DIVERSITY OF INDONESIAN MANGO (*Mangifera indica*) CULTIVARS BASED ON MORPHOLOGICAL AND RAPD MARKERS

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SUMMARY

Indonesia has a mango germplasm garden called KP Cukur Gondang Gardens since 1941, which has 208 native cultivars. Genetic diversity was estimated by morphology and RAPD markers. The number of cultivar observed based on morphology, DNA and both characters were 82, 76, and 72 cultivars respectively. Morphological clustering analysis classified the cultivars into three major groups Kidang Kencono, Kopek and Carang. The coefficients of genetic similarity within cultivars by using morphological characters were 0.38 to 0.85. RAPD markers using 8 primers gave reproducible DNA polymorphisms, with the number of polymorphic bands ranging from 1 to 13. A total of 65 distinct DNA fragments ranging from 0.20 to 2.0 Kb were amplified by using seven selected primers. The coefficient of genetic similarity within mango cultivars was 0.69 to 0.98 and the smallest similarity represent by the cultivar Santok with Cengkir, whereas the cultivars Jenis Baru and Ndok 181 have the highest similarity coefficient. There is 2 to 31% range of genetic variability in 82 accession mango cultivars from Cukur-Gondang germplasm. The cluster analysis indicated that the 83 cultivars of mangoes formed nine major clusters with 75% similarity. The first major cluster had nine accessions. Meanwhile, 76 cultivars from combined markers had nineteen major clusters similarity coefficients of 0.69 to 0.88. According to this result, both molecular and morphological data sets were not equally effective to quantify and organize the genetic diversity of mango cultivars.

Keywords: Indonesian mango cultivars, germplasm, morphology, RAPD

High genetic variability of Indonesian mangoes can be exploited by breeding programs to produce high quality mangoes. The management of mangoes would be effective and efficient if the characterization is accurate, so that it results in clear grouping which can be used as reference for the breeders, farmers, traders, certification bodies, and in intellectual property rights, and trade agreements (Anand, 2000). Furthermore, it is important to protect the Indonesian mango cultivars from defraud and guaranty the originality. The use of progenitors based on genetic distance among cultivars is important for plant breeding program. In addition, the understanding of intraspecific genetic variation patterns is important for genetic resource management and conservation.

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On the other hand, the Indonesian mango cultivar diversity is threatened by natural habitat loss. During less than a quarter century, tens and even hundreds of wild mangoes became extinct, but has not been explored and identified. Identification, characterization, and evaluation of the mangoes either cultivated or wild mangoes had not been well done, especially those from outside of Java. Some of the mango cultivars in Java had been identified based on morphological characters by Kusumo *et al.* (1975) and Efendy *et al.* (2003).

Limited morphological variation makes mango cultivar discrimination difficult based on visible attributes. The use of molecular markers is useful for cultivar identification and to estimate the genetic similarity among the cultivars. Thus, genotype variation between cultivars can be distinguished clearly and duplication of accessions can be avoided.

Random amplified polymorphic DNA *(*RAPD*)* is a marker genotyping technique which is technically simple, quick and produces a relatively high level of polymorphism. In addition, this technique is useful to analyze genetic diversity within species without prior sequence information (Williams *et al.*, 1990). The RAPD markers can discriminate between species and it is possible to show the phylogenetic relationships among cultivars with similar morphology (Kusch and Heckmann, 1996). RAPD analysis had been performed in mango accessions from Australia (Bally *et al.*, 1996), Florida (Schnell *et al.*, 1995), Venezuela (Lo'pez-Valenzuela *et al.*, 1997), and India (Kumar and Narayanaswamy, 2001; Karihaloo and Divedi, 2003).

The aim of this research was to classify Indonesian mango cultivars by using morphological and RAPD markers. This information could be used as a reference system to: avoid accession duplication; guarantee the cultivar purity; give accurate information about mango genetic diversity for management and conservation; and to provide information on cultivar similarity which can easily be used to select the progenitors in mango breeding program.

MATERIALS AND METHODS

Plant Materials

The materials were collected from Cukur Gondang Collection Garden, Pasuruan East Java. Detail materials which were collected could be seen in Table 1. All materials were observed based on morphology, DNA and both characters were 82, 76, and 72 cultivars respectively. The cultivars without morphological analysis included 'Gandewo25', 'Kapuk Randu', Glembo 361', 'Gambir 263', 'Madu Lumut', and 'Slendro'.

Morphological characters were observed following Rifai (1976), Vogel (1987), and Haris and Haris (1994) with 92 characters namely: canopy shape, branching patterns, flowering rotation, leaf density, leaf position, leaf forms, leaf surface, leaf vernation, leaf base and apex, leaf length and width, inflorescence shape, pedicel hairs, stipule type, stipule shape, hairs on stipule, stipule size, calyx length and width, calyx shape, hairs on calyx, perianth color, perianth shape, hairs on perianth, position of vein branches on perianth, curve on perianth, perianth length and width, pistil and stamen orientation, ovary orientation, fruit fall percentage, fruit shape, pedicel position, fruit base shape, fruit apex shape, hollow and half fruit shape, seed shape, weight, fruit length and width, mature fruit skin color, thickness and flesh color, fruit fibre, fruit water content, taste and fruit aroma, fruit skin thickness, dots on fruits, and skin wax.

Tabel 1. Eighty two mango cultivars from KP Cukurgondang-Pasuruan East Java

No.	Cultivar names	Origin	No.	Cultivar names	Origin
1	'Cantel 71'	Probolinggo EJ	42	'Kidang Kweni'	Cirebon WJ
2	'Arumanis'	Probolinggo EJ	43	'Cengkir 103'	Indramayu WJ
3	'Gandik'	Madura EJ	44	'Kepodang 45'	Probolinggo EJ
4	'Cuncung 201'	Pasuruan EJ	45	'Madu Senggoro'	Pasuruan EJ
5	'Beluk7'	Probolinggo EJ	46	'Ra'dhera 257'	Madura EJ
6	'Beruk II	Pasuruan EJ	47	'Gayer 213'	Semarang CJ
7	'Tabher 23'	Situbondo EJ	48	'Gadoh 345'	Cirebon WJ
8	'Madu 65'	Pasuruan EJ	49	'Glembo 361'	Cirebon WJ
9	'Duren 375'	Cirebon WJ	50	'Nanas 71'	Probolinggo EJ
10	'Kapal395'	Cirebon WJ	51	'Berem 10'	Probolinggo EJ
11	'Kidang Kencono'	Cirebon WJ	52	'Polok 157'	Probolinggo EJ
12	'Gendruk 75'	Pasuruan EJ	53	'Kopyor Wedus'	Pasuruan EJ
13	'Dodol Wirosongko'	Cirebon WJ	54	'Lalijiwo 91'	Semarang CJ
14	'Gandariya '	Cirebon WJ	55	'Kopek Mundu 329'	Cirebon WJ
15	'Manila 337'	Pohjontrek EJ	56	'Kecik 47'	Probolinggo EJ
16	'Beku 279'	Jati Roto CJ	57	'Carang'	Cirebon WJ
17	'Dodol pijet'	Tegal CJ	58	'Kotak 59'	Probolinggo EJ
18	'LahangII'	Cirebon WJ	59	'Cempora 215'	Yogjakarta DIY
19	'Krasak 327'	Semarang CJ	60	'Lampeni 63'	Probolinggo EJ
20	'Banyak 345'	Cirebon WJ	61	'Buaya 371'	Cirebon WJ
21	'Musuh 341'	Cirebon WJ	62	'Kebo 109'	Cirebon WJ
22	'Endok Asin'	Cirebon WJ	63	'Limun 197'	Pasuruan EJ
23	'Danas Madu'	Cirebon WJ	64	'Sophia 243'	Pasuruan EJ
24	'Daging 379'	Pasuruan EJ	65	'Dodol Birowo'	Pohjontrek EJ
25	'Gandewo 25'	Pasuruan EJ	66	'Trapang III'	Pohjontrek EJ
26	'Kates 43'	Madura EJ	67	'Mangkok'	Pohjontrek EJ
27	'Gedong 289'	Cirebon WJ	68	'Kopek'	Pohjontrek EJ
28	'Jelali 253'	Madura EJ	69	'Gurih Panjang'	Probolinggo EJ
29	'Santok 89'	Magetan EJ	70	'Randu'	Pasuruan EJ
30	'Bubut 367'	Cirebon EJ	71	'Nanas 93'	Probolinggo EJ
31	'Welulang 81'	Probolinggo EJ	72	'Guling 97'	Bangil EJ
32	'Pandan147'	Probolinggo EJ	73	'Randu 411'	Pasuruan EJ
33	'Canting 137'	Probolinggo EJ	74	'Soho 199'	Pasuruan EJ
34	'Delima 209'	Pasuruan EJ	75	'Janis 17'	Bangil EJ
35	'Bapang Lumut'	Pasuruan EJ	76	'Pasir 167'	Probolinggo EJ
36	'Jenis Baru 2'	Pasuruan EJ	77	'Gandewo 25'	Pasuruan EJ
37	'Wajik 423'	Pohjontrek EJ	78	'Gambir 263'	Madura EJ
38	'Kapuk Randu'	Cirebon WJ	79	'Madu Anggur '	Probolinggo EJ
39	'Dodol Jembar '	Tegal CJ	80	'Madu Lumut 163'	Probolinggo EJ
40	'Golek 35'	Pasuruan EJ	81	'Endok 181'	Cirebon WJ
41	'Kates 277'	Pasuruan EJ	82	'Slendro 203'	Pasuruan EJ

Molecular Analysis

CTAB procedure was used for DNA extractions (Carmen del Castillo *et al.*, 2006) with some modifications. Selection made on 10 OPA primers (OPA 10-20), 10 Primer OPB (OPB 2-12) and 4 primary OPH, then acquired by 8 primary that gives the best band. The DNA was amplified with 8 random primers of 10 base pairs in length (OPA 14, OPA 16, OPA 17, OPA 18, SBH 12, SBH 13, SBH 14, dan SBH 19) following Williams *et al.* (1990). The PCR fragments were then electrophoresed with a 1 kb molecular weight ladder (Promega) using agarose gels (1.2%) in TBE buffer. Electrophoresis was done for 150 minutes with 60 volts, at room temperature.

Data analysis

Clustering was arranged based on morphological characters and DNA fragments can be amplified for each accession. Synonyms, different cultivars of the same name (homonyms), and phylogeny among accession was known from similarity analysis by using NTSYS pc 2.02k version (Rolf, 2004). Morphological and genetic similarity of mango based on morphology and RAPD was analyzed by using SAHN clustering with Unweighted Pair Group Method with Arithmatic Average (UPGMA). The result of this analysis was indicated using a dendrogram. Genetic relationships were interpreted from a dendrogram, with genetic distances between 0.00 (0%) to 1.00 (100%).

RESULTS AND DISCUSSION

Clustering and diversity of mango cultivars based on morphological characters

Mango diversity can be identified by using morphological characters because they can be simply observed. Results based on morphological characters on 76 mango cultivars in Cukur Gondang Pasuruan showed that there was high variability for fruit shape, mature fruit skin color, mature flesh color, aroma, and fruit size. Mango 'Golek', 'Kepodang' and 'Bapang', are characterized by long shaped fruits. 'Madu', 'Kebo', 'Gedong' are globose, while 'Arumanis', 'Kopyor', 'Gendruk', 'Delima' are long globose. The variation in fruit skin color suggest that 'Kepodang', 'Delima', 'Sengir', 'Madu Nangka', 'Mangkok', 'Urang' and 'Beruk' are yellowish orange to red, while those of 'Berem', 'Golek' dan 'Madu' are green to yellowish green. On the other hand, 'Thaber', 'Gurih Panjang', 'Manalagi Probolinggo' are dark green when mature. Based on flesh color, there are two groups i.e. whitish yellow ('Berem', 'Madu' dan 'Nanas'), yellow to orange ('Arumanis', 'Beruk', 'Gedong').

The results of cluster analysis based on morphological similarity matrices, suggest that there is no cluster based on area of origin or fruit shape. The clusters were formed based on similarity of 92 morphological characters. All individuals clustered into one group at 38% morphological similarity. Clusters based on morphological characters suggests that there are 76 mango cultivars with similarity range of 0.38 to 0.85. Cultivar 'Limun197' and 'Nanas93' has the highest similarity value (0.85). Cultivar 'Lalijiwo' and cultivar 'Trapang' has the lowest similarity value (0.38). Both cultivars are different species.

Cluster analysis for morphological data resulted in a dendrogram (Figure 1) with similarity coefficients between 0.38 and 0.85 or with morphological diversity of 0.15 to 0.62. in the similarity 50% there are three main groups i.e. group I consists of 10 cultivars i.e. 'Jenis Baru', 'Cuncung201', 'Kidang Kencono', 'Daging379', 'Manila337',
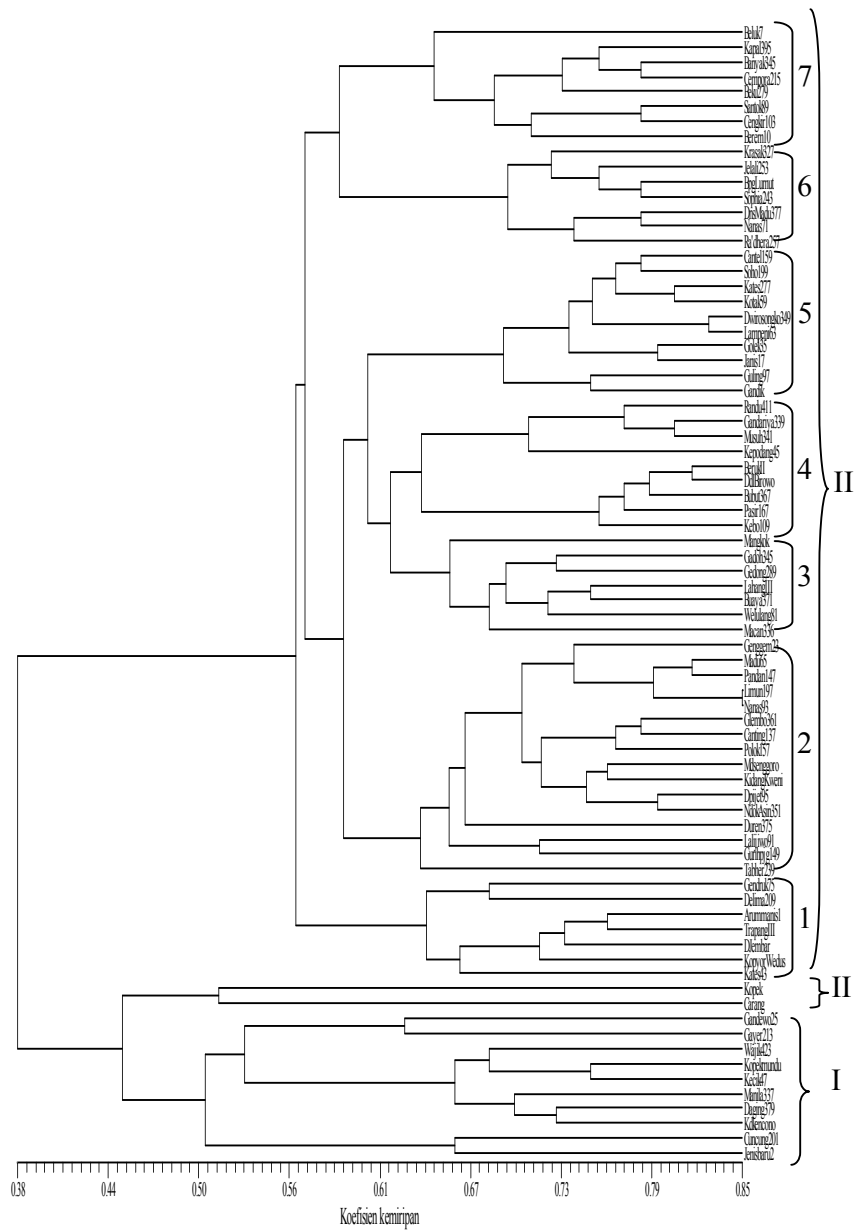
Figure 1. Indonesian mango dendrogram based on morphological markers

'Kecik47', 'Kopek Mundu', 'Wajik423', 'Gayer213', and 'Gandewo25'. This group represents cultivars which are similar to 'Kidang Kencono'. This main group has a similarity level lower than the other two main groups.

The main group II consists of 2 cultivars namely 'Kopek' and 'Carang'. This group is characterized by the same leaf form, areole, inflorescence width, hair on the inflorescence, hair position on flowers, sepal width, petal apex, petal vernation, ovary position, fruit skin size and color.

The group 1 cultivars includes 'Kopyor Wedus', 'Dodol Jembar', 'Arumanis', 'Delima209' and 'Gendruk'. The fruit shape is like 'Arumanis' where the fruit is long oval, fruit apex flat to rounded, with a shallow tip or no tip, oblong leaf, acute apex, and presence of hairs on the main peduncle. Cultivar 2 is closely related to 'Madu65' which is characterized by oval fruit shape, fruit base and apex rounded. The main similar characters are the bract size 0.6 to 16 mm, lamina length 8.5 to 17 cm, narrowly triangular sepal, no hairs on the bracts, and the vein branches on petals one-third of the way towards the base. This group consisted of 'Tabher', 'Gurih Panjang', 'Lalijiwo91', 'Duren375', 'Ndok Asin351', 'Dodol Pijet', 'Kidang Kweni', 'Madu Senggoro', 'Pelok157', 'Canting137', 'Glembo361', 'Nanas93', 'Limun197', 'Pandan147', 'Madu65', and 'Gengem23'.

Group 3 is 'Gedong' characterized by bracts between 0.6 to 16 mm, lamina 8.5 to 17 cm, narrowly triangular sepals, no hair on bracts, vein branches one-third towards the base. This group consist of 'Gedong289', 'Mangkok', 'Pasir167', 'Bubut367' dan 'Welulang81', 'LahangIII', 'Gadoh345', 'Buaya371', and 'Macan336'. This group represents the small sized mangoes with average weight of > 250 g/fruit. This group comes from West Java and Central Java. On the other hand, large mangoes come from East Java. This observation can be explained with the different geographical position which influences the rainfall and dry season which are different on both areas. In East Java the dry season is relatively longer, consequently the photosynthate is more for fruit growth.

Group 4 consists of two mango subgroups i.e. Kepodang and Kebo. Kepodang has acute fruit base, flat fruit apex, no concavity and beak in the fruit apex, leaf lateral veins of 20 to 26 pairs, medium areole density, inflorescence width <11 cm, widely triangular bract, apex acuminate bract, widely triangular sepals, petal length 2 to 2.5 mm, and petal venation branched at base. This subcultivars includes 'Kepodang45', 'Musuh', 'Gandariya339', and 'Randu411'.

Subcultivar Kebo is characterized by the round fruit, base and apex, narrowly oblong leaves, widely triangular bracts (0.6 to 1.6 mm long), acuminate bract apex, hairs present in ventral bracts, narrowly triangular sepals, oblong petals (2 to 2.5 mm long), lateral ovary, 6 to 12 cm long fruits, curved fruit base, (similarity level 75.2%). This cultivars included 'Dodol Birowo', 'Beruk', 'Bubut', 'Pasir', and 'Kebo'.

Group 5 consists of 'Gandik', 'Guling97', 'Janis17', 'Golek35', 'Lampeni63', 'Dodol Wirosongko349', 'Kotak59', 'Kates277', 'Soho199', and 'Cantel159'. This cultivar is called Golek, and is characterized by long fruits, the young fruit skin is whitish-green, the flesh color is yellow to orange. The distinguishing characters are oblong leaf shape with acute apex, leaves >35 cm long, oblong petals, perpendicular petal orientation.

Group 6 (Bapang), which is the intermediate between Golek and Arumanis, is characterized by the dark green young fruit, yellow colored mature flesh, acute to acuminate fruit base, fruit apex acuminate to flat, and shallowly beaked, venation 0.76 to 1.5 cm apart, hairs present on main peduncle, widely triangular bracts, 0.6 to 1.2 mm long. This group consists of Ra'dhera257, Nanas71, Danas Madu377, Sophia243, Bapang Lumut, Jelali253, and Krasak327.

Group 7 consists of 'Berem10', 'Cengkir103', 'Santok89', 'Beku279', 'Cempora215', 'Banyak345', 'Kapal395', and 'Beluk'. These cultivars represent the large mangoes which can reach 1.5 to 2 kg/fruit such as Berem, which is characterized by a flat fruit base, pedicel position lining to the front, and acuminate fruit apex. The discriminating characters are the young fruit skin color which is whitish-green, the mature flesh color whitish yellow to yellow, fruits rounded, acuminate fruit apex, beaked, hairy main peduncle, narrowly triangular sepals, oblong petals, vein branches in petal base, and petals 2.0 to 2.5 mm long.

Grouping and cultivar diversity based on RAPD markers

Based on RAPD markers, the closely related mangoes are 'Janis' and 'Ndok181' with a similarity coefficient of 0.98, followed by 'Kidang Kweni' with 'Mangkok' and 'Kidang Kweni' with 'Lahang' 0.97 each. These three cultivars also have a high morphological similarity. The most diversely-related species were between 'Madu67' and 'Manila' with similarity value 0.60; both cultivars are different. Madu67' is a member of *M. lalijiwa* and Manila is a member of *M. indica*. Furthermore, the lowest similarity value is between 'Trapang' and 'Glembo' (0.62). These lower values of genetic similarity based on RAPD markers support findings by Kostermans and Bompard (1993) that Madu is a different group included in *M. lalijiwa*.

The dendrogram produced with RAPD markers on the 82 mangoes showed 69% to 98% similarity (Figure 2). The cluster resulted is different from those based on morphology. The main group of mango cultivars based on RAPD markers cannot be separated. The differences in the DNA markers especially the number and size, play an important role in determining the genetic diversity level. The number of polymorphic DNA bands can represent the mango genomic profile, due to the site distribution of primer attachment.
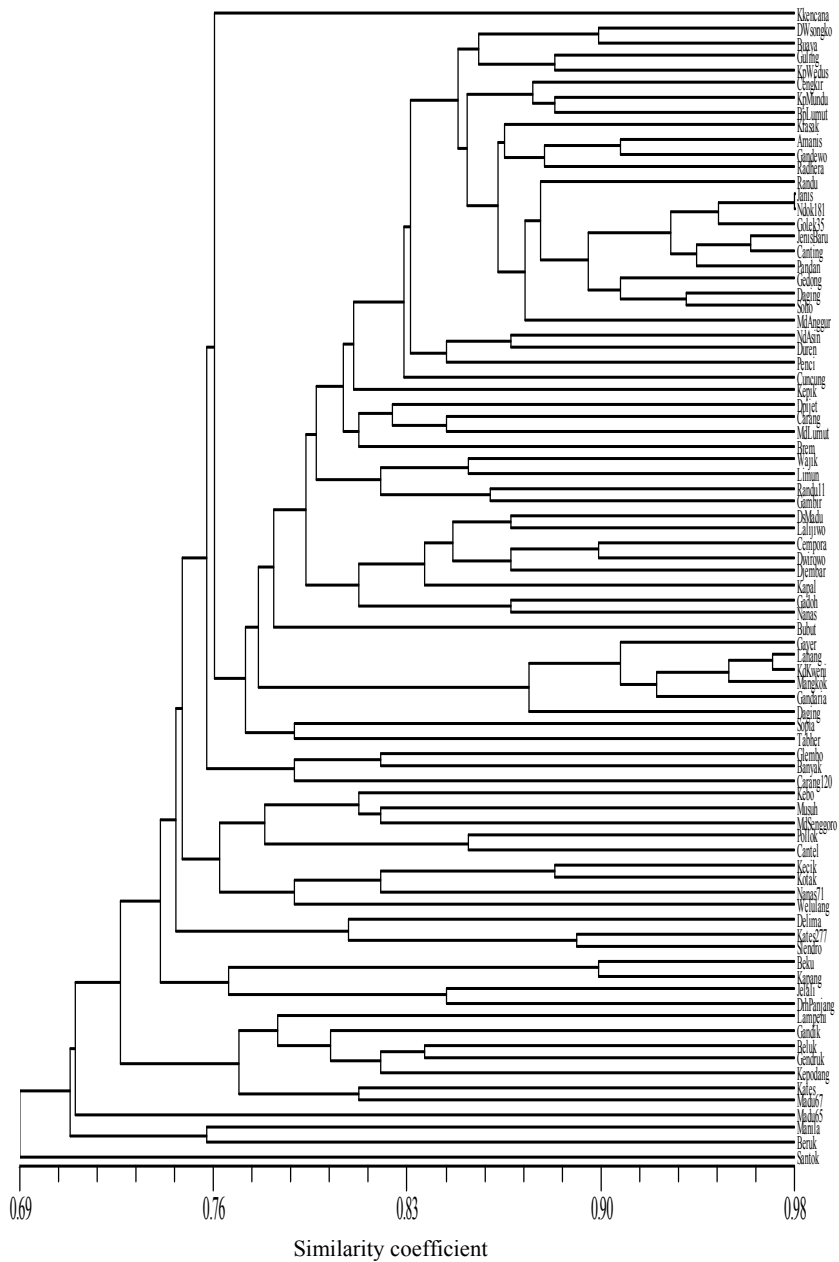
Figure 2. Indonesian mango dendrogram based on RAPD markers

The differences and polymorphism of DNA markers produced by each primer depict the complexity of plant genome (Grattapaglia *et al.*, 1992) because the DNA bands are the results of primer nucleotide binding on plant chromosomes. Therefore, the more primers used, the better the real genome represented.

At the 76% similarity level, the dendrogram can be divided into 10 main groups. The main first, second, third, and fourth consist of only one cultivar ('Santok', 'Beruk', 'Manila', and 'Madu65' respectively). The fifth group consists of seven cultivars (i.e. 'Madu67', 'Kates', 'Kepodang', 'Gendruk', 'Beluk', 'Gandik', and 'Lampeni'). Group 6 consists of 'Durih Panjang', 'Jelali', 'Trapang', and 'Beku'. Group 7 comprises 'Slendro', 'Kates277', and 'Delima'. Group 8 consists of 'Welulang', 'Nanas71', 'Kotak', 'Kecik', 'Cantel', 'Pelok', 'Madu Senggoro', and 'Musuh'. Group 9 includes 'Carang120', 'Banyak, dan 'Glembo'. Group 10 includes 53 cultivars. RAPD markers can group 'Kidang Kweni', 'Lahang', and 'Mangkok' within one group with 96.4% similarity. The clusters formed by these three cultivars agrees with grouping based on morphological markers.

The dendrogram based on DNA markers does not give a clear cluster (Figure 2). The low similarity among indicates the limitation of these markers for determining mango phylogeny, but indicates that they are suitable to search variability within cultivars to select the best progenitors.

Grouping based on RAPDs is different from those based on morphology. The mutation rate in the DNA and morphology levels take place in different speed. Therefore, the speciation process within the species is still proceeding with different rates. Fertilization systems for mangoes, which is open pollinated, and the chromosome number which is similarly allotetraploid has caused high level cross adjustment among the cultivars or within cultivars can cause high variation in the mangoes.

Clustering based on mango cultivar diversity based on morpology and RAPD combination

Based on combination of morphological and RAPD markers, the similarity of 72 mango cultivars ranges between 60 to 88% (Figure 3). Cultivars with the highest similarity are between 'Golek' and 'Janis' (88%). Clustering with combination of morphology and RAPD is almost similar to that based on morphology. However, there are some distinctions in the similarity values in grouping. At the 65% similarity there are main groups namely: the first main group consists of 10 cultivars (i.e. 'Gayer', 'Gandewo', 'Jenis Baru', 'Cuncung', 'Manila', 'Kidang Kencono', 'Wajik', 'Kopek Mundu', 'Kecik', and 'Daging').

The second main group consists of two cultivars (i.e. 'Kopek' and 'Carang'), with which they are separated from the 76 other cultivars. The third main group consists of 64 cultivars forming 7 subgroups which are similar to those based on morphology. The analysis results of morphology, RAPD, and combination showed some differences in value range of similarity coefficient: 47, 29, and 28% respectively (Table 2). These differences suggest that variation in morphological markers is larger than for RAPDs at the DNA level. While the value of dissimilarity or variability resulting from morphological markers, RAPD and in combination is 15 to 62%, 2 to 31%, and 12 to 40% respectively. The range of genetic variability is narrower than that of the morphological variation, there is possibility that the environmental factors play an important role beside genetic factors (Allard 1960), however, the DNA variation range of 2 to 40% is wide within the cultivars and can be used by breeding programs.

Table 2. Similarity coefficient and cophenetic coefficient of Indonesia mangoes based on morphology, RAPD, and combination of both

Similarity coefficient	Morphology	RAPD	Combination
Highest value	0.85	0.98	0.88
Lowest value	0.38	0.69	0.60
Cophenetic coefficient (r)	0.89	0.77	0.77
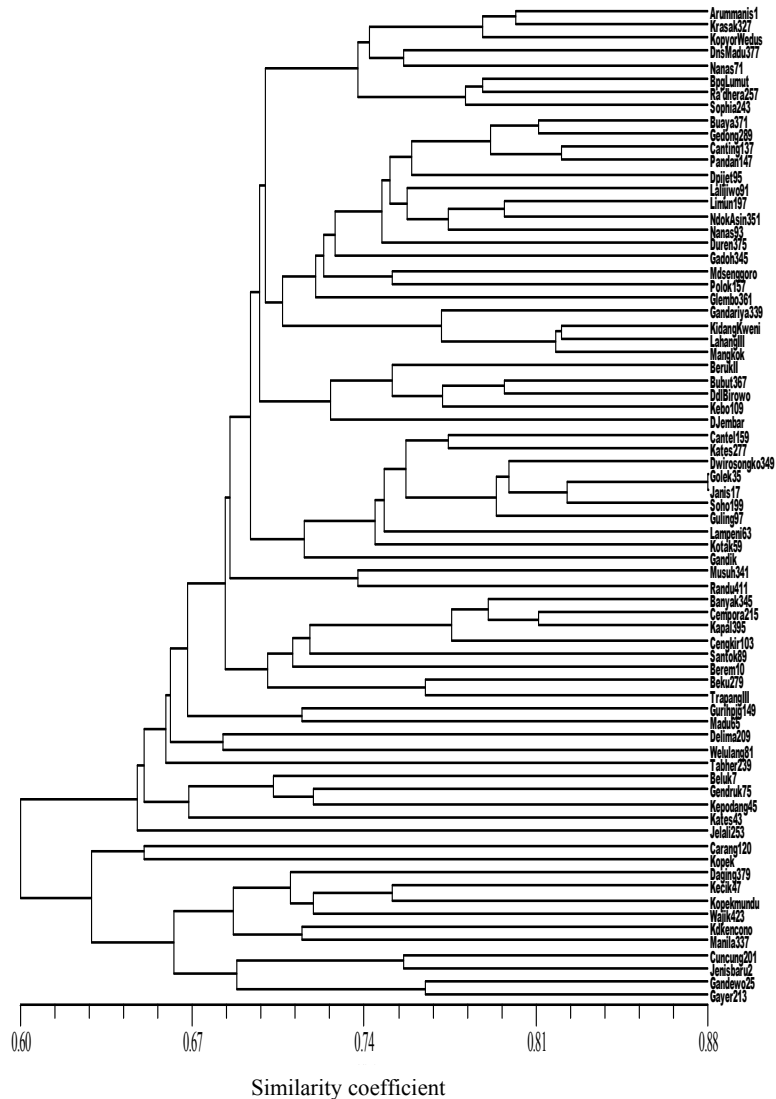
Figure 3. Dendrogram of Indonesian mangoes based on combination of morphology and RAPD markers

The genetic diversity of the Indonesian mangoes is wide and based on the result of clustering, there are no duplicate accessions, so that, to conserve the mangoes, all of the accessions in Cukurgondang should be conserved. Collection consisting of smaller populations will make it easier to manage, so that genetic information can be obtained. Good collections from the garden has to represent all species, areas of origin, and the environment (Brown, 1989). The availability of germplasm with large amount and wide genetic diversity support mango breeding programs especially in the selection of the acurate progenitor candidates.

Indonesian mango classification based on 92 morphological characters and 8 primers of RAPD is different from classification which is based on the fruit characters

Correlation of morphological characters and DNA band characteristics

Correlation among RAPD markers OPA 18-18 with rounded canopy is 70% with Pearson's Correlation Analysis (99% confidence). It means, the mango canopy rounded can be characterized by OPA18-18 markers. Correlation among morphological characters was found between large bracts and thick seeds (80%), thick seeds and fruit flat beak shape (100%) and concavity in fruit apex and water content (80%).

CONCLUSION

Cluster analysis of Indonesian mango based on 92 morphological characters and RAPD markers provided very important information regarding genetic diversity of Indonesian mangoes. The Indonesian mango genetic diversity is wide namely 15-62% (morphology), 2-31% (RAPD), and 12-40% (combination of both markers). Based on mango clustering there is no accession duplication so that all acessions should be conserved. DNA bands of primer OPA 18-18 can be used as the indicator of rounded canopy.

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