Integrated propagation strategies for the conservation and commercialization of Blood Fruit (Haematocarpus validus Miers)

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Research Article

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Abstract

Blood Fruit or Khoon Phal (*Haematocarpus validus*) is an underutilized wild fruit climber of the Menispermeaceae family, which faces difficulty in regenerating and maintaining stable populations due to its irregular and slow germination, unsustainable harvesting, habitat destruction, and lack of conservation awareness. This study evaluated seed and softwood cutting-based propagation methods under nursery conditions at the Department of Forestry, NEHU, Tura Campus, Meghalaya. Pre-sowing treatments, including water soaking, chilling, thiourea, KNO_3, and gibberellic acid (GA_3) at different concentrations, were tested in a Completely Randomized Design (CRD). Conversely, vegetative propagation was assessed using softwood cuttings categorized into three diameter classes (< 1.5, 1.5-3.0, and > 3.0 mm), three node levels (one, two, and three), and ten growth regulator treatments (nine auxin treatments: IAA, IBA, and NAA at 250, 500, and 1000 ppm, along with a control) under a three-factorial CRD. Results revealed that the highest germination (95.56%) was observed in seeds soaked in water for 24 hours, while the earliest germination was reported in GA_3 500 ppm (28.67 days). Seed treated with 0.1% thiourea showed the highest seedling vigour indices, and seedling growth parameters were significantly enhanced by thiourea 0.1% and GA_3 500 ppm. In vegetative propagation, softwood cuttings (< 1.5 mm) showed significantly higher propagation success. Among the auxin treatments, 500 ppm NAA was found to be the most effective, and three-node cuttings performed significantly better than others. In conclusion, thiourea 0.1%, GA_3 500 ppm, and water soaking for 24 hours were effective for improving seed germination and seedling growth, while softwood three-node cuttings with 500 ppm NAA emerged as an easy and effective vegetative propagation method. These findings provide practical and sustainable propagation strategies for the large-scale cultivation and conservation of this nutritionally rich and medicinally valuable forest climber in Meghalaya.

1. Introduction

Blood fruit or *Khoon Phal* (*Haematocarpus validus* (Miers) Bakh. f. ex-Forman) is an underutilized wild edible fruit of the family Menispermaceae. The genus name derives from “haem,” denoting iron content, and “carpus,” meaning fruit. It is an evergreen woody climber that naturally grows across tropical and subtropical regions of South and Southeast Asia [1, 2]. In India, blood fruit occurs naturally in the Northeastern and Andaman and Nicobar regions, grows under diverse climatic conditions, including arid regions and acidic soils, at elevations up to 1000 m [3].

The plant has dark green, glabrous, branched stems and climbs with the support of large trees such as banyan, jackfruit, and Baccaurea [4]. It grows up to 100 m, flowers during August-September, and fruits ripen from March to May [2, 3]. The leaves are simple, alternate, elliptic, and three-veined, while the inflorescence is cauliflorous. The fruits are drupes with a stalked base, smooth endocarp, and a style scar near the base. Seeds are curved, non-endospermic, with thick cotyledons and a short radicle. Seed dispersal occurs through gravity (barochory), animals (zoochory), and humans (anthropochory).

The Fruits are rich in antioxidants, iron, Vitamin C, β-carotene, phenols, flavonoids, and anthocyanins [5]. The vibrant red berries produce a natural dye, offering a sustainable option for textile dyeing [2]. It is valued in folk medicine to treat fever, anemia, and digestive problems; the roots are used to relieve itching; and the tender shoots are utilized to cure jaundice [6, 7, 8]. Despite its ecological and ethnobotanical importance, the species remains underutilized and poorly studied for domestication, propagation, and large-scale cultivation [4].

Even though there are several review articles, little research exists on the regeneration of this species. Diocious nature, recalcitrant seeds, and underdeveloped embryos remain major challenges associated with seed-based propagation, leading to irregular and slow germination and delayed natural emergence. Habitat destruction, unsustainable harvesting, and climate change further threaten natural populations and limit regeneration potential. Under such conditions, the development of robust, high-quality planting material becomes essential for conservation and large-scale cultivation.

To date, there have been only two studies focused on the propagation of blood fruit via seed, which investigated the presowing treatment [9] and the standardization of growing media [7]. However, seed-based propagation alone is constrained by poor germination behaviour (37–58% at 90 days after sowing) and slow establishment. The earlier findings of Bohra et al. [9] reported that germination initiated only after 45 days, with the control requiring even longer duration (52.50 days).

Vegetative propagation offers an alternative approach for rapid multiplication, yet systematic studies on cutting type, node number, and auxin treatments in *H. validus* are scarce. Seed propagation ensures genetic variability, whereas vegetative propagation enables rapid and uniform multiplication. Moreover, no study has attempted to integrate and compare seed and vegetative propagation approaches for developing a comprehensive propagation strategy for this species.

Therefore, the present study was undertaken to standardize and compare seed and vegetative propagation techniques for improving germination, rooting, seedling growth, and the development of robust quality planting stock of *H. validus* under nursery conditions. Such integrated approaches are needed for domestication and agroforestry adoption of this nutritionally rich and medicinally valuable forest climber in Meghalaya.

2. Materials and Methods

2.1 Study site and nursery condition

The present study was conducted in the nursery of the Department of Forestry at NEHU, Tura Campus, West Garo Hills, Meghalaya, from March 2025 to April 2026. The region experiences a subtropical to temperate climate, with temperatures generally ranging from 12 to 34°C [8]. The sexual (seed-based) and vegetative (cutting-based) propagation approaches were evaluated to identify suitable techniques for mass multiplication and conservation. Both experiments were conducted under similar nursery conditions using a vermicompost-based growing medium comprising soil, farmyard manure, and vermicompost in a 2:1:1 ratio, as suggested by Bohra et al. [7]. The nursery was maintained under a green shade net providing 50% shading.

2.2 Seed propagation experiment

For the seed germination study, uniform-sized mature blood fruits were collected from a 17-year-old *H. validus* climber during mid-April 2025 in Chasingre village (25.563710, 90.234675), Tura, Meghalaya (Fig. 1). For the cutting-based study, cuttings were collected in Mid-February 2026 (Pre-flowering stage). The plant *H. validus* was taxonomically identified and authenticated by Prof. Jadeyegowda Madegowda, Professor, College of Forestry, Ponnampet. A voucher specimen was deposited at the Herbarium of the Department of Forestry, North-Eastern Hill University, Tura Campus, Meghalaya, India, under accession number NEHU-DF-DS-07.

The healthy and viable seeds were manually extracted by removing the adhering pulp, and then thoroughly washed to remove traces of pulp around the seed coat, thereby minimizing secondary fungal contamination. A total of sixteen seed treatments, including control, normal water soaking (12, 24 and 48 hours), chilling at 4°C (12, 24 and 48 hours), thiourea (0.1, 0.2 and 0.3%) for 24 hours, KNO_3 (2, 5 and 10%) for 24 hours, and GA_3 (500, 1000 and 1500 ppm) for 24 hours, were used in a Completely Randomized Design (CRD) with three replications. After seed treatment, the seeds were dipped in 0.2% Bavistin and then sown at a depth of 1 cm in the prepared vermicompost-based growing medium. Interestingly, dipping seeds in 0.2% Bavistin significantly reduced fungal infection by up to 85%, even when small amounts of pulp remained adhered to the depulped seeds.

Observations on germination were recorded at 15-day intervals for 90 days after sowing (DAS), following the International Seed Testing Association (ISTA) guidelines [10, 11]. The germination parameters, such as initiation of germination (IG), completion of germination (CG), germination percentage (GP), mean daily germination (MDG), peak value (PV), and germination value (GV), were recorded. Seedling growth parameters like shoot length (SL), root length (RL), number of leaves (NOL) per seedling, collar diameter (CD), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), and root dry weight (RDW) were measured from the randomly selected five seedlings from each treatment. Seedling vigour index (SVI), seedling vigour index-mass (SVI-m), and survival percentage were also calculated.

Germination per cent (GP) was calculated as the proportion of seeds germinated from the total number of seeds treated and expressed as a percentage. Mean daily germination (MDG) was estimated following the formula given by Czabator [12] as the total number of germinated seeds divided by the total number of days taken for germination. Peak value (PV) was estimated following the formula given by Czabator [12] as the highest number of seeds germinated in a given day divided by the number of days at this peak value. Germinative value (GV) was estimated following the formula given by Czabator [12].

GV = PV × MDG, where PV is the peak value, and MDG is the mean daily germination.

Shoot and root lengths were measured using a graded scale. The seedling dry weight of 10 seedlings from each treatment was quantified using an electronic weighing balance after desiccating them in a hot air oven at 60°C for 48 hours [12]. Seedling vigour index (SVI) was estimated by multiplying the standard germination (%) by the average seedling length (cm) on the 90th day of germination [13, 14]. Seedling vigour index-mass (SVI-m) was estimated by multiplying the standard germination (%) by the mean seedling dry weight (mg) on the 90th day of germination [13]. The collar diameter (mm) of the seedlings in the nursery was measured using a digital vernier caliper. The Root: Shoot Ratio (R: S) and Survival rate (%) were recorded at 90 DAS following standard procedures [15, 16]. The root-to-shoot ratio was calculated as R: S = (root dry weight in grams) / (shoot dry weight in grams). A moderate-to-high R: S ratio reflects greater root development relative to shoot biomass, which enhances water and nutrient acquisition under harsh conditions [17].

2.3 Vegetative propagation experiment

Softwood cuttings representing three diameter classes (< 1.5 mm, 1.5–3.0 mm, and > 3.0 mm) were selected (Fig. 2.b) for the present study based on preliminary observations indicating diameter-dependent variation in rooting response.

The experiment was designed to evaluate the combined effects of cutting diameter, node number, and auxin treatments on rooting and subsequent establishment. A three-factorial Completely Randomized Design (CRD) was adopted with three replications. The factors included: (i) three diameter classes of softwood cuttings (< 1.5 mm, 1.5–3.0 mm, and > 3.0 mm), (ii) three levels of node number (one, two, and three nodes per cutting), and (iii) ten growth regulator treatments (nine auxin treatments comprising Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), and Naphthalene acetic acid (NAA) at concentrations of 250, 500, and 1000 ppm, along with an untreated control).

Cuttings were collected from healthy, disease-free mother plants and prepared to the required size by maintaining the specified number of nodes (1 to 3 nodes). The basal ends of the cuttings were treated with respective auxin concentrations using the basal quick dip method, wherein the cut ends were dipped in the hormone powder before planting [18]. Treated cuttings were then planted in a vermicompost-based growing medium comprising soil, farmyard manure, and vermicompost in a 2:1:1 ratio under uniform nursery conditions to facilitate rooting and growth (Fig. 2.c).

Cuttings were covered with transparent polythene for up to 7 days, with brief ventilation on alternate days to allow air exchange, until rooting began or new leaf emergence was observed. Necessary intercultural operations such as watering, shading, and protection from pests and diseases were carried out regularly. Observations on rooting and shoot growth parameters, including initiation of sprouting in DAP (IS), completion of sprouting in DAP (CS), shoot length (cm), root length (cm), number of shoots, and number of leaves, were recorded at 30 and 60 days after planting (DAP). Additionally, growth parameters such as fresh weight, dry weight, Root: Shoot ratio (R: S), and survival percentage were also recorded at 30 and 60 DAP to assess the effectiveness of different treatments.

2.4 Statistical Analysis

The data were analyzed using IBM SPSS (version 2020) to perform analysis of variance (ANOVA) under a completely randomized design (CRD) [19]. The significance of differences among treatments was tested using Fisher's (F) test, and means were compared using Duncan's multiple range test (DMRT) at $p \leq 0.05$ [19, 20]. The percent values were angular transformed (arc-sine) before analysis. If the F calculated value was found to be non-significant, it was designated as NS.

3. Results

3.1 Seed germination and seedling performance

3.1.1 The effect of pre-sowing treatments on seed germination and seedling growth parameters

The seed germination parameters, such as initiation of germination (IG), completion of germination (CG), seedling vigour index (SVI), and seedling vigour index-mass (SVIm), showed significant ($p \leq 0.05$) differences. In contrast, non-significant differences were observed for germination percentage (GP), mean daily germination (MDG), peak value (PV), germinative value (GV) (Table 1; Fig. 3). The earliest germination was recorded in the GA_3 500 ppm treatment (28.67 days), while delayed germination occurred to 47.00 days in the control, indicating that GA_3 significantly increased the germination process. Completion of germination was highest (86.33 days) in thiourea 0.1%, followed by chilling treatment (85.67 days), and lowest (71.67 days) was in water soaking for 24 hours. The highest germination percentage (95.56%) was observed in 24 hours of water soaking, whereas the lowest was 77.79% in the control, although differences were statistically non-significant (Table 1). The seedling vigour index-mass (SVI-m) was found to be statistically significant, with the highest value noted under GA_3 500 ppm (253.96), followed by thiourea 0.1% (248.51), whereas the lowest was in the control (127.98). The differences among the mean daily germination (MDG), peak value (PV), and germinative value (GV) were not statistically significant. Overall, seeds treated with growth regulators (GA_3 500 ppm and thiourea 0.1%) and water soaking for 24 hours significantly improved seedling vigour compared to the control, signifying their positive effect on early seed germination and seedling establishment (Table 1).

Table 1
Seed germination parameters at 90 days after sowing

Treatment		IG	CG	GP	MDG	PV	GV	SVI (0.000)	SVI-m
Control		47.00^{a}	84.33^{ab}	77.79	0.13	0.03	0.004	6.74^{e}	127.98^{d}
Water soaking	12 hrs	41.33^{abcd}	78.67^{b}	82.22	0.13	0.04	0.005	10.56^{ab}	182.33^{bcd}
	24 hrs.	34.67^{de}	71.67^{c}	95.56	0.16	0.03	0.005	10.70^{ab}	231.91^{ab}
	48 hrs.	36.00^{d}	73.00^{c}	84.45	0.14	0.03	0.005	9.55^{abcd}	190.98^{bc}
Chilling at 4°C	12 hrs	37.33^{cd}	85.67^{a}	86.67	0.14	0.05	0.008	9.79^{abcd}	185.51^{bc}
	24 hrs.	44.33^{ab}	84.67^{ab}	84.44	0.14	0.03	0.004	10.67^{ab}	206.18^{abc}
	48 hrs.	43.67^{abc}	82.33^{ab}	82.22	0.13	0.03	0.004	8.47^{bcde}	181.80^{bcd}
Thiourea for 24 hrs.	0.1%	40.33^{abcd}	86.33^{a}	88.89	0.15	0.04	0.006	11.18^{a}	221.96^{abc}
	0.2%	42.00^{abcd}	79.00^{b}	86.67	0.14	0.05	0.006	10.10^{abc}	207.58^{abc}
	0.3%	40.67^{abcd}	83.67^{ab}	83.33	0.13	0.03	0.004	9.32^{abcd}	186.69^{bc}
GA_3 for 24 hrs.	500 ppm	28.67^{e}	73.33^{c}	86.67	0.14	0.04	0.006	11.12^{a}	253.96^{a}
	1000 ppm	44.33^{abc}	81.67^{ab}	84.44	0.14	0.03	0.004	9.01^{abcde}	207.98^{abc}
	1500 ppm	39.33^{bcd}	82.00^{ab}	81.11	0.13	0.03	0.004	8.54^{bcde}	188.29^{bc}
KNO_3 for 24 hrs.	2%	35.67^{d}	72.67^{c}	83.33	0.13	0.04	0.005	9.21^{abcd}	209.83^{abc}
	5%	43.67^{abc}	84.33^{ab}	80.00	0.13	0.02	0.003	8.05^{cde}	173.98^{bcd}
	10%	45.33^{ab}	85.67^{a}	78.90	0.13	0.02	0.003	7.55^{de}	165.66^{cd}
Mean		40.27	80.56	84.17	0.14	0.03	0.005	9.41	195.16
SE		0.55	0.46	0.96	0.00	0.00	0.00	0.17	4.36
F-value.		4.79	7.96	1.25	1.35	1.64	1.86	3.36	2.76
Sig. ($p \leq 0.05$)		0.00	0.00	0.29^{NS}	0.23^{NS}	0.12^{NS}	0.07^{NS}	0.00	0.01

IG- Initiation of germination; CG- Completion of germination; GP- Germination per cent; MDG- Mean daily germination; PV- Peak value; GV- Germinative value; SVI- Seedling vigour index; SVIm- Seedling vigour index-mass; SE- Standard Error; NS- Non-Significant ($p \leq 0.05$); Means with the same alphabet are not significantly different from each other at $p \leq 0.05$ using DMRT test.

Pre-sowing treatments significantly ($p \leq 0.05$) influenced seedling growth and survival parameters at 90 DAS, except root length and root fresh weight (Table 2). Among the treatments, water soaking for 24 hours, thiourea (0.1%), and GA_3 at 500 ppm exhibited superior performance across most growth and survival parameters compared to the control (Fig. 4).

Table 2
Seedling growth and survival parameters at 90 DAS

Treatments		SL (cm)	RL (cm)	CD (mm)	NOL	SFW (g)	RFW (g)	SDW (g)	RDW (g)	R:S	Survival (%)
Control		68.83^{f}	18.17	1.32^{e}	11.50^{f}	2.97^{e}	0.77	1.16^{c}	0.49^{h}	0.42^{abcde}	88.33^{ef}
Water soaking	12 hrs.	91.50^{abcde}	21.00	1.44^{bcde}	13.83^{bcde}	3.78^{bcd}	1.13	1.61^{b}	0.62^{efg}	0.39^{cde}	91.67^{abcdef}
	24 hrs.	106.83^{a}	21.83	1.42^{cde}	16.33^{a}	4.20^{ab}	1.17	1.74^{ab}	0.70^{bcdef}	0.41^{abcde}	95.33^{abcde}
	48 hrs.	92.50^{abcde}	20.33	1.35^{e}	14.67^{abcd}	3.87^{abcd}	1.14	1.64^{b}	0.62^{efg}	0.37^{de}	91.00^{bcdef}
Chilling at 4ºC	12 hrs.	90.17^{bcde}	22.83	1.36^{e}	13.83^{bcde}	4.00^{abc}	1.00	1.48^{b}	0.66^{cdefg}	0.45^{abcd}	92.00^{cdef}
	24 hrs.	102.50^{abc}	22.83	1.44^{bcde}	15.50^{ab}	4.44^{ab}	1.23	1.71^{ab}	0.72^{bcde}	0.42^{abcde}	89.67^{abcd}
	48 hrs.	83.33^{def}	19.50	1.32^{e}	14.83^{abcd}	4.19^{ab}	1.17	1.63^{b}	0.58^{gh}	0.35^{e}	87.67^{f}
Thiourea for 24 hrs.	0.1%	103.83^{ab}	21.77	1.56^{bc}	15.22^{abcd}	4.59^{a}	1.23	1.74^{ab}	0.76^{bc}	0.44^{abcd}	95.33^{a}
	0.2%	96.17^{ab}	20.53	1.53^{bcd}	15.33^{abc}	4.07^{abc}	1.27	1.64^{b}	0.74^{bcd}	0.45^{abcd}	93.00^{abcd}
	0.3%	90.17^{bcde}	21.50	1.42^{cde}	15.00^{abcd}	4.16^{ab}	1.24	1.61^{b}	0.63^{defg}	0.39^{bcde}	90.33^{bcdef}
GA_3 for 24 hrs.	500 ppm	107.17^{a}	20.67	1.79^{a}	16.33^{a}	4.51^{ab}	1.50	2.04^{a}	0.88^{a}	0.43^{abcd}	94.00^{ab}
	1000 ppm	86.67^{cde}	20.27	1.58^{b}	14.17^{bcde}	3.38^{cde}	1.27	1.67^{b}	0.76^{bc}	0.47^{ab}	92.00^{abcde}
	1500 ppm	85.50^{de}	19.67	1.43	13.33^{cdef}	3.36^{cde}	1.15	1.58^{b}	0.74^{bcd}	0.47^{a}	91.33^{abcdef}
KNO_3 for 24 hrs.	2%	87.83^{bcde}	22.50	1.52^{bcd}	14.50^{abcd}	4.05^{abc}	1.19	1.72^{ab}	0.79^{ab}	0.46^{abc}	93.66^{abc}
	5%	81.17^{def}	19.33	1.37^{de}	13.17^{def}	3.25^{de}	1.10	1.56^{b}	0.62^{efg}	0.40^{abcde}	88.33^{ef}
	10%	76.17^{ef}	19.17	1.32^{e}	12.33^{ef}	3.16^{de}	1.07	1.51^{b}	0.59^{fgh}	0.39^{cde}	89.00^{def}
Mean		90.65	20.74	1.45	14.37	3.87	1.16	1.63	0.68	0.42	91.29
SEd		1.22	0.35	0.01	0.16	0.06	0.03	0.02	0.01	0.01	0.31
F-cal.		4.97	0.98	6.71	4.43	5.14	1.94	2.93	7.93	2.34	3.25
Sig. ($p \leq 0.05$)		0.00	0.50^{NS}	0.00	0.01	0.00	0.06^{NS}	0.01	0.00	0.02	0.00

SL- Shoot length (cm); Root length (cm); Collar diameter (mm); Number of leaves; Seedling fresh weight (SFW); Seedling dry weight (SDW); R:S– Root-shoot ratio; SE- Standard Error; NS- Non-Significant ($p \leq 0.05$); Means with the same alphabet are not significantly different from each other at $p \leq 0.05$ using DMRT test.

The differences in shoot length ranged from 68.83 cm in the control to 107.17 cm in the GA_3 500 ppm treatment, representing a 55% improvement over the control and statistically similar to water soaking for 24 hours (106.83 cm). Root length, however, did not show significant ($p \leq 0.05$) variation among pre-sowing seed treatments (Table 2). Similarly, GA_3 500 ppm showed the maximum collar diameter (1.79 mm) and root dry weight (0.88 g). The maximum number of leaves was observed in GA_3 500 ppm and water soaking 24 h treatments (16.33), while the minimum was (11.50) in the control (Fig. 4). Seedling fresh and dry weights were highest under GA_3 500 ppm (6.01 g and 2.92 g, respectively) and lowest in the control (3.74 g and 1.65 g). Additionally, Thiourea, chilling, and KNO_3 treatments also showed moderate improvements over the control. The root-to-shoot ratio did not differ significantly among treatments, ranging between 0.35 and 0.47. The survival percentage was significantly higher in water soaking for 24 hours (95.33%) and thiourea 0.1% (95.33%), followed by GA_3 500 ppm (94.00%), compared to the control (88.33%). Overall, GA_3 at 500 ppm and thiourea at 0.1% significantly enhanced seedling vigor, biomass accumulation, and survival under nursery conditions (Table 2).

3.2 Rooting, Growth, and Survival of Cuttings

The results demonstrated that cutting diameter classes (CDCs), number of nodes (NON), and auxin concentration (AC) significantly ($p \leq 0.05$) influenced rooting, vegetative growth, and survival parameters of cuttings at both 30 and 60 days after planting (DAP) (Table 3; Fig. 5).

The results showed a consistent trend across most parameters, indicating the importance of selecting suitable planting material and hormonal (auxin) treatment for successful propagation through cuttings (Table 4). Among the different CDCs, the smallest (cuttings with a diameter < 1.5 mm) performed significantly better than medium (1.5–3.0 mm) and thicker (> 3 mm) cuttings (Table 3). The results indicated a diameter-dependent response in rooting, growth, and survival of cuttings. The small diameter cuttings recorded the highest shoot length (15.24 cm at 30 DAP and 61.64 cm at 60 DAP) and root length (8.40 cm and 13.80 cm, respectively). They also produced a greater number of shoots (1.38 and 1.45) and leaves (3.35 and 9.10), along with higher

fresh (0.51 and 4.39 g) and dry (0.15 and 1.77 g) biomass accumulation at 30 and 60 DAP, respectively (Fig. 5). Survival percentage was also highest in this small diameter category (99.22% at 30 DAP and 96.72% at 60 DAP). In contrast, thicker cuttings (> 3 mm) showed comparatively lower growth and survival (Table 3).

The number of nodes (NONs) on cuttings also significantly ($p \leq 0.05$) improved all growth parameters and survival rate at 30 DAP, except survival at 60 DAP (Table 3). Two-node cuttings emerged as the most effective for most parameters at 60 DAP, recording maximum shoot length (59.81 cm) and root length (12.56 cm) (Fig. 5). Three-node cuttings recorded higher growth and biomass values at the initial stage (30 DAP), whereas two-node cuttings showed greater growth and biomass accumulation at 60 DAP. One-node cuttings consistently performed the poorest across all growth parameters. Survival percentages were relatively high across all node categories, though two-node cuttings maintained slightly better growth and performance. Interestingly, three-node cuttings showed a lower survival rate compared with the control (Table 4).

Auxin concentration (AC) treatments significantly ($p \leq 0.05$) enhanced rooting and growth compared to untreated control cuttings, except for the number of shoots parameter (Table 3).

Table 3
The effect of softwood cutting diameter, number of nodes, and auxin concentration on rooting and growth parameters of the cuttings

Treatments		IS (DAP)	CS (DAP)	Shoot length (cm)		Root length (cm)		Number of shoots		Num
				30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP	30 D
Cutting diameter class (CDC)	< 1.5 mm	8.83^{b}	16.92^{c}	15.24^{a}	61.64^{a}	8.40^{a}	13.80^{a}	1.38^{a}	1.45^{a}	3.35
	1.5-3 mm	9.23^{b}	19.22^{b}	14.05^{b}	58.14^{b}	7.42^{b}	11.56^{b}	1.32^{ab}	1.37^{a}	3.27
	> 3 mm	10.40^{a}	21.50^{a}	11.76^{c}	51.98^{c}	6.83^{c}	10.01^{c}	1.18^{b}	1.30^{a}	3.22
Number of nodes (NON)	One-node	11.10^{a}	21.27^{a}	11.12^{c}	53.37^{c}	6.75^{c}	10.85^{c}	1.02^{b}	1.07^{b}	2.22
	Two-node	7.97^{c}	18.00^{b}	14.68^{b}	59.81^{a}	8.09^{a}	12.56^{a}	1.42^{a}	1.53^{a}	3.37
	Three-node	9.40^{b}	18.37^{b}	15.25^{a}	58.57^{b}	7.81^{b}	11.97^{b}	1.45^{a}	1.52^{a}	4.25
Auxin concentration (AC) in ppm	Control	10.61^{ab}	21.06^{a}	10.72^{f}	54.10^{e}	5.73^{e}	8.53^{e}	1.11^{b}	1.33^{ab}	3.03
	IBA 250	9.39^{cd}	19.67^{b}	14.74^{bc}	58.74^{b}	7.74^{b}	12.09^{bc}	1.28^{ab}	1.28^{ab}	3.28
	IBA 500	7.83^{e}	17.28^{d}	14.89^{bc}	58.77^{b}	8.10^{b}	12.30^{c}	1.28^{ab}	1.44^{ab}	3.33
	IBA 1000	10.11^{bc}	19.56^{b}	12.26^{e}	55.04^{e}	7.03^{d}	11.36^{d}	1.33^{ab}	1.44^{ab}	3.06
	IAA 250	9.72^{bc}	19.78^{b}	13.28^{d}	57.28^{cd}	7.22^{cd}	11.60^{d}	1.22^{ab}	1.39^{ab}	3.33
	IAA 500	9.39^{cd}	18.44^{c}	14.02^{cd}	58.02^{bc}	8.01^{b}	12.41^{bc}	1.33^{ab}	1.33^{ab}	3.33
	IAA 1000	10.06^{bc}	20.06^{b}	11.58^{e}	54.45^{e}	6.72^{d}	11.26^{d}	1.11^{b}	1.11^{b}	3.06
	NAA 250	8.61^{de}	18.00^{cd}	15.16^{b}	59.16^{b}	8.19^{b}	12.63^{b}	1.39^{ab}	1.56^{a}	3.50
	NAA 500	7.83^{e}	17.78^{cd}	16.52^{a}	60.39^{a}	9.04^{a}	13.47^{a}	1.56^{a}	1.56^{a}	3.72
	NAA 1000	11.33^{a}	20.50^{ab}	13.67^{d}	56.58^{d}	7.70^{bc}	12.26^{bc}	1.33^{ab}	1.28^{ab}	3.17
Grand Mean		9.49	19.21	13.68	57.25	7.55	11.79	1.29	1.37	3.27
SEd		0.10	0.10	0.09	0.13	0.05	0.05	0.03	0.04	0.04
Sig. ($p \leq 0.05$)	CDC	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.23^{NS}	
	NON	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	AC	0.00	0.00	0.00	0.00	0.00	0.00	0.16^{NS}	0.17^{NS}	
	CDC*NON	0.31^{NS}	0.00	0.00	0.00	0.48^{NS}	0.25^{NS}	0.56^{NS}	0.84^{NS}	
	CDC*AC	0.99^{NS}	0.99^{NS}	0.01	0.11^{NS}	0.00	0.00	0.82^{NS}	0.97^{NS}	
	NON*AC	0.98^{NS}	0.99^{NS}	0.00	0.09^{NS}	0.01	0.00	0.35^{NS}	0.55^{NS}	
	CDC*NON*AC	1.00^{NS}	1.00^{NS}	0.77^{NS}	0.99^{NS}	0.00	0.00	0.95^{NS}	0.62^{NS}	

IS: Initiation of sprouting; CS: Completion of sprouting; DAP: Days after planting; Means with the same alphabet are not significantly different from each other at $p \leq 0.05$ using DMRT test.

Table 4
The effect of softwood cutting diameter, number of nodes, and auxin concentration on growth and survival parameters of the cuttings

Treatments		Fresh weight (g)		Dry weight (g)		Root shoot ratio (R:S)		Survival (%)	
		30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP
Cutting diameter class (CDC)	< 1.5 mm	0.51^{c}	4.39^{a}	0.15^{c}	1.77^{a}	20.85^{a}	41.57^{a}	99.22^{a}	96.72^{a}
	1.5-3 mm	1.23^{b}	4.31^{b}	0.41^{b}	1.69^{b}	18.80^{b}	39.63^{b}	98.67^{a}	96.22^{a}
	> 3 mm	1.72^{a}	3.86^{c}	0.64^{a}	1.40^{c}	14.31^{c}	32.33^{c}	95.28^{b}	88.11^{b}
Number of nodes (NON)	One-node	0.82^{c}	3.72^{c}	0.28^{c}	1.43^{c}	12.29^{c}	33.60^{c}	97.00^{b}	93.72^{a}
	Two-node	1.17^{b}	4.57^{a}	0.40^{b}	1.76^{a}	18.79^{b}	41.52^{a}	98.39^{a}	94.17^{a}
	Three-node	1.48^{a}	4.26^{b}	0.51^{a}	1.66^{b}	22.89^{a}	38.42^{b}	97.78^{ab}	93.17^{a}
Auxin concentration (AC) in ppm	Control	1.03^{e}	4.05^{d}	0.35^{e}	1.57^{e}	17.96^{ab}	36.90^{bc}	96.30^{bc}	93.52^{ab}
	IBA 250	1.12^{d}	4.16^{c}	0.39^{d}	1.60^{cd}	16.93^{b}	37.70^{ab}	97.96^{ab}	93.33^{ab}
	IBA 500	1.16^{c}	4.18^{bc}	0.40^{cd}	1.62^{cd}	18.92^{a}	38.09^{ab}	98.52^{a}	94.07^{ab}
	IBA 1000	1.05^{e}	4.10^{d}	0.36^{e}	1.59^{de}	17.94^{ab}	35.57^{c}	96.11^{bc}	92.04^{b}
	IAA 250	1.17^{c}	4.19^{bc}	0.40^{cd}	1.61^{cd}	18.23^{ab}	37.89^{ab}	99.07^{a}	94.45^{ab}
	IAA 500	1.14^{cd}	4.16^{c}	0.40^{cd}	1.61^{cd}	17.93^{ab}	37.97^{ab}	97.59^{abc}	93.15^{b}
	IAA 1000	1.16^{cd}	4.20^{bc}	0.40^{cd}	1.62^{bc}	17.37^{b}	38.13^{ab}	95.93^{c}	91.85^{b}
	NAA 250	1.21^{b}	4.24^{b}	0.42^{b}	1.64^{b}	17.84^{ab}	38.55^{ab}	98.52^{a}	94.08^{ab}
	NAA 500	1.34^{a}	4.37^{a}	0.46^{a}	1.67^{a}	18.97^{a}	39.51^{a}	99.26^{a}	95.93^{a}
	NAA 1000	1.17^{c}	4.21^{bc}	0.41^{bc}	1.62^{bc}	17.79^{ab}	38.14^{ab}	97.96^{ab}	94.45^{ab}
Grand Mean		1.15	4.19	0.40	1.62	17.99	37.85	97.72	93.69
SEd		0.01	0.03	0.01	0.02	0.14	0.20	0.20	0.27
Sig. ($p \leq 0.05$)	CDC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	NON	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.32^{NS}
	AC	0.00	0.00	0.00	0.00	0.05	0.01	0.00	0.05
	CDC*NON	0.00	0.00	0.00	0.00	0.00	0.00	0.99^{NS}	0.75^{NS}
	CDC*AC	0.00	0.01	0.00	0.02	0.23^{NS}	0.79^{NS}	0.22^{NS}	0.61^{NS}
	NON*AC	0.00	0.01	0.01	0.07^{NS}	0.85^{NS}	0.38^{NS}	0.82^{NS}	0.92^{NS}
	CDC*NON*AC	0.12^{NS}	0.89^{NS}	0.11^{NS}	0.17^{NS}	0.99^{NS}	0.47^{NS}	0.92^{NS}	1.00^{NS}

DAP: Days after planting; Means with the same alphabet are not significantly different from each other at $p \leq 0.05$ using DMRT test.

Among all treatments, NAA at 500 ppm was proven to be the most effective. It resulted in the highest values for shoot length (16.52 cm and 60.39 cm), root length (9.04 cm and 13.47 cm), number of shoots (1.56 at both stages), number of leaves (3.72 and 8.94), fresh weight (1.34 g and 4.37 g) dry weight (0.46 g and 1.67 g), root shoot ratio (18.97 and 39.51), and Survival percentage (99.26 and 95.93%) at 30 and 60 DAP, respectively (Table 3). In comparison, control (untreated) cuttings showed the lowest performance in almost all growth and survival parameters.

Moderate concentrations of IBA and IAA (250–500 ppm) also improved growth and rooting compared to the control, but their effects were less than NAA at 500 ppm. Higher concentrations (1000 ppm) of all auxin treatments showed a declining trend. Overall, the findings suggest that the combination of thinner cuttings (< 1.5 mm), two nodes, and treatment with NAA at 500 ppm results in better rooting efficiency, enhanced vegetative growth, greater biomass accumulation, and higher survival rates under nursery conditions.

4. Discussions

4.1 Seed propagation efficiency

Seed-based propagation of *H. Validus* showed significant ($p \leq 0.05$) improvement in seed germination and seedling vigour under pre-sowing treatments such as thiourea (0.1%), GA_3 (500 ppm), and water soaking for 24 hours. Results indicate that physiological dormancy can be partially overcome through chemical and hydration treatments [21]. The earlier germination observed under GA_3 treatments (28.67 days) compared to the control (47.00 days) highlights the role of gibberellic acid in breaking physiological dormancy by stimulating enzymatic activity, promoting embryo growth, and promoting reserve mobilization [11, 22]. Similarly, thiourea treatments also accelerated germination as well as seedling vigour parameters, which might be due to the promotion of seed respiration and mobilization of stored reserves. These results align with the findings in *Phyllanthus emblica* [23] and *Terminalia arjuna* [24]. Additionally, the superior germination observed with simple water soaking (95.56%) underscores the importance of hydration-mediated metabolic activation [21, 25]. The effectiveness of water soaking in enhancing seed germination has been reported in other Menispermaceae climber species, such as *Coscinium fenestratum* [26]. Another study reported that KNO_3 (2–10%) resulted in 67–95% germination in *Coscinium fenestratum* by enhancing seed imbibition and stimulating metabolic activity related to dormancy breaking [27].

Further, GA_3 500 ppm and thiourea 0.1% treatments effectively involved in the improvement of seedling growth parameters, hence significantly enhanced seedling vigour and establishment. GA_3 stimulates cell elongation, division, and stem growth, increases nutrient mobilization, and enhances photosynthetic efficiency and chlorophyll content, resulting in longer, more robust seedlings and increased biomass accumulation [22]. The seedlings raised from thiourea treatment exhibited enhanced growth performance, improved survival and collar diameter, and higher physiological activity and root growth, likely due to increased nitrogen metabolism, enzymatic activation, respiration, and dormancy breaking that enable cell division and elongation [24, 28].

To date, only one study has evaluated seed-based propagation of *H. validus*, focusing on presowing treatments [9]. The present study reported much earlier germination, initiating at 28.67 days under GA_3 500 ppm treatment, compared to 47.00 days in the control. This contrasts with the earlier sole findings of Bohra et al. [9], who reported that germination initiated only after 45 days, with the control requiring even longer duration (52.50 days). It is noteworthy that water soaking for 24 hours resulted in a higher germination (95.56%) and improved seedling growth compared to 75.00% reported in an earlier study by Bohra et al. [9]. The findings relevant to seed germination and seedling establishment with 0.1% thiourea were consistent with earlier findings, but in GA_3 treatments, the present study recorded higher germination and seedling performance at 500 ppm compared to earlier reported results at 1000 ppm. These findings support that moderate concentrations of growth regulators may be more effective than higher doses, possibly due to reduced hormonal imbalance or inhibitory effects at supra-optimal levels [29, 30].

4.2 Vegetative propagation efficiency

Vegetative propagation through cuttings exhibited comparatively faster establishment and higher uniformity, particularly in the two nodal cuttings treated with NAA (500 ppm). The interaction of CDCs, NONs, and ACs indicates that successful vegetative propagation depends on a synergistic relationship between physical characteristics of cuttings and hormonal regulation.

The superior performance of thinner cuttings (< 1.5 mm) suggests that juvenile tissues possess a higher rooting potential compared to thicker and mature cuttings. Juvenile tissues are generally characterized by higher metabolic activity, greater cell division, and increased sensitivity to endogenous auxins, which collectively enhance adventitious root formation. Similar trends of diameter-dependent responses have been reported in members of Menispermaceae, such as *Tinospora cordifolia* and *Cissampelos pareira*, where juvenile softwood cuttings showed improved rooting compared to mature stem segments.

Several woody and horticultural species with thinner cuttings exhibited better rooting and growth due to reduced lignification and improved carbohydrate mobilization [29, 31]. In contrast, thicker cuttings (> 3 mm), although containing more stored reserves, often show reduced rooting efficiency due to increased lignifications [32], lower permeability to water and hormones, and reduced physiological activity [33]. The lower survival rate observed in thicker cuttings in the present study supports the decline in rooting ability of woody plants with increasing tissue maturity and stem diameter, due to reduced meristematic activity and hormonal sensitivity [29, 34].

The number of nodes per cutting also significantly affected growth and rooting responses. Two-node cuttings performed best for most parameters, indicating an optimal balance between resource availability and transpiration demand. Nodes serve as critical sites for meristematic activity and endogenous hormone synthesis, particularly auxins and cytokinins, which regulate the development of both roots and shoots [35]. The superior performance of two-node cuttings at later stages (60 DAP) may be attributed to efficient allocation of assimilates between root and shoot systems, resulting in sustained growth and biomass accumulation [36]. In contrast, One-node cuttings resulted in poor growth performance, likely due to insufficient stored reserves and limited meristematic sites [36, 37].

Varying concentrations of auxin treatments significantly enhanced rooting, growth, and survival compared to untreated control cuttings. The results show the pivotal role of exogenous auxins in stimulating adventitious root formation [37]. Among the auxin treatments, NAA at 500 ppm proved to be the most effective treatment in producing superior growth, biomass, and survival. This may be attributed to the stability and prolonged activity of NAA in plant tissues, which promote cell elongation, differentiation, and the initiation of root primordia [38]. The growth hormone NAA treatment may improve the mobilization of carbohydrates and nitrogenous compounds towards the basal portion of cuttings, thereby facilitating root development [39]. In addition to NAA, the effect of IBA on rooting has been documented in other Menispermaceae climber species, including *Cissampelos pareira* [40]. Moderate concentrations of IBA and IAA (250–500 ppm) also improved rooting and growth compared to the control, but their comparatively lower effectiveness may be due to differences in uptake, transport, and metabolic stability [35]. The decline in performance at higher concentrations (1000 ppm) across all treatments suggests potential inhibitory or phytotoxic effects, as supra-optimal levels can disrupt cellular metabolism and hinder root initiation [29, 39].

4.3 Comparative Propagation Efficiency and Biological Basis

The present study clearly demonstrates that both seed and vegetative propagation methods significantly improve the regeneration potential of *H. validus*, although their efficiency differs in terms of establishment rate, uniformity, and propagation success. The contrasting performance of seed and vegetative

methods highlights the role of dormancy versus juvenility, where seed propagation is influenced by dormancy-related constraints, while vegetative propagation depends on the physiological status of the cutting material [33, 41].

Similarly, hormonal regulation also differs between the two approaches. In seed germination, GA_3 promotes germination processes by activating metabolic pathways and overcoming dormancy, whereas in vegetative propagation, auxins such as NAA induce adventitious root formation and subsequent development [42]. These variations reflect distinct biological processes governing propagation, with seed-based methods favouring genetic variability and adaptability, and vegetative methods ensuring clonal fidelity and uniform growth. Thus, the contrasting performance of the two approaches can be attributed to the interplay between dormancy versus juvenility and differential hormonal control mechanisms, which ultimately determine propagation success and plant establishment [43].

4.4 Practical Implications and Propagation Strategy

From an applied perspective, both propagation methods offer distinct advantages for the domestication and large-scale cultivation of *H. Validus*, but also have certain drawbacks. Seed propagation is advantageous for maintaining genetic diversity, producing rootstocks, and supporting conservation-oriented large-scale plantation programmes, especially in degraded and marginal lands. The effectiveness of simple treatment such as water soaking for 24 hours further enhances its suitability for field-level adoption by farmers and local communities.

In contrast, vegetative propagation provides a reliable method for the rapid multiplication of selected superior genotypes with uniform growth characteristics, facilitating the establishment of orchards with desirable traits for commercial cultivation. The use of softwood cuttings treated with NAA (500 ppm) offers a practical and efficient approach for clonal propagation, ensuring consistency in yield and quality.

However, the choice of propagation method ultimately depends on factors such as the availability of seeds and planting material, cost, space, and the intended objectives of cultivation. Therefore, an integrated propagation approach that combines both seed and vegetative approaches is recommended. This type of strategy allows for the maintenance of genetic diversity through seed propagation while enabling the multiplication of elite genotypes through clonal methods.

Further studies are underway to evaluate seed treatment with Beejamrutha and a microbial consortium, as well as the effects of combined applications of IBA and NAA at varying concentrations on vegetative propagation of softwood cuttings. In addition, grafting and layering techniques are also being systematically investigated to further enhance propagation efficiency and support the large-scale domestication of this underutilized fruit species.

5. Conclusion

Blood fruit is an underutilized yet highly nutritious and medicinally important wild climber of Northeast India. The present study highlights that both seed and vegetative propagation methods are effective for improving the regeneration and establishment of *H. validus*, each offering distinct advantages depending on the intended application. Rather than relying on a single method, the findings support a complementary propagation approach for sustainable utilization and conservation of this underutilized forest climber. Significant differences among treatments indicated that pre-sowing treatments with thiourea 0.1%, water soaking for 24 hours, and GA_3 500 ppm enhance germination rate, seedling vigour, and other growth parameters compared to the control. The shoot length, collar diameter, biomass accumulation, and survival rate were effectively enhanced in seedlings treated with GA_3 and thiourea, which demonstrates their potential to produce physiologically vigorous seedlings. However, for field-level adoption, simple pre-sowing treatments such as water soaking for 24 hours provide a low-cost and practical method for improving germination and seedling establishment, making them suitable for farmers and community-based planting programmes.

For nursery-based multiplication, the use of juvenile softwood cuttings treated with NAA (500 ppm) offers a reliable technique for rapid and uniform propagation of planting material, particularly for the multiplication of superior genotypes. From a conservation perspective, integrating both propagation methods is essential. Seed propagation can be utilized to maintain genetic diversity and support large-scale plantation efforts, while vegetative propagation can ensure the multiplication of elite and high-performing individuals. This combined strategy enables the development of robust planting stock while preserving the genetic base of the species.

Overall, the study provides a practical and scalable propagation framework that can facilitate the domestication, large-scale cultivation, and conservation of *H. validus* in the North Eastern region of India. Adoption of these standardized techniques can enhance livelihood opportunities, promote agroforestry integration, and contribute to biodiversity conservation. Further research focusing on seed physiology, *in vitro* propagations, and field establishment will be valuable for commercialization and conservation of this nutritionally rich species.

Declarations

Competing interest

The authors have no relevant financial or non-financial interests to disclose.

Ethical approval

This study did not involve human participants or animals. The plant parts and seed collection were conducted in accordance with local regulations and the guidelines of the International Union for Conservation of Nature (IUCN). Prior permission was obtained from the landowners for photographing and collecting plant materials.

Consent to participate

Not applicable

Consent to publish

Informed consent to publish was obtained from all participants whose identifiable images or personal data were included in the manuscript.

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Author Contribution

Study conception, design, and material preparation by Dinesha S. Data collection and analysis were performed by Sanren Pungjung, Ajijul Mir, and Gongthuiliu Kamson. The first draft of the manuscript was written by Dinesha S, Sanren Pungjung, and Baluti Passah and edited by Dinesha S. All authors read and approved the final manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

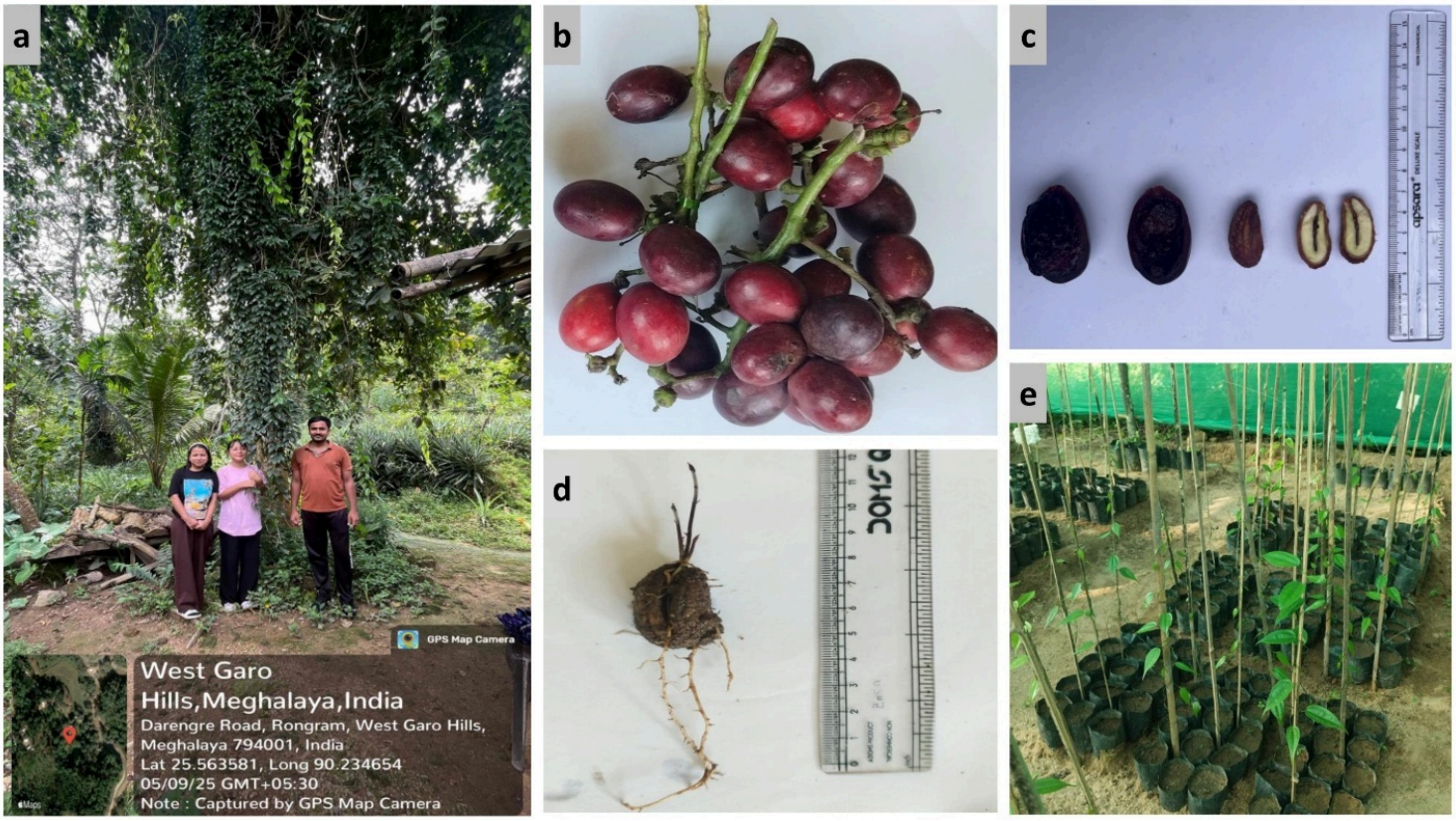

Figure 1

a. Blood fruit, a woody climber, spreads over a large *Artocarpus chama* tree; **1.b.** Ripe fruits; **1.c.** Dissected fruit and seed;**1.d.** Polyembryonic seed germination; **1.e.** Propagation of blood fruit in the nursery

Figure 2

a. Softwood, semi-hardwood, and hardwood cuttings; **2.b.** Preparation of softwood cuttings with diameters of <1.5 mm, 1.5-3.0 mm, and >3.0 mm; **2.c.** Planting of softwood cuttings in polybags under nursery conditions.

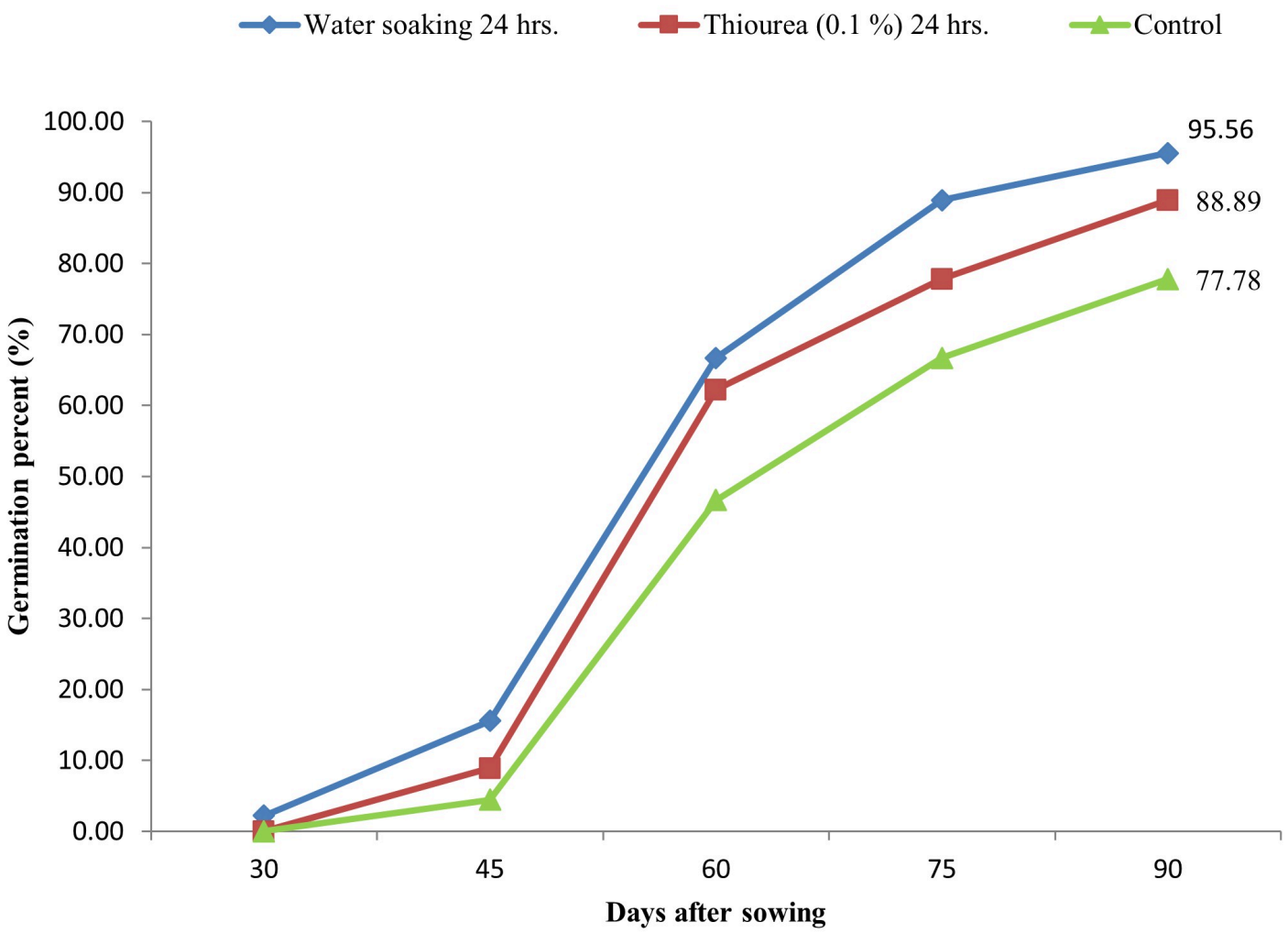

Figure 3

Germination percentage at 15-day intervals

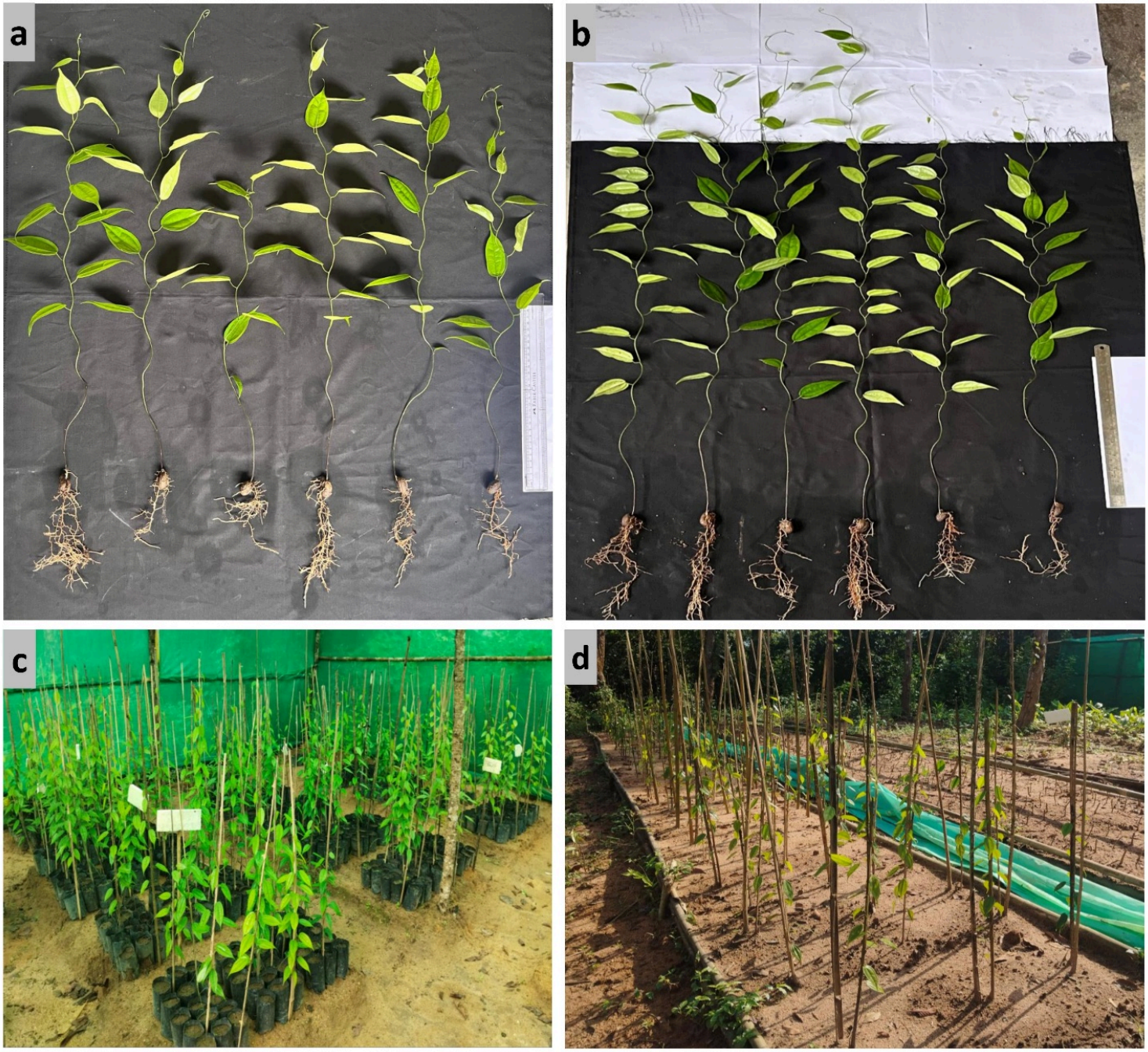

Figure 4

a. Seedling growth in the control treatment at 90 DAS; **3.b.** Seedling growth in Thiourea 0.1 % treatment at 90 DAS; **4.c.** Blood fruit seed germination and seedling growth under nursery conditions at 90 DAS; **4.d.** Outplanting of seedlings for annual assessment of growth parameters

Figure 5

a. Sprouting of juvenile < 1.5 mm softwood cuttings at 15 DAP; **5.b.** Rooted cutting at 20 DAP;**5.c.** Established seedling via juvenile two-node cuttings treated with 500 ppm NAA at 30 DAP.