ANALYZING POLYMORPHISM AND PHYLOGENETIC OF FIVE HOT CHILI VARIETIES FROM SUMATRA ISLAND, INDONESIA

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Abstract. The objective of this study was to investigate polymorphism and phylogenetic of hot chili varieties from different provinces in Indonesia. Five different varieties of hot chili from different provinces (Jambi, West Sumatra and Aceh) were grown and maintained in field including Loker Telun Berasap, Ahang Adro, Kopay, Awe and Udeng. Eight sample plants from each cultivar were selected from 8-weeks old plants after transplanting. Morphological traits were evaluated on stems, leaves, flowers, fruits and seeds. A cluster analysis using Minitab® (Version 18) statistical software was carried out to evaluate phylogenetic relationship among genotypes. The clustering was based on similarities of their morphological traits. In addition, leaf samples were collected from sample plants and submitted to genetic analysis using RAPD markers. The results of this study revealed that there were variations in qualitative and quantitative characters as well as growth habit and morphology of the five hot chili varieties. RAPD markers and cluster analysis showed that Loker Telun Berasap or Ahang Adro morphologically different from other three varieties. Therefore, Loker Telun Berasap and Ahang Adro are two varieties that can be proposed to be released as national superior hot chili cultivars.

Key words: *Capsicum annuum*; plant breeding; marker assisted selection; genomic selection; DNA sequencing.

INTRODUCTION

Hot chili (*Capsicum annuum* L.) is one of economically important vegetable crops in Indonesia, in which it contributes significantly to Indonesian national income. The development of high-yielding chili has been greatly supported by plant breeding programs which are conducted conventionally or using modern technologies or by combining the two methods. High-yielding varieties were developed for their important traits such as high productivity, pests and diseases resistance, and responsiveness to environmental stresses [37].

The superior hot chili varieties developed are those with high productivity, favored by consumers, adaptable to climatic change, resistant to pests and diseases attacks as well as being resistant to biotic and abiotic environmental stresses. Unfortunately, chili plants cultivated in the highlands and lowlands are generally planted in open fields. Chili production in open fields often faces many obstacles, such as ever-changing climate, limited water availability, and pest and disease attacks. These disrupt plant growth so that production is not optimal. Therefore, chili plants are developed to create adaptive varieties to grow and produce optimally. This work is related to the availability of genetic variability of chili germplasm in various regions in Indonesia, including local chilies in Jambi Province, Indonesia [28]. The role of local farmers is very important in selection and collection of superior germplasm. They help the government and plant breeders to find important local cultivars that can be used as a source of superior characteristics. The more the diversity in phenotypic and genotypic characteristics of the plants, the greater gene pools of superior characteristics/features that can be used in assembling superior varieties [27].

Phenotypic diversity is morphological appearance of plants that can be evaluated from their qualitative and quantitative characteristics. Plant morphology is one of common ways of determining relationships between groups of plants, and comparisons of reproductive structures is often made to determine relationships. Therefore, morphological analysis is advantageous method of determining the range of descent of various species. Meanwhile, genetic diversity is a variation at DNA level that is not affected by the growing environment. The evaluation of genetic diversity can be done with the help of DNA molecular markers. Such techniques are able to detect varietal differences at DNA or gene levels with speed and with high level of accuracy [32]. One of the widely used molecular markers to test genetic diversity is Random Amplified Polymorphic DNA (RAPD) [5, 9, 17]. The RAPD markers were first proposed by Williams *et al.* [36] was a method to identify many DNA polymorphisms quickly and efficiently.

The RAPD markers has been used quite efficiently and effectively in studying genetic variation and genomes identification in many chili cultivars [11, 17]. The main advantage of RAPD is this technology is quite simple and easy to assay [3], therefore, RAPD has become very popular in many laboratories. Studies on the use of RAPD molecular markers have been reported on various plants for different breeding purposes. Gusmiaty *et al.* [8] used RAPD molecular markers to investigate the genetic diversity of pine. The same technology also was used by Fatmawati *et al.* [6] in their study on the relationship among various maize varieties. Hanum *et al.* [9] used the RAPD markers for analyzing polymorphism of red rice variety from South Sumatra. In chili, [26] used the RAPD molecular marker to determine the resistance of various genotypes against ChiVMV (chili veinal mottle virus), and Sikora and Nowaczyk [29] used the same technique to calculate genetic distance and kinship of three interspecific chili hybrids (*C. frutescens* × *C. annuum*), (*C. frutescens* × *C. chinense*) and (*C.*

frutescens × *C. baccatum*). However, to our knowledge this is the first report on the evaluation of genetic variation of hot chili peppers from different regions of Sumatra Island, Indonesia.

Further, the Regulation of Indonesian Minister of Agriculture No. 38/2019 on the Release of Plant Varieties stated that the release of a new crop variety is possible if it advances productivity as well as improving farmers' welfare, is distinct from other varieties, and its quality and agronomic performances are uniform and stable. The enaction of this regulation convinced farmers and seed producers that they are supplied with authentic and assured quality of seed materials with known identity of a specific variety. Keeping this in mind, the objective of present study was to evaluate genetic variation of five hot chili varieties from three provinces in Indonesia (Jambi, West Sumatra, and Nangroe Aceh Darussalam). Specifically, this study aimed to 1) determine the genetic diversity of five local chili varieties from Sumatra, Indonesia, to obtain particular superior characteristics, and 2) formulate a description of the five local chili varieties by ascertaining the correlation of morphological and genetic diversity using RAPD molecular markers.

MATERIALS AND METHODS

Plant Materials

Chili samples were obtained from farmers' fields from three different provinces in Indonesia. Loker Telun Berasap and Ahang Adro varieties were from Jambi Province, Kopay variety was from West Sumatra Province, and Awe and Udeng varieties were from Nangroe Aceh Darussalam Province. These five varieties are local superior varieties that have the potential to be released as national superior varieties.

Field Assessment

Seeds were sown on soil with trichoderma-based compost (2:1) and kept under 50% shading. Four weeks after sowing, plants were transferred to experimental plots prepared with 25 tons·ha^{-1} trichoderma-based compost and 2 tons·ha^{-1} dolomite. Black plastic mulch was used to cover the plots.

The trial used 5 replication each consisted of 5 plots, so there were 25 experimental plots. Each experimental plot consisted of 25 plants from which 16 individuals were taken as sample plants. Following two weeks of transplanting, 8 plants were selected for morphology evaluation, and other 8 plants were selected for DNA analysis to determine their genetic variability.

Routine plant care including water supply, control on pests and diseases attacks, weed control, and biourine (concentration of 60%) spray every 2 weeks. Ripe fruits were harvested during the first flowering period (4 - 6 weeks). Leaf samples were analyzed at the Plant Breeding and Tissue Culture Laboratory, Tropical Fruit Research Institute Solok, West Sumatra, Indonesia.

Observation of plant morphological diversity was carried out on qualitative and quantitative characters, referring to the Guidelines for The Conduct of Test for Distinctness, Homogeneity and Stability of Pepper (*Capsicum annuum* L.) [22] and the Regulation of Indonesian Minister of Agriculture Number 61/2011 on the Testing, Assessment, Release and Withdrawal of Varieties.

RAPD Analysis

DNA isolation and purification

Five sample plants from each cultivar were selected from 8-weeks old plants after transplanting. Specimens were submitted to genetic analysis using RAPD markers. RAPD primers that were used to evaluate genetic diversity in different varieties of chili in this study were OPA2, OPA3, OPA13, RAPD2 and RAPD5. This was done by the following steps:

DNA isolation following the CTAB method [5]. Samples of young leaves (top 3 leaves from plants aged 4 weeks old or 1 g of leaf sample) which have been cut into pieces were placed in a mortar, then 500 µL of extraction buffer (200 mM Tris-HCl, 250 mM NaCl, 25 mM EDTA) was added, and leaf samples were macerated. The sample was transferred into a microtube and 500 µL of extraction buffer which had been heated to 65°C was added. Then the sample was incubated in a water bath for 45 minutes at 65°C while gently shaken every 15 minutes and cooled down to room temperature.

DNA extraction and purification followed the protocol described by Green and Sambrook [7]. The DNA samples obtained at step 1 (above), were placed into 500 µL of chloroform-IAA solution chloroform : isoamyl alcohol at a ratio of 24 : 1 plus 200 µL of 0.2% polyvinylpyrrolidone (PVP). The samples were then centrifuged at 4000 rpm for 10 minutes. The supernatant was transferred into a new microtube, and 500 µL of chloroform-IAA solution and 200 µL of 0.2% PVP were added, centrifuged again at 4000 rpm for another 10 minutes and then the supernatant was transferred into a new microtube. A 500 µL cold isopropanol and 200 µL 5 M NaCl were added to the supernatant, then stored at -25°C for 45 minutes. Following this step, the sample was centrifuged at 4000 rpm for 10 minutes, then the liquid phase was removed leaving DNA pellet. The DNA pellet was washed with 300 µL 96% ethanol then centrifuged at 4000 rpm for 10 minutes, and the liquid phase was removed. The DNA pellet was washed again with 300 µL of 70% ethanol and centrifuged at 4000 rpm for 10 minutes. The pellets formed were dried for 5 minutes, then dissolved in 50 µL Tris-EDTA (TE) and stored in a freezer until the DNA was used.

Quantitative DNA testing using UV-Vis spectrophotometry. Desjardins and Conklin [4] claimed that genomic DNA extract which has an A260/A280 ratio between 1.8 – 2.0 indicated that the DNA met the purity requirements required in molecular analysis. Pure DNA could absorb ultraviolet light because of the presence of purine and pyrimidine bases. DNA double

bands absorbed ultraviolet light at 260 nm wavelength, whereas phenol or protein contaminants absorbed light with 280 nm wavelength. DNA purity is measured by dividing the absorbance value of DNA double bands (260 nm wavelength) by the absorbance value of phenol or protein contaminants (280 nm wavelength). The DNA purity value ranges from 1.8 to 2.0. If DNA purity is less than 1.8 indicates that the DNA sample contains protein contaminants, and proteinase enzymes should be added to purify it. On the other hand, if the value is more than 2.0 it means the DNA sample contains RNA contaminants, and a ribonuclease enzyme is applied to remove them.

PCR amplification

DNA amplification and electrophoresis followed the method of Williams *et al*. (1990) [36] using a CFX 9600 PCR machine (Biorad). For polymorphism analysis, we first provide DNA templates. This DNA template was obtained from the extraction of genomic DNA from 5 varieties of chili plants (Loker Telun Berasap, Ahang Adro, Kopay, Awe and Udeng). Furthermore, the templates were used in DNA amplification process by PCR using five selected primers. Meanwhile, the quality of extracted genomic DNA was analyzed using a spectrophotometer. The quality of this extraction indicates the purity of the DNA as seen from the value of the ratio A260/A280.

Electrophoresis was performed by inserting the sample DNA (PCR results) into a 1.2% (w/v) agarose gel in 1x TAE buffer (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA) with Ethidium bromide (Etbr) dye. A total of 3 µL of DNA samples were electrophoresed at 100 volts for 30 minutes. The electrophoretic visualization was carried out with a UV transilluminator.

Molecular analysis was carried out on leaf samples to see the genetic characters. Observation was made by visualizing DNA bands on an UV transilluminator. The profile was then photographed through a red filter for documentation.

Data Analysis

Observations were made on 8 randomly taken samples on 21 characters at specified growth stages when the characteristics showed full expression. Among 41 morphological characters studied, 20 were visually assessed and 21 were measured. While charaterization of leaf and flower colour was done as per Royal Horticulture Society Colour Charts (RHCC) Sixth Edition [23]. Analysis of measurable characters was carried by developing cluster analysis using Minitab statistical software [15] to determine the phylogenetic relationship among cultivars based on similarity of morphological traits.

RESULTS

Evaluation on qualitative and quantitative characters

Results of evaluation on qualitative characters presented on Table 1 show that morphological characters are almost the same in leaves, flowers, and fruits. However, there was a somewhat difference in the color series number, in which Udeng has a different color and leaf shape from other four varieties. Meanwhile, Ahang Adro shows differences in the color of young fruit and ripe fruit compared to other four cultivars.

Evaluation on quantitative characters (Table 2), shows differences among varieties on leaf area, flower size, fruit weight, fruit size, dichotomous height, plant height, and plant productivity. Observation on the adaptability of the varieties to the lowland agroclimate shows an average of good adaptability and resistance to environmental stress. This can be seen from the average Leaf Relative Water Content (LRWC) value which exceeds 60%, even in Telun Berasap and Ahang Adro (both are Kerinci local) the average LRWC value is higher than Awe and Udeng (national superior cultivars from Aceh). Furthermore, the resistance of local varieties from Kerinci against dominant pest attacks (leaf curl and fruit rot) also shows an average of mild to moderate (10-30%). This indicates that these local varieties from Kerinci can grow and produce optimally in different environmental conditions.

Table 1. Qualitative characters of five hot chili varieties from different provinces of Sumatra Island, Indonesia.

Varieties	Leaf Color	Leaf Shape	Leaf Edge	Color of Young Fruit	Color of Ripe Fruit
TB	139 dark yellowish green A	oval and tapered tip	choppy	moderate yellowish green 139 c	61 B strong purplish red
AA	NN 137 greyish olive green A	oval and tapered tip	choppy	dark yellowish green 139 a	61 A deep purplish red
KY	YG 147 moderate olive green A	oval and tapered tip	a little choppy	moderate yellowish green 139 c	61 B strong purplish red
AW	YG 147 moderate olive green A	oval and tapered tip	a little choppy	moderate yellowish green 139 b	61 A deep purplish red
UD	NN 137 greyish olive green A	lancet	a little choppy	moderate yellowish green 139 b	61 B strong purplish red

Varieties	Plant Habitus	Flower Petal Color	Flower Stem Color	Flower Crown Color	Flower Position
TB	upright	green	green	white	duck
AA	upright	dark green	dark green	white	duck
KY	upright	green	green	white	duck
AW	upright	green	green	white	duck
UD	upright	green	green	white	duck

Table 1 (continued)

Varieties	Cross Slice of Fruit	Fruit Tip Shape	Fruit Base Shape	Shape the Edges of the Fruit Petals	Fruit Position
TB	a little choppy	tapered	blunt	a bit jagged	hang
AA	a little choppy	tapered	blunt	a bit jagged	hang
KY	a little choppy	tapered	blunt	a bit jagged	hang
AW	a little choppy	curved & tapered	blunt	a bit jagged	hang
UD	a little choppy	curved & tapered	blunt	a bit jagged	hang

Varieties	Fruit Surface	Fruit Shape	Fruit Petals	Seed color	Seed Surface
TB	a bit wrinkled	elongated	close	yellow	rough
AA	a bit wrinkled	elongated	close	yellow	rough
KY	a bit wrinkled	elongated	close	yellow	rough
AW	a bit wrinkled	elongated	close	yellow	rough
UD	a bit wrinkled	elongated	close	yellow	rough

Note: TB = Loker Telun Berasap, AA = Ahang Adro, KY = Kopay, AW = Awe, UD = Udeng .

Table 2. Quantitative characters of five hot chili varieties from different provinces of Sumatra Island, Indonesia.

Quantitative Character	Varieties				
	TB	AA	KY	AW	UD
1. Leaves:					
a. Length (cm)	11.0 - 11.6	11,2 - 11,8	10,4 - 11,8	11,0 - 11,6	8,1 - 9,0
b. Width (cm)	4.0 - 4.2	3.9 – 4.1	3.3 – 4.1	3.4 – 4.0	1.0 – 1.2
2. Flowers:					
a. Flowering age (DAP)	29 - 32	33 - 35	29 - 32	30 - 33	26 - 29
b. Stalk length (cm)	1.9 - 2.8	1.3 - 2.4	2.2 - 3.1	1.6 - 2.0	1.3 - 2.0
c. Stalk diameter (mm)	7.0 - 11.4	6.5 - 9.8	6.5 - 13.2	6.7 - 10.8	5.6 - 9.6
d. Crown diameter (mm)	11.1 - 17.3	14.3 - 17.5	13.9 - 21.3	13.2 - 23.7	11.8 - 16.2
3. Fruit:					
a. Age of fruiting (DAP)	36 - 40	38 - 40	35 - 40	37 - 40	33 - 35
b. Harvesting age (DAP)	60 - 70	65 - 75	65 - 70	67 - 70	60 - 65
c. Weight per fruit (g)	4.48 - 5.29	5.23 - 6.15	6.29 - 10.17	6.70 - 8.09	5.29 - 6.31
d. Fruit length (cm)	17.4 - 18.7	13.0 - 15.4	21.2 - 24.3	16.8 - 20.3	17.0 - 22.0
e. Fruit diameter (mm)	5 - 8	6 - 8	6 - 7	6 - 8	6 - 7
f. Fruit stalk length (cm)	4.2 -7.3	5.0 - 6.2	4.3 - 5.9	4.0 - 5.6	4.8 - 5.4
g. Stalk diameter (mm)	5 - 6	7 - 8	7 - 9	5 - 8	5 - 8
4. Seed diameter (mm)	3 - 4 mm	3 - 4 mm	3 - 4 mm	3 - 4 mm	2 - 3 mm
5. Stem:					
a. Plant height (cm)	71.6 - 98.7	74.3 - 94.6	74.5 - 95.8	68.7 - 94.8	84.6 - 105.8
b Dichotomous height (cm)	24.3 - 27.7	20.8 - 23.2	21.0 - 26.3	20.2 - 26.7	26.5 - 30.7
c. Stem diameter (mm)	12.0 - 16.4	11.5 - 14.8	11.5 - 18.2	11.7 - 15.8	10.6 - 14.6
6. Plant productivity (ton ha^{-1}).	25.80	30.45	22.64	24.18	20.87
7. Plant Adaptability					
a. LRWC Value (%)	69.29 - 76.68	66.06 - 75.56	71.33 - 80.59	62.03 - 85.82	53.16 - 74.19
b. Fruit Rot (%)	20.6 - 28.2	18.4 - 20.7	45.6 - 59.8	5.6 - 7.2	19.8 - 21.4
c. Leaf Curling (%)	11 - 22	11 - 33	56 - 78	0	0 - 11

Note: DAP = Days After Planting, TB = Loker Telun Berasap, AA = Ahang Adro, KY = Kopay, AW = Awe, UD = Udeng.

Analysis of measurable characters was carried by developing cluster analysis using Minitab statistical software [15] for 21 measurable characters revealed that 3 of the 5 cultivars were closely related for the characters under study. Out of visually assessed descriptors studied, 11 were found to be monomorphic, the rest were polymorphic. These visually characters when grouped together fell into 2 distinctive groups. Data also shows that Awe and Udeng were uniform for all the seven measurable characteristics. However, Telun Berasap and Kopay remained distinct.

The relationships among the five chili cultivars was determined by a cluster analysis (grouping analysis), and cultivars were grouped based on their characteristic similarity. The cluster analysis resulted in the grouping as indicated in Figure 1.

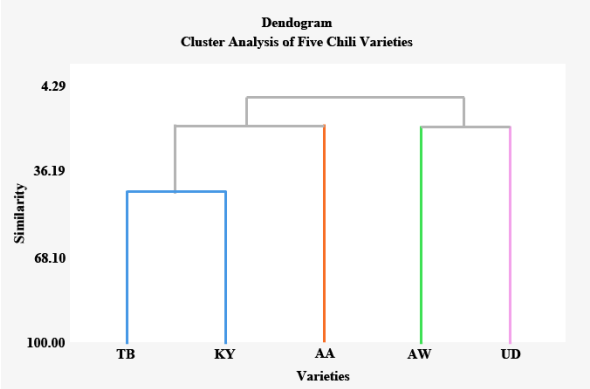

Figure 1. Dendogram analysis showing phylogenetic diversity of 5 chili cultivars identified by RAPD markers (TB = Loker Telun Berasap, AA = Ahang Adro, KY = Kopay, AW = Awe, UD = Udeng).

Genetic variability

Results of DNA aplification showed that four out of five primers had capability to produce polymorphic bands of chili, especially OPA 3, OPA 13, RAPD 2 and RAPD 5. Polymorphism was showed in Telun berasap and Ahang Adro samples. However, with OPA 2 primer, samples from the five chili varieties produced the same banding pattern (monomorphic). In OPA 3, OPA 13 and RAPD 2, Telun Berasap and Ahang Adro has polymorphism in the 0.6 kb band, while in RAPD 5 primer, the polymorphism was in the 0.25 kb band.

DISCUSSION

Evaluation on morphological variability

A detailed morphological, geographical and molecular characterization is necessary to support chili breeding and conservation [10]. Morphological characterization is a method of describing plant morphological characters based on the rubric issued by the International Plant Genetic Resources Institute [12], to provide information about a particular germplasm. In this way, genotypes can be differentiated and made more useful for various purposes.

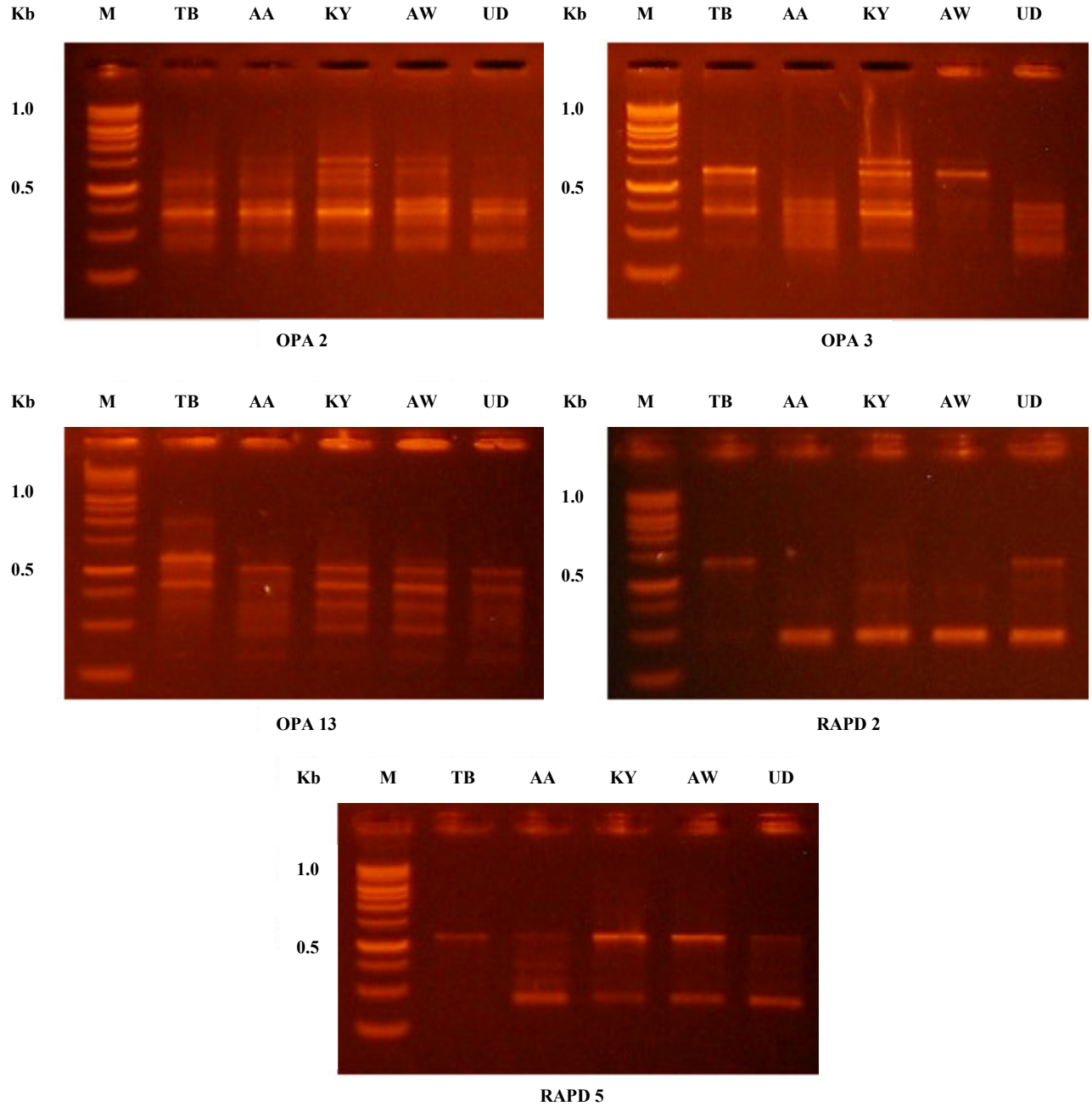

Figure 2. Band pattern obtained from RAPD markers for five primers in five chili cultivars (TB = Loker Telun Berasap, AA = Ahang Adro, KY = Kopay, AW = Awe, UD = Udeng). M = 1 kb DNA ladder.

Our study revealed that morphological characters among five tested chili varieties were almost the same in leaves, flowers, and fruits, except in color series number which showed a slight difference as demonstrated by Udeng and Ahang Adro varieties (Table 1). Differences on quantitative characters were detected on leaf area, flower size, fruit weight, fruit size, dichotomous height, plant height, and plant productivity (Table 2). The diversity in all evaluated characters confirmed Pickersgill's findings [19] who suggested that there was a large genetic variation in *Capsicum* genus. Further, Sinha and Mishra [31] suggested the important of qualitative over quantitative characters to identify a particular plant variety because they are genetically controlled and less dependent to environment.

Leaf area of Telun Berasap and Ahang Adro is relatively the same, but larger than other three cultivars. Leaf area is an important morphological character related to the photosynthetic ability of plants, since it is directly related to the amount of light that can be captured and utilized by plants. Leaf area is also a determinant factor in biomass accumulation, transpiration, and energy transfer by crop canopies. Therefore, leaf surface area as morphological predictor is important in understanding how different plant species have different photosynthetic capacities.

Harvest age or numbers of days from seed sowing until the first fruit harvest remained the same among five cultivars which is between 60 - 70 dayas after planting. Slight different was found in Udengan which was 60 - 65 dayas after planting.These is an important attribute to monitor the time of seed development, because this period influences directly some traits such as physiological quality and seed viability [14]. All cultivars showed different harvest times, indicating variability in reproductive cycle. Harvest age is an important character in the development of new chili varieties, allowing farmers to have wider options to choose cultivars with shorter or longer reproductive cycles.

Morphological characters such as fruit diameter, number of fruits per plant and plant height are factors that influence plant yields [2]. Fruit characteristics varied based on each genotype. Fruit stalks that are too tough will make harvesting difficult. In general, chili breeding is designed to obtain a moderate level of fruit stalk toughness to facilitate harvest. Breeding is also directed at other characteristics such as plant productivity, architecture, and resistance to pests and diseases [13].

Plant plant size indicated by plant height, dichotomous height and stem diameter are important traits because plant size affects directly their indication of cultivation as pot plants or garden or field crops. In field cultivation, size of plant guides the planning of distance among plants, thus affected the number of plants per growing area. There was variation among the size of evaluated cultivars. Similar condition was also observed in other studies in *Capsicum* [30, 33]. Furthermore, Pedó *et al.* [18] suggested that plant size, especially canopy diameter is an important attribute to photosynthetic rate, influencing indirectly in plant production.

Evaluation on genetic variability

The RAPD marker is an effective complementary technique in exploiting the germplasm diversity to boost agricultural production, provide food and nutrition in sustainable way, as well as to document the release of new varieties [1, 16]. The technique was developed by Williams *et al.* [36] and by Welsh and McClelland [35]. In chili, the technique was proven to be effective to test the presence of genetic diversity among varieties as reported by Purnomo and Ferniah [21] who tested the genetic diversity between Habanero (*Capsicum chinense*) and Bird's Eye chili (*Capsicum frutescens*) using Primer OPA 8. Furthermore, using 16 random primers, Rosmaina *et al.* [25] employed RAPD markers to test the genetic diversity of six genotypes of chili treated with gamma irradiation and control plants.

The results of the analysis using the RAPD technique for five primers in Figure 2 shows that the Telun Berasap (Kerinci local) and Ahang Adro (Kerinci local) are not the same as the three other varieties, namely Kopay (from West Sumatra), Awe (from Aceh), and Udeng (from Aceh). Based on the amplification results, it was also found that the DNA samples of Telun Berasap and Ahang Adro were genetically different from the other three varieties.

From Figure 2 it can be seen that the results of the analysis using the RAPD technique for five primers show that the Telun Berasap (Kerinci local) and Ahang Adro (Kerinci local) are not the same as the three other varieties, namely Kopay (from West Sumatra), Awe (from Aceh), and Udeng (from Aceh). Based on the amplification results, it was also found that the DNA samples of Telun Berasap and Ahang Adro were genetically different from the three varieties.

The results indicated that the value of genomic DNA extracts from the 5 chili varieties (Loker Telun Berasap, Ahang Adro, Kopay, Awe and Udeng) were in the range of 1.8 – 2.0. This value indicates that the sample DNA meets the requirements for purity for molecular analysis [4]. If the value is below 1.8, the extracted DNA still contains polyphenol contamination. On the other hand, if the value is above the maximum limit (> 2.0) then the resulting DNA is contaminated with protein, phenol, or other compounds and RNA. RNA contamination in DNA extraction will result in the appearance of many and unclear RNA bands. According to Wang *et al.* [34], DNA is said to be of good quality if the DNA extract is free from contamination such as polyphenols, proteins, and polysaccharides.

The results of genetic analysis using RAPD molecular markers in the form of screening primers showed polymorphisms on chili DNA banding pattern. Further, five primers (OPA 2, OPA 3, OPA 13, RAPD 2 and RAPD 5) were used in the genetic diversity test of the five chili varieties. The test results showed the

spread of DNA band polymorphisms as presented in Figure 2. In the OPA 2 primer, the five varieties showed the same banding pattern (monomorphic), while with other four primers polymorphism was found in Telun Berasap and Ahang Adro. These monomorphic band was thought to be part of genes that encode for the same character in all samples.

Polymorphism occurs as a result of changes in nucleotide bases which result in changes to the primer binding site within the amplified region [36]. Further, Poerba and Ahmad [20] suggested that the differences in polymorphism could be caused by genetic variation that exist among the different accessions. According to Roldán-Ruiz *et al.* [24], the appearance of high polymorphic bands on PCR amplification indicated high genetic diversity exists within the samples studied. Thus, our study provides evidence which indicates that 5 chili cultivars examined have genetic diversity.

Cluster analysis

The relationship among the five tested varieties was determined by a cluster analysis to separate them into groups based on the similarities in their characteristics. Data on quantitative and qualitative characters were analysed by a grouping analysis program using Minitab-18 (MTB-18) [15] to determine their phylogenetic relationships based on morphological similarity. The result of the grouping analysis showed that Telun Berasap and Ahang Adro (Jambi local varieties) were in the same cluster as Kopay (West Sumatra local variety) but in different cluster and show different morphological characters from Awe and Udeng (from Aceh). In addition, Telun Berasap and Ahang Adro are adaptable to different agroclimate conditions as indicated by higher value of LRWC (Table 1). Therefore, they have the potential to be proposed as important sources of superior hot chili germplasm from Jambi Province and to be continued to the stage of "Variety Release".

It was concluded that knowledge of polymorphisms of hot chili varieties from Sumatra Island, Indonesia, is of great importance for the source of genetic variability for breeding purposes. Five cultivars (Loker Telun Berasap, Ahang Adro, Kopay, Awe, and Udeng) have diversity in their qualitative and quantitative characters. Based on RAPD marker and cluster analysis, Loker Telun Berasap and Ahang Adro showed a considerable amount of diferences from other three varieties. These two varieties, therefore, are proposed be listed as elite hot chili varieties and can proceed to the stage of the release of new national superior hot chili varieties.

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