**Introduction**

Mango (*Mangifera indica* L.) is a juicy stone fruit in the Anacardiaceae, which includes about 850 species of tropical fruit trees (Bompard 2009), and is an economically important cash crop produced about 40 Mt in 2012 (Mitra 2016). Mango is grown widely in the world’s tropical and subtropical regions, as well as in a wide range of more marginal areas; India, China, Thailand, Mexico, Pakistan and Indonesia are the major producers (Mitra 2016). It is believed to have originated in the areas from India, where it has been grown for more than 4000 years and considered to be a primary center of diversity, to the Malay Peninsula in Southeast Asia.

More than 1000 mango cultivars exist around the world (Mukherjee 1953). They can be divided into two cultivar groups based on their embryo type: the monoembryonic (Indian) type is predominantly distributed in the subtropics, and the polyembryonic (Southeast Asian) type is most common in the tropics (Iyer and Degani 1997, Viruel et al. 2005). The polyembryony trait is dominant (Aron et al. 1998, Mukherjee and Litz 2009). The Indian type has a zygotic (sexually produced) embryo, and the fruit skin is mainly red, whereas the Southeast Asian type has several nucellar embryos (produced from the mother plant), and the skin is mainly green to yellow (Iyer and Degani 1997, Viruel et al. 2005).

During the 20th century, mango germplasms were introduced into Florida, USA, from the Caribbean Islands, Southeast Asia (the Philippines, Cambodia), India, and whole area extending from India to the Malay Peninsula, creating a secondary center of genetic diversity (Mukherjee and Litz 2009). In 1910, a seedling of ‘Mulgoba’ came into production in Florida, and the attractive selection was named ‘Haden’. Genetic relatedness and diversity analyses revealed three distinct clusters. Two clusters correspond to the groups of USA and India, which are closely related. The other includes accessions from Southeast and East Asia. The results agree with the previous identification of genetically distinct Indian and Southeast Asian types, and suggest that the Florida accessions, which originated from hybrids between these two types, are more closely related to the Indian type.

**Key Words:** genetic diversity, genetic resources in Japan, *Mangifera indica*, mango, parentage.
Isozyme markers were initially used in a survey of genetic variation (Gan et al. 1981) and for the identification of cultivars (Degani et al. 1990). Schnell et al. (1995) used random amplified polymorphic DNA (RAPD) markers to fingerprint cultivars and estimate the genetic relationships among a group of putative ‘Haden’ seedlings. López-Valenzuela et al. (1997) used RAPD markers to estimate the genetic diversity of 15 mango cultivars and identified a specific RAPD band that was associated only with the polylembozy type. Kashkush et al. (2001) used amplified-fragment-length polymorphic (AFLP) markers to estimate the genetic relationships among 16 cultivars and 7 rootstocks. These markers have been used to identify cultivars, evaluate their genetic relationships, and confirm that cross-pollination has occurred (Arias et al. 2012, Krishna and Singh 2007).

Simple sequence repeat (SSR), or microsatellite, markers have advantages over many other marker types: they are highly polymorphic, have multiple alleles, and are co-dominant. SSRs have been widely used for the conservation of genetic resources and in population genetics, molecular breeding, and paternity testing studies (Ellegren 2004). In mango, SSR markers are particularly important in the identification of cultivars, determination of genetic variability, conservation of germplasm, and identification of the domestication and movement of germplasm (Viruel et al. 2005). More than 100 SSR markers have been developed from various mango germplasms (Chiang et al. 2012, Dillon et al. 2014, Duval et al. 2005, Honsho et al. 2005, Ravishankar et al. 2011, Schnell et al. 2005, Viruel et al. 2005), and there are some studies on regional genetic diversity of mango using SSRs, e.g. Schnell et al. (2006) for Florida mango cultivars, Hirano et al. (2010) for Myanmar mango landraces, Tsai et al. (2013) for Taiwanese cultivars.

In Japan, cleaved amplified polymorphic sequence markers (Shudo et al. 2013) and retrotransposon-based insertion polymorphism markers (Nashima et al. 2017) were developed for marker-assisted selection and construction genetic linkage map in mango breeding program. Although these practical molecular tools have been developed, information of mango genetic resources in Japan is still meager.

To obtain the information for cultivar identification and diversity of Japanese mango genetic resources, in this study, we analyzed genetic diversity and relatedness of 120 accessions of mango which cover almost all mango collection in Japan, using 46 polymorphic SSR markers. Accurate percentages of many commercially grown cultivars were identified or reconfirmed. Phylogeographic relationships were discussed in comparison with previous studies.

**Materials and Methods**

**Plant materials and DNA extraction**

We analyzed 120 mango genetic resources held in Japan. They originated from the USA (Florida, Hawaii), Australia, Colombia, Egypt, Haiti, Honduras, India, Israel, Mexico, Panama, the Philippines, South Africa, Taiwan, Thailand, Trinidad and Tobago, Vietnam, and the West Indies (Table 1) (Campbell 1992, Hamilton 1993, Knight et al. 2009, Olano et al. 2005, Schnell et al. 2006). The origins of six accessions (‘Barl’, ‘Khom-JIRCAS’, ‘Khom-OPARC’, ‘Mayer’, ‘Turpin’, and ‘Yu-Win #6-JIRCAS’) are unknown. Eighty-three mango accessions were collected and maintained at the Japan International Research Center for Agricultural Sciences, Tropical Agriculture Research Front (JIRCAS, Ishigaki, Okinawa, Japan), and 37 accessions were at the Okinawa Prefectural Agricultural Research Center Nago Branch (OPARC, Nago, Okinawa, Japan).

Ninety-six F1 individuals from the cross of ‘Irwin’ × ‘Keitt’ were used for evaluation of segregation of SSR genotypes. Plant materials were grown and maintained at the OPARC.

Genomic DNA was isolated from young leaves with a DNasey Plant Mini Kit (Qiagen, Germany) according to the manufacturer’s instructions.

**SSR analysis**

We preliminary tested 67 SSR markers that originated from mango. Of those, 21 were excluded because of no amplification, unstable amplification of the target band or the presence of monomorphic fragments. We used the remaining 46 SSR markers (Table 2), comprising 26 from Ravishankar et al. (2011), 6 from Schnell et al. (2005), and 14 from Viruel et al. (2005).

SSR markers were amplified in a 5-μL reaction mixture, containing 2.5 μL of Multiplex PCR Master Mix with HotStar Taq DNA Polymerase (Qiagen), 5 pmol of each primer (forward, fluorescently labeled with FAM or HEX; R, unlabeled), and 5 ng of genomic DNA. The PCR profile consisted of initial denaturation for 15 min at 95°C; 35 cycles of denaturation for 60 s at 94°C, annealing for 60 s at 55°C, and extension for 60 s at 72°C; and a final extension for 7 min at 72°C. The amplified PCR products were separated and detected in a PRISM 3130xl DNA sequencer (Applied Biosystems, USA). The sizes of the amplified bands were scored against internal standard DNA (400HD-ROX, Applied Biosystems) in GeneScan software (Applied Biosystems).

**Data analysis**

Using CERVUS v. 2.0 (Marshall et al. 1998) and MarkerToolKit v. 1.0 software (Fujii et al. 2008), we estimated the expected (H\textsubscript{E}) and observed heterozygosity (H\textsubscript{O}) at SSR marker loci in the cultivars. H\textsubscript{E} was calculated from allele frequencies using an unbiased formula as 1 – Σp\textsuperscript{i} \textsuperscript{2} (1 ≤ i ≤ m), where m is the number of alleles at the target locus and p\textsubscript{i} is the allele frequency of the i\textsuperscript{th} allele at the target locus. H\textsubscript{O} was calculated as the number of heterozygous individuals divided by the total number of individuals.

Parent–offspring relationships were tested by comparing the SSR alleles in each accession with those of its reported parents; the data were analyzed in MARCO software (Fujii
Table 1. Mango accessions used and their assessed parentage in this study

| No. | Accession name | Origin (abbreviation) | Embryo-ny* | Source** | Accession nos.*** | Parentage assessed by SSR markers in this study | Parentage from literatures**** |
|-----|----------------|-----------------------|------------|----------|------------------|-----------------------------------------------|--------------------------------|
| 1   | Ah Ping        | Hawaii, USA (HI)      | M          | JIRCAS   | JTMG-001         | offspring of Haden                            |                                |
| 2   | Al             | Taiwan (TW)           | M          | JIRCAS   | JTMG-002         | Lippens × Haden                               |                                |
| 3   | Alphonso       | India (IN)            | M          | JIRCAS   | JTMG-003         | offspring of Haden                            |                                |
| 4   | Anderson       | Florida, USA (FL)     | M          | JIRCAS   | JTMG-004         | offspring of Haden Sandersha × Haden (d)      |                                |
| 5   | Bailey’s Marvel| Florida, USA (FL)     | M          | JIRCAS   | JTMG-005         | offspring of Haden Haden × Bombay (d)         |                                |
| 6   | Barl           | unknown (?)           | U          | OPARC    | Barl (OPARC)     | Keitt × Tommy Atkins                          |                                |
| 7   | Becky-JIRCAS   | Florida, USA (FL)     | M          | JIRCAS   | JTMG-006         | offspring of Haden                            |                                |
| 8   | Becky-OPARC    | Florida, USA (FL)     | M          | OPARC    | Haden (OPARC)    | Haden × Brooks (d)                            |                                |
| 9   | Beverly        | Florida, USA (FL)     | M          | JIRCAS   | JTMG-007         | offspring of Haden                            |                                |
| 10  | Carabao        | Philippines (PH)      | P          | JIRCAS   | JTMG-008         | offspring of Haden                            |                                |
| 11  | Carrie         | Florida, USA (FL)     | M          | JIRCAS   | JTMG-009         | offspring of Julie (d)                        |                                |
| 12  | Cat For Rock   | Vietnam (VI)          | U          | JIRCAS   | JTMG-010         |                                |                                |
| 13  | Choke Anan     | Thailand (TH)         | P          | JIRCAS   | JTMG-011         |                                |                                |
| 14  | Cushman        | Florida, USA (FL)     | M          | OPARC    | Cushman (OPARC)  | offspring of Haden                            |                                |
| 15  | Dot-JIRCAS     | Florida, USA (FL)     | M          | JIRCAS   | JTMG-013         | Carrie × Spirit of ‘76 (one discrepancy of LMMA11) |                                |
| 16  | Dot-OPARC      | Florida, USA (FL)     | M          | OPARC    | Dot (OPARC)      | Carrie × Spirit of ‘76 (one discrepancy of LMMA11) | offspring of Zill (d) |
| 17  | Duncan         | Florida, USA (FL)     | M          | JIRCAS   | JTMG-014         |                                | offspring of Nam Doc Mai (d) |
| 18  | Edward-JIRCAS  | Florida, USA (FL)     | M          | JIRCAS   | JTMG-015         | offspring of Haden                            |                                |
| 19  | Edward-OPARC   | Florida, USA (FL)     | M          | OPARC    | Edward (OPARC)   | offspring of Haden                            |                                |
| 20  | Fahlan         | Thailand (TH)         | U          | JIRCAS   | JTMG-016         |                                |                                |
| 21  | Fairchild      | Panama (PA)           | U          | OPARC    | Fairchild (OPARC)| offspring of Alphonso                         |                                |
| 22  | Facsell        | USA                   | M          | JIRCAS   | JTMG-017         | Lippens × Haden                               |                                |
| 23  | Fukuda-JIRCAS  | Hawaii, USA (HI)      | M          | JIRCAS   | JTMG-018         | offspring of Haden                            |                                |
| 24  | Fukuda-OPARC   | Hawaii, USA (HI)      | M          | OPARC    | Fukuda (OPARC)   | offspring of Haden                            |                                |
| 25  | Glenn-JIRCAS   | Florida, USA (FL)     | M          | JIRCAS   | JTMG-019         | offspring of Haden                            |                                |
| 26  | Glenn-OPARC    | Florida, USA (FL)     | M          | OPARC    | Glenn (OPARC)    | offspring of Haden                            |                                |
| 27  | Golden Lippens-JIRCAS | Florida, USA (FL) | M | JIRCAS | JTMG-020 | offspring of Lippens                            | offspring of Lippens (a, d) |
| 28  | Golden Lippens-OPARC | Florida, USA (FL) | M | OPARC | Golden Lippens (OPARC) | offspring of Lippens                             |                                |
| 29  | Golden Nugget-JIRCAS | Florida, USA (FL) | M | JIRCAS | JTMG-021 | offspring of Haden                            | offspring of Kent (d)         |
| 30  | Golden Nugget-OPARC | Florida, USA (FL) | M | OPARC | Golden Nugget (OPARC) | offspring of Haden                             |                                |
| 31  | Gouvrea        | Hawaii, USA (HI)      | U          | JIRCAS   | JTMG-023         | offspring of Haden                            |                                |
| 32  | Graham         | Trinidad Tobago       | M          | JIRCAS   | JTMG-024         |                                | offspring of Julie (a) |
| 33  | Haden-JIRCAS   | Florida, USA (FL)     | M          | JIRCAS   | JTMG-027         | offspring of Turpentine-JIRCAS                 |                                |
| 34  | Haden-OPARC    | Florida, USA (FL)     | M          | OPARC    | Haden (OPARC)    | offspring of Turpentine-JIRCAS                 |                                |
| 35  | Hatcher        | Florida, USA (FL)     | M          | JIRCAS   | JTMG-028         | offspring of Haden                            |                                |
| 36  | Hodson         | Florida, USA (FL)     | M          | JIRCAS   | JTMG-029         | offspring of Haden                            |                                |
| 37  | Honglong-JIRCAS| Taiwan (TW)           | U          | JIRCAS   | JTMG-041         | offspring of Haden                            |                                |
| 38  | Honglong-OPARC | Taiwan (TW)           | U          | OPARC    | Honglong (OPARC) | offspring of Haden                            |                                |
| 39  | Irwin          | Florida, USA (FL)     | M          | JIRCAS   | JTMG-030         | Lippens × Haden                               | Lippens × Haden (b, d)         |
| 40  | Jacquelin-OPARC| Florida, USA (FL)     | M          | OPARC    | Jacquelin (OPARC)| offspring of Haden or Pruter Haden × Tommy Atkins |                                |
| 41  | Jacquelin-JIRCAS| Florida, USA (FL)   | M          | JIRCAS   | JTMG-031         | offspring of Haden                            |                                |
| 42  | Jakarta        | Florida, USA (FL)     | M          | JIRCAS   | JTMG-032         | offspring of Haden                            |                                |
| 43  | Jewel          | Florida, USA (FL)     | M          | JIRCAS   | JTMG-033         | offspring of Haden                            |                                |
| 44  | Jinhuang-JIRCAS| Taiwan (TW)           | U          | JIRCAS   | JTMG-040         | White × Kent (one discrepancy of LMMA9)        |                                |
| 45  | Jinhuang-OPARC | Taiwan (TW)           | U          | OPARC    | Jinhuang (OPARC) | White × Kent (one discrepancy of LMMA9)        |                                |
| 46  | Jinfong        | Taiwan (TW)           | U          | OPARC    | Jinfong (OPARC)  | offspring of Irwin                            |                                |
| 47  | Jubilee        | Florida, USA (FL)     | M          | JIRCAS   | JTMG-034         | Sensation × Irwin                             | Sensation × Irwin (d)          |
| 48  | Keitt          | Florida, USA (FL)     | M          | OPARC    | Keitt (OPARC)    | offspring of Haden                            |                                |
| 49  | Keitt Red-JIRCAS| Taiwan (TW)           | U          | JIRCAS   | JTMG-036         | Irwin × Keitt                                 |                                |
| 50  | Keitt Red-OPARC| Taiwan (TW)           | U          | OPARC    | Keitt Red (OPARC)| Irwin × Keitt                                 |                                |
| 51  | Kensington     | Australia (AU)        | P          | JIRCAS   | JTMG-037         |                                |                                |
| 52  | Kensington Pride| Australia (AU)      | P          | OPARC    | Kensington Pride (OPARC) |                                |                                |
| 53  | Kent           | Florida, USA (FL)     | M          | JIRCAS   | JTMG-038         | offspring of Haden                            |                                |
| 54  | Khom-JIRCAS    | unknown (?)           | U          | JIRCAS   | JTMG-039         | offspring of Haden                            |                                |
| 55  | Khom-OPARC     | unknown (?)           | U          | OPARC    | Khom (OPARC)     |                                |                                |
| 56  | Lancellita     | Honduras (HN)         | M          | JIRCAS   | JTMG-043         |                                |                                |
| 57  | Lily-JIRCAS    | Florida, USA (FL)     | M          | JIRCAS   | JTMG-044         | Springfels × Sensation                        | Springfels × Sensation (d)    |
| 58  | Lily-OPARC     | Florida, USA (FL)     | M          | OPARC    | Lily (OPARC)     | Springfels × Sensation                        |                                |
| 59  | Lippens-JIRCAS | Florida, USA (FL)     | M          | JIRCAS   | JTMG-045         | offspring of Haden                            |                                |
| 60  | Lippens-OPARC  | Florida, USA (FL)     | M          | OPARC    | Lippens (OPARC)  | offspring of Haden                            |                                |
| 61  | Madame Francis | Haiti (HT)            | P          | JIRCAS   | JTMG-046         |                                |                                |
| 62  | Magshamim      | Israel (IL)           | M          | JIRCAS   | JTMG-047         |                                |                                |

*Note: *Embryo-ny* indicates the type of embryo used in the study.
*Note: **Source** indicates the source of each accession.
*Note: ***Accession nos.*** indicates the accession numbers used.
*Note: ****Parentage from literatures indicates the parentage assessed from literature sources.

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Table 1. (continued)

| No. | Accession name (abbreviation) | Origin | Embryo* | Source** | Accession nos.*** | Parentage assessed by SSR markers in this study | Parentage from literatures**** |
|-----|-------------------------------|--------|---------|----------|-------------------|-----------------------------------------------|----------------------------------|
| 63  | Maha Chanok                    | Thailand (TH) | U       | JIRCAS   | JTMG-048          | offspring of Neelumlate (one discrepancy of LMMA9) | Neelum × Dashehari (a, b)       |
| 64  | Mallika                       | India (IN) | M       | JIRCAS   | JTMG-049          |                                                |                                 |
| 65  | Manilaita                      | Mexico (MX) | P       | JIRCAS   | JTMG-050          |                                                |                                 |
| 66  | Manzanillo                    | Mexico (MX) | M       | JIRCAS   | JTMG-051          | Haden × Kent                                   |                                 |
| 67  | Mapulehu                      | Florida, USA (FL) | M   | JIRCAS   | JTMG-052          | offspring of Turpentine-JIRCAS                  |                                 |
| 68  | Mayer                          | unknown (?) | M       | JIRCAS   | JTMG-053          | offspring of Step (d)                          |                                 |
| 69  | Momi-K                         | Hawaii, USA (HI) | U      | JIRCAS   | JTMG-054          | offspring of Haden                             |                                 |
| 70  | N-13                           | Israel (IL) | U       | OPARC    | N-13 (OPARC)      |                                                |                                 |
| 71  | Nam Doc Mai #2-JIRCAS          | Thailand (TH) | M      | JIRCAS   | JTMG-056          |                                                |                                 |
| 72  | Nam Doc Mai #2-OPARC          | Thailand (TH) | M       | OPARC    | Nam Doc Mai #2 (OPARC) |                                                |                                 |
| 73  | Nam Doc Mai #4-JIRCAS          | Thailand (TH) | P      | JIRCAS   | JTMG-057          |                                                |                                 |
| 74  | Nam Doc Mai #4-OPARC          | Thailand (TH) | P       | OPARC    | Nam Doc Mai #4 (OPARC) |                                                |                                 |
| 75  | Naomi                          | Israel (IL) | M       | JIRCAS   | JTMG-058          | offspring of Palmer (e)                        |                                 |
| 76  | Neelumlate                     | India (IN) | M       | JIRCAS   | JTMG-059          |                                                |                                 |
| 77  | Niku                           | Taiwan (TW) | U       | JIRCAS   | JTMG-060          |                                                |                                 |
| 78  | Oro                            | Mexico (MX) | M       | JIRCAS   | JTMG-061          |                                                |                                 |
| 79  | Osteen                         | Florida, USA (FL) | U | JIRCAS   | JTMG-062          | offspring of Haden                             | offspring of Haden (a, b, d)    |
| 80  | Palmer                         | Florida, USA (FL) | M | JIRCAS   | JTMG-063          | offspring of Haden                             | offspring of Haden (a, b, d)    |
| 81  | Parvin                         | Hawaii, USA (HI) | P      | JIRCAS   | JTMG-064          | offspring of Turpentine                         |                                 |
| 82  | Parvin                         | Florida, USA (FL) | U       | OPARC    | Parvin (OPARC)    | offspring of Haden                             | offspring of Haden (a)          |
| 83  | Piva-JIRCAS                    | South Africa (ZA) | M      | JIRCAS   | JTMG-065          |                                                |                                 |
| 84  | Piva-OPARC                     | South Africa (ZA) | M       | OPARC    | Piva (OPARC)      |                                                |                                 |
| 85  | Pruter                         | Florida, USA (FL) | U       | JIRCAS   | JTMG-066          | offspring of Haden                             |                                 |
| 86  | R2E2                           | Australia (AU) | P       | JIRCAS   | JTMG-067          | Kensington Pride × Kent                        |                                 |
| 87  | Rad                             | Thailand (TH) | P       | JIRCAS   | JTMG-068          |                                                |                                 |
| 88  | Rapoza                         | Hawaii, USA (HI) | M       | JIRCAS   | JTMG-069          | Irwin × Kent or offspring of Haden             |                                 |
| 89  | Ruby                            | Florida, USA (FL) | M     | JIRCAS   | JTMG-070          | offspring of Haden                             | offspring of Haden (d)          |
| 90  | S-01                           | Florida, USA (FL) | U     | OPARC    | S-01 (OPARC)      | offspring of Haden                             | offspring of Haden (d)          |
| 91  | Sensation                      | Florida, USA (FL) | M     | JIRCAS   | JTMG-071          | offspring of Haden                             | offspring of Haden (d)          |
| 92  | Shiba                          | Taiwan (TW) | U       | JIRCAS   | JTMG-072          |                                                |                                 |
| 93  | Sonsien-JIRCAS                 | Taiwan (TW) | U       | JIRCAS   | JTMG-073          |                                                |                                 |
| 94  | Sonsien-OPARC                  | Taiwan (TW) | U       | OPARC    | Sonsien (OPARC)   |                                                |                                 |
| 95  | Spirit of ’76-JIRCAS           | Florida, USA (FL) | M | JIRCAS   | JTMG-074          | offspring of Haden                             | Zill × Haden (a, d)            |
| 96  | Spirit of ’76-OPARC            | Florida, USA (FL) | M       | OPARC    | Spirit of ’76 (OPARC) | offspring of Haden                             | Zill × Haden (a, d)            |
| 97  | Springfields-JIRCAS            | Florida, USA (FL) | M       | JIRCAS   | JTMG-075          | offspring of Haden                             | offspring of Haden (a, d)       |
| 98  | Springfields-OPARC             | Florida, USA (FL) | U       | OPARC    | Springfields (OPARC) | offspring of Haden                             | offspring of Haden (a, d)       |
| 99  | Tahar                          | Israel (IL) | M       | JIRCAS   | JTMG-076          | offspring of Irwin                             |                                 |
| 100 | Tainoung No. 1-JIRCAS          | Taiwan (TW) | M       | JIRCAS   | JTMG-077          |                                                |                                 |
| 101 | Tainoung No. 1-OPARC          | Taiwan (TW) | M       | OPARC    | Tainoung No. 1 (OPARC) |                                                |                                 |
| 102 | Taiwan                         | Taiwan (TW) | U       | JIRCAS   | JTMG-078          |                                                |                                 |
| 103 | Tommy Atkins                   | Florida, USA (FL) | M     | JIRCAS   | JTMG-079          | offspring of Haden                             | offspring of Haden (a, b, d)    |
| 104 | Turpentine-JIRCAS              | West Indies (WI) | P      | JIRCAS   | JTMG-081          |                                                |                                 |
| 105 | Turpentine-OPARC               | West Indies (WI) | P      | OPARC    | Turpentine (OPARC) |                                                |                                 |
| 106 | Turpin                         | unknown (?) | P       | JIRCAS   | JTMG-082          |                                                |                                 |
| 107 | Valencia Pride-JIRCAS          | Florida, USA (FL) | M     | JIRCAS   | JTMG-082          | offspring of Haden                             | offspring of Haden (a, d)       |
| 108 | Valencia Pride-OPARC           | Florida, USA (FL) | M     | OPARC    | Valencia Pride (OPARC) | offspring of Haden                             | offspring of Haden (a, d)       |
| 109 | Vallenato                      | Colombia (CO) | P       | JIRCAS   | JTMG-083          | offspring of Haden                             | offspring of Haden              |
| 110 | Van Dyke-JIRCAS                | Florida, USA (FL) | M       | JIRCAS   | JTMG-084          | offspring of Haden                             | offspring of Haden (b, d)       |
| 111 | Van Dyke-OPARC                 | Florida, USA (FL) | M       | OPARC    | Van Dyke (OPARC)  | offspring of Haden                             | offspring of Haden (b, d)       |
| 112 | White-JIRCAS                   | Taiwan (TW) | P       | JIRCAS   | JTMG-085          |                                                |                                 |
| 113 | White-OPARC                    | Taiwan (TW) | P       | OPARC    | White (OPARC)     |                                                |                                 |
| 114 | White Pirie                    | Jamaica (JA) | P       | JIRCAS   | JTMG-086          |                                                |                                 |
| 115 | Yu-Win                         | Taiwan (TW) | U       | JIRCAS   | JTMG-025          | offspring of Irwin                             |                                 |
| 116 | Yu-Win #2                      | Taiwan (TW) | U       | OPARC    | Yu-Win #2 (OPARC) | Jinhuang × Irwin                               |                                 |
| 117 | Yu-Win #6-JIRCAS               | unknown (?) | U       | JIRCAS   | JTMG-026          | Jinhuang × Irwin                               |                                 |
| 118 | Yu-Win #6-OPARC                | Taiwan (TW) | U       | OPARC    | Yu-Win #6 (OPARC) | Jinhuang × Irwin                               |                                 |
| 119 | Zebeda                         | Egypt (EG) | M       | JIRCAS   | JTMG-087          |                                                |                                 |
| 120 | Zillate                        | Florida, USA (FL) | M     | JIRCAS   | JTMG-088          | offspring of Keitt                             | offspring of Keitt (d)          |

* M: monoembryony; P: polyembryony; U: unknown.

** JIRCAS: Japan International Research Center for Agricultural Sciences, Tropical Agriculture Research Front; OPARC: Okinawa Prefectural Agricultural Research Center Nago Branch.

*** Accessions of OPARC are maintained using cultivar name.

**** Parentage was described in literatures of a: Campbell (1992), b: Knight et al. (2009), c: Lee et al. (2009), d: Schnell et al. (2006), and e: Tomer et al. (1993).
Segregation of SSR alleles were evaluated for 46 SSR loci used in this study to validate if each SSR is derived from single locus or multiple ones, by using 96 F₁ individuals obtained from the cross of ‘Irwin’ × ‘Keitt’. JoinMap ver. 4.1 software (Kyazma B.V., the Netherlands; Van Ooijen 2011) was used. We also picked up significant linkages between two SSR loci for alleles of ‘Irwin’ as well as ‘Keitt’, calculated by JoinMap ver. 4.1 software.

### Results

**Genetic identification of mango accessions using SSR markers**

We identified 274 putative alleles in the 120 accessions (Table 2). The number of alleles per locus ranged from 2 at 3 of the loci (MiIIHR10, MiIIHR13, MiSHRS-26) to 11 at 3 of the loci (MiIIHR17, LMMA6, LMMA10), with an average value of 6.0 (Table 2). \( H_E \) ranged from 0.024 at MiIIHR10 to 0.834 at MiIIHR30 and LMMA1, with an average value of 0.577. \( H_O \) ranged from 0 at MiIIHR10 to 0.880 at MiIIHR30, LMMA1, and LMMA10, with an average value of 0.569. The 120 accessions could be differentiated and classified into 83 genotypes excluding identical accessions by the 46 SSR markers (Fig. 1).

Thirteen groups showing identical SSR genotypes were found in this study (Table 3). Twenty-three out of 30 groups included accessions with the same names maintained at different organizations, JIRCAS and OPARC. On the other hand, 13 groups included synonymous accessions. For example, three accessions (‘Ai’, ‘Fascell’, and ‘Irwin’) were identified as the same genotype 1. Similarly, ‘Bailey’s Marvel’ vs. ‘Beverly’ (Genotype 2), ‘Duncan’ vs. ‘Nam Doc Mai #2-JIRCAS’ (Genotype 5), ‘Gouviea’ vs. ‘Momi-K’ (Genotype 11), ‘Haden-JIRCAS’ vs. ‘Mayer’ (Genotype 12), ‘Honglong-JIRCAS’ vs. ‘Jinlong’ (Genotype 13), ‘Jakarta’ vs. ‘Valencia Pride-JIRCAS’ (Genotype 14), ‘Kensington’ vs. ‘Kensington Pride’ (Genotype 17), ‘Nam Doc Mai #4-JIRCAS’ vs. ‘Turpin’ (Genotype 21), ‘Nam Doc Mai #4-OPARC’ vs. ‘Paris’ (Genotype 22), ‘Osteen’ vs. ‘Springfels-OPARC’ (Genotype 23), ‘White-JIRCAS’ vs. ‘White Pirie’ (Genotype 29), and ‘Yu-Win #2’ vs. ‘Yu-Win

### Table 2. Characteristics of SSR markers applied for mango accessions

| SSR loci       | No. of alleles | \( H_E \) | \( H_O \) | References (Genbank accession nos.) |
|----------------|----------------|---------|---------|-------------------------------------|
| MiIIHR01       | 4              | 0.372   | 0.349   | Ravishankar et al. (2011), EF592181 |
| MiIIHR02       | 8              | 0.734   | 0.590   | Ravishankar et al. (2011), EF592182 |
| MiIIHR03       | 3              | 0.547   | 0.675   | Ravishankar et al. (2011), EF592183 |
| MiIIHR05       | 6              | 0.756   | 0.843   | Ravishankar et al. (2011), EF592185 |
| MiIIHR07       | 4              | 0.521   | 0.482   | Ravishankar et al. (2011), EF592187 |
| MiIIHR10       | 2              | 0.024   | 0.000   | Ravishankar et al. (2011), EF592190 |
| MiIIHR11       | 3              | 0.330   | 0.386   | Ravishankar et al. (2011), EF592191 |
| MiIIHR12       | 6              | 0.530   | 0.530   | Ravishankar et al. (2011), EF592192 |
| MiIIHR13       | 2              | 0.493   | 0.494   | Ravishankar et al. (2011), EF592193 |
| MiIIHR14       | 4              | 0.428   | 0.422   | Ravishankar et al. (2011), EF592194 |
| MiIIHR16       | 5              | 0.544   | 0.554   | Ravishankar et al. (2011), EF592196 |
| MiIIHR17       | 11             | 0.826   | 0.867   | Ravishankar et al. (2011), EF592197 |
| MiIIHR20       | 5              | 0.473   | 0.386   | Ravishankar et al. (2011), EF592200 |
| MiIIHR21       | 5              | 0.116   | 0.072   | Ravishankar et al. (2011), EF592201 |
| MiIIHR22       | 5              | 0.637   | 0.482   | Ravishankar et al. (2011), EF592202 |
| MiIIHR24       | 8              | 0.758   | 0.747   | Ravishankar et al. (2011), EF592203 |
| MiIIHR25       | 7              | 0.727   | 0.735   | Ravishankar et al. (2011), EF592209 |
| MiIIHR30       | 9              | 0.834   | 0.880   | Ravishankar et al. (2011), EF592210 |
| MiIIHR32       | 8              | 0.641   | 0.663   | Ravishankar et al. (2011), EF592212 |
| MiIIHR33       | 4              | 0.590   | 0.554   | Ravishankar et al. (2011), EF592213 |
| MiSHRS-26      | 2              | 0.193   | 0.217   | Schnell et al. (2005), AY942818    |
| MiSHRS-29      | 5              | 0.560   | 0.590   | Schnell et al. (2005), AY942822    |
| MiSHRS-32      | 7              | 0.535   | 0.482   | Schnell et al. (2005), AY942824    |
| MiSHRS-33      | 5              | 0.355   | 0.434   | Schnell et al. (2005), AY942825    |
| MiSHRS-39      | 7              | 0.616   | 0.639   | Schnell et al. (2005), AY942829    |
| LMMA1          | 9              | 0.834   | 0.880   | Viruel et al. (2005), AY628373     |
| LMMA2          | 7              | 0.650   | 0.458   | Viruel et al. (2005), AY628374     |
| LMMA4          | 5              | 0.663   | 0.554   | Viruel et al. (2005), AY628376     |
| LMMA5          | 3              | 0.307   | 0.289   | Viruel et al. (2005), AY628377     |
| LMMA6          | 11             | 0.694   | 0.735   | Viruel et al. (2005), AY628378     |
| LMMA7          | 6              | 0.716   | 0.687   | Viruel et al. (2005), AY628379     |
| LMMA8          | 9              | 0.747   | 0.747   | Viruel et al. (2005), AY628380     |
| LMMA9          | 7              | 0.806   | 0.711   | Viruel et al. (2005), AY628381     |
| LMMA10         | 11             | 0.799   | 0.880   | Viruel et al. (2005), AY628382     |
| LMMA11         | 6              | 0.764   | 0.735   | Viruel et al. (2005), AY628383     |
| LMMA12         | 7              | 0.713   | 0.747   | Viruel et al. (2005), AY628384     |
| LMMA14         | 4              | 0.400   | 0.301   | Viruel et al. (2005), AY628386     |
| LMMA15         | 6              | 0.561   | 0.566   | Viruel et al. (2005), AY628387     |
| LMMA16         | 6              | 0.748   | 0.843   | Viruel et al. (2005), AY628388     |
| Average        |                | 0.577   | 0.569   |                                        |
Fig. 1. Phenogram of the 120 mango genetic resources evaluated. The phenogram was produced using the UPGMA method based on Dice’s coefficient. Origins of accessions are indicated as two-letter ISO 3166 codes or US state abbreviations; “?” = unknown.
### Table 3. Mango accessions showing identical genotypes

| Genotype | Accession name (Code No.)* |
|----------|----------------------------|
| 1        | Ai (2), Fascell (22), Irwin (39) |
| 2        | Bailey’s Marvel (5), Beverly (9) |
| 3        | Becky-JIRCAS (7), Becky-OPARC (8) |
| 4        | Dot-JIRCAS (15), Dot-OPARC (16) |
| 5        | Duncan (17), Nam Doc Mai #2-JIRCAS (71), Nam Doc Mai #2-OPARC (72) |
| 6        | Edward-JIRCAS (18), Edward-OPARC (19) |
| 7        | Fukuda-JIRCAS (23), Fukuda-OPARC (24) |
| 8        | Glenn-JIRCAS (25), Glenn-OPARC (26) |
| 9        | Golden Lippens-JIRCAS (27), Golden Lippens-OPARC (28) |
| 10       | Golden Nugget-JIRCAS (29), Golden Nugget-OPARC (30) |
| 11       | Gouviea (31), Momi-K (69) |
| 12       | Haden-JIRCAS (33), Haden-OPARC (34), Mayer (68) |
| 13       | Honglong-JIRCAS (37), Honglong-OPARC (38), Jinlong (46) |
| 14       | Jakarta (42), Valencia Pride-JIRCAS (107), Valencia Pride-OPARC (108) |
| 15       | Jinhuang-JIRCAS (44), Jinhuang-OPARC (45) |
| 16       | Keitt Red-JIRCAS (49), Keitt Red-OPARC (50) |
| 17       | Kensington (51), Kensington Pride (52) |
| 18       | Khom-JIRCAS (54), Khom-OPARC (55) |
| 19       | Lily-JIRCAS (57), Lily-OPARC (58) |
| 20       | Lippens-JIRCAS (59), Lippens-OPARC (60) |
| 21       | Nam Doc Mai #4-JIRCAS (73), Turpin (106) |
| 22       | Nam Doc Mai #4-OPARC (74), Paris (81) |
| 23       | Osteen (79), Springfels-OPARC (98) |
| 24       | Piva-JIRCAS (83), Piva-OPARC (84) |
| 25       | Sonsien-JIRCAS (93), Sonsien-OPARC (94) |
| 26       | Spirit of ‘76-JIRCAS (95), Spirit of ‘76-JIRCAS (96) |
| 27       | Tainoung No. 1-JIRCAS (100), Tainoung No. 1-OPARC (101) |
| 28       | Van Dyke-JIRCAS (110), Van Dyke-OPARC (111) |
| 29       | White-JIRCAS (112), White-OPARC (113), White Pirie (114) |
| 30       | Yu-Win #2 (116), Yu-Win #6-JIRCAS (117), Yu-Win #6-OPARC (118) |

*Representative accessions of identical genotypes group were indicated underlined.

_outlined: #6-JIRCAS* (Genotype 30), showed identical SSR genotypes (Table 3). These synonymous accessions should be carefully identified by using genetic resources maintained at the different organizations. One representative accession was chosen from each genotype group by taking into account the record of introduction background of each genetic resources such as passport data, and used for further analysis.

Out of 27 homonymous cultivars maintained in both JIRCAS and OPARC with same cultivar name, four cultivar sets (‘Jacquelin’, ‘Nam Doc Mai #4’, ‘Springfels’, ‘Turpentine’) showed different SSR genotypes between the two organizations. These accessions should be treated and counted the record of introduction background of each genetic resources such as passport data, and used for further analysis.

Parentage analysis

We analyzed the parentages of the 120 accessions by using 274 putative alleles at 46 polymorphic SSR loci. Many accessions were identified as offspring of ‘Haden-JIRCAS’ crossed with unidentified cultivars not tested in this study (‘Ah Ping’, ‘Anderson’, ‘Bailey’s Marvel’, ‘Becky-JIRCAS’, ‘Cushman’, ‘Edward-JIRCAS’, ‘Fukuda-JIRCAS’, ‘Glenn-JIRCAS’, ‘Golden Nugget-JIRCAS’, ‘Gouviea’, ‘Hatcher’, ‘Hodson’, ‘Jacquelin-OPARC’, ‘Jacquelin-JIRCAS’, ‘Keitt’, ‘Kent’, ‘Lippens-JIRCAS’, ‘Osteen’, ‘Palmer’, ‘Pravin’, ‘Pruter’, ‘Ruby’, ‘S-01’, ‘Sensation’, ‘Spirit of ’76-JIRCAS’, ‘Springfels-JIRCAS’, ‘Tommy Atkins’, ‘Valencia Pride-JIRCAS’, ‘Vallenato’, ‘Van Dyke-JIRCAS’; Table 1). The results revealed both parents of 11 accessions: ‘Barl’ (‘Keitt’ × ‘Tommy Atkins’), ‘Dot-JIRCAS’ (‘Carrie’ × ‘Spirit of ’76-JIRCAS’, except for one discrepancy at LMMA11), ‘Irwin’ (‘Lippens-JIRCAS’ × ‘Haden-JIRCAS’); ‘Jinhuang-JIRCAS’ (‘White-JIRCAS’ × ‘Kent’, except for one discrepancy at LMMA9); ‘Jubilee’ (‘Sensation’ × ‘Irwin’), ‘Keitt Red-JIRCAS’ (‘Irwin’ × ‘Keitt’), ‘Lily-JIRCAS’ (‘Springfels-JIRCAS’ × ‘Sensation’), ‘Manzailllo’ (‘Haden-JIRCAS’ × ‘Kent’), ‘R2E2’ (‘Kensington’ × ‘Kent’), ‘Rapoza’ (‘Irwin’ × ‘Kent’ or offspring of ‘Haden-JIRCAS’), and ‘Yu-Win #6-JIRCAS’ (‘Jinhuang-JIRCAS’ × ‘Irwin’) (Table 1). The single discrepancies in ‘Dot-JIRCAS’ and ‘Jinhuang-JIRCAS’ may be due to allele mutations. Since there were no discrepancies at the other 45 SSR loci, we assumed that the parentages of ‘Dot-JIRCAS’ and ‘Jinhuang-JIRCAS’ were correct.

Genetic relatedness

We constructed a phenogram of the 120 accessions based on SSR analysis (Fig. 1). Many accessions from Florida were grouped in the upper part of the phenogram, while accessions from India (‘Alphonso’, ‘Mallika’, ‘Neelumlate’), Thailand (‘Choke Anan’, ‘Fahlan’, ‘Nam Doc Mai #2-JIRCAS’, ‘Nam Doc Mai #4-JIRCAS’, ‘Rad’), Vietnam (‘Cat For Rock’), and Egypt (‘Zebda’) were grouped in the lower part. Nevertheless, the accessions were mingled.

Genetic diversity of mango genetic resources

For further genetic diversity analyses to characterize mango genetic resources in Japan, we also employed 83 independent accessions selected by SSR genotyping in this study as a representative collection in Japan. As for the PCoA, the first and second principal components explained 14.25% and 7.17% of the variation, respectively. Overall, all 83 accessions distributed sparsely on the scatter plot, suggesting that genetic resources in Japan possess a certain level of genetic diversity in terms of SSR variation. Based on their origin, it was revealed that they tended to form three groups: “USA”, “India”, and “Thailand, Taiwan, the Philippines and Vietnam” (Fig. 2), in contrast to the UPGMA
Genetic diversity and relatedness of mango

Cluster I included accessions from India (‘Alphonso’, ‘Mallika’, and ‘Neelumlate’), suggesting that typical Indian type accessions were included.

In the analysis of population structure, $\Delta K$ showed a maximum at $K = 3$, suggesting three genetically distinct clusters (I, II, and III in Fig. 3). Cluster I included accessions from India (‘Alphonso’, ‘Mallika’, and ‘Neelumlate’), suggesting that typical Indian type accessions were included.

Fig. 2. Scatter plot of 83 mango genetic resources based on principal coordinates analysis. For accession numbers, see Table 1. Origins of accessions are indicated as two-letter ISO 3166 codes or US state abbreviations; “?” = unknown.

Fig. 3. Bar plot of 83 mango genetic resources by structure analysis ($K = 3$) with 46 SSR loci. Origins of accessions are indicated as two-letter ISO 3166 codes or US state abbreviations; “?” = unknown.
Cluster II included predominantly US accessions from Florida and Hawaii. Cluster III included mostly Asian accessions from Thailand, Vietnam, and Taiwan, in which accessions of Southeast Asian type were predominant. These clusters were generally consistent with the groups obtained from PCoA as mentioned above. As for the relationship between population structure and embryo types of the seed, monoembryonic accessions were predominant in clusters I and II, showing a relationship between embryony and cultivar clusters identified by population structure analysis (Supplemental Fig. 1). Polyembryonic accessions were predominant in cluster III and also featured in cluster II.

**Segregation of SSR loci**

In order to characterize whether SSR alleles were derived from single locus or multiple loci used in this study, segregations of SSR genotypes were evaluated by using 96 F₁ individuals obtained from the cross of ‘Irwin’ × ‘Keitt’ (Table 4). Thirty-five SSR loci showed segregations of SSR genotypes in the 96 F₁ individuals of ‘Irwin’ × ‘Keitt’, whereas no segregation was observed for 11 SSR loci. Eighteen SSR loci showed binary segregations (a/a; a/b, a/c; b/c, a/b: a/c), and 17 of them fitted to the expected segregation ratio of 1:1, whereas only one SSR locus MiIIHR13 showed skewed segregation at 5% level. Out of the 13 SSR

| SSR loci | SSR genotypes of Irwin (bp) | Segregation for F₁ hybrids of Irwin × Keitt | Expected ratio | chi-square value | Signif. |
|----------|-----------------------------|---------------------------------------------|----------------|-----------------|--------|
| MiIIHR01 | 252/252                     | 246/252                                     | 1:1            | 0.00            | ns     |
| MiIIHR02 | 171/175                     | 175/199                                     | 1:1:1:1        | 0.25            | ns     |
| MiIIHR03 | 235/235                     | 235/235                                     | 1:1:1:1        | 0.04            | ns     |
| MiIIHR05 | 209/216                     | 209/216                                     | 1:1:1:1        | 2.75            | ns     |
| MiIIHR07 | 170/170                     | 170/170                                     | 1:1:1:1        | 0.38            | ns     |
| MiIIHR10 | 190/190                     | no segregation                              |                |                 |        |
| MiIIHR11 | 221/221                     | 212/221                                     | 1:1            | 2.04            | ns     |
| MiIIHR12 | 177/177                     | no segregation                              |                |                 |        |
| MiIIHR13 | 190/197                     | 190/197                                     | 1:1            | 3.38            | *      |
| MiIIHR14 | 354/354                     | 342/354                                     | 1:1:1:1        | 1.50            | ns     |
| MiIIHR16 | 208/208                     | no segregation                              |                |                 |        |
| MiIIHR17 | 244/247                     | 244/247                                     | 1:1:1:1        | 8.08            | **     |
| MiIIHR20 | 190/190                     | no segregation                              |                |                 |        |
| MiIIHR21 | 239/239                     | no segregation                              |                |                 |        |
| MiIIHR22 | 227/241                     | 227/241                                     | 1:1:1:1        | 3.92            | ns     |
| MiIIHR24 | 247/247                     | 247/247                                     | 1:1:1:1        | 1.50            | ns     |
| MiIIHR25 | 151/151                     | no segregation                              |                |                 |        |
| MiIIHR26 | 145/164                     | 145/149                                     | 1:1:1:1        | 0.58            | ns     |
| MiIIHR27 | 197/197                     | no segregation                              |                |                 |        |
| MiIIHR28 | 112/120                     | 112/114                                     | 1:1:1:1        | 1.58            | ns     |
| MiIIHR29 | 157/157                     | 153/157                                     | 1:1:1:1        | 2.67            | ns     |
| MiIIHR30 | 202/204                     | 198/202                                     | 1:1:1:1        | 2.67            | ns     |
| MiIIHR32 | 188/190                     | 188/180                                     | 1:1:1:1        | 2.67            | ns     |
| MiIIHR33 | 168/180                     | 168/180                                     | 1:1:1:1        | 0.67            | ns     |
| MiIIHR34 | 236/246                     | 236/246                                     | 1:1:1:1        | 0.67            | ns     |
| MiIIHR35 | 193/201                     | 193/201                                     | 1:1:1:1        | 1.04            | ns     |
| MiSHRS-4 | 135/130                     | 133/133                                     | 1:1:1:1        | 1.33            | ns     |
| MiSHRS-26 | 281/281                    | 281/281                                     | 1:1:1:1        | 0.38            | ns     |
| MiSHRS-29 | 186/188                    | 186/186                                     | 1:2:1:2        | 0.56            | ns     |
| MiSHRS-32 | 211/211                    | 207/211                                     | 1:2:1:2        | 0.67            | ns     |
| MiSHRS-33 | 254/257                    | 254/257                                     | 1:2:1:2        | 0.67            | ns     |
| MiSHRS-39 | 374/374                    | 374/374                                     | 1:2:1:2        | 0.67            | ns     |
| LMMA1    | 208/210                     | 206/208                                     | 1:1:1:1        | 0.67            | ns     |
| LMMA2    | 285/297                     | 285/297                                     | 1:1:1:1        | 0.67            | ns     |
| LMMA4    | 237/237                     | 231/237                                     | 1:1:1:1        | 2.67            | ns     |
| LMMA5    | 288/288                     | no segregation                              |                |                 |        |
| LMMA6    | 112/131                     | 112/112                                     | 1:2:1:2        | 0.67            | ns     |
| LMMA7    | 206/206                     | 206/212                                     | 1:2:1:2        | 0.67            | ns     |
| LMMA8    | 263/263                     | 263/263                                     | 1:2:1:2        | 0.67            | ns     |
| LMMA9    | 178/178                     | 178/178                                     | 1:2:1:2        | 0.67            | ns     |
| LMMA10   | 162/181                     | 177/181                                     | 1:2:1:2        | 0.67            | ns     |
| LMMA11   | 238/246                     | 238/255                                     | 1:2:1:2        | 0.67            | ns     |
| LMMA12   | 211/211                     | 207/211                                     | 1:2:1:2        | 0.67            | ns     |
| LMMA14   | 177/177                     | no segregation                              |                |                 |        |
| LMMA15   | 217/225                     | 217/217                                     | 1:2:1:2        | 0.67            | ns     |
| LMMA16   | 240/245                     | 245/250                                     | 1:2:1:2        | 0.67            | ns     |

* and ** showed distortion at 5% and 1% level.
Mango shows the third biggest production of tropical fruits in the world, next to the bananas and the pineapples (FAOSTAT), and has been cultivated world-wide in the tropical and subtropical areas. In contrast to bananas and pineapples, however, mango has not been comprehensively studied as industrial plantations led by major commercial companies. Therefore, there have been conserved hundreds of mango cultivars which may possess a certain genetic diversity with regionally uniqueness in the production areas. In Japan, mango commercial production started in 1980s. Because of the limited cultivation history and production areas in Japan, mango has not yet become major fruit crop in Japan (Ogata et al. 2016).

In this study, 120 mango accessions in Japan were clearly distinguished into 83 genotypes excluding synonymous and identical accessions by the SSR markers. The use of SSR markers can differentiate mango cultivars and identify genetic diversity (Chiang et al. 2012, Duval et al. 2005, Honsho et al. 2005, Ravishankar et al. 2011, Schnell et al. 2005, Viruel et al. 2005). Some synonymous (identical SSR genotypes with different cultivar names) and homonymous (different SSR genotypes with the same cultivar name) accessions were pointed out in this study. Therefore, introduction background of mango accessions such as passport data should be carefully examined and considered again for validation as genetic resources, which will be utilized for breeding programs.

Using 11 SSR markers, Dillon et al. (2013) determined genetic diversity of 254 M. indica accessions maintained in the Australian National Mango Genebank, but found it difficult to identify parentage. Olano et al. (2005) analyzed 63 Florida cultivars to identify their pedigrees by using SSR markers, and Schnell et al. (2006) performed DNA analysis of 203 cultivars using SSR markers. The pedigree data that we obtained are in good accordance with those of Olano et al. (2005) and Schnell et al. (2006), including the many off-spring of ‘Haden’ and the parentages of ‘Irwin’, ‘Jubilee’, and ‘Lily’. The parentage of ‘Dot-JIRCAS’ (‘Carrie’ × ‘Spirit of ’76-JIRCAS’) was newly identified in this study, confirmed by all loci except LMMA11. Similarly, the parentage of ‘Jinhuang-JIRCAS’ (‘White-JIRCAS’ × ‘Kent’) was confirmed by all loci except LMMA9. These discrepancies may be due to high mutation rates of SSR loci: estimates of mutation rates among loci vary over the range of $10^{-3}$ to $10^{-5}$ (Weber and Wong 1993) in human SSRs, exceeding mutation rates for non-SSR loci by up to four orders of magnitude (Lacy 1987). Moriya et al. (2011) likewise concluded that allele mutation occurred at one out of 46 SSR loci in ‘Ozenokurenai’ apple and its parents ‘Morioka #47’ × ‘Morioka #46’.

PCoA indicated that accessions from India had a close

| SSR locus 1  | SSR locus 2  | Recombination frequency | LOD score |
|--------------|--------------|-------------------------|-----------|
| MiIIHR05     | MiIIHR26     | 0.031                   | 23.06     |
| MiIIHR17     | MiIIHR32     | 0.094                   | 15.55     |
| MiSHRS-4     | LMMA2        | 0.115                   | 14.07     |
| MiIIHR22     | LMMA10       | 0.156                   | 10.10     |

| SSR locus 1  | SSR locus 2  | Recombination frequency | LOD score |
|--------------|--------------|-------------------------|-----------|
| MiIIHR14     | MiIIHR24     | 0.000                   | 28.57     |
| MiIIHR14     | LMMA16       | 0.052                   | 20.05     |
| MiIIHR24     | LMMA16       | 0.052                   | 20.05     |
| MiIIHR01     | MiSHRS-39    | 0.073                   | 18.04     |
| MiIIHR05     | MiIIHR26     | 0.094                   | 16.29     |
| MiIIHR07     | LMMA12       | 0.094                   | 16.09     |
| MiIIHR02     | MiSHRS-32    | 0.115                   | 15.45     |
| MiSHRS-4     | LMMA2        | 0.125                   | 13.13     |
| MiIIHR22     | LMMA10       | 0.146                   | 11.51     |
| MiIIHR29     | LMMA11       | 0.208                   | 7.63      |
| MiIIHR29     | MiIIHR33     | 0.229                   | 6.31      |
relationship with accessions from the USA, while accessions from Thailand, Taiwan, the Philippines, and Vietnam seemed to be genetically separate (Fig. 2). These groupings appear to correspond to the previously defined Indian and Southeast Asian types (Iyer and Degani 1997, Viruel et al. 2005). Structure analysis also identified three clusters: cluster I included accessions from India and some of Florida, cluster II contained most accessions from the Florida and Hawaii of USA, and cluster III included many accessions from Southeast Asia. Moreover, monoembryonic accessions predominated in clusters I and II, and polyembryonic accessions predominated in cluster III (Supplemental Fig. 1).

These results were in good accordance with previous studies (Iyer and Degani 1997, Viruel et al. 2005).

Unstable flowering is one of the most important issues in mango cultivation and production to be solved, not only in Japan but also in Southeast Asia. It may be due in part to unstable climatic conditions such as obscurity seasonal change from rainy to dry period, and in part to higher temperatures during the flower initiation period as influenced by global warming (Normand et al. 2015). The mechanism of flower initiation tends to differ between the Indian and Southeast Asian types, reflecting the climate features of each region (Davenport 2009): flower initiation in the Indian type is induced mainly by low temperature, whereas that in the Southeast Asian type is induced mainly by drought stress in the dry season. It is important to understand cultivar characteristics and genetic diversity for choosing the appropriate genetic resources in order to maintain stable flowering in the practical field. Our results reveal the genetic structural distribution of the Indian and Southeast Asian types of mango genetic resources in Japan.

There has been no practical information about genetic diversity of mango in Japan. It is partly because the commercial production in Japan is quite recently (started from 1980s) and substantially monoculture of ‘Irwin’ (occupies >90% production in Japan), so there had been no strong interest about characteristics among genetic resources and also no intensive introduction of other new cultivar. However, recently, mango has been focused as one of the potential cash crops for premium fruit with high price in the commercial markets in Japan. The accessions that we examined cover almost all mango cultivars in Japan, therefore, their genetic information will pave the way to the use of the genetic resources for breeding and/or direct use of domestic production in Japan. Since the mango accessions used in this study have been mainly selected and established in Florida, and disseminated to the major production countries/areas (Mukherjee and Litz 2009), it is considered that mango accessions evaluated here could reflect the representative genetic diversity among major cultivars in the world.

Molecular markers have been used to create genetic linkage maps of mango (Arias et al. 2012, Kashkush et al. 2001, Kuhn et al. 2017, Luo et al. 2016). Although a lot of SSR markers have been developed (Chiang et al. 2012, Dillon et al. 2014, Duval et al. 2005, Honsho et al. 2005, Ravishankar et al. 2011, Schnell et al. 2005, Viruel et al. 2005), SSR-based genetic linkage maps were not constructed and reported. In this study, we evaluated 46 SSR markers with 96 F1 individuals from ‘Irwin’ × ‘Keitt’, and identified that 35 SSR markers might be mapped in the genetic linkage maps of ‘Irwin’ and/or ‘Keitt’. Four SSR combinations showing significant linkages for alleles of ‘Irwin’, i.e., MiIIHR05 vs. MiIIHR26, MiIIHR17 vs. MiIIHR32, MiSHRS-4 vs. LMMA2, and MiIIHR22 vs. LMMA10, could be positioned in the same linkage groups of ‘Irwin’. Eleven SSR combinations showing significant linkages for alleles of ‘Keitt’ could be used for genome mapping of ‘Keitt’. SSR markers provide a reliable method for evaluation of genetic diversity and construction of genetic maps because of their co-dominant inheritance and the allelic abundance (Weber and May 1989). Reference genetic linkage maps constructed with genome-wide molecular markers such as SSR markers are important for many genetic and breeding applications in fruit trees including marker-assisted selection (MAS), mapping of quantitative trait loci, and map-based gene cloning (Yamamoto and Terakami 2016). MAS can accelerate the selection process and reduce the number of progeny needed and thus the cost of raising individuals to maturity in the field (Luby and Shaw 2001).

Recently, high-density, almost saturated linkage maps in mango were developed through the use of next-generation sequencing-based and transcriptome-based single nucleotide polymorphism markers (Kuhn et al. 2017, Luo et al. 2016). Genetic maps are valuable tools for quantitative trait locus mapping and MAS of plants with desirable traits. Significant associations between traits and single nucleotide polymorphism markers for branch habit and for fruit bloom, ground skin color, blush intensity, beak shape, and pulp color (Kuhn et al. 2017) will be valuable for MAS in mango breeding programs.

With these advantages of recent molecular tools, mango genetic resources characterized in this study will be utilized to accelerate for promotion of mango cultivation in Japan and will contribute to provide information for breeding and/or adoption appropriate cultivar for stable production in the world.

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