Genetic Diversity Based on RAPD Marker of Ramie Plants (Boehmeria nivea [L.] Gaud) In West Sumatera

IOP Conf. Series: Earth and Environmental Science **327** (2019) 012014 doi:10.1088/1755-1315/327/1/012014

Genetic Diversity Based on RAPD Marker of Ramie Plants (Boehmeria nivea [L.] Gaud) In West Sumatera

R Mayerni[1], Yusniwati[2], D Yulfa[2], and S R O S Chan[2]

[1,2] Faculty of Agriculture ,Andalas University, Limau Manis Padang, 25163 Indonesia

Corresponding author's e-mail address: renimayerniagr@unand.ac.id

Abstract. This research has been conducted at the Experimental Farm of Agricultural Faculty of Andalas University, two districts in West Sumatera (Lima Puluh Kota and Agam) and the Laboratory of the Department of Agronomy and Horticulture Agricultural Faculty, Bogor. The purpose of this study was to determine the genetic diversity of the ramie plants collection Experimental Farm, Agricultural Faculty of Andalas University and exploration at two districts in West Sumatera. This research was conducted by molecular characterization, using RAPD markers *(Random Amplified Polymorphic DNA*) with 5 primers (OPF 4, OPF5, OPH7, OPX2, and OPX 17). The samples used were 4 clones of ramie plants (Padang 3, Ramindo, Matur and Situjuah) with 3 replications. Fragments were sized with reference to the size markers and scored (present/absent). The similarity coefficients calculated and dendograms produced using NTSYS software (version 2.02). From the results of the study the results of the similarity level coefficient were 0.56-0.78, and formed 2 large groups (I, II). M1 and M2 clones are in group I, while in groups II (IIa and IIb). Group IIa (R1, R2, R3, P3, M1) and Group IIb (S1, S2, S3, P1).

1. Introduction

Ramie plants (*Boehmeria nivea* (L.) Gaud.) is one of the annual plants that have many benefits. Ramie plants as raw material in the textile industry [8], pulp [5], paper [25], land conservation [2], as compost material [19], traditional medicine [9], and animal feed [11].

Fiber from ramie plants as a raw material for the textile industry, because produce high-quality fibers [21]. In addition, its fiber has a smooth, long and strong texture [14,15,26]. Rami bast fibre clearly shows extra xilary fibre that is found in the cortex around floem [17].

According from [16,4,22], ramie plants are easy to grow in the tropics and will high produce if planted in the lowlands to highlands (10 - 1500 m above sea level). In West Sumatra forest, ramie plants are still found wildly. Knowledge of genetic diversity is very important in plant breeding activities to determine steps to improve the quality and quantity of ramie plants.

And to know genetic diversity can used molecular characterization observations, information about molecular character is needed as a differentiator between plant accession and estimation of distance or genetic diversity in kinship analysis so that it can be used as basic material for improving ramie plants.

The further the genetic distance between parents in one species, the greater the chance to produce new varieties [24]. [12] state that to improve the genetic properties of wild ramie germplasm better through molecular characterization.

RAPD technique (*Random Amplified Polymorphism* DNA) is one of the PCR-based molecular methods that can be used to identify individuals at the DNA level [6,7]. This method is also relatively

cheaper and popular [10] and ability to quickly detect polymorphisms at a number of band analyzed [1].

This can be seen in the results of research by [13] that of the 37 ramie accessions using 31 primers with the RAPD technique which included 29 wild accessions and 8 commercial accessions with polymorphism levels of 95.5%. As well as based on research by [18], out of 5 clones of ramie plants using 10 primers showed a level of polymorphism of 93%. [21] also added that the high level of polymorphism with molecular markers in ramie plants would be very useful in analyzing population structure and effective in managing plant genetic resources.

2. Material and Methods

2.1. Materials

Fresh leaf samples collected from 4 ramie clones (Ramindo 1, Padang 3, Matur and Situjuah), CTAB extraction buffer (Tris-HCl (1 M, pH 8), NaCl (1.4 M), EDTA (0.5 M, pH 8)) was used for DNA extractions. DNA Taq Polymerase was used for amplification reactions.

2.2. Methods

Sample from leaf segments 3-5 (15-20) grams. Leaf samples were sterilized using 70% alcohol and washed using distilled water. DNA Isolation was performed using the CTAB method. amplification DNA was performed using PCR. Reaction mixtures contained: Taq polymerase reaction mixture 6.25 µL, DDH_2O 4.25 µL, ramie DNA 1 µL and primer 1 µL. Primers used were either from [3], OPX 17, OPX 02, OPH 07, OPF 04, OPF 05 and were purchased from QIAGEN. PCR conditions were as follows: initial denaturation at 94 ^{0}C for 5 min followed by 45 cycles at 94 ^{0}C, 1.5 min at 37 ^{0}C, 1 minute at 72 ^{0}C and a final extension reaction at 72 ^{0}C for 5 minutes. PCR products were analyzed by agarose gel electrophoresis following staining with ethidium bromide

2.3. Data Analysis

Fragments were sized with reference to the size markers and scored (present / absent). The similarity coefficients calculated and dendograms produced using the *Unweighted Pair Group Method with the Arithmetic Average* (UPGMA) and the NTSYSpc software (version 2.02) [20].

3. Result and Discussion

Identification by morphological characters is still influenced by the environment. So as to support the results of the analysis, kinship analysis can be carried out based on molecular character using the RAPD technique. This analysis was carried out on 4 clones of ramie plants using 5 primers [18]. The primers used are OPX 17, OPH 07, OPF 04, OPH 05 and OPX 02. Of the 5 RAPD primers used there are differences in the produced of bands (Table 1).

Table 1. Profile of bands

Primary Code	Monomorphic	Polymorphic	Number of Band	Polymorphism(%)
OPX 17	2	9	11	81
OPH 07	0	13	13	100
OPF 04	0	10	10	100
OPH 05	0	9	9	100
OPX 02	0	11	11	100
Total	2	42	54	96.2

It can be seen that the total bands produced from PCR amplification in 4 clones of ramie plants is 54 bands. From the samples tested each one primer produces a range of 9-13 polymorphic ribbon PCR fragments, so that it can be said that the RAPD technique can select polymorphism in a fast time. From the results of testing using 5 primers showed a high value of polymorphism which is indicated by a total of polymorphic bands of 42 bands and a level of polymorphism of 96.2%. The high value of

the resulting polymorphism shows the high diversity in the samples tested. In addition, the results of PCR amplification using 5 primers only produced 1 monomorphic band in the use of OPX 17 primers, whereas for the other 4 primers it did not produce monomorphic bands. To visualize DNA bands formed from the results of amplification and electrophoresis (Figure 1-5).

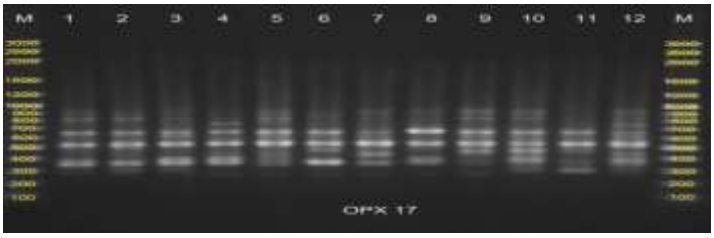

Figure 1. Visualization of Gel Electrophoresis Using OPX 17

Description: Column 1-12 is a clone, (M = DNA *Ladder*), 1,2,3 (Ramindo 1), 4,5, 6 (Matur), 7,8,9 (Situjuah), P1, P2, P3 (Padang 3)

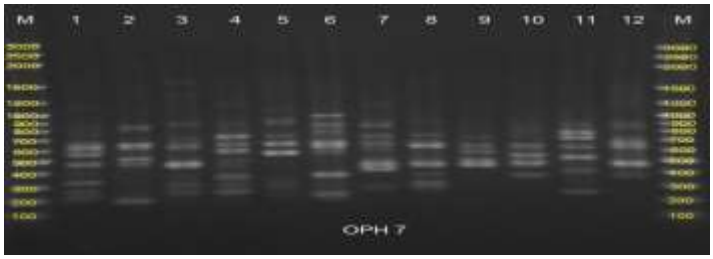

Figure 2. Visualization of Gel Electrophoresis Using OPH 07

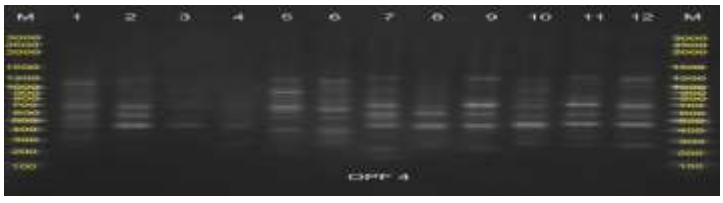

Figure 3. Visualization of Gel Electrophoresis Using OPF 04

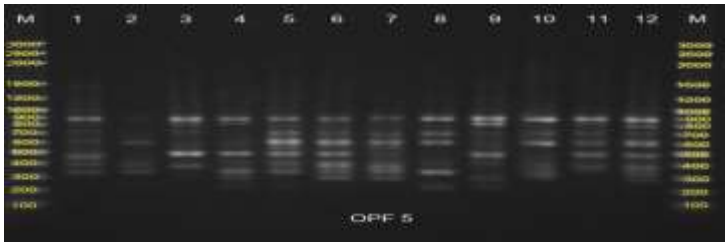

Figure 4. Visualization of Gel Electrophoresis Using OPF 05

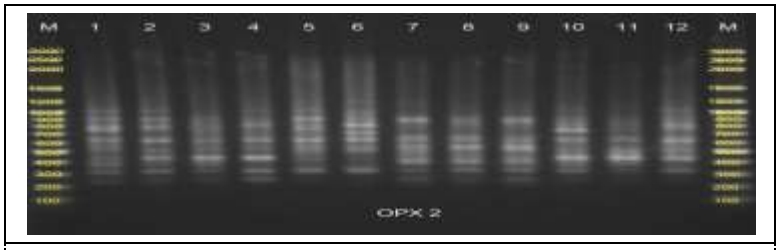

Figure 5. Visualization of gel electrophoresis using OPX 02

RAPD technique as a molecular marker in this study shows very high polymorphism. From 5 primers used, all produced polymorphisms> 50% (Table 1.). The number of bands produced ranged from 9-13 polymorphic bands, with the most bands produced by OPH 07 and the least by OPH 05. Furthermore the percentage of polymorphism ranged from 81-100%. The lowest percentage of polymorphism (81%) is produced by OPX 17 primer while the other 4 primers produce 100% polymorphism.

The ability of the 5 primers used in this study to detect polymorphisms has also been reported from [3], it was reported that the results of analysis of 5 clones using 10 RAPD primers with the 5 best primers of which were tested in the study produced a total band, 101 bands with a total of 94 polymorphic bands and the percentage 93%.

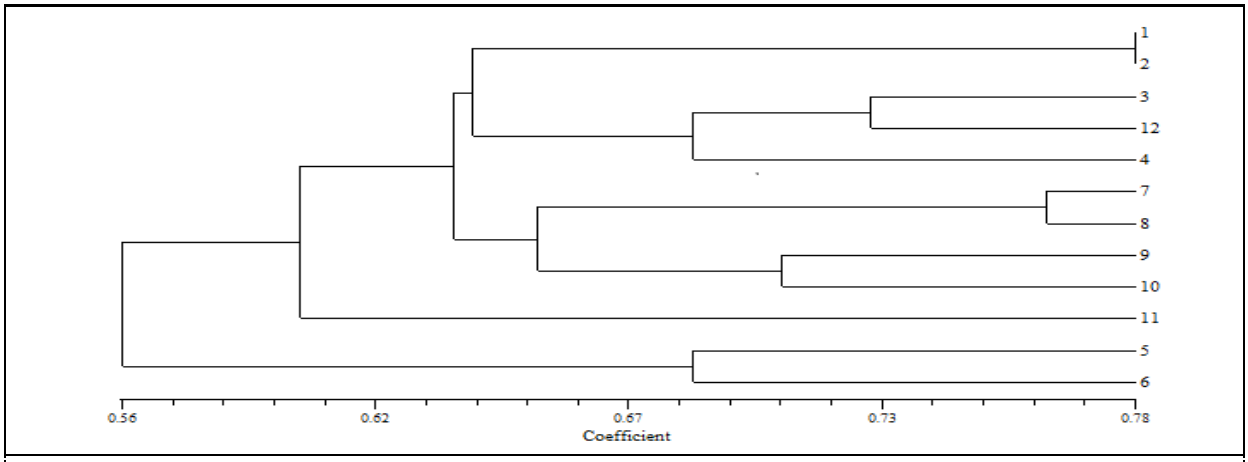

Figure 6. Dendogram of 4 Clones Rami Plants
(1, 2,3 = Ramindo, 4,5,6 = Matur, 7,8,9 = Situjuah and 10, 11, 12 = Padang 3)

Similarity analisis of ramie plants produces a dendogram with a coefficient of 0.56-0.78 or 56-78%, the overall character is molecularly united at a coefficient of 0.56, there are 2 main groups namely groups I and II. Group I consists of clones 5 and 6. Group II into 2 groups again IIA (1,2,3,12,4), IIB (7,8,9,10) and IIC (only 11). The closest similar clones are 1 and 2 (Ramindo replicates 1 and 2). Ramindo, Situjuah, Padang 3 and Matur clones (1 replication) are included in one group while Matur clones (2 replications) form their own groups.

[23] stated that the greater the value of the similarity coefficient, the greater the level of similarity between plants compared. Conversely the smaller the number of similarity coefficients, the smaller the level of similarity of these plants. That is, the greater the number of similarities, the closer the level of kinship and vice versa if the smaller the number of similarities, the further the relationship. Implications in plant breeding, with the existence of a long-standing family relationship, high heterotic, high recombinant values are very important for assembling hybrid seeds or plants. High genetic diversity is more desirable because closely related individuals will have a close genetic distance, whereas if they are distant, they will have a far genetic distance.

4. Conclusion

The results of molecular analysis using RAPD markers showed that the high level of diversity produced by the primer with a percentage of polymorphism was 96.2%. As for the similarity analysis, the coefficient value is 56-78% which indicates that between clones have close kinship.

References

[1] Azrai, M. 2005. Pemanfaatan Markah Molekuler dalam Proses Seleksi Pemuliaan Tanaman. *Jurnal Agro Biogen* **1 (**1): 26-37.

[2] Banerjee, P., D. P. Ray., S. Debnath., dan P. K. Biswas. 2015. Quality Attributes of North-East Himalayan Soil for Cultivation of Ramie (*Boehmeria nivea* L. Gaudich.) *International Journal of Agriculture, Environment and* Biotechnology **8** (4): 879-884.

[3] Chan, S. R. O. S. 2018. Identifikasi Tanaman Rami (*Boehmeria nivea* (L.) Gaud.) Koleksi Kebun Percobaan Fakultas Pertanian Universitas Andalas. Tesis. Program Pascasarjana Universitas Andalas. Padang.

[4] Dahlan, D. 2011. Buku Ajar: Mata Kuliah Budidaya Tanaman Industri. Universitas Hasannudin. Makassar.

[5] Dewi, T. K., Dandy., dan W. Akbar Pengaruh Konsentrasi NaOH, Temperatur Pemasakan, dan Lama Pemasakan Pada Pembuatan Pulp dari Batang Rami dengan Proses Soda. *Jurnal Teknik Kimia* **17 (2)**: 68-74.

[6] Dwiatmini, K., N. A. Mattjik., H. Aswidinnoor., dan N.L. T. Matius. 2003. Analisis Pengelompokan dan Hubungan Kekerabatan Spesies Anggrek Berdasarkan Kunci Determinasi Fenotipik dan Marka Molekuler RAPD.*Jurnal Hortikultura* **13**(1):16-27

[7] Eltanti, F. 2015. Karakteristik Morfologi dan Molekuler 18 Genotipe Cabai Hias (*Capsicum* spp.).Skripsi. Fakultas Pertanian Institut Pertanian Bogor. Bogor.

[8] Fan, P., F. He., Y. Yang., M. Ao., J. Ouyang., Y. Liu., dan L. Yu. 2015. *In-Situ* Microbial Degumming Technology with *Bacillus* sp. HG-28 for Industrial Production Of Ramie Fibers. *Biochemical Engineering Journal* **97**: 50–58.

[9] Huang, C. J., G. Wei., Y. C. Jie., J. J. Xu., S. Y. Zhao., L. C. Wang., dan S. A. Anjum. 2015. Responses of Gas Exchange, Chlorophyll Synthesis and ROS-Scavenging Systems to Salinity Stress in Two Ramie (*Boehmeria nivea* L.) *Cultivars. Photosynthetica* **53** (3): 455-463.

[10] Jamsari. 2007. Bioteknologi Pemula: Prinsip Dasar dan Aplikasi Analisis Molekuler. UNRI Press.Pekanbaru.

[11] Kipriotis, E., X. Heping., T. Vafeiadakis., M. Kiprioti., dan E. Alexopoulou. 2015. Ramie and Kenaf as Feed Crops. *Industrial Crops and Products* **68**: 126–130.

[12] Liu, F. H., Z. Li., Q. Liu., H. He., X. Liang., dan Z. Lai. 2003. Introduction to the Wild Resources of the Genus *Boehmeria* Jacq. in China. *Genetic Resources and Crop Evolution* **50** (8) : 793-797.

[13] Liu, L. J., Z. Q. Meng., B. Wang., X. X. Wang., J. Y. Yang., dan D. X. Peng. 2009.Genetic Diversity among Wild Resources of the Genus Boehmeria Jacq. FromWest China Determined Using Inter-simple Sequence Repeat and Rapid Amplification of Polymorphic DNA Markers. *Plant Production Science* **12** (1): 88-96.

[14] Liu, T., S. Tang., S. Zhu., dan Q. Tang. 2014. QTL Mapping for Fiber Yield-Related Traits by Constructingthe First Genetic Linkage Map in Ramie (*Boehmeria nivea* L. Gaud). *Molecular Breeding* **34** (3): 883-892.

[15] Liu, T., S. Zhu., Q. Tang., dan S. Tang. 2015. Identification of a *CONSTANS* Homologous Gene with Distinct Diurnal Expression Patterns in Varied Photoperiods in Ramie (*Boehmeria nivea* L. Gaud). *Gene* **560**: 63–70.

[16] Mayerni, R. 2006. Prospek dan Peluang Tanaman Rami di Indonesia.Andalas University Press. Padang.

[17] Mayerni, R, T. Maideliza. D. Rezki. 2017. Comparative Study of Length and Growth Rate of Ramie (*Boehmeria nivea* L. Gaud.*)* Bast Fiber of Indonesian Clones. *International Journal on Advanced Science Engineering Information Technology*.7 (6) : 2273-2278.

[18] Mayerni, R, Gustian, SROS Chan. 2018. Morphology and Molecular Identification Of Indonesia Ramie Clones *(Bohmeria nivea (L.)* Gaud.). Accepted in *International Journal on Advanced Science Engineering Information Technology*

[19] Mitra, S., M. Kumar., M. Saha., dan B. S. Mahapatra. 2014. Effect of Irrigation and Nutrient Management on Growth, Fibre Yield and Water use of Ramie (*Boehmeria nivea*). *Indian Journal of Agricultural Sciences* **84** (5): 595–601.

[20] Rohlf, F.J. (1998) NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System Version 2.0. Applied Biostatistics Inc., Exeter Software, Setauket, New York.

[21] Satya, P., S. Mitra., D. P. Ray., B. S. Mahapatra., M. Karan., S. Jana., dan A. K. Sharma. 2013. Rapid And Inexpensive Naoh Based Direct PCR For Amplification Of Nuclear And Organelle DNA From Ramie (*Boehmeria nivea*), A Bast Fibre Crop Containing Complex Polysaccharides. *Industrial Crops and Products* **50**: 532–536.

[22] Subandi, M. 2011. Budidaya Tanaman Perkebunan. Gunung Djati Press. Bandung.

[23] Syukur, M. , Sujiprihati and R. Yunianti. 2012. Teknik Pemuliaan Tanaman. Penebar Swadaya. Jakarta.

[24] Tenda, E., M. Tulalo., dan Miftahorrachman. 2009. Hubungan Kekerabatan Genetik Antar Sembilan Aksesi Kelapa Asal Provinsi Sulawesi Utara. *Jurnal Littri* **15** (3): 139-144

[25] Yang, B., M. Zhou., W. S. Shu., C. Y. Lan., Z. H. Ye., R. L. Qiu., Y. C. Jie., G. X. Cui., dan M. H. Wong. 2010. Constitutional Tolerance to Heavy Metals of a Fiber Crop, Ramie (*Boehmeria nivea*), and its Potential Usage. *Environmental Pollution* **158**: 551–558.

[26] Zhu, S., S. Tang., Q. Tang, dan T. Liu. 2014. Genome-Wide Transcriptional Changes of Ramie (*Boehmeria nivea* L. Gaud) in Response to Root-Lesion Nematode Infection. *Gene* **552**: 67–74.