

Review

Seedlessness in Citrus

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ABSTRACT

The presence of a large number of seeds in citrus fruits is a big hindrance in consumer acceptability even if fruit possesses high organoleptic properties. Seedy cultivars are accepted only if seedless cultivars are not present or they are much superior in fruit characteristics than seedless cultivars. Therefore, horticulturists and breeders have been involved in the development of seedless cultivars since very long. Different approaches have been made by numerous researchers. These strategies include conventional and non conventional breeding techniques. Conventional techniques, mostly used in past, face many problems and complications due to specific reproductive biology of citrus and is also time consuming. Non conventional techniques include embryo rescue, protoplast fusion, production of cybrids, using inter specific and inter generic or even wide crosses/fusions. Development of flow cytometry and molecular markers has enabled the identification of the new genomes even at early stage. Thus reducing complications faced in conventional breeding.

Key Words: Seedlessness; Citrus; Cultivar

INTRODUCTION

Natural and induced ploidy manipulations have played a significant role in the domestication and improvement of crops. As mutations polyploidy have great potential to bring about sudden changes in genotypes and phenotypes. The change in chromosome number per cell can be caused by such treatments as application of extremely high or low temperature or application of auxins as indole-3 acetic acid (Hartman & Kester, 1959), endosperm derived calli (Gmitter *et al.*, 1990), somatic hybridization (Oiyama *et al.*, 1981), gamma irradiation (Fry, 1963; Karkadze, 1986) and interloidal hybridization (Jaskani, 1998).

Among polyploids, triploids are of utmost importance as they produce seedless fruits and vegetables (Raza, 2001). Production of seedless (triploid) citrus fruits could lead to the improvement of quality of fresh fruits. Seedless citrus varieties have a distinct commercial advantage. Seediness is considered as an obstacle in releasing newly selected high quality mandarins (Vardi, 1996). There are a number of cultivars with desirable horticultural characteristics which have not attained commercial importance because of their seediness (Fatta-Del-Bosco *et al.*, 1992). In the developed countries seedy cultivars are grown where they fill a maturity season gap or for other superior traits or for simple lack of seedless varieties.

Breeders have been investigating methods that allow sexual recombination that result in progeny with nearly seedless fruit. Various methods have been used in this regard.

Crossing Diploids with Tetraploids (2n x 4n). Crossing diploid plants using female parents that are genetically able to produce a high percentage of unreduced megaspores

(Geraci *et al.*, 1982) is a method to get triploids. These crosses have the limitation of producing a low number of triploid hybrids which are difficult to detect and isolate from the diploid or from the small number of hybrids obtained in the case of a polyembryonic female parent (Geraci, 1978). Triploid embryos from monoembryonic species are easily identified because the seeds carrying them are 1/3 to 1/6 times smaller than diploid seeds (Esen & Soost, 1973).

The problem of citrus embryo abortion in 2n x 4n crosses has been a limitation for recovering triploids by this method (Esen *et al.*, 1979; Oiyama *et al.*, 1981). Many triploid plants can be produced which may combine the desirable characteristics of diploid and tetraploid parents if triploids can be rescued using embryo rescue technique *i.e.* culturing immature embryos *in vitro* 12 to 15 weeks after pollination (Satarrantino & Resupero, 1981; Oiyama & Kobayashi, 1990) following 2n x 4n hybridization. Even in some crosses, when Marsh seedless grapefruit, Tarocco, Sanguinello, Ovaletto, Sanguigno and Biondo sweet orange, Avana mandarin, Femminello lemon and Hong Kong Kumquat (as tetraploid polyembryonic male parent) were crossed with diploid monoembryonic female parents (Clementine, tangor, pummelo, Meyer lemon and citron). The resultant triploids not only produced seedless fruits but also have excellent organoleptic characters and are easy to peel (Satarrantino, 1992). It is also observed that culturing of immature embryos of underdeveloped seeds are triploid while embryos from fully developed seed are found to be tetraploid (Oiyama *et al.*, 1991).

Crossing Tetraploids with Diploids (4n x 2n). Triploids of citrus can also be produced by crossing tetraploid seed parent with diploid pollen parent (Cameron & Burnett, 1978; Esen *et al.*, 1979). Triploid plants were recovered by

Table I. Some seedless varieties of *Citrus*

Common Name	Botanical Name	Varieties
Sweet Orange	<i>Citrus sinensis</i>	Hamlin, Washington Navel, Pera, Valencia, Shamouti
Mandarin/Tangerine	<i>Citrus reticulata</i>	Satsuma mandarin (Owari & Wase), Clementine, Sue Linda
Grape Fruit	<i>Citrus paradisi</i>	Marsh, Thompson, Redblush
Lime	<i>Citrus aurantifolia</i>	Tahiti lime
Wilking x Mineola (Mandarin x Tangelo)	<i>C. reticulata</i> x (<i>C. reticulata</i> x <i>C. paradisi</i>)	Winola

excising seeds from mature fruits of $4n \times 2n$ crosses. Vigour was very high although the initial survival of small germinating seeds was low. It was also observed that $4n \times 2n$ crosses had higher seed set than that of reciprocal crosses (Esen & Soost, 1972; Cameron & Burnett, 1978). This is due to the unfavorable ratio, 4:4, of embryo to endosperm chromosomes in such triploids as compared to a normal ratio, 4:6, in the tetraploids (Esen & Soost, 1973a).

Embryo Rescue Technique. Embryo culture has been practiced by plant breeders for over half a century. In recent years, technical considerations relating to the composition of the medium and excision of the embryo have improved the ability to utilize zygotic embryo culture to rescue intervarietal, interspecific and intergeneric crosses (Bhojwani & Razdan, 1983). Hybrid seed often abort because the endosperm fails to develop normally (Cocking, 1986). The rescue of hybrid embryos by *in vitro* culture was first demonstrated by Laibach (1925) and now is a well established procedure with extensive use in plant breeding. The problem of citrus embryo abortion in $2n \times 4n$ crosses has been a limiting factor for recovering triploid plants by this method. This is due to altered ratio of number of chromosomes of embryo and endosperm or may also be caused by unknown factors in the tetraploid pollen parent as suggested by Oiyama *et al.* (1981).

The culture of zygotic embryos is regularly used to rescue immature hybrid embryos which resulted from interspecific or other wide crosses where pollination and fertilization occur normally but endosperm development fails and mature embryos are not produced. This involves excision from immature seeds (100-120 days after pollination) which usually reached heart shaped zygotic embryos and then subsequent culture of these embryos on suitable medium *in vitro* (Wen-Cai, 1981; Oiyama & Kobayashi, 1990; Soares *et al.*, 1992).

Spontaneous Triploids. In citrus spontaneous triploids and tetraploids also exist as sexual zygotic seedlings. The frequency of triploids was as high as 5% among seedling progeny (Lapin, 1937). In spite of low seed content and seedlessness, only the 'Tahiti lime' attained commercial significance among naturally occurring triploids (Vardi & Spiegel-Roy, 1978; Vardi, 1996). Among some open pollinated citrus, 'Lisbon Lemon', 'Eureka Lemon', 'Ruby' sweet orange and 'Imperial' grapefruit triploids progenies were observed suggesting that unexpected triploids are common among progenies of citrus cultivars (Esen & Soost, 1971). Spontaneous triploids may arise by

the production of $(2x)$ egg cells by diploid seed parent or $2x$ pollen grains and their union with a monoploid (x) gamete. However, most probably the double chromosome number is commonly provided by egg (Vardi, 1992).

These unexpected triploids seeds can be distinguished from diploids with considerable accuracy because the triploids were $1/3$ to $1/6$, the size of diploids. It is also observed that there is precocity in development of their embryos and they also germinate early than diploid seed of same age (Esen & Soost, 1973).

Triploids plants in addition to seedlessness have some other desirable characters, e.g. 'Winola', a spontaneous triploid hybrid from a cross between 'Wilking and Minneola', fruit is above medium size round to oblate, red skin and of course seedless, occasionally one seed per fruit. Trees are typically spreading with some drooping branches and thorns at the non fruiting parts but fruit bearing branches tend to become nearly thorn less (Spiegel-Roy & Vardi, 1992).

Irradiation. Irradiation may be of value as a tool to enhance the frequency of mutation of vegetatively propagated plants. Mutation breeding through irradiation is also a way to seek triploids, since natural mutations and sports were often found in citrus. Gamma irradiation and chemical mutagens were used to observe somatic mutation as a means to study the evolution of various citrus species and varieties. Irradiation with Co^{60} at 7 Ki with 127 R/min produced about 300 times more mutants of Jincheng orange than the natural mutation (Zhang *et al.*, 1988).

Endosperm Culture. The endosperm is a unique tissue. Being the fusion product of three haploid nuclei; two polar nuclei and one pollen nucleus, in most angiosperms, is triploid. With the development of tissue culture techniques it is now possible to get embryogenesis from cultured endosperm. This technique enables the production of triploid (seedless) plants. Recovery of triploid plants from *in vitro* endosperm culture following controlled pollination could be a useful breeding strategy for citrus, a vegetatively propagated perennial crop, for which seediness is undesirable and unnecessary. It can also overcome barriers to sexual hybridization resulting from apomixis and embryo abortion.

Embryoid culture had been made possible by peeling the nucellus off under dissecting microscope to remove embryoid and embryoid tissue. It was done two months after flower anthesis and endosperm cultured in MT medium + 2 ppm 2, 4-D + 5 ppm Benzyl adenine + 1000

ppm casein hydrolysate (Wang *et al.*, 1975). Similarly embryogenesis in endosperm of sweet tanger (*C. reticulata* x *C. sinensis*) was also induced and triploid plants were regenerated (Mooney *et al.*, 1996).

Use of Recent Biotechnological Tools. Conventional breeding has many difficulties in citrus triploid production due to the reproductive complications of the plant. To expedite genetic improvement, new approaches have been explored. Among them is somatic hybridization via protoplast fusion and development of cybrids. The first somatic hybrid of citrus was produced by Ohgawara *et al.* (1985) and by now numerous inter and intrageneric somatic hybrids have been produced. This approach is now becoming the most important in the creation of triploids and novel germplasm for improving rootstock and scion varieties.

Transgenic lime (*C. aurantifolia*) plants containing gene for decreased seed set were obtained from seedling hypocotyl and epicotyl segments by *Agrobacterium* mediated transformation. Putative transformants were identified by polymerase chain reaction, PCR (Koltunow *et al.*, 1995). If haploid lines are obtained and then crossed with diploid lines via protoplast fusion, triploids can also be produced. This allows the insertion of a haploid genome to the whole diploid genome of high organoleptic quality cultivars. It can be done via electrofusion of protoplasts (Ollitrault *et al.*, 1995). The regenerated calli can be studied using flow cytometry, isozyme banding and molecular markers; RAPD, RFLP, AFLP etc (Ollitrault *et al.*, 1995; Guo *et al.*, 1996; Jumin & Nito, 1996; Guo & Deng, 1998; Scarano *et al.*, 2002)

CONCLUSIONS

With the increasing demand for seedless cultivars of citrus it has been made possible to get maximum number of seedless cultivars. There has been various ways to get seedless cultivars. In past conventional breeding techniques had been used that face many obstacles also, thus limiting the production of seedless cultivars. With the advancement in science and technology especially biotechnology, it is now more easy to get seedless citrus fruits (plants) with all desirable characters like easy peeling, improved juice and TSS. These techniques also made possible to get seedless cultivars from an existing one without altering its other characters which was not possible through conventional breeding.

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