Morphological and molecular characterization of melon landraces (*Cucumis melo* L.) from central and southern Vietnam

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Chapter 1. General introduction

Origin and intraspecific classification of melon

Melon (*Cucumis melo* L.) is one of the most important and diverse vegetable crops of Cucurbitaceae family and is classified into two subspecies, *C. melo* ssp. *agrestis* and *C. melo* spp. *melo*. The former has short and appressed hair on the surface of the ovary and mostly distributed in Africa, Central America, and Asia from India to Far-East, while the latter has long and spreading ovary's hair and is commonly found in India, Central and Western Asia, Africa, Europe and America (Kirkbride 1993; Pitrat 2013). The origin of melon remains controversial with multiple studies reaching different conclusions. The long-standing hypothesis is that Africa is the origin of *C. melo* (Whitaker and Davis 1962; Kirkbride 1993). This hypothesis is encouraged by the richness of *Cucumis* species, which have the same chromosome number with *C. melo* (2n=24), in Africa (Ghebretinsae *et al.* 2007). However, the current studies pointed to an Asian origin of melon, since closely related wild species of *Cucumis* have been found in Asia/Australia (Renner *et al.* 2007; Schaefer *et al.* 2009; Sebastian *et al.* 2010; Endl *et al.* 2018). Sequence analysis of chloroplast genome revealed that cultivated melon consists of three maternal lineages, suggesting their independent origin (Tanaka *et al.* 2013; Endl *et al.* 2018). Recently, Zhao *et al.* (2019) presumed that three distinct lineages of melon were domesticated independently in India and Africa. The two different Indian lineage gave rise to spp. *agrestis* mainly distributed in East and South Asia and spp. *melo* typically grown in Europe and the United States, and African lineage gave rise to Sudanese *tibish* and *seinat* types (Serres-Giard and Dogimont 2012; Pitrat 2013; Zhao *et al.* 2019). Gonzalo *et al.* (2019) also suggested Indian origin of Occidental and Oriental cultivars.

Melon is known to be a highly variable species of *Cucumis* genus with distinct morphological types that led botanists to propose intraspecific classification schemes (Stepansky *et al.* 1999; Pitrat 2016). Naudin (1859) laid the foundation for such intraspecific grouping when try to group the melon into the complex taxa under the species. This French botanist's classification was based the observed variation characters of living melon plants grown in the

garden of the Muséum d'Histoire Naturell, Paris (France) and he proposed 10 tribes/groups of melon. The recent classification schemes were originally developed based on this classification. According to Munger and Robinson (1991), melon was divided into seven groups*;* groups Agrestis, Cantalupensis, Inodorus, Flexuosus, Conomon, Chito and Dudaim, and Momordica. On the contrary, Pitrat (2008) classified into two subspecies and 16 botanical varieties: ssp. *melo,* which includes vars. *cantalupensis*, *reticulatus*, *adana*, *chandalak*, *ameri*, *inodorus*, *chate*, *flexuosus*, *dudaim, chito* and *tibish* , and ssp. *agrestis*, which includes vars. *momordica*, *conomon*, *chinensis*, *makuwa* and *acidulus*. Among them, vars. *chito* and *tibish* were later reclassified as ssp. *agrestis* by Esteras *et al.* (2013).

The confusion which has existed in the term using for under species level classification. Pitrat (2008) used term *varietas* (*vars*.) as botanical variety while Munger and Robinson (1991) used term *Group* as horticultural group as botanical taxa under species. However, according to the International Code of Nomenclature for Cultivated Plants (ICNCP), the term "Group" should be used for taxon at or below the rank of species and "Group" comprise cultivars with similarity morphological traits by which that Group is defined (Brickell *et al.* 2009). Therefore, Pitrat (2016) was revised his intraspecific classification of melon and horticultural group and in some cases the sub-group as botanical taxa were used to classify the melon relies on morphological variation in some key characters. He assumed 19 intraspecific horticultural groups of melon, their morphological character and geographical distribution are summarized in Table 1.1. However, the current taxonomy scheme based on morphological characters still remain the mismatch with their genetic structure (Blanca *et al*. 2012; Esteras *et al*. 2013; Hu *et al*. 2015; Sanseverino *et al.* 2015; Jung *et al*. 2020), in addition, the assignment of a cultivar to a specific horticultural group did fit well to the key characters (Jung et al., 2020). Thus, combination of morphological characters and molecular markers as more efficient methods need to be considering for intraspecific classification, especially in studying melon domestication. The phenotypically difference could be the result of geographical clines during their expansion.

Table 1.1. A short description of main botanical group of *Cucumis melo* L. following Pitrat (2016) classification

Group	Distribution	Sex expression	Hairs on ovary	Fruit (shape/color)	Flesh color	Placenta (color/ number)	Sweetness
Agrestis	Africa, Asia (from Turkey to Japan) and Australia	M	Short or long	Round - oval – elliptic. Immature: light-green Light-green without or with dark-greens spots or stripes	Light - green	White 3	-
Kachri	India	M	Short or long	Round - oval – elliptic. Immature: light-green Yellow or cream without or or with dark-greens or orange spots or stripes	Light – green (somtimes slightly orange)	White 3	-
Chito	Central America and the Caribbean Islands	M	Short	Round – ovoid Immature: light-green Yellow	Light - green	Light-green 3	-
Tibish	Sudan	A	Short	Elliptical or pyriform Dark green with light green stripes or spots	Light green	Light-green 5	-
Acidulus	India and Sri Lanka	M	Short	Oval – Elliptic Immature: light-green Yelow - orange - ochre with stripes	White	White 3	-
Momordica	India and south-east Asia	M	Short	Flat – oval -elongated Uniform colour or with speckles, spots or stripes	light-green (sometimes white or slightly orange)	Orange or white 3	+ (Low)
Conomon	Far - East (China, Korea, Japan)	A	Short	Oval or elongated Immature: light-green Light-green or white (sometimes dark-green)	light-green or white	White or orange 3	-
Makuwa	Far - East (China, Korea, Japan)	A	Short	Round to oval	green (sometimes white or orange)	5	+ (Medium)
Chinensis	Far-East	A	Short	Pear – shape Light-green with dark-green spots	Green – orange	Orange 5	+ (Low)
Flexuosus	Morocco to India and the northern Mediterranean shore (Spain, Italy, Greece)	M	Long (usually) or short (sometimes)	Very long Immature: light-green or dark-green Cream (sometimes orange)	Light-green or slightly orange	Orange or light-green 3	-
Chate	Mediterranean basin and Western Asia	M	Long	Round to oval Cream	Light-orange (sometimes white or light-green)	Orange (sometimes white) 3	+ (very low)
Dudaim	Turkey to Afghanistan, and north to Turkmenistan	A	Long	Round to ovoid Immature: light-green with dark- green spots Yellow colour	White	White 5	-

				with orange/ochre spots or stripes			
Chandalak	Central Asia to India	A	Long	Round or flat Cream to orange/brown	green (sometimes orange or white)	Orange or white 3	+ (medium)
Indicus	Central India	A	Long	Elliptical Immature: light-green Grey/orange/brown/cream	Orange (rarely green)	Orange (rarely green) 3	+ (High)
Ameri	Turkey to western China	A	Long	Oval or cylindrical Yellow – light green	White, light-green or light-orange	White or orange 3	+ (High)
Cassaba	Western and central Asia	A	Long	Pear-shaped Yellow/ dark-green	Light-green	White 5	+ (High)
Ibericus	Mediterranean area (Spain), North and South America	A	Long	Elliptical or acorn (sometimes round)	Light-green (sometimes light-orange) juicy	White or orange 3	+ (High)
Inodorus	Central Asia, Meditterranean area, North and South America	A	sLong	Round White – yellow – dark green	light-green	White 3	+ (High)
Cantalupensis	European, Western Asia, North and South America	A usually	Long	Flat –round – oval. White - light-green - dark-green	Orange (sometimes green)	Orange (sometimes white) 3	+ (High)

Eastern Asian melon

In this thesis, eastern Asia is flexibly used to refer equal geographical area of the Far East which include China, North and South Korea, and Japan and the countries in mainland of Southeast Asia consist of Myanmar (Burma), Peninsular Malaysia, Thailand, Laos, Cambodia, and Vietnam.

Due to the great commercial importance, numerous comprehensive studies have been done on melon germplasm from India, West Asia and Spain (López-Sesé *et al.* 2003; Paris 2012; Zhang *et al.* 2016; Pavan *et al.* 2017). On the contrary, the melon from eastern Asia, especially Southeast Asia, has been little accessed and remains underexploited for evolutionary study and practical breeding, although they have been recognized as an important sources for pest and disease resistance (Wolukau, et al. 2007; Pitrat 2013).

Taxonomic and distribution

Traditional melon cultivars in eastern Asia belonged in five groups as Table 1.1, Momordica, Acidulus, Conomon, Makuwa, and Chinensis. All of them belong to *agrestis* subspecies with short hair on the surface of the ovary. The sex expression was correlated with geographical distribution of these groups; Group Momordica and Acidulus are monoecious type (male flower and female flower in the same plant) which mainly distributed from India to Southeast Asia; Group Conomon, Makuwa and Chinesis are andromonoecious type (both bisexual flowers and male flowers in the same plant) commonly found in China, Korean and Japan (Pitrat 2016)

A possible key for the determination of these Groups is suggested by Pitrat (2016) as following:

1. Monoecious, flat to elongate fruit shape, very thinned skin fruit, fruit bursting at maturity, mealy flesh.	**(1) Momordica**
Other	
2. Monoecious, elliptical, ovate or elongate fruit shape, brightly colored (yellow, orange, brown) fruit skin, very firm white acidic flesh.	**(2) Acidulus**
Other	
3. Pyriform fruit shape, fruit has light-green skin color with dark-green spots, bumps, non-dehiscent peduncle, small round (section) seeds.	**(3) Chinensis**
Other	
4. Non-sweet flesh at maturity	**(4) Conomon**
Sweet flesh at maturity	**Makuwa**

As mentioned above, the intraspecific classification based only on the morphological characters was not fit well to genotype data. The admixture of horticultural groups occurred in some studies as the gene flow among different intraspecific taxa when the crossing is not impeded (Blanca *et al.* 2012; Esteras *et al.* 2013; Hu *et al.* 2015; Sanseverino *et al.* 2015). Therefore, with the

combination of morphological and molecular-based classifications, the eastern Asian melon cultivars could belong to three gene pools, sometimes each gene pool comprise some different botanical varieties inside but its genotype is homologous.

- Momordica gene pool (*Cucumis melo* ssp. *agrestis* var. *momordica* – Phut or snap melon). Grown in India and other Asian countries and distinct from any other Oriental melon groups (Wang *et al.* 2018). The smooth surface of the fruit cracks as maturity approaches and the fruit disintegrates when barely ripe. Most melons in this group are monoecious. When using the seed length as a typical character for classifying melon subspecies, subspecies *melo* has the seed length longer than 9.0mm and subspecies *agrestis* has shorter seed length (< 9.0mm), the seeds of some *Momordica* are the exception - belonged *agrestis* subspecies but its seed length is longer than 9.0 mm
- Conomon gene pool (*Cucumis melo* ssp. *agrestis* var. *conomon* – pickling melon, *Cucumis melo* ssp. *agrestis* var. *makuwa* – sweet melon, makuwa melon, and *Cucumis melo* ssp. *agrestis* var. *chinesis*). The members of this gene pool have thinned fruit skin and andromonoecious type. The morphological variation of melons in this group were quite high but the genetic background of var. *makuwa*, var. *conomon*, and var. *chinensis* were nearly homologous (Akashi *et al.* 2001; Tanaka *et al.* 2007; Blanca *et al.* 2012; Wang *et al.* 2018). These results leading to the conclusion that they were descendants of a common ancestral lineage. Therefore, they were put together in one group.
- Acidulus gene pool (*C. melo* ssp. *agrestis* var. *acidulus* and *C. melo* ssp. *agrestis* var. *agrestis*) is native India, currently grown as vegetables. Its vines are monoecious, mostly non-climacteric, no aroma, no sugar, low pH (acidic fruits) (Blanca *et al.* 2012).

After ca. 1900, various types of melon cultivars of ssp. *melo* have been introduced to eastern Asian countries from Europe and USA (Hu, 2005, Dung *et al.* 2016) and the role of traditional melon cultivars was declining (Pitrat 2008).

Genetic diversity and the origin of Far Eastern melon

The highest genetic diversity was observed in the germplasm collected from India and supports the earlier that this is the primary center of diversity of Oriental melon. Least genetic diversity found in the Far – East (Akashi *et al.* 2001; Tanaka *et al.* 2007; Blanca *et al.* 2012; Esteras *et al.* 2013; Gonzalo *et al.* 2019). The decrease genetic variation from south to east Asia, from India, Myanmar to Vietnam to Far East, supported the hypothesis that the melon distribution in eastern Asia is the result of the eastward expansion (Yi *et al.* 2009; Nhi *et al.* 2010). The general picture is that the central of India is the origin of these taxa, from there, the eastward transmission happened, which was driven by wet condition, has established the small seed in eastern India (seed length is less than 9.0 mm), and subsequent diffusion to Far-East (Akashi *et al.* 2001; Katsunori Tanaka *et al.* 2007; Yi *et al.* 2009). During this expansion, they have still been selected mainly due to the geographical differences and be diverted into different landraces with different morphology (Nakata *et al.* 2005; Tanaka *et al.* 2015). Therefore, the lowest genetic variation of Far East melon as the result of founder effect. However, the recent carbonized melon seeds discovered at Late Neolithic sites in the Lower Yangtze Valley of eastern China and dated to 6500 year ago (Zhang *et al.* 2016) that time before of any cultural contacts between China and India (Fuller 2011). Therefore, the earliest activities of eastern Asian melon domestication was concentrated in Lower Yangtze Valley of eastern China and China is probably one of several melon domestication centers.

Chinese melon was divided into two groups based on the thickness of the fruit skin. The thick-skinned melon is mainly cultivated in Xinjiang (north-west China) and thin-skinned melon in eastern China. Production areas in Qinghai, Gansu, and Shaanxi provinces have both thick – skin and thin – skin varieties of melon (Aierken et al., 2011). In Chinese, the 瓜 (*kua*/*gua*) is the general term for melon and cucumber. The Chinese melon landraces divide into four types: "*Tian – gua*" (sweet melon), which sweet and crisp flesh, "*Mian – gua*" (Mealy melon), which large melon with yellow skin, soft and less sweet flesh, and "*Yue – gua*" or "*Cai – gua/Bai - gua"* (Melon from

South China), which was the earliest melon being traced back to the fifth century, and *"Hami - gua*" (Hami melon) (Hu 2005). The thinned – skin melon is mostly produced in the eastern and central provinces in China which fit with the region of the oldest archeological excavation in China (Zhang *et al.* 2016). The "*Yue – gua*" in Chinese together with "*shiro – uri*" in Japanese mentioned to the Baiyue land or simply Yue land (百越/百粵, referred as 越, 粵, 鉞) which was the ancient area elongated from Zhejiang in Southeast China to Jiaozhi, in Northern Vietnam along shelf coast. This give hypothesis that Conomon domestication took place in a region between Northern Vietnam and Southeast China.

Vietnamese melons

Only limited melon remains have been identified in Southeast Asian. In Vietnam, 50 melon seeds had been excavated in Viet Bac and Dong Xa sites of Red River Delta region. The archeological discoveries suggest that the melon has been cultivated in Vietnam at least from 2 CE. These seed remains belong to small seed size with seed length range from 6 – 8mm and seed width 3 – 5 mm (Nguyen 2010).

The morphological and molecular characterization of Vietnamese melon landraces have been reported firstly by Nhi *et al.* (2010), for five cultivar groups, "Dua le", "Dua vang", "Dua bo", "Dua gang" and "Dua thom", collected from the northern part of Vietnam. "Dua le" has globular fruits with crispy flesh and white epicarp, while "Dua vang" has yellow epicarp. "Dua bo" has powdery flesh and less sweet flesh, and "Dua thom" has oblong fruit with aroma and diverse flesh color from white to yellow and orange. For these four groups, mature fruits are consumed as dessert. "Dua gang" has elongate fruit with vertical stripe and immature fruits are generally used as vegetable. Their materials of "Dua le", "Dua vang", "Dua bo", and "Dua gang" were mainly collected in the midland and lowland of Northwest, Red River Delta and Northern Central Coast, while "Dua thom" was collected in the highland of the Northeast. The results from molecular marker analysis identified two distinct groups of the Northern Vietnamese melon population, the lowland, and the highland melon group. The lowland melons were much closer to

group Conomon vars. *conomon* and *makuwa* in Far-East than Vietnamese highland melons. By comparing with reference accessions of var. *makuwa* and var. *conomon*, “Dua le” and “Dua vang” were suggested as *makuwa* variety, “Dua gang” belonged var. *conomon*, “Dua bo” with the softer and mealy flesh, sometimes having peeling skin at mature stage which is the typical character of *momordica* variety, therefore, Nhi et al (2010) considered “Dua bo” belonging to the Momordica group, although their sex type was andromonoecious while Momordica group was monoecious. However, with the limitation of materials studied and area coverage, the diversity of Vietnamese melon is still unclear and the origin of Vietnamese melons and Conomon group were still unsolved.

Chapter 2. Melon resource exploration and collecting in central and southern Vietnam

Introduction

Vietnam is located on the eastern margin of Southeast Asian mainland and lied in the last chains of two big mountain systems, the Chinese and end the India-Myanmar. Plant genetic resources in Vietnam are greatly influenced by South Asian and Chinese plants, therefore, this country provided an interesting scenario for studying the domestication and diversification of Oriental melon.

Vietnam territory stretches over 15 degrees of latitude from 8º27' to 23º23' North, the wide range of latitudes make Vietnam´s climatic diversified and complicated. In general, the natural climate boundary, Hai Van pass at 16th North parallel, and essentially separates the monsoonal climate in the north from the tropical monsoon in the south. The north Hai Van pass comprising northwest, northeast, Red River Delta, and Northern central coast had two annual distinct seasons – the wet, cold winter and humid, hot summer, while south Hai Van pass had two seasons - rainy and dry, weather is humid and hot all year round (Trinh 1996). In addition, the existence of the mountainous areas also lead to climatic conditions varying significantly between regions.

Based on its climate and biophysical environment, the Vietnamese government groups the provinces into eight regions along three parts of country. The north consists four regions namely Northwest, Northeast, the Red River Delta; the central part includes 3 regions, namely North-Central Coast South-Central Coast and Central Highlands (Tay Nguyen, in this study, is denoted as Southern highlands to distinguish with the highland from the north, in the northeast); the south is divided into Southeast and Mekong River Delta.

In 2008, during a collecting mission in Northern part and northern central coast region of Vietnam, five types of melon (*Cucumis melo)* were observed and collected, "Dua le" is small sweet melon with white skin, "Dua vang" is melon with yellow smooth skin fruit, "Dua gang" is non-sweet melon, pale skinned fruit, "Dua bo" has mealy fruit flesh and the dominant fruit skin color is orange with green striped; the fifth type is "Dua thom" with thin and smooth skin, little aroma,

and a variable flesh color including orange. The four former types - “Dua le”, “Dua vang”, “Dua gang”, “Dua bo”- have genetically related with Group Makuwa and Conomon in East Asia, while the latter – “Dua thom”- has genetical similarity to those in mountainous area of South Asia (Nhi *et al.* 2010). As the results, differentiation within *Cucumis melo* accessions from Vietnam, the relationship among two group types and the transmission of group Conomon are still not clear. Therefore, the purpose of the present study was to expand the collection into different parts of Vietnam to clarify the variability of Vietnamese melon germplasm.

Method of field study and character investigation

Study area

The Central Vietnam is located between latitudes $19^0$48’ and $11^0$30’ North, and longitudes $105^0$46’ and $108^0$16 East. Their provinces are clustered together geographically into three regions: 1) Northern Central Coast with Thanh Hoa, Nghe An, Ha Tinh, Quang Binh, Quang Tri and Thua Thien Hue province, 2) Southern Central Coast comprising Da Nang city, Quang Nam, Quang Ngai, Binh Dinh, Phu Yen, Khanh Hoa, Ninh Thuan and Binh Thuan province and Central Highlands (Tay Nguyen) including Kon Tum, Gia Lai, Dak Lak, Dak Nong, Lam Dong provinces. Because of differences in latitudes and the marked variety of topographical relief, the climate tends to vary considerably from place to place. Central Vietnam experiences a tropical monsoon in the north, and tropical savanna climate in the south and highlands area. The coastal strip of Southern Central Coast is usually dry and hotter than the rest in the summer season (from April to October), however, it is wetter and colder in winter-monsoon season (from November to March). In this time, there is much rain but distributed unevenly, heavy rainfall usually occurs in October - November. The temperature of the south coastal cities is hot all year round, around 22^oc to 31^oc. Its rainy season lasts from September to December, the dry season lasts for 3 - 6 months with the rain per month fall less than 100mm. Annual temperature of the Central Highlands is from 21^0C to 23^0C. This zone, in particular, enjoys nearly double the average rainfall of the country. Almost of the

precipitation occurs during the summer time, from April to October. In many places of the Central Highlands, it rains continuously for six moths with more than 200mm/month

The Southern Vietnam comprises two regions– Southeast and Mekong River delta. The southern area also belongs to tropical savanna climate, predominantly with high humidity and a distinct wet and dry season. The wet season is from April to October and dry season is from November to March of next year. However, all months, the temperature is above 25^{o}c. (Takagi, Esteban, and Thao 2014)

Site and sampling

Germplasm exploration and collecting activities were undertaken in 2014 and 2015 from twelve and two provinces in central and southern part of Vietnam, respectively. The regions covered by the exploration missions include the Northern Central Coast (from Thanh Hoa to Thua Thien Hue province); Southern Central Coast region in Quang Nam, Quang Ngai, Binh Dinh, Phu Yen, Khanh Hoa, and Ninh Thuan province; Central Highlands (Tay Nguyen), parts of Dak Lak and Gia Lai province; and Southern area (parts of Ho Chi Minh city and Long An province. The geographical distribution of germplasm exploration is described in Fig.2.1.

The preliminary information about cultivation place was taken from local market or roadside vegetable stands. From this information, we try to find the melon field and directly interview the melon farmer. Thus, samples were collected from local markets, roadside vegetable stands, farmers' houses, and cropping fields. Detailed passport data on place of collection, sample type, local crop names and related information of cultivation methods (e.g., cultivation place, sowing and harvest times, fertilizer application, and fruit usage), etc., for collected samples were recorded.

Seeds from each fruit or one seed-storage bag (in farmers' houses) were registered as one sample. Mixed stored seeds as the interview information were separated based on the different seed character and registered independently as corresponding to the individual seed samples

Fruit and seed investigation

For fruit samples, the fruit traits including fruit weight, fruit length, fruit diameter, flesh thickness, outer rind, stripe on rind, and spot on rind, flesh color, and Brix were evaluated.

Seed length and seed width were measured on 10 seeds of each collected samples. The seed samples were classified as large (≥9.0 mm length) or small seeds (<9.0 mm length), according to Akashi *et al.* (2002). Seed weight were measured 10 seeds with 10 replication for each sample. The seeds were selected randomly.

Results

Collected samples

A total of 98 melon samples were collected from fourteen provinces in five regions (Northern Central Coast, Southern Central Coast, Central Highlands (Tay Nguyen), Southeast, and Mekong River delta) of Vietnam during a collection trip undertaken in 2014 and 2015. Of the 97 samples, 61 and 37 were collected as seeds and fruits, respectively. These melon accessions comprised landrace cultivars, improved varieties, and netted melon. Detailed passport data was shown in table 2.1 and the summary of collected samples was shown in Table 2.2.

In Vietnam, Vietnamese (King people) use term “Dua” as prefix for watermelon (Dua hau), cucumber (Dua leo/Dua chuot) and melon. In addition, in northern Vietnam, many local names of melon were used such as “Dua le”, Dua vang”, “Dua gang”, “Dua bo”, “Dua nut”, “Dua”, while in southern Vietnam, “Dua gang” is the only one local name was used. In Tay nguyen region (Central highland), Jarai and H’mong call “Dua” is “Mon” and “tok” means yellow. The local name deeply entrenched in the language of a region and descriptive the typical character to distinguish with other crops, example “Dua nut” was used in Nghe An and Ha Tinh provinces to indicate the melon that were broken when its ripen, “Dua bo” that mean mealy melon were used in Thanh Hoa, Quang Binh, Quang Tri. Many local names easily make misleading, therefore, by comparing with melon types have been published in Nhi *et al.* (2010) and the local name and related information about usage and fruit traits in this field study, were categorized collected

melons from central and northern Vietnam into the following five groups, “Dua le”, “Dua vang”, “Dua bo” (including “Dua bo”, “Dua nut” and “Dua”), “Dua gang”, and “Montok”. Two samples VNC3 and VNC23 belong subspecies *melo* (explained below). The former three groups are used for dessert, whereas “Montok” used both, mature fruits used as desert and immature fruits used as vegetable. Interestingly, the usage of “Dua gang” is different in north and south Vietnam. Southern “Dua gang” is mainly grown to harvest mature fruits as dessert, while immature fruits of northern “Dua gang” are often used as vegetable like cucumber. The vegetable and dessert melon could be harvested the first fruit after sowing 30 - 35 and 60 - 75 days, respectively. The harvested period (the first harvested time to the last harvested time) was about 30 to 45 days.

“Montok” is Tay Nguyen’s melon and was cultivated by ethnic minorities (H’mong and Jarai People). Only one fruit sample of “Dua le” and one “Dua gang” sample were collected in this region. However, the fruit sample “Dua le” here was brought from Ninh Binh province of Red Delta River region. “Dua gang” which collected in Dak lak province also was brought here in 1985 when the farmer move from Tam ky, Quang Nam province of southern central coast region to Dak Lak province following the “New Economic Program” of the Vietnamese government. They used this fruit of “Dua gang” in immature stage for pickled purposes.

Table 2.2. The geographical distribution and the samples in five melon groups

Melon type	VNC	VSC	VSH (Tay Nguyen)	VSE	VSD	Number samples
Dua le	16	1	1	-	-	18
Dua vang	1	-	-	-	-	1
Dua bo	16	-	-	-	-	17
Dua gang	11	21	1	3	5	41
Montok	-	-	20	-	-	20
Ssp. *melo*	2	-	-	-	-	2

Cultivation methods

The growing season and the growing period varies from different region and different melon cultivar groups. In northern central coast and southern central coast, the growing season of melon from March to July. However, northern “Dua gang” as the vegetable usage was usually sown from March to May and harvested from May to July, because of the short growing period - first

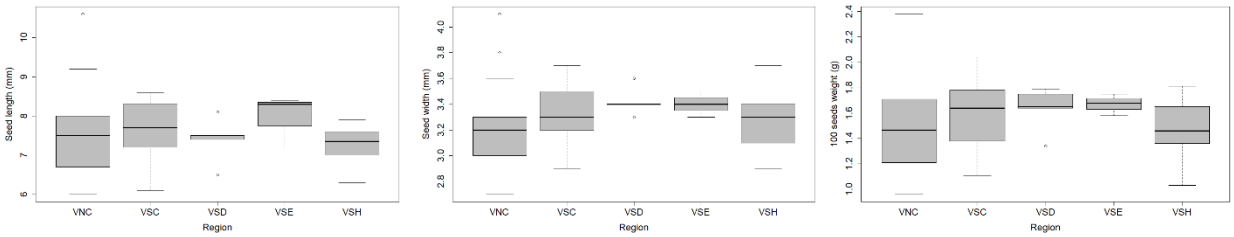
harvested, Northern “Dua gang” could be grown two to three times in growing seasons. Southern “Dua gang”, "Dua bo" and "Dua le" are sown in March - April and harvested in June - July. In fact, the local farmers usually based on the lunar calendar, they are sown in February after Tet holiday. "Montok" had two growing seasons related to elevation in Tay Nguyen region (VSH), the sites which elevation higher than 200m such as the collected sites Duc Co district and K’bang district of Gia Lai province (356m to 532m above sea level), “Montok” was sown in June and harvested in September, while the remaining sample sites with elevation lower than 200m, “Montok” was sown in April and harvested in June or July. The “Dua gang” in Tay Nguyen region was sown in December and harvested in February. In the southeast region, melon was cultivated from December to March in rice field after harvesting, making the bed doesn’t necessary for landrace varieties, while southern delta region, the growing season is from March to May and making the bed is needed. As the evaluation of the farmer, if the landrace melon in southeast region is grown later, the much rainy in April will make fruit having lower quality, the flesh is less soft and sticky. “Montok” was cultivated by mixed cropping with maize and/or upland rice, another melon groups are cultivated as monoculture. In southern east and Mekong Delta, the chemical fertilizers are applied during the cultivation.

Seed and fruit characters

Collected Vietnamese melon accessions were almost classified as small-seed type (seed length is less than 9mm), except two subspecies *melo* accessions, VNC3 – Dua le Kim co nuong and VNC23 – “Dua luoi” (netted melon).

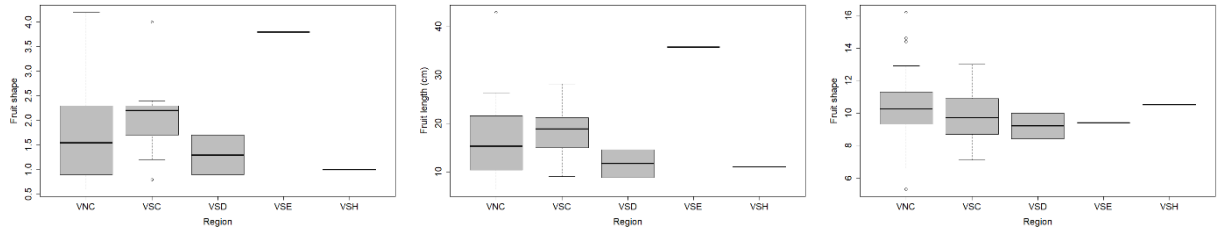
In seed size, seed length showed wide variation ranging from 6.0 to 10.6 mm. Out of 98 samples, the seed characters - seed length, seed width and 100 – seeds weight of 44 samples (excluded 2 samples of the subspecies *melo*) from Central coast (Northern and southern) had wide range than remaining samples from other regions. Especially in Ngu My village, Ninh Xuan commune, Ninh Hoa district, Khanh Hoa province, three seed samples (VSC70, VSC 71, and VSC72) with different seed length were observed from a farmer. As the farmer information, the traditional

cultivar had green color epicarp, soft flesh, fruit over ripping could be cracking makes it difficult to harvest and transport therefore the local farmer brought the seed with yellow epicarp from the south which could grow whole year and no broken. These two types were grown together and the next seasons variate fruit shape and fruit color skin were observed. Farmers selected and keep seed separately.



(A) (B) (C)

Fig 2.2. Variation of melon seed traits in five collected regions. (A) Seed length, (B) Seed width, (C) 100 – seeds weight



(A) (B) (C)

Fig 2.3. Variation of melon fruit traits in five collected regions. (A) Fruit shape, (B) Fruit length, (C) Fruit diameter

In fruit traits, variation was observed in fruit and seed characters. Fruit shape varied from round to elongated shape. Fruit length range from 7.3 to 43.0 cm and the wide range occurred in Northern Central Coast. However, no statically difference detected in seed and fruit characters detected of 5 collected region (Fig 2. 2 and Fig 2.3).

Discussion

Vietnamese use the term “Dua” as prefix to call water melon (Dua hau), cucumber (Dua leo/Dua chuot) and melon. The sound of “Dua” is similar to Chinese “Kua/gua”. The historical evidence also showed that Viet dwellers in the past had lived in the southern part of the Yangtze River Delta, then due to war conditions, they moved southward to establish the Red River Delta

civilization (Trinh 1996). Therefore, the lowland Vietnamese melons which cultivated by King people seem to be related to Chinese melon.

In central and southern Vietnam, there are five melon cultivar groups, “Dua le”, “Dua vang”, “Dua bo, “Dua gang”, and “Montok”. The melon had the local name “Dua thom” which collected in northern highland as in Nhi *et al.* (2010) couldn’t be observed in this area. The growing season of highland melon - “Montok” in this study and “Dua thom” in the study of Nhi *et al.* (2010) start at the beginning of the rainy season (sown from April), and intercrop with upland rice, corn or cassava. Growing season of in lowland from Red Delta River, Central Coast and southern fall in summer/dry season. Interestingly, “Dua gang” sample cultivated by King people in Tay Nguyen region have a season like the lowland Southeast and Mekong River Delta (from December to March).

Improved variety is more popular in Southern part (Southern Central Coast, Southeast and Mekong River delta) than the Northern Central Coast. The farmers buy seed in market and they could be cultivated whole year. The mainly improved varieties is the F1 hybrids imported from Thailand and Taiwan seed companies, because the convenience in the whole year production in southern part, these improved varieties is widely used and step by step replace the local varieties, which have caused genetic erosion. Furthermore, melon of subspecies *melo* (seed length is longer than 9.0 mm) also occurred in the northern central coast. The farmers tend to cultivate these melons mixed with landraces cultivars, example, in Quang Binh province, VNC22 - Dua bo and VNC23 – Dua luoi (netted melon) were grown in same place, VNC3 – Dua le Kim co nuong, VNC4 – Dua gang thai xanh, and VNC5 – Dua gang thai trang were grown in same field in Nghe An province. In southern, melons of subspecies *melo* also were cultivated but separated with landraces in greenhouse or in open field. Based on information of seed origin and by analyzing seed traits, we classified 8 samples as improved varieties and excluding for further experiment.

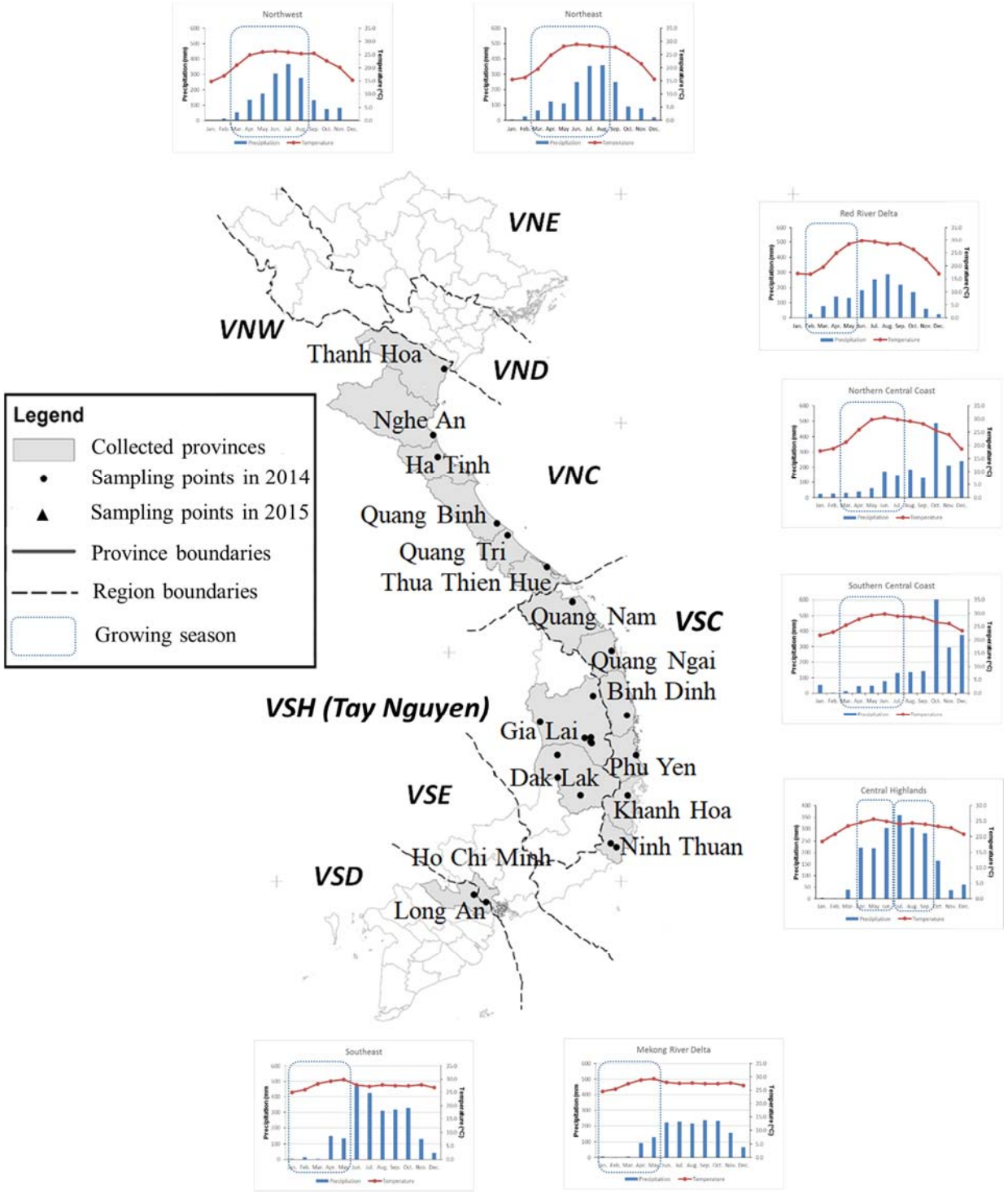


Fig. 2.1. Sampling sites of central and southern melons and the monthly rainfall and temperature of eight regions in Vietnam (compiled from IMHEN 2014).

Table 2.1. Detailed passport data of collected melon samples and their seed characters

Accession code[1]	Local name	Collected site				Sample type[2]	Source[3]	Fruit usage[4]	Seed length (mm)	Seed width (mm)	100 seed weight (g)
		Village	Cummune	District	Province						
VNC1	Dua bo	7	Lien Loc	Hau Loc	Thanh Hoa	S	FH	F	7.6	3.0	1.44
VNC2	Dua bo	7	Lien Loc	Hau Loc	Thanh Hoa	S	FH	F	8.0	3.1	1.50
VNC3*	Dua le Kim co nuong	Long Tan	Nghi Khanh	Cua Lo	Nghe An	F	F	F	10.6	4.1	2.38
VNC4	Dua gang thai xanh	Long Tan	Nghi Khanh	Cua Lo	Nghe An	F	F	F	7.5	3.2	1.63
VNC5	Dua gang thai trang	Long Tan	Nghi Khanh	Cua Lo	Nghe An	F	F	F	7.8	3.1	1.69
VNC6	Dua le		Nghi Huong	Cua Lo	Nghe An	S	FH	F	6.5	2.9	1.04
VNC7	Dua nut	10	Nghi Huong	Cua Lo	Nghe An	F	R	F	7.7	3.4	1.67
VNC8	Dua nut	Long Tan	Nghi Khanh	Cua Lo	Nghe An	F	R	F	7.7	3.3	1.74
VNC9	Dua nut	Long Tan	Nghi Khanh	Cua Lo	Nghe An	S	FH	F	8.0	3.2	1.86
VNC10	Dua nut		Nghi Huong	Cua Lo	Nghe An	S	FH	F	8.4	3.5	1.97
VNC11	Dua nut		Nghi Huong	Cua Lo	Nghe An	S	FH	F	8.1	3.5	2.03
VNC12	Dua nut	3	Nghi Huong	Cua Lo	Nghe An	F	R	F	8.0	3.5	1.86
VNC13	Dua gang	Dong Hai	Thach Hai	Thach Ha	Ha Tinh	S	FH	Veg	7.5	3.3	1.71
VNC14	Dua bo	Thọ	Thach Lien	Thach Ha	Ha Tinh	S	FH	F	7.1	3.1	1.39
VNC15	Dua le		Thach Hai	Thach Ha	Ha Tinh	S	FH	F	6.6	2.9	1.21
VNC16	Dua le		Thach Khe	Thach Ha	Ha Tinh	F	R	F	6.9	2.7	1.05
VNC17	Dua le	Thọ	Thach Lien	Thach Ha	Ha Tinh	S	FH	F	6.1	3.0	1.12
VNC18	Dua le	Thọ	Thach Lien	Thach Ha	Ha Tinh	S	FH	F	6.6	3.0	1.11
VNC19	Dua le	Thọ	Thach Lien	Thach Ha	Ha Tinh	S	FH	F	6.2	2.9	0.96
VNC20	Dua le		Thach Lien	Thach Ha	Ha Tinh	F	R	F	6.7	2.8	1.12
VNC21	Dua le		Thach Lien	Thach Ha	Ha Tinh	F	R	F	6.8	3.0	1.15
VNC22	Dua bo	1	Cam Thuy	Cam Lo	Quang Binh	F	F	F	8.3	3.6	2.03
VNC23*	Dua luoi (Netted melon)	1	Cam Thuy	Cam Lo	Quang Binh	F	F	F	9.2	3.8	2.17
VNC24	Dua gang			Cua Viet	Quang Tri	F	LM	Veg	7.7	3.4	1.32
VNC25	Dua gang		Gio Hai	Gio Linh	Quang Tri	S	FH	Veg	7.1	3.4	1.71
VNC27	Dua vang		Vinh Tu	Vinh Linh	Quang Tri	F	F	F	7.7	2.8	1.38
VNC28	Dua le		Vinh Tu	Vinh Linh	Quang Tri	F	F	F	6.6	3.0	1.24
VNC29	Dua le		Vinh Tu	Vinh Linh	Quang Tri	S	FH	F	6.4	2.9	1.03
VNC30	Dua le		Vinh Tu	Vinh Linh	Quang Tri	S	FH	F	6.0	2.8	1.02
VNC31	Dua le		Vinh Tu	Vinh Linh	Quang Tri	S	FH	F	6.3	3.0	1.10
VNC32	Dua bo		Vinh Tu	Vinh Linh	Quang Tri	F	F	F	7.4	3.3	1.43
VNC33	Dua bo		Vinh Tu	Vinh Linh	Quang Tri	S	FH	F	7.6	3.2	1.53
VNC34	Dua bo		Vinh Tu	Vinh Linh	Quang Tri	S	FH	F	7.7	3.3	1.49
VNC35	Dua gang ray	Dien Dai	Phu Xuan	Phu Vang	Thua Thien Hue	F	LM	Veg	8.1	3.3	1.25
VNC36	Dua gang	Dien Dai	Phu Xuan	Phu Vang	Thua Thien Hue	S	FH	Veg	7.8	3.3	1.65
VNC37	Dua gang soc		Vinh Thanh	Phu Vang	Thua Thien Hue	F	LM	Veg	7.5	3.4	1.50
VNC38	Dua gang		Vinh Thanh	Phu Vang	Thua Thien Hue	F	F	Veg	6.8	3.2	1.57
VNC39	Dua gang	Quang Xuyen	Phu Xuan	Phu Vang	Thua Thien Hue	F	F	Veg	8.1	3.3	1.70
VNC40	Dua		Vinh Hien	Phu Vang	Thua Thien Hue	S	FH	F	7.2	3.1	1.42
VNC41	Dua		Vinh Hien	Phu Vang	Thua Thien Hue	S	FH	F	8.0	3.3	1.83
VNC42	Dua le		Vinh Thanh	Phu Vang	Thua Thien Hue	F	LM	F	6.7	2.9	1.26

VNC43	Dua le		Vinh Thanh	Phu Vang	Thua Thien Hue	S	LM	F	6.2	2.9	1.04
VNC44	Dua le		Vinh Thanh	Phu Vang	Thua Thien Hue	S	LM	F	6.9	3.3	1.36
VNC45	Dua le		Vinh Xuan	Phu Vang	Thua Thien Hue	F	F	F	6.3	2.9	1.23
VNC46	Dua		Huong Long	Kim Long	Thua Thien Hue	F	LM	F	8.8	3.5	1.80
VSC47	Dua gang		Dien Hong	Dien Ban	Quang Nam	F	F	Veg	7.9	3.3	1.22
VSC48	Dua gang		Dien Hong	Dien Ban	Quang Nam	S	FH	Veg	7.2	3.2	1.35
VSC49	Dua gang		Nghia My	Tu Nghia	Quang Ngai	F	F	F, Veg	8.6	3.3	1.80
VSC50	Dua gang		Nghia My	Tu Nghia	Quang Ngai	S	FH	F	8.6	3.4	2.04
VSC51	Dua gang		Nhon Hung	An Nhon	Binh Dinh	F	LM	F	8.3	3.2	1.30
VSC52	Dua gang		Nhon Hung	An Nhon	Binh Dinh	F	LM	F	7.3	3.2	1.40
VSC53	Dua gang		Nhon Hung	An Nhon	Binh Dinh	F	LM	F	6.6	2.9	1.36
VSC54	Dua gang		An My	Tuy Hoa	Phu Yen	F	R	F	8.3	3.5	2.03
VSC55	Dua gang		An My	Tuy Hoa	Phu Yen	F	R	F	7.9	3.3	1.71
VSH56	Montok		So	Chuse	Gia Lai	S	FH	Veg	7.5	3.5	1.81
VSD57*	Dua gang	4	Luong Binh	Ben Luc	Long An	F	R	F	7.5	3.6	1.75
VSD58*	Dua gang	4	Luong Binh	Ben Luc	Long An	F	R	F	6.5	3.4	1.34
VSD59*	Dua gang	Chanh	Duc Lap Ha	Duc Hoa	Long An	S	FH	F	7.5	3.4	1.65
VSC60	Dua gang	Tan Xuan	Ninh Xuan	Ninh Hoa	Khanh Hoa	S	FH	F	7.5	3.4	1.66
VSD61	Dua gang	Chanh	Duc Lap Ha	Duc Hoa	Long An	S	FH	F	8.1	3.3	1.79
VSE62	Dua gang	4	Quy Duc	Binh Chanh	Ho Chi Minh	S	FH	F	8.3	3.5	1.75
VSE63	Dua gang	4	Quy Duc	Binh Chanh	Ho Chi Minh	S	FH	F	8.4	3.4	1.68
VSE64*	Dua gang	4	Quy Duc	Binh Chanh	Ho Chi Minh	F	R	F	7.2	3.3	1.58
VSC65	Dua gang	My Loi	Ninh Loc	Ninh Hoa	Khanh Hoa	S	FH	F	6.1	3.2	1.11
VSC66*	Dua gang	My Loi	Ninh Loc	Ninh Hoa	Khanh Hoa	F	R	F	7.7	3.3	1.68
VSC67	Dua gang	My Loi	Ninh Loc	Ninh Hoa	Khanh Hoa	S	FH	F	7.3	3.2	1.68
VSC68	Dua gang	My Loi	Ninh Loc	Ninh Hoa	Khanh Hoa	S	FH	F	7.5	3.6	1.38
VSC69*	Dua gang	Ngu My	Ninh Xuan	Ninh Hoa	Khanh Hoa	F	R	F	7.7	3.7	1.78
VSC70	Dua gang	Ngu My	Ninh Xuan	Ninh Hoa	Khanh Hoa	S	FH	F	7.0	3.2	1.86
VSC71	Dua gang	Ngu My	Ninh Xuan	Ninh Hoa	Khanh Hoa	S	FH	F	8.3	3.6	1.55
VSC72	Dua gang	Ngu My	Ninh Xuan	Ninh Hoa	Khanh Hoa	S	FH	F	6.3	3.5	2.02
VSH73	Dua gang	Thanh Phu	Hoa Son	K'rong bong	Dak Lak	S	FH	Veg	7.6	3.5	1.52
VSH74	Dua le			Buôn Đôn	Dak Lak	F	LM	F	7.1	3.2	1.77
VSH75	Montok	Buon Mong	Ea Krok	Ea sup	Dak Lak	S	FH	Veg, F	7.2	3.2	1.67
VSH76	Montok	Buon Mong	Ea Krok	Ea sup	Dak Lak	S	FH	Veg, F	7.0	3.6	1.24
VSH77	Montok	Buon Mong	Ea Krok	Ea sup	Dak Lak	S	FH	Veg, F	7.9	3.3	1.39
VSH78	Montok	Buon Pleiku	Ia k'dam	Ia pa	Gia Lai	S	FH	Veg	7.4	3.4	1.36
VSH79	Montok	K'dam 2	Ia K'dam	Ia pa	Gia Lai	S	FH	F	6.9	3.1	1.64
VSH80	Montok	Amada	Chu mo	Ia pa	Gia Lai	S	FH	F	7.6	3.5	1.42
VSH81	Montok	Amada	Chu mo	Ia pa	Gia Lai	S	FH	Veg	6.3	2.9	1.65
VSH82	Montok	Amanhan	Ia piar	Phu Thien	Gia Lai	S	FH	F	7.0	3.2	1.43
VSH83	Montok	Bon Bir	Chu Bah	Ayun pa	Gia Lai	S	FH	F	7.0	3.1	1.23

VSH84	Montok	Buon Hiao	Chu Bah	Ayun pa	Gia Lai	S	FH	F	6.3	2.9	1.75
VSH85	Montok	Buon Hoanh	Iarbol	Ayun pa	Gia Lai	S	FH	F	7.3	3.0	1.03
VSH86	Montok	Buon Hoanh	Iarbol	Ayun pa	Gia Lai	S	FH	F	7.3	3.0	1.42
VSH87	Montok	Buon Hiao	Chu Bah	Ayun pa	Gia Lai	S	FH	Veg, F	7.2	3.3	1.30
VSH88	Montok	Mook Den	Ia Tom	Duc Co	Gia Lai	S	FH	Veg, F	7.5	3.3	1.45
VSH89	Montok	Mook Den	Ia Tom	Duc Co	Gia Lai	S	FH	Veg, F	7.7	3.2	1.22
VSH90	Montok	Mook Den	Ia Tom	Duc Co	Gia Lai	S	FH	Veg, F	7.7	3.4	1.47
VSH91	Montok	Lang Kroi	Daksmar	K'bang	Gia Lai	S	FH	Veg	7.5	3.3	1.64
VSD94*	Dua gang				Tien Giang	S	LM	F	7.4	3.4	1.64
VSC95	Dua gang		Van Phuoc	Ninh Phuoc	Ninh Thuan	S	FH	Veg, F	8.4	3.7	1.64
VSC96	Dua le		Tu Tam	Ninh Phuoc	Ninh Thuan	S	FH	F	6.3	2.9	1.64
VSC97	Dua gang		Nha Ho	Nhon Son	Ninh Thuan	S	FH	Veg, F	7.9	3.4	1.64
VSC98	Dua gang	Dong Ne	My Son	Ninh Son	Ninh Thuan	S	FH	F	7.8	3.7	1.64
VNC99	Dua gang		Phu Mau	Phu Vang	Thua Thien Hue	S	FH	Veg	8.1	3.1	1.64
VSH100	Montok				Gia Lai	S	FH	Veg, F	7.9	3.4	1.63
VSH101	Montok		Lang Chuoi	Chuse	Gia Lai	S	FH	Veg, F	7.8	3.7	1.66

1 * considered as improved variety
2 F – Fruit, S – Seed
3 FH- Farmer's house, F-Field, R- Roadside vegetable stand, F - Cropping fields, LM - Local market
4 Veg – Vegetable, F - Fruit
.

Table 2.3. Fruit characters of melon from central and northern Vietnam

Accession code	Local name	Collected province	Fruit size			Fruit epicarp			Fruit flesh		
			Weight (kg)	Length (cm)	Width (cm)	Color	Stripe on rind[1]	Spot on rind[1]	Color	Thickness (cm)	Brix (°)
VNC3	Dua le Kim co nuong	Nghe An	0.5	11.5	9.6	G	-	-	LG	3.7	5.0
VNC4	Dua gang thai xanh	Nghe An	2.1	26.4	14.4	DG	LG	-	LG	3	2.0
VNC5	Dua gang thai trang	Nghe An	1.7	20.5	12.7	W	-	G	W	3	3.0
VNC7	Dua bo	Nghe An	1.1	22.0	10.9	Y	-	DG	LG	2.5	2.5
VNC8	Dua bo	Nghe An	0.8	16.7	10.9	Y	-	DG	W	2.4	5.0
VNC12	Dua bo	Nghe An	1.3	20.7	10.9	Y	-	DG	W	3.8	3.2
VNC16	Dua le	Ha Tinh	0.4	7.6	9.3	W	-	-	W	1.9	7.0
VNC20	Dua le	Ha Tinh	0.6	8.5	10.3	W	-	-	W	3	7.0
VNC21	Dua le	Ha Tinh	0.7	10.3	10.9	LG	-	-	LG	2.1	9.0
VNC22	Dua bo	Quang Binh	1.9	26.2	11.3	Y	-	DG	LG	3.8	3.7
VNC23	Dua luoi	Quang Binh	1.6	15.6	14.6	Br	Br	Br	O	3.1	10.2
VNC24	Dua gang	Quang Tri	0.3	21.6	5.3	LG	-	DG	W	1.5	3.0
VNC27	Dua vang	Quang Tri	1.4	12.9	16.2	O	-	-	W	3.1	5.2
VNC28	Dua le	Quang Tri	0.4	6.4	10.2	W	-	-	W	2	9.6
VNC32	Dua bo	Quang Tri	0.8	11.4	12.9	Y	-	DG	LG	2.9	3.2
VNC35	Dua gang ray	Thua Thien Hue	0.7	25	7.8	G	G	-	LG	1.8	3.0
VNC37	Dua gang soc	Thua Thien Hue	0.5	19.5	6.9	DG	G	-	G	1.8	5.0
VNC38	Dua gang	Thua Thien Hue	0.4	14.9	6.6	LG	G	-	LG	1.3	4.0
VNC39	Dua gang	Thua Thien Hue	2.9	43.0	10.2	G	G	-	LG	2.9	5.0
VNC42	Dua le	Thua Thien Hue	0.3	7.3	9.8	LY	-	-	W	1.9	8.0
VNC45	Dua le	Thua Thien Hue	0.5	10.4	9.3	W	-	-	W	2.0	8.0
VNC46	Dua	Thua Thien Hue	0.4	15.0	7.7	Y	-	DG	LG	2.0	2.4
VSC47	Dua gang	Quang Nam	0.7	28.2	7.1	LG	G	-	W	2.3	5.0
VSC49	Dua gang	Quang Ngai	0.5	14.8	8.7	Y	G	-	LG	2.2	5.0
VSC51	Dua gang	Binh Dinh	0.5	18.4	10.6	G	-	DG	LG	2	3.6
VSC52	Dua gang	Binh Dinh	0.8	18.9	8.4	Y	-	DG	LG	2.1	3.8
VSC53	Dua gang	Binh Dinh	1.0	20.8	9.5	Y	-	DG	LG	3.4	5.8

VSC54	Dua gang	Phu Yen	1.1	25.8	10.9	Y	-	DG	G	2.4	4.3
VSC55	Dua gang	Phu Yen	1.0	21.2	9.7	Y	-	DG	G	2.3	5.1
VSD57	Dua gang	Long An	0.9	14.6	8.4	O	-	-	LG	2.8	3.0
VSD58	Dua gang	Long An	0.6	8.8	10	O	-	-	LG	3.0	3.0
VSE64	Dua gang	Ho Chi Minh	1.4	35.8	9.4	Y	PG	-	LG	30	4.0
VSC66	Dua gang	Khanh Hoa	0.6	9.0	11.0	Y	-	-	LG	2.9	3.2
VSC69	Dua gang	Khanh Hoa	2.5	15.0	13.0	LY	-	-	W	5.0	3.0
VSH74	Dua le	Dak Lak	0.6	11.0	10.5	W	-	-	LG	2.5	7.0

1 G: Green, LG: Light green, DG: Dark green, Br: Brown

Chapter 3. Morphological characterization of melons (*Cucumis melo* L.) collected in Central and Southern Vietnam

Introduction

Melon (*Cucumis melo* L.) is one of the most important fruit vegetables of tropical, sub-tropical and warm temperate areas. This is the most variable species of the genus *Cucumis* in term of sex expression, fruit traits, and seed size. Cultivated melons predominantly belong to andromonoecious (bisexual flowers and male flowers in the same plant) or monoecious (male flower and female flower in the same plant) sex type (Boualem *et al.* 2008). More two different types of sex expression can be distinguished; gynoecious (bear only female flower) and hermaphrodite (bear only bisexual flowers). Regarding fruit morphology, the size varies from very small (less than 100 g) to very large (more than 4 kg, up to 10 kg), and fruit shape varies from slightly flat, ellipsoid, obovoid, round, and long to extremely long. Other fruit traits such as rind color, flesh content and color, sweetness, sourness, aromatic compounds also show highly polymorphic (Monforte *et al.* 2014).

Therefore, the method most frequently used to primary evaluate genetic diversity in melon is based on morphological characters. Later, when molecular markers were introduced as a convenient tool to assess the genetic diversity of a population, the molecular marker becomes more popular. However, due to the varies in phenotype of melon, until now, the molecular markers could not explain all case of genetic diversity and still remain different in genetic diversity estimated by two approaches (Stepansky *et al.* 1999, Jung *et al.* 2020). Therefore, the morphological characters are still widely used to supporting information in conjunction with molecular marker for the analysis of melon diversity (Jung *et al.* 2020). Variations in melon morphological features, distribution patterns, and adaptive and agronomic characters are well documented by International Plant Genetic Resources Institute, Rome, Italy (IPGRI 2003). Geographically distinct populations can differ in their levels of genetic diversity or in the spatial distribution of that diversity (Costa *et al.* 2016). The study of diversity among different varieties from different geographical regions elucidate the relationships among the cultivated melon for further conservation and breeding program.

In 2008, during a collecting mission in Northern part of Vietnam, five types of melon (*Cucumis melo)* were observed and collected, "Dua le", "Dua vang", "Dua bo", "Dua gang" and "Dua thom". The former four types - "Dua le", "Dua vang", "Dua gang", "Dua bo"- have genetically related with vars. *makuwa* and *conomon* in East Asia, while the latter – "Dua thom"- has genetical similarity to those in the mountainous area of South Asia. Beside of these five melon types, we occurred two more cultivar types in Central and Southern Vietnam. One is "Montok" cultivated mainly by Jarai people - a minor ethnic people living in Central Highland of Vietnam, "Mon" is "Dua" and "tok" is yellow, this name indicates the yellow skin melon with stripe usual used as vegetable at immature stage and in case of harvesting too late, the ripen fruit could be used as fruit – directly eaten. Another type is "Dua gang", same local name but different use purpose and fruit character which one had been recorded by Nhi et al., 2010. One kind of "Dua gang" dominate with the elongated shape and to be used as vegetable in immature stage for raw eaten or pickle as Nhi et al., 2010 reported; another has elliptical shape, less sweet, this ripen melon commonly served with sugar and blender, similar ways to consume "Dua bo". The former was collected mainly in Northern Central Coast, the latter commonly distributed in the south (Southern Central Coast, Southeast, and Mekong Delta River) (Fig 3.1). Therefore, the purpose of the present study was to expand the collection into Central and Southern Vietnam to clarify the variability of these cultivar types.

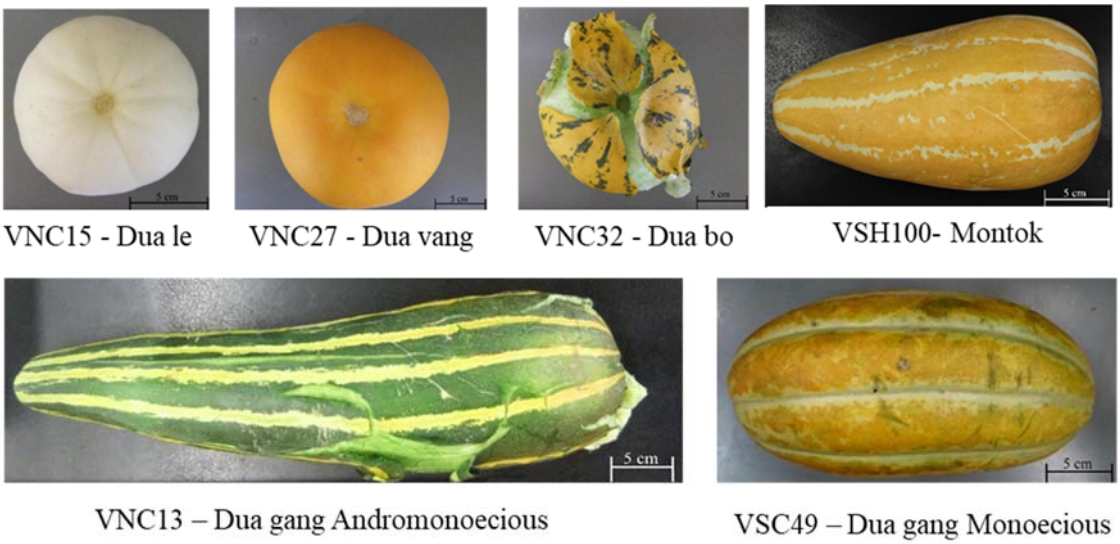


Fig 3.1. Photographs of typical fruit of six melon types from central and southern Vietnam

Materials and methods

Plant materials

A total of 73 melon landrace accessions were collected from a collection trip undertaken in 2014 and 2015 after removing the improved seeds and the seeds with low vigor

Morphological characterization

The original collections were grown in greenhouse of Okayama University for morphological characterization during the 2015 and 2016 cropping season (April to August). The morphological characterization of melon accessions was carried out using 16 selected descriptors for fruit, seed and inflorescence, mainly established by International Plant Genetic Resources Institute (IPGRI) and European Cooperative Programme for Plant Genetic Resources (ECPGR) (Table 3.1). Two plants for each accession were used for evaluating fruit characters. Length and width of seed were measured for ten seeds of each accession by digital Nogis scale.

Statistical analyses

Data of 16 phenotypic characters were subjected to ANOVA, principal components analysis (PCO) and clustering analysis using R software (R Development Core Team 2012). ANOVA were carried out to determine the relative variance among localities and among melon types for each trait. PCO was performed to identify accession groups and to determine the axes and the characters significantly contributing to the variation. In this procedure, the similarity matrix was used to generate eigenvalues and scores for the accessions. The first two principal components, which accounted for the highest variation, were then used to plot two-dimensional scatter plots. The Gower's algorithm was used to determine the distance matrix of quantitative and qualitative descriptors (Gower 1971). From the dissimilarity matrix, the clustering of accessions was performed by average linkage method of *hclust* function in R software (R Development Core Team 2012).

Table 3.1. Fruit, seed and inflorescence characteristic used for melon varieties characterization

Descriptor	Acronym	State/Unit
Fruit characteristics		
Fruit weight	FW	g
Fruit height	FH	cm

Fruit diameter	FD	cm
Fruit shape	FS	FS=FH/FD 1. Globular, 2. Flattened, 3. Oblate, 4. Elliptical, 5. Pyriform (pear – like), 6. Ovate, 7. Acorn, 8. Elongate 9. Scallop.
Predominant fruit skin color	FC	1. White, 2. Light yellow, 3. Cream, 4. Pale green, 5. Green, 6. Dark green, 7. Blackish – green, 8. Orange, 9. Brown, 10. Grey
Secondary fruit skin color	FSC	0. No secondary skin color, 1. White, 2. Light yellow, 3. Cream, 4. Pale green, 5. Green, 6. Dark green, 7. Blackish – green, 8. Orange, 9. Brown, 10. Grey
Secondary skin color pattern	SCP	0. No secondary skin color, 1. Spackled (spot < 0.5cm), 2. Spotted, blotchy (spots > 0.5 cm), 3. Striped, 4. Short streaked, 5. Long streaked
Main color of flesh	FLC	1. White, 2. Yellow, 3. Cream, 4. Pale green, 5. Green, 6. Orange, 7. Salmon
Flesh thickness	FLT	cm
Number of placentas	PC	1. Three, 2. Five, 99. Other
Brix	Bx	Degree
Seed characteristics		
Seed length	SL	cm
Seed width	SW	cm
Seed shape	SSp	SSp=SL/SW 1. Roundish (length/width < 2.0), 2. Elliptical (length/width between 2.1 and 2.5), 3. Oval (length/width >2.5), 4. Triangular, 5. Pinonet type
100 – seeds weight	100W	g
Inflorescence		
Sex expression type	SexGH	1. Monoecious, 2. Andromonoecious, 3. Gynoecious, 4. Male sterile, 5. Female sterile

Results

The morphological characteristics of the fruit, inflorescence, and seed of 73 melon accessions were presented in table 3.2 and summarized in table 3.3. The principal component analysis revealed that fruit shape, weight, length, and width; flesh sugar content by brix, flesh thickness; seed length; and seed width were varied. However, obvious difference was detected among five collected regions by PCO1 and PCO2 (Fig 3.2). In this, PCO1 was related to fruit shape, weight, length, seed length and flesh sugar content, while PCO2 related to fruit width and flesh thickness.

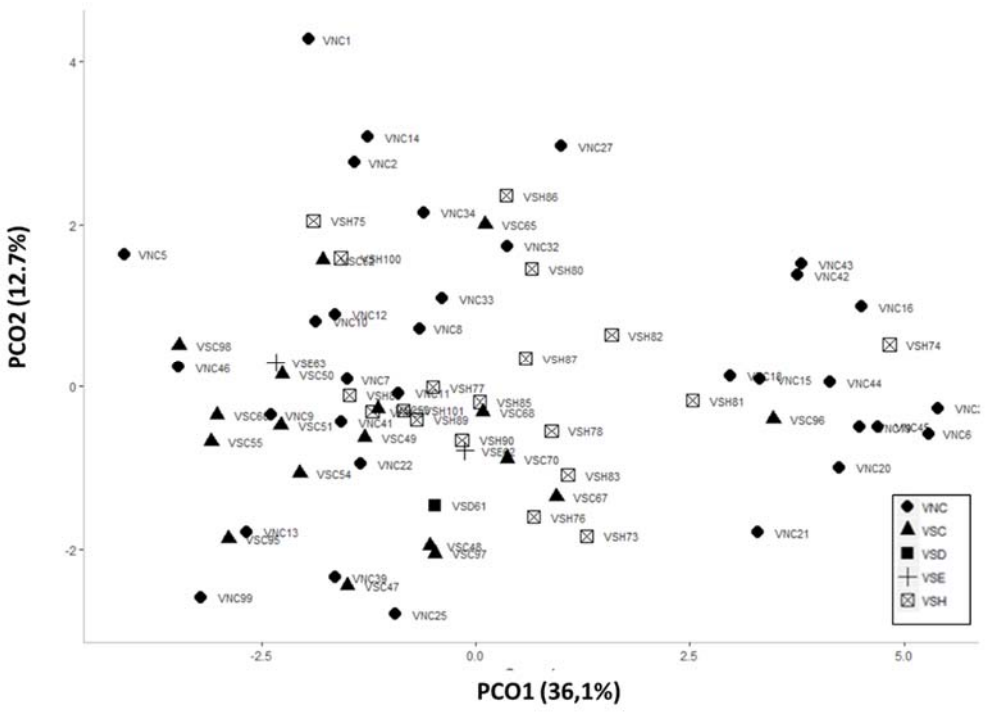


Fig. 3.2. Distribution on the first two principal coordinates of 73 melon accession from five regions in Central and southern Vietnam. Principal component score of each accession was calculated in PCO analysis by 16 morphological characters. Contribution rate of their total variance is indicated in parentheses under the two axes.

In chapter 2, the Vietnamese melon landraces from central and southern were categorized into five groups based on the local name and the fruit characters as “Dua le”, “Dua vang”, “Dua bo” and “Dua gang”. The morphological results further point the differences of sex expression showed in these five groups, “Dua le” and “Dua vang” were andromonoecious, while “Montok” was monoecious. “Dua gang” and “Dua bo” proved to be the admixture. Notably, the sex type expression in “Dua gang” correlated to collected sites, northern “Dua gang” collected from Ha tinh province to Quang Nam province was Andromonoecious, while the southern “Dua gang” was Monoecious which collected from Quang Ngai province of Southern central coast to Long An province of Mekong Delta region, the fruit usage also different in two “Dua gang” groups, Andromonoecious “Dua gang” was used as vegetable and Monoecious “Dua gang” was used as dessert. Thus, in this chapter “Dua gang” was divided into two types, “Dua gang-andromonoecious” and “Dua gang-monoecious” based on the sex expression.

Fruit characters and seed size varied among six cultivar types (Table 3.3). “Dua le” and “Dua vang” had higher soluble solid content, and shorter seed compared with other groups. As clearly indicated by fruit shape index was 0.8, they were characterized by round-shaped fruits. Average fruit height (35.1cm) and fruit shape index (3.8) was the largest in “Dua gang- Andromonoecious” among six melon cultivar types, followed by “Dua gang- Monoecious” (21.8cm, 2.1). The difference among two types of “Dua gang” was statistically significant (P<0.01). “Dua bo” and “Montok” had oblong fruits with similar fruit height (17.8 cm and 21.8 cm) and fruit shape index (1.7 – 1.8). Interestingly, peeling skin in mature fruit, the typical character of Momordica group was observed not only in accession of “Dua bo” (VNC32) but also in one accession of “Dua gang – Andromonoecious” (VNC13) (Fig 3.1)

Table 3.3. The average performance of six melon types in central and northern Vietnam

Melon type	No. of accessions	Seed[a]		Fruit[a]				Flesh[a]		Sex expression[b]
		Length (mm)	Width (mm)	Weight (g)	Height (cm)	Diameter (cm)	Shape	Thickness (cm)	Brix	
Dua le	14	6.3 c	3.1 b	421 b	8.3 c	9.8 b	0.8 c	1.9 b	9.0 a	A
Dua vang	1	7.0	3.2	1010 a	10.7	14.2	0.8	3.1	3.8	A
Dua bo	15	7.4 ab	3.4 ab	1296 a	20.0 b	12.0 a	1.7 b	2.8 a	3.1 c	A (13) M (2)
Dua gang – Andro	7	7.0 ab	3.3 ab	1361 a	35.1 a	9.2 b	3.8 a	2.1 ab	3.7 bc	A
Dua gang - Mono	19	7.6 a	3.4 a	1245 a	21.8 b	10.4 ab	2.1 b	2.5 ab	4.7 b	M
Montok	17	6.9 bc	3.2 ab	966 a	17.8 b	10.2 ab	1.8 b	2.5 ab	4.8 b	M

[a] Mean value with the same lettr indicate non – significant differences at 0.01 level by Tukey – Kramer test. “Dua vang” was not included for statistical analysis because only one accession was analyzed

[b] Sex expression is indicated by: A – Andromonoecious; M - Monoecious

The cluster performed by average linkage method based on Gower’s coefficient was dissected central and southern Vietnamese melons into 2 clusters (Fig 3.3), cluster I is sweet melon cluster which consisted all members of “Dua le” and “Dua vang”, cluster II is the non –sweet melons . Cluster II further could be classified into 4 sub-clusters, in which sub – cluster IIb was “Dua gang – Andromonoecious” when all “Dua gang – Andromonoecious” accessions presented in this sub - cluster, sub – cluster IIc consisted of 13 “Dua bo” accesions out of 15 accessions, “Dua gang – Monoecious” mixed in sub-cluster IIa with “Dua bo” and IId with “Montok”

Discussions

There are six melon types in central and southern Vietnam. “Dua le” has white smooth skin, fruit small fruit, round shaped and sweet, “Dua vang” has yellow smooth skin, round shaped fruit, “Dua bo” which have oblong in fruit shape and the dominant fruit skin is orange with green striped or short streaked, less sweet, “Montok” has oblong fruit shape, yellow skin with striped, “Dua gang – Andromonoecious” mainly distributed from Nghe An province to Quang Nam province has elongate fruit with pale green to green colour, intermediate fruit size; “Dua gang – Monoecious” varied in fruit traits, elliptical to elongate fruit, yellow to green epicarp color, less sweet.

The sex expression is the key character to distinguish Conomon, Makuwa, and Chinesis Groups with Mormodica and Acidulus Groups in eastern Asia (Pitrat 2016). Conomon, Makuwa, and Chinesis Groups are andromonoecious while Mormodica and Acidulus Groups are monoecious. The difference in sexual of flower also had been reported between Chinese thin-skinned melon and vegetable melon types (Luan, Delannay, and Staub 2008). The dendrogram using genetic similarities (Jaccard’s coefficient) revealed by RAPD markers in the study of Luan, Delannay, and Staub (2008) indicated that both Chinese thin-skinned melon had genetic affinities with Indian accession, while they were most distant from Chinese net or non-netted thick skin. The results of Nhi et al. (2010) also pointed the close genetic relationship of “Dua gang – andromonoecious” (from northern Vietnam), “”Dua bo”, “Dua vang”, and “Dua le” with Group Conomon. The cluster in this study showed that the “Dua gang – Monoecious” established the distinguish sub-group with Vietnamese *conomon* and same group with highland melon – “Montok”. Therefore, comparative analysis of genetic variability of central and southern Vietnamese melon and melon germplasm of diverse origins, especially Far East countries is need to clarify genetic diversity of Vietnamese melon and their genetic relationship with melon from South and East Asia region.

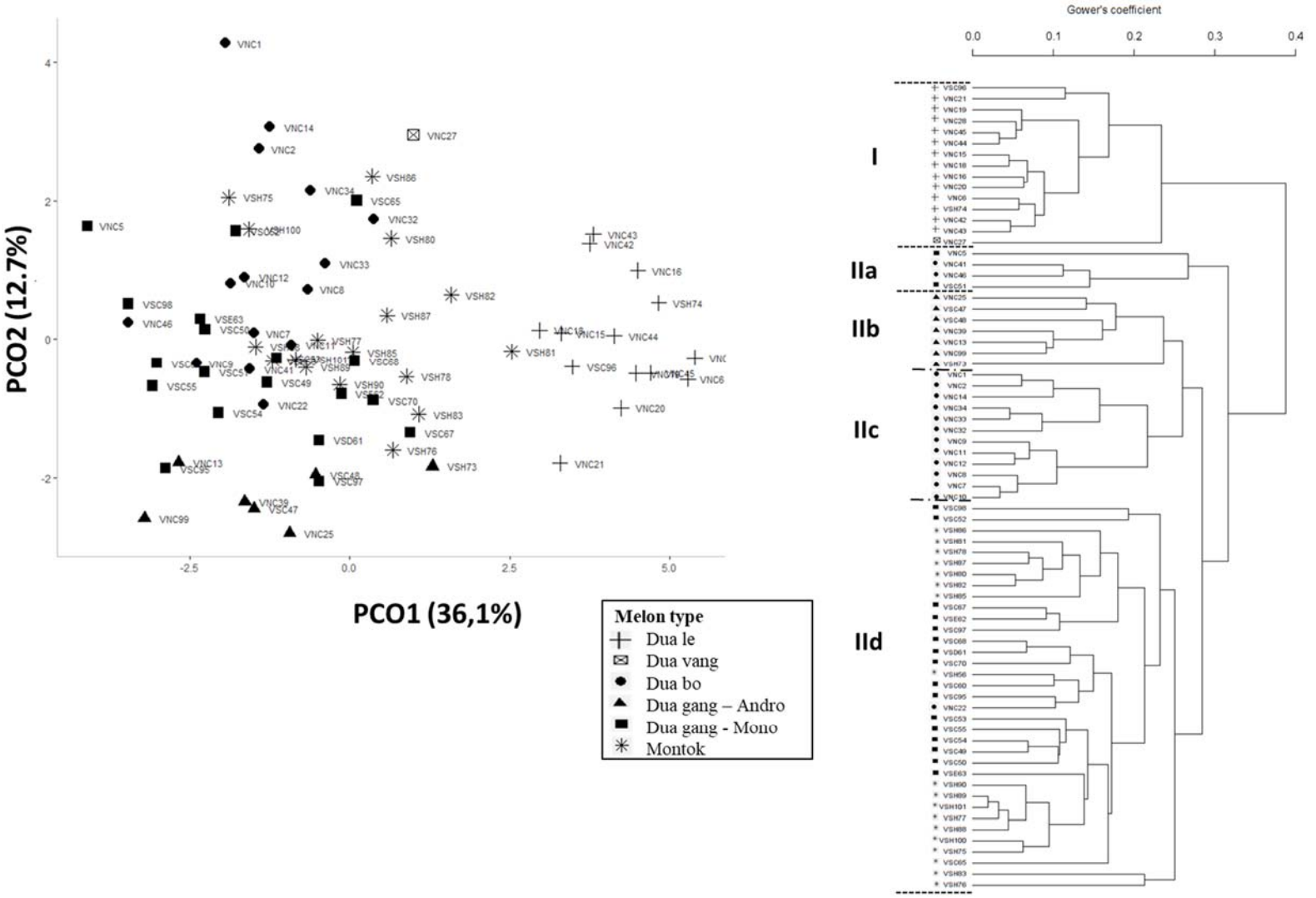


Fig 3.3. Distribution on the first and second principal coordinates and cluster of central and southern Vietnamese melons

Table 3.2. Fruit, seed and inflorescence characteristics of 73 central and northern Vietnamese melons

Code	Common name	Cultivar type	FS	FW	FL	FD	FT	FC	FSC	SCP	FLC	Bx	CN	SL	SW	SSp	100W	Sex
VNC1	Dua bo	Dua bo	1	1728	14.5	16.2	4.4	3	7	5	4	3.2	4	7.38	3.18	2	1.51	0
VNC2	Dua bo	Dua bo	1	1385	12.5	14.3	3.3	3	7	5	4	3	4	7.56	3.3	2	1.5	0
VNC5	Dua gang	Dua gang - Monoecious	2	3350	33.3	14.8	3.3	8	0	0	1	6	3	9.06	3.87	2	1.69	1
VNC6	Dua le	Dua le	1	240	6.5	8.4	1.1	1	0	0	1	12.5	5	6.53	3.08	2	1.04	0
VNC7	Dua bo	Dua bo	2	1115	23.0	9.4	2.6	8	6	2	1	2.8	5	7.52	3.67	2	1.88	0
VNC8	Dua bo	Dua bo	2	1075	20.6	10.7	2.5	8	6	2	1	2.5	5	7.12	3.31	2	1.74	0
VNC9	Dua bo	Dua bo	3	1450	25.1	9.7	2.5	8	6	2	4	3	5	7.74	3.57	2	1.86	0
VNC10	Dua bo	Dua bo	2	1140	21.2	11.2	2.7	8	6	2	1	2	5	7.67	3.64	2	1.97	0
VNC11	Dua bo	Dua bo	2	1050	23.8	9.8	2.1	8	6	2	4	3.5	5	7.03	3.46	2	1.64	0
VNC12	Dua bo	Dua bo	2	1370	21.6	11.6	2.5	8	6	2	4	2	5	7.31	3.43	2	1.71	0
VNC13	Dua gang	Dua gang - Andromonoecious	4	1950	44.8	10.3	2.3	6	3	3	4	3	3	7.1	3.31	2	1.61	0
VNC14	Dua bo	Dua bo	1	1910	22.1	15.1	3.3	5	6	5	4	4.5	4	7.06	3.13	2	1.33	0
VNC15	Dua le	Dua le	1	425	9.0	9.7	1.8	1	0	0	1	6	5	6.61	3.27	2	1.31	0
VNC16	Dua le	Dua le	1	500	9.0	11.0	2.0	1	0	0	1	7.3	5	5.87	2.84	2	1.05	0
VNC18	Dua le	Dua le	1	540	11.0	10.9	2.1	1	0	0	1	5.5	3	6.37	3.16	2	1.11	0
VNC19	Dua le	Dua le	1	310	6.5	9.2	2.3	1	0	0	1	7	4	6.13	3.1	1	1	0
VNC20	Dua le	Dua le	1	230	7.1	7.7	1.7	1	0	0	1	6.6	3	6.33	2.93	2	0.98	0
VNC21	Dua le	Dua le	1	260	7.4	8.2	1.8	2	0	0	4	8	3	6.66	3.38	1	1.21	0
VNC22	Dua bo	Dua bo	3	950	25.8	9.2	3.1	3	5	2	4	3.5	3	7.35	3.25	2	1.66	0
VNC25	Dua gang	Dua gang - Andromonoecious	4	1360	32.8	9.1	2.1	6	3	3	4	3.5	3	6.69	3.35	1	1.46	0
VNC27	Dua vang	Dua vang	1	1010	10.7	14.2	3.1	8	0	0	1	3.8	5	6.96	3.24	2	1.42	0
VNC28	Dua le	Dua le	1	345	7.2	10.4	1.6	1	0	0	1	10.5	5	5.99	3.07	1	1.03	0
VNC32	Dua bo	Dua bo	1	605	9.3	11.7	2.5	8	6	2	4	3	5	6.96	3.27	2	1.31	0
VNC33	Dua bo	Dua bo	1	560	8.3	12.2	2.0	8	6	5	4	3.8	3	7.39	3.19	2	1.53	0
VNC34	Dua bo	Dua bo	1	780	10.1	14.2	2.6	8	6	5	4	3.8	3	7.04	3.14	2	1.49	0
VNC39	Dua gang	Dua gang - Andromonoecious	4	1100	33.3	9.2	1.9	5	4	3	4	5	3	7.48	3.31	2	1.7	0
VNC41	Dua bo	Dua bo	2	1840	26.8	12.1	2.2	3	0	0	4	4	3	7.69	3.47	2	1.8	1
VNC42	Dua le	Dua le	1	575	8.5	11.6	2.5	1	0	0	1	11	5	6.52	3.06	2	1.43	0

VNC43	Dua le	Dua le	1	720	10.8	11.9	2.5	2	0	0	1	11.5	5	6.45	3.18	2	1.04	0
VNC44	Dua le	Dua le	1	590	9.9	10.7	2.2	1	0	0	1	10.2	5	6.28	3.28	1	1.36	0
VNC45	Dua le	Dua le	1	400	8.5	9.2	2.1	1	0	0	1	10.8	5	6.28	3.24	1	1.23	0
VNC46	Dua bo	Dua bo	3	2480	35.2	13.2	3.6	2	0	0	4	2	3	7.78	3.58	2	1.8	1
VSC47	Dua gang	Dua gang - Andromonoecious	4	1955	36.7	9.7	2.5	3	4	3	1	2	3	6.77	3.45	1	1.58	0
VSC48	Dua gang	Dua gang - Andromonoecious	4	945	31.9	9.1	2.3	4	4	3	1	2.5	3	6.52	3.24	2	1.35	0
VSC49	Dua gang	Dua gang - Monoecious	2	1010	19.7	9.9	2.0	8	3	3	4	3.3	3	7.85	3.25	2	1.5	1
VSC50	Dua gang	Dua gang - Monoecious	2	1300	22.0	10.8	3.0	6	4	3	4	4	3	7.49	3.34	2	2.04	1
VSC51	Dua gang	Dua gang - Monoecious	3	2160	32.2	11.0	3.1	2	3	2	4	5.5	3	7.77	3.47	2	1.3	1
VSC52	Dua gang	Dua gang - Monoecious	2	2005	25.8	13.0	2.9	8	7	2	1	5	4	7.32	3.28	2	1.49	1
VSC53	Dua gang	Dua gang - Monoecious	2	1250	15.8	9.6	2.4	8	6	2	4	6	3	7	3.29	2	1.79	1
VSC54	Dua gang	Dua gang - Monoecious	2	800	22.2	8.9	2.4	8	3	3	4	4	3	7.79	3.71	2	1.71	1
VSC55	Dua gang	Dua gang - Monoecious	2	1240	22.9	9.6	2.5	8	7	3	5	3.8	3	7.62	3.65	2	2.01	1
VSH56	Montok	Montok	2	930	20.5	9.6	3.1	3	2	5	4	3.8	3	7.4	3.42	2	1.43	1
VSC60	Dua gang	Dua gang - Monoecious	2	1310	25.4	10.2	2.9	3	6	5	4	3.8	3	8.07	3.5	2	2.03	1
VSD61	Dua gang	Dua gang - Monoecious	2	700	19.2	8.7	2.0	3	4	3	4	5	3	7.02	3.39	2	1.76	1
VSE62	Dua gang	Dua gang - Monoecious	2	850	21.7	9.4	2.4	3	4	3	1	5	3	7.38	3.5	2	1.09	1
VSE63	Dua gang	Dua gang - Monoecious	2	1435	26.0	11.0	2.3	8	7	5	4	4	4	8	3.53	2	1.09	1
VSC65	Dua gang	Dua gang - Monoecious	1	1125	12.7	12.7	3.3	7	3	3	4	6.3	3	6.41	3.09	2	1.33	1
VSC67	Dua gang	Dua gang - Monoecious	2	600	18.5	8.3	1.7	5	4	3	1	4.8	3	6.93	3.13	2	1.18	1
VSC68	Dua gang	Dua gang - Monoecious	1	575	9.5	10.5	1.9	3	4	3	4	6	3	7.16	3.48	2	1.75	1
VSC70	Dua gang	Dua gang - Monoecious	2	600	19.0	9.0	2.0	4	6	4	4	6	3	6.82	2.94	2	1.38	1
VSH73	Dua gang	Dua gang - Andromonoecious	2	200	18.0	7.5	1.5	4	4	3	4	6.5	3	7.21	3.04	2	1.35	0
VSH74	Dua le	Dua le	1	360	8.0	9.6	2.1	1	0	0	1	11	5	6.1	3.04	2	1	0
VSH75	Montok	Montok	2	2040	24.3	13.1	3.9	8	2	5	4	6	3	6.84	3.22	2	1.28	1
VSH76	Montok	Montok	3	1200	25.0	9.4	2.6	8	0	0	1	4	3	6.33	3.2	1	1.27	1
VSH77	Montok	Montok	2	1050	20.0	10.6	2.4	8	2	5	4	6.3	3	6.75	3.23	2	1.41	1
VSH78	Montok	Montok	2	600	14.1	8.7	2.0	5	1	5	1	4.5	4	7.12	3.22	2	1.27	1
VSH80	Montok	Montok	1	580	10.3	10.8	2.7	8	2	5	1	4	4	7.02	3.12	2	1.27	1
VSH81	Montok	Montok	1	450	11.0	9.0	1.7	5	1	5	1	5	3	6.32	2.82	2	0.99	1
VSH82	Montok	Montok	1	500	8.7	10.1	1.8	8	1	5	1	6	4	7.02	3.06	2	1.28	1

VSH83	Montok	Montok	2	600	13.9	9.1	2.5	3	3	5	4	5	4	5.98	3.48	1	1.22	1
VSH85	Montok	Montok	2	725	13.9	8.8	2.1	7	8	5	1	4.3	4	6.88	3.34	2	1.05	1
VSH86	Montok	Montok	2	1550	20.7	12.4	3.2	8	1	5	1	4	5	7.07	2.92	2	0.63	1
VSH87	Montok	Montok	2	745	16.8	10.0	2.1	8	2	5	1	4.5	5	6.69	3.25	2	1.35	1
VSH88	Montok	Montok	2	1050	21.4	10.8	2.4	8	2	5	4	4	3	7.08	3.42	2	1.56	1
VSH89	Montok	Montok	2	950	20.1	9.6	2.3	8	2	5	4	5.5	3	7.18	3.29	2	1.3	1
VSH90	Montok	Montok	2	750	17.7	9.1	2.3	6	1	5	4	5	3	7.06	3.21	2	1.37	1
VSC95	Dua gang	Dua gang - Monoecious	3	1000	24.9	8.7	2.3	4	5	3	4	3.5	3	8.52	3.57	2	1.98	1
VSC96	Dua le	Dua le	1	400	7.1	9.1	1.2	3	0	0	4	8	5	6.57	3.2	2	1.28	0
VSC97	Dua gang	Dua gang - Monoecious	3	710	22.4	7.9	2.0	2	5	3	1	4.5	3	7.87	3.25	2	1.47	1
VSC98	Dua gang	Dua gang - Monoecious	2	1625	21.6	12.8	2.9	4	5	4	1	3.5	3	8.18	3.82	2	2.31	1
VNC99	Dua gang	Dua gang - Andromonoecious	5	2015	48.3	9.6	2.0	5	5	3	4	3.5	4	7.49	3.54	2	1.51	0
VSH100	Montok	Montok	2	1675	23.8	12.1	3.1	8	2	5	4	4.5	5	6.95	3.3	2	1.61	1
VSH101	Montok	Montok	2	1025	20.7	9.5	2.6	8	2	5	4	6	3	7.09	3.32	2	1.39	1

Chapter 4. Vietnamese genetic diversity inferred by molecular markers and their relationship with Southeast and East Asian melon germplasm

Introduction

Oriental melon consists of two botanical groups of Munger and Robinson (1991), groups Momordica and Conomon, or five varieties of Pitrat (2008), vars. *momordica*, *conomon, makuwa, chinensis,* and *acidulus*. Group Momordica (snap melon) is native to India and is characterized by smooth fruit surface, mealy, non-sweet flesh, fruit cracks at maturity, and monoecious type of sex expression. Group Conomon, known as Oriental small-seed type melon (seed length is less than 9mm), is popular in Far-East (China, Korea, and Japan) (Tanaka *et al*. 2007) and is andromonoecious. Group Conomon is further divided into two varieties, *conomon* and *makuwa*. Var. *conomon*, called as "Shirouri" and "Cai-gua" in Japan and China, respectively, is non-sweet type and young fruits are used mainly as vegetable, while var. *makuwa* is sweet type and mature fruits are consumed as dessert. However, genetic diversity analysis of Asian melon populations, using isozyme (Akashi *et al*. 2002), RAPD (Tanaka *et al.* 2007; Yi *et al.* 2009), and SSR (Nhi *et al.* 2010), clearly showed that vars. *conomon* and *makuwa* shared the common gene pool and were not genetically differentiated. Furthermore, it was hypothesized that vars. *conomon* and *makuwa* had been originated from Indian melon landraces and differentiated somewhere in Southeast Asia during the eastward transmission of melon (Akashi *et al.* 2002; Tanaka *et al.* 2007).

Vietnam is located in the areas spanning from India to China, and thus the analysis of Vietnamese melon landraces is indispensable to uncover the origin of vars. *conomon* and *makuwa*. The morphological and molecular characterization of melon landraces have

been reported firstly by Nhi *et al.* (2010), for five cultivar groups, “Dua le”, “Dua vang”, “Dua bo”, “Dua gang” and “Dua thom”, collected from the northern part of Vietnam. “Dua le” has globular fruits with crispy flesh and white epicarp, while “Dua vang” has yellow epicarp. “Dua bo” has powdery flesh and less sweet flesh, and “Dua thom” has oblong fruit with aroma and diverse flesh color from white to yellow and orange. For these four groups, mature fruits are consumed as dessert. “Dua gang” has elongate fruit with vertical stripe and immature fruits are generally used as vegetable. Their materials of “Dua le”, “Dua vang”, “Dua bo”, and “Dua gang” were mainly collected in the midland and lowland of Northwest, Red River Delta and Northern Central Coast, while “Dua thom” was collected in the highland of the Northeast. By molecular marker analysis, they showed that “Dua thom” was genetically differentiated from other four “Dua” groups which were closely related with vars. *conomon* and *makuwa*. However, with the limitation of materials studied and area coverage, the diversity of Vietnamese melon is still unclear.

Hence, in an attempt to evaluate genetic diversity and genetic differentiation in Vietnamese melon, we collected landraces from central and southern part of Vietnam and detected molecular polymorphism, together with landraces from northern part of Vietnam collected by Nhi *et al.* (2010). Genetic diversity and genetic structure in Vietnamese melon were also compared with landraces from South and Southeast Asia as well as reference accessions of vars. *cantalupensis*, *inodorus*, *conomon*, and *makuwa*.

Materials and methods

Plant materials

Sixty-four melon accessions (*Cucumis melo* L.) were primarily selected from 73 melon accessions in chapter 3, the represented accessions were selected since the no genetic difference of the samples in the same collected locality occurred. As reference accessions,

31 accessions of vars. *conomon*, *makuwa, cantalupensis, inodorus*, and *agrestis* and 48 accessions from nearby countries, Yunnan (China), Thailand, Myanmar, Bangladesh, and India were used. Two accessions of wild cucumber, *Cucumis sativus* var. *hardwickii*, were also used as an outgroup. In addition, to provide wide geographical coverage of Vietnam, trait data and molecular data of 23 accessions of northern Vietnamese landraces selected from materials used by Nhi *et al.* (2010) were added for data analysis. The detail of materials was given in Supplementary Table 4.1 and was summarized in Table 4.1, and. "Dua gang" accessions were divided into two groups, "Dua gang-andromonoecious" and "Dua gang-monoecious", based on their sex expression type. Indian melon landraces were classified into two seed type groups, that is, large- and small-seed types, whose seed length was longer and smaller than 9mm, respectively.

DNA extraction and PCR amplification

Seeds were sown on a wet filter paper in the petri dish and grown at 28^{o}C under 16h light – 8h dark cycle at light intensity 46.5 $\mu Ms^{-1}m^{-2}$. Total DNA was extracted from young leaves of seedling using CTAB (cetyl-trimethyl-ammonium bromide) method as described by Murray and Thompson (1980) with minor modifications. The quality and quantity of each DNA samples were evaluated with spectrophotometer.

PCR based molecular markers including random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and allele specific markers for *CmACS7* and *CmPH* were used in this study. Twelve RAPD and seven SSR markers were selected for their reproducibility and their ability to detect distinct polymorphism in East Asian melon (Aierken *et al.* 2011; Nhi *et al.* 2010; Tanaka *et al.* 2007; Yi *et al.* 2009). *CmACS7* genotype was determined for sex expression type analysis using a CAPS marker developed by Boualem *et al.* (2008). *CmPH* genotype was determined for sourness of

fruit flesh using allele specific marker developed based on the sequence polymorphism reported by Cohen *et al.* (2014). The detail of the primer set was shown in Supplementary Table 4. 2.

PCR amplification was done in a 10µl PCR mixture consisted of 50ng of genomic DNA; 1µl PCR buffer (Sigma, USA: 10 mM Tris-HCl [pH 8.3], 50 mM KCl); 0.25 mM $MgCl_2$ for *CmACS7* and 0.2mM for RAPD, SSR and *CmPH*; 0.1 mM dNTP; 0.5 µM primers, and 0.25U Taq polymerase (Sigma, USA).

Amplification reactions were performed using an iCycler (Bio-Rad, USA). The PCR cycle for SSR, *CmACS7* and *CmPH* started with an initial denaturing step at 95ºC for 3 min, followed by 35 cycles of 95ºC for 1 min, 56ºC-57ºC for 1 min (60ºC for 30 sec for *CmPH*) and 72ºC for 2 min, and final extension at 72ºC for 5 min. The PCR cycle for RAPD was as follows: initial denaturing at 95ºC for 3 min, 40 cycles of 93ºC for 1 min, 40ºC for 2 min, 72ºC for 2 min and final extension at 72ºC for 5 min.

PCR products of *CmACS7* digested with *AluI* (New England BioLabs, Ipswich, MA, USA) and of RAPD were electrophoresed on 1.5% agarose gels at constant voltage 50V, while that of *CmPH* on 3% agarose gel. For SSR, PCR products were electrophoresed on nondenatured 10% polyacrylamide gel at constant voltage 240V.

Data analysis

Marker bands of RAPD were scored as 1 for presence and 0 for absence. For SSR, marker fragments were scored based on their size from the smallest (1) to the largest (6-18 depending on the marker). The output profile after scoring was used to calculate number of effective alleles (Ne), the polymorphic index content (PIC) (Botstein *et al.* 1980), gene diversity (D) within each group (Weir 1996) and genetic distance (GD) among groups (Nei 1972). Genetic similarity (GS) among accessions was calculated as

described by Apostol et al. (1993), and their genetic distance (GD) was calculated using the formula GD = 1-GS. A dendrogram was constructed based on the genetic distances using the PHYLIP program with the unweighted pair group method by arithmetic averages (UPGMA) cluster analysis. Principal coordinates analysis (PCoA) based on the genetic similarity matrix was done to show multiple dimensions of each group and accession on a scatter plot (Gower 2016). Population structure was analyzed using a model-based approach available in the software STRUCTURE 2.3.4 (Pritchard *et al.* 2000). In this analysis, we used the admixture model without prior grouping assumptions and evaluated 1 to 10 genetic clusters (K) with 20 permutation for each K value. Each run was implemented with a burn-in period of 25,000 steps followed by 100,000 Monte Carlo Markov (MCM) chain.

Results

Genetic diversity of Vietnamese melon and reference accessions

Vietnamese melon landraces of seven "Dua" groups were mostly classified as small-seed type, except one accession of "Dua gang" (VNC5) (Table 4.1). In contrast, sex expression type and flesh sourness estimated by *CmACS7* and *CmPH* genotype proved to be diversified in Vietnamese landraces. "Dua le" and "Dua vang" were andromonoecious with non-sour flesh, while "Dua thom", "Montok" and "Dua dai" were monoecious with sour flesh. "Dua gang" and "Dua bo" proved to be the admixture in terms of sex expression and flesh sourness.

Among reference accessions, small-seed type which is characteristic of vars. *conomon* and *makuwa* was common in areas from India to Yunnan (China), showing gradual decrease of large-seed type to the eastward. Geographical variation in sex expression type was not simple. Accessions of two contrasting variety groups, vars. c*antalupensis* and

indonorus with large-seed and vars. *conomon* and *makuwa* with small-seed, were andromonoecious, while small-seed type accessions from India to Yunnan (China) were mostly monoecious. Flesh sourness also showed similar geographical variation, showing frequent distribution of sour type in small-seed type accessions from India to Yunnan (China). The exceptional case is vars. *conomon* and *makuwa* which were not discriminated with each other and both sour and non-sour type presented in each variety.

Seven SSR and 12 RAPD markers generated a total of 100 alleles in 168 accessions studied, among which 61 alleles were detected in 87 accessions of Vietnamese landraces. For three RAPD markers, A41-930, B32-700, and B86-1350, no polymorphism was detected in Vietnamese landraces, and PIC among 168 accessions was also smaller than 0.01. Number of polymorphic SSR bands ranged from 6 (CMN 4-07) to 18 (CMN 61-44), and that of effective alleles was from 2.1 to 5.1. PIC value ranged from 0.05 (B32-700) to 0.78 (CMBR 2), and the average was 0.39 (Table 4. 2).

Gene diversity calculated by RAPD and SSR data was 0.35 in 87 Vietnamese landraces, and as high as those in areas from India to Thailand. It was also shown that Vietnamese landraces were more diversified compared with vars. *conomon* and *makuwa*. However, gene diversity in each "Dua" group was smaller than 0.23, with an exception of "Dua gang" (0.30), and nearly equivalent to those of vars. *conomon* and *makuwa*. These results indicated that genetic diversity was enriched in Vietnam by the presence of seven groups of melon, each of which is rather less diversified within group except "Dua gang".

Genetic relationship and population structure

The dendrogram obtained from UPGMA cluster analysis based on genetic distance separated 168 accessions into four major clusters, and further into 17 sub-clusters (Fig.

4.1). The outgroup, two accessions of *C. sativus* var. *hardwickii*, formed a distinct cluster I. All accessions of vars. c*antalupensis* and *indonorus* formed cluster IV, together with 13 accessions from India-L (large-seed type), Bangladesh, Myanmar, and Thailand, and one accession of var. *agrestis* from India. Vietnamese landraces were classified into Clusters II and III. Cluster II was characterized by the presence of vars. *makuwa*, *conomon*, and *agrestis*, and contained Vietnamese accessions of "Dua le"(13/13), "Dua vang"(3/3), "Dua bo"(16/18), "Dua gang"(18/27), and each one accession of "Dua thom" and "Dua dai". Cluster III consisted of Vietnamese accessions of "Dua thom"(7/8), "Montok"(16/16), "Dua gang"(9/27), "Dua bo"(2/18), and "Dua dai"(1/2). Majority of melon accessions from India to Yunnan (China) was also classified into cluster III. Although "Dua gang" contained accessions of Clusters II and III, andromonoecious type accessions were mostly classified into Cluster II. These results together with sex expression type clearly indicated that Vietnamese melon was comprised of two major groups. The first consisted of "Dua le", "Dua vang", "Dua bo", and part of "Dua gang", being andromonoecious and genetically related to vars. *makuwa* and *conomon*. The other included "Dua thom", "Montok", and part of "Dua gang", being monoecious and closely related to landraces from India to Yunnan (China).

Genetic relationship uncovered by cluster analysis was reproduced on the PCO plot (Fig. 4.2). Vietnamese accessions of "Dua le", "Dua vang", and "Dua bo" were plotted closely with vars. *conomon* and *makuwa*, and clearly separated from other accessions by PCO1. PCO2 axis was considered to explain relatedness of "Dua thom" and "Montok" with landraces from India to Yunnan (China), showing similarity with geographically closer populations such as Yunnan (China) and Thailand. As expected from Table 4, "Dua gang" accessions were widely scattered along PCO1 axis, and those of clusters IIa, IIb

and IId were plotted together with “Dua le”, “Dua vang”, and “Dua bo”. On the contrary, those of clusters III were close to “Dua thom” and “Montok”, while those of IIe and IIg were located at intermediate position between two major groups.

To understand the genetic structure of Vietnamese melon, population structure was performed using the STRUCTURE software. The delta K suggested the presence of three main populations in 168 accessions. The population structure and classification of 168 samples into three populations was shown in Fig. 4.3. Out of 166 accessions studied, 137 accessions (82.5%) were assigned to one of the three populations using Q-value threshold of 70% (Table 4.3, Supplementary Table 4.1). Population P1 consisted of 10 accessions of vars. c*antalupensis* and *indonorus* and 23 accessions from India, Bangladesh, Myanmar, and Thailand (diagonal line, Fig. 4.3.). No Vietnamese accessions belonged to population P1. Although one accession of var. c*antalupensis*, “Melon Cantalupo di Charentais” was classified in cluster IIg (Table 4.3), it was also assigned to population P1. Population P2 included accessions of vars. *conomon* and *makuwa*, and of “Dua le”, “Dua vang”, “Dua bo”, and “Dua gang”. Population P3 consisted of landraces from India to Yunnan (China) and of “Dua gang”, “Dua thom”, and “Montok”. The remaining 29 accessions (17.3%) were categorized as having mixed ancestry with admixture in genetic composition. In Vietnamese landraces, 10 accessions of “Dua bo”, “Dua gang”, and “Dua thom” proved to be the admixture type. The result of STRUCTURE analysis also demonstrated the presence of two major groups and admixture between them in Vietnam.

Genetic relationship among cultivar groups

The pairwise genetic distance (GD) among 19 cultivar groups was shown in Table 5, and the greatest distance (0.842) was observed between var. *makuwa* and var. *inodorus* and the lowest (0.014) was between “Dua vang” and “Dua le”. GD among “Dua le”, “Dua

vang", "Dua gang-andromonoecious", and "Dua bo" was smaller than 0.110, indicating that these four cultivar groups are genetically close. Similarly, GD was small (0.090) between "Dua thom" and "Montok". Furthermore, GD between the former four groups and the latter two groups ranged from 0.315 to 0.665, indicating distinct genetic differentiation between them. Among the former four groups, "Dua le" and "Dua vang" related most closely with var. *makuwa* (GD=0.094-0.101), while "Dua gang-andromonoecious" and "Dua bo" with var. *conomon* (GD=0.088-0.097). In contrast, "Dua thom" and "Montok" were distantly related with vars. *conomon* and *makuwa* (GD=0.451-0.682), and closely with cultivar groups from Yunnan (China) to India-small (GD=0.096-0.236). "Dua gang-monoecious" was rather unique in Vietnamese cultivar groups. Although this group was most close with "Dua bo", GD with other Asian groups except "Dua dai" was intermediate ranged from 0.112 to 0.297.

Based on genetic distance among 19 groups (Table 4.4), UPGMA cluster analysis and PCoA were performed and genetic relationship was visualized in Figs. 4.4 and 4.5. In good accordance with accession based analysis, 19 groups were separated into three groups, that is, ssp. *melo* group, South and South-East Asian group, and vars. *conomon* and *makuwa* group. Among Vietnamese cultivar groups, "Dua le", "Dua vang", "Dua bo", and "Dua gang-andromonoecious" fell into vars. *conomon* and *makuwa* group, while "Dua thom", "Montok", and "Dua dai" into South and South-East Asian group. "Dua gang-monoecious" was located at intermediate position between these two groups on PCO plot (Fig. 4.5).

Discussion

Genetic structure of Vietnamese melon was first reported by Nhi *et al.* (2010), in which local landraces collected from northern Vietnam were studied and classified as ssp.

agrestis which had short hairs on the ovary. They also indicated two distinct groups of melon landraces which distributed allopatrically and were genetically differentiated with each other. In this study, we collected melon landraces from southern and central Vietnam, and revealed diversity and genetic structure of local melon in whole Vietnam based on the analysis of fruit traits and molecular polymorphism. In addition to five cultivar groups, "Dua le", "Dua vang", "Dua bo", "Dua gang" and "Dua thom", an additional group "Montok" was recognized by field research in Tay Nguyen region (Central Highlands). Furthermore, monoecious type of "Dua gang" was recognized in southern Vietnam, and thus "Dua gang" was divided into two groups, "Dua gang-andromonoecious" and "Dua gang-monoecious". Among these seven groups of cultivated melon, "Dua thom" and "Montok" proved to be common in highland areas of northern (H'mong and Thai) and southern (Jarai and H'mong) Vietnam, respectively, where ethnic group people shown in parenthesis grow melon in upland rice field as mixed cropping. The others were mainly grown as mono cropping in plain areas of whole Vietnam, mainly by Kinh, Muong, and Khmer people.

Reflecting such differences, Vietnamese melon was genetically separated into two distinct geographical groups, "Dua thom" and "Montok" of highland areas (Cluster III and population P3) and "Dua le", "Dua vang", "Dua bo", and "Dua gang-andromonoecious" of plain areas (Cluster II and population P2), by the analysis of molecular markers (Table 4.3, Supplementary Table 4.1). These two geographical groups were also different in sex expression type and flesh sourness. "Dua thom" and "Montok" were monoecious with sour flesh, while the others were andromonoecious with non-sour flesh with some exceptions in "Dua bo" (Table 4.1). In contrast, "Dua gang-monoecious" was polymorphic for flesh sourness, assigned to populations P1, P2 and admixture (Table

4.3), and scattered between two geographical groups on PCO plot (Figs. 4.2 and 4.5), suggesting their hybrid origin between the two geographical groups. One of the parents for hybridization should be monoecious and could be "Montok", since "Dua gang-monoecious" accessions except VNC5 were collected from southern Vietnam, mostly from VSC region adjacent to VSH (Tay Nguyen) region where ethnic group people grow "Montok" (Supplementary Table 4.1).

As to the origin of vars. *conomon* and *makuwa*, molecular-based phylogenetic relationship among East and South Asian melon inferred that vars. *conomon* and *makuwa* descended from the small seed size Indian melons which adapted to wet condition in the eastern (Akashi *et al.* 2002; Tanaka *et al.* 2007). Furthermore, the population-level genetic diversity analyses in global, geographic regional, and national scales also showed that the genetic variability was high in Indian melon landraces and quite low in Conomon group of Far – East (Akashi *et al.* 2002; Tanaka *et al.* 2007; Luan *et al*. 2008; Nhi *et al*. 2010; Leida *et al.* 2015). The decrease of genetic diversity from Indian melon landraces to Far-Eastern Conomon melons combined with a large differentiation existence in vars. *conomon* and *makuwa* with other botanical varieties reflected the genetic bottleneck took place, subsequent genetic drift and/or inbreeding during the eastward spread of Far-eastern melon from their primary center of diversity (India) as the result of geographical differences (Serres-Giardi and Dogimont 2012; Tanaka *et al.* 2015, Gonzalo *et al.* 2019). A hypothesis was generally been accepted that the progenitor of vars. *makuwa* and *conomon* had been introduced from India to China (at least by 100BC) via Myanmar, Lao, eastern China and they have been established during the eastward spread or in Yue land which was the ancient area elongated from Zhejiang in Southeast China to Jiaozhi, in Northern Vietnam along shelf coast as their Japanese and Chinese name mentioned

(Akashi *et al.* 2002; Tanaka *et al.* 2007; Luan *et al*. 2008, Wang *et al*. 2018). Extremely interesting that Japanese *conomon* variety posed a weakly separate from the accessions of the vars. *chinensis* and *makuwa* in molecular – based genetic distance tree and grouped with vars. *flexuosus* and *momordica* in metabolomic – based phylogenetic tree in study of Moing *et al*. (2020). Tanaka *et al*. (2015) also demonstrated the artificial selection in the Japanese melon domestication process than weaker natural selection in order to created varieties suited to local agricultural and cultural condition and their genetic erosion from early Japanese melon. A similar situation could be seen in this study, Vietnamese *conomon* variety. "Dua bo" and "Dua gang – Andromonoecious", grouped with Japanese *conomon* in sub-cluster IIa while *makuwa* varieties belonged subclusters IIc and IId (Table 4.3), the genetic diversity of Vietnamese Conomon group (vars. *conomon* and *makuwa*) was not higher than Far-eastern one. These were taken together to hypothesize of secondary diversity center and even tertiary diversity center of vars, *conomon* and *makuwa* had been established some whether in China, northern Vietnam, and Japan. More evidences from historical record and archeological remain, especially from Vietnam are needed to clarify if and when the new types of Far-eastern melon (vars. *conomon* and *makuwa*) have come into existence.

Highland melon groups, "Dua thom" and "Montok", proved to be closely related with each other (GD=0.090), as also shown in Figs. 4.2, 4.4, and 4.5. They also showed similarity in fruit traits and usage as dessert, though immature fruit of "Montok" was sometimes used as vegetable. It was thus indicated that they can be regarded as the same group, in spite that they are grown in geographically distant areas by different groups of ethnic people. "Dua thom" and "Montok" proved to be closely related with cultivar groups from Yunnan (China) to India-small (Table 4.4), and shared the same traits of

monoecious and sour flesh. It was therefore suggested that “Dua thom” and “Montok” had been introduced from west. However, little is known about melon populations of neighboring countries, with an exception of Myanmar reported by Yi et al. (2009). Recently, field research of vegetable crops including melon have been conducted in Laos (Matsunaga *et al*., 2010) and Cambodia (Tanaka *et al*., 2016). Genetic diversity of these melon landraces remained to be studied.

Although Nhi *et al*. (2010) reported that genetic variation in Vietnamese melon landraces was smaller compared with those from India (Tanaka et al., 2007), Myanmar (Yi et al., 2009), and Iran (Soltani et al. 2010), the result of this study also showed the lower genetic diversity of panel Vietnamese melon landraces than India and Myanamar, however the genetic diversity of whole Vietnamese melon germplasm was higher than Thailand, Bangladesh, Yunnan (China) and Vietnamese melon in previous study. It is due to besides of the two Vietnamese groups as reported before, Conomon group and Highland group, there was the presence of “Dua gang” in southern Vietnam. This type of “Dua gang” is the likely the inter-group hybridization of northern “Dua gang” with andromonoecious sex expression and “Montok” with monoecious expression. Therefore, the genetic diversity of Vietnamese melon was enhanced by the presence of this additional hybridization group.

Table 4.1. List of melon types, number of accessions in each class of seed length *CmPH* genotype, *CmACS7* genotype and their genetic diversity

Melon group	No. of accessions	Seed length		*CmPH* genotype		*CmACS7* genotype		Genetic diversity		
		Large - seed	Small - seed	Not-sour	Sour	Andromonoecious	Monoecious	RAPD+SSR	RAPD	SSR
Vietnam										
Dua le	*13*	-	*13*	*13*	-	*13*	-	*0.14*	*0.05*	*0.30*
Dua vang	*3*	-	*3*	*3*	-	*3*	-	*0.13*	*0.04*	*0.29*
Dua bo	*18*	-	*18*	*15*	*3*	*15*	*3*	*0.19*	*0.07*	*0.40*
Dua gang - Andro	*8*	-	*8*	*8*	-	*8*	-	*0.17*	*0.08*	*0.33*
Dua gang - Mono	*19*	*1*	*18*	*7*	*12*	-	*19*	*0.30*	*0.16*	*0.53*
Dua thom	*8*	-	*8*	-	*8*	-	*8*	*0.21*	*0.14*	*0.33*
Montok	*16*	-	*16*	-	*16*	-	*16*	*0.21*	*0.10*	*0.42*
Dua dai	*2*	-	*2*	-	*2*	-	*2*	*0.23*	*0.17*	*0.34*
Subtotal	87	1	86	46	41	39	48	0.35	0.21	0.58
var. *conomon*	6	-	6	2	4	6	-	0.22	0.19	0.29
var. *makuwa*	8	-	8	5	3	7	1	0.17	0.10	0.27
var. *agrestis*	7	-	7	2	5	2	5	0.38	0.22	0.66
Yunnan (China)	5	-	5	-	5	-	5	0.08	0.00	0.23
Thailand	5	-	5	2	3	-	5	0.32	0.17	0.57
Myanmar	18	2	16	2	17	2	16	0.41	0.24	0.69
Bangladesh	7	2	5	-	7	-	7	0.34	0.15	0.65
India-small	7	-	7	2	5	-	7	0.37	0.18	0.71
India-large	6	6	-	1	1	3	3	0.39	0.26	0.62
var. *cantalupensis*	5	5	-	5	-	5	-	0.26	0.12	0.51
var. *inodorus*	5	5	-	5	-	5	-	0.31	0.19	0.53
Total	166	21	145	72	91	69	97	0.44	0.28	0.70

Table 4.2. The number of effective alleles (Ne) and polymorphic information content (PIC) at 19 marker loci of Vietnamese melons and168 studied accessions

	Vietnam (n=87)		All (n=168)	
Markers	Ne	PIC	Ne	PIC
A20_1100	1.91	0.36	1.97	0.37
A20_800	1.91	0.36	1.99	0.37
A22_800	1.52	0.28	1.45	0.26
A31_800	1.28	0.20	1.35	0.23
A41_930	1.00	0.00	1.17	0.13
A57_800	1.43	0.26	1.38	0.24
B32_900	1.67	0.32	1.73	0.33
B32_700	1.00	0.00	1.05	0.05
B68_1068	1.34	0.22	1.43	0.26
B71_1220	1.07	0.06	1.45	0.26
B86_1350	1.00	0.00	1.07	0.07
B99_1400	1.02	0.02	1.32	0.21
CMN 04-03	2.19	0.44	3.47	0.67
CMN 04-07	2.77	0.56	3.16	0.63
CMN 04-40	1.39	0.27	2.09	0.50
CMN 61-44	2.17	0.50	3.35	0.68
CMBR 2	3.51	0.68	5.13	0.78
CMBR 83	3.22	0.64	4.57	0.75
CMBR 120	2.96	0.61	3.63	0.68
Mean	1.81	0.30	2.25	0.39

Table 4.3. Number of melon accessions classified into 4 major clusters, 17 sub-clusters and 4 populations of reference and Vietnamese melon types.

Melon group	No. of acc.	Cluster No.																	Population structure			
		I	II a	II b	II c	II d	II e	II f	II g	III a	III b	III c	III d	III e	III f	IV a	IV b	IV c	P1	P2	P3	Mix
Vietnam																						
Dua le	13	-	-	1	10	2	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-
Dua vang	3	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-
Dua bo	18	-	12	1	2	-	1	-	-	-	-	1	-	1	-	-	-	-	-	15	-	3
Dua gang - Andro	8	-	5	1	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	7	-	1
Dua gang - Mono	19	-	4	2	-	-	4	-	1	-	3	5	-	-	-	-	-	-	-	7	7	5
Dua thom	8	-	-	-	-	-	1	-	-	-	-	-	6	1	-	-	-	-	-	-	7	1
Mon tok	16	-	-	-	-	-	-	-	-	-	-	6	8	2	-	-	-	-	-	-	16	-
Dua dai	2	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	2
Subtotal	87	0	21	6	14	3	6	0	2	0	4	12	14	5	0	0	0	0	0	45	30	12
var. *conomon*	6	-	3	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	5	-	1
var. *makuwa*	8	-	-	-	1	4	-	3	-	-	-	-	-	-	-	-	-	-	-	8	-	-
var. *agrestis*	7	-	-	-	-	3	-	-	1	-	1	-	-	1	-	-	-	1	4	3	-	-
Yunnan (China)	5	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	5	-
Thailand	5	-	-	-	-	-	-	-	-	-	-	-	2	1	-	2	-	-	2	-	3	-
Myanmar	18	-	-	-	-	-	-	-	-	4	1	-	4	1	2	4	2	-	6	-	3	9
Bangladesh	7	-	-	-	-	-	-	-	-	-	3	-	-	1	1	2	-	-	3	-	1	3
India-small	7	-	-	-	-	-	-	-	1	1	2	-	1	-	2	-	-	-	4	-	1	2
India-large	6	-	-	-	-	-	-	-	-	-	2	-	-	-	1	2	-	1	4	-	-	2
var. *cantalupensis*	5	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2	2	-	5	-	-	-
var. *inodorus*	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	3	-	5	-	-	-
Total	166	0	24	6	15	10	6	5	6	5	13	12	21	14	6	14	7	2	33	61	43	29

Table 4.4. Pairwise genetic distance between 19 groups of melon

Melon type	Dua le	Dua vang	Dua bo	Dua gang-Andro	Dua gang-Mono	Dua thom	Montok	Dua dai	var. *conomon*	var. *makuwa*	var. *agrestis*	Yunnan (China)	Thailand	Myanmar	Bangladesh	India-small	India-large	var. *cantalupensis*
Dua le																		
Dua vang	0.0138																	
Dua bo	0.1104	0.0854																
Dua gang-Andro	0.1021	0.1086	0.0352															
Dua gang-Mono	0.2033	0.1823	0.0970	0.1116														
Dua thom	0.6652	0.6315	0.3776	0.4202	0.2484													
Montok	0.5738	0.5438	0.3151	0.3458	0.1661	0.0896												
Dua dai	0.6609	0.6447	0.3929	0.4629	0.3224	0.2838	0.2614											
var. *conomon*	0.1660	0.1431	0.0880	0.0974	0.1484	0.5215	0.4511	0.4724										
var. *makuwa*	0.1014	0.0935	0.1461	0.1273	0.2568	0.6815	0.5827	0.6591	0.0979									
var. *agrestis*	0.1646	0.1657	0.1115	0.1125	0.1274	0.3481	0.3146	0.3139	0.1649	0.1610								
Yunnan (China)	0.6887	0.6798	0.4460	0.4563	0.2970	0.1540	0.1559	0.2519	0.5305	0.6934	0.3948							
Thailand	0.6057	0.5868	0.3989	0.4450	0.2521	0.1693	0.1627	0.2886	0.4665	0.5751	0.2639	0.2566						
Myanmar	0.5749	0.5477	0.3145	0.3638	0.2213	0.0956	0.1334	0.2926	0.4108	0.5543	0.2377	0.2093	0.1241					
Bangladesh	0.4898	0.4638	0.2920	0.3379	0.1732	0.2359	0.2160	0.2927	0.3026	0.4405	0.1766	0.3221	0.1164	0.1220				
India-small	0.4303	0.3828	0.2164	0.2748	0.1791	0.2002	0.1828	0.3434	0.2586	0.3645	0.1628	0.3329	0.1621	0.0906	0.1290			
India-large	0.4553	0.4297	0.2601	0.2818	0.1882	0.3238	0.2755	0.3403	0.3546	0.4288	0.1739	0.4620	0.2071	0.1821	0.1797	0.1712		
var. *cantalupensis*	0.5648	0.5939	0.4634	0.4443	0.3098	0.4623	0.3866	0.5084	0.4982	0.5572	0.3033	0.5723	0.2205	0.2628	0.2207	0.3027	0.1552	
var. *inodorus*	0.7788	0.7918	0.5811	0.6335	0.3930	0.4114	0.3962	0.4297	0.6673	0.8424	0.4162	0.5255	0.2041	0.2629	0.2285	0.3393	0.2099	0.1062

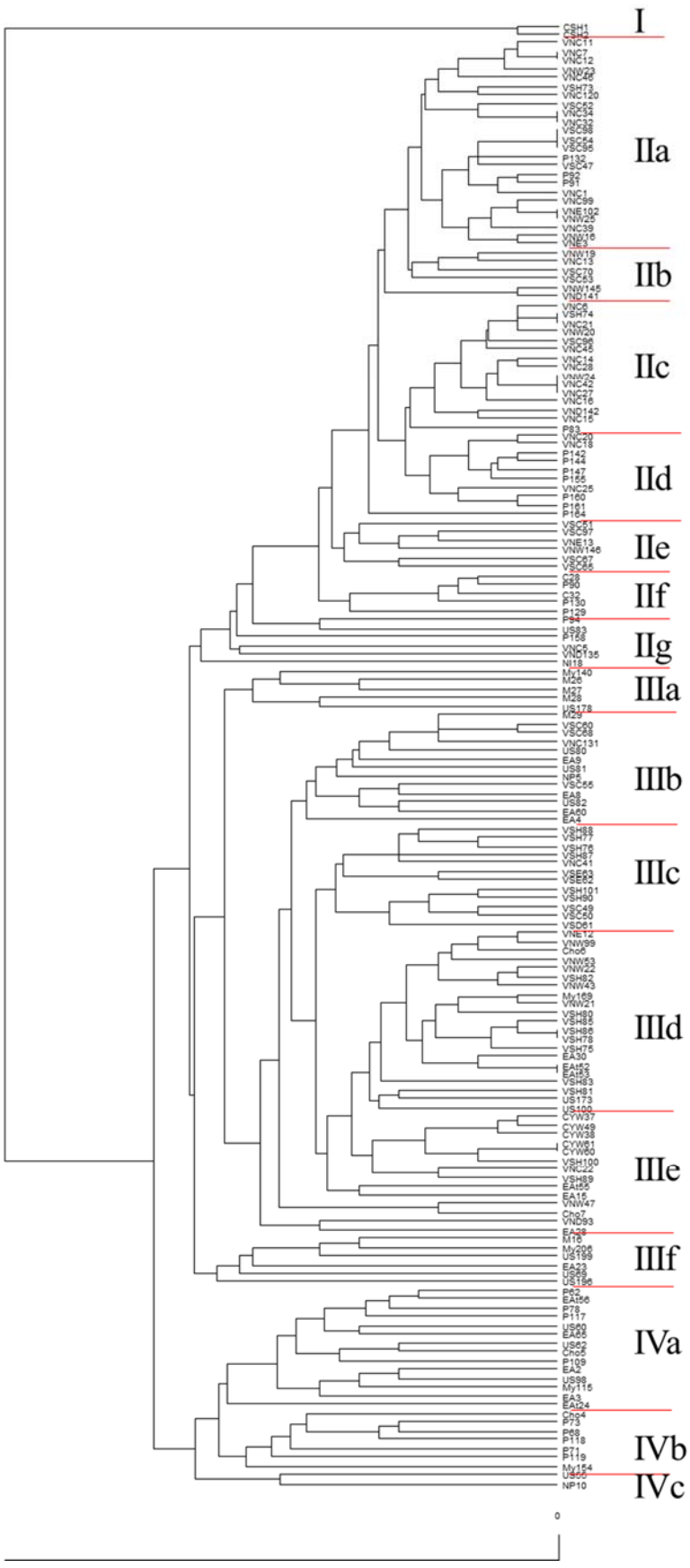


Fig 4.1. Genetic relationship between 168 studied accessions shown by UPGMA cluster analysis based on GD

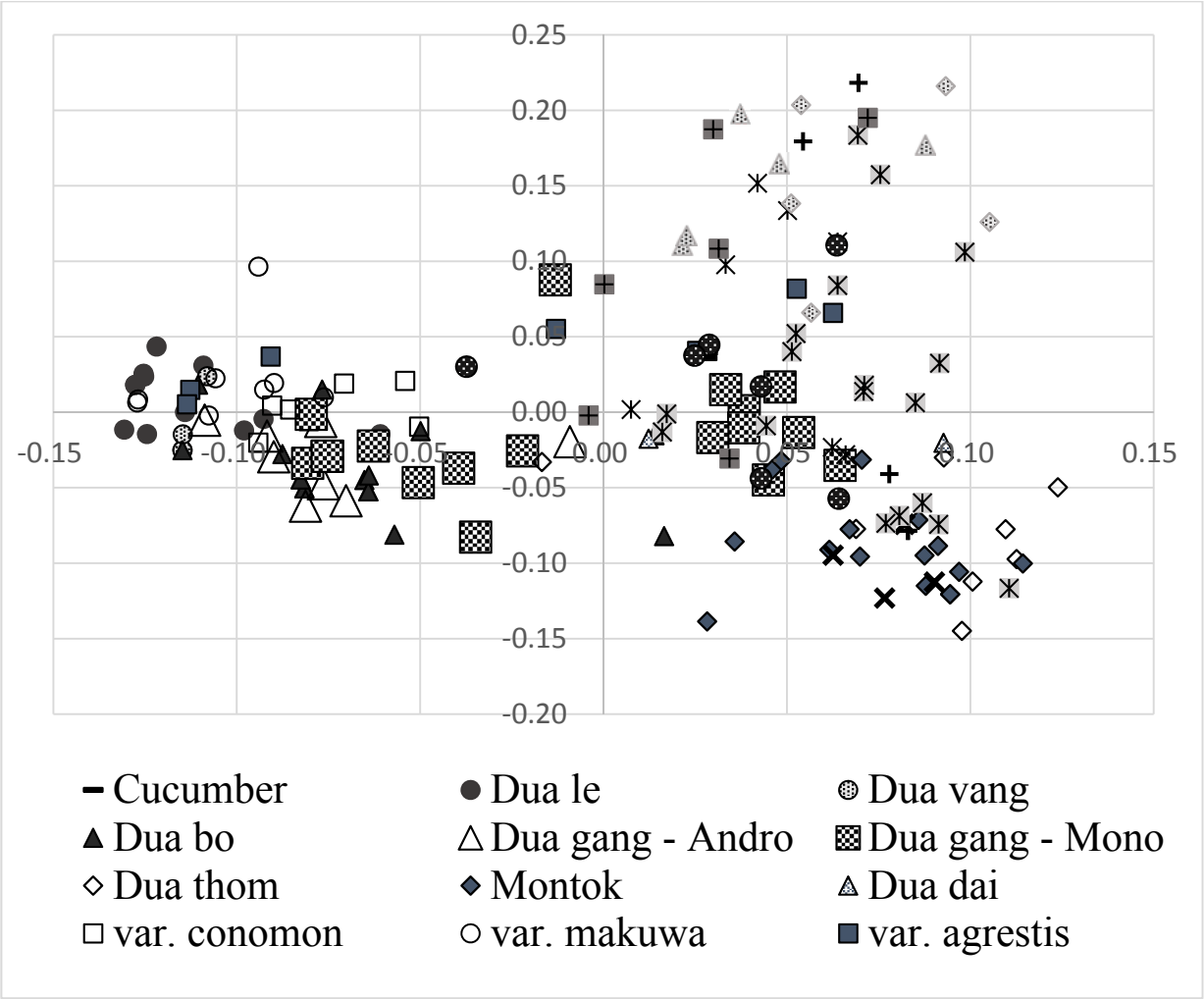


Fig 4.2. Distribution of the first two principal co-ordinates of 168 studied accessions

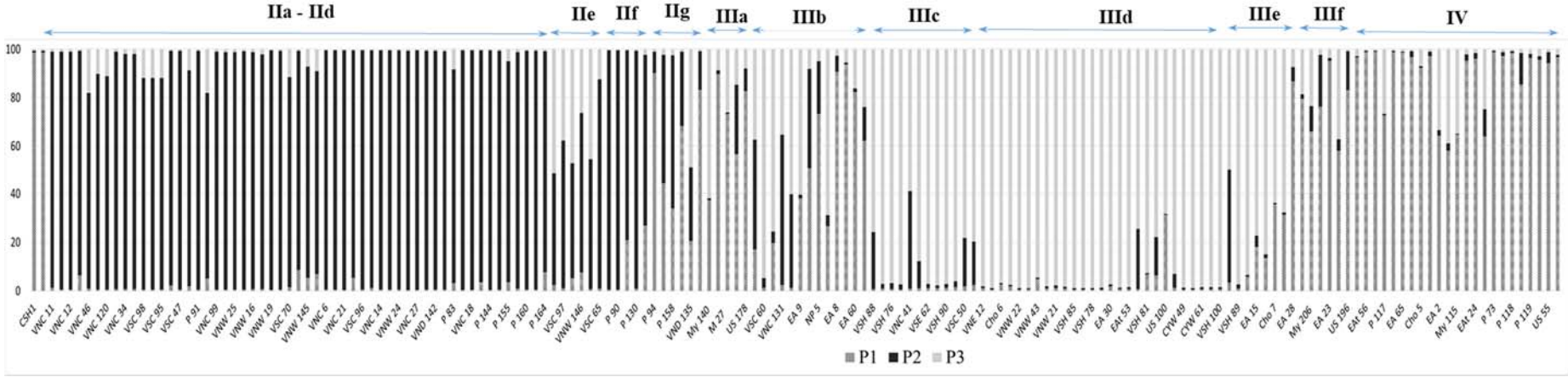


Fig 4.3. Bar plots for individual melon accessions by STRUCTURE using the admixture model based on 19 markers

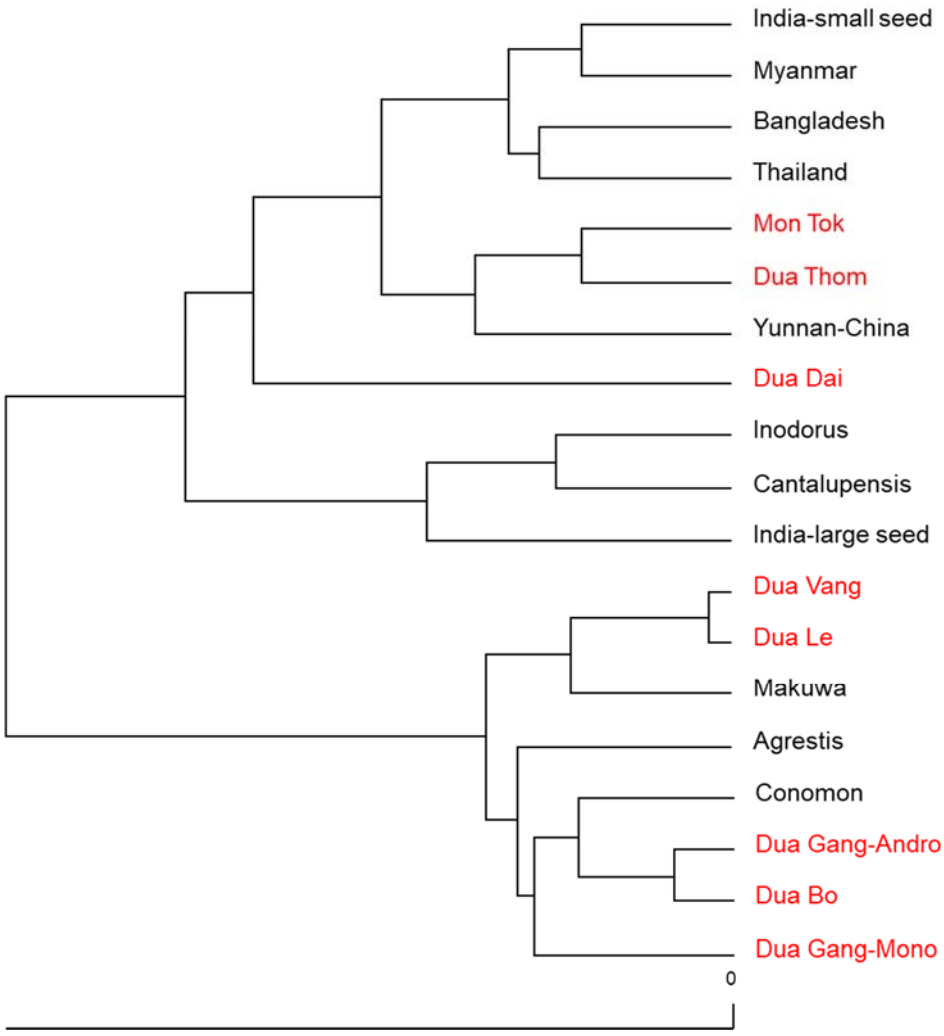


Fig 4.4. Genetic relationship between 19 groups of melon landraces, revealed by UPGMA cluster analysis based on GD

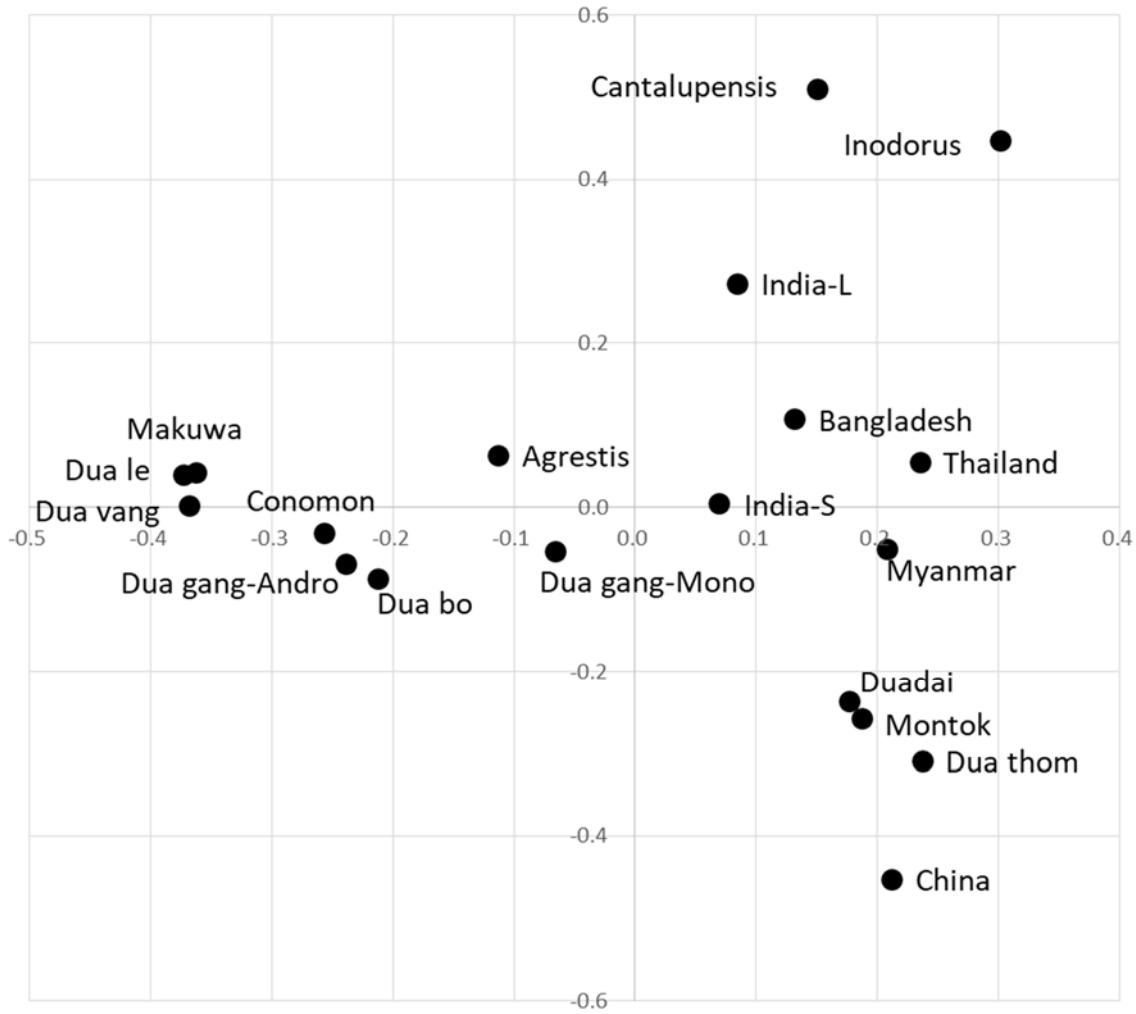


Fig 4.5. Distribution on the first two principal co-ordinates of 19 groups of melon

Chapter 5. General conclusion

Out of seven cultivar type of melon in Vietnam, six types were collected in central and southern Vietnam, “Dua le”, “Dua vang”, “Dua bo”, “Dua gang – Monoecious”, “Dua gang – Andromonoecious”, and “Montok. Only “Dua thom”, northern highland melon couldn’t be occurred in the field trip taken in 2014 and 2015. The morphological traits were different in these melon types. “Dua le” and “Dua vang” were characterized by higher Brix value and smaller fruit with round shape, and was used as dessert, as already reported by Nhi *et al.* (2010). In contrast, “Dua gang-andromonoecious” showed lower Brix value and larger fruit with oblong shape, and immature fruit was used as vegetable. Mature fruit of “Dua bo” was eaten as dessert, usually with sugar or condensed milk, though Brix value was not high. Although the usage was different, “Dua gang-andromonoecious” and “Dua bo” had similarity in texture of flesh which was mealy at mature stage. The analysis of genetic relationship with groups outside Vietnam revealed that these four groups, “Dua le”, “Dua vang”, “Dua bo”, and “Dua gang – Andromonoecious”, related very closely with vars. *conomon* and *makuwa*. Among them, “Dua le” and “Dua vang” were grouped in the same sub-cluster with var. *makuwa*, while “Dua bo” and “Dua gang-andromonoecious” were clustered with var. *conomon*. Nhi *et al.* (2010) suggested hybrid origin of “Dua bo” between var. *momordica* and var. *conomon*, since peeling skin or fruit splitting at the mature stage were commonly observed in these three groups. However, the result in this study was concluded that “Dua le” and “Dua vang” should be classified as var. *makuwa*, while “Dua bo” and “Dua gang-andromonoecious” as var. *conomon*.

Highland melon groups, “Dua thom” and “Montok”, showed similarity in fruit traits and usage as dessert, though immature fruit of “Montok” was sometimes used as vegetable. It was thus indicated that they can be regarded as the same group, in spite that they are grown in geographically distant areas by different groups of ethnic people. “Dua thom” and

"Montok" proved to be closely related with cultivar groups from Yunnan (China) to India-small, and shared the same traits of monoecious and sour flesh. It was therefore suggested that "Dua thom" and "Montok" had been introduced from west.

In overall, two distinct groups of Vietnamese melon landraces were described. One group was andromonoecious and genetically close to vars. *conomon* and *makuwa*, while the other was monoecious and related with melon landraces from India to Yunnan (China). Furthermore, "Dua gang-monoecious" was suggested as the hybrid origin between the two geographical groups , the presence of this inter-group hybrids contributed to enhance genetic diversity in Vietnamese melon.

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