Polyembryony in Undeveloped Monoembryonic Diploid Seeds Crossed with a Citrus Tetraploid

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Abstract. Some undeveloped seeds from mature *Citrus* fruit of monoembryonic diploid cultivars crossed with a tetraploid selection were observed to be polyembryonic. The multiple embryos formed a small mass at the micropylar end. Plants regenerated *in vitro* from the embryos in polyembryonic seeds were triploid and showed identical peroxidase banding patterns on acrylamide gels. These results indicate that the multiple embryos found in the undeveloped seed from monoembryonic diploid × tetraploid crosses are genetically identical and of zygotic origin.

Polyembryony is a common feature in *Citrus*. Multiple embryos are produced from nucellar cells. However, some cultivars have been reported to produce two or more sexual embryos in one seed (Bacchi, 1943; Cameron and Garber, 1968; Frost, 1926; Ozsan and Cameron, 1963; Parlevliet and Cameron, 1959; Ueno et al., 1967). Sexual polyembryony may be due to multiple fission of the zygotic embryo or the presence of more than one egg in an ovule (Bacchi, 1943). Cameron and Garber (1968) presented cytological evidence that supernumerary sexual embryos could occur by budding from the primary embryo. We describe polyembryony in undeveloped seeds from mature fruit of monoembryonic diploid cultivars crossed with a tetraploid and discuss its origin.

Two monoembryonic diploid cultivars, Clementine mandarin (*Citrus clementina* Hort. ex Tanaka) and Miyaruchi Iyokan (*C. iyo* Hort. ex Tanaka) were hand-pollinated with tetraploid 'Kawano Natsudaikai' (*C. natsudaikai* Hayata) pollen. Hand pollination was carried out immediately following emasculation of flowers, and all the pollinated flowers were covered with bags. Seeds were extracted from mature fruit 7 months after pollination and were classified as well-developed or undeveloped (Fig. 1). Embryo formation in undeveloped seeds was observed under a binocular dissecting microscope.

Most of the seeds produced by each seed parent in the cross with the tetraploid were undeveloped (Table 1). The frequency of normal seed development was greater for 'Miyaruchi Iyokan' than for 'Clementine'. The high frequency of undeveloped seeds from 2x-4x crosses, already reported by several workers (Cameron and Soost, 1969; Esen and Soost, 1972, 1973; Oiyama et al., 1981; Tachikawa et al., 1961), has been attributed

to abortion of triploid embryos at various stages of embryogenesis (Esen and Soost, 1973).

The undeveloped seeds from these crosses were classified as monoembryonic, polyembryonic, or embryoless. The percentage of polyembryonic seeds was higher in the cross using 'Miyaruchi Iyokan' as the seed parent (Table 2). The embryos in polyembryonic seeds formed a small mass at the micropylar end (Fig. 2). These embryos were nearly globular in shape but very dissimilar in size. The number of embryos differed greatly among seeds in each cross. The mean number of embryos per seed was about half as great in 'Clementine' as in 'Miyaruchi Iyokan' (Table 3).

Fig. 1. Normal (left) and undeveloped (right) seeds from mature fruit of 2x 'Clementine' × 4x 'Kawano Natsudaikai'.

The origin of these embryos was verified by determining chromosome number and analyzing the isozyme patterns of plants regenerated from eight polyembryonic seeds having two to 11 embryos. The embryos of these seeds were cultured on Murashige and Tucker (1969) medium containing 500 mg malt extract and 40 mg adenine/liter to induce plantlet formation. Chromosome counts carried out by the root tip squash technique of Oiyama (1981) indicated that all plants produced from any one polyembryonic seed were triploid ($2n = 3x = 27$) (Table 4). Multiple embryos formed in undeveloped seeds are therefore of zygotic origin. Acrylamide gel electrophoretic analysis of peroxidase isozymes according to the method described by Kobayashi (1987) showed no differences in banding patterns among the triploid plants from the same polyembryonic seed (Fig. 3). This similarity suggests that the embryos in one undeveloped seed are

Table 1. Seed set in monoembryonic diploid × tetraploid *Citrus* crosses.^a

Seed parent	No. fruits	No. seeds			Undeveloped seeds (%)
		Fully developed	Undeveloped	Total	
Clementine	20	3	427	430	99
Miyaruchi Iyokan	12	24	128	151	85

^aSeed parents were pollinated with 4x 'Kawano Natsudaikai'.

Table 2. Embryo formation in undeveloped seeds from monoembryonic diploid × tetraploid *Citrus* crosses.^a

Seed parent	No. undeveloped seeds	Distribution of undeveloped seeds on the basis of embryo formation					
		Monoembryonic		Polyembryonic		Embryoless	
		No.	%	No.	%	No.	%
Clementine	427	248	58.1	71	16.6	108	25.3
Miyaruchi Iyokan	128	71	55.5	27	21.1	30	23.4

^aSeed parents were pollinated with 4x 'Kawano Natsudaikai'.

Table 3. Number of embryos in polyembryonic undeveloped seeds from monoembryonic diploid × tetraploid *Citrus* crosses.^a

Seed parent	No. undeveloped seeds	No. embryos/seed	
		Range	Mean ± SD
Clementine	40	2-17	4.8 ± 3.1
Miyaruchi Iyokan	21	3-20	9.2 ± 6.1

^aSeed parents were pollinated with 4x 'Kawano Natsudaikai'.

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Table 4. Chromosome number of regenerated plants from multiple embryos in undeveloped seeds from 2x 'Clementine' crossed with 4x 'Kawano Natsudaidai'.

No. embryos in examined seeds	No. regenerated plants	
	Chromosome number (2n)	
	27	Others
2	2	0
3	3	0
4	2	0
5	3	0
6	3	0
7	3	0
8	4	0
11.	5	0

Fig.2. Single (left) and multiple (right) embryos formed in undeveloped seed from 2x 'Clementine' × 4x 'Kawano Natsudaidai'.

Fig. 3. Isoelectric focusing profiles of peroxidase isozymes in triploid plants produced from one polyembryonic seed.

genetically identical and derived from a single fertilized egg cell.

The present study demonstrates that sexual polyembryony of citrus occurs at the triploid level. This sexual polyembryony seems to be a specific phenomenon related to abnormal development of triploid embryos in monoembryonic diploid × tetraploid crosses.

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