

Fruit Science**Polyembryony in Mandarin: Its Role in True-to-Type Propagation, Genetic Conservation and Citrus Improvement**

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Abstract: -

Mandarin orange (*Citrus reticulata* Blanco) is an economically important citrus fruit crop with considerable nutritional and commercial value. One of its important biological characteristics is polyembryony, particularly nucellar embryony, in which several embryos may develop within a single seed. Nucellar embryos originate from maternal nucellar tissue and are generally genetically similar to the mother plant, making them valuable for the production of true-to-type seedlings. Polyembryony therefore has considerable importance in citrus propagation, preservation of desirable genotypes and production of vigorous orchard plants. However, it also creates difficulties in conventional citrus breeding because nucellar and sexually produced zygotic seedlings can occur together in the same seed. Morphological identification of these seedlings is often unreliable, particularly in open-pollinated populations. Molecular markers such as RAPD and SSR have consequently become useful tools for differentiating nucellar and zygotic seedlings and for assessing genetic diversity. This article summarizes the significance of polyembryony in mandarin, its variation among cultivars, its advantages and limitations, and the role of molecular approaches in citrus improvement.

Keywords: *Citrus reticulata*, mandarin, polyembryony, nucellar embryo, zygotic embryo, true-to-type propagation, RAPD, SSR.

1. Introduction:

Mandarin orange (*Citrus reticulata* Blanco) is an important citrus fruit crop valued for its nutritional, medicinal and commercial properties. Citrus fruits contain ascorbic acid, essential oils and antioxidant compounds, which contribute to their dietary and commercial importance. Mandarin is particularly notable for its high degree of

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polyembryony, a reproductive phenomenon in which more than one embryo develops within a seed (Pal, 2017). Polyembryony in citrus generally involves the development of nucellar embryos in addition to a sexually produced zygotic embryo. Nucellar embryos arise from somatic cells of the nucellus and are therefore generally similar to the maternal plant. This characteristic makes them useful for obtaining true-to-type planting material and conserving desirable characteristics of selected mother plants (Button and Kochba, 1993). The phenomenon has both beneficial and challenging consequences. On one hand, nucellar seedlings can provide vigorous, uniform and potentially disease-free planting material. On the other hand, the simultaneous occurrence of nucellar and zygotic seedlings makes it difficult to identify hybrids and sexually derived plants during citrus breeding. Because citrus has a long juvenile period and high heterozygosity, reliable identification of seedling origin is particularly important (Pal, 2017).

2. Understanding Polyembryony in Mandarin

Polyembryony refers to the formation of multiple embryos within a single seed. In mandarin, these embryos may include a zygotic embryo produced through sexual reproduction and one or more nucellar embryos originating from maternal tissue. The

proportion of nucellar and zygotic embryos differs among species and cultivars. Mandarin is generally considered to possess a high and variable degree of polyembryony (Pal, 2017). Nucellar embryony is especially significant because the embryos arise from the maternal nucellus rather than from the fusion of male and female gametes. Consequently, nucellar seedlings are expected to retain the desirable genetic constitution of the mother plant. This makes nucellar propagation useful where uniformity and maintenance of elite genotypes are important. The degree of polyembryony in mandarin cultivars is highly variable. Earlier studies reported polyembryony ranging from approximately 10 to 100% among different mandarin types. Some cultivars produce predominantly zygotic seedlings, whereas others produce mainly nucellar seedlings (Toxopeus, 1930; Torres, 1936). Frost and Soost (1968) reported differences among cultivars such as King, Kishiu, Ponkan, Satsuma and Szinkom. For example, Kishiu was reported as monoembryonic, whereas several other mandarin types showed a high proportion of polyembryony. This variation is important for both propagation and breeding. Highly polyembryonic cultivars can be useful sources of nucellar seedlings for orchard establishment, while cultivars with greater production of zygotic seedlings may be more

useful where sexual variation is required for breeding.

3. Importance of Nucellar Embryony for Citrus Propagation

One of the greatest advantages of nucellar embryony is its potential to produce true-to-type seedlings. Since nucellar embryos originate from maternal tissue, they are theoretically genetically identical or very similar to the mother plant. Therefore, desirable characteristics of selected elite trees can be maintained through nucellar seedlings (Button and Kochba, 1993). Nucellar seedlings also possess several advantages over conventional vegetative propagation. According to the reviewed literature, nucellar seedlings develop a tap root and can establish a stronger root system than plants raised through cutting or air layering. They may also show restoration of vigour that can be lost after repeated vegetative propagation. In addition, seed propagation can reduce some risks associated with the movement of propagative material (Pal, 2017). Another important advantage is the potential reduction of certain propagule-borne disease risks. The source article notes that citrus viruses associated with vascular tissues may be absent from nucellar seedling material, making nucellar embryony useful for raising healthier planting material. However, this should not be interpreted as a guarantee that every seedling

is free from every pathogen; appropriate nursery sanitation and pathogen-indexing practices remain important. Nucellar embryony can also help preserve the heterozygous condition and desirable genetic characteristics of elite mother plants. Thus, it has significance not only for multiplication but also for conservation of valuable citrus germplasm.

4. Polyembryony and Citrus Breeding

Although polyembryony is highly useful for propagation, it presents a major challenge in citrus breeding. Conventional citrus breeding is complicated by several biological characteristics, including nucellar polyembryony, high heterozygosity, long juvenile periods and incompatibility problems (Pal, 2017). During a breeding programme, the desired hybrid is produced through sexual reproduction and is therefore represented by a zygotic embryo. However, in polyembryonic citrus seeds, nucellar embryos may develop alongside the zygotic embryo. If the seedlings are selected only on the basis of general appearance, a nucellar seedling may be mistakenly identified as a hybrid. This problem is particularly serious because citrus seedlings may require several years to reach reproductive maturity. Waiting until flowering and fruiting to confirm the identity of a seedling greatly increases the time and cost of breeding. Therefore, early and reliable identification of zygotic and nucellar seedlings

is essential. Polyembryony has also been exploited indirectly in citrus improvement. The source article notes that crosses involving diploid and tetraploid plants can be used to produce triploid plants, which have potential for development of seedless citrus varieties (Soost and Cameron, 1985). However, the occurrence of nucellar embryos can interfere with the identification and selection of desired sexual progenies.

5. Genetic Diversity of Mandarin

Mandarin cultivars exhibit considerable diversity, although the degree of variation differs among groups. Some mandarin groups are thought to have originated through hybridization, whereas others, including several Satsuma and Clementine types, show relatively low genetic diversity and are associated with mutation-derived variation (Breto *et al.*, 2001; Barkley *et al.*, 2006). Molecular studies have helped clarify relationships among mandarin cultivars. RAPD and other molecular marker approaches have been used to identify genotypes, investigate relationships and assess genetic diversity. The source article reports that RAPD profiling of 25 elite *C. reticulata* clones from the North Eastern Himalayan region revealed approximately **65% genetic variability** and grouped the genotypes into two major clusters (Das *et al.*, 2004). Such information is important for germplasm conservation,

selection of elite planting material and breeding programme design. Understanding genetic relationships can help breeders select diverse parents and identify genetically similar or duplicate accessions.

6. Molecular Identification of Nucellar and Zygotic Seedlings

Traditional methods for distinguishing nucellar and zygotic seedlings have included morphological characters, biochemical analysis, chromatography, spectrophotometry and isozyme analysis. However, these methods have limitations because morphological characters may be influenced by environmental conditions and developmental stage. Biochemical markers may also produce variable results because they represent products of gene expression (Pal, 2017). DNA-based molecular markers provide a more reliable approach. Random Amplified Polymorphic DNA (RAPD) markers have been widely used because they are relatively simple and do not require prior knowledge of the target DNA sequence. RAPD analysis has been applied for genotype identification, genetic relationship studies, phylogenetic analysis, mapping and mutant identification (Luro *et al.*, 1995; Das *et al.*, 2004).

Simple Sequence Repeat (SSR) markers are another useful molecular tool. SSRs are co-dominant markers and can provide more reliable discrimination among

genotypes. The source article notes their application for distinguishing sexual from nucellar citrus seedlings (Oliveira et al., 2002). However, SSR analysis can be more expensive and technically demanding than RAPD, which may limit its routine application in resource-constrained situations.

Molecular identification is particularly important in open-pollinated mandarin populations. The source review points out that morphological identification of nucellar seedlings is not always reliable, and the occurrence of zygotic twins or triplets further complicates identification. Therefore, DNA-based markers can provide an effective method for distinguishing hybrids from nucellar seedlings at an early stage.

7. Significance for North Eastern and Himalayan Citrus

The identification and utilization of nucellar seedlings have particular importance in the North Eastern and Himalayan citrus-growing regions. Farmers commonly raise citrus seedlings from seed and may select seedlings based on visible morphological differences. However, morphological identification cannot always reliably distinguish nucellar and zygotic plants. The RAPD-based characterization of elite *C. reticulata* clones from the North Eastern Himalayan region demonstrated substantial genetic variability and provided useful

clustering of genotypes (Das *et al.*, 2004). Such molecular characterization can support the identification and conservation of elite local citrus resources.

For regions where farmers depend on locally adapted mandarin types, combining nucellar propagation with molecular characterization can help maintain desirable planting material while avoiding confusion between true-to-type nucellar seedlings and genetically different zygotic progenies.

8. Limitations and Future Prospects

Despite its advantages, polyembryony presents several limitations. The major challenge is the simultaneous development of nucellar and zygotic embryos, which complicates hybrid identification. The long juvenile phase of citrus further delays confirmation of plant identity. Conventional morphological and biochemical approaches are therefore insufficient for rapid and accurate identification in many situations. Future citrus improvement can benefit from combining nucellar propagation, tissue culture, molecular markers and modern biotechnological approaches. RAPD can provide a comparatively simple method for preliminary characterization, whereas SSR and other DNA-based markers can provide more precise genetic discrimination. Somatic hybridization and genetic transformation have also been applied in citrus improvement programmes

(Pal, 2017). For mandarin improvement programmes, an integrated approach should therefore include identification of elite mother plants, production of nucellar seedlings, molecular confirmation of seedling identity, conservation of genetically valuable germplasm and selection of suitable parents for hybridization.

Conclusion

Polyembryony is one of the most important biological characteristics of mandarin orange and has major implications for both propagation and breeding. Nucellar embryony provides an efficient natural mechanism for producing seedlings that retain the desirable characteristics of elite mother plants. Nucellar seedlings may also possess vigorous root systems and can contribute to the production of uniform orchard planting material.

At the same time, polyembryony creates serious difficulties in citrus breeding because nucellar and zygotic seedlings may develop together. Morphological identification is not always reliable, particularly in open-pollinated populations. Molecular markers such as RAPD and SSR provide useful approaches for early and accurate identification of seedlings and for assessing genetic diversity. Therefore, the effective utilization of polyembryony, combined with molecular characterization, can contribute

significantly to the conservation, propagation and genetic improvement of mandarin, particularly in citrus-growing regions of North Eastern and Himalayan India.

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