



CHROMOSOME NUMBER AND TIME PERIOD OF MITOTIC CYCLE OF FESTIVAL AND CALIFORNICA STRAWBERRY CULTIVARS (*Fragaria x ananassa* AND *Fragaria vesca*)

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ABSTRACT

The cultivated strawberries, *Fragaria x ananassa* and *Fragaria vesca*, are the most economically-important softfruit species. *F x ananassa* and *F vesca*, both diploid ($2n=2x=14$) relatives of the commercial octoploid strawberry, are an attractive model for functional genomics research in Rosaceae. Its small genome size, short reproductive cycle, and facile vegetative and seed propagation make *F. x ananassa* and *F. vesca* a promising candidates for forward and reverse genetics experiments. In order to determine their genetic differences in more detail, chromosome characterization of the two strawberry cultivars was investigated. A method used for chromosome slides in this research was a squash method with modification in pre-treatment. The result showed *Fragaria x ananassa* had ($2n = 4x = 28$) chromosome number is 28 and *Fragaria vesca* had ($2n = 2x = 14$) chromosome number is 14. The time of mitotic that both strawberry cultivars was similar at 7 to 8.30 am. In addition, mixoploid cells were found in both strawberry cultivar indicating that these cultivars had been treated by mutagenic agents for a breeding program.

Key words : *Fragaria*, chromosome, mitotic

INTRODUCTION

The cultivated strawberries, *Fragaria x ananassa* and *Fragaria vesca*, are the most economically important soft fruit species and belong to the Rosaceae which known to be a large and diverse family including *Malus* (apples), *Prunus* (the stone fruits and almonds), *Rubus* (the blackberries and raspberries), *Pyrus* (pears) and many ornamental species including the genus *Rosa* (the roses). *Fragaria x ananassa* is an allo-octoploid species ($2n = 8x = 56$) derived from artificial hybridisation between two progenitor species *F. chiloensis* and *F. virginiana* (Darrow, 1966). The genus *Fragaria* has a basic chromosome number of seven ($x=7$) (Ichijima, 1926), and four main fertility groups are recognized: the diploids ($2n=2x=14$) which include the model species for the genus, *F. vesca*; the tetraploids ($2n=4x=28$) including *F. orientalis*; the single hexaploid species *F. moschata* ($2n=6x=42$); and four octoploid species ($2n=8x=56$): *F. chiloensis*, *F. iturupensis*, *F. virginiana* and the hybrid cultivated strawberry, *F. x ananassa*. The octoploid species are thought to be allo-octoploids, with a proposed genome composition of AAA'A'BBB'B' implying contributions from at least four distinct ancestral diploid genomes. However, the prior genome composition models (AAAABBCC and AAA'A'BBBB) of (Fedorova, 1946) and (Senanayake & Bringhurst, 1967), respectively, recognized the possibility of an autopolyploid component, and the genome compositions of the octoploid species have yet to be rigorously established.

The chromosomes of *Fragaria* are rather small, ranging from 0.9 – 1.7 microns in length (Yarnell, 1928) and display very little variation in morphology between species (Iwatsubo &

Naruhashi, 1989). Ichijima (1926) studied the cytology of *Fragaria* chromosomes at various levels of ploidy. In the diploid species, no irregular chromosomal behavior was observed in the course of heterotypic division and the 14 somatic chromosomes were clearly seen and could be readily counted at metaphase. In the octoploid *Fragaria*, it was difficult to count the somatic chromosomes because of the closely and irregularly packed character. The count was best done during late diakinesis. Chromosome number in these experiments could not be determined definitely, but it was reported that it appeared to be 56, which would be expected for an octoploid species with a haploid chromosome number of $x=7$. Byrne & Jelenković (1976) observed only bivalents in meiotic spreads from nine cultivated genotypes. More recently, (10) applied fluorescent *in situ* hybridization (FISH) to *F. vesca* using the 45S and 5S rRNA genes, which revealed six 45S and two 5S sites within the 14 somatic chromosomes of *F. vesca*. The experiments of (Lim, 2004) enabled the construction of an *F. vesca* karyotype with three pairs of marker chromosomes, but the technique has yet to be extended to the genetically and cytologically more complex genomes of the octoploid *Fragaria* species. In order to determine their genetic differences in more detail, chromosome characterization of the two strawberry cultivars was investigated.

MATERIALS AND METHODS

Plant material

F. x ananassa and *F. vesca* accessions used in this study was obtained from the Banyuroto Agrotourism at Sawangan Magelang, Central Java. Seedlings were taken from young plants that germinated from one month old of stolon.

Chromosome preparation

Root tips from germinating seedling from field-grown was used for chromosome preparation. Seedling germination from *F. x ananassa* and *F. vesca* was conducted in a growth chamber maintained at 25°C. Germinating root of stolon derived from seedling with 1 – to 5 – mm radicles and cutted at root tip. The root tips were placed in freshly prepared 45% glacial acetic acid and stored in a refrigerator for at least 15 min. Subsequently, the root tips were washed three times with aquadest, and were excised and hydrolyzed in 1N HCl for 3 min at 56°C. The tips were washed with aquadest three times, and then were stained in 2% aceto orcein at room temperature for 2h. A root was transfered to the center of a clean microscope slide and added a drop of glicerol onto the root. Using a razor blade cut off most of the unstained part of the root, and discarded it. Cover the root tip with a cover slip, and then carefully push down on the cover slide with the wooden end of a dissecting probe. Push hard, but do not twist or push the cover slide sideways. The root tip should spread out to a diameter about 0.5 – 1 cm.

RESULTS AND DISCUSSION

Chromosomes consider small in all *Fragaria* species, and only minor variation in chromosome morphology has been described (Ichijima, 1926; Senanayake & Bringhurst, 1967; Iwatsubo & Naruhashi, 1989). *F. x ananassa* had tetraploid ($2n = 4x = 28$) and *F. vesca* had diploid ($2n = 2x = 14$) (Figure 1). Mitotic phase were obtained for prophase, prometaphase,

metaphase, anaphase, telophase and interphase (Figure 2).

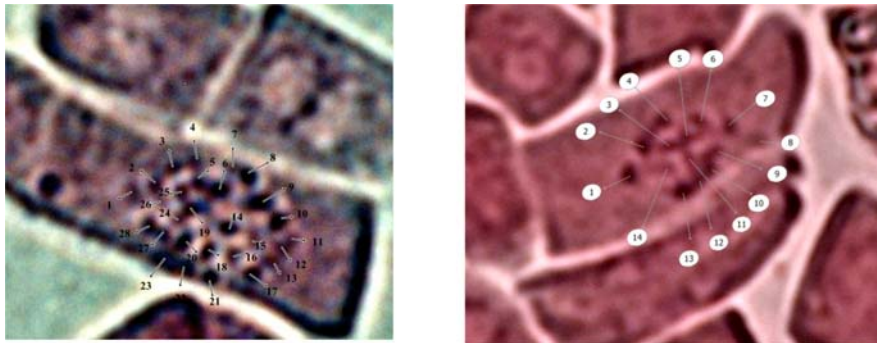


Figure 1. Chromosome number from *F. x ananassa* ($2n = 4x = 28$) (left) and *F. vesca* ($2n = 2x = 14$) (right). The number indicates the count of chromosome

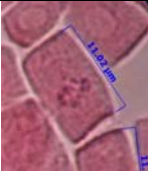




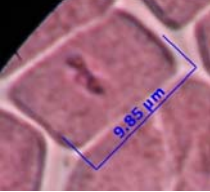
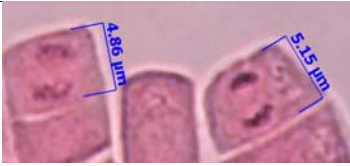

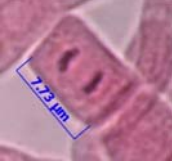
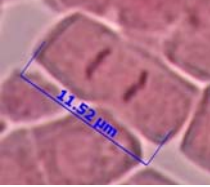
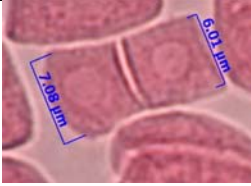
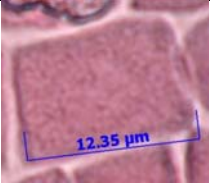
Mitotic Phases	<i>F. x ananassa</i> ($2n = 4x = 28$)	<i>F. vesca</i> ($2n = 2x = 14$)
Prophase		
Prometaphase		
Metaphase		
Anaphase		
Telophase		
Interphase		

Figure 2. Mitotic phase of *Fragaria x ananassa* (left) and *Fragaria vesca* ($2n = 2x = 14$) (right).

Time periods for *F. x ananassa* were at 06.00 – 07.00 am and *F. vesca* at 07.00 – 08.00 am. The basic chromosome number in *Fragaria* is $x = 7$ (Ichijima, 1926). The recognized *Fragaria* species comprises an apolyploid series, including twelve diploid ($2n = 2x = 14$) species, four tetraploids ($2n = 4x = 28$), one hexaploid ($2n = 6x = 42$: *F. moschata*), and four octoploids ($2n = 8x = 56$). Synthetic octoploids have been constructed via controlled, interspecific hybridizations accompanied by chromosome doubling, in an effort to broaden the octoploid gene pool available to strawberry breeders (Evans, 1977; Bors, 2000). The decaploid level has been obtained in controlled crosses accompanied by chromosome number manipulation (Ahmadi & Bringham, 1992; Scott, 1951). Decaploids referred to as *Fragaria x vescana* were derived from crossing *F. x ananassa* ($2n = 56$) with tetraploid forms of *F. vesca* var. *semperflorens* ($2n = 28$) followed by backcrossing to *F. x ananassa* (2).

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