Domestication and conservation efforts in *Haematocarpus validus* (Miers.) Bakh. f. ex Forman (Menispermaceae): an underutilized fruit species and natural colourant

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Blood fruit is a promising anti-oxidant rich underutilized fruit species with potential as a natural colourant for food, beverage and handicraft industries. The species has been used for fresh consumption, treating ailments and as a dyeing agent by local people including regional tribes in tropical Asian countries. However, at present, fruits are harvested from the wild and there is considerable scope for promoting their cultivation in homestead gardens. Owing to limited population of the species, seed germination studies were conducted. Treatment of seeds with thiourea (0.1%) or GA_3 (1000 mg/l) for 24 h was most congenial for obtaining healthy seedlings in large numbers. To extract chlorophylls and total carotenoids, dimethylsulphoxide was found to be a superior solvent over acetone (80%). Differences in fruit sizes were noticed in fruits grown in two states of India. These findings form the basis for conservation as well as domestication of this multipurpose fruit species in the tropical Asian countries.

Keywords: Chlorophyll, germination, home garden, liana, Menispermaceae, regeneration, tropical islands.

BLOOD FRUIT (*Haematocarpus validus* (Miers.) Bakh. f. ex Forman, Menispermaceae) is a woody climber that grows by taking support of large trees and yields edible fruits[1]. Immature fruits, a source of vitamin C, are used locally for preparation of value-added products like pickles and chutney[2]. Fully ripe fruits are relished mainly by the Bengali community in Andaman islands[3] and ethnic communities of Bangladesh[4]. Acidic sweet fruits turn dark red on ripening, which can also be dried and stored for future consumption. Antioxidant rich ripe fruits contain iron, ascorbic acid, β-carotene, phenols, flavonoids, minerals and anthocyanins, while anti-nutritional factors are meagre[3,4]. In Tripura, it is used to prepare processed products such as squash and for dyeing local handicrafts. Tribes in Assam and Meghalaya are reported to use this species for its juice[5]. Fruits contain bright red juice, which could be utilized as a natural colourant in the preparation of desserts and beverages[6]. Use of natural colourants for improving the appearance of processed products is a known phenomenon, e.g. use of kokum (*Garcinia indica*) as natural colouring agent in the pummelo (*Citrus grandis*) based beverages has been successfully demonstrated[7]. Such natural colourants have been well accepted alternatives as they are healthy and free from other harmful effects as artificial colourants. Various plant parts such as fruits and seeds (for treating anemia), roots (for curing itching) and tender shoots (for treatment of jaundice) are also being used in traditional medicines[6]. Nicobarese tribes use leaf decoction for treating body ache[8].

The species is known to tolerate varied conditions including dry climate and highly acidic soils[4]. In addition, it thrives well in Andaman and Nicobar Islands (ANI), which receive heavy rains of about 3100 mm, with a distinct dry phase of 3–4 months. In Meghalaya, the species has been categorized as Critically Endangered[5]. In Bangladesh[9], the species had attained the Vulnerable status; however a recent report categorized it as highly threatened[6]. Nevertheless, both reports suggest that increasing anthropological activities and climatological factors are major causes responsible for it, and there is need to conserve the species on an urgent basis. Similarly, in ANI, loss of biodiversity due to natural calamities and anthropological pressure (mainly destructive harvesting) is becoming a rising concern[10]. Furthermore, fruit predation by forest fauna, which hinders the natural regeneration process in many underutilized species[11], could not be ruled out. In Middle Andaman island, local people suggested forage of blood fruit by deer when fruits were borne near the ground.

Presently most of the produce is harvested from the wild, and no systematic efforts have been made so far to promote its domestication. Its domestication could help in reducing the burden on natural stock and the produce

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would serve as a source of nutrition to the local people. Hence, the present study was undertaken as a basic step towards the domestication of this species on a small scale. Seed germination is one of the quickest means to multiply any species in large number, which can assist in replenishing the wild stocks[11,12] apart from providing planting material to the local farmers. Further, variability generated through sexual propagation could be assessed for identification of horticulturally superior types, thereby assisting the crop improvement process, the basis of domestication, in this novel fruit. Hence, the first experiment aimed at standardization of seed germination technique in blood fruit. Secondly, photosynthetic pigments play a vital role in primary metabolism of any plant. These pigments would be required to be estimated during various future studies pertaining to crop physiology, biochemistry, crop production and crop improvement in this species. Selection of the method for pigment determination varies with the species in question. Hence, in the second experiment, we attempted to identify a suitable method for determination of photosynthetic pigments in this species. This article also highlights the importance of this species, covering its distribution, morphology and future prospects.

Materials and methods

Local names and distribution of the species

Blood fruit is known by various names[6], viz. *Khoon Phal*, *Rakta Phal* (Hindi), *Roktogula*, *Lalgola* (Bengali), *Rosco* (Chakma), *Ranguichi* (Marma), *Thaichak* (Kokborok), etc. In ANI, blood fruit has been reported to occur in forests of North and Middle Andaman islands and North Nicobar islands[3,13]. It has also been reported from Tripura, Meghalaya, Assam, Arunachal Pradesh, Sikkim and West Bengal[6,14]. Preliminary studies conducted at ICAR Research Complex for North Eastern Hill Region, Tripura centre revealed that it is mostly found in Gomati and Dhalai districts, Tripura. Apart from India, it is also reported from Bangladesh, Indonesia, Singapore, Pakistan and Thailand[5,6].

Morphological studies

Fruits were collected from local markets of North and Middle Andaman, ANI and Tripura and brought to the laboratory, wherein they were allowed to ripen naturally. Morphological parameters were recorded on 10 fruits using standard procedures. Ten fully developed leaves were used for recording their morphological features.

Seed germination study

Seeds were extracted from fully ripe fruits from ANI, washed and divided into different lots with 48 seeds in each treatment before imposing soaking treatments, viz. T_1: water soaking; T_2: GA_3 (500 mg/l); T_3: GA_3 (1000 mg/l); T_4: thiourea (0.1%) and T_5: thiourea (0.2%). Treated seeds were sown in pro-trays filled with coir pith and germination was observed till 115 days after sowing. Data obtained was used for calculation of germination characteristics. Germination (%) at different intervals was calculated by dividing the number of seeds germinated by the total number of seeds sown and multiplied by 100. Mean daily germination (MDG = cumulative germination/total number of days), germination index[15] and days for first germination[16] were also calculated. Ten plants from each treatment were randomly selected after 115

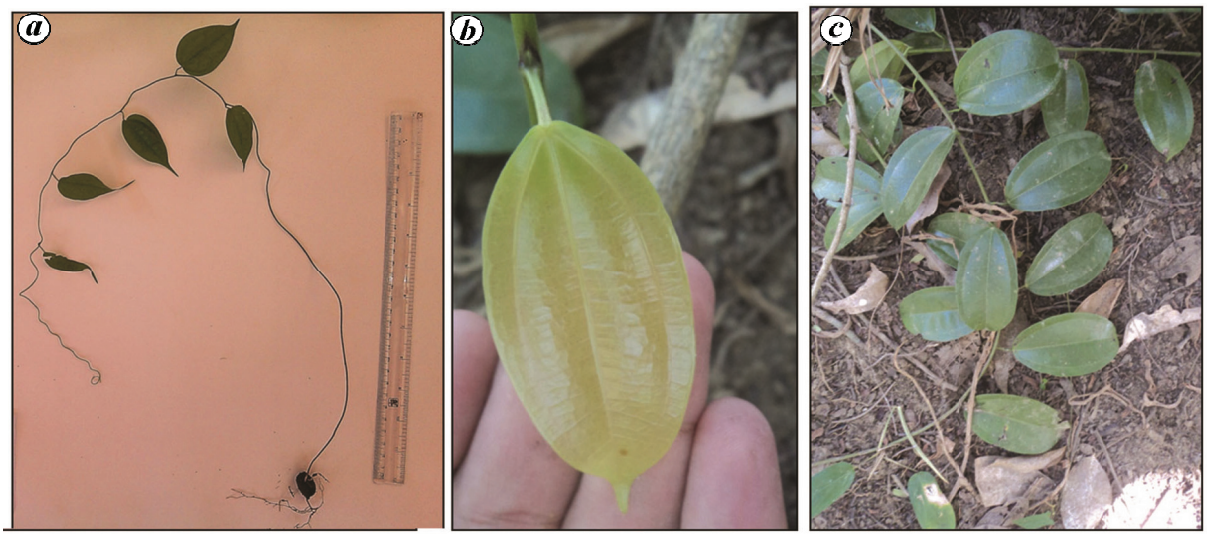

Figure 1. ***a***, Nine-week-old seedling; ***b***, a young leaf; ***c***, mature leaves borne on the well grown plant.

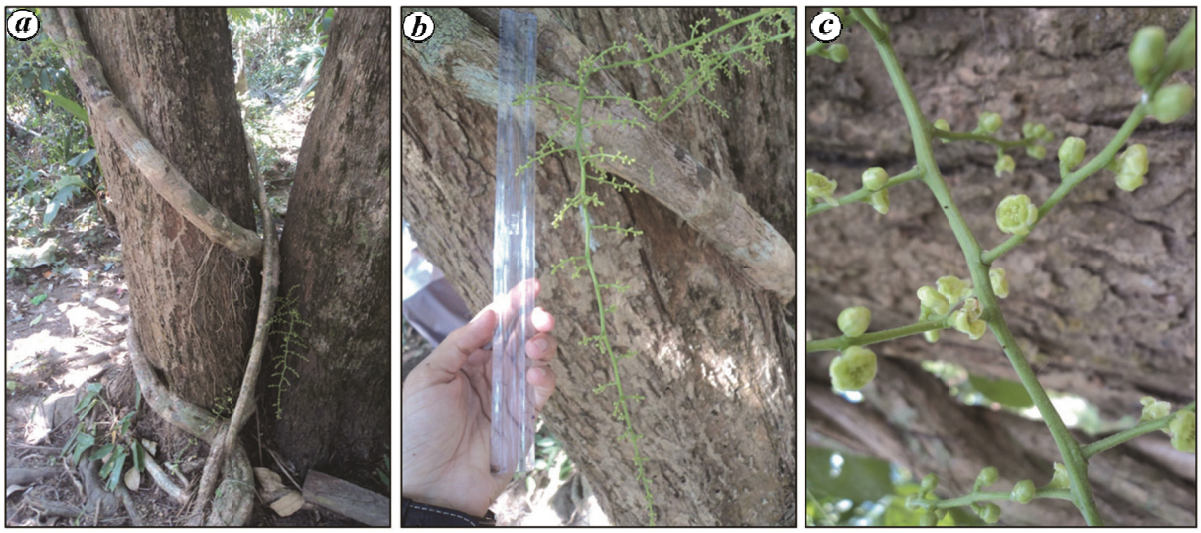

Figure 2. ***a***, Blood fruit liana growing on a tree; ***b***, inflorescence borne on the main trunk of the liana; ***c***, close view of an inflorescence.

days of sowing for recording growth parameters. Seedling vigour index was estimated by multiplying seedling length (cm) with germination (%) in the respective treatment[17]. Regenerated seedlings were transplanted in black poly-bags (30 cm × 22.5 cm) filled with soil and farmyard manure (1 : 1) and were staked using wooden sticks till field transplanting. Data was analysed using Web Agri Stat Package ver. 2.0 (ICAR-RC for Goa, Old Goa, India).

Comparison of methods for pigment determination

Two methods were compared for determination of photosynthetic pigments. In the first method, recently matured leaf sample (1 g) was extracted with pre-chilled acetone (80%) till the residue became colourless and the volume was made up to 10 ml. The extract was centrifuged at 4°C for 10 min at 5000 rpm and the supernatant was used for spectrophotometric determination. In the second method, leaf sample (125 mg) was incubated in 6.25 ml of dimethylsulphoxide for 48 h in dark at ambient temperature. The supernatant was used for recording absorbance using spectrophotometer. Calculations were done using Wellburn formulae[18].

Results

Morphological observations

Leaves are elliptic, ovate-elliptic or obovate-elliptic with obtuse base (Figure 1). The average length and width of leaves collected from ANI during the present study were 12.50 cm and 5.61 cm respectively, with 1.6 to 2.5 cm long petioles. Though systematic studies have not been conducted on phenology, it has been found to produce flowers more than once a year. Inflorescence (Figure 2), borne on the main trunk or in axis, is a panicle of up to 40 cm length with flowers having six ovate petals. Due to light greenish to yellowish colour, flowers in the upper branches are not distinguishable from the leaves of supporting trees. Drupaceous fruits are borne in clusters and exhibit unsynchronized ripening in a bunch (Figure 3 *a* and *b*). Fruit colour changes from green to reddish and finally turns dark red or purple (Figure 3 *c*). Pericarp is generally thick but inedible. Owing to blood red colour of pulp upon ripening (Figure 4), the species is known as blood fruit.

The main fruit ripening season is April to June in ANI and Tripura. Fruits are sometimes sold in local markets at about INR 100–150 per kg (2017 price at Middle Andaman markets). Fruits obtained from Andaman and Tripura markets were studied for morphometric parameters (Table 1). Fruits from Tripura were lighter (12.78 g) when compared to those produced in Middle Andaman (15.11 g). A similar trend was noticed for fruit length and width. Pulp content of 18.38% with total soluble solid content of 18 to 19° Brix was recorded from Andaman grown fruits.

Seed germination study

Germination as affected by different treatments is presented in Figure 5. During the initial 45 days, no germination was noticed in any of the treatments. Most seeds started germinating thereafter and a linear trend was observed. At 50 days, varying degree of germination was recorded in all treatments except control. This clearly indicates the presence of dormancy in this species.

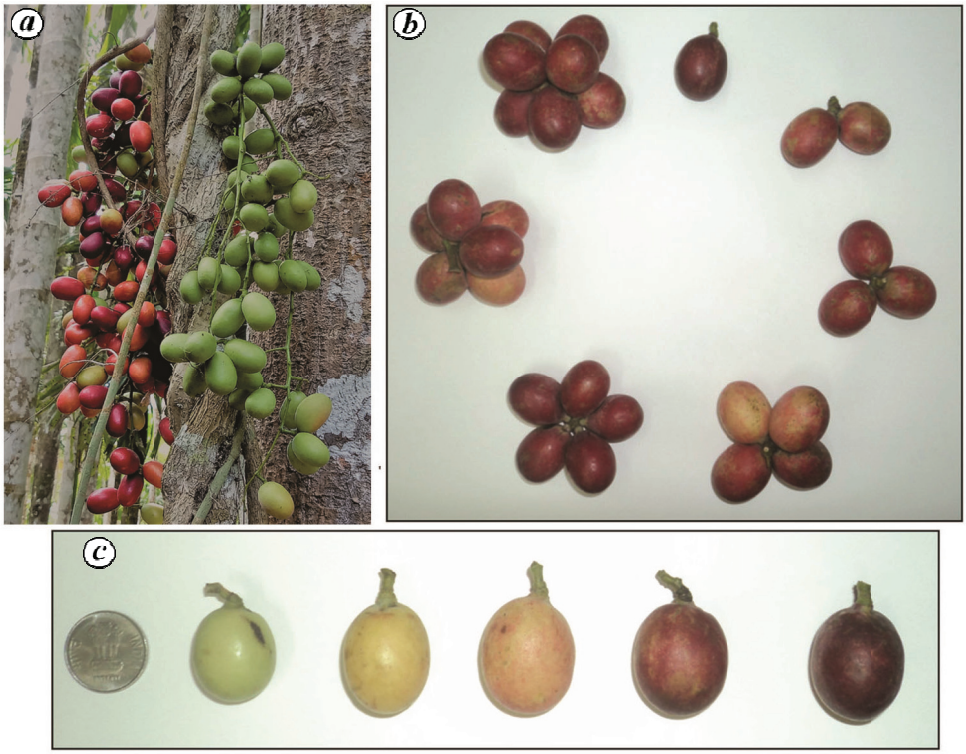

Figure 3. ***a***, Bunch exhibiting uneven ripening of the fruits; ***b***, clusters of fruits borne in a bunch; ***c***, colour change during fruit development.

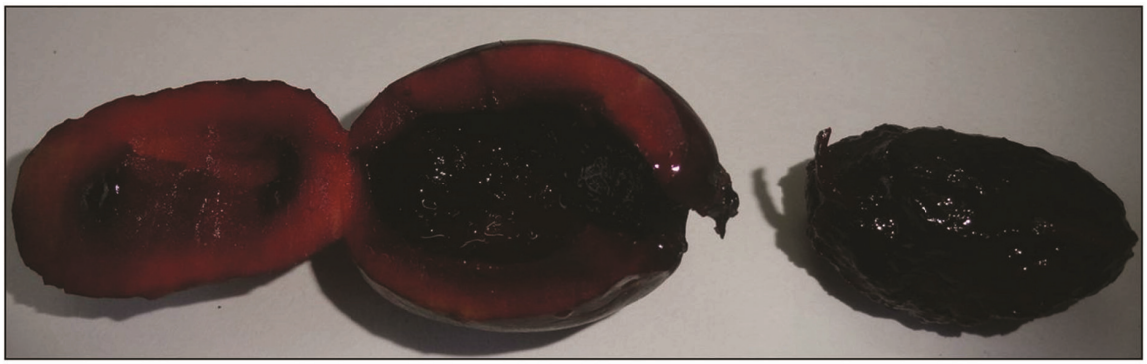

Figure 4. A cut open fruit showing attractive coloured pulp and seed.

Table 1. Comparative morphological parameters of blood fruit grown in different regions; ANI and Tripura (India) and Bangladesh

Parameters	ANI	Tripura	Bangladesh
Fruit weight (g)	15.11 ± 0.403	12.78 ± 0.519	28.0
Fruit length (mm)	35.82 ± 0.455	32.20 ± 0.450	40.0–50.0
Fruit width (mm)	27.26 ± 0.409	25.30 ± 0.380	20.0–25.0
Pulp (%)	18.38 ± 1.899	–	22.0
TSS (°B)	19.0	–	18.0
Reference	Present study	Present study	Khatun *et al.*[6]

Interestingly, treatment with 0.1% thiourea could induce germination in only 2.1% seeds by 50 days of sowing and it remained the slowest until 70 days. However, at 115 days, germination was maximum (85.4%) in this treatment. This did not differ significantly from those treated with 1000 mg/l GA_3 and 0.2% thiourea at 115 days.

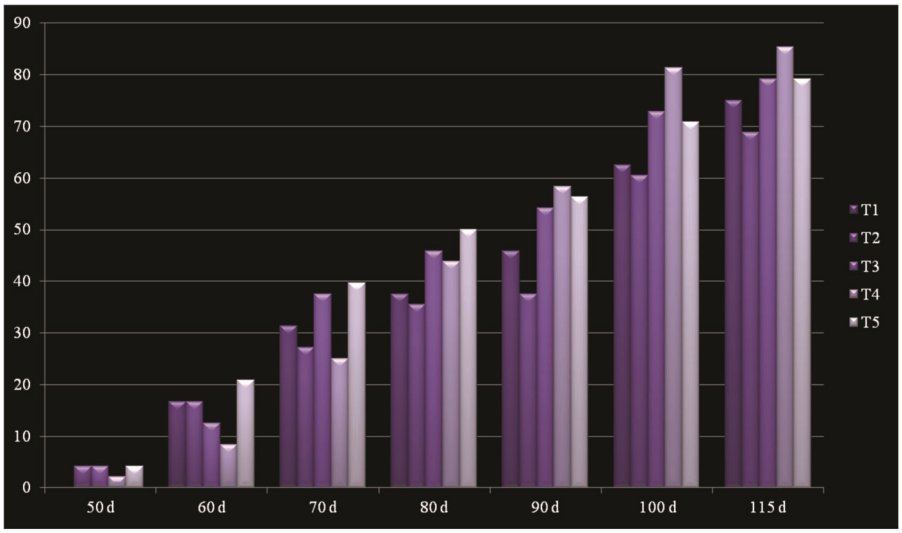

Figure 5. Percentage of seed germination in different pre-treatments during observation period. T_1: Water soaking; T_2: GA_3 (500 mg/l); T_3: GA_3 (1000 mg/l); T_4: Thiourea (0.1%); T_5: Thiourea (0.2%).

Table 2. Effect of different pre-treatments on vine growth parameters in blood fruit (*Haematocarpus validus*) after 115 days of sowing

Treatment	Final germination (%)	Vine length (cm)	Root length (cm)	No. of primary roots	No. of secondary roots	Number of nodes	Number of leaves	Collar thickness (mm)
T_1: Water soaking	75.0bc	81.93b	7.98a	1.0a	12.5a	11.9c	4.2c	1.02a
T_2: GA_3 (500 mg/l)	68.8c	75.76c	7.82a	1.0a	9.0c	11.9c	4.8b	1.08a
T_3: GA_3 (1000 mg/l)	79.2ab	85.71a	7.03b	1.0a	11.1b	14.8a	5.6a	1.09a
T_4: Thiourea (0.1%)	85.4a	70.58d	7.75a	1.0a	12.6a	15.1a	5.4a	1.05a
T_5: Thiourea (0.2%)	79.2ab	64.71e	6.14c	1.0a	12.1a	13.3b	5.0b	1.06a

Mean values followed by the same lower-case letter in a column did not differ significantly at $P \leq 0.05$ using least significant difference.

About 75% of seeds germinated in control (Table 2). Treatment with 0.2% thiourea was found the most efficient in inducing significantly earlier germination (47 days), while it was the slowest (52.5 days) in control (Figure 6 *a*). Non-significant differences were noticed for mean daily germination, germination index and seedling vigour index (Figure 6 *b–d*). This meant that though delayed germination was noticed in control, it had no negative effect on germination characteristics studied.

Newly germinated seedlings had glabrous green stem. Vine length after 115 days of sowing varied between 64.71 cm and 85.71 cm (Table 2). Longest vines were recorded from seeds treated with 1000 mg/l GA_3 (T_3), which was significantly superior to control (81.93 cm). Length of the primary roots varied between 6.14 cm and 7.98 cm, however the root length in control was statistically comparable with those produced in treatments T_2 (500 mg/l GA_3) and T_4 (0.1% thiourea). The number of primary roots, however, differed significantly amongst the treatments. Though a maximum number of primary roots was produced in thiourea treatments (T_4 and T_5), it remained on par with the control.

Germinated seedlings are generally leafless during the initial stage, and leaf development occurs with seedling age. The number of nodes was maximum (15.1) in seedlings obtained from thiourea (0.1%) treatment, closely followed by those treated with 1000 mg/l GA_3 (14.8). The number of leaves was also the highest in these two treatments. For both number of nodes and leaves, the lowest values were recorded in control. Thickness of the vines at collar region did not differ significantly amongst the treatments. Considering the germination and overall performance, soaking of seeds in thiourea (0.1%) or GA_3 (1000 mg/l) could be recommended for obtaining healthy seedlings in large numbers (Figure 7 *a*).

Ex situ conservation

Thirty seedlings (obtained from the germination study) were planted in the germplasm block of ICAR-CIARI, Port Blair for conservation. Seedlings were also planted in Dhanikhari Experimental Garden cum Arboretum of Botanical Survey of India (Port Blair), Department of Agriculture's Organic Horticulture Farm (Sippighat) and Krishi Vigyan Kendra (Middle Andaman Island). In order to promote homestead cultivation, seedlings were also distributed to farmers in South Andaman during this year.

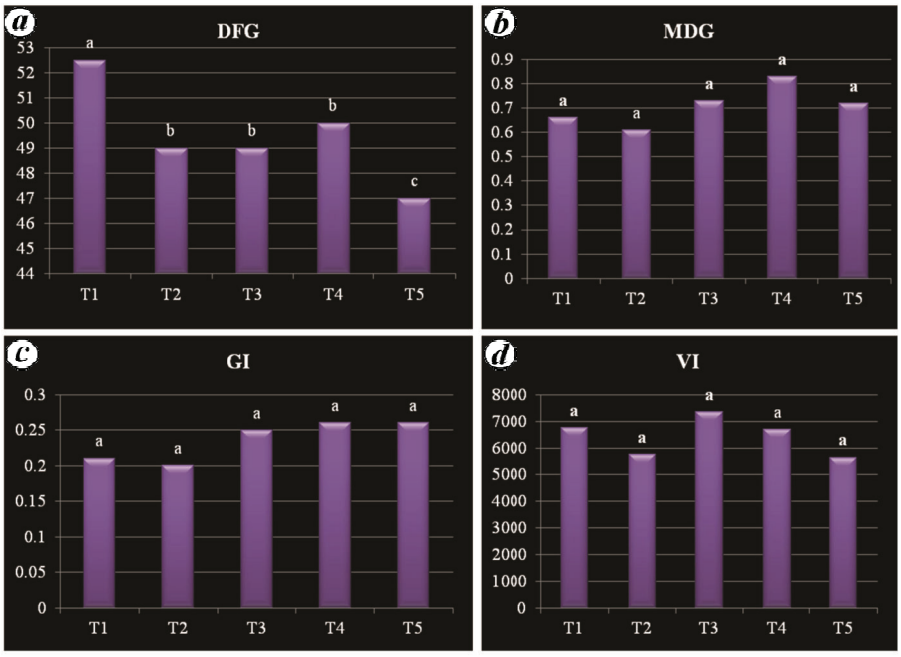

Figure 6. ***a***, Days for first germination (DFG); ***b***, mean daily germination (MDG); ***c***, germination index (GI); ***d***, seedling vigour index (VI) in blood fruit as influenced by different pre-treatments. T_1: Water soaking; T_2: GA_3 (500 mg/l); T_3: GA_3 (1000 mg/l); T_4: Thiourea (0.1%); T_5: Thiourea (0.2%). Same lower-case letter on different bars in a graph indicates no significant difference at $P \leq 0.05$ using least significant difference.

Figure 7. (***a***) Germinated seedlings of blood fruit; seedlings staked with wooden sticks; (***b***) polyembryonic seedling.

A custodian farmer has also been recognized in the North and Middle Andaman. These seedling progenies would form the base population for identification of superior type of blood fruit in future.

Comparison of methods for pigment determination

The method involving incubation of leaf tissues in dimethylsulphoxide was found superior for estimation of chlorophylls and total carotenoids in blood fruit (Table 3). All the parameters studied, viz. chlorophyll *a*, chlorophyll *b*, total chlorophylls, chlorophyll *a* : chlorophyll *b* and total carotenoids were significantly lower in acetone method (0.087 mg/g, 0.036 mg/g, 0.123 mg/g, 2.402 mg/g and 0.038 mg/g respectively), when compared to dimethylsulphoxide method (0.405 mg/g, 0.132 mg/g, 0.537 mg/g, 3.059 and 0.071 mg/g respectively).

Discussion

Comparison of fruits collected from two states of India and Bangladesh revealed considerable variations in fruit

Table 3. Comparative performance of two methods on extraction efficiency of photosynthetic pigments

Pigment	Concentration (mg/g, FW)		*t* test
	80% Acetone method	DMSO method	
Chlorophyll *a*	0.087 ± 0.002	0.405 ± 0.002	**
Chlorophyll *b*	0.036 ± 0.001	0.132 ± 0.002	**
Total chlorophylls	0.123 ± 0.002	0.537 ± 0.004	**
Chl *a* : Chl *b*	2.402 ± 0.085	3.059 ± 0.046	**
Total carotenoids	0.038 ± 0.0003	0.071 ± 0.0005	**

morphology. Fruits from Tripura and ANI were comparatively lighter and smaller than those reported from Bangladesh[6]. Pulp content, which is the economic portion of the fruit, varied between 18.38% (ANI) and 22.0% (Bangladesh). With the natural population in all these regions being seedling originated, considerable scope exists for identification of superior types with bigger size fruits and higher pulp recovery. Systematic surveys could be taken up in all the growing areas to tap the diversity.

As water soaking resulted in appreciable seed germination, it could be assumed that under wild conditions of evergreen tropical forests, the species can regenerate on its own, following a few showers of monsoon, as it would largely simulate water soaking treatment. However, collection of fruits from the wild by the local people and predation by forest fauna hinders the natural regeneration process[11]. Hence, assisted regeneration through nursery raising is of paramount importance in its conservation. Thiourea and gibberellic acid are amongst the most commonly used chemicals for seed germination. In blood fruit, both chemicals were efficient enough to improve germination and seedling growth. Positive effects of thiourea could be attributed to its efficiency in overcoming dormancy and its cytokinin activity[19]. Earlier reports indicated that GA_3 promoted germination process, post-germinative development and conversion into plantlets[20,21] through activation of hydrolytic enzymes that are known to increase the osmotic content of seeds[22]. Seeds treated with 1000 ppm GA_3 produced the longest seedlings, which could be due to cell elongation activity of gibberellic acid[23,24]. Comparison of methods for estimation of photosynthetic pigments revealed significant differences and the method involving dimethylsulphoxide was found to be superior. Efficacy of pigment extraction method and the solvent employed varies greatly with the species[25], wherein photosynthetic pigments of non-course textured tissues (such as blood fruit) could be more efficiently extracted using dimethylsulphoxide[18].

The way forward

Considering the nutritional importance and potential uses, systematic efforts have been initiated to collect and evaluate the diversity, standardize mass multiplication, cultivation techniques, etc. for promoting commercial scale cultivation in Bangladesh[6]. In other places of distribution too, it needs to be conserved on priority[26]. Systematic surveys could help in identification of types with higher pulp content, precocity and tolerance to biotic and abiotic stresses. Polyembryony was reported[27] for the first time in the species (Figure 7 *b*), which could further be studied in detail. Efforts have been initiated at ICAR-CIARI (Port Blair) for standardization of micropropagation protocol. Procedures for isolation of anthocyanins and utilization as natural colourant for preparing products need to be standardized. Sensitization of stakeholders holds the key to open up the vistas of domestication and sustainable utilization in such species.

1. Forman, Menispermaceae. *Flora Malesiana* (ser. I), 1986, **102**, 157–253.
2. Anonymous, Annual Report for 2013–14, Indian Council of Agricultural Research – Central Island Agricultural Research Institute, Port Blair, India.
3. Singh, D. R., Singh, S. and Shajeeda Banu, V., Estimation of phytochemicals and determination of beta carotene in *Haematocarpus validus*, an underutilized fruit of Andaman and Nicobar Islands. *Eur. J. Environ. Ecol.*, 2014, **1**(1), 12–15.
4. Rahim, M. A., Khatun, M. J. M., Rahman, M. M., Anwar, M. M. and Mirdah, M. H., Study on the morphology and nutritional status of Roktogota (*Haematocarpus validus*) – an important medicinal fruit plant of hilly areas of Bangladesh. *Int. J. Minor Fruits Med. Arom. Plants*, 2015, **1**(1), 11–19.
5. Singh, B. and Bedi, Y. S., Rediscovery, taxonomic history and extended enumeration of *Haematocarpus validus* Bakh. f. ex Forman (Menispermaceae) to Indo-Myanmar biodiversity hotspot. *Nat. Acad. Sci. Lett.*, 2016; doi:10.1007/s40009-016-0483-8.
6. Khatun, M. J. M., Rahman, M. M., Rahim, M. A. and Mirdah, M. H., Study on the taxonomy and nutritional status of lalgula (*Haematocarpus validus*): a promising endemic ethnic fruit of Bangladesh. *G-Science*, 2014, 1–4.
7. Bohra, P., Sreenivas, K. N. and Sreeramu, B. S., Development of a cost-effective, palatable and shelf stable blended beverage of pummelo (*Citrus grandis* Linn.). *Fruits*, 2012, **67**, 249–256.
8. Kumar, K., Kumar, B., Selvun, T., Sajibala, B., Jairaj, R. S. C., Mehrotra, S. and Pushpangadan, P., Ethnobotanical heritage of Nicobarese tribe. *J. Econ. Taxon. Bot.*, 2006, **30**, 331–348.
9. Sarwar, A. K. M. G., Genera represented by single species in the flora of Bangladesh and their conservation needs – a review. *J. Today's Biol. Sci. Res. Rev.*, 2013, **2**(1), 68–83.
10. Gautam, R. K. *et al.*, Utilization of agri-horticultural biodiversity for food and nutritional security in Andaman and Nicobar Islands. In Souvenir, National Seminar on Harmonizing Biodiversity and

Climate Change: Challenges and Opportunity, Port Blair, India, 17–19 April 2015, pp. 55–65.
11. Sundriyal, M. and Sundriyal, R. C., Seed germination and response of stem cuttings to hormonal treatment in six priority wild edible fruit species of Sikkim Himalaya. *Indian For.*, 2001, **127**, 695–706.
12. Sundriyal, M. and Sundriyal, R. C., Underutilized edible plants of the Sikkim Himalaya: need for domestication. *Curr. Sci*., 2003, **85**(6), 731–736.
13. Rao, P. S. N., *Haematocarpus* Miers. In *Flora of Andaman and Nicobar Islands* (eds Hazra, P. K., Rao, P. S. N. and Mudgal, V.), Botanical Survey of India, Kolkata, 1999, pp. 98–99.
14. http://indiabiodiversity.org
15. AOSA, In *Seed Vigor Testing Handbook. Contribution No. 32 to the Handbook on Seed Testing*, Association of Official Seed Analysis, East Lansing, 1983, p. 88.
16. Al-Mudaris, M. A., Notes on various parameters recording the speed of seed germination. *Der Tropenlandwirt Beitrage zur tropischen Landwirtschaft Veterinarmedizin*, 1998, **99**, 141–154.
17. Abdul Baki, A. A. and Anderson, J. D., Vigor determination in soybean by multiple criteria. *Crop Sci*., 1973, **13**, 630–633.
18. Wellburn, A. R., The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol*., 1993, **144**, 307–313.
19. Cetinbas, M. and Koyuncu, F., Improving germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea. *Hortic Sci.* (*Prague*), 2006, **33**, 119–123.
20. Evans, A. S., Mitchell, R. J. and Cabin, R. J., Morphological side effects of using gibberellic acid to induce germination–consequence for the study of seed dormancy. *Am. J. Bot.*, 1996, **83**, 543–549.
21. Rascio, N, Mariani, P., Dalla, V. F., La Rocca, N., Profumo, P. and Gastaldo, P., Effects of seed chilling or GA_3 supply on dormancy breaking and plantlet growth in *Cercis siliquastrum* L. *Plant Growth Regul*., 1998, **25**, 53–61.
22. Paleg, L. G., Physiological effect of gibberellic acid II on starch hydrolyzing enzymes of barley endosperm. *Plant Physiol.*, 1960, **35**, 902–996.
23. Gul, H., Khattak, A. M. and Amin, N., Accelerating the growth of *Araucaria heterophylla* seedlings through different gibberellic acid concentrations and nitrogen levels. *J. Agric. Biol. Sci.*, 2006, **1**, 25–29.
24. Taylor, A. and Cosgrove, D. J., Gibberellic acid stimulation of cucumber hypocotyls elongation. *Plant Physiol*., 1989, **90**, 1335–1340.
25. Barua, U., Das, R. P. and Gogoi, B., Chlorophyll estimation in some minor fruits of Assam. *Ecol. Environ. Conserv*., 2016, **22**(4), 1787–1789.
26. Kalkame, C. M., Suresh, C. P., Somi, Y. S. and Baggio Ch. Momin, Biodiversity and conservation of wild horticultural plants in Meghalaya, North East India. In *Book of Abstracts, International Symposium on Next Generation Approaches for Sustainable Development of Hill and Upland Horticulture*, Sikkim University, Gangtok, India, 6–7 November 2015, p. 10.
27. Bohra, P. and Waman, A. A., Observations on occurrence of polyembryony in four species from Bay Islands, India. *Indian For*., 2018, **144**(3), 311–312.

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