Exploring natural variability in the mulberry (Morus indica) cultivar, Kanva-2 through clonal selection

RAVI KUMARA R
CSB - Muga P-3 Seed Station
MANJUNATHA H.B.
manjunathahb@gmail.com
University of Mysore

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Abstract

Mulberry (*Morus* spp.) is a crucial plant in sericulture, serving as the sole food source for the silkworm, *Bombyx mori*. Clonal selection in mulberry is a strategic approach to leveraging natural variability for the development of improved cultivars. This method enables the identification and propagation of superior clones that exhibit desirable traits, such as enhanced leaf quality, growth vigor, and adaptability to varying environmental conditions. In the present study; we identified 11 clonal variants from the cultivar Kanva-2 (K-2) through field surveys. These clonally selected genotypes were designated as CS-1, CS-2, CS-3, CS-4, CS-5, CS-6, CS-7, CS-8, CS-9, CS-10, and CS-11, and underwent detailed characterization of their morphometric and molecular traits. The clonally-derived genotypes exhibited distinct features compared to their mother plant, K-2. Notably, genotypes CS-1, CS-4, CS-5, and CS-6 demonstrated improved leaf yield and quality traits, highlighting their potential advantages over the original cultivar. These genotypes can be utilized in breeding programs or to enrich germplasm resources.

1. Introduction

In India, the mulberry is cultivated about 2, 63,302 ha for rearing the silkworm *Bombyx mori* and produced 29892 MT of mulberry raw silk during 2023-24 [1]. Many improved mulberry varieties with respect to different agro-climatic zones as well as tolerance against various stressesare available in India, and their leaf yielding potential is upto 80 MT/ha/year. Mainly conventional breeding methods are used to develop many popular mulberry varieties such as introduction of foreign varieties (S-1, Goshoerami, and Kosen), selection through open pollination (Kanva-2, MR-2, S-13, S-34, RFS-135, RFS-175, AR-11, S-146, and Ber. S-799), clonal selection (TG-1, Chinese White, Anantha, Vishwa, and Vishala), systematic hybridization (RC-1, RC-2, Sahana ,V-1, G-2, G-4, MSG-2, AGB-8, BC2-59, C-763, C-776, C-2016, C-2017, C-2028, C-2038, C-2058, C-2060, and C-1360), mutation induction (S-30, S-36, S-41, and S-54), and development of triploids (Tr-10, C-1730, Tr-23, S-1635, S-1608, AR-12, and Suvarna-2) by crossing between diploid and tetraploid parents [2].

Genetic variability plays a crucial role in enhancing mulberry plant improvement. It contributes to increased yields, improved leaf quality, adaptability to diverse stress conditions, and heightened resistance to pests and diseases. The genetic resources of mulberries serve as a foundation for effective crop management. Activities such as collection, introduction, and exchange of genetic materials are essential for enriching the existing gene pool, providing breeders with extensive opportunities for further advancements in mulberry cultivation [3]. Currently, India maintains 1,317 mulberry accessions in an *ex-situ* field gene bank at the Central Sericultural Germplasm Resources Centre (CSGRC) in Hosur. These accessions are evaluated and characterized, with useful traits documented [4]. Mulberry is a perennial, cross-pollinated plant characterized by high heterozygosity and notable inbreeding depression during sexual reproduction. To address this, clonal propagation is used to generate genetically identical plants. However, the morphological variations often observed in mulberry gardens among plants of the same variety are primarily due to phenotypic plasticity, which refers to changes induced by environmental factors or mechanical mixtures that can occur during collection and propagation. In contrast, genetic variation within a clone can result from mutations, contributing to genetic diversity even within the same variety [5].

Exploring clonal variants is a crucial breeding strategy for developing new cultivars with superior traits in mulberry cultivation in India. Bud mutation serves as an important source of plant variation, allowing for the direct selection of new varieties and providing valuable germplasm for hybrid breeding. This method is both simple and effective for generating new cultivars [6, 7]. Additionally, the unique traits arising from bud mutations can be reliably maintained through asexual reproduction methods, such as grafting and cutting, and can be inherited by subsequent generations [8]. In this context, the present study focuses on clonal selection in mulberry, with special emphasis on shade tolerance. It involves exploration and surveying in coconut orchards, where mulberry is cultivated as an intercrop, with the aim of characterizing, conserving, and utilizing the diverse genetic resources available.

2 Materials and methods

2.1 Exploration, survey, and collection

The study was conducted considering > 20 years old mulberry gardens, wherein cv. Kanva-2 (K-2) was intercropped with coconut palms in the traditional sericulture areas. The region has a dry climate with temperatures ranging from 19–36°C, humidity of 63–77%, and average rainfall of 868.7 mm. Soils are primarily deep red clay, shallow red, and red sandy loam. The mulberry variants were identified through visual observation of the existing cultivars in the gardens. To ensure a proper comparative analysis, selected variants were propagated from cuttings, maintaining both the variant and its corresponding mother plant over a one-year period. The study considered 26 different morphotypes of mulberry, of which 11 exhibited clear morphological differences from the standard K-2 variety. The remaining 15 variants showed similar traits when exposed to full sunlight but exhibited notable variations when grown under the shade of coconut palms, indicating phenotypic plasticity. Out of the 11 variants that exhibited differences from K-2, further studies were conducted on 11 specific genotypes, which were selected and named CS-1 to CS-11 for detailed analysis.

2.2 Establishment of evaluation plot and management

The clonally selected 6-month-old mulberry saplings were planted at the experimental plot, Department of Sericulture Science, University of Mysore, Mysuru, using a randomized block design (RBD) with three replications, consisting of six plants/genotype/replication, at a spacing of 90 x 90 cm under irrigated conditions. The soil was red loam with a pH of 6.9 and standard package of practices were followed commonly. After one year of establishment, eight successive harvests were taken with an interval of 65–70 days, and all the harvests were made by shoot pruning at 45 cm height. Farmyard manure was applied at a rate of 20 MT/ha/yr in two split doses. Chemical fertilizers were also applied at a rate of 350:140:140 kg NPK/ha/yr, divided into five split doses. Plant protection measures were ensured throughout the experiment.

2.3 Morpho-metric analysis

Morphological, growth, yield, reproductive, and anatomical parameters of the mulberry variants were recorded according to established descriptors [9]. For biochemical analyses, protein content was estimated using the method of Lowry *et al.* [10], carbohydrate levels following Plumer [11], and chlorophyll content was measured based on the method by Hiscox and Israelstam [12]. Samples were taken from leaves in the 7th to 9th order, following the respective protocols. The Data were analyzed adopting the method of one-way analysis of variance using the Statistical Tool for Agricultural Research (STAR). Tukey's Honest Significant Difference (HSD) was used for testing of significance

2.4 Cytological studies

The somatic chromosome number was determined in root and shoot tip cells using standard cytological techniques [13, 14]. Mulberry root tips were treated with 2% colchicine for 3 hours, hydrolyzed in 1N HCl for 10 minutes at 40°C, stained with 2% aceto-orcein, and squashed in 45% acetic acid. For terminal shoots, pre-treatment involved 0.1% colchicine + 0.001 M 8-hydroxyquinoline for 4 hours, followed by hydrolyzation in 1N HCl for 20 minutes at 25°C. The shoot tips were digested in 5% cellulose for 3–4 hours at 37°C, stained with 2% aceto-carmine, and squashed in 45% acetic acid. Chromosomes were examined under a microscope and photomicrographed. Chromosome length was estimated (6 cells/genotype) during metaphase stage by using IdeoKar software [15] and mean values were calculated.

2.5 Determination of nuclear DNA content

The nuclear DNA content of mulberry genotypes was estimated using flow cytometry (FCM) as described by Galbraith *et al.* [16]. Nuclei were isolated from 40 mg of fresh mulberry leaves, using *Pisum sativum* (2C = 9.09 pg) as the reference standard. The tissue was chopped in a hypotonic propidium iodide buffer, filtered, and incubated at 37°C. FCM analysis was performed on a BD LSR Fortessa™ cell analyzer, with data processed using BD FACS Diva 8.0.3 Software. Ploidy was calculated using the formula: Ploidy = Reference ploidy × (mean G1 peak position of sample / mean G1 peak position of reference), with 1 pg = 980 Mbp.

2.6 Molecular studies

2.6.1 Genomic DNA extraction, Primer selection, PCR amplification, and Gel electrophoresis

Genomic DNA was extracted from young mulberry leaves using the cetyl trimethyl ammonium bromide CTAB method [16] and a plant genomic DNA purification kit (HIPurATM, HIMEDIA). DNA quantity was assessed using a Nanodrop Spectrophotometer (Thermo Scientific) and quality confirmed on a 1.0% agarose gel. ISSR markers (Table 1) were used for PCR amplification [17], with thermal cycling on an Applied Biosystems cycler: initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 1 minute, annealing at 48–60°C for 45 seconds, extension at 72°C for 2 minutes, a final extension at 72°C for 7 minutes, and cooling at 4°C. PCR products were separated on a 2% agarose gel in 1X TAE buffer, with a 100 bp DNA ladder (Gene DireX and Himedia) as a size reference. Gel images were captured under UV light, and molecular sizes were estimated using a 2000 bp ladder.

Table 1: List of ISSR primers and their sequences

Sl.No.	**Primers**	Nucleotide Sequence (5'- 3')	Size (bp)
1	**UBC-812**	GAGAGAGAGAGAGAGAA	300–1500
2	**UBC-813**	CTCTCTCTCTCTCTCTT	300–1500
3	**UBC-815**	CTCTCTCTCTCTCTCTG	300–1500
4	**UBC-824**	TCTCTCTCTCTCTCTCG	300–2000
5	**UBC-834**	AGAGAGAGAGAGAGAGYT	200–1500
6	**UBC-840**	GAGAGAGAGAGAGAGAYT	300–1000
7	**UBC-843**	CTCTCTCTCTCTCTCTRA	300–1500
8	**UBC-844**	CTCTCTCTCTCTCTCTRC	300–2000
Where Y = C and T & R= A/G			

2.6.2 Analysis of genetic diversity

The reproducible amplified bands were manually scored as present (1) or absent (0) for each primer and genotype, creating a binary matrix. This data was used to calculate similarity coefficients, which were then applied to construct a dendrogram using the UPGMA method in NTSYS software. Genetic diversity of genotypes was analysed by calculating the percentage of polymorphic bands (PPB), percentage of monomorphic bands (PMB), and the Polymorphism Information Content (PIC) of each primer [18]. Resolving power was computed as per Prevost and Wilkinson [19]. All statistical analyses were performed using NTSYS Software [20].

3 Results

3.1 Plant morphology

Morphological traits are essential for breeding programs aimed at improving mulberry varieties. The assessment of morphological traits in the mother plant K-2 and its clonally derived genotypes reveals variability for some characters (Table 2). K-2 exhibited medium growth vigor, while the clonally derived genotypes showed a range of growth potentials: high vigor in CS-1, CS-4, CS-5, and CS-6; low vigor in CS-2, CS-8, and CS-9; and medium vigor in CS-3, CS-7, CS-10, and CS-11. Morphologically, most genotypes, including K-2, displayed an erect growth habit and straight branching. In contrast, CS-5 exhibited a spreading growth habit with slightly curved branches. All genotypes shared a consistent shoot color, characterized by greyish-green hues, and featured medium-sized, acute triangular buds. Accessory buds were present across all genotypes. The stipule characteristics were uniform, with all genotypes exhibiting free-lateral stipules that are caduceus. Notably, the shape of lenticels varied between the mother plant and its clones. K-2 had oval and elliptical lenticels, while CS-2 and CS-5 presented round and oval shapes, respectively (Fig. 1).

Table 2
Morphological traits of clonally selected mulberry genotypes and their mother plant, K-2

Genotypes	Vigour	Growth habit	Branch nature	Colour of mature shoot	Lenticel shape	Mature bud size	Bud shape	Bud attachment	Accessory bud	Stipule nature	Stipule duration	Petiole groove
K – 2	Medium	Erect	Straight	Greyish green	Oval & Elliptical	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent
CS – 1	High	Erect	Straight	Greyish green	Oval & Elliptical	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent
CS – 2	Low	Erect	Straight	Greyish green	Round & Oval	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent
CS – 3	Medium	Erect	Straight	Greyish green	Oval & Elliptical	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent
CS – 4	High	Erect	Straight	Greyish green	Oval & Elliptical	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent
CS – 5	High	Spreading	Slightly curved	Greyish green	Round & Oval	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent
CS – 6	High	Erect	Straight	Greyish green	Oval & Elliptical	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent
CS – 7	Medium	Erect	Straight	Greyish green	Oval & Elliptical	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent
CS – 8	Low	Erect	Straight	Greyish green	Oval & Elliptical	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent
CS – 9	Low	Erect	Straight	Greyish green	Round & Elliptical	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent
CS – 10	Medium	Erect	Straight	Greyish green	Oval & Elliptical	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent
CS – 11	Medium	Erect	Straight	Greyish green	Oval & Elliptical	Medium	Acute triangle	Adhering to branch	Present	Free-lateral	Caducous	Absent

3.2 Leaf morphology

Leaf morphological traits are essential for distinguishing among mulberry variants, as evidenced by the characteristics of the mother plant K-2 and its clonal derivatives. K-2 features light green, non-glossy leaves with a truncate base and a slightly rough surface. In contrast, clonal variants display a range of colours, from light green in CS-1 and CS-9 to dark green in CS-5, CS-10, and CS-11. Variants like CS-1 and CS-6 exhibit strongly glossy leaves. Additionally, CS-8 and CS-9 show wrinkled leaves with impressed veins, diverging from K-2's smooth texture. Variation in leaf base shapes among different clones of a plant, where the clones (CS-1, CS-3, CS-4, CS-5, and CS-6) show cordate leaf bases, while the mother plant has a truncate leaf base (Fig. 2). The combination of leaf color, texture, shape, and venation provides clear morphological markers that can be used to identify different mulberry clones.

3.3 Propagation, growth and yield parameters

The assessment of various growth and yield parameters among genotypes reveals significant variations across multiple traits. Sprouting percentage ranged from 66.31 to 95.33%, with K-2 and genotypes CS-1, CS-3, and CS-8 showing the highest rates, while CS-9 had the lowest. For survivability, percentages varied from 57.24 to 91.49%, with CS-8 demonstrating the highest survivability. The number of branches per plant ranged from 5 to 11, with CS-7 having the most branches, exhibiting an 8.27% increase over K-2. In terms of shoot length, genotypes ranged from 80.01 to 132.12 cm, with CS-7 showing the greatest length. Inter-nodal lengths varied from 4.68 to 6.64 cm, with CS-3 exhibiting the longest internodes. The petiole lengths ranged from 3.78 to 5.80 cm, with CS-11 measuring the longest. Leaf area varied from 147.76 to 379.62 cm², with CS-5 recording the largest area. The shoot girth size ranged from 0.53 to 1.30 cm, with CS-5 being the highest girth. The leaf-specific weight ranged from 14.23 to 20.09 mg, with CS-6 being the thickest. Total leaf yield per plant varied significantly, from 101.71 to 648.24 g, with CS-5 achieving the highest yield. The moisture content of fresh leaves ranged from 72.78 to 77.34%, with CS-6 having the highest percentage, while moisture retention after six hours varied from 59.78–73.34%, with CS-6 again leading in retention (Table 3).

Table 3
Leaf morphological characteristics of clonally selected mulberry genotypes and their mother plant, K-2

Genotypes	Leaf colour	Leaf glossiness	Leaf wrinkles	Leaf apex	Leaf base	Leaf margin	Leaf surface	Leaf texture	Leaf shape	Leaf angle	Leaf nature
K – 2	L Light G green	Non-glossy	Non-wrinkle	Acuminate	Truncate	Crenate	Slightly rough	Charataceous	Ovate	Acute	Homophyllo
CS – 1	Light green	Strongly-glossy	Non-wrinkle	Acuminate	Cordate	Crenate	Smooth	Charataceous	Cordate	Acute	Homophyllo
CS – 2	Green	Glossy	Non-wrinkle	Acuminate	Truncate	Crenate	Smooth	Charataceous	Narrow ovate	Acute	Homophyllo
CS – 3	Green	Glossy	Non-wrinkle	Acuminate	Cordate	Crenate	Smooth	Charataceous	Cordate	Acute	Homophyllo
CS – 4	Green	Glossy	Non-wrinkle	Acuminate	Cordate	Crenate	Smooth	Charataceous	Wide ovate	Acute	Homophyllo
CS – 5	Dark-green	Non-glossy	Non-wrinkle	Acuminate	Cordate	Crenate	Smooth	Charataceous	Cordate	Acute	Homophyllo
CS – 6	Green	Strongly-glossy	Non-wrinkle	Acuminate	Cordate	Crenate	Smooth	Charataceous	Cordate	Acute	Homophyllo
CS – 7	Dark-green	Glossy	Non-wrinkle	Acuminate	Truncate	Crenate	Smooth	Charataceous	Narrow ovate	Acute	Homophyllo
CS – 8	Green	Glossy	Wrinkle with impressed veins	Acuminate	Truncate	Crenate	Smooth	Charataceous	Narrow ovate	Acute	Homophyllo
CS – 9	Light green	Non-glossy	Wrinkle with impressed veins	Acuminate	Truncate	Crenate	Slightly rough	Charataceous	Cordate	Horizontal	Homophyllo
CS – 10	Dark green	Glossy	Non-wrinkle	Acuminate	Truncate	Crenate	Smooth	Charataceous	Narrow ovate	Acute	Homophyllo
CS – 11	Dark green	Non-glossy	Non-wrinkle	Acuminate	Truncate	Crenate	Slightly rough	Charataceous	Narrow ovate	Acute	Homophyllo

3.4 Reproductive traits

Most mulberry genotypes, including the mother plant K-2, displayed a gynoecious nature with pubescent, spreading stigmas and black fruits, except for CS-2 (androecious) and CS-5 (bisexual). Significant variation was noted in the average number of inflorescences per plant, with CS-4 producing the highest at 49, while CS-11 had the lowest at 21; K-2 had 45 inflorescences. Inflorescence lengths varied, measuring 0.31 to 1.87 cm for females, 2.25 cm for males, and 1.44 cm for bisexuals. Additionally, the number of flowers per inflorescence ranged from 12 to 20 for female catkins, 16 for male catkins, and 14 for bisexual catkins (Table 4).

Table 4
Propagation, growth and yield parameters of clonally selected mulberry genotypes and their mother plant, K-2

Genotypes	Sprouting (%)	Survivability (%)	No. of branches/ plant	Shoot length (cm)	Internode length (cm)	Petiole length (cm)	Leaf area (cm)	Shot girth (cm)	Leaf specific weight (mg/cm)	Leaf moisture content (%)	Leaf moisture retention (%)	Le Yie Pla (g)
K – 2	95.33^{a}	87.44^{abc}	10.76^{ab}	99.11^{bcd}	5.51^{bcd}	4.35^{ef}	148.24^{f}	0.85^{cd}	16.47^{cd}	72.78^{d}	62.78^{de}	32
CS – 1	94.51^{ab}	88.56^{abc}	9.45^{bc}	106.74^{b}	5.99^{abc}	4.82^{cd}	338.23^{b}	1.09^{b}	16.92b^{c}	76.07^{abc}	66.07^{cd}	42
CS – 2	86.68^{d}	78.04^{de}	6.48^{efg}	99.68^{bcd}	5.00^{de}	3.78^{g}	236.59^{d}	0.82^{cd}	14.23^{f}	74.78^{bcd}	59.78^{e}	22
CS – 3	94.13 ^{ab}	89.17^{ab}	7.94^{de}	91.58^{cdef}	6.64^{a}	4.56^{def}	181.04^{e}	0.85^{c}	15.71^{e}	75.66^{abc}	68.66^{c}	32
CS – 4	88.01^{cd}	82.09^{cd}	9.92^{b}	87.65^{def}	4.68^{e}	4.63^{de}	273.77^{c}	1.06^{b}	16.28^{d}	76.67^{ab}	66.67^{cd}	42
CS – 5	93.17 ^{abc}	83.78^{bcd}	6.08^{fg}	109.06^{b}	6.2^{ab}	5.25^{bc}	379.62^{a}	1.30^{a}	17.36^{b}	74.42^{bcd}	64.42^{d}	64
CS – 6	93.00 ^{abc}	89.14^{ab}	8.39^{cd}	80.01^{f}	5.21^{cde}	4.26^{ef}	335.66^{b}	1.00^{b}	20.09^{a}	77.34^{a}	73.34^{a}	41
CS – 7	89.04 ^{bcd}	84.36^{bcd}	11.65^{a}	132.12^{a}	5.01^{de}	5.10^{bc}	250.39^{cd}	0.80^{cd}	16.4^{cd}	75.39^{abc}	71.39^{b}	32
CS – 8	94.62 ^{ab}	91.49^{a}	9.55^{bc}	82.66^{ef}	5.63^{bcd}	5.30^{b}	147.76^{f}	0.53^{e}	14.47^{f}	73.95^{cd}	63.95^{d}	10
CS – 9	66.31^{e}	57.24^{f}	7.41^{def}	96.9^{bcd}	6.30^{ab}	4.15^{fg}	240.02^{d}	0.77^{cd}	15.63^{e}	75.64^{abc}	65.64^{d}	24
CS – 10	85.66^{d}	74.66^{e}	6.44^{efg}	104.38^{bc}	4.70^{e}	5.46^{ab}	262.41^{cd}	0.85^{c}	16.51^{cd}	74.65^{bcd}	64.65^{d}	32
CS – 11	91.11 ^{abcd}	85.19^{abc}	5.55^{g}	95.96^{bcde}	5.03^{de}	5.80^{a}	254.29^{cd}	0.75^{d}	15.53^{e}	76.34^{abc}	66.34^{cd}	25
Mean	**89.30**	**82.50**	**8.30**	**98.81**	**5.49**	**4.79**	**254.00**	**0.89**	**16.29**	**75.31**	**66.14**	**33**
SD±	**8.04**	**9.32**	**1.97**	**13.83**	**0.65**	**0.60**	**72.80**	**0.19**	**1.50**	**1.26**	**1.55**	**13**
Significance	*	*	**	*	**	**	*	**	**	**	**	*

3.5 Leaf anatomical features

The leaf anatomical traits in clonally selected mulberry genotypes revealed significant variations against their mother plant. Leaf thickness ranged from 53.83 to 173.52 µm, with CS-6 being the thickest, while CS-8 was the thinnest. Cuticle thickness varied from 2.95 to 8.11 µm, with CS-6 again showing the highest value. The palisade proportion ranged from 27.54 to 42.33%, while the spongy proportion varied from 17.16 to 48.33%, with CS-6 having the highest spongy tissue (Fig. 3). Stomatal frequency ranged from 357 to 762 no./mm^2, with CS-4 leading, and stomatal size varied from 193.56 to 254.16 µm, with CS-6 having the largest. Chloroplast numbers per guard cell ranged from 6 to 16, with CS-6 showing the most, and trichome counts varied from 6 to 57 no./mm^2, with CS-8 having the highest (Table 5).

Table 5
Reproductive traits of clonally selected mulberry genotypes and their mother plant, K-2

Genotypes	Sex expression	No. of inflorescence /plant	Inflorescence length (cm)	No. of flowers /inflorescence	Stigma nature	Stigma type	Fruit colour
K – 2	Gynoecious	45.23^{b}	1.76^{b}	17.36^{bc}	Pubescent	Spreading	Black
CS – 1	Gynoecious	36.04^{d}	1.25^{cd}	18.63^{b}	Pubescent	Spreading	Black
CS – 2	Androecious	31.58^{e}	2.25^{a}	16.37^{c}	NA	NA	NA
CS – 3	Gynoecious	26.87^{f}	0.98^{e}	16.59^{c}	Pubescent	Spreading	Black
CS – 4	Gynoecious	49.37^{a}	1.11^{cd}	18.36^{b}	Pubescent	Spreading	Black
CS – 5	Bisexual	28.74^{f}	1.44^{c}	14.38^{d}	Pubescent	Spreading	Black
CS – 6	Gynoecious	31.05^{e}	1.56^{c}	20.17^{a}	Pubescent	Spreading	Black
CS – 7	Gynoecious	42.22^{bc}	1.22^{cd}	15.36^{d}	Pubescent	Spreading	Black
CS – 8	Gynoecious	44.18^{b}	0.89^{e}	16.35^{c}	Pubescent	Spreading	Black
CS – 9	Gynoecious	39.33^{c}	1.87^{b}	12.26^{e}	Pubescent	Spreading	Black
CS – 10	Gynoecious	27.54^{f}	0.31^{e}	15.34^{d}	Pubescent	Spreading	Black
CS – 11	Gynoecious	21.16^{g}	1.41^{c}	17.36^{bc}	Pubescent	Spreading	Black
Mean	NA	35.27	1.33	16.54	NA	NA	NA
SD±		8.77	0.50	2.09			
Significance		**	**	**			

3.6 Leaf biochemical parameters

Nutritional and biochemical analyses of clonally evolved mulberry genotypes revealed significant variations in key traits. Protein content ranged from 22.79 to 27.22%, with CS-6 showing the highest levels, surpassing the mother plant K-2. Carbohydrate content varied from 13.17 to 15.33%, also peaking in CS-6. Chlorophyll a and b levels ranged from 2.57 to 3.88 mg/g and 1.19 to 2.36 mg/g, respectively, with CS-6 leading in both categories. Total chlorophyll content varied from 3.93 to 6.17 mg/g, again favoring CS-6 (Table 6).

Table 6
Leaf anatomical features of clonally selected mulberry genotypes and their mother plant, K-2

Genotypes	Leaf thickness (µm)	Upper cuticle thickness (µm)	Palisade tissue thickness (%)	Spongy tissue thickness (%)	Stomatal frequency (no./mm^2)	Stomatal size (µm)	Chloroplast No. /stomata	Trichome density (no./mm^2)
K – 2	96.52^{d}	5.53^{c}	30.33^{e}	36.53^{c}	574.56^{c}	229.66ef	10.55^{e}	36.56^{c}
CS – 1	112.83^{bc}	5.28^{cd}	33.56^{d}	34.55^{cd}	673.45^{b}	217.16^{h}	7.26^{h}	36.83^{c}
CS – 2	63.01^{h}	3.31^{h}	35.27^{c}	17.16^{f}	741.83^{a}	193.56^{i}	8.45^{g}	44.33^{b}
CS – 3	96.16^{d}	5.03^{de}	42.33^{a}	43.33^{b}	576.66^{c}	238.52^{bc}	15.55^{b}	14.52^{d}
CS – 4	86.18^{e}	4.73^{ef}	37.56^{b}	36.36^{c}	762.33^{a}	222.83^{gh}	7.48^{h}	37.65^{c}
CS – 5	76.83^{g}	3.95^{g}	27.54^{f}	36.56^{c}	756.52^{a}	237.23^{cd}	9.31^{f}	45.33^{b}
CS – 6	173.52^{a}	8.11^{a}	33.33^{d}	48.33^{a}	443.26^{d}	254.16^{a}	16.93^{a}	6.16^{f}
CS – 7	116.12^{b}	6.46^{b}	36.16^{c}	41.16^{b}	578.31^{c}	237.33^{cd}	15.19^{b}	9.83^{e}
CS – 8	53.83^{i}	2.95^{h}	34.66^{d}	24.54^{e}	357.16^{e}	225.16^{fg}	6.48^{i}	57.24^{a}
CS – 9	75.73^{g}	4.71^{ef}	37.33^{b}	34.66^{cd}	656.83^{b}	232.33^{de}	8.73^{fg}	44.66^{b}
CS – 10	112.52^{c}	5.48^{cd}	32.59^{d}	35.16^{cd}	731.17^{a}	243.55^{b}	12.16^{c}	36.83^{c}
CS – 11	82.16^{f}	4.55^{f}	37.24^{b}	32.56^{d}	759.36^{a}	234.33^{cde}	11.34^{d}	46.33^{b}
Mean	**95.44**	**5.00**	**35.55**	**35.02**	**634.18**	**230.41**	**10.77**	**34.61**
SD±	**31.46**	**1.38**	**3.07**	**8.12**	**132.55**	**15.29**	**3.50**	**15.94**
Significance	**	**	**	**	**	**	**	**

Table 7
Leaf biochemical parameters of clonally selected mulberry genotypes and their mother plant, K-2

Genotypes	Protein (%)	Carbohydrate (%)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
K – 2	25.52^{c}	13.25^{c}	3.24^{b}	1.24^{bc}	4.48^{cd}
CS – 1	26.36^{b}	14.21^{b}	3.18^{b}	1.19^{c}	4.37^{cde}
CS – 2	24.46^{e}	13.35^{c}	2.71^{c}	1.22^{c}	3.93^{e}
CS – 3	25.62^{bc}	14.24^{b}	3.15^{b}	2.36^{a}	5.51^{b}
CS – 4	25.29^{cd}	14.53^{b}	3.12^{b}	1.28^{bc}	4.41^{cd}
CS – 5	25.77^{bc}	14.41^{b}	3.21^{b}	1.18^{c}	4.39^{cd}
CS – 6	27.22^{a}	15.33^{a}	3.88^{a}	2.29^{a}	6.17^{a}
CS – 7	26.28^{b}	14.36^{b}	3.18^{b}	2.11^{a}	5.28^{b}
CS – 8	22.79^{g}	13.17^{c}	2.57^{c}	1.56^{b}	4.07^{de}
CS – 9	23.50^{fg}	13.28^{c}	3.17^{b}	1.35^{bc}	4.53^{c}
CS – 10	24.67^{de}	14.45^{b}	3.25^{b}	1.26^{bc}	4.52^{cd}
CS – 11	23.63^{f}	14.28^{b}	3.66^{a}	1.50^{b}	5.16^{b}
Mean	**25.09**	**14.07**	**3.19**	**1.54**	**4.74**
SD±	**1.31**	**0.64**	**0.34**	**0.44**	**0.65**
Significance	**	**	**	**	**

3.7 Chromosome studies

Chromosome counting during mitotic cell division revealed a consistent number of 28 somatic chromosomes in root and shoot tip cells across all clonally selected genotypes (CS-1 to CS-11) and their mother plant K-2 (Fig. 4a), confirming a diploid ploidy level (2n = 2x = 28). During prophase, clonal variants (CS-3, CS-7, CS-9, and CS-10) exhibited varying degrees of chromosome condensation compared to K-2. Normal chromosome segregation was observed at anaphase, with proper formation of daughter nuclei in all genotypes. No variations in chromosomal complements were detected, indicating that the regular mitotic processes in both shoot and root meristematic tissues reflect the genetic stability of these clonal variants.The chromosome analysis of this mulberry genotypes revealed a pair of small chromosomes measuring between 0.96 and 1.42 µm, alongside a pair of larger chromosomes ranging from 2.87 to 3.27 µm among the total of 28 chromosomes. The average length of these chromosomes varied from 1.98 to 2.52 µm, with the mother plant K-2 having a mean length of 2.21 µm. Notably, CS-10 exhibited increased chromosome lengths, while CS-2 displayed reduced lengths compared to the mother plant (Table 8).

Table 8. Ploidy level, chromosome length, and nuclear DNA content of clonally selected mulberry genotypes and their mother plant, K-2

Genotypes	Chromosome numbers	Mean of amount Nuclear DNA (pg/c)	Chromosome length (µm)	
			Range	Mean
K - 2	2n=2x=28	0.82	1.14 - 3.11	2.21
CS - 1	2n=2x=28	0.94	1.11 - 3.16	2.32
CS - 2	2n=2x=28	0.83	0.96 - 2.98	2.11
CS - 3	2n=2x=28	0.93	1.23 - 3.24	2.38
CS - 4	2n=2x=28	0.74	1.04 - 2.87	1.98
CS - 5	2n=2x=28	0.95	1.18 - 3.21	2.41
CS - 6	2n=2x=28	0.89	1.22 - 3.14	2.13
CS - 7	2n=2x=28	0.78	1.32 - 2.77	2.03
CS - 8	2n=2x=28	0.94	1.14 - 3.27	2.48
CS - 9	2n=2x=28	0.89	1.42 - 3.21	2.12
CS - 10	2n=2x=28	0.95	1.16 - 3.18	2.52
CS - 11	2n=2x=28	0.88	0.98 - 3.14	2.31

3.8 Nuclear DNA content

Flow cytometry analysis of the ploidy status and genome size of all clonally selected genotypes, including the mother plant K-2 (Fig. 4b), confirmed again that all are diploid. The nDNA content for K-2 was determined to be 0.82 pg. Notably, several genotypes exhibited increased genome sizes: CS-1 (0.94 pg), CS-2 (0.83 pg), CS-3 (0.93 pg), CS-5 (0.95 pg), CS-6 (0.89 pg), CS-8 (0.94 pg), CS-9 (0.89 pg), CS-10 (0.95 pg), and CS-11 (0.88 pg). Conversely, CS-4 and CS-7 showed reduced genome sizes of 0.74 pg and 0.78 pg, respectively. The nuclear DNA content remained consistent across replicates of the same genotype, varying only from 0.01 to 0.08 pg (Table 8).

3.9 Genetic diversity analysis

The study utilized ISSR markers to assess genetic diversity among 11 clonally selected genotypes, along with the mother plant K-2. The results revealed distinct genetic variations across all genotypes, providing valuable insights into their genetic relationships and the overall diversity within the group (Table 9 and Fig. 5). Using 8 ISSR primers, a total of 52 amplified products (fragments) were obtained across the 12 genotypes. Of these, 41 (80.04%) were polymorphic, indicating genetic variation among the genotypes. These polymorphic bands are crucial for assessing genetic diversity. The remaining 11 products (19.96%) were monomorphic, as they were consistent across all genotypes and showed no genetic variation.The highest level of polymorphism (100%) was observed with the primers UBC-812 and UBC-844, while the lowest level (60%) was observed with UBC-813. The Polymorphism Information Content (PIC), which measures the informativeness of a marker, ranged from 0.11 for the UBC-840 primer to 0.38 for UBC-844, with an average of 0.25 per primer pair. The Resolving Power (RP), which indicates the discriminatory potential of the primers, was highest for UBC-824 (RP = 5.50) and lowest for UBC-840 (RP = 2.67), with an average RP of 3.83 across all primers.The Effective Multiplex Ratio (EMR), which describes the efficiency of primers in generating polymorphic bands, ranged from 4.44 for UBC-813 to 8.00 for UBC-844, with an average EMR of 6.61 for all 8 primers. The Marker Index (MI), which reflects the discriminatory power of the markers, ranged from 0.60 for UBC-813 to 3.07 for UBC-844, with an average MI value of 1.72.A UPGMA dendrogram was constructed to illustrate the genetic relationships among the clonally selected genotypes and their mother plant, K-2. The 12 genotypes were grouped into two clusters based on genetic similarity values ranging from 0.82 to 0.98 (Table 10). Cluster 1, comprising CS-7, CS-8, and K-2, shows lower genetic similarity around 0.84, indicating greater genetic diversity. Cluster 2, which includes the remaining genotypes starting with CS-5, displays higher genetic similarity, suggesting a closer genetic relationship among these genotypes (Fig. 6).

Table 9. Genetic diversity parameters in 12 mulberry genotypes calculated for ISSR primers

Primers	TB	PB	MB	PPB	PMB	Mean	PIC	RP	EMR	MI
UBC-812	5.00	5.00	0.00	100.00	0.00	7.40	0.31	3.83	7.40	2.32
UBC-813	5.00	3.00	2.00	60.00	40.00	7.40	0.14	3.50	4.44	0.60
UBC-815	8.00	6.00	2.00	75.00	25.00	9.00	0.28	4.00	6.75	1.88
UBC-824	7.00	5.00	2.00	71.43	28.57	7.29	0.18	5.50	5.20	0.92
UBC-834	7.00	6.00	1.00	85.71	14.29	9.14	0.29	3.33	7.84	2.24
UBC-840	8.00	5.00	3.00	62.50	37.50	10.00	0.11	2.67	6.25	0.69
UBC-843	7.00	6.00	1.00	85.71	14.29	8.14	0.29	4.50	6.98	2.01
UBC-844	5.00	5.00	0.00	100.00	0.00	8.00	0.38	3.33	8.00	3.07
Total	**52.00**	**41.00**	**11.00**	**80.04**	**19.96**					
Mean	**6.50**	**5.13**	**1.38**	**80.04**	**19.96**	**8.30**	**0.25**	**3.83**	**6.61**	**1.72**

Where: TB=Total band, PB=Polymorphic Band, MB=Monomorphic Band, PPB=Percentage Polymorphic Band, PMB=Percentage Monomorphic Band, PIC=Polymorphic Information Content,

RP= Resolving Power of primer, EMR=Effective Multiple Ratios, MI=Marker index

Table 10. Genetic similarity coefficients among 12 mulberry genotypes obtained using ISSR markers

Genotypes	K-2	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7	CS-8	CS-9	CS-10	CS-11
K-2	1											
CS-1	0.850	1										
CS-2	0.861	0.861	1									
CS-3	0.767	0.900	0.833	1								
CS-4	0.824	0.941	0.824	0.767	1							
CS-5	0.846	0.897	0.917	0.900	0.971	1						
CS-6	0.784	0.865	0.861	0.900	0.912	0.946	1					
CS-7	0.844	0.844	0.813	0.700	0.750	0.844	0.813	1				
CS-8	0.889	0.861	0.806	0.800	0.824	0.944	0.833	0.844	1			
CS-9	0.806	0.861	0.861	0.867	0.882	0.972	0.944	0.813	0.833	1		
CS-10	0.842	0.842	0.889	0.867	0.853	0.895	0.865	0.844	0.889	0.889	1	
CS-11	0.825	0.875	0.889	0.867	0.912	0.897	0.865	0.844	0.889	0.861	0.895	1

4 Discussion

Clonally propagated crop species exhibit reduced adaptability to environmental changes compared to sexually propagated species, making clonal variation the primary source of adaptive potential for vegetatively propagated plants [21]. While mutations serve as a key driver of clonal variation independent of sexual reproduction and environmental factors [22]. Clonal selection remains the principal method for modifying cultivar characteristics without significantly altering varietal integrity; however, this progress is limited to the genetic diversity generated within a crop and its existing clones. Mutations accumulated since a cultivar's inception constitutes the majority of this variation, and once a mutation occurs, cuttings from the mutated clone replicate it almost exactly. Consequently, older cultivars are more likely to exhibit a greater number of clones with distinct mutations due to their slower rate of mutation accumulation, resulting in increased variance compared to newer cultivars [23]. Plants that undergo bud mutations often display unique traits relative to their parent, including changes in shoot morphology, inflorescence, and fruit, along with modifications in external appearance, internal structure, physiology, and biochemistry [24, 25]. This source of novel variation has frequently led to the development of improved fruit and ornamental plant varieties [22].

The mulberry variety (*Morus indica*) K-2, also known as M-5 (Mysore-5), is an open-pollinated hybrid developed from Mysore local variety seeds at the Sericulture Farm in Channapatna, Ramanagara District, Karnataka, India. Since its introduction in 1990, the K-2 variety has been cultivated under both irrigated and rain-fed conditions across the southern states of India, yielding approximately 30–35 MT/ha/year [26]. However, it is now largely being replaced by the rolling variety V-1 in traditional sericulture areas of Karnataka. The K-2 cultivar has served as a foundational material for developing improved varieties through hybridization (e.g., var. RFS-135, RFS-175, AR-1, S-13, Sahana, and Suvarna), clonal selection (e.g., var. TG-1), and research into both natural [27] and induced mutants [28, 29]. In this study, we report on the morphometric and genetic variability among clonally-derived genotypes, highlighting their distinct characteristics compared to the mother plant, K-2.

The first step in characterizing and categorizing mulberry germplasm is phenotypic characterization [30], with leaf morphological variation serving as a crucial indicator for detecting differences among plants in mulberry gardens. Key features such as leaf surface, margin, and apex are essential for distinguishing between different mulberry varieties [31]. Natural mutants of mulberry exhibit alterations in leaf color, surface texture, shape, and size [27, 5]. In our study, clonally derived genotypes displayed dark green leaves (CS-5, CS-7, CS-10, and CS-11) and green leaves (CS-2, CS-3, CS-4, CS-6, and CS-8), in contrast to the light green leaves of the mother plant K-2. Chlorophyll content, an indicator of photosynthetic capacity, correlates with leaf color; dark green leaves (CS-5, CS-10, and CS-11) have higher chlorophyll levels [32]. This characteristic is stable across different environmental conditions [33] and most improved mulberry varieties feature either dark green or green leaves [34] .Additionally, genotypes CS-1, CS-2, CS-3, CS-4, CS-6, CS-7, CS-8, and CS-10 exhibit glossy leaves, contrasting with the non-glossy nature of K-2. A previous report identified a glossy leaf mutant in a K-2 (M-5) mulberry garden [27], glossy leaves are often more palatable to silkworms (*Bombyx mori*), leading to improved feeding rates and better growth [35]. Furthermore, CS-8 and CS-9 exhibit wrinkled leaves with prominent veins, unlike the smooth leaves of the mother plant, resembling the Venosa leaf mutant characterized by uneven growth and prominent veins [27]. The variation in leaf base and shape among clonally derived genotypes (CS-1, CS-2, CS-3, CS-4, CS-5, CS-7, CS-8, CS-9, CS-10, and CS-11) reflects their adaptation to different ecological conditions [36], while the smooth surface of the leaves in genotypes CS-1, CS-2, CS-3, CS-4, CS-6, CS-8, and CS-10 enhances palatability for silkworms [37].

Morphologically, most genotypes, including K-2, displayed an erect growth habit and straight branching, which is beneficial for maximizing light capture and promoting healthy growth [38]. Accessory buds were present across all genotypes, which is advantageous for potential branching and vegetative propagation [39]. Identifying the types, traits, and morphological structures of the buds is crucial for effective variety cultivation, selection, and breeding [40]. Plant vigor is a vital consideration in mulberry breeding, influencing not only the success of the breeding program but also the sustainability and profitability of mulberry cultivation. Breeding for vigor helps produce resilient, high-quality cultivars that can thrive in diverse environmental conditions [41]. The plant growth vigor observed in genotypes CS-1, CS-4, CS-5, and CS-6 is significantly higher than that of their mother plant, characterized by greater shoot girth, leaf area, leaf specific weight, and leaf yield. In the clonally derived genotypes, petiole length showed a negative correlation with leaf yield, whereas branch length, internodal distance, leaf area, leaf specific weight, moisture content, moisture retention capacity, and leaf thickness all demonstrated significant positive correlations with leaf yield per plant [42]. Reproductive traits demonstrated notable diversity, particularly with the predominance of gynoecious forms, which can influence pollination and fruiting patterns. The higher number of inflorescences in CS-4 indicates its potential for increased fruit yield, a vital consideration for mulberry's economic value [43].

Understanding the anatomical structure of mulberry leaves can provide insights into their physiological functions, adaptability to environments, and nutritional value.The quality of a leaf is influenced by its structural features and the presence of trichomes, which are associated with various physiological processes [44]. In mulberry genotypes that have undergone clonal evolution, anatomical changes in leaves are often observed to facilitate growth under diverse agro-climatic conditions [37, 17]. Increased leaf thickness (CS-1, CS-6, CS-7, and CS-10), cuticle thickness (CS-6 and CS-7), and the proportion of palisade tissue were noted in clonally derived genotypes compared to their mother plant. The mesophyll tissue, comprising palisade and spongy parenchyma, serves as the primary photosynthetic zone, contributing significantly to leaf thickness [45]. Conversely, reduced stomatal frequency (CS-6 and CS-8) and smaller stomatal size (CS-2) were observed in clonally evolved genotypes relative to K-2. Leaf moisture content and retention capacity are influenced by factors such as cuticle thickness, leaf thickness, mesophyll tissue, stomatal size, and frequency [46]. (Mulberry genotypes with smaller stomata and lower frequency demonstrate reduced water loss, thereby exhibiting enhanced tolerance under stress conditions [47, 45]. Additionally, the decreased number of trichomes in CS-6 and CS-7 renders the leaves more palatable to silkworms [48]. Variations in anatomical characteristics provide robust evidence for adaptation to challenging environments [49].

The nutritional status of mulberry leaves, determined by moisture, protein, carbohydrates, and minerals, directly affects silkworm productivity [50]. Feeding trials indicate that nutrient levels in mulberry significantly influence silkworm growth and cocoon production [51, 52, 53, 54]. Moisture content is crucial for enhancing nutritional quality, with higher levels facilitating better feeding and digestion in silkworm larvae [55, 56, 57]. In this study, genotypes CS-1, CS-3, CS-4, CS-6, CS-7, CS-9, and CS-11 showed over 75% moisture content, compared to 62.78% in K-2. Ideal moisture content for Chawki silkworms is 75–80% [26]. The moisture retention capacity is also vital, as it affects leaf freshness and palatability [32]. Higher chlorophyll content (6.17 mg/g) in CS-6 mulberry leaves enhances the leaf's photosynthetic efficiency, leading to greater production of sugars and other essential nutrients that are crucial for silkworms. Proteins and carbohydrates are fundamental macronutrients for the optimal growth, development, and silk production of silkworms [58, 59]. CS-6 stands out due to its superior nutritional profile, with protein (27.22%) and carbohydrates (15.33%) content, making it an ideal choice for silkworm feeding. This high nutrient content promotes healthier silkworm growth, improved silk yield, and better silk quality.Due to its exceptional nutrient profile, CS-6 is of great importance in mulberry breeding programs, where it can be utilized to develop new varieties with enhanced nutritional value for silkworm rearing. The adoption of such high-nutrient genotypes can significantly boost the efficiency and profitability of sericulture operations by improving both silkworm productivity and silk quality, ensuring sustainable and high-yielding sericulture practices [60].

Clonally propagated plants exhibit clonal diversity due to molecular processes such as transposon activity, gene mutation, somatic recombination, and DNA methylation [61]. One method to assess genetic variability in these variants is through flow cytometry analysis of genome size. Clonal variants displayed a range of 2C DNA contents, with CS-4 and CS-7 showing lower values, while CS-1, CS-2, CS-3, CS-5, CS-6, CS-8, CS-9, CS-10, and CS-11 exhibited higher values compared to the mother plant K-2. Spontaneous mutations often lead to cultivars with altered DNA content [62]. The plasticity of nuclear genomes at the chromosomal level is evidenced by changes in chromosomal number, structure, and DNA composition, with repetitive DNA sequences playing a significant role in plant chromosomal evolution [63]. Variations in karyotype between genotypes are generally attributed to the acquisition, deletion, alteration, and rearrangement of nuclear DNA [64, 65]. Banding pattern polymorphism in clonal genotypes is often due to restriction enzyme target site mutations, which can result in three-band differences, or DNA rearrangements such as deletions or insertions, leading to two-band differences [66, 67]. In this context, the clonal genotypes of the mulberry cultivar K-2 have evolved through natural bud mutations, resulting in novel phenotypes that are distinctly different from those of the mother plant K-2.

5 Conclusion

The exploration of natural variability in mulberry through clonal selection offers a promising pathway for developing improved cultivars that meet the demands of sericulture.The findings from this study present a comprehensive understanding of the genetic diversity among the mulberry genotypes. This diversity can be strategically utilized in breeding programs to select for traits that enhance growth, productivity, and nutritional quality, ultimately improving the sustainability and profitability of mulberry cultivation in sericulture. Future research should focus on elucidating the genetic basis of these traits and exploring their interactions with environmental factors to fully realize the potential of these promising genotypes.

Declarations

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Plant ethics: The experiments conducted on the studied plant were in compliance with all relevant institutional, national, and international guidelines and legislation.

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References

1. Central Silk Board. Sericultural statistics in India - A glance. Available from: https://www.cabdirect.org/cabdirect/abstract/19780559488https://csb.gov.in/wpcontent/uploads/2022/07/Raw-Silk-Production.pdf. 2023 [Accessed 2024 Apr 10].
2. Tikader A, Kamble C. Mulberry breeding in India: A critical review. Sericologia, 2007;47:359–366.
3. Sung GB. (2002) Conservation status of mulberry genetic resources in Korea. Paper contributed to Expert Consultation on Promotion of Global Exchange of Sericulture Germplasm Satellite Session of XIXth ISC Congress, September 21st -25th Bangkok, Thailand. Conservation Status of Sericulture Germplasm Resources in the World - I. Conservation Status of Mulberry (*Morus* spp.) Genetic Resources in the World.
4. CSGRC (2022) Annual Report, CSB-Central Sericultural Germplasm Resources Centre, Hosur, India p. 3.
5. Kumara RR, Kumar MGP. Bud sports in the popular mulberry cultivar, Victory-1 and their characteristics. International Journal of Industrial Entomology and Biomaterials, 2024;48(2):78–85.
6. Wang S. M., Li W. J., Liu Y. X., He L., Yue M. and Zhang Z. H. (2017) Comparative transcriptome analysis of shortened fruit mutant in woodland strawberry (Fragaria vesca) using RNA-Seq. J. Integr. Agric., 16: 828–844.
7. Du X., Wang Y., Liu M., et al. (2020) The assessment of epigenetic diversity, differentiation, and structure in the ‘Fuji’ mutation line implicates roles of epigenetic modification in the occurrence of different mutant groups as well as spontaneous mutants. PLoS One, 15: e0235073.
8. Chen M. S., Zhao M. L., Wang G. J., He H. Y., Bai X. and Pan B. Z. (2019) Transcriptome analysis of two inflorescence branching mutants reveals cytokinin is an important regulator in controlling inflorescence architecture in the woody plant Jatropha curcas. BMC Plant Biol., 19: 468.
9. Sahay A, Jhansi Lakshmi K, Saraswathi P, Sekar S (2016) Manual of mulberry gene bank operations and procedures. Technical Manual, CSB-CSGRC, Hosur, India.
10. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265–75.
11. Plummer, D. T. (1971). *Introduction to practical biochemistry.* McGraw Hill Co. Ltd, UK.
12. Hiscox, J.D. and G.F. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57: 1332–1334.
13. Sharma, A.K. and Sharma, A. (1980) Chromosomes techniques.Butterworth & Co., London.
14. MaY, Islam-Faridi M.N, Crane C.F., Stelly D M., Price H. J and Byrne D H.. (1996). A New Procedure to Prepare Slides of Metaphase Chromosomes of Roses. HORTSCIENCE 31(5):855–857.
15. Mirzaghaderi G and Marzangi K. (2015) IdeoKar: An ideogram constructing and karyotype analyzing software. Caryologia 68 (2015):31–35.
16. Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP, Firoozabady E. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science. 1983. Jun 3;220(4601):1049–51. doi: 10.1126/science.220.4601.1049

17. Kumara RR, Reddy TH, Swamy Gowda MR, Devamani M, Radhakrishna PG (2022) Genotypic and phenotypic variability in a clone of mulberry cultivar, Vishala. Sericologia 62, 9–17.
18. Roldan R, Dendauw J, Vanbockstaele E, Depicker A, De Loose M. AFLP markers reveal high polymorphic rates in ryegrasses (Lolium spp.) Mol Breed. 2000;6:125–134.
19. Prevost A. and Wilkinson M.J. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor. Appl. Genet., 98 (1) (1999), pp. 107–112
20. Rohlf, F.J. (1992) NTSYS-pc Numerical taxonomy and multivariate analysis system. Exeter Software, New York.
21. Lebot V., Tuia V., Ivancic A., et al. (2018) Adapting clonally propagated crops to climatic changes: a global approach for taro (*Colocasia esculenta* (L.) Schott). Genet. Resour. Crop Evol., 65: 591–606.
22. Harten, V.A.M. & Broertjes, C. 1988. Induced mutations in vegetatively propagated crops. Plant Breeding Review, 6: 55–91.
23. Jackson R S., Grape Species and Varieties, Editor(s): Ron S. Jackson, In Food Science and Technology, Wine Science (Second Edition), Academic Press, 2000, Pages 13–44,
24. Li S.F, Su T, Cheng GQ, Wang BX, Li X, Deng CL, Gao WJ. Chromosome Evolution in Connection with Repetitive Sequences and Epigenetics in Plants. Genes. 2017; 8(10):290.
25. Foster T. M. and Aranzana M. J. (2018) Attention sports fans! The far-reaching contributions of bud sport mutants to horticulture and plant biology. Hort. Res., 5: 44.
26. Dandin S. B. and Giridhar K. (2014) *Hand book of Sericulture Technologies*. Central Silk Board, Bangalore, India.
27. Dandin SB, Rajan MV, Mallikarjunappa RS (1996) Mutant forms in mulberry (Morus spp.). Sericologia 36, 353–358.
28. Murthy V. N. Y, Ramesh H. L. and Munirajappa. Ethyl Methane Sulphonate induced morphological variations in mulberry (Morus) variety M5. Journal of Applied and Natural Science 3 (1): 114–118 (2011).
29. Ramesh H L, Murthy V N Y, Munirajappa, "Gamma Ray Induced Radio Sensitivity in Three Different Mulberry (Morus) Genotypes"American Journal of Plant Sciences, Vol.4 No.7, 2013.
30. Tikader, A., Thangavelu, K. and Rao, A. A., 2004. Characterization and evaluation of mulberry (Morus spp.) germplasm. Indian J. Seric., 43(1):106–110.
31. Peris N. W., Gacheri K. M., Theophillus M. M. and Lucas N. (2014) Morphological characterization of mulberry (Morus spp.) accessions grown in Kenya. Sustain. Agric. Res., 3:10–17.
32. Sujathamma, P. and Dandin,S.B. 2000. Leaf quality evaluation of mulberry (Morus spp.) genotypes through chemical analysis. Indian J. Seric., 39(2): 117–121.
33. M S, Rukmangada & MG, Sabitha & Sivaprasad, Vankadara & Naik, Vorkady. (2016). Genetic variation in photosynthetic parameters and SPAD chlorophyll content among a diverse set of mulberry (Morus spp.) germplasm. 56(3). 139–148.
34. Saratchandra B., Vijayan K., Srivastava P. P., Jayaram Raju P., Mahanta J. C. and Giridhar K. (2011) New mulberry varieties authorized for cultivation. Indian Silk, 2: 14–15.
35. Susheelamma BN and MS Jolly (1986) Evaluation of morpho-physiological parameters associated with drought resistance in mulberry. Indian J. Seric. 25: 6–14.
36. Kidner C A and Umbreen S. (2010).Why is Leaf Shape so Variable?. International Journal of Plant Developmental Biology. 4 (Special Issue 1), 64–75.
37. Kumara R. R., Ramesh H. L. and Manjunatha H. B. (2021) Morpho-biometric and cytogenetic analysis of clonally evolved mulberry cultivars (Morus Spp.). Acta. Sci. Agric., 5: 92–101.
38. Schneider S, Ziegler C, Melzer A. Growth towards light as an adaptation to high light conditions in Chara branches. New Phytol. 2006;172(1):83–91.
39. Mohamed A. Hussein1, Emad Eldin H. Abd-Elall1, Esraa, M.E. Hussein1 And Fayza W. Sbralla. (2021). Studies on vegetative propagation of mulberry (*Morus* sp.) by hardwood cutting (178–198) Journal of Scientific Development for Studies and Research (JSD), Volume 2, Issue 5, 2021.
40. Petkov Z. (2007) Morphological characteristics of the shoots and buds from local Bulgarian mulberry varieties. Proceedings of International Conference on “Sericulture Challenges in the 21st Century” (Serichal 2007) & the 3rd BACSA meeting,18–21 September 2007, Vratza, Bulgaria, p. 110.Plummer, D.T. (1971) Introduction to practical biochemistry.McGraw Hill Co. Ltd, UK.
41. Vijayan, K., Das, K. K., Chakraborti, S. P., and Roy, B. N. (1998). Heterosis for yield and related characters in mulberry. Indian Journal of Genetics and Plant Breeding, 58(3), 369–374.
42. Kumara R. R. and Ramesh H. L. (2022) Genetic variation, heritability, correlation and path analysis for leaf yield traits in clonally selected mulberry genotypes (*Morus* spp.). J. plant dev. sci., 14:587–591.
43. Vijayan, K., Ravikumar, G., Tikader, A. (2018). Mulberry (*Morus* spp.) Breeding for Higher Fruit Production. In: Al-Khayri, J., Jain, S., Johnson, D. (eds) Advances in Plant Breeding Strategies: Fruits. Springer, Cham. https://doi.org/10.1007/978-3-319-91944-7_3
44. Laltanmawii and Roychowdhuri S. Effects of chromosomal variations on morphology and leaf anatomical behaviours in mulberry (*Morus* sp.). Journal of Crop and Weed 6(2)35–39: (2010).
45. Rao A A, A Tikader, Ravindran, V Girish Naik and P Mukherjee. Leaf Morpho-Anatomical Variability in Mulberry (Morus spp.) Germplasm. Indian Journal of Plant Genetic Resources, Vol. i6, No.1 (2003).
46. Ninge Gowda KN, Sudhakar (2002) Studies on the leaf moisture of some exotic mulberry varieties. In: Advances in Sericulture Research, Proceedings (National Conference on Strategies for Sericulture Research and Development, 16–18, November, 2000). India: Central Sericultural Research and Training Institute, Central Silk Board, Ministry of Textiles, Government of India.

47. Susheelamma, K., & Jolly, M. S. (1986). Evaluation of morpho-physiological parameters associated with drought resistance in mulberry, 25, 06–14.
48. Kesavacharyulu K., Kumar V. and Sarkar A. (2004) Scanning electron microscopic studies on leaf surface trichomes in mulberry and its influence on rearing performance of silkworm, Bombyx mori L. Int. J. Ind. Entomol., 8: 33–41.
49. Karakurt H. Aslantaş R. (2008) The Formation and Changing Physiology of Plant Colour Pigments, Alatarım. 7 (2); 34–41.
50. Bongale, U. D. and Chaluvachari (1995) Evaluation of eight mulberry germplasm varieties by leaf biochemical and bioassay moulting studies. Sericologia, 35(1): 83–94.
51. Susheelamma BN, Geethadevi RG, Jolly Jalaja MS, et al. Effect of different mulberry varieties on silkworm, Bombyx mori (L.). Insect Science and Its Application. 1989;10(3):359–363.
52. Kumar H, Priya Y S, Kumar M and Elangovan V, 2013. Effect of Different Mulberry Varieties and Seasons on Growth and Economic Traits of Bivoltine Silkworm (Bombyx mori). Journal of Entomology, 10: 147–155.
53. Yogan V, Narayanappaa M, Ramesh H L, Lokesh G, Muni Rajappa, Yadav BRD Assessment of six Mulberry (Morus) Germplasm varieties through Moulting and Bioassay parameters by using Crossbreed Silkworms Bombyx mori L. for Commercial Exploitation in Kolar District, Karnataka, India. Int. Res. J. Biological Sci., Volume 2, Issue (9), Pages 69–75, September,10 (2013).
54. Alipanah, Masoud, Abedian, Zabihollah, Nasiri, Abdolazim, Sarjamei, Farid, Nutritional Effects of Three Mulberry Varieties on Silkworms in Torbat Heydarieh, Psyche: A Journal of Entomology, 2020, 6483427, 4 pages, 2020. https://doi.org/10.1155/2020/6483427
55. Paul, D. C., Subba Rao, G. and Deb, D. C (1992) Impact of dietary moisture on nutritional indices and growth of Bombyx mori and concomitant larval duration. J. Insect. Physiol., 38, 229–235.
56. Rahmathulla V. K., Himantharaj M.T., SRINIVASA G., RAJAN R.K. Association of moisture content in mulberry leaf with nutritional parameters of bivoltine silkworm (*Bombyx mori L.*).. Acta Entomologica Sinica, Acta Entomologica Sinica, 2004, 47(6): 701–704
57. Rahmathulla V.K., R. Tilak and R. K, Rajan. Influence of Moisture Content of Mulberry Leaf on Growth and Silk Production in Bombyx mori L. Caspian J. Env. Sci. 2006, Vol. 4 No.1 pp. 25–30.
58. Horie Y. (1978) Quantitative requirements for growth of the silkworm *Bombyx mori* L. JARQ, 12:211–217.
59. Hiratsuka E (1917) Researches on the nutrition of the silkworm. Shanghi Shikenjo Hokoku Tech. Bull., 2: 353–412.
60. Machii, H. and Katagiri, K. 1991. Varietal differences in nutritive values of mulberry leaves for rearing silkworms. JARQ, 25: 202–208.
61. Breto M. P., Ruiz C., Pina J. A. and Asins M. J. (2001) The diversification of *Citrus clementina* Hort. ex Tan., a vegetatively propagated crop species. Mol. Phylogenet. Evol., 21: 285–293.
62. Ortega-Ortega J, Ramirez-Ortega F, Ruiz-Medrano R, Xoconostle Cazares B (2019) Analysis of genome size of sixteen *Coffea arabica* cultivars using flow cytometry. Hort Sci 54, 998–1004.
63. Li SF, Su T, Cheng GQ, Wang BX, Li X, Deng CL, Gao WJ. Chromosome Evolution in Connection with Repetitive Sequences and Epigenetics in Plants. Genes (Basel). 2017;8(10):290.
64. Eichler E.E., Sankoff D. Structural dynamics of eukaryotic chromosome evolution. Science. 2003;301:793–797.
65. Dobigny G., Britton-Davidian J., Robinson T.J. Chromosomal polymorphism in mammals: An evolutionary perspective. Biol. Rev. 2017;92:1–21.
66. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DP, et al. (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33, 2233–2239.

Figures

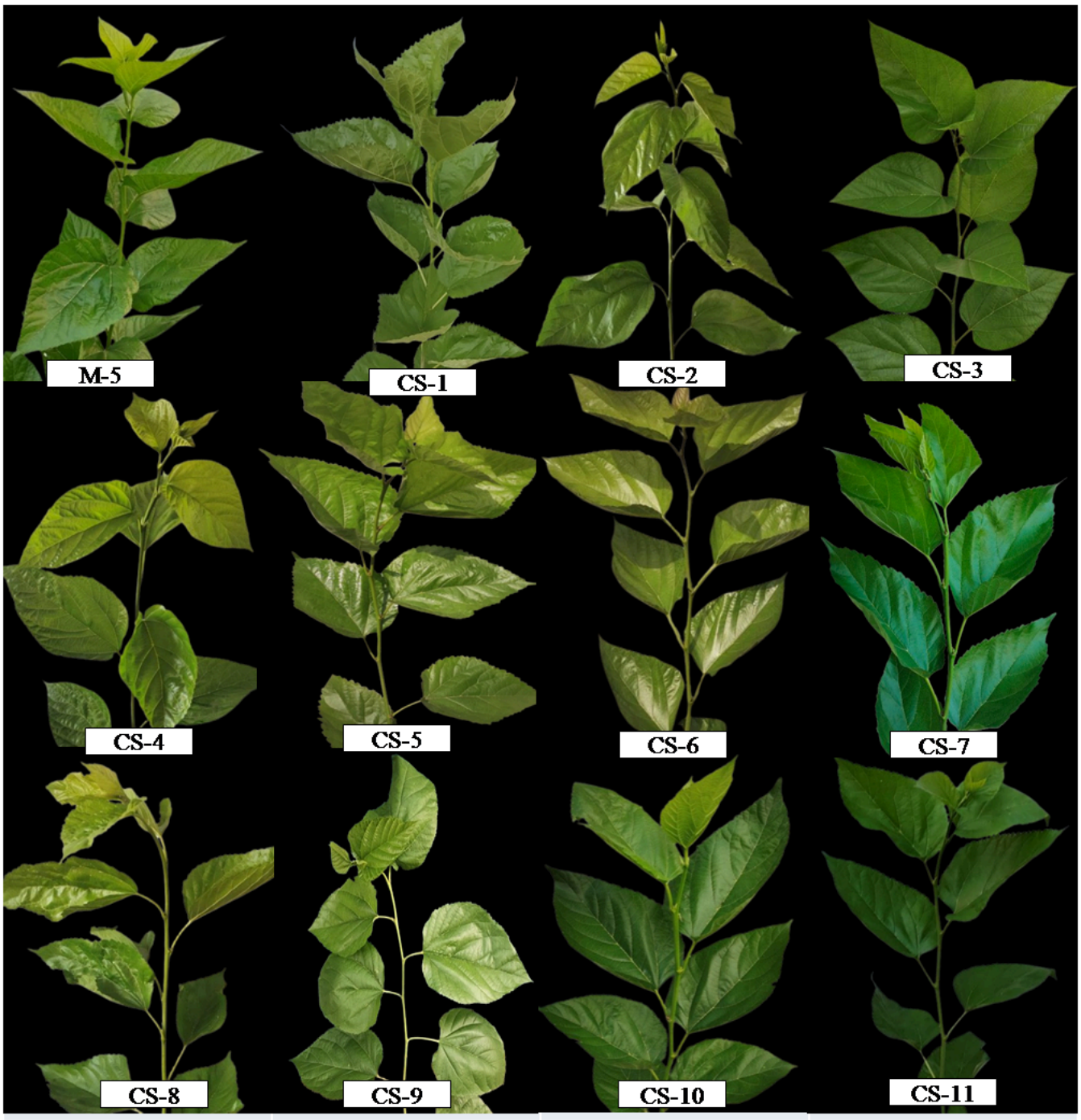

Figure 1

Morphological view of clonally selected mulberry genotypes and their mother plant, K-2

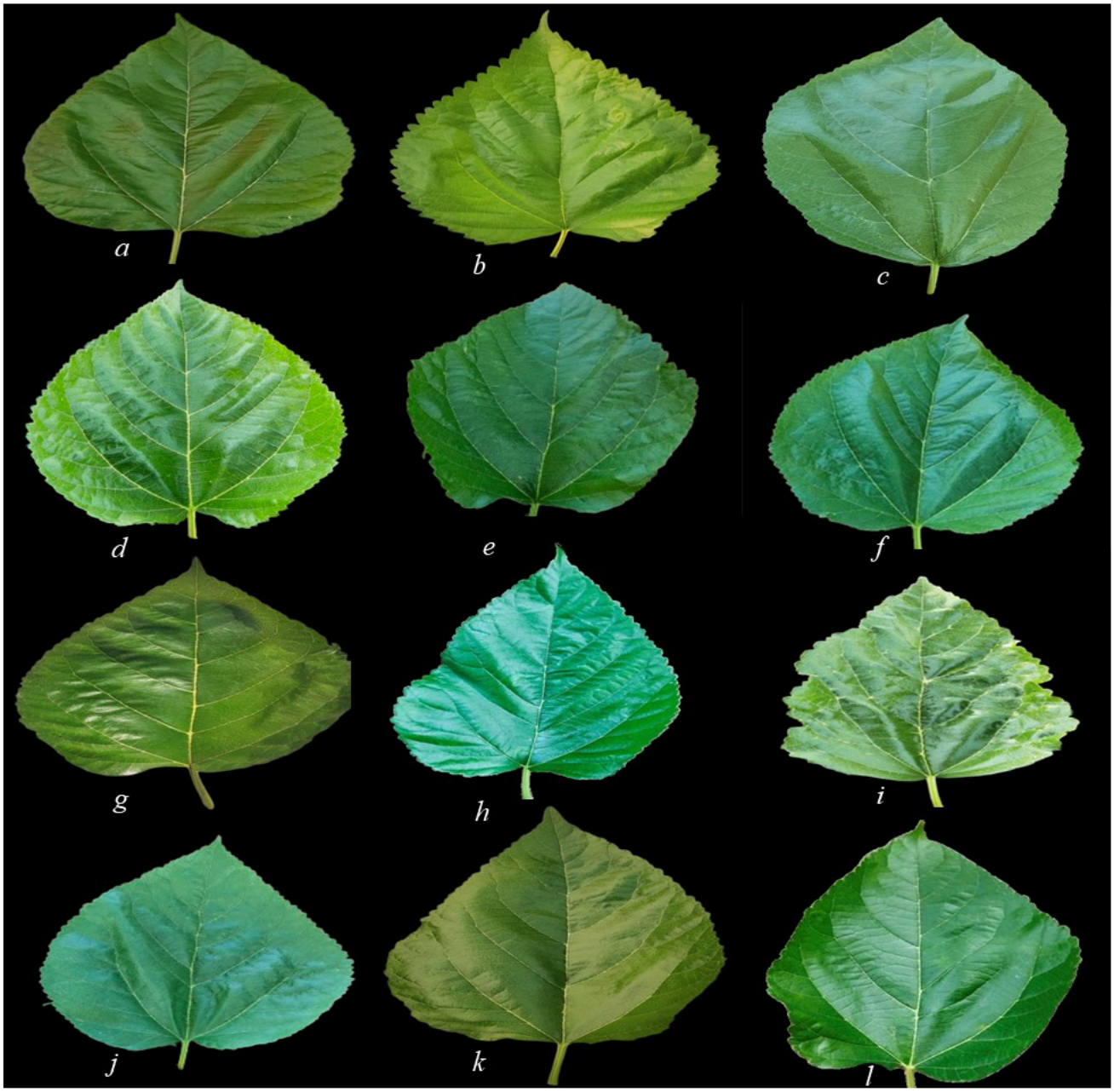

Figure 2

Leaf morphological view of mother plant K-2 (a) and its clonally derived genotypes *viz.,* CS-1 (b), CS-2 (c), CS-3 (d), CS-4 (e), CS-5 (f), CS-6 (g), CS-7 (h), CS-8 (i), CS-9 (j), CS-10 (k), and CS-11 (l)

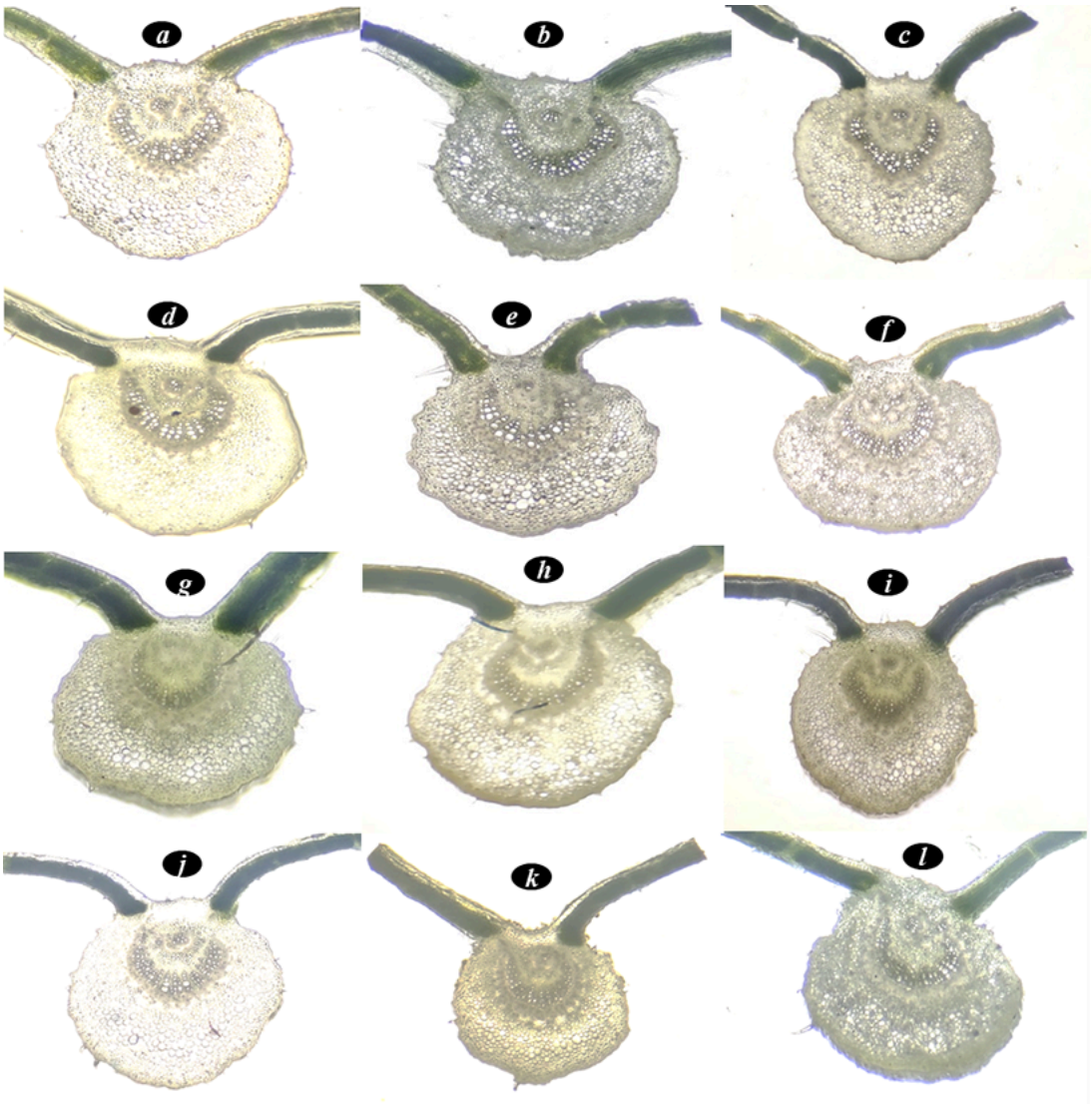

Figure 3

Leaf anatomical view (50 µm) of mother plant K-2 (a) and its clonally derived genotypes *viz.,* CS-1 (b), CS-2 (c), CS-3 (d), CS-4 (e), CS-5 (f), CS-6 (g), CS-7 (h), CS-8 (i), CS-9 (j), CS-10 (k), and CS-11 (l)

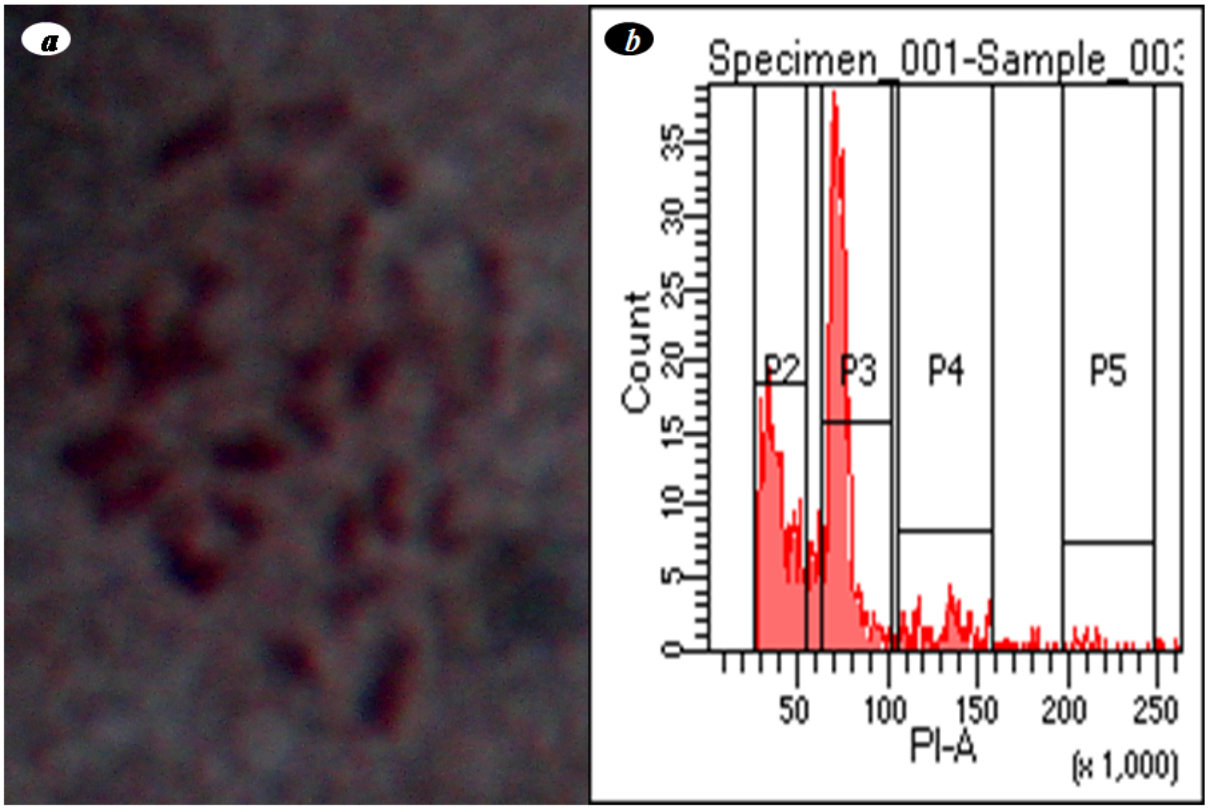

Figure 4

Somatic chromosomes of K-2 (a); Representative histogram of the flow cytometry analysis in K-2 (b)

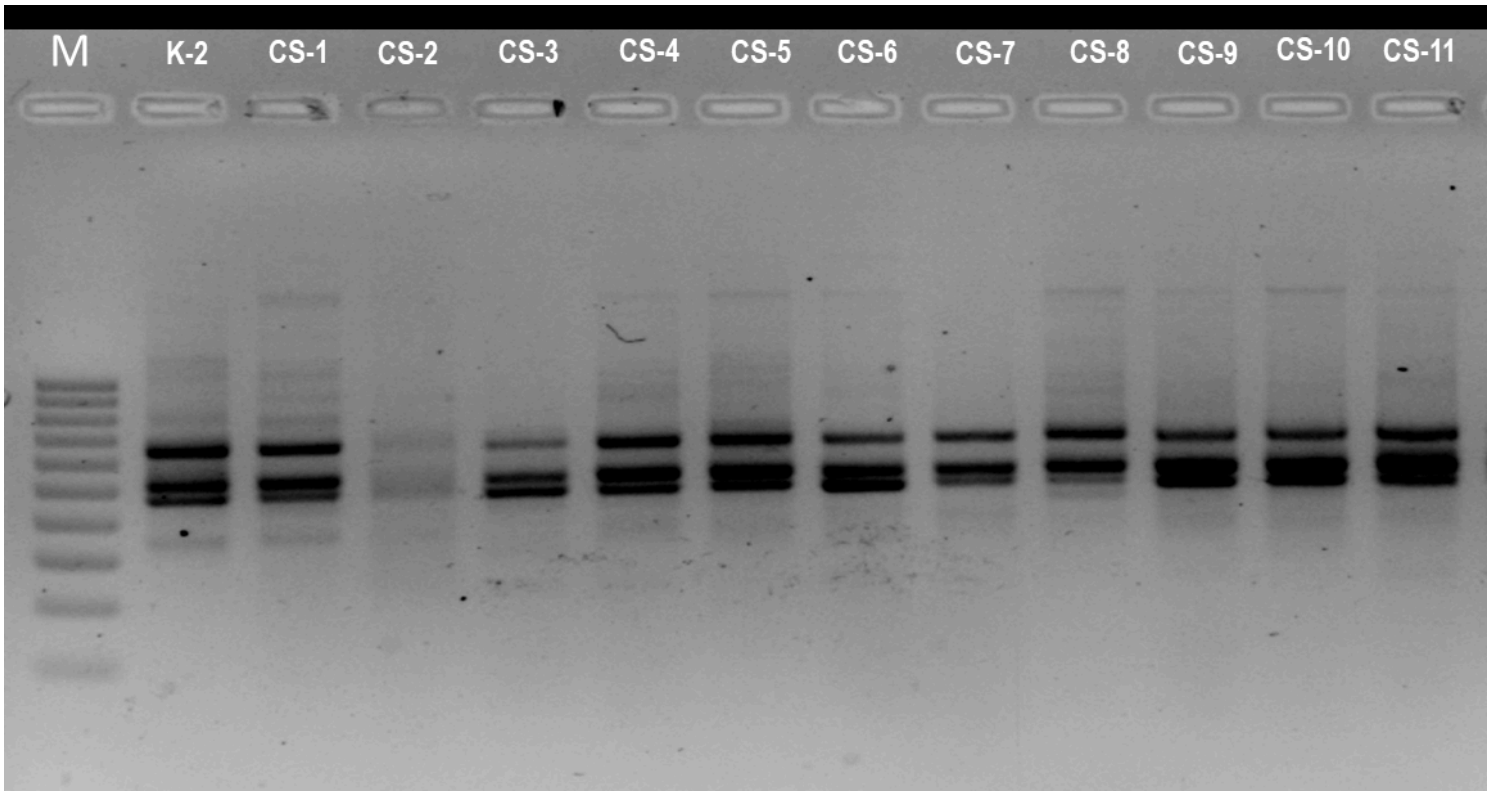

Figure 5

ISSR banding patterns between the mother plant, K-2 and its clonal variants

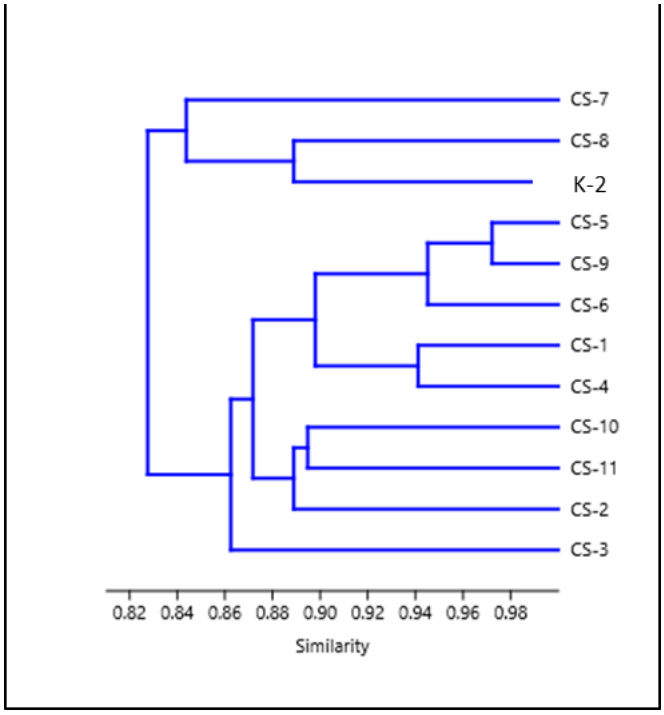

Figure 6

UPGMA Dendrogram depicting relationships among 12 genotypes