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MOLECULAR CHARACTERIZATION OF *Piper retrofractum* Vahl IN JAVA USING INTER SIMPLE SEQUENCE REPEATS (ISSR) MARKERS

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ABSTRACT

Piper is a genus of the family Piperaceae which has a very large number, in the tropics and sub-tropics. There are about 22 species of Piper which are registered in medicinal herbs and world herbs, one of which is Java chili (*Piper retrofractum* Vahl.). The morphological characters of P. *retrofractum* are different in various planting centers, especially the leaf and fruit characters vary. Limitations of morphological characters encourage the use of molecular characters. The purpose of this study was to analyze the molecular character of Java chilli (*P. retrofractum*) on the island of Java using ISSR (Inter simple sequence repeat) markers. Molecular markers can provide relatively more accurate information because genetic traits tend to be stable in environmental changes and not influenced by age. Leaves from 12 different locations were isolated using the SDS method. Amplification was carried out using 12 ISSR polymers. Data is analyzed using the NTSYS version 2.02 program. The results showed that the amplification of *P. retrofractum* DNA produced 70 polymorphic DNA bands. The dendrogram results from 12 sampling locations showed that *P. retrofractum* on Java Island had a similarity coefficient of 0.69-0.93. This shows that genetic diversity based on ISSR markers from *P. retrofractum* Vahl is relatively low.

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1. INTRODUCTION

Piper is a genus of the family Piperaceae which has a very large number, both in tropical and sub-tropical (Jaramillo & Manos, 2001). The most well-known member of the community is sirih (*Piper betle* L.) which is used in various cultural rituals and as medicines. Pepper (P. *nigrum* L.) and Java chili (P. *retrofractum* Vahl.) Are members of Piper who have economic value. There are around 22 species of Piper listed in the ingredients of herbs and world herbs, one of which is P. *retrofractum* Vahl (Khare C. P., 2004). P. *retrofractum* Vahl is currently widely cultivated in various regions in Indonesia, starting from Java, Madura, Bali, Nusa Tenggara, Lampung and some in Kalimantan and Sulawesi (Haryudin & Rostiana, 2009). These plants are native plants originating from Southeast Asia, such as Indonesia, Thailand, and Malaysia (Soetarto, 1986).

Based on the results of the initial (unpublished) research regarding the morphological character of P. *retrofractum* Vahl, it was found that there were several different morphological characters in various planting centers, especially the characters of leaves and fruit. Leaves have different base shapes and the size of leaves and fruit also vary. The results of these initial studies are in line with the results of Rostiana *et al.*, (1994) study, that agroecological conditions influence the genetic expression of P. retrofractum Vahl plants. Evidence of the morphological differences in P. *retrofractum* Vahl. also supported by research by Haryudin and Rostiana (2009) which stated that P. *retrofractum* Vahl. varied in the form of leaves, fruits, stems, and branches morphology of 23 plant accessions characterized in several planting centers.

Differences in morphological characters from P. *retrofractum* Vahl. it proves the existence of diversity at the species level. This diversity is evidence of the existence of genetic diversity that makes the species have many variations. Diversity information is needed for future plant development efforts and management of germplasm sources. Information on diversity can be obtained by analysing P. *retrofractum* Vahl plants using certain markers, which are based on morphological, anatomical, molecular, and chemical characters.

The study that developed at this time was a study with a molecular character approach. Molecular approaches include primary genetic information (DNA), secondary (RNA), and tertiary or protein (Radford, 1986). The molecular approach in taxonomic studies can be done at protein levels and DNA levels. At the DNA level it is known as RFLP markers (Restriction Fragment Lenght Polimorphism), Random Amplified Polimorphic DNA, AFLP (Amplified Fragment Lenght Polimorphism), SSR (Simple Sequence Repeat / Microsatelite) and ISSR (Inter Simple Sequence Repeats) (Ninot and Aleta, 2003; Mondini *et al*., 2009). Molecular markers can provide relatively more accurate information because genetic traits tend to be stable in environmental changes and not influenced by age (Julisaniah *et al*., 2008).

Inter-simple sequence repeats (ISSR) markers are methods based on ubiquitous microsatellite and hypervariable nature in the eukaryotic genome. ISSR is a multilocular marker based on amplification of DNA fragments flanked by simple recurrent nucleotide sequences with inverse orientation (Besse, 2014). This recurrent area is spread throughout the chromosome genome. The role of repetitive fragments on the chromosome can be a high chance area of crossing in the event of chromosome reduction (meiosis). In addition, binding of DNA strands can also occur (self-dimer) or looping) which affects genetic diversity. This marker is a dominant marker that has several advantages, compared to other dominant markers such as RAPD (Zietkiewicz *et al*., 1994). ISSR reveals high discriminant ratios and genetic variability (Moulin *et al*., 2012), as well as high levels of polymorphism (Djè *et al*., 2010). ISSR markers in certain circumstances can act as codominant markers capable of identifying individuals with heterozygous alleles (Pandit *et al*., 2007; Besse, 2014). In the PCR process, oligonucleotide primers can be randomly determined based on di-, tri- or tetra-nucleotide repetitive, which at the primary end 3 'or 5', can be added with 1-3 bases.

Analysis using ISSR markers has been carried out on many plant species, with various purposes, such as for the purpose of collection and conservation of African oranges (Dje *et al*., 2010), conservation of Japonica tea in China and Japan (Lin *et al*., 2013), plasma characterization Brazilian sweet potato nutrition (Moulin *et al*., 2012), sex determination of green potato plants from India (Nanda *et al*., 2013), determination of uniformity of wine invitro clones (Nookaraju and Agrawal 2012), and study of evolution and speciation in Asteraceae (Archibald *et al*., 2006). ISSR markers were also used to assess the genetic diversity of durian cultivars in Thailand (Vanijajiva 2012) and to analyze genetic diversity from Piper spp. on Hainan Island in China (Jiang and Liu, 2011). The use of ISSR markers to analyze the molecular character of P. retrofractum Vahl. in Indonesia it has not been done, so it is important to do this as a way to determine genetic diversity and the selection of the best germplasm for the purpose of conserving natural resources, increasing production from plantation products and improving the quality of breeding. P. Retrofractum Vahl is one of the native plants of Indonesia, especially many spreads on Java (Aspan, 2008; Haryudin and Rosdiana, 2009), so that the conservation of these plants is absolutely necessary. The purpose of this study was to analyze the molecular characterization of P. retrofractum Vahl on Java Island using ISSR (Inter simple sequence repeat) markers.

2. RESEARCH METHOD

Plant materials

The tools used are mortar, pestle, a set of electrophoresis devices, PCR machines, nano drop, micropipette (20, 200, 1000), refrigerator, centrifuge, centrifuge tube, eppendorf tube, nanodrop, Geldoc Major Science, PCR BioRad. The material used was leaves from the P. retrofractum Vahl plant obtained from farmers in 10 districts in East Java and Central Java.

The sampling locations were different, there were 12 locations, two districts in Central Java and eight districts in East Java (Figure 1.). Sampling in Banyuwangi and Jember districts was carried out in two places that were far apart and both had different morphological characters (the results of morphological observations). Determination of the location is based on the provisions, namely the place is a cultivation center, the area of the garden is approximately one hectare acre (ha), and the plant life is more than 1 year.

Picture 1. Sampling Location
Source: https://www.google.co.id/maps/@-7.3438565,110.5030146,7z

Table 1. Shows Sampling locations. The altitude of the place varies, the average air temperature is 29.3 ° C, the average humidity is 71.7% and the soil pH averages 6.78.

Table 1. Sampling Location

No.	Location	Position	Humidity	Air temperature	soil pH	Height
1.	Jember 1(Tegalgede)	8º09'57"S 113º42'54'E	78%	28ºC	6,8	97 m
2.	Jember 2 (Andongrejo)	8º24'17"S 113º44'30'E	70%	30ºC	6,8	54m
3.	Banyuwangi 1 (Silir)	8º32'17" S 114º07'49" E	75%	29ºC	6,8	226 m
4.	Banyuwangi 2 (Songgon)	8º12'26" S 114º09'12" E	74%	30ºC	6,8	158m
5.	Lamongan	7º16'22" S 112º21'16" E	66%	31ºC	6,5	93 m
6.	Kediri	7º54'50" S 112º05'07" E	80%	28ºC	7	222 m
7.	Bangkalan	6º53'21"S 113º19'59'E	63%	32ºC	6,8	16 m
8.	Sampang	6º53'07"S 113º01'48'E	65%	32ºC	6,8	60 m
9.	Pamekasan	7º06'41"S 113º33'51'E	65%	30ºC	6,8	120 m
10.	Sumenep	7º05'49"S 113º49'03'E	70%	29ºC	6,7	83 m
11.	Wonogiri	8º09'53"S 110º51'20'E	75%	28ºC	6,8	298 m
12.	Temanggung	7º21'13"S 110º16'19'E	65%	28ºC	6,8	558 m

DNA extraction

DNA isolation uses SDS (sodium dodecyl sulphate). Young leaf samples from P. *retrofractum* Vahl as much as 0.3 grams which have been soaked with liquid N2 are crushed using mortar until smooth. The fine powder was transferred to the eppendorf tube which had been filled with 600 µl of extraction buffer then added with 30 µl SDS 20% and 1.25 µl β-mercaptoethanol then incubated at the waterbath at 65oC for 10 minutes. After incubation, 300 mL of potassium acetate (5 M) was added and then vortexed until homogeneous and then incubated on ice for 10 minutes and separated by centrifugation at a speed of 12,000 rpm at 4oC for 10 minutes. The supernatant was then taken with a micropipette and transferred to a new eppendorf tube and added with 375µl of cold isopropanol swirled and then incubated at -20oC for 1 hour. The excreted tube is then centrifuged at a speed of 12,000 rpm at 4oC for 10 minutes. The supernatant was then discarded and the remaining pellets were added with 300 µl TE buffer and 9 µl RNase, then incubated at 37 ° C for 1 hour. After incubation, it was added with 300 µl PCI and a cortex, then centrifuged at a speed of 12,000 rpm at 4oC for 10 minutes. The supernatant was transferred to a new eppendorf tube then added with chloroform with the same volume, then centrifuged at a speed of 12,000 rpm at 4oC for 10 minutes. The supernatant was then transferred to a new eppendorf tube and added with 0.8 x isopropanol, 0.2 x sodium acetate and swirled and then incubated at -20oC for 1 hour. After incubation then centrifuged at a speed of 12,000 rpm at 4oC for 10 minutes, the supernatant was then discarded and the pellet was washed using 600 µl of 70% alcohol. The pellets which have been washed with alcohol are then centrifuged at a speed of 12,000 rpm at 4oC for 10 minutes, then the supernatant is removed and the pellets are dried with vacuum dry for 5 minutes then added 30 µl buffer TE and stored at -20oC.

DNA amplification

DNA from P. *retrofractum* Vahl leaves were amplified using 12 ISSR markers. The PCR process was carried out on a volume of 20 µl with a composition of 4 µl DNA templates, 10 µl master mix, 2 µl primer, and 4 µl sterile free ions. DNA amplification stages were carried out 40 cycles. Pre-denaturation at 95oC for 1 minute, denaturation at 95oC for 15 seconds, annealing temperature according to the primary character (the temperature of each primer annealing is presented in Table 2), and extension at 72oC for 10 seconds. The PCR results of each primer were put into 2% agarose gel wells and electrophoresed at 100 volts for 60 minutes. Electrophoresis results are then visualized using Geldoc. DNA bands from PCR results are then observed as molecular characters.

Data analysis

Electrophoresis data were analyzed using Gel Analyzer 2010a. The ribbon of DNA fragments that appear are written in number 1 and those that do not appear are numbered 0. Data is then analyzed by NTSYSpc 2.02 (Numerical Taxonomy and Multivariate Analysis System) version 2.02. The grouping results with SIMQUAL (Similarity of Qualitative Data) are based on the Simple Matching coefficient value. Similarity value using SAHN (Sequential Agglomerative Hierarchial and Nested Clustering) analysis to construct dendrogram using UPGMA method (Unweighted Pair Group Method with Arithmetic Mean) (Rohlf, 1998).

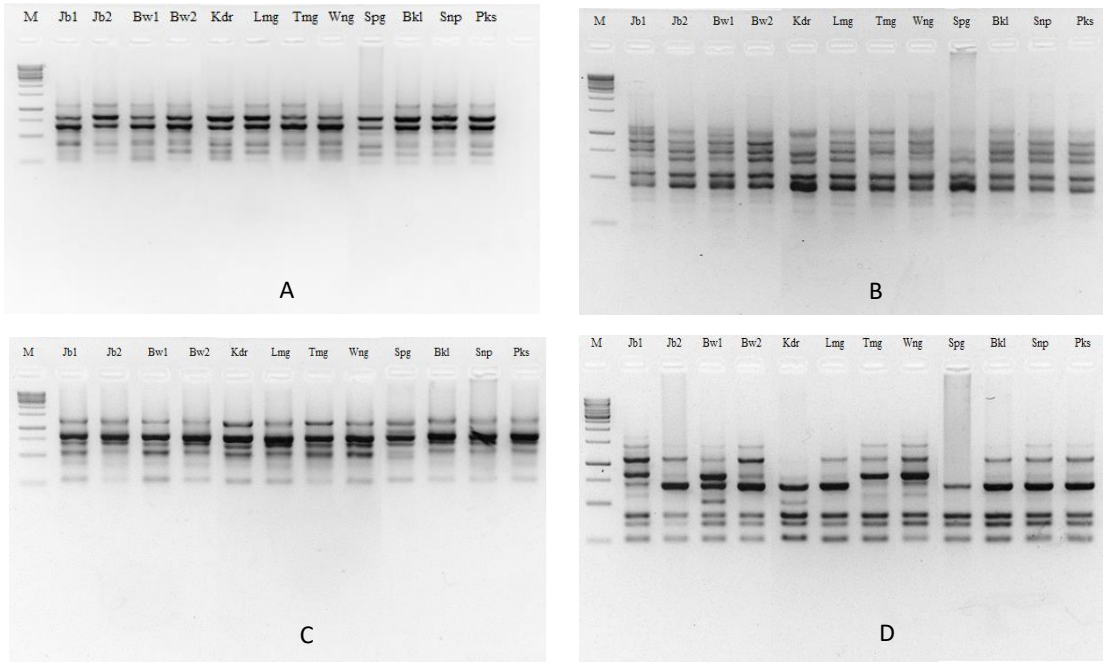
3. RESULTS AND DISCUSSION

Amplification of DNA by PCR

To get optimal results in the PCR process, the results of DNA isolation are optimized first. Optimization is carried out at the annealing temperature of the primers used. The results of the optimization of the attachment temperature are presented in Table 2.

No.	Primer	Primer sequence 5'-3'	Suhu *annealing* (ºC)
1.	UBC-808	AGAGAGAGAGAGAGAGC/ (AG)8C	45
2.	UBC-809	AGAGAGAGAGAGAGAGG/ (AG)8G	45
3.	UBC-826	ACACACACACACACACC/ (AC)8C	48
4.	UBC-829	TGTGTGTGTGTGTGTGC/ (TG)8C	48
5.	UBC-834	AGAGAGAGAGAGAGAGYT/ (AG)8YT	45
6.	UBC-835	AGAGAGAGAGAGAGAGYC/ (AG)8YC	46
7.	UBC-836	AGAGAGAGAGAGAGAGYA/ (AG)8YA	45
8.	UBC-842	GAGAGAGAGAGAGAGAYG/ (GA)8YG	45
9.	UBC-844	CTCTCTCTCTCTCTCTGC/ (CT)8RC	46
10.	UBC-846	CACACACACACACACART/ (CA)8RT	48
11.	UBC-854	TCTCTCTCTCTCTCTCRG/ (TC)8RG	46
12.	UBC-856	ACACACACACACACACYA/ (AC)8YA	49

The results of observations from the electrophoresis process show that different primers produce different DNA bands, this can be seen from Figure 2. Twelve primers used produced 70 DNA bands. Each primer produced an average of 5.8 DNA bands. The number of DNA bands in each primer is different, this is influenced by the way the primary recognizes the homologue in the DNA print. DNA bands produced from the PCR process are 50 bp up to 1000 bp (Table 3.).



Picture 2. PCR results from 12 sampling locations, (A) using 808 UBC primer, (B) using 809 UBC primer, (C) using 826 UBC primer, (D) using 834 UBC primer

Table 3. List of primers, size and number of amplified bands on ISSR markers

Primary name	The size of the amplified DNA band	Total Ribbons
UBC-808	75, 100, 150, 300, 400, 500	6
UBC-809	100, 200, 350, 400, 450, 500	6
UBC-826	250, 400, 450, 500, 600	5
UBC-829	600, 700	2
UBC-834	50, 100, 150, 250, 300, 350, 400, 550, 750, 1000	10
UBC-835	75, 150, 175, 225, 300, 350, 400, 500	8
UBC-836	450, 500, 600, 650, 750	5
UBC-842	350, 450, 500, 600, 650, 675, 700, 725	8
UBC-844	200, 400, 450, 600	4
UBC-846	375, 500, 550, 650, 700	5

ISSR primers are polymorphic bands to analyse the genetic diversity of various plant species such as Piper species (Jiang and Liu, 2011), Alpinia oxyphylla (Wang et al., 2012), and Solanum tuberosum (Bornet et al., 2002). ISSR primers were first used by Zietkiewicz *et al*. (1994) to differentiate genetically related individuals quickly. One advantage of this technique is that no previous genome sequence is needed (Bornet et al, 2002).

The most abundant primer is the UBC 834 ISSR, while the least ribbon primary is UBC 829. The number of bands produced in each primer indicates that the primary sequence is abundant in the plant genome (Powell et al., 1996). DNA bands that appear from the PCR results shown in the electrophoresis gel are considered as one allele. DNA bands with the same migration rate are considered to be homologous loci. DNA bands from electrophoresis showed different thicknesses, this was due to differences in the intensity of primer attachment to the DNA. One DNA band can be amplified more than the other band (Kumar, et al., 2009).

The profile of the DNA band pattern that has been interpreted into binary data is then done by cluster analysis. Cluster analysis of P. *retrofractum* in 12 sampling locations is presented in Figure 3. Grouping was formed on the similarity coefficient of 0.69-0.93. This shows that genetic diversity based on ISSR markers of P. *retrofractum* is relatively low.

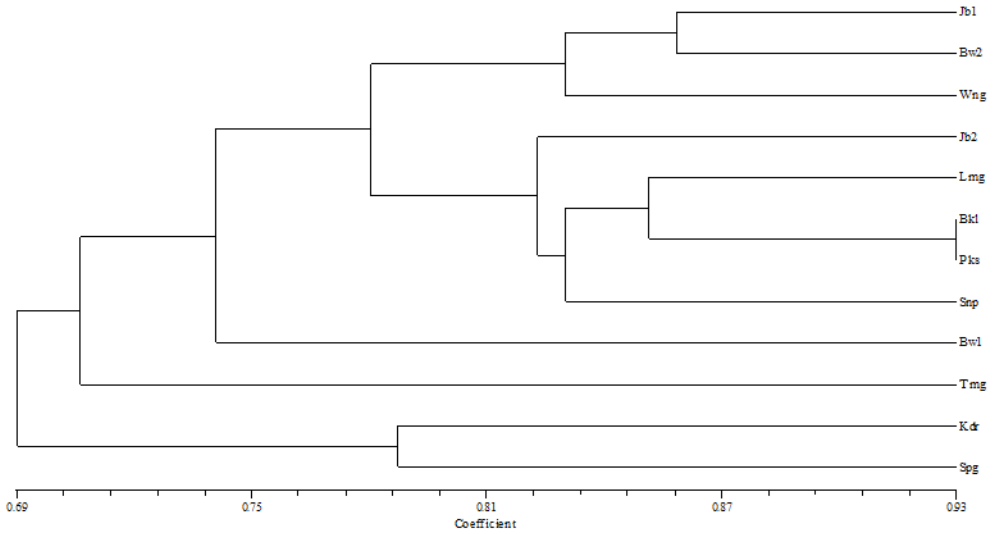


Figure 3. Dendrogram of P. *retrofractum* based on DNA profile with ISSR markers in Java with the similarity coefficient of SM and UPGMA method

The dendrogram results showed that there were 3 groups of P. retrofractum species, the first were groups from Jember 1, Banyuwangi 2, and Wonogiri with a similarity coefficient of around 0.83. The second group consisted of Jember 2, Lamongan, Bangkalan, Pamekasan, and Sumenep with a similarity coefficient of 0.82. The third group consists of Kediri and Sampang with a similarity value of around 0.79. There are two origin samples which are not included in the three groups, namely Banyuwangi 1 and Temanggung.

P. *retrofractum* originating from Bangkalan and Pamekasan has one similarity coefficient, which means that based on 12 ISSR markers used both have similar molecular or identical characters. Molecular similarity can be indicated that both of these plants originate from the same parent, although morphologically both have little difference. This difference can be caused by environmental factors. Genetic changes can be caused by an interaction between genetic and environmental factors that cause physical changes (phenotypes) that are temporary or can be permanent. Permanent changes can occur due to mutations.

Location for sampling P. *retrofractum* Vahl plants. Located at an altitude of 16 to 558 masl with temperatures ranging from 28oC to 32oC, the pH of the soil is around 6.5 to 7 with air humidity of 63% to 80%. The sampling location is in a relatively diverse geographical area with different microclimate conditions, especially water availability and ambient temperature. Plant adaptation to the environment can affect physiological and chemical changes.

Genetic diversity is likely caused by the multiplication of these plants. Java chili plants (P. *retrofractum* Vahl) are plants that can grow vegetatively and generatively. Farmers in Java increase their vegetative vegetation by stem cuttings, mounding, and separation of tillers. Plants that grow vegetatively have relatively low genetic variations, whereas plants that grow generatively have high genetic diversity.

Low genetic variation based on ISSR markers used in the study may be caused by less informative allele frequencies. ISSR markers are the dominant markers so little informative alleles are formed (Bolaric et al., 2005). The difference in the number of DNA bands produced by each amplification illustrates the level of complexity of the genome of a plant. The amplified DNA bands are the result of pairing plant nucleotides with nucleotide markers. The more markers and the number of DNA bands, the more representative parts of the genome will be, and the plant genome will be more clearly depicted.

An interesting finding from this study is that all P. *retrofractum* plants amplified with 12 primers produced polymorphic DNA bands. Of the 12 sampling locations 6 of them had specific bands that were not owned by other locations, namely P. *retrofractum* from Banyuwangi 1, Jember 2, Kediri, Sumenep, and Wonogiri. Specific bands found can be used as ideal plant identities because they are only found in one sampling location (Zulfahmi, 2013). Specific tape can be used as a genetic profile for identification of a species or a cultivar.

4. CONCLUSSION

Molecular characterization of P. *retrofractum* Vahl plants in Java can be detected using ISSR markers. The ISSR primers used were twelve and produced 70 DNA bands. The dendrogram results show that the similarity coefficient is around 0.69-0.93. This 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

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6. REFERENCES

Archibald, F.S., Bourbonnais, R., Jurasek, L., Paice, M.G., and Reid, I.D., 1997. Kraft pulp bleaching and delignification by *Trametes versicolor*. *Journal of Biotechology* 53: 215-336.

Aspan, R., 2008. Taksonomi Koleksi Tanaman Obat Kebun Tanaman Obat Citeureup. BPPOM RI. Jakarta.

Besse, P. (ed), 2014. Molecular Plant Taxonomy Methods and Protocols. Spinger. London.

Bolaric S, Barth S, Melchinger A, Posselt U. 2005. Genetic Diversity among Polish landraces of common out (Avena sativa L.) Genet Resour Crop Evol. 60:2157-2169.

Djè Y., Tahi C.G., Zoro Bi AI, Baudoin J.P., Bertin. P., 2010. Use of ISSR markers to assess genetic diversity of African edible seeded *Citrullus lanatus* landraces. *Sci Hortic.* 124:159–164. doi: 10.1016/j.scienta.

Dyer, L. A., and Palmer, A. D. N., 2004. *Piper:* A Model Genus for Studies of Phytochemistry, Ecology, and Evolution. Kluwer Academic / Plenum Publishers. New York

Haryudin, W., dan Rostiana, O., 2009. Karakteristik Morfologi Cabe Jawa (Piper retrofractum. Vahl. Di Beberapa Sentra Produksi. *Buletin. Littro. Vol 20 No.1, hal.1-10.*

Hesse, M., Halbritter, H., Zetter, R., Weber, M., Buchner R., Radivo A. F., Ulrich S. 2009. Pollen Terminology An illustrated handbook. Springer. Austria

Jaramillo, M.A., Callejas, R., Davidson, C., Smith, J.F., Stevens, A.C., Tepe, E. J., 2008. *Systematic Botany* 33 (4): pp. 647-660.

Jiang, Y. dan Liu, Jin-Ping. 2011. Analysis of Genetic Diversity of Piper spp. In Hainan Island (China) using Inter-Simple Sequence Repeat (ISSR) markers. *African Journal of Biotechnology* Vol. 10 (66) pp.14731-14737.

Julianisah, I.N., Sulistyowati, L., Sugiharto, N.A., 2008. Analisis kekerabatan mentimun (*Cucumis sativus* L) menggunakan metode RAPD-PCR dan Isozim.*Biodiversitas.* 9:99–102.

Khare, C.P., 2007. *Indian medicinal plants*. 1st edition. Springer Science Business Media, LLC., 16–17.

Kumar P, Gupta VK, Misra AK, Modi DR, Pandey BK. 2009. Potential of molecular markers in plant biotechnology. Plant Omi J. 2:141-162.

Mondini L., Noorani A., Pagnotta M.A., 2009. Assesing Plant Genetic Diversity by Molecular Tools. *Diversity* 1: 19-35.

Moulin MM, Rodrigues R, Simoes L, Goncalves A, Pereira G. 2012. A comparison of RAPD and ISSR markers reveals genetic diversity among sweet potato landraces (*Ipomoea batatas* (L.) Lam.). *Acta Sci.* 34:139– 147.

Ninot, A. and Aletta, N., 2003. Identification and genetic relationship of persian walnut genotypes using isozyme markers. *Journal of the American Pomological Society* 57:106-114.

Pandit SS, Mitra S, Giri AP, Pujari KH, Patil BP, Jambhale ND, Gupta VS. 2007. Genetic diversity analysis of mango cultivars using inter simple sequence repeat markers. *Current Sci.* 93:1135–1141.

Parmar. V. S., Jain, S.C., Bisht, K S., Jain R., Taneja, P., Jha A, Tyagi. O. D., Prasad, A. K., Wengel, J, E T Carl. Olsen And Per. 1997. Phytochemistry of The Genus Piper. *Phytochemisrry, Vol. 46, No. 4, pp. 591-673, 1997.* Elsevier Science. Pergamon.

Prasad, M.P., Shekhar, S., Amit, B., 2012. Phytochemical Analysis and Antioxidant potential of *Piper species* and its Molekuler Characterization by RAPD Markers. *International Jounal of Fundamental & Applied Sciences*. Vol 1. No.4 p. 71-73.

Radford, A.E., 1986. *Fundamental of Plant Systematics*. London: Longman Higer Education.

Rohlf FJ. 1998. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. New York (US): Exeter software.

Ziekiewicz, E., A. Rafalski, D. Labuda. 1994.Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genome*, 20: 176-183.

Zulfahmi. 2013. Penanda DNA untuk analisis genetic tanaman. Jrnal Agroteknologi 3(2): 41-52.