Genetic diversity based on RAPD F2 generation of apple cactus (Cereus spp.)

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Abstract. Apple cactus consists of 2 types, long spines (Cereus jamacaru) and short spines (C. peruvianus); both are self-incompatible. So that only artificial crosses between species that produce fruit. But some fruits are found from natural crosses of long spines. The three results of these crosses, long spines with short spines; short spines with long spines; and natural crosses on long spines, have produced F2 seedlings. The purpose of this study was to study genetic diversity based on the RAPD of the F2 seedlings. DNA isolation was carried out using Doyle and Doyle method modified on the addition of Polivinilpirolidon. The RAPD technique uses 3 primers: OPD-11, OPM-10, and OPN-5. Analysis of genetic diversity using Simple Matching coefficient using NTSys 2.02 software. Based on the three primers, each F2 offspring had genetic diversity but was grouped according to the parent. Showed that the three RAPD primers were effective for markers of genetic diversity in apple cactus.

1. Introduction

Cactus is one type of ornamental plant that is known and widely planted in Indonesia. Cactus or Cactaceae have various kinds of spines, shapes, and sizes, and the appearance of cactus plants has aesthetic value. One kind of ornamental cactus is Cereus. The physical appearance of Cereus looks like a tree that has a grayish-green cylindrical trunk and can reach 10 meters or 33 feet in height with a trunk diameter of 10-20 cm [1]. Cereus or as known as Apple Cactus, produces large cream-colored flowers that bloom only one night. It produces fruit with fruit skin colors that vary from purple-red to yellow. The flesh of the fruit produced is white and has small seeds in large numbers [2].

Two types of Apple Cactus grown in Indonesia, there are short spines clone type (spine size smaller than 20 mm) and long spines clone type (spine size more than 20 mm) [3]. Based on the research done before, Apple Cactus are self-incompatible plant or means that unable to be fertilized by its own pollen [4], found out when revealed the result that the only pollination between different types that produced fruit [3]. Apple cactus currently has a high economic value, but research on Apple Cactus, especially regarding its genetic diversity, has not been done much.

Analyzing the genetic diversity of each genetic resource needs to be done to obtain specific characters of each genotype both morphologically and molecularly. Morphological characters result from interactions between genes and the environment, so morphological markers lack inconsistent traits because they are influenced by environmental factors [5]. Molecular markers can overcome these
shortcomings by carrying out DNA-based analysis. DNA markers are more consistent and have high accuracy [6].

PCR-RAPD is one of the tools in molecular analysis by using specific markers in studying genetic diversity. DNA-based RAPD using PCR (Polymerase Chain Reaction) engine is based on amplifying random DNA segments using primers whose nucleotide sequences are determined randomly. RAPD can determine the genetic similarity of a species and genetic diversity between species [7]. RAPD marker has the advantages that it is cheaper, easy to perform, can quickly provide output, can produce a large number of band polymorphisms, and is easy to obtain the primers needed to analyze the genome. RAPD does not require basic information about the base sequence of a species but requires only a small amount of pure DNA compared to other molecular markers [8]. This study aims to examine the genetic diversity F2 Generation of Apple Cactus using RAPD markers.

2. Methods

2.1. Experimental material
This research was carried out at the Laboratory of Genetics and Plant Breeding, Faculty of Agriculture Gadjah Mada University, in October - November 2020. The materials used were fifteen assesis from the cross of the Apple Cactus (5 samples each from the cross of long × short spines (J×P), the reciprocals (P×J), and long-spines natural cross (JOP)) selected from the greenhouse of the Faculty of Agriculture, Sebelas Maret University, Surakarta. Materials were prepared to analyze molecular characters following Doyle and Doyle's DNA isolation protocol by adding modifications with polyvinylpyrrolidone (PVP). The sample weight is below 0.05g, so all sample parts are used without any waste. The results of DNA isolation were carried out by PCR-RAPD reactions with PCR composition per reaction using 12.5 µl, consisting of GoTaq Green PCR Mastermix 6.25 µl; primer 2.5 µl; Nuclear Free Water (NFW) 3.5 µl; and 2.5 µl DNA samples. The RAPD primers used were OPN-05 (ACTGAACGCC), OPM-10 (TCTGGCGCAC), and OPD-11 (AGCGCCATTG). PCR amplification was performed using a Biorad T100 Thermal cycler, observed by electrophoresis on 1.5% agarose gel (GeneDirex). Electrophoresis is regulated with a voltage of 100 V; 400 mA for 60 minutes. FloroSafe staining was applied so that the DNA banding pattern could be seen under UV light. DNA Ladder (SMOBIO) was used as a size marker.

2.2. Statistical analysis
Data analysis was carried out by scoring for the emerging bands. Score 1 was given to the band that appeared, and score 0 if not appeared. Furthermore, the scoring data were analyzed using NTSYS-pc software version 2.02 to determine genetic similarity or dendrogram.

3. Results and discussion
Modification on isolation protocol of Doyle and Doyle by addition of Polyvinylpyrrolidone (PVP) is to reduce phenolic [9]. This modification affects the result of purity ratio of the DNA among 1.5-2.2. DNA purity belonging to the good category of it ranged from 1.8 to 2.0. If the DNA purity value is less than 1.8, it means that contaminants in the form of protein are still found, while a DNA purity value is more than 2.0 means that contaminants are still found in the form of phenol [10]. Binary data obtained from scoring on amplification result, a score of 1 given to the loci that had appeared band while a score of 0 was given to the loci that the band did not appear on each base pairs (bp) as shown on Figure 1. The scoring result then calculated the polymorphic percentage as shown in Table 1.

Based on Figure 1, electrophoresis was carried out after successful DNA amplification. It is marked by the appearance of a clear DNA band. The electrophoresis stage used agarose gel with a concentration of 1.5%. Agarose was stained with Florosafe as much as 5 µl so that further DNA band patterns could be seen. The result of the band pattern has various; therefore, the scoring is done on a band pattern with strong intensity and brightness [11]. The results of the electrophoresis of the three primers used produced different numbers of bands or loci. Based on the visualization of DNA bands by the three primers, it can be seen that the intensity of the light produced is different, some bands are faint, and some are bright.
The intensity difference in the resulting band can be influenced by the distribution of primer attachment, concentration, and genome purity [12].

![Figure 1](image1.png)

**Figure 1.** Result of electrophoresis with primer A) OPN-05, B) OPM-10, C) OPD-11

Based on Table 1, the total number of bands generated from the 3 primers used is 28 bands with sizes ranging from 300 bp to 1800 bp. The percentage of polymorphism bands was 83% and 100%. The 100% polymorphism percentage belonged to the OPN-05 and OPD-11 primers, while the 83% polymorphism percentage was found in the OPM-10 primer. The results of a high percentage of polymorphisms indicated that the three primers were suitable to be used as markers in the genetic diversity analysis of Apple Cactus. This result indicates that a high level of polymorphism (<50%) means that the primers used can show the molecular diversity of the tested samples. Genetic polymorphism is defined as individuals with different genetic traits but coexists in a population [13].

| Primary   | Ribbon size(bp) | Polymorphic Ribbon | % Polymorphic |
|-----------|-----------------|--------------------|---------------|
| OPN-05    | 450 – 1800      | 13                 | 100           |
| OPM-10    | 300 – 1300      | 6                  | 83            |
| OPD-11    | 330 – 1300      | 9                  | 100           |

Based on Figure 2, the clustering analysis resulted in 4 clusters. The samples in cluster 1 were natural crosses of long spines 1st sample (JOP1), 2nd sample (JOP2), and 4th sample (JOP4). Cluster 2 is filled with natural crosses of long spines 3rd sample (JOP3), and 5th sample (JOP5), and long × short spines cross 1st sample (J×P1), 2nd sample (J×P2), and 3rd sample (J×P3). Cluster 3, the smallest cluster compared to other clusters, only contains the 4th sample (J×P4) and the 5th sample (J×P5) of long × short spines crosses. The fourth cluster contains all samples of short × long spines crosses which are grouped. The five samples of short × long spines cross formed a group because they had a similar coefficient of 0.69. The 2nd and 3rd samples have the highest coefficient of similarity among all cross-assessments, which is 0.89. This clustered reciprocal of short × long spines cross differs from the results of the F2 cross and its monohybrid cross. This is because, in a reciprocal F2 cross, there is a combination of genes that carry superior traits from male and female F1 parents, resulting in characters that appear to be better characters than their parents [14].

Figure 2 showed that the three samples of 1st, 2nd, and 4th natural crosses of long spines formed one group with a similarity coefficient of 0.82. The 3rd and 5th samples of natural crosses of long spines clustered in cluster 2 with a similarity coefficient of 0.75. The three samples of 1st, 2nd, and 3rd long ×
short spines cross formed a group with a similarity coefficient of 0.82. The 4th and 5th of long × short spines cross sample formed a small cluster with a similarity coefficient of 0.71. This grouping can occur because of the similarity of morphological characters. Morphological characters caused by the samples that grow close together, or come from the same parent can trigger grouping of the samples, both from the same or different cross groups [15].

Table 2. The similarity value of 15 accesses from crosses of Apple Cactus based on the three primers tested (OPN-05, OPM-10, OPD-11)

|       | JOP1 | JOP2 | JOP3 | JOP4 | JOP5 | J×P1 | J×P2 | J×P3 | J×P4 | J×P5 | P×J1 | P×J2 | P×J3 | P×J4 | P×J5 |
|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| JOP1  | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| JOP2  | 0.82 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |
| JOP3  | 0.75 | 0.71 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |
| JOP4  | 0.82 | 0.86 | 0.71 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |
| JOP5  | 0.68 | 0.71 | 0.71 | 0.79 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |
| J×P1  | 0.68 | 0.79 | 0.71 | 0.79 | 0.71 | 1.00 |      |      |      |      |      |      |      |      |      |      |
| J×P2  | 0.64 | 0.75 | 0.75 | 0.75 | 0.82 | 0.82 | 1.00 |      |      |      |      |      |      |      |      |      |
| J×P3  | 0.64 | 0.68 | 0.82 | 0.68 | 0.75 | 0.82 | 0.86 | 1.00 |      |      |      |      |      |      |      |      |
| J×P4  | 0.50 | 0.61 | 0.61 | 0.61 | 0.68 | 0.75 | 0.79 | 0.71 | 1.00 |      |      |      |      |      |      |      |
| J×P5  | 0.64 | 0.68 | 0.68 | 0.68 | 0.75 | 0.61 | 0.71 | 0.57 | 0.71 | 1.00 |      |      |      |      |      |      |
| P×J1  | 0.46 | 0.50 | 0.57 | 0.50 | 0.64 | 0.64 | 0.54 | 0.68 | 0.61 | 0.46 | 1.00 |      |      |      |      |      |
| P×J2  | 0.43 | 0.46 | 0.61 | 0.54 | 0.68 | 0.61 | 0.71 | 0.71 | 0.64 | 0.57 | 0.68 | 1.00 |      |      |      |      |
| P×J3  | 0.54 | 0.57 | 0.64 | 0.64 | 0.79 | 0.71 | 0.71 | 0.71 | 0.64 | 0.57 | 0.68 | 0.89 | 1.00 |      |      |      |
| P×J4  | 0.43 | 0.54 | 0.54 | 0.54 | 0.61 | 0.61 | 0.64 | 0.71 | 0.57 | 0.61 | 0.71 | 0.75 | 1.00 |      |      |      |
| P×J5  | 0.50 | 0.61 | 0.68 | 0.54 | 0.68 | 0.68 | 0.71 | 0.79 | 0.71 | 0.50 | 0.75 | 0.71 | 0.75 | 0.71 | 1.00 |

Description: JOP: natural crosses of long spines, J×P: long spines × short spines, P×J: short spines × long spines

Figure 2. Dendrogram of grouping 15 accesses of Apple Cactus crosses based on the 3 primers tested (OPN-05, OPM-10, OPD-11)

4. Conclusion
RAPD can be used to classify Apple Cactus based on their genome. All samples of short × long spines crosses (P×J) were clustered in the same cluster. The dendrogram with the Simple Matching (SM)
coefficient resulted in the similarity coefficient of the 3 primers ranging from 0.61 to 0.89. The more primers used, the more visible the diversity displayed because, the more genomic segments read by the primers.

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