



Morpho-metric Analysis of Leaf and Fruit of Selected Mulberry Accessions: A Preliminary Measure for Diversity Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. Author SC designed the experiment. Author PS conducted the field experiment. Author MAB, collected the literature. Author JA drafted the manuscript. Author GRV finalized the data. Author SL finalized the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Morus spp. exhibits wide variation in leaf morphology among different species and accessions within the same species. Some of the important silent morphological characteristics pertaining to mulberry leaf and fruit have been selected for the current study. Among all the ten selected genotypes ovate leaf shape was recorded to be the most common ones. Maximum leaf size of leaf was recorded in case of genotype S-1635 as 187cm and minimum in case of Kanva-2 as 28cm.

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Most of the genotypes were recorded with serrated to parted margins except C-4 with parted margin and smooth leaf texture with exceptions of coarse leaf in C-4, Chinese white and C-2038. Two most common types of leaf apex have been recorded as acuminate and acute with cordate, hastate and truncate leaf base. Maximum leaf length was recorded in S-1635 as 17 cm followed by Chakmajra as 15 cm and S-13 as 14 cm and 7 cm was recorded in C-4 and Kanva-2 and maximum in S-1635 as 11cm followed by S-13 and chakmajra as 8 cm and least in G-4 and Kanva-2 as 4 cm. Similarly maximum actual leaf area was recorded in genotype S-1635 as 71 cm² and minimum in genotype C-2038 as 19.5 cm². Fresh and dry weight of the leaf was recorded to be maximum in S-1635 as 11.4 g and minimum in Kanva-2 as 3.5 g and V-1 as 5.2 g respectively. In addition to fresh and dry leaf weight, moisture percentage and moisture retention capacity was recorded to be maximum of 72.4% in C-4 and minimum of 54.26% in S-1635 and maximum moisture retention capacity of 64% was recorded for G-4 genotype and minimum of 50% in C-2038. Maximum stomatal frequency was observed to be highest of 156 mm² was recorded in S-1635 and least in Chinese white as 84 mm² and stomatal index of 16% in V-1 and least in genotype S-13 as 11.1% and Cystolyth frequency was observed to be highest S-13 as 52% and minimum in genotype C-2038 as 24%. Thus, the current study could be used by plant breeders for genetic characterization and evaluation of different genotypes.

Keywords: Mulberry; accessions; germplasm; cystolyth; stomata; moisture; breeding.

1. INTRODUCTION

Mulberry is a fast growing, deciduous, woody perennial tree, which belongs to the family Moraceae [1]. Mulberry has been cultivated since the ancient times, and has economic importance today. It is use as feed to silkworm, fodder to animal, and also used in fruit production [2]. Mulberry is the main food source for silkworm and the sericulture is mainly based on the mulberry for the production of silk. The chemical composition of mulberry leaf constitutes 70-80% moisture, 19-25% protein, 10-15% minerals and 10-15%of sugars [3]. Genus *Morus* exhibits morphological and genetic diversity among various species [1]. Some varieties are homophyllous, having only one type of leaf either entire or lobed, while other are heterophyllous, having both entire and lobed leaves with glossy texture. Inflorescence is of catkin type having large male catkinsand small rounded female catkinswhich later develops in multiple fruit.The fruits are edible and contain water, protein, fat, carbohydrate, vitamins, fibre and mineral etc [4].

The morpho-anatomical characteristics are frequently utilized by the plant breeders for differentiation and evaluation of genotypes. On the other hand, genetic diversity is one of the key elements for the effective breeding programme in any crop. Moreover, genetic diversity within and among the population is the backbone of conservation of plant genetic resources for both

present and future use. Mulberry breeder's will require as much genetic diversity as possible from which to select and recombine favourable traits through cross breeding. Improvement through breeding or clonal selection depends on the extent of magnitude of diversity between the genotypes [5]. Thus the current study is based on morphological and anatomical assessment of mulberry genotypes available in Mulberry Germplasm Bank of Poonch Campus, University of Jammu. Therefore, the current investigation was formulated aiming at characterization of selected mulberry accessions on the basis of morpho-metric aspects of leaf and fruit.

2. MATERIALS AND METHODS

The current experiment was carried out at Mulberry Germplasm Bank, Poonch campus, University of Jammu-Indi during the month of March-May, 2022 with an objective to draw conclusions on morphological and physiological variations among the selected genotypes (Table 1). For the current study 10 different mulberry genotypes established in Germplasm Bank of Poonch campus, University of Jammu, India were selected and utilized for data collection pertaining to leaf and fruit parameters for morpho-physiological identification of the selected genotypes. The data thus generated was subjected to stastical analysis (ANOVA) on SPSS software.

Table 1. Pedigree record of 10 selected mulberry accessions

S.no.	Name of the mulberry genotype	Germplasm accession no.	Origin	Source of procurement
1	S-1635	R1	(CENTRAL SERICULTURAL GERMPLASM RESEARCH STATION (CSGRS), HASUR, TAMIL NADU	STATE SERICULTURE DEVELOPMENT DEPARTMENT (SSDD), JHULAS STATION, POONCH, J&K
2	S-13	R3		
3	S-146	R5		
4	Chakmajra	R7		
5	C-4	R9		
6	Chinese White	R11		
7	C-2038	R13		
8	G-4	R15		
9	Kanva-2	R17		
10	V-1	R19		

3. RESULTS AND DISCUSSION

3.1 Morphology of Leaf Selected Genotypes

3.1.1 Leaf shape, margins, apex and base

The leaf shape of any plant genotype is very important for morphological studies as it helps to identify and differentiate among different accessions. For the selected 10 mulberry genotypes of the Mulberry Germplasm Bank different types of leaf shapes have been recorded ranging from ovate, wide-ovate to lobate and palmatipartite with serrated and parted leaf margins (Fig. 1), acuminate and acute leaf apex and cordate, hastate and truncate leaf bases have been recorded and presented Table 2. Thus revealing significant variability in mulberry genotypes which shows close conformity to the earlier results of Chanotra *et al* [6]. Erarslan *et al.* [7] studied species of *Morus alba*, *Morus nigra* and *Morus rubra* and reported the genotypes to exhibit ovate to broadly ovate and ovate to oblong-ovate leaf shapes respectively and also showed that the leaf size varied from 20-35 cm² for respective species.

3.1.2 Leaf texture, colour and pubescence

The texture was observed in 60-70 days old leaves for selected genotypes and majority of them were recorded to exhibit smooth leaf texture with few exceptions of coarse leaf in case of C-4, Chinese white and C-2038 (Table 2). Generally mulberry leaves were observed to possess light green when young and dark green (Figs. 2&3) on full maturity i.e. 70 day old leaf as shown in Table 2. Similarly, different types of Leaf pubescence viz. strigose, hispid, and hirsute were recorded for different selected

genotypes (Table 3). Binaco *et al.* [2] recorded the variations in leaf characteristics including size and texture as an attribute of mulberry genotypes towards its native environmental conditions in which the genotype is acclimatized. Sikdar *et al.*, [8] described G-4 genotypes leaves to possess dark green colour for C-2038 and S-1635.

3.1.3 Leaf length, width and size

For the studied genotype maximum length and width was recorded in genotype S-1635 as 17cm, 11cm followed by chakmajra as 15cm, 8cm and S-13 as 14cm, 8cm respectively. Whereas least length of 7cm was recorded in C-4 and Kanva-2 as 7cm and least width in case of genotypes G-4 and Kanva-2 as 4cm. Maximum leaf size was recorded in S-1635 as 187cm² and minimum in Kanva-2 as 28 cm² (Table 3). Similar experiment was performed by Kala *et al.*, 2016 in which they demonstrated the genotype S-799 to showed maximum leaf length of 34.8 cm followed by S-146 as 22.2 cm and Chakmajra 22.0 and minimum in genotype Tr-10 as 16.0 cm.

3.1.4 Actual leaf area

In current experiment Actual leaf area is calculated by graph method (Fig. 5) and maximum values were recorded in case of genotype S-1635 followed by Chakmajra, G-4 and V-1 as 71, 41, 34 and 31 cm² respectively. Whereas, minimum values were recorded in Kanva-2 and C-2038 as 22 and 20 cm² respectively. Moreover, Rao *et al.* [9] also obtained widest range of diversity for leaf area, leaf length and leaf width. Peris *et al.* [10] studied the morphology of different genotypes and showed that largest leaf size of 32 cm² in Kanva-2.

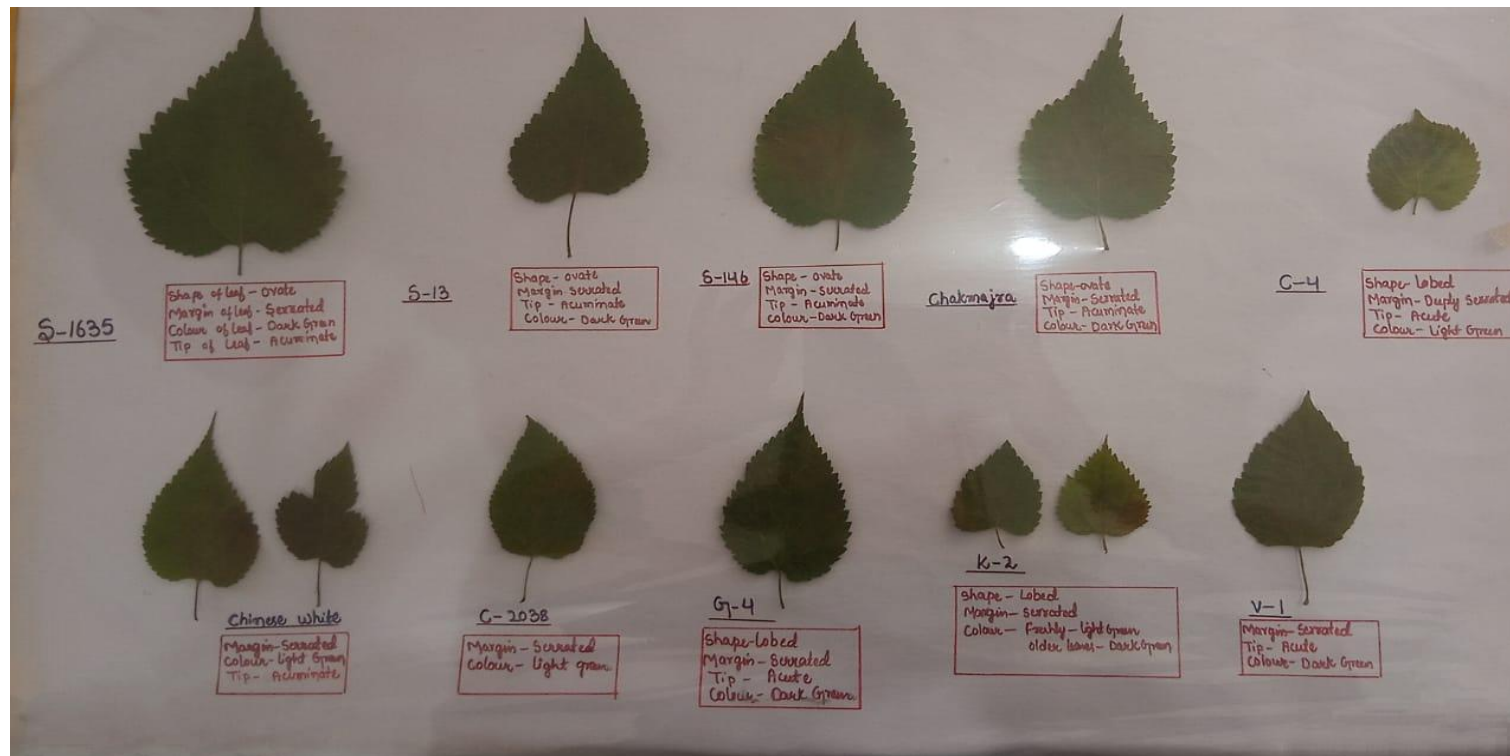


Fig. 1. Collection of leaves of selected 10 mulberry accessions grown at Germplasm Bank of Poonch Campus

Table 2. Different shapes of mulberry leaves selected genotypes

S. No.	Name of the Genotype	Accession No.	Leaf Shape	Leaf margin	Leaf tip	Leaf base	Leaf texture	Leaf pubescence	Leaf color	
									Young	Mature
1	S-1635	R1	Ovate	Serrated	Acuminate	Cordate	Succulent	Strigose	Light green	Dark green
2	S-13	R3	Ovate	Serrated	Acuminate	Cordate	Succulent	Strigose	Light green	Dark green
3	S-146	R5	Ovate	Serrated	Acuminate	Cordate	Succulent	Hispid	Light green	Dark green
4	Chakmajra	R7	Ovate	Serrated	Acuminate	Cordate	Succulent	Strigose	Light green	Dark green
5	C-4	R9	Lobed	Parted	Acuminate	Hastate	Coarse	Strigose	Light green	Dark green
6	Chinese white	R11	Ovate	Serrated	Acuminate	Hastate	Coarse	Hirsute	Light green	Dark green
7	C – 2038	R13	Ovate	Serrated	Acuminate	Truncate	Coarse	Strigose	Light green	Dark green
8	G-4	R15	Lobed	Serrated	Acute	Cordate	Succulent	Strigose	Light green	Dark green
9	Kanva-2	R17	Ovate	Serrated	Acute	Cordate	Succulent	Hispid	Light green	Dark green
10	V-1	R19	Ovate	Serrated	Acute	Cordate	Succulent	Strigose	Light green	Dark green



Fig. 2. Assessment of Young and mature leaf colour

Table 3. Details of leaf length, width and size of selected mulberry genotypes

S. No.	Name of the genotypes	Accession No.	Leaf length (cm)	Leaf width	Size of leaf (cm ²)	Actual leaf area(cm ²)	Fresh leaf weight (in grams)	Dry leaf weight (g)	Moisture percentage (%)	Moisture Retention Capacity%
1	S-1635	R1	17	11	187	71	11.4	5.1	55.26	63.2
2	S-13	R3	14	8	112	28	8	2.9	63.75	62.5
3	S-146	R5	10	7	65	27	10.2	3.2	68.6	59.9
4	Chakmajra	R7	15	8	116	41	7.6	2.4	68.4	56.6
5	C-4	R9	7	5	35	23	5.8	1.6	72.4	60.4
6	Chinese white	R11	11	5	47	27	5.7	1.7	70.17	56.2
7	C-2038	R13	12	5	50	20	6	1.8	70	50
8	G-4	R15	9	4	33	34	7.5	2.5	66.6	64
9	Kanva-2	R17	7	4	28	22	3.5	1	71.42	53.2
10	V-1	R19	10	6	58	31	5.2	1.5	71.15	53.9
Average	-		11.2cm	6.3cm	73.1cm²	32.4 cm²	7.09g	2.37g	67.77%	57.99%
S.D.	-		3.32	2.21	50.47	14.83	2.374	1.175	5.077	4.734

3.1.5 Fresh and dry leaf (10) weight

For calculation of fresh leaf weight of 10 healthy leaves of each genotypes were randomly selected and the samples were weighted in electronic weighing balance for their fresh leaf weight. Maximum fresh and dry weight was recorded for genotypes S-1635 as 11.4g, 5.1g and minimum in Kanva-2 as 3.5g and 1.0g respectively (Table 3). In mulberry genotypes presented by Chanotra *et al.* [1] maximum fresh leaf weight of 494.8 g was recorded in Tr-4 and minimum of 98.3 g in Kokuso-27. Maximum dry leaf weight of 91.9 g was recorded in Tr-4 and minimum in Kokuso-27 as 14.1g, which supported the values of current investigation.

3.1.6 Moisture percentage and moisture retention capacity (MRC)

Highest moisture percentage with 72.4% was recorded for genotype C-4 and least as 55.26% in S-1635. Moreover, maximum MRC percentage was recorded in case of G-4 as 64% and minimum in C-2038 as 50% (Table 3). Chikkalingaiah *et al.* [11] and Banerjee *et al.* [12] had also recorded values for moisture percentage as 63.7% and MRC as 78%, which lies in close conformity with the current findings. Shivadhankar [13] conducted the similar experiment and observed MRC for mulberry leaves in two spring and two autumn crops. The moisture content in fresh leaf with more MRC of 70.83% was recorded in spring than autumn season. MRC after 6 hours was recorded as 93.09% in V-1 higher than the other genotypes where the values ranged from 87.34% in S-13 to 75.15% in Mysore local.

3.1.7 Anatomical parameters of leaf

Stomatal frequency: The cytogenetic information as an important tool for identifying the different mulberry cultivars. Maximum stomatal frequency with 156 mm² was recorded in genotype S-1635 followed by genotypes C-2038 as 144 mm², S-13 as 128 mm², G-4 as 122 mm², Chakmajra as 121 mm², S-146 as 115 mm², V-1 as 102 mm², C-4 and Kanva-2 as 84 mm² and least in genotype Chinese white as 84 mm². Venkatesh K.H [14] studied the stomatal frequency of three genotypes namely; MR-2, C-763 and *Morus macroura* and maximum stomatal frequency with 206.66 mm² was recorded for genotype C-763 followed by genotype MR-2 as 199.87mm⁻² and genotype *Morus macroura* as 159.65mm².

Stomatal index and Cystolyth frequency:

Stomatal index indicated the density of stomata available in the selected leaves. For the studied genotypes maximum value of stomatal index was recorded to be in genotype V-1 as 16% stomatal index and least in genotype S-13 as 11.1% (Table 4). The same slides were used to calculate the cystolyth frequency in the leaves of selected genotypes. The cystolyth cells visual under microscope were manually calculated so as to determine their frequency in the whole leaf lamina and maximum number of cystolyth cells was recorded in genotype Chinese white and Kanva-2 as 51% and minimum in genotype C-2038 as 24%. Erarsalan *et al.* [7] studied species of *Morus alba*, *Morus nigra*, *Morus rubra* and calculated the stomatal index & Cystolyth frequency and found that the *Morus nigra* showed maximum stomatal index & Cystolyth frequency as 13.26% followed by *Morus rubra* as 11.11% and *Morus alba* as 19.71% respectively.

3.2 Morpho-anatomical Parameters of Fruit

3.2.1 Nature of genotype: Monoecious or Dieocious

In current study 10 germplasm accessions originated from different species of *Morus* have been studied for observing gametophytic nature and different varieties for their reproductive nature have been recorded as (Table 5). Tikader *et al.* [15] worked on same parameter and they recorded 39% varieties collected from diverse geographical regions were found 16% male, 53% female, 17 monoecious and 13 was bisexual.

3.2.2 Size of catkin (male and female)

Mulberry Inflorescence is of catkin type male catkin are generally large in size with drooping peduncle loosely arranged while female catkin are small in size, very compactly arranged. After pollination and fertilization the entire inflorescence becomes a multiple fruit. Largest size of male and female catkin was recorded for C-2038 as 4.2 cm and 2.5 cm and smallest for genotype S-1635 and S-13 as 2 cm and 0.5cm respectively. Tikader *et al.* [15] also recorded that inflorescence of *Morus lavigata* was found to be the longest among the inflorescence of mulberry species with catkin 5-12cm long in males and 5-6 cm long in females showing similarity to present observations.

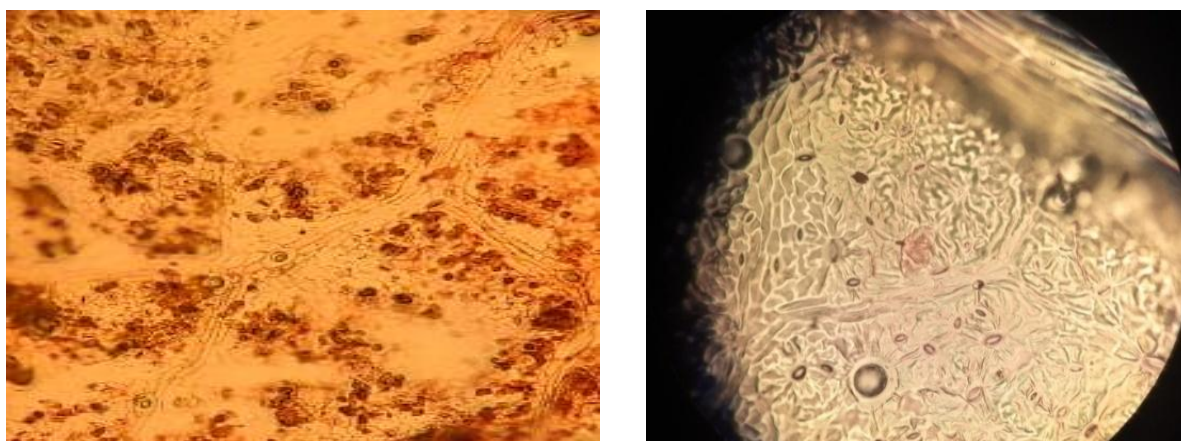


Fig. 3. T.S. of upper and lower epidermis of mulberry leaf

Table 4. Stomatal frequency, stomatal index and cystolith frequency of selected genotypes

S. No.	Name of the genotype	Accession No.	Stomatal frequency (mm ²)	Stomatal Index (%)	Cystolith frequency (%)
1	S-1635	R1	156	14.2	45
2	S-13	R3	128	11.1	52
3	S-146	R5	115	13	48
4	Chakmajra	R7	121	12	40
5	C-4	R9	94	12.7	45
6	Chinese white	R11	84	13.2	51
7	C-2038	R13	144	13.7	24
8	G-4	R15	122	14.4	27
9	Kanva-2	R17	94	23.5	51
10	V-1	R19	102	16	30
Average	-	-	116 mm²	14.38%	41.3%
S.D.	-	-	23.041	3.477	10.583

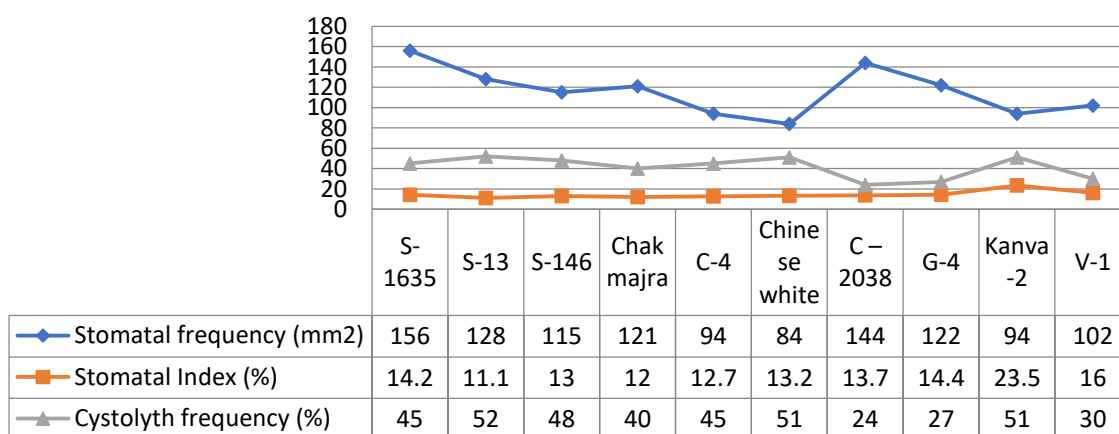


Fig. 4. Stomatal frequency, stomatal Index and cystolith frequency in selected mulberry genotypes

Table 5. Reproductive Nature of selected genotype

S. No.	Name of the genotype	Accession No.	Nature of genotype	Size of male catkin (cm)	Size of female catkin (cm)	Male catkin	Female catkin	Compactness Male catkin	Compactness Female catkin	Male catkin	Female catkin	Color of Male catkin	Color of Female catkin
1	S-1635	R1	Monoecious	2	1	Dark green with purplish tinge	Black	Loose	Stiff	Sweet	Sweet	Dark purple	Light purple
2	S-13	R3	Monoecious	2	0.5	Dark green to brownish tinge	Black	Loose	Stiff	Sweet	Sweet	Dark purple	Light purple
3	S-146	R5	Dioecious	3	1.5	Dark green	Black	Loose	Stiff	Sweet	Sweet	Dark purple	Light purple
4	Chakmajra	R7	Monoecious	2	1	Dark green	Black	Loose	Stiff	Sweet	Sweet	Dark purple	Light violet
5	C-4	R9	Monoecious	3	1	Dark green	Black	Loose	Stiff	Sweet	Sweet	Dark purple	Light violet
6	Chinese white	R11	Dioecious	4	2	Dark green	Black	Loose	Stiff	Sweet	Sweet	Dark purple	Light purple
7	C-2038	R13	Monoecious	4.2	2.5	Dark green	Black	Loose	Stiff	Sweet	Sweet	Dark purple	Light purple
8	G-4	R15	Monoecious	4	2	Dark green	Black	Loose	Stiff	Sweet	Sweet	Dark purple	Light purple
9	Kanva-2	R17	Monoecious	3	1.5	Dark green	Black	Loose	Stiff	Sweet	Sweet	Dark purple	Light purple
10	V-1	R19	Monoecious	4.2	4	Dark green	Black	Loose	Stiff	Sweet	Sweet	Dark purple	Light purple

3.2.3 Colour, compactness and taste of catkin (male and female)

The mulberry fruit exhibits different shades of darker to lighter colour depending upon the concentration of phytochemical and stage of maturity of the fruit. Colour of catkin varies from dark green to light green with brownish or pinkish tinge that changes to purplish to violet black on maturity (Fig. 5). In mulberry generally the male catkin are longer than female catkins and are loosely attached as compared to female ones. The ethno-botanical usage of mulberry involves consumption of ripe fruits, which are highly appreciated for their delicious taste and are consumed either fresh or after extraction of juice. The taste of mulberries depends on the presence of various antioxidants and phyto-chemicals in varied concentration. For the studied genotypes, mature and fully ripened mulberries were recorded to be sweet in taste. Tikader et al. [15] also worked on same parameter and found that female inflorescence to be usually short and the flower to be compactly arranged and after shedding the pollen, the inflorescence dries and falls.

3.2.4 Weight and juice of 10 mature catkins (male and female)

For the current study 10 male catkins were randomly picked up and weighted on electronic weighing balance for quantification of their weight. Maximum weight of male catkin was recorded for the genotype S-1635 as 18.2 g and

minimum in genotype Chinese white as 6.2 g (Table 6). Whereas, maximum weight in case of female catkin was recorded for the genotype Chinese white as 10.5g and minimum in genotype Chakmajra as 3.9 g (Table 6). For extraction of juice mature female and male catkins were randomly selected and maximum volume of 10 fruit juice was recorded for the genotype S-1635 as 250 ml and 180 ml for male and female catkins resp. and least in Chinese white as 130ml for male and S-13 and Kanva-2 as 120ml for female catkins resp. Xiao et al. [16] *Morus nigra* fruit that showed 0.75 ± 0.004 mg/g while that for *Morus alba* fruit was recorded with weight of 0.93 ± 0.003 mg/g. Maximum volume of juice of male catkin was obtained from genotype S-1635 as 250 ml and least in genotype Chinese white as 138 ml. While in female maximum juice was recovered from C-1635 as 180 ml and least in genotype Chinese white as 120 ml. Vijayan et al. [4] worked on the quality of mulberry juice collected from 100 mulberry fruits and recorded to recover maximum volume of 250 ml of juice from S-1635 fruits.

3.2.5 Color of the juice (male and female catkin)

For the selected genotypes the colour was observed as dark to purplish violet in shade. Wendy et al., [17] worked on the evaluation of mulberry fruit juice extracted by different methods and found mostly purplish colour fruit juice.

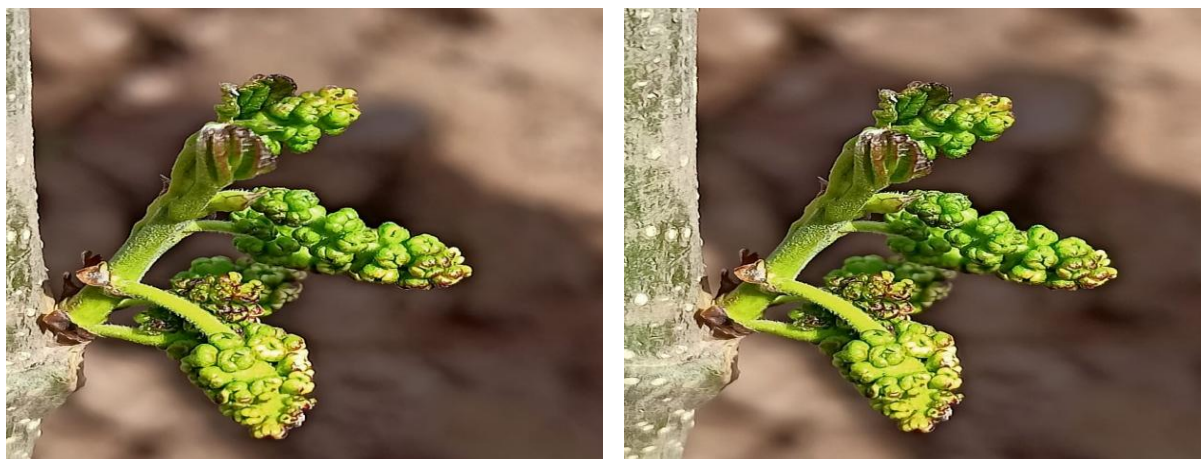


Fig. 5. Young catkins of mulberry showing compactness of male and female catkin

Table 6. Volume of juice extracted from male and female catkin

S. No.	Accession no.	Name of the genotype	Weight of male catkin	Weight of female catkin	Volume of Juice: Male catkin (ml)	Volume of Juice: Female catkin (ml)
1	R1	S-1635	18.2	10.5	250	180
2	R3	S-13	8.4	6.1	180	120
3	R5	S-146	7.9	5.4	160	140
4	R7	Chakmajra	5.8	3.9	140	130
5	R9	C-4	6.8	4.7	145	128
6	R11	Chinese white	6.2	5.5	138	130
7	R13	C-2038	8.7	5.1	182	156
8	R15	G-4	7.4	5.2	155	158
9	R17	Kanva-2	6.4	4.8	140	120
10	R19	V-1	8.0	6.4	174	140
Average	-	-	8.38g	5.76g	166.4 ml	140.2 ml
S.D.	-	-	3.585	1.808	33.83	19.21



Fig. 6. Stages of maturity of mulberry fruits: male & female catkins

4. CONCLUSIONS

The current experiment was conducted for easy identification of different genotypes based on the morphological and anatomical parameters of leaf and fruit of different genotypes for drawing accurate conclusion related to leaf and fruit morphology and anatomy of selected genotypes namely; S-1635, S-13, S-146, Chakmajra, C-4, Chinese white, C-2038, G-4, Kanva-2 and V-1 established at Germplasm Bank of Poonch Campus, University of Jammu. Among all the ten genotypes ovate leaf shape with maximum size

of genotype S-1635 as 187cm and minimum in case of Kanva-2 as 28cm having serrated to parted leaf margins with acuminate and acute leaf apex and cordate, hastate and truncate leaf bases. Maximum actual leaf area was recorded in genotype S-1635 as 71cm² and minimum in C-2038 as 19.5 cm² with fresh and dry weight of 11.4 g and minimum in Kanva-2 as 3.5 g and V-1 as 5.2 g respectively. Maximum moisture percentage of 72.4% was recorded in C-4 and minimum 54.26% in S-1635. Whereas, maximum MRC of 64% was recorded for G-4 and minimum of 50% in C-2038. The maximum stomatal

frequency of the selected genotypes was 156 mm² in S-1635 and least in Chinese white as 84 mm². For the studied genotypes maximum value of stomatal index was recorded to be of 16% in V-1 and least in S-13 as 11.1%. Thus, morpho-anatomical characteristics were frequently utilized by the plant breeders for differentiation and evaluation of genotypes. Morphological traits exhibiting utmost importance for assessment of extent of diversity on preliminary level and could be used by plant breeders for genetic characterization and evaluation of different genotypes. This could also be helpful for the selection of mulberry accessions to be used in further breeding programme for making effective selection of the potential material. Thus, offering new opportunities for development of new mulberry genotypes with improved agronomical traits [18,19].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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