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Morphological and physicochemical studies in blood fruit (*Haematocarpus validus* Bakh. f. ex Forman): A tropical fruit and natural colorant

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ABSTRACT

Blood fruit is a tropical liana bearing edible fruit rich in anthocyanins. The species is distributed in many Asian countries, wherein it provides livelihood and nutritional security to native dwellers. However, in many of these regions produce is harvested from the wild by employing destructive harvesting practices, thereby damaging the natural stand. Systematic studies were initiated to popularize this species as a novel crop in the humid tropical parts of the world. Present study aims at studying four collections (HV/NE/TRI, HV/MA/KAL, HV/MA/SNU and HV/MA/SND) for morphological and biochemical traits. Results revealed considerable variability for fruit and seed traits, total soluble solids and pH. Total anthocyanin content among the collections varied between 183.63 and 328.28 mg CE/ 100 g pulp. Strong positive correlation was noticed between fruit dimensions with fruit weight, while negative correlation was noticed between pulp content and peel thickness. Ratio of material: solvent for obtaining better anthocyanins recovery was optimized to 1:15 (w/v). Promising genotype (HV/MA/SND) with superior fruit size, TSS content and total anthocyanins content was identified for further exploitation.

1. Introduction

Blood fruit (*Haematocarpus validus* Bakh. f. ex Forman) is a tropical fruit bearing species of Menispermaceae family. It is a liana that climbs on large trees in its natural habitat and fruit are borne in bunches which hang directly on the stem (Fig. 1a). Occurrence of the species has been reported from tropical countries such as India, Bangladesh, Indonesia, Pakistan, Singapore and Thailand (Singh and Bedi, 2016; Khatun et al., 2014). In India, natural populations of the species have been reported from the Andaman and Nicobar Islands, Arunachal Pradesh, Assam, Meghalaya, Sikkim, Tripura and parts of West Bengal (Bohra et al., 2018; Khatun et al., 2014; Singh and Bedi, 2016).

Fruit (Fig. 1b) and other parts of the species have traditionally been used for variety of purposes in regions of its native distribution. Fruit are climacteric in nature and color changes from green to dark red during fruit ripening (Fig. 1c). Fruit contain dark red edible pulp (Fig. 2a) and that is the reason for its name – blood fruit. Fruit have been known to be rich sources of vitamin C, iron, β-carotene, phenols, flavonoids and minerals (Rahim et al., 2015; Singh et al., 2014). Our recent studies have suggested that dark red pulp of blood fruit (Fig. 2a) is a novel source of anthocyanins major being Pelargonidin and Cyanidin (Bohra et al., 2020a). These compounds have been reported to have a number of medicinal properties (Konczak and Zhang, 2004). Presence of antioxidant rich phenolic compounds (Bohra et al., 2020a) and negligible amounts of anti-nutritional factors in the pulp (Singh et al., 2014) adds up to the nutritional importance of the species.

Blood fruit is not only valued nutritionally, but it has also been contributing towards economic and livelihood security of the farmers in its native regions of distribution. In local markets of the Andaman Islands, fruit were being sold (Fig. 2b) at the rate of INR 300 per kg during 2019. Until recently, majority of the fruit were harvested from the forests by following destructive harvesting practices. Due to faulty harvesting practices and occurrence of natural disasters, wild population of blood fruit was dwindling in nature and hence, habitat enrichment activities were carried out to conserve it (Bohra et al., 2018; Bohra et al., 2021). Considering the potential of this species, scientific studies were initiated to facilitate its cultivation as a novel crop in the humid tropical regions of India (Bohra et al., 2018, 2020a, 2020b). Vines laden with bunches look very attractive and hence, the species is also becoming a popular choice for inclusion in the agro-tourism activities in the Bay Islands. Also, identification of potential of this species as natural colorant for various industries has also triggered interest amongst the farmers for its cultivation.

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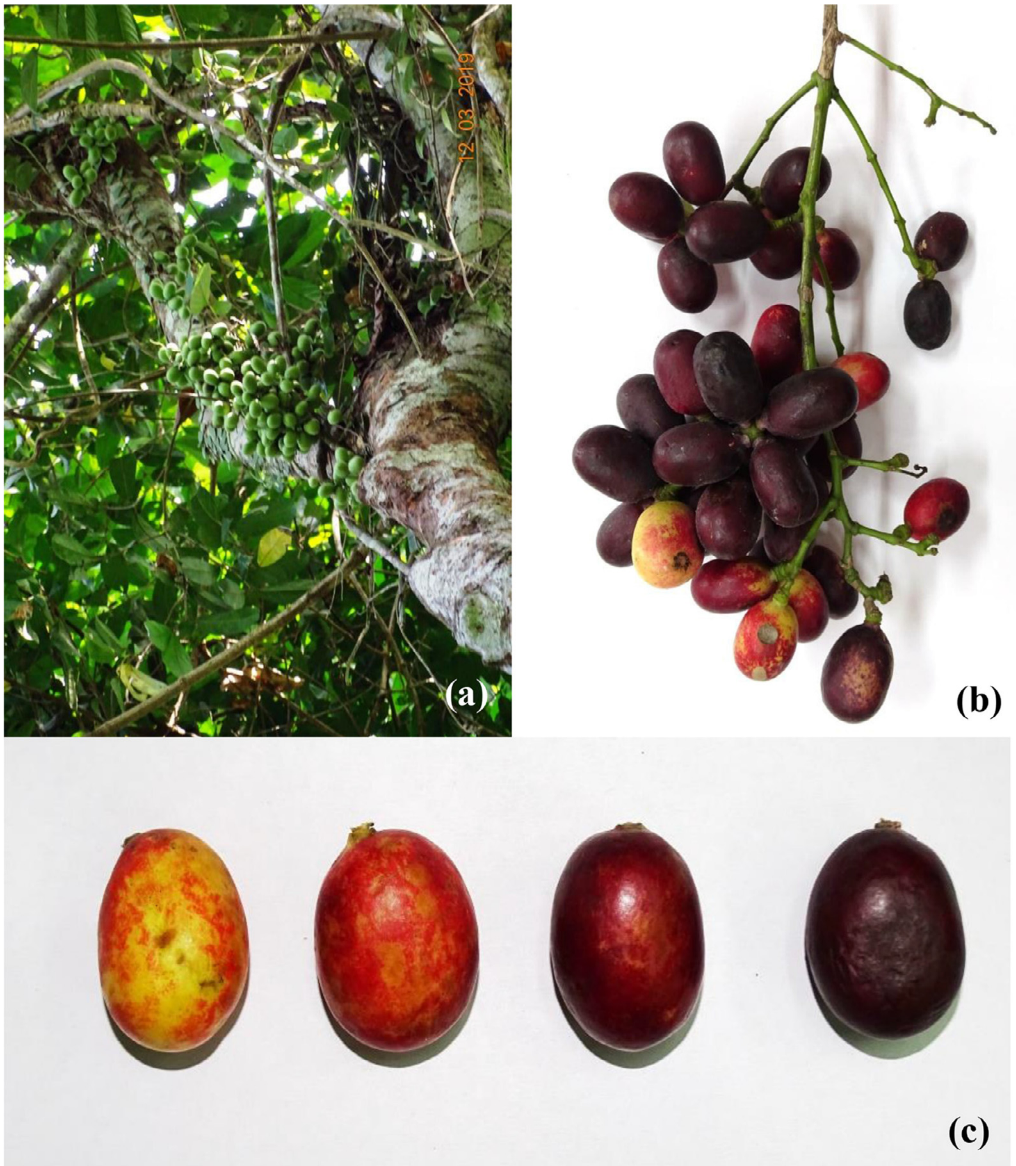

Fig. 1. Fruits of blood fruit. a Vine laden with developing fruits; b harvested bunch segment and c stages of fruit ripening (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.).

Natural stands of blood fruit are of seedling origin and variability is noticed among them. Identification of superior types is an important step in the popularization of new species. Considering this, field surveys were carried out in Andaman group of islands and Tripura, and selected collections were evaluated for morphological and biochemical parameters for identification of useful genotypes. In order to understand the relationship between various traits, correlation analysis was carried out using important traits. Furthermore, to improve extraction efficiency of total anthocyanins, effect of material: solvent ratio was also studied.

2. Materials and methods

2.1. Collection of samples

The present investigation was carried out during 2017–2019 during which surveys were conducted and blood fruit bunches were collected at color break stage from three identified locations of North and Middle Andaman Islands and one from Tripura (Table 1). Further, two collections were observed at South Andaman and North Andaman Islands, which produced only male flowers. Hence, they were not considered for analysis. Fruit were transported to laboratory at ICAR-Central Island Agricultural Research Institute, Port Blair, India (11°36′49.85″ N and 92°42′58.42″ E). These fruit were used for assessing morphological parameters and analysis of biochemical parameters.

2.2. Assessment of morphological parameters

Fully developed fruit of stage 3 (Fig. 1c) were separated from bunches, washed to remove adhering dust particles and surface dried at room temperature. At this stage, fruit were ripe and had uniform red/ maroon color peel (as per genotype). For assessing morphological characters, ripe fruit (characterized by dark red to maroon peel) were selected. Fruit polar (FPL) and equatorial (FEL) length were determined using a measuring scale (Camlin, Mumbai, India). Fruit polar circumference (FPC) and fruit equatorial circumference (FEC) were measured using a thread and scale; while peel thickness (PLT), seed length (SL), width (SW) and thickness (ST) were assessed using a digital Vernier caliper (Yamayo Classic, Yamayo Measuring Tools

Fig. 2. Blood fruit- View of cut open fruit showing dark red pulp (a) and ripe fruits harvested from home garden of Middle Andaman Island for sale in local market (b) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Co. Ltd., Tokyo, Japan). Fruit weight (FWt), peel weight (PLWt), pulp weight (PWt) and seed weight (SWt) were assessed using analytical balance (CY-104, Citizen, Mumbai, India). Peel (PEEL) and pulp (PULP) percentage were calculated on w/w basis.

2.3. Analysis of biochemical parameters

Fruit were cut opened; pulp was extracted manually and used for biochemical analysis. Total soluble solids content (°Brix) was determined using hand held refractometer of 0–55 °Brix range (Optics Technology, Delhi, India). pH was measured using digital bench top pH meter (HI2211, Hanna Instruments, RI, USA). Total anthocyanins were extracted from pulp using methanol acidified with HCl (99:1, v/v). Determination was carried out using spectrophotometric method (Shivashankara et al., 2017) at 540 nm (Biospectrometer, Eppendorf, Hamburg, Germany) and results were presented as mg/100 g Cyanidin equivalent.

2.4. Optimization of material: solvent ratio for extraction of anthocyanins

Fully ripe fruit were cut and pulp was scraped out manually from the peel and seeds. Two gram pulp was accurately weighed and transferred to test tubes under dark condition. Deionized water was acidified (1%) using citric acid and used for extraction at four ratio viz. 1:5, 1:10, 1:15 and 1:20 (w/v). Tubes were covered with aluminum foil and incubated for 72 h. After incubation, extracts were homogenized and absorbance was recorded using Biospectrometer at 540 nm (Shivashankara et al., 2017).

2.5. Statistical analysis

Data was subjected to analysis of variance (ANOVA) using Web Agri Statistical Package (WASP 2.0, ICAR-CCARI, Ela, India) and mean separation was carried out using least significant difference. Data were presented as mean ± standard error of mean, which was calculated using Microsoft Excel. Correlation analysis between ten selected morphological characters was carried out using WASP 2.0.

3. Results and discussion

It has been suggested that diversity of tropical and subtropical plants could offer promising compounds, which could be used for various industrial purposes (Wissgott and Bortlik, 1996) and hence, promotion of newer sources of such compounds has been envisaged. Considering the potential of blood fruit as novel source of natural colorant and antioxidants (Bohra et al., 2020a), present study was carried out.

3.1. Morphological traits of blood fruit

In general, for all the parameters studied, except for seed width, collection from Tripura (HV/NE/TRI) showed significant superiority. Fruit polar length varied between 3.5 cm in HV/MA/SND and 4.3 cm in HV/NE/TRI, while polar circumference varied between 10.5 cm and 12.4 cm in these collections. Lowest fruit equatorial length and circumference were observed in collection HV/MA/KAL (2.7 cm and 9.2 cm) which remained on par with other collections from Andaman Islands (Table 2). Size of fruit is one of the most important characteristic of a fruit crop and larger sized fruit are generally preferred in

Table 1.
Details of collection locations of blood fruit from Andaman Islands and Tripura.

Collection	District	State/ Union Territory	Geographical coordinates	
			Latitude (N)	Longitude (E)
HV/NE/TRI	Gomati	Tripura	23° 54′ 16.3″	91° 18′ 54.8″
HV/MA/SNU	North and Middle Andaman	Andaman and Nicobar Islands	12° 37′ 57.50″	92° 53′ 08.64″
HV/MA/KAL	North and Middle Andaman	Andaman and Nicobar Islands	–	–
HV/MA/SND	North and Middle Andaman	Andaman and Nicobar Islands	12° 37′ 58.22″	92° 53′ 08.24″

Table 2.
Variability for fruit morphological parameters in blood fruit collections from Andaman Islands and Tripura.

Variable	HV/NE/TRI	HV/MA/KAL	HV/MA/SNU	HV/MA/SND
FPL (cm)	4.3 ± 0.04 a	3.6 ± 0.05 c	3.5 ± 0.04 d	3.8 ± 0.04 b
FPC (cm)	12.4 ± 0.18 a	10.6 ± 0.17 c	10.5 ± 0.11 c	11.1 ± 0.10 b
FEL (cm)	3.1 ± 0.04 a	2.7 ± 0.03 c	2.8 ± 0.02 bc	2.9 ± 0.06 b
FEC (cm)	10.2 ± 0.08 a	9.2 ± 0.08 b	9.3 ± 0.06 b	9.3 ± 0.06 b
FWt (g)	21.17 ± 0.568 a	12.68 ± 0.341 d	15.38 ± 0.291 c	17.42 ± 0.276 b
PLWt (g)	11.71 ± 0.597 a	7.20 ± 0.192 c	8.56 ± 0.229 b	8.63 ± 0.158 b
PWt (g)	5.52 ± 0.288 a	2.91 ± 0.197 c	4.20 ± 0.097 b	4.24 ± 0.115 b
PEEL (%)	54.77 ± 1.584 a	56.99 ± 0.969 a	55.53 ± 0.713 a	49.58 ± 0.569 b
PLT (mm)	4.4 ± 0.18 a	3.4 ± 0.13 b	3.1 ± 0.11 c	3.2 ± 0.08 bc
PULP (%)	26.17 ± 1.412 ab	22.72 ± 1.165 c	27.40 ± 0.689 a	24.28 ± 0.501 bc
SL (cm)	3.1 ± 0.05 a	2.3 ± 0.04 c	2.3 ± 0.03 c	2.8 ± 0.04 b
SW (cm)	1.5 ± 0.02 b	1.3 ± 0.04 c	1.5 ± 0.02 b	1.7 ± 0.02 a
ST (cm)	1.3 ± 0.02 a	1.0 ± 0.02 c	1.2 ± 0.02 b	1.3 ± 0.03 a
SWt (g)	4.0 ± 0.18 a	2.4 ± 0.11 b	2.6 ± 0.09 b	3.7 ± 0.14 a

All values are mean ± standard error of mean. Values followed by similar letter in a row did not differ significantly at 5% level of significance. FPL: fruit polar length; FPC: fruit polar circumference; FEL: fruit equatorial length; FEC: fruit equatorial circumference; FWt: fruit weight; PLWt: peel weight; PWt: pulp weight; PEEL: peel (%); PLT: peel thickness; PULP: pulp (%); SL: seed length; SW: seed width; ST: seed thickness and SWt: seed weight.

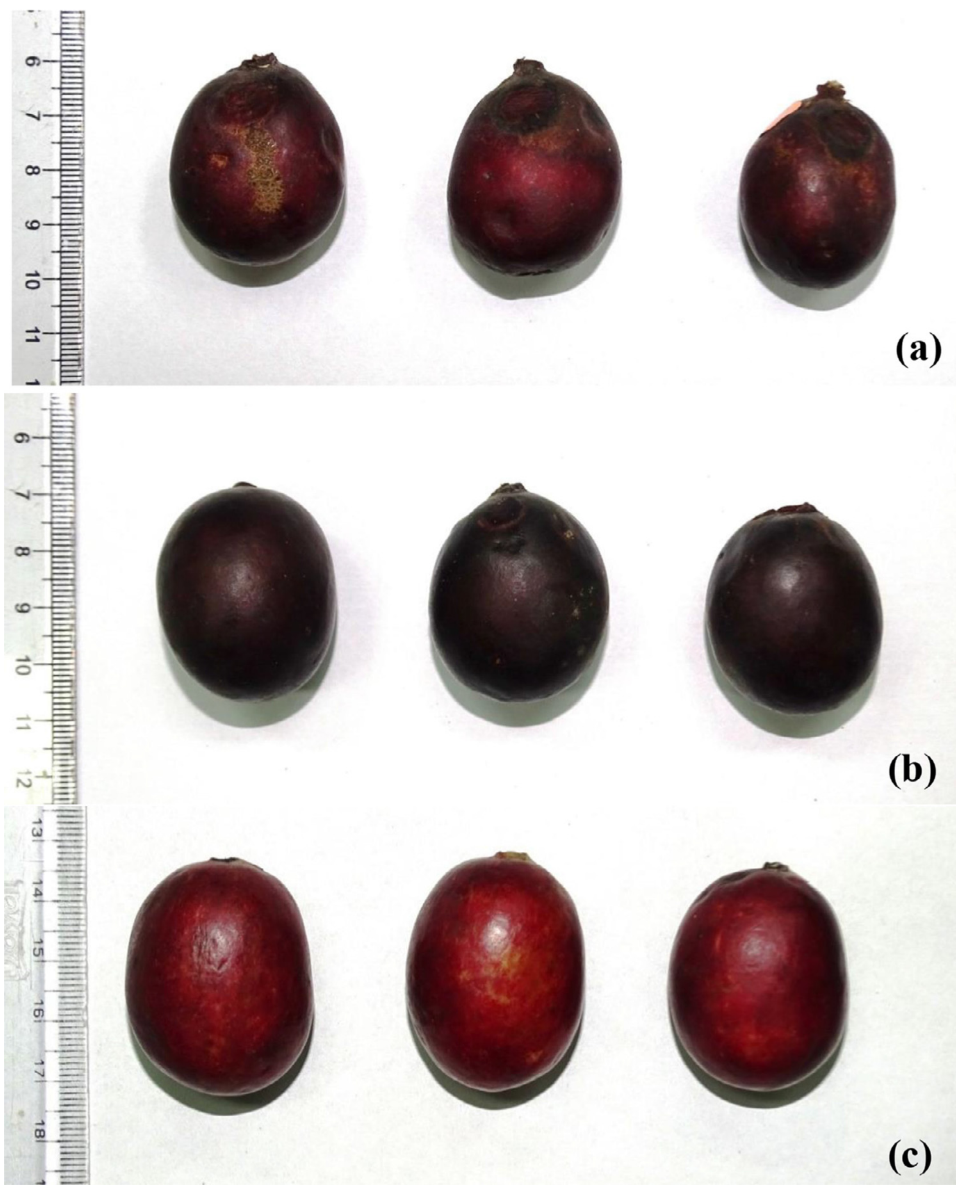

Fig. 3. Fruits of collections from Andaman islands. a HV/MA/KAL; b HV/MA/SNU and c HV/MA/SND.

most fruit bearing species (Sameer et al., 2019). In present study, mean fruit weight ranged from 12.68 g (HV/MA/KAL) to 21.17 g (HV/NE/TRI). Fruit of collections from Andaman Islands (Fig. 3) were smaller than that of collection from Tripura. However, among Andaman collections mean fruit weight of collection HV/MA/SND was the highest.

In earlier study (Bohra et al., 2018), average weight of fruit collected from Tripura market was 12.78 g. In general, fruit are harvested from the wild and bulked before selling. As a result, produce reaching markets is of variable sizes. Identification of genotypes with better fruit parameters (larger fruit size, higher pulp content, TSS and anthocyanins content *etc.*) and promotion of their cultivation could help in getting uniform quality produce in the markets. Due to genotype or microclimate or both, bigger fruit of 28.0 g and 30.9 g have been reported from Bangladesh (Khatun et al., 2014) and Garo Hills of Meghalaya (Momin et al., 2018), respectively. Systematic exploration in these regions and subsequent evaluation of the collections could be helpful for identification of superior genotypes.

Due to larger sized fruit in collection HV/NE/TRI, weight of peel (11.71 g) and weight of pulp (5.52 g) was also found to be the highest in this collection (Table 2). Collection HV/MA/KAL had mere 2.91 g pulp in each fruit. Interestingly, in case of collection HV/NE/TRI and HV/MA/SNU, pulp was easily detachable from the seed; however, in latter case, pulp was difficult to separate from the peel. Genotypes with easily detachable seeds have been reported in peaches and nectarines (Ali et al., 2017). In rambutan also, identification of free aril types has been considered advantageous owing to better pulp recovery and ease of separation of pulp from the seed (Sameer et al., 2019). This character could be useful especially in obtaining better recovery of anthocyanins from blood fruit. Further, thicker peels observed in HV/NE/TRI could provide advantage in harvesting as the liana grows very high and harvesting using pole could result in impact injury to the fruit. Further, such genotype could have higher shelf life as reported by Danner et al. (2011) in case of jabuticaba.

Pulp is the economic part of this species and its content varied between 22.72% (HV/MA/KAL) and 27.40% (HV/MA/SNU). Earlier study suggested that sample from Bangladesh had heavier fruit (Khatun et al., 2014); however, their pulp content was lower (22.0%) than that observed in the present study. Considering the limited pulp content, this character could be given priority for future collection missions. Seed length (3.1 cm) was found to be highest in HV/NE/TRI collection, while seeds in collection HV/MA/SND were the widest (1.7 cm). Seed thickness and seed weight in these two collections remained comparable. In seeds of HV/NE/TRI, a typical projection was observed at the proximal end of the seed (Fig. 4), which was completely absent in all the collections from Andaman Islands. Possible role of this character needs to be studied. Variations for various morphological characters observed in the present study could help in determining the ideotype of blood fruit in future.

3.2. Biochemical characteristics

Even though the collection HV/NE/TRI showed superior morphological parameters, its biochemical parameters were not always superior (Figs. 5–7). Total soluble solids content in the fruit is an indication of their degree of sweetness. The TSS content amongst the studied blood fruit collections varied considerably (Fig. 5) and fruit from HV/NE/TRI had the lowest total soluble solids content of 11 °Brix. All the collections from the Andaman Islands had distinctly higher TSS content. The collection HV/MA/SND was found to have the highest TSS content of 18 °Brix, which was followed by that in HV/MA/KAL (17 °Brix) and HV/MA/SNU (16 °Brix). During our previous study (Bohra et al., 2018), we had reported TSS content of 19 °Brix in the fruit samples collected from Island market. Considering this variation, there is large scope for identification of further sweeter genotypes from native regions. In case of pH, most acidic fruit were observed in collection HV/MA/KAL with 3.01 pH, while as high as 3.61 pH value was observed in collection HV/NE/TRI (Fig. 6). This means, fruit of blood fruit are generally acidic in nature and acid blend with TSS determines the overall taste of the fruit.

Anthocyanins are the most important bioactive compounds present in blood fruit, considering its use as natural colorant. Highest total anthocyanins content (Fig. 7) of 328.38 mg CE/100 g was present in pulp of HV/MA/SND, which was followed by that in HV/MA/KAL (301.895 mg CE/100 g). Total anthocyanins content in HV/NE/TRI collection was 245.85 mg CE/100 g. Interestingly, both highest and lowest contents were observed in collections from Andaman Islands (Fig. 7). It is known that anthocyanins are synthesized through flavonoid biosynthetic pathway and several regulatory and structural genes are involved in this process (Kayesh et al., 2013). Different genotypes are known to have differential gene expression patterns (Karanjalker et al., 2018). Further, interaction of genotypes with their

Fig. 4. Presence of typical projection in the seed of HV/NE/TRI (left), which is completely absent in collections from Andaman- HV/MA/KAL (right).

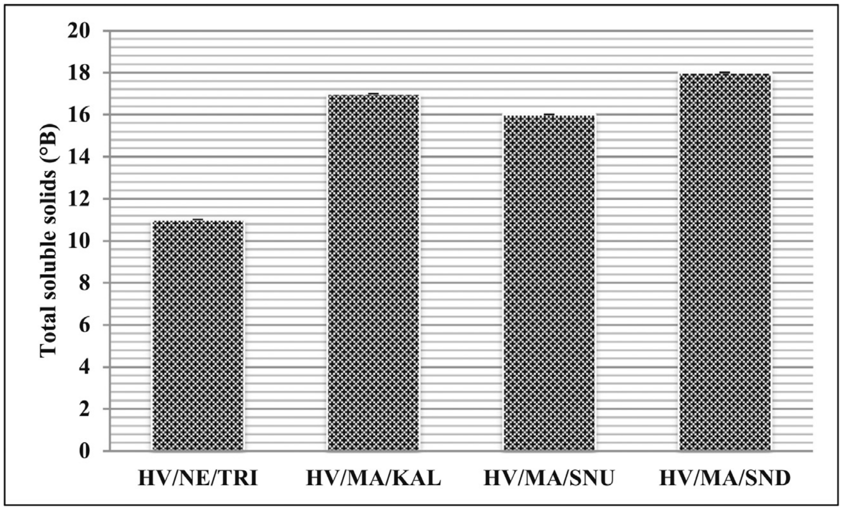

Fig. 5. Variation in total soluble solids (°Brix) content in pulp of blood fruit collections from Andaman islands and Tripura.

growing environment also causes differences in biochemical characteristics. Hence, variability in the amount of anthocyanins synthesized in fruit of different genotypes could be justified.

Genotype dependent variations as seen in present study have also been reported in wild collections of *Rubus coreanus* in which total anthocyanins varied between 6.7 and 312.2 mg CE/ 100 g (Lee et al., 2013). Distinct variations in 26 seedling populations of black raspberry have also been reported by Dossett et al. (2010). Species that are sources of anthocyanins are of great potential in food, pharmaceutical and cosmetic industries due to their medicinal and nutritional properties (Danner et al., 2011; Kamei et al., 1995; Giusti et al., 1998) and hence, identification of genotypes with superior anthocyanins content particularly in underutilized species has been emphasized (Bohra et al., 2018; Dossett et al., 2010; Danner et al., 2011). Considering superior fruit size, TSS content and total anthocyanins content in collection HV/MA/SND, the germplasm could be promoted for cultivation in the humid tropical conditions.

3.3. Pigment extraction studies

Optimization of material: solvent ratio is important to obtain better recovery of the pigments besides avoiding environmental impact due to use of excessive solvents (Pham et al., 2019). In order to optimize the ratio for better recovery of total anthocyanins from the fruit pulp of blood fruit, four ratios were studied. Total anthocyanins recovery was found to be the lowest (229.6 mg CE/100 g) at 1:05 ratio, which increased gradually to 261.4 mg CE/100 g at 1:10 and further to 302.9 mg CE/100 g at 1:15 ratio (Fig. 8).

When solvent is insufficient to fill up the material, pigments could not be extracted optimally from the vacuoles due to non-availability of hypertonic environment (Pham et al., 2019). Increase in solvent proportion causes higher-density gradient and higher distribution coefficient, which results in increased recovery of anthocyanins content (Khazaei et al., 2016), as also evident in the present study. At optimum point, the cells swell to the maximum capacity due to the filling up of solvent, thereby causing them to burst and release pigments from the vacuole. During present study this optimum ratio of material: solvent was found to be 1:15. However, this increment continues only up to a point beyond which the extract recovery declines due to lower solute weight ratio (Pham et al., 2019). This was evident in the present study as the ratio of 1:20 resulted in lower recovery of the pigments (272.4 mg CE/100 g).

Optimization of material: solvent ratios have been attempted in other species with similar results. In *Clitoria ternatea*, anthocyanins recovery increased from 103.5 mg/L to 121.6 mg/L with increase in liquid: solid ratio from 15:1 to 25:1 and increasing solvent proportion beyond this resulted in drastic reduction of anthocyanins recovery (Pham et al., 2019). Oancea et al. (2013) has also reported optimum pulp: solvent ratio of 1:10 in blackberry, while it was 1:15 in sweet cherry. Profound influence of this ratio on extraction efficiency of

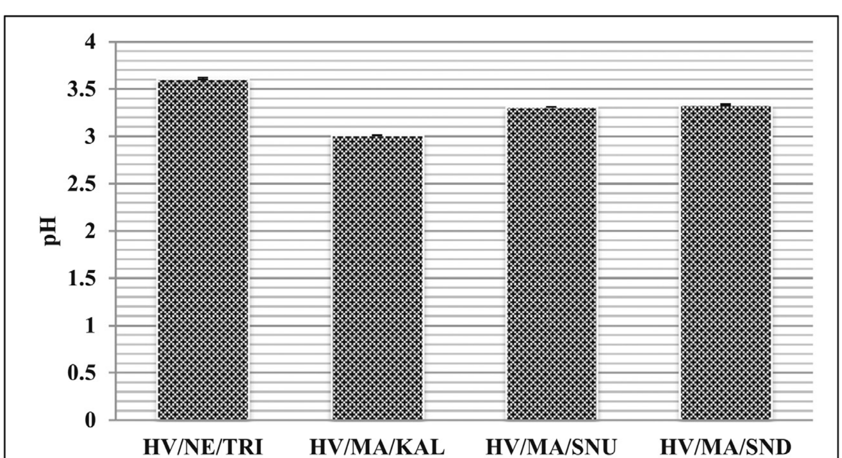

Fig. 6. Variation in pH of pulp of blood fruit collections from Andaman islands and Tripura.

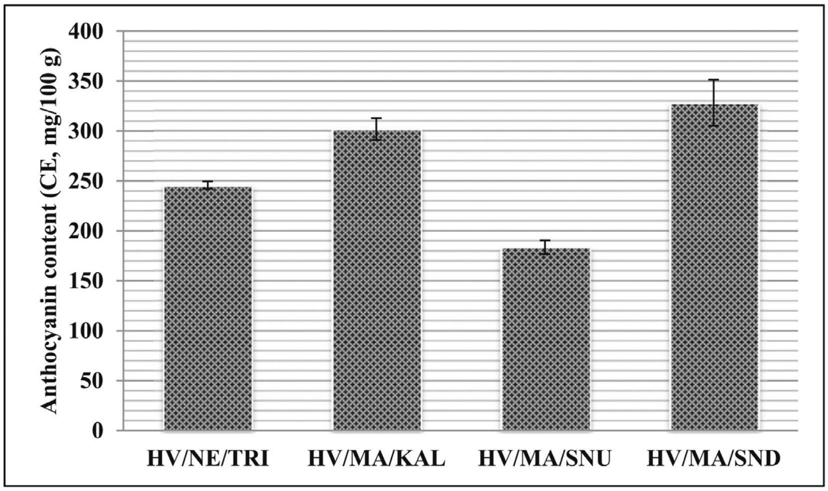

Fig. 7. Variation in total anthocyanin content in pulp of blood fruit collections from Andaman islands and Tripura.

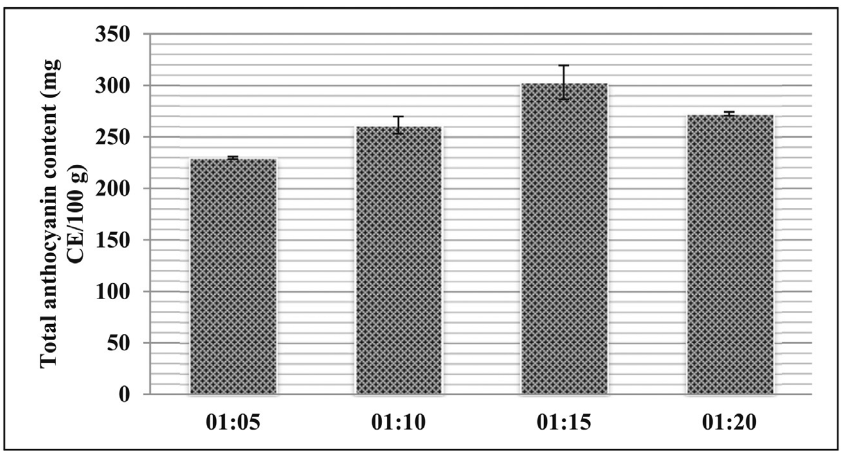

Fig. 8. Total anthocyanin content in pulp of blood fruit as influenced by solute: solvent ratio.

pigments has been reported in saffron, in which the optimum ratio was 1:77.5 (Jafari et al., 2019). For extraction of anthocyanins from banana bracts, 1:30 ratio was found to be optimum (Begum and Deka, 2017). As the optimum ratio varies with the species, it was also optimized for blood fruit as 1:15 in the present study.

Anthocyanins have been widely used as natural colorants in food and other industries. Apart from coloring the products, anthocyanins are known to provide medicinal benefits such as antioxidant properties, which are known to counteract the risk of various ailments including cancer, diabetes and cardiovascular illness (Konczak and Zhang, 2004). Identification of pigment rich natural sources could not only help in diversifying the product base, but would also help local farmers in getting remunerative prices to their produce. Recovery of anthocyanin pigments from plant tissues has been a complex process and a number of factors, including material: solvent ratio, are known to affect the efficiency of the method (Arici et al., 2016; Khazaei et al., 2016; Pham et al., 2019). Information reported in present study could help in obtaining better recovery of pigments from blood fruit.

Table 3.
Correlation matrix of different characters in blood fruit.

	FPL	FEL	FWt	PLT	PULP	PEEL	SL	SW	ST	SWt
FPL	1.000									
FEL	0.819	1.000								
FWt	0.845	0.888	1.000							
PLT	0.738	0.677	0.641	1.000						
PULP	−0.103	−0.033	−0.007	−0.399	1.000					
PEEL	0.003	0.002	−0.067	0.414	−0.671	1.000				
SL	0.777	0.651	0.740	0.483	0.107	−0.347	1.000			
SW	0.113	0.192	0.267	−0.201	0.212	−0.613	0.464	1.000		
ST	0.666	0.665	0.721	0.286	0.106	−0.368	0.755	0.588	1.000	
SWt	0.758	0.713	0.841	0.464	−0.095	−0.235	0.783	0.469	0.798	1.000

FPL: fruit polar length; FEL: fruit equatorial length; FWt: fruit weight; PLT: peel thickness; PULP: pulp (%); PEEL: peel (%); SL: seed length; SW: seed width; ST: seed thickness and SWt: seed weight.

3.4. Correlation studies

Correlation studies have been known to help in future breeding program of any crop (Rekha et al., 2011). Correlation studies in blood fruit suggested relations among various morphological traits (Table 3). Strong positive correlation was noticed for fruit polar length with fruit equatorial length (r = 0.819), fruit weight (r = 0.845), peel thickness (r = 0.738), seed thickness (r = 0.666) and seed weight (r = 0.758). Similar trend was observed for fruit equatorial length with these characters and the correlation values ranged from r = 0.651 to r = 0.888. Positive correlation between fruit dimensions and fruit weight have been reported in other vine fruit crop- grapes (Khadivi-Khub et al., 2014) and sapota (Rekha et al., 2011). In apple cultivars Golden Delicious and Red Chief also, strong positive correlation was noticed between fruit size and fruit weight (De Salvador et al., 2006).

Fruit weight was highly correlated with seed parameters such as seed weight (r = 0.841), seed length (r = 0.740), seed thickness (r = 0.721) and peel thickness (r = 0.641). It means that inedible parts are the major contributors of fruit weight. Pulp content was negatively correlated (r = - 0.671) with peel content (Table 3). Hence, further explorations should be carried out to identify genotypes with thinner peels.

4. Conclusion

1 Variability existed in the natural progenies of blood fruit for studied morphological and biochemical traits.
2 Based on superior fruit size, TSS content and total anthocyanin content, collection HV/MA/SND was identified for promoting cultivation in humid tropical conditions.
3 Material: solvent ratio had profound influence on recovery of anthocyanins from fruit pulp and the ratio of 1:15 was found to be optimum.
4 Correlation studies revealed strong positive correlation among fruit morphological parameters.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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