Journal of Tropical Forest Science 35(3): 331–338 (2023)
https://doi.org/10.26525/jtfs2023.35.3.331
ISSN: 0128-1283, eISSN: 2521-9847

DEVELOPMENT OF AGROTECHNIQUES AND RESTOCKING OF *PERSEA MACRANTHA*—A JIGAT YIELDING PLANT FOR SUSTAINABLE UTILISATION

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Submitted September 2022; accepted November 2022

The stem bark of *Persea macrantha were* extensively harvested as raw material and is commercially known as jigat for the preparation of agarbatti incense stick in India. Due to the unscientific and over harvesting of the bark, their populations are declining apart from the other biological constraints of the plant. Restrictions imposed on bark harvesting from the natural forests lead to a severe shortage in raw materials. The current study showed that the application of auxins such as IBA and NAA at 1000 ppm resulted in 80% stem rooting on young stands about 5 years old within a period of 45–50 days of planting. Air layerings produced at 100% success in rooting with the aid of IAA 1000 ppm after 2 months of girdling. Harvested fresh seeds displayed 44% germination with moisture content of 47%. Enhanced seed germination up to 80% was recorded after removing the seed coat followed by soaking with GA_3 at 500 ppm in a dipping duration of 24 hours. The seeds were categorised as recalcitrant and critical moisture content was found to be 32–34%. Restocking of the seedlings among the plantation areas was found promising and resulted in 65–80% seedling survival. The agrotechniques developed and promising seedling survival is to ensure the large-scale multiplication and establishment of the species outside the forests for raw materials sustainability.

Keywords: Jigat species, agrotechniques, augmentation, outside the forest, management, resource utilisation.

INTRODUCTION

Persea macrantha belongs to the family Lauraceae and is distributed in the Western Ghats of India and Sri Lanka. In India, the tree can be found in evergreen, semi-evergreen and riparian ecosystems as a medium to tall tree, 30 m in height and distributed up to an altitude of 2100 m asl (Kulkarni et al. 2011). The species is classified in the vulnerable conservation status and is prioritised for conservation (Ravikumar & Ved 2000). The tree has many folk's uses in various states of India for example, the bark is used for the treatment of asthma and rheumatism (Kirtikar & Basu 1999). The leaves are used externally in ulcers (Nadkarni 2000). The alcoholic extract of the limed roots indicated the presence of major alkaloids, machiline and macranthine which have wide applications in modern medicine (Tomita et al. 1963).

Jigat is the powdered stem bark of *Persea macrantha* which is the main ingredient for agarbatti incense stick production (Salim 2017, Prasad & Bhatnagar 1991). The agarbatti incense stick production is a traditional industry in India and plays an important role in India's rural economy, providing employment opportunities to the rural societies (Hazarika et al. 2018). In Kerala, stakeholders depend on the trees grown outside the forest, mainly in plantation areas of Idukki and Wayanad districts as state forest department-imposed restrictions on bark harvesting and obtaining supply from the natural forests. The overexploitation of the species has drastically reduced the genetic stock and caused a severe critical situation in raw material availability for the industry. Currently, the beneficiaries import 50% of jigat material from the countries like Malaysia and Thailand (Hazarika et al. 2018).

Persea macrantha is grown traditionally as a shade tree in the coffee, cardamom and turmeric plantations in the high ranges of Kerala (Kumar 2007). The overexploitation and subsequent degeneration of the stands resulted in a loss in suboptimal levels of shade required for the crops which further lead to low productivity. Maintenance of optimal shade levels is critical for ensuring sustained productivity of crops (Kumar 2007).

Current knowledge of the artificial regeneration of the species is inadequate which further affected the multiplication and paucity of *ex-situ* planting materials. The restriction on bark harvesting from the natural forests not only affected the employment opportunities and subsequently reduced production in the industry. Development of agrotechniques through stem rooting and air layering methods with the aid of auxins and enhanced seed germination practices standardised through suitable seed pretreatments could be adopted as a low-cost propagation technology for large scale multiplication of the species. The seedling augmentation carried out among the plantation areas was found to be a promising practice to increase the number of plants outside forests and thereby improve crop productivity by serving as a shade tree. The success of augmentation practice also could be extended in agroforestry systems to meet the escalating demand for raw materials as part of conservation, management and resource-based sustainable utilisation of the species for the dependent societies.

MATERIALS AND METHODS

Study area

Plant propagules were collected from Nelliyampathy in the Palakkad District (10° 29.213' N; 76° 40.356' E) and Vazhachal forest areas in the Thrissur District (10° 17.837' N; 076° 9.573' E) of Southern Western Ghats, Kerala (Figure 1). Nelliyampathy forest area has an average annual rainfall of 3000 mm and the maximum atmospheric temperature varied from 28–29 °C from March–April and the minimum temperature from 15–17°C from December–January. Vazhachal forest has an average annual rainfall of 2700 mm and the maximum atmospheric temperature varied from 30–32 °C in March–April and minimum from 19–21 °C in December–January. Vegetative propagation and seed germination experiments were conducted at the Campus nursery of the Kerala Forest Research Institute (KFRI), Peechi, Kerala, South India (Figure 2). KFRI is situated about 20 km east of Thrissur district, spreading over a 28 ha reserve forest area

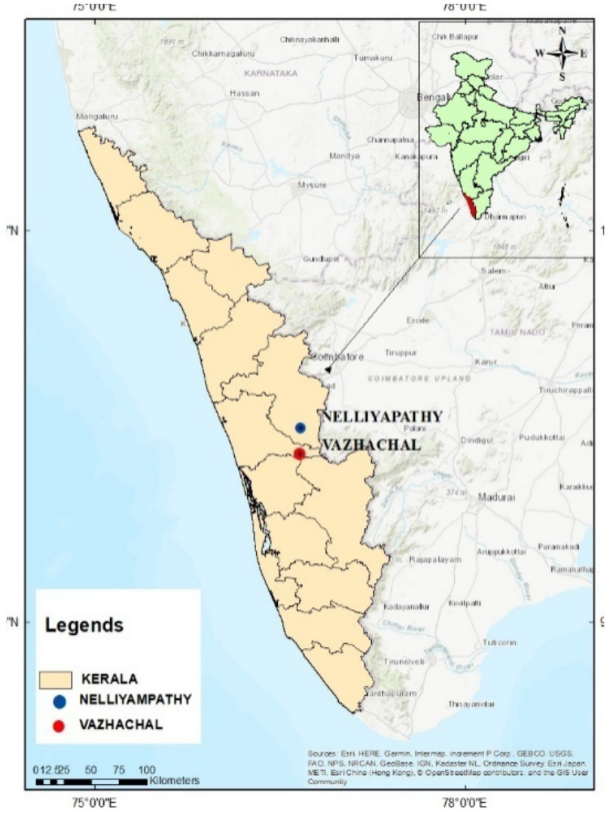

Figure 1 The Western Ghats showing collection area

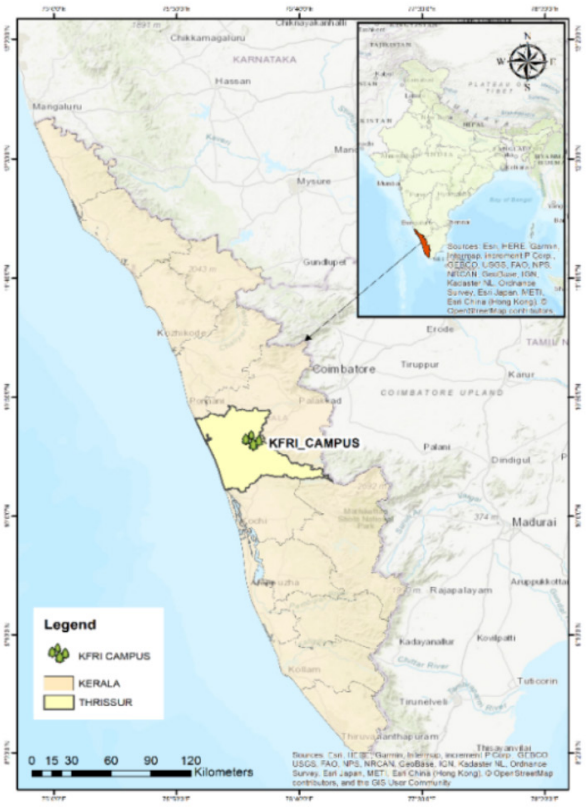

Figure 2 Kerala state showing the study propagule area at KFRI Campus

adjacent to Peechi-Vazhani Wildlife Sanctuary at 10.530° N latitude and 76.347° E longitude at an altitude of 186 m asl.

Seed propagation

Ripened fruits were collected from the natural species populations located in the Nelliyampathy and Vazhachal forest areas during March and April 2021. Fruit and seed characteristics such as average weight, length and thickness were recorded. The Moisture Content (MC) of fresh seeds was calculated by oven dry method, using the formula:

$$\text{Moisture Content (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100$$

The fresh seeds were collected in triplicates and 10 seeds for each group were sown in coarse river sand as a sowing medium and kept in low poly tunnels of the nursery and maintained at 28 ± 2 °C and 80 ± 5% relative humidity. The fresh seeds were subjected to desiccation and chilling temperature conditions to assess the germinability and category of the seed.

Seed pretreatments

The processed seeds were undergone pretreatments to improve their germination performance (Warakagoda & Subasinghe 2015). The seeds were subjected to the following treatments along with control sets, namely, (a) seeds soaked in concentrated sulphuric acid for 1 minute, (b) seeds soaked in cold water for 24 hr, (c) seed coats removed by a sharp knife, (d) seeds soaked in potassium nitrate (KNO_3) (2% and 10%) and disodium hydrogen phosphate (Na_2HPO_4) (10%) for 24 hr, (e) seeds soaked in gibberellic acid (GA_3) at 500 and 1000 ppm concentrations for 24 hr and (f) treatments in combination of seed coat removed and soaked in KNO_3 (10%) for 24 hr, seed coat removed and soaked in Na_2HPO_4 (10%) for 24 hr, seed coat removed and soaked in GA_3 (500 ppm) for 24 hr, seed coat removed and soaked in GA_3 (1000 ppm) for 24 hr and seed coat removed and soaked in distilled water for 24 hr.

After each set of treatments, the seeds were washed in running tap water and sowed under nursery conditions. The height, collar diameter and the number of leaves of the seedlings were recorded after attaining two pair of leaves.

Vegetative propagation through stem cuttings

Stem propagules were collected from Vazhachal forest areas. Stem cuttings of 12–15 cm in length with 2–3 nodes with one or two leaves at the tip were prepared from young stands below five years old and adult trees above five years old. Type of auxins such as IAA, IBA and NAA were prepared in liquid form after dissolving absolute ethyl alcohol and then diluted to 50% with distilled water at concentrations of 1000, 2000, 3000 and 5000 ppm. The hormone solutions were applied to the cutting base by dipping each concentration for one minute. The control set of cuttings was dipped in distilled water. Each set of stem cuttings was immediately planted in river sand medium and kept in the mist house of the nursery maintained at 28 ± 2 °C and 80 ± 5% relative humidity. Data on rooting such as by the percentage of rooting, number of roots per cutting, length of roots was taken after 45 days of planting and tabulated as per standard statistical methods. The rooted plants or ramets were transplanted into polybags and their survival was recorded after three months.

Vegetative propagation through air layering

Air layering was conducted in nursery-raised saplings below 5 years old. A small strip of bark was girdled out just below the nodal region, 15–20cm below the shoot tip of lateral branches, using a sharp knife. Auxins such as IAA, IBA, and NAA at concentrations 1000 ppm were applied in the girdled region. The girdles were immediately covered with white polythene sleeves containing moist rooting compost consisted of coco peat, river sand, dried and sieved cow dung (2:1:1), and then tied firmly at both ends to avoid drying of the medium. A total of 32 layered plants were regularly observed for the initiation of roots without opening the polythene sleeves. The rooting percentage was calculated by using the formula:

$$\text{Rooting per cent} = \frac{\text{Number of layer}}{\text{Total number of shoots air layered}} \times 100$$

Planting stock and insect-pest incidence

The plants produced as resulted from the propagation study were maintained in polybags and used as planting stock for augmentation (Jose et al. 2010). The seedlings were monitored and recorded for insect-pest incidence.

Selection of agroclimatic zones and seedling augmentation

Based on the altitudinal variations, 4 agroclimatic zones which include plantations of coffee, cardamom and turmeric were selected in three districts of Kerala for augmentation of seedlings. Planting was done during the south-west monsoon in the months of June–July (Jose & Pillai 2014). An equal number of tagged and untagged seedlings were planted in subplots marked on the planting site to facilitate seedling survival data collection. Seedling survival was recorded from respective sites on half yearly basis.

RESULTS AND DISCUSSION

Seed germination

Many of the tropical forest tree species do not germinate readily even under favourable conditions, hence the need for pretreating seeds (Olayode & Gbadamosi 2009). The fresh seeds with moisture content of 47%, displayed 44% germination. However, enhanced germination at 80% was achieved after removing the seed coat and soaking in GA_3 of 500 ppm for duration of 24 hours (Table 1). The seeds were categorised as recalcitrant where chilling temperature conditions and desiccation found to affect the seed viability. The possible fact for better percent germination by priming may be due to the stimulation on series of biochemical changes in the seed that are essential to initiate the emergence process such as breaking down dormancy, hydrolysis and metabolism of growth inhibitors, imbibition and activation of enzymes (Ajouri et al. 2004). A comparison between each treatment was done using one-way ANOVA and followed by Duncan Multiple Range Test (DMRT). F-value was found to be significant for stem girth ($P < 0.05$) and leaf number ($P < 0.001$) and higher stem girth was observed in treatment 3. Results of DMRT show that treatment 3 is significantly higher than treatments 7 and 9. No significant difference was noted between other treatments. A higher leaf number was observed in treatment 1 and it showed no significant difference with treatments 3 and 13. Treatment 13 had significantly higher leaf number compared

Table 1 Details of seed pretreatments and seedling attributes

No	Treatment	Root length	Stem girth	Stem length	Leaf number
1	T1 (GA_3 (500 ppm))	16.43 ± 0.77	2.03 ± 0.07^{ab}	20.98 ± 0.85	5.01 ± 0.35^{a}
2	T2 (Scarification)	16.00 ± 0.73	2.00 ± 0.07^{ab}	20.00 ± 0.85	3.29 ± 0.52^{bcd}
3	T3 (Seed coat removed + Na_2HPO_4)	17.61 ± 0.95	2.19 ± 0.07^{a}	20.90 ± 0.12	4.16 ± 0.33^{abc}
4	T4 (KNO_3 (10%))	17.50 ± 0.35	1.98 ± 0.04^{ab}	20.75 ± 1.14	3.60 ± 0.27^{bc}
5	T5 (GA_3 (1000 ppm))	19.23 ± 0.83	2.07 ± 0.07^{ab}	23.87 ± 0.85	3.70 ± 0.37^{bc}
6	T6 (H_2SO_4 (1%))	18.33 ± 5.36	2.01 ± 0.12^{ab}	19.17 ± 1.74	3.33 ± 0.33^{bcd}
7	T8 (KNO_3 (2%))	17.75 ± 2.18	1.82 ± 0.08^{b}	19.75 ± 1.42	3.43 ± 0.36^{bcd}
8	T9 (Seed coat removed + KNO_3 (2%))	15.50 ± 1.67	2.00 ± 0.09^{ab}	18.17 ± 0.66	3.75 ± 0.45^{bc}
9	T10 (Na_2HPO_4 (10%))	17.06 ± 0.93	1.83 ± 0.06^{b}	22.83 ± 0.94	2.17 ± 0.17^{d}
10	T11 (Seed coat removed + GA_3 (500ppm))	19.77 ± 1.39	2.10 ± 0.06^{ab}	21.33 ± 0.71	3.68 ± 0.23^{bc}
11	T12 (Seed coat removed + GA_3 (1000ppm))	18.48 ± 0.66	1.99 ± 0.08^{ab}	21.64 ±1.04	2.89 ± 0.31^{cd}
12	T13 (Cold water)	19.19 ± 1.91	1.99 ± 0.08^{ab}	22.42 ± 1.76	2.85 ± 0.34^{cd}
13	T14 (Seed coat removed + distilled water)	17.75 ± 0.71	2.00 ± 0.09^{ab}	21.77 ± 1.63	4.36 ± 0.56^{ab}
14	Control	18.42 ± 0.72	2.05 ± 0.06^{ab}	20.57 ± 0.75	3.50 ± 0.31^{bc}
	F-value	0.944^{ns}	1.820*	1.568^{ns}	5.230**
	(P-value)	(0.508)	(0.043)	(0.098)	(<0.001)

** = significant at 0.01 level, * = significant at 0.05 level, ns = non-significant
Different letters as superscripts differ significantly

to treatments 9, 11 and 12. Treatment 9 had significantly lower leaf number compared to all other treatments except in treatments 2, 6, 7, 11 and 12. The highest root length (19.77 cm) was recorded in treatment 10 and lowest (16.00 cm) were recorded in treatment 2. In this study, the increase in length of root was recorded in the similar treatment to which has boosted the growth of the seedling above ground level and it might have given the same effect for the below ground such as in the root. Plant growth regulators in overcoming the harmful effect on growth may be due to the change in endogenous growth regulators (Thomas et al. 2005).

Vegetative propagation through stem cuttings

The stem rooting ability of *P. macrantha* was found significantly affected by auxin treatment. Stem cuttings collected from the juvenile plants were more responsive to rooting than from the adult trees. It was reported that the application of auxin was found to stimulate the cambial activity in juvenile plants at a higher rate than that of aged trees and thereby resulting in the mobilisation of reserve food materials at the nodal region and inducing rooting of stem cuttings or layers (Kamlesh et al. 1995, Jose & Thomas 1998, Singh et al. 2004). The juvenile stem cuttings of *P. macrantha* produced root with a comparatively success rate with different auxin treatments. A maximum rooting of 80% was achieved with IBA and NAA at 1000 ppm along with control sets within 59 days of planting (Table 2). The higher concentrations of auxins caused decrease rooting performance. Thimann (1956) suggested that different organs responded differently to types and ranges of auxin concentration, each having a promontory and inhibitory range and the type and concentration of auxin that promoted shoot growth might inhibit root growth or vice versa. The maximum root number at 4.25 ± 1.89 and the highest means root length at 8.23 ± 2.76 cm resulted during treatment with IBA 1000 ppm. The ramets exhibited 100% survival for a monitoring period of 3 months. Since clonal propagation merits a quick and economically useful technique for producing true-to-type progenies of superior quality; the method would be supplemented the deficit faced in the seed regeneration of the species (Jose et al. 2000, Aslam et al. 2007). Uniyal et al. (1993) found that the formation of roots on cuttings treated with auxins was important for survival, as cuttings in the control that did not form roots wilted and died. The stem cuttings of adult trees responded with a maximum rooting of 33% with a mean root number of 3 ± 2.61 with the aid of IAA 1000ppm. No rooting was observed in the control set and in other auxin-treated sets.

Vegetative propagation through air layering

The success of air layering was significantly affected by various auxin treatments. The method of girdling during air layering stimulates mobilisation of carbohydrates and auxins which accumulate in the basal portion of girdled branches. These added food reserves favoured the formation of callus, development of adventitious root primordia and subsequent root formation (Adriance & Brison 1955, Hartmann et al. 2010). In air layering, the rooting percentage varied from 75 to 100% under different treatments. The highest layering success at 100% was achieved in IAA 1000 pm compared to 75% in control sets within 63 days of girdling. Differences in uptake and metabolism of the three auxins might be a cause for difference in rooting behaviour (Krisantini et al. 2006).

Table 2 Vegetative propagation through stem cuttings

Treatment (ppm)	No. of cuttings	Rooted Cuttings (%)	Mean number of roots ± SD (cm)	Mean length of roots ± SD (cm)	Survival of rooted plants (%)
Control	10	80	3 ± 2.70	4.03 ± 4.15	100
IAA 1000	10	60	3.33 ± 1.15	5.05 ± 4.18	100
IBA 1000	10	80	4.25 ± 1.89	8.23 ± 2.76	100
NAA 1000	10	80	2.5 ± 1.91	5.05 ± 3.55	100
IAA 2000	8	50	1.5 ± 0.70	2.33 ± 1.52	100
IBA 2000	8	50	2.5 ± 2.12	4.4 ± 2.07	100
NAA 2000	8	Nil	-	-	-

The successful adventitious root formation and the subsequent developments were also highly dependent on interaction between endogenous and exogenous auxins (Kochhar et al. 2005). Air layered branches showed 100% success survival (Table 3). The success of air layering therefore can be treated as an additional clonal multiplication of the species for its conservation and to ensured availability of new plantlets.

Insect-pest incidence

Harvested seeds recorded the presence of insect larval infestations. The larva was reared and adults were identified as weevils (Coleoptera). During the vegetative phase, the leaves were eaten by larvae of the adult butterfly, *Papilio clytia,* which served as the host plant for the butterfly species.

Planting activity

The established seedlings in polybags with a mean height of 30 cm were transferred for field planting. Each seedling was planted at a distance between 5 m. Half-yearly data were collected on the survival of planted seedlings from respective sites at Karappara/Nelliampathy and Siruvani/Attapady Forest Ranges, Palakkad District, Pambadumpara plantations in Idukki District and Vanamoolika herbals in Wayanad District (Table 4).

CONCLUSION

The vegetative propagation methods for *Persea macrantha* developed through rooting of stem cuttings and air layering could be utilised for large scale quality *ex-situ* plant production of the species. The seed pretreatment standardised was found to nearly double the germination performance of the non-treated plant sets, facilitating mass seedling production for the cultivation of species. Known as a recalcitrant seed, critical moisture content determined and enabled safe level storage of seeds at seed bank and supply of viable seeds for future requirement. Due to the restriction on harvesting and gaining supply from the natural forests, the success of seedling augmentation could be extended to other plantation areas to enhance the genetic stock apart from only serving as a shade tree beneficial for other agriculture crop productivity. The study further increases the cultivation of species in agroforestry ecosystems to meet the increasing demand for raw materials for the industry and the subsequent benefit of the dependent societies.

Table 3 Vegetative propagation through air layering

Sl. No.	Number of branches	Treatment	Rooting (%)	Survival (%)
1	8	Control	75	100
2	8	IAA 1000	100	100
3	8	IBA 1000	75	100

Table 4 Details of augmentation

Sl. No.	Planting site	GPS	Altitude (m)	No. of seedlings planted	Survival percentage (%)
01	Coffee estate at Karappara	10˚ 27.611' N 76˚ 38.748' E	949	50	80
02	Coffee and Cardamom estate at Siruvani	11˚ 02.027' N 76˚ 40.675' E	993	100	65
03	Coffee and cardamom estate at Pambadumpara	9˚ 47.491' N 77˚ 09.476' E	1076	100	5*
04	Coffee and turmeric estate at Vanamoolika	11˚ 48.666' N 76˚ 9.934' E	790	100	72

* = Seedling survival was biased due to the replanting activity of the Cardamom plants

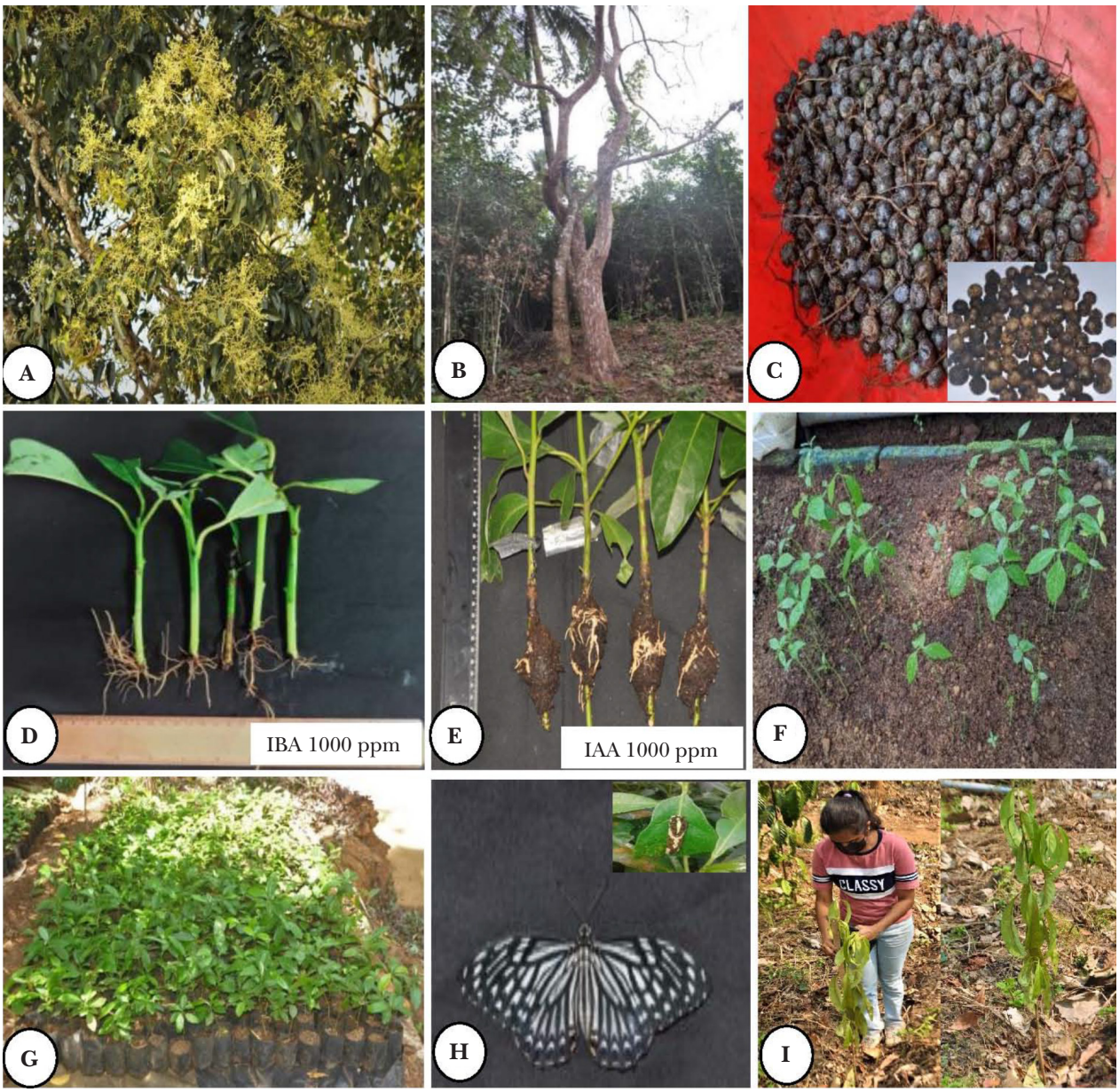

Figure 3 Plantation technology of *Persea macrantha* (A) Tree during peak flowering, (B) Over exploited tree, (C) Harvested fruits and processed seeds (inset), (D) Rooted stem cuttings (IBA 1000 ppm) (E) Air-layered plantlets (IAA 1000 ppm), (F) Germinating seeds (G) Planting stock (H) *Papilio clytia* butterfly (I) Post planting seedling evaluation

ACKNOWLEDGMENTS

The authors thanked Syam Viswanath, Director of KSCSTE-Kerala Forest Research Institute, Peechi for the facilities and encouragement provided throughout the study. V.B. Sreekumar, Principal Scientist at KFRI for academic support of the study. Our appreciations extended to Divisional and Deputy Forest Officers of Nelliyampathy and Vazhachal Forest Divisions, Kerala Forest and Wildlife Department, Thiruvananthapuram for logistic support in the field. Our gratitude also given to plantation owners for their support in the augmentation activity and the National Bamboo Mission, Ministry of Agriculture & Farmers Welfare, Government of India for financial support of the Project.

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