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COMPARATIVE ANALYSIS ON DIVERSITY PATTERN OF PINEAPPLE BASED ON PHENOTYPIC AND RAPD MARKERS

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obir, Siska Indriajaya Apriyani, Hajrial Aswidinnoor & Yuniarti. 2006. Analisis Perbandingan Pola eanekaragaman Nenas Berdasarkan Penanda Fenotipik dan RAPD. *Floribunda* 3(2): 44–51. — Nenas iperbanyak secara vegetatif sehingga keragaman genetiknya cenderung rendah, oleh karena itu dibutuhkan iatu pendekatan yang akurat untuk mendeteksi keragaman genetik koleksi plasma nutfah nenas. Pada enelitian ini dilakukan analisis keragaman genetik 20 aksesi dari Indonesia dan dua aksesi dari Pantai Gading. engamatan 27 karakter morfologi fenotipik dan 29 pita hasil amplifikasi analisis RAPD bertujuan untuk iengetahui jarak genetik dan kekerabatan antar aksesi yang diuji. Analisis gerombol dengan berdasarkan arakter morfologi mampu mendeteksi keragaman genetik sebesar 0.36 hingga 0.84 berdasarkan koefisien esamaan, dan pada tingkat koefisien kesamaan 0.35, aksesi yang diuji dapat dipisahkan menjadi tiga kelompok tama. Selanjutnya berdasarkan 23 pita polimorfis analisis RAPD mampu menduga keragaman genetik sebesar .62-1.00 berdasarkan koefisien kesamaan. Pada tingkat koefisien kesamaan 0.67, aksesi yang diuji dapat ipisahkan menjadi tiga kelompok utama dan mampu memisahkan aksesi tipe Queen dan aksesi tipe Smooth ayenne. Analisis konkurensi menghasilkan nilai korelasi Rohlf yang rendah (r=0.0747). Nilai ini menunjukkan ahwa pengelompokkan sebaiknya menggunakan data gabungan morfologi dan RAPD, dan analisis gerombol abungan menduga keragaman genetik sebesar 0.49–0.81 berdasarkan koefisien kesamaan, dan pada tingkat oefisien kesamaan 0.56, aksesi yang diuji dapat dipisahkan menjadi empat kelompok utama dan mampu iemisahkan aksesi tipe Queen dan aksesi tipe Smooth Cayenne.

Kata kunci: Nenas, keragaman genetik, RAPD,morfologi, korelasi Rohlf.

obir, Siska Indriajaya Apriyani, Hajrial Aswidinnoor & Yuniarti. 2006. Comparative Analysis on Diversity attern of Pineapple Based on Phenotypic and RAPD Markers. *Floribunda* 3(2): 44–51. — Pineapple is iostly propagated through vegetative mean, and tend to have narrow genetic variability; therefore, powerful iethod is required to differentiate their genetic variability, and consequenly 20 *Ananas commosus* accessions om nine locations in Indonesia and two accessions from Ivory Coast, were subjected to genetic variability nalysis. This research utilizes their phenotypic performance and RAPD markers to evaluate the genetic istance and relationship among those accessions. Twenty-seven morphological characters and 29 RAPD ands had been utilized in this study. Cluster analysis revealed that based on morphological markers the ccessions were separated accessions from 0.36 to 0.84 of similarity coefficient, and three primary groups ould be distinguished at similarity coefficient of 0.35. Subsequently RAPD analysis has been separated the ccessions at 0.62–1.00 of coefficient of similarity. From this analysis, three primary groups constructed at .67, and it is able to differentiate Queen and Smooth Cayenne type accessions. The concurrence analysis on iorphological and RAPD analysis, showed the very weak Rohlf correlation value (r=0.0747), which indicated iat grouping of pineapple accessions should be combination of morphological characters and RAPD analysis. ombination morphological showed that accessions distributed from 0.49 to 0.81 of similarity coefficient level, nd at 0.56 level similarities, the pineapple accessions were clustered into four primary groups and it is able to ifferentiate Queen and Smooth Cayenne type accessions.

Key words: Pineapple, genetic variability, RAPD, morphology, Rohlf correlation.

Pineapple [*Ananas commosus* (L.) Merr.] production is predominantly asexual and varieties of *A. commosus* are self-incompatible (seedless when self-pollinated), so that it made low variation in pineapple. Seeds may be produced by artificial cross-pollination or assisted by humming bird, but this rarely happen. Under this condition, breeding by human is needed to increase the variation. In a breeding program, breeders need high variation of introduction or local germplasm. The variation can be increased by hybridization, mutation, somaclonal variation and other techniques (Collins 1968).

According to Bai et al. (2000), germplasm collections are useful in characterizing individual accessions and cultivars, in detecting duplications of genetic materials, as a general guide in selecting

parents for crossing in breeding programs and in developing informative mapping populations for genome mapping. In order to asses the genetic resources for breeding program, however, a genetic variation analyses is needed on pineapple collection, to elucidate potential parents for hybridization as well as inheritance analysis (Allard 1960).

Genetic variation analyses can be observed from morphological characters and other markers, such as protein or deoxyribonucleic acid (DNA) based markers. Pineapple characterization that often accomplished was based on morphological descriptions. However, the evaluation of morphological characters requires the plants have to be mature prior to identification. Subsequently genetics resources characterization also needed to establish development of genetic markers for valuable traits (Paterson et al. 1991).

Molecular markers provide a quick and reliable method for estimating genetic relationships among genotypes of any organism (Thormann et al. 1994). Random amplified polymorphic DNA (RAPD) analysis (Williams et al. 1990) has been used for diversity analysis in a vast array of crops, i.e. used for the determination of genotypes (Hasizume et al. 1993) and QTL analysis (Grandillo & Tanskley 1996). This approach is based on the polymerase chain reaction (PCR) (Saiki et al. 1988). The amplification of template DNA genome using short, synthetic deoxyribonucleotides of random sequence as primers. Each primer can direct the amplification of several unrelated regions of the genome (Sondur et al. 1996). The resolving power of RAPD technique is several folds higher than visual and protein markers and is much simpler and technically less demanding than RFLP and other similar techniques (Williams et al. 1990).

MATERIALS AND METHODS

Plant Materials

Pineapple germplasm collections of CETROFS (Center for Tropical Fruit Studies) that included 20 accessions collected from Java and Sumatra island, and two accessions introduced from Ivory Costa, Africa (Table 1). They were grew in Pasirkuda Field Station, Bogor, located 250 m above sea level.

Table 1. List of pineapple accessions with their origin subjected in the study.

No.	Name	Origin	Accesion Code
1	Queen Hijau, Crgn	Bogor, West Java	JBBMQH–6
2	Queen Hijau, Bgr	Bogor, West Java	JBBMQH–7
3	Queen Hijau Kartasura	Sukoharjo, Central Java	JTSLQH–3
4	Queen Hijau Batu Pml	Pemalang, Central Java	JTPLQH–8
5	Queen Hijau Gnb	Gunung Batin, Lampung	SLLLQH–4
6	Queen Hijau Tns	Tanjungsari, Lampung	SLLLQH–5
7	Queen Hijau Ckp	Cikampek, West Java	JBKLQH–1
8	Queen Hijau Pwj	Purworejo, Central Java	JTPMQH–2
9	Baby Pineapple	Ivory Coast	LNPCBP
10	Queen Kuning Blt	Blitar, East Java	JTBLQK
11	Queen Merah Pwj	Purworejo, Central Java	JTPMQM–3
12	Queen Merah Ts	Tanjungsari, Lampung	SLLLQM–2
13	SC Supersweet	Ivory Coast	LNPCSS
14	Queen Merah Bgr	Bogor, West Java	JBBMQM–1
15	SC Cimanglid	Subang, West Java	JBSMSC–1
16	SC Kumpay	Subang, West Java	JBSMSC–2
17	SC Curug Rendeng	Subang, West Java	JBSMSC–3
18	SC Tambakan	Subang, West Java	JBSMSC–4
19	SC Minyak Bgr	Bogor, West Java	JBBMCM
20	SC Leksono Wnb	Wonosobo, Central Java	JTWHSC
21	SC Merah Wnb	Wonosobo, Central Java	JTWHSCM
22	Queen Hijau PLb	Plembang, South Sumatra	SSSPMQH

Morphological Analysis

Examination on 27 morphological characters was done in August 2003 to February 2005. It consists of 27 characters, consist of plant height, number of leaves, leaf length, leaf width, canopy diameter, leaf color, spine position, spine density, leaf color, number of suckers, number of slips, penduncle length, external fruit color, fruit weight (with or without crown), fruit length, fruit diameter, core diameter, core length, blossom end cup depth, number of blossom end cup, flesh total soluble solid, flesh pH, flesh total acid, number of crown leaf, and crown length.

RAPD Analysis

Four random primers of 10 bases in length with GC base content ≥ 60% of each primer was selected. Single arbitrary 10-base primer from series OPD, OPI, and OPN (Operon Technologies, Almeida, California USA) were tested for their ability to amplify scorable and reproducible DNA fragment. PCR reactions were carried out in a 25 µl reaction mix containing approximately 25–50 ng template DNA, 10X PCR buffer (100 mM Tris-HCl, pH 9.0 at 25°C, 500 mM KCl, 1.0 Triton X-100), 2 mM $MgCl_2$, 0.2 mM of each dATP, dCTP, dGTP and dTTP, 0.4 pmol of a single 10-base primer, and 1 unit of Taq DNA polymerase (Promega). Amplification was performed in ASTEC Thermal Cycler PC 707, programmed for 45 cycles of each of the following: 95 °C for 1 min, 35 °C for 1 min, and 72 °C for 2 min. A final elongation step of 7 min at 72 °C was included. Reaction products were mixed with 2.5 µl of loading dye (0.25% bromphenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) and spun briefly in a microtube before loading (Sambrook et al. 1989), then separated on 1.2 % agarose gel (Promega) and stained with ethidium bromide, and the gels were illuminated under UV light.

RESULT

Data Analysis

The morphological characters scoring data were analyzed by The SIMQUAL, SAHN and TREE programs from Numerical Taxonomy and Multivariate Analysis System version 2.02 (NTSYS-pc 2.02). The similarity degrees were calculated according to Simple Matching coefficient in the SAHN program. The scoring data then transferred into a binary data with the same programs, but the similarity degrees were calculated according to Dice coefficient.

DNA fragments from the amplification products were transferred into a binary data ('1' for presence and '0'for absence). The SIMQUAL, SAHN and TREE programs from Numerical Taxonomy and Multivariate Analysis System version 2.02 (NTSYS-pc 2.02) packages were used to obtain the genetic relationship. Similarity Degree (SD) were calculated according to Dice coefficient that principally the same with Nei & Li coefficient (1979): SD= 2Nab/ (Na+Nb), which Nab=Number of band presents in a and b, Na=Number of bands present in a and Nb=number of bands presents in b. Grouping was carried out using Unweighted Pair Group Method and Arithmetic Average (UPGMA) cluster analysis and principal component analysis (PCA). Goodness of fit for cluster analysis was revealed by cophenetic correlation. According to Rohlf (1993) the degree of fit can be interpreted subjectively as shown on Table 2.

Table 2. Goodness of Fit (Rohlf 1993).

Level	Interpretation
0.9?r	Very good fit
0.8?r<0.9	Goodfit
0.7?r<0.8	Poorfit
R<0.7	Very poor fit

Morphological Markers

Variability of morphological characters significantly revealed by spine characters, leave coloration, ratoon number, fruit shape and flesh color. Variation on spine characters observed on presence and position of the spine. Leave coloration show variation on middle leaves on upper surface from greenish, green with yellow mottling, green with red mottling, and reddish orange. Sucker numbers vary from few in Queen type and abundant in Smooth Cayenne type. Fruit shape vary from pyriform (pear shaped), cylindrical sharp taper, cylindrical slight taper conical to long conical. Subsequently, flesh color varies from light cream, pale yellow, yellow, golden yellow to deep golden yellow.

Cluster analysis of twenty-seven morphological characters, genetic variability was detected at genetics distance of 0.64–0.16 or at coefficient of similarity 0.36–0.84. The variability closest accessions are JBSMSC-3 with JTWHSCM with 0.84 similarity level, those two accessions were collected from separated area, which JBSMSC-3 from Subang West Java and JTWHSCM from Wonosobo Central Java, indicated that both

accessions should be had same origin, since pineapple is introduced species from Latin America (Collins 1968).

Grouping performed at 0.45 coefficient of similarity level, the evaluated accessions separated into three primary groups. First group consist of 16 accessions of JBBMQH-6, JBBMQH-7, JTSLQH-3, JTPMQM-3, JTPMQH-2, JTBLQK, JTPLQH-8, SLLLQM-2, JBBMQM-1, SLLLQH-5, SSSPMQH, SLLLQH-4, LNPCSS, LNPCBP, JBSMSC-4, and JBBMCM. The group II covered four accessions of JBKLQH-1, JBSMSC-1, JBSMSC-3, and JTWHSCM. The third group III consists of two members JBSMSC-2, and JTWHSC (Figure 1).

Since we have difficulties to identify morphological characters of each primary group, subsequently Principle Component Analysis (PCA) also conducted to elucidate grouping pattern of the accessions. The analysis result indicated that 70 % variability among accessions described at cumulative of eight principle components and the first three principle components describe only 51.73%, which is not eligible to construct fine grouping among 22 pineapple accessions (Table 3).

RAPD Analysis

A total of 29 fragments were successfully amplified from genomic DNA of 22 accessions of pineapple by 6 random primers OPG-02, OPG-11,

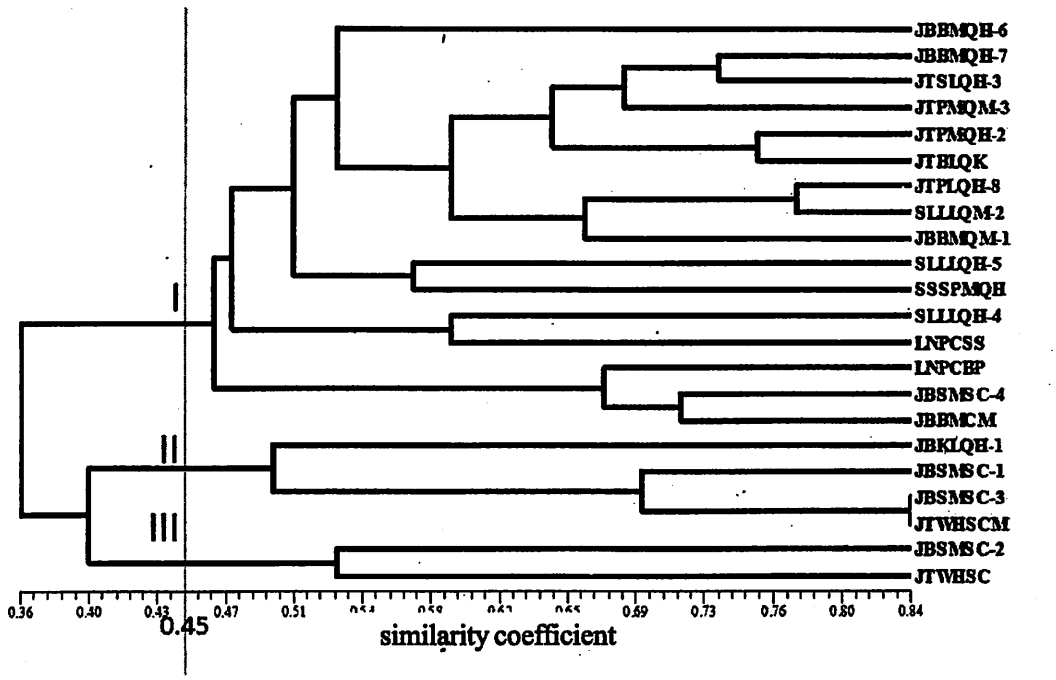

Fig. 1. A dendogram of three primary groups based on scored data of 27 morphological characters of 22 accessions of pineapple.

Table 3. Eigen Value based on morphological characters of 22 pineapple accessions.

Component	Eigen Value	Percent	Cumulative
I	17,81	36,35	36,35
II	4,66	9,51	45,87
III	2,87	5,86	51,73
IV	2,69	5,49	57,22
V	2,34	4,78	62,01
VI	1,77	3,62	65,63
VII	1,68	3,43	69,06
VIII	1,56	3,20	72,26

OPE-07 and OPE-11with GC bases content > 60% of each primer. The fragment number of each primer ranging from 5 to 10, on average 7.25 fragments per primer (Table 4). Out of total fragments, 79% showed as polymorphic with ranged from 4 to 9, on average was 5.75 fragments per primer (Table 4).

A dendogram based on the UPGMA-link method using Nei & Li similarity (1979) was constructed and presented in Figure 2. RAPD analysis by using four primers has revealed genetics distance range 0.00 to 0.38 or at coefficient of similarity 1.00 to 0.62. The dendogram also revealed that JBBMQH-6 and JBBMQH-7 shared same genetic properties since both of the originated from same area at Bogor. The variability which detected by RAPD analysis lower to those of morphological markers, as well as detected by isozymes marker of 0.25 coefficient of similarity (Hadiati & Sukmadjaja 2002). However the 23 polymorphic band of four utilized primers detected variability higher to those

Table 4. Sequence of RAPD primers used and number of fragment amplification products.

No	Primer	Sequence	Amplified Band	Polymorphic Band
1	OPG-02	GGCACTGAGG	7	5
2	OPG-11	TGCCCGTCGT	7	4
3	OPE-07	AGATGCAGCC	10	9
4	OPE-11	GAGTCTCAGG	5	5
	Total		29	23

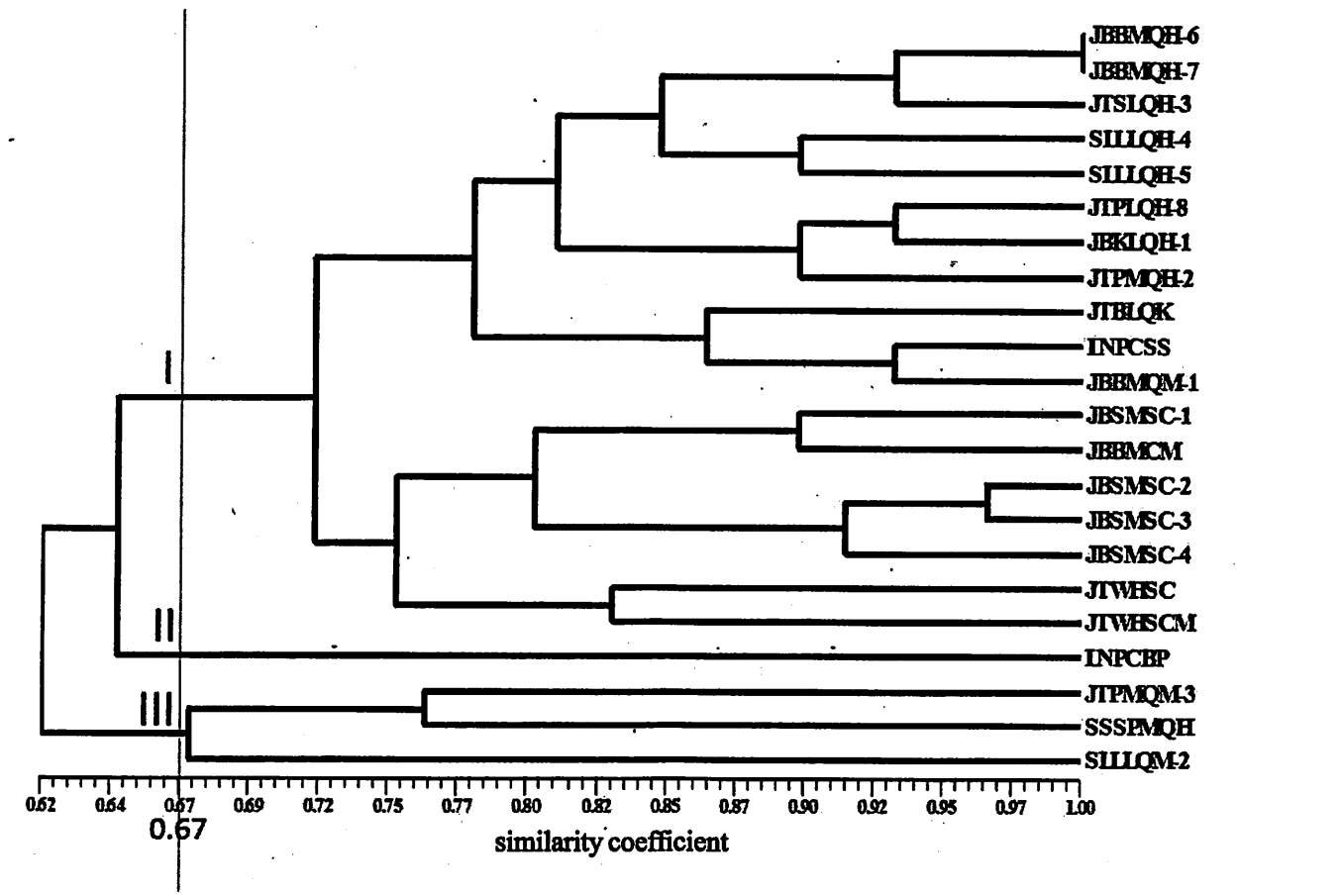

Fig. 2. A dendogram based 23 RAPD polymorphic bands on 22 accessions of pineapple.

of Ruas et al. (2002) that using 132 polymorphic RAPD marker revealed coefficient of similarity at 0.75.

At 0.67 similarity coefficient level, the dendogram constructed from cluster analysis separated the accessions into three primary groups. Group I included 18 accessions, and divided into two sub-groups at 0.73 similarity coefficient level. First subgroup (Ia) consists of 11 Queen type accessions of JBBMQH-6, JBBMQH-7, JTSLQH-3, SLLLQH-4, SLLLQH-5, JTPLQH-8, JBKLQH-1, JTPMQH-2, JTBLQK, and JBBMQM-1 except for Smooth Cayenne LNPCSS, meanwhile subgroup Ib consist of 7 Smooth Cayenne type accessions of JBSMSC-1, JBBMCM, JBSMSC-2, JBSMSC-3, JBSMSC-4, JTWHSC, and JTWHSCM. Accession Queen type LNPCB solitary in Group II. Subsequently Group III shared by three Queen type accessions of JBBMQM-3, SSSPMQH, and SLLLQM-2. These results indicated that RAPD markers more powerful to differentiate Queen type and Smooth Cayenne type compare to those of morphological markers.

Principal Component Analysis (PCA) based on 23 polymorphic RAPD markers revealed that at first two principle components described 76.87% of genetic variability among 22 pineapple accessions (Table 5). Those result indicated among 23

Table 5. Eigen Value based 23 polymorphic RAPD markers on 22 pineapple accessions.

Component	Eigen Value	Percent	Cumulative
I	15.65	53.98	53.98
II	6.64	22.89	76.87
III	1.65	5.71	82.59

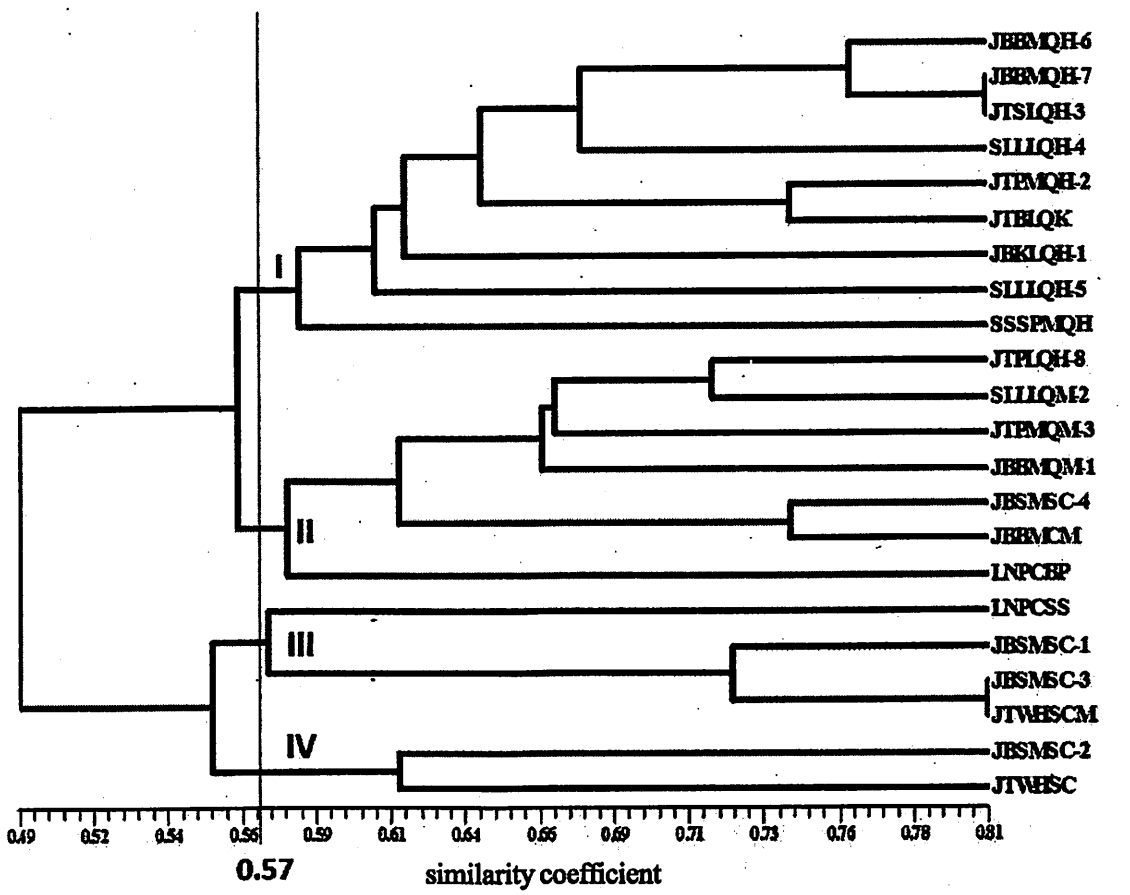

Fig. 3. A dendogram based on joint morphological-RAPD markers cluster analysis of 22 accessions of pineapple.

polymorphic RAPD markers and there were at least two potential markers for differentiating between Queen and Smooth Cayenne type.

DISCUSSION

Association between grouping in morphological characters with grouping by cluster analysis was evaluated by using *goodness of fit* base on Rohlf correlation value (1993), calculated *MxComp* correlation function with NTSYSpc program version 1.80 (Exeter software, New York). The concurrence analysis on morphological and RAPD analysis result, showed the very weak Rohlf correlation value (r=-0, 0747). This result was indicated that grouping of 22 pineapple accessions should be using combination of morphological-RAPD analysis.

A dendogram base on combination morphological-RAPD markers cluster analysis showed that genetic variability among evaluated accessions ranged from 0.19 to 0.51 or at coefficient of similarity 0.81 to 0.69 (Figure 3). The dendogram also revealed that the closest relationship showed by two pairs of JBBMQH-7 with JTSLQH-3 and JBSMSC-3 with JTWHSCM whose both shared 0.81 similarity coefficient. At similarity coefficient level of 0.57, the 22 pineapple accessions separated into four groups. Group I shared by 9 Queen type members of JBBMQH-6, JBBMQH-7, JTSLQH-3, SLLLQH-4, JTPMQH-2, JTBLQK, JBKLQH-1, SLLLQH-5, and SSSPMQH. Group II consist of 6 accessions of Queen type of JTPLQH-8, SLLLQM-2, JTPMQM-3, JBBMQM-1, JBBMCM, LNPCBP, and one accessions of Smooth Cayenne type JBSMSC-4. Group III has three Smooth Cayenne type members of LNPCSS, JBSMSC-1, JBSMSC-3, and JTWHSCM, and Group IV also shared by Smooth Cayenne type member of JBSMSC-2, and JTWHSC. The grouping pattern also revealed that cluster analysis based on combination morphological-RAPD markers is powerful to differentiate Queen type accessions from Smooth Cayenne type accessions.

RAPD analysis detected duplicity of accessions inside of the collection JBBMQH-6 and JBBMQH-7, however that duplicity was not reflected in morphological phenotype. Moreover showed higher genetic distance to the closest relationship that detected by morphological clustering as well as combiantion morphological and RAPD analysis clustering. This result indicated that the RAPD markers used in this study has not yet reflected genetic variability as detected in morphological analysis.

ACKNOWLEDGEMENT

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REFERENCES

Allard RW. 1960. *Principles of Plant Breeding.* John Willey and Sons. Inc. New York. 485 pp.

Bai G, Ayele M, Tafera H & Nguyen HT. 2000. Genetic diversity in tef (*Eragrotis tef* (Zucc) Trotter) and its relatives as revealed by Random Amplified Polymorphic DNAs. *Euphytica* 112: 15–22.

Collins JL. 1968. *The Pineapple.* Leonard Hill. London. 294 p.

Grandillo S & Tanskley SD. 1996. QTL analysis of horticultural traits differentiating the cultivated tomato from the closely related species *Lycopersicon pimpinellifolium. Theor. Appl. Genet.* 92: 935–951.

Hadiati S & Sukmadjaja D. 2002. Keragaman pola pita berbagai aksesi nenas berdasarkan analisis isozime. *J. Bioteknologi Pertanian* 7: 62–70.

Hasizume T, Sato T & Hirai M. 1993. Determination of genetic purity of hybrid seed in watermelon (*Citrullus lanatus*) and tomato (*Lycopersicon esculentum*) Using Random Amplified Polymorphic DNA (RAPD). *Japan J. Breed.* 43: 367–375.

Nei M & Li WH. 1979. Mathematical model for studying genetics variation in terms of restriction Endonucleases. *Proc. Nat. Acad. Sci.* 74: 5269 –5273.

Paterson AH, Tanskley SD & Sorrels ME. 1991. DNA markers in plant improvement. *Adv. Agron.* 44: 39–90.

Rohlf FJ. 1993. Ntsys-pc: *Numerical Taxonomic and Multivariate Analysis System. Version 2.0.* User Guide, Exeter software. Exeter Publishing Co.Ltd.

Ruas CF, Ruas PM & Cabral JRS. 2001. Assessment of genetic relatedness of the genera *Ananas* and *Pseudananas* confirmed by RAPD markers. *Euphytica* 119: 245–252.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB & Erlich HA. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239: 487–491.

Sambrook J, Fritsch EF & Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual.* (Second Edition). Cold Spring Harbor Laboratory Press. New York.

Sondur SN, Manshardt RM & Stiles JI. 1996. A genetic linkage map of papaya based on randomly amplified polymorphic DNA markers. *Theor. Appl. Genet.* 93: 547–553.

Thormann CE, Forreira ME, Camango LEA, Tivang JG. & Osborn TC. 1994. Comparison of RFLP and RAPD markers to estimating genetic relationships within and among Cruciferous species. *Theor. Appl. Genet.* 88: 973–980.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA & Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res.* 18(22): 6531–6535.