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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF LOCAL CHILI ACCESSIONS IN NORTH SUMATRA, INDONESIA

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SUMMARY

Red chili (*Capsicum annuum* L.) is a crucial vegetable crop in North Sumatra, Indonesia, facing challenges of inadequate seed quality. The seed origin also influences the quality and subsequent development of the plant. This study aimed to characterize local red chili accessions. The latest research consisted of two phases. The first phase involved morphological identification (February to May 2023) in Lubuk Cuik Village, North Sumatra, Indonesia. The second phase probed molecular characterization, conducted from March to September 2023. Morphological identification followed IPGRI (International Plant Genetic Resources Institute) descriptors, while molecular characterization employed simple sequence repeat (SSR) markers (CAMS-390, ASSR-281, and EPMS-335). The results demonstrated diverse morphological traits among the local chili accessions. Several morphological characters obtained could be useful as a source of genetic diversity. The SSR analysis revealed monomorphic bands with CAMS-390 (547–569 bp), ASSR-281 (731–758 bp), and EPMS-335 (587–625 bp). Importantly, all accessions exhibited resistance to anthracnose, with five genotypes also displaying resistance to fusarium. The dendrogram showed a similarity coefficient of 0.4 for all chili genotypes analyzed and divided into two groups. Based on phylogenetic analysis, a kinship relationship existed between local cultivars and hybrid varieties of one group. This research contributes valuable insights into the genetic diversity and disease resistance of local chili varieties, vital for future breeding and conservation efforts.

Keywords: Red chili (*C. annuum* L.), local chili, accessions, genetic linkage, phylogenetic analysis, morphological and molecular markers

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Key findings: Characterization is the initial stage to obtain good quality red chili (*C. annuum* L.) cultivars through plant breeding. The genotype's success is partly determining the availability of gene sources contained in germplasm.

INTRODUCTION

Red chili (*Capsicum annuum* L.) is a crucial horticultural crop, cultivated commercially due to its comprehensive nutritional content and high economic value. It has wide utilization in household consumption and the food industry (Nurlenawati and Nimih, 2010). According to Marliah *et al.* (2011), red chilies provide color and taste that can arouse appetite, contain numerous vitamins, which can also serve as medicines, food mixtures, and for livestock. In Batu Bara Regency, Indonesia, red chili production was stable at 111,446 tons in 2019. It decreased to 101,956 tons in 2020, but increased again to 156,547 tons in 2021 (Central Bureau of Statistics, 2022).

The disparity between current productivity and the genetic potential of chili genotypes suggests several limiting factors, including inadequate knowledge of advanced farming technologies, low adoption levels, limited capital, and crop failure risks due to pests and diseases in red chili, lettuce, and onion (Miskiyah and Munars, 2009). One of the efforts to overcome these problems and increase the red chili productivity is to use quality seeds of superior cultivars (Syukur *et al.*, 2010). The success of cultivars results partly from the availability of gene sources contained in germplasm. Genetic variability found in the germplasm is a source of genes with a strategic role in the improvement of genotypes. However, to obtain the necessary gene sources in cultivars, characterization of the germplasm is necessary for optimal use (Mejaya *et al.*, 2010).

In obtaining the diverse genetic resources, characterization is the initial step to acquire good quality chili cultivars. Characterization depends on easily observed characters, which are inherited, and mostly without influences from environmental factors. The recording of the expression of these qualitative features are quite difficult; therefore, morphological characterization is

vital. Morphological characteristics are considerably insufficient to find an evident position, therefore, requiring other techniques as a complement to evaluate the kinship. However, morphological characterization provides the basic information that helps recognize the superior traits and their diversity levels (Santos *et al.*, 2011).

In chili, the characteristics of stamen and pistil color, growth type, unripe and ripe fruit color, and the shape of chili fruit tip are the qualitative attributes (Widyawati *et al.*, 2014). For genotypes, identifying the yield-contributing traits, including qualitative ones, plays an essential role in producing cultivars with better adaptability to existing environmental conditions (Ezatollah *et al.*, 2011). In North Sumatra, Indonesia, especially Batu Bara, the cultivation of various local red chili peppers is due to high productivity, good quality, long life, and more favorable with consumers. The farming community hopes to achieve higher production; however, the optimal production is difficult to obtain due to pests and diseases. Therefore, it is necessary to identify the desirable morphological characteristics and choose such traits, which are beneficial as selection criteria to enhance the chili fruit yield.

Chili is a plant that is susceptible to different diseases. The chili cultivation carried out by farmers receives complaints of the crop often attacked by diseases and pests in Batu Bara Regency, Indonesia. Therefore, research on chili plants related to their adaptation level, disease resistance, quality improvement, and increased production need earnest attention. Some farmers plant local or 'kopekan' chili cultivars, obtaining seeds from previous crops or fellow farmers. The origin of these seeds is often uncertain, as it is unclear whether they are native to the area or introduced from elsewhere. Molecular analysis can help analyze the chili genotype based on their genetic background. This method can determine the genetic variations in the germplasm and

genetic relationship between genotypes and also ensure which can be better in developing the chili cultivars with superior properties.

Molecular analysis is crucial in the genetic information of chili genotypes. While substantial research has progressed on morphology, anatomy, physiology, biochemistry, and post-harvest handling, genetic studies remain limited. The simple sequence repeats (SSR) markers are the mostly used technique in plant breeding. The SSRs are repetitive sequences of DNA that represent a significant part of the eukaryotic genome and can provide the genetic markers, which can differentiate the genotypes. The SSR markers are relatively fast, simple, polymorphic, codominant, and stable (Swapna and Srivastava, 2012). Based on the above discussion, this study aims to identify both morphological and molecular markers in local red chili accessions in North Sumatra, Indonesia.

MATERIALS AND METHODS

Study site, experimental material, and procedure

The conduct of this research had two phases. The first phase involved morphological identification, conducted from February to May 2023 in Lubuk Cuiik Village, Lima Puluh Pesisir District, Batu Bara Regency, North Sumatra, Indonesia. The second examined molecular characterization, held from March to September 2023 at the Laboratory of Biotechnology, Faculty of Agriculture, University of Sumatera Utara, Indonesia.

The materials used for morphological identification included seven red chili accessions (Table 1) owned by the farming community of Lubuk Cuiik Village, Batu Bara Regency, as identified and supplemented with secondary data from the Central Bureau of Statistics, Indonesia. For molecular characterization, the chili material used was the six chili genotypes, consisting of local cultivars (Lokal Hijau, Lokal Putih, and Indrapura),

hybrids (Djitu and Laju), and the check cultivar (Akar Variety), with resistance to anthracnose and fusarium for comparison. The chili genotype's young leaves at 3–4 weeks of age underwent analysis, with specific primers related to the fusarium pathogen (ASSR-281), Anthracnose (CAMS-390), genetic relationship (EPMS-335), and other materials.

Morphological identification

The conduct of morphological identification used a survey method. Data collection on red chili genotypes relied on the descriptor guidebook of the International Plant Genetic Resources Institute (IPGRI) for qualitative and quantitative characters. Primary data directly came from respondents in the field through observations and questionnaire results. The additional secondary data needed were in the form of descriptions, numerical data, and maps regarding the state research area to support the primary data obtained from various sources, including literature studies and other related agencies. Sampling occurred using a purposive sampling method (intentionally) based on the information from the local farming community and some other considerations, especially about the plant's age and its physical conditions. Observations materialized by visiting each chili field owned by different farmers in several villages as sample location points. Direct interviews with the farming community employed a questionnaire. The observed parameters consisted of morphological traits, such as, stem, leaf, fruit, flower, and seed.

Molecular characterization

The process for identifying chili accessions related to genetic kinship, using SSR markers, involved six important stages. These are the DNA isolation from chili leaves, qualitative and quantitative tests of isolated DNA, the PCR simple sequence repeat (SSR), 1.5% agarose electrophoresis, analysis of SSR markers, determination of base pair size (allele length), and genetic relationship analysis.

Data analysis

Determination of PCR band size

The determination of PCR band size continued using the UVITEC Cambridge FireReader, with a 100 bp DNA ladder used as the standard. Processing the electrophoresis images detected band presence, with assessments based on the marker values entered.

Genetic linkage analysis

For the determination of genetic kinship, the resulting DNA profile reached translating into binary data: if a DNA band exists (assigning a value of 1), and if no DNA band (assigning a value of 0). Then, the binary data's processing used the Multivariate Statistical Package (MVSP ver. 3.2) software (Kovach, 1989). The genetic dissimilarity analysis proceeded with a descriptive analysis of diversity, i.e., Unweighted Pair Group Method Arithmetic (UPGMA) to obtain an overview of the kinship among the chili accessions.

RESULTS AND DISCUSSION

Morphological identification

Farmers typically purchase certified hybrid seeds of chili (*Capsicum annum* L.) varieties, i.e., Djitu, Laju, and CMK Tavi, from agricultural stores due to their large fruit size and high productivity. However, some farmers plant local or 'kopekan' chili cultivars, obtaining seeds from previous crops or fellow farmers. The origin of these seeds is often uncertain, as it is unclear whether they are native to the area or introduced from elsewhere. According to survey reports, the local chili genotypes were also comparable to chili hybrids; however, they still have deficiencies in several plant morphological characteristics.

Among the 23 observed qualitative characters, four were consistent across all chili accessions: shape of the stem, fruit, fruit base, and fruit tip (Tables 1 and 2; Figure 1). The 19 other qualitative features have variations either across the entire chili accessions or only

in some landraces. Variations and similarities in phenotypic traits incur influences from both the genetic makeup and environmental conditions, such as, temperature, humidity, water availability, altitude, and land slope, as well as, farming practices like fertilization. Syukur *et al.* (2010) also reported the chili genotypes' phenotypic appearance showed distinction from genetic factors, the environment, and their interactions, which directly affected the qualitative and quantitative characteristics of the plants. Genetic factors indicate the inheritance from chili parental genotypes to the offspring, while environmental factors can be the manipulated conditions for the plants to grow as optimally as possible.

In Lubuk Cuik Village, the chili local cultivars were the A1P1S1-A1P1S3, which are the Lokal Hijau or local green, and the genotype A1P2S4-A1P2S6 as the Lokal putih or local white. The Lokal Putih genotype was visible with taller plants than the chili Lokal Hijau genotype. The Lokal Putih genotype had characteristics of a brighter color of ripe fruit, with larger fruit size and weight. However, at the younger stage, the chili Lokal Hijau genotype is greener than the Lokal Putih genotype (whitish-green). The green chilies' demand is always rising versus the demand for Lokal Putih genotype. Based on survey interviews, both Lokal Hijau and Lokal Putih genotypes occurred resistant to the diseases in Lubuk Cuik Village, Indonesia.

The chili national cultivars Djitu hybrid, CMK Tavi, and Laju also gained scrutiny. Kartikasari *et al.* (2016) stated the phenotypic diversity value describes the state of character diversity visually. A low phenotypic diversity value indicates the character being tested has a uniform phenotypic appearance and a high phenotypic value, and the characters have a diverse phenotypic appearance. In the studied chili genotypes, the local chili cultivars also bore observations, where origins were also unknown. Among the local cultivars, the chili genotypes like Lokal Hijau and Lokal Putih have not received distinctive local names, and only has the common name of Kopekan chili

Table 1. Phenotypic appearance of the chili genotypes in Lubuk Cuik Village, Batubara Regency, North Sumatra, Indonesia.

Phenotypic Appearance	Accessions						
	Lokal Hijau (A1P1S1)	Lokal Hijau (A1P1S2)	Lokal Hijau (A1P1S3)	Lokal Putih (A1P2S4)	Lokal Putih (A1P2S5)	Lokal Putih (A1P2S6)	Djitu (A1P2S7)
Traits	Lokal Hijau (A1P1S1)	Lokal Hijau (A1P1S2)	Lokal Hijau (A1P1S3)	Lokal Putih (A1P2S4)	Lokal Putih (A1P2S5)	Lokal Putih (A1P2S6)	Djitu (A1P2S7)
Stem color	Green with purple	Green with purple	Green with purple	Green with purple	Green with purple	Green purple line	Green
Rod Shape	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical
Bristles on the stem	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Growth type	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
Leaf color	Dark green	Dark green	Dark green	Dark green	Dark green	Dark green	Dark green
Leaf shape	Ovate	Ovate	Ovate	Ovate	Lanceolate	Lanceolate	Lanceolate
Bristles on leaves	Medium	Medium	Rare	Medium	Medium	Rare	Medium
Flower position	Pendant	Pendant	Pendant	Pendant	Pendant	Pendant	Pendant
Crown color	White	White	White	White	White	White	White purple edge
Anther color	Purple	Purple	Purple	Purple	Purple	Purple	Purplish blue
Stalk color	White	White	White	White	White	White	Purple
Stigmatized position	Same height	Same height	Same height	Higher	Higher	Higher	Higher
Node color	Deep purple	Deep purple	Deep purple	Purple	Purple	Purple	Purple
Crown shape	Play	Play	Play	Play	Play	Play	Play
Petal type shape	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
Young fruit color	Green	Green	Green	Whitish green	Whitish green	Whitish green	Dark green
Ripe fruit color	Red	Bright red	Red	Bright red	Bright red	Red	Red
Fruit shape	Elongate	Elongate	Elongate	Elongate	Elongate	Elongate	Elongate

Table 2. Phenotypic appearance of the chili genotypes in Lubuk Cuik Village, Batubara Regency, North Sumatra, Indonesia.

Phenotypic Appearance	Accessions						
	Lokal Hijau (A1P1S1)	Lokal Hijau (A1P1S2)	Lokal Hijau (A1P1S3)	Lokal Putih (A1P2S4)	Lokal Putih (A1P2S5)	Lokal Putih (A1P2S6)	Djitu (A1P2S7)
Traits	Lokal Hijau (A1P1S1)	Lokal Hijau (A1P1S2)	Lokal Hijau (A1P1S3)	Lokal Putih (A1P2S4)	Lokal Putih (A1P2S5)	Lokal Putih (A1P2S6)	Djitu (A1P2S7)
Shape of fruit base	Obtuse	Obtuse	Obtuse	Obtuse	Obtuse	Obtuse	Obtuse
Fruit tip shape	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed
Fruit cross section	Slightly bumpy	Slightly bumpy	Slightly bumpy	Slightly bumpy	Slightly bumpy	Slightly bumpy	Slightly bumpy
Fruit skin surface	Half-wrinkled	Half-wrinkled	Smooth	Half-wrinkled	Half-wrinkled	Half-wrinkled	Half-wrinkled
Seed color	Yellowish	Yellowish	Yellowish	Yellowish	Yellowish	Yellowish	Yellowish
Seed surface texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Plant height (cm)	93.4	101.3	91.2	97.9	99.5	91.4	101.3
Number of seeds per fruit	48	51	44	54	53	51	55
Rod diameter (mm)	12.29	11.81	10.33	10.91	12.1	11.5	11.43
Fruit length (cm)	16.2	15.6	14.9	16.1	16.9	17.1	16.7
Canopy width (cm)	78.3	81.4	74.1	79.4	72.6	75.2	84.1
Leaf length (cm)	9.2	9.5	9.7	8.4	9.1	9.2	8.8
Leaf width (cm)	3.3	3.6	3.2	3.1	3.1	3.2	3.1
Fruit width (cm)	10.25	9.93	11.01	10.26	10.09	10.17	10.21
Weight per fruit (g)	7.33	7.09	7.33	7.43	7.23	6.97	7.48
Number of fruits per plant	2	1	1	2	1	2	3
Harvest age (DAP)	88 HST	88 HST	88 HST	88 HST	88 HST	88 HST	80 HST

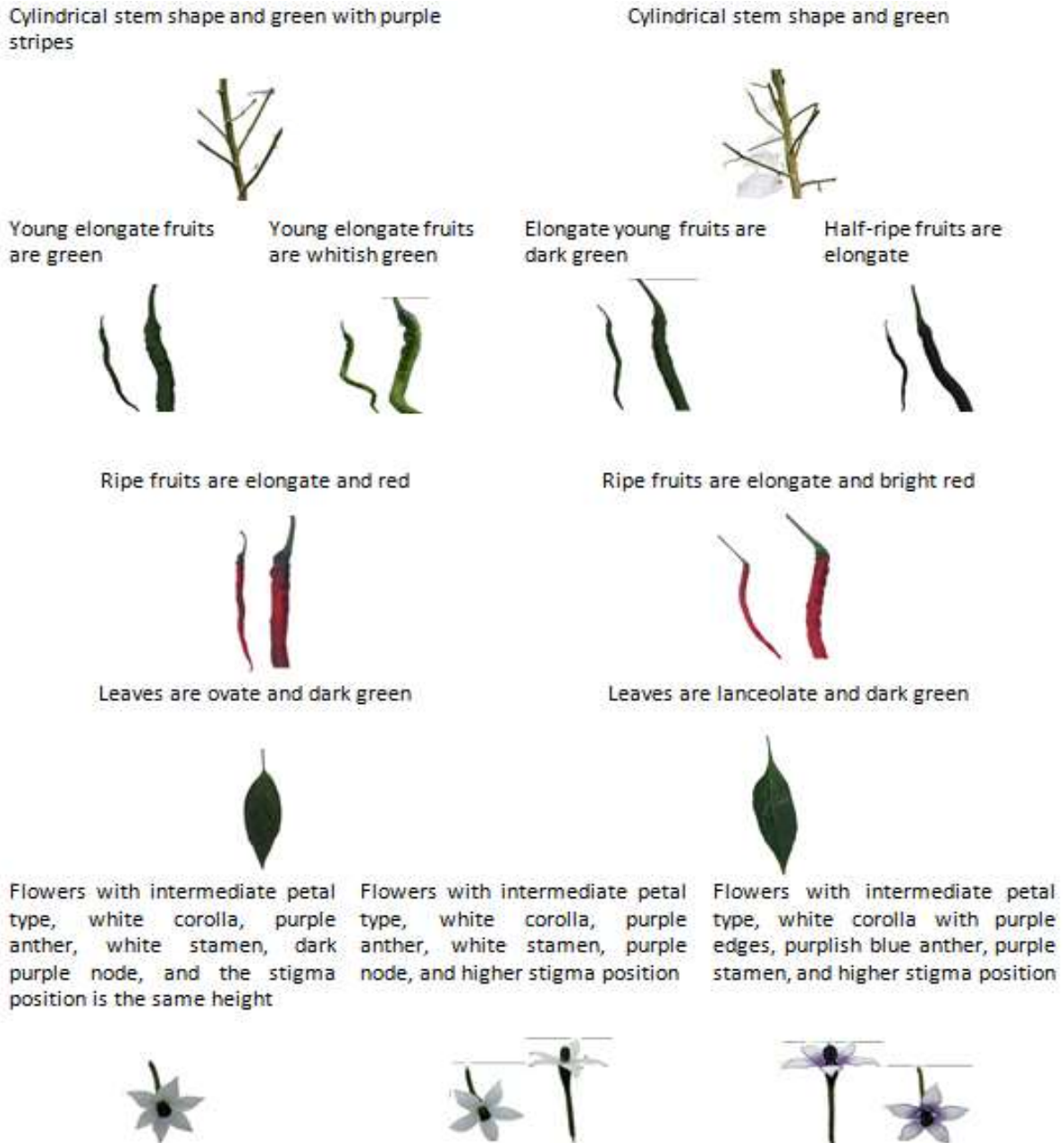


Figure 1. Phenotypic appearance of red chili genotypes in Lubuk Cuiik Village, Indonesia.

(meaning using the seeds from the previous crop). Based on the morphological characterization, several morphological qualities obtained could be useful as a source of genetic diversity. The important characteristics necessary in the development of superior chili cultivars for highest production were fruit weight, length, and width, number of seeds per fruit, and maturity.

Apart from quantitative features, potential qualitative characteristics include fruit surface texture and ripe fruit color. The surface texture of the chili fruit has quite a smooth texture, which are favorable with consumers, including the Lokal Hijau accession A1P2S4. The ripe fruit color can be effective as a criterion, as the color of ripe chili fruit is bright red. Some farmers said the local chili

genotypes are disease-resistant. However, on average, the farmers said local chilies were more resistant than hybrid chilies. After harvesting, the local chili genotypes appeared stronger than hybrid chilies, which immediately showed the physiological and morphological changes just after harvest, where the leaves quickly experienced chlorosis. Similarly, local chilies have the same fruit length and width as in the hybrid chilies if cared properly, including Lokal Putih chili genotypes.

However, local chili genotypes have some shortcomings needing attention. These include taking long time in the intermediate phase for the fruit to turn red and require more care. Moreover, some farmers noticed chili fruits acquire pockmarks easily, do not survive unsuitable climate, and have a longer harvest time. Apart from that, the local chilies' fruits become whitish green at the younger stage, which is also unattractive with the end users in the market.

Molecular characterization

DNA amplification of red chili

DNA amplification of six chili cultivars (Djitu, Laju, Lokal Hijau, Lokal Putih, Akar, and Indrapura) ensued to identify resistance to fusarium wilt and anthracnose for genetic relationship analysis using the primers ASSR-281, CAMS-390, and EMPS-335, respectively.

The amplified DNA sustained electrophoresis on a 1.5% agarose gel. However, one primer (ASSR-281) did not amplify the DNA (Table 3). Amplification of six chili cultivars used three SSR primers, and all the primers showed monomorphic bands. The observations on the number of DNA fragments and the percentage of monomorphic of each primer appear in Table 4.

The banding patterns produced by the three primers varied, with band sizes ranging from 547 to 758 bp (Table 4). Each primer produced one band, resulting in a total of three bands, all of which were monomorphic. Consequently, no polymorphic bands were evident, and the percentage of polymorphic bands was zero. The highest number of banding patterns emerged in all the three primers (CAMS-390, ASSR-281, and EPMS-335).

Primer CAMS-390 proved useful for analyzing anthracnose resistance and amplifying the DNAs of six chili accessions (Figure 2a). The banding pattern was homozygous, with one monomorphic band, ranging from 547 to 569 bp. The polymorphic bands' percentage was zero. Primer ASSR-281 was effective for analyzing the resistance to fusarium and amplifying the DNAs of five chili accessions, with one unamplified DNA, namely,

Table 3. Amplification results of the three SSR primers.

No.	Primers	Number of Amplified Grows	Number of Unamplified Germplasm	Unamplified Lineage Name
1	CAMS-390	6	-	-
2	ASSR-281	5	1	Akar variety
3	EPMS-335	6	-	-

Table 4. Polymorphic percentage.

No.	Primers	Band Size (bp)	Number of Band	Polymorphic Bands	Monomorphic Bands	Polymorphic (%)
1	CAMS- 390	547-569	1	0	1	0
2	ASSR-281	731-758	1	0	1	0
3	EPMS-335	587-625	1	0	1	0
Total			3	0	3	0
Average			1	0	1	0



Figure 2. Amplification pattern of SSR markers of red chili genotypes with primers a) CAMS – 390, b) ASSR – 281, c) EPMS – 335; M = *Marker Ladder* 100 bp, in Lubuk Cuik Village, Indonesia.

the Akar variety (Figure 2b). The banding pattern was homozygous, with one monomorphic band, and the band size was around 731–758 bp. Primer EPMS-335 was capable of analyzing the genetic relationship and amplifying the DNAs of six chili accessions (Figure 2c). The banding pattern was also homozygous, with one monomorphic band, ranging from 587 to 625 bp. The percentage of polymorphic bands was 0%.

DNA amplification of chili peppers showed relatedness to fusarium and anthracnose resistance, using three SSR primers, primer CAMS-390 (analyzing resistant to anthracnose), ASSR-281 (analyzing resistant to fusarium), and EPMS-335 (analyzing genetic relationship) in Lubuk Cuik village, Indonesia. The SSR primers used can amplify red chili DNA accessions and produce the monomorphic/polymorphic banding patterns, varied by different base pair sizes. The polymorphic banding pattern owned two or more alleles in one locus, and that was not evident in each primer, while the monomorphic bands were notable in each primer. This is because the SSR markers have codominant properties (which can distinguish homozygous alleles from heterozygous) and showed capable to accurately detect the polymorphic and monomorphic loci (Chawla, 2002; Varshney *et al.*, 2004).

Based on the results, each primer produces a different number of DNA bands (Table 4). The bands also emerged with varying base sizes and intensities. Differences in DNA band intensity sustained influences

from the distribution of primer attachment sites on the genome, the purity, and concentration of the genome in the reaction. The existence of different banding patterns based on the number and size of bands illustrates the manifestation of a very complex plant genome. The number of bands produced by each primer depends upon the distribution of homologous sites on the genome (Williams *et al.*, 2000).

Primers CAMS-390, ASSR-281, and EPMS-335 showed monomorphic banding patterns and no polymorphic bands. DNA samples that can produce bands indicate the DNA has sequences complementing with the primers. Therefore, not all DNA samples can produce bands at a particular locus. This difference can serve as an indication of genetic diversity both within and among the chili populations. The difference in DNA banding patterns also attained influences from differences in genomic DNA amplification results. The polymorphic DNA banding patterns' existence resulted from alterations in the arrangement of bases in each DNA sample.

Primers CAMS-390 and EPMS-335 can amplify all the red chili DNA, while the primer ASSR-281 can only amplify five red chili DNA samples (Table 3). The primer ASSR-281 has undergone repetitions for PCR optimization twice, but was unable to amplify the entire red chili genotypes. This may be due to some other factors, like the absence of suitable DNA sequences as primer attachment sites on the DNA template. Some experimental evidence showed a difference of just one base pair is

enough to cause a mismatch in the primer mold, which then prevents amplification. Ariani (2014) stated nucleotide bases will only attach to the target sequence. The primer CAMS-390 was capable to show amplification of six individual red chili genotypes tested with homozygous banding patterns, with one banding ranging from 547 to 569 bp. Minamiyama's *et al.* (2007) findings revealed using the primer CAMS-390 to analyze the resistant genes to anthracnose disease.

The primer ASSR-281 was capable to show the amplification of five individual red chili accessions tested with homozygous banding patterns, and revealed one band, ranging from 731 to 758 bp. The absence of polymorphic banding pattern was due to the absence of the formation of differences in the size and number of bands formed in each sample studied, which illustrated the existence of a very complex plant genome. The primer ASSR-281 has been used in previous studies to analyze the resistant genes to fusarium disease (Singh *et al.*, 2013).

Phylogenetic analysis

The phylogenetic analysis aimed to determine the primary separation of six red chili genotypes based on genetic similarity coefficients. The analysis of local red chilies from Lubuk Cuik village, compared with

hybrids and standard cultivars, is available in Figure 3. Phylogenetic analysis with UPGMA showed the coefficient of genetic similarity in all tested individuals ranges from 0.4 to 1.0. At a similarity coefficient of 0.4, the analyzed chili genotypes bore dividing into two groups. The first group consisted of two subgroups, namely, IA consisting of Djitu and Group IB consisting of the genotypes Indrapura, Laju, and Lokal Putih. Meanwhile, the second group comprised the chili genotypes Lokal Hijau and Akar.

The dendrogram showed the genetic similarity between the tested chili genotypes, ranging from 0.4–1 (40% similarity), and each red chili genotype attained grouping based on genetic similarity. The highest genetic distance is one, while the lowest genetic distance is 0.1 (attachment 10). Martono and Syfaruddin (2018) reported the lower genetic similarity coefficient value indicates the two genotypes have a close relationship, and these genotypes might be identical. Conversely, the higher the genetic similarity coefficient value, the genotypes have a distant kinship relationship.

At a similarity coefficient of 0.4, all the analyzed chili genotypes underwent division into two groups. From the UPGMA dendrogram, it reveals the Lokal Hijau cultivar (from Lubuk Cuik village) and the Akar variety (from West Sumatra), belonged to one group. Based on the interview results, the cultivation and

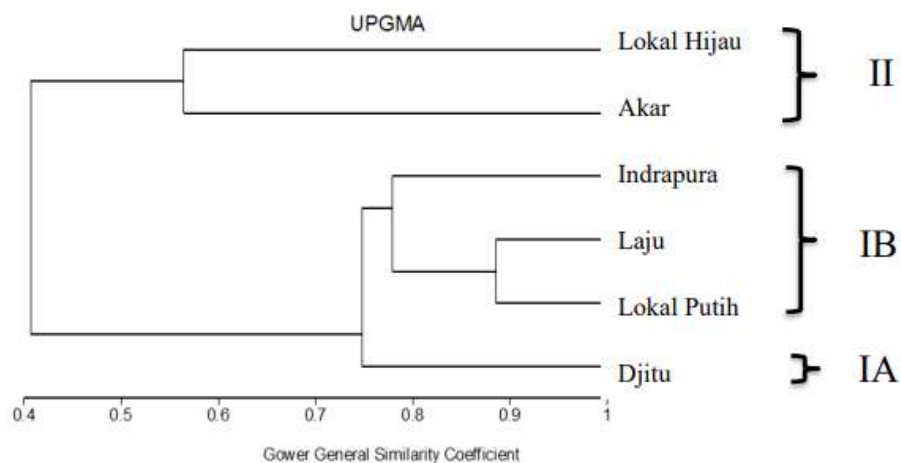


Figure 3. UPGMA phylogenetic similarity of red chili genotypes on Fusarium and Anthracnose resistance characters in Lubuk Cuik Village, Indonesia.

development of the local chili genotypes began in 2011 and 2012 in Lubuk Cuik village, Indonesia. The chili products mostly sold to the local areas comprised Batubara, Medan, Pekanbaru, Riau, West Sumatra, Dumai, and Batam. Based on this, one can assume the spread of Lokal Hijau chili cultivars in the Western Sumatra area, causing a genetic relationship between the Lokal Hijau cultivars from Lubuk Cuik village, and distribution to Western Sumatra with the Akar variety from Payakumbuh, Western Sumatra. Based on the decision of the Ministry of Agriculture of Indonesia (2022), the Akar variety is a local chili cultivar originating from the City of Payakumbuh, West Sumatra Province, Indonesia. The said chili cultivar development resulted through the selection and purification of the cultivars. Likewise, with the same grouping of Lokal Putih, Indrapura, and Laju varieties, one can assume the close relationship of the three genotypes.

Based on the results, six chili genotypes emerged with resistance to anthracnose. The same can be evident from the results of electrophoretic visualization of the CAMS-390 primers used to analyze the presence of resistant characters to anthracnose pathogens, showing DNA bands. The primer ASSR-281 used to analyze all the chili genotypes for the presence of resistant characters to fusarium pathogens showed only five chili genotypes with the DNA bands, while one sample (Akar variety) did not show the presence of DNA bands. This indicates the Akar variety does not contain genes with resistant traits against fusarium (Deka *et al.*, 2016; Amas *et al.*, 2023; Maulani *et al.*, 2023).

CONCLUSIONS

Lokal Hijau chili (*C. annuum* L.) has medium-sized fruit, is dark green at the younger stage, with branches characterized by a dark purplish color. The Lokal Putih chili genotype has a large fruit, almost equivalent to the hybrid variety Djitu. It also has a distinct characteristic of the fruit as whitish green at the younger stage. Lokal Hijau and Lokal Putih

genotypes take longer to ripen than the hybrid Djitu. The brown/black to red phase of the fruit takes quite longer, before making the first harvest. Monomorphic bands resulted from three primers, primer CAMS-390 with a DNA band length of 547–569 bp, primer ASSR-281 with a band length of 731–758 bp, and primer EPMS-335 with a band length of 587–625 bp. From the results, all the chili genotypes showed resistance to anthracnose, while for fusarium, only accessions revealed resistance, except the Akar variety. Based on the phylogenetic analysis, a kinship relationship existed between local cultivars and hybrid varieties of one group.

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