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Morpho-physiological characterization of mulberry genotypes (*Morus* spp.) for inheritance in future breeding programme

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Abstract
In present investigation, genetic diversity of 44 mulberry genotypes was determined by using fourteen different phenotypic characters *viz*. sprouting time, leaf shape, apex, base, margins, pubescence, leaf length, leaf width, actual leaf area, fresh leaf weight, dry weight, moisture percentage, internodal distance and number of leaves per meter twig were and revealed significant variability among the genotypes. PCA revealed maximum variation of 50.56 per cent by PC1 and least of 0.05 per cent by PC8. Cluster dendrogram grouped 44 genotypes under two main clusters A and B with sub-clusters and sub-sub clusters. Kokuso-27 was identified as the most diverse genotype forming an individual sub-sub-cluster alone. Behrampur, BhremC-776, Chinese white, Dhar local, LF-1, Miuraso, S-41, S-54, S-146, S-799, S-1608, S-1635, S-1708, Tr-4, Tr-8 and Kokuso-27 were identified as promising genotypes. This investigation suggested that the *Morus* germplasm is quite diverse and can be utilized for future breeding programmes.

Keywords: *Morus*, morphology, diversity, cluster analysis, PCA

Introduction

Mulberry is a deep rooted perennial plant with wide range of adaption under diverse climatic conditions and exhibits numerous morphological and physiological characteristics. Mulberry is a hard woody native plant of Asia falling under genus *Morus,* family Moraceae, division Magnoliophyta, class Magnoliopsida and under order Urticales. It has the ability to survive under diversified climatic conditions ranging from temperate to subtropical regions of the Northern hemisphere to the tropics of the Southern hemisphere and in different soil types including clayey to loamy type. The variation in its morphological characteristics can be attributed to various disruptive environmental conditions. Knowledge of morphology and physiology is a perquisite for a breeder before starting any breeding programme. Thus morpho-physiological characteristics are frequently utilized by the plant breeders for differentiation and evaluation of genotypes.

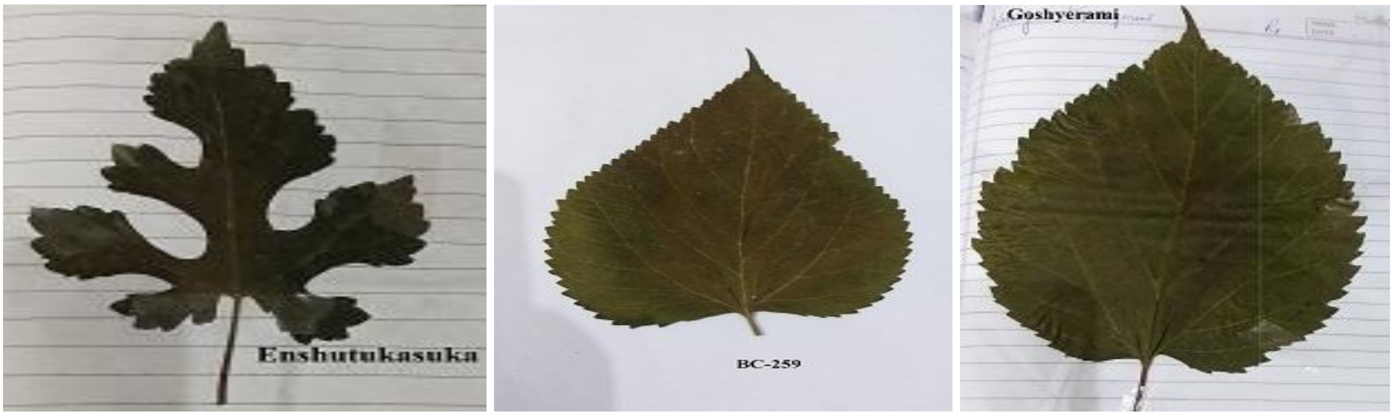
Morphological traits are of utmost importance for assessment of extent of diversity on preliminary level and are used by plant breeders for genetic characterization and evaluation of plants genotypes.

Material and Methods

Morpho-physiological measurements were taken at mulberry germplasm bank of Divison of Sericulture, Udheywala campus, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India. The plant material for the present study comprised of forty four mulberry genotypes maintained at 1x1 meter spacing in bush type plantation, including both temperate and tropical varieties (Table 1). Foliar characters were recorded after 60-70 days of pruning. Three replications were taken from each accession for data collection. For morpho-physiological characterization observations were recorded for fourteen characters including six phenotypic parameters namely sprouting time, leaf shape, apex, base, margin and leaf pubescence and eight quantative traits namely leaf length (cm), width (cm), actual leaf area (cm^2), fresh leaf weight (g), dry leaf weight (g), moisture percentage (%), internodal distance (cm) and number of leaves per meter twig. Data obtained for various morphological parameters was classified as per visual analysis method (Lawrence, 1967) [4] and quantative traits was subjected to statistical analysis using the R software version 3.5.1 2018 and results were obtained.

Results

Results obtained for six phenotypic characters are presented in Table 1. Sprouting period in studied mulberry varieties exhibited varied pattern. Early sprouting was observed in Behrampur by 15th of January while as late sprouting was observed in Shimanouchi and S-41 in last week of March. Among morphological characters, leaf apex, leaf base, leaf margin and leaf shape showed variation among the genotypes. Leaf shape varied from ovate, wide ovate to palmatipartite (Plate 1), leaf apex varied from acuminate to acute (Plate 2), leaf base varied from cordate, truncate to hastate (Plate 3) and leaf margins varied from serrate to parted (Plate 4). Nine different types of Leaf pubescence *viz*. strigose, puberulous, sericeous, hispid, tomentose, pilose, scabrous, wooly and hirsute were recorded for different genotypes.



(a) Palmatipartite (b) Ovate (c) Wide-ovate

Plate 1: Types of leaf shapes

(a) Acuminate (b) Acute

Plate 2: Types of leaf apices

(a) Cordate (b) Hastate (c) Truncate

Plate 3: Types of leaf bases.

(a) Serrate (b) Parted

Plate 4: Types of leaf margins

Table 1: Leaf morpho-physiological parameters of mulberry genotypes for the year 2017-18.

S. No.	Genotype	Sprouting Time (2017-18)	Leaf Shape	Leaf Apex	Leaf Base	Leaf Margin	Leaf Pubescence
1	Asayuki	23rd of March	Palmatipartite	Acuminate	Cordate	Serrate	Pilose
2	Enshutukasuka	23rd of March	Palmatipartite	Acute	Hastate	Serrate	Hirsute
3	Fukushima	14th of March	Ovate	Acuminate	Cordate	Serrate	Sericeous
4	Goshyerami	20th of March	Wide-ovate	Acuminate	Cordate	Serrate	Pilose
5	Ichinose	23rd of March	Palmatipartite	Acute	Cordate	Parted	Strigose
6	Kairyoroso	23rd of March	Palmatipartite	Acute	Cordate	Parted	Wooly
7	Kamabori	23rd of March	Ovate	Acuminate	Cordate	Serrate	Scabrous
8	Kokuso-20	16th of March	Ovate	Acute	Cordate	Parted	Puberulous
9	Kokuso-27	26th of March	Palmatipartite	Acute	Cordate	Parted	Strigose
10	Limencina	19th of March	Palmatipartite	Acute	Cordate	Parted	Strigose
11	Miuraso	31st of January	Palmatipartite	Acuminate	Cordate	Serrate	Strigose
12	Rokokyoso	14th of March	Palmatipartite	Acute	Hastate	Serrate	Wooly
13	Shimanouchi	28th of March	Palmatipartite	Acuminate	Truncate	Serrate	Strigose
14	BC-259	31st of January	Ovate	Acuminate	Cordate	Serrate	Puberulous
15	Bhrem C-776	23rd of January	Ovate	Acuminate	Truncate	Serrate	Puberulous
16	Behrampur	15th of January	Ovate	Acuminate	Cordate	Serrate	Pilose
17	C-763	20th of January	Ovate	Acuminate	Cordate	Serrate	Scabrous
18	Chakmajra	19th of January	Ovate	Acuminate	Truncate	Serrate	Strigose
19	Chinese white	23rd of March	Ovate	Acuminate	Cordate	Serrate	Hirsute
20	Dhar local	2nd of February	Ovate	Acuminate	Cordate	Serrate	Scabrous
21	Kanva-2	23rd of January	Ovate	Acuminate	Truncate	Serrate	Hispid
22	KNG	20th of March	Palmatipartite	Acute	Cordate	Parted	Scabrous
23	LF-1	23rd of January	Ovate	Acuminate	Cordate	Serrate	Strigose
24	LF-2	31st of January	Palmatipartite	Acuminate	Cordate	Serrate	Strigose
25	NS-1	31st of March	Ovate	Acute	Cordate	Serrate	Strigose
26	NS-2	14th of March	Ovate	Acute	Cordate	Serrate	Strigose
27	NS-3	23rd of March	Palmatipartite	Acuminate	Cordate	Serrate	Strigose
28	S-1	19th of January	Ovate	Acuminate	Cordate	Serrate	Scabrous
29	S-30	2nd of February	Ovate	Acuminate	Cordate	Serrate	Strigose
30	S-36	27th of February	Wide-Ovate	Acuminate	Cordate	Serrate	Strigose
31	S-41	28th of March	Ovate	Acuminate	Truncate	Serrate	Scabrous
32	S-54	19th of January	Ovate	Acuminate	Truncate	Serrate	Scabrous
33	S-146	2nd of February	Wide-Ovate	Acuminate	Cordate	Serrate	Hispid
34	S-799	18th of January	Ovate	Acuminate	Cordate	Serrate	Tomentose
35	S-1531	23rd of January	Ovate	Acuminate	Cordate	Serrate	Strigose
36	S-1608	26th of January	Ovate	Acuminate	Cordate	Serrate	Pilose
37	S-1635	23rd of January	Ovate	Acuminate	Cordate	Serrate	Strigose
38	S-1708	19th of January	Wide-Ovate	Acuminate	Cordate	Serrate	Strigose
39	Sujanpur	20th of January	Ovate	Acuminate	Cordate	Serrate	Pilose
40	Tr-1	17th of January	Ovate	Acuminate	Cordate	Serrate	Scabrous
41	Tr-4	19th of January	Ovate	Acuminate	Cordate	Serrate	Strigose
42	Tr-8	19th of January	Ovate	Acuminate	Cordate	Serrate	Strigose
43	Tr-10	19th of January	Ovate	Acuminate	Cordate	Serrate	Sericeous
44	V-1	23rd of January	Ovate	Acuminate	Truncate	Serrate	Strigose

Results obtained for eight quantitative traits are presented in Table 2. Highest value for leaf length was recorded in S-799 (34.8 cm) and least in Kairyoroso (4.8 cm), whereas maximum leaf width of 13 cm was recorded in Behrampur and minimum 6.9 cm in Kokuso-27. Maximum leaf area was recorded in S-54 as 295 cm^2 and minimum in Kokuso-27 as

80.1 cm^2. 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 14.1 g. Leaf moisture percentage showed variation among the studied genotypes with an average of 80.2 per cent. Highest moisture percentage of 90.1 per cent was found in S-1708 and lowest in Tr-10 as 65.7 per cent. Minimum internodal distance (cm) and maximum number of leaves per meter twig was recorded in genotype Kokuso-27 as 3.2 cm and 30 respectively.

Table 2: Pooled mean values of eight quantative traits of mulberry genotypes for the year 2017-18.

S. No.	Genotype	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Fresh weight 100 leaves (g)	Dry weight 100 leaves (g)	Leaf moisture (%)	Internodal distance (cm)	Leaves/ meter twig (no.
1	Asayuki	16.8	11.2	191.7	141.4	22.8	83.8	4.0	23
2	Enshutukasuka	15.1	08.1	123.9	156.7	21.9	86.0	4.6	19
3	Fukushima	19.1	11.1	213.0	177.5	20.4	88.5	3.8	17
4	Goshyerami	21.8	12.6	278.0	240.3	52.4	78.1	4.6	17
5	Ichinose	16.2	09.6	156.5	205.4	35.2	82.8	4.3	17
6	Kairyoroso	4.8	09.6	141.0	102.9	24.2	76.4	4.5	18
7	Kamabori	17.5	10.6	188.9	133.1	26.4	80.1	4.7	18
8	Kokuso-20	18.1	10.9	197.5	155.4	30.2	80.5	5.0	17
9	Kokuso-27	11.4	6.9	80.1	98.3	14.1	85.1	3.2	30
10	Limencina	14.8	09.8	147.5	125.8	22.4	82.1	4.7	19
11	Miuraso	19.3	09.3	181.1	362.4	69.6	80.7	6.3	14
12	Rokokyoso	18.0	8.8	159.4	161.7	37.3	77.0	5.1	18
13	Shimanouchi	17.6	12.1	215.7	149.9	26.2	82.5	3.4	19
14	BC-259	16.0	10.9	175.9	235.3	44.2	81.2	5.4	12
15	Bhrem C-776	19.6	09.7	190.6	306.8	42.6	86.1	9.0	10
16	Behrampur	21.1	13.0	276.0	216.7	24.9	88.5	4.7	11
17	C-763	21.8	10.7	237.0	217.2	32.7	85.0	6.9	12
18	Chakmajra	22.0	11.8	261.5	258.6	36.7	85.8	3.9	11
19	Chinese white	19.5	11.1	222.3	356.9	64.4	82.0	7.0	11
20	Dhar local	16.6	10.3	172.5	346.0	63.7	81.5	6.6	12
21	Kanva-2	18.5	10.4	195.4	243.5	77.2	68.2	5.0	12
22	KNG	15.0	08.2	125.6	191.7	29.5	84.6	4.1	19
23	LF-1	16.5	09.9	163.7	306.1	84.8	72.2	4.7	18
24	LF-2	20.6	10.5	216.8	208.1	63.7	69.3	6.8	12
25	NS-1	23.2	11.5	271.6	232.1	30.5	86.8	4.8	12
26	NS-2	22.9	12.5	288.4	244.6	38.9	84.0	9.7	12
27	NS-3	18.1	12.0	218.4	279.1	48.6	82.5	5.8	13
28	S-1	17.5	09.1	161.4	178.9	31.3	82.5	5.6	14
29	S-30	18.1	10.4	191.8	266.3	60.0	77.4	4.7	15
30	S-36	16.8	09.3	158.3	233.4	72.2	69.0	4.9	15
31	S-41	21.7	11.6	253.4	322.9	54.7	83.0	5.2	13
32	S-54	23.3	12.5	295.0	400.6	64.1	84.1	5.1	17
33	S-146	22.2	12.5	272.6	299.1	79.8	73.3	5.6	14
34	S-799	34.8	08.4	122.4	126.6	38.6	69.5	5.0	13
35	S-1531	19.3	10.1	194.6	225.3	57.8	74.3	6.5	12
36	S-1608	21	09.8	206.6	341.4	76.2	77.6	7.1	13
37	S-1635	19.6	11.6	227.7	376.6	81.3	78.4	6.7	14
38	S-1708	19.6	11.7	233.1	320.1	31.4	90.1	7.0	13
39	Sujanpur	20.6	11.2	232.0	246.2	24.9	89.8	5.6	11
40	Tr-1	17.1	09.6	164.7	216.7	28.0	87.0	8.0	14
41	Tr-4	18.8	12.1	228.5	494.8	91.9	81.4	7.4	13
42	Tr-8	18.5	11.8	223.4	358.1	75.8	78.8	8.4	12
43	Tr-10	16.0	10.7	172.3	153.8	52.6	65.7	5.8	13
44	V-1	20.4	10.3	212.1	333.2	47.7	85.6	6.4	14
Mean		18.6	10.6	201.0	224.1	46.7	80.2	5.6	15.2
S.D.		3.09	1.78	60.41	96.36	22.16	7.41	1.67	4.20

Principle component analysis of eight quantitative traits.
PCA analysis of eight quantative traits of mulberry genotypes was carried out using R software (Version-3.5.1 2018).

Scree plot
Table 3 explains that first principal component (PC1) accounts for maximum variation of 50.56 per cent of the total variability alone, followed by PC2 (21.73%), PC3 (12.23%), PC4 (8.53%), PC5 (3.65%), PC6 (3.06%), PC7 (0.15%) and PC8 accounts least variation of 0.05 per cent. Figure 1 evident graphically that PC1 extracted maximum amount of variation among studied characteristics.

Table 3: Total variance explained by principal component analysis (PCA).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigen value	4.04	1.73	0.97	0.68	0.29	0.24	0.12	0.00
Percentage of variance	50.56	21.73	12.23	8.53	3.65	3.06	0.15	0.05
Cumulative percentage	50.56	72.30	84.54	93.07	96.73	99.79	99.94	100

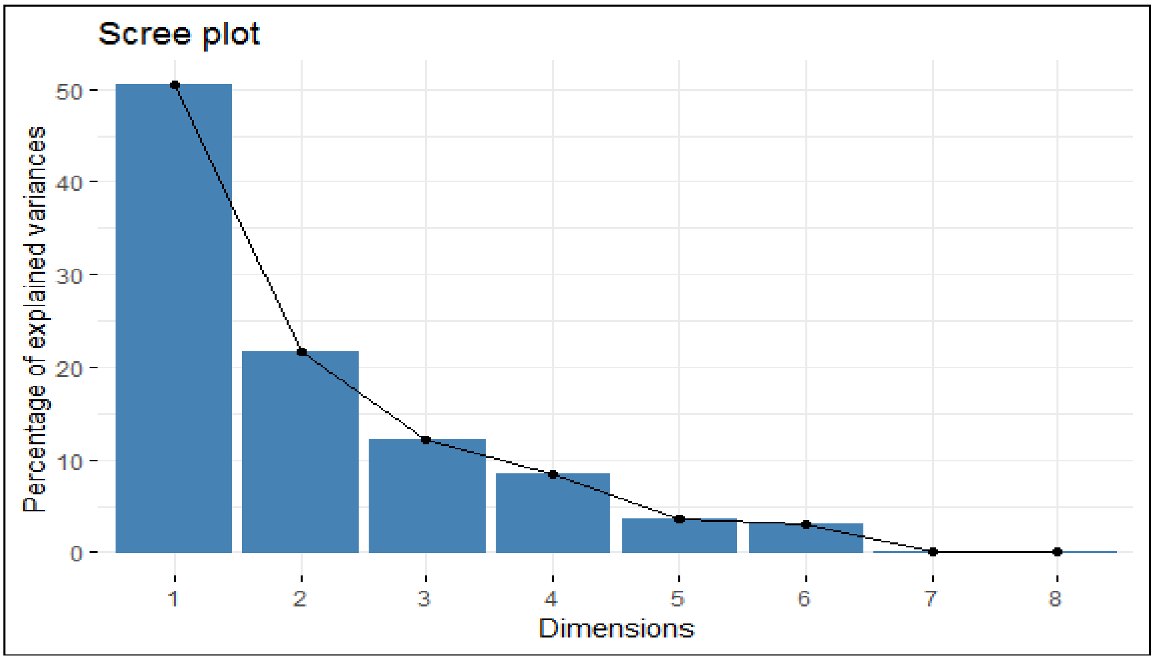


Fig 1: Scree plot of eight Principal Components indicating the percentage of variation contributed by each component

Bi-plot of genotypes

Bi-plot of genotypes represented 44 genotypes on the basis of variation range indicates the intensity of four different colors of blue, brown, yellow and orange (0-0.25, 0.25-0.50, 0.50-0.75 and above 0.75 per cent) respectively. Genotype, Behrampur, NS-1, Sujanpur, Chakmajra, NS-2, S-41, Chinese-white, S-1635, Tr-4, Tr-8, S-1608 and Kanva-2 depicted higher scores on first principal component and indicated superiority over other genotypes (Figure 2).

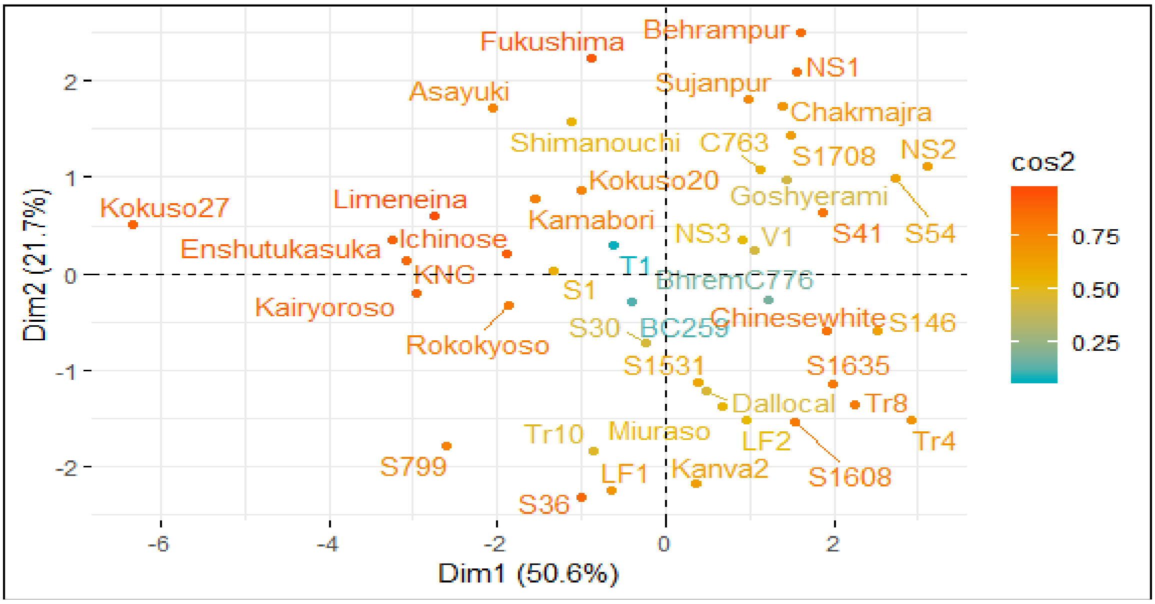


Fig 2: Bi-plot of genotypes

PCA Bi-plot of characteristics (variables)

Length of arrows showed the proportion of contribution by eight characters *viz.* fresh leaf weight, dry leaf weight, moisture percentage, inter-nodal distance, number of leaves per meter twig, leaf length, leaf width and actual leaf area in the principal component and direction of arrows indicated whether the proportion was positive or negative as shown in Figure 6. Among studied characteristics highest loading was observed in case of actual leaf area and leaf length (0.87) followed by fresh leaf weight and leaf width (0.78), dry leaf weight (0.57) and moisture percentage (0.08) with respect to PCA-I. Whereas number of leaves per meter twig contributed minimum to the first principal component (-0.76) as shown in Table 4 and Figure 3.

Table 4: Loadings; Fresh leaf weight, dry leaf weight, moisture percentage, Inter-nodal distance, number of leaves per meter twig, leaf length, leaf width and actual leaf area.

	Component	
	1	**2**
Fresh leaf weight	0.78	-0.28
Dry leaf weight	0.57	-0.74
Moisture percentage	0.08	0.79
Internodal distance	0.61	-0.28
Number of leaves per meter twig	-0.76	0.16
Leaf length	0.87	0.29
Leaf width	0.78	0.36
Actual leaf area	0.87	0.38

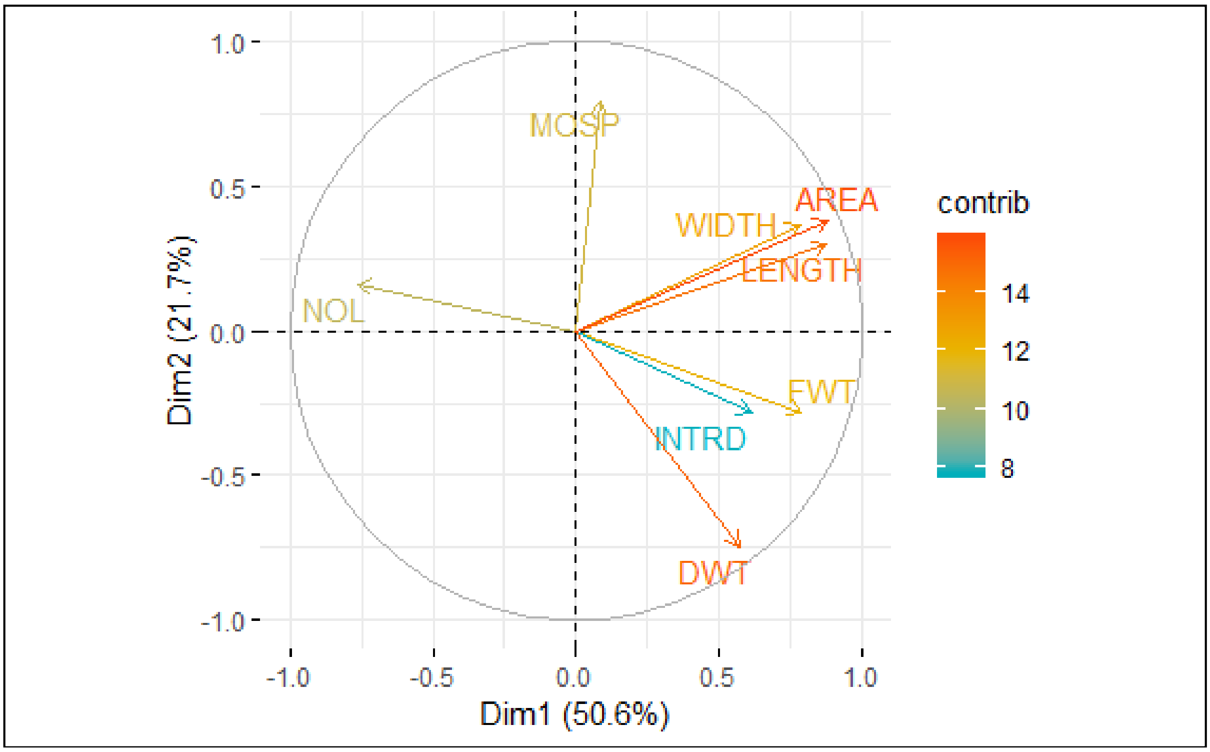


Fig 3: PCA Bi-plot of characteristics (variables) indicating the proportion of contribution in the principal component by the length of arrows and direction of arrows indicates whether the proportion is positive or negative.

Cluster plot

Cluster plot grouped 44 genotypes under 5 clusters each having different genotypes (Fig. 4). The varieties within individual cluster represented close association with each other and diversity between the clusters represented by five different colors (red, brown, green, blue and purple).

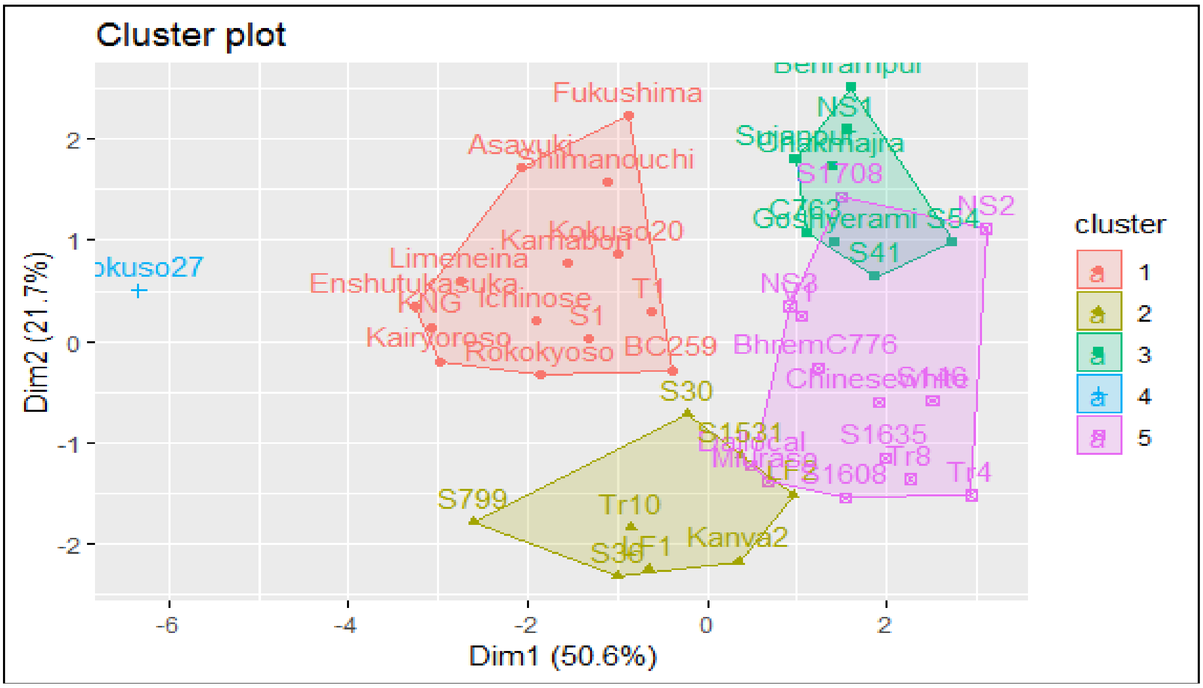


Fig 4: Cluster plot representing the intensity of diversity or association among 44 genotypes grouped under five clusters.

Cluster dendrogram

Cluster dendrogram grouped fourty four genotypes under two main clusters A and B representing the intensity of diversity and closeness among them. Cluster-A had two sub-clusters A_1 and A_2. Sub-cluster A_1 further divided into two sub-sub clusters A_{1a} and A_{1b}. Sub-cluster A_2 further divided into two sub-sub clusters A_{2a} and A_{2b}. Cluster-B had two sub-clusters B_1 and B_2. Sub-clusters B_1 further divided into two sub-sub clusters B_{1a} and B_{1b}. Sub-cluster B_2 further divided into two sub-sub clusters B_{2a} and B_{2b}. Among 44 genotypes represented under cluster dendrogram on the basis of morphological data, Kokuso-27 was found to be the most diverse genotype forming individual sub-sub cluster alone (Fig. 5).

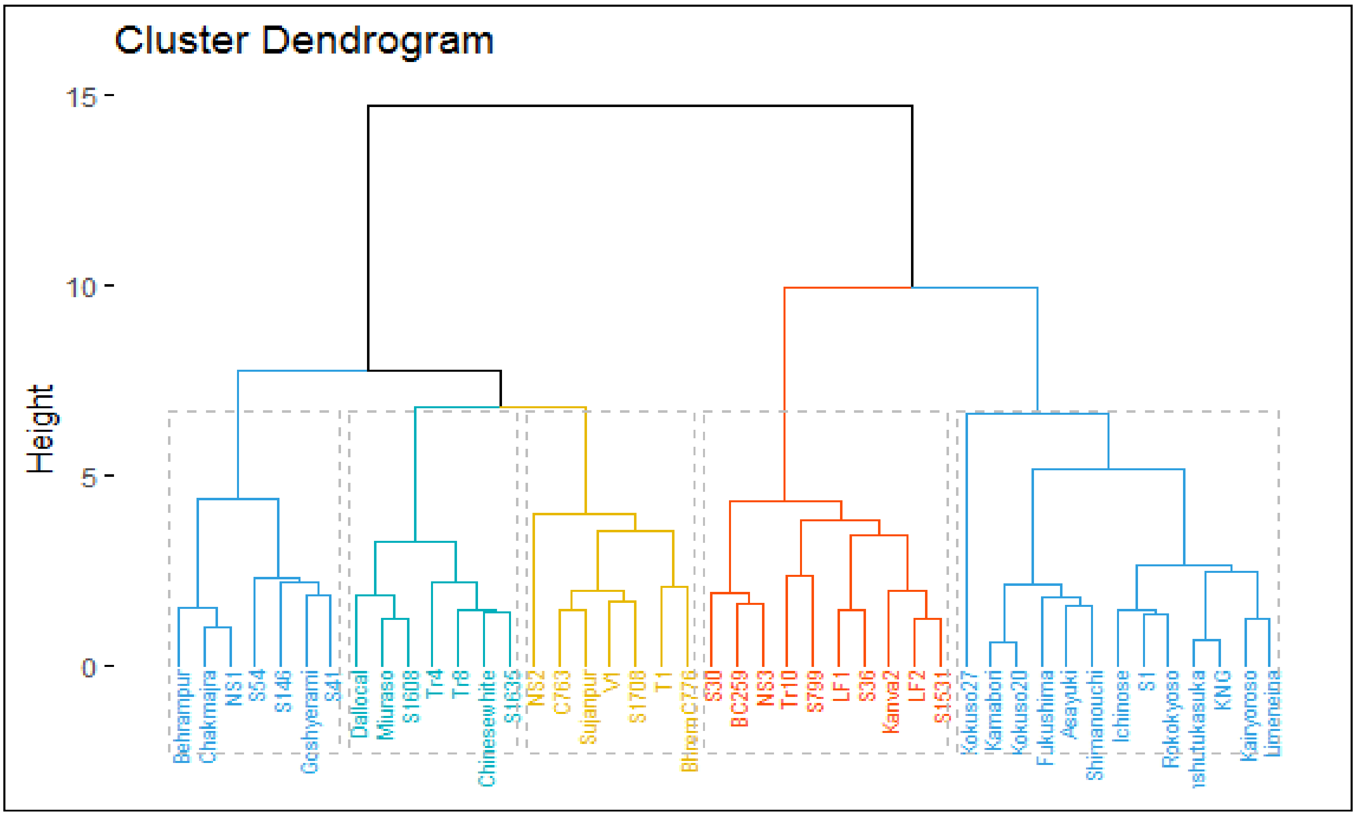


Fig 5: Cluster dendrogram of 44 mulberry genotypes based on morpho-physiological characters.

Discussion

Cordell *et al.*, 1998 [2] reported numerous morphological and physiological characteristic in plant species including mulberry. Gray (1990) [3] suggested phenotypic plasticity as the contributory factor to mulberry's growth and survival under disruptive environmental conditions. Pandey and Nagar, (2002) [6] 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. 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) [5]. Widest range of diversity (98.3 to 494.8 g) was recorded for fresh leaf weight followed by actual leaf area (80 to 295 cm^2), moisture percentage (65.7 to 90.1 %), dry leaf weight (14.1 to 91 g), number of leaves per meter twig (10 to 30), leaf width (6.9 to 13 cm), leaf length (4.8 to 34.8 cm) and internodal distance (3.2 to 9.7 cm) which are in accordance with the results obtained by Tikader and Roy (2001) [8] and Rao *et al.* (2004) [27]. 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 (Adolkar *et al.*, 2007) [1].

Conclusion

It is concluded that morphological traits clustered the fourty four mulberry accessions into two main groups which further formed sub-clusters and sub-sub clusters. Genotype Kokuso-27 was identified as the most diverse one forming individual sub-sub cluster. Widest range of diversity was recorded for fresh leaf weight followed by actual leaf area, moisture percentage, dry leaf weight, number of leaves per meter twig, leaf width, leaf length and internodal distance, 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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