Citrus Pentaploids from Small Seeds of Diploid $\times$ Diploid Crosses

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Abstract. Seedlings from some small seeds of diploid $\times$ diploid crosses using 'Miyauchi iyokan' (*Citrus iyo* Hort. ex Tanaka) as the seed parent were found to be pentaploid ($2n = 5x = 45$). The growth of pentaploid seedlings was extremely weak on their own roots, but was much more vigorous when micrografted on seedlings of tetraploid trifoliate orange [*Poncirus trifoliata* (L.) Raf.]. Diploid 'Miyauchi iyokan' produced hexaploid seedlings from small seeds in addition to triploid and tetraploid seedlings from normal seeds when crossed with tetraploid 'Funadoko' (*C. funadoko* Hort. ex Y. Tanaka) and 'Trovita' sweet orange [*C. sinensis* (L.) Osbeck]. Based on this phenomenon, the origin of pentaploids is discussed.

Citrus and related genera are generally diploid with a somatic chromosome number of 18 (Frost, 1925). Certain diploid $\times$ diploid crosses produce triploid plants from small seeds (Esen and Soost, 1971, 1973a; Geraci et al., 1975; Oiyama and Okudai, 1983). Esen and Soost (1971, 1973a, 1973b) concluded that triploid seedlings arose from small seeds of diploid $\times$ diploid crosses that originated from the union of unreduced female gametes and reduced male gametes, and hypothesized that the 3:5 ratio of embryo to endosperm ploidy was the factor responsible for the reduced seed size. Triploids have promise as commercial cultivars because they are essentially seedless (Soost and Cameron, 1980, 1985). The occurrence of spontaneous triploids among the progeny of diploid cultivars provides a direct approach to breeding seedless cultivars. Polyploids may also provide promising materials for genetic and cytogenetic studies. In the course of producing triploid seedlings from diploid $\times$ diploid crosses, we obtained pentaploid seedlings from small seeds. We give herein a brief description of such pentaploid seedlings and describe their possible origin.

The monoembryonic diploid cultivar Miyauchi iyokan was hand-pollinated with pol-

len from diploid trifoliate orange, 'Kawano natsudaidai' (*C. natsudaidai* Hayata), and tetraploid 'Funadoko' (*C. funadoko* Hort. ex Y. Tanaka) and 'Trovita' sweet orange that were obtained from nucellar seedlings. The crosses with tetraploid cultivars were made to determine the occurrence of sexual polyploids. Hand pollination was carried out immediately following emasculation of flowers, and all pollinated flowers were covered with bags. Fully developed seeds were collected from each fruit of diploid $\times$ diploid crosses at maturity; the seeds, substantially smaller than normal (Fig. 1), were selected visually. The embryos were excised from those seeds and germinated on moistened filter paper in petri dishes placed in darkness at 28 to 30°C. Somatic chromosome counts were performed on root-tip cells according to the procedure described by Oiyama (1981). Root tips were pretreated with an aqueous solution

Fig. 1. Small (top row) and normal (bottom row) seeds from diploid 'Miyauchi iyokan' $\times$ diploid trifoliate orange cross.

of 2 mM 8-hydroxyquinoline for 20 h at 10°C, and then fixed in a 3 ethanol : 1 acetic acid solution for 24 h. For staining, root tips were immersed in 1% lacto-propionic orcein for 3 h immediately following fixation.

Small seeds were obtained from both crosses, although the frequency was much higher when trifoliate orange was used as the male parent (Table 1). Chromosome counts showed that six seedlings derived from eight small seeds in the cross with trifoliate orange and three seedlings derived from five seeds in the cross with 'Kawano natsudaidai' were pentaploid, with 45 chromosomes (Table 1, Fig. 2). Pentaploid embryos germinated more slowly than diploid ones. The growth of pentaploid seedlings was extremely weak on their own roots. Hence, there was a marked difference in height between diploid and pentaploid seedlings (Fig. 3A). These pentaploid seedlings, however, grew much more vigorously when micrografted on seedlings of tetraploid trifoliate orange according to the method of Takahara et al. (1986) (Fig. 3B). The plants obtained from the cross with trifoliate orange had trifoliate-shaped leaves; this is a dominant trait that is expressed in

Fig. 2. Metaphase chromosomes in root-tip cell of a pentaploid seedling ($2n = 45$).

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Table 1. Occurrence of small seeds and chromosome number of seedlings from these seeds in diploid x diploid crosses using 'Miyauchi iyokan' as the seed parent.

Pollen parent	No. fruit	No. developed seeds			Small seeds (%)	No. seedlings with indicated chromosome no. ²	
		Small	Normal	Total		27 (3x)	45 (5x)
Trifoliolate orange	15	8	180	188	4.3	2	6
Kawano natsudaidai	19	5	322	327	1.5	2	3

²Emerged from small seeds only.

Table 2. Chromosome number of seedlings from fully developed seeds of diploid 'Miyauchi iyokan' x tetraploid crosses.²

Pollen parent	No. fruit	No. seeds		No. seedlings with indicated chromosome no.		
		Fully developed ²	Undeveloped	27 (3x)	36 (4x)	54 (6x)
Funadoko	17	24 (3)	145	13	8	3
Trovita sweet orange	15	78 (1)	159	19	8	1

²Chromosome counts were made according to the method described in the text.

²Number of small seeds are shown in parentheses.

Fig. 3. (A) Pentaploid seedling from diploid 'Miyauchi iyokan' x diploid trifoliolate orange cross compared with diploid seedling (right) 3 months after sowing and (B) micrografted onto tetraploid trifoliolate orange seedling.

trifoliolate orange and its F₁ hybrids (Cameron and Frost, 1968).

Certain diploid cultivars of citrus, such as 'Sukega' [*C. paradisi* Macf. x *C. sinensis* (L.) Osbeck] and 'Hyuganatsu' (*C. tamurana* Hort. ex Tanaka), have been reported to produce hexaploid seedlings in addition to triploid and tetraploid seedlings when crossed with tetraploids (Ren and Soost, 1972, 1973b; Oiyama et al., 1981). These hexaploid seedlings originated from small seeds, and it was concluded that they were derived

from the functioning of doubly unreduced female gametes (Esen and Soost, 1973b). Doubly unreduced gametes have also been reported in strawberry (Bringhurst and Gill, 1970). Diploid 'Miyauchi iyokan' produced a mixture of triploid, tetraploid, and hexaploid seedlings from fully developed seeds when crossed with pollen from tetraploid cultivars, and all the hexaploid seedlings emerged from small seeds (Table 2). This result indicates that doubly unreduced female gametes were formed in diploid 'Mi-

yauchi iyokan'. From this evidence, we conclude that small pentaploid seeds produced by diploid 'Miyauchi iyokan' crossed with diploids might arise from the union of doubly unreduced female gametes with normal reduced male gametes.

Citrus pentaploids themselves do not have potential value for cultivar improvement, but they would be useful sources for study of genetics or cytogenetics at the polyploid level.

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