing rooting	Cuttings showing rooting (%)	No. of primary roots ^a	Max. no of primary roots	Length of the roots (cm) ^a	Max. length of the primary root (cm)
Control (0)	3	20.00	3.25	3.33	4.88	9.23
IBA (1.23)	2	13.33	7.85	9.66	11.50	20.20
IBA (2.46)	3	20.00	8.30	11.00	14.47	17.66
IBA (4.92)	10	66.67	8.44	11.66	11.16	20.50
IBA (7.38)	1	6.67	2.75	3.00	13.23	13.90
NAA (1.34)	6	40.00	5.70	10.33	7.14	7.80
NAA (2.68)	3	20.00	2.12	2.66	9.20	11.20
NAA (5.37)	2	13.33	1.50	1.66	4.94	7.50
IAA (1.42)	4	26.67	2.46	2.66	9.75	11.43
IAA (2.85)	3	20.00	2.00	2.60	12.03	24.80
IAA (5.70)	1	6.67	3.75	4.33	3.87	6.16
NAA (1.34) + IBA (1.23)	6	40.00	4.15	6.33	3.97	5.03
NAA (1.34) + IBA (2.46)	9	60.00	4.50	8.33	8.03	15.30
NAA (1.34) + IBA (4.92)	11	73.33	7.81	10.33	7.07	15.83
NAA (1.34) + IAA (2.85)	4	26.67	1.45	2.00	1.05	1.26
NAA (1.34) + IAA (5.70)	7	46.67	3.19	4.33	5.95	9.96
IAA (1.42) + IBA (1.23)	3	20.00	3.70	4.66	7.04	9.23
IAA (1.42) + IBA (2.46)	7	46.67	4.19	5.66	7.29	12.30
IAA (1.42) + IBA (4.92)	9	60.00	8.89	15.66	7.65	15.20
IAA (1.42) + NAA (2.68)	6	40.00	2.75	4.00	10.12	10.53
IAA (1.42) + IBA (4.92) + NAA (1.34)	13	86.67	8.87	11.00	7.14	16.23
α	0.05	0.05	0.05	0.05	0.05	0.05
Error <i>df</i>	42	42	42	42	42	42
Error mean square	0.47	35.26	1.05	0.77	0.92	0.72
Critical value of <i>t</i>	2.01	2.01	2.01	2.01	2.01	2.01

Each value = treatment mean of three replicates (each replicate contains 15 cuttings). Bold indicates best responses

^a Subsampling data 15 cuttings within each replicate

by threefold, root length by twofold as compared to control (Fig. 1). IBA is the best auxin for general use because it is nontoxic to plants over a wide concentration range than NAA or IAA (Hartmann et al. 2002) and also effective in promoting rooting of a large number of plant species (Teklehaimanot et al. 1996; Henrique et al. 2006). The average number of roots per cutting was in the range 1 (NAA 5.37 mM) to 8 (IBA 4.92 mM). A maximum of 24.80 cm root length was observed with 2.85 mM IAA (Table 2). The differences among auxins could also be related to other factors such as higher stability and a slow rate of conjugation of IBA, so that the free IBA required to induce rooting will be available over a longer period of time than IAA or NAA (Krisantini et al. 2006).

Higher doses (above than 7 mM) of all three auxins tested further inhibited sprouting. Inhibitions were more pronounced with NAA (8.05 mM), where only 8.90%

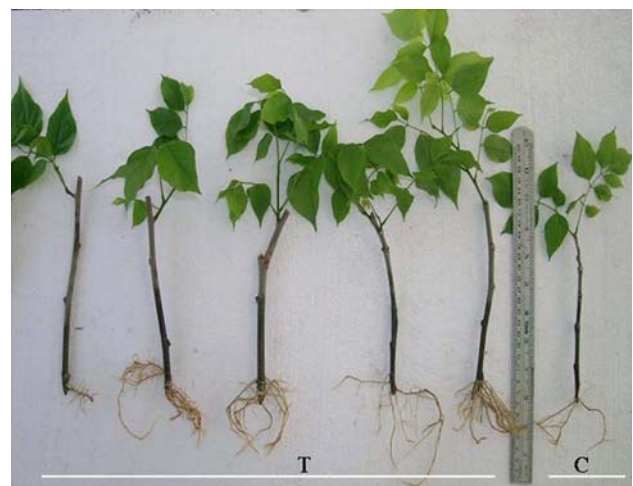


Fig. 1 Effect of IBA (4.92 mM) on the rooting of cuttings of candidate plus tree *P. pinnata*. T treated, C control

sprouting was observed and no rooting response until 6 weeks of insertion. In general, there were significant differences between the treatments with regard to sprouting. IBA at 4.92 mM concentration produced maximum number of shoots (=4) same as control (Fig. 2a), but the average length of the shoots (21.47) was thrice that of the control (7.61) (Fig. 2b).

Varied combination and concentration of different hormones were also taken with the aim to evaluate their effect in stimulating the rhizogenesis of elite genotype of *Pongamia* cuttings. With the two-component and three-component mixture, the percentage increase in rooted cuttings was in range of 20.0–73.33 and 86.67%,

respectively (Table 2). A higher yield of rooted cuttings was achieved in three-component combinations (NAA 1.34 mM + IAA 1.42 mM + IBA 4.92 mM) enhancing their yield up to four times as compared to control. IBA in combination exerted a synergistic action on the effectiveness of rooting, which became evident not only in a higher yield of rooted cuttings but also in an improved root system of stimulated cuttings (Table 2). Auxins in combination also had stimulating effect on shooting particularly in the number of cuttings showing sprouting, although the total number of shoots was not significantly different from control as far as three-component combination was concerned (Fig. 2a). Similar results of synergism between IBA

Fig. 2 Effect of auxins on sprouting; **a** shoot number, **b** shoot length. Each value = mean ± SD

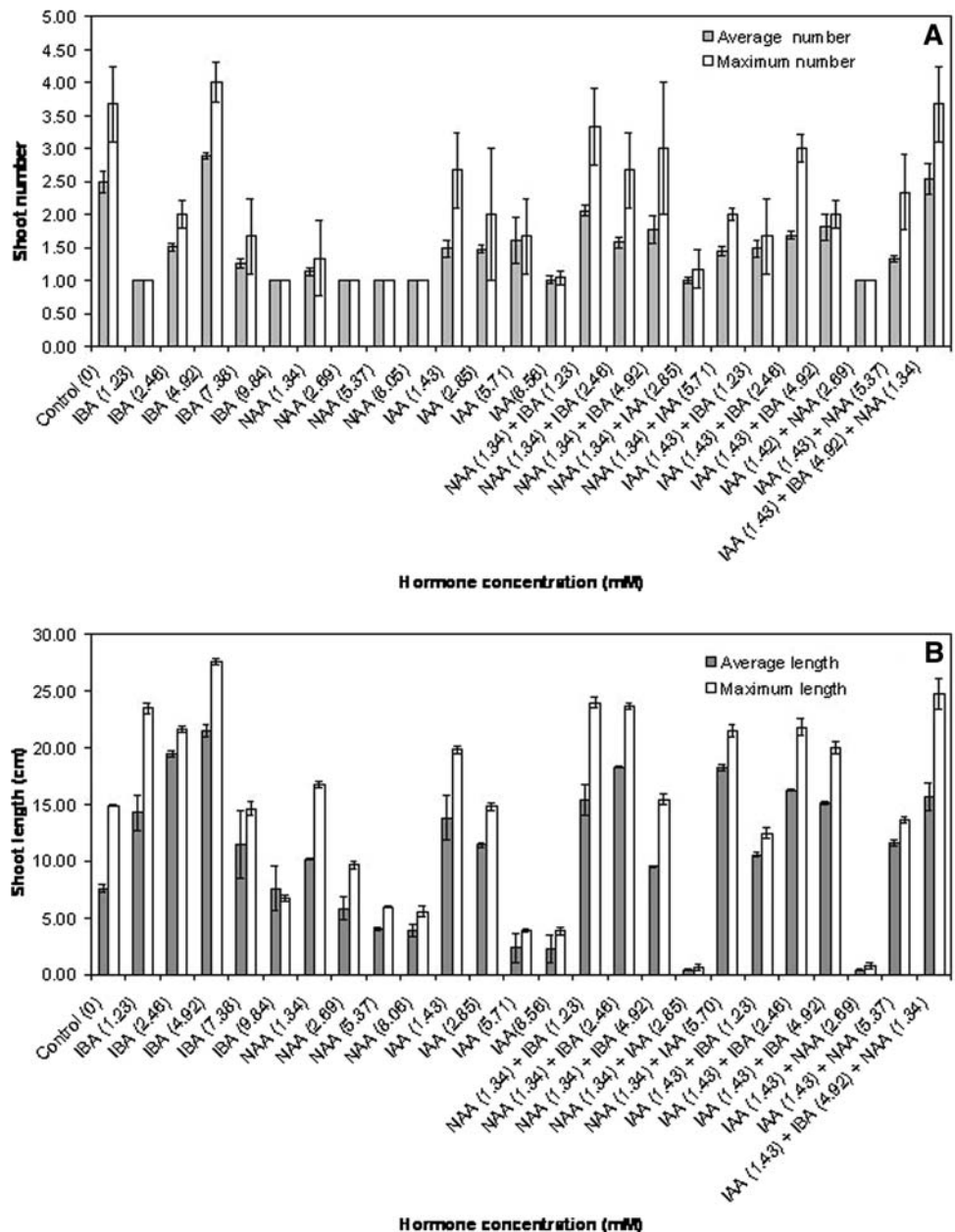


Table 3 Effect of 4.92 mM IBA on rooting of juvenile and mature cuttings of *P. pinnata*

Source	Cuttings showing rooting (%)	No. of primary roots ^a	Max. no of primary roots	Length of the roots (cm) ^a	Max. length of the primary root (cm)
Mature field grown cuttings	66.67 ± 3.85	8.44 ± 0.12	11.66 ± 0.88	11.16 ± 0.31	20.50 ± 0.51
2-month-old nursery raised cuttings	80.00 ± 3.85	6.03 ± 0.16	8.13 ± 0.33	8.00 ± 0.07	12.35 ± 0.40

Each value = treatment mean of three replicates (each replicate contains 15 cuttings). Each value = treatment mean ± SE (standard error)

^a Subsampling data 15 cuttings within each replicate

and synthetic auxins were reported in woody plants (Henselova 2002). Interestingly flowering was also observed in three-component mixture by the end of 3 month period (Fig. 3). Flowering capacity of cuttings by the end of 3 month period indicates the maintenance of mature state and lack of rejuvenation of cuttings. Reserve food materials in the cuttings support flowering and sprouting, but they are not the only cause (De Vier and Geneve 1997; Roh et al. 2005; Thiele et al. 2008). The maximum sprouting (86.67%) in *P. pinnata* was also observed in this mixture, although the maximum number of roots and maximum length of root was slightly less than that in IBA at 4.92 mM.

The relatively poor rooting with IAA treated stem cuttings of *P. pinnata* (6.67% for IAA) in comparison to IBA could be explained by the sensitivity of IAA to light (Hartmann et al. 2002), production of more ethylene which is known to inhibit the root production (Mullins 1972) and could also be due to the higher metabolic turnover. IBA is less sensitive than IAA to non-biological degradation such as photo-oxidation (Epstein and Ludwig-Muller 1993; De

Klerk et al. 1997). Alternatively, there may be a difference in rate of uptake and metabolism of the two auxins as demonstrated in apple and *Grevillea* microcuttings (De Klerk et al. 1997; Krisantini et al. 2006). Evaluating the total effectiveness of all the mixtures tested, the three-component combinations that had synergistic effect in enhancing rooting response were found to be the best.

Death of some cuttings commenced from the fifth week of propagation and by the 11th week of recording, the sprouted shoots had already senesced and eventually died. Cutting death was 17 and 25% on 5th week and 33.3 and 42.7% on 11th week in NAA at 1.34 mM and IAA at 1.42 mM, respectively. Death was more pronounced in two-component mixture. Cutting death recorded on 5th, 7th and 11th week was 27, 43 and 58% in IAA (1.42 mM) + IBA (2.46 mM), whereas in IAA (1.42 mM) + IBA (1.23 mM) cutting death recorded on 11th week (83%) was almost three times that of 5th week. In all experiments, cutting death was largely due to water deficits incurred as a result of physiological shock while the cuttings are taken. The result emphasizes the need to maintain high relative humidity around the cuttings during propagation, in order to minimize the water loss. It is accepted that a positive effect of stimulators on the rooting process in plants is achieved if other conditions are adhered to optimum temperature, humidity and also the type of cuttings (Henselova 2002).

Many reports exist for differences in rooting frequency depending on the exogenous auxin or combination of auxins being used. We may state from the results obtained that the concentration of IBA per se, as well as its mixture with lower concentrations of IAA and NAA has an essential impact on the percentage yield of rooted cuttings.

Effect of maturation on rooting

It has been reported in woody tree species that the rooting potential of the cuttings is a juvenile characteristic and that the rooting capacity declines after maturation (House et al. 1996; Kibbler et al. 2004). Stem cuttings from 2-month-old raised seedling of elite genotype NGPP46 were also subjected to exogenous application of IBA at 4.92 mM that exerted the best stimulating action on plant rhizogenesis. The results indicate that there are differences in rooting



Fig. 3 Flowering in rooted cuttings of *P. pinnata* in three-component combination (NAA 1.34 mM + IAA 1.42 mM + IBA 4.92 mM) maintained in mist chamber

percentage between the seedling raised in nursery bed and the cuttings obtained from field-grown trees. The cuttings obtained from young seedlings in nursery bed had a high rate of rooting (almost 80%) in comparison with cuttings raised from the field (66.67%) (Table 3). Similar observations were reported in *P. cineraria* which showed better rooting; up to 60% in cuttings taken from 6-month-old plants in comparison to 8 year old cuttings which showed a maximum rooting of only 35% (Arya et al. 1994). It was observed that there was a decrease in rooting percentage with the increase in age of the parent plant and our findings in *P. pinnata* are in conformation with reports for other species where a decline in rooting was observed with age of the parent plant (Abramovich et al. 1980; Oduol and Akunda 1988). There was no difference recorded between cuttings taken from the base of the mature plants propagated from cuttings and those taken from the apex (data not shown) indicating that juvenility (age of the plant material) and not the position of the cutting on the stock plant affects rooting capacity. Similar observations were recorded in recalcitrant woody tree species (Kibbler et al. 2004). The cuttings raised through seedlings were found to induce multiple stems when their tips were cut and the resultant multiple shoots could be used for rooting. Profuse rooting of cuttings is of practical importance especially when planting in denuded and marginal areas. The fast developing root system will lead to successful establishment of the plants which was the case in the present study.

Conclusion

In view of limited supply of natural fossil fuel, *Pongamia* is undoubtedly one of the key source-species and a potential source of viable biodiesel. The study reported here has applied significance. Standardization of the vegetative propagation techniques and establishment of PGRC (Pongamia Genetic Resources Center) can be a timely step in this direction for large-scale production of genetically superior saplings throughout the year. The results of our current study on adventitious rooting from candidate plus tree varying different natural and synthetic auxins would encourage tree breeding and its commercialization in areas suitable for its growth. It also has important implications for germplasm utilization and may establish a case study of genetic enhancement of biodiesel species (Rangan, personal communication). With an overall rooting success of 40–60% and with a reasonably good growth rate, production of planting material from stem cuttings of *P. pinnata* through vegetative means offers opportunities for a cheaper, practically feasible and technically less demanding alternative means of propagation.

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