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Mulberry wild species in India and their use in crop improvement – A review

A.Tikader[1] and C.K. Kamble[2]

[1]*Central Sericultural Germplasm and Resources Centre, Hosur – 635 109, Tamil Nadu, India*
[2] *Central Sericultural Research and Training Institute, Mysore – 570 008, Karnataka, India*

Corresponding author email: atikader_csgrc@yahoo.co.in

Abstract

Exploitation of wild relatives of crop plants to a large extent depends on the efficient use of germplasm resources available in natural habitat and the centre of diversity. The primary objective for breeding is to identify the mulberry germplasm, which will be used as a parent. This includes the performance of individual accessions with regards to their origin, passport data, characterization, and evaluation through a series of tests in field. The crop improvement is the transfer of desired genes and gene combination from unadapted sources into most usable breeding materials. To bring greater diversity into the breeding pool, it requires introduction of exotic and wild materials. The breeders find it difficult to use unadapted materials from wild source. The intermediate materials produced after incorporating the new genes is treated as genetically enhanced materials, which may be useful to develop desired lines. The use of wild mulberry species is limited and concentrated effort requires utilizing them. The variability, secondary and tertiary characters may be identified from the wild gene pools to develop resistant genotypes and to utilize the resources efficiently for crop improvement.

Key words: Wild gene pools, breeding, secondary, tertiary genes, and crop improvement

Introduction

Improvement of mulberry (*Morus* spp.) for its foliage characters both in quantitative and qualitative aspects is the long-term goal for mulberry breeders. The genetic improvement of mulberry depends on the availability of genetic variability in germplasm. Selection of suitable genotypes from gene pool requires a thorough knowledge on foliage characters for utilizing them in hybridization. The breeding efforts made so far were restricted to cultivated species for first generation only (Hamada, 1963; Das, 1984). In India, *Morus* is represented by four species i.e., *M.indica* L., *M.alba* L., *M.laevigata* Wall. and *M.serrata* Roxb. (Tikader and Dandin, 2005a). The distribution map of this genus in India is presented in Fig. 1. The characteristic features of four species available in India are also presented in Table 1.

The mulberry improvement programme on sound scientific base have started in the early 1960’s in India, the scientist could be able to identify some promising clones from the open pollinated hybrid population and also from introductions. Different research institutes under Central Silk Board developed improved mulberry varieties through, conventional, polyploid and backcross breeding. The institutes evolved high yielding mulberry varieties like S1, S1635, S799, C776, C763, BC259, TR10,

Table 1. Characteristic feature of *Morus* species available in India.

***Morus* species**	Characteristic features
Morus indica	Teeth of leaves unequal, the lateral nerves running straight into the teeth or forked within the margin, leaves usually medium to long, male spike less than 2.5 cm long, style is long, hairy, fruit ovoid or cylindrical, unripe fruit colour red, becomes black when ripe. Bud colour dark brown, elongated, triangular in shape.
Morus alba	Teeth of leaves uniform, usually blunt, segments of the perianth of female flowers in four numbers, the two outer keeled, style very short, fruit colour white, pink and black. Bud colour brown, oval round in shape.
Morus laevigata	Teeth of leaves fine, the lateral nerves abruptly curved upwards within the margin, style is very short. Fruit is long greenish, white, dark purple. Bud colour brown, elongated, lengthy and elliptical in shape.
Morus serrata	Teeth of leaves usually coarse, somewhat unequal and sharp, leaves velvety, coarse, full of minute hair on both the side, segments of the perianth of female flowers 2-4, usually 3, all similar, style is medium, fruit colour white, pink and mucilage fruit. Bud colour dark brown, round and bigger in size.

Somaclonal variant (SV1) etc. Through mutation breeding S30, S36, S41 and S54 varieties were developed for irrigated conditions (Yadav, 2004). Considering the need for rainfed mulberry under rainfed selection programmes (RFS) developed two varieties namely, RFS – 135 and RFS – 175. During 1997, the variety V-1 having a yield potential of 70 MT/ha/year with superior quality leaf was released to the field (Jalaja et al. 1994). The variety performed better in the Southern States, Maharastra and Orissa.

Different Research Institutes are involved in developing superior variety suitable for the sericulture industry as a whole. In some institutes, the improved varieties are selected based on superior clone and natural variability. To develop the present day improved varieties, the scientist have to exercises a lot of permutation and combination of various mulberry germplasm accessions available in their working germplasm but extensive efforts are required to utilize various wild *Morus* species. Using the same parents or related parents are creating narrow genetic base knowingly or unknowingly. Though the scientists are observing more leaf yield/ quality aspects but in long term this may not be suitable for specific condition. To bring greater diversity into the breeding pools, it requires introduction of unadapted and productive exotic or wild species. The exotic or wild species (*M.serrata and M.laevigata*) maintained at Central Sericultural Germplasm Resources Centre, Hosur may be utilized for the purpose. The wildmulberry germplasm available in the *ex-situ* field gene bank is a good source for exploitation in crop improvement and hunt for new recombination of genes (Tikader and Thangavelu, 2002b). Until and unless the species are characterized and evaluated, it is not possible to understand the traits of interest available and utilize them properly for crop improvement programme and other related purpose. The exploitation of wild species is rather scanty in India and elsewhere and concentrated efforts are to be made to utilize the germplasm.

Biodiversity of wild species

M. serrata, the natural Himalayan mulberry is confined to North West India. Depending on the agro-climatic distribution, the morphological parameters showed wide variation. The bark colour varied from red, brown to dark brown and blackish brown. Leaf varied from unlobed to multilobed, thin to thick, rough, tomentose and velvety. Phyllotaxy is mixed type i.e., 1/2, 1/3, 2/5. Internodal distance varied from 3.5 to 7.5cm. In *M. laevigata* the bark colour varied from grayish to reddish and leaf is mostly unlobed. Phyllotaxy is unique i.e., 1/2. Internodal distance is more i.e., from 6.0 cm – 8.0 cm (Tikader and Thangavelu, 2000b).

Ploidy status of wild species

Mulberry the most important crop for sericulture is having different ploidy levels. Most of the genotypes available are diploids (2x, 2n=28) but exhibits higher ploidy i.e., 3x, 3n=42, 4x, 4n=56, 6x, 6n=84 and docasaploid 22x, 22n=308 (Basavaiah et al*.,* 1989). Haploid mulberry through in vivo anther culture was developed in China (Shoukang et al., 1987). Hence wide ranges of mulberry genotypes of different ploidy levels are available for utilization in crop improvement. Due to low leaf yield, slow growth rate and poor feeding performance, genotypes with higher ploidy are not suitable for silkworm rearing. The higher ploidy genotypes having the undesirable characters like coarse leaf, more hairs and less succulence of leaves and therefore most of the tetraploids are not used for bioassay (Hamada, 1963; Das et al., 1970; Tojyo, 1985). The origin of species and forms in polyploid mulberry was studied intensively (Abdullaev, 1965). The mulberry is diploid with 28 chromosomes but the balanced and unbalanced number is also available. The balanced form starts with 2n = 28, 56, 84, 112, 140, 168, 196, 224, 252, 208 and 308 whereas unbalanced number is 2n =14, 42, 70, 98, 126, 154, 182, 210, 238, 266 and 294. A study was carried out with 2n= 28, 42, 56, 70, 84, 98, 112, 166 and 308. The 308 chromosomes bearing plant gives higher yield of berries with good palatability (Abdullaev, 1965). The naturally available mulberry is having higher ploidy i.e., *M. tiliaefolia* (84), *M. cathyana* (56, 84), *M.nigra* (28, 308), *M. serrata* (28, 42, 56, 84), *M. laevigata* (28, 42, 56) and even haploid mulberry are available in natural condition (*M. notabilis* = 14) (Maode, 1996).

The chromosome count of the collected samples indicates that *M. serrata* is available in polyploid form in nature. Mulberry in general is diploid (2n=28). But in nature, the species are found in diploid (2n=28), triploid (2n=42), tetraploid (2n=56) and hexaploid (2n=84). Ploidy level has been used to trace the genesis of plant endemism. The palaeo endemics have high chromosome number and are assumed to be ancient polyploids and grow in higher altitude (Dhar, 2002). The similar case also observed for *M. serrata*. The ploidy level of *M. laevigata* Wall. varies from diploid to tetraploid and grows in higher altitude ranges from 700 – 2200 m.

Reproductive variability of wild species

The inflorescence length is higher in *M. serrata* Roxb. than other cultivated species in India. The maximum collections of *M. serrata* possess male flower. The male inflorescence of *M.serrata* varies from 2.4 – 4.25 cm and female 1.3 – 3.8 cm. If the chromosome number increases, the pollen size also increases in *M. serrata*. The pollen study indicates that the diameter of the pollen spores varies from 16.0 – 25.7, 21.0 – 28.0 and 23.0 – 30.5, 25.0 – 33.0 µm in diploid, triploid, tetraploid and hexaploids, respectively. The number and diameter of the pollen pores showed 2- 5 porate pollen grain (Tikader et al., 2000). Male and female flowers are in separate plant i.e., dioecious. Fruit colour varied from black, creamish and pink. The fruit is very sweet to taste after ripening. Mucilage juice is the special character of the species (Tikader et al., 2002c).

M. laevigata shows dioecious flower i.e., male and female in separate plant. Male inflorescence length varies from 3.72 – 11.30 cm. The anther length varies from 0.90 – 1.16mm and stamen length from 2.02 – 5.19 mm. The pollen diameter and viability varies from 15.32 – 21.54 µm and 55.21 – 94.25%. *M. laevigata* is having lengthy inflorescence and used as key characters to identify the species. The female inflorescence length varied from 5.00 – 12.00cm. The flower/catkin varies from 132.00 – 244.00, style length 0.02 – 2.35mm, fruit length 6.10 – 10.27cm and fruit weight 2.02 – 6.37g. Fruit colour varied from black, white, pink and greenish. The fruit is usually lengthy and seedless, very sweet to taste (Tikader et al., 2000c). Rare abnormality in male inflorescence of *M. laevigata* is recorded which was collected from Andaman and Nicobar Islands (Tikader and Thangavelu, 2003).

Leaf histological variability in wild species

The stomata frequency ranges from 255.00 – 416.00 per mm² whereas idioblast frequency 14.00 – 36.00 per mm². The palisade and spongy layer thickness plays a vital role for keeping moisture in leaf during adverse climatic condition. Total leaf thickness indicates the quality of leaf. *M. serrata* possesses coarse, hairy leaf suitable to adjust in adverse condition i.e. cold, drought etc. The chloroplast number in guard cells of stomata is the indicator of ploidy level, which ranges from 12– 32 The chloroplast number per stomata is the indirect way to group mulberry accessions in different ploidy levels (Tikader et al., 1999a; Tikader and Rao, 2001a, Yang and Yang, 1995). In *M. serrata*, the chloroplast number per stomata varies from 10–20 in triploid, 10–26 in tetraploid and 12–30 in hexaploid. The variation in chromosome number, chloroplast number per stomata and pollen diameter are useful for grouping the germplasm accessions and also in mulberry crop improvement programme (Tikader and Rao, 2001a). The chloroplast count is used for primary grouping of the accessions and finally chromosome count is considered to ascertain the ploidy level.

The variation of leaf anatomical parameters in *M. laevigata* ranges from 18.95–30.38 µm stomata length, 15.55–20.65 µm and stomata width, 266.90–943.70 mm² in stomatal frequency, idioblast length (4.25–39.35 µm), idioblast width (8.67–36.68 µm) and idioblast frequency (10.73–23.66 mm²). The palisade layer thickness (49.03–96.53 µm), spongy layer thickness (55.57 – 128.66 µm) and leaf thickness (140.90 – 267.75 µm) showed variation among the accessions. The chloroplast number / stomata in *M. laevigata* varies from 9.50-16.20 and indicates some of the accessions are diploid, triploid and tetraploid also.

Growth performance of wild species

The growth behaviour is important for effective utilization of mulberry germplasm for sericultural purposes. Wide range of variation was observed in *M. serrata* among the different growth traits. Among the parameters, the moisture content (68.10 – 80.22%) and moisture retention (55.13 – 75.00%) showed consistent and higher value than the commercial varieties, but the coefficient of variation (CV) is 3.08 – 8.17 %, respectively. The wide CV indicates the variation among the traits and useful for utilization in crop improvement programme (Tikader and Dandin, 2005a,b).

Different growth traits of *M. laevigata* showed variation among the accessions. Number of shoot varies from 10.17 – 23.33 followed by length of the longest shoot (80.83 – 205.33cm), inter nodal distance (4.56 – 9.15cm), 100 leaf weight (381.73 – 1093.02g), total shoot length (572.67-3277.83cm), moisture content (68.97 – 73.30%), moisture retention (61.11-83.62%) and leaf yield per plant (0.39 –3.00kg). The mulberry accessions react with the environment and express the different traits. The fluctuation of environment also effects the expression of individual parameter.

Information on the field performance of *M. serrata* was assessed and found significant variation among the accessions. The accession provides the information on variability, association and grouping pattern. As the mulberry grows in natural condition with the component of stress related factors, it might be utilized in mulberry crop improvement programme through breeding (Tikader and Dandin, 2005a,b).

Genetic variability of wild species

Divergence analysis at species level in mulberry was conducted on morphological, anatomical and reproductive traits. Based on these traits the species are grouped into different clusters. *M. alba* alone formed individual cluster for all the traits where *M. laevigata* formed separate cluster for morphological and reproductive traits. *M. macroura* and *M.* rotundiloba are grouped in one cluster for anatomy and reproductive traits except morphological traits that shows their close association (Tikader et al*.,* 1999b).

When the genetic distance was calculated between species, it is seen that the inter-specific distance was highest between *M. serrata* Roxb. and *M. indica* L. (0.465) that is followed by *M. serrata* and *M. alba* L. (0.457), *M. serrata* and *M. macroura* Miq. (0.401). The genetic distance between *M. macroura* Miq. and *M. indica* L. was 0.307, which was higher than that of *M. macroura* Miq, and *M. alba* L. (0.339). The interspecific distance between *M. indica* L. and *M. alba* L. was 0.168. The intraspecific variability as calculated by averaging the genetic distance among the genotypes of a single species was highest in *M. serrata* Roxb. (0.180) and lowest in *M. alba* L. (0.147).

The genotypes of *M. serrata* Roxb. and *M. macroura* Miq. can be used for breeding with cultivated varieties, which are capable of high yielding but are sensitive to biotic and abiotic stress to confer stress resistance to these varieties (Vijayan et al*.,* 2006). The promising genetic resources of *M. serrata* Roxb. and *M .macroura* Miq. should preserve properly

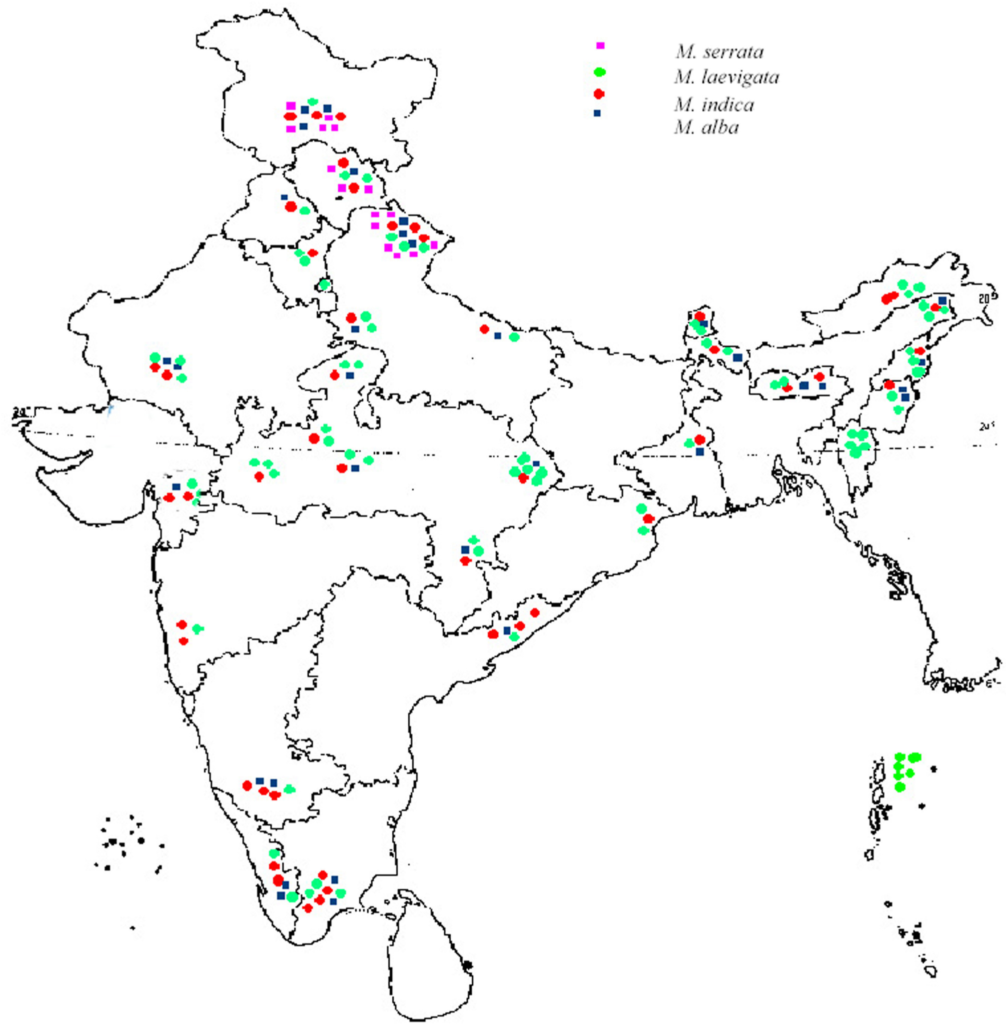

Fig 1. Distribution of *Morus* species in India

for the genetic advancement of the presently cultivated varieties of mulberry. Sixteen populations of the wild mulberry, *M. serrata* Roxb. were analysed for their genetic diversity with the aim to use them in introgressive breeding programme with cultivated relatives. Significant amount of genetic diversity was observed among these populations for morpho-anatomical as well as DNA markers.

The results indicates significant amount of genetic diversity among the collections of *M. serrata* the 17 ISSR primers generated a total of 95 DNA markers, 51 of which were polymorphic revealing 67% polymorphism among the populations (Vijayan et al., 2004). The use of RNA markers is limited in case of mulberry and more work is required to be carried out.

Breeding performance of wild species

The breeding objective is to produce/ create a broadly adapted population with sufficient genetic buffering to face a wide variety of environmental conditions producing good yield. In order to broaden the genetic base, new gene pools have to be incorporated into the mulberry varieties. Earlier the crossability among the different *Morus* species and its inheritance pattern were studied (Dandin et al*.,* 1987; Dwivedi et al., 1989; Tikader and Dandin, 2001b). *M. serrata* possess several agronomically important traits such as higher leaf thickness, greater leaf moisture content, moisture retention and resistance to abiotic and biotic stresses. Among the abiotic factors, the species are resistance to drought and frost (Tikader and Thangavelu, 2002d). Initial breeding performance of *M. serrata* at inter-specific level with *M. indica* found suitable for crop improvement. More number of crosses at inter-specific and intra-specific level may be tried with *M. laevigata* and *M. serrata* through introgression breeding. F1 hybrid thus developed of *M. indica* and *M. laevigata*, *M. indica* and *M. serrata* should be studied for further utilization (Tikader and Thangavelu, 2005c, Tikader and Rao, 2002a). *M. serrata* and *M. laevigata* provides scope for target oriented selective breeding to incorporate the characters of drought, frost, and disease resistance into cultivated varieties. Moreover, the breeding performance of *M. laevigata* showed positive results (Tikader and Thangavelu, 2005c). The wild species retain their characteristics after crossing and expressed in hybrids. So the present results showed the utilization of *M. laevigata* and scope for selection in crop improvement programme.

The inter-specific hybridization studies in mulberry were conducted involving *M. alba* with *M. australis*, *M. latifolia*, *M. cathayana*, *M. nigra* and *M. sinensis*. The poor seed setting in some cross combinations involving *M. cathayana*; *M. laevigata* and *M. nigra* with diploid, mutant of *M. alba* can be attributed to the differences in chromosome numbers and ploidy levels (Das and Krishnaswami, 1965; Katagiri et al., 1982; Dzhafrov and Nadzhafov, 1984).

The intra-specific hybridization helps in developing homozygous lines that will prove the way for carrying studies for understandings the various inheritance pattern and genetics involved in the manifestation of different traits in mulberry. In this regard report on haploid mulberry (Maode et al., 1996; Thomas et al., 1999) is available which may be followed for studying the inheritance pattern.

Propagation of wild species

In general mulberry is propagated through vegetative cuttings, but the wild species are poor in rooting and shows limitation to study the material. The alternative method of propagation is grafting (Tikader and Thangavelu, 2006). *M. laevigata* and *M. serrata* are wild species and very poor in rooting showed survival after grafting on local shoot stock. Maximum survival % was recorded 57.30 % followed by 12.00 % in *M. laevigata*. *M. serrata* showed highest survival 75.00 % followed by 46.60 % and lowest 10.65 %. During the grafting period, the temperature recorded which varied from 15 – 26 degree C and relative humidity varies from 30 – 90%. The successful graft union depends on the close matching of the callus producing tissues near the cambium layers and similarly the incompatibility also depends on the materials, which is used as stock. The wild collection of *M. serrata* is generally higher ploidy i.e., triploid, tetraploid, hexaploid etc. When graft is made, the adjustment of chromosome number poses incompatibility and result in unsuccessful grafting. The graft union formed at the initial stage with apparent success, gradually develop distress symptoms with time due to failure of producing the callus parenchyma which is the main reason for low percentage of successful grafts and difficult in multiplication. *M. laevigata i*s also a wild species, poor rooter and difficult to multiply through vegetative cuttings. When grafting is made on good rooting variety (Kanva-2 and V1), maximum survival % was recorded 57.30 and lowest 12.00. *M. laevigata* also showed grafting incompatibility like *M. serrata*. Therefore, all the grafting was not successful due to graft incompatibility.

Specific characters of wild species

M. serrata is known as Himalayan mulberry due to its origin. The wild species possesses drought tolerant characters like leaf rolling, abundant xylem, less stomata per unit area (255.82 – 416.30 / mm^2) and slow growth in response to moisture stress which are useful in breeding to develop stress tolerant varieties etc. are the information's gathered during collection of materials (Tikader and Thangavelu, 2002b).

M. laevigata harbors a number of agronomical important traits such as bigger leaf size, higher leaf thickness, moisture retention, resistance to biotic and abiotic stress like drought, saline and frost (Ravindran *et al*., 1999). Due to above positive characters, *M. laevigata* showed scope for introgression breeding between wild and cultivated species (Tikader and Dandin, 2005a, b; Vijayan et al., 2006).

Bioassay of wild species

M. serrata was used for bioassay study and results indicate that it is suitable for late stage and from hatching to cocoon harvest; the crop yielded 32 kg/100 disease free layings. The silkworm race was used as PM x CSR2 hybrid. The bioassay was conducted in Gohar block of Mandi district in Himachal Pradesh where abundant *Morus serrata* is available. The farmers can get additional income from the silkworm rearing as they are using the plant as fodder. The plants are growing in the bund areas and landless farmers can take up rearing on such species to supplement their income as additional crop or as alternate food during the last stage of rearing (Singh *et. al*., 2006).

A 20 to 40 years old tree can yield leaves from 500 kg to 2000 kg/ crop respectively (Singh et al., 2006). Similar report was made available from Chamoli district of Uttarkhand where more than 20 kg cocoons / 100dfls were harvested after feeding the *M. serrata* leaves to silkworm at late age with bivoltine hybrids (Tikader and Thangavelu, 2002d).

The rearing performance of *M. laevigata* was assessed throughout rearing and was found that larval period (26 days), weight of 10 mature larvae (32.02 g), yield/10000 larvae (5077 no.), 6400 g by weight, single cocoon weight (1.28 g), shell weight (0.223 g) and silk ratio (17.33%) (Tikader, 1993). The leaves of *M. laevigata* were utilized for silkworm rearing at late age (Ravindran et al., 1999; Tikader and Rao, 2002a). The quality of leaf is good but mortality of the worm was little high. Effect of feeding leaves of *M. alba* and *M. laevigata* were assessed on larval growth and silk yield of *Bombyx mori* L. The result indicated that *M. laevigata* leaf *was* better in rearing performance than *M. alba* (Mahmood et al., 1987).

Utilization of genetic resources

Most of the wild and weed relatives of domesticates have not been collected, studied and conserved. Wild progenitors and other close relatives of crop species – the secondary gene pools have assumed an important role as genetic resources used by plant breeders. Gene transfer by genetic engineering is opting up tertiary gene pools.

The utilization of different *Morus* species was attempted and inter-specific hybridization was conducted to incorporate the desirable characters for crop improvement. But sometimes, it is observed that interspecific crosses do not perform well due to various reasons. The main reason may be incompatibility, geographical isolation among the species. The performance of untried wild species like *M. serrata*, *M. laevigata* and *M. tiliaefolia* were tried at inter and intra-specific level. At national and international level *M. alba*, *M. indica*; *M. multicaulis* and *M. bombycis* are extensively used for commercial purposes. Both *M. laevigata* and *M. serrata* are growing as tree in forest area and people are using these trees for multi purpose use other than sericulture. But till date these species have not been used for mulberry crop improvement due to its non-availability or suitability for 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 the gene pool of cultivated forms.

M. serrata and *M. laevigata* possess several agronomic important traits including resistance to abiotic stresses like drought and frost. Some authors tried to study the crossability among different *Morus* species and its inheritance pattern. All the reports are of preliminary in nature and have attempted to get successful hybrids of wild species of *M. laevigata* and *M. serrata* with cultivated species like Kanva –2 and Kajli (Tikader and Dandin, 2007: Tikader and Thangavelu, 2005c).

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, leaf is soft, palatable to silkworm and rearing performance is like commercial varieties. The vigour and growth performance are better in male parents than female parents and suitable for selection. The rooting performance on lesser side, which has to be improved by back crossing or treatment with root hormones like IBA, IAA and commercial hormones.

Thus the pre-breeding effort highlights the possibility of using wild *M. laevigata* and *M. serrata* effectively and efficiently (Tikader and Dandin, 2007). Similar result was also reported in cotton. Stewart (1994) indicated possibility of getting recombinants through back cross for various traits. It is also possible to get recombinants through backcross in mulberry. Efforts in this regard are under progress to get abundant population and isolating the desired plant through constant observations. It is well known fact that the perennial crops require more gestation period for establishment and expression of characters. The F_1 plants also showed the characters of high biomass, vigorous growth and profuse fruit formation, timber yield that can be exploited for non-sericulture use.

Wide genetic variability between *M. laevigata* and *M. serrata* ranges from 0.951 to 0.422. Systematic studies pertaining to the diversity of these two wild mulberry species are inadequate. To study the representative collections of two wild mulberry species in India for assessment of their genetic structure, diversity and interrelationship, which is very important from the point of view of their utilization in crop improvement programme and conservation.

This study gives an insight into the broad genetic structure of these two wild species. The information generated from the study will be useful for further in depth analysis of these species towards utilization in mulberry improvement and conservation programme (Naik et al., 2006).

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