Genetic Diversity of Local Rice (*Oryza sativa* L.) from North Sumatera Using Simple Sequence Repeat (SSR)

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**Abstract.** Genetic diversity of rice in Sumatera Utara have been reported by using any molecular markers, and one of the markers is Simple Sequence Repeat (SSR). The research used 4 SSR primers such as RM 20, RM 580, RM 413 and RM 131 to analysis the genetic diversity analysis from 12 collection of rices (11 local cultivars from North Sumatera and 1 cultivar of hybrid rice). The alleles were amplified using Polymerase Chain Reaction (PCR), and amplicons were visualized in agarose gel-electrophoresis. The result showed that 82 DNA bands were detected, with 200-1769 bp from all primer s being used. The highest number of polymorphic bands were from RM 20 primer with 19 DNA bands, and the lowest from RM 131 with 9 DNA bands. From Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis, 12 collection of rices was clustered in similarity coefficient value of 0,58, on similarity coefficient value of 0,74 the collections were further clustered into 5 groups. Two groups was clustered based on their distribution area, Kabupaten Labuhan Batu Utara and Kabupaten Karo, while 3 other groups were clustered randomly.

1. Introduction

North Sumatra has many valuable natural resources and is known as one of the Indonesian rice granaries [1]. North Sumatera local rice cultivars are quite diverse, and optimally utilized in plant breeding. However, hybrid rice is tend to be more cultivated than local rice in North Sumatera nowadays. The cultivars has declined and certain cultivar has lost from circulation and no longer can be found. The switch of land use from highland paddy field ecosystem into vegetable and other horticulture plantation, became the trigger of declining local rice cultivars population. In the future, local cultivars will face genetic erosion and in worst scenario, extinction [2]. One of valuable traits that local cultivars possessed are their superior adaptability compared to modern or hybrid cultivars, especially towards resistance to biotic stress (pests and diseases) and abiotic stress (nutrient toxicity, suboptimal biophysical environment), high grain quality and unique tastes.

Genetic traits determination can be obtained from agronomic properties, morphological characters and molecular markers. Molecular markers can be used to give results of rapid genetic diversity. Until now, there are many DNA-based molecular markers, such as the Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), and Single Nucleotide Polymorphism (SNP) [3]. The SSR markers are being widely used in genetic analysis due to their following features: codominant marker, able to detect high-level allele diversity, ease of use, cheap and in compatible use with Polymerase Chain Reaction (PCR) [4].
The SSR markers have been reported to distinguish among local black rice cultivars and also among other local aromatic and non-aromatic rice cultivars [5,6]. However, the information regarding the application of SSR markers for genetic analysis of local cultivars from North Sumatera is still limited. Hence, the research will provide additional information to our knowledge about the local endangered cultivars based on their localities.

2. Materials and Methods

2.1 Samples Collection

The plant materials used in this study were 12 rice collections consisting of 11 local rice collection of North Sumatera namely Sigudang, Sigambi, Siasahan, Siorsik, Ramos, Kukubalam, Talipuyu, Sigambiri Merah, Sigambiri Putih, Martabe and Mandailing, and 1 hybrid rice namely IR64. Samples were collection from Balai Pengkajian Teknologi Pertanian (BPTP) Medan, North Sumatra, from Balai Benih Padi Sukamandi, Subang sub-district, West Java and from the local farmers (Labuhan Batu Utara and South Tapanuli district). All seedlings up to 2-3 weeks old were isolated for their DNAs.

2.2 DNA Isolation

DNA isolation was performed using a modified CTAB (Cetyl Methyl Ammonium Bromide) method [7]. Leave samples weighing 0.1 g were cut into small pieces and placed into mortar containing 700 μL 2% CTAB extraction buffer (20 mM EDTA, 0.1 M Tris-HCL pH 8.0, 1.4 M NaCl, 2% CTAB, and 0.4% β-mercaptoethanol) pre-heating at 65 °C, then crushed by pestle. The samples then were filled into 1.5 mL of microtubes, and incubated at 65 °C for 45 minutes then mixed with 500 μL phenol-chloroform-isoamyl alcohol (25:24:1) following homogenization. The samples were centrifuged at 12,000 rpm for 10 minutes at 4 °C. The supernatant was transferred to a new tube and 500 μL phenol-chloroform-isoamyl alcohol (25:24:1) was added. During this stage, the work was done twice. The supernatant was transferred to a new tube and added with 700 μL cold isopropyl alcohol following incubation in the freezer for 2 hours. The sample was centrifuged at 12,000 rpm for 10 minutes at 4 °C. The pellets were washed with 70% alcohol and centrifuged at 12,000 rpm for 10 minutes at 4 °C. The pellet was dried and later dissolved with 100 μL ddH2O and incubated for 1 hour at 37 °C, then the solution was stored at -20 °C as stock solution.

2.3 DNA Amplification

DNA amplification was performed in PCR within a volume of 25 μL with a reaction mixture consisted of GoTaq® Green Master Mix solution, RNAse free aquadest, Primers (Macrogen, Inc. Korea), and DNA template. The PCR program used was specified as follows: 35 cycles, initial denaturation at 94 °C for 1 minute, annealing at 51.3-55.5 °C for 51 minutes, extension at 72 °C for 1 minute and final extension at 72 °C for 5 minutes. The primers used in this study were: RM 20, RM 580, RM 413 and RM 131 with distinct specification (Table 1).

| Primers   | Primer Sequence (F: Forward/ R: Reverse) | Annealing Temperature (°C) |
|-----------|-----------------------------------------|---------------------------|
| RM 580    | F: GATGAACCTGAAATTTGAGATCC R: CACTCCCATGTATTGAACTCC | 54.7°C                    |
| RM 131    | F: TCCCTCCCTCCCTCGCCCACTG R: CGATGTTCGAGCATTGTCCTCC | 54.7°C                    |
| RM 413    | F: GCCTCCCTTCGGATGAGAG R: TCCCACAACTGCTTCCT | 55.5°C                    |
| RM 20     | F: ATCTTGTCCCTGCAAGGTCAT R: GAAACAGGAGCAGATTTTCAT | 51.3°C                    |
2.4 DNA Visualization & Bands Scoring

The PCR amplicons were pipetted 10 μL and mixed with loading dye prior insertion into agarose gel wells. Electrophoresis was run at 100 V for 45 min. The gel then was soaked within 10 μL Ethyidium Bromide (EtBr) solution (10 μL in 1 L distilled water) for 10 minutes. The gel was visualized under UV trans-illuminator and documented for the presence of DNA or polymorphic bands. The resulting DNA profile was translated into binary data, in which score 1 (one) indicating presence of band and 0 (zero) indicating absence of band.

2.5 Data Analysis

Estimation of genetic diversity was analyzed from dendrogram synthesized using Numerical Taxonomy and Multivariate Analysis System (NTSys) software Version 2.02. Data were grouped by using Unweighted Pair Group Method with Arithmetic Mean Analysis (UPGMA) method based on Dice- Similarity Coefficient, in view of genetic distance among samples.

3. Results and Discussion

3.1 DNA Profile of 12 Rice Collections

Amplification of total DNA from local and hybrid rice samples by using 4 SSR markers: primers RM 580, RM 413, RM 131 and RM 20 are shown in Figure 1. The four primers used in this study were adapted from a study in analyzing the diversity of international rice collections [8].

All amplicon size were ranging between 603 to 1769 bp with number of bands formed during amplification as many as 89 bands. The amplicon bands of the entire rice collection using the 4 primers showed the different patterns on each primer. This indicated the presence of polymorphism in all resulting bands (Table 2). DNA Polymorphism is characterized by the presence and absence of bands in a sample as well as the difference in the size of the bands produced [9].

| Primers | Size Range of Bands (bp) | Number of Polymorphic Bands | Number of Monomorphic Bands | Total Bands | Percentage of Polymorphic Bands (%) |
|---------|--------------------------|------------------------------|-----------------------------|-------------|------------------------------------|
| RM 580  | 603 – 1578               | 12                           | 12                          | 24          | 50                                 |
| RM 20   | 200 – 1769               | 19                           | 10                          | 29          | 65,52                              |
| RM 131  | 793 – 1685               | 9                            | 7                           | 16          | 56,25                              |
| RM 413  | 1431 – 1706              | 11                           | 2                           | 13          | 84,61                              |
| Total   | 51                       | 31                           | 82                          | 64,1        |                                    |
The highest percentage of polymorphic bands were produced from RM 413 primers with value of 84.61% while the lowest was produced from RM 580 primers with value of 50%. The largest number of bands were produced from RM 20 primers as many as 29 DNA bands while the lowest from RM 413 producing only 13 DNA bands. In general, the four primers used in this analysis can be categorized as primers that have high polymorphism, yet were applicable primers.

### 3.2 Genetic Similarity Analysis from 12 Rice Collections

The genetic relationship between each of rice collections used in this study can be determined by analyzing genetic similarity between samples, from the value of the similarity coefficient. The coefficient values of similarity are presented in the form of dendogram (Figure 2).

![Dendrogram clustering of rice cultivars, as determined by SSR analysis](image)

**Figure 2.** Dendrogram clustering of rice cultivars, as determined by SSR analysis

The dendrogram showed that 12 rice collections were grouped in similar coefficient of 0.58. The 12 collections were further grouped into 5 groups in similar coefficient of 0.74. Two groups were clustered based on the origins of distribution: Group 1 and 3. Group 1 consisted of IR64 (hybrid cultivar), Sigambi, Ramos and Kukubalam collected from Labuhan Batu Utara, while Group 3 consisted of Sigambiri Merah and Sigambiri Putih originating from Karo and Simalungun districts.

Group 2 consisted of Sigudang, Siasahan and Talipuyu rice cultivars. Group 4 consisted of only Siorsik, while Group 5 consisted of Martabe and Mandailing’s. The three groups are clustered randomly and not based on the origins of distribution. Furthermore, Group 2 is the only group in which entire collections were originating from the same region of South Tapanuli. But, rice collections from South Tapanuli are not only Sigudang, Siasahan and Talipuyu but also the Siorsik and Martabe’s. Yet, the latter two were not clustered together in Group 2. The Siorsik clustered on its own, while Martabe clustered together with Mandailing originating from Mandailing Natal district. The grouping of rice collections in Group 2, 4 and 5 occurred randomly and were from the same origins, North Sumatera, then the rice grains might have been mixed with other regions’. The rice grains were brought by the farmers and then cultivated it in farmers area.

As a result, the genetic diversity of rice collection is not based on the distribution of its territory. The dendrogram analysis that showed cultivars clustered into one group, depicted a large genetic similarity or a small genetic distance [5]. In general, North Sumatra rice cultivars or collections had a high genetic diversity, as shown from the similarity coefficient obtained in the value of 0.58-1.00. In addition, the similarity coefficient in the value of 1, showed absolute similarity while approaching
value of 0, means the further differences occurred. The further the genetic distance owned by an individual, the higher the genetic diversity.

Other study recommended the use of numerous SSR primers to produce more significant polymorphism. The study of genetic diversity of rice cultivars from India, were conducted using RAPD and SSR markers. The study concluded that the combined use of different genetic markers may help to minimize error in estimating genetic similarity between samples [10]. Although the resulting polymorphic bands might be different among cultivars in which other marker such as RAPD produced more polymorphic features than SSR [11]. The robust nature of SSR markers still provided better estimation of genetic diversity from aromatic and elite rice cultivars [12,13]. Although the trend of using SSR markers have been reported extensively by other countries, the future application in Indonesia is still needed to reveal the genetic diversity of local rice in other region.

4. Conclusions

Twelve local rice cultivars from North Sumatera showing considerable genetic diversity based on SSR markers. The total number of DNA bands resulted in 51 polymorphic and 31 monomorphic bands with band sizes ranging between 200 – 1769 bp. Five groups were clustered based on the similarity coefficient from dendrogram analysis. The results may be used for further rice breeding program or information regarding accurate identification of certain threatened rice cultivars.

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References

[1] Siregar H 1981 . Rice cultivation in Indonesia (Jakarta: PT. Sastra Husada)
[2] Daradjat AA, Silitonga, S and Nafisah 2015. Balai Besar Penelitian Tanaman Padi 1-10
[3] Susanto U, Sutrisno and Aswidinnoor H 2015 Balai Besar Penelitian Tanaman Padi 353-365
[4] Moeijopawiro S 2010 Buletin Plasma Nutfah 16
[5] Kristamtini, Taryono, Basunanda P and Murti RH 2014 Jurnal AgroBiogen. 10 69
[6] Padmadi B 2009 Identification of Aromatic Rice Cultivar Using Aromatic gene-based Markers (Bogor: Bogor Agricultural Institute)
[7] Borges A, Rosa MS, Recchia GH, de Queiroz-Silva JR, Bressan EA and Veasey EA 2009 Sci. Agric. 66 529
[8] Singh A, Saini R, Singh J, Arya M, Ram M, Pallavi, Mukul & Singh PK 2015 International journal of Agriculture, Eniroment and Biotechnology. 8 143
[9] Suryatini KY 2011. Genetic Diversity Analysis of Castor (Jatropha curcas L.) Using Simple Sequence Repeats (SSR). (Bali: Udayana University)
[10] Bansal H, Kumar R, Vivek and Sanjay 2013 African Journal of Biotechnology 12 5404
[11] Kanawapee N, Sanitchon J, Srihaban P and Theerakulpisut P 2011 Electronic Journal of Biotechnology 14 6
[12] Meti, N, Smala KC, Bastia DN and Rout GR 2013 African Journal of Biotechnology 12 4238
[13] Chakravarthi, BK and Naravaveni R 2006 African Journal of Biotechnology 5 684