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Pre-breeding efforts to utilize two wild *Morus* species

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Among the four species of mulberry available in India, *Morus laevigata* and *M. serrata* are wild and possess unique features of bigger leaf size, higher leaf moisture and moisture retention, higher protein and carbohydrate with greater adaptability to adverse climatic condition. In an effort to transfer these traits to cultivated species, inter-specific hybridization was effected between *M. indica* (var. Kanva-2) and *M. laevigata* as well as *M. indica* (var. Kajli) and *M. serrata*. The present communication reports the successful hybridization of mulberry involving wild and cultivated species. *M. laevigata* was collected from natural populations of Andaman Islands and *M. serrata* from northwestern Himalayan belt. After repeated trial of hybridization, successful F_1 seeds were obtained in both the crosses. The F_1 seeds were sown for seedling and behaviour studies. The F_1 plant (*M. indica* × *M. laevigata*) showed better performance than the female parent in most of the characters, while it was better than male parent for a few characters. In another cross, the F_1 plant (*M. indica* × *M. serrata*) showed better performance than both parents for most of the characters. The crosses are expected to carry some genetic load, as the wild species were genetically and geographically distant and carry valuable genes.

Keywords: Hybridization, *Morus* sp., pre-breeding, wild species.

MULBERRY is the sole food plant of silkworm. The quality and quantity of cocoon production depend on the quality leaf of mulberry. Among the four species of mulberry reported[1,2] in India, the present cultivated form of mulberry belongs to *Morus alba* and *M. indica*[3]. Propagation of mulberry through stem cuttings of a particular variety/cultivar makes the plantation almost homogenous. *M. laevigata* collected from Andaman Islands represents the wild species from the mainland[4], which is found in diploid to tetraploid ($2n = 4x = 56$) forms. *M. serrata* is another wild species found in India, which is endemic to northwestern Himalayas[4,5]. In general, mulberry is diploid ($2n = 2x = 28$), but in natural population of *M. serrata*, the ploidy level varies from diploid to hexaploid ($2n = 6x = 84$)[6,7]. Both *M. laevigata* and *M. serrata* trees grow in forest areas and are used for a variety of purposes other than sericulture. Till date, these species have not been used for mulberry crop improvement due to their non-availability or suitability for the silkworm industry. The cultivated species exhibits considerable genetic diversity, but the diploid mulberry showed narrow genetic base and threat to genetic erosion. In order to broaden the genetic base, new gene pools have to be incorporated into those of the cultivated forms.

M. serrata and *M. laevigata* possess several agronomically important traits, including resistance to abiotic stresses like drought and frost[5]. Earlier attempts of interspecific crosses involving *M. laevigata* and *M. serrata* with cultivated mulberry species showed a reproductive barrier. Some researchers studied the crossability among different *Morus* species and their inheritance pattern[7–11]. All the reports are preliminary in nature. This study is an attempt to obtain successful hybrids of wild species of *M. laevigata* and *M. serrata* with cultivated species.

The study was undertaken to assess the performance of F_1 hybrids obtained from crosses of wild species, *M. laevigata* and *M. serrata* with *M. indica*, the cultivated form, in an attempt to transfer desirable traits from wild to cultivated species. A comparison between parents and hybrids in respect of morphological, anatomical, reproductive and growth traits was made.

Morus species, viz. *M. indica* (var. Kanva-2), *M. indica* (var. Kajli), *M. laevigata* and *M. serrata* maintained in the field gene bank at Central Sericultural Germplasm Resources Centre, Hosur were used for this study. The

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cultivated species were collected from Central Sericultural Research and Training Institute, Mysore and Berhampore, whereas the wild species were obtained from Andaman Islands and Uttarakhand through survey and exploration. Materials procured from different geographical zones that are genetically distinct were used in this study. *M. indica* (var. Kanva-2), a popular commercial cultivar from South India was used as female parent and *M. laevigata* collected from Lamia Bay (Andaman and Nicobar Islands) was used as pollinator.

In another set of crosses, *M. indica* (var. Kajli), a popular cultivar from West Bengal was used as female parent and *M. serrata* collected from Uttarakhand was used as pollinator. In the hybridization programme, crosses were effected during normal flowering season i.e. February–March. Before hybridization, flowering of both parents was synchronized to match for effective breeding. Both parents produced sufficient flowers to carry out the experiment. The female catkins of the parents were covered with pergamin paper bags after anthesis and within a week the stigma becomes receptive, as evidenced by its white colour. Pollen was collected from the male inflorescence in a petri plate and kept under cover just before dehiscence of the anthers. The pollen grains were dusted over the receptive stigmas with the help of a camel hairbrush. Pollination was repeated 2–3 times for one week to pollinate flowers of all ages inside the paper bags[12]. In each cross, 20 female inflorescences were pollinated.

The cross becomes effective if pollen fertility is higher. Pollen fertility was studied by standard 2% acetocarmine staining method[13]. Pollen grains that had taken the stain were considered as fertile and those that were not stained properly and had irregular shapes were considered as sterile. Observations were made on more than 350 pollens grains. Hanging drop method in 2% sucrose solution was used to test pollen germination[14].

Seeds collected from the cross after 4–5 weeks were germinated and planted in the nursery to obtain seedlings. After establishment, a comparative assessment of parents and F_1 was made. The observations presented in Tables 1 and 2 were recorded for parents and wherever possible for F_1 plant. The morphological parameters were recorded based on visual observation[15]. Leaf anatomical parameters were recorded according to the standard procedure. Growth traits were recorded following standard procedure for mulberry germplasm[16].

Table 1. Performance of parents and hybrid plants for morphological, anatomical, reproductive and growth traits (*Morus indica* × *Morus laevigata*)

Characters	*M. indica* var. K2 (female)	*M. laevigata* var. Lamia bay (male)	Hybrid (female)	Percentage heterosis over	
				Female	Male
Morphological traits					
Branch nature	Erect	Semi-erect	Erect	–	–
Leaf lobation	Unlobed	Unlobed	Unlobed	–	–
Leaf colour	Pale green	Dark green	Dark green	–	–
Leaf surface	Smooth	Smooth	Smooth	–	–
Leaf margin	Serrate	Repand	Serrate	–	–
Leaf length (cm)	15.00	32.00	25.00	+66.67	–21.88
Leaf width (cm)	13.00	28.00	23.00	+76.92	–17.86
Phyllotaxy	Mixed	1/2	Mixed	–	–
Anatomical traits					
Stomata size (sq, µm)	229.00	365.00	585.28	+160.00	+60.27
Stomata frequency (sq. mm)	675.00	520.00	275.00	–59.26	–47.12
Leaf thickness (µm)	155.45	145.65	185.10	+19.07	+27.08
Reproductive traits					
Sex	Female	Male	Female	–	–
Inflorescence length (cm)	3.00	11.30	5.50	+83.38	–42.48
Fruit length (cm)	3.50	–	6.50	+85.71	–
Fruit colour	Black	–	Pinkish	–	–
Fruit taste	Sweet	–	Sweet	–	–
Seed setting (%)	95.00	–	75.00	–21.05	–
Growth traits					
Single leaf wt (g)	4.00	12.00	10.00	+150.00	–16.67
100 leaf wt (g)	350.00	900.00	850.00	+142.86	–5.56
Leaf area (sq. cm)	275.00	790.00	650.00	+136.36	–13.33
Inter nodal distance (cm)	5.00	7.50	6.00	+20.00	–20.00
Leaf moisture (%)	70.00	71.00	78.80	+12.57	+11.00
Leaf moisture retention (%)	72.00	83.00	82.00	+13.89	–1.20
Leaf yield/plant (kg)	1.55	1.70	2.00	+83.25	+17.65
Rooting (%)	92.00	< 5.00	40.00	–56.52	+35.00

Table 2. Performance of parents and hybrid plants for morphological, anatomical, reproductive and growth traits (*Morus indica* × *Morus serrata*)

Characters	*M. indica* var. Kajli (female)	*M. serrata* (male)	Hybrid (female)	Percentage heterosis over	
				Female	Male
Morphological traits					
Branch nature	Spreading	Semi-erect	Erect	–	–
Leaf lobation	Deep lobed	Medium lobed	Medium lobed	–	–
Leaf colour	Green	Dark green	Dark green	–	–
Leaf surface	Smooth	Rough	Smooth	–	–
Leaf margin	Serrate	Serrate	Serrate	–	–
Leaf length (cm)	17.00	16.00	15.00	–11.76	–6.25
Leaf width (cm)	15.00	14.50	13.50	–10.00	–6.89
Phyllotaxy	Mixed	1/2	Mixed	–	–
Anatomical traits					
Stomata size (sq. µm)	260.00	540.00	320.00	+23.08	–40.74
Stomata frequency (sq. mm)	950.00	335.00	450.00	–2.63	+34.33
Leaf thickness (µm)	145.00	285.00	210.00	+44.83	–26.32
Reproductive traits					
Sex	Female	Male	Male	–	–
Inflorescence length (cm)	2.30	5.10	6.50	+182.60	+27.45
Fruit length (cm)	2.60	–	–	–	–
Fruit colour	Black	–	–	–	–
Fruit taste	Sweet sour	–	–	–	–
Seed setting (%)	90.00	–	–	–	–
Growth traits					
Single leaf wt (g)	1.40	4.50	4.00	+185.70	–11.10
100 leaf wt (g)	125.00	400.00	390.00	+212.00	–2.50
Leaf area (sq. cm)	130.00	240.00	210.00	+ 61.54	–12.50
Internodal distance (cm)	4.40	5.00	4.25	–3.40	–15.00
Leaf moisture (%)	65.75	71.00	76.00	+15.59	+7.04
Leaf moisture retention (%)	72.00	73.00	74.00	+2.78	+1.37
Leaf yield/plant	0.65	1.20	1.35	+107.69	+12.50
Rooting (%)	65.00	<5.00	95.00	+46.15	+90.00

Morphological performance indicated that F_1 hybrid between *M. indica* and *M. laevigata* was like the male parent except for branch nature, phyllotaxy and leaf margin, which is like the female parent. Leaf length and width were more than the female parent but less than the male parent. Stomata size and leaf thickness of F_1 hybrid showed heterosis over both parents, whereas stomata frequency was less than both parents. However, for practical use less stomata per unit area is desirable for any stress-related experiments. The reproductive behaviour showed higher performance than female parent. Catkin length and fruit length of F_1 hybrid increased up to 83.33 and 85.71% over female parent. The colour of the fruit changed from black to pink in F_1 hybrid. In general, *M. laevigata* does not combine with other species. For this reason most of the *M. laevigata* accessions produce seedless fruits. Even if a seed is formed, i.e. pseudo seed, it will not have an embryo and will not germinate. But the F_1 hybrid produced in this experiment exhibited more than 75% seed set. Profuse flower and fruit formation was observed like in the female parent (Figure 1 *a–c*).

All the growth traits of the F_1 hybrid were better than the female parent, except rooting percentage. The growth parameters of F_1 hybrid were less than the male parent, except leaf moisture (78.70%), leaf yield/plant (2.00 kg) and rooting percentage for which F_1 showed the heterosis over the male parent. In any improved variety, leaf yield is the ultimate goal while high leaf moisture percentage helps in silkworm rearing.

In sericulture, the mulberry variety plays an important role. The leaf of *M. laevigata* is not used for silkworm rearing due to its thick and rough nature. But in the F_1 hybrid, the leaf is soft, palatable to the silkworm and rearing performance is like in commercial varieties. Vigour and growth performance are better than female parent and suitable for selection. However, the rooting performance has to be improved by backcrossing or treatment with root hormones like IBA, IAA and other commercial hormones.

The morphological parameters of F_1 hybrid between *M. indica* and *M. serrata* were like male parent except leaf surface, which is like female parent. The leaf length and width of F_1 was less than both parents. The anatomical parameters showed mixed result. Stomata size and leaf thickness is better in F_1 than female parent. The stomata frequency is better in F_1 than male parent. On the whole

Figure 1. Morphological variations among parents and inter-specific hybrid for leaf (***a***), inflorescence (***b***) and fruit (***c***). ***a***, Leaf of *Morus indica* var. Kanva-2; *M. indica* × *M. laevigata* (F_1); *M. laevigata*. ***b***, Inflorescence of *M. indica* var. Kanva-2 (♀); *M. indica* × *M. laevigata* (♀); *M. laevigata* (♂). ***c***, Fruit of *M. indica* var. Kanva-2; *M. indica* × *M. laevigata* (F_1).

Figure 2. Same as in Figure 1, but for different parents and inter-specific hybrid. ***a***, Leaf of *Morus indica* var. Kajli; *M. indica* × *M. serrata* (F_1); *M. serrata*. ***b***, Inflorescence of *M. indica* var. Kajli (♀); *M. indica* × *M. serrata* (♂); *M. serrata* (♂). ***c***, Fruit of *M. indica*.

the anatomical parameters of F_1 were better with medium range of value and suitable for selection. The F_1 had male inflorescence like male parent but bigger in size. The pollen viability of male parent was 75–80% whereas in F_1, the pollen viability ranged from 90 to 95%. In higher ploidy, pollen viability is generally low, but F_1 hybrid showed higher pollen viability with better pollen germination (80%). In case of growth traits all the parameters showed higher value in F_1 hybrid compared to the female parent. The F_1 hybrid showed heterosis over both parents in respect of leaf moisture percentage, leaf moisture retention percentage, leaf yield/plant and rooting percentage. The parent *M. serrata* is poor in rooting percentage and showed less than 5% rooting (Figure 2 *a–c*).

But the hybrid plant exhibited more than 95% rooting. The female parent is good in quality parameters, but not widely accepted due to its low yield and moderate rooting (65%). Performance of the F_1 hybrid is comparatively better than both parents. *M. serrata* is not generally used for sericulture due to its rough, thick and tomentose leaf. The F_1 hybrid showed smooth leaf, like the female parent with more leaf area and was thus suitable for silkworm rearing.

Thus the pre-breeding effort highlights the possibility of using wild *M. laevigata* and *M. serrata* effectively and efficiently. A similar result was also reported in cotton[17]. Stewart and Mc[18] indicated the possibility of obtaining recombinants through backcross for various traits. It is also possible to obtain recombinants through backcross in mulberry. Efforts in this regard are under progress to obtain abundant population and isolate the desired plant through careful observations. The perennial crops require more gestation period for establishment and expression of characters, which is also to be noted. The F_1 plants also showed the characters of high biomass, vigorous growth, profuse fruit formation, and timber yield that can be exploited for non-sericulture use.

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Current status, distribution and conservation of rare and endangered medicinal plants of Kedarnath Wildlife Sanctuary, Central Himalayas, India

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Assessment of population structure on the basis of density, distribution and diversity-dominance pattern was carried out in Kedarnath Wildlife Sanctuary, Uttarakhand, India. Besides, distribution pattern, population structure and conservation status of ten rare and endangered medicinal plants were also evaluated. Different habitat types for these species were identified and sampled using vertical belt transects. Out of ten habitats identified, distribution of most of the species was found to be restricted in 2–3 habitats. However, *Picrorhiza kurrooa* showed wide distribution in six habitats, while *Swertia chirayita* was restricted to a single habitat. On the basis of density, occurrence in different habitats and level of pressure, we have

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