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Evaluation of mulberry cultivars (*Morus* spp.) in Western Uganda

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Abstract
Mulberry (*Genus morus*) is an economically important plant used for sericulture, as it is the sole food plant for the domesticated silkworm, *Bombyx mori*. The genus *Morus*, which is widely distributed in Asia, Europe, North and South America, and Africa, is cultivated extensively in East, Central and South Asia for silk production. Attempts have been made to characterise the genetic diversity in mulberry. This experiment was set up in a randomized complete block design with three replications for each variety. Ten agronomic traits to include Plant Height (HT), Internode Distance, (ID), number of branches per plant, Lamina Length (LL), lamina width (LW), leaf area, leaf yield/plant were studied, data was analyzed statistically using R version 4.0.1 (R Core Team, 2014) and significant differences were considered at $P< 0.05$. There were significant variations in ten agronomic traits among the tested mulberry accessions. Genetic background and environment are the main factors influencing leaf yield. Correlation matrix of different traits showed that leaf yield is a combination of multiple traits and plays a significant role. Leaf yield per plant significantly differed across all the varieties but branching varieties such as S.36 recorded highest leaf yield compared to other varieties studied.

Keywords: Diversity, mulberry, cultivar, morphology, accession, sericulture

Introduction
Mulberry (*Morus* spp.) is an economically important tree cultivated for its leaves used as a unique food source for domesticated silkworm (*Bombyx mori* L.). It is a perennial plant propagated exclusively through stem cuttings and thus the acquired true-to-type plant population. The vegetative reproduction leads to the perpetuation of the characters with great precision. Mulberry leaves with superior quality and higher yield are the essential requirement for quality cocoon production. *Morus* spp. is a widely distributed crop in the temperate, sub-tropical and the tropical regions of the world and is highly adaptable to a wide range of climatic, topographical and soil conditions (Hosseini *et al.*, 2018) [11].

Due to its considerable importance in the sericulture industry across the world, development of high yielding mulberry varieties through various breeding methods has been going on for many years and is mainly based on indirect selection for yield growth characters. A large number of improved varieties have evolved for commercial purpose in recent years. These varieties registered their superiority over traditional local varieties in exhibiting increased yield with good quality leaves, wide adaptability and resistance to stress condition. This has led to the release of cultivars with varying agronomic traits to the farmers. Recent studies confirm the expectation that these genotypes differ extensively in the physiological process determining yield. It was widely accepted that genetic variation was of fundamental importance for species' conservation (Banerjee *et al.* (2007) [2]; Gilpin *et al.* (1986) [9]; Barrett *et al.* (1991) [4]; Lande (1999) [17]. Sarkar *et al.* (1992) reported that in mulberry variety selection, total shoot length, total weight of all branches and weight of 100 leaves were considered as important parameters which had direct effects on leaf yield. The main objective of evaluation of mulberry genetic resources was to promote utilization directly by the breeders for the improvement of programme. In order to achieve such objectives, it was very essential to evaluate mulberry genetic resources for growth and yield traits. Hence the present studies were undertaken to evaluation of elite mulberry genotypes in different seasons.

Materials and Methods
Plant materials: Eight mulberry genotypes (All foreign accessions as shown in Table 1) were evaluated for their genetic diversity using agronomically important traits. All the genotypes used in the study originated from China, India and Kenya (Table 1).

Study site

This study was carried out at the National Sericulture Resources Development Centre, Rubaare, Sheema, Uganda. The centre is situated at 00 32S, 30 24E and 942 m altitude with tropical dry climate. The soils are generally acidic but fertile with soil pH of 6.5–7.5. The average rainfall ranges from 1000 – 1500 mm per annum with an average annual temperature of 26 degrees Celsius (78° Fahrenheit).

Experimental design

The experiment was set up in a randomized complete block design with three replications. The genotypes were planted in 4.0 m × 5.0 m plots at a spacing of 1.0 M × 1.0 M. Throughout the experimental period, the plots were kept free of weeds by hoeing. No Fertilizers or supplementary water through irrigation was applied during the trials. Major pests and diseases (Leaf spot, leaf rust and powdery mildew) were controlled by 1-2 sprays (depending on pest pressure) using Dimethoate 40%EC and Rocket 44 EC (Profenofos 40% + Cypermethrin 4%) at recommended rates.

Data collection

After one year of establishment in the field, data was recorded by following a 5-crop schedule. Data from the 5 plants were recorded on various yield attributing traits such as plant height (HT), Internode distance (ID), number of branches per plant, Lamina length (LL), lamina width (LW), leaf area, leaf yield/plant and were recorded. The leaf yield per plant was recorded after 90 days of pruning. These measurements were adopted from Adolkar *et al*. (2007) [1], Thangavelu *et al*. (2002) [26]; Machii *et al*. (2001) [19] and Food and Agriculture organization (FAO), (2007) [8].

Data Analysis

The experimental data obtained throughout the whole study period was analyzed statistically using R version 4.0.1 (R Core Team, 2014). Significant differences were considered at $P < 0.05$. The mean values for all the agronomic traits were later used for correlation matrix studies.

Table 1: Cultivars used in the study

S. NO	Genotype	Species	Country of origin
1	Thika	*M. alba L.*	Kenya
2	Kanva2	*M. indica L.*	India
3	Embu	*M. alba L.*	Kenya
4	Thailand	*M. alba L.*	Thailand
5	Mysore Local	*M. indica L.*	India
6	S.36	*M. indica L.*	India

Adopted from Vijayan *et al.*, 2003 [33] and Wangari Peris and Ngode Lucas (2016) [21].

Table 2: Quantitative phenotypic characters recorded on 6 genotypes of mulberry

S. No	Qualitative character
1	Plant Height
2	Number of Branches
3.	Bud length
4.	Internode distance
5.	Lamina length
6.	Lamina width
7.	Leaf Area
8.	Leaf Yield
9.	Petiole Length
10.	Total Biomass

Table 3: Means of eleven phenotypic traits of six mulberry accessions

Cultivar	Bud length (cm)	Internode distance (cm)	Lamina length (cm)	Lamina width (cm)	Leaf area (cm)	Leaf yield per plant (g)	Number of branches (cm)	Petiole length (cm)	Plant height (cm)	Total biomass per plant (g)	Hundred leaf weight (g)
Embu	3.22 ± 0.97	3.13 ± 0.71	17.72 ± 1.3	13.44 ± 1.42	239.78 ± 41.24	0.335 ± 0.10	4.11 ± 1.69	4.17 ± 0.35	281.78 ± 49.63	1.20 ± 0.55	0.45 ± 0.19
Thika	4.89 ± 0.78	3.80 ± 0.53	16.33 ± 2.45	11.78 ± 2.22	196.44 ± 60.75	0.45 ± 0.19	3.77 ± 1.56	3.94 ± 0.53	237.78 ± 105.42	2.16 ± 0.96	0.64 ± 0.05
Kanva 2	2.33 ± 0.71	3.33 ± 1.15	12.92 ± 1.43	8.89 ± 0.93	115.93 ± 24.61	0.32 ± 0.33	3.44 ± 1.67	2.56 ± 0.39	206.89 ± 47.50	0.649 ± 0.22	0.47 ± 0.25
Local Variety	3.22 ± 0.83	3.87 ± 1.00	19.17 ± 2.28	13.11 ± 1.90	254.78 ± 64.74	0.54 ± 0.22	4.0 ± 1.41	4.09 ± 0.68	277.44 ± 64.92	1.838 ± 0.85	0.70 ± 0.32
S-36	3.44 ± 1.13	3.89 ± 0.89	18.17 ± 0.71	15.56 ± 0.88	282.94 ± 25.17	0.83 ± 0.28	4.3 ± 2.78	3.56 ± 0.46	232.22 ± 82.88	2.31 ± 0.84	0.92 ± 0.28
Thailand	3.56 ± 0.52	3.44 ± 0.73	17.56 ± 2.96	14.39 ± 2.55	258.33 ± 80.74	0.14 ± 0.05	5.0 ± 2.00	3.61 ± 0.86	270.89 ± 56.44	1.416 ± 0.742	0.54 ± 0.22
F Values	8.609	1.23	10.685	15.56	11.387	10.465	10.6962	9.639	12.6199	6.6156	12.478
P values	0.0000070 47	0.3098	0.000000613 4	0.000000004 377	0.00000002825	0.000000078 6	0.0006289	0.000000203 6	0.00001728	0.0000931	0.00000007 825

Results and Discussion

The mulberry germplasm accessions tested in this experiment are presented in Table 3. These accessions were collected from different sources.

Morphological Variability among Mulberry Accessions

The agronomic variation among the accessions is presented in Table 3. A high variation was observed in different growth and yield related traits. The results obtained in this study indicate that all the mulberry accessions were significantly different in all the major agronomic traits. Means of the different morphological parameters assessed i.e., plant height, number of branches, bud length, lamina length, lamina width, leaf area, leaf yield per plant, petiole length, pant height and total biomass per plant were significantly different across the different cultivars of the mulberry ($P \leq 0.05$). However, the internode distance (F=1.23) was not statistically significant across the different cultivars of mulberry. Thika had the highest bud length whereas the least bud length was observed in Kanva 2 variety. The long internode distance was relatively uniform for the six mulberry cultivars though S.36, Local variety and Thika genotypes had the longest respectively. The highest lamina length was recorded with the local variety and S.36 whereas least lamina length was observed in the Kanva 2 variety. Highest lamina width was observed in S-36 whereas least lamina width was observed in Kanva 2. S-36 and Thika varieties had the highest total biomass respectively whereas Kanva 2 had the least biomass. Maximum leaf yield was observed in S-36 whereas the least leaf yield was observed in the Thailand variety. Highest number of branches was noted on accessions Thailand, S.36, Embu and Local variety respectively. Plant height of the different accessions ranged from 206.89±47.50 cm-281.783±49.63 cm with Embu being the tallest accession followed by Local variety, Thailand, Thika and S.36, respectively (Table 3).

Petiole length ranged from 2.56±0.39-4.17±0.35 cm with Embu, Local variety sand Thika accessions having longest petiole length while the shortest petioles were noticed from Kanva-2 followed by S.36 respectively. On calculating the leaf area, it was discovered that S.36 genotype had the highest (282.94 ± 25.17), followed by Thailand (258.33 ± 80.74) and Local variety (254.78 ± 64.74). The least leaf area was noticed in Thika (196.44 ± 60.75) and Kanva-2 (115.93 ± 24.61). Weight of a hundred was high in S.36 (0.92 ± 0.28), Local variety (0.70 ± 0.32) and Thika (0.64 ± 0.05).

Mulberry (*Morus* spp.) is grown under varied climatic conditions ranging from temperate to tropical (Kafkas *et al.*, 2008) [14]. Plant species with a wide range of environmental adaptations like mulberry; have been found to exhibit numerous morphological and physiological characteristics across the environment. 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) [18]. 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) [32, 1]. In this study, six mulberry genotypes were evaluated for their morphological diversity in Uganda. All the genotypes showed significant variations in all the main agronomic traits. Significant differences were observed the number of primary branches per plant, internodal distance, Plant height, number of branches, lamina length, lamina width, petiole length, bud length and leaf area except for internode distance. The F-ratio indicated that all the accessions were highly significant for all the traits. These results agree with the observations made by Tikader and Roy (2001) [31] and Tikader and Dandin (2005) [27]. Leaf yield per plant significantly differed across all the varieties. Many authors have reported that, mulberry leaf yield largely depends on various leaf yield contributing characters, genotype and agronomic practices followed (Kasivishwanathan and Iyengar, 1970; Rangaswami *et al*., 1976; Shastry *et al*., 1980; Jolly and Dandin, 1986; Bari *et al*., 1989; Bindroo *et al*., 1990; Dandin *et al*., 1993) [16, 22, 25, 12, 3, 5, 7]. Yokoyama (1963) [35] further reported that mulberry leaf yield depends on the number and length of shoots, internodal distance and leaf weight/plant. Present findings in this study are in conformity with these observations from different authors. In profusely branching varieties such as S.36, yield was high.

The results obtained from this study slightly deviated from similar studies conducted from the other countries. Chambel *et al*. (2004) [6] found that the environmental changes were 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. These could be used to explain the significant variations in all the varieties studied. Similar results were reported by (Karst & Lechowicz, 2007) [15] where they found differences in plasticity within species in relation to environmental factors. 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) [10]. The breeders have the opportunity to select a suitable group of germplasm for further utilization. The genetic diversity is being assessed by single trait leaf yield which is the end product used by farmers for silkworm rearing to produce cocoon. This confirms the importance of understanding how individual trait or farmers use group of traits to identify different genotypes.

Simple Correlation coefficient matrix

Simple correlation coefficient was carried out among the accessions on different growth and yield traits (Table 4). Correlation studies were important in understanding the relationships (phylogeny) between morphological/agronomic traits of accessions. This helped breeders to formulate appropriate breeding strategies for selection of desired traits (Herbert *et al.,* 1994). It was revealed from the results obtained that lamina length was positively and significantly correlated to lamina width (*r*= 0.8557***). Bud length expressed a positive correlation with laminar length (*r*= 0.1696) and lamina width (*r*= 0.1739). Internode distance expressed a significant positive correlation with lamina length (*r*= 0.3102*). However, it showed non-significant correlation with lamina width and bud length. Correlations of petiole length to lamina length (*r*= 0.6800***), lamina width (*r*= 0.5370***), bud length (*r*= 0.3727**) and internode distance (*r*= 0.3984**) were positive and significant. Plant height was significantly and positively correlated to petiole length (*r*= 0.2912**) and the number of branches (*r*= 0.3516**), while internodes distance (*r*=-0.0073) was negatively correlated. Lamina length (*r*= 0.2767), lamina width (*r*= 0.1261), Bud length (*r*= 0.1572), plant biomass (*r*= 0.4553), leaf yield (*r*= 0.1052) and the leaf area (*r*= 0.1493) were positively correlated with the plant height.

In this study, differences in relationship of growth and yield parameters associated to yield were noticed. All the traits in this study showed association with leaf yield. Several authors have reported similar findings (Sarkar *et al*. 1987, Vijayan *et al*. 1997; Tikader & Roy 1999) [23, 34, 30]. The relationship of different traits indicated that all the traits were associated either directly or indirectly with leaf yield. The leaf yield was a complex trait which is contributed by other traits. Leaf yield was directly related with the number of primary branches per plant, length of the longest shoot, total shoot length, internodal distance, leaf moisture content, leaf moisture retention capacity, and single leaf weight. Other traits were associated with each other and formed a complex relationship among them. The highly correlated traits should be considered during selection. Morphological identity along with growth and yield traits has direct relevance to the farmers as well as plant breeders to select, utilize and conservation of germplasm (Tikader and Kamble 2008c) [28]. It was important to note that growth and yield traits have a number of limitations to express in different environmental conditions and influence the performance either positive or negative (Tikader and Kamble 2008d) [29]. Thus, mulberry genotypes which have performed well can be suitable for further utilization and conservation of genetic resources in Uganda.

Table 4: Correlation coefficient matrix

Trait	Lamina length	Lamina width	Bud length	Internode distance	Petiole length	Leaf area	Leaf yield	Number of branches	Plant biomass	Plant height
Lamina length										
Lamina width	0.8557***									
Bud length	0.1696	0.1739								
Internode distance	0.3102*	0.1963	0.1943							
Petiole length	0.6800***	0.5370 ***	0.3727**	0.3984**						
Leaf area	0.9454***	0.9714***	0.1647	0.2679*	0.6021					
Leaf yield	0.2436	0.3278**	0.1369	0.1052	0.1092***	0.2812*				
Number of branches	0.0742	0.1483	0.0392	0.2176	0.2106	0.0879	0.1023			
Plant biomass	0.1795*	0.2897* *	0.3437*	0.1349**	0.2723*	0.2676*	0.6779***	0.3561**		
Plant height	0.2767	0.1261	0.1572	-0.0073	0.2912*	0.1493	0.1052	0.3516**	0.4553	
Hundred weight	0.2669*	0.3847**	0.2830*	0.2011	0.1635**	0.3294**	0.7084***	0.1781	0.4595***	0.1302

Conclusion

Since the introduction of Silk production in Uganda several years ago, the industry is still undeveloped. This is attributed to several challenges faced by the farmers, one being lack of desirable mulberry varieties to provide quality leaves for feeding the silkworms. In the present study, different mulberry varieties were evaluated for their morphological performance in Uganda. Morphological variations were noticed in all the key agronomic traits in all the six mulberry varieties. The identification of elite varieties or promising varieties of mulberry is important because it can ultimately have a direct effect on silk production and quality. One of the important ways by which these can be identified is to screen the mulberry germplasm for range and extent of genetic diversity present in it. Significant variation across the accessions for traits such as plant height, number of branches, leaf yield, 100 leaf weight, lamina length, lamina width, petiole length and leaf area can be utilized in selection of mulberry accessions for future mulberry improvement and breeding of high yielding varieties. This study was also helpful for the selection of mulberry accessions for use in sericulture.

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