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Mulberry advances in plant tissue culture and molecular biology-A review

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Abstract
Mulberry is the most economical species in sericulture industry. The leaves of mulberry are used for silk production. It also has many other medicinal values present in it such as anti-inflammatory, antidiabetic, anticancer, and many more. Whereas, its other parts are also useful in making paper, furniture, textile, sculptures, medicines and musical instruments (Jang *et al*., 2002). So, conservation of this elite germplasm is mandatory. So, different kind of approaches has been reached out to maintain the broad genetic base and mass production of this species via conventional and biotechnological tools. To keep increasing the qualitative and quantitative value of this tree species.

Keywords: Plant tissue culture, mulberry, micropropagation, molecular biology

Introduction

Morus alba l. (mulberry) is a woody perennial deciduous tree species that belongs to the order Rosales and the family Moraceae. The plant is known by various names in different languages: English: silkworm mulberry, white mulberry; Sanskrit: Tula; Hindi: Shah Toot; Malayalam: Malbari; Tamil: kamblichedi; other: kiskuri, musukotta (Orwa *et al*. 2009) [6].

The history of this species traces back to China, then extends to other parts of the world such as Europe, Japan, and North America, including India. It is believed that mulberry originated in the northern hemisphere, particularly in the Himalayan foothills, and spread to the tropics of the southern hemisphere (Benavides *et al*., 1994). The white mulberry has a wide range of usefulness, which includes primarily silk production by its leaves for silk-producing insects (*Bombyx mori L.)* and ethnomedicinal uses. Apart from this, it also has phytochemical constituents, which are of great importance. Mulberry fruit is a traditional medicine for hypoglycemia, dysentery, and constipation (Lee *et al*., 2011), and it is bestowed with phenolic acids and flavonoids (Arfan *et al*., 2012). The white mulberry is versatile both commercially and economically. More than 68 species of Morus have been widely recognised (Datta, 2000), of which M. alba, M. latifolia, and M. mutlicaulis are grown for leaves, while M. nigra is grown for fruit and M. serrata for timber (Vijayan *et al*., 2011a) [4]. As mentioned earlier, the white mulberry is a lucrative species. So, it raises a concern about sustaining the species for further advancement and use. But the variation in Shah toot (mulberry) can be found in different zones around the world depending on the phenotype of it. Which occur due to the environment and genotype influence, as phenotype=genotype + environment. No two trees can have the same phenotype completely; there is a colossal amount of phenotypic variation. So, the traditional method and biotechnological tools are two modus operandi by which one can approach expanding further to improve the species quantity and quality as later methods become more feasible and attainable.

Morphology

Kingdom: plantae
Class: angiosperms
Subclass: eudicots
Order: Rosales
Family: Moraceae
Genus: Morus
Species: white mulberry

Silkworm mulberry is a ring porous woody perennial rapid growing deciduous flowering tree species.

The tut can obtain a height around 6-9 meter. The crown of a tree is condensed and dropping downwards branches with dense foliage. Leaves vary tremendously, ovate or broadly ovate, distichous, simple to 3- lobed, dentate, palmately 3-veined at base; stipules lateral, caducous, coriaceous (Orwa *et al*.2009) [6]. musukotta forms cylindrical and straight trunk the bark of a tree consists of a slender squamous ridges and colour vary from greyish to brown sometimes appears taupe without buttress formation. fruit a syncarp, consisting of several drupes encircled in a fleshy perianth up to 5 cm long; white, pinkish- white, purple or black (Orwa *et al*. 2009) [6]. The Mulberry time of flowering is from October-February; seeds can be collected in a phase of April-May, weight of mulberry seed is (455 seeds/gm) can vary not only from tree to tree in the same species but also on the same tree and dispersal of seed is done via birds(ornithophily) as the seeds are recalcitrant in nature. The method of planting is naked roots with suitable size(height) of 1 meter and approximate age of 9 months.

The silvicultural characteristics of a tree follows such as Morus alba is a light demander in addition to drought sensitive and frost tender species which can coppice easily.

Conventional method

Mulberry is one of the most important tree species, and growing it with the help of vegetative propagation techniques is a cumbersome process. The method used in this long-generation plant is stem cutting. However, the success of this method depends upon various biotic and abiotic factors like age, genetic makeup, and the

environmental condition of the plant. Additionally, newly developed mulberry varieties cannot be immediately propagated through stem cuttings, as at least 6–7 months of maturity are required before cuttings can be isolated from the parental plant (Kapur *et al*., 2001). Which makes it tedious, time-consuming, and labour-intensive. So, moving towards a biotechnological approach is much more suitable.

Biotechnological tools

Mulberry is of great importance as mentioned earlier. Almost all the parts of the plants contain beneficial bioactive compounds. The mulberry shows high heterozygosity and improving it via conventional approach is very difficult. So, shifting towards modern biotechnological tools such as plant tissue culture and molecular biology can yield great genetic improvement.

Plant tissue culture

Tissue culture is a term used for asexual *in vitro* propagation or clonal propagation. in mulberry different areas have been explored and improvised such as micropropagation, callus culture, somatic embryogenesis, organogenesis and many more. Plant tissue culture Simply put, if a portion of the plant body is divided into a small portion known as an explant, that portion can be developed into a complete plant. *In vitro*, the explant demonstrates a very high degree of flexibility, allowing it to evolve into another kind and therefore regenerate an entire new plant.

The future perspective of plant tissue culture has been adjusted in recent decades to promote plant growth, biological activity, transformation, and secondary metabolite production due to development and a desire to grow on a large scale. A considerable advancement in strategies has been sought to deal with the problem of low concentrations of secondary metabolites in whole plants. The sterile plantlets will overcome the contamination problem and shorten the sterilisation process. Secondary metabolites and medicinally relevant chemicals have been found to propagate *in vitro* quite effectively for selective metabolite formation.

Micropropagation in mulberry

Many attempts have been made to improve the quality of tree species via micropropagation, mass scale production can help in the study of genetic improvement, morphological characteristics and also contamination free production of plants. Mulberry *in vitro* regeneration has been attempted with many degrees of success. Since there are variations in regeneration among mulberry varieties (Bhau and Wakhlu, 2003; Rao *et al*., 2010). Many targeted attempts have been made for standardization of mulberry *in vitro* regeneration protocol. Mulberry is a recalcitrant species in terms of tissue culture, and shoot regeneration is greatly dependent on the genotype, type of explant and combination of growth regulator used in the culture media (Feyissa *et al*., 2005). Different kind of explants are used for the micropropagation of morus species such as shoot apices (Ivani~ka, J., 1987), buds (Attia, Sdessoky, El-Hallous & Shaaban., 2014) [11], leaf (Kapur *et al*., 2001; Vijaya Chitra and Padmaja, 2005), nodal segments (Yadav *et al*., 1990; Vijaya Chitra and Padmaja, 1999). A wide variation of success has been observed in mulberry *in vitro* regeneration.

Molecular biology approach

The study of plants at the DNA, RNA, and protein levels is known as plant molecular biology. Molecular or genomic level study requires the isolation of pure DNA as it is the most challenging factor for molecular biologist while dealing with plants. Plants can be studied using advanced molecular biotechnology techniques to learn about their unique properties. Once this is recognised, plants can be genetically modified to produce 'designer plants' (i.e., GMOs) that are tailored to certain requirements. The high genetic variety seen in plant populations allows for the discovery of DNA changes (i.e., genetic mutations) that are responsible for quantitative and qualitative variation in traits of interest, providing knowledge that will be valuable in plant breeding efforts.

In recent advance methods biotechnological aspect has come out to be the most reliable methods in scientific society. Mulberry, as a tree with high heterozygosity, makes it challenging to improve economically important features through conventional breeding. Selection and breeding Environmentally less impacted and developmentally stable molecular markers provide breeders with dependable tools for characterising germplasm and selecting parents and offspring via marker-assisted selection. As a result, it would be sensible to employ biotechnological technologies to maximise the tremendous benefits that mulberry provides to humanity.

Mulberry molecular study

Genomic study requires to isolate the pure quality of DNA foremost. As, high quality DNA from mulberry leaves containing higher amounts of polysaccharides, polyphenols, resins, and other viscous contaminants (Suraksha Chanotra *et al*. 2019). Which hinders the extraction of pure quality of DNA. The procedures for the extraction of plant DNA are modified continuously to get quality DNA (H. Jingade Anuradha *et al*. 2013). Molecular markers are foremost important for the study of genetic constituents, molecular marker applications used in mulberry includes, SSR Genetic diversity among mulberry genotypes maintained in temperate climatic conditions in India (Wani *et al*., 2013); SSR, RAPD,

ISSR Testing the quality of genomic DNA extracted with a new protocol (Anuradha *et al*., 2013); RAPD Used for estimating the genetic diversity of EMS treated mulberry

Genotypes (Anilkumar *et al*., 2012); RAPD Worked out the relationship among 47

mulberry genotypes (Ozrenk *et al*., 2010). This all studies shows that latest biotechnologies tools are useful for the study of mulberry at molecular level.

Conclusion

Mulberry biotechnology has improved significantly in areas such as tissue culture and molecular biology and has contributed to micropropagation. Isolation of somaclonal variants, screening of germplasm for stress tolerance, level of polyploids, production of synthetic seeds, cryopreservation of genetic resources, development of transgenic plants, characterization of germplasm accessions, and identification of markers associated with economically important traits. Regarding the molecular marker systems, only a few SSR primers are still available for use. These few primers are not enough to make saturated linkage maps to identify QTLs tightly linked to economically important traits.

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