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Genetic diversity in Vietnamese melon landraces revealed by the analyses of morphological traits and nuclear and cytoplasmic molecular markers

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Genetic diversity among 59 melon landraces from Vietnam was studied by analyzing morphological traits and molecular markers. The morphological characters of the melon landrace fruits were highly diversified. Among the five types of cultivated melon, "Dua le" and "Dua vang" were classified as Conomon var. *makuwa*, whereas "Dua gang" was classified as Conomon var. *conomon*, and "Dua bo" was classified as Momordica. However, "Dua thom" could not be classified into a proper group or variety. The gene diversity based on random amplified polymorphic DNA (RAPD) and single sequence repeat analyses was small and equivalent to that of Chinese Conomon. A cluster analysis revealed that "Dua bo", "Dua le", "Dua vang", and "Dua gang" were grouped in cluster II. Clusters III and IV consisted mainly of Conomon accessions from China and Japan. "Dua thom" was classified into cluster V with landraces from Yunnan Province, China. The comparison of a RAPD profile with 291 melon accessions from Africa and Asia clearly showed that "Dua thom" and Yunnanese landraces were closely related with the small-seed type melons from Myanmar, Bangladesh, and northeastern India. The other four types were related closely with Conomon and Agrestis accessions from China, Korea, and Japan, indicating their involvement in the differentiation and establishment of the Conomon group in East Asia.

Key Words: chloroplast genome, Conomon, *Cucumis melo*, genetic diversity, RAPD, SSR, Vietnam.

Introduction

Melon (*Cucumis melo* L.) is an important horticultural crop in tropical and subtropical regions and is also grown extensively in temperate climate countries (Pech *et al.* 2007). *Cucumis melo* is one of the most polymorphic species among the major cucurbit vegetables and has rather complicated systematics. Based on morphological characteristics, Naudin (1859) subdivided this species into ten groups. Munger and Robinson (1991) later reclassified the species into seven groups: Agrestis (wild melon), Flexuosus (snake melon), Conomon (pickling melon, Chinese white cucumber), Cantalupensis (cantaloupe or muskmelon), Inodorus (winter melon, honeydew, casaba), Chito (mango melon), Dudaim (Queen's pocket melon), and Momordica (Phoot or snap melon). Jeffrey (1980) proposed a division of *C. melo* into two subspecies according to the hairiness of the ovary: subsp. *agrestis* with short hairs found throughout India and eastern Asia and subsp. *melo* with long hairs found throughout India and central and western Asia, Europe, and the New World.

According to Fujishita (1983) and Akashi *et al.* (2002), seed length is highly variable among melon varieties, and melon groups can be classified into large-seed types (seed length ≥ 9.0 mm) and small-seed types (seed length < 9.0 mm). The former includes the groups Cantalupensis and Inodorus with sweet flesh, which are of commercial importance in the United States and Europe as well as in Mediterranean and Asian countries. The latter includes the groups Conomon and Agrestis with low sugar content and smooth skin, which are cultivated mainly in South and East Asia (Robinson and Decker-Walters 1997, Tanaka *et al.* 2007). The Conomon group is divided into var. *conomon* and var. *makuwa* which are cultivated in India, China, Korea, Japan, and Southeast Asia (Hammer *et al.* 1986). The fruit of var. *conomon* is neither sweet nor aromatic and is eaten raw as salad or pickled like cucumber. In contrast, the fruit of var. *makuwa* is sweet and fragrant when fully ripened and is eaten raw as a dessert. The Conomon group is an important genetic resource for disease resistance and is often utilized in melon breeding, as reviewed by Akashi et *al.* (2002).

Genetic diversity in melon has been analyzed using several types of molecular markers such as isozymes (Akashi *et*

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al. 2002, McCreight *et al.* 2004), amplified fragment length polymorphism (AFLP; Yashiro *et al.* 2005), random amplified polymorphic DNA (RAPD; Mliki *et al.* 2001, López-Sesé *et al.* 2003, Staub *et al.* 2004, Sensoy *et al.* 2007, Tanaka *et al.* 2007, Yi *et al.* 2009, Soltani *et al*. 2010), and simple sequence repeat (SSR; Monforte *et al.* 2003). Tanaka *et al.* (2007) conducted RAPD analysis of melon landraces from Asian countries and showed that the Japanese melon varieties *makuwa* and *conomon* are closely related with the small-seed type (< 9.0 mm) melon in east India. They suggested that the Conomon group vars. *makuwa* and *conomon* might be differentiated from the small-seed type melon in east India by its further eastward transmission. An analysis of melon landraces from Myanmar, sharing the border with India in the west and China in the east, revealed a genetic similarity among small-seed type accessions from India and Myanmar (Yi *et al.* 2009). These results highlight the importance of a germplasm diversity analysis of melon from Southeast Asia.

A crop's name often reflects its history. In the case of melon, the Conomon group var. *conomon* is called "Yue Gua" in Chinese. The Chinese character "Yue" represents Vietnam, and it is believed that "Yue Gua" was introduced to China from Vietnam. Vietnam shares a border with China in the north and with Laos and Cambodia in the west. Northern Vietnam is characterized by high and rugged mountains that are last parts of the Himalayan range. In this area, reflecting the geographical complexity, many kinds of indigenous crops, such as rice, maize, and cucumber are grown by ethnic minorities. Cucurbitaceae crops are considered to be one of the most important horticultural crops in Vietnam. Different types of landrace melons are also cultivated and are called "Dua thom" (melon with aroma), "Dua bo" (melon with powdery flesh), "Dua vang" (melon with yellow skin), "Dua le" (round shape, white epicarp color generally), and "Dua gang" (elongated fruit) depending on their fruit characteristics. The former four types are used as a dessert. In contrast, "Dua gang" is mainly used as a vegetable and looks quite similar to the Japanese "Shirouri", which is classified in the Conomon group var. *conomon*. Besides these, weedy melon also grows in Vietnam and is called "Dua dai". However, irrespective of their importance as genetic resources for disease resistance (Daryono *et al.* 2003) and wet tolerance (Akashi *et al.* 2002), less attention has been paid to the Vietnamese melon and little is known about their genetic diversity and their relationship with melon landraces of the surrounding countries.

Therefore, in the present study, the genetic diversity among melon landraces collected in Vietnam was studied by analyzing the morphological traits of the fruit. The melon landraces were also analyzed with RAPD and SSR markers to uncover genetic diversity in the nuclear genome. For the cytoplasm genome analysis, a single nucleotide polymorphism (SNP) in the plastid subtype ID sequence (PS-ID), the linker sequence between the genes *rpl16* and *rpl14* (Nakamura *et al.* 1997), and the consensus chloroplast SSR marker (ccSSR7) were employed. On the basis of these results, the genetic relationship with melon landraces from other regions of the world, especially the Conomon group, is also discussed.

Materials and Methods

Plant materials

A total of 86 accessions of melon (*C. melo* L.) were used in this study, including landraces from Vietnam (59 accessions) and China (Yunnan Province; five accessions, the Conomon group from east China; 14 accessions), and reference accessions from Japan (groups Conomon [2] and Cantalupensis [1]), the US (groups Cantalupensis [1] and Inodorus [1]), Italy (group Cantalupensis [1]), Spain (group Inodorus [1]), and Russia (group Inodorus [1]) (Table 1). Vietnamese melon landraces were collected from 18 provinces mainly in northern and central Vietnam (Fig. 1). As mentioned above, five types of cultivated melon, "Dua thom", "Dua bo", "Dua vang", "Dua le", and "Dua gang" as well as weedy melon (Agrestis group, "Dua dai") are known in Vietnam; this study examined 20, 15, 2, 8, 13, and 1 accessions of these melon types, respectively.

Evaluation of morphological traits

Length and width of the three representative seeds were

Fig. 1. Map of Vietnam. Melon landraces were collected from the 18 provinces indicated.

Table 1. List of melon accessions analyzed in this study

Accession No.	Province/Cultivar	Country of origin	Group/Variety[a]	Melon type	Seed			Sex Expression[b]	Chloroplast marker		Cluster[d]
					Class	Length (mm)	Width (mm)		PS-ID	ccSSR7	
Vietnamese melon landraces											
VN 3	Cao Bang	Vietnam	Momordica	Dua bo	small	7.3	3.6	A	A	338 bp	IIc
VN 16	Hoa Binh	Vietnam	Momordica	Dua bo	small	7.5	3.1	A	A	338 bp	IIc
VN 23	Son La	Vietnam	Momordica	Dua bo	small	6.5	2.8	A	A	338 bp	IIf
VN 24	Son La	Vietnam	Momordica	Dua bo	small	7.8	2.8	A	A	338 bp	IIe
VN 25	Son La	Vietnam	Momordica	Dua bo	small	8.1	3.4	A	A	338 bp	IIc
VN 102	Lang Son	Vietnam	Momordica	Dua bo	small	7.8	3.3	A	A	338 bp	IIc
VN 145	Dien Bien	Vietnam	Momordica	Dua bo	small	6.5	3.0	A	A	338 bp	IIf
VN 146	Dien Bien	Vietnam	Momordica	Dua bo	small	7.9	3.0	A	A	338 bp	IIc
VN 147	Dien Bien	Vietnam	Momordica	Dua bo	small	7.5	3.1	A	A	338 bp	IIc
VN 170	Ha Tinh	Vietnam	Momordica	Dua bo	small	7.3	3.1	A	A	338 bp	IIc
VN 171	Ha Tinh	Vietnam	Momordica	Dua bo	small	7.5	3.5	A	A	338 bp	IIc
VN 175	Nghe An	Vietnam	Momordica	Dua bo	small	7.7	3.5	A	A	338 bp	IIc
VN 176	Nghe An	Vietnam	Momordica	Dua bo	small	8.2	3.5	A	A	338 bp	IIc
VN 178-1	Ninh Binh	Vietnam	Momordica	Dua bo	small	7.9	3.5	A	A	338 bp	IIc
VN 178-2	Ninh Binh	Vietnam	Momordica	Dua bo	small	6.1	2.8	A	A	338 bp	IIc
VN 135	Hung Yen	Vietnam	Agrestis	Dua dai	small	4.2	2.1	M	A	338 bp	I
VN 115	Thua Thien Hue	Vietnam	Conomon var. *conomon*	Dua gang	small	7.2	3.2	A	A	338 bp	IIc
VN 117	Thua Thien Hue	Vietnam	Conomon var. *conomon*	Dua gang	small	7.7	3.3	A	A	338 bp	IIc
VN 118	Thua Thien Hue	Vietnam	Conomon var. *conomon*	Dua gang	small	7.8	3.3	A	A	338 bp	IIc
VN 120	Quang Tri	Vietnam	Conomon var. *conomon*	Dua gang	small	7.1	3.4	A	A	338 bp	IIe
VN 130	Thua Thien Hue	Vietnam	Conomon var. *conomon*	Dua gang	small	7.9	3.6	A	A	338 bp	IIc
VN 131	Thanh Hoa	Vietnam	Conomon var. *conomon*	Dua gang	small	6.8	3.0	A	A	338 bp	IIb
VN 133	Thua Thien Hue	Vietnam	Conomon var. *conomon*	Dua gang	small	6.6	3.1	A	A	338 bp	IId
VN 134	Thua Thien Hue	Vietnam	Conomon var. *conomon*	Dua gang	small	7.8	3.7	A	A	338 bp	IId
VN 137	Quang Nam	Vietnam	Conomon var. *conomon*	Dua gang	small	6.7	3.2	A	A	338 bp	IIc
VN 138	Quang Nam	Vietnam	Conomon var. *conomon*	Dua gang	small	7.8	3.2	A	A	338 bp	IIc
VN 139	Phu Yen	Vietnam	Conomon var. *conomon*	Dua gang	small	8.5	3.3	M	A	338 bp	IIa
VN 173	Ha Tinh	Vietnam	Conomon var. *conomon*	Dua gang	small	7.2	3.4	A	A	338 bp	IIc
VN 177	Nghe An	Vietnam	Conomon var. *conomon*	Dua gang	small	8.0	3.7	A[c]	A	338 bp	IIc
VN 20	Son La	Vietnam	Conomon var. *makuwa*	Dua le	small	5.9	2.5	A	A	338 bp	IIe
VN 111	Thua Thien Hue	Vietnam	Conomon var. *makuwa*	Dua le	small	6.6	3.0	A	A	338 bp	IIe
VN 113	Thua Thien Hue	Vietnam	Conomon var. *makuwa*	Dua le	small	5.2	2.9	A	A	338 bp	IIe
VN 123	Thua Thien Hue	Vietnam	Conomon var. *makuwa*	Dua le	small	5.2	2.7	A	A	338 bp	IIf
VN 140	Phu Yen	Vietnam	Conomon var. *makuwa*	Dua le	small	6.2	2.8	A	A	338 bp	IId
VN 141	Hai Duong	Vietnam	Conomon var. *makuwa*	Dua le	small	6.2	2.5	A	A	338 bp	IIf
VN 172	Ha Tinh	Vietnam	Conomon var. *makuwa*	Dua le	small	6.4	3.0	A	A	338 bp	IId
VN 174	Ha Tinh	Vietnam	Conomon var. *makuwa*	Dua le	small	6.3	3.0	A	A	338 bp	IId
VN 9	Cao Bang	Vietnam	—	Dua thom	small	6.9	2.8	M	A	338 bp	Vb
VN 11	Bac Kan	Vietnam	—	Dua thom	small	6.1	2.9	M	A	338 bp	Vb
VN 12	Bac Kan	Vietnam	—	Dua thom	small	7.0	2.8	M	A	338 bp	Vb
VN 13	Tuyen Quang	Vietnam	—	Dua thom	small	7.9	3.4	M	A	338 bp	Va
VN 21	Son La	Vietnam	—	Dua thom	small	7.1	3.1	M	A	338 bp	Vb
VN 22	Son La	Vietnam	—	Dua thom	small	6.5	3.0	M	A	338 bp	Vb
VN 26	Son La	Vietnam	—	Dua thom	small	7.1	3.0	M	A	338 bp	Vb
VN 32	Son La	Vietnam	—	Dua thom	small	7.5	3.3	M	A	338 bp	Vb
VN 33	Son La	Vietnam	—	Dua thom	small	7.1	3.2	M	A	338 bp	Vb
VN 35	Son La	Vietnam	—	Dua thom	small	7.3	2.8	M[c]	A	338 bp	Vb
VN 36	Son La	Vietnam	—	Dua thom	small	6.6	3.2	M	A	338 bp	Vb
VN 43	Son La	Vietnam	—	Dua thom	small	6.4	3.0	M	A	338 bp	Vb
VN 44	Son La	Vietnam	—	Dua thom	small	7.3	3.0	M	A	338 bp	Vb
VN 47	Dien Bien	Vietnam	—	Dua thom	small	6.9	3.1	M	A	338 bp	Vb
VN 53	Son La	Vietnam	—	Dua thom	small	7.3	3.1	M	A	338 bp	Vb
VN 56	Son La	Vietnam	—	Dua thom	small	7.2	3.1	M	A	338 bp	Vb

Table 1. (continued)

Accession No.	Province/Cultivar	Country of origin	Group/Variety[a]	Melon type	Seed			Sex Expression[b]	Chloroplast marker		Cluster[d]
					Class	Length (mm)	Width (mm)		PS-ID	ccSSR7	
VN 57	Son La	Vietnam	—	Dua thom	small	6.8	2.9	M	A	338 bp	Vb
VN 66	Son La	Vietnam	—	Dua thom	small	7.4	3.6	M	A	338 bp	Vb
VN 99	Lao Cai	Vietnam	—	Dua thom	small	6.6	3.0	M	A	338 bp	Vb
VN 160	Dien Bien	Vietnam	—	Dua thom	small	6.8	3.0	M	A	338 bp	Vb
VN 19	Son La	Vietnam	Conomon var. *makuwa*	Dua vang	small	7.3	2.6	A	A	338 bp	IIe
VN 142	Hai Duong	Vietnam	Conomon var. *makuwa*	Dua vang	small	5.3	2.5	A	A	338 bp	IId
Chinese melon landraces											
CYW 37	Yunnan	China	—	—	small	7.0	3.0	M[c]	A	338 bp	Vc
CYW 38	Yunnan	China	—	—	small	8.0	3.0	M	A	338 bp	Vc
CYW 49	Yunnan	China	—	—	small	8.0	3.5	M[c]	A	338 bp	Vc
CYW 60	Yunnan	China	—	—	small	7.0	3.0	M	A	338 bp	Vc
CYW 61	Yunnan	China	—	—	small	7.0	3.0	M	A	338 bp	Vc
P 83	Mitanggang	China	Conomon var. *makuwa*	—	small	7.4	3.4	A	A	338 bp	IIIa
P 142	Damiangua	China	Conomon var. *makuwa*	—	small	7.0	4.0	A	A	338 bp	IIIb
P 143	Shidaodaqinggua	China	Conomon var. *makuwa*	—	small	8.0	3.0	A	A	338 bp	IIIa
P 144	Shilinghuangjingua	China	Conomon var. *makuwa*	—	small	6.0	3.0	A	A	338 bp	IIIa
P 147	Wengua	China	Conomon var. *makuwa*	—	small	7.0	3.0	A	A	338 bp	IIIb
P 153	Chi-86-56	China	Conomon var. *makuwa*	—	small	7.5	3.5	A	A	338 bp	IIIb
P 155	Qianzhong-5	China	Conomon var. *makuwa*	—	small	7.5	3.5	A	A	338 bp	IIIb
P 208	Chi-87-12	China	Conomon var. *makuwa*	—	small	7.0	3.0	A	A	338 bp	IV
C 28	Xingtangmiangua	China	Conomon var. *makuwa*	—	small	7.0	3.0	A	A	338 bp	IV
C 32	Heipilengzisudigua	China	Conomon var. *conomon*	—	small	7.0	3.5	A	A	338 bp	IV
P 154	Chi-86-61	China	Conomon var. *conomon*	—	small	7.1	3.5	A	A	338 bp	IIIb
P 158	Qingpilürouxianggua	China	Conomon var. *conomon*	—	small	7.0	4.0	A	A	338 bp	IIIb
Tan 4	Caigua	China	Conomon var. *conomon*	—	small	8.0	3.0	A	A	338 bp	IIIa
Tan 6	Qingpicaigua	China	Conomon var. *conomon*	—	small	8.0	3.0	A	A	338 bp	IIIa
Reference accessions											
P 90	Kinpyo	Japan	Conomon var. *conomon*	—	small	6.0	3.0	A	A	338 bp	IV
P 130	Karimori	Japan	Conomon var. *conomon*	—	small	6.0	3.0	A	A	338 bp	IV
P 62	Earl's Favourite	Japan	Cantalupensis	—	large	10.0	5.0	A	T	338 bp	VI
P 68	Homegarden	USA	Cantalupensis	—	large	11.2	4.6	A	T	333 bp	VI
P 94	Charentais[e]	Italy	Cantalupensis	—	large	10.2	4.5	A	T	338 bp	VI
P 73	Honey Dew	USA	Inodorus	—	large	11.5	5.2	A	T	333 bp	VI
P 117	Tendral	Spain	Inodorus	—	large	9.6	4.3	A	T	333 bp	VI
P 118	Kokand	Russia	Inodorus	—	large	10.0	4.9	A	T	333 bp	VI

[a] —; Horticultural group/variety was not specified as shown in the text.
[b] Sex expression type determined by CAPS analysis is indicated by: A Andromonoecious; M Monoecious.
[c] Determined only by the flower morphology.
[d] Cluster number shown in Fig. 4.
[e] Melon Cantalupo di Charentais.

measured for all accessions, which were then classified into a large-seed type (≥ 9.0 mm) and a small-seed type (< 9.0 mm), according to Akashi *et al.* (2002) (Table 1). Qualitative and quantitative traits of the fruits were measured for 34 melon fruits, obtained from local markets in Vietnam, from which we harvested the original seed samples of 34 melon accessions listed in Table 4. Table 4 also summarizes the seven traits assessed in this study: (1) weight, (2) length, (3) diameter of fruit, (4) color of the exocarp skin, (5) color of fruit flesh, (6) color of placenta, and (7) soluble solid content (Brix grade).

DNA extraction

Seeds of each accession were sown on wet filter paper in a Petri dish and germinated in an incubator maintained at 30°C with a 16 h light and 8 h dark cycle at a light intensity of 46.5 $\mu M\ s^{-1}\ m^{-2}$. After 2 weeks, cotyledons from one seedling of each accession were ground individually in liquid nitrogen. Total DNA was extracted using the cetyl-trimethyl-ammonium bromide method (Murray and Thompson 1980) with minor modifications. The quality and quantity of each DNA sample were determined with a spectrophotometer.

Cleaved amplified polymorphic sequence (CAPS) analysis for sex expression type

According to Boualem *et al.* (2008), the difference between the andromonoecious type and the monoecious type is caused by a SNP in *CmAcs7* gene, and they established a CAPS marker to distinguish the two types. Therefore, the sex expression type was determined for 86 melon accessions using their primer set: Alu1CAPS_F (5′-ACATTCAAT TCAACAAATCTTCAGTTC-3′) and Alu1CAPS_R (5′-GGGTATAGTAATTACAGTAAAGAGTGG-3′).

The PCR conditions for the CAPS analysis were as follows: a 10 µl mixture containing 50 ng genomic DNA, 1 µl PCR buffer (Sigma, St. Louis, MO, USA: 10 mM Tris-HCl; pH 8.3, 50 mM KCl), 2.5 mM $MgCl_2$, 0.1 mM dNTP, 0.25 µM of each primer, and 0.25 U *Taq* polymerase (Sigma). Amplification reactions were performed using i-Cycler (Bio-Rad, Hercules, CA, USA). The PCR cycle was as follows: an initial denaturing step at 95°C for 3 min, 35 cycles at 95°C for 1 min, 57°C for 1 min, and 72°C for 2 min. The final extension step was at 72°C for 5 min. PCR products were digested with *Alu* I (New England BioLabs, Ipswich, MA, USA), and then electrophoresed on a 1.5% agarose gel (GenePure LE, BM Bio, Tokyo, Japan) at a constant voltage of 100 V using a horizontal gel electrophoresis system (Mupid-2, Cosmo Bio, Tokyo, Japan). Gels were stained with ethidium bromide and visualized by illumination with UV light.

Chloroplast genome analysis

A SNP (A/T) in the PS-ID sequences was analyzed using dCAPS primers. The nucleotide sequence of each primer was as follows: Psid2F—5′-AAAAAAAAACAATTGCA GATTRAATT-3′ and Psid1R—5′-AGCATTTAAAAG GGTCTGAGGT-3′ (R = A or G). The PCR mixture and PCR cycle were the same as for the CAPS analysis of *CmAcs7*, but annealing was done at 52°C for 2 min. The PCR products were digested with *Apo* I (New England BioLabs), and then electrophoresed on a 3% agarose gel at a constant voltage of 100 V. The gels were stained as described above.

As another marker of the chloroplast genome, the ccSSR7 consensus chloroplast SSR marker (Chung and Staub 2003) was employed in this study, because Tanaka *et al.* (2006) reported that ccSSR7 was polymorphic following a 5 bp deletion (ATATT). The nucleotide sequence of each primer was ccSSR7-F 5′-CGGGAAGGGCTCGKGCAG-3′ and ccSSR7-R 5′-GTTCGAATCCCTCTCTCTCCTTTT-3′ (K = T or G). The PCR mixture and PCR cycle was the same as the CAPS analysis of *CmAcs7*, but annealing was done at 56°C for 1 min. The PCR products were electrophoresed on a 10% nondenatured polyacrylamide gel at a constant voltage of 260 V. The gels were stained as described above.

RAPD analysis

Eighteen random primers (12-mer, Bex, Tokyo, Japan), which were selected for their ability to detect polymorphisms by Tanaka *et al.* (2007), were used in this study (Table 2). The PCR mixture was the same as the CAPS analysis of *CmAcs7*, but the primer concentration was changed to 0.5 µM. The PCR cycle was as follows: an initial denaturing step at 95°C for 3 min, 40 cycles at 93°C for 1 min, 40°C for 2 min, and 72°C for 2 min. The final extension step was at 72°C for 5 min. After amplification, electrophoresis and gel staining were conducted in the same way as for the CAPS analysis of *CmAcs7*.

Table 2. Eighteen random primers used in this study and the size of polymorphic fragments

Primer number	Sequence (5′ → 3′)	Size of polymorphic fragments (bp)
A07	GATGGATTTGGG	872
A20	TTGCCGGGACCA	1100, 800
A22	TCCAAGCTACCA	1520
A23	AAGTGGTGGTAT	1200*
A26	GGTGAGGATTCA	1400*
A31	GGTGGTGGTATC	800
A39	CCTGAGGTAACT	2027*
A41	TGGTAGGTAACT	930*
A57	ATCATTGGCGAA	800*
B15	CCTTGGCATCGG	600*
B32	ATCATCGTACGT	900, 700*
B68	CACACTCGTCAT	1078
B71	GGACCTCCATCG	1220
B84	CTTATGGATCCG	700*, 600*, 550*
B86	ATCGAGCGAACG	1500*, 1350*
B96	CTGAAGACTATG	850*, 750*
B99	TTCTGCTCGAAA	1400*
C00	GAGTTGTATGCG	1350*

*; No polymorphism among 59 Vietnamese melon landraces.

SSR analysis

Sixteen SSR markers exhibiting distinct and stable polymorphisms selected by Aierken *et al.* (2010) were used for the analysis of Vietnamese melon landraces (Table 3). These SSR markers were developed by Akashi *et al.* (2001), Ritschel *et al.* (2004), and Fukino *et al.* (2007). The protocol for the ccSSR7 analysis was applied for the SSR analysis.

Data analysis

The RAPD marker band was scored as 1 for present and 0 for absent. The marker fragments of SSR were scored based on their size from the smallest (1) to the largest. From these data, 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. The gene diversity (D) within each group and genetic distance (GD) among groups were calculated as described by Weir (1996) and Nei (1972), respectively. A dendrogram was constructed by the PHYLIP program using the unweighted pair group method with arithmetic mean (UPGMA) method.

Table 3. Primer sequences of 16 SSR markers used in this study and the expected size of PCR products

Marker	F primer (5′ → 3′)	R primer (5′ → 3′)	Expected fragment size (bp)
ACS2-ms1	TCTTTTGTTCTTGGTTGTGAGT	GATTGCTTTATTTTGAATCTTTTG	200
CMBR 2	TGCAAATATTGTGAAGGCGA	ATCCCCACTTGTTGGTTTG	114
CMBR 12	ACAAACATGGAAATAGCTTTCA	GCCTTTTGTGATGCTCCAAT	134
CMBR 22	TCCAAAACGACCAAATGTTCC	ATACAGACACGCCTTCCACC	177
CMBR 53	GCCTTTTGTGATGCTCCAAT	AAACAAACATGGAAATAGCTTTCA	134
CMBR 83	CGGACAAATCCCTCTCTGAA	GAACAAGCAGCCAAAGACG	142
CMBR 105	TGGTAAGCATTTTGAAATCACTTTT	TTCCAGACATCTAAAGGCATTG	139
CMBR 120	CTGGCCCCCTCCTAAACTAA	CAAAAAGCATCAAAATGGTTG	167
CMN 04-03	ATCACAGAGACCGCCAAAAC	GGTTGAAGATTGCGCTTGAT	218
CMN 04-07	GAAAGCATTAAATATGGCATTGG	AAGCTTAACAGCTTCCAGGG	286
CMN 04-40	CACCTGACGATAGGGGTGTT	AGTATTCGGGTTGGCAAAAA	212
CMN 08-22	CATCCTCCTCATCCTCCTCA	ACGGATGAATCGGAACTTCA	223
CMN 08-90	CCACGCCCTCTATACCCATA	GGGACTGTTGGGTTTTCTGA	210
CMN 21-41	GAGGAAATTTTGGAGTTTTTCAA	TTCCAGACATCTAAAGGCATTG	281
CMN 22-16	CAGAGGAGGTGGAACTAACCA	CCATTTTCAACCTCCCAAGA	233
CMN 61-44	TGTTGGAGTTTAATGAGGAAGGA	AGAGAAGATGAATGGGGCAC	233

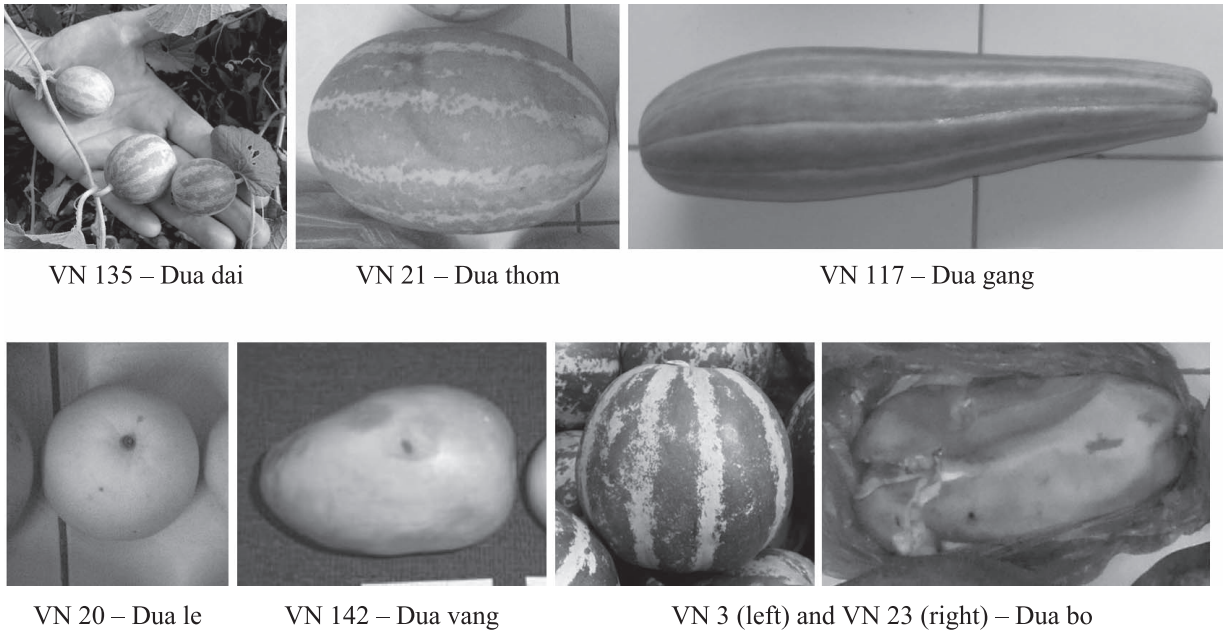

Fig. 2. Photographs of typical examples of six types of Vietnamese melon landraces.

Results

Morphological characteristics of fruit samples

The morphological characteristics of the fruit and the Brix grade of flesh juice measured in 34 fruit samples obtained at the local market in Vietnam are summarized in Table 4. Photographs of typical fruits are shown for each melon type in Figure 2. All of the landraces had smooth skin and were classified as small-seed type (5.2–8.1 mm). A comparison among the four melon types, except "Dua vang" in which only two accessions were examined, showed that "Dua le" had smaller seeds ($P \leq 0.01$) (Table 5). Fruit weight ranged from 160 to 2700 g among accessions, and it was clear that "Dua thom" had heavier fruit ($P \leq 0.01$). The height and diameter of the fruit and their ratio also differed among melon types ($P \leq 0.01$). "Dua gang" and "Dua thom" had elongated and oblong fruits, respectively, whereas "Dua bo" (except VN 23) and "Dua le" had round-shaped fruits. Some "Dua thom" accessions had orange-colored exocarp skin and/or flesh, while such fruits were not found in other types. Most of the "Dua thom" accessions had orange-colored placenta, whereas white was predominant in "Dua gang" and "Dua le". The Brix degree ranged from 2° to 7° among accessions, and the difference among melon types was statistically significant ($P \leq 0.01$). The highest value was observed in "Dua le" (5.9°), followed by "Dua thom" (4.3°), "Dua gang" (3.1°), and "Dua bo" (2.7°). Flesh texture was mealy in all accessions of "Dua bo" and in half of the

Table 4. Seed size, fruit characters and sex expression of 34 melon landraces collected in Vietnam

Accession No.	Seed		Fruit			Color of[a]			Brix (°)	Cluster[b]	Group/Variety[c]	Melon type
	Length (mm)	Width (mm)	Weight (g)	Length (cm)	Diameter (cm)	Exocarp skin	Flesh	Placenta				
VN 3	7.3	3.6	1550	13.0	15.0	Y (G stripe)	YW	O	2.0	IIc	Momordica	Dua bo
VN 16	7.5	3.1	1600	14.0	16.0	G (GY stripe)	GY	O	4.0	IIc	Momordica	Dua bo
VN 23	6.5	2.8	700	20.0	10.0	Y	W	O	3.0	IIf	Momordica	Dua bo
VN 24	7.8	2.8	800	14.0	14.0	YG	W	O	3.0	IIe	Momordica	Dua bo
VN 25	8.1	3.4	800	12.0	12.0	G (GY stripe)	W	O	3.5	IIc	Momordica	Dua bo
VN 145	6.5	3	300	10.0	8.5	Y (G stripe)	W	W	2.0	IIf	Momordica	Dua bo
VN 146	7.9	3	340	9.5	9.0	Y (G stripe)	GW	W	2.0	IIc	Momordica	Dua bo
VN 147	7.5	3.1	300	6.5	8.5	Y (G stripe)	GW	W	2.0	IIc	Momordica	Dua bo
VN 115	7.2	3.2	370	20.0	6.0	GW (rib)	W	W	4.0	IIc	Conomon var. *conomon*	Dua gang
VN 117	7.7	3.3	750	32.0	8.0	GW (rib)	W	W	2.5	IIc	Conomon var. *conomon*	Dua gang
VN 118	7.8	3.3	630	26.0	8.0	GW (rib)	W	W	3.0	IIc	Conomon var. *conomon*	Dua gang
VN 120	7.1	3.4	170	16.5	4.5	GW	W	W	3.0	IIe	Conomon var. *conomon*	Dua gang
VN 20	5.9	2.5	300	8.0	9.0	W	W	O	6.5	IIe	Conomon var. *makuwa*	Dua le
VN 111	6.6	3.0	470	9.0	10.0	WG	W	W	7.0	IIe	Conomon var. *makuwa*	Dua le
VN 113	5.2	2.9	250	7.0	8.0	W	W	W	7.0	IIe	Conomon var. *makuwa*	Dua le
VN 141	6.2	2.5	400	13.0	8.0	Y	W	W	3.0	IIf	Conomon var. *makuwa*	Dua le
VN 19	7.3	2.6	800	12.0	12.0	Y	W	O	3.0	IIe	Conomon var. *makuwa*	Dua vang
VN 142	5.3	2.5	160	5.5	6.5	WG	WG	W	7.0	IId	Conomon var. *makuwa*	Dua vang
VN 11	6.1	2.9	1600	20.0	12.0	Y (W stripe)	GY	W	4.0	Vb	—	Dua thom
VN 12	7.0	2.8	1800	21.0	13.0	G (W stripe)	GY	W	5.0	Vb	—	Dua thom
VN 21	7.1	3.1	2000	21.0	15.0	Y (W stripe)	G	O	4.0	Vb	—	Dua thom
VN 22	6.5	3.0	1000	13.0	12.0	OY (W stripe)	W	O	4.0	Vb	—	Dua thom
VN 26	7.1	3.0	1700	19.0	14.0	OY (W stripe)	O	O	4.0	Vb	—	Dua thom
VN 32	7.5	3.3	2700	24.0	15.0	WG (W stripe)	O	O	3.0	Vb	—	Dua thom
VN 33	7.1	3.2	1800	17.0	15.0	O	OG	O	4.0	Vb	—	Dua thom
VN 35	7.3	2.8	1300	17.0	12.0	G (WY stripe)	G	O	4.0	Vb	—	Dua thom
VN 36	6.6	3.2	1100	12.0	13.0	W	W	W	4.5	Vb	—	Dua thom
VN 43	6.4	3.0	1500	17.0	13.0	WY	W	O	5.1	Vb	—	Dua thom
VN 44	7.3	3.0	1600	21.0	13.0	G (W stripe)	OG	O	4.0	Vb	—	Dua thom
VN 47	6.9	3.1	800	12.0	11.0	OY (W stripe)	GO	O	4.0	Vb	—	Dua thom
VN 53	7.3	3.1	1300	20.0	13.0	GO	OG	O	4.0	Vb	—	Dua thom
VN 56	7.2	3.1	1900	21.0	14.0	G (W stripe)	OG	O	4.0	Vb	—	Dua thom
VN 57	6.8	2.9	1300	19.0	12.0	OY (W stripe)	OG	O	5.0	Vb	—	Dua thom
VN 66	7.4	3.6	1000	10.5	11.5	OG (W stripe)	G	Y	6.0	Vb	—	Dua thom

[a] Colors are indicated by: G green; O orange; Y yellow; W white. Intermediate colors are indicated by the combination of two characters, with the first character representing the predominant color.

[b] Cluster number shown in Fig. 4.

[c] —; Horticultural group/variety was not specified as shown in the text.

Table 5. The average performance of five types of Vietnamese melon landraces

Melon type	No. of accessions	Seed[a]		Fruit[a]				Brix[a] (°)	Sex Expression[b]	Group/Variety[c]
		Length (mm)	Width (mm)	Weight (g)	Length (cm)	Diameter (cm)	Shape			
Dua bo	8	7.4 b	3.2 bc	799 a	12.4 a	11.6 bc	1.09 a	2.7 a	A	Momordica
Dua gang	4	7.5 b	3.3 c	480 a	23.6 b	6.6 a	3.56 b	3.1 ab	A	Conomon var. *conomon*
Dua le	4	6.0 a	2.8 a	355 a	9.3 a	8.8 ab	1.07 a	5.9 c	A	Conomon var. *makuwa*
Dua vang	2	6.3	2.6	480	8.8	9.3	0.92	5.0	A	Conomon var. *makuwa*
Dua thom	16	7.0 b	3.1 ab	1525 b	17.8 ab	13.0 c	1.36 a	4.3 bc	M	—

[a] Mean values with the same letter indicate non-significant differences at 0.01 level by Tukey-Kramer test. “Dua vang” was not included for statistical analysis, since only two accessions were analyzed.

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

[c] —; Horticultural group/variety was not specified as shown in the text.

"Dua thom" accessions. Fruit skin crack, a typical trait of the Momordica group, was observed in one accession of "Dua bo" (VN 23).

CAPS analysis for sex expression type

The expected size (772 or 777 bp) of the PCR products was amplified in 57 accessions, whereas amplification was not successful in two accessions (VN 35 and VN 177). After the *Alu* I digestion, four fragments of 116, 137, 197, and 327 bp were detected in 21 accessions (Fig. 3A). These accessions were regarded as monoecious, according to Boualem *et al.* (2008). Thirty-six accessions, of which the PCR product was digested into 116, 137, and 519 bp, were regarded as andromonoecious. The sex expression type of VN 35 and VN 177 was confirmed by observing flower morphology in the greenhouse (Table 1).

Sex expression type proved to be clearly different among melon types. All "Dua thom" accessions were monoecious, while all accessions of other types except VN 139 were andromonoecious. Eight reference accessions and most of the Conomon accessions from China were andromonoecious, as expected. However, in the Yunnan Province landraces, three accessions proved to be monoecious, and PCR amplification was not successful in two accessions (CYW 37 and CYW 49).

Genetic characteristics of Vietnamese melon landraces

Chloroplast genome analysis: The analysis of the PS-ID sequence and ccSSR7 showed that all of the Vietnamese landraces were the A-type and 338 bp type, respectively (Table 1). This chloroplast genome type was the same as that of the Conomon accessions from China and Japan. In contrast, in large-seed type reference accessions (Cantalupensis and Inodorus groups), the PS-ID sequence was T type and the ccSSR7 was the 333 bp type (deletion of 5 bp) in Inodorus and the 338 bp type in Cantalupensis except in "Homegarden".

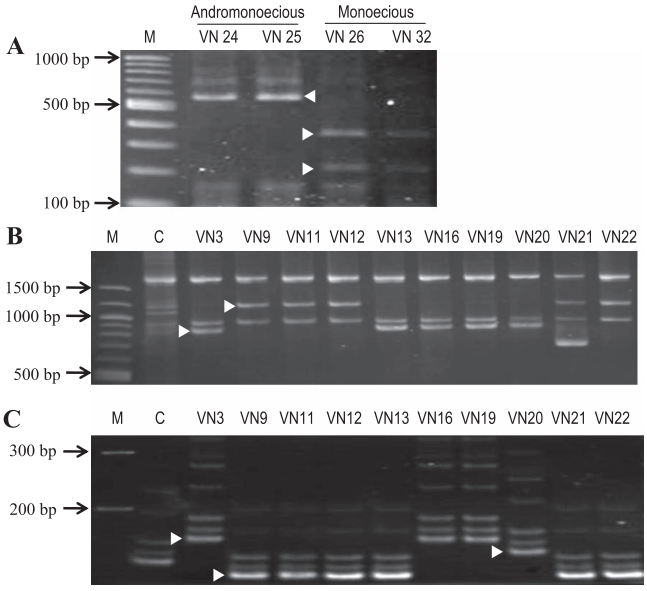

Fig. 3. Gel profile of Vietnamese melon landraces. (A) Cleaved amplified polymorphic sequence profile of *CmAcs7* on 1.5% agarose gel. (B) Random amplified polymorphic DNA with the A20 primer on 1.5% agarose gel. (C) Simple sequence repeat with the primer CMBR 83 on 10% nondenatured polyacrylamide gel. Arrows show polymorphic bands. Lane M, 100 bp DNA ladder marker; lane C, control DNA.

RAPD and SSR analysis: genetic relationship between Vietnamese melon and reference accessions

Among 24 markers analyzed, only eight markers were polymorphic in the Vietnamese melon landraces (Table 2). Among the other markers, nine and seven markers were absent and present in all accessions, respectively. The most polymorphic marker band was generated by primer A20, and marker bands of 1100 bp and 800 bp, referred to as A20-1100 and A20-800, were amplified in 20 and 39 accessions, respectively, among 59 accessions from Vietnam (Fig. 3B). The frequency of six markers was different among four melon types except "Dua vang" ($\chi^2 = 18.4–47.8$, $df = 3$, $P \leq 0.01$). Gene diversity (D) was 0.106 in 59 accessions from Vietnam and was equivalent to that in Chinese Conomon except for accessions from Yunnan Province ($D = 0.118$) and much smaller than that in the reference accessions ($D = 0.314$) (Table 6). In addition, the D within each melon type except "Dua vang" was much smaller and ranged from 0.030 to 0.039.

Among 16 SSR markers examined, five (ACS2-ms1, CMBR 22, CMBR 53, CMN 08-22, and CMN 22-16) were monomorphic in Vietnamese melon landraces. Among them, three markers (ACS2-ms1, CMBR 53, and CMN 08-22) were monomorphic also in the Chinese Conomon. The number of alleles detected in polymorphic markers ranged from two (CMN 08-90 and CMBR 12) to five (CMN 61-44). The most polymorphic marker was CMBR 83 ($D = 0.675$), followed by CMN 04-03 ($D = 0.608$) (Fig. 3C). Two markers (CMBR 2, CMBR 83) proved to be polymorphic within each melon type. The frequency of seven markers, CMN 04-03, CMN 04-07, CMN 08-90, CMN 61-44, CMBR 2, CMBR 83, and CMBR 120, was different among four melon types except "Dua vang" ($P \leq 0.01$). The value of D was 0.308 in 59 accessions from Vietnam and ranged from 0.151 to 0.236 in four melon types except "Dua vang" (Table 6). These values were smaller than those in Chinese Conomon ($D = 0.310$) and the reference accessions ($D = 0.553$).

The GD among accessions calculated from the RAPD and SSR data ranged from 0 to 0.800 (P 117, P 118 vs. P 154, P 158) in 86 accessions and was 0.290 on average (data not shown). In contrast, GD ranged from 0 to 0.400 (VN 20 vs. VN 43) in the Vietnamese landraces and averaged 0.188.

To determine the genetic relationship among melon landraces, a dendrogram was constructed based on the GD values calculated from the RAPD and SSR data, using the UPGMA method (Fig. 4). The 86 accessions of melon were grouped into six major clusters, and further into 14 subclusters. Table 6 summarizes the number of melon accessions

Table 6. Gene diversity and number of melon accessions classified into six clusters in each type of Vietnamese landraces, 19 accessions from China and eight reference accessions

Melon type	No. of accessions	Cluster No.													Gene diversity		
		I	IIa	IIb	IIc	IId	IIe	IIf	IIIa	IIIb	IV	Va	Vb	Vc	VI	RAPD	SSR	RAPD + SSR
Dua dai	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dua bo	15	—	—	—	12	—	1	2	—	—	—	—	—	—	—	0.030	0.199	0.098
Dua gang	13	—	1	1	8	2	1	—	—	—	—	—	—	—	—	0.032	0.175	0.089
Dua le	8	—	—	—	—	3	3	2	—	—	—	—	—	—	—	0.030	0.236	0.113
Dua vang	2	—	—	—	—	1	1	—	—	—	—	—	—	—	—	0.021	0.188	0.088
Dua thom	20	—	—	—	—	—	—	—	—	—	—	1	19	—	—	0.039	0.151	0.084
Yunnan	5	—	—	—	—	—	—	—	—	—	—	—	—	5	—	0.013	0.140	0.064
Chinese Conomon	14	—	—	—	—	—	—	—	5	6	3	—	—	—	—	0.118	0.310	0.195
Reference accessions	8	—	—	—	—	—	—	—	—	—	2	—	—	—	6	0.314	0.553	0.409

classified into each subcluster. Cluster I was composed of one accession of Agrestis (VN 135), which was collected on a river bank in Hung Yen Province. Cluster II was further divided into six subclusters. Subclusters IIa and IIb consisted of one accession each of "Dua gang". Twelve of 15 "Dua bo" accessions were grouped into subcluster IIc, together with eight "Dua gang" accessions. This result suggested a close genetic relationship between the Momordica group and Conomon group var. *conomon* in Vietnam. Two melon types of Conomon var. *makuwa*, "Dua le" and "Dua vang", were classified into subclusters IId–IIf together with three accessions each of "Dua gang" and "Dua bo". Clusters III and IV consisted mainly of Chinese landraces of the Conomon group and two reference accessions of the Japanese Conomon. "Kinpyo" and "Karimori" were included in cluster IV.

Cluster V was rather distantly related to clusters I–IV and divided into three subclusters. All "Dua thom" accessions were classified into subcluster Vb, with the exception of VN 13 (subcluster Va). In contrast to the Chinese landraces of the Conomon group, small-seed type accessions from the Yunnan Province of China formed subcluster Vc, indicating a close relationship with the Vietnamese "Dua thom". Cluster VI was distantly related with other clusters and consisted of the large-seed type reference accessions.

Discussion

This is the first report on the genetic diversity of Vietnamese melon landraces, which are now being replaced by F_1 hybrids imported from Thailand and Taiwan seed companies, especially in the southern part of Vietnam. Information obtained by field research revealed that five types of cultivated melon ("Dua bo", "Dua le", "Dua vang", "Dua thom", and "Dua gang") are known in Vietnam (Fig. 2). The former four types are used for dessert, whereas "Dua gang" is mainly used as a vegetable and is very popular in the central part of Vietnam from Quang Tri to Phu Yen Provinces. The growing season also differs by area and/or melon type. "Dua thom" is mainly cultivated by ethnic minorities during the rainy season (sown in March–May and harvested in July–August) in the highland area of northern Vietnam. As this type can grow normally under humid conditions, its tolerance to biotic and abiotic stresses should be surveyed to utilize these melons as novel genetic resources. The other types are cultivated during the dry season in midland and lowland areas. In areas around Hanoi, melon is sown in February–March and harvested in April–May, while the growing season is from March–April to June–July in the central part of Vietnam. In addition to the cultivated melon, weedy melon of Agrestis group was also found (Fig. 2).

Vietnamese local melons were largely diversified in fruit morphological characters such as size and shape of fruit and color of skin and flesh (Table 4 and Table 5). Diversity was also observed in sex expression type. All "Dua thom" accessions were monoecious, whereas the others were andromonoecious with the exception of VN 139 ("Dua gang"). This diversification has been confirmed by analysis of the *CmAcs7* gene encoding 1-aminocyclopropane-1-carboxylic acid synthase (Boualem *et al.* 2008). Additional sequence variation in the *CmAcs7* gene was also suggested, as PCR amplification was not successful in two accessions: VN 35 ("Dua thom") and VN 177 ("Dua gang").

The molecular genetic analysis revealed that the chloroplast genome type is common in Vietnamese local melon and is the same as that of the group Conomon (Table 1, Tanaka *et al.* 2006). Genetical similarity among landraces was also confirmed by the RAPD analysis. Sixteen of 24 RAPD markers were monomorphic as shown in Table 2, and 59 accessions were classified into 11 RAPD types by eight polymorphic markers. In the most extreme case, for example, 17 accessions including "Dua bo", "Dua le", and "Dua gang" showed an identical RAPD type. The D value calculated by the RAPD data was 0.106 in 59 landraces from Vietnam and equivalent to that in the Chinese Conomon except accessions from Yunnan Province (D = 0.118; Table 6). Tanaka *et al.* (2007) analyzed the same RAPD markers and reported that D ranged from 0.148 to 0.305 in Indian melon populations. Similarly, D was 0.218 in small-seed type melons from Myanmar (Yi *et al.* 2009), and 0.201 in Flexuosus accessions from Iran (Soltani *et al.* 2010), respectively. In addition, D within each melon type was much smaller

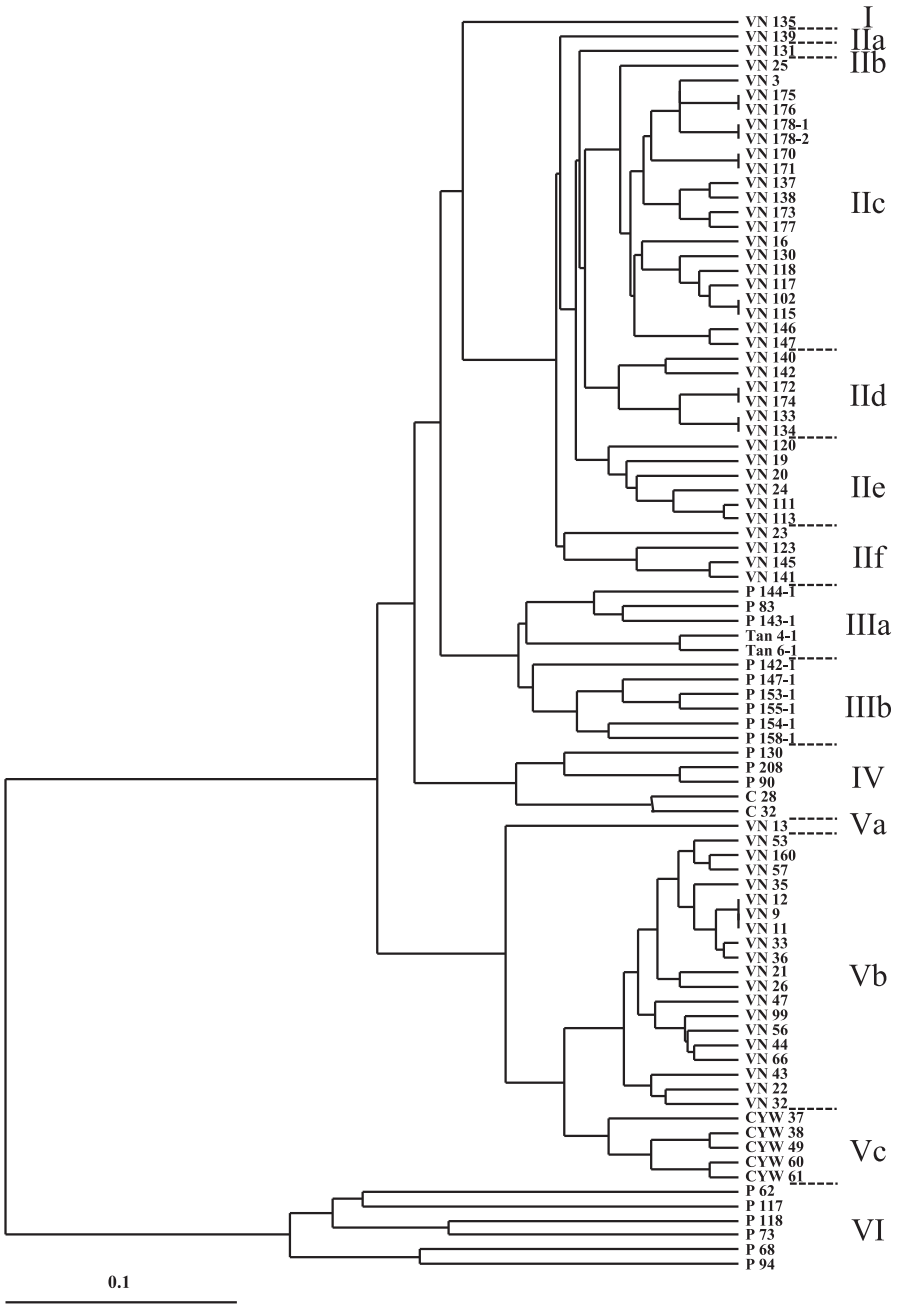

Fig. 4. Genetic relationship between 86 melon accessions shown by UPGMA cluster analysis based on genetic distance calculated from RAPD and SSR.

(Table 6). Thus, genetic variation in Vietnamese melon landraces studied here was small, although they were differentiated into different melon types and morphologically diversified. This was also true for the Japanese accessions of group Conomon (Akashi *et al.* 2002). However, further analysis should be conducted to determine the genetic diversity in Vietnamese melon by including melon landraces from the southern part of Vietnam.

All of the Vietnamese landraces studied here had short hairs on the ovary and were classified as ssp. *agrestis* Jeffrey. In addition, they had common characteristics such as non-netted fruit skin, relatively low sugar content, and seeds shorter than 9.0 mm (Table 4). Among five melon types, "Dua le" and "Dua vang" were classified as Conomon var. *makuwa*, with andromonoecy, thin and smooth skin, sweet flesh, small seeds, and fragrance when fully ripened. "Dua gang", mainly used as a vegetable, was classified as Conomon var. *conomon*, with andromonoecy, thin and smooth skin, nonsweet flesh, small seeds, and no aroma. "Dua bo" could be tentatively classified as group Momordica because the fruit flesh was mealy in all accessions, and the fruit skin cracked in two-thirds of accessions (Fig. 2). "Dua

thom" had thin and smooth skin, small yellow seeds, little aroma, and a variable flesh color including orange. In addition, fruit skin crack was not observed, and all accessions were monoecious. This type of melon accession is also popular in Laos (Saito *et al.* 2009), Myanmar (Yi *et al.* 2009), and India (Kato *et al.* 2006). However, the characteristics mentioned above do not fit the description of known varieties or horticultural groups (Pitrat *et al.* 2000, Munger and Robinson 1991), and their classification proved to be difficult. Therefore, this type could not be classified in the present study.

As shown in Figure 4, Vietnamese melon landraces were clearly divided into two groups. The first group included four melon types grown in midland and lowland areas, ("Dua bo", "Dua le", "Dua vang", and "Dua gang"), and they were clustered with the Chinese Conomon vars. *makuwa* and *conomon*. The second group consisted of one melon type grown in highland areas, "Dua thom", and was clustered with the Yunnanese landraces from China. Akashi *et al.* (2006) analyzed 291 melon accessions from Africa and Asia, using the 18 primers of the present study. Their RAPD data were combined with the present results to uncover the genetic relationship between Vietnamese melon and melon from other countries. Using a UPGMA cluster analysis, "Dua thom" and Yunnanese landraces were grouped into cluster III together with small-seed type accessions from Thailand, Myanmar, Bangladesh, northeastern India, and Nepal (data not shown). Therefore, this type of melon, which was not classified to a known variety or group, was transmitted from India through Myanmar to southwestern China and northwestern Vietnam.

In contrast, 39 accessions of "Dua bo", "Dua le", "Dua vang", "Dua gang" and "Dua dai" were all grouped into cluster II with the Agrestis and Conomon accessions from China, Korea, and Japan. This result indicated that these five melon types are not genetically differentiated from each other, although they are clearly different in fruit morphology, sex expression, and usage (Fig. 2 and Table 5). Akashi *et al.* (2006) and Yi *et al.* (2009) also could not distinguish among melon accessions of the Japanese Conomon vars. *makuwa* and *conomon*, and among those of Conomon, Agrestis, and Momordica in Myanmar, respectively. Possible explanations for such a genetic similarity among melon varieties or groups are as follows: (1) five types have been differentiated from a common ancestor, (2) genetic introgression between these types often occurred through spontaneous hybridization because of their out-crossing nature, and/or (3) the classification of these types into different varieties or species was improper. Although this problem remains to be solved, special attention should be paid to the classification of "Dua bo". This type of melon was classified as Momordica mainly because of mealy flesh and the presence of fruit skin crack (Fig. 2). However, all "Dua bo" accessions were andromonoecious, which was not common in group Momordica (Table 1 and Table 5). In addition, their seed length (6.1–8.2 mm) was in the range of group Conomon and was shorter than that of Momordica in Myanmar (7.0–10.0 mm after Yi *et al*. 2009) and in India (6.0–9.9 mm after Kato *et al.* 2006), suggesting a hybrid origin between Momordica and Conomon that was andromonoecious.

A phylogenetic relationship between Vietnamese melon and group Conomon of China and Japan was also indicated by the RAPD data of Akashi *et al.* (2006). Three "Dua bo" accessions were grouped into subcluster IIa together with the Japanese and Chinese Conomon vars. *makuwa* and *conomon* (data not shown). The other 36 accessions were grouped into subcluster IIb together with Agrestis from Korea and Japan and Conomon vars. *conomon* and *makuwa* from Japan and China, including P208 (Chi-87-12), C28 (Xingtangmiangua), and C32 (Heipilengzisudigua) examined in this study. Therefore, the five types of Vietnamese melon, "Dua bo", "Dua le", "Dua vang", "Dua gang" and "Dua dai", were not genetically differentiated from group Conomon of China and Japan. This is consistent with the deduced meaning of the Chinese character for "Yue Gua". These results thus strongly suggest that group Conomon vars. *makuwa* ("Dua le", "Dua vang", and possibly "Dua bo") and *conomon* ("Dua gang") were established in or around Vietnam, transmitted to China, Korea, and Japan, and shared a common gene pool with group Agrestis. However, the genetic variation in Conomon landraces was smaller in those of Vietnam than those of China, and this result does not rule out the possibility that the group Conomon was established in China and transmitted to Vietnam (Table 6). Therefore, further study, including more landraces from China and Vietnam, is required.

Acknowledgments

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