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Morphological Characterization of Mulberry (*Morus spp.*) Accessions Grown in Kenya

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Abstract

Genetic divergence of five mulberry accessions including Embu, Thika, Thailand (*M. alba*), Kanva-2 and S41 (*M. indica*) grown in Kenya were examined using twelve phenotypic traits. The assessment of phenotypic traits was done in a field study in two localities, Nairobi and Eldoret. The traits that were significantly different across the mulberry accessions included lamina width and petiole length ($P \leq 0.01$), petiole width and growth height ($P \leq 0.05$), internodes distance and number of branches ($P \leq 0.001$). The Duncan's Multiple Range Test (DMRT) results were used to generate a dendrogram derived from hierarchical cluster analysis that further partitioned the mulberry accessions into four groups. Embu and Thailand accessions grouped together while S41, Thika and Kanva-2 accessions grouped separately. Embu and Thailand accessions were characterized by fewer numbers of branches than the rest of the accessions. Thika accession had high number of branches and short internode distance. Significant and positive correlations were found between leaf yield traits except in internode distance and number of branches which were significant and negatively correlated. Significant and positive correlations can be utilized since they are rewarding for mulberry leaf yield improvement.

Keywords: mulberry, phenotypic traits, genetic divergence, correlation, hierarchical cluster analysis

1. 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).

Plant species with a wide range of environmental adaptations like mulberry; have been found to exhibit numerous morphological and physiological characteristics (Cordell et al., 1998). Morphological variability in mulberry is said to have contributed to its growth and survival under various disruptive environmental conditions (Gray, 1990). Phenotypic plasticity is the ability to develop different phenotypes in response to environmental conditions (Winn, 1996), is heritable and plays an important role in species evolutionary strategy (Agrawal, 2001).

Phenotypic plasticity lies at the intersection of a variety of disciplines like ecology, physiology, developmental morphology, genetics and evolution. Differentiation of plant genotypes for plant breeding programs has been utilizing phenotypic characters for evaluation of plant genotypes (Mace et al., 2005). Morphological characterization of mulberry has been used as a tool to examine possible genetic relationships, and this information used in its improvement (Tikader, 1997; Adolkar et al., 2007). Therefore in this study, morphological traits were studied in order to distinguish five of the mulberry accessions grown in Kenya.

2. Materials and Methods

Five mulberry accessions [Embu, Thika, Thailand (*M. alba*), S41 and Kanva-2 (*M. indica*)] were evaluated in this study. These were obtained from International Centre of Insect Physiology and Ecology (ICIPE) germplasm site at Nairobi and Kenya Agricultural Research Institute (KARI) Thika.

2.1 Experimental Site

The assessment of the field morphological traits was carried out in 2009-2010 in two locations; Moi University Chepkoilel Campus in Eldoret and at ICIPE headquarters in Nairobi, Kenya. Eldoret is located between latitude $0^{\circ} 30'$ North and longitude $35^{\circ} 15'$ East. The altitude is 2180 m ASL and it receives an annual rainfall that ranges between 624-1622 mm. The mean annual temperature is 17°C . Nairobi is located between latitude $1^{\circ} 16' 60''$ South and longitude $36^{\circ} 49' 0''$ East. The altitude varies from 1680 m-1728 m ASL while the mean annual rainfall is 925mm falling in a bimodal pattern. The annual temperature ranges from 11.8°C to 27.85°C .

2.2 Nursery Preparation

Cuttings of size 15-20 cm with 4-6 healthy buds were made. The cuttings were planted in a rooting media of sand and soil at a ratio of 2:1, respectively. This was done in wooden boxes of 75 X 75 X 50 cm each for every variety. The cuttings were planted in a diagonal format leaving only two buds above the surface of the soil. After planting, the soil around the cuttings was pressed firmly and watered to field capacity. Thereafter watering of cuttings was done as and when required.

2.3 Field Layout and Experimentation

A randomized complete block design (RCBD) with three replications was used in this experiment. Cuttings of the five mulberry accessions which included; Thailand, Thika, Embu, S41, and Kanva-2 were planted at a distance of 3×3 m in pit holes of $30 \times 30 \times 30$ cm deep. Each experimental unit consisted of six mulberry cuttings and a guard row surrounding the whole experimental plot. Farm yard manure was applied at the rate of 7 tons ha^{-1} prior to planting. NPK was applied in order to supply 16.8 kg N, 7.4 kg P_2O , 13.8 kg K_2O ha^{-1} using the ring method, 60 days after transplanting. All plants were bottom pruned 30cm from ground level after six months of planting as per the procedure certified by Raina, (2000). A booster dose of urea was applied at 45 kg N ha^{-1} 35 days post-pruning. Plots were maintained weed free to the end of the experiment by hand weeding.

2.4 Field Measurement of Morphological Traits

Morphological measurements at ICIPE were made on four randomly selected and tagged plants per accession and replicated thrice based on Randomized Complete Block Design. In Eldoret, measurements on morphological traits were carried out on four selected plants per accession across the replicates, in an experiment consisting of five mulberry selections planted in a randomized complete block design, with three replicates. Twelve parameters were measured and data used for diversity analysis. The quantitative traits were lamina length (cm), lamina width (cm), petiole length (cm), petiole breadth (cm), and internodes distance (cm), plant height (cm), bud length (cm) and number of branches. The Qualitative traits were leaf apex, leaf margin, leaf surface, and leaf texture.

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. The breadth was measured by cutting the petiole portion from the base of the leaf blade and measuring its breadth. These parameters were recorded after 82 days of pruning by taking measurements from the fully grown leaves from the 7th to 9th positioned leaves in the longest shoot. Leaf apex was denoted as acute; the tip portion was straight to convex margin forming an angle of less than 90° , acuminate; leaf tip was slightly extended with margins markedly concave, caudate; the leaf tip extended greatly forming a tail like structure, obtuse; the leaf had straight to convex margins forming an angle more than 90° . Leaf texture was denoted as membranous; leaves were thin and semi transparent like a fine membrane, charatacious; leaves were opaque, coriaceous; leaves were leathery, thick and with a stiff shoot. Leaf surface was felt by rubbing the upper surface of the leaf blade gently with the fingers, the feeling could either be smooth, slightly rough, rough and hairy. The leaf margin was recorded as crenate; crenations were smoothly rounded without a pointed apex, serrate; serrations were pointed with their axes inclined forming oblique angles, dentate; dentations were pointed with axes approximately perpendicular to the trend of the margin, repand; margins forming a smooth line. These parameters were recorded after 63 days of pruning in the middle portion of the longest shoot. Counts of the number of branches were done while growth height was measured on the longest shoot. Bud length was measured on the matured bud from the basal portion to the tip of the bud. Internodes distances were measured between the 8th-9th nodes. These parameters were recorded at full growth stage of the plant after 101 days of pruning. Shape and colour of fruits were determined during the period of data collection. These measurements have been adopted from Adolkar et al. (2007) and Food and Agriculture organization (FAO), (2007).

2.5 Data Analysis

Data were analyzed by GenStat statistical package using General Linear Model (GLM); $Y_{ijk} = \mu + \beta_i + \gamma_j + \sum_{ijk}$ where μ was the general mean, β_i was the block effect, γ_j was the treatment effect and \sum_{ijk} was the error term. 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 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.

3. Results

3.1 Morphological Parameters

Qualitative results showed leaf apex of Thika, S41 and Kanva-2 accessions as being acute while Embu and Thailand accessions had a caudate apex. The leaf margins of Embu, Thailand and Kanva-2 accessions were dentated while those of Thika and S41 were serrated. The different mulberry accessions varied in their leaf surface where the Embu accession was rough, Thika; slightly rough, Thailand; smooth, S41; hairy and Kanva-2; smooth. The leaf texture of accessions Thika and Kanva-2 were membranous while those of Embu and Thailand accessions were charatacious.

Table 1 shows the means of the different morphological parameters based on LSD showed that the lamina length, lamina width, petiole length, petiole width and growth height were significantly different across the two environments with Eldoret having higher parameters than ICIPE, Nairobi. However, the number of branches, internode distance and bud length were not significantly different across the two environments.

Means of the different parameters across the different accessions showed that lamina width, internode distance, number of branches varied significantly with accessions while lamina length, petiole length, growth height, bud length and petiole width were not significant across the accessions. Nevertheless, large leaf sizes were noted in accessions S41, Thika, Embu, Kanva-2 and Thailand, respectively. Petiole length ranged from 2.82-3.575 cm. Thika accession had the shortest petioles followed by Kanva-2, Thailand, S41 and Embu, respectively. Long internode length was noted in Embu, Kanva-2, S41, and Thailand and Thika accessions, respectively. Growth heights of the different accessions ranged from 97.0 cm-123.5 cm with Kanva-2 being the tallest accession followed by Thika, Thailand, Embu and S41, respectively. Highest branching was noted on accessions Kanva-2, S41, and Thika, Thailand and Embu, respectively (Table 1).

The ANOVA results revealed that the mean squares of lamina length, lamina width, petiole length, and petiole width and plant height differed significantly ($p \leq 0.001$) across the environments (Appendix 2a). On the other hand, there were highly significant ($p \leq 0.05$) differences in petiole width and growth height across the accessions. Internode distance and number of branches also differed significantly ($p \leq 0.001$) among the accessions. There were highly significant differences ($p \leq 0.01$) in lamina width and petiole length across the accessions. Results also showed significant differences ($p \leq 0.05$) in lamina length, petiole length, petiole width and high significant difference ($p \leq 0.01$) in internode distance and number of branches between the locations (Table 2).

Table 1. Means of eight phenotypic traits of 5 mulberry accessions measured across two environments

Traits	Location		Accessions					S.E	CV%
	Nairobi	Eldoret	Thailand	S41	Kanva-2	Embu	Thika		
Lamina length (cm)	11.98b	17.78a	13.79a	15.96a	14.08a	15.31a	15.26a	1.14	7.60
Lamina width (cm)	9.72b	14.00a	10.45b	12.59a	10.45b	12.63a	13.20a	0.91	7.70
Petiole length (cm)	2.91b	3.59a	3.27a	3.52a	3.06a	3.57a	2.82a	0.31	9.40
Petiole Width (cm)	0.35b	0.52a	0.39a	0.42a	0.43a	0.47a	0.47a	0.04	9.40
Internode distance (cm)	5.25a	4.74a	4.78a	4.92a	5.54a	5.67a	4.07b	0.55	10.90
plant height (cm)	76.70b	148.00a	113.50a	97.00a	123.50a	107.90a	119.80a	17.37	15.50
Number of branches	18.07a	19.06a	14.79b	22.42a	22.42a	11.29b	21.91a	3.96	21.30
Bud length (cm)	1.80a	1.83a	1.75a	1.70a	1.95a	1.90a	1.76a	0.20	11.30

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

SE = Standard Error. CV= Coefficient of Variation.

3.2 Correlation Between Morphological Traits of the Mulberry Accessions Across the Two Environments

Table 2 shows that lamina length was positively and significantly correlated to lamina width ($r = 0.941^{**}$), petiole length ($r = 0.614^{**}$), plant height ($r = 0.798^{**}$), and petiole width ($r = 0.800^{**}$). Lamina width was positively and significantly correlated to petiole length ($r = 0.558^{**}$), plant height ($r = 0.718^{**}$) and petiole width ($r = 0.822^{**}$). Correlations of petiole length to plant height ($r = 0.391^*$) and petiole width ($r = 0.582^{**}$) were positive and significant. plant height was significantly and positively correlated to petiole width ($r = 0.693^{**}$) while internodes distance ($r = -0.363^*$) was negative and significant. Significant, negative correlations were also found between internodes distance and number of branches ($r = -0.342^*$).

Table 2. Pearson correlation coefficient matrix for quantitative variables of 5 mulberry accessions

	Lamina length	Lamina width	Petiole length	Plant height	Internodes distance	N ^o of branches	Bud length	Petiole width
Lamina length								
Lamina width	0.941**							
Petiole length	0.614**	0.558**						
Plant height	0.798**	0.718**	0.391*					
Internodedistance	-0.222	-0.25	0.253	-0.363*				
N ^o of branches	0.279	0.202	-0.29	0.184	-0.342*			
Bud length	0.073	0.081	0.131	0.189	0.135	-0.13		
Petiole width	0.800**	0.822**	0.582**	0.693**	-0.095	0.085	0.061	

*, **, ***; significant at $p = 0.05, 0.01, 0.001$, respectively.

Cluster analysis of the different mulberry (*Morus spp*) accessions based on morphological traits.

A more specific grouping was done to cluster the five mulberry accessions (Figure 1) Results revealed four groups where Thailand and Embu accessions were clustered tightly while each of the Thika, Kanva-2 and S41 accessions were grouped separately. Nonetheless, S41 was distantly related to the other accessions.

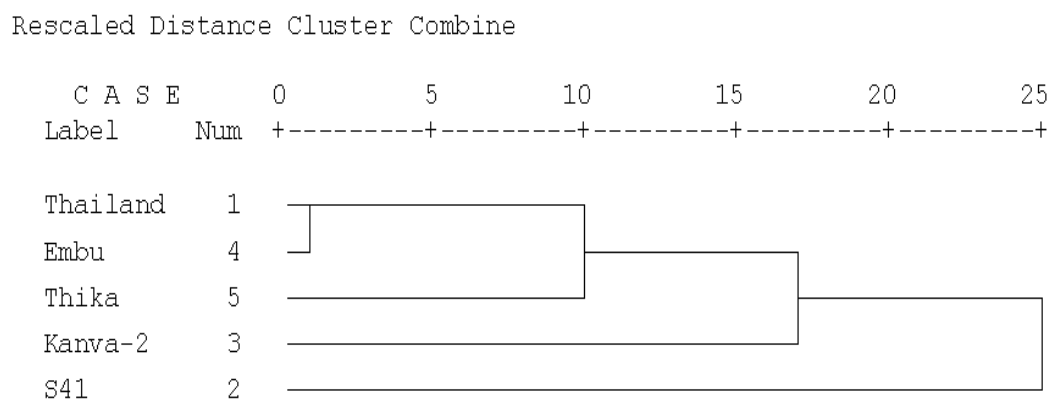


Figure 1. Dendrogram derived from hierarchical cluster analysis of combined 8 morphological traits of *Morus spp*

4. Discussion

Mulberry (*Morus spp.*) is grown under varied climatic conditions ranging from temperate to tropical (Sedat et al., 2008). The mulberry accessions investigated in this study were grown in two different environments in different seasons. Mean separation of the morphological traits according to LSD showed that the two environments were

significantly different in lamina length, lamina width, petiole length, and petiole width and growth height. This could have been attributed to differences in environmental conditions. Similar results were reported by Karst and Lechowics (2007) where they found differences in plasticity within species in relation to environmental factors. Chambel et al. (2004) reported response of pine trees population to environmental changes as based on the amount of genetic variability to adapt to the new conditions by not only altering population structure, but also on the extent in which each individual is able to change its phenotype according to the environment. Phenotypic plasticity in response to temperature and photoperiod changes has been noted on European tree species (Kramer, 1995). Gray (1990) suggested phenotypic plasticity as the contributory factor to mulberry's growth and survival under disruptive environmental conditions.

The wide natural distribution of mulberry supports the idea that plasticity operates in these species. Lamina length, lamina width, petiole length and width as well as growth height varied across the environments. Gray (1990) also observed plasticity in leaf and fruit characteristics of mulberry. On the other hand, Kitajima et al. (1997) reported seasonal variation in size and stomatal conductance in tropical canopy trees. Leaf polymorphism is a result of great phenotypic plasticity. Gabersick and Martinac (1992) studied leaf characteristics of amphibious bistort (*Polygonum amphibium* L., Polygonaceae) in an environmental gradient and found great phenotypic plasticity on leaf characters. Pandey and Nagar, (2002) also reported variation in leaf traits to be as a result of plant adaptation to their growth. It is therefore true that inherent phenotypic differences allow a plant to survive in a wide range of environmental conditions by altering leaf morphology.

In the two environments the morphological traits showed better performance at Eldoret than at Nairobi. These could have been attributed to water stress experienced at ICIPE immediately after pruning and therefore the crop did not perform well. Similar results were reported by Singh and 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 (Meiri & Poljakoff, 1970; Susheelamma et al., 2000; Mujeeb et al., 2004).

The analysis of variance (ANOVA) showed a wide range of variation and significant difference ($p \leq 0.01$) in lamina width and petiole length, ($p \leq 0.05$) in petiole width and growth height, ($p \leq 0.001$) in internode distance and number of branches across the different accessions. Similar results were reported by Tikader and 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 and 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).

Correlations are important in understanding the relationships (phylogeny) between morphological/agronomic traits of accessions. This helps breeders to formulate appropriate breeding strategies for selection of desired traits (Herbert et al., 1994). In some cases, differences in relationship of growth and yield parameters associated to yield are often noticed (Vijayan et al., 1997; Tikader & Dandin, 2005, 2008).

In the present study, lamina length was positively and significantly correlated to lamina width, petiole length, growth height and petiole width. Significant positive correlations were also found between lamina width and petiole length, growth height and petiole width. Petiole length was positively and significantly correlated to growth height and petiole width. These findings are similar to those reported by Banerjee et al. (2007) in mulberry. Leaf size, internodes distance, shoot length, lamina weight and fresh leaf weight were found to have a positive effect on yield (Tikader & Roy, 2001). Negative and significant correlations were found between growth height and internodes distance. Internode distance was also negatively associated to the number of branches. However, positive correlation of leaf yield/plant is mainly contributed via number of branches and internodes distance (Banerjee et al., 2007). The highly correlated traits can therefore be considered during selection for their high yields since the mulberry leaves are of economic importance in the sericulture industry.

In this study, Thika accession has large leaf sizes, short internode distance and high branching thereby having the best traits that contribute to leaf yield. Kanva-2 and S41 accessions can be selected for their high branching and large leaf sizes. Additionally, S41 can be selected for its short height which is suitable for leaf picking. The Embu accession has large leaf sizes, although its internode distance are long and its branches are also few. Likewise, for Thailand accession despite its short internode distance, its branches are few while the leaf sizes are small. Therefore selection of mulberry accessions with the best traits that contribute to leaf yield should be

accessions Thika, Kanva-2, S41, Embu and Thailand, respectively.

Based on the hierarchical cluster analysis, four groups were evident from the dendrogram. Embu and Thailand accessions clustered tightly while the other accessions clustered, separately. Thailand, S41 and Kanva-2 accessions have been introduced to Kenya; however it is evident that the accession did not cluster according to their geographical origin (Fotedar & Dandin, 1998; Tikader & Roy, 2002; Tikader et al., 2003). On the other hand, S41 was distantly related to the other accessions which could have been attributed to its short height. At species level, *M. alba* accessions (Thailand, Thika and Embu) grouped separately morphologically from *M. indica* (Kanva-2 and S41) accessions, however the overall distance between them was not wide. Similar results were reported by Gururajan (1960); where considering morphological features he grouped *M. alba* and *M. indica* species as one species. Further, Sharma et al. (2000) reported no significant morphological differences among genotypes of *M. alba* and *M. indica* species.

5. Conclusion

Morphological traits clustered the five mulberry accessions into four groups with Thailand and Embu accessions grouping together. These were characterized by few branches while the rest of the accessions grouped separately. Thika, S41 and Kanva-2 accessions were characterized by many branches and large leaf sizes. In addition, significant variation across the accessions was contributed by internode distance, lamina width and number of branches. Consequently, these traits can be utilized in selection of mulberry accessions for future mulberry improvement and breeding of high yielding varieties.

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