Euphytica (2007) 153:233–247
DOI 10.1007/s10681-006-9259-4

# Molecular characterization of South and East Asian melon, *Cucumis melo* L., and the origin of Group Conomon var. *makuwa* and var. *conomon* revealed by RAPD analysis

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Received: 26 September 2005 / Accepted: 11 August 2006 / Published online: 28 September 2006


**Abstract** The genetic diversity and relationship among South and East Asian melon *Cucumis melo* L. were studied by using RAPD analysis of 69 accessions of melon from India, Myanmar, China, Korea, and Japan. The genetic diversity was large in India, and quite small in Group Conomon var. *makuwa* and var. *conomon* from East Asia, clearly indicating a decrease in genetic variation from India toward the east. Cluster analysis based on genetic distance classified 17 groups of accessions into two major clusters: cluster I comprising 12 groups of accessions from India and Myanmar and cluster II that included five groups of accessions of Group Conomon var. *makuwa* and var. *conomon* from East Asia. Cluster I was further divided into three subclusters, of which subclusters Ib and Ic included small- and large-seed type populations, respectively. Therefore, this division was based on their seed size, not cultivation area. The large-seed type from east India was differently included in the subcluster of small-seed type (Ib). A total of 122 plants of 69 accessions were classified into three major clusters and subclusters: clusters I and II comprised melon accessions mostly from India and Myanmar, and cluster III comprised Group Conomon var. *makuwa* and var. *conomon* from East Asia. The frequency of large- and small-seed types was different between clusters I and II, also indicating genetic differentiation between large- and small-seed types. One plant of the small-seed type from east India was differently included in cluster III, and two plants from east India were classified into subcluster IV. These results clearly showed that South Asian melon is genetically differentiated by their seed size, and that small-seed type melon in east India is closely related to Group Conomon var. *makuwa* and var. *conomon*.

**Keywords** *Cucumis melo* · Var. *conomon* · Var. *makuwa* · Genetic variation · RAPD · Phylogenetic relationship

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## Introduction

Melon (*Cucumis melo* L.) is an important horticultural crop, and is consumed as dessert, vegetable, and ornamental fruit, depending on the area and type of melon. A great variation exists in fruit characters, such as size, color and taste of melon fruit, and *C. melo* is considered the most diversified species in the genus *Cucumis* (Jeffrey 1980; Mallick and Masui 1986; Bates and Robbinson 1995). Naudin (1859) defined nine tribes of cultivated melon mainly from fruit characters and cultivation area. However, because of the large variation, intraspecific classification is complicated. Various modifications have been proposed (Munger and Robbinson 1991). *C. melo* has been classified into seven groups and this taxonomic classification has been used in various analyses (Mliki et al. 2001; Nakata et al. 2005). Wild melons with slender vines and small, inedible fruit is assigned to subsp. *agrestis*, and cultivated melon is classified into various horticultural groups of subsp. *melo*. Groups Cantalupensis and Inodorus have sweet flesh and are cultivated in Europe and USA, and Groups Momordica and Conomon have low sugar content and a smooth skin and are cultivated in South and East Asia. These two geographical origins are also different in seed length: the former is longer 9.0 mm and the latter, except group Momordica, is less than 8.5 mm (Fujishita 1983). Group Conomon, as defined by Munger and Robbinson (1991), is divided into var. *conomon* (Group Conomon var. *conomon*) and var. *makuwa* (Group Conomon var. *makuwa*) has been cultivated in Japan and used as different crops (Kitamura 1950). Group Conomon var. *makuwa* is also cultivated in Korea and rarely cultivated in South China. Its fruits with a smooth skin are sweet and fragrant when fully ripened, and they are eaten raw. Group Conomon var. *conomon* fruits with a smooth skin are sometimes eaten raw, but are usually cooked or pickled. Although they do not taste sweet, fully ripened fruits are slightly sweet and fragrant.

More recently, in addition to the classical classification based on morphology, cross compatibility, etc., molecular markers have been used to assess genetic diversity and phylogenetic relationship in melon. Genetic diversity in the United States and European melon has been examined by using restriction fragment length polymorphism (RFLP) analysis (Neuhausen 1992). Garcia et al. (1998) successfully used random polymorphic DNA (RAPD) analysis to evaluate melon germplasm of Galia and Piel de Sapo market classes. RAPD analysis has been used also to evaluate genetic diversity in germplasm of melon from Africa, Spain, Greece, and Japan (Mliki et al. 2001; Lopez-Sese et al. 2003; Nakata et al. 2003; Staub et al. 2004). RAPD analysis is more efficient than isozyme analysis to detect polymorphism on a single band basis (Staub et al. 1997), and its efficiency is as high as RFLP analysis (Silberstein et al. 1999). Simple sequence repeat (SSR) analysis (Staub et al. 2000) and amplification fragment length polymorphism (AFLP) analysis (Garcia-Mas et al. 2000) are more efficient than RAPD analysis, and thus are suitable to detect polymorphism among closely related breeding materials. However, the genetic relationship among melon germplasm shown by these analyses was similar irrespective of the marker systems used, indicating the usefulness of RAPD technology for phylogenetic analysis in melon. Phylogenetic analysis of melon using their phenotypic and molecular variation successfully separated Western melon from Asian melon, indicating genetic differentiation within cultivated melon (Silberstein et al. 1999; Stepansky et al. 1999). However, most of these studies focused mainly on the United States and European melon.

Less attention has been on East and South Asian melon, but various types of novel genetic resources are included and used in breeding programs. Akashi et al. (2002) and McCreight et al. (2004) evaluated genetic variation in East and South Asian melon by analysis of isozyme polymorphism, and showed that Indian melon is rich in genetic diversity compared with East Asian melon. Akashi et al. (2002) also analyzed the phylogenetic relationship among South and East Asian melon, and showed a difference in seed length between Group Conomon var. *makuwa* and var. *conomon* (4.5–8.5 mm) and Indian cultivated melon (4.0–13.0 mm). The frequency of the small-seed type melon (<9.0 mm)

varied between areas in India, being frequent in the central and eastern areas. Among Indian accessions, genetic differentiation was not detected between local populations, but was detected between large- and small-seed type melons. Their conclusion was also supported by AFLP analysis of East and South Asian melon (Yashiro et al. 2005). According to their report, seven accessions of the small-seed type from east and central India and Myanmar and two accessions of the large-seed type from east India were clustered together with most of the accessions of Group Conomon var. *makuwa* and var. *conomon*, suggesting the existence of the primitive type of Group Conomon var. *makuwa* and var. *conomon* in east India. However, RAPD analysis of melon cultivars from Japanese seed companies, suggested a relationship between melon accessions of Group Conomon and from the southern part of Africa (Nakata et al. 2005). This might be explained either by the introduction of Asiatic Group Conomon to South Africa or by their independent domestication from the same taxa, i.e., subsp. *agrestis*.

The origin of Group Conomon var. *makuwa* and var. *conomon* cultivated in East Asia, genetic diversity and differentiation in Indian melon, and the genetic relationship between Indian melon and Group Conomon var. *makuwa* and var. *conomon* requires further investigation. In this study, therefore, the genetic relationship among South and East Asian melon was studied by using RAPD analysis of 69 accessions of melon landraces from India, Myanmar, China, Korea, and Japan.

## Materials and methods

### Plant materials

Table 1 summarizes the 69 accessions of melon landraces (*C. melo* L.) used in this study. These accessions were selected mainly from East and South Asia, being 27 accessions from China, Korea and Japan, five accessions from Myanmar, and 37 accessions from India. The number of plants analyzed differed among accessions. From the 69 accessions, only one plant was examined in 16 accessions of Group Conomon var. *makuwa* and var. *conomon*, while two plants were examined in all other accessions, to give a total of 122 plants. Only one plant was necessary for some accession because they were maintained as pure lines in the National Institute of Vegetable and Tea Science (NIVTS), Japan.

Seeds of these accessions were provided by NIVTS, Japan, and the North Central Regional Plant Introduction Station, Iowa State University (USDA), USA. These accessions were first cultivated in the field or glasshouse of Okayama University, and selfed seeds of each accession were used in the experiments. Of the 69 accessions, 64 and 49 accessions were used by Akashi et al. (2002) and Yashiro et al. (2005), respectively.

India is a large country with large geographical and environmental diversity and is generally dry in the western region and wet in the eastern region (Hatakeyama 1964). In this study, Indian accessions were divided into five geographical groups after Akashi et al. (2002) based on annual precipitation in each area: west area (Punjab, Rajasthan, Gujarat, and Maharashtra, IW1-IW12), central area (Madhya Pradesh, IC1-IC16), north area (Uttar Pradesh, IN1-IN12), south area (Tamil Nadu and Andhra Pradesh, IS1-IS14) and east area (Bihar, Meghalaya, and Assam, IE1-IE20). A total of 106 plants from 53 accessions of unknown variety were classified into large-seed type (≥9.0 mm) and small-seed type (<9.0 mm) based on their seed length (Table 1).

### DNA extraction

Seeds were sown on filter paper and were grown at 26°C in a 16 h light–8 h dark cycle at light intensity 46.5 $\mu$M $s^{-1}$ $m^{-2}$. Ten-day-old seedlings were individually ground in liquid nitrogen, and total DNA was extracted by using the procedure of Murray and Thompson (1980) with minor modifications.

### RAPD analysis

Random primers (176; 12 mer, Bex) were tested by using five cultivars of melon: Group Canta-

**Table 1** Melon accessions analyzed in this study

| Plant number | Accession | Country of origin | Area/variety[a] | Seed size[b] | Seed source[c] | Samples studied by | | Cluster number[e] |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Akashi[d] | Yashiro[d] | |
| JM1 | New melon | Japan | var. *makuwa* | S | 1 | + | + | III |
| JM2 | New melon | Japan | var. *makuwa* | S | 1 | + | - | III |
| JM3 | Kanro | Japan | var. *makuwa* | S | 1 | + | + | III |
| JM4 | Kinpyo | Japan | var. *makuwa* | S | 1 | + | + | III |
| JM5 | Seikan | Japan | var. *makuwa* | S | 1 | + | - | III |
| JM6 | Nanbukin | Japan | var. *makuwa* | S | 1 | + | - | III |
| JC1 | Takada-shiro-uri | Japan | var. *conomon* | S | 1 | - | + | III |
| JC2 | Tokyo-wase-shiro-uri | Japan | var. *conomon* | S | 1 | - | - | III |
| JC3 | Nakasaki-tsuke-uri | Japan | var. *conomon* | S | 1 | + | - | III |
| JC4 | Karimori | Japan | var. *conomon* | S | 1 | + | + | III |
| JC5 | Hyougo-aoshima-uri | Japan | var. *conomon* | S | 1 | + | - | III |
| KM1 | 630044 | Korea | var. *makuwa* | S | 1 | + | - | III |
| KM2 | 630044 | Korea | var. *makuwa* | S | 1 | + | - | III |
| KM3 | 630047 | Korea | var. *makuwa* | S | 1 | + | + | III |
| KM4 | 630047 | Korea | var. *makuwa* | S | 1 | + | - | III |
| KM5 | 940147 | Korea | var. *makuwa* | S | 1 | + | + | III |
| KM6 | 940215 | Korea | var. *makuwa* | S | 1 | + | + | III |
| CM1 | PI 136173 | China | var. *makuwa* | S | 2 | + | + | II |
| CM2 | PI 136173 | China | var. *makuwa* | S | 2 | - | + | III |
| CM3 | PI 157070 | China | var. *makuwa* | S | 2 | + | + | III |
| CM4 | PI 157070 | China | var. *makuwa* | S | 2 | - | + | III |
| CM5 | 76007 | China | var. *makuwa* | S | 1 | + | + | III |
| CM6 | 76007 | China | var. *makuwa* | S | 1 | - | - | III |
| CM7 | 760008 | China | var. *makuwa* | S | 1 | + | - | III |
| CM8 | 760008 | China | var. *makuwa* | S | 1 | - | - | III |
| CM9 | 780143 | China | var. *makuwa* | S | 1 | + | + | III |
| CM10 | 910055 | China | var. *makuwa* | S | 1 | + | - | III |
| CM11 | 910055 | China | var. *makuwa* | S | 1 | + | - | III |
| CM12 | 940178 | China | var. *makuwa* | S | 1 | + | - | III |
| CM13 | 940184 | China | var. *makuwa* | S | 1 | + | - | III |
| CM14 | Mi-tang-tin | China | var. *makuwa* | S | 1 | + | - | III |
| CC1 | 940182 | China | var. *conomon* | S | 1 | + | - | III |
| CC2 | 940307 | China | var. *conomon* | S | 1 | + | - | III |
| CC3 | 940307 | China | var. *conomon* | S | 1 | + | - | III |
| CC4 | P169-1 | China | var. *conomon* | S | 3 | + | + | III |
| CC5 | P169-2 | China | var. *conomon* | S | 3 | + | - | III |
| CC6 | P171-1 | China | var. *conomon* | S | 3 | + | + | III |
| CC7 | P171-2 | China | var. *conomon* | S | 3 | + | - | III |
| IW1 | PI 116738 | India | Punjab | S | 2 | + | - | I |
| IW2 | PI 116738 | India | Punjab | S | 2 | + | - | I |
| IW3 | Ames 20522 | India | Rajasthan | S | 2 | + | + | II |
| IW4 | Ames 20522 | India | Rajasthan | S | 2 | + | + | II |
| IW5 | PI 164796 | India | Maharashtra | S | 2 | + | + | I |
| IW6 | PI 164796 | India | Maharashtra | S | 2 | + | + | II |
| IW7 | PI 182952 | India | Gujarat | L | 2 | + | + | II |
| IW8 | PI 182952 | India | Gujarat | L | 2 | + | - | II |
| IW9 | PI 164825 | India | Maharashtra | L | 2 | + | + | VI |
| IW10 | PI 164825 | India | Maharashtra | L | 2 | - | + | VI |
| IW11 | PI 116666 | India | Punjab | L | 2 | + | + | I |
| IW12 | PI 116666 | India | Punjab | L | 2 | + | + | I |
| IC1 | Ames 20981 | India | Madhya Pradesh | S | 2 | - | + | II |
| IC2 | Ames 20981 | India | Madhya Pradesh | S | 2 | - | + | II |
| IC3 | Ames 20982 | India | Madhya Pradesh | S | 2 | + | + | II |

**Table 1** continued

| Plant number | Accession | Country of origin | Area/variety[a] | Seed size[b] | Seed source[c] | Samples studied by | | Cluster number[e] |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Akashi[d] | Yashiro[d] | |
| IC4 | Ames 20982 | India | Madhya Pradesh | S | 2 | + | + | II |
| IC5 | Ames 20974 | India | Madhya Pradesh | S | 2 | + | + | II |
| IC6 | Ames 20974 | India | Madhya Pradesh | S | 2 | - | + | II |
| IC7 | Ames 20979 | India | Madhya Pradesh | S | 2 | + | + | I |
| IC8 | Ames 20979 | India | Madhya Pradesh | S | 2 | + | + | II |
| IC9 | Ames 20947 | India | Madhya Pradesh | S | 2 | + | + | II |
| IC10 | Ames 20947 | India | Madhya Pradesh | S | 2 | + | + | I |
| IC11 | Ames 21007 | India | Madhya Pradesh | L | 2 | + | + | II |
| IC12 | Ames 21007 | India | Madhya Pradesh | L | 2 | - | + | II |
| IC13 | PI 124435 | India | Madhya Pradesh | L | 2 | + | + | I |
| IC14 | PI 124435 | India | Madhya Pradesh | L | 2 | + | + | I |
| IC15 | Ames 20967 | India | Madhya Pradesh | L | 2 | + | + | I |
| IC16 | Ames 20967 | India | Madhya Pradesh | L | 2 | + | + | I |
| IN1 | PI 175109 | India | Uttar Pradesh | S | 2 | + | + | V |
| IN2 | PI 175109 | India | Uttar Pradesh | S | 2 | + | + | V |
| IN3 | PI 179666 | India | Uttar Pradesh | S | 2 | + | + | I |
| IN4 | PI 179666 | India | Uttar Pradesh | S | 2 | + | + | I |
| IN5 | PI 165508 | India | Uttar Pradesh | L | 2 | + | + | I |
| IN6 | PI 165508 | India | Uttar Pradesh | L | 2 | + | + | I |
| IN7 | PI 116479 | India | Uttar Pradesh | L | 2 | + | + | I |
| IN8 | PI 116479 | India | Uttar Pradesh | L | 2 | + | - | I |
| IN9 | PI 124109 | India | Uttar Pradesh | L | 2 | + | + | I |
| IN10 | PI 124109 | India | Uttar Pradesh | L | 2 | + | - | I |
| IN11 | PI 116490 | India | Uttar Pradesh | L | 2 | + | - | II |
| IN12 | PI 116490 | India | Uttar Pradesh | L | 2 | + | - | II |
| IS1 | PI 164320 | India | Tamil Nadu | S | 2 | + | + | II |
| IS2 | PI 164320 | India | Tamil Nadu | S | 2 | - | + | II |
| IS3 | PI 164323 | India | Tamil Nadu | S | 2 | + | + | II |
| IS4 | PI 164323 | India | Tamil Nadu | S | 2 | + | + | II |
| IS5 | PI 124096 | India | Andhra Pradesh | S | 2 | - | - | II |
| IS6 | PI 124096 | India | Andhra Pradesh | S | 2 | - | - | II |
| IS7 | PI 123501 | India | Tamil Nadu | L | 2 | - | + | I |
| IS8 | PI 123501 | India | Tamil Nadu | L | 2 | + | + | I |
| IS9 | PI 164585 | India | Tamil Nadu | L | 2 | + | - | I |
| IS10 | PI 164585 | India | Tamil Nadu | L | 2 | + | - | II |
| IS11 | PI 123684 | India | Andhra Pradesh | L | 2 | - | - | I |
| IS12 | PI 123684 | India | Andhra Pradesh | L | 2 | - | - | I |
| IS13 | PI 124105 | India | Andhra Pradesh | L | 2 | - | - | I |
| IS14 | PI 124105 | India | Andhra Pradesh | L | 2 | - | - | I |
| IE1 | PI 210542 | India | Meghalaya | S | 2 | + | + | III |
| IE2 | PI 210542 | India | Meghalaya | S | 2 | - | - | II |
| IE3 | PI 210541 | India | Meghalaya | S | 2 | + | + | I |
| IE4 | PI 210541 | India | Meghalaya | L | 2 | + | + | I |
| IE5 | PI 124113 | India | Bihar | S | 2 | + | + | I |
| IE6 | PI 124113 | India | Bihar | S | 2 | + | + | I |
| IE7 | PI 124112 | India | Bihar | S | 2 | + | + | IV |
| IE8 | PI 124112 | India | Bihar | L | 2 | + | + | IV |
| IE9 | PI 166125 | India | Bihar | S | 2 | + | + | II |
| IE10 | PI 166125 | India | Bihar | S | 2 | + | + | II |
| IE11 | PI 124111 | India | Bihar | L | 2 | + | + | I |
| IE12 | PI 124111 | India | Bihar | L | 2 | - | + | I |
| IE13 | PI 124207 | India | Bihar | L | 2 | + | + | I |
| IE14 | PI 124207 | India | Bihar | L | 2 | + | + | I |

**Table 1** continued

| Plant number | Accession | Country of origin | Area/variety[a] | Seed size[b] | Seed source[c] | Samples studied by | | Cluster number[e] |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Akashi[d] | Yashiro[d] | |
| IE15 | PI 124208 | India | Bihar | L | 2 | + | + | I |
| IE16 | PI 124208 | India | Bihar | L | 2 | + | + | I |
| IE17 | PI 210076 | India | Assam | L | 2 | + | - | II |
| IE18 | PI 210076 | India | Assam | L | 2 | + | + | II |
| IE19 | PI 210077 | India | Assam | L | 2 | + | + | II |
| IE20 | PI 210077 | India | Assam | L | 2 | - | + | II |
| MY1 | PI 200816 | Myanmer | Mandalay | S | 2 | + | + | II |
| MY2 | PI 200816 | Myanmer | Mandalay | S | 2 | + | + | II |
| MY3 | PI 200819 | Myanmer | Mandalay | S | 2 | + | - | II |
| MY4 | PI 200819 | Myanmer | Mandalay | S | 2 | + | - | II |
| MY5 | PI 200814 | Myanmer | Mandalay | S | 2 | + | + | II |
| MY6 | PI 200814 | Myanmer | Mandalay | S | 2 | + | + | II |
| MY7 | PI 200817 | Myanmer | Kachin | S | 2 | + | + | II |
| MY8 | PI 200817 | Myanmer | Kachin | S | 2 | + | + | II |
| MY9 | PI 200813 | Myanmer | Mandalay | L | 2 | + | - | I |
| MY10 | PI 200813 | Myanmer | Mandalay | L | 2 | + | - | I |

[a] Var. *makuwa* and var. *conomon* indicate Group Conomon var. *makuwa* and var. *conomon*, respectively

[b] Accessions of unknown variety were classified as large-seed type (≥9.0 mm) and small-seed type (<9.0 mm), and indicated by "L" (large-seed type) and "S" (small-seed type)

[c] *1* National Institute of Vegetable and Tea Science (NIVTS), Japan, *2* North Central Regional Plant Introduction Station, Iowa State University (USDA-ARS), USA, *3* Okayama University, Japan

[d] Plants also analyzed by Akashi et al. (2002) and Yashiro et al. (2005) are indicated by "+"

[e] Cluster number shown in Fig. 3

lupensis cv. 'Earls' Favourite' (netted), Group Cantalupensis cv. 'Rocky Ford' (netted), Group Cantalupensis cv. 'Charentais', Group Conomon var. *makuwa* cv. 'Kinpyo', Group Conomon var. *conomon* cv. 'Takada-shiro-uri'. Eighteen random primers selected for their ability to detect polymorphism and for the stability of PCR amplification were used for RAPD analysis (Table 2). PCR amplification was done in a 10 $\mu$l mixture containing 50 ng genomic DNA, 1 $\mu$l PCR buffer (Sigma®, St. Louis, MO, USA: 10 mM Tris–HCl (pH 8.3), 50 mM KCl), 2.5 mM $MgCl_2$, 0.25 U *Taq* polymerase (Pharmacia for primer A07 and Sigma for others), 0.1 mM dNTP and 0.5 $\mu$M primer by using i-Cycler (Bio-Rad Laboratories, Hercules, CA, USA), and PC–707 (ASTEC, Tokyo, Japan). An initial denaturing step at 95°C for 3 min, 40 PCR cycles at 94°C for 1 min, 40°C for 2 min, and 72°C for 2 min were done, and then a final extension at 72°C for 5 min. After the amplification, samples underwent electrophoresis on 1.5% agarose gel (Takara, Tokyo, Japan) at constant voltage 100 V (Mupid-2, Cosmo Bio, Tokyo, Japan). Then the PCR products were visualized with ethidium bromide staining and their polymorphisms were evaluated.

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

| Primer number | Sequence (5′ → 3′) | Polymorphic fragments | |
|---|---|---|---|
| | | Number | Size (bp) |
| A07 | GATGGATTTGGG | 2 | 1,353, 872 |
| A20 | TTGCCGGGACCA | 2 | 1,100, 800 |
| A22 | TCCAAGCTACCA | 1 | 1,520 |
| A23 | AAGTGGTGGTAT | 1 | 1,200 |
| A26 | GGTGAGGATTCA | 1 | 1,400 |
| A31 | GGTGGTGGTATC | 1 | 800 |
| A39 | CCTGAGGTAACT | 1 | 2,027 |
| A41 | TGGTACGGTATA | 3 | 1,353, 1,020, 930 |
| A57 | ATCATTGGCGAA | 1 | 800 |
| B15 | CCTTGGCATCGG | 1 | 600 |
| B32 | ATCATCGTACGT | 2 | 900, 700 |
| B68 | CACACTCGTCAT | 1 | 1,078 |
| B71 | GGACCTCCATCG | 1 | 1,220 |
| B84 | CTTATGGATCCG | 3 | 700, 600, 550 |
| B86 | ATCGAGCGAACG | 2 | 1,500, 1,350 |
| B96 | GTGAAGACTATG | 2 | 850, 750 |
| B99 | TTCTGCTCGAAA | 1 | 1,400 |
| C00 | GAGTTGTATGCG | 1 | 1,350 |
| Total | | 27 | |

### Data analysis

DNA fragments were scored as present (1) or absent (0) for 27 markers. Genetic similarity (GS) measured according to Apostol et al. (1993) represented the similarity between two accessions and was calculated by the formula GS = (N11 + N00)/T, where N11 and N00 were the number of positive and null bands, respectively, shared between two accessions, and T was the total number of bands scored. The genetic distance (GD) between two accessions was calculated by using the formula GD = 1-GS. Gene diversity (D) within each group and GD between each group were calculated according to Weir (1996) and Nei (1972), respectively. A dendrogram was constructed by using Phylip programs (http://bioweb.pasteur.fr/seqanal/phylogeny/phylip-uk.html), based on the GD matrix, by using the un-weighted pair group method with arithmetic averages (UPGMA) cluster analysis. Principal coordinate analysis (PCO; Gower 1966) based on the GS matrix was done to show multiple dimensions of each group and the accessions in a scatter-plot.

## Results

### RAPD analysis

Eighteen primers produced 27 polymorphic marker bands whose sizes were 550–2,027 bp, and the average number of marker bands of each primer was 1.5 (Table 2). The most polymorphic band was A20–800 amplified from 60 of the 122 plants examined. Polymorphism was detected in two plants of each accession, except two accessions of Group Conomon var. *makuwa* from China (PI 157070 (CM3, CM4), 910055 (CM10, CM11)) and one accession of Group Conomon var. *makuwa* from Korea (630047 (KM3, KM4)). The number of polymorphic marker bands detected within each accession was 1–10. The average number of polymorphic marker bands detected in each population was higher in large- (18.6) and small- (16.0) seed types from South Asia than from Myanmar (9.0) and Group Conomon var. *makuwa* and var. *conomon* (6.8) from East Asia.

### Genetic relationship between melon populations

Genetic diversity (D) within each geographical region was calculated from the frequency of 27 RAPD markers (Table 3). This ranged from 0.253 to 0.305 (average = 0.281) in large-seed types from India to 0.148–0.278 (average = 0.221) in small-seed types from India, and were higher than for the Myanmar region (average = 0.128). In contrast, the populations of Group Conomon var. *makuwa* and var. *conomon* from China, Korea, and Japan were less diversified (max. = 0.119) than those from India and Myanmar, indicating decreasing genetic diversity from India toward the east.

The GD between the 17 groups of accession S ranged from 0.01 to 0.51 and was 0.21 on average (Table 4). By using cluster analysis based on GD and using the UPGMA method, they were classified into two major clusters: Cluster I comprised 12 accession groups from India and Myanmar, cluster II that included five accession groups of Group Conomon var. *makuwa* and var. *conomon* (Fig. 1). Cluster I was further divided into subclusters from their seed size, not their cultivation area: Ib of small-seed type and Ic of large-seed type. One exception was the large-seed type from east India that was included in subcluster Ib of the small-seed type.

The genetic relationships between the 17 groups of accessions were further analyzed by using PCO based on a similarity matrix. Up to 61.5% of the total variation was explained by the first two axes that explained, respectively, 47.1 and 14.4% (Fig. 2). The genetic relationship shown by the PCO plot in Fig. 2 was similar to that of cluster analysis. The groups of accessions were divided into two groups by the first principal axis (PCO1), one group consisted of Group Conomon var. *makuwa* and var. *conomon* and the other group consisted of accessions from Myanmar and India. The populations from Myanmar and India were separated into large- and small-seed type groups by the second principal axis (PCO2). The large-seed type melon from

**Table 3** Gene diversity and number of melon plants classified into six clusters in each group of melon landraces

| Country | Variety[a]/seed size | Area | Number of accessions | Cluster | | | | | | Gene diversity |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I | II | III | IV | V | VI | |
| India | Large-seed type | West | 6 | 2 | 2 | – | – | – | 2 | 0.253 |
| | | North | 8 | 6 | 2 | – | – | – | – | 0.278 |
| | | Center | 6 | 4 | 2 | – | – | – | – | 0.294 |
| | | South | 8 | 7 | 1 | – | – | – | – | 0.273 |
| | | East | 12 | 7 | 4 | – | 1 | – | – | 0.305 |
| | Small-seed type | West | 6 | 3 | 3 | – | – | – | – | 0.239 |
| | | North | 4 | 2 | – | – | – | 2 | – | 0.218 |
| | | Center | 10 | 2 | 8 | – | – | – | – | 0.224 |
| | | South | 6 | – | 6 | – | – | – | – | 0.148 |
| | | East | 8 | 3 | 3 | 1 | 1 | – | – | 0.278 |
| Myanmar | Large-seed type | | 2 | 2 | – | – | – | – | – | 0.093 |
| | Small-seed type | | 8 | – | 8 | – | – | – | – | 0.163 |
| China | var. *makuwa* | | 14 | – | 1 | 13 | – | – | – | 0.104 |
| | var. *conomon* | | 7 | – | – | 7 | – | – | – | 0.076 |
| Korea | var. *makuwa* | | 6 | – | – | 6 | – | – | – | 0.091 |
| Japan | var. *makuwa* | | 6 | – | – | 6 | – | – | – | 0.119 |
| | var. *conomon* | | 5 | – | – | 5 | – | – | – | 0.071 |
| Total | | | 122 | 38 | 40 | 38 | 2 | 2 | 2 | |

[a] Var. *makuwa* and var. *conomon* indicate Group Conomon var. *makuwa* and var. *conomon*, respectively

east India was plotted close to the small-seed type populations.

All groups of accessions from South Asian were clearly separated from groups of Group Conomon var. *makuwa* and var. *conomon* by cluster analysis (Fig. 1) and by PCO analysis (Fig. 2). To identify South Asian melon populations most closely related to those of Group Conomon var. *makuwa* and var. *conomon*, the GD between five accession groups of Group Conomon var. *makuwa* and var. *conomon* and 12 accessin groups from South Asia was compared (Table 5). The GD was smaller in the populations of small-seed type melon (average distance = 0.25) compared with populations of large-seed type melon (average distance = 0.33); the difference was statistically significant ($t = 1.986^{*}$, $df = 10$; $t = 4.480^{**}$, $df = 58$). The smallest value was in the small-seed type population from east India (0.20), and then in populations from Myanmar (0.21) and central India (0.22). Among the large-seed type melon, the population from east India showed the smallest value (0.24). These results showed that small-seed type melon from central and east India is related closely to Group Conomon var. *makuwa* and var. *conomon*.

## Genetic relationships between 122 plants of melon landraces

The GDs between 122 plants of 69 accessions were calculated from the presence or absence of 27 RAPD markers, and their relationship was analyzed. The average GD was 0.33 and ranged from 0 to 0.74. The largest GD was recorded between Seikan (JM5), Group Conomon var. *makuwa* of Korea, and two accessions of large-seed type melon (PI 124105 (IS13, IS14)) from south India (data not shown). The smallest GD was between 18 pairs of accessions of Group Conomon var. *makuwa* and var. *conomon* from China, Korea, and Japan.

By using cluster analysis and the UPGMA method, 122 plants were classified into six clusters, of which 116 plants were included in clusters I–III (Fig. 3, Table 3). In cluster I, 28 of 38 plants (73.7%) were large-seed type. In cluster II, 29 of 40 plants (72.5%) were small-seed type and one plant (PI 136173; CM1) of Group Conomon var. *makuwa* from China was also included. In Cluster III, 37 plants of Group Conomon var. *makuwa* and var. *conomon* and the accessions of two varieties were not separated. Plant PI 210542 (IE1) of small-seed type

**Table 4** Genetic distance between 17 groups of melon landraces of Asian melon, calculated from the frequency of 27 RAPD marker bands

| Accession group number | Country | Variety/seed size | Area | Cluster number[a] | Accession group number | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| 1 | India | Large-seed type | West | Ic | – | | | | | | | | | | | | | | | | |
| 2 | | | North | Ic | 0.10 | – | | | | | | | | | | | | | | | |
| 3 | | | Center | Ic | 0.12 | 0.07 | – | | | | | | | | | | | | | | |
| 4 | | | South | Ic | 0.16 | 0.09 | 0.08 | – | | | | | | | | | | | | | |
| 5 | | | East | Ib | 0.15 | 0.12 | 0.07 | 0.12 | – | | | | | | | | | | | | |
| 6 | | Small-seed type | West | Ib | 0.18 | 0.13 | 0.11 | 0.19 | 0.08 | – | | | | | | | | | | | |
| 7 | | | North | Ia | 0.17 | 0.17 | 0.14 | 0.17 | 0.14 | 0.16 | – | | | | | | | | | | |
| 8 | | | Center | Ib | 0.14 | 0.23 | 0.11 | 0.22 | 0.09 | 0.13 | 0.25 | – | | | | | | | | | |
| 9 | | | South | Ib | 0.15 | 0.19 | 0.09 | 0.26 | 0.12 | 0.14 | 0.20 | 0.06 | – | | | | | | | | |
| 10 | | | East | Ib | 0.13 | 0.15 | 0.12 | 0.22 | 0.06 | 0.09 | 0.14 | 0.10 | 0.09 | – | | | | | | | |
| 11 | Myanmar | Large-seed type | | Ia | 0.24 | 0.17 | 0.11 | 0.18 | 0.18 | 0.24 | 0.21 | 0.25 | 0.29 | 0.24 | – | | | | | | |
| 12 | | Small-seed type | | Ib | 0.19 | 0.19 | 0.09 | 0.26 | 0.10 | 0.08 | 0.24 | 0.08 | 0.07 | 0.09 | 0.27 | – | | | | | |
| 13 | China | var. *makuwa* | | II | 0.28 | 0.33 | 0.26 | 0.46 | 0.24 | 0.28 | 0.37 | 0.22 | 0.25 | 0.22 | 0.30 | 0.22 | – | | | | |
| 14 | | var. *conomon* | | II | 0.30 | 0.37 | 0.28 | 0.48 | 0.24 | 0.27 | 0.35 | 0.24 | 0.24 | 0.19 | 0.38 | 0.20 | 0.05 | – | | | |
| 15 | Korea | var. *makuwa* | | II | 0.28 | 0.37 | 0.29 | 0.50 | 0.27 | 0.29 | 0.41 | 0.24 | 0.29 | 0.23 | 0.36 | 0.23 | 0.01 | 0.05 | – | | |
| 16 | Japan | var. *makuwa* | | II | 0.35 | 0.40 | 0.30 | 0.51 | 0.24 | 0.29 | 0.34 | 0.23 | 0.24 | 0.20 | 0.34 | 0.21 | 0.05 | 0.10 | 0.06 | – | |
| 17 | | var. *conomon* | | II | 0.31 | 0.36 | 0.25 | 0.44 | 0.21 | 0.25 | 0.29 | 0.18 | 0.20 | 0.17 | 0.29 | 0.18 | 0.08 | 0.16 | 0.11 | 0.03 | – |

[a] Cluster number shown in Fig. 1

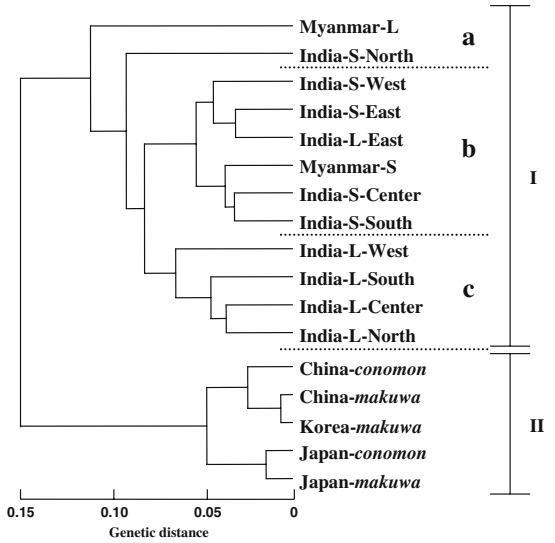


**Fig. 1** Genetic relationship between 17 groups of melon landraces, revealed by UPGMA cluster analysis based on GD. *L* Large-seed type, *S* Small-seed type

from east India (Meghalaya) was included in cluster III, and plant PI 210542 (IE2) was included in cluster II. Cluster IV was closely related to cluster III, and comprised plants of

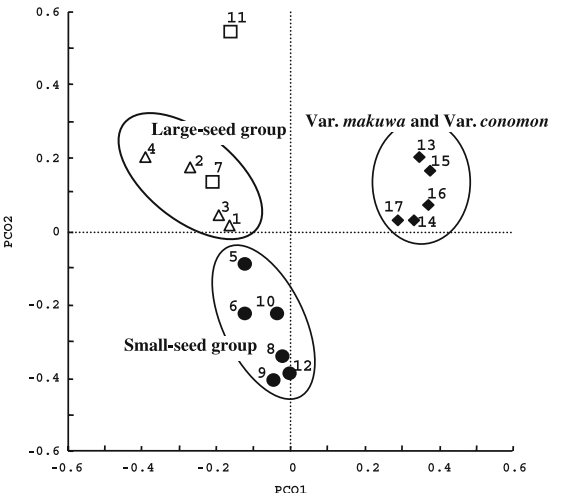


**Fig. 2** Distribution on the first two principal co-ordinates of 17 groups of melon landraces from South and East Asia. *Symbols* represent cluster number of each group of accessions shown in Fig. 1 (*Open square box* Ia, *filled circle* Ib, *open triangle* Ic, *filled diamond* II). Numeric characters represent the accession group number listed in Table 4

PI 124112 (small-seed type; IE7, large-seed type; IE8) from east India (Bihar). These results, therefore, indicate that South Asian melon is genetically differentiated by its seed size, and that small-seed type melon from east India is closely related to Group Conomon var. *makuwa* and var. *conomon*.

By using PCO analysis based on a similarity matrix between each plant, up to 35.8% of the total variation was explained by the PCO1 and PCO2 axes, which explained 24.5 and 11.3%, respectively (Fig. 4). Most plants of Group Conomon var. *makuwa* and var. *conomon* were closely grouped in the left upper quadrant, and were distinguished from those of South Asia by the PCO1 axis. Melon accessions from South Asia were scattered widely, and most large- and small-seed type accessions were at different positions on the PCO plot separated by the PCO1 and PCO2 axes, indicating the richness in genetic variation and genetic differentiation between large- and small-seed type melons. One plant of small-seed type, PI 210542 (IE1) from east India, which was included in cluster III (Fig. 3), was also included in Group Conomon var. *makuwa* and var. *conomon* on the PCO plot. Plant PI 210542 (IE2) and plants PI124112 (IE8), PI 210076 (IE18), and PI 210077 (IE19) of the large-seed type were closely related to Group Conomon var. *makuwa* and var. *conomon*.

## Discussion

Although the ancestral species of cultivated melon is unknown, cultivated melon originated in Africa, was first domesticated in Middle and Near East and spread throughout the world (Robinson and Decker-Walters 1997). Various types of melon have been established in different parts of the world, and their seed sizes are different. The seed length is longer than 9.0 mm in Groups Cantalupensis and Inodorus cultivated in the Middle and Near East, Europe, and USA (Fujishita and Oda 1965). Group Conomon var. *makuwa* and var. *conomon* cultivated in East Asia are small-seed type (<9.0 mm). Both large- and small-seed types are common in South Asia (Akashi et al. 2002).

**Table 5** Genetic distance between groups of melon landraces from India and Myanmar and var. *makuwa* and var. *conomon* in East Asia

| Accession group number | China | | Korea | Japan | | Average |
|---|---|---|---|---|---|---|
| | var. *makuwa* | var. *conomon* | var. *makuwa* | var. *makuwa* | var. *conomon* | |
| India (Large-seed type) | | | | | | |
| West | 0.28 | 0.30 | 0.28 | 0.35 | 0.31 | 0.30 |
| North | 0.33 | 0.37 | 0.37 | 0.40 | 0.36 | 0.36 |
| Center | 0.26 | 0.28 | 0.29 | 0.30 | 0.25 | 0.27 |
| South | 0.46 | 0.48 | 0.50 | 0.51 | 0.44 | 0.48 |
| East | 0.24 | 0.24 | 0.27 | 0.24 | 0.21 | 0.24 |
| India (Small-seed type) | | | | | | |
| West | 0.28 | 0.27 | 0.29 | 0.29 | 0.25 | 0.28 |
| North | 0.37 | 0.35 | 0.41 | 0.34 | 0.29 | 0.35 |
| Center | 0.22 | 0.24 | 0.24 | 0.23 | 0.18 | 0.22 |
| South | 0.25 | 0.24 | 0.29 | 0.24 | 0.20 | 0.24 |
| East | 0.22 | 0.19 | 0.23 | 0.20 | 0.17 | 0.20 |
| Myanmar | | | | | | |
| Large-seed type | 0.30 | 0.38 | 0.36 | 0.34 | 0.29 | 0.33 |
| Small-seed type | 0.22 | 0.20 | 0.23 | 0.21 | 0.18 | 0.21 |

Melon accessions from Europe and USA and those from East Asia were classified into separate clusters by using RAPD analysis (Silberstein et al. 1999; Stepansky et al. 1999). Indian melon accessions were classified into two clusters, Euro-American melon and East Asian melon, by using SSR analysis (Monforte et al. 2003). Fujishita et al. (1993) classified melon accessions into three types by analysis of bitterness of young fruit placenta of the inter-varietal $F_1$ hybrid: Group Conomon var. *makuwa* and var. *conomon* (East Asia), Group Momordica (South Asia), and Group Cantalupensis and Inodorus (Europe and USA). These results clearly indicate genetic and geographical differentiation in melon, and suggest that seed length of each type of melon is related to such a differentiation.

In this study, the average D was 0.281 and 0.221, respectively, for large- and small-seed types in five geographical groups of accessions from India (Table 3). Group Conomon var. *makuwa* and var. *conomon* from China, Korea and Japan were less diversified (0.092) than South Asian melon, as also indicated by isozyme analysis (Akashi et al. 2002; McCreight et al. 2004) and AFLP analysis (Yashiro et al. 2005). The average GD among South Asian melon accessions (0.15) was larger than among accessions of Group Conomon var. *makuwa* and var. *conomon* (0.07). Therefore, a specific type of melon may have been selected in East Asia from genetically diversified melon introduced from South Asia, and then differentiated into var. *makuwa* and var. *conomon* as a bottleneck effect, as also suggested by the above studies (Akashi et al. 2002; Yashiro et al. 2005). India has various climate zones. Western India is dry with intermittent rain from June to September, and the east is wet with continuous rainy days from April to early October. A large genetic variation detected in South Asian melon could be partly explained by cultivation under diverse climatic condition and by cultivation of different types of melon in the rainy or dry season. The D may also have been increased by occasional hybridization among large- and small-seed type melons.

In this study, by using cluster analysis and PCO analysis of 17 populations, South Asian melon, except those from east India and Myanmar, were differentiated by their seed size (Figs. 1, 2), as also indicated by isozyme and AFLP analyses (Akashi et al. 2002; Yashiro et al. 2005). Why is South Asian melon, rich in genetic diversity, differentiated by their seed size, and why was small-seed type melon selectively transmitted to East Asia? Small-seed type melons are mostly wet-tolerant under field conditions, and the frequency

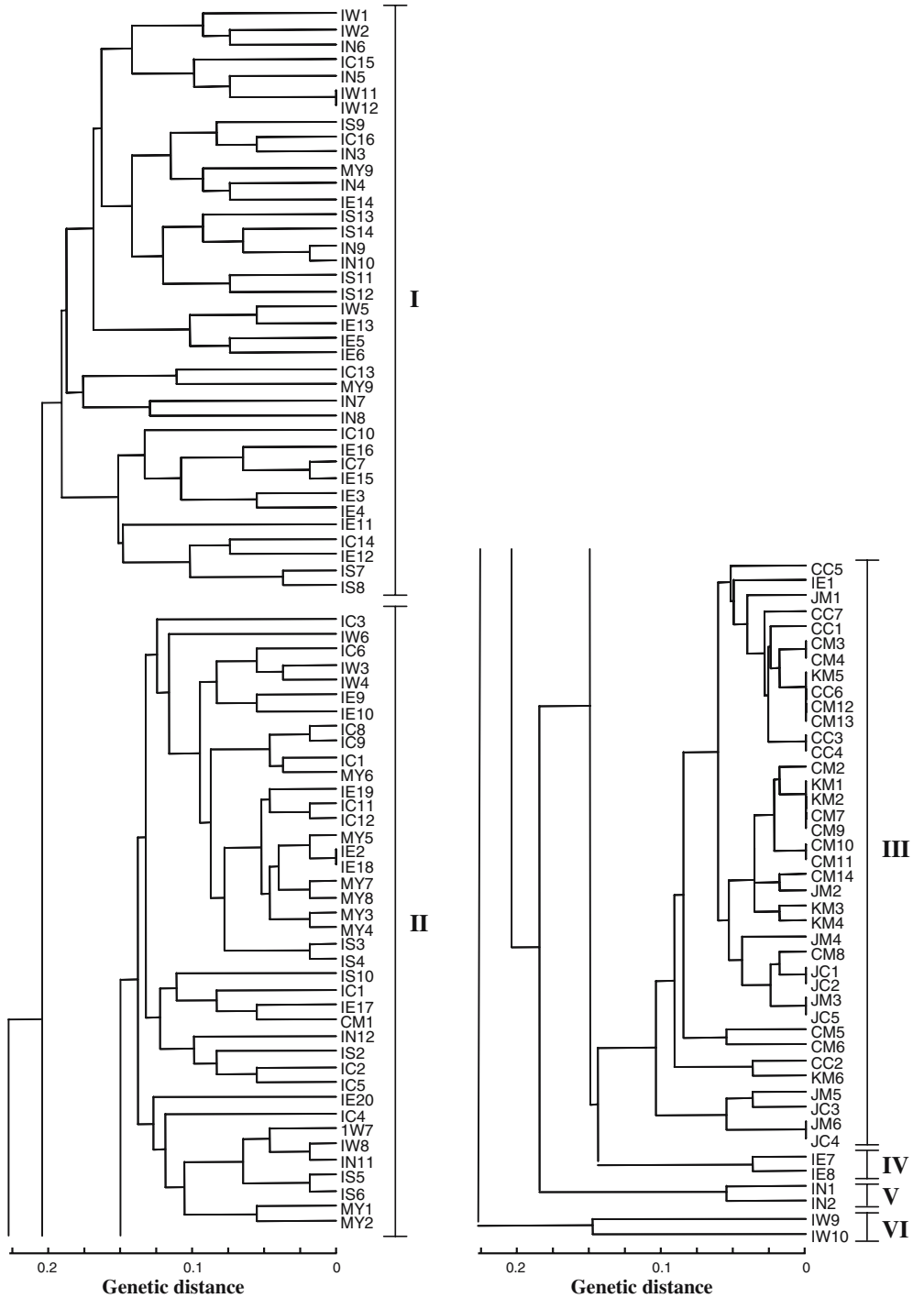


**Fig. 3** Genetic relationship between 122 plants of melon landraces from South and East Asia, revealed by UPGMA cluster analysis based on GD. Each plant was indicated by plant number listed in Table 1

of wet-tolerant accessions increases from west to east India (Akashi et al. 2002). This was confirmed by our field trip that mainly small-seed type melon grows during the rainy season in east India (Kato et al. 2006). Group Conomon var. *makuwa* and var. *conomon* showed a high ability

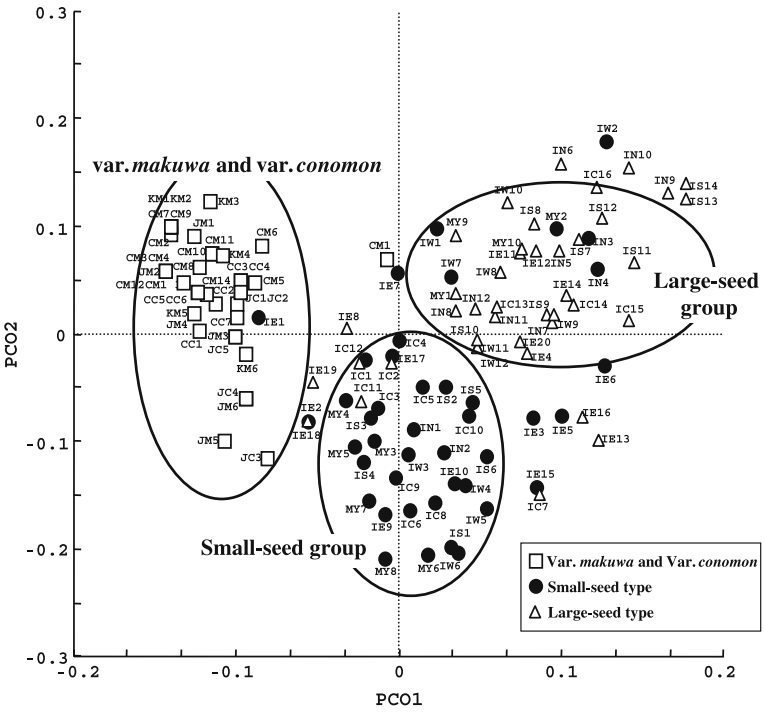


**Fig. 4** Distribution on the first two principal co-ordinates of 122 plants of melon landraces from South and East Asia. *Symbols* represent group/type of each plant, and plant number (see Table 1) is indicated

to germinate under anaerobic conditions in shallow water of depth 10 mm, and melon accessions that showed a high ability to germinate were all of the small-seed type among South Asian melon of this group (Tanaka et al. 2002). Group Conomon var. *makuwa* and var. *conomon* are closely related to the small-seed type of east and central India and Myanmar (Table 5), confirming earlier reports using isozyme analysis (Akashi et al. 2002). Therefore, small-seed type melon with wet tolerance may have originated in central India and was selected under wet condition in east India, resulting in the establishment of Group Conomon var. *makuwa* and var. *conomon* with further eastward transmission. Group Conomon var. *makuwa* and var. *conomon* were genetically inseparable, which can be explained by assuming that Group Conomon var. *makuwa* and var. *conomon* originated from the same gene pool. To confirm this hypothesis, additional melon germplasm should be collected in east India and South east Asia, and be evaluated for molecular polymorphism and wet tolerance.

In the case of identification of the primitive type of Group Conomon var. *makuwa* and var. *conomon* in India, by using AFLP analysis, two plants of central India, five plants of east India and two plants of Myanmar were clustered with Group Conomon var. *makuwa* and var. *conomon* (Yashiro et al. 2005). In this study, three plants each of large-seed type (PI 124112 (IE8), PI 210076 (IE18), PI 210077 (IE19)) and small-seed type (PI 210542 (IE1, IE2), PI 124112 (IE7)) from east India were closely related to Group Conomon var. *makuwa* and var. *conomon* (Figs. 3, 4), and thus these plants, especially the small-seed type, could be the primitive type of Group Conomon var. *makuwa* and var. *conomon*. Four plants of two accessions (PI 210542 (IE1, IE2), PI 124112 (IE7, IE8)), whose relationship with Group Conomon var. *makuwa* and var. *conomon* was confirmed by both RAPD and AFLP analyses, showed similarity with Group Conomon var. *makuwa* and var. *conomon*, also by fruit characters such as weight (0.3–1.0 kg), length (13.0–20.0 cm) and width (8.5–10.0 cm) of fruit, smooth skin, and brix of flesh juice (4.0–5.0°). A small-seed type melon from Meghalaya (PI 210542; IE1), which is most closely related to Group Conomon var. *makuwa*

and var. *conomon* by cluster and PCO analyses, showed perfect germination under anaerobic conditions in shallow water of depth 10 mm (Tanaka et al. 2002), and was wet tolerant in the field (Akashi et al. 2002).

The seed length of wild species of *Cucumis* is shorter than 9.0 mm and of cultivated melon in the areas surrounding the Mediterranean Sea is mostly longer than 9.0 mm. Therefore, seed length is reasonably assumed to have increased over the long history of domestication. However, little is known about the origin of small-seed type melon. Nakata et al. (2005) even suggested a relationship between South African melon germplasm and Group Conomon var. *makuwa* and var. *conomon* by using analyses of RAPD and SSR polymorphisms, but Indian melon germplasm was not included. Therefore, further study is necessary to uncover the origin of small-seed type melon. RAPD markers analyzed in this study were mostly nuclear markers suitable to detect polymorphism between cultivars or accessions of the same species. However, cytoplasmic markers should be used to analyze long-term evolution, because the cytoplasmic genome is genetically invariable and is transmitted maternally. Chloroplast SSR markers (ccSSR) applicable to a wide range of plant species have been developed by Chung and Staub (2003), and phylogenetic relationships among *Cucurbitaceae* species have been successfully analyzed (Chung et al. 2003). Analysis of the chloroplast genome by using these markers could be effectively used to uncover the origin and differentiation of large- and small-seed type melons and the evolution of *Cucumis* species. Furthermore, the mitochondrial genome is inherited paternally in *Cucumis* species (Havey et al. 1998), and so interesting information should be obtained by analysis of the cytoplasmic genome.

**Acknowledgments** We would like to thank Dr. K. R. Reitsma, Iowa State University, for kindly supplying the seeds. This study was partly supported by a Grant-in-Aid for International Scientific Research of Ministry of Education, Science, Culture and Sports, Japan (No. 15255011), entitled ''Genetic assay and study of crop germplasm in and around China''. This is Contribution number 2 from the Sato Project of Research Institute for Humanity and Nature (RIHN), Japan.

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