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Morphological characterization, antioxidant capacity and diversity of Syzygium cumini trees from Sri Lanka

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ABSTRACT

Syzygium cumini (L.) Skeels (Madan) is widely distributed in South Asian countries, including Sri Lanka, where it was naturally found. There were only few studies reported on *S. cumini* for its values and other benefits in Sri Lanka. This study focuses on morphological characterization and the diversity of *S. cumini* trees in seven locations of Sri Lanka. Data were collected on different morphological traits. Leaf, flower and fruit morphology showed significant differences among locations. Further, trees from Batticaloa region showed different morphology, which would potentially open a different avenue for further study to trace different cultivars across the country. Results of the diversity index and multivariate analyses indicated high diversity in morphology and antioxidant capacity of trees. The variables such as leaf area, inter-nodal length, petiole length, anther length, flament length, fruit size and pericarp thickness could be suitable candidates to investigate the morphological variation of *S. cumini* any further. Antioxidant capacity studies also showed significant differences among locations and trees. Fruit hue angle was positively correlated to antioxidant capacities. Finding of this study concluded that the wide distribution of *S. cumini* trees throughout Sri Lanka in different geographical locations showed high diversity in leaf, flower and fruit morphology together with antioxidant capacity.

Keywords: Syzygium cumini; Morphological diversity; Antioxidant capacity; Sri Lanka

1. Introduction

Syzygium cumini (L.) Skeels (Madan) is a large evergreen multipurpose tree of Myrtaceae family (Samba-Murthy and Subrahmanyam, 1989). It is commonly known as Madan in Sri Lanka and Jamun, Black plum, Indian blackberry, Java plum, jambolan elsewhere (Sivasubramaniam and Selvarani, 2012; Steiner et al., 2017). The tree is recorded as native plant to Bangladesh, India, Pakistan, Sri Lanka, Malaysia, Philippines, and Indonesia, also found in many other regions of the world with rich variability and wide adaptability to tropics, subtropics as well as temperate zone (Singh et al., 2013). S. cumini is known for its wide use as a medicinal plant for the treatment of various diseases (Patil et al., 2012). However, it is categorized as an underutilized crop in Sri Lanka (Dahanayake, 2015) and attention on this fruit crop is very poor to upgrade it from underutilised to well utilise status. The tree is naturally distributed in low and intermediate elevations of Sri Lanka. The genetic diversity among species is highly governed by ecological factors and micro-geographical differentiation (Reisch et al., 2003), which could lead to the high diversity of fruit quality and taste (Dissanayake et al., 2019) and possible morphological and genetic diversity in S. cumini trees.

S. cumini fruits are highly valued by different researches on underutilised wild fruits. It is proven that S. cumini contains phyto-

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chemicals with rich properties to fight against Diabetics (Kumar et al., 2008). Studies in India on *S. cumini* showed that seeds are rich in flavonoids and total phenolic contents, which could scavenge free radicals and protect antioxidant enzymes (Ravi et al., 2004; Bajpai et al., 2005) and also contain protein and calcium in fairly rich level. Therefore, as a fruit crop it has high potential to contribute to healthy life of the consumers. Further, wide variation of the tree in nature could potentially be support to reinforce the food security even in occurrence of global climate change scenario. Over the past century, many plant genetic resources have been lost and one third of today's diversity could disappear by 2050 (FAO, 2011). Hence, extensive study on this neglected tree as a food crop is very important to understand the variationn and diversity of the crop as genetic resources.

Studying morphological diversity with understanding of how diversity is structured is a prerequisite in designing conservation strategies. Further, it helps to answer taxonomical, phylogenetical, demographical, and ecological questions of great relevance for conservation. Basic morphological differences are important to know plant identifications for their varietal characters. But few investigations have been undertaken to study the morphological variation of *S. cumini* trees in Sri Lanka. In this study, we are looking for *S. cumini* for morphological characterization, and antioxidant capacity from different locations of Sri Lanka.

2. Materials and methods

2.1. Location and tree selection

Naturally grown 20 S. *cumini* trees were randomly selected from each geographical location of Sri Lanka for morphology and phytochemical study. Details of seven locations were as follows: Ampara (7°18' N, 81°41' E, 39 m AMSL), Batticaloa (7°43' N, 81°41' E, 15 m AMSL), Hambanthota (6°08' N, 81°04' E, 57 m AMSL), Kalpitiya (8°12' N, 79°42' E, 59 m AMSL), Knuckles (7°32' N, 80°45' E, 776 m AMSL), Belihuloya (6°41' N, 80°47' E, 587 m AMSL), Udawalawa (6°28' N, 80°42' E, 197 m AMSL). Geo-coordinates of selected trees were also recorded and named separately. Trees from Ampara were named as A series (A1, A2...), Batticaloa as B series (B1, B2...), Hambanthota as H series (H1, H2...), Kalpitiya as K series (K1, K2...), Knuckles as N series (N1, N2...), Belihuloya as SC series (SC1, SC2...) and Udawalawa as UW series (UW, UW2...). Diameter at breast height (DBH) of selected trees was 20–30 cm.

2.2. Leaf morphology

Four to five leaf branches were randomly selected from each tree and measurements were recorded from four mature leaves of each branch. All leaf branches were photographed using Nikon camera (Nikon D7200). Leaf area, leaf length and leaf maximum with were recorded using a portable leaf area meter (Portable Area Meter, Model LI-3000C, USA). Petiole length of four leaves of branch and internodal length were also recorded. Angle between leaf midrib and leaf veins was measured using ImagJ software (ImageJ 1.45s, USA) by analysing the images.

2.3. Floral morphology

Five flowers were randomly selected from each tree from different geographical locations of Sri Lanka and observed under a dissecting microscope (Stemi 305, Carl zeiss Microscopy, Germany) attaching a digital camera. Length of floral cup (Hypanthium), width of floral cup (Hypanthium), length of style, width of style, length of filament, width of filament, length of anther and width of anther were recorded by analysing microscopic photographs using ImagJ software (ImageJ 1.45s, USA) (Fig. 1).

2.4. Fruit morphology

Photographs of randomly selected ripen fruits with scale in the side, were captured using Nikon camera D7200 and images were analysed using ImagJ software (ImageJ 1.45s, USA) to get values of fruit length and fruit width.



Fig. 1 Floral measurement of Syzygium cumini

Cross sections of ripen fruits were prepared by cutting fruits with sharp laboratory knife and observed under dissecting microscope (Stemi 305, Carl zeiss Microscopy, Germany). Each cross section of fruits was captured using camera attached to the microscope. Pericarp thickness was measured by analysing photographs with ImageJ software.

2.5. Fruit ripening

All fruits including immature, different stages of ripening and fully ripen were harvested from different geographical locations of Sri Lanka namely Ampara, Batticaloa, Hambanthota, Kalpitiya and Belihuloya. Harvested fruits of each tree were visually observed and identified during different fruit maturity stages. Each stage of each tree was carefully observed for different colour development separately. Hue angle of colour was measured using a chroma meter (Chroma meter CR-400, Konica Minolta Inc., Japan).

2.6. Antioxidant capacity

Healthy fruits were collected from Ampara, Batticaloa, Hambanthota, Kalpitiya and Belihuloya regions of Sri Lanka. Fruits were harvested from randomly selected five trees in each location. Fruit samples were air dried sealed, and stored in –80 °C until analysis.

2.6.1. Sample preparation

Seed and pericarp of air-dried ripen fruits were separated. Five gram sample from each pericarp and seed were taken for the study. Samples were finely ground to homogenous powder by using cooled mortar and pestle and transferred to 50 mL conical flasks which has wrapped with Aluminium foil to not to penetrate light. Chemical contents of samples were extracted into 50 mL methanol by sonication using digital heated Ultrasonic Bath (Soner 206H, German) and filtrated using Buchner funnel with Whatman No. 1 filter papers. Sonication was done at 35-40 °C for about 30 min to accelerate the extraction process. Filtration was concentrated to dryness under reduced pressure at 32 °C using a Rotavapor® (J.P.SELECTA-RS 3000V, Spain) to obtain crude methanol extracts. Dried extracts were transferred to Eppendorf tubes (1.5 mL) and stored in -80 °C until use. One millilitre of 10 mg•mL⁻¹ stock solutions were prepared for all the seed and pericarp samples and then kept in -80 °C until further use.

2.6.2. Total polyphenolic content assay

The Folin-Ciocalteu method (Singleton et al., 1999) was adopted for UV-spectrophotometer to determine total phenolic content in extracted S. *cumini* seed and pericarp of ripen fruits. The reaction mixture contained 125 μ L of crude sample, 1 mL of Folin-Ciocalteu reagent, 625 μ L of distilled water, and 750 μ L of 10% sodium carbonate solution. Reaction mixture was incubated at room temperature [(25 ± 3)°C] for 30 min and absorbance was recorded at 765 nm using spectrophotometer (GENESYS 10S UV-Vis, USA). Total phenolic contents were calculated using gallic acid standard curve and expressed as mg gallic acid equivalents per gram of dried fruit.

2.6.3. Total flavonoid content assay

Total flavonoid content was determined by Aluminium chloride method (Siddhuraju and Becker, 2003) using UV spectrophotometer (GENESYS 10S UV-Vis, USA). The reaction mixture contained 100 μ L 2% Aluminium Chloride, 20 μ L of crude sample and 80 μ L of methanol. Absorbance was recorded at 415 nm after 10 min of incubation at room temperature [(25 \pm 3)°C]. Total flavonoid contents were calculated using quercetin standard curve and expressed as mg quercetin equivalents per gram of dried fruit.

2.6.4. ABTS free radical scavenging assay

Free radical scavenging activity was determined using 2,2azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS⁺) radicals. Five concentrations (0.5, 0.25, 0.125, 0.0625, 0.03125 mg·mL⁻¹) of crude samples were prepared in 50 mmol·L⁻¹ Phosphate Buffer Saline (PBS, pH 7.4). ABTS⁺ radical was generated by incubating equal quantities of 10 mmol·L⁻¹ ABTS and 2.5 mmol·L⁻¹ Potassium persulphate solution at 37°C for 16 h (Re et al., 1999). Then fresh ABTS⁺ solution was diluted seven times with 50 mmol·L⁻¹ PBS just before use. In the assay, 50 µL sample was mixed with 110 µL of 50 mmol·L⁻¹ PBS in cuvette and 40 µL of ABTS⁺ solution was added in dark and incubated at (25 ± 3)°C for 10 min.

2.7. Data analysis

Morphological data were analysed by using analysis of variance procedure (ANOVA) followed by Fisher comparison for grouping in order to separate different categories.

All the measurements of each morphological trait were divided into same number of classes depending on the range of values obtained from whole trees. Then number of trees belonging to each class of each character was used in order to calculate the Shannon–Wiener Diversity index (H') (Shannon, 1948) according to the following equation: $H' = H/H_{max}$. Where $H = \Sigma^n_i \ln p_i$, n is the number of phenotypic classes for a characteristic and p_i is the percentage proportion of the total number of entries in the ith classes. $H_{max} = \ln(n)$ in order to express the values of the H' in the range of 0 and 1.The Shannon–Wiener diversity index was categorized as low (0.10–0.40), intermediate (0.40–0.70) and high (> 0.70). Shannon Wiener diversity index for leaf traits, floral traits, fruit traits and antioxidant properties was calculated separately.

Principal component analysis (PCA) was performed based on the morphological characters and antioxidant properties irrespective the geographical locations.

3. Results

3.1. Morphological characteristics

3.1.1. Leaf traits

Leaf area: Average leaf area of S. cumini trees across the country was recorded as 31.23 cm² (Fig. 2) with the smallest leaf area in a tree from Hambanthota (12.12 cm²), whereas the highest in a tree from Batticaloa (82.23 cm²) (Fig. 3). This emphasizes that across the country there was huge variation of leaf area. However, the significantly lowest average leaf area (21.95 cm²) was recorded in Belihuloya. The highest average leaf area was recorded in Batticaloa (48.18 cm²) and it was significantly higher than the rest of the locations (Fig. 2). Further, leaf area of trees from Ampara was also significantly higher than that of other locations except Batticaloa. Both locations are in Eastern side of the country.



Fig. 2 Leaf area of Syzygium cumini trees from different locations of Sri Lanka Vertical bars represent mean \pm SE (n = 25). Means that do not share a letter are significantly different. Solid line across the graph shows mean leaf area of all locations, whereas dotted lines represent SE of leaf area. CV=37.27.



Fig. 3 Syzygium cumini leaves with the and the smallest leaf area from Batticaloa and Hambanthota regions, respectively

Leaf length: Average leaf length of S. cumini trees across the country was recorded as 9.27 cm (Fig. 4). The lowest average leaf length (8.20 cm) was recorded in Belihuloya. The highest average leaf length was recorded in Batticaloa region (10.47 cm) and it was significantly higher than the rest of the locations (Fig. 4).

Leaf width: Average leaf width across the country was recorded as 4.78 cm (Fig. 5) with the smallest leaf width in a tree from Hambanthota (2.30 cm), whereas, the highest was from Batticaloa (8.52 cm). This is similar to the other leaf characters across the country. The lowest leaf width (3.91 cm) was recorded in tree from Belihuloya. The highest average leaf width was recorded from Batticaloa (6.54 cm) and it was significantly higher than the rest of the locations (Fig. 5). Leaf length/width ratio: Leaf length/width ratio in certain extent represents and gives glimpse about leaf shape. Average leaf length/width ratio in Batticaloa was significantly lower compared to the rest of the locations. This leads most tree leaves close to round shapes (Fig. 6). Ampara, which is also located in the East of the country similar to Batticaloa, also showed significantly lower ratio than other locations but higher than Batticaloa. Almost in all other locations, in average, ratio was in the range of 2.

Leaf shape: There was much of the visually striking diversity of different leaf shapes among collected trees in the country (Fig. 7). Those were comparatively drastic differences. The differences were not localized and some shapes found in throughout such as elliptic but some shapes belonged to few trees but will not represent particular geographical location such as obtuse with parallel margins.

Petiole length, internodal length and leaf vein angle: Petiole length, internodal length and leaf vein angle also showed significant differences among locations (Table 1). Significantly longer petiole length (16.15 mm) was found in trees from Batticaloa. The lowest petiole length was recorded in trees from Belihuloya (13.28 mm). However, this was not significantly lower than other locations except Batticaloa and Udawalawa. Average internodal length of total plants was 38.69 mm. However, Ampara, Batticaloa and Kalpitiya represented significantly higher internodal length than rest of the locations. Internodal length and leaf vein angle showed close relationship between locations. Ampara, Batticaloa and Kalpitiya showed significantly higher internodal length and leaf vein angle compared to other locations. Value of all these three traits was higher in Batticaloa region.

Clusters for leaf morphology: Three different clusters were identified on leaf morphological characteristics at the similarity level of 82. Cluster 1 was prominent in all the locations except Batticaloa and Kalpitiya that contained 62% of the trees sampled. Cluster 3 was prominent in Batticaloa, whereas in Kalpitiya it was cluster 2. Distribution of number of clusters in all locations was varying (Fig. 8).



Fig. 4 Leaf length of Syzygium cumini trees from different locations of Sri Lanka $\label{eq:CV} CV = 15.51.$



Fig. 5 Leaf width of Syzygium cumini trees from different geographical locations of Sri Lanka CV = 22.37.

Table 1 Petiole length, internodal length and leaf vein angle of Syzygium cumini trees (n=20) from different geographical locations of Sri Lanka

	(====)		
Location	Petiole length/mm	Internodal length/ mm	Leaf vein angle
Ampara Batticaloa Hambanthota Kalpitiya Knuckles Belihuloya Udawalawa Mean	$\begin{array}{c} 13.79 \pm 0.49 \text{ BC} \\ 16.15 \pm 0.77 \text{ A} \\ 13.96 \pm 0.81 \text{ BC} \\ 13.71 \pm 0.51 \text{ BC} \\ 13.47 \pm 0.47 \text{ BC} \\ 13.28 \pm 0.53 \text{ C} \\ 15.18 \pm 0.73 \text{ AB} \\ 14.23 \pm 0.24 \end{array}$	$\begin{array}{c} 45.39 \pm 1.65 \text{ A} \\ 45.46 \pm 1.40 \text{ A} \\ 31.44 \pm 1.58 \text{ BC} \\ 47.15 \pm 2.20 \text{ A} \\ 33.50 \pm 2.12 \text{ BC} \\ 31.10 \pm 1.79 \text{ C} \\ 36.19 \pm 1.75 \text{ B} \\ 38.69 \pm 0.83 \end{array}$	$\begin{array}{c} 63.68 \pm 1.20 \text{ A} \\ 64.93 \pm 1.04 \text{ A} \\ 54.51 \pm 0.86 \text{ C} \\ 64.43 \pm 0.69 \text{ A} \\ 59.77 \pm 0.69 \text{ B} \\ 58.27 \pm 0.71 \text{ B} \\ 59.28 \pm 0.64 \text{ C} \\ 60.72 \pm 0.41 \end{array}$
CV	22.51	28.22	8.96

Note: Data were presented as mean \pm SE. Values with similar letters are not significantly different in one column.



Fig. 6 Leaf length/width ratio of Syzygium cumini trees from different locations of Sri Lanka $\mathrm{CV}=17.34.$



Fig. 7 Different shapes of Syzygium cumini leaves



Fig. 8 Distribution of leaf morphological clusters among different locations of Sri Lanka

3.1.2. Floral morphology

Flowering occurred in *S. cumini* trees from April to May and lasted for 1,2 weeks. During experimental period, flowers bloomed only in few trees and the rest would come to bearing stage in future years due to erratic or irregular bearing behaviour (based on our past observations). Therefore, for floral morphology study, floral samples were used from available trees.

Length of floral cup: Average length of floral cup of S. cumini trees across the country was recorded as 3.06 mm (Fig. 9) with the shortest in a tree from Batticaloa (1.38 mm), whereas, the highest from Kalpitiya (5.45 mm). There were variations of average length of floral cup with more than 3 times higher length in the biggest flower than the smallest. When regions were considered, the lowest average length of floral cup was recorded in Batticaloa (2.13 mm). The highest average length of floral cup was recorded in trees from Kalpitiya region (3.65 mm) and it was significantly higher than Ampara, Batticaloa and Hambanthota (Fig. 9).

Width of floral cup: Average width of floral cup of S. cumini trees across the country was recorded as 2.99 mm (Fig. 10) with the



Fig. 9 Length of floral cup of Syzygium cumini from different locations of Sri Lanka CV = 25.32.



Fig. 10 Width of floral cup of Syzygium cumini trees from different locations of Sri Lanka CV = 21.25.

shortest width of floral cup in a tree from Batticaloa (1.79 mm), whereas, the highest from Hambanthota (5.02 mm). There were variations of width of floral cup more than 2 times in the biggest flower than that of the smallest. The lowest average width of floral cup was recorded in Batticaloa (2.28mm). The highest average width of floral cup was recorded in Kalpitiya region (3.60 mm) and it was significantly higher than Ampara, Batticaloa and Belihuloya (Fig. 10).

Style, anther and filament: Style length and width also showed significant differences among all locations (Table 2). In both situations Kalpitiya and Udawalawa showed significantly higher values than Ampara. The lowest length and width were recorded from Batticaloa and Ampara, respectively.

All measurements of pistil and stamen structures of S. cumini showed significant differences among all locations except width of filament. Some floral characteristics showed higher variation than that of others and length of anther showed very high variation of 66.1%.

The cluster analysis was performed based on morphological differences of eight floral characteristics of S. *cumini*. Three clusters were formed with Euclidean distance (complete Linkage, correlation, coefficient distance) ranged between 0.216 and 1.10.

Cluster 1 was the major one for floral morphology which occupied 72% of the trees, whereas cluster 3 with the least number of trees of 11% were distributed in all geographical locations. All three clusters can be found in all locations except Knuckles and Hambanthota (Fig. 11).

3.1.3. Fruit morphology

Peak period for fruit availability was from August to September. Fruit length, fruit width, lengh/width ratio, fruit size and

1401	Table 2 Tiolar morphological characteristics of Tany openca nowers of 5/2/yium canna nom on hanka (n=10)								
Location	Style length/mm	Style width/mm	Anther length/mm	Anther width/mm	Filament length/mm	Filament width/mm			
Ampara	$3.00\pm0.17~\text{DE}$	$0.26\pm0.12~\text{C}$	$0.29\pm0.02~\text{C}$	$0.39\pm0.036~\text{B}$	$3.18\pm0.24~\text{C}$	$0.15\pm0.03~\text{A}$			
Batticaloa	$2.56\pm0.18~\text{E}$	$0.32\pm0.019~\text{ABC}$	$0.54\pm0.16~\text{ABC}$	$0.29\pm0.025~\text{AB}$	$3.01\pm0.25~\text{C}$	$0.16\pm0.02~\text{A}$			
Hambanthota	3.75 ± 0.26 BC	$0.33\pm0.021~\text{AB}$	$0.37\pm0.03~BC$	$0.22\pm0.03~B$	3.57 ± 0.37 C	$0.14\pm0.02~\text{A}$			
Kalpitiya	$4.39\pm0.19~\text{A}$	$0.37\pm0.02~\text{A}$	$0.54\pm0.05~\text{AB}$	$0.44\pm0.044~\text{A}$	4.99 ± 0.27 A	$0.14\pm0.01~\text{A}$			
Knuckles	$2.62\pm0.02~\text{CDE}$	$0.30\pm0.07~ABC$	$0.41\pm0.01~\text{ABC}$	$0.23\pm0.02~\text{AB}$	$3.40\pm0.21~\text{BC}$	$0.11\pm0.01~\text{A}$			
Belihuloya	$3.45\pm0.35~\text{CD}$	$0.30\pm0.021~B$	$0.36\pm0.03~BC$	$0.25\pm0.02~B$	3.56 ± 0.34 C	$0.14\pm0.01~\text{A}$			
Udawalawa	$4.13\pm0.23~\text{AB}$	$0.37\pm0.02~\text{A}$	$0.60\pm0.21~\text{A}$	$0.27\pm0.016~B$	$4.50\pm0.33~\text{AB}$	$0.15\pm0.01~\text{A}$			
Mean	3.62 ± 0.09	0.32 ± 0.07	0.41 ± 0.03	0.30 ± 0.14	4.00 ± 0.12	0.14 ± 0.06			
CV	26.23	22.8	66.1	46.88	30.4	46			

Table 2 Floral morphological characteristics of fully opened flowers of Syzygium cumini from Sri Lanka (n-10)

Note: Data were presented as mean ± SE. CV (coefficient of variance) was calculated considering all trees irrespective to the geographical location.

Table 3 Fruit morphologi	cal characteristics of a	Svzvaium cumini from	different locations of	of Sri Lanka <i>(n</i>	$=10^{1}$
Tuble 5 Trait morphologi	cui characteribtico or i	oyzygiuni cuntin nom	amercine locadono e	n on Dunna (m	

Location	Fruit length/ mm	Fruit width /mm	Fruit length/width ratio	Fruit size/ mm ²	Pericarp thickness / μ m	Pericarp thickness/%
Ampara	$14.20\pm0.37~\text{C}$	$10.68 \pm 0.25 \text{ B}$	$1.33\pm0.03~\text{B}$	152.512 ± 9.71 B	$1292.42 \pm 75.41 \text{ B}$	31.86 ± 1.20 B
Batticaloa	$17.95 \pm 0.59 \text{ A}$	$13.78\pm0.39~\text{A}$	$1.32\pm0.04~\text{BC}$	$251.43 \pm 16.29 \text{ A}$	$1740.04 \pm 34.59 \text{ A}$	$34.95\pm1.07~\text{AB}$
Hambanthota	$13.40\pm0.33~\mathrm{C}$	$11.71\pm0.28~\mathrm{B}$	$1.15\pm0.02~\mathrm{D}$	$159.02 \pm 13.28 \text{ B}$	$1328.07 \pm 95.37 \text{ B}$	25.56 ± 6.63 C
Kalpitiya	$17.36\pm0.46~\text{AB}$	$14.28\pm0.27~A$	$1.23\pm0.03~\text{CD}$	$250.24 \pm 10.24 \; \text{A}$	$1932.08 \pm 48.98 \text{ A}$	$37.57 \pm 4.49 \text{ A}$
Belihuloya	$13.56 \pm 0.27 \; \text{C}$	$11.36\pm0.21~\text{B}$	$1.20\pm0.02~\text{CD}$	$156.41 \pm 8.48 \text{ B}$	$1316.62 \pm 66.58 \text{ B}$	$32.22 \pm 7.52 \text{ B}$
Udawalawa	$15.48\pm0.61~\text{BC}$	$10.58 \pm 0.26 \text{ B}$	$1.47 \pm 0.05 \text{ A}$	$165.29 \pm 14.11 \text{ B}$	714.29 ± 79.59 C	$18.54\pm9.82~\text{C}$
Mean	15.83 ± 0.34	12.64 ± 0.23	1.28 ± 0.02	202.75 ± 7.75	1491.55 ± 62.32	$\textbf{32.13} \pm \textbf{1.04}$
CV	18.24	15.14	13.35	32.22	32.18	25.65

Note: CV (coefficient of variance) was calculated considering all trees irrespective to the geographical location. Means that do not share a letter are significantly different.



Different locations in Sri Lanka

Fig. 11 Distribution of Syzigium cumini trees under three clusters and different locations based on floral morphological characters

pericarp thickness were studied as fruit morphological characteristics.

There were significantly differences in average fruit length of S. *cumini* across the locations (Table 3). The lowest average fruit length was recorded from Hambanthota (13.40 cm). The location that recorded the highest average fruit length was Batticaloa (17.95 cm) and it was significantly higher than the rest of the locations except Kalpitiya. When individual trees were considered, the lowest fruit length was in a tree from Hambanthota (10.82 mm) while the highest was from Batticaloa (24.85 mm). The grand mean of fruit length of all locations was 15.83 mm (Table 3).

Fruit width was significantly different among locations similar to fruit length. Fruits from Kalpitiya and Batticaloa regions showed significantly higher fruit width (14.28 mm and 13.78 mm, respectively) compared to the rest of the regions. Mean value of fruit width of all locations was 12.64 mm.

Fruit size, calculated by multiplying the fruit length and fruit width, was significantly different among locations. Significantly large fruits were recorded from Kalpitiya and Batticaloa. Meanwhile, there were trees with small fruit size of 97.37 mm^2 and very big fruits of 372.98 mm^2 .

Thickness of pericarp was also varied among trees and significant high variation was recorded among locations. Similar to the fruit size, pericarp thickness of fruits was also high in trees from Kalpitiya and Batticaloa regions.

According to cluster analysis, fruit morphological traits were formed into clusters. Cluster 1 was prominent in two locations such as Hambanthota and Belihuloya. Cluster 2 was prominent in Ampara and Udawalawa. Cluster 3 was prominent in Batticaloa and Kalpitiya (Fig. 12).

3.2. Fruit ripening

Fruit ripening stages were identified as Stage 1, Stage 2, Stage 3, Stage 4 and Stage 5 (Fig. 13). Patel and Rao (2014) also explained five ripening stages of S. cumini fruits and accordingly



Fig. 12 Distribution of Syzygium cumini trees under three clusters and different geographical locations based on fruit morphological traits

these five stages can be named as Young, Premature, Mature, Preripened and Ripened. Our previous study in a different location was also recorded the same (Dissanayake et al., 2019). Stage 1 was green for all trees and identified two green colours such as dark green and light green (Fig. 13). Dark green fruits were recorded in 36% of total fruit bearing trees of all locations, whereas, light green recorded in 64% of trees. Stage 5 of every tree was dark black purple. Stage 2 (Premature stage) was colour breaking stage which started changing green to pink. Portion of fruit became pink and some trees showed pink and green in one fruit. Some fruits showed no clear line between pink and green, mostly dull pink. In stage 3 (Mature) whole fruit colour became pink or magenta. Values of hue angle also showed variation among different ripening stages (Fig. 14). Stage 1 showed less variation among whole plant, however stage 5 showed extremely high variation of hue angle. Stage 2, Stage 3 and Stage 4 also showed high deviations from the mean.

3.3. Variation in antioxidant capacity

As phytochemical and antioxidant capacities, total polyphenolic content (TPC), total flavonoid content (TFC) and ABTS+ radical scavenging activity [as inhibitory concentration of 50% (IC_{50})] in fruit pericarp and in seed were analysed. Average TPC, TFC



Fig. 14 Hue angle of fruit colour in five stages of Syzygium cumini from different locations

and ABTS+ radical scavenging activities of seed and pericarp from each location were significantly different (Table 4). Kalpitiya, Hambanthota, Belihuloya and Batticaloa region showed no significant difference among TPC in pericarp but Ampara recorded higher (20.82 mg•g⁻¹ gallic acid). This value was around 4 times higher than the lowest in Belihuloya region (5.15 mg•g⁻¹ gallic acid). Compared with seed, TPC was significantly lower in pericarp, which is similar to the previous findings (Dissanayake et al. 2018). TPC in seed also varied significantly among regions. Further, the highest TPC in seed extract was from Belihuloya (62.05 mg•g⁻¹ gallic acid), whereas the lowest in Batticaloa (21.74 mg•g⁻¹ gallic acid).

Values of TFC in seed and pericarp were significantly lower in Batticaloa than the rest of the region (Table 4). TPC was also very low in Batticaloa region. Though the TPC in seed of *S. cumini* was significantly higher than pericarp, the TFC content was more or less similar in seed and in pericarp.

ABTS+ radical scavenging activity, expressed as IC_{50} of a compound, was inversely related to its antioxidant capacity. A lower IC_{50} indicates a higher antioxidant activity. S. cumini seeds were with significantly higher antioxidant activity than pericarp irrespective to the tree or geographical location (Table 4). Radical scavenging activity of pericarp showed significantly different result among locations. The highest activity was recorded in Belihuloya (56.35 mg•g⁻¹ trolox), whereas Batticaloa was the lowest (13.02 mg•g⁻¹ trolox).



Fig. 13 Different fruit ripening stages of Syzygium cumini In stage I number of Dark green and light green fruits counts indicated as percentage.

Location	TPC /(mg•g-1 gallic acid)		TFC /(mg•g-1 quer	cetin)	ABTS+ IC50 /(mg•g-1 Trolox)	
	Seed	Pericarp	Seed	Pericarp	Seed	Pericarp
Ampara	$35.76 \pm 6.13BC$	$20.82\pm6.84\text{CD}$	2.59 ± 0.5 AB	$2.28\pm0.33\text{ABC}$	$1.05\pm0.52\text{D}$	$24.76 \pm 3.69B$
Batticaloa	$21.74 \pm 3.98 \text{CD}$	$7.94 \pm 1.48 \mathrm{D}$	0.80 ± 0.11 CD	$0.45\pm0.08 \mathrm{D}$	$0.43\pm0.11\text{D}$	$13.02 \pm 2.66 BCD$
Hambanthota	$46.19\pm9.91\text{AB}$	$7.76\pm2.36D$	$2.20\pm0.35 \mathrm{ABC}$	$2.95\pm0.91A$	2.29 ± 0.89 CD	$29.29\pm6.16\mathrm{B}$
Kalpitiya Belihuloya	$\begin{array}{c} 33.16 \pm 6.13 BC \\ 62.05 \pm 9.77 \ A \end{array}$	$\begin{array}{c} 8.68 \pm 1.70 \text{D} \\ 5.15 \pm 0.91 \text{D} \end{array}$	$\begin{array}{c} \textbf{3.18} \pm \textbf{1.20A} \\ \textbf{1.96} \pm \textbf{0.14ABCD} \end{array}$	$\begin{array}{c} \textbf{2.45} \pm \textbf{0.78ABC} \\ \textbf{1.03} \pm \textbf{0.16BCD} \end{array}$	$\begin{array}{c} 0.64 \pm 0.33 \text{D} \\ 22.72 \pm 13.63 \text{BC} \end{array}$	$\begin{array}{c} \text{20.75} \pm \text{1.40BCD} \\ \text{56.35} \pm \text{17.84A} \end{array}$

Table 4 Antioxidant properties of fruit pericarp and seed extracts of Syzyajum cumini from different locations of Sri Lanka (n=10)

Note: TPC: Total phenolic contents; TFC: Total flavonoid contents. Means that do not share a letter are significantly different.

Table 5 Shannon-Wiener diversity index (H') for phenotypic traits and antioxidant properties for different locations of Sri Lanka

Phenotypic trait	All locations	Ampara	Batticaloa	Hambanthota	Kalpitiya	Knuckles	Belihuloya	Udawalawa
Leaf traits								
Leaf area	0.82	0.80	0.70	0.64	0.51	0.44	0.50	0.55
Leaf length	0.96	096	0.88	0.99	0.76	0.68	0.72	0.87
Leaf width	0.86	0.83	0.62	0.69	0.56	0.52	0.43	0.61
Leaf length/width ratio	0.92	0.74	0.72	0.75	0.79	0.72	0.81	0.76
Petiole length	0.61	0.56	0.60	0.77	0.41	0.38	0.50	0.63
Internodal length	0.87	0.71	0.67	0.67	0.68	0.60	0.70	0.78
Angle of vein	0.88	0.72	0.65	0.79	0.56	0.72	0.66	0.60
Floral traits								
Floral cup length	0.92	0.76	0.63	0.64	0.74		0.86	0.75
Floral cup width	0.90	0.66	0.54	0.61	0.50		0.74	0.66
Style length	0.96	0.92	0.54	0.78	0.53		0.97	0.55
Style width	0.98	0.70	0.68	0.87	0.88		0.90	0.83
Anther length	0.92	0.74	0.83	0.66	0.49		0.72	0.60
Anther width	0.82	0.90	0.83	0.37	0.91		0.62	0.45
Filament length	0.92	0.74	0.59	0.71	0.72		0.80	0.79
Filament width	0.78	0.72	0.65	0.45	0.82		0.77	0.43
Fruit traits								
Fruit length	0.85	0.50	0.78	0.42	0.73		0.43	0.82
Fruit width	0.85	0.53	0.73	0.59	0.32		0.58	0.61
Fruit length/width ratio	0.79	0.58	0.78	0.25	0.70		0.43	0.88
Fruit size	0.92	0.53	0.76	0.62	0.73		0.43	0.65
Pericarp thickness	0.92	0.67	0.69	0.56	0.58		0.85	0.56
Percentage of pericarp thickness	0.76	0.62	0.41	0.49	0.53		0.70	0.83
Antioxidant properties								
Total phenolic contents of seed	0.98	0.83	0.66	0.42	0.83		0.84	
Total phenolic contents of pericarp	0.86	0.66	0.59	0.66	0.66		0.31	
Total flavonoid contents of seed	0.81	0.66	0.42	0.42	0.83		0.59	
Total flavonoid content of pericarp	0.89	0.66	0	1.00	0.83		0.42	
ABTS ⁺ radical scavenging activity of seed	0.91	0.66	0.42	0.66	0.59		1.00	
ABTS ⁺ radical scavenging activity of pericarp	0.93	0.66	0.66	0.66	0.42		0.59	

The Shannon-Wiener Diversity Index for all morphological traits was varied across the geographical locations (Table 5). Leaf length showed the highest diversity index (0.96) over other leaf traits. However, it varied across the locations and the highest was recorded in Hambanthota (0.99) and the lowest in knuckles (0.68). In average, the leaf trait having the lowest diversity index was petiole length (0.61), which varied from 0.38 (Knuckles) to 0.77 (Hambanthota). All leaf traits in Ampara showed high diversity index with more than 0.7 except petiole length (0.56). In Batticaloa only two leaf traits exceeded 0.7, such as leaf length (0.87) and leaf length/width ratio (0.72). In Hambanthota 4 leaf traits showed high diversity index, such as leaf length (0.99), length/width ratio (0.75), petiole length (0.77) and angle of vein (0.79). Only two leaf traits showed diversity index above 0.7 in Kalpitiya, such as leaf length (0.76) and leaf length/width ratio (0.79). In Knuckles also two leaf traits showed high diversity index above 0.7, leaf length/width ratio ((0.72) and angle of vein (0.72). Both in Belihuloya and Udawalawa three traits showed high diversity above 0.7, such as leaf length, leaf length/width ratio and internodal length.

Floral traits also showed varied Shannon-Wiener Diversity Index in all locations. All eight floral traits showed high diversity index. However, it varied in different locations. Very low diversity index such as 0.37 showed for anther width in Hambanthota. In Ampara, Kalpitiya and Belihuloya, there were no less than 5 floral traits showed diversity index more than 0.7 (Table 5). All fruit traits showed high diversity, but in location wise it showed lower diversity.

Antioxidant properties showed high Shannon-Wiener Diversity Index. However, in some locations some traits showed very low diversity index, such as total flavanoid content in pericarp in Batticaloa (0). Very high diversity of ABTS scavenging activity of pericarp was recorded for all locations (0.93). But when dif-



Fig. 15 Principal component analysis of leaf morphology (A), floral morphology (B), fruit morphology (C), and antioxidant properties (D) of Syzygium cumini across Sri Lanka

ferent locations were considered separately it showed low diversity index. Total phenolic content in seed, total flavonoid content in seed, ABTS scavenging activity of seed in different locations showed high diversity index.

3.4. Principle component of morphological characteristics

For leaf morphological traits, first four principal components explained 92.2% of the total variation for the whole locations. The first principal component alone explained 50.2% of total leaf variation and was related mainly with leaf area, leaf width, and leaf length. The second principal component explained 19.7% of total leaf variation and was related with angle of veins (Fig. 15).

First four principal components explained 80.9% of the total variation for floral morphological traits from whole locations. The first principal component explained 45.1% of total floral variation and was related mainly with flower cup width, style length, style width, filament length and flower cup length. The second principal component explained 13.7% of total leaf variation and was related with anther length and filament width. The third principal component explained 11.4% of total floral traits variation and was related with anther width (Fig. 15).

For fruit morphological traits, first three principal components explained 92.7% of the total variation for the whole locations. The first principal component alone explained 52.3% of total fruit trait variation and was related mainly with fruit length, fruit width, and fruit size. The second principal component explained 24.9% of total leaf variation and was related with pericarp thickness and percentage of pericarp thickness (Fig. 15). First six components of antioxidant properties explained 96% of the total variation of trees. The first principal component explained 33.2% of variation by antioxidant properties such as total polyphenol content of seed, total flavonoid content of seed, total flavonoid content of pericarp, ABTS⁺ radical scavenging activity of seed, and ABTS⁺ radical scavenging activity of pericarp (Fig. 15).

3.5. Correlation among morphological characters and antioxidant capacity

Some morphological characteristics and antioxidant properties irrespective to the locations showed significant correlation among them (Table 6). Fruit size was positively correlated to leaf area, however it was mild correlation. Pericarp thickness also showed very high correlation to fruit size with P-value of 0.015. Total flavonoid content in pericarp negatively correlated to pericarp thickness. Other morphological features were not significantly correlated to total polyphenolic content, total flavonoid content, and ABTS⁺ radical scavenging activity. Hue angle, which represented fruit colour, significantly correlated to antioxidant properties in different ripening stages of the fruit, such as stage 3, stage 4 and stage 5. Total flavonoid content in pericarp of the fruit in ripening stage 4, showed significantly high positive correlation to hue angle changes. Total phenolic content in pericarp showed significantly high positive correlation only to hue angle in stage 3. ABTS⁺ radical scavenging activity in pericarp showed significantly high correlation to hue angle of ripening fruit in stage 4 and 5.

Table 6 Significantly correlated morphological and phytochemical properties of S. cumini.

Morphological/phytochemical property	Morphological/phytochemical property	Correlation	P-Value
Fruit size	Leaf area	0.437	0.037
Fruit size	Pericarp thickness	0.501	0.015
Total flavonoid content (Pericarp)	Pericarp thickness	-0.452	0.030
Hue angle (Stage 4 of fruit ripening	Pericarp thickness	-0.648	0.023
Hue angle (Stage 4 of fruit ripening)	Total flavonoid content (Pericarp)	0.717	0.009
Hue angle (Stage 3 of fruit ripening)	Total phenolic content (Pericarp)	0.834	0.001
Hue angle (Stage 4 of fruit ripening)	ABTS ⁺ radical scavenging activity (Pericarp)	0.580	0.048
Hue angle (Stage 5 of fruit ripening)	ABTS ⁺ radical scavenging activity (Pericarp)	0.712	0.009
ABTS ⁺ radical scavenging activity (Pericarp)	Total phenolic content (Seed)	0.580	0.004
ABTS ⁺ radical scavenging activity (Seed)	Total phenolic content (Seed)	0.588	0.003
ABTS ⁺ radical scavenging activity (Seed)	ABTS [‡] radical scavenging activity (Pericarp)	0.701	0

4. Discussion

4.1. Leaf morphological characteristics and diversity

Batticaloa region showed higher values of all leaf traits than other locations. This indicated that there were high influences of location on leaf characteristics and Batticaloa was with different trees. Further, leaf shape can be considered as a valid clue for high variation among S. cumini trees in the country. These different trees might be different varieties of S. cumini but it needs further extensive study for confirmation.

As indicated by cluster analysis, Cluster 2 and 3 were deviated from main cluster and having low number of trees, implied the existence of potential different cultivars. Further, there were possible effects of geographical differences on this cluster variation. Especially, in Batticaloa less prominent cluster 3 became prominent cluster and in Kalpitiya it was cluster 2.

Leaf size is developmentally related to the venation architecture (Sack and Scoffoni, 2013). Larger leaves mostly have larger petioles with more veins, which contain many larger xylem and phloem conduits, enhance the transport capacity per unit leaf area (McKown et al., 2010). Larger leaves may be vulnerable to drought and other climatic changes and this can be overcame by having multiple veins, or by the accessory transport and support tissues (Brodribb et al., 2010). Accordingly, larger vein angle of larger leaves especially in Ampara and Batticaloa could be due to having smaller veins and other accessory tissues in between bigger side veins.

Leaf area may reflect plant photosynthetic capacity on large geographical scales (Wright et al., 2004; Wang et al., 2011). Relatively high leaf area may enhance plant photosynthetic capacity and primary productivity (Wilson et al., 1999). This could help improve fruit productivity of S. cumini, which required separate study in broad scale. Furthermore, leaf morphological traits may better reflect the changes in environmental factors such as temperature (Hultine and Marshall, 2000; Li and Bao, 2014; Moles et al., 2014), light intensity (Lusk and Warton, 2007), and water status (Kooyers et al., 2015) and could lead to possible S. cumini trait development (Moles et al., 2014). These morphological studies in addition to characterization of a tree morphology as leaves are directly subjected to these environmental variations (Givnish, 1984) and are, thus, important probes for autecological or synecological studies because they evolve specific strategies to certain environmental characteristics (Pyykko, 1979).

Moreover, according to multivariate analysis, leaf area, leaf width, and leaf length can be taken as candidate leaf traits for diversity study of S. *cumini*.

4.2. Floral morphological characteristics and diversity

The floral cup, botanically known as hypanthium, is a cupshaped tube like structure formed by uniting basal portions of the calyx, the corolla, and the stamens (Hickey and King, 2000; Beentje and Williamson, 2010). It is present in most flowering species, although varies in structural dimensions and appearance. Differentiation between the hypanthium in particular species is useful for identification (Takhtajan and Cronquist, 1981). To detect the differences or variation among S. cumini trees, dimensions of hypanthium could be used. However, according to our observation, the maximum difference in the shortest and the longest hypanthium was around 3 mm. Though it was very small difference, there was significant difference of floral cup length and width among different locations from which it emphasised that there was higher variation among S. cumini trees in the country. When consider overall population, length and width of flower cup was about 3 mm, which is aligned with findings of Orwa et al. (2009). But the present study showed that it varied from location to location. Average filament length was relatively shorter when compared to previous studies (6 and 7 mm) (Orwa et al., 2009).

Results of cluster analysis implied that there were high variations among floral morphological characteristics irrespective to the geographical locations. The result of floral morphology changes is supportive to the previous morphological characteristic diversity which plant breeders have huge potential to go for further investigation to screen out the best cultivar. According to Diversity index and multivariate analysis, the best characteristic for evaluating the variation of *S. cumini* trees for possible different cultivar was filament length, style width, floral cup width, style length and floral cup length which could be a suitable candidate to investigate the floral morphological variation of *S. cumini* in future study. But it needs careful attention as there were no uniform-size anther and filament in one flower. Better to select the longest filaments out of all.

4.3. Fruit morphological characters and diversity

S. cumini fruit is botanically described as a berry or resembles large berries with relatively big seed, whereas Chen and Craven

(2015) mentioned that the fruit of *Syzygium* species as "drupaceous". Distal end of the fruit slightly deep cup, formed due to remaining parts of hypanthium, is a significantly prominent structure making fruits slightly curved shape when cup settled to the side of fruit.

This study gave clear evidence for, that geographical locations have significant effect on fruit length. However, the fruit length in the country is relatively shorter when compared to previously data in India and in Indonesia which showed some fruits with 35 mm and 50 mm length, respectively (Ayyanar and Subash- Babu, 2012; Bijauliya et al., 2017). However, this disclosed that variation of fruit length of selected trees was very high giving insight of having high potential to have fruits with more long length as well there was huge variation can see if consider each location separately (Dissanayake et al., 2019).

Nevertheless, variation of fruit length and fruit width make more differences and variation in fruit size. Especially, when the ratio is around 1, fruits are globular in shape. In our record in some cases the ratio was less than 1 which led to fruit shape flat round or oblate. Normal description of fruit shape of *S. cumini* is ovoid-oblong or elliptical (Warrier et al., 1996). However, the present study showed significantly different fruit shapes such as flat round or oblate through globular or spheriod according to the description about avocado fruit shapes (IPGRI, 1995) and then ovoid-oblong. Further, THE present study indicated that good germplasm for big fruit size lied under Northern part of the country. Similarly, pericarp thickness of fruits was also high in trees from Kalpitiya and Batticaloa, which was important for commercially valued trees for different fruit productions including juice.

Cluster analysis of fruit indicated that Batticaloa and Kalpitiya shared common morphologies in fruits and would give promising background information for plant breeders to produce commercially viable fruit bearing trees. Further, we suppose that there was interesting botanical value for botanists to identify ecologically adapted characteristics. Diversity index of fruit morphological traits varied from location to location, which emphasized more effect on geographical location on fruit traits. Low diversity index of some fruit morphological traits in some locations indicated that the characteristic of the trait was specific to the location and could be used as breeding purpose. According to multivariate analysis of principal component of fruit morphological traits, fruit length, fruit width, fruit size and pericarp thickness explained first component variation and could be considered as candidate traits for future studies on diversity.

The high phenotypic diversity could be ascribed to the different genetic constitution of the tree genotypes as well as to the environmental conditions.

4.4. Fruit ripening

Generally fully ripe fruit of S. cumini, irrespective to the location, was dark purple (almost black). Different authors have also described similar colour changes during fruit ripening of S. cumini (Ayyanar and Subash-Babu, 2012; Steiner et al., 2017). However, Morton (1987) reported white fruits in Indonesia. When ripe, presence of intense purple colour is very attractive for industrial processing (Steiner et al., 2017).

The fruit colour change during ripening is most probably as a consequence of chlorophyll degradation similar with senescence

of other horticultural crops (Dissanayake et al., 2008, 2012). Intense purple colour, which is characteristics of ripen fruit, is results of decrease in chlorophyll and carotenoids content parallel to increase in amount of anthocyanin (Brandão et al., 2011; Patel and Rao, 2014). As there was no uniform colour variation in some stages such as stage 2, stage 3 and Stage 4, chlorophyll degradation and other chemical reaction process could vary from tree to tree.

4.5. Antioxidant capacity and diversity

Values of total flavonoid content in seed and pericarp were significantly lower in Batticaloa region than the rest of the region. Total phenolic content was also very low in Batticaloa region. This indicated that there was a possible effect of geographical changes or genetic differences on these total phenolic content and total flavonoid content in *S. cumini* fruits. Though the total phenolic content in seed of *S. cumini* was significantly higher than pericarp, the total flavonoid content was more or less similar in seed and in pericarp. In Hambanthota, total flavonoid content in pericarp was higher than seed.

This variation could be a reason of variation in chemical contents of fruit among regions (Widyawati et al., 2014). Phenolic compounds are vital plant constituents and are major factors responsible for biological activities such as antioxidant, antimicrobial, antiviral and anticancer activities (Küçük et al., 2007). Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids (Wojdyło et al., 2007). As this is the factor, when consider for selecting trees for further improvement for healthy food, separate investigation required parallel with morphological changes and different geographical locations.

S. cumini seed extracts had higher total phenolic content, total flavonoid content and ABTS⁺ radical scavenging activity compared to pericarp extracts. This may be attributing the content of more phenolic compounds in seed extracts such as alkaloid, jambolin, glycoside jambolin, gallic acid, than pericarp extracts (Ayyanar and Babu, 2012). Previous studies also reported that S. cumini seed extracts gave higher total phenolic content, total flavonoid content and antioxidant activity compared to pericarp (Antolovich et al., 2000; Saha et al., 2013; Margaret et al., 2015; Dissanayake et al., 2018).

In this study, high variation was observed within *S. cumini* trees from different locations (Kalpitiya, Ampara, Hambanthota, Batticoloa). According to recent reports, polyphenolic content, responsible for antioxidant capacity in plant foods, varies greatly even among cultivars of the same species (Urquiaga and Leighton, 2000). The polyphenolics were largely influenced by genetic factors, environmental conditions, variety processing and storage conditions (Bravo, 1998).

Traditionally, S. cumini was propagated by seeds, hence there was considerable variation among the trees, due to its cross pollination in nature (Purseglove, 1981). Thus, a great deal of antioxidant variation is observed in trees and fruits within S. cumini population. It is evident that S. Cumini possess diverse antioxidant activity among the trees and among the regions determined by ABTS radical scavenging assay. The results of diversity index and multivariate analysis indicated that total flavonoid content in pericarp, total flavonoid content in seed, ABTS⁺ radical scavenging activity in pericarp, total phenolic content in seed and ABTS⁺ radical scavenging activity in seed could be candidates to investigate the antioxidant property variation of the species in future.

4.6. Correlations among some morphological characteristics and antioxidant capacity

In this study, only few fruit-related characteristics showed correlation with other morphological characteristics and some chemical properties related to antioxidant properties. Fruit size positively correlated to leaf area. This was basically due to high storage in the tree as a consequences of high photosynthesis rate in larger leaf area. Hence, larger leaf area tended to result in a larger fruit and trees with small leaf would result in smaller fruits. Nevertheless, fruit size was also directly related to pericarp thickness which is commercially important as a food. On other hand, pericarp thickness was negatively correlated to total flavonoid content in the pericarp. However, it expressed nutritionally negative impact to the fruit. By all this mean, it can be highlighted that selection of better commercial varity should be based on size of *S. cumini* leaves.

Fruit colour, which is represented by hue angle, showed positive correlation to antioxidant properties in three different ripening stages. This can be used to predict the antioxidant properties in the fruit during ripening.

5. Conclusions

This study emphasized that S. cumini trees across the country were highly varied and diverse based on morphological traits and antioxidant properties of fruits. The average traits of morphological features could be used for future studies on S. cumini trees. Leaf shape of S. cumini was varied in the country indicating possible ecotypes of trees. The variations in floral morphology and fruit morphology were also supportive to the above conclusion. Diversity index and multivariate analysis indicated that leaf area, leaf width, leaf length, fruit length, fruit width, fruit size, pericarp thickness, floral filament length, style width, floral cup width, style length, and floral cup length could be used as candidate traits for future tree diversity studies. Further, total flavonoid content in pericarp, total flavonoid content in seed, ABTS⁺ radical scavenging activity in pericarp, total phenolic content in seed and ABTS⁺ radical scavenging activity in seed were suitable candidates to investigate the antioxidant property variation of the species in future studies in fruit crop development. The present study indicated that good germplasm for big fruit lied under Northern part of the country. Further, the selection of trees for big fruit can be detected by having relatively large leaf area. As this study was basically on morphological characterization of S. cumini trees, it will give substantial knowledge base to botanist and plant breeders for the future research.

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