Genetic Relationship of *Hibiscus* spp. Based on DNA bands Using RAPD Technique

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**Abstract.** The variety of *Hibiscus* can be categorized based on genetic relationship. This research aimed to figure out genetic relationship of *Hibiscus* spp. (*Hibiscus rosa-sinensis* (Hrs), *Hibiscus schizopetalus* (Hs), and *Hibiscus tiliaceus* (Ht)) based on DNA bands using Random Amplified Polymorphic DNA technique and find out the band of DNA to categorize *Hibiscus* spp. in dendrogram. The leaves of the samples were collected from Kebun Raya Purwodadi – Pasuruan, Indonesia and then used as DNA sources. The primer used in this study were OPA-1, OPA02, and OPA-3. The experiment resulted 56 bands of DNA which were obtained from amplification process. Subsequently, we performed cluster analysis by applying Unweighted Pair Group Method with Arithmetic Mean (UPGMA), in which simple matching, and Principal Component Analysis (PCA) analysis were selected to obtain dendrogram. The resulted dendrogram exhibited that *H. rosa-sinensis* possess a closed genetic relationship (index similarity 0.65) with *H. schizopetalus* but showed far genetic relationship with *H. tiliaceus* (index similarity 0.40).

**Keyword:** RAPD Technique, *Hibiscus* spp, DNA, genetic similarity

1. **Introduction**

Genetic relationship study of a species is an essential research that can be used as a tool for conservation, for example finding a species genetically closed to endangered species. Malvaceae is a high diversity group of flowering plant which is broadly distributed especially in tropical region, consisting of 245 genera. One of genera of Malvaceae is *Hibiscus* [1]. Beside it used as ornamental plants, Hibiscus show also pharmacological benefit, such as antibacterial, anti-inflammatory, treats boils, coughs, heat, urinary tract infections, normalizes menstrual cycles, expectorants, and stops bleeding [2]. *Hibiscus* is one genera of Malvaceae with high diversity, therefore it is chosen as our research project to find out its ancestral variety – monophyletic or polyphyletic. Studying genetic relationship a species is a critical research. Moreover, this study stage becomes an important process in germplasm characterization and conservation to maintain genetic erosion more effective, design a sampling strategy underlying breeding process [3]. These can be studied through Random Amplified Polymorphic DNA (RAPD).

Random amplified polymorphic DNA (RAPD) techniques is one technique frequently used to analyze genetic relationships based on molecular approaches. This technique involves polymerization of DNA or polymerase chain reaction (PCR) assisted by oligonucleotide primers for amplification [3]. It is a rapid and efficient technique to identify a large number of DNA, especially identification of polymorphisms in the genome leading to information of genetic diversity, genetic relationship, genetic mapping, and DNA fingerprints [4].

Based on the above mentioned background, this study was conducted to determine the relationship between *Hibiscus* spp. based on DNA banding pattern with Random Amplified Polymorphic DNA (RAPD) technique. In addition, it also aims to find out DNA bands that affect the grouping of *Hibiscus* spp. into the dendrogram. This research was conducted by observing DNA banding patterns with RAPD techniques. DNA banding patterns usage in genetic studies will improve data accuracy and efficiency, because the process is not influenced by environmental factors or plant development stages [4,]. The use of RAPD techniques is because this technique is able to quickly and effectively identify DNA polymorphisms, making it suitable for the study of genetic relationships [5].
2. Experimental Method

2.1. Materials and instruments

The leaves sample (Hibiscus rosa-sinensis, Hibiscus schizopetalus, and Hibiscus tiliaceus) were collected from Kebun Raya Purwodadi – Pasuruan - East Java. The chemicals were provided from commercial sources (p.a grade or biochemical grade). Instrument used in this study were vortex, UV-Vis spectrophotometer, electrophoresis, micropipettes, centrifuge, PCR machine (Thermal cycler).

2.2. DNA isolation

DNA isolation was performed according to the protocol of Porebski et al [6].

2.3. DNA amplification

DNA amplification was performed according to the protocol of Porebski et al [6] using 3 different primers, that were OPA-1 (5’-CAG GCC CTT C-3’), OPA-2 (5’-TGC CGA GCT G-3’), dan OPA-3 (5’-AGT CAG CCA C-3’). The results of DNA amplification was analyzed using Multi-Variate Statistical Principle (MVSP) version 3.22 program for clustering and similarity index, and the last to obtain dendrogram [7].

3. Results and discussion

Genetic relationship of Hibiscus spp based on DNA bands pattern.

DNA band pattern from amplification was used as characteristic for Hibiscus spp clustering into dendogram. Results of amplification of three samples using OPA-1, OPA-2, and OPA-3 as primer showed a clearly separated DNA bands (Fig. 1), and totally 54 DNA bands were observed. Then we made scoring to the DNA bands as presented in Table 1.

![Figure 1. Results of DNA amplification using RAPD technique.](image_url)

Legend: A = using OPA-1; B = using OPA-2; C = using OPA-3; Hrs= H. Rosa-sinensis; Hs= H. Rosa-sinensis; Hs = H. schizopetalus; Ht = H. tiliaceus; M = marker 10 kb
Table 1. DNA bands scoring of *Hibiscus rosa-sinensis* (Hrs), *Hibiscus schizopetalus* (Hs), and *Hibiscus tiliaceus* (Ht)

| No | DNA bands (bp)/OPA-1 | Sample | DNA bands (bp)/OPA-2 | Sample | DNA bands (bp)/OPA-3 | Sample |
|----|----------------------|--------|----------------------|--------|----------------------|--------|
| 1  | 4000                 | Hs 0 | 0 0 1                | 2000   | 1 0 0                | 1600   | 0 1 0                |
| 2  | 3100                 | Hs 1 | 1 0 1                | 1600   | 0 1 0                | 1500   | 1 0 1                |
| 3  | 1700                 | Hs 1 | 0 1 0                | 1500   | 1 0 0                | 1100   | 1 0 0                |
| 4  | 1600                 | Hs 1 | 1 1 1                | 1400   | 0 1 0                | 770    | 1 1 0                |
| 5  | 1400                 | Hs 1 | 1 1 0                | 1050   | 1 0 1                | 750    | 1 1 1                |
| 6  | 1100                 | Hs 0 | 1 0 0                | 1010   | 1 1 0                | 550    | 0 1 1                |
| 7  | 1050                 | Hs 0 | 1 1 0                | 1000   | 1 1 1                | 510    | 0 1 1                |
| 8  | 1010                 | Hs 1 | 1 1 0                | 750    | 0 1 1                | 300    | 0 1 0                |
| 9  | 1000                 | Hs 1 | 1 1 0                | 550    | 0 1 0                | 260    | 1 0 0                |
| 10 | 770                  | Hs 1 | 1 1 1                | 510    | 0 1 1                | 240    | 0 1 0                |
| 11 | 550                  | Hs 1 | 1 1 0                | 500    | 1 1 0                |        |                    |
| 12 | 510                  | Hs 0 | 0 0 0                | 490    | 1 1 0                |        |                    |
| 13 | 490                  | Hs 0 | 1 1 0                |        |                      |        |                    |

As shown in Figure 1, OPA-1, OPA-2, and OPA-3 were capable to amplified DNA satisfactorily, as could be seen with the emergence of polymorphic patterns with good clarity of DNA bands. This is in accordance with criteria of Haris et al. [8]. The DNA bands obtained in the experiment were characteristic shown by polymorphism pattern which lead to the assumption that the genotype character of the sample *Hibiscus* spp can be grouped in a close genetic relationship. However, this assumption should be proved through a dendrogram formation. The detail of these observations are presented in Table 2.

Table 2. The results of amplification using OPA-1, OPA-2, and OPA-3 primers

| No | Primer | Sequence (5’-3’) | DNA band pattern | % Polymorphic | Total DNA band | Size of DNA band (kb) |
|----|--------|------------------|------------------|---------------|----------------|-----------------------|
| 1  | OPA-1  | CAG GCC CTT C     | Polymorphic      | 10            | 79,92%         | 21                    | 0,49 - 4             |
| 2  | OPA-2  | TGC CGA GCT G     | Monomorphic      | 11            | 91,67%         | 20                    | 0,49 - 2             |
| 3  | OPA-3  | AGT CAG CCA C     | Polymorphic      | 9             | 90%            | 15                    | 0,24 - 1,6           |

Subsequently, we analyzed similarity index as presented in Table 3. The results showed that *Hibiscus rosa-sinensis* and *Hibiscus schizopetalus* exhibited the highest similarity (0.650), while *Hibiscus tiliaceus* and *Hibiscus rosa-sinensis* *Hibiscus rosa-sinensis* exhibited the lowest similarity (0.400), and similarity of *Hibiscus schizopetalus* and *Hibiscus tiliaceus* was between the others (0.450). Based on similarity index, we obtained the dendrogram as presented in Figure 2. Based on the obtained dendrogram (Fig. 2), *Hibiscus* spp can be divide into 2 groups, that are *Hibiscus tiliaceus* group, while *Hibiscus rosa-sinensis* and *Hibiscus schizopetalus* belongs to another group.

Table 3. Similarity value of *Hibiscus rosa-sinensis* (Hrs), *Hibiscus schizopetalus* (Hs), and *Hibiscus tiliaceus* (Ht)

| No | Sample | Hrs | Hs   | Ht   |
|----|--------|-----|------|------|
| 1  | Hs     | 1,000 |      |      |
| 2  | Hrs    | 0,650 | 1,000 |      |
| 3  | Ht     | 0,450 | 0,400 | 1,000 |
**Figure 2.** Genetic Relationship *Hibiscus* spp. shown through the dendrogram. Hrs = *Hibiscus rosa-sinensis*, Hs = *Hibiscus schizopetalus*, Ht = *Hibiscus tiliaceus*

The following table, we grouped the DNA bands determined the similarity

| No | DNA Band (bp) | Component 1 | Component 2 |
|----|--------------|-------------|-------------|
| 1  | 4000         | 0.318       | -0.042      |
| 2  | 3100         | -0.318      | 0.042       |
| 3  | 2000         | -0.133      | 0.399       |
| 4  | 1700         | 0.318       | -0.042      |
| 5  | 1600         | 0.000       | 0.000       |
| 6  | 1500         | 0.185       | 0.357       |
| 7  | 1400         | -0.318      | 0.042       |
| 8  | 1100         | -0.318      | 0.042       |
| 9  | 1050         | 0.185       | 0.357       |
| 10 | 1010         | -0.318      | 0.042       |
| 11 | 1000         | 0.000       | 0.000       |
| 12 | 770          | 0.000       | 0.000       |
| 13 | 750          | 0.000       | 0.000       |
| 14 | 550          | 0.000       | 0.000       |
| 15 | 510          | 0.133       | -0.399      |
| 16 | 500          | -0.318      | 0.042       |
| 17 | 490          | -0.318      | 0.042       |
| 18 | 300          | -0.185      | -0.357      |
| 19 | 260          | -0.133      | 0.399       |
| 20 | 240          | -0.185      | -0.357      |

*Eigen value* 3.264 1.736

The high similarity of *Hibiscus rosa-sinensis* and *Hibiscus schizopetalus* in molecular level was also observed in morphological level, such as the similarity of their leaves and flowers, but not their habitus.
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Figure 3. Leaf and flower *Hibiscus rosa-sinensis* (A), Leaf and flower *Hibiscus shizopetalus* (B), Leaf and flower *Hibiscus tiliaceus*

Figure 4. Habitus *Hibiscus rosa-sinensis* (A), Habitus *Hibiscus shizopetalus* (B), Habitus *Hibiscus tiliaceus* (C)

While *Hibiscus tiliaceus* is quite differ from *Hibiscus rosa-sinensis* and *Hibiscus shizopetalus*, both from the molecular or morphological level. As knowledge of the researchers, there is no article reported the taxonomy genetic relationship of *Hibiscus* spp.

4. Conclusion
In conclusion, we have successfully determined the genetic similarity of *Hibiscus* spp based on DNA bands using RAPD methods. Hrs and Hs showed a close genetic similarity, whereas *Hibiscus tiliaceus* exhibited far genetic similarity compared to *Hibiscus rosa-sinensis* and *Hibiscus shizopetalus*.

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