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# DIVERSITY ANALYSIS OF TUBERED-BEARING *Ipomoea trifida* (H.B.K.) G. DON. ORIGINATED FROM CITATAH WEST JAVA INDONESIA BASED ONCHROMOSOME TRAITS

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***Abstract***

*Ipomoea trifida is considered as a wild crop relative to sweet potato Ipomoea batatas. A set of 10 accessions was selected for tubered-bearing I. trifida originated from Citatah West Java was evaluated on their diversity based on chromose traits. A field trial and laboratory analysis were conducted at Universitas Padjadjaran Bandung Indonesia. Relationship between species identified by cluster analysis and Principal Component Analysis (PCA). The results showed that the observation on the 10 accession of tubered-bearing I. trifida using nine chromosome traits produced dissimilarities distance (Euclidean coefficient) ranging from 1.75 to 6.22. Dendogram generated at a dissimilarity distance of 5.23 showed the formation of three main clusters. Principal Component Analysis (PCA) produced first two principal component ($PC_1$ and $PC_2$), which has been able to explain 89.64% of the total variation. It is concluded that thare are highy diveristy between 10 accession of tubered-bearing I .trifida based on chromosomes traits.*

***Keywords:*** *Chromosome, Ipomoea trifida, Principal Component Analysis.*

## INTRODUCTION

Wild relative of sweet potato *Ipomoea trifida* is considered as having a potential source of genes to support plant breeding programs of domesticated sweet potato (*Ipomoea batatas* (L.) Lam.). 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 in 2010 have 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 *etal*., 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, Universitas Padjadjaran. 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$^0$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 root 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), shape of chromosomes, haploid complement chromosome lenght (HCL), the value of intrachromosomal index ($A_1$) and interchromosomal index ($A_2$). Analysis of genetic diversity performed using cluster analysis and Principal Component Analysis (PCA) with the software of XLstat 2009.

## RESULTS AND DISCUSSIONS

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

Dissimilarities distance (Euclidean coeffisien) between ten accession of tubered-bearing *I. trifida* based on nine chromosome traits ranged from 1.755 to 6.224 (Figure 1).

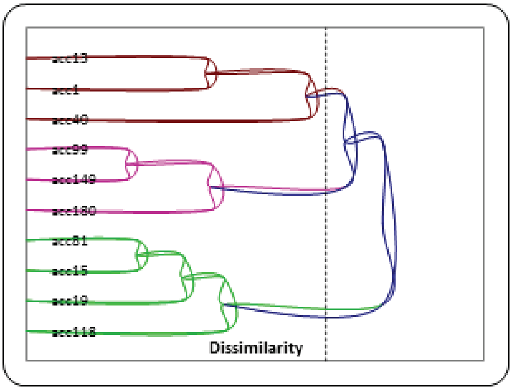

Figure 1. Dendogram of clustering on ten accession of tubered-bearing I. trifida

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 is 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 accessions 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 accessions 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 accessions. Based on Eigen value > 1, then there are two pricipal components that have been able to explain 89.64% of the variation total of accessions tested (Table 1.).

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

| No | Traits | Principal Component (PC) | |
|---|---|---|---|
| | | 1 | 2 |
| 1 | Lenght of the long arm (q) | **-0.892** | 0.414 |
| 2 | Lenght of the short arm (p) | -0.014 | **0.988** |
| 3 | Total lenght of chromosome (TL) | **-0.660** | **0.735** |
| 4 | Centomere Index (CI) | **0.863** | 0.484 |
| 5 | Number of metacentric chromosome (m) | **0.903** | 0.332 |
| 6 | Number of submetacentric chromosome (sm) | **-0.903** | -0.332 |
| 7 | haploid complement chromosome lenght (HCL) | **-0.660** | **0.733** |
| 8 | Intrachromosomal index ($A_1$) | **-0.851** | -0.500 |
| 9 | Interchromosomal index ($A_2$) | -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). The first Principal Componen ($PC_{1)}$ 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 ($A_2$). The traits of the short arm (p), centromere index (IS), the number of metacentric chromosomes (m), the number of submetacentric chromosome (sm) and intrakchromosomal asymmetry index ($A_1$) on $PC_2$ contributes for 36.393% of the variation that arises among the tested accessions. Therefore, until the second principal component ($PC_2$) was able to explain 89.640% of the total variation (Table 1.). To see the pattern of distribution of ten accessions tubered-bearing *I. trifida* can be seen in biplot graphic (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 > $90^0$ then the accessions have a distant relationship.

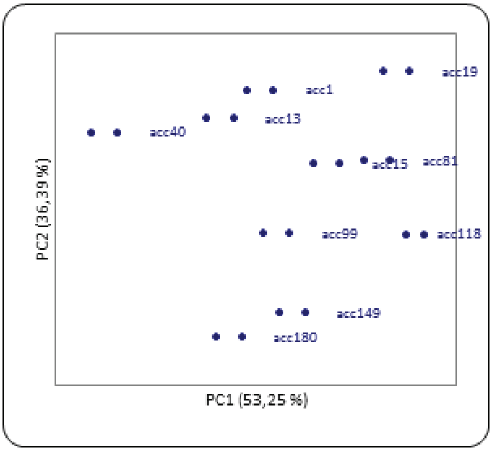

Figure 2. Biplot of PC1 and PC2 of ten accession tubered-bearing I. trifida base on chromosome traits

Ten accessions spread in four quadrants of biplot. Accession 15, 19, and 81 are in the same quadrant, indicating that the three accessionshave a close relationship. The three accessions also have close relationship with accession 118 that was in different quadrants as forming an acute angle (<$90^0$).

## CONCLUSIONS

Ten accessions of tubered-bearing I. trifida originated from Citatah West Java has a broad genetic diversity.Tests on the ten accession of tubered-bearing I. trifida using nine chromosome traits produced dissimilarities 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.Principal Component Analysis (PCA) produced two first principal component (PC1 and PC2), which has been able to explain 89.64% of the total variation.

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