Unit 1: Plant Tissue Culture

Lecture: Triploids and Cybrids
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Cybrids, in contrast to conventional hybrids, possess a nuclear genome from only one parent, but cytoplasmic genes from both parents. The process of protoplast fusion resulting in the development of cybrids is known as cybridization. In cybridization, heterozygosity of extrachromosomal material can be obtained.

Methods to produce cybrids

Cybrids may arise through:

a. Fusion of a normal protoplast with an enucleated protoplast;
b. Fusion between a normal protoplast and a protoplast containing non-viable nucleus;
c. Elimination of one of the nuclei after heterokaryon formation; or
d. Selective elimination of chromosomes after fusion.

Refer to 2nd slide cybrid advantage figure provided to you on your whatsapp group/ mail
Triploid Production

Pious Thomas and JB Mythili

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Triploid plants are seed-sterile and they are valued for seedless fruits and better vegetative growth. Triploids can be isolated by screening natural populations, or could be generated by various approaches such as in vitro culturing of endosperm tissue, crossing a tetraploid genome obtained either through colchicine treatment or somatic hybridization with a diploid genome, protoplast fusion between haploid and diploid genomes or selection in anther culture-derived population. Triploids are commercially exploited in citrus and watermelon for seedless fruits, better yield in sugarbeets and better vegetative growth in timber-yielding plants.

Triploid, by definition, is an organism with three basic sets of chromosomes. Due to irregular distribution of chromosomes at meiosis, triploids produce non-functional gametes leading to failure of embryo formation and seed development. Triploid plants are valued for the seedless fruits. They are a great boon in fruits where the seeds are dispersed in the flesh such as in watermelon (see Fig. 8.1) and citrus. Seedlessness in most of the cultivated bananas is due to the natural triploidy. In certain instances, triploids may be more vigorous as in sugarbeet or they may show better vegetative growth by conserving the photosynthates normally consumed in seed and fruit production. This is of great value in tree species, which are grown for biomass production such as tea, acacia and sandalwood. Triploids are also valued for ornamental purposes as demonstrated in petunias.

Triploids, however, are undesirable in plants where seeds are of commercial importance.

Triploids can be generated in different ways. These include

- Selection from natural population.
- In vitro culture of endosperm.
- Making a tetraploid parent through colchicine treatment followed by hybridization between tetraploid female and diploid male.
- Production of a tetraploid through somatic hybridization between two diploid lines followed by crossing with a diploid male line.
- Protoplast fusion between haploid and diploid genomes.
- Selection in anther culture derived population.

Selection from natural population

Banana is a typical example of naturally existing triploid. Most of the cultivated bananas are triploids derived from the two wild species Musa acuminata (A' genome) and M. balbisiana (B' genome). The best known bananas belong to pure acuminata AAA group (Gros Michel, Cavendish, Robusta, Chenkadali). M. balbisiana genome is associated with greater drought hardness and disease resistance. Edible triploids with mixed genome include AAB group (Rasthall, Poovan, Nendran, Pachanadan, Nendrapadathi) and ABB group (Nalla Bontha, Monthan, Karpuravalli).
In citrus, Tahiti lime is an example of a spontaneous triploid cultivar, which has been commercialized, and this cultivar is entirely seedless. The frequency of naturally occurring triploids in citrus is, however, low. Occasional triploid progenies are selected from conventional crosses between diploid cultivars. The spontaneous natural process could be exploited with more efficiency by combining embryo rescue and triploid selection.

Cultivated potato (*Solanum tuberosum*) is a tetraploid (2n = 4x = 48) while majority of the naturally occurring potato species are diploid or tetraploid. Other polyploid species such as triploid, pentaploids and hexaploids also occur in nature.

**In vitro culture of triploid endosperm**

Endosperm is the nutritive tissue formed as a result of double fertilization. It is a triploid tissue which results from the fusion of a sperm nucleus from the pollen grain with two previously fused antipodal cells. Under normal conditions, endosperm acts as a source of nutrients for the developing embryo. More than 80% families of flowering plants are known to possess endosperm in their developing seeds. During the development of the embryo, the nutritive endosperm may be consumed resulting into non-endospermous (exalbuminous) seeds or it may persist as a reservoir of food and utilized during seed germination in endospermous (albuminous) seeds.

Through in vitro culturing at an appropriate stage, it has been possible to induce tissue proliferation and triploid plant regeneration from endosperm tissue. Regeneration is achieved either through embryogenesis (*Citrus, Santalum*) or through organogenesis (*Putrenjiva*). Following the first successful attempt to culture maize endosperm on a medium supplemented with the extracts of potato or young corn kernels, success in about 40 species of angiosperms have been reported. Most of these belong to Euphorbiaceae, Loranthaceae, Rutaceae and Santalaceae. Initially, considerable difficulty was encountered in getting regeneration from endosperm tissue. Use of nitrogen-rich Murashige and Skoog medium, compared to the previously used media, helped in promoting the response in endosperm cultures. A detailed list of plants where endosperm culture
has been attempted can be found in Srivastava and Purohit. Some selected crops of commercial or horticultural significance are listed in Table 8.1.

The use of triploid endosperm tissue has proved a quick and efficient method of obtaining triploid citrus plants. The disadvantage of using this method is that the window for obtaining endosperm tissue from immature citrus seeds is limited to only a few weeks in each year, and success rates have been very low. The parenchymatous nature and lack of vascular tissue also make the endosperm an excellent experimental system for biological studies.

Making a polyploid through colchicine treatment followed by hybridization between tetraploid female and diploid male

The classical method for breeding triploids is crossing tetraploid and diploid genotypes and this has been demonstrated in developing seedless watermelon and seedless citrus. Seedless watermelon is one classical example of practical use of colchicine-induced polyploidy in triploid production.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Endosperm stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia nilotica</td>
<td>Immature</td>
</tr>
<tr>
<td>Ammona squamosa</td>
<td>Mature</td>
</tr>
<tr>
<td>Asparagus officinalis</td>
<td>Immature</td>
</tr>
<tr>
<td>Citrus grandis</td>
<td>Immature</td>
</tr>
<tr>
<td>Citrus sinensis</td>
<td>Immature</td>
</tr>
<tr>
<td>Emblica officinalis</td>
<td>Mature</td>
</tr>
<tr>
<td>Juglans regia</td>
<td>Mature</td>
</tr>
<tr>
<td>Hordeum vulgare</td>
<td>Immature</td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>Mature and Immature</td>
</tr>
<tr>
<td>Putranjiva roxburghii</td>
<td>Mature</td>
</tr>
<tr>
<td>Santalum album</td>
<td>Mature</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Immature</td>
</tr>
</tbody>
</table>

Table 8.1 Examples of endosperm cultures in economically important plants that have regenerated plantlets

| Source: Adapted from Ref [6]

There are several steps in producing triploid watermelon.

**Production of tetraploid line through colchicine treatment:** Application of colchicine 0.2%–0.4% to the growing point of young seedlings at 1 to 2 true-leaf stages for 2 to 3 days successively is generally practised.

**Production of 3x seeds:** This is done by either hand pollinating the tetraploid female with diploid male, or in open fields with adjacent rows of 4x female and 2x male and pinching of male buds in 4x plants.

**Germination of 3x seeds:** Triploid seeds are poor in germination and hence the seeds are to be germinated under controlled conditions followed by transplantation to field.

**Production of 3x fruits:** Triploid plants are raised along with diploid plants, which serve as pollinators.

Many triploid watermelon lines are commercially available in the USA and Japan. In India, a triploid watermelon was generated from the cross between Tetra 2 and Pusa Bedana. However, this variety has not been a big success commercially. Triploid production is hampered by the need for seed production every season, high cost of seeds, poor seed germination and low seed viability. Micropropagation has been developed as an alternative to seed propagation, circumventing the problems encountered in triploid seed production. Triploid watermelon is micropropogated either through multiple shoot induction from cotyledonary nodes in a BAP (5–10 μM) supplemented medium or through the use of shoot tip and nodal microcuttings in a low BAP (1 μM) supplemented medium.

By in vitro culturing of apparently aborted seeds from a cross between tetraploid Arka Manik and diploid Arka Manik, a seedless triploid with good fruit size, fruit quality and attractive crimson flesh has been evolved at the Indian Institute of Horticultural Research, Bangalore (see Fig. 8.1). The micropropagation approach optimized for this variety using microcuttings in low BAP.
sugar per unit area than diploids.

Triploids are valued for their seedlessness, better vigour, greater biomass production and for their ornamental value. Seedlessness is a great advantage in fruits that have many seeds dispersed in the flesh. Sterility in triploid also helps in conserving the photosynthates normally consumed in seed and fruit development, thus contributing to better biomass production. This would be of much significance in instances where the vegetative parts such as tuber (potato, sugarbeet) or timber (acacia, sandalwood and poplar) have economic value. The size in seedless fruits is not affected as observed in case of triploid watermelon and citrus. Seedlessness in triploids comes in the way of their propagation.

Reference:
Pious Thomas and J.B. Mythili- Triploid Production. Division of Biotechnology
Indian Institute of Horticultural Research, ICAR