Distribution of two groups of melon landraces and inter-group hybridization enhanced genetic diversity in Vietnam

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To understand the genetic diversity and differentiation of Vietnamese melon (Cucumis melo L.), we collected 64 landraces from the central and southern parts of the country and assessed molecular polymorphism using simple sequence repeat and random amplified polymorphic DNA markers. The Vietnamese melon was divided into seven cultivar groups, namely “Dua le”, “Dua vang”, “Dua bo”, “Dua gang-andromonoecious”, “Dua gang-monoecious”, “Dua thom”, “Montok”, and the weedy-type melon “Dua dai”. Among these, Dua le, Dua vang, Dua bo, and Dua gang-andromonoecious are cultivated on plains and they formed cluster II along with the reference accessions of Conomon and Makuwa. Based on genetic distance, Dua le and Dua vang were regarded as Makuwa and Dua bo and Dua gang-andromonoecious as Conomon. In contrast, Dua thom and Montok are cultivated in highlands, and they formed cluster III along with landraces from the southern and eastern foot of the Himalayas. Dua gang-monoecious which is commonly cultivated in the southern parts of Vietnam, exhibited the greatest genetic diversity, as explained by its possible origin through the hybridization between Dua gang-andromonoecious and Montok. Genetic differences in melon landraces between plains and highlands and hybridization between these two geographical groups have contributed to the enhancement of genetic diversity in Vietnamese melon.

Key Words: Cucumis melo, genetic diversity, molecular polymorphism, population structure, Conomon, Makuwa, Vietnam.

Introduction

Melon (Cucumis melo L.) is one of the most important vegetable crops of the Cucurbitaceae family, and it is cultivated worldwide. Although it is widely accepted that cucumber (C. sativus), another important member of the genus Cucumis, was domesticated at the foothills of the Eastern Himalayas (Whitaker and Davis 1962), the origin of melon remains controversial. The long-standing hypothesis is that Africa is the origin of C. melo (Kirkbride 1993, Whitaker and Davis 1962), and this hypothesis is supported by the richness of African Cucumis species, which possess the same chromosome number as C. melo (2n = 24) (Kirkbride 1993). However, recent studies have indicated the Asian origin of melon, since closely related wild species of Cucumis have been found in Asia and Australia (Endl et al. 2018, Renner et al. 2007, Schaefer et al. 2009, Sebastian et al. 2010). Sequence analysis of the chloroplast genome revealed that cultivated melon comprises of three maternal lineages, suggesting an independent origin (Endl et al. 2018, Tanaka et al. 2013). Recently, Zhao et al. (2019) suggested that three melon lineages were domesticated independently in India and Africa.

Subsequently, cultivated melon with three maternal lineages spread to the other parts of the world, where various types of melon were selected and utilized during the long history of cultivation. As a result, melon is now known to be the most diversified among the Cucurbitaceae crops. Due to this great diversity, melon classification is challenging and has been revised several times since its first classification by Naudin (1859) into 10 varieties (Kirkbride 1993, Munger and Robinson 1991, Pitrat 2008). More recently, Pitrat (2016) classified melon into 19 horticultural groups based primarily on geographical origin, morphology and horticultural traits: Agrestis, Kachri, Chito, Tibish, Acidulus, Momordica, Conomon, Makuwa, Chinensis, Flexuosus, Chate, Dudaim, Chadalak, Indicus, Ameri, Cassaba, Ibericus, Inodorus, and Cantalupensis.

Melon landraces grown in East and Southeast Asia are
mostly classified into four groups: Momordica, Conomon, Makuwa, and Chinensis (Pitrat 2016, Yi et al. 2009). The Momordica group (snap melon) is characterized by mealy, non-sweet flesh, fruit crack at maturity, and monoecious sex expression (M type), and it is mainly grown in India (Dhillon et al. 2012). It is also common in Myanmar (Yi et al. 2009) and the Hachijojima Island of Japan. Although Momordica is not grown on the main islands of Japan, archeological evidence suggests its production around 1,000 years ago (Tanaka et al. 2016b). The Conomon and Makuwa groups are characterized by small seeds (<9 mm long) and andromonoecious sex expression (A type), and they are popular in the Far-East (China, Korea, and Japan) (Tanaka et al. 2007). The Conomon group, called “Shirouri” in Japan and “Cai-gua” in China, is characterized by non-sweet type fruits, and the young fruits are mainly used as a vegetable. The Makuwa group is characterized by sweet fruits and mature fruits are consumed as dessert. However, genetic diversity analyses of Asian melon populations using isozymes (Akashi et al. 2002) or random amplified polymorphic DNA (RAPD) (Tanaka et al. 2007, Yi et al. 2009) and simple sequence repeat (SSR) (Nhi et al. 2010) markers have proven that Conomon and Makuwa share a common gene pool and are not genetically distinct. In this light, Conomon and Makuwa were hypothesized to have originated from Indian melon landraces and to have differentiated somewhere in Southeast Asia during the eastward transmission of melon (Akashi et al. 2002, Tanaka et al. 2007).

Vietnam is in Southeast Asia, roughly east of India and south of China; thus, the analysis of Vietnamese melon landraces is indispensable to uncover the origin of Conomon and Makuwa. Using accessions of five cultivar groups, namely “Dua le”, “Dua vang”, “Dua bo”, “Dua gang”, and “Dua thom”, collected from the northern parts of Vietnam (NW and NE in Fig. 1), Nhi et al. (2010) performed the first morphological and molecular characterization of Vietnamese melon landraces. Dua le group is characterized by globular fruits with crispy flesh and white epicarp, whereas Dua vang groups is characterized by yellow epicarp. Dua bo group is characterized by fruits with powdery, less sweet flesh. Dua thom group has oblong, aromatic fruits, with diverse flesh color from white to yellow and orange. Mature fruits from all four groups are consumed as dessert. Dua gang group has elongated fruits with vertical stripes and young fruits are generally used as a vegetable. Nhi et al. (2010) collected the material of Dua thom from the highlands in northwestern regions (NW in Fig. 1), and that of other groups mainly from the mid- and lowlands of northeastern, northern, and northern central coastal regions (NE, ND, and NC in Fig. 1) of Vietnam. Using molecular marker analysis, the authors demonstrated that Dua thom group is genetically distinct from the other four groups, which are closely related to Conomon and Makuwa. However, given the limitations of material and small area coverage, the diversity of Vietnamese melon remains unclear.

Materials and Methods

Plant material

Sixty-four melon accessions (Cucumis melo L.), primarily...
of local landraces, were collected from southern and central Vietnam during surveys undertaken in 2014 and 2015 (Fig. 1). They were categorized into the following six cultivar groups: “Dua le”, “Dua vang”, “Dua bo”, “Dua gang”, “Dua thom”, “Montok”, and the weedy-type melon “Dua dai” based on the information provided by the local people, including the purpose of use and local name (Supplemental Fig. 1). Montok is cultivated in the Tay Nguyen region (southern highlands, SH) by ethnic minorities (Jarai and H’mong) (Fig. 1). Thirty-one accessions of Conomon, Makuwa, Cantalupensis, Ameri, Ibericus, Inodorus, and Agrestis as well as 48 accessions from nearby countries [China (Yunnan), Thailand, Myanmar, Bangladesh, and India] were used as reference. Two accessions of the wild cucumber C. sativus var. hardwickii were also used as the outgroup. In addition, to widen the 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 included in the data analysis. The melon accessions studied here are summarized in Table 1, and the details of the materials are provided in Supplemental Table 1. Dua gang accessions were divided into two groups based on seed expression, namely Dua gang-andromonoecious (Dua gang-A) and Dua gang-monoecious (Dua gang-M). Indian melon landraces were classified into two groups based on seed size, namely large and small seed-types, with seed length above and below 9 mm, respectively. Since the number of accessions was three or less for Ameri, Ibericus, and Inodorus, they were grouped as Inodorus for analysis.

For the 63 accessions of Vietnamese cultivated melon collected in 2014 and 2015, fruit characteristics, including the Brix of flesh juice, fruit length and diameter, and fruit shape index (fruit length/fruit diameter), were evaluated by growing them in a greenhouse at the Okayama University, Japan, in 2015 and 2016. Two plants of each accession were used to evaluate the fruit characteristics. Length and width were measured for 10 seeds of each accession using a digital Nogis scale.

DNA extraction and PCR amplification

Seeds were sown on wet filter papers in petri dishes and grown at 28°C and 46.5 μM·s⁻¹·m⁻² light intensity under a 16/8 h light/dark cycle. Total DNA was extracted from young leaves of seedlings using the cetyltrimethylammonium bromide (CTAB) method, as described by Murray and Thompson (1980), with minor modifications. The quality and quantity of each DNA sample were evaluated using a spectrophotometer (DU-530, Beckman, USA).

PCR-based molecular markers, including RAPD, SSR, and allele-specific markers for CmACS7 and CmPH, were used. Twelve RAPD and seven SSR markers were selected for their reproducibility and ability to detect distinct polymorphisms in East Asian melon (Aierken et al. 2011, Nhi et al. 2010, Tanaka et al. 2007, Yi et al. 2009). The CmACS7 genotype was determined for sex expression analysis using

### Table 1. Number of accessions examined in each group and inter-group difference in seed length, marker genotypes and gene diversity

| Melon group | No. of accessions | Seed length | CmPH genotype | CmACS7 genotype | Gene diversity |
|-------------|-------------------|-------------|----------------|------------------|---------------|
|             |                   | Large-seed  | Small-seed     | Not-sour         |               |
| Vietnam     |                   |             |                |                  |               |
| Dua le      | 13                | –           | 13             | –                | 0.14          |
| Dua vang    | 3                 | –           | 3              | –                | 0.13          |
| Dua bo      | 18                | –           | 15             | 3                | 0.19          |
| Dua gang-A  | 8                 | –           | 8              | –                | 0.17          |
| Dua gang-M  | 19                | 1           | 18             | 7                | 0.30          |
| Dua thom    | 8                 | –           | 8              | –                | 0.21          |
| Montok      | 16                | –           | 16             | –                | 0.23          |
| Dua dai     | 2                 | –           | 2              | –                | 0.35          |
| Subtotal    | 87                | 1           | 86             | 46               | 0.52          |
| Conomon     | 6                 | –           | 6              | 2                | 0.22          |
| Makuwa      | 8                 | –           | 8              | 5                | 0.17          |
| Agrestis    | 7                 | –           | 7              | 2                | 0.38          |
| Yunnan (China) | 5         | –           | 5              | –                | 0.08          |
| Thailand    | 5                 | –           | 5              | 2                | 0.32          |
| Myanmar     | 18                | 2           | 16             | 2                | 0.41          |
| Bangladesh  | 7                 | 2           | 5              | –                | 0.34          |
| India-small | 7                 | –           | 7              | 2                | 0.37          |
| India-large | 6                 | 6           | –              | 1                | 0.39          |
| Cantalupensis | 4          | 4           | –              | 4                | 0.24          |
| Inodorus    | 6                 | 6           | –              | 6                | 0.30          |
| Total       | 166               | 21          | 145            | 72               | 0.44          |
the CAPS marker developed by Boualem et al. (2008). The CmPH genotype was determined for the sourness of fruit flesh using an allele-specific marker developed based on the sequence polymorphism reported by Cohen et al. (2014). The details of the primer sets are presented in Supplemental Table 2.

PCR amplification was performed using a 10 μL reaction mixture containing 50 ng genomic DNA; 1 μL PCR buffer (10 mM Tris–HCl [pH 8.3] and, 50 mM KCl; Sigma, USA); 0.25 mM MgCl₂ for CmACS7 and 0.2 mM for RAPD, SSR, and CmPH; 0.1 mM dNTP; 0.5 μM primers, and 0.25 U Taq polymerase (Sigma).

The reactions were performed using iCycler (Bio-Rad, USA). The PCR conditions for SSR, CmACS7, and CmPH markers were as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of 95°C for 1 min, 56°C–57°C for 1 min (60°C for 30 s for CmPH), and 72°C for 2 min, and final extension at 72°C for 5 min. The PCR conditions for RAPD markers were as follows: initial denaturation at 95°C for 3 min, followed by 40 cycles of 93°C for 1 min, 40°C for 2 min, and 72°C for 2 min, and final extension at 72°C for 5 min.

The PCR product of CmACS7 digested with Alu I (New England BioLabs, USA) and that of RAPD were electrophoresed on 1.5% agarose gels at a constant voltage of 50 V. The PCR product of CmPH was electrophoresed on 3% agarose gel at a constant voltage of 50 V. The PCR product of SSR was electrophoresed on a non-denatured 10% polyacrylamide gel at a constant voltage of 260 V.

Data analysis

RAPD marker bands 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 the number of effective alleles (Ne), polymorphic information 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 GD was calculated as GD = 1–GS. A dendrogram was constructed based on the GD values using the PHYLIP program with unweighted pair group method with arithmetic mean (UPGMA) cluster analysis. Principal coordinates analysis (PCoA) based on the GS matrix was applied to illustrate the multiple dimensions of each group and accession on a scatter plot (Gower 2016). The population structure was analyzed using a model-based approach available in STRUCTURE 2.3.4 (Pritchard et al. 2000). In this analysis, the admixture model without prior grouping assumptions was used, and 1 to 10 genetic clusters (K) with 20 permutations for each K value were evaluated. Each run was implemented with a burn-in period of 25,000 steps, followed by 100,000 Markov chain Monte Carlo (MCMC) simulations.

Results

Genetic diversity of Vietnamese melon and reference accessions

Most Vietnamese melon landraces from the seven Dua groups, except one Dua gang-M accession, were classified as small seed-type (Table 1). In contrast, sex expression type and flesh sourness estimated based on the CmACS7 and CmPH genotypes varied across the tested Vietnamese landraces. Dua le and Dua vang were A type with non-sour flesh, while Dua thom, Montok, and Dua dai were M type with sour flesh. Dua gang and Dua bo showed varying sex expression and flesh sourness. Thus Dua gang was divided into two groups, namely Dua gang-A (A type) and Dua gang-M (M type). Since only three accessions of Dua bo were classified as M type, they were included in Dua bo together with the A type accessions for analysis.

Furthermore, fruit characteristics and seed size varied among the cultivar groups. Dua le and Dua vang showed higher soluble solid content and shorter seeds than the other groups, though pairwise difference was partly insignificant (Table 3). Dua le and Dua vang were characterized by round fruits based on the fruit shape index ranging from 0.86 to 0.91. The average fruit length (36.7 cm) and fruit shape index (3.88) were the highest in Dua gang-A, followed by Dua gang-M (21.8 cm, 2.14, respectively). The differences in these traits between Dua gang-A and Dua gang-M were statistically significant (P < 0.01). Dua bo, Dua thom, and Montok were characterized by oblong fruits of comparable length (17.2–18.9 cm) and fruit shape index (1.60–1.73). The purpose of use also differed among the cultivar groups. Dua le, Dua vang, Dua bo, Dua gang-M, and Dua thom are consumed as dessert, while young fruits of Dua gang-A are used as a vegetable. Montok is mainly grown to harvest mature fruits as dessert, but immature fruits are often used as a vegetable similar to Dua gang-M. Since Brix value was low in Dua bo, Dua gang-M, Dua thom and Montok, they were usually eaten with sugar or condensed milk.

Among reference accessions, the small seed-type characteristic to Conomon and Makuwa was common in areas from India to Yunnan (China), with a gradual eastward decrease in seed size (Table 1). Geographic variation in the sex expression type is not straightforward, and different combinations of sex type and seed size were observed. Accessions of two contrasting groups, including Cantalupensis and Inodorus with large seeds and Conomon and Makuwa with small seeds, were A type, with the exception of one Makuwa accession, whereas most small seed-type accessions in areas from India to Yunnan (China) were M type. Same appears to be true for flesh sourness and seed size, with the small seed-type accessions in areas from India to Yunnan (China) often being the sour-type. Conomon and Makuwa were exceptions presenting both sour and non-sour types.
The seven SSR and 12 RAPD markers used generated a total of 100 alleles in the 168 accessions studied, of which 61 were detected in the 87 accessions of Vietnamese landraces. No polymorphism was detected in Vietnamese landraces for the three RAPD markers A41-930, B32-700, and B86-1350, and PIC among the 168 accessions was also below 0.13 (Table 2). The number of polymorphic SSR bands ranged from six (CMN 4-07) to 18 (CMN 61-44), and the number of effective alleles ranged from 2.09–5.13. PIC ranged from 0.05 (B32-700) to 0.78 (CMBR 2), with an average of 0.39.

Gene diversity calculated by RAPD and SSR data was 0.35 among the 87 Vietnamese landraces, being as high as that among landraces in areas from India to Thailand and that among Agrestis which were collected from five countries (Table 1, Supplemental Table 1). In addition, the Vietnamese landraces are more diversified than Conomon and Makuwa. However, gene diversity within each Dua group did not exceed 0.23, with the exception of Dua gang-M (0.30), being nearly equivalent to that within Conomon and Makuwa. These results indicated that the seven cultivar groups, each rather less diversified within group (except Dua gang-M), enhanced the genetic diversity of Vietnamese melon.

**Genetic relationship and population structure**

The dendrogram obtained using UPGMA cluster analysis based on GD separated the 168 accessions into four major clusters and 17 sub-clusters (Fig. 2). Two accessions of *C. sativus var. hardwickii*, the outgroup formed a distinct cluster I. All but one accessions of Cantalupensis and Inodorus formed cluster IV, together with 13 accessions in areas from India (large seed-type), Bangladesh, Myanmar, and Thailand and one accession of Agrestis from India. The Vietnamese landraces were classified into clusters II and III. The Vietnamese accessions of Dua le (13/13), Dua vang (3/3), Dua bo (16/18), Dua gang-A (7/8), Dua gang-M (11/19), Dua thom (1/8) and Dua dai (1/2) formed cluster II, along with Conomon, Makuwa, and Agrestis. Meanwhile, the Vietnamese accessions of Dua thom (7/8), Montok (16/16), Dua gang-M (8/19), Dua gang-A (1/8), Dua bo (2/18), and Dua dai (1/2) formed cluster III, along with majority of the accessions in areas from India to Yunnan (China). Although Dua gang accessions were placed in both cluster II and III, Dua gang-A accessions were mostly classified into cluster II. These results, together with the sex expression type, demonstrate that Vietnamese melon comprises two major groups. The first group includes Dua le, Dua vang, Dua bo, and Dua gang-A, which are genetically related to Conomon and Makuwa, and the second group includes Dua thom, Montok, and part of Dua gang-M, which are closely related to landraces in areas from India to Yunnan (China).

The genetic relationships uncovered by cluster analysis were reproduced on the PCO plot (Fig. 3). The Vietnamese

| Markers | Vietnam (n = 87) | All (n = 168) |
|---------|-----------------|--------------|
|         | Ne   | PIC | Ne   | PIC |
| **RAPD** |      |     |      |     |
| 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 |
| **SSR**  |      |     |      |     |
| CMN 4-03 | 2.19 | 0.44 | 3.47 | 0.67 |
| CMN 4-07 | 2.77 | 0.56 | 3.16 | 0.63 |
| CMN 4-40 | 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 2.** The number of effective alleles (Ne) and polymorphic information content (PIC) at 19 marker loci of Vietnamese melon and all accessions studied.

| Melon group | No. of accessions | Fruit usage | Brix (%) | Fruit length (cm) | Fruit diameter (cm) | Fruit shape | Seed length (mm) | Seed width (mm) |
|-------------|-------------------|-------------|---------|------------------|---------------------|-------------|------------------|-----------------|
| Dua le      | 13                | 13          | –       | 8.5 a            | 8.4 c               | 9.4 b       | 6.3 b            | 3.0 b           |
| Dua vang    | 3                 | 3           | –       | 6.0 ab           | 10.5 bc             | 12.2 a      | 6.5 b            | 2.8 b           |
| Dua bo      | 18                | 17          | –       | 3.6 b            | 18.9 b              | 12.3 a      | 7.4 a            | 3.3 ab          |
| Dua gang-A  | 8                 | –           | 8       | 3.8 b            | 36.7 a              | 9.4 b       | 7.1 a            | 3.3 ab          |
| Dua gang-M  | 19                | 19          | 3       | 4.7 b            | 21.8 b              | 10.3 ab     | 2.14 b           | 7.6 a           |
| Dua thom    | 8                 | 8           | –       | 5.4 b            | 17.2 bc             | 10.3 ab     | 1.65 bc          | 7.0 ab          |
| Montok      | 16                | 14          | 11      | 4.9 b            | 17.6 b              | 10.2 b      | 1.73 b           | 6.8 b           |

Mean values with the same letter indicate insignificant differences at 0.02 level, by Tukey–Kramer test.

The Vietnamese group includes Dua le, Dua vang, Dua bo, and Dua gang-A, which are genetically related to Conomon and Makuwa, and the second group includes Dua thom, Montok, and part of Dua gang-M, which are closely related to landraces in areas from India to Yunnan (China). The genetic relationships uncovered by cluster analysis were reproduced on the PCO plot (Fig. 3). The Vietnamese.
accessions of Dua le, Dua vang, Dua bo, and Dua gang-A were clustered with Conomon and Makuwa but clearly separated from the other accessions by PCO1. PCO2 explained the relatedness of Dua thom and Montok with landraces in areas from India to Yunnan (China), showing similarities with geographically closer populations, such as those in Yunnan (China) and Thailand. Dua gang-M accessions were widely scattered along PCO1, and the accessions of clusters Ila and IIb were plotted together with Dua le, Dua vang, and Dua bo, as expected from results in Table 4. On the contrary, the accessions of clusters III were close to Dua thom and Montok, while those of Ile and IIg were placed medially between the two major groups.

The population structure was analyzed to understand the genetic structure of Vietnamese melon. The Delta K value suggested the presence of three main populations in the 168 accessions studied. The population structure and classification of the 168 accessions into three populations are shown in Fig. 4. Of the 166 melon accessions studied, 137 (82.5%) were assigned to one of the three populations using a Q-value threshold of 70% (Table 4, Supplemental Table 1). Population (P) 1 included 10 accessions of Cantalupensis and Inodorus, and 23 accessions from India, Bangladesh, Myanmar, and Thailand. No Vietnamese accessions belonged to P1. Although one accession of Cantalupensis, ‘Melon Cantalupo di Charentais’ was classified in cluster IIg (Table 4), it was also assigned to P1. P2 included accessions of Conomon and Makuwa and most of Dua le, Dua vang, Dua bo, and Dua gang-A accessions. P3 included landraces in areas from India to Yunnan (China) and Dua gang-M, Dua thom, and Montok accessions. The remaining 29 accessions (17.3%) were categorized as having mixed ancestry, with admixture in their genetic composition. Ten accessions of Dua bo, Dua gang-A, Dua gang-M, and Dua thom proved to be the admixture type among the studied Vietnamese landraces. The STRUCTURE analysis also demonstrated the presence of admixture in two major groups of Vietnam melon.

**Genetic relationship among cultivar groups**

The pairwise GD among the 19 groups is shown in Table 5. The greatest distance (0.842) was observed between Makuwa and Inodorus and the lowest (0.014) between Dua vang and Dua le. GD among Dua le, Dua vang, Dua gang-A, and Dua bo did not exceed 0.110, indicating that these four cultivar groups are genetically closely related. Similarly, GD between Dua thom and Montok was small (0.090). Furthermore, GD between the former four groups and the latter two groups ranged from 0.315 to 0.665, indicating distinct genetic differentiation among them. Among the former four groups, Dua le and Dua vang were the most closely related to Makuwa (GD = 0.094–0.101), while Dua gang-A and Dua bo were the most closely related to Conomon (GD = 0.088–0.097). In contrast, Dua thom and Montok were distantly related to Conomon and Makuwa (GD = 0.451–0.682) but closely related to the cultivar groups from Yunnan (China) to India (small seed-type) (GD = 0.096–0.236). Dua gang-M was rather unique among the Vietnamese cultivar groups. Although this group was the closest to Dua bo (GD = 0.097), its GD from the other Asian groups, except Dua dai was moderate, ranging from 0.112 to 0.297.

UPGMA cluster analysis and PCoA were performed based on the GDs among the 19 groups (Table 5), and the
genetic relationships are shown in Figs. 5 and 6. Consistent with the accession-based analysis, the 19 groups were separated into three clusters: the South and Southeast Asian group (cluster I), the Cantalupensis and Inodorus group (cluster II), and the Conomon and Makuwa group (cluster III). Among the Vietnamese cultivar groups, Dua le, Dua

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Table 4. Number of melon accessions classified into 17 sub-clusters in Fig. 2 and four populations in Fig. 4

| Melon group   | No. of accessions | Cluster No. | Population structure |
|---------------|-------------------|-------------|----------------------|
|               |                   | I 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-A    | 8                 | – – 5 1 – 1 – – – – 1 – – – – – 7 – 1 |
| Dua gang-M    | 19                | – – 4 2 – 4 – 1 – 3 5 – – – – – 7 7 5 |
| Dua thom      | 8                 | – – – – – – 1 – – – – – – 6 1 – – – – – – – 7 1 |
| Montok        | 16                | – – – – – – – – – – – – 1 1 – – – – – – – – 16 – |
| Dua dai       | 2                 | – – – – – – – – – – – – – – – – – – – 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 |
| Conomon       | 6                 | – – 3 – – – – 2 1 – – – – – – – – – – – – – – 5 – 1 |
| Makuwa        | 8                 | – – 1 4 – 3 – – – – – – – – – – – – – – – 8 – – |
| Agrestis      | 7                 | – – – – 3 – 1 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 |
| Cantalupensis | 4                 | – – – – – – – – – – – – – – – – – – – 1 – – – – – 1 2 – 4 – – |
| Inodorus      | 6                 | – – – – – – – – – – – – – – – – – – – 3 3 – 6 – – |
| Total         | 166               | 0 24 6 15 10 6 5 6 5 13 12 21 14 6 14 7 2 33 61 43 29 |

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Fig. 3. Distribution of 168 accessions on the first two principal coordinates. Accessions are indicated with symbols unique to each group.
Fig. 5. Genetic relationship between 19 groups of melon, revealed by UPGMA cluster analysis based on genetic distance.

Fig. 4. Inferred population structure of 168 melon accessions by STRUCTURE using the admixture model based on 19 molecular markers (K = 3). Accessions are sorted from the left to right, according to the order in Fig. 2.

Table 5. Pairwise genetic distance between 19 groups of melon

| Melon group | Dua le | Dua vang | Dua bo | Dua gang-A | Dua gang-M | Dua Thom | Montok | Dua dai | Conomon | Makuwa | Agrestis | Yunnan (China) | Thailand | Myanmar | Bangladesh | India-small | India-large | Cantalupensis |
|-------------|--------|----------|--------|------------|------------|----------|--------|--------|---------|---------|---------|----------------|----------|---------|------------|-------------|-------------|--------------|
| Dua le      | 0.014  |          |        |            |            |          |        |        |         |         |        |                 |          |         |            |             |             |              |
| Dua vang    | 0.110  | 0.085   |        |            |            |          |        |        |         |         |        |                 |          |         |            |             |             |              |
| Dua bo      | 0.102  | 0.109   | 0.035  |            |            |          |        |        |         |         |        |                 |          |         |            |             |             |              |
| Dua gang-A  | 0.203  | 0.182   | 0.097  | 0.112      |            |          |        |        |         |         |        |                 |          |         |            |             |             |              |
| Dua gang-M  | 0.665  | 0.632   | 0.378  | 0.420      | 0.248     |          |        |        |         |         |        |                 |          |         |            |             |             |              |
| Montok      | 0.574  | 0.544   | 0.315  | 0.346      | 0.166     | 0.090   |        |        |         |         |        |                 |          |         |            |             |             |              |
| Dua dai     | 0.661  | 0.645   | 0.393  | 0.463      | 0.322     | 0.284   | 0.261  |        |         |         |        |                 |          |         |            |             |             |              |
| Conomon     | 0.166  | 0.143   | 0.088  | 0.097      | 0.148     | 0.522   | 0.451  | 0.472  |        |         |        |                 |          |         |            |             |             |              |
| Makuwa      | 0.101  | 0.094   | 0.146  | 0.127      | 0.257     | 0.682   | 0.583  | 0.659  | 0.098  |        |        |                 |          |         |            |             |             |              |
| Agrestis    | 0.165  | 0.166   | 0.112  | 0.127      | 0.348     | 0.315   | 0.314  | 0.165  | 0.161  |        |        |                 |          |         |            |             |             |              |
| Yunnan (China) | 0.689  | 0.680   | 0.446  | 0.456      | 0.297     | 0.154   | 0.156  | 0.252  | 0.531  | 0.693  | 0.395  |                 |          |         |            |             |             |              |
| Thailand    | 0.606  | 0.587   | 0.399  | 0.445      | 0.252     | 0.169   | 0.163  | 0.289  | 0.467  | 0.575  | 0.264  | 0.257  |                 |          |         |            |             |             |              |
| Myanmar     | 0.575  | 0.548   | 0.315  | 0.364      | 0.221     | 0.096   | 0.133  | 0.293  | 0.411  | 0.554  | 0.238  | 0.209  | 0.124  |                 |          |         |            |             |             |              |
| Bangladesh  | 0.490  | 0.464   | 0.292  | 0.338      | 0.173     | 0.236   | 0.216  | 0.293  | 0.303  | 0.441  | 0.177  | 0.322  | 0.116  | 0.122  |                 |          |         |            |             |             |              |
| India-small | 0.430  | 0.383   | 0.216  | 0.275      | 0.179     | 0.200   | 0.183  | 0.343  | 0.259  | 0.365  | 0.163  | 0.333  | 0.162  | 0.091  | 0.129  |                 |          |         |            |             |             |              |
| India-large | 0.455  | 0.450   | 0.260  | 0.324      | 0.188     | 0.324   | 0.276  | 0.340  | 0.355  | 0.429  | 0.174  | 0.462  | 0.207  | 0.182  | 0.180  | 0.171  |                 |          |         |            |             |             |              |
| Cantalupensis | 0.565  | 0.594   | 0.463  | 0.444      | 0.310     | 0.462   | 0.387  | 0.508  | 0.498  | 0.557  | 0.303  | 0.572  | 0.221  | 0.263  | 0.221  | 0.303  | 0.155  |                 |          |         |            |             |             |              |
| Inodorus    | 0.779  | 0.792   | 0.581  | 0.634      | 0.393     | 0.411   | 0.396  | 0.450  | 0.667  | 0.842  | 0.416  | 0.526  | 0.204  | 0.263  | 0.229  | 0.339  | 0.210  | 0.106  |                 |          |         |            |             |             |              |

Discussion

Nhi et al. (2010) reported the genetic structure of Vietnamese melon for the first time and indicated two distinct groups of melon landraces that were allopatrically distributed and genetically differentiated from each other. In the present study, we collected melon landraces from southern and central Vietnam (Fig. 1) and revealed the diversity and genetic structure of local melon based on the analysis of fruit traits and molecular polymorphism. In addition to the five cultivar groups, namely Dua le, Dua vang, Dua bo, Dua gang and Dua Thom, we recognized Montok based on materials collected during field surveys in the Tay Nguyen region (SH). Furthermore, M type of Dua gang was recognized in southern Vietnam. Thus, Dua gang was divided into two groups, namely Dua gang-A and Dua gang-M. Among the seven groups of cultivated melon, Dua Thom and Montok proved to be common in highlands of northern Vietnam, Dua bo, and Dua gang-A were categorized into the Conomon and Makuwa group, whereas Dua Thom, Montok, and Dua Dai were categorized into the South and Southeast Asian group. Dua gang-M placed medially between these two groups on the PCA plot (Fig. 6).
H’mong and Thai) and southern (Jarai and H’mong) Vietnam, respectively, where ethnic groups (indicated in parenthesis) cultivate melon in upland rice fields as a mixed crop. The other cultivar groups are mainly cultivated in mono cropping in the lowland areas of Vietnam, mainly by the Kinh, Muong, and Khmer people.

Reflecting such diversity, Vietnamese melon could be genetically separated into two distinct geographical groups, including Dua thom and Montok of highland areas (cluster III and P3) and Dua le, Dua vang, Dua bo, and Dua gang-A of lowland plains (cluster II and P2), through the analysis of molecular markers (Table 4, Supplemental Table 1). Furthermore, these two geographical groups differed in terms of sex expression type and flesh sourness. While Dua thom and Montok are M type with sour flesh, the rest are A type with non-sour flesh, albeit with some exceptional Dua bo accessions (Table 1). Of note, Dua gang-M is polymorphic for flesh sourness (Table 1), assigned to P2, P3, and admixture population (Table 4) and placed medially between the two geographical groups on the PCO plot (Figs. 3, 6). These results suggest the hybrid origin of Dua gang-M from the two geographical groups. One of the parents for hybridization should be M type, probably Montok, since most Dua gang-M accessions, except VNC5, were collected from southern Vietnam, mostly from the southern central coastal areas adjacent to the Tay Nguyen region (SH), where ethnic groups grow Montok (Fig. 1, Supplemental Table 1). Dua gang-M showed the second highest fruit length and fruit shape index, following Dua gang-A, and these values were higher than those in Montok, although the difference was insignificant (Table 3). Therefore, Dua gang-A may be the other parent for hybridization from which the oblong fruit shape was inherited. Although these three groups were genetically distinct (Figs. 3, 6), they were similar in terms of the use of young fruits as a vegetable (Table 3).

Although the four groups of lowland plains, namely Dua le, Dua vang, Dua bo, and Dua gang-A, are closely related with one another (GD = 0.014–0.110, Table 5), they differed in terms of fruit traits, seed size, and usage (Table 3). Dua le and Dua vang are characterized by a high Brix value (6.0–8.5) and small, round fruits, which are consumed as dessert, as already reported by Nhi et al. (2010). In contrast, Dua gang-A is characterized by a low Brix value (3.8) and large, oblong fruits, which are used as vegetable when young. For use as a vegetable, the capacity to produce larger fruits is considered essential, since the size of young fruits sold in the local market in the Hue city ranged from 15.5 to 35.0 cm, with an average of 25.1 cm (Nhi, unpublished). The mature fruits of Dua bo are consumed as dessert, usually with sugar or condensed milk, despite the low Brix value (3.6, Table 3). Although they differed in terms of usage, Dua gang-A and Dua bo showed similar flesh texture, mealy at the mature stage. The analysis of genetic relationships with groups outside Vietnam revealed that these four groups are closely related to Conomon and Makuwa. Specifically, Dua le and Dua vang were clustered with Makuwa, whereas Dua bo and Dua gang-A were clustered with Conomon (Fig. 5). These relationships were reproduced on the PCO plot (Figs. 3, 6). According to Nhi et al. (2010), Dua bo likely originated through hybridization between Momordica and Conomon, since peeling skin or fruit splitting at the mature stage is characteristic to these three groups. However, 12 accessions of Momordica from India and Myanmar were assigned to clusters III and IV or to P1, P3, and admixture population (Table 4), distinct from Dua bo. Based on these results, Dua le and Dua vang should be classified as Makuwa and Dua bo and...
Dua gang-A as Conomon.

The highland melon groups, namely Dua thom and Montok, were closely related to each other (GD = 0.090, Figs. 3, 5, 6). They were similar in terms of fruit traits and usage as dessert, although young fruit of Montok are sometimes used as a vegetable (Table 3). Thus, these can be regarded as the same group, regardless of being grown in geographically distant areas by different ethnic groups. Further, Dua thom and Montok were closely related to the cultivar groups from Yunnan (China) to India (small seed-type) (Table 5, Fig. 5) and shared the same traits of M type and sour flesh. Therefore, Dua thom and Montok are likely introduced to Vietnam from the West. However, little is known regarding the melon populations of the neighboring countries, with the exception of Myanmar populations studied by Yi et al. (2009). Recently, field studies of vegetable crops, including melon, have been undertaken in Laos (Matsunaga et al. 2010) and Cambodia (Tanaka et al. 2016a). Nonetheless, the genetic diversity of these melon landraces remains unexplored as yet.

Furthermore, regarding their origin, Conomon and Makuwa were likely domesticated in India (Kitamura 1950) or China (Jeffrey 1980, Robinson and Decker-Walters 1997). Specifically, Makuwa was established in North China and Conomon in South China (Kitamura 1950). Using molecular-based phylogenetic studies, Akashi et al. (2002) and Tanaka et al. (2007) indicated a single and common origin of these two groups, suggesting that they originated from the India small seed-type melon through intense selection under wet conditions at the southern and eastern foot of the Himalayas. McCreight et al. (2004) have supported this hypothesis based on the results of their diversity analysis of Indian and Chinese melon accessions. In the present study, landraces in areas from India to Yunnan (China) were mostly classified into clusters III and IV along with the Vietnamese highland melon groups Dua thom and Montok, and were not closely related to the Vietnamese Conomon and Makuwa (Table 3, Figs. 5, 6). In addition, the small seed-type Indian accession PI 210542 of cluster IIg (Supplemental Table 1) that was plotted close to Conomon and Makuwa (Fig. 3) showed M type, unlike Conomon and Makuwa. Therefore, the direct genetic relationships of landraces from the southern and eastern foot of the Himalayas with Conomon and Makuwa could not be verified in the present study. Conomon and Makuwa are widely grown and exhibit rich morphological and genetic diversity in China (Luan et al. 2008). In contrast, the Vietnamese Conomon and Makuwa are considered less diversified than the reference accessions from China and Japan, evidenced by the analysis of gene the diversity and CmPH genotype (Table 1). Therefore, it seems reasonable to conclude that Conomon and Makuwa were introduced from China to Vietnam, similar to amaranths (Nguyen et al. 2019).

Author Contribution Statement

TTD, KT, PTPN, and KK designed the study; TTD, TPD, and ONI conducted molecular and field experiments; TTD, KT, GS, and KK analyzed molecular data, TTD, HN, and KK interpreted results and drafted the manuscript.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Number 26257409. The authors also acknowledge the partial support of the University of Agriculture and Forestry, Hue University, Vietnam.

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