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Study of Genetic Variability 30 Rice Genotypes *(Oryza sativa* L.*)* Using Some SSR (Simple Sequence Repeat) Markers Adrift Zn

Studi Variabilitas Genetik 30 Genotipe Padi (*Oryza Sativa* L.) Menggunakan Beberapa Marka SSR (*Simple Sequence Repeat*) Terpaut Zn

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ABSTRACT

Rice is one of the important commodities in indonesia. Analysis of rice genetic diversity is necesarry for the succes of the local rice variety program. This study aims to identify the genetic variability and kinship patterns of 30 rice genotypes (Oryza sativa L.) using six zinc-linked SSR (Simple Sequence Repeat) markers. The research was conducted in September - December 2021 at the DNA Laboratory of the Sukamandi Rice Plant Research Center. A total of 30 local rice genotypes with diverse Zinc content have been analyzed using laboratory experiments. The results showed that there were different allele variations (2 – 8) among the genotypes tested with an average number of alleles of 4.5, while the average value of Polymorphism Information Content (PIC) amounted to 0.48 (0.20 - 0.70). 4 SSR markers have PIC values of > 0.5 (RM162, RM38, RM30, and RM80) which show that the markers are informative for the study of rice genetic diversity with Zinc content variety with an average gene diversity value of 0.53. The results of the phylogenetic analysis showed that the 30 genotypes clustered into five clusters with a similar coefficient of 0.68.

Keywords: *Rice, genetic diversity, SSR markers, Zn*

ABSTRAK

Padi merupakan salah satu komoditas penting di Indonesia. analisis keragaman genetik padi diperlukan untuk keberhasilan program pemuliaan varietas padi lokal. Penelitian ini bertujuan untuk mengidentifikasi variabilitas genetik dan pola kekerabatan tiga puluh genotipe padi (*Oryza sativa* L) menggunakan beberapa marka SSR terpaut Zink. Penelitian dilaksanakan pada bulan September - Desember 2021 di Laboratorium DNA Balai Besar Penelitian Tanaman Padi, Sukamandi. Sebanyak 30 genotipe padi lokal dengan kandungan Zn yang beragam telah dianalisis menggunakan eksperimen laboratorium. Hasil penelitian menunjukkan bahwa terdapat variasi alel yang berbeda (2 - 8) di antara genotipe-genotipe yang di uji dengan rerata jumlah alel 4,5, sedangkan rerata nilai *Polymorphism Information Content* (PIC) sebesar 0,48 (0,20 - 0,70). Terdapat 4 marka SSR yang memiliki nilai PIC > 0,5 yaitu RM162, RM38, RM30 dan RM80 yang menunjukkan bahwa marka-marka tersebut informatif untuk studi keragaman genetik padi dengan ragam kandungan Zn dengan rerata nilai diversitas gen sebesar 0,53. Hasil analisis filogenetik menunjukkan bahwa 30 genotipe tersebut mengelompok menjadi lima kelompok dengan koefisien kemiripan 0,68.

Kata Kunci: padi, keragaman genetik, marka SSR, zn

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the important food crops in human life. As the main food of the community, rice serves as a provider of nutrients for humans (Hamam et al., 2017). One of the nutrients that humans need is micronutrients such as Fe and Zn.

However, rice is known to have inadequate Zn nutrients that have the potential to cause malnutrition for people who consume (Ahmadi & Rukadi, 2019). The optimum daily zinc dose for humans is at least 34.7-43.4 ppm; while the zinc content in VUB rice (New Superior Variety) is 23.9 ppm, this dose has not even reached the optimal daily dose of Zinc for humans (Hamam et al., 2017). In addition to resulting in decreased endurance, productivity and quality of human life, Zn deficiency is also one of the factors that cause stunting or dwarfism.

According to Welch & Graham (2004) nutritional deficiencies especially iron (Fe), zinc (Zn) and vitamin A are the cause of nearly two-thirds of children's deaths in the world. Therefore, efforts to improve the quality of rice containing Zn need to be improved and more vigorously so that it can contribute to increasing nutritional value and public health (Liyanan et al., 2015).

The nutritional content of foodstuffs can be improved through plant breeding, both conventionally and non-conventionally (biotechnology) (Indrasari, 2018). Plant breeding is a series of plant genetic research and development activities to assemble cultivars / superior varieties that are useful to humans (Carsono et al., 2014). Plant breeding is divided into conventional and non-conventional plant breeding.

Non-conventional plant breeding in the form of DNA-based molecular markers has been widely used in plant breeding because it has several advantages that can be taken into account including having the speed to identify different species and being able to distinguish analyzing kinship more efficiently and accurately and not affected by the environment (Rohaeni et al., 2016).

The molecular marker SSR is one of the most widely used markers for genetic mapping, diversity analysis and evolutionary studies (Pasaribu et al., 2017). SSR markers have several advantages compared to other markers, namely codominant because they can distinguish heterozygous alleles and homozygous alleles, loci are evenly distributed in the genome, have high polymorphisms, and require only a small amount of DNA.

Molecular markers in the form of SSR microsatellite marks (Simple Sequence Repeat) used in this study aimed to identify genetic variability and kinship patterns of thirty local rice genotypes (*Oryza sativa* L).

METHODS

The research was carried out from September 2021 to December 2021 at the DNA Laboratory, Rice Plant Research Center located on Jl. Raya 9 Sukamandi Village, Ciasem District, Subang Regency, West Java. The research location is located at the coordinate point 6021'18"S 170038'44"E with a height of 16 meters above sea level.

The materials used are 70% cold isopropanol, Red PCR master mix (thermo scientific), acrylamide 8%, Acrylamid 40%, Tsp 10x, bisacrylamide, APS, TEMED, PCRBIO Ladder III 50 - 1500 bp parafilm, aluminum foil, alcohol, aquades, aquabidesr (ddH2O), tissue paper, rubber gloves, Gel red, mineral oil, and DNA extract 30 rice genotypes (Oryza sativa L) (table 1) : Fatmawati, Mahakam, Asahan, Semeru, Barito, Pepe, Gilirang, Tapus, Tondano, Konawe, Seratus Malam, Batang Ombilin, Batang Lembang, Lalan, Cipunegara, Diah Suci, Seililin, Barumun, Batang Sumani, Wera, Ciapus, Singkil, Mendawak, Cikapundung, Batang Piaman, Air Tenggulang, Situ Gintung, Walanay, Batanghari, Maninjau and 6 microsatellite markings (SSR) : RM162, RM3331, RM80, RM30, RM38, RM439.

The tools used are micro tube 96 well, vortex, freezer, refrigerator, electrophoresis (polyacrylamide), PCR (Therma Cycler), PCR Rotate, Chambell well, micropipet size

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1-50 µl, 100-500 µl, and 200-1000 µl, micro tip (white, yellow, and blue), measuring glass cup, UV Tray, silk, Taper tooth, Glass mold, gel doc/UV transilluminator, mobile phone, scales, magnetic stirrer, mask and stationery.

Table 1. List of rice varieties used in research.

No.	Accession Number	Varieties	Fe (ppm)	Zn (ppm)*
1	1692	Fatmawati	15.10	23.30
2	4180	Mahakam	12.50	22.80
3	1551	Asahan	13.50	22.70
4	7901	Semeru	13.90	22.70
5	1717	Barito	14.20	22.40
6	5893	Pepe	11.60	22.20
7	1205	Gilirang	12.70	22.10
8	4468	Tapus	12.60	22.10
9	4940	Tondano	13.50	22.00
10	1197	Konawe	11.00	21.90
11	723	Seratus Malam	12.20	21.60
12	1757	Batang Ombilin	10.60	21.30
13	6661	Batang Lembang	15.80	21.20
14	1470	Lalan	12.10	21.20
15	4186	Cipunegara	13.20	21.10
16	1664	Diah Suci	12.90	21.10
17	7323	Seililin	12.50	21.10
18	1140	Barumun	13.10	20.80
19	6665	Batang Sumani	11.70	20.70
20	1170	Wera	13.20	20.70
21	1719	Ciapus	13.20	20.60
22	1190	Singkil	13.30	20.50
23	1171	Mendawak	12.30	20.50
24	7309	Cikapundung	13.20	20.10
25	6662	Batang Piaman	12.70	20.00
26	7308	Air Tenggulang	14.50	19.90
27	1725	Situ Gintung	15.10	19.90
28	1202	Walanay	12.20	19.90
29	1153	Batanghari	12.80	19.80
30	1189	Maninjau	11.80	19.80

Description: *Zn content in Ppm (data obtained from the Rice Plant Research Center)

PCR (Polymerase Chain Reaction)

PCR is performed using a total volume of 31.50 µl consisting of 10 µl Master Mix, 0.25 µl primer forward, 0.25 µl reverse primer, 3 µl DNA template, 8 µl ddH2O and 10 µl Mineral Oil.

The PCR program is run with a predefined temperature setting. Pre-denaturation temperature regulation is carried out at 95^{o}C for 3 minutes, then followed by denaturation stage with a temperature of 95^{o}C for 15 seconds per cycle for 35 cycles, the 55^{o}C annealing stage for 15 seconds per cycle, then the extension stage of 72^{o}C for 15 seconds per cycle, ending with a 72^{o}C final extension stage for 1 minute.

Electroforesis (PAGE)

PCR DNA band separation is performed by electrophoresis using 8% PAGE (Polyacrilamide Gel Electrophoresis). Each well contains 3 µl samples of each DNA and two wells containing a DNA

ladder of 1 µl. Electrophoresis is run with an electric current of 60 V for 120 minutes. Visualization of the detected fragments is carried out using immersion in a solution containing 200 ml of ddH2O and 20 µl of red gel. Furthermore, the DNA band is visualized using a UV Transilluminator connected to the computer.

Data Analysis

Analysis of electrophoresis data is carried out based on the results of scoring or assessing the appearance of DNA band values which are binary data in microsoft excel programs. Value (1) is given for positive amplification describing observed alleles, while value (0) is given for negative amplification describing the absence of observed alleles. The allele diversity profiles i.e. the number of alleles per locus, the frequency of major alleles, the diversity of alleles, and the value of polymorphism information content (PIC) are performed using Power Marker 3.25 software. Then, made kinship tree / philogram between each accession. The software used is NT Edit to import scoring data and cluster analysis is carried out based on the results of molecular marking scores using NTSys software.

PIC quantity is based on the number of alleles produced by a mark and the frequency of each allele tested. The PIC value indicates that the SSR used is informative enough to see the diversity between genotypes. The greater the PIC value in a primer, the better the primer for the SSR mark.

Mathematically, the calculation of pic values is as follows (Liu, n.d.).

$$PIC_i = 1-\sum^{n}_{j=1} Pij^2$$

Pij^2 = frequency of allele j on locus i and n = the number of alleles on the locus.

The coefficient of genetic distance is represented by the value of geometric distance (Nei & Takezaki, 1994). Calculations are carried out assuming there are no mutations and all allele frequency changes occur due to genetic deviations.

$$D_A = 1 - \frac{1}{m}\sum_{j=1}^{m}\sum_{t=1}^{aj}\sqrt{\rho_{ij}q_{ij}}$$

DA = coefficient of genetic distance, ρij = allele frequency i at locus j in population P, qij = allele frequency i at locus j in population Q, m = number of locus examined, and αj = number of alleles at locus j.

RESULTS AND DISCUSSIONS

DNA Visualization

DNA visualization is done using Doc Gel uv transilluminator after previously soaking the gel in a coloring tray containing red gel (dye). The results of the DNA visualization showed that the amplification of the SSR mark adrift Zn in the test had different characteristics and amplification capabilities (Figure 1).

DNA amplification results showed the presence of DNA bands smeared on some of the markings tested. Smears show that the DNA tape is dirty because it is contaminated by other compounds that are also extracted so that when visualized the resulting DNA tape is not good. In addition, smears can occur due to several other factors, namely the UV wavelength used and the time used when electrophoresis (Juniar, 2021).

Diversity of SSR Markings

Based on the scoring results, the allele produced by the 6 primary SSRs used gives the results of the polymorphic band. The allele diversity profile in the form of the number of alleles per locus, the frequency of major alleles, the diversity of alleles, and the value of polymorphism information content (PIC) is presented in table 2.

Each primer has a different profile. The results of the analysis showed that the number of alleles produced from the 6 SSR markings used was 2 – 8 with an average of 4.5 alleles per locus. Locus RM162 is the mark that produces the most allele variations, namely as many as 8 alleles with a primer of RM3331 and RM439 being the primary which produces the lowest allele

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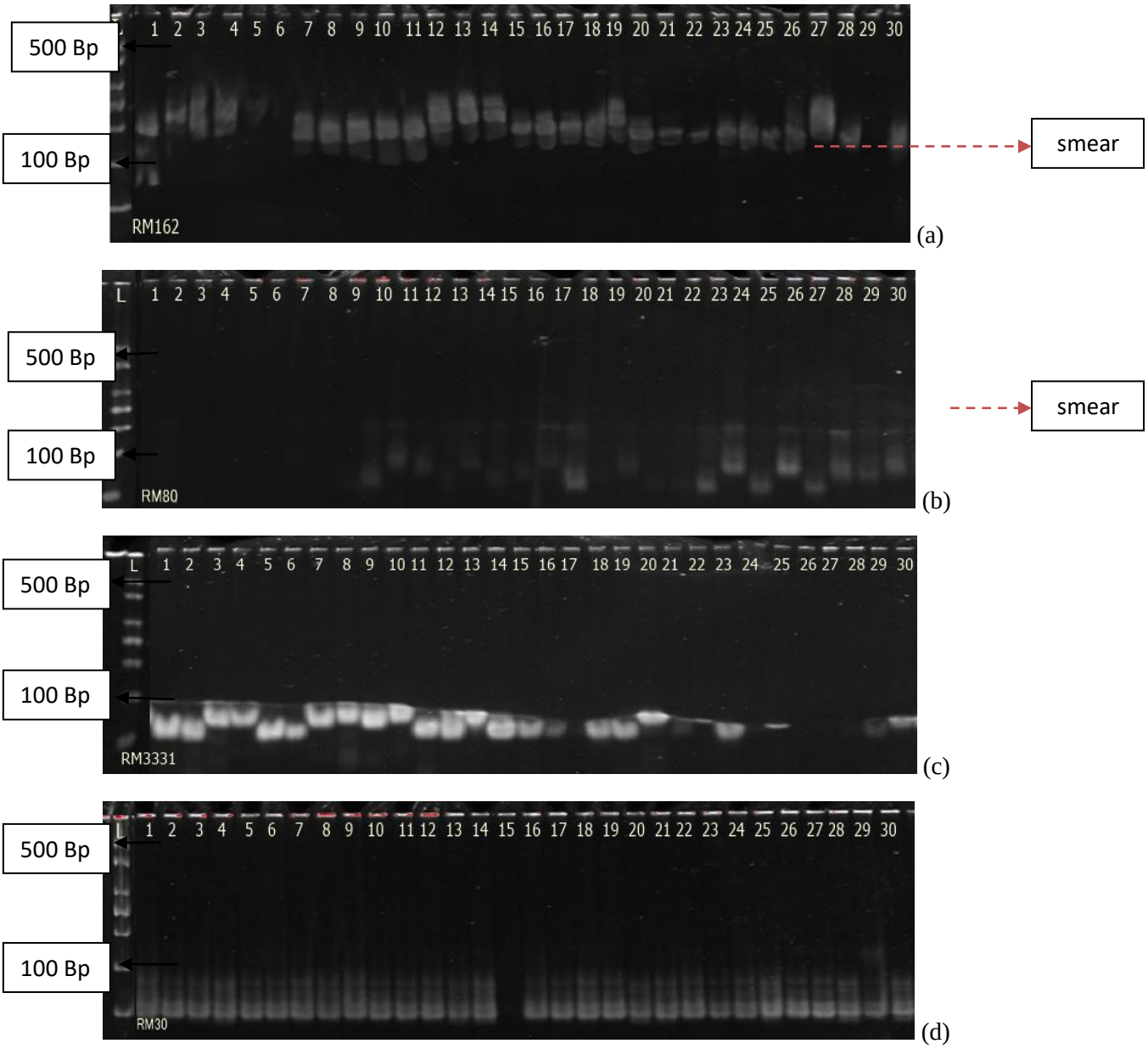
variation, which is as much as 2 alleles per locus. This shows that the RM162 mark is the best marker for identifying genetic variations when viewed from the variations of the allele identified.

The average frequency of the main allele obtained is 59% with the lowest value of 40% on the RM162 mark and the highest value of 86% on the RM439 mark. Gene diversity values show the level of genetic uniformity in a population ranges from 0.2778 (RM3331) to 0.7400 (RM162) with an average of 0.5307.

Pic scores of the 6 primers tested ranged from 0.20 - 0.70 with an average of 0.48. This indicates that the SSR mark used is able to detect polymorphism in a population by 20-70%. The lowest PIC value is found in the primary RM439 of 0.20, while the highest PIC value which has a value of > 0.5 is generated by the primary RM162, RM38, RM30 and RM80. This indicates that the four primers are polymorphic and informative primers.



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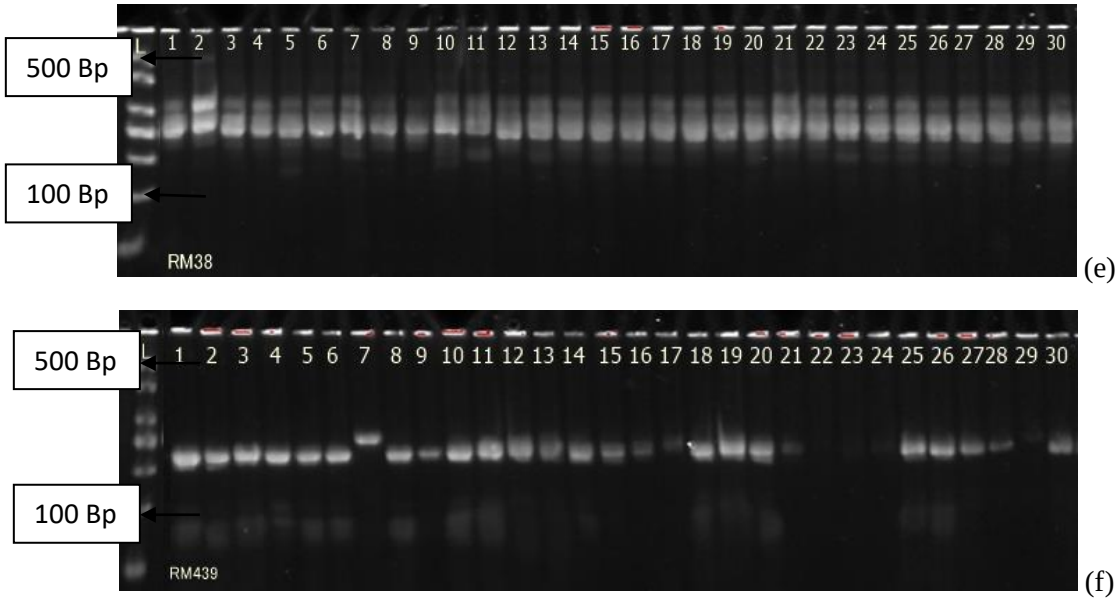

Figure 1. DNA Visualization Results 30 Rice genotypes using the RM162 (a); RM80 (b); RM3331 (c); RM30 (d); RM38 (e); and RM439(f) on 8% Acrylamide gel.

Table 2. Profile of 6 SSR markers adrift Zn on 30 rice genotypes

Markers	Number of Alleles	Main Allele Frequency	Gene diversity	PIC
RM162	8	0,4000	0,7400	0,70
RM3331	2	0,8333	0,2778	0,24
RM80	3	0,5000	0,6111	0,53
RM38	7	0,4833	0,6928	0,65
RM439	2	0,8667	0,2311	0,20
RM30	5	0,4833	0,6317	0,56
Mean	4,5	0,5944	0,5307	0,48

Sulistyawati (2017) stated that the amount of genetic diversity in the population is determined by the number of polymorphic genes and the number of alleles produced in each of these genes. Xing et al. (2005) states that the value of polymorphic information is directly proportional to the number of alleles produced on each locus. The higher the number of alleles, the higher the PIC value. Markers with a large number of alleles tend to have a higher PIC value, so they are considered more informative.

The results of the Powermarker analysis showed that the number of alleles produced from the 6 SSR markers used was 2 – 8 with an average of 4.5 alleles per locus. Of the 6 SSR markings tested, the RM162 locus was the marking that produced the most allele variation is as many as 8 alleles, then the RM38 locus produces 7 alleles, the RM30 locus produces 5 alleles, the RM80 locus produces 3 alleles, then the locus RM3331 and RM439 produces 2 alleles each which makes RM3331 and RM439 the lowest allele variation generating markers. This shows that the RM162 mark is the best mark for identifying the presence of genetic variation in terms of the variation in the alleles identified.

The Polymorphic Informative Content (PIC) value is used as a standard for evaluating genetic markings based on DNA bands resulting from PCR amplification. According to Botstein et al. (1980), PIC

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values are divided into 3 parts i.e. PIC > 0.5 = markings used are very informative, 0.25 > PIC > 0.5 = the markings used have a moderate level of informativeness and PIC < 0.25 = low level of informativeness of the markings.

The PIC values of the 6 primers tested ranged from 0.20 - 0.70 with an average of 0.48. This indicates that the SSR markings used are able to detect polymorphism in a population of 20-70%. The lowest PIC value is found in the RM439 primary of 0.20, while the highest PIC value having a value of > 0.5 is generated by the RM162, RM38, RM30 and RM80 primers (Table 2). This shows that the four primers are polymorphic and informative primers.

Genetic Diversity 30 Rice Genotypes

Genetic diversity analysis is analyzed using the UPGMA method. The matrix of genetic similarity values (genetic distance) is further determined using the Unweighted Pair Group Method Arithmetic (UPGMA) cluster analysis method which will then obtain genotype grouping with the formation of kinship dendograms between the 30 rice genotypes tested.

The genetic diversity of 30 rice genotypes using 6 SSR markings (Figure 2) has a coefficient of similarity that ranges from 0.51 – 0.97 (51% - 97%) or with a diversity value of 0 - 46%. At a similarity level of 0.68 or 68% obtained by five groups, group I consists of Fatmawati, Mahakam, Barito, Pepe, Asahan, Semeru, Tapus, Tondano. Group II consists of Gilirang, Konawe, Seratus Malam. Group III consists of Batang Ombilin, Batang Lembang, Batang Sumani, Lalan. Group IV consists of Ciapus, Singkil, Mendawak, Cikapundung, Air Tenggulang, Diah Suci, Barumun, Batang Piaman, Seililin, Situ Gintung, Batang Hari, Wera, Walanay, Maninjau. Group V consists of only one genotype, Cipunegara.

The degree of cophysien similarity can determine the degree of kinship of the genotype tested. The similarity coefficient of 0.51 – 0.97 indicates that the 30 genotypes tested had kinship levels from highly related to distant relatives and had wide genetic diversity. According to Lucasz (2015), if the coefficient of genetic similarity >0.6 then the similarity of the genotype to each other is closely related or very related, while the coefficient of genetic similarity with the value of <0.6 then it can be said that the genotype in the test has a distant kinship.

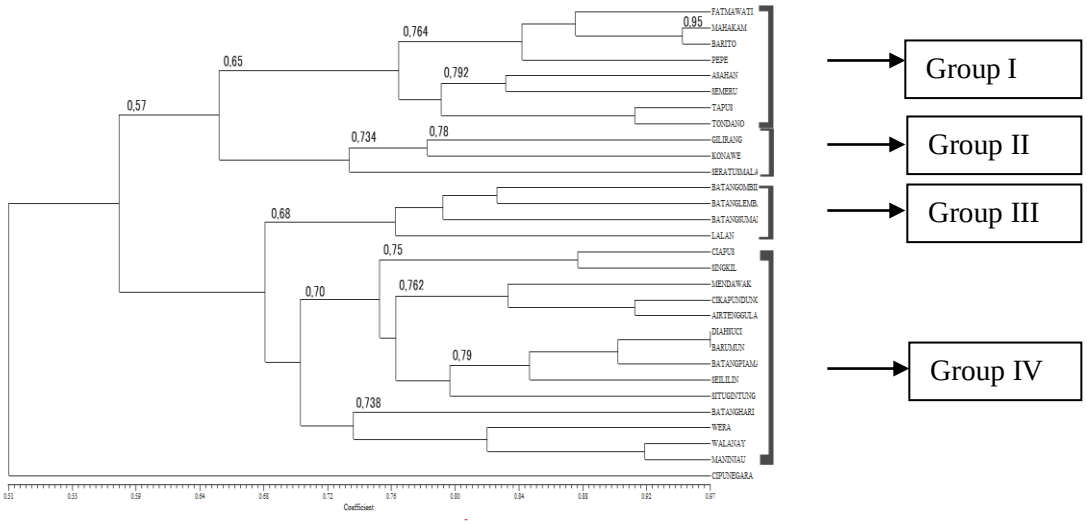

Figure 2. UPGMA Dendogram Shows Genetic Link Between 30 Rice Genotypes Using 6 SSR Markings

The formed dendogram describes the closely related kinship relationship between genotypes with each other (the coefficient value of genetic similarity >0.6). Judging from the Zn content owned by each genotype, groups I and II have a higher Zn

content compared to groups III and IV (Table 1).

From the dendogram formed, the Cipunegara variety is separated into one different group from the other genotype, this shows that Cipunegara has a different character with all the genotypes tested. Based on genetic distance analysis, Diah Suci and Barumun varieties have the highest genetic distance of 97%, indicating that molecularly these two varieties have a high genotype similarity. The high value of the coefficient of similarity indicates that there are similarities between variables so that there can be a close kinship relationship. Acquaah (2007) explained that the farther the genetic distance, the easier the crossing process. Varieties that have the furthest level of kinship will have the potential to be used as prospective crossing elders. Crossing rice genotypes with the farthest genetic distance, even between species, has the potential to obtain progeny with superior more traits (Terryana, 2020). According to Sutoro et al. (2017), crossing between prospective elders with a long genetic distance will maximize the opportunity in obtaining transgressive aggression among cross progeny.

CONCLUSION

Based on the results, it can be concluded that the 30 rice genotypes tested showed a polymorphism level of 0.20-0.70 with an average of 0.48 and had 27 alleles with an average value of 4.5 alleles per locus. The coefficient of genetic similarity of 0.68 formed 5 groups with the highest number of genotypes being Group IV which consisted of 14 genotypes. Meanwhile, the group with the few genotypes is the Group V which consisted of 1 genotype.

Based on the kinship of the 30 genotypes, the genetic distance values ranging from 0.51 to 0.97 were obtained. This indicates that the genetic variability of 30 rice genotypes based on Zn-linked SSRs (Simple Sequence Repeats) markers can be said to be quite wide.

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