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Polyploidy Breeding in Mulberry

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Polyploidy is the heritable condition in which a normally diploid cell or organism acquires one or more additional sets of chromosomes. It is a kind of numerical change in a whole set of chromosomes. This phenomenon is present mainly in plants than in animals. A few animals exhibiting polyploidy are: fishes, lizards, amphibians and some insects. Polyploid types are labeled according to the number of chromosome sets in the nucleus. Polyploidy is most commonly observed in the plant kingdom. Thousands of years of selective cultivation and plant breeding have resulted in vigorous food plants that are commonly tetraploid and hexaploid. If you compare diploid and tetraploid varieties of the same type of plant, very often the tetraploid plants grow larger and more vigorously. Among animals, polyploidy is often observed in bony fish and amphibians. In general, there is a genetic bias for even ploidy numbers in animals. Polyploid is the multiplication of entire sets of chromosomes. In other words, polyploid genotype has more than two homologous sets of chromosomes in its cell. For example, tetraploid plants have four sets of chromosomes in their cells. Polyploidy is common among flowering plants (angiosperms) and is a major force in plant speciation (Grant, 1981). Almost 47%-70% of angiosperms are polyploid (Ramsey & Schemske, 1998).

Types of Polyploidy

1. **Autopolyploidy:** Autopolyploidy are also referred to as autoploids. They contain multiple copies of the basic set (x) of chromosomes of the same genome (Acquaah, 2007; Chen, 2010). Autoploids occur in nature through union of unreduced gametes and at times can be artificially induced (Chen, 2010). Natural autoploids include tetraploid crops such as alfafa, peanut, potato and coffee and triploid bananas. They occur spontaneously through the process of chromosome doubling. Chromosome doubling in autoploids has varying effect based on the species. Spontaneous chromosome doubling in ornamentals and forage grasses has led to increased vigour. For instance, ornamentals such as tulip and hyacinth, and forage grasses such as ryegrasses have yielded superior varieties following spontaneous chromosome doubling (Acquaah, 2007). Due to the observed advantages in nature, breeders have harnessed the process of chromosome doubling in vitro through induced polyploidy to produce superior crops. For example, induced autotetraploids in the watermelon crop are used for the production of seedless triploid hybrids fruits (Wehner, 2008). Such polyploids are induced through the treatment of diploids with mitotic inhibitors such as dinitroaniles and colchicine (Compton *et al.*, 1996). To determine the ploidy status of induced polyploids, several approaches may be used. These include, chloroplast count in guard cells, morphological features such as leaf, flower or pollen size (gigas effect) and flow cytometry (Brummer et al., 1999; Heping *et al.*, 2008).
2. **Allopolyploid:** Allopolyploids are also called alloploids. They are a combination of genomes from different species (Acquaah, 2007). They result from hybridization of two

or more genomes followed by chromosome doubling or by the fusion of unreduced gametes between species (Acquaah, 2007; Chen, 2010; Jones *et al*., 2008; Ramsey and Schemske, 1998). This process is key in the process of speciation for angiosperms and ferns (Chen, 2010) and occurs often in nature. Economically important natural alloploid crops include strawberry, wheat, oat, upland cotton, oilseed rape, blueberry and mustard (Acquaah, 2007; Chen, 2010).

Polyploidy In Mulberry

Sericulture is the science of rearing silkworms for the production of silk fibres. Sericulture is one of the major employment providers in India and several other Asian countries (Vijayan 2010). *Bombyx mori* can grow well only on mulberry leaves. Hence, to enhance sericulture productivity mulberry leaf production has to be increased, which can be made possible by developing new varieties with higher leaf yield and better adaptability. In asexually propagated perennial crops, where vegetative organs are of economic use, the polyploidy breeding method has been utilized successfully for their improvement. Being a vegetatively propagated perennial foliage crop with comparatively less number of chromosomes, mulberry is suitable for the induction of polyploidy for its improvement. Mulberry (*Morus* spp) is a multipurpose dioecious, heterozygous and out breeding tree. Nearly 70% of the mulberry leaf protein is converted into silk protein through biosynthesis in silkworm. Thus, mulberry leaf is the central dogma in sericulture and the increased biomass (leaves) in mulberry variety is the principal determining factor of higher cocoon yield. Thus there is an immense need for improvement of mulberry varieties in terms of nutritive value and increased biomass (leaves) to ensure profitable production of cocoon.

Many mulberry genotypes are available in nature, but all are not utilized for rearing silkworms since they lack in one or the other required beneficial trait. In case of mulberry, triploids are usually preferred over diploids and tetraploids because of the desirable traits like higher foliage yield, better silkworm palatability, better quality and better adaptability to environmental stimuli and resistance to cold and stress. It has also been found that silkworms fed with the leaves of polyploid mulberry plants resulted in higher egg production, larval weight, good cocoon harvest and longer cocoon fibre length. Triploids are usually produced by crossing diploids and artificially induced tetraploids. The artificially induced tetraploids are also used for silkworm feeding. However, they are actually used as a source of breeding material for the production of triploid varieties. Furthermore, the frequency of stomata per unit area is significantly less in triploid and tetraploid compared to diploids. Stomatal frequency is an important parameter in selecting moisture retenting varieties and drought resistant genotype (Nautiyal *et al.*, 1994). Most of the species of Morus are diploid having 28 chromosomes, but a few polyploid species, namely, *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 under natural condition (M. notabilis = 14). *M. laevigata* Wall. is a natural tetraploid occurring in the wild and in the cultivated forms in the eastern Himalayas (Datta 1954; Das 1961); its leaves are unsuitable for silkworm feeding. The occurrence of natural tetraploids of Morus has not been reported from any other part of the world other than India.

Methods of inducing polyploidy in mulberry

a. **Treatment of seeds and protocorms:** Seeds plants are either pre-soaked in aqueous solutions of colchicine and sowed in nutrient agar or sowed without pretreatment into colchicine-incorporated nutrient agar. Different aqueous colchicine concentrations for pre-treatment can be used especially in the range of 0.05 to 1.0 percent. All seeds should first be sterilized and then used for sowing. In the case of pro to corm treatments, seeds should first be sown and germinated in nutrient agar. When green protocorms are formed,

required quantities of sterilized colchicine should be swirled around in the flask each day to wet the protocorms. When seedlings become approximately 1.5 to 2 cm. high with sufficient roots and leaves, they should be removed from the flasks and transplanted into plots containing disinfected peat moss as the planting medium.

b. **Treatment of seedlings:** Seedlings about 3 to 4 cm tall are soaked in aqueous colchicine of different concentration as per requirement of the experiment. This can be done by two methods. The first method involves the immersion of seedlings for 3 hours in vials containing colchicine solution. The second method, designated as infiltration method after Braak and Zeilinga, consists of immersing the seedlings in vials containing colchicine solution and placing the vials in an exsiccator in which a vacuum is created by means of a water vacuum pump. In 7 minutes the solution began to bubble. The plants are allowed to remain in vacuum for 10 minutes. This procedure is supposed to evacuate the air from the plants and allow the solution to penetrate the tissues more readily than by soaking without the vacuum. At the end of the treatment period for both methods, plants are removed from the vials, washed under tap water, and planted in peat-moss flats for later chromosome counts.

c. **Treatment of inflorescence, cuttings, young shoots, and apical meristems of mature plants:**

i. **Treatment of inflorescence-** Immature inflorescence of the plant should be selected and the apical section of the inflorescence should be covered with absorbent cotton saturated with aqueous colchicine solution ranging in concentration as per requirement. Polyethylene bags are to be placed over the cotton to prevent drying. Duration of treatments may extend from 8 hours to 5 days. At the end of the treatments, the cotton applicators should be removed and the inflorescence is left to develop.

ii. **Treatment of tip-** Tip cuttings of the plant should be selected. The cuttings should be approximately 8 to 10 inches long, and the basal ends are then to be immersed in vials containing aqueous solutions of colchicine. Different Concentrations as per requirement can be used and the duration of treatments may range from 1 to 20 days. At the end of the treatment period, cuttings are to be planted in cutting boxes containing wood shavings as medium. The root tips for chromosome counts can be taken from the roots which will develop on new growth above the original treated apices. In some instances, apical growth after treatment may be arrested for prolonged periods and axillary shoots will emerge from the first or second node below the treated apex. In these cases, root tips can be taken from the axillary growth. In a few cases, chromosome counts can also be made from bud materials.

iii. **Treatment of young shoots-** Young shoots about 3 to 4 inches long, should either be excised at the base or with part of the parent stem still attached to the base of the young shoots. The base of these shoots or of the mature stems is immersed in colchicine solutions of various concentrations for different durations. At the end of the treatment period, each cutting is to be planted in a 5-inch clay pot for growth and further observations.

iv. **Treatment by incision of apical area-** The apical region of the plant should be incised longitudinally to expose the apical meristem, and colchicine-lanolin paste of different concentrations has to be administered into the incision with a toothpick. Controls can be treated with lanolin paste without colchicine. Sometimes, instead of using colchicine -lanolin paste, glycerin -colchicine solution can also be applied into the incisions with a camel-hair brush. To account for the possible effects of time of exposure to the colchicine and the possible drying of the solution, colchicineglycerine

solution has to be applied 2, 4, and 6 times, each application being given at 2-day interval.

v. **Treatment by injection of colchicine-** The apical regions young shoots (3 to 15 inches long) are treated by injecting aqueous-colchicine or glycerin-colchicine solution with capillary pipettes. These pipettes are made by stretching glass tubing to a thin point. the excess terminal leaves have to be cut off to facilitate the penetration of the pipette into the meristematic region between the folds of the leaf sheaths. The capillary pipettes can either be inserted or left in the plant for gradual release of the solution or 3 - 6 drops of colchicine solution should be injected and the pipette has to be removed immediately. Injections have to be given 1, 3, and 6 times at 3-day intervals (H. Y. Nakasone and H. Kamemoto, 1961).

Varieties developed by Poliploidy Breeding

1. Das *et al*. (1970) developed a number of triploids mulberry varieties viz. Tỉ 8, Tr 10, Tr 4 which were-released in the field and found to be suitable in the hilly areas of eastern India.
2. A triploid variety S1635 was isolated from the selection of a population raised by uncontrolled pollination in CSR-II at Berhampore which showed a very high adaptability with good yield.
3. Palksina and Dzahafarov (1967) developed some tetraploids viz. 16/29, 16/33, 16/7, 16/31 and 16/9 which became commercially popular in Russia.
4. Abdullaev (1972) developed three triploids and two tetraploids which showed higher yield in comparison with diploid control.
5. Later Abdullaev and Aliev (1984) developed five triploid varieties of *Morus alba* suited for cultivation in Azerbizan.
6. In Japan, out of nineteen mulberry varieties registered and released for cultivation in field from 1958-1993 five varieties viz. Shinkenmochi, Aobanezumi, Mitsushigeri, Yukimasari and Yukia: ahi are triploids and the first three varieties are recommended for cold regions and the other two varieties can tolerate extreme cold climate and recommended for snowy areas.
7. At Central Sericultural Researh and Training Institute, Mysore, India a number of tetraploid mulberry varieties has been developed from the high yielding diploid genotypes of Victory-1, S-13 and S-34 and are being maintained as breeding resource material.

Conclusion

Although there are good number of breeding techniques available in various agricultural crops; including mulberry, yet each of these methods suffers from one drawback or the other. Polyploidy has been intensively used in improving the mulberry crop, and there are good numbers of reports available which have indicated improvement in various commercial characters of the plant. However the technique has not been popularized in mulberry due to many reasons. The suitability of breeding method usually depends upon the nature of crop. So selection of the breeding method depends upon the kind and nature of crop which is under study.

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