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REVIEW ARTICLE

MODERN PLANT BREEDING TECHNIQUES IN MULBERRY CROP IMPROVEMENT

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ABSTRACT

Mulberry is a perennial plant that maintains high heterozygosity due to the out breeding reproductive system. It is recalcitrant to most of the conventional breeding methods to overcome of this problem non-conventional breeding methods such as polyploidy, endosperm culture, micro propagation and induction of mutations too have some long- range advantages such as showing better performance over the original parental characters with passage of time and for the production of good qualitative and quantitative characteristics of mulberry leaf along with the vision of overcoming biotic and abiotic stress. In- vitro techniques such as tissue and organ culture offer the plant breeder new openings in the colonel propagation, genetic manipulation and production of homozygous inbred lines to improve the nutritive value in Mulberry leaves.

Key words: Heterozygosity, Mulberry.

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INTRODUCTION

Mulberry leaf is food for Mulberry silkworm Bombyxmori (L), to improve the nutritional status of the plant non-conventional breeding attempts are being made i.e. breeding, hybridization, tissue culture for the production of nutritive Mulberry leaf. In-vitro techniques such as tissue and organ culture offer the plant breeder wider role in the colonel propagation, genetic manipulation and production of homozygous inbred lines to improve the nutritive value in Mulberry leave. This article enumerates the available nonconventional breeding methods for mulberry improvement.

Evaluation of haploid through pollen culture in mulberry

Production of haploid plants is the easiest and most rapid method for producing those homozygous lines. Its use in Morusspp was first reported by Shoukang *et al*. (1987). MS media with the supplemental hormones in the media play a very important role in determining the success of culturing. When BA and IBA were added simultaneously, the maximum rate of induction was 13.6%. Anthers harvested at the mid-uninucleate stage showed better frequencies of induction (22 to 24%) for embryoids than did those taken at the early- and late-uni nucleate stages. Treatment with low temperatures did not prompt any induction. Differentiation in the embryoids was best when the concentration of auxin was fairly reduced and that of cytokinin was slightly increased. Tewary *et al.* (1994) also cultured Morus pollen in vitro. Pollen were isolated from inflorescences that were starved for periods ranging from 24 h to 10 d. afterward, the pollen suspensions were cultured in drops on MS media.

Evaluation of haploid through Ovule culture in mulberry

Culturing unfertilized ovule/ovary/flower buds, and then inducing a haploid cell of the female gamete (egg or synergid) to develop into an apogamous sporophyte. For example, Lakshmi Sita and Ravindran (1991) were able to produce gynogenic plants from ovary cultures of *M. indica*. Individual ovaries, harvested either before or after fusion to form sorosis, were cultured on MS media supplemented with growth regulators. Thomas *et al*. (1999). They adopted an approach of obtaining haploid plants by culturing unpollinated ovaries. Segments from in vitro-developed inflorescences were cultured. After three weeks, individual ovaries were transferred to the same medium supplemented with various growth regulators. On an MS medium fortified with 4.5 pM 2, 4-D, 500 mg/L glycine, and 200 mg/L proline, ovaries developed gynogenic seedlings. Out of the 20 plantlets analyze dcytological for ploidy, 12 were haploids, the other 8,

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aneuploids. The gynogenic plants were then established in soil (Thomas, 1999).

Induction of polyploidy through colchicine treatment in mulberry

Mulberry bushes were given middle pruning to hasten the sprouting of axillary buds. Five different concentrations of aqueous colchicine (C22H25NO5) viz., 0.1%-0.5% were used to treat the vegetative buds. Selected buds were thoroughly washed in distilled water before the application of colchicine. Buds were covered with cotton swabs and colchicine solution was applied from 8am-5pm for three consecutive days at an interval of one hour and control buds were treated with distilled water. Cotton swabs were removed after the treatment and buds were thoroughly washed in distilled water. Buds were allowed to grow by providing required agricultural inputs. Untreated axillary buds situated on the treated portion of shoots were removed periodically so as to maintain only treated axillary buds. Some triploid viz., Tr-10, Tr-8 and Tr-4 verities developed through Colchicine treatment to auxiliary buds.

Production of triploids through Hybridization

Hybridization between one parent with unreduced gametes (2n gametes) and another diploid parent (n gamete) is the typical way to triploid formation. The female parent with unreduced gametes plays a particularly important role in triploid plant formation (Ramsey & Schemske 1998). These various triploid formation mechanisms result in different levels of offspring fertility and phenotypes (Lu *et al.* 2013). Triploid plants can be recovered by 2x × 2x, 2x × 4xs, 4x × 2x or 2x × 3x sexual hybridization. Most conventional methodology is from 2x × 4x and 4x × 2x hybridization (Ji*et al.* 2013).

Endosperm culture in vitro

As endosperm is a triploid tissue, it would be reasonable to assume that natural triploids could be successfully regenerated plants from endosperm tissues. The first attempts at endosperm culture in vitro took place in the 1930s (Lampe & Mills 1936).Genotype, sampling times, and culture media are important aspects of endosperm culture systems. First, the efficiency of endosperm response has been found to be genotype-dependent in many species (Popielarska-Konieczna *et al.* 2013).

In vitro induction of tetraploidy in mulberry

Apical buds (3–5 mm) excised from in vitro-grown plants and cultured on MS medium containing BA (8.8 μM) with four concentrations of colchicine (0.0, 0.05, 0.1 and 0.2%). The buds were kept on this medium for 24 h under a 16-h irradiance of 45 μmol m–2 s –1 in the culture room at 25°±1°C. After 24 h of treatment the buds were transferred to MS medium containing BA (8.8 μM) without colchicine. These were transferred on day 30 to fresh medium. For root induction, the growing micro cuttings were transferred to MS medium containing 2.6 μM NAA on the 60th day. For cytological confirmation of tetraploids, shoot tips of normal and tetraploid plants were pretreated separately with a saturated solution of paradichloro benzene (PDB) for 3 h. The pretreated shoot tips after washing were fixed in 1:3 proprionic-ethanol solution overnight. Staining was done with proprionic-orcein (2%) and squashed in 45% proprionic acid. Somatic chromosomes were studied using temporary slides. Leaf size and thickness were measured from mature leaves. Stomatal frequency and size and number of chloroplasts per stoma of mature leaf were studied by fixation in 1:3 acetic-ethanol solution followed by staining in a 2% KI3 solution for 1 min.

Colchicine Induced Tetraploids of Mulberry

Bud treatment: Cuttings of *M. indica* L. were planted in earthen pots and all but the terminal bud were removed. When buds started sprouting, they were treated with 0.1, 0.2 and 0.4% aqueous solution of colchicine each for 2, 3, 4, 5 and 6 hours daily for three consecutive days. The bud was wrapped with a piece of cotton and colchicine solution was given from a glass dropper from time to keep it moist After the treatment cotton was removed and the bud was washed with distilled water.

Mutation breeding in mulberry: Mulberry exhibits plasticity and is a versatile plant. Successful exploitation of various mutagenic agents for inducing aberration has become one of the most important lines of contemporary research. Mutation induction techniques such as radiation or chemical mutagens are good tools for increasing variability in crop species because spontaneous mutations occur with an extremely low frequency. Mutation techniques have significantly contributed to plant improvement worldwide and have made an outstanding impact on the productivity and economic value of some crops. Mutation breeding has been widely employed in recent times for improving vegetatively propagated crop plants and mutation breeding employing radiation (X-rays, Gamma rays, Fast neutrons, etc.) was used to explore the feasibility of developing new varieties and gamma rays have been proved to be highly potent in inducing variability in mulberry plant. Verities $\mathbf{S_{30}}$, $\mathbf{S_{36}}$, $\mathbf{S_{41}}$and S_{54} are released through mutation breeding in mulberry.

Somatic hybridization: The protoplasts of different plant species fuse together to form hybrids is known as somatic hybridisation and the hybrids so produced is known as somatic hybrids. Somatic hybridization through protoplast fusion has opened a new avenue for developing new characteristics, which are not possible through conventional breeding .There are only a few reports dealing with plant regeneration from protoplasts in mulberry (Tewary and Sita, 1992; Umate et al., 2005). A combination of 2% cellulase, 1% macerozyme and 0.5% macerase is found optimal for better isolation of viable protoplast. Protoplast fusion in mulberry was successfully achieved using chemical fusogen (Ohinshi and Kiyama, 1987) and electro-fusion (Ohinshi and Tanabe, 1989). Although protoplast isolation and regeneration was achieved, development of somatic hybrids in mulberry could not be achieved. Hence, efforts in this end need to be continued.

Micropropagation in Mulberry: Success of propagation through stem cuttings, grafting or budding in mulberry depends on a number of factors such as genetic makeup of the plant, age and physiological conditions of the parental cutting, climatic conditions and cultural practices. Furthermore, newly developed mulberry varieties cannot be immediately propagated through stem cuttings as at least 6-7 months of maturity is required before cuttings can be isolated from the parental plant (Kapur et al., 2001). Micro propagation on the

other hand, allows multiplication of the plant in a short period under the controlled conditions. Further, in conventional method of propagation through stem cuttings, each stem cutting produces only one plant, whereas in micro propagation thousands of plants can be produced from a single plant piece (explant). Moreover micro propagation can provide plantlets throughout the year irrespective of seasonal variations. Thus, micro propagation is an efficient and cost effective method for rapid multiplication of mulberry in a relatively short time and limited space. Micro propagation also facilitates production of virus free plants from the apical meristematic tissues. Ohyama (1970) initiated mulberry micro propagation by regenerating whole plants from axillary buds of *M. alba*. Later, a number of investigators used different media and explants. Shoot tips and dormant axillary buds were found suitable for mulberry micro propagation.

Agrobacterium-mediated gene transfer: various vectors used in plant transformation, the Ti plasmid of Agro bacterium tumefactions has been widely used. This bacterium is known as “natural genetic engineer” of plants because it has natural ability to transfer T-DNA of its plasmids into plant genome upon infection of cells at the wound site and causes an unorganized growth of a cell mass known as crown gall. Ti plasmids are used as gene vectors for delivering useful foreign genes into target plant cells and tissues. The foreign gene is cloned in the T-DNA region of Ti plasmid. To transfer the foreign DNA in host plants, leaf discs, embryo genic callus or other suitable explants are collected and infected with Agro bacterium carrying recombinant disarmed Ti plasmid vector. In general, briefly the vector tissue is then cultured on a shoot regeneration medium for 2-3 days during which the transfer of T-DNA along with foreign genes takes place. After this, the transformed tissues are transferred onto a regeneration medium supplemented with usually lethal concentration of an antibiotic to selectively eliminate non transformed tissues. After 3-5 weeks, the regenerated shoots are transferred to root -inducing medium, root shoots are acclimatized and after 3-4 weeks, complete plants are transferred to soil. Molecular techniques like polymerase chain reaction (PCR) and Southern hybridization are used to detect the presence of foreign genes in the transgenic plants (Reviewed by Shabir *et al*., 2013).

Conclusion

Great efforts were made to study different in vitro cultivation methods for mulberry. The several of these protocols already served as useful experimental systems for cell culture and transformation studies in mulberry. Although used for tissue culture and nuclear transformation studies, the use of mulberry for chloroplast transformation has not been reported. Consequently, the next few years are likely to see large and rapid changes in mulberry breeding.

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