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Genetic Diversity of Sweet Potato (*Ipomoea batatas* L.) in East Java, Indonesia

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ABSTRACT

Genetic diversity is a major source of germplasm for the selection and assembly of new clones. Genetic diversity determination within a population can be observed on the information on the genetic relationship of organisms concerned. This genetic relationship between two individuals can be measured based on the similarity of characters. Molecular markers have become a reference for information on genetic relationships more accurately and quickly. Sweet potatoes that are categorized as hexaploid having the high enough potential diversity, but often not exposed due to the phenotypic appearance similar to each other. Research was held in Plant Biotechnology Laboratory of Faculty of Agriculture, University of Brawijaya, Indonesia. Molecular analysis carried out by using four RAPD markers released by MDBio, Inc., i.e., SP20, SP06, A69, and A71. DNA was extracted with the CTAB method based on the modification of Doyle and Doyle[1]. Electrophoration was done on 0.8% agarose gel, and then visualized on UV transilluminator. Polymorphism of amplified DNA was photographed with Polaroid camera. The results of molecular analysis using RAPD markers showed that the clones Biru Ungu and Bestak had a genetic similarity of 78%, despite having different phenotype. Clones Mantang and Supra were distantly related based on the results of molecular analysis which showed the genetic similarity value of only 15%. RAPD Molecular marker produced dendrogram of 12 clones tested which was separated into 2 groups with the level of genetic similarity of 39% and 15%, respectively. The first group consisted of Mantang Kuning, Biru Ungu, Ase, Shogun, IR Melati, Pak Ong, Stemped and Bestak, while the second group consisted of Supra. There were three clones that did not produce DNA band amplification results, i.e., Mongkrong Putih, NK and Biru Mangsi so the genetic relationship of these three clones could not be identified.

Key words: Genetic diversity, Sweet potato, RAPD molecular marker analysis.

INTRODUCTION

Sweet potato is an important crop which ranks sixth of the world after wheat, rice, corn, potatoes and barley. Sweet potato germplasm is estimated at more than 1,000 species in the world but only 142 species are identified by researchers. Although the potential is quite large, but genetic studies as a basis for the development of cultivars is still limited [2]. Most plants in Indonesia are endemic, which means that its distribution is limited to certain geographical areas, or specific ecological units. This limited distribution of plant that makes endemism will reveal unique genetic diversity or biodiversity [3]. Patterns of endemism in the Indonesian archipelago are unique and depend on the history of a particular geographical area. The highest endemism of species found in Borneo and was followed by Sulawesi, Sumatra and the last one is Java [4]. The successful of genetic improvement through artificial crosses needs the knowledge about the genetic relationship between parental clones that will be selected as a source of genes [5].

Genetic relationship between two individuals can be measured based on the similarity of character with the assumption that the different character due to differences in genetic makeup. There are three types of markers that can be used to obtain information about the genetic relationship, i.e. biochemical markers, molecular markers and morphological markers. Molecular markers analysis needs to be done in order to know whether the sweet potato has a near or distant genetic relationship with the phenotype that vary due to environmental influences. Sweet potatoes generally have a fairly high diversity but are often not known because it looks similar to each other. Molecular markers have become a reference for information on genetic relationships more accurately and quickly. RAPD as one of some molecular markers analysis is not influenced solely by the environment, but it is easier to do and get results faster without the need for high skill such as use of other molecular markers analysis.

DNA sequences will allow scientists to discover the genetic relationship between organisms.

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Based on the research result, it is noted that organisms with a close relationship has the same DNA sequence, the more distantly related the same sequence number will be less [6]. Polymerase Chain Reaction (PCR) is a technique used to amplify specific location of DNA, using primer sets to synthesize DNA and initiated by a thermo stable DNA polymerase which is stable at high temperatures. Typically, the amplification result of specific DNA sites is abundant that can be detected using gel electrophoresis [7]. RAPD is one of amplification technique using the primers and PCR (gene-Cycler). DNA fragment was amplified by Polymerase Chain Reaction (PCR) using synthetic short primer (usually 10 bp) of randomly arranged sequences. These oligonucleotides serve as forward and reverse primer and can usually be amplified 30-10 genomic locations simultaneously. Amplified fragment was separated using gel electrophoresis and polymorphism will be detected as the presence or absence of bands. RAPD has been used for many purposes, varies from individual studies (eg genetic identity) to studies involving species that have a close relationship. Due to the abundance of the amplified genome, then RAPD has also been applied to studies of gene mapping [8]. Molecular markers such as DNA markers are suitable tools for determining genetic distances because of the polymorphic markers can be obtained more quickly than morphological markers [9]. RAPD procedure is cheaper, faster, requiring small DNA samples (0.5 to 50 ng) [10]. RAPD markers have been used to analyze the genetic variability among clones of sweet potato in America [11], in Chilli [12], in Malaysia [13] and detect linkage between RAPD markers with sweet potato resistance genes against nematodes [14].

MATERIALS AND METHODS

The research was conducted by taking samples in several centers of sweet potato planting area in East Java, Indonesia. Molecular marker analysis was conducted in Plant Biotechnology Laboratory, Faculty of Agriculture, and University of Brawijaya, Indonesia. The research was conducted during the period from January to May 2007.

The tools used during the research were mortar and pestle, PCR (gene-Cycler), Erlenmeyer tubes, Beaker glasses, Eppendorf tubes, micropipettes, Stirrer, hotplates, digital scales, Polaroid camera, water bath, centrifuge, spectrophotometer, cuvette, refrigerator, microwave, electrophoresis apparatus and UV transilluminator.

The material used for DNA stocks were 12 cultivars of sweet potato grown by farmers in sweet potatoes cultivation area in East Java, Indonesia. The twelve DNA stock materials were sweet potato clones Mongkrong Putih, Mantang Kuning, Biru Ungu, Biru Mangsi, Ase, NK, Bestak, Shogun, IR Melati, Stemped, Pak Ong, and Supra.

The materials used for DNA isolation were liquid nitrogen, CTAB buffer, β-Mercaptoethanol, PCI (Phenol, Chloroform, Isoamil alcohol), CI (Chloroform, Isoamil alcohol), ammonium acetate, absolute ethanol, ethanol 70%, TE (Tris-Cl, EDTA), TBE (Tris-Cl, Boric acid, Na2EDTA). Materials used for agarose gel electrophoresis were 1x TBE, ethidium bromide (EthBr), and loading dye.

PCR-RAPD technique performed using 4 types of primary, as presented in table 1.

Table1 The type and bases sequence of the four primers used

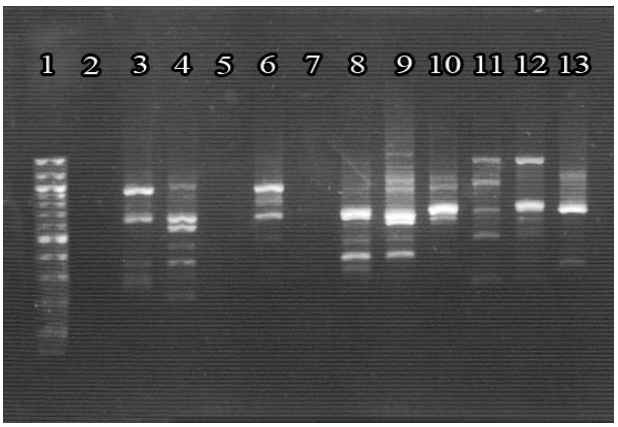
No	Type of Primer	Bases sequence (5'→3')
1.	SP20	GGC ACT GAG G
2.	SP06	CCG TGA CTC A
3.	A69	TGG TAC GGT ATA
4.	A71	ACT CTT CTA CAA

Molecular data was converted into binary data with an agreement that DNA band appearance in gel noted as 1 and 0 for the absent of the band as the results of electrophoresis.

RESULTS AND DISCUSSION

A result of molecular markers is to compare the DNA bands amplified from several clones with the assumption that each band carrying one particular trait. Genetic relationship can be explained in the form of dendrogram. Among the many existing molecular markers, RAPD-PCR markers used for several reasons, such as that it does not need a high level of purity, less expensive, produces more rapid polymorphic DNA band and all PCR-based molecular markers are not sensitive to changes in the surrounding environment [15].

Amplification products by PCR-RAPD can be observed using 0.8% agarose gels and 1x TBE solution. The result of electrophoresis of 12 sweet potato clones using the SP20 primer is presented in Figure 1:



1: DNA Ladder 100 bp
2: Mongkrong Putih
3: Mantang Kuning
4: Biru Ungu
5: Biru Mangsi
6: Ase
7: NK
8: Bestak
9: Shogun
10: IR Melati
11: Stemped
12: Pak Ong
13: Supra

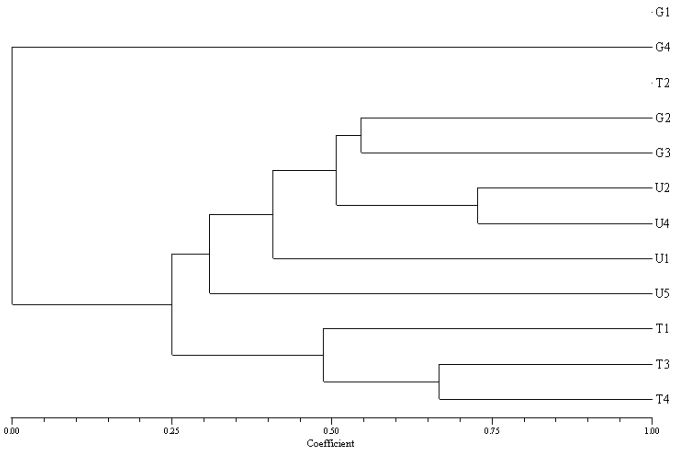
Figure 1 Electrophoresis of DNA Amplified by SP20 Primer

It showed that the present of amplified bands appeared mostly monomorphic. There were a total of 22 bands of amplified DNA, and 20 of them were monomorphic while only 2 of them were polymorphic bands. Some clones had DNA bands with the same size and supposed that these clones were related. DNA bands of different sizes resulting from the amplification using the same primer of RAPD were assumed to derive from different specific locations. Theoretically, the amount of amplified fragment length depends on the primer used and the size of the target genome. There were also seven bands sized 500 bp DNA in the clones Mantang Kuning, Ase, Bestak, Shogun, IR Melati, Stemped and Supra. These seven clones were supposed also had close genetic relationship. Clones Ase, Bestak, Shogun, Stemped and Pak Ong were member of one related genetic, because they contained at least three similar monomorphic bands at 300, 500, and 1000 bp.

There were 4 clones that were not amplified from 12 clones tested using the SP06 primer. It was supposed that the base pair was mismatch between this primer and the genome. Variations in band intensity of DNA amplification with two primers used looked very diverse. The amplified DNA bands used in this study was the DNA band that looked clear. The size of DNA bands that appeared using SP20 primer ranged between 200 bp to 1500 bp. While the size of DNA amplified bands using SP06 primer ranged between 300 bp to 1200 bp. This was consistent with that stated by Grattapaglia *et al* (1992) [16], which stated that in general, the number of base pairs that could be amplified in the genomes of plants ranging from 200-2000 bp and sometimes even up to 5000 bp.

There were 4 types of primers used in this research, i.e., SP06, SP20, A69 and A71 primers. However, only SP20 and SP06 primer produced bands of DNA amplification on clones tested. It was because the GC ratios of these primers were above 50%. GC ratio of SP20 primer was 70% while SP06 primer was 60%. A69 and A71 primer were only 33% and 34%, respectively.

The results obtained in elektroferogram were converted into a data matrix with 0 for the absence of amplified DNA bands, and 1 for the band present. Data matrix for the SP20 and SP06 primer were used to create the dendogram as shown in figure 2 and 3, respectively.



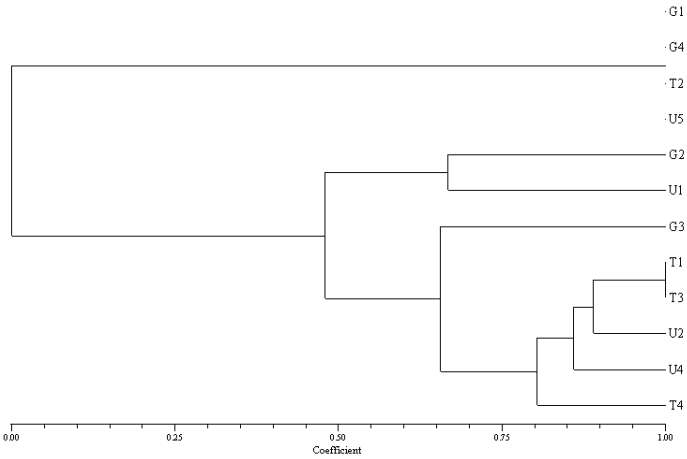
G1: Mongkrong Putih
G2: Mantang Kuning
G3: Biru Ungu
G4: Biru Mangsi
T1: Ase
T2: NK
T3: Bestak
T4: Shogun
U1: IR Melati
U2: Stemped
U4: Pak Ong
U5: Supra

Figure 2. Dendrogram for SP20 Primer

Figure 2 showed that SP20 primer resulted many polymorphic bands and separated the 12 clones tested into two major groups on the genetic similarity value of 25%.

- Group 1 consisted of clone Mantang Kuning, Biru Ungu, Stemped, Pak Ong, IR Melati, and Supra with the genetic similarity value of 31%.
- Group 2 consisted of clone Ase, Bestak, and Shogun with the genetic similarity value of 49%.

Mongkrong Putih, Biru Mangsi and NK clones were not included in any group in the dendrogram and were considered not related to other clones. Group 1 tend to be more diverse and the genetic relationships were more distant than group 2. It is shown by genetic similarity values of the group which was less than 50%. Group 2 was more closely related and was identified by genetic similarity value of 49% and 67%.



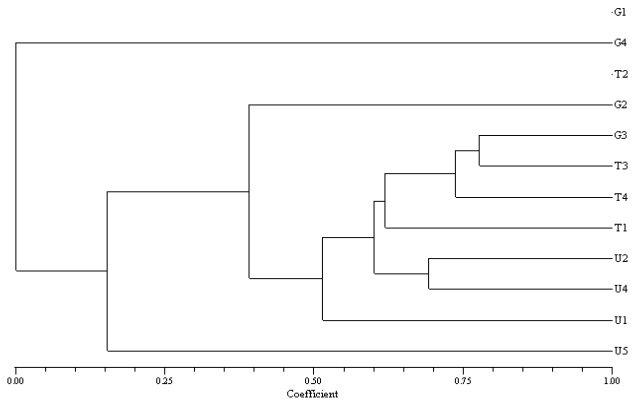
G1: Mongkrong Putih
G2: Mantang Kuning
G3: Biru Ungu
G4: Biru Mangsi
T1: Ase
T2: NK
T3: Bestak
T4: Shogun
U1: IR Melati
U2: Stemped
U4: Pak Ong
U5: Supra

Figure 3. Dendrogram for SP06 Primer

Figure 3 showed that Ase and Bestak clones had genetic similarity value equal to 1, which could make them actually considered to be closely related or identical clones based on DNA band amplification result. The other clones tested were divided into two major groups on the value of genetic similarity of 48%, i.e.:

- Group 1 consisted of Mantang Kuning, and IR Melati clone with the genetic similarity value of 67%.
- Group 2 consisted of Biru Ungu, Stemped, Pak Ong, Shogun, Ase and Bestak clone with genetic similarity value of 66%.

Most of the clones had a fairly close genetic relationship, especially Ase and Bestak clone that had the genetic similarity values equal to 100%. Ase, Bestak, Stemped, Pak Ong, and Shogun clone proved to have a close genetic relationship. The value of genetic similarity among the five clones, which had three monomorphic bands, was 80%. The monomorphic band was assumed carried the same genes. If many clones had the same monomorphic bands, it was presumably corresponding clones had a closer genetic relationship. These genes can be expressed in the morphology of the clones in question, such as the dominant color of the vine.
Dendrogram of combined primer is presented in Figure 4 below:



G1: Mongkrong Putih
G2: Mantang Kuning
G3: Biru Ungu
G4: Biru Mangsi
T1: Ase
T2: NK
T3: Bestak
T4: Shogun
U1: IR Melati
U2: Stemped
U4: Pak Ong
U5: Supra

Figure 4 Dendrogram of Combined Primer

There are 3 clones, namely Mongkrong Putih, Biru Mangsi and NK that did not express DNA amplification and therefore could not be related genetically with other clones. Dendrogram in figure 4 above provide information that:

- Biru Ungu and Bestak clone had high levels of genetic similarity at 78%, both of which were related to Shogun at 74% similarity. These three clones were grouped together with Ase at the genetic similarity value of 62%.
- Stemped and Pak Ong had a degree of genetic similarity of 70% and then allied with the four other clones at the level of similarity above 60%. These six clones were grouped with IR Melati in a genetic similarity value of 52%.
- Mantang Kuning was grouped with 7 other clones at the level of similarity of 39% and most distant genetic relationship with Supra clone in a genetic similarity value of 15%.

CONCLUSION

Biru Ungu and Bestak clone had a value as high as 78% similarity in molecular basis. Both had different phenotypes but proved to be close related genetically. Mantang Kuning and Supra clone had a genetic similarity value of 15% genetic similarity in molecular basis. Both clones had different phenotypes and proved distant related genetically.

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