DIVERSITY ANALYSIS ON TUBERED-BEARING Ipomoea trifida (H.B.K.) G.Don.
BASED ON CHROMOSOME TRAITS

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ABSTRACT

Wild relatives of sweet potato Ipomoea trifida was a potential source of genes to support plant breeding programs of sweet potato (Ipomoea batatas (L.) Lam.) To determine the genetic diversity I. trifida, observations on the the chromosomes traits was conducted. Plant materials used ten accession of tubered-bearing I. trifida. Relationship between species identified by cluster analysis and Principal Component Analysis (PCA). The results showed the observation on the ten accession of tubered-bearing I. trifida using 9 chromosome traits produces dissimilarities distance (Euclidean coeffisien) ranging from 1.755 to 6.224 and showed a broad diversity. Dendogram generated on dissimilarities distance of 5.228 showed the formation of three main clusters. Principal Component Analysis (PCA) produced first two principal component (PC₁ and PC₂), which has been able to explain 89.64% of the total variation.

Keywords: Ipomoea trifida, chromosome traits, cluster analysis, PCA

INTRODUCTION

Hambali (1988) reported that the highest genetic and phenotypic diversity of wild relatives of sweet potato in Indonesia is in the Citatah-West Java. The results of field observations in Citatah by a Padjadjaran University Team has collected 168 accessions of wild relatives of sweet potato that have not been identified. Based on morphological variation in flowers and leaves, supposedly 168 accessions comprising I. trifida and I. triloba (Agung Karuniawan, personal communication, 2010). Wild relatives of sweet potato were found in Citatah known by local residents as “huhuian” and “boled areuy”. Naturally wild relatives of sweet potato were found in Citatah grow as weeds in agricultural land of sweet potatoes, and other calcareous slope areas. Wild relatives of sweet potato I. trifida has been used as a source of genes in sweet potato breeding to improve the character of the yields, levels of dry matter,
starch, increased levels of protein (Kobayashi & Miyazaki, 1976) and resistance to certain pests and diseases as black rot root diseases (Shiotani and Kawase, 1989; Komaki, 2001) and scab disease (Hartana, 1994).

Wild relatives *I. trifida* originated from Citatah West Java has morphotype variations, so to determine the level of genetic diversity and relationship among accessions need to be done the clustering by morphological or cytogenetic studies (chromosome traits). Morphological characterization done through observation of the phenotypic appearance, while cytogenetic studies done through observation of chromosome number or ploidy level and form of chromosomes. These characters can be used as a chromosome differentiating factor for identifying genetic variation in plants that will be useful for breeders in developing and improving the quality of crops.

Most wild relatives of sweet potato are found of tetraploid or diploid (Renwarin *et al.*, 1994) and have not been characterized, so many potential sources of genetic diversity is unknown. So far it is not known the level of morphotype variation, genetic diversity and relationship among accessions of wild relatives of sweet potato originated from Citatah West Java. This information is necessary to support the management and utilization of germplasm. Information of the level of genetic diversity on germplasm material is needed by breeders to identify potential progenitor and will be useful also to prevent the use of closely related progenitor closely in crossing.

MATERIALS AND METHODS

Materials

Research materials consisted of ten accessions of tubered-bearing *I. trifida* collection of the Faculty of Agriculture, Padjadjaran University. Root tips used as materials for preparations for observation of chromosomes. The chemicals required include a solution of 0.002 M 0.8-hydroksiquinolin, fixative solution (ethanol: glacial acetic acid = 3:1), a solution of 4N HCl, solution of 45% acetic acid, and 2% orcein.

Methods

Chromosome preparations

Root tips that meristimatis obtained from stem cuttings grown on medium (soil: compost: manure = 1:1:1). Chromosome preparations made using the squash method of Darnaedi (1990). The root was cut 1 cm from the root tip and soaked in a solution of 0.002 M 8-hydroksiquinolin for 3-5 hours at 18-20°C. Subsequently the roots were fixed in a mixture solution of ethanol: glacial acetic acid (3:1) for 48 hours and transferred to a solution of 4N HCl for 10 minutes. Subsequently the roots was immersed in 45% acetic acid solution for 10 minutes. Staining of
preparations carried out using 2% orcein for 10 minutes on top of a glass object, then closed, heated and pressed.

Observations and data analysis

Observation of chromosome using light microscopy. Chromosomes on prometafase or early metaphase stage photographed and made the micrography. Chromosome captured images magnified and printed with a computer program, then print out of the chromosome picture was used for observation of chromosome number, chromosome size (the length of the long arm (q), the length of the short arm (p) and total length (q + p)), centromere index (CI), haploid complement chromosome lenght (HCL), shape of chromosome was determined by chromosome arm ratio (r = p / q), the value of intrachromosomal index (A₁) and interchromosomal index (A₂). Analysis of genetic diversity performed using cluster analysis and Principal Component Analysis (PCA) with the software of XLstat 2009.

RESULT AND DISCUSSION

Cluster analysis on ten accession of tubered-bearing *I. trifida* based on chromosome traits.

The result of cluster analysis on ten accession of tubered-bearing *I. trifida* based on nine chromosome traits can be seen in Fig.1.

![Dendogram of clustering on ten accession of tubered-bearing *I. trifida*](image)

Dissimilarity distance (Euclidean coeffisien) ranged from 1.755 to 6.224. Dendogram generated at a dissimilarities distance of 5.228 showed the formation...
of three main clusters, namely cluster I consists of 4 accessions (accession 118, 19, 15, and 81); Cluster II consists of 3 accessions (accession 180, 149, and 99); and cluster III consists of 3 accessions (accession 40, 1 and 13). Highest dissimilarities distance of 6.224 possessed by the accession of 118, 19, 15, and 81 are joined in cluster I. Thus it can be assumed the four accession have the most distant relationship to other accession based on the chromosome traits were observed. The lowest dissimilarities distance of 1.755 are owned by the accession of 99 and 149, showed that the two accessions have a high similarity of chromosome traits so that the realtionship between the two accessions are very close. The high degree of similarity between the two accessions probably that the two accession is the same material. Afuape et al. (2011) state that the genotypes that showed high similarity can be expected as duplicate genotypes so besides morphological characterization, molecular characterization is needed to confirm whether these genotypes are the same material with a different name or whether these genotypes came from the same parent.

**Principal component analysis on ten accession of tubered-bearing *I. trifida* based on chromosome traits.**

Principal component analysis performed on ten accessions of tubered-bearing *I. trifida* to see relationship and chromosome traits affecting variation appeared between accession. Based on Eigen value > 1, then there are two principal components that have been able to explain 89.64% of the variation total of accessions tested (Table 1).

In Table 1 showed that PC1 contributes the proportion of variation by 53.247% in ten accessions of tubered-bearing *I. trifida* provided by almost all the tested chromosome traits except the traits of the short arm (p) and interchromosomal asymmetry index (A2). The traits of the short arm (p), centromere index (IS), the number of metacentric chromosomes (m), the number of submetacentric chromosome (sm) and intrachromosomal asymmetry index (A1) on PC2 contributes for 36.393% of the variation that arises among the tested accessions. Therefore, until the second principal component (PC2) was able to explain 89.640% of the total variation.

**Table 1. Principal component analysis (PCA) on ten accession of tubered-bearing *I. trifida* based on nine chromosome traits**

<table>
<thead>
<tr>
<th>No</th>
<th>Traits</th>
<th>Principal Component (PC)</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lenght of the long arm (q)</td>
<td>-0.892</td>
<td>0.414</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Lenght of the short arm (p)</td>
<td>-0.014</td>
<td>0.988</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Total lenght of chromosome (TL)</td>
<td>-0.660</td>
<td>0.735</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Centomere Index (CI)</td>
<td>0.863</td>
<td>0.484</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Number of metacentric chromosome (m)</td>
<td>0.903</td>
<td>0.332</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Number of submetacentric chromosome (sm)</td>
<td>-0.903</td>
<td>-0.332</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>haploid complement chromosome lenght (HCL)</td>
<td>-0.660</td>
<td>0.733</td>
<td></td>
</tr>
</tbody>
</table>
Intrachromosomal index (A₁) -0.851 -0.500
Interchromosomal index (A₂) -0.155 0.589

Eigen 4.792 3.275
Proportion (%) 53.247 36.393
Cumulative (%) 53.247 89.640

Note: The bold is the value of an influential traits because diskriminant > 0.5 (Zubair, 2004).

To see the pattern of distribution of ten accessions tubered-bearing *I. trifida* can be seen in Figure 2. Accessions that are in the same quadrant indicates that the accessions have a very close relationship, however if they are in a different quadrant with an angle > 90° then the accessions have a distant relationship.

![Figure 2. Biplot of PC₁ and PC₂ of ten accession tubered-bearing I. trifida base on chromosome traits](image)

In Figure 2 showed that the ten accessions spread in four quadrants of biplot. Accession 15, 19, and 81 are in the same quadrant, indicating that the three accessions have a close relationship. The three accessions also have close relationship with accession 118 that was in different quadrants as forming an acute angle (<90°).

**CONCLUSION**

1. Ten accessions of tubered-bearing *I. trifida* originated from Citatah West Java has a broad genetic diversity.
2. Tests on the ten accession of tubered-bearing *I. trifida* using 9 chromosome traits produced dissimilarities distance (Euclidean coefficients)
ranged from 1.755 to 6.224. Dendogram generated at a dissimilarities distance of 5.228 showed the formation of three main clusters.

3. Principal Component Analysis (PCA) produced 2 first principal component (PC₁ and PC₂), which has been able to explain 89.64% of the total variation.

REFERENCES


