Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 5 Issue 6 June 2021

Research Article

Morpho-biometric and Cytogenetic analysis of Clonally Evolved Mulberry Cultivars (*Morus* Spp.)

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Received: May 03, 2021

Published: May 26, 2021

Abstract

Genetic variation is uncommon in mulberry (*Morus* spp.) cultivars due to its asexual propagation and shows the considerable phenotypic plasticity to adopt different agro-climatic conditions. These open an ample scope for the selection of superior clones for better leaf yield and quality. To uncover this cryptic feature, we have selected clonally evolved mulberry cultivars TG-1, Anantha, and Vishala and compared them with their putative mother plants M-5, RFS-135, and S-1635 respectively. Interestingly, significant variations are obvious in all clonally evolved mulberry cultivars with their respective putative mother plants. Comparatively, the plant height, the number of branches, leaf yield, and survival percentage were higher in TG-1, Anantha, and Vishala over M-5, RFS-135, and S-1635 cultivars respectively. Furthermore, altered anatomical structures - thick cuticle, fewer and smaller stomata in TG-1, Anantha, and Vishala leaves are positively correlated with moisture conservation and drought adaptation. Besides, clonally evolved mulberry cultivars had significantly higher leaf moisture, soluble protein, and sugar contents. The cytological evidence shows that RFS-135 and Anantha have 2n=2x=28 (Diploids), M-5 and TG-1 also exhibit 2n=2x=28; but Vishala and S-1635 possess 2n=3x=42 (Triploids). We hypothesize that discrete morphological, anatomical, and biochemical variations in clonally evolved mulberry cultivars compared to their putative mother plants are due to no gain or loss in chromosomal complements, rather to repetitive DNA sequence or related epigenetic modifications.

Keywords: Mulberry; Chromosome; Clonal Selection; TG-1; Anantha; Vishala

Introduction

The mulberry plant constitutes an exclusive food plant of the silkworm, *Bombyx mori* L. by having unique secondary metabolites like morin, β-sitosterol, etc. Chiefly, most of the mulberry cultivars are diploid in nature with 2n=2x=28, but few have 2n=3x (42), 4x (56), 6x (84), 8x (112), and Decosaploid with 22x=308 chromosomes. The optimum level of ploidy considered from a biological standpoint is triploidy, but as the ploidy level increases, the leaf surface becomes rougher, making the foliage unsuitable for silkworm rearing [1]. Moreover, a vast majority of asexually propagated *Morus* species are diploids, perennials, cross-pollinated, highly heterozygous, and display severe inbreeding depression. Contrastingly, sexual propagation through seeds seldom shows true-to-typeness due to its heterozygous nature. As a result, mulberry is highly amenable to vegetative propagation through stem cuttings followed by periodic pruning/training practices to facilitate leaf plucking and branch feeding for silkworm rearing.

Over the period, vegetatively propagated plant varieties exhibit significant genotypic and phenotypic variability that is induced due to somatic mutation caused by various factors like, point mutation, somatic recombination, retrotransposon activity, and virus infection. In addition, repeated pruning also results in genetic and morphological variations, while significant genetic variability emerges in the clonal population of vegetatively propagated crops [2-5]. So, marked variations in clonally-selected varieties over their parents for morphological, anatomical, and cytological traits are due to natural somatic bud mutation or epigenetic effect.

Variability for the desirable characters is heritable in nature in plants [6]. Many cultivars selected based on superior clones and natural variability are potato [7], sugarcane [8], mango [9], banana [10], which show resistance against biotic as well as abiotic stresses with enhanced crop yield and quality. Similarly, many mulberry cultivars, *viz.,* Chinese white, Mandalaya (S-1), Vishwa (DD), Vishala, TG-1 (Talaghattapura-1), and Anantha with promising higher leaf yield reported from different geographical regions of India have evolved through clonal selection [11,12]. Interestingly, clonally-selected varieties TG-1, Vishala, and Anantha exhibit morphological changes as well as varying leaf-bearing capacity when grown in seven test centers in India's Eastern and North-eastern zones [13]. Thus, clonally-selected varieties are comparatively productive because they are stable, ideal for breeding experiments, and easy to maintain in the field. Considering the significance of mulberry varieties in the rearing of silkworms, and due to the paucity of information except for morphological and yield parameters, the present investigation has been undertaken to uncover chromosomal variations if any in comparison with that of morphological, anatomical, and biochemical changes in the clonally evolved cultivars and their putative mother plants.

Materials and Methods

Three clonally evolved genotypes *viz.,* Talaghattapura-1 (TG-1), Anantha, and Vishala were selected for a comparative analysis along with their mother plants M-5 (Kanva-2), RFS-135, and S-1635 respectively. TG-1 is recommended for rain-fed (Malnad region and Southern red soils) sericulture areas in Karnataka and yields 35-40 MT/ha/year [11]. The cultivar Anantha is recommended for semi-arid conditions of South India and the leaf yield potential is 65 - 70 MT/ha/year [14]. Vishala is recommended for assured irrigation conditions of South India and the leaf yield potential is 60 - 65 MT/ha/year [15]. The M-5 (Kanva-2) variety has been cultivated both under irrigated as well as rain-fed conditions in the Southern states of India and yields 30-35 MT/ha/year. The cultivar RFS-135 is a popular cultivar for the rain-fed conditions in India and leaf yield is 10-12 MT/ha/year. The cultivar S-1635 is a popular cultivar in India's Eastern and Northern states and the leaf yield is 40 - 45 MT/ha/year in irrigated conditions [11].

Morphological, growth, and yield parameters were analyzed as per set descriptors [16]. Leaf anatomy and stomatal studies were carried out following the method described by Mallikarjunappa., *et al.* [17]. Trichomes were observed by staining the decolorized leaf segment with 1% Methylene blue for one minute. Estimation of soluble protein [18], total soluble sugar [19], leaf moisture content, and retention capacity [20] was performed using tender, medium and coarse leaves separately following respective standard protocols. For chromosome studies, mulberry root tips were treated with colchicine for 3 hrs followed by hydrolyzation in 1N HCl for 10 minutes at 40°C. The resultant root tips were stained with 2% aceto-orcine and squashed using 45% acetic acid [21]. The chromosomes were screened under a microscope and photomicrographed. Chromosome length was estimated by using IdeoKar software [22]. The nuclear DNA (2C) amount was estimated by flow cytometry from the relative fluorescence strength of sample peaks and internal standards as previously described by Yamanouchi., *et al* [23]. Data were analyzed by adopting standard deviation (±) and mean values were expressed.

Results

Discrete variations between clonally evolved mulberry cultivars and their respective putative mother plants with respect to phenotypic traits and nuclear DNA (2C) contents recorded are elucidated.

Morphological characteristics

Morphological characterization is in use as a tool to evaluate the possible genetic relationships between different varieties in plant improvement. Thus, we have used this yardstick to assess the clonally-derived mulberry cultivars and their respective putative mother plants. Accordingly, semi-erect nature and slightly curved branches, with greenish-purple in the young and purplish-brown color on the mature shoot were noticed in clonally-derived TG-1 (Figure 1B), whereas its mother plant M-5 has semi-erect and

straight branching nature, with green in the young and greyish-green color in the mature shoot (Figure 1A). TG-1 has dark green, smooth leaf surface with serrate margin and truncate leaf base (Figure 1C), whereas M-5 has light green, slightly rough surface with serrate margin and truncate leaf base. Both cultivars exhibited free-lateral and caducous stipules. Interestingly, both the cultivars bear only female flowers, and the color of the ripened fruit was black in M-5 and red in TG-1 respectively (Table 1).

Cultivar Anantha exhibits luxuriant growth with erect and straight branches having green and greyish-brown color in young and mature shoots respectively (Figure 1E). Both cultivars exhibited free-lateral and caducous stipules. The RFS-135 has medium growth with semi-erect and slightly curved branches. The young and mature shoots were green and greyish-brown in color (Figure 1D). Anantha leaves are unlobed, light green, with slightly rough surface, crenate margin, and wide ovate leaf base, whereas RFS-135 leaves are green with a slightly rough and serrate leaf margin and cordate leaf base (Figure 1F). Interestingly, changes in sex expression were observed with male flowers alone in Anantha throughout all seasons, whereas RFS-135 bears the only females, with a pubescent, spreading stigma and black-colored fruit.

Vishala also exhibits luxuriant growth with spreading, and slightly curved branches coupled with dark green and greyish-brown color of young and mature shoots (Figure 1H). The S-1635 has shown vigorous growth with semi-erect and straight branches, while the color of the young shoot was green and yellow-green on the mature shoot (Figure 1G). In Vishala, the stipule was foliaceous and caducous, whereas, in S-1635, it was free-lateral and caducous. S-1635 has a green, slightly rough surface, dentate leaf margin, and cordate leaf base with wide ovate leaf shape, whereas Vishala has unlobed, dark-green, rough surface, crenate margin leaf with broadly ovate leaf shape (Figure 1I). Discrete changes in sex expression were also observed in Vishala and S-1635 bearing only female and male flowers respectively.

Figure 1: Clonally evolved cultivars and their putative mother mulberry plants. A) M-5 variety; B) TG-1 variety; C) Leaf of M-5 and TG-1; D) RFS-135 variety; E) Anantha variety; F) Leaf of RFS-135 and Anantha; G) S-1635 variety; H) Vishala variety; I) Leaf of S-1635 and Vishala variety.

Growth and yield parameters

Leaf yield in mulberry is a complex trait, highly influenced by not only the environment but also depends on the plant height, number of branches, leaf area, weight, and water content. As a consequence, the parameter varies among clonally-derived mulberry cultivars and their mother plants. So, the number of branches per plant recorded was 30%, shoot length 14.09%, leaf area length 6.50% and width 17.83%, leaf yield 16.20%, rooting percentage 12.5%, and the number of roots 50% increased in TG-1 than its mother plant M-5. Besides, it has short intermodal length (-31.09%) and petiole length (-3.97%); lower number of flowers in a catkin (-50%), inflorescence length (-40%), inflorescence width (-37.5%), fruit length (-36.06%) and weight (-30.48%) compared to M-5 (Table 2). In Anantha, the number of branches per plant (45.33%), shoot length (23.43%), length (49.02%), and width (43.03%) of leaf area were higher than the RFS-135 cultivar. While the internode (-20.82%) and petiole length (-18.52%) in the Anantha cultivar were lower than in its parent, the leaf yield (88.23%), rooting percentage (11.76%), and the total number of roots per plant (30.15%) were higher. Even in Vishala, ~16.66% higher number of branches per plant, 18.87% in shoot length, 12.46% in leaf length, and 4.69% width of leaf than the S-1635 cultivar. Furthermore, while internode length was reduced by -31.42% and petiole length was reduced by -4.36%, the Vishal cultivar had a higher leaf yield of 33.33%, rooting of 22.66%, and the total number of roots per plant of 23.80% than its parental plant.

Leaf anatomical characters

Leaf anatomical characters were also found to vary between clonally-derived and their mother plants (Table 3). Interestingly,

Qualitative Traits	M-5	TG-1	V (1/0)	RFS –135	Anantha	V (1/0)	S-1635	Vishala	V (1/0)
Plant vigor	Medium	Medium	0	Medium	High	1	High	Very High	1
Growth nature	Semi-Erect	Semi-Erect	0	Semi-Erect	Erect	1	Semi-Erect	Spreading	1
Branch nature	Straight	Slightly Curved	1	Slightly Curved	Straight	1	Straight	Slightly Curved	1
Color of young shoot	Green	Greenish Purple	1	Green	Dark Green	1	Green	Dark Green	1
Color of mature shoot	Greyish Green	Purplish Brown	1	Greyish Brown	Greyish Brown	0	Yellow Green	Greyish Green	1
Stipule nature	Free-lateral	Free-lateral	0	Free-lateral	Free-lateral	0	Free-lateral	Foliaceious	1
Stipule duration	Caducous	Caducous	0	Caducous	Caducous	0	Caducous	Caducous	0
Lobation type	Unlobed	Unlobed	0	Unlobed	Unlobed	0	Unlobed	Unlobed	0
Leaf color	Light Green	Dark Green	1	Green	Light Green	1	Green	Dark Green	1
Leaf nature	Homo phyllous	Homo phyllous	0	Homo phyllous	Homo Phyllous	0	Homo phyllous	Homo phyllous	0
Leaf surface	Slightly Rough	Smooth	1	Slightly Rough	Smooth	1	Slightly Rough	Rough	1
Leaf texture	Charata cious	Charata cious	0	Coriaceous	Charata Cious	1	Coriaceous	Coriaceous	0
Leaf apex	Acuminate	Acuminate	0	Acuminate	Acuminate	0	Acuminate	Acuminate	0
Leaf margin	Serrate	Serrate	0	Serrate	Crenate	1	Dentate	Crenate	1
Leaf base	Truncate	Truncate	0	Cordate	Cordate	0	Cordate	Cordate	0
Leaf shape	Ovate	Narrow Ovate	1	Wide Ovate	Ovate	1	Wide Ovate	Broadly Ovate	1
Leaf hairiness	Glabrous	Glabrous	0	Glabrous	Sparsely Hairy	1	Sparsely Hairy	Hairy	1
Sex	Female	Female	0	Female	Male	1	Male	Female	1
Stigma nature	Pubescent	Pubescent	0	Pubescent	NA	-	NA	Pubescent	-
Stigma type	Spreading	Spreading	0	Spreading	NA	-	NA	Spreading	-
Fruit color	Black	Red	1	Red	NA	-	NA	Red	-
* Significant morphological variations indicated as Present (1)/Absent (0)									

Table 1: Morphological characteristics of clonally evolved mulberry cultivars and their mother plants.

Qualitative Traits (Mean values)	M-5	TG-1	V (%)	RFS -135	Anantha	V (%)	S-1635	Vishala	V (%)
No. of branches/ plant	10.00 ± 1.23	13.00 ± 2.34	30	12.00 ± 0.21	17.44 ± 2.44	45.33	18.00 ± 4.12	21.00 ± 2.74	16.66
Shoot length (cm)	512.04 ± 1.11	584.21 ± 0.27	14.09	521.01 ± 4.21	643.12 ± 1.51	23.43	721.11 ± 0.71	857.19 ± 1.08	18.87
Inter-nodal length (cm)	6.11 ± 1.41	4.21 ± 4.21	-31.09	6.34 ± 0.00	5.02 ± 3.41	-20.82	7.32 ± 0.11	5.02 ± 2.43	-31.42
Petiole length (cm)	3.02 ± 2.11	2.90 ± 2.14	-3.97	3.94 ± 1.32	3.21 ± 2.41	-18.52	4.12 ± 1.41	3.94 ± 1.83	-4.36
Leaf length(cm)	17.84 ± 3.26	19.00 ± 4.17	6.50	15.34 ± 2.14	22.86 ± 4.21	49.02	35.22 ± 0.00	39.61 ± 1.52	12.46
Leaf width (cm)	14.30 ± 2.24	16.85 ± 3.41	17.83	13.64 ± 0.41	19.51 ± 1.34	43.03	31.08 ± 1.64	32.54 ± 2.47	4.69
Leaf area /cm^2	139.25 ± 2.41	151.47 ± 3.74	8.77	121.44 ± 1.24	193.67 ± 1.74	59.47	304.29 ± 4.27	396.24 ± 0.43	30.21
Fresh weight of 100 leaves (g)	394.21 ± 2.18	420.05 ± 2.43	6.55	296.31 ± 1.94	562.23 ± 0.21	89.74	600.32 ± 7.25	842.81 ± 0.47	40.39
Leaf yield /plant (Kg)	2.53 ± 2.41	2.94 ± 3.14	16.20	2.04 ± 0.47	3.84 ± 1.24	88.23	3.96 ± 2.05	5.28 ± 1.21	33.33
Number of roots/ plant	12.32 ± 1.54	18.14 ± 1.47	50.00	17.31 ± 2.47	22.53 ± 0.41	30.15	21.07 ± 0.21	26.16 ± 1.24	23.80
Rooting (%)	>80	>90	12.5	>85	>95	11.76	>75	>92	22.66
No. of flowers in a catkin	46.08 ± 2.14	23.11 ± 2.43	-50	28.34 ± 0.14	NA	-	NA	36.24 ± 1.24	-
Inflorescence length (cm)	1.5 ± 3.12	0.9 ± 2.32	-40	2.14 ± 1.42	NA	-	NA	2.00 ± 3.21	-
Inflorescence width (cm)	0.8 ± 2.14	0.5 ± 3.24	-37.5	1.22 ± 0.00	NA	-	NA	1.5 ± 2.24	-
Fruit length (cm)	2.44 ± 0.14	1.56 ± 3.22	-36.06	2.91 ± 3.31	NA	-	NA	3.01 ± 2.14	-
Fruit weight (g)	1.64 ± 2.17	1.14 ± 1.28	-30.48	2.65 ± 2.43	NA	-	NA	3.12 ± 1.43	-

Table 2: Growth and yield parameters of clonally evolved cultivars and their putative mother mulberry plants.

the thickness of the leaf (10.13%), cuticle (37.08%), palisade parenchyma (8.23%), and spongy parenchyma cells (20.39%) were remarkably higher in TG-1 than its mother plant M-5. On the other hand, a decrease in the frequency of stomata (-35.81%), stomata size (-30.86%), and the number of trichomes (-48.36%) was observed in TG-1 compared to its mother plant cultivar. The Anantha cultivar produced more trichomes (103.66%) than its mother plant, RFS-135. Furthermore, while the leaf thickness (43.32%), cuticle (19.36%), palisade parenchyma (36.15%), and spongy parenchyma (72.16%) increased, the number (-33.51%) and size (-7.41%) of stomata were declined compared to its mother plant RFS-135. The clonally-selected Vishala also showed higher leaf (10.87%), cuticle (21.21%), palisade parenchyma (30.35%), and spongy parenchyma (23.03%) thickness and less number (-20.73%) and size (-24.03%) of stomata, and trichomes (-16.06%) compared to its mother plant S-1635.

Parameters	M-5	TG-1	V (%)	RFS-135	Anantha	V (%)	S-1635	Vishala	V (%)
Leaf thickness (µm)	180.54 ± 1.23	198.25 ± 2.31	10.13	152.22 ± 1.14	218.17 ± 2.31	43.32	285.41 ± 1.02	316.45 ± 1.22	10.87
Cuticular thickness (µm)	7.01 ± 2.54	9.61 ± 3.08	37.08	12.24 ± 2.41	14.61 ± 3.00	19.36	10.51 ± 4.11	12.74 ± 2.42	21.21
Thickness of palisade parenchyma(µm)	87.21 ± 4.23	94.39 ± 2.14	8.23	75.14 ± 2.11	102.31 ± 1.34	36.15	121.51 ± 5.31	158.39 ± 2.65	30.35
Thickness of spongy parenchyma (µm)	65.32 ± 3.24	78.64 ± 8.41	20.39	53.21 ± 4.21	91.61 ± 0.00	72.16	98.11 ± 3.21	120.71 ± 3.44	23.03
Stomatal frequency (per mm^2 area)	327.54 ± 1.32	210.23 ± 2.42	-35.81	356.84 ± 3.25	237.23 ± 2.14	-33.51	677.84 ± 3.25	537.32 ± 5.41	-20.73
Stomatal size (µm)	306.24 ± 3.24	211.71 ± 4.21	-30.86	312.36 ± 1.00	289.21 ± 1.11	-7.41	512.36 ± 1.00	389.21 ± 1.11	-24.03
Number of chloroplast/ stomata	28.32 ± 3.12	34.84 ± 4.12	23.02	14.32 ± 3.12	19.84 ± 3.12	38.54	10.26 ± 2.30	18.54 ± 1.21	80.70
Number of trichomes (per mm^2 area)	52.31 ± 3.41	27.01 ± 1.22	-48.36	36.05 ± 4.12	73.42 ± 2.48	103.66	91.05 ± 4.12	76.42 ± 2.48	-16.06

Table 3: Leaf anatomical parameters of clonally evolved cultivars and their putative mother mulberry plants.

Leaf biochemical contents

A comparison of clonally-derived mulberry plants with their putative mother plants revealed significant differences in biochemical components. The leaf moisture plays a vital role in improving the quality and palatability of mulberry leaf for silkworms by favoring the ingestion, digestion, and assimilation of nutrients. Accordingly, water content and retention capacity for six hours were found higher in the TG-1 variety, measuring 72.35% and 54.17% as against 67.89% and 49.13% respectively in the mother plant M-5. Whereas, both the traits were also found higher in the clonally-selected variety Anantha (water content 74.34%; water retention capacity-53.54%) compared to the RFS-135 cultivar. Even in the case of Vishala, a clonally-selected variety, the leaf water content and water retention capacity were 71.34% and 49.54%, which is higher than the S-1635 cultivar (Figure 2).

As the protein in the leaves of mulberry is the major source for bio-synthesis of silk protein in the silk gland of the silkworm, we have estimated the protein content available in the leaves of clonally-selected mulberry varieties and its mother plant. Interestingly, the quantity of soluble protein content in the mulberry leaf was significantly elevated in clonally-derived

cultivars *viz.,* TG-1 (25.06%), Anantha (24.44%), and Vishala (28.44%) compared to its mother plants *viz.,* M-5 (23.88%), RFS-135 (23.10%) and S-1635 (26.77%).

Concomitantly, as silkworms require a sufficient supply of carbohydrates as an energy source that resulted in good cocoon yield carbohydrate content in mulberry leaves was estimated. As a result, higher total sugar contents were recorded in TG-1 (12.60%), Anantha (14.23%), and Vishala (15.23%) over its mother plants *viz.,* M-5 (11.62%), RFS-135 (13.61%), and S-1635 (13.54%) respectively.

Figure 2: Biochemical analysis of leaf from clonally evolved cultivars and their putative mother mulberry plants.

Cytogenetic analysis

Notably, both M-5 and TG-1 have 2n=28 (Figure A and B), but total chromosome length and nuclear DNA (2C) content differ, with TG-1 having a higher value (3.32µm and 0.476 pg) than M-5 (3.21µm and 0.403 pg). So as in Anantha and RFS-135 (2n=28, Figure D and C) with 3.26µm and 0.432 pg and 3.13µm and 0.414 pg respectively. Despite the fact that both the S-1635 and Vishala cultivars had 2n=3x=42 chromosomes (Figure E and F), the Vishala (3.28µm and 0.692 pg) had a longer total chromosome length and DNA content than the S-1635 (3.19µm and 0.653 pg). However, interestingly, no gain or loss in the chromosomal complement of clonally-derived mulberry cultivars when observed, but it is obvious that chromosome length and DNA contents were higher compared to their respective mother plants.

Varieties	Chromosome Number	Mean length of Total Chromosomes (µm)	Mean amount of Nuclear DNA (pg/c)
M-5	2n=2x=28	3.21	0.403 (2Cx)
TG-1	2n=2x=28	3.32	0.476 (2Cx)
RFS-135	2n=2x=28	3.13	0.414 (2Cx)
Anantha	2n=2x=28	3.26	0.432 (2Cx)
S-1635	2n=3x=42	3.19	0.653 (3Cx)
Vishala	2n=3x=42	3.28	0.692 (3Cx)

Table 4: Cytogenetic analysis of clonally evolved cultivars and their putative mother mulberry plants.

Figure 3: Chromosomal compliments of clonally evolved cultivars and their putative mother mulberry plants. A) M-5; B) TG-1; C) RFS-135; D) Anantha; E) S-1635; F) Vishala.

Discussion

Many fruit tree species that have crossed in cultivation have shifted from sexual to vegetative propagation [24]. As such, somatic mutations are an important factor of "clonal evolution"

in plants, which depends on the age of the clone, various environmental stresses, and genotype [25]. Mulberry, which is vegetatively propagated and pruned more frequently, increasing the likelihood of occurrence of sectoral mutants or chimeric forms [26] and detection of such mutants, is one of the key channels in mulberry breeding. As a result, vegetatively propagated plants are emerging as an important source of epigenetic modifications [27], as observed in the current study of clonally-derived mulberry cultivars compared to their putative parents for the first time.

Interestingly, significant variations between clonally-derived mulberry cultivars and their mother plants with respect to morphology, anatomy, biochemical, growth, and cytological traits were recorded in the present investigation. These modifications could be due to repetitive sequences leading to epigenetic modification as a fact of "clonal evolution" which is influenced by environmental stresses and the genotype [25]. In plants, a spectrum of epigenetic modification is greatly reliant on the contribution of repetitive DNA sequence and shape of the chromosome [28]. Besides, total chromosome length is also positively correlated with the number of repetitive sequences that consecutively determine the total nuclear DNA content [29]. Towards this end, despite the fact that the chromosome number remained constant between all clonally-derived mulberry cultivars and their putative parents with no "loss or gain" theory, its length and DNA content were found to be higher in clonally-derived mulberry cultivars than in their putative parents. This change in chromosome length and DNA composition suggests the high plasticity of nuclear genomes at the chromosome level [30] in the clonally-derived mulberry cultivars than their putative parents for the first time. This genome duplication not only leads to genetic flexibility and allows an increased level of mutation [31], but also aids in the evolution of novel functions and stress adaptation [32].

As a consequence, the variations in leaf morphology-leaf size, shape, color, texture, and margins were observed in clonally-derived mulberry cultivars from that of their mother plants depict its adaptation to varying eco-climates [33]. These variations have greater significance, because broad leaves in clonal cultivars usually grow quickly, and are very efficient in photosynthesis due to large leaf surface area [34], and the smooth surface of the leaf [TG-1 and Anatha] is more palatable to the silkworm. Furthermore, the acuminate leaf apex of clonal varieties is appropriate for the rapid removal of rain droplets, which is a critical issue in mulberry to avoid damage during rainfall [35]. The shoot color variations from juvenile phase to mature phase might help in defensive coloration for herbivores [36]. Moreover, a predominant change in the sex expression of clonally-derived mulberry cultivars is similar to that of sex reversal in the clonal population of *Decodon verticillatus* [37].

In addition, the clonally-derived mulberry cultivars exhibit significant anatomical modifications in the leaf as an adaptive response not only manages drought situation and moisture conservation capacity but also facilitates them to grow better in different agro-climatic conditions. Variability in anatomical characteristics is strong evidence for adaptation to stress environments [38]. This shift is consistent with the current study's findings, which show that clonally-derived mulberry cultivars have longer chromosomes and higher DNA contents than their putative parents as a result of genome duplication, which leads to genetic flexibility, increased mutation [31], the evolution of novel functions, and stress adaptation [32]. Taken together, it is quite evident from the present study that all clonally-derived cultivars possess higher leaf yield traits-plant height, the number of branches, leaf length, weight, and water content [39-41]; reduced internodes and petiole length [42]. Thus, clonally-derived mulberry cultivars grow faster and produce more leaves of good quality and quantity than the mother plant [15], which is a unique feature of the study.

Further, increased leaf cuticular thickness in all the clonally-derived mulberry cultivars is directly correlated with the higher leaf moisture content and its retention that facilitates absolute consumption of leaf by the larvae, leading to increased larval growth, which is an important parameter of silkworm rearing [43]. Besides, higher protein and carbohydrate contents recorded in the clonally-derived mulberry cultivars than mother plants of the present study is due to the higher rate of photosynthesis because of the large lamina [34], which is influenced by genetic plasticity via genome duplication [31] and stress adaptation [32] as evident from the present findings on increased chromosome length and DNA contents in the clonally-derived mulberry cultivars than their putative parents. However, it opens ample scope to uncover genome organization leading to genetic plasticity employing comparative and genomic in-situ hybridization techniques.

Conclusion

Mulberry's genetic improvement is hampered by its long juvenile phase, dioecious nature, and a lack of genetic linkage to

better understand desirable and weak traits. However, clonal selection in mulberry offers ample scope, as clonal variety is stable and retains its original traits like pure line variety, to detect genome duplication leading to genetic flexibility. Since no comparative chromosomal studies between clonally-derived cultivars and their putative mother plants have been reported so far, this being the first report, we suppose that discrete morphological, anatomical, and biochemical variations observed in them are not due to gain or loss in chromosome number but might be due to presence of repetitive sequence and related epigenetic modifications (28). Furthermore, changes in chromosome length and DNA content improved drought adaptation characteristics in TG-1 and Anantha, resulting in faster growth and higher quality and quantity of leaves than their mother plants.

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