KnE Social Sciences

4th International Conferences in Social Sciences (4th ICONISS): Urban Innovation

KNE Publishing

Research Article

Genetic Diversity of Local Chili (*Capsicum annum* L.) from West Sumatra Indonesia Based on Inter Simple Sequences Repeat (ISSR) Markers

Tri Budiyanti[1*], Riska[1], Rinda Kirana[1], NL. Putu Indriyani[1], E. Darma Husada[2], Sri Wahyuni[3], Irfan Suliansyah[4], and Dini Hervani[4]

[1]Research Centre for Horticulture, National Research and Innovation Agency (BRIN), Bogor, Indonesia
[2]Research Centre for Applied Microbiology, National Research and Innovation Agency (BRIN), Bogor, Indonesia
[3]Research Center for Plants Conservation, Botanic Gardens and Forestry, National Research and Innovation Agency (BRIN), Bogor, Indonesia
[4]Department of Agronomy, Faculty of Agriculture, Universitas Andalas, Padang, Indonesia

ORCID
Tri Budiyanti: https://orcid.org/0000-0001-7630-0366

Corresponding Author: Tri Budiyanti; email: trib006@brin.go.id

Published: 2 October 2024

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the 4th ICONISS Conference Committee.

Abstract.
Capsicum annum L. is the most widely cultivated species due to its economic importance and health benefits. West Sumatra is one of the largest chili pepper producers in Indonesia, so it has a lot of local chili genetic resources. There is inadequate information on the genetic diversity of local chilies in West Sumatra. The purpose of this research was to analyze the genetic diversity of 23 genotypes of chili pepper from West Sumatra amplified with 10 markers of ISSR. The material used was leaves from 23 genotypes of West Sumatra's local chili which are collections of the Agriculture Faculty, Andalas University, Padang, West Sumatra. The results showed that ISSR markers (ISSR primer 12, ISSR UBC, and ISSR 3M) had produced 84 polymorphic DNA bands with a total polymorphic percentage of 61.45%. The dendrogram based on 10 ISSR markers showed that the 23 chili genotypes were retrieved into three main groups (I, II, III, IV) with genetic similarity coefficient values ranging from 0.67 to 0.97 or a diversity of features of 0.03–0.33 (30%). Group I consisted of 1 genotype, group II consisted of 3 genotypes, and group III consisted of 14 genotypes. Kopay chili (G20) has a large genetic distance compared to the other genotypes, while Lolai and Gero chilies have a very high similarity. This genetic diversity information can be used as a basis for generating crosses between genotypes in a breeding program. The farther the genetic distance will produce a high population diversity, the greater the chance of getting superior hybrids.

Keywords: *Capsicum annum*, ISSR, local chili, West Sumatra

OPEN ACCESS

1. Introduction

Capsicum spp. is a horticulture plant native to tropical and subtropical America, consisting of more than 30 species. The five main cultivated species are C. annum L., C. frutescens L., C. Chinense Jacq., C. baccatum L., and C. pubescens [1,2]. C. annum is the most widely cultivated commodity and is an economically important species due to its sweet and spicy flavor [2]. In Indonesia, C. annum is often called as red chili or curly chili. Chili is a frequently consumed commodity as it is a major source of provitamins A (carotene), E (α-tocopherol) and vitamin C (ascorbic acid). Red chilies contain carotenoids, compounds with anticarcinogenic and antioxidant properties. Both ripe and unripe fruits contain a high content of phenolic, especially flavonoids which are reported to have antioxidant and other bioactive properties [3,4].

Indonesia is the third largest producer of chili in the world after China and Turkey. The national production of red chilies in Indonesia in 2021 was 2747018.03 tons. Indonesia has a high diversity of red chilies, both from local chili populations and from breeding programs.

West Sumatera is a province with the highest consumption of red chilies in 2021, namely 0.59 kg/capita/month. The next largest province consumes chilli pepper is Bengkulu, namely 0.58 kg/capita/month, followed by Jambi 0.46 kg/capita/month, and Riau 0.37 kg/capita/month. West Sumatra is one of the national chili producer with production in 2020 reaching 166,245 tons [5]. This causes West Sumatera to potentially have a high genetic diversity of local chili peppers, but its advantages have not been utilized and highlighted. The results of exploration done by Suliansyah et al [5] have obtained several local chili genotypes from West Sumatra. Studies on the genetic information of local West Sumatran chilies are still very limited. Currently, few studies on the morphological characterization and yield test of several local West Sumatran chilies. To increase the commercial value and support the development of local West Sumatran chilies, the genetic information needs to be investigated.

The germplasm characterization is the initial stage of a variety assembling program in breeding [6]. Characterization is carried out to determine the superior traits of the germplasm in order to obtain potential varieties for further development. The characterization of chili varieties in Indonesia are commonly based on morphological characters thus far, which require an intensive observation and it is difficult to differentiate individually on a fairly close relationship [7]. Morphological characters or phenotypes are the result of interactions between genotype and environment. This makes it difficult to

distinguish whether a character is genetic or more influenced by environmental factors [8].

Application of molecular markers DNA in characterization is one solution to overcome the problem. The technique can mark certain gene directly thus it can help to select the desired traits and provide more precise results that are not influenced by the environment [9]. Utilization of technology in the form of molecular markers is a more efficient approach in analysing genetic diversity. The results of cluster analysis based on SSR markers show that there is a fairly high diversity and level of genetic similarity among the 27 chili genotypes used in this study, where the genotypes are divided into two main groups with a genetic similarity level of 74% [9].

The molecular markers tool that commonly used to study the genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology in various plants are inter simple sequence repeat (ISSR). The ISSR molecular marker that uses a single primer to target identical regions between microsatellites is the simple sequence repeat (SSR) region. ISSR primers consist of 8 repeating dinucleotide units (or 6 repeating trinucleotide units) and one or more nucleotide anchors which are designed to target the end of the microsatellite region and preventing dimerization of primer. Repetitive sequences in SSR that are not separated by ISSR will tend to experience self-annealing. ISSR primers produce polymorphisms whenever one of the genomes is missing a repeat sequence or when a deletion, insertion or translocation occurs that changes the distance between repeat sequences. Usually the dinucleotide sequence hooks at the 3' or 5' end showing high polymorphism [10,11].

Based on literature studies, it shows that research on the genetic diversity of local chilies from West Sumatra, Indonesia using ISSR markers has never been carried out. The purpose of the research activity was to analyse the genetic diversity of twenty-three chilli pepper genotypes from West Sumatera by using ten ISSR markers.

2. Material and Methods

2.1. Methods

The research was conducted at the Quality and Molecular Testing Laboratory, Agency for Standardization and Instrument of Tropical Fruit, Ministry of Agriculture. The chili plant material used was 23 local West Sumatran chili genotypes (Table 1). The sample

consisted of 23 genotypes from the collection of the Agriculture Faculty, Andalas University, Padang, West Sumatra, Indonesia.

2.2. DNA isolation

Total DNA isolation from leaf samples that had been stored in silica gel was extracted by using the Zymo Research Quick-DNA TM plant/seed MiniPrep Kit. Extraction begins by adding beta-mercaptoethanol into the lysis buffer up to 0.5% (v/v) final dilution, namely 250 µl per 50 ml or 500 µl per 100 ml. Then 150 mg of finely grinded leaves or seed samples were added to a ZR BashingBead^{TM} lysis tube (2.0 mm) and add 750 μl of BashingBead^{TM} Buffer into the tube and close tightly. The mixture was sealed in a bead beater provided with a 2 ml tube holder assembly and processed at maximum speed for $\geq$ 5 min. ZR BashingBead^{TM} lysis tubes were centrifuged in a microcentrifuge at $\geq$10,000 xg for 1 min. of 400 μl supernatants were transferred to the Zymo-Spin^{TM} III-F Filter and centrifuged for 1 minute at 8,000 xg, then the filter was removed. 1,200 μl of Genomic Lysis Buffer were added into the collection tube from the previous step and mixed well. 800 μl of the mixture in the collection tube was transferred to column II CR Zymo-Spin^{TM} and centrifuged at 10,000 xg for 1 min.

The flow from the collection tube is removed and the previous steps are repeated. 200 μl of pre-wash DNA were added onto column II CR Zymo-Spin and centrifuged at 10,000 xg for 1 minute. 500 μl gDNA was added to the Zymo-Spin^{TM} II CR column and centrifuged at 10,000 xg for 1 min.

The Zymo-Spin^{TM} II CR column was transferred to a clean 1.5 ml microcentrifuge tube and add 100 μl (minimum 50 μl) of elution buffer directly to the column matrix. Centrifuged at 10,000 xg for 30 seconds to elute DNA. The Zymo-Spin^{TM} III-HRC filter was placed in a clean collection tube and 600 μl of prep solution was added. Centrifuged at 8,000 xg for 3 minutes. The eluted DNA was transferred to a Zymo-Spin^{TM} III-HRC Spin Filter that had been prepared with a clean 1.5 ml microcentrifuge tube and centrifuged at exactly 16,000 xg for 3 minutes. The filtered DNA can be followed up with PCR and other downstream applications.

The activity began with the selection and optimization of 19 ISSR primers, then 11 primers were selected that had the best polymorphic bands and specific annealing temperatures for each primer. The PCR mix solution was made with a volume of 6 µl in a 0.2 ml tube with a PCR mix composition consisting of 3 µl KOD, 0.5 µl ddH2O, 1 µl ISSR primer (Table 2), and 1.5 µl DNA, then spindown. The PCR process is carried out

through 6 stages, namely pre-denaturation, denaturation, annealing, extension, final extension and storage. PCR reaction conditions were predenaturation at 98°C for 3 minutes, denaturation at 98°C for 10 seconds, annealing at 40-60°C for 3 seconds, and elongation at 68°C for 1 second, final elongation at 68° C for 7 minutes and storage at 4°C were carried out in a thermal cycler for 35 cycles. The list of ISSR primers used can be seen in Table 1. The PCR products were separated in 1% agarose gel dissolved in 80 ml TAE buffer using electrophoresis at 100 v for 30 min. The electrophoresed agarose gel was then visualized using Gel Doc Bio-Rad.

2.3. Data analysis

Molecular characterization data in the form of DNA bands/fragments were analysed using the NYSYSpc program. The results obtained are in the form of a dendrogram which shows the relationship between characters from DNA obtained through a series of analyses.

3. Results and Discussion

The genotypes used in this research were local chilies originating from several locations in West Sumatra (Table 1). Twenty-three of these genotypes are curly chili types grown by farmers in their original locations.

The results shows that the ten ISSR primers used could amplify the DNA and produce polymorphic fragments (See Figure 1 and Table 2). Each primer produces at least 4 to 13 fragments. Characterization of 23 local West Sumatran chili genotypes using 10 primers on ISSR resulted in a total of 84 bands of which 54 bands were considered polymorphic. The percentage of polymorphic loci was 61.45% indicating a rather high level of polymorphism. These results are higher than other studies [12]. This result is lower than the characterization of 16 chili rootstock genotypes using 15 primers resulting in a total of 136 different bands with an average score of 9.06 ability bands per primer of which 102 were considered polymorphic. The percentage of polymorphic loci was 83.3% indicating a higher level of polymorphism [13].

The highest polymorphism resulted from amplification using ISSR 12 primer, namely 100%, the lowest polymorphism was 16.67% when amplified using Primer 5 ISSR. ISSR marker can detect molecular characterization of P. retrofractum Vahl plants in Java. The ISSR primers used were twelve and produced 70 DNA bands. The dendrogram results

TABLE 1: Twenty-three genotypes of local chili collected from West Sumatra.

No	Genotype Name	Collecting Locations
1	Tali	Solok Selatan
2	Ateng Maninjau	Agam
3	Kampung Manangah	Solok Selatan
4	Randah	Agam
5	Sijunjung	Sijunjung
6	Paijan	Pasaman
7	Bonsai hijau Tanah Datar	Tanah Datar
8	Ateng Pasbar	Pasaman Barat
9	Kaput	Solok Selatan
10	Lontabar Payakumbuh	Limapuluh Kota
11	Keriting Bukitinggi	Bukitinggi
12	DM-2	Damasraya
13	Lokal Tanah Datar	Tanah Datar
14	Damasraya 1	Damasraya
15	Pasir Pasaman Timur	Pasaman
16	Kuhay	Padang Pariaman
17	Limersi	Tanah Datar
18	Lolai	Pasaman Barat
19	TDS	Tanah Datar
20	Kopay	Payakumbuh
21	Aka Solsel	Solok Selatan
22	Lokal Maninjau	Agam
23	Gero Tanah Datar	Tanah Datar
24	Lokal Pesel	Pesisir Selatan

show that the similarity coefficient is around 0.69-0.93. This result shows that genetic diversity based on ISSR markers from P. retrofractum Vahl is relatively low. To obtain a more comprehensive picture it is necessary to use more primers and samples so that a picture of the molecular character of P. retrofractum Vahl can be clearly observed.

The dendrogram based on 10 ISSR markers shows that at 0.78 genetic distance, 23 chili genotypes are split into four main groups (I, II, III and IV) with genetic similarity coefficient values ranging from 0.67-0.97 or there is a diversity of characteristics of 0.04-0.32 (27%). Group I consist of 1 genotype, group II consists of 3 genotypes, group III consists of 14 genotypes and group IV consists of 4 genotypes (see Figure 2).

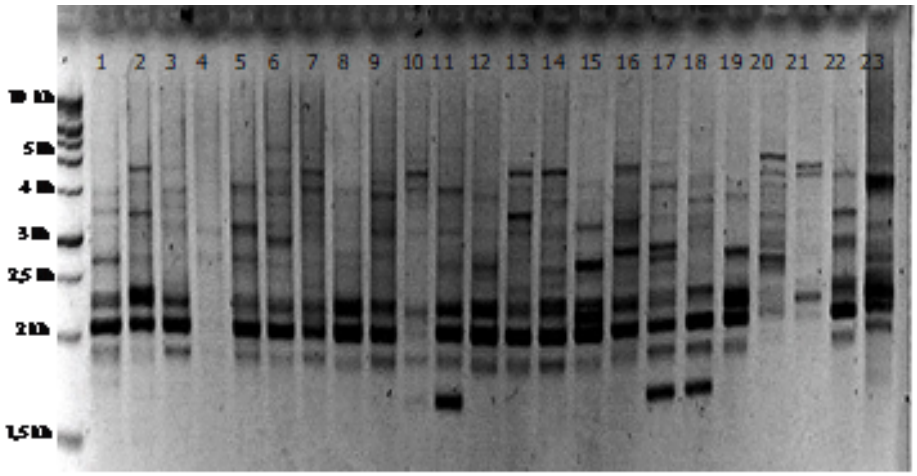

Figure 1: ISSR banding patterns in 23 genotypes of local chili from West Sumatra as revealed by ISSR 12 primer.

Table 2: List of primer, their sequences, number of band and polymorphic features used to amplify 23 genotypes of local chilli, West Sumatera Indonesia.

No	Primer	Nucleotide Sequence (5'-3')	NB	NPB	PPB (%)
1	ISSR1	5'YAY GYA CAY (TG)7 T 3'	4	2	50,00
2	ISSR3 M	5' (CA)6 AG 3'	9	8	88,89
3	ISSR5M	5' TCC TCC TCC TCC TCC 3'	8	5	62,50
4	ISSR.12	5'CTCCTCCTCGC 3'	13	13	100,0
5	ISSR16	(GA)8YT	6	4	66,67
6	ISSR A2		11	6	54,55
7	UBC-809	AGAGAGAGAGAGAGAGG/ (AG)8G	9	7	77,78
8	ISSR3	5'(AC)8YG3'	10	6	60,00
9	ISSR 5		6	1	16,67
10	ISSR27	5'GTCACCACCACCACCACC 3'	8	3	37,50
Total			84	55	61,45

Information: NB = Number of bands, NPB = Number of polymorphic bands, PPB = Percentage of polymorphic bands.

The similarities or differences in banding patterns possessed by 23 chili genotypes do not mean that the ISSR locus refers directly to certain morphological characters. However, the grouping of 23 local West Sumatran chili genotypes uses ISSR markers which are analysed among the local West Sumatran chilies. Grouping analysis showed that the chili genotypes tested tended to have a high similarity trait. The possible assumption is because all of them have type of curly.

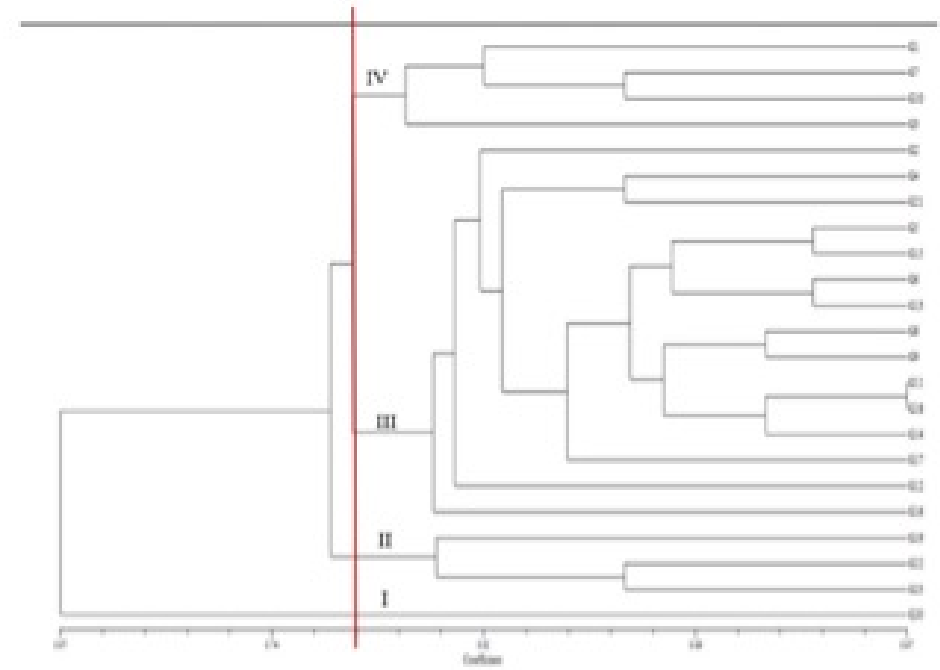

Figure 2: UPGMA phenogram showing genetic diversity of the 23 genotypes local chili West Sumatera based on ISSR bands.

The information on the genetic diversity level and genetic distance that has been obtained can be used as initial information to select diverse parents of the same genotype or to select parents which are the most closely related to increase heterosis or combine desired genes from more diverse backgrounds in producing elite genotypes in crosses. In addition, this information can be applied to minimize the high similarity germplasm on the genotypes collection in Indonesia. Kopay chilies is self-clustered and has the farthest genetic distance compared to the other 22 genotypes. This shows that Kopay chilies are different genetically from other local chilies. Kopay chilies is native to Payakumbuh district with specific characteristics in the size of the fruit, which is between 20-33 cm [14].

4. Conclusion

Molecular characterization of local chili from West Sumatra can be detected using ISSR markers. The ISSR primers used were twenty-three with a total of 84 bands DNA produced. The dendrogram results show that the similarity coefficient is approximately 0.67-0.97 and based on ISSR markers, genetic diversity of local chilli pepper is quite high.

References

[1] Bosland PW, Votava EJ, Votava EM. Peppers: vegetable and spice capsicums. UK: Cabi; 2012. https://doi.org/10.1079/9781845938253.0000.

[2] Wang D, Bosland PW. The genes of Capsicum. HortScience. 2006;41(5):1169–87.

[3] Rahmah IN, Sulistyono A, Makhziah M. Growth response and yield of eggplant (Solanum melongena L.) to Paklobutrazol and water hyacinth liquid organic fertilizer. PLANTROPICA: Journal of Agricultural Science. 2021;6(2):154–62.

[4] Cho SY, Kim HW, Lee MK, Kim HJ, Kim JB, Choe JS, et al. Antioxidant and anti-inflammatory activities in relation to the flavonoids composition of pepper (Capsicum annuum L.). Antioxidants. 2020 Oct;9(10):986.

[5] Suliansyah I, Ekawati F, Hariandi D. Identification and morphological characterization of local red chilies from West Sumatra. InIOP Conference Series: Earth and Environmental Science 2023 Apr 1, Vol. 1160, No. 1, p. 012013. England: IOP Publishing; 2023. https://doi.org/10.1088/1755-1315/1160/1/012013.

[6] Terryana RT, Nugroho K, Rijzaani H, Lestari P. Karakterisasi keragaman genetik 27 genotipe cabai berdasarkan marka SSR (Simple Sequence Repeat). Berita Biologi. 2018;17(2):183–94.

[7] da Costa FR, Pereira TN, Vitória AP, de Campos KP, Rodrigues R, da Silva DH, et al. Genetic diversity among Capsicum accessions using RAPD markers. Crop Breed Appl Biotechnol. 2006;6(1):18–23.

[8] Kang MS. Genotype-environment interaction and stability analyses: an update. Quantitative genetics, genomics and plant breeding. 2020;9:140-61. https://doi.org/10.1079/9781789240214.0140.

[9] Terryana RT, Ningrum ND, Nugroho K, Saptadi D, Kurniawan H, Lestari P. Analisis keragaman genetik dan pengembangan profil sidik jari DNA 20 varietas cabai lokal Indonesia berdasarkan marka SSR. Jurnal AgroBiogen. 2020;16(2):45–58.

[10] Bani PW, Daryono BS, Purnomo P. Penanda molekuler inter simple sequence repeat untuk menentukan ketahanan tanaman jagung terhadap penyakit bulai. J Fitopatologi. 2017;13(4):127–35.

[11] Hariyani SA, Zubaidah S. Molecular Characterization of Piper Retrofractum Vahl in Java using Inter Simple Sequence Repeats (ISSR) Markers. BIOEDUKASI. 2022;20(1):1–7.

[12] Olatunji TL, Afolayan AJ. Evaluation of genetic relationship among varieties of Capsicum annuum L. and Capsicum frutescens L. in West Africa using ISSR markers. Heliyon. 2019 May;5(5):e01700.

[13] Vo TB, Huynh K, Tran TB, Swee KY, Nguyen LH. Assessment of genetic diversity of chili rootstock using ISSR marker. CTU J Innov Sustain Dev. 2016;(03):7–13.

[14] Noli ZA, Labukti HV. Pengaruh Ekstrak Paku Resam (Gleichenia linearis) sebagai Biostimulan terhadap Pertumbuhan dan Hasil Cabai Keriting (Capsicum annum L.). Kultivar Kopay. Agro Bali: Agricultural Journal. 2022;5(3):492–7.