



Artículo Original | Original Article

## Morphological diversity of *Morus* spp. (Mulberry accessions) grown in Muzaffarabad, Azad Jammu & Kashmir, Pakistan

[La diversidad morfológica de *Morus* spp. (accesiones de mora) cultivados en Muzaffarabad, Azad Jammu y Cachemira, Pakistán]

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**Abstract:** Genetic diversity of thirty mulberry accessions was determined by using the eleven different phenotypic characters. The study was conducted in field areas of Azad Jammu and Kashmir. The main objective of this study was to find out the diversity in morphological characters of Mulberry accessions found in Azad Jammu and Kashmir and Pakistan. The results showed that there is a significant difference in quantitative parameters among the thirty accessions ( $p \leq 0.001$ ). The cluster analysis showed that the data is divided into two main groups at near 80 dissimilarity level. This study suggests that the *Morus* germplasm is quite diverse.

**Keywords:** Mulberry species; Morphology; Genetic diversity; Cluster analysis; Principal component analysis

**Resumen:** Se determinó la diversidad genética de treinta accesiones de mora utilizando once caracteres fenotípicos diferentes. El estudio se realizó en áreas de campo de Azad Jammu y Cachemira. El objetivo principal de este estudio fue conocer la diversidad en los caracteres morfológicos de las accesiones de mora encontrados en Azad Jammu, Cachemira y Pakistán. Los resultados mostraron que hay una diferencia significativa en los parámetros cuantitativos entre las treinta accesiones de mora ( $p \leq 0.001$ ). El análisis de conglomerados mostró que los datos se dividen en dos grupos principales a un nivel de disimilitud cercano a 80. Este estudio sugiere que el germoplasma de *Morus* es muy diverso.

**Palabras clave:** Especie de Multiberry; Morfología; Diversidad genética; Análisis de conglomerados; Análisis de componentes principales

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## INTRODUCTION

Many plant species occupy a variety of contrasting habitats within a limited area and therefore plants must deal with these contrasting environmental conditions. Mechanisms by which a species may occupy a wide habitat range include reversible modifications to environmental conditions such as deficiency of nutrients, water, soil salinity or alkalinity, temperature and light levels, where plants attain a high degree of phenotypic plasticity (Williamson *et al.*, 1995; Fukui *et al.*, 2000; Vijayan 2009) Phenotypic plasticity is therefore an important means by which individual plants respond to environmental heterogeneity (Guo *et al.*, 2007).

*Morus* is a complex genus, which belongs to Moraceae. According to plant scientists, it's includes 12 (Freeman, 1978); 14 (Zhang *et al.*, 2002); 30 (Martín *et al.*, 2002) or 68 species (Datta, 2002). Among species, *Morus alba* (white mulberry), *Morus nigra* (black mulberry) and *Morus rubra* (red mulberry) are wide spread throughout world. *Morus alba* originated in Southwest China, *Morus rubra* originated in North America and *Morus nigra* originated in Iran (Datta, 2002; Ercisli, 2004; Machii *et al.*, 2000).

Moraceae unveil a remarkable diversity of morphological and life history traits, particularly inflorescence architectures, breeding systems, and pollination syndromes (Berg, 1990; Sakai *et al.*, 2000; Sakai, 2001; Zerega *et al.*, 2004; Clement & Weiblen, 2009).

Mulberries are found from temperate to subtropical regions of the Northern hemisphere to the tropics of the Southern hemisphere and they can grow in a varied range of climatic, topography and soil conditions. They are widely spread throughout the world Tutin (1996).

Genotypic classification of plants can be identified by using their phenotypic characters. The local farmers identify the cultivars diversity by botanical traits. Morphological characters are the most valuable tools for studying genetic diversity in the absence of DNA markers. These morphological traits are quite variable and are being used by the farmers for identification of plant materials for example for studying various genetic variation patterns, identifying the duplicates and correlation among the various morphological characteristics. Morphological traits are worthwhile for preliminary assessment, because they offer a quick tactic for evaluating the extent of diversity (Asare *et al.*, 2011).

Plant breeders use the morphological and agronomic traits for the genetic characterization of plants. For this reason, there is need to collect, characterize and evaluate remnant local genotypes before they disappear. According to (Aliyu & Fawole, 2000), cluster analysis has the singular efficacy and ability to identify crop accessions with highest level of similarity using the dendrogram. These results of the classification of the cultivars can predict groups by discriminate analysis. The cluster analysis can classify different genotypes on the basis of similarity and thus provides a hierarchical classification (Arslanoglu *et al.*, 2011).

## MATERIALS AND METHODS

Morphological measurements were taken at the Sericulture research center Patika and surroundings of Muzaffarabad Azad Jammu and Kashmir. The mulberry accessions include both cultivated and wild varieties of mulberry. Three replications were taken from each accession. Ten parameters were measured for data analysis. The qualitative traits were leaf color, shape, margins, tip and base. While the quantitative aspects were lamina length (cm), lamina width (cm), petiole length (cm), petiole width (cm) and leaf area index (cm). The parameters were recorded when the leaves are in fully expanded state.

Leaf length was measured from the leaf base at the juncture of the petiole attachment to the leaf tip leaving the extended portion of the tip. Lamina width was measured from the widest point of the leaf. Petiole length was measured by cutting the petiole portion of the leaf from the base of the leaf blade and measuring its length. Leaf area index was measured by using the formula given by Turner, 1974. i.e. (length  $\times$  width  $\times$  0.75) (Turner, 1974). These parameters were recorded from the fully expanded leaves. These measurements have been adopted from (Adolkar *et al.*, 2007) and Food and Agriculture organization.

### Data analysis

Data was analyzed by using the software Mathematical Statistical Tool for Analysis in Social Sciences (MSTAT). Significant differences in each of the morphological parameters were tested using analysis of variance (ANOVA). Means that were significantly different were separated using Least Significance Difference (LSD). The significant differences between means were calculated by a one-way analysis of variance (ANOVA) using Duncan's

multiple-range test at  $P < 0.001$  The parameters were also subjected to correlation and hierarchical cluster analysis using SPSS 12.0 statistical package to

determine the relatedness of the mulberry accessions with respect to the different parameters.

## RESULTS AND DISCUSSION

The quantitative characters of mulberry accessions are given in the Table No. 1

**Table No. 1**  
**Qualitative characters of Mulberry accessions**

Accessions	Leaf Color	Shape	Tip	Base	Margins	Surface
Punjab II punjab	Fresh green	Cordate	acuminate	cordate	Serrate	Coarse
Gumgi Srilanka	Fresh green	Cordate	acuminate	cordate	Serrate	Soft
Japan ealy	Fresh green	Cordate	acuminte	cordate	Serrate	Soft
Pak I punjab	dark green	Cordate	acumunate	truncate	Serrate	Soft
Punjab I	Fresh green	Cordate	acuminate	cordate	Doubly serrate	Soft
Morus latefolia Srilanka	Light green	Cordate	acute	cordate	Dentate	Soft
Morus latefolia Late	Light green	Lobate	acuminate	cordate	Serrate	Soft
husang china	Light green	Cordate	acute	cordate	Serrate	Soft
PFI	Light green	Cordate	acute	cordate	Serrate	Soft
Kanmasi Japan	Light green	Cordate	acute	cordate	Serrate	Soft
Kanmasi Japan late	Light green	Cordate	acute	cordate	Serrate	Soft
Lun-40-punjab	Light green	Cordate	acute	cordate	Serrate	Soft
Korean subni	Light green	Cordate	acute	cordate	Serrate	Soft
Gumgi korean early	Light green	Cordate	acute	cordate	Serrate	Soft
Morus latefolia early	Light green	Cordate	acute	cordate	Serrate	Soft
Morus latefolia	Dark green	Cordate	acute	cordate	Serrate	Soft
Morus alba I	Light green	Cordate	acute	cordate	Serrate	Soft
Kanmasi Japan early	Dark green	Cordate	acute	cordate	Serrate	Coarse
Pak II Punjab	Light green	Cordate	acute	cordate	Serrate	Coarse
Husang china early	Light green	Cordate	acute	cordate	Serrate	Coarse
Husang china late	Light green	Cordate	acute	cordate	Serrate	Soft
NARC local	Light green	Cordate	acute	cordate	Serrate	Soft
Gumgi korean late	Light green	Cordate	acute	cordate	Serrate	Soft
Punjab II	Dark green	Cordate	acute	cordate	Serrate	coarse
Morus Indica	Light green	Lobate	acuminate	cordate	Serrate	Soft
Morus alba	Light green	Cordate	acuminate	cordate	Serrate	Soft
Morus macorura	Light green	Cordate	acuminate	cordate	Dentate	Soft
Morus nigra	Light green	Cordate	acuminate	cordate	Serrate	Soft
Morus serrata	Dark green	lobed	acuminate	cordate	Serrate	Soft
Japan late	Light green	Cordate	acuminate	cordate	Serrate	Soft

Means of the different morphological parameters based on LSD showed that the lamina length, lamina width, petiole length, petiole width and leaf area index were significantly different across the thirty accession of Mulberry leaf (Table No. 2). The maximum lamina length was noted in Accession Punjab II, *Morus latefolia* Late and Pak II Punjab (19.6 cm), (16.63 cm), and (14.60 cm) respectively

while accession Kanmasi Japan early has minimum lamina length (7.933 cm). Lamina width varied across all the accessions, lamina width ranged from 5-15 cm. Accession Punjab II had highest lamina width (15.37 cm), and accession Pak II Punjab had the lowest lamina width (5.833 cm). Largest petiole length was noted in accessions *Morus serrata*, *Morus nigra*, Kanmasi Japan and Korean subni respectively.

Petiole width ranged from 0.366-0.1000 cm. Punjab II and *Morus latefolia* late had the highest petiole width (0.3667 cm) followed by Korean subni and Gumgi korean early (0.3333 cm). Pak I punjab had the lowest petiole width followed by *Morus Indica*, *Morus alba* and *Morus macorura*. Leaf area ranged

from 193.6-34.9 cm, Punjab II had the highest leaf area followed by *Morus latefolia* Late and Gumgi korean early while shortest leaf area Pak II Punjab, *Morus nigra*, *Morus serrata* and Japan early, Pak I Punjab and Punjab I respectively (Table No. 2).

**Table No. 2**  
**Means of five morphological traits of thirty mulberry accessions**

Accessions	Lamina length	Lamina width	Petiole length	Petiole width	Leaf area index
Punjab II punjab	10.77DEFGH	9.700EFGHI	2.333CDE	0.2333CDE	79.47EFG
Gumgi Srilanka	10.83DEFGH	8.767FGHIJ	3.733ABCD	0.2000DEF	71.61EFG
Japan early	9.533EFGH	7.700JK	3.333ABCDE	0.2333CDE	57.83FG
Pak I punjab	10.33DEFGH	7.567JK	2.200DE	0.1000 G	58.69FG
Punjab I	10.17DEFGH	7.733JK	3.367ABCDE	0.2333CDE	58.92FG
<i>Morus latefolia</i> Srilanka	9.900DEFGH	8.167IJ	2.767BCDE	0.2000DEF	65.64EFG
<i>Morus latefolia</i> Late	16.63AB	11.50BCD	3.500ABCDE	0.3667 A	146.8 AB
Husang China	10.03DEFGH	9.467EFGHI	3.300ABCDE	0.2000DEF	71.42 EFG
P.F.I	10.30DEFGH	8.233HIJ	2.633BCDE	0.1333FG	67.31 EFG
Kanmasi Japan	12.33CDEF	10.33DEF	4.033AB	0.2333CDE	99.04 CDEF
Kanmasi Japan late	12.63CDE	9.833EFGH	3.033ABCDE	0.2333CDE	92.99 DEF
Lun-40-punjab	9.667EFGH	8.633GHIJ	2.267DE	0.2000DEF	63.59 EFG
Korean subni	13.03CD	10.97CDE	3.967ABC	0.3333AB	108.2 BCDE
Gumgi korean early	14.77BC	12.63B	3.400ABCDE	0.3333AB	140.6 BC
<i>Morus latefolia</i> early	11.40DEFG	8.433GHIJ	3.033ABCDE	0.2000DEF	73.10 EFG
<i>Morus latefolia</i>	11.17DEFG	9.900EFG	3.000ABCDE	0.3000ABC	82.11 EFG
<i>Morus alba</i> I	9.667EFGH	8.767FGHIJ	3.133ABCDE	0.1667EFG	74.68 EFG
Kanmasi Japan early	7.933H	8.433GHIJ	2.900ABCDE	0.2000DEF	61.55 EFG
Pak II Punjab	14.60BC	5.833L	3.233ABCDE	0.1333FG	34.98 G
Husang china early	12.00CDEFG	12.03BC	2.567BCDE	0.2667BCD	132.7 BCD
Husang china late	14.8BC	9.700EFGHI	3.300ABCDE	0.2333CDE	87.80 DEF
NARC local	10.33DEFGH	8.833FGHIJ	3.367ABCDE	0.2333CDE	72.84 EFG
Gumgi korean late	11.00DEFGH	9.000FGHIJ	2.067E	0.2333CDE	74.75 EFG
Punjab II	19.6A	15.37A	3.667ABCDE	0.3667A	193.6 A
<i>Morus Indica</i>	9.900DEFGH	6.333KL	3.567ABCDE	0.1000G	53.97 FG
<i>Morus alba</i>	9.000GH	8.867FGHIJ	3.333ABCDE	0.1000G	59.91 FG
<i>Morus macorura</i>	10.87DEFGH	10.57CDE	3.467ABCDE	0.1000G	88.03 DEF
<i>Morus nigra</i>	9.833EFGH	8.433GHIJ	4.133AB	0.1333FG	65.16 EFG
<i>Morus serrata</i>	10.63DEFGH	7.700JK	4.500A	0.2000DEF	61.33 EFG
Japan late	9.267EFG	8.633GHIJ	3.367ABCDE	0.1000G	60.37FG

Means having a common letter are not significantly different at the 5% level of significance according to LSD.

*Correlation between morphological traits of mulberry accessions*

**Table No. 3**  
Pearson correlation coefficient matrix for qualitative variables of thirty accessions

	Lamina length	lamina width	petiole length	petiole width	leaf area index
Lamina length	1	0.702	-0.199	-0.162	0.762
Lamina width	0.702	1	-0.30	0.384	0.993**
Petiole length	-0.199	-0.30	1	0.355	-0.108
Petiole width	-0.162	0.384	0.355	1	0.132
Leaf area index	0.764 *	0.993**	-0.108	0.312	1

\*, \*\* shows significance at 0.01, 0.001 respectively.

It is revealed from the table that lamina length is positively and significantly correlated to lamina width ( $r = 0.70$ ) and leaf area index ( $r = 0.762$ ) while significant negative correlation was found in petiole length ( $r = -0.199$ ) and petiole width ( $-0.162$ ). Lamina width is positively correlated to the lamina length ( $r = 0.702$ ), petiole width ( $r = 0.384$ ) and leaf area index ( $r = 0.993^{**}$ ). Petiole length is negatively and significantly correlated to lamina length ( $r = -0.199$ ), lamina width ( $r = -0.30$ ) and leaf area index ( $r = -0.108$ ).

**Cluster and principal component analysis of morphological parameters**

It is obvious from the cluster analysis that the genotypes that are showing similarity with each other form two clusters (Figure No. 1). These two groups are divided into sister groups and sub sister groups. The outer groups are formed by Punjab II and Pak II Punjab and different from rest of the genotypes. The accessions in the same cluster show similarity to one another. The principal component analysis shows that the accessions, *Morus serrata*, *Morus nigra*, Gumgi Korean and pak I Punjab have the more different phenotypic characters than the other accessions.

**DISCUSSION**

Mulberry (*Morus* spp.) is grown under varied climatic conditions ranging from temperate to tropical (Kafkas et al., 2008). Plant species with a wide range of environmental adaptations like mulberry; have been found to exhibit numerous morphological and physiological characteristics. Thirty mulberry accessions were investigated in this study were taken randomly. All accession showed

varied response in leaf morphology. The means of all the traits according to its LSD had significant different lamina length, lamina width, petiole length, and petiole width and leaf area.

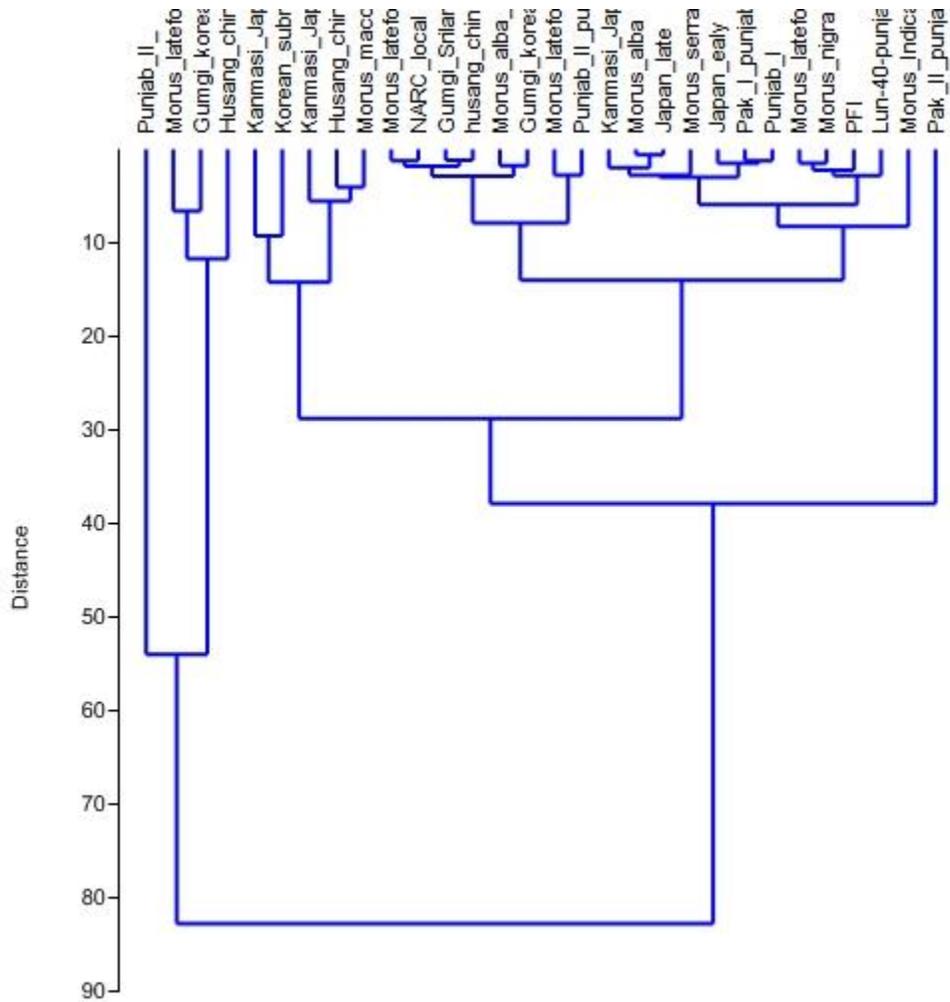
Chambel et al. (2004) found that the environmental changes are not only responsible for changing the population structure based on its genetic makeup to survive in the new environment and the level of each individual to alter its phenotype according to its environment. Similar results were reported by (Karst & Lechowicz, 2007) where they found differences in plasticity within species in relation to environmental factors.

Many plant species occupy a variety of contrasting habitats within a limited area and therefore plants must deal with these contrasting environmental conditions. Mechanisms by which a species may occupy a wide habitat range include reversible modifications to environmental conditions such as deficiency of nutrients, water, soil salinity or alkalinity, temperature and light levels, where plants attain a high degree of phenotypic plasticity (Fukui et al., 2000; Vijayan, 2009). Phenotypic plasticity is therefore an important means by which individual plants respond to environmental heterogeneity (Guo et al., 2007).

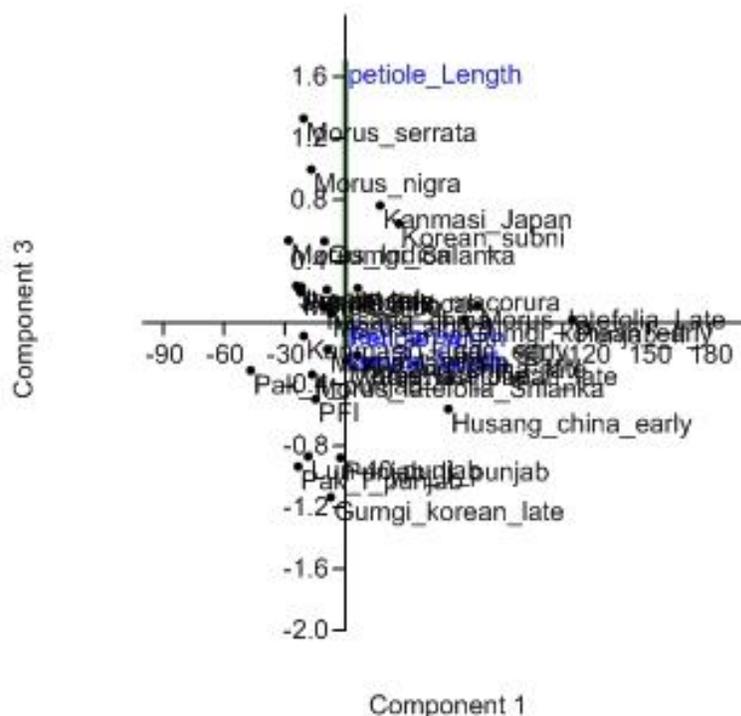
Phenotypic attributes are important to study the morphological characters in leaf yield. To evaluate the plant genotypes, the plant breeders use the phenotypic characters to study them (Mace et al., 2010). Morphological characterization of mulberry has been used as a tool to examine possible genetic relationships between different varieties that will be fruitful in its improvement (Tikader, 1997; Adolkar

et al., 2007). Mulberry is found in a wide range of distribution and it is thought to be having a lot of plasticity in its species. So, the leaf characters like lamina length, width, petiole length, width and height changes as the environment changes. (Gray, 1990)

also revealed the plasticity in mulberry fruit and leaf characters. While, (Kitajima et al., 1997) studied the topical canopy trees and found that there seasons also affect the size and stomatal functioning.



**Figure No. 1**  
**Dendrogram derived from hierarchical cluster analysis of combined 5 morphological traits of 30 Mulberry accession**



**Figure No. 2**  
**Principal Component analysis based on morphological characters of 30 Mulberry accession**

Phenotypic plasticity causes differential leaf forms. (Jones & Corlett, 1992) worked on the *Polygonium amphibium* L., Polygonaceae in various environments and came with a conclusion that there was a high phenotypic plasticity there in leaf characters. Pandey & Nagar, (2002) found that leaves can be different depending on their growth adaptations. Therefore it is pointed out that the ability to withstand different environmental scenarios, natural phenotypic differences is at top role. Similar results were concluded by Singh & Singh (2003) on decreased height of plant, girth, leaf area and cane yield in sugar cane (*Saccharum officinarum*). Reduction in leaf area, leaf growth and development was also reported in water stressed corn (Jones, 1992). Drought was also found to reduce leaf area, and vary mulberry morphological characters (Susheelamma *et al.*, 2000; Mujeeb *et al.*, 2004).

In current study, the analysis of variance (ANOVA) showed a wide range of variation and significant difference ( $p \leq 0.05$ ) in Lamina length,

width and petiole length, and width and leaf area. Similar results were reported by Tikader & Kamble, (2008) with highly significant differences of growth and yield traits of mulberry. Phenotypic variability of mulberry germplasm has been detected (Thangavelu *et al.*, 2000; Tikader & Rao, 2002). This kind of performance was reported by Ogunbodede & Ajibade, (2001) to be a function of environmental adaptation as well as genetic component. The leaf apex, margin, surface and texture could be used for identification purpose. Stem, young shoot, and newly sprouted leaf colors are also forms of identification of the different mulberry accessions (Adolkar *et al.*, 2007).

Based on the hierarchical cluster analysis, three groups were evident from the dendrogram. Pak II punjab, Lun-40-punjab, *Morus Indica*, PFI, *Morus latifolia* Srilanka, Japan late, *Morus alba* clustered. Similarly, Punjab II Punjab, *Morus latifolia* Late, Gumgi korean late form tight cluster while the other accessions clustered, separately. However it is

evident that the accession did not cluster according to their geographical origin (Tikader & Roy, 2002; Tikader *et al.*, 2003). Cluster analysis based on morphology was studied by Sharma *et al.* (2000) reported that based on morphological differences among accession they form cluster. Principal component analysis for relationship among different accessions showed most of the variance explained by bipolt charting. According to first and second components, component near to origin has higher positive coefficient with respect to the desirability of high level of these indicators.

## CONCLUSIONS

It is concluded that morphological traits clustered the thirty mulberry accessions into two main groups which further form sub clusters. In addition, significant variation across the accessions was contributed by lamina length, lamina width, petiole length, width and leaf area, these traits can be utilized in selection of mulberry accessions for future mulberry improvement and breeding of high yielding varieties. This study is also helpful for the selection of mulberry accessions for use in sericulture.

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