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# FREQUENCY OF TRIPLOIDS IN DIFFERENT INTERPLOIDAL CROSSES OF CITRUS

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**Abstract**

The frequency of triploids in progenies from 2x x 4x and 4x x 2x crosses of Kinnow mandarin and Succari sweet orange was analysed. Chromosome counts indicated that crosses among tetraploid x diploid Kinnow yielded maximum triploids while reciprocal crosses of Kinnow exhibited a mixed population of diploid, triploid and tetraploid seedlings. The occurrence of triploids varied from 25% to 83.4% depending on the pistillate parents used. Crosses of diploid Succari with tetraploid Kinnow also produced nucellar diploid, sexual triploid and tetraploid seedlings. The highest number of tetraploids (71.4%) were produced in cross of tetraploid Kinnow with diploid Succari.

## Introduction

Citrus chromosomes are small and not easy for extensive studies, but usable preparations for routine examination can be made from pollen mother cells, root tips, shoot tips and embryos (Soost & Cameron, 1975). The basic chromosome number in the genus *Citrus* and other members of the subfamily Aurantioideae is x=9 (Frost, 1925). Virtually all wild and cultivated forms of citrus are diploids (Krug, 1943). Polyploids, mostly triploids and tetraploids are also known to occur either spontaneously or after certain crosses. Russo & Torrisi (1951) reported an exceptional case where they found three hybrid tetraploids (allotetraploid) from 2x x 2x crosses. Such tetraploids are used in triploid breeding programs (Jaskani & Khan, 2000).

Triploid plants have been produced by crossing tetraploid female parents with diploid pollen parents (Esen *et al*., 1979). Cameron & Burnett (1978) obtained high proportions of 3x hybrids from crosses of 4x sexual seed parents by 2x pollen parents in *Citrus*. This is in contrast to the low proportions of 3x usually obtained with 4x pollen parents (Esen & Soost, 1972). Production of triploids by crosses of diploid seed parents with tetraploid parents has also been reported (Esen & Soost, 1972; Oiyama & Kobayashi, 1990); however, low set of fully developed seeds was a common feature (Cameron & Soost, 1969; Esen & Soost, 1972; Jaskani *et al.*, 2005). Similarly, Oiyama *et al.*, (1991) used pollen from a tetraploid somatic hybrid for triploid production but maximum seedlings developed from developed seeds were tetraploids. Tetraploidy in diploid x tetraploid crosses of *Citrus* results from the union of unreduced female gametes and reduced male gametes (Esen & Soost, 1972). Geraci (1978) counted chromosomes of immature embryos at the cotyledonary stage and on seedlings coming from the same crosses of 2x x 4x lemons and observed percentage of triploids from 2% to 4% in the embryos and from 0 to 0.56% in the seedlings. The frequency of triploids and tetraploids in 2x x 4x crosses is affaected by the staminate parents used. Oiyama *et al*., (1981) investigated the ploidy levels of seedlings obtained from 7 diploid monoembryonic seed parents crossed by 3 tetraploid pollen parents and reported that pollen parents affected on ratio of triploid and tetraploid embryos. This study elaborates the occurrence and frequency of triploids and tetraploids in interploid reciprocal crosses of Kinnow mandarin and Succari sweet orange.

## Materials and Methods

**Sexual hybridization:** Controlled hand pollinations were made using 2x x 4x and 4x x 2x crosses. Parents used were diploid and tetraploid Kinnow mandarin (*Citrus reticulata* Blanco) and diploid Succari sweet orange [*Citrus sinensis* (L.) Osbeck]. Unopened or partially opened flower buds were collected from pollen parent trees one day before anthesis. In the laboratory, petals and styles were removed with forceps and the flowers were placed under a fluorescent lamp to promote anthers dehiscence for crosses to be made next morning. Before pollination, the flowers of desired stage i.e., those that were expected to open within the next 24 hours or with one petal open, were emasculated on seed parent plants. Pollen was applied on emasculated flowers by touching the dehisced anthers of the pollen parent gently to the stigma and bagged to exclude contamination with stray pollen. The replicated experiment was laid out according to randomized complete block design. One hundred and eighty crosses were made in each parent. The embryos were germinated as described by Jaskani *et al.*, (2005). Chromosome counts were made in young leaves of progeny seedlings as described under.

**Preparation of samples:** Tender leaves of hybrid population and parents were collected from the orchard at 8:30 to 11:00 a.m. Plant material was pre-treated with saturated solution of 1,4-dichlorobenzene for two hours. Samples were fixed for 2 hours in 100% ethyl alcohol, glacial acetic acid and chloroform (6:1:3 v/v). Then the material was transferred to isolation solution of 5N HCl for 20 minutes. Mordant solution (4% $FeNH_4(SO_4)_2.12H_2O$) was prepared and the samples were treated for 1 hour followed by 4-5 washing with distilled water. Staining was made with 0.5% Haematoxylin solution for 2 hours.

**Microscopy:** A drop of acetic acid (45%) was put on the glass slide before placing the sample. A piece of filter paper was used for the absorption of extra acetic acid. The sample was squashed by knocking with the back of needle and was examined under the light microscope at x100 magnification.

## Results

**Kinnow (4x) x Kinnow (2x) progeny:** Two types of seeds were found while culturing embryos *In vitro*, single and multiple embryos, and both were compared for their chromosome counts. The detail of chromosome numbers of seedlings recovered from Kinnow (4x) x Kinnow (2x) seeds having single embryo is presented in Table 1. The seedlings germinated from polyembryonic seeds could not survive during transplanting. The accession numbers $TS_3$, $TS_4$, $TS_6$, $TS_9$ and $TS_{10}$ showed 27 numbers of chromosome count and confirmed triploidy. Only the accession number $TS_8$ was tetraploid having 36 numbers of chromosomes.

**Table 1. Ploidy level in Kinnow (4x) X Kinnow (2x) progeny.**

| Accessions | No. of Chromosomes |
|---|---|
| $TS_3$ | 27 |
| $TS_4$ | 27 |
| $TS_6$ | 27 |
| $TS_8$ | 36 |
| $TS_9$ | 27 |
| $TS_{10}$ | 27 |

**Table 2. Ploidy level in Kinnow (2x) X Kinnow (4x) progeny.**

| Accessions | No. of Chromosomes |
|---|---|
| $KM_{1-1}$ | 36 |
| $KM_{4-1}$ | 18 |
| $KM_{4-2}$ | 27 |
| $KM_{9-2}$ | 36 |
| $KM_{10-1}$ | 36 |
| $KM_{11-2}$ | 18 |
| $KM_{12-2}$ | 27 |
| $KM_{13}$ | 18 |
| $KM_{19}$ | 18 |
| $KM_{22-1}$ | 18 |
| $KS_2$ | 36 |
| $KS_3$ | 27 |

**Table 3. Ploidy level in Succari (2x) X Kinnow (4x) progeny.**

| Accessions | No. of Chromosomes |
|---|---|
| $SM_{5-1}$ | 27 |
| $SM_{6-1}$ | 27 |
| $SM_{6-2}$ | 18 |
| $SM_{7-1}$ | 18 |
| $SM_{8-1}$ | 18 |
| $SM_{8-2}$ | 18 |
| $SM_{9-4}$ | 36 |
| $SM_{10-1}$ | 27 |
| $SM_{11-1}$ | 27 |
| $SM_{13-1}$ | 36 |
| $SM_{13-2}$ | 27 |
| $SM_{14-1}$ | 18 |
| $SS_1$ | 27 |
| $SS_2$ | 36 |
| $SS_3$ | 18 |

**Kinnow (2x) x Kinnow (4x) progeny:** Chromosomes counts presented in Table 2 refers to the seedlings recovered from Kinnow (2x) x Kinnow (4x) crosses. Among the accession numbers germinated from polyembryonic seeds, the accession number $KM_{1-1}$, $KM_{9-2}$ and $KM_{10-1}$ and from monoembryonic seeds $KS_2$ produced 36 numbers of chromosomes unexpectedly. $KM_{4-2}$, $KM_{12-2}$ and $KS_3$ proved as triploid hybrid seedlings with 27 numbers of chromosomes. Accessions $KM_{4-1}$, $KM_{11-2}$, $KM_{13}$, $KM_{19}$ and $KM_{22-1}$ were diploid. Accessions $KM_{4-1}$ and $KM_{4-2}$ were from a polyembryonic seed and exhibited 18 and 27 chromosomes, respectively.

**Succari (2x) x Kinnow (4x) progeny:** The progeny germinated from the crosses of Succari (2x) x Kinnow (4x) produced a mixture of diploid, triploid and tetraploid seedlings (Table 3). Accession number $SS_1$, $SS_2$ and $SS_3$, germinated from monoembryonic seeds, were triploid, unexpected tetraploid and diploid, respectively. $SM_{5-1}$, $SM_{6-1}$, $SM_{10-1}$, $SM_{11-1}$ and $SM_{13-2}$ were the seedlings germinated from polyembryonic seeds and yielded 27 numbers of chromosomes. $SM_{13-1}$ and $SM_{13-2}$ were from polyembryonic seed material and produced unexpected tetraploid and triploid progeny from diploid Succari sweet orange. Similarly accession $SM_{9-4}$ was also unexpected tetraploid. Other accessions were nucellars with 18 chromosomes.

**Kinnow (4x) x Succari (2x) progeny:** Chromosome counts of the seedlings germinated from Kinnow (4x) x Succari (2x) crosses are presented in Table 4. $TS_2$, $TS_4$, $TS_7$ and $TS_{10}$ produced triploid with 27 number of chromosome. Accession numbers TS, $TS_1$, $TS_5$, $TS_6$, $TS_9$, $TS_{11}$, $TS_{12}$ and $TS_{13}$ were germinated from the monoembryonic seeds but accomplished unexpected tetraploids rather than triploids, may be of nucellar origin. Accessions $TM_{2-3}$ - $TM_{10}$ were from the polyembryonic seed. Only $TM_{2-3}$ and $TM_{10}$ were triploid while others resulted in normal nucellar tetraploids.

## Discussion

Following chromosomal analysis of seedlings from 2x x 4x crosses, a high number of tetraploids, in addition to triploids, was observed. However, the proportions fluctuated depending on the pistillate parents but staminate parents seem to have little effect on proportion of triploids and tetraploids (Tables 1 & 2). When comparisons were made on the basis of different parents progeny, Kinnow (4x) x Succari (2x) produced the highest percentage of tetraploids (71.4%) followed by Kinnow (2x) x Kinnow (4x) (33.3%), Succari (2x) x Kinnow (4x) (20%) and Kinnow (4x) x Kinnow (2x) (16.6%). The occurrence of tetraploids in high frequencies from 2x x 4x crosses was expected, resulted from the mechanisms such as doubling of the chromosome complement in the haploid egg cell due to delayed fertilization by diploid male gametes, or preferential fertilization of diploid egg cells by diploid male gametes in the presence of certain combinations of incompatible alleles (Cameron & Soost, 1969; Esen & Soost, 1972). Tachikawa *et al.*, (1961) suggested chromosomal aberration to explain the occurrence of such tetraploids in frequencies as high as 70 to 80%.

High proportions of triploid (83.4%) were obtained in crosses of 4x Kinnow crossed with 2x Kinnow (Table 1). This was in contrast to the low proportions (25%) obtained with 4x Kinnow used as pollen parents with 2x Kinnow seed parents (Table 2). These results are similar to that of Cameron & Burnett (1978) who obtained high proportions of 3x hybrids from crosses of 4x seed parents by 2x pollen parents in *Citrus*. It was also noted that the frequency of triploids in crosses where tetraploids were used as seed parents affected by the staminate parents. Tetraploid Kinnow seed parent crossed with diploid Succari yielded low number of triploids but high proportion of tetraploids (Table 4).

**Table 4. Ploidy level in Kinnow (4x) X Succari (2x) progeny.**

| Accessions | No. of Chromosomes |
|---|---|
| TS | 36 |
| $TS_1$ | 36 |
| $TS_2$ | 27 |
| $TS_4$ | 27 |
| $TS_5$ | 36 |
| $TS_6$ | 36 |
| $TS_7$ | 27 |
| $TS_9$ | 36 |
| $TS_{10}$ | 27 |
| $TS_{11}$ | 36 |
| $TS_{12}$ | 36 |
| $TS_{13}$ | 36 |
| $TM_{2-3}$ | 27 |
| $TM_{2-6}$ | 36 |
| $TM_{3-2}$ | 36 |
| $TM_{5-2}$ | 36 |
| $TM_6$ | 36 |
| $TM_{6-1}$ | 36 |
| $TM_{8-2}$ | 36 |
| $TM_{9-1}$ | 36 |
| $TM_{10}$ | 27 |

The recovery of triploid hybrids obtained from interploid sexual hybridization using different parents, gives the opportunity to increase the genetic variability among seedless citrus types and to select important fruit characteristics. Tetraploid plants obtained in this manner, however, increase the genetic variability and, if fertile, can be used as parents for polyploid breeding programs to produce triploids. It will enhance the citrus gene pool for further exploitation.

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