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Review Article

# Role of Biotechnology in Genetic Conservation of Mulberry Plants for Crop Improvement

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## ABSTRACT

Mulberry (*Morus L*) is an important tree crop that provides sustainable economic and environmental benefits to a large number of people who live in rural and suburban areas of Asian countries especially of China and India. The sustainability of sericulture in a region is largely dependent on the mulberry leaf productivity as mulberry leaf production alone cost more than 60% the total silkworm cocoon cost. Thus, all sericulturally important countries have been striving to develop varieties that adopt well to the agroclimatic conditions and respond well to the cultural practices. Although the species delimitation in mulberry is still a point of great debate, it is believed that more than 68 species exist under the genus Morus and out them only a few species such as M. alba, M. bombycis, M. indica, M. latifolia, M. multicaulis (for foliage) and M. nigra (for fruit) are cultivated. The remaining species along with many landraces of the cultivated species are considered as wild, therefore, they have been mostly neglected. The recent observation that the genetic pool of the domesticated species is shrinking and the wild species M. serrata, M. laevigata, and M. tartarica hold genes for several important traits like drought, salinity and frost resistance has generated on conservation and utilization of the wild mulberry genetic resources. Thus, countries across the globe have been adopting both conventional and modern biotechnological methods to collect, characterize, conserve and utilize large amount of genetic resources in their crop improvement programs. This, review is, thus, undertaken to give an overview of the role of biotechnology on exploration, characterization, and conservation of wild germplasm for crop improvement in mulberry.

**Keywords:** Germplasm; Biotechnology; Conservation; Crop improvement

## INTRODUCTION

Mulberry (*Morus*; Moraceae), is a tree crop being cultivated widely in Asian countries to feed the monophagous Silkworm (Bombyx mori L). Since mulberry leaf production cost along covers more than 60% of the total production cost of silkworm cocoons, mulberry cultivation is considered as one of the vital components of sericulture activities [1]. China and India being the top silk producing countries have developed a number of mulberry varieties suitable for a wide range of agro climatic conditions [2]. Generally, the plant has three main species ostensibly named after the fruit color such as white (*Morus* alba), red (M. rubra) and black mulberry (M nigra) with numerous cultivars [3], but most of these mulberry varieties were developed from a few species such as M. alba, M. atropupurea, M. bombycis, M. indica, M. latifolia and M. multicaulis though more than 68 species have been reported from the genus *Morus* [4]. The major reasons for the lack of utilization of other species are the poor leaf quality due to the, coarseness of the leaf, low moisture content and its retention after leaf harvest, low protein content, and the low leaf yield [5]. Thus, it is imperative to develop qualitatively and qualitatively superior mulberry through identification of better parents and their utilization in breeding.

## ORIGIN AND DISTRIBUTION OF *MORUS*

Mulberry (*Morus*) is understood to have originated in the northern hemisphere, particularly in the Himalayan foothills and extended into the tropics of southern hemisphere [6,7]. While reviewing the centers of origin, Vavilov [8] placed Morus

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L. in China-Japan centre of plant origin. Excavations of early tertiary Moraceae fossils [9] and contemporary molecular studies [10] revealed an early diversification of Moraceae in Eurasia and subsequent migration into the southern hemisphere. The genus *Morus* is exists in all regions between 50oN Latitude and 10oS Latitude, from sea level to altitudes as high as 4000 m [11], which include Asia, Europe, North and South America, and Africa. Continental America has four species *viz*., M. insignis, M. celtidifolia, M. corylifolia and M. mexicana. China has 24 species but only four species *viz*., M. alba, M. multicaulis, M. atropurpurea and M. *mizuho* are largely cultivated for sericulture purposes and the remaining are considered wild species. In Japan, out of the 19 species only three species are mostly cultivated *viz*., M. alba, M. bombycis, M. latifoila. In India, there are many species, of which *Morus* Alba and M. indica are fully domesticated while M. serrata and M. laevigata grow wild in the Himalayas. In Africa, M. mesozygia has been reported to occur in humid, sub-humid and semi-arid areas, it grows well from sea level to an altitude up to 1000 m [12]. The common names attached with some of these species also indicate either their origin or their morphological distinctiveness. For example, M. alba is called 'white mulberry' because of the fruit and bark color, white mulberry is native to China but has spread into several other countries [13]. The M. rubra is called 'red mulberry' due to stem and fruit color, red mulberry is native to North America, and it has been cultivated in America since colonial times and its fruit is made into wine and also the fruit is considered a valuable agricultural and wildlife feed. The M. nigra is called 'black mulberry' due to the black fruit it bears. Black mulberry, a native of Iran, is cultivated for its fruits in South Europe, Southwest Asia and is the most important species in the Mediterranean countries [14]. The black mulberry (Turkish name 'Kara Dut') is widely grown in Turkey for its delicious edible fruits [15]. Owing to the mediterranean conditions, the northeastern part of Turkey, in particular Coruh valley has notable populations of black mulberry. Similarly, based on the place of origin M. serrata as 'Himalayan mulberry', M. australis as 'Chinese mulberry', M. mesozygia as 'African mulberry', M. celtidifolia as 'Mexican mulberry', M. *microphylla* as 'Texas mulberry' and M. tartarica is as 'Russian mulberry'.

### GENETIC RESOURCES AND THEIR CONSERVATION

The genetic resources of mulberry comprise the domesticated and the wild species including traditional varieties, land races, elite lines and special varieties developed by breeders/researchers, and their wild relatives. Since many of these valuable genetic resources are disappearing rapidly due to destruction of habitats, exhaustive utilization for human benefits, and increased vulnerability to the severely changing environmental conditions, appropriate actions need to be taken immediately for conserving them for the future needs. Since mulberry exists in different ploidy levels ranging from haploidy with 14 chromosomes to decasoploidy with 308 chromosomes and most of the cultivating varieties of mulberry are either diploids or triploids, the wild species with higher ploidy number get very little attention from both breeders and conservationists, resulting in loss of considerable genetic diversity [16]. Further, due to the increased industrialization and urbanization, large areas of the natural habitat of mulberry have been destroyed and many populations have been reduced below the minimum size required for their continued survival without management. Nonetheless, recent findings that the genetic resources of the wild mulberry species such as M. serrate, M. leavigata, M. tiliaefolia and M. tartarica have a number of agronomically important traits like resistance to abiotic and biotic stresses and faster growth [17] have ignited the awareness to conserve these valuable genetic resources for better crop improvement [16]. Consequently, countries across the world have collected and conserved a large number of mulberry accessions in the germplasm (Table 1).

| Country | Mulberry accession |
|---|---|
| Japan | 1375 |
| China | 3000 |
| South Korea | 208 |
| India | 1254 |
| Bulgaria | 140 |
| Italy | 50 |
| France | 70 |
| Indonesia | 5 |
| Taiwan | 5 |
| Argentina | 2 |
| Colombia | 4 |
| Mexico | 5 |
| Peru | 2 |
| USA | 23 |

**Table 1:** Mulberry accessions in the germplasm banks of a few countries (Source: Vijayan et al. [2]).

## COLLECTION AND CHARACTERIZATION OF GENETIC RESOURCES

In order to collect right samples from populations and species, it is necessary to make adequate preparation in the form of extensive surveys on distribution, extent of genetic diversity within and between populations, occurrence of new and rare alleles etc.. Most of the mulberry species are hard to collect in the form of seeds as they are highly heterozygous, hence, only stem cuttings are collected either for direct planting or for grafting. As the stem cuttings may not remain viable for a long period, they are preserved properly and transported to the conservation site immediately. While collecting the samples, proper records such as name, environment conditions, geographic features of the collection site, race name, variety

name, species name and other important morphological features are recorded as passport data of the accession as documentation is the most critical activity of germplasm conservation and utilization.

## CONVENTIONAL METHODS FOR CONSERVATION OF MULBERRY GENETIC RESOURCES

The conservation of mulberry genetic resources involves several activities ranging from establishment of protected areas to building of DNA libraries. Being a highly heterozygous, perennial tree propagated mainly through vegetative means a number of strategies have been adopted to conserve the precious gene pools safely and efficiently as shown in Fig. 1 [18]. The most important and widely adopted conventional conservation strategies such as (i) *in-situ* conservation, (ii) *ex-situ* conservation, (iii) *in-vitro* conservation (iv) cryopreservation and (v) DNA banking are described below.

## *IN-SITU* CONSERVATION

In *situ* conservation is defined as the conservation of plants in their original habitats, and the planned area serves as the home land of the plant species. Thus, it should be protected from all destructive and disturbing activities. The area may be well protected by fencings to prevent grassing, cutting of trees and exhaustive utilization, etc. The major benefit of *in situ* conservation, it permits continued activities evolutionary forces like mutation, natural selection and population structuring etc., thereby promoting free evolution of the species. Accordingly, efforts have now been made to collect information on the location of availability of mulberry genetic resources with details on the "declared protected area network of India" including biosphere reserves, national parks and botanical gardens, wild life sanctuaries etc. [19]. The important *in situ* conservations are:-

## HABITAT CONSERVATION

Many species survive and best perpetuate only in their own niche or microclimate available in the wild habitat itself. The advantages of such approach are that it does not require detailed knowledge of the flora available in that habitat. The Namdapha Biosphere Reserve of India in Arunachal Pradesh for Coptis teeta, Demabeyang Valley in North Sikkim for Panax pseudo ginseng and Nanda Devi Biosphere for Rhododendron, pine etc. including Morus serrata, which is available in that area.

## BIOSPHERE RESERVES

The biosphere reserve establishes a balanced relationship between human and biosphere. The biosphere is the site of excellence for foster economic and human development, which is a socio-culturally and ecologically sustainable with a logistic support for demonstration projects, environmental education and training cum research. Considering the importance of mulberry as potential resources for employment in the rural areas of India, identified 14 biosphere reserves in the country by the national committee on environmental planning and coordination (NCEPL) and United Nations Educational, Scientific and Cultural Organization (UNESCO), among them territories like Kaziranga, Manas, Nandadevi, Namdapha, Nokrek, North Andaman, Great Nicobar and Uttarkhand [18] for *in situ* conservation of mulberry. In Canada, red mulberry has been declared as the most endangered tree species by the committee on status of endangered wildlife in Canada (COSEWIC). Red mulberry is also listed as "threatened" or "rare" in three northern United states. Efforts to conserve this species have received strong support from land managers and naturalists and a recovery plan has been developed for the species M. rubra in Hamilton's Royal Botanical Gardens, Ball's Falls Conservation Area, Niagara Glen, Rondeau Provincial Park, Point Pelee National Park, Fish Point Provincial Nature Reserve, Pelee Island, Middle Island and East Sister Island.

## GENE SANCTUARIES/NATIONAL PARKS

The significant approaches for *in situ* conservation are declaring the highly rich communities and habitats under protected areas. The protected area can be easily maintained with the help of communities residing in that area. India has one of the largest networks of protected areas including 81 National parks, 441 wild life sanctuaries and 31 wetland and mangroves providing in-situ conservation of many plant species including Morus species. Five of the protected areas have been designated as world's Heritage Sites under UNESCO's World Heritage Programme namely Kaziranga National Park, Keoladeo Ghana national park, Manas wild life Sanctuary, Nanda Devi National Park and Sunderban National Park.

## SACRED GROOVES

From time immemorial plant are worshiped like Gods or their blessings in India. Many tribal communities live in complete harmony with nature; their feeling to cut a plant might cause evil effects on their family. These communities have developed their own system of conservation forests or habitats by naming sacred grooves. These are untouched virgin forests with a taboo that even taking a dead wood or fallen fruits may cause harm to the person. There exist more than 500 sacred grooves in the tribal inhabited area of North Eastern region; Maharashtra, Western Ghats, Nilgiri, Orissa and Uttarakhand, Khasi and Jaintia hills and Baster area in Central India are rich in such diversity. Morus serrata is being worshiped in Uttarakhand; Himachal Pradesh as sacred grooves and don't uses its wood leaves fruits etc. The oldest mulberry tree of about 1200 years old is being worshiped in Joshimath of Uttarakhand states [20].

## *EX- SITU* CONSERVATION

*Ex-situ* conservation is defined as the conservation of the genetic resources outside of their original habitats, conservation of plants in botanical gardens, experimental stations, research institute, on-farm conservation by farmers with traditional agricultural systems, nursery or home garden or field gene banks are all come under the kinds of ex-situ conservation. Previously, arboreta and botanical gardens play significant roles in collection and conservation of wild species [21]. Although, conservation of seeds at low temperature is the most ideal method of germplasm conservation, clonally propagated crops

like mulberry with long juvenile period and high genetic heterozygosity, conservation of the germplasm accessions through preservation of seeds is not practiced as seeds do not replicate the true genetic constitution of the plant. Therefore, mulberry genetic resources are conserved mainly through preserving vegetative parts in a viable form or maintenance of the whole plant either in the field or using both ways. Ex-situ field gene banks are developed through planting of stem cuttings/saplings or by grafting the buds on appropriate rootstocks. The ideal genetic resource conservation programs generally have an active collection of germplasm that is used for evaluation of accessions for economic traits and distribution of genetic resources for breeders and other research groups, and a base collection used exclusively for the purpose of long-term preservation. In order to have more security on the genetic resources, duplicates of base collections are usually kept in geographically diverse locations.

## *EX-SITU* FIELD GENE BANK MANAGEMENT IN INDIA

The management of collections is important, as it has to be preserved for posterity. The utility of germplasm is only realized if the germplasm stored in, has been clearly defined, evaluated, characterized scientifically and documented [22]. The development of an effective data management, therefore also forms gene bank manager's responsibility. Several countries/ research institutes conserve mulberry germplasm in the field gene bank as ex-situ conservation and pruning followed once in a year for conservation and twice for recording data.

## ON-FARM PARTICIPATORY CONSERVATION

The other form of conservation is the on-farm conservation linked with Farmers Participatory Breeding (FPB), which gives special emphasis on sustaining and utilizing on-farm biodiversity by the farmers. In India, rich Morus diversity exists under managed habitats i.e. in the backyards, kitchen gardens, farmhouses, horticultural gardens, agricultural lands and roadside plantations. These are the first hand selections of the farmers and rural folks for varied utilizations. Therefore, conservation of these potentially useful genetic resources is being promoted in most of the sericulturally important countries. In mulberry, the wild species like M. laevigata, M. serrata, M. tartarica, M. cathayana and many others do not get much attention in the formal sector for cultivation for sericulture purposes. However, these wild species have been used for other non-sericultural purposes such as horticulture and agro forestry. Farmers/aboriginals largely use fruits and timbers of these species as a livelihood. Thus, the biodiversity of these species are conserved through the on-farm participation of aboriginals and farmers.

## BOTANICAL GARDEN/HERBARIA

Botanical gardens are being maintained from thousands of years when herbal doctors, healers, sages maintained gardens of their own for medicinal plants. Even today, one can notice many ashrams in and around Haridwar, Rishikesh (Uttarakhand) and Himalayan region where medicinal plants have been maintained since ages including mulberry. The major botanical gardens in India are Tropical Botanical Garden and Research Institutes (TBGRI), Thiruvananthapuram (Kerala); Medicinal and Aromatic Plant Garden and Herbarium Pune, Lal Bagh Botanical Garden (LBG), Bangalore; Royal Botanical Garden (RBG), Kolkata; Llyod Botanical Gardens (BG), Darjeeling (West Bengal). The Govt. of India and Provincial Govt. together run and maintain 33 Botanical gardens, which maintain the diversity in the form of plants or plant population. Biodiversity has also been preserved in the form of Herbarium specimens. Botanical Survey of India (BSI) has the largest holding of 1500000 specimens. There are many more herbaria in India maintained in different Research Institutes where Morus species is also stored. The National Germplasm on mulberry also maintained more than 1000 samples of different Morus species.

## ARBORETA

Traditionally arboreta have been regarded as assemblages of tree for scientific purposes generally with some sort of economic imperative in the not too distant back ground. The arboreta and botanical gardens had more utilitarian purpose-to find species that would benefit the new colonies and to establish which trees would provide wood, fruit, foliage for future needs. Community can get use of such arboretum in different ways. The arboreta help in maintaining genetic conservation of valuable rare and endangered trees. Arboreta can reflect changing values in society where they are becoming something for all to enjoy rather than the domain of select few and serve the purpose of society.

## BIOTECHNOLOGICAL TOOLS FOR CONSERVATION OF MULBERRY GENETIC RESOURCES

With the advent of biotechnology, the conservation strategies for plant genetic resources undergone significant changes as it facilitated conservation of genetic resources more economically with fewer genetic changes, especially for those which are propagated through vegetative mans. The following advanced conservation technologies have been adopted for mulberry.

## *IN VITRO* CONSERVATION

Mulberry being a vegetatively propagated plant is conserved under *in-vitro* conditions by culturing shot-tips and axillary buds. Somatic embryogenesis and organogenesis from callus obtained from different explants could also be conserved. Some of the major advantages of this technique is to conserve and propagate pathogen free plants as under sterile conditions the plants multiplied would be free of pathogens [23] and also the plants can be multiplied irrespective of the weather conditions.. Since all the regenerants originating from cloning are identical to the mother plant, a small piece of plant material can be considered as a germplasm and it can be used for conservation. Two types of conservation strategies have been used mulberry such as the slow growth method and the cryopreservation method. In slow growth method, shoot tips or axillary buds are cultured under conditions which keep the plant alive but retard the growth significantly. In general, shoot tips and nodal segments of juvenile shoots are used for micropropagation in mulberry as

they limits the incidence of contaminations [24], besides, Embryos, hypocotyls, cotyledons and leaf tips are also be used as explants for micropropagation [25,26]. The most widely used medium is the MS medium [27] and other media like AE [28], B5 [29] and *woody plant medium* (WPM) [30] and growth hormones such as 2mg L-1 BAP, NAA, IAA, IBA, 2,4-D alone or in various combinations [31]. However, for long term preservation of genetic materials slow growth method is not useful as it required repeated regeneration and may suffer from genetic changes and contaminations from pathogens [32]. Thus for long term preservation cryopreservation methods are used.

## CRYOPRESERVATION OF DORMANT BUDS

Cryopreservation involves storage of plant material at ultra-low temperatures in liquid nitrogen (-196°C). At this temperature, cell division and metabolic activities remain suspended and the material can be stored without changes for long periods. It also takes very little space for storage of large number of genetic resources. In mulberry the ideal plant part for cryopreservation was found to be winter buds, though embryonic axes, pollen, synthetic seeds have also been used [33-37]. There two type of cryopreservation techniques suitable for mulberry. Under the conventional method, cryopreservation fo material is achieved by slow cooling down of the material at a controlled rate (usually 0.1-4°C/min) down to about –40°C, followed by rapid immersion of samples in liquid nitrogen. It is operationally complex and requires sophisticated and expensive programmable freezers. On the other hand, the new method called vitrification procedure, the cell dehydration is achieved by subjecting the samples to physical or osmotic dehydration. This is followed by ultra-rapid freezing which results in vitrification of intracellular solutes, i.e. formation of an amorphous glassy structure without occurrence of ice crystals, which are detrimental to cellular structural integrity. These techniques are less complex and do not require a programmable freezer, hence are suited for use in any laboratory with basic facilities for tissue culture [38]. preserved 908 mulberry germplasm accessions belonging to *M. indica, M. alba, M. latfolia, M. cathayana, M. laevigata, M. nigra, M. australis, M. bombycis, M. sinensis,* M *multicaulis and M. rotundioba.*

## DNA BANKING

Another method of genetic resource preservation is the DNA banking wherein the genetic information encoded in the genome of the plant conserved for long by isolating the nucleic acid and preserving it in -80°C in deep freezers. Mulberry genomic DNA can be extracted from leaf samples using different routinely used techniques [39]. Although the pure DNA can be stored in several ways, the most commonly used method is dissolving the DNA in Tris TE buffer (10 mM Tris-HCl and 1.0 M EDTA) and storing at -80°C in deep freezers. The purified DNA can also be stored in alcohol indefinitely as DNA is highly stable and it is estimated that a fully hydrated DNA can be kept at room temperature about 10,000 years to depolymerise into small fragments [40]. Lyophilized DNA (dried) can also be stored indefinitely without any damage. Since DNA can be stored in small vials, it is presumed that the genetic resources of all the plant species in the world can be stored in a single building [41]. An alternative to preservation of isolated DNA is direct storage of cells and tissues under low temperature (-80°C). This method has the advantage of providing a continuous supply of materials for extraction of DNA and also provides materials for biochemical and molecular studies. DNA banking is considered as the most economical way of conserving the plant genetic resources (Table 2).

| *In situ* | *Ex situ* | *In vitro* | **DNA banking** |
|---|---|---|---|
| Apt for forest species and wild crop relatives | Only option for the asexually reproducing plants | Suitable for both sexually and asexually reproducing plants | Suitable for both sexually and asexually reproducing plants |
| Field oriented, laborious and expensive | Field oriented, laborious and expensive | Laboratory oriented minimum space and less laborious | Laboratory oriented minimum space and less laborious |
| Allows evolution to continue | Evolution restricted | No chance of evolution | No chance of evolution |
| Increases genetic diversity. | Less prone to genetic variability | No genetic variation | No genetic variation |
| Vulnerable to disease and other natural calamities | Vulnerable to disease and other natural calamities | Well protected against disease and other natural calamities | Well protected against disease and other natural calamities |
| Strengthens the link between conservationists and local people who traditionally maintain the plant | Minimum interactions | No interactions | No interactions |
| Exchange of materials is difficult | Exchange of materials possible but needs extra care | Easy exchange of materials | Easy exchange of materials |

**Table 2:** Merits and demerits of different methods used for conserving the genetic resources of mulberry (Source: Vijayan et al. [18]).

### USE OF MOLECULAR MARKERS FOR CHARACTERIZATION OF GENETIC RESOURCES FOR BETTER CONSERVATION AND UTILIZATION

Since mulberry is a highly heterozygous perennial tree with long juvenile period and expression of most of the economically important traits are under the influence of environmental and stages of growth and development, absolute dependence on these traits for characterization and evaluation of the genetic

resources is highly laborious, time taking and information generated may vary greatly between locations and plant with different ages. Thus, it was imperative to adopt the modern biotechnological tools to characterize and evaluate them for proper conservation and utilization. Unlike phenotypic traits, molecular markers enjoy the advantages of stability, independence of environmental factors and age of the plant, availability in plenty and are mostly of objective rather than subjective. Thus, different types of molecular markers have been used for germplasm characterization, identification of duplicated, identification of unique genes and alleles, unraveling of genetic diversity among accessions of both domesticated and wild species. Application of DNA finger printing in mulberry was initiated in Japan [42,43]. RAPD was the first DNA based marker used in mulberry, using which the genetic diversity among a few cultivars was initially tested [44-47]. Later, several researchers used RAPD markers for assessing the interspecific and the intraspecific relationships in mulberry [48-51]. However, considering the inconsistency in the result from RAPD markers, even in the same laboratory, attempts were made to explore other DNA markers in mulberry. ISSR markers were used to estimate the genetic diversity among indigenous cultivars of M. alba and M. indica [52-58] and among different ecotypes of China [59]. Relationships between temperate and tropical mulberry species [39] and interspecific variability [55] were also investigated in detail using ISSR and RAPD markers. Sharma et al. [13] used AFLP for the first time to elucidate the interrelationships of different mulberry species. In the meantime, efforts were made to develop microsatellite primers for mulberry. Accordingly, researchers developed several SSR primers for mulberry, using these SSR primers, able to discriminate the wild genetic resources from the domesticated ones [60,61]. Wild species such as M. laevigata, M. cathayana, M. nigra, M. mongolica and M. wittiorum grouped together into a cluster separated from the cluster formed by M. alba and other cultivated varieties. At present, a number of molecular markers such as RAPD, ISSR, AFLP, SSR, EST and DNA sequences from both nuclear and plastid genes are used for elucidating genetic diversity among cultivars of the same species, interspecific variations, relationships among geographically divergent species and cultivars. Diversity analysis with these DNA based markers is of much help to define core collections, which provides a user-friendly entry point for breeders to access large and varied germplasm collections, and to identify duplicates in the germplasm accessions. The genetic relationships between cultivated and wild mulberry species was also investigated using molecular markers [62] and found that M. laevigata is closer to M. indica and M. alba than M. serrata. In a related investigation, the internal transcribed spacers of nuclear ribosomal RNA (nrITS) and the cpDNA gene (trnL-F) were used to elucidate the phylogenetic relationships among different species of mulberry [63]. This study, further, confirmed the closer relationship of M. laevigata with M. alba. These studies generated significant information to help plan breeding strategies to utilize the wild genetic resources of mulberry for the crop improvement in mulberry. Recently, whole genome of mulberry was sequenced to find out gene sequences and molecular markers, [64,65]. The whole genome sequencing has significant impact on characterization of germplasm as illustrated with 134 mulberry accessions by Jiao et al [65]. Using the newly identified 14,273,912 high-quality SNPs, the phylogenetic relationship among 132 cultivars using 2 wild mulberry genotypes was assessed and found that the phylogenetic tree based on whole-genomic SNPs was not in consistent with the traditional delimitations of mulberry species. Likewise, genome-wide 2229 SNPs were used for assessing the genetic relationship among 54 mulberry accessions from seven species viz., M. alba, M. indica, M. bombycis, M. acidosa, M. latifolia, M. kagayamae, and M. rotundiloba [66,67] and found that only three species could group into monophyletic clades viz, M. acidosa M. kagayamae and M. rotundiloba, while all other species formed non-monophyletic groups indicating admixture among them through natural hybridizations. Thus, the classification of the genus Morus is not an easy task even with genome-wide DNA markers. Besides exposing the inefficiency of the current species delimitations of the genus Morus, the above studies also brought out the usefulness of the whole genome sequencing and DNA markers for germplasm characterization and crop improvement.

## CONCLUSION

Plant Genetic Resources (PGR) is highly valuable for the current and future research for utilization and maintenance of sustainable food production for the benefit of human growing population. They have been used worldwide for genetic improvement of crops and contributed to major increases in crop productivity and resistance to pests, diseases and adverse climatic growing conditions. The mulberry germplasm resources are the building blocks/gene pool for genetic improvement/enhancement; genes for adaptation, endurance for biotic, abiotic stresses/environments; to develop high yielding varieties; to tackle pest and disease management; to reduce dependency on external inputs and conserved future use and posterity. The efficient utilization depends largely on appropriate characterization, evaluation, documentation and conservation of the mulberry genetic resources using both by conventional and biotechnological approaches. Biotechnology has been applied for better conservation and utilization of genetic resources through mass *in-vitro* clonal propagation, genetic diversity analysis to identify promising parents, speeding up of breeding through indemnification and selection of potential progenies, elimination of pathogens for safe distribution and conservation of germplasm. Thus, sericulture in general and mulberry cultivation in particular got much benefit from modern biotechnology to boost the productivity.

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