Developing protocols for the propagation and multiplication of *Baccaurea courtallensis* (Wight) Mull. Arg. for domestication – a wild edible fruit tree of the Western Ghats, India

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Inadequate know-how on seed and nursery technology of wild edible fruits is a bottleneck for their commercialization. The seeds of *Baccaurea courtallensis* lose their viability within six days under ambient conditions and are found to be sensitive to desiccation and low-temperature conditions revealing their recalcitrant nature. The longevity of the seeds could extend up to six months during storage in polycarbonate bottles maintained at 20°C and 40% relative humidity. Coarse river sand was optimized as the ideal sowing medium for seed germination. The application of 3000 ppm naphthalene acetic acid was standardized for clonal propagation for selective breeding of female genetic stock.

Keywords: *Baccaurea courtallensis*, domestication, multiplication, propagation protocols, wild edible fruits.

BACCAUREA COURTALLENSIS (Wight) Mull. Arg. (Phyllanthaceae), is a lesser-known wild edible fruit tree, endemic to the Western Ghats, India, distributed in the evergreen and semi-evergreen forests up to an altitude of 1000 m asl. The cauliflorous flowers are crimson red in colour. Fruits are locally known as 'mootilpazham' (in Malayalam), owing to the cluster fruit arrangement at the base of the trunk. Traditionally, the tribal communities consume fully ripened fruits and the rind is used to prepare pickles. Seed oil is a lubricant used as an additive in several industries[1]. The fruits contain protein, potassium, reducing sugar, non-reducing sugar, carbohydrates, total phenols, antioxidants and flavonoids[2,3]. The whole plant is a rich source of medicinally important components. Roots and leaves have antioxidant radical scavenging properties, which protect against oxidative damage[4]. The ripened fruits are largely consumed by mammals and birds, including elephants, giant squirrels, lion-tailed macaques, tortoises, etc.

Despite the proven nutritive quality of the species, commercialization efforts have not been undertaken so far due to inadequate know-how of nursery and seed technology. The present study thus aimed to develop protocols for the large-scale, vegetative and seed propagation, and subsequent multiplication of plants *ex-situ* in view of domestication and popularization of the species as an additional fruit resource from the Western Ghats, India. The study also generates the fruit and seed morphometrics for the commercial prospects of the species.

Seed germination is the cheapest and easiest method for the large-scale production and multiplication of several species, which can assist in the regeneration and replenishment of wild stocks[5,6]. The method also helps produce a large number of seedlings and detects genetically superior accessions for boosting crop improvement programmes[7]. The seed germplasm storage enables the supply of quality seeds as and when required for various purposes[8], apart from conserving the genetic resources of the species[9]. Vegetative propagation techniques standardized could serve multiple purposes. They help identify the suitable hormones, medium and climatic conditions for producing superior-quality plants of the species[10]. The method is also significant when dioecy exists among plants. The multiplied plants developed using this study can be utilized for planting in home gardens and agrosystems for the domestication and subsequent popularization of the species.

Materials and methods

Sample collection and morphological studies

Ripened fruits were collected from the Vazhachal forest area (10°17.707′N; 076°39.263′E; altitude 542 m amsl), Thrissur district, Kerala, India. The fruits were brought to the laboratory of the Tree Physiology Department of Kerala Forest Research Institute, Peechi. The morpho-metrics of fruits and processed seeds were recorded. Moisture content (MC) of the seeds was determined by oven-dry method using the following formula

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times 100.$$

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Seed viability and storage

The critical moisture content (CMC) of the seeds was detected using the field germination method with the respective MC at each sowing. Seeds were categorized in response to desiccation and chilling temperature storage conditions. As part of storage practices, surface-dried seeds were maintained under ambient conditions (28°C and 75% relative humidity (RH)) and at controlled seed-banking conditions (20°C and 40% RH) using polycarbonate bottles as a container. The viability of seeds under storage conditions was checked at biweekly intervals.

Effect of sowing media

The processed seeds were sown in four media, S_1–S_4, viz. vermiculate (S_1), sieved leaf litter compost (S_2), sieved topsoil + sieved leaf litter compost (S_3) and coarse river sand (S_4), later kept in the mist house of the nursery (maintained at 30°C and 85% RH). The germination percentage, type of germination, days taken for initial germination, shoot length, root length, number of roots, number of leaves and stem girth were recorded.

Vegetative propagation

Clonal propagules (apical stem cuttings) were collected from the adult female trees identified among the populations of the species. The stem cuttings were planted in pots containing coarse river sand. The ring air layering was carried out among saplings maintained in the nursery as physical vagaries were found to disrupt the layering experiment *in situ*. Both the experimental sets were kept in the mist house maintained at 28° ± 2°C and 80% ± 5% RH. The auxins indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA), ranging from 1000, 3000 and 5000 ppm respectively were applied to optimize the concentration. The number of roots and root length of each stem cutting were recorded according to standard statistical methods.

Data analysis

Data recorded for fruit and seed morphological parameters were represented as mean ± standard error (SE). Data on seed germination with respective sowing medium and rooting parameters were analysed by ANOVA using SPSS.

Results

Phenology, fruit and seed morphometrics

In dioecious trees, flowering starts in February and extends up to March. The fruits usually mature from July to August, coinciding with the southwest monsoon months. Fruits and inflorescence are borne on the main trunk or at the base of the trunk (Figure 1 *a* and *b*). Flowers are dark crimson in colour, which provides a feast for the eyes in

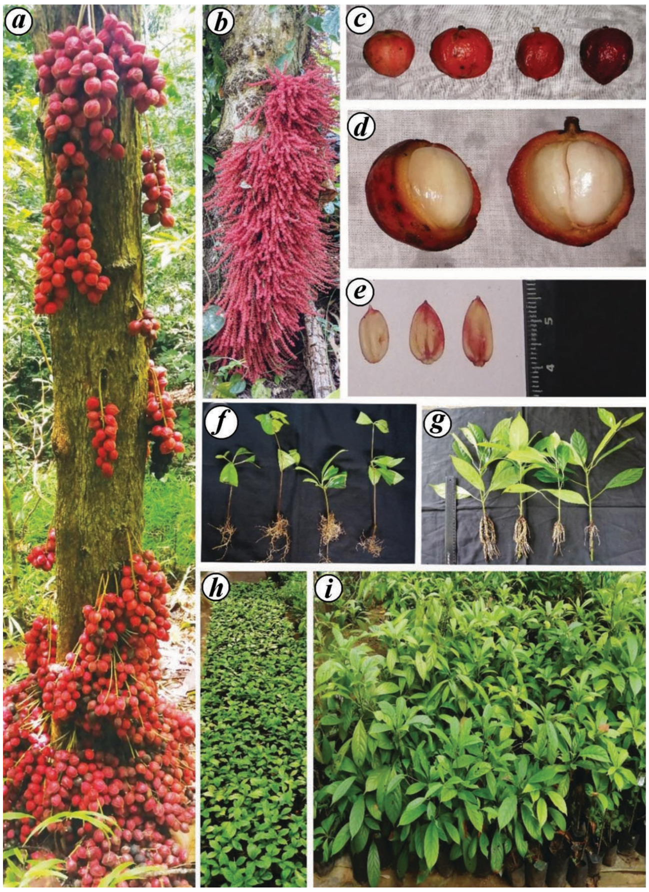

Figure 1. ***a***, Ripened fruits of *Baccaurea courtallensis* borne on the tree trunk. ***b***, Male inflorescence. ***c***, Fruit colour change during maturity. ***d***, Single fruit showing rind and pulp. ***e***, Seeds. ***f*** and ***g***, Rooted stem cuttings and layers treated with 3000 ppm NAA. ***h*** and ***i***, Views of established planting stock.

Table 1. Fruit attributes

Parameters	Mean ± SE
Fruit weight (g)	12.10 ± 0.56
No. of fruits per kg	84 ± 15.15
Fruit length (mm)	28.86 ± 0.62
Fruit width (mm)	28.73 ± 0.77
Pulp (g)	3.9 ± 0.24

Table 2. Seed attributes

Characteristics	Mean ± SE
Weight of 100 seeds (g)	31.10 ± 0.003
No. of seeds per kg	3210 ± 28.93
Seed length (mm)	13.086 ± 0.27
Seed width (mm)	9.534 ± 0.200
Seed thickness (mm)	4.312 ± 0.17
Moisture content in fresh seeds (%)	60

full bloom. Fruit colour changes from orange to crimson red on ripening (Figure 1 *c*). The fruit rind is semi-hard, pulp is white in colour and juicy in nature (Figure 1 *d*). Fruits are sweet and sour; 1–3 seeds are usually contained in fruit (Figure 1 *e*). Tables 1 and 2 list the morphometric parameters of fruits and seeds respectively.

Desiccation and low-temperature response of seeds

The graph plotted with seed germination against MC was analysed using ordinary least square regression analysis, revealing a significant strong positive correlation ($r^2 = 0.765$, $P = 0.000$) between these two variables (Figure 2). Fresh seeds with 60% moisture content showed 100% germination. The normal viability period lasted for six days with CMC at 21% with 30% seed germination, indicating seed intolerance to desiccation. The seeds kept under low-temperature conditions also affected the viability and hence categorized as recalcitrant-type.

Seeds of *B. courtallensis* kept in polycarbonate bottles at ambient conditions (28° ± 2°C and 75 ± 5% RH) maintained viability for up to five months with 50% germinability (Figure 3). Seeds under storage in controlled seed-banking conditions (20°C and 40% RH) showed viability for up to six months with 30% germinability (Figure 4).

Effect of sowing media

Seed germination and seedling attributes showed significant variations among the media. River sand was found to be the most suitable sowing medium with 100% germination within a germination duration of 15 days, along with the highest values of shoot length (10.10 cm), root length (13.50 cm), number of roots (9.23), number of leaves (4.86) and stem girth (2.12 mm). The sieved topsoil and sieved leaf litter compost at 3 : 1 ratio resulted in 74% germination with a shoot length of 9.37 cm, root length 12.82 cm, number of roots 8.32, number of leaves 4.09 and stem girth 2.06 mm. Seeds sown in vermiculite took more days for germination (21 days) than sieved leaf litter compost (19 days); however, germination performance in both media was identical (57%) (Table 3).

Clonal propagation

Clonal propagation through stem cuttings and ring air layering with the aid of 3000 ppm NAA showed maximum rooting @ 67% and 100% respectively (Tables 4 and 5). The maximum number of roots and root length was also recorded in the treated sets (Figure 1 *f* and *g*). The rooting response of stem cuttings on IBA was the least in comparison with other auxins. In layering, all the treatments could induce roots, and the application of NAA, in particular, was more effective than IBA and IAA. The rooting of stem cuttings and layering was observed on the 25th and 35th day respectively. The control sets did not show any rooting.

Discussion and conclusion

Recalcitrant seed behaviour is common among the species distributed in evergreen and riparian forests. The seeds of these species are well adapted for quick germination as the soil moisture is high during the senescence of fruits[11]. The large fruit size and high MC are key features of the recalcitrant seeds. The long-term storage of recalcitrant seeds is also difficult as they are sensitive to desiccation and low-temperature conditions[12]. The CMC of recalcitrant seeds varied from 21% to 31%, depending on the nature of the species[13–15]. The seeds of *B. courtallensis* were sensitive to desiccation and chilling temperature (0°C), revealing

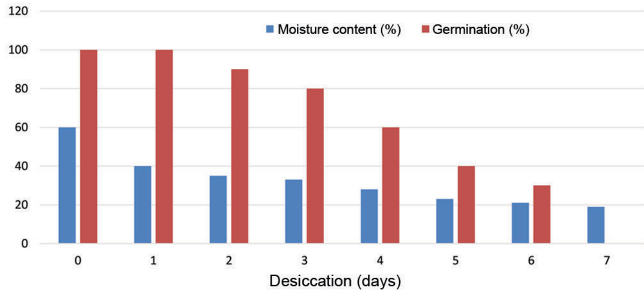

Figure 2. Effect of desiccation on moisture content and germination of seeds.

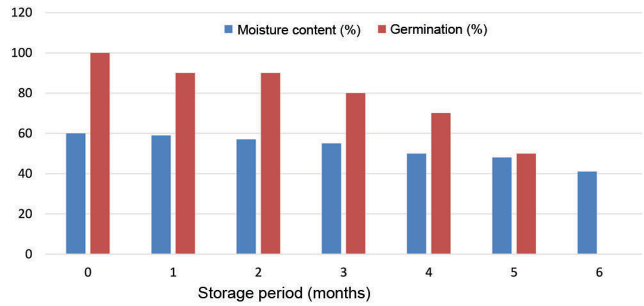

Figure 3. Moisture content versus germination at ambient conditions.

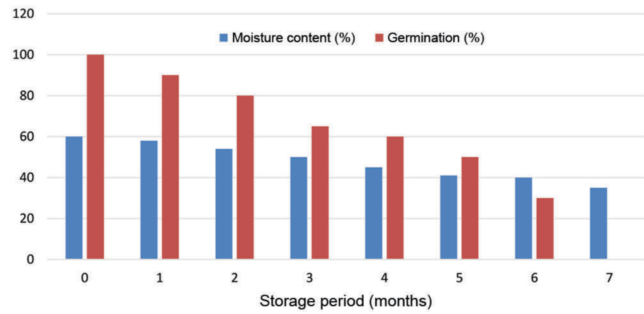

Figure 4. Moisture content versus germination at controlled seed-banking conditions.

Table 3. Effect of sowing media on seed germination and seedling growth

Substrate	Initial germination	Germination (%)	Shoot length (cm)	Root length (cm)	No. of roots	No. of leaves	Stem girth (mm)
Vermiculite (S_1)	21	57	6.30	10.35	6.70	3.06	1.86
Sieved leaf litter compost (S_2)	19	57	8.88	11.06	7.64	3.88	1.95
Sieved topsoil + sieved leaf litter compost (3 : 1; S_3)	16	74	9.37	12.82	8.32	4.09	2.06
River sand (S_4)	15	100	10.10	13.50	9.23	4.86	2.12
CD (0.05)	1.1022	0.5385	3.3370	6.188	3.9013	1.905	0.4557
F-test	*	*	*	*	*	*	*

Table 4. Vegetative propagation through stem cuttings

Auxin	Concentration of auxin (ppm)	Rooting (%)	Average number of roots per cutting (mean ± SE)	Average length of roots (mean ± SE)
IAA	1000	34	0.33 ± 0.21^{a}	1.86 ± 1.59^{abc}
	3000	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	5000	34	0.33 ± 0.21^{a}	0.42 ± 0.33^{a}
IBA	1000	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	3000	34	0.33 ± 0.21^{a}	2.48 ± 1.61^{abc}
	5000	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
NAA	1000	34	0.67 ± 0.45^{a}	1.13 ± 0.98^{ab}
	3000	67	3 ± 1.25^{b}	4.38 ± 1.59^{c}
	5000	67	1.50 ± 0.77^{a}	3.95 ± 1.33^{bc}
Control	0	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Note: Level of significance, $P < 0.05$. The mean values shown in the corresponding columns followed by the same alphabet are not significantly different. Here 'a' represents the lowest value and 'c' the highest value. IAA, Indole-3-acetic acid; IBA, Indole-3-butyric acid; NAA, Napthalene acetic acid.

Table 5. Vegetative propagation through air layering

Auxin	Concentration of auxin (ppm)	Rooting (%)	Average number of roots per layer (mean ± SE)	Average length of roots (mean ± SE)
IAA	1000	100	15.25 ± 3.43^{bc}	7.04 ± 0.82^{cd}
	3000	75	5.25 ± 2.14^{ab}	3.51 ± 1.19^{abc}
	5000	25	3.00 ± 3.00^{a}	0.63 ± 0.63^{a}
IBA	1000	25	6.25 ± 6.25^{ab}	1.11 ± 1.11^{ab}
	3000	75	7.25 ± 3.32^{ab}	5.52 ± 1.97^{bcd}
	5000	50	5.00 ± 3.00^{ab}	4.16 ± 2.41^{abc}
NAA	1000	50	4.75 ± 3.82^{ab}	2.46 ± 1.46^{ab}
	3000	100	24.76 ± 4.45^{c}	9.68 ± 0.52^{d}
	5000	25	2.25 ± 2.25^{a}	1.07 ± 1.07^{ab}
Control	0	25	0.75 ± 0.75^{a}	1.59 ± 1.59^{ab}

Note: Level of significance, $P < 0.05$. The mean values shown in the corresponding columns followed by the same alphabet are not significantly different. Here 'a' represents the lowest value and 'd' the highest value.

their recalcitrant nature. The seeds lost their viability when the MC reduced from an initial 60% to 21%. Several studies have reported that germination of desiccation-sensitive seeds declines rapidly as seed MC decreases[16–20]. Reduction in the MC of seeds leads to their decay and death of recalcitrant seeds. If the seed MC reduces below the lowest safe moisture content, seed viability decreases rapidly[21].

Seeds of *B. courtallensis* maintained viability for up to six months under controlled storage conditions (20°C and 40% RH). Storage temperature, MC of seeds and storage time determine the shelf life of seeds[22]. Several studies have shown that medium–low temperature and humidity conditions are favourable to maintaining seed viability for a considerable period of time owing to the reduced seed dehydration under controlled climatic conditions[23–27]. Chilling temperatures were reported to have a determined effect on the seed viability of many recalcitrant seeds, which might be due to freezing injuries[28]. Thus both higher ambient temperatures (28° ± 2°C) as well as very low temperatures

(0°–10°C) were detrimental to the storage life of recalcitrant seeds. The storage of seeds with low MC and at low temperatures will enhance viability loss among recalcitrant seeds[29]. The reduction of RH *in situ* was also found to decrease seed MC, and it negatively influenced the germination capacity[30]. It is known that during exposure of seeds, even at constant RH, the seed MC also decreases. Thus, maintaining optimum temperature and RH under controlled seed-banking conditions enables extended seed viability of recalcitrant seeds.

Appropriate growing media or substrates for sowing seeds directly affect the germination, functional root and shoot systems[31]. The quality of seedlings is significantly affected by the growth media in the nursery[32]. River sand is found to be the ideal sowing medium for seed germination and establishment of the seedlings, followed by top soil and leaf litter in the ratio 3 : 1. Several studies have reported that sand is the ideal medium for raising seedlings because of its good drainage and aeration ability[33,34]. The general potting mix used in the nursery is considered optimum for field growth and establishment[35]. Vermiculite and leaf litter compost were poor substrates for *B. courtallensis* as they resulted in lower germination and growth.

Vegetative propagation is a commonly used technique for the multiplication of quality planting stock among forestry and fruit plants, as the method involves the selection of plus trees for the development of superior clones of true to type[36,37]. Auxins are effective in the induction of adventitious rooting in most tree species[38,39]. Vegetative propagation by stem cuttings and air layering using 3000 ppm NAA exhibited maximum rooting in *B. courtallensis*. The positive effect of NAA on rooting in *Bauhinia retusa*, *Gluta travancorica*, and *Humboldtia vahliana* supports the present findings in *B. courtallensis*[40–42]. It has been confirmed that a specific auxin at an optimum concentration level is required for adventitious root formation in *B. courtallensis*. The effect of ideal auxin at the right concentration has been well studied[43,44]. Among the vegetative methods, air layering with the help of auxins was the most convenient and successful method of propagation in fruit trees, like guava and litchi[45,46] as well as forestry species[47] as it is a quick, efficient, simple and inexpensive technique for early flowering/fruiting of species.

The vegetative and seed propagation protocols, including seed germplasm storage developed for *B. courtallensis* could be utilized for the large-scale multiplication and subsequent domestication, popularization and commercialization of this lesser-known species.

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