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CHARACTERIZATION OF LEAF, FLOWER, AND POD MORPHOLOGY AMONG VIETNAMESE COCOA VARIETIES (*THEOBROMA CACAO* L.)

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Abstract

This study examined the morphological traits of 63 cocoa varieties that have been imported and cultivated in Vietnam. These cocoa varieties were collected from five regions in Southern Vietnam and were classified into three groups based on their phylogenetic relationships. Their morphological features were individually evaluated and analysed. This included leaf characteristics (leaf and stem anatomy) and flower features (ligule shape, anther number, pollen, stamen and ovule, fruit, seed). The results of this study showed a large variation across all morphological characteristics of the evaluated cocoa varieties. The Vietnamese cocoa flower showed a diversity of morphological characteristics including five shapes of ligule (oval, broad, deltoid, elliptic and sub-lanceolate) and each stamen also has a bilobed anther with the exception of trilobed anther for TD11. Furthermore, the shape of pollen grains was found homogeneous in all 63 varieties. The colour of the stamens and ovules was purple and white respectively for all examined samples. Three kinds of fruit shapes were identified, namely Angoleta, Amelonado and Cundeamor, and these were of various colours. Additionally, an anatomical analysis on the midrib structure of the leaves from the 63 varieties showed the highest similarity, likewise the stem structure. The colour of young leaves was observed as being green and red. This is the primary research that scrutinizes the morphological biodiversity of Vietnamese cocoa varieties during the three-decade development of the Vietnam cocoa project. The results have a practical applicationsfor cocoa cross-breeding and botanical taxonomy.

Key words: Cocoa, Floral description, Fruit shape, Reproductive organ, *Theobroma cacao* L.

Introduction

Cocoa trees (*Theobroma cacao* L.) have recently been cultivated in some areas of Vietnam, showing themselves to be a potential crop for economic growth. Cocoa trees are well-adapted to Southern Vietnam because of the suitable climate and soil conditions, especially in the Highland areas and the Mekong Delta provinces (Phuoc, 2009). Vietnamese cocoa cultivations originated from two groups including the domestic group grafted by Trinitario cultivars in Vietnam (CT and TD varieties) and the cocoa imported group composed of international varieties namely SCV, ICS, SCA, POUND, MAN, PA, LCTEEN, IMC, UIT, APA, AMAZ, NA, MO, SIAL, EET, IFC. These imported varieties came from Peru, Costa Rica and Malaysia. Uyen & Sum (Uyen & Sum, 1996) and Phuoc (Phuoc, 2009) identified all 63 varieties as belonging to the Trinitario group. However, the diversity of hybrids also causes some difficulties in taxonomic research. Currently, cultivated cocoa trees have been categorized into three main groups. The first majority group is Criollo, which has a nicely flavored bean but is susceptible to diseases. The second group is Forastero, which has a high yield and is highly tolerant to diseases, although the bean aroma is weak. The third group is Trinitario which is the hybrid group of both Criollo and Forastero, thus it has inherited the high quality traits of both groups (Hardy, 1960; Wood & Lass, 1985; Laurent *et al*., 1994; Bekele *et al*., 2006; Hamon *et al*., 2003; Sounigo *et al*,. 2005; Bekele *et al*., 2006; Motamayor *et al*., 2008; Jain & Priyadarshan, 2009, Efombagn *et al*., 2009).

The floral organization of *T. cacao* is basically conserved across the genus *Theobroma*. Studies on morphological divergence have been performed on flowers, fruits and leaves in order to identify and classify different cocoa varieties (Cuatrecasae, 1964; Braudeau, 1969, Soria & Enríquez, 1981; Engels, 1983, N'Goran *et al*., 1994; Lachenaud *et al*., 1999; Bekele *et al*., 2006, Jahan *et al*., 2014). Studies on morphological divergence revealed the classification of two morphological groups: the first group includes the Criollo varieties and the second group is of the Forastero varieties (Engels, 1983). This result was later confirmed by N'Goran (N'Goran, 1994) who applied morphological investigations to the characteristics of seed (bean) and fruit (fruit) traits. Enríquez and Soria (Soria & Enríquez, 1981) also classified the diversity of cocoa varieties by describing several anatomical traits such as flower, fruit, and leaf. The fully developed flowers have 5 free sepals, 5 free petals, 10 stamens (5 fertile and 5 nonfertile staminodes) and an ovary of 5 united carpels (Majer *et al*., 1994). The petals are narrow at the base, but extend into cup-shaped pouches. They are usually pink and white, but the precise

coloration and pattern may vary slightly and can indicate a certain genotype. The stamens are arranged in two whorls; the outer consists of five nonfertile staminodes and the inner consists of five fertile stamens. The stamens have two anthers that lie in the pouch of the corresponding petal. The style is twice as long as the ovary and consists of five parts around an axis (Wood & Lass, 1985). The fused style has the appearance of being single but is divided at the tip into five stigmas (Wood & Lass, 1985; Majer *et al*., 1994).

Lachenaud (Lachenaud *et al*., 1999) introduced the identification of large varieties of cocoa cultivars based on their morphological features (ligule, sepal). In addition, the types and populations of cocoa varieties are also defined by phenotypic appearance of cocoa fruits (pods) (Efombagn *et al*., 2009). This corresponds with previous studies by Cuatrecasae (Cuatrecasae, 1964); Uyen and Sum (Uyen & Sum, 1996); Lachenaud (Lachenaud *et al*., 1999); Bartley (Bartley, 2005); Phuoc (Phuoc, 2009); Santos (Santos *et al*., 2012) and Garcia (Garcia *et al*., 2014). These studies also examined the taxonomy of cocoa varieties by evaluating morphological traits of different organs such as cocoa reproductive organs (ligule, ovary, and fruit shape) or plant organs (leaf shape and leaf anatomy). Moreover, the pre-existing research was based on non-natural populations, which were represented either by the morphology of their geographic groups – their country of origin which were previously distinguished by geographical factors - their country of origin (Lachenaud *et al*., 1999; Bartley, 2005; Lachenaud *et al*., 2007; Swanson *et al*., 2008; Santos *et al*., 2012; Garcia *et al*., 2014). In addition, Ha (Ha *et al*., 2015a) only analysed the anatomical traits (fruit shape and leaf size) of the fourteen main cocoa varieties in Vietnam and classified them into 2 groups - the hybrid Criollo group and the hybrid Forastero group. The assessment of the fourteen cocoa varieties in Vietnam was conducted to clarified the genetic relations with some results related pod shape characterizations (Ha *et al*., 2015b). Therefore, this study elucidates the morphological features of natural *T. cacao* populations in Vietnam which was very limited until now.

The Vietnamese cocoa breeding programme is significant for the further development of the cocoa industry in Vietnam. Therefore, this study focused on examining the morphological features of the 63 cocoa varieties currently cultivated in Southern Vietnam based on plant organs and reproductive organs. To the best of our knowledge, there are no international publications on the morphological diversity of Vietnamese cocoa varieties. Consequently, this report provides vital information on the morphology collection of Vietnam which in turn can assist the development of improved cocoa breeding techniques.

Materials and Methods

In this study 63 cocoa varieties were collected from five typical cocoa regions in Southern Vietnam, including Dak Lak province, Dong Nai province, Ben Tre province, Nong Lam University-Ho Chi Minh city, and Can Tho City (Fig. 1). Table 1 presents an overview of the analyzed varieties by name code, institute code, and sampling sites.

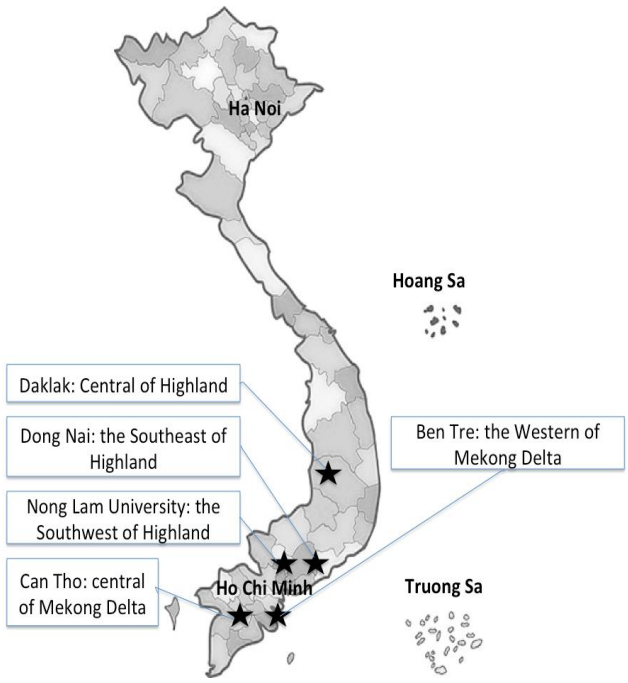

Fig. 1. The location of cocoa plantations in the South of Vietnam where the 63 cocoa varieties were collected for examination. Collected cocoa regions have been marked with black stars.

Cocoa harvest season: In Vietnam two crops of cocoa are harvested in May-July and October-December annually. Samples for the present study (flower, leaf and fruit) were collected in both of these periods.

Sampling collection: An examination of all the cocoa trees was undertaken during the two main flowering periods. Three trees were identified and used during both harvest seasons (6 trees in total per accession) for the collection of flowers and buds. The reproductive organs were packed into small glass bottles and put in cold boxes before observation.

Cocoa flower: Ten newly bloomed flowers per accession were collected at random from trunks and branches as described by Lachenaud (Lachenaud *et al*., 1999). In each flower only one of the floral parts to be studied was measured. In each accession studied the ligule shape was the most representative in a sample of six structure ligules observed under binocular microscope (Motic SMZ-168 Stereo zoom Microscope). Three measurements were taken for each flower: flower bud length (distal tip of the sepals to the base of the receptacle), flower width at its widest point, and the pedicel length (from the base of the flower bud to the tree). Measurements were calibrated from the images using a ruler (Paint software, adobe Photoshop ruler tool), flower bud measurements were converted to millimeters.

Cocoa bud (unopened): Per accession five buds 5-6 cm in length and 3-4 cm in width were chosen at random from the tree trunks. The number of anther inside the ovary was counted under a binocular microscope (Motic SMZ-168 Stereo zoom Microscope). This preliminary study showed that all the descriptors were usable for clonal characterization and that ten flowers per clone provided sufficient relative accuracy on the means.

Table 1. List of Vietnamese cocoa varieties used in this study with information of their accession name, genebank accession number and collection code.

Collection code	Institute varieties code	Imported country	Genebank accession number	Sampling location
DK1	CT3	Columbia	KR864819	EaKar-Daklak province (Highland)
DK2	CT5	Columbia	KR864820	EaKar-Daklak province (Highland)
DK3	CT6	Columbia	KR864758	EaKar-Daklak province (Highland)
DK4	CT7	Columbia	KR864815	EaKar-Daklak province (Highland)
CT1	CT8	Columbia	KR864816	Phongdien-Cantho province (Mekong Delta)
CT2	CT9	Columbia	KR864806	Phongdien-Cantho province (Mekong Delta)
CT3	CT21	Columbia	KR864814	Phongdien-Cantho province (Mekong Delta)
BT1	TD1	Malaysia	KR864779	Chauthanh-Bentre province (Mekong Delta)
BT2	TD2	Malaysia	KR864780	Chauthanh-Bentre province (Mekong Delta)
BT3	TD3	Malaysia	KR864781	Chauthanh-Bentre province (Mekong Delta)
BT4	TD5	Malaysia	KR864782	Chauthanh-Bentre province (Mekong Delta)
BT5	TD6	Malaysia	KR864783	Chauthanh-Bentre province (Mekong Delta)
BT6	TD7	Malaysia	KR864784	Chauthanh-Bentre province (Mekong Delta)
BT7	TD8	Malaysia	KR864785	Chauthanh-Bentre province (Mekong Delta)
BT8	TD9	Malaysia	KR864786	Chauthanh-Bentre province (Mekong Delta)
BT9	TD10	Malaysia	KR864787	Chauthanh-Bentre province (Mekong Delta)
BT10	TD11	Malaysia	KR864788	Chauthanh-Bentre province (Mekong Delta)
BT11	TD12	Malaysia	KR864789	Chauthanh-Bentre province (Mekong Delta)
BT12	TD13	Malaysia	KR864790	Chauthanh-Bentre province (Mekong Delta)
BT13	TD14	Malaysia	KR864791	Chauthanh-Bentre province (Mekong Delta)
DN1	TD15	Vietnam	KR864792	Trangbom-Dongnai province (Highland)
DN2	LCTEEN37/A	Ecuador	KR864759	Trangbom-Dongnai province (Highland)
DN3	LCTEEN62/S	Ecuador	KR864760	Trangbom-Dongnai province (Highland)
DN4	POUND16/B	Peru	KR864775	Trangbom-Dongnai province (Highland)
DN5	POUND16/A	Peru	KR864774	Trangbom-Dongnai province (Highland)
DN6	MAN15/6	Trinidad	KR864762	Trangbom-Dongnai province (Highland)
DN7	IMC105	Trinidad	KR864809	Trangbom-Dongnai province (Highland)
DN8	IMC67	Trinidad	KR864810	Trangbom-Dongnai province (Highland)
DN9	IFC5	Amazon	KR864807	Trangbom-Dongnai province (Highland)
DN10	UIT1	Amazon	KR864805	Trangbom-Dongnai province (Highland)
DN11	PA88	Peru	KR864767	Trangbom-Dongnai province (Highland)
DN12	IMC53	Peru	KR864808	Trangbom-Dongnai province (Highland)
DN13	PA137	Peru	KR864771	Trangbom-Dongnai province (Highland)
DN14	PA70	Peru	KR864768	Trangbom-Dongnai province (Highland)
DN15	PA120	Peru	KR864769	Trangbom-Dongnai province (Highland)
DN16	ICS1	Trinidad	KR864813	Trangbom-Dongnai province (Highland)
DN17	ICS43	Trinidad	KR864811	Trangbom-Dongnai province (Highland)
DN18	APA4	Amazon	KR864818	Trangbom-Dongnai province (Highland)
DN19	AMAZ1515	Ecuador	KR864817	Trangbom-Dongnai province (Highland)
DN20	SCA6	Peru	KR864776	Trangbom-Dongnai province (Highland)
DN21	SCA9	Peru	KR864777	Trangbom-Dongnai province (Highland)
DN22	PA169	Peru	KR864773	Trangbom-Dongnai province (Highland)
DN23	NA33	Peru	KR864765	Trangbom-Dongnai province (Highland)
DN24	MA12	Ecuador	KR864761	Trangbom-Dongnai province (Highland)
DN25	MO81	Peru	KR864763	Trangbom-Dongnai province (Highland)
DN26	TD32	Vietnam	KR864798	Trangbom-Dongnai province (Highland)
DN27	TD60	Vietnam	KR864802	Trangbom-Dongnai province (Highland)
DN28	TD57	Vietnam	KR864801	Trangbom-Dongnai province (Highland)
DK5	TD17	Vietnam	KR864793	EaKar-Daklak province (Highland)
DK6	TD18	Vietnam	KR864794	EaKar-Daklak province (Highland)
DK7	PA127	Peru	KR864770	EaKar-Daklak province (Highland)
DK8	TD24	Vietnam	KR864796	EaKar-Daklak province (Highland)
DK9	NA149	Peru	KR864766	EaKar-Daklak province (Highland)
DK10	TD77	Vietnam	KR864804	EaKar-Daklak province (Highland)
DK11	TD42	Vietnam	KR864799	EaKar-Daklak province (Highland)
DK12	TD31	Vietnam	KR864797	EaKar-Daklak province (Highland)
DK13	TD52	Vietnam	KR864800	EaKar-Daklak province (Highland)
DK14	TD70	Vietnam	KR864803	EaKar-Daklak province (Highland)
DK15	TD20	Vietnam	KR864795	EaKar-Daklak province (Highland)
DK16	SIAL339	Amazon	KR864778	EaKar- Daklak province (Highland)
DK17	NA32	Amazon	KR864764	EaKar-Daklak province (Highland)
DK18	EET376	Amazon	KR864812	EaKar-Daklak province (Highland)
DN14	PA156	Amazon	KR864772	Trangbom-Dongnai province (Highland)

Cocoa leaf: Ten branches of young leaves in one accession were observed. The samples taken from the mature cocoa leaves were then organised according to shape and size.

Cocoa fruit (pod): Per accession, three fruits were collected at random for the examination. The fruit was collected when ripe (yellow-green or yellow-red)

Flower structure observation: Flower's particles were collected by testing cocoa buds with forceps and needles. The ligule, sepal length, ovule height and diameter, stamen, spitil and anther were observed using Motic SMZ-168 Stereo zoom Microscope and camera Olympus CX41- C5050. Pollen grains were collected from the anther. The scanning electron microscopy of pollen grains were taken by using an Tabletop Microscope TM-1000 (Hitachi High Technology). Stems and midribs were stained by Carmin alune' 0.1N and vertd'iod 0.01N before anatomical observation (Nguyen, 2006).

Data (leaf width and height, sepal length, ovule heigh and diameter) were statistically analyzed using the computer software program SPSS 20 and the difference between means was adjudged by Duncan's New Multiple Range Test (DMRT). The null hypotheses were controlled with the Duncan test, which can be considered as an extension of the *F* test, used in the univariate analysis of variance.

Seed shape and color: Cocoa fruit was cut with vertical trend for seed shape observation.

Results

63 morphological features were recorded and the results are presented in Tables 2 and 3. Sample data including leaf, ligule, fruit traits were defined according to the identification.

Leaf characters: This study focused on the plant organs, including stem and leaf. Their anatomy was observed under a binocular microscope.

During observation it was noticed that the colour of the cocoa leaves were all dark green in mature cocoa plants while the immature plants were presented as two different colours: red purple (TD3, TD6, TD10, TD15, ICS1, TD31, TD17) and yellow green (data not shown). The leavas were ellipitically shaped.

Leaf anatomy: For all 63 cocoa varieties collected, the midrib in the cross section was concave-convex in structure. The vascular system of the midrib of *T. cacao* was collateral, surrounded by a sclerenchyma system. The gland is visible in the middle of the midrib (Fig. 2).

Fruit shape: The 63 cocoa collected varieties were determined to be Amelonado (oval shape, smooth skin, dark purple colytedon), Angoleta (elongated shape, rough, narrow neck, dark purple colytedon, shape in bottom) and Cundeamor (elongated shape, rough, dark purple colytedon) fruit shapes (Fig. 3).

Seed shape: The 63 collected cocoa varieties showed only one oval seed shape (data not shown) with purple colytedon in young and dark brown colytedon in mature period.

Flower description

Ligule: The data collected showed the diversity amongst the Vietnamese cocoa varieties in relation to their morphology flower description features. The variation of ligule shapes includes five variations namely oval, broad, deltoid, elliptic and sub-lanceolate (Fig. 4).

Table 2. Summary of morphological characteristics of ligule shape and leaf colour of Vietnam cocoa varieties.

Morphology features	Ligule shape					Young leaf color	
	Oval	Broad	Deltoid	Elliptic	Sub-lanceolate	Red purple	Yellow green
Accession	TD3, SIAL339, NA33, AMAZ1515, UIT1, LCTEEN62/S, ICS63, MO81, PA169, TD27, MA12, SCA6, TD15, TD7, PA120, PA137, TD9, SCA9, LCTEEN62/S,	TD5, MAN15/2, POUND16/B, POUND16/A, PA156, APA4,	TD1,TD3, SIAL339, NA32, TD2, IMC53, PA88, NA147, PA127, MO81, PA137, PA70, EET367, TD5, TD12, IMC67,	SIAL339, CT9, IFC5,	SCA9, TD31,CT3, TD8, TD27,	TD3, TD6, TD10, TD15, ICS1, TD31, TD17	the rest 56 varieties

Table 3. Morphological characteristics of fruit shape and color of Vietnam cocoa varieties.

Morphology features	Fruit shape			Fruit color	
	Cundeamor	Amelonado	Angoleta	Red purple	Yellow
Accession	TD2, TD3, TD8, LCTEEN37/A, POUND16/B, IMC67, PA88, IMC53, PA137, PA70, PA120, ICS1, ICS43, APA4, PA169, TD18, PA127, TD24, TD42, TD31, TD52, TD20, SIAL339, EET376, PA156	TD1,TD6,TD10,TD 12, TD14, CT7, TD15, AMAZ1515, SCA6, SCA9, NA33, NA149, NA32	TD3, TD5, TD6, TD7, TD8, TD9, TD11, TD13, CT3, CT6, CT8, CT9, CT21, LCTEEN62/S, POUND16/A, MAN/2, IMC105, IFC5, UIT1, MA12, MO81, TD32, TD60, TD57, TD17, TD77, TD70, TD20,	TD17, TD31, TD3, TD5, TD6, TD10, TD15, ICS1	TD3, TD5, TD6, TD7, TD8, TD9, TD11, TD13, CT3, CT6, CT8, CT9, CT21, LCTEEN62/S, POUND16/A, MAN/2, IMC105, IFC5, UIT1, MA12, MO81, TD32, TD60, TD57, TD17, TD77, TD70, TD20

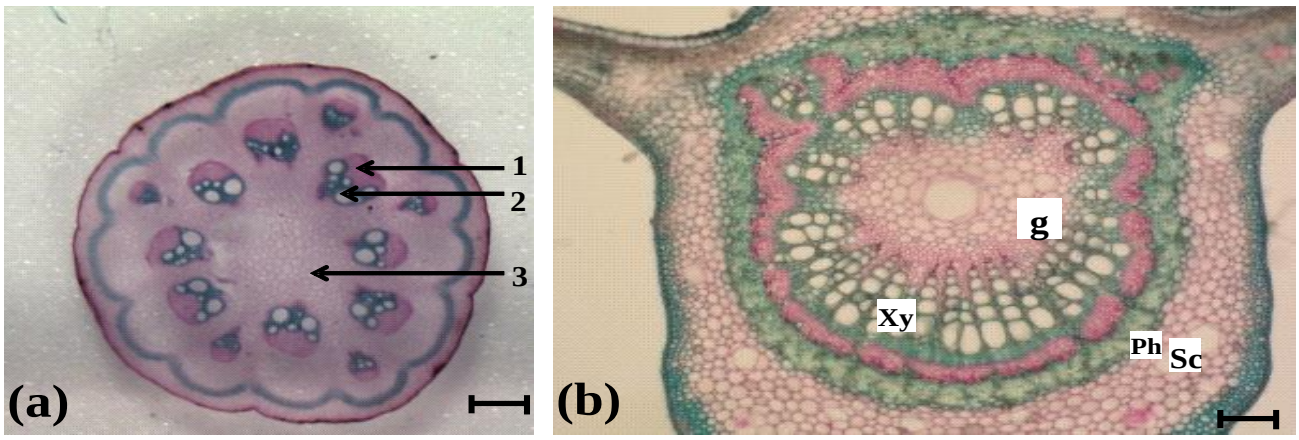

Fig. 2. Leaf anatomical features of *Theobroma cacao* L. (a) Secondary stem of TD11 (1: Phloem, 2: Xylem, 3: Pith). (b) Midrib of CT3 clone (g: gland, Xy: xylem, Ph: phloem, Sc: schlerenchyma). Scale bar = 0.02mm (a), 0.267mm (b). This figure is in the colour of the microscope observation.

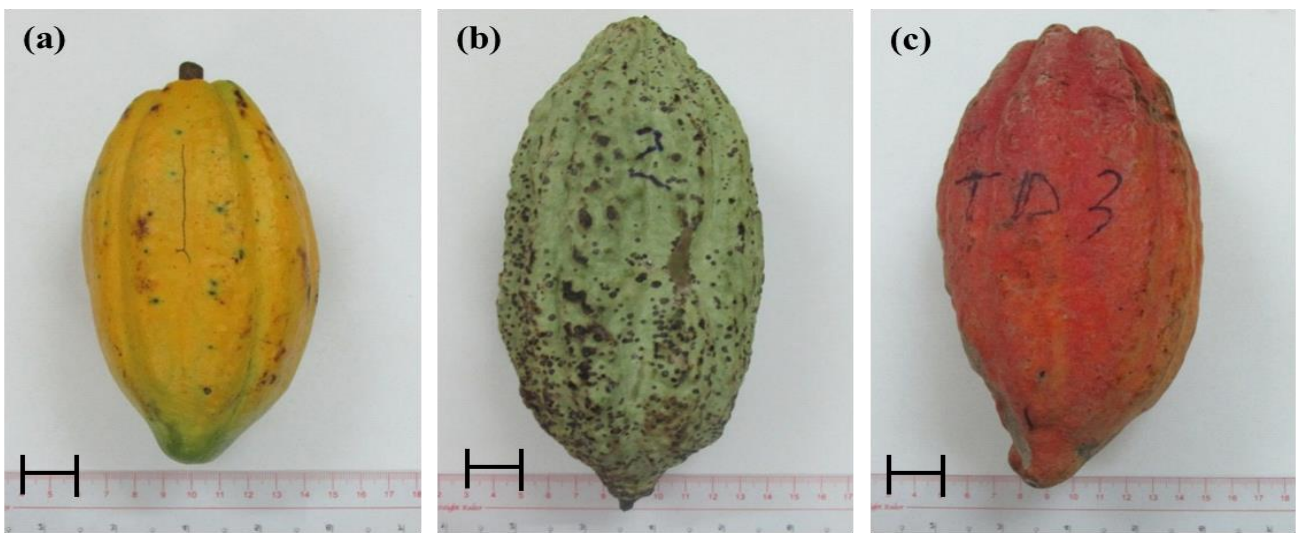

Fig. 3. Vietnamese cocoa fruit shapes Amelonado (a), Angoleta (b), Cundeamor (c). Scale bar = 2cm.

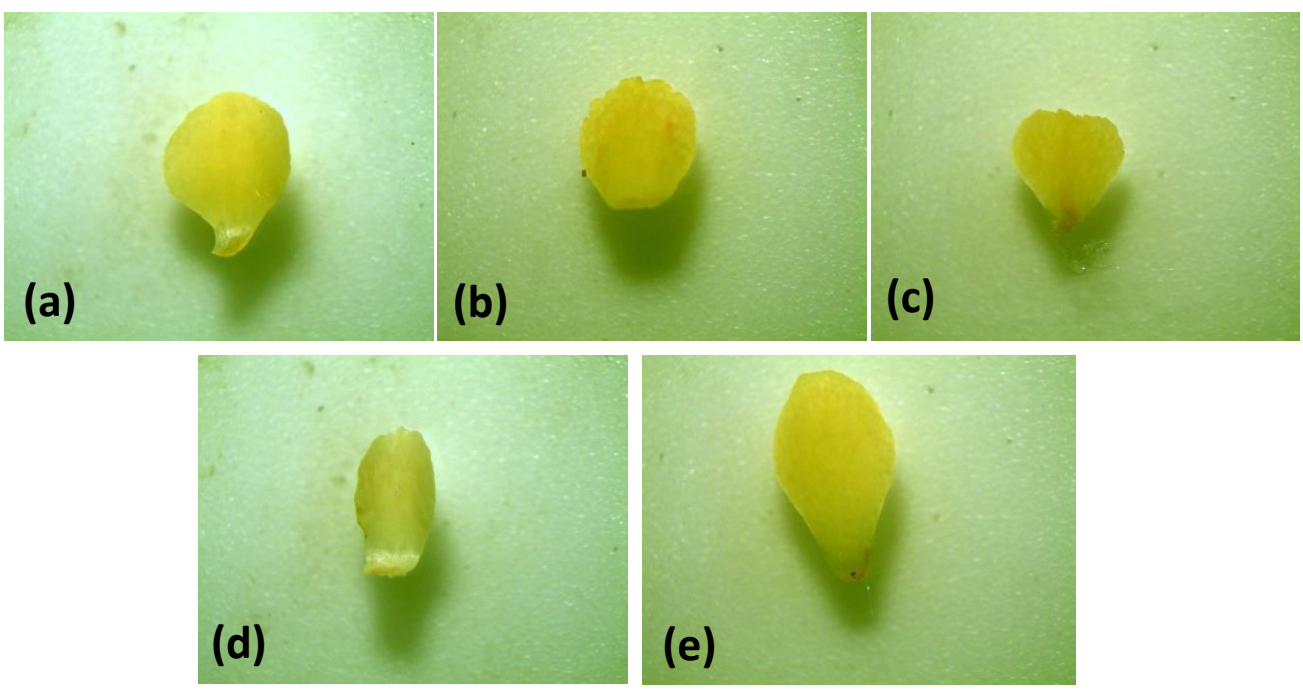

Fig. 4. The various of cocoa ligule shapes: (a) oval, (b) broad, (c) deltoid, (d) elliptic, (e) sub-lanceolate.

Stamen and ovule: The staminode, stamen and ovule were observed by a binocular. Inall 63 samples thestamenwasdarkpurple and the ovule was white (Fig. 5).

Anther: The arrangement of anthers (Fig. 6) was different with TD11 having a trilobed anther whilst the rest had bilobed anthers.

Pollen shape: It was observed that the reproductive organ pollen grains, in all 63 varieties, had a round shape. Usually the pollen grains can be used to classify species diversity but not in this study (Fig. 7).

Discussion

This study illustrates clarifies the anatomical diversity of the leaves and the flowers of Vietnamese *Theobroma cacao* L. based on morphological comparison.

Leaf characteristics: According to Bartley (Bartley, 2005), the colour of the fresh (young, developing) leaves is considered to be a distinctive trait for taxonomy. For example, the *Criollo* group and certain Amazon populations are found to have light green shades. Our result showed that 56 varieties in Vietnam were identified with green shades and therefore could be categorized into the Criollo group. The other 7 varieties hav red leaves whereby it is likely they originate from the Forastero group. Enriquez and Soria (Soria & Enriquez, 1981) indicated that there are 3 phenotypes of cocoa foliage including lanceolate, elliptical and oval patterns. In regardstoour analysis, 63 Vietnamese varieties were observed to have an elliptical shape. Another foliage classification is also mentioned by Bartley (Bartley, 2005) regarding the leaf shape, namely spherical and cylindrical shapes. However, neither of the authors was able to clarify which group these foliage shapes could be sorted into. Therefore, it is difficult to discriminate the cocoa populations based on leaf colour because of the expression of progeny characteristics between Criollo and Forastero.

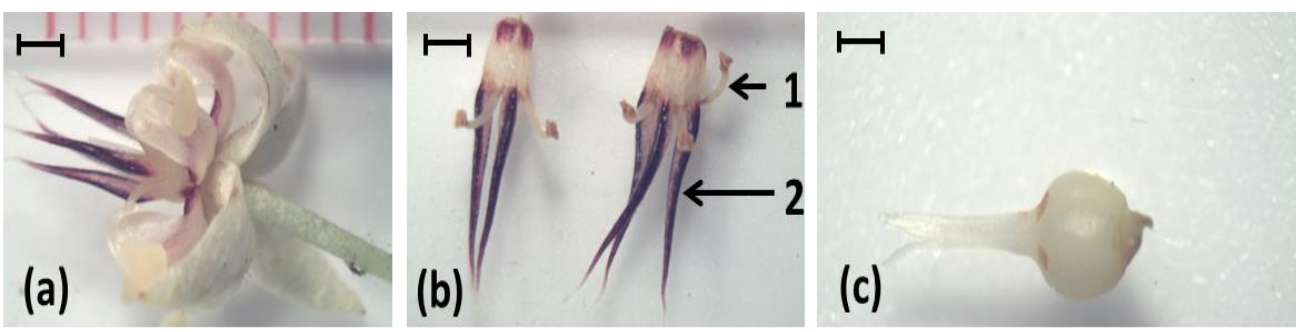

Fig. 5. Blossom cocoa flower (a); Staminodes and stamen (b), staminode in purple (1), stamen in light pink (2); Ovule in white colour (c). The figure is in colour in binocular. Scale bar = 0.05mm (a), (b); = 0.1mm (c).

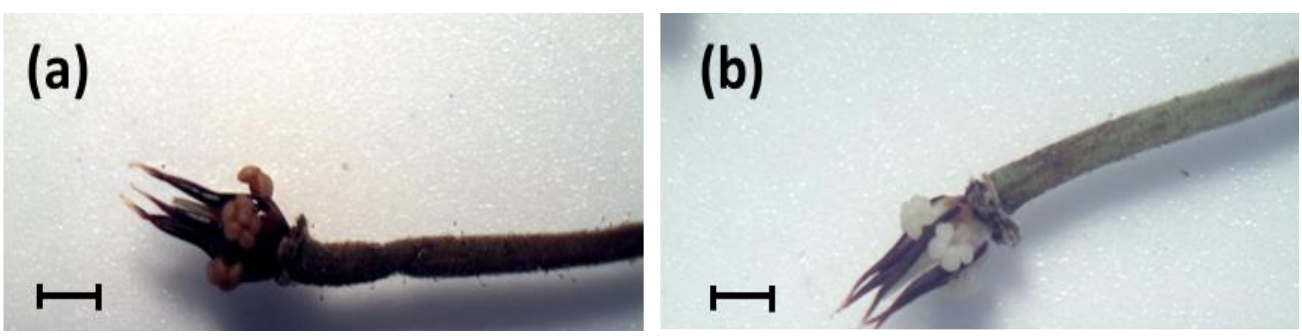

Fig. 6. The structure arrangement of anthers, trilobed anther (a) and bilobed anther (b). Scale bar = 0.1mm.

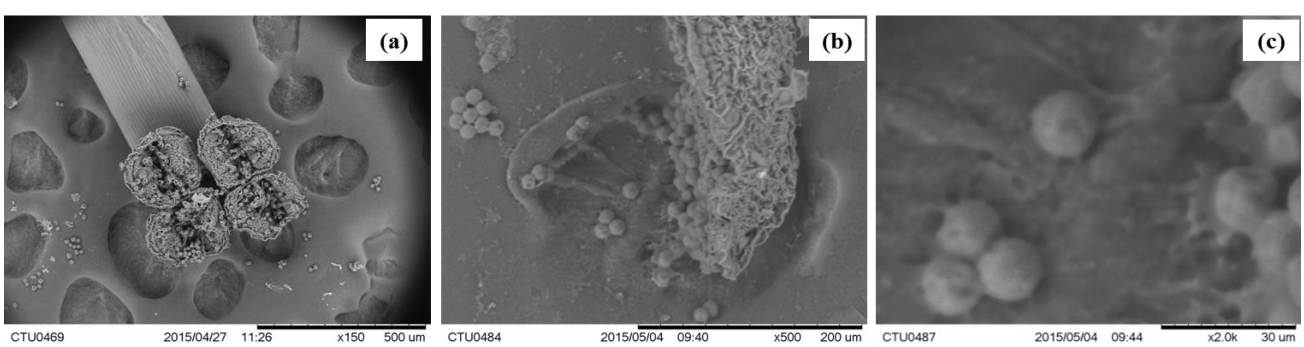

Fig. 7. Scanning electron micrographs of bilobed of anther (a), pollen grains (a), pollen grains in round shape (b).

Foliage and stem anatomy: A cross section shows that the structure of leaf and stem hold the highest similarity among 63 varieties. This could be due to the independency of the vascular system of plants ton environmental changes (Easu, 1964).

Fruit traits: The shape and colour of the fruits is considered to be the most significant characteristic to separate individual genotypes from the diversity of the species.

Fruit shape: There are many studies about thecharacteristics of cocoa fruits. It has been proposed thatthereare four distinguishable shapes in cocoa fruit namely Amelonado, Calabacillo, Angoleta, and Cundeamor (Pound, 1932; Chessman, 1944; Cuatrecasae, 1964; Bartley 2005). Amelonado is usedtodescribe fruits which are ovoid without a prominent point while Cundermor/Cundeamor is used for elongated cylindrical fruits with a pronounced bottleneck and a sharp point. Calabacillo indicates round fruits but no point whereas Angoleta is specified as long fruits without a bottleneck and pointed ends (Soria & Enriquez, 1981; Bartley, 2005). According to Cuatrecasae (1964), Braudeau (1969) and Bartley (2005), Amelonado and Callabacillo are attributed to fruits that belong to Forastero group which has a high yield whilst the titles of Angoleta and Cundeamor belong to the two fruit shapes of the Criollo group. The result of shape analysis in this study identified three shapes of cocoa fruit including Amelonado, Angoleta and Cundeamor (Table 3). This result confirmed the variation of examined cocoa varieties that originated from the Trinitario hybrid group. Phuoc (Phuoc, 2009) showed the Trinitario TD origin has characteristics combining the characteristics of Criollo and Forastero. For example, TD1 varieties are a hybrid progeny of PA35 x NA32 (Phuoc, 2009; Ha *et al*., 2015b), two offprings - PA and NA presented the Criollo characteristics of elongated - green fruit and oval colytedon (Bartley, 2005).

Fruit color: The coloration of cocoa fruit is a notable characteristic for classification. During maturation, the green cocoafruitwill turn into yellow and the red fruit into orange (Soria & Enriquez, 1981). Aprevious report from Bartley (Bartley, 2005) revealed that the red fruit colour could be representative to the Criollo group and as a result the offspring of this group has inherited this trait.

Additionally, Cuatrecasae (1964) and Braudeau (1969) also agreedthat the Criollo group has red leaves. Our data clearly report that there are 2 different colours amongst the fruits of Vietnamese cocoa. The orange hue was found in 8 varieties and the yellow hue was found in the remaining 55 varieties. To be more detailed, TD varieties (TD10, TD15, TD17, and TD31) have red fruit and red young leaves. These traits might prove that these varieties belong to the Criollo group. Therefore, plantbreeders should take into accountthese traits since the Criollo group is noticeably favoured because of its seed qualification (Lachenaud *et al*., 2007; Beleke *et al*., 2006,Jain & Priyadarshan, 2009). For instance, TD3 has the Criollo characteristics, which produces the decent flavored beans, but the fruit size is small (Ha *et al*., 2015b). This disadvantage could be improved by hybridization with TD9 (Forastero group), which yields bigger fruits. The selection for hybrid progenies should be more easily approached because of the distinguishable characteristics between the Criollo and Forastero group.

Seed shape and color: Our examination showed that the shape and colour of the seeds werenot significantly different across samples. Previous studies have shown that the cocoa seed colour was found tobe white, pale purple, intermediate purple and dark purple and cocoa seed shapes varied from almost spherical to cylindrical (Soria & Enriquez, 1981; Bartley, 2005). Thus, classification based on shape and colour is difficult in our investigation since all samples have a spherical shape and a dark purple color.

Flower description

Ligule: Previous studies showed that cocoa ligule has a yellow hue and varies in shape (Soria & Enriquez, 1981; Lachenaud *et al*., 1999). Our result is coherent with those studies as the examined ligule of 63 varieties diversified in shape (Fig. 4) and the colour was yellowish. The obtained results showed five shapes of ligule (oval, broad, deltoid, elliptic, and sub-lanceolate). Therefore, this characteristic could be a useful feature for further classification.

Stamen and ovule color: Our study showed that 63 examined varieties of stamenoids were darkish purple while their stamens were pinkish. These results are similar to Soria and Enriquez (Soria & Enriquez, 1981) whichshowed that most of the floral elements acquire a pink pigment except for the ligule which hasa yellow pigment. Moreover, Bartley (Bartley, 2005) also described the variation of colouration ranging from pink to very dark red or purple. As for the ovule colour, all ovule samples were whitish which was also recorded by Soria and Enriquez (Soria &Enriquez, 1981).

Anther: The examination of anthers indicated that the majority of cocoa accesions have a dilobed anther structure, with the exception of TD11 which has a trilobed anther structure. The TD11 has a greater amount of anther which means it has more pollen grains. The more pollen grains, the more fruit is formed in the cocoa tree. This information is significant to support cocoa breeders in their activity.

Pollen grains: Pollen grains are one of themost important elements in studies concerning flowers, but results are limited. With the aim to have a detailed report about the morphology of cocoa varieties in Vietnam, this work focused oninvestigatingvariationsin pollen grain shape. The results showed that there is only one round shape of pollen grain among 63 collected cocoa samples. This study didnotfocusonthe number of pollen grains because of the different sizes of collected cocoa bulb acrossed the 2 cocoa seasons. Thus, an examination of the number of pollen grains needs to be further investigated.

Leaf size, sepal length, ovule length and diameter: Our work showed showed no significant difference in the measurement for all surveyed data (data not shown) among the 63 examined cocoa varieties. This can explain that these cocoa clones has classify in *Theobroma cocoa* L. subspecies. Moreover the work was performed in one season from May to July. An examination of the other season (from October to December) could be the subject of further research.

Based on our morphological analysis, mainly focusing on fruit shape, the 63 varieties are divided into two groups - Criollo-Trinitario and Forastero-Trinitario. The former group, Criollo-Trinitario, appears with typical features including either the fruit colour being orange (TD17, TD31, TD3, TD5, TD6, TD10, TD15, ICS1) or its shape having an Amelonado genotype (TD1, TD6, TD10,TD12, TD14, CT7, TD15, AMAZ1515, SCA6, SCA9, NA33, NA149, NA32). The latter group, Forastero-Trinitario, have an Angoleta and/or Cundeamor fruit shape and a yellow fruit colour. The Forastero-Trinitario represents the rest of the varieties. The ligule shape features, young leaf colors and fruit shapes of Vietnamese *Theobroma cocoa* L. varieties were summarized in Table 2. This table shows the main similarities and differences between the features in the examined varieties. This result corresponds with previous research into the morphological and anatomical traits of cocoa trees.

Conclusion

Several cocoa morphological characteristics were investigated in this study such as ligule shape, anther number, pollen, stamen and ovule, pod, seed, leaf color and leaf anatomy. The examination of reproductive organs showed a diversity of morphological characteristics (five ligule shapes, two young leaf colours, the unique structure of midrib and secondary stem among 63 examined cocoa varieties). Significantly, three kinds of fruit shapes (Angoleta, Amelonado and Cundeamor) were identified in the Vietnamese cocoa varieties, with each a different colour. The Vietnamese cooca varieties can therefore be divided into two groups: Criollo-Trinitario and Forastero-Trinitario. The TD11 accession displayed an interesting results with a trilobed antherstructure. This study significantly contributes to the development and conservation of biodiversity among Vietnamese cocoa. This work provides an important contribution to morphological characterization of polymorphic patterns from several organs of *Theobroma* species in the Southern Vietnam, which can help breeders in the future for interspecific crossing attempts.

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