

Original Articles

Morphological characteristics of fruit in a haploid pummelo

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(Accepted on December 27, 2010)

Summary : The ploidy level of several tissues and the morphological characteristics in the fruit of a haploid plant derived from seedlings of 'Banpeiyu' pummelo (*Citrus maxima* (Burm.) Merr.) were investigated. Flow cytometric analysis revealed that the relative nuclear DNA content of the haploid was half those of the juice sac, pulp segment, albedo, and flabedo of the 'Banpeiyu' pummelo, and the haploidy was maintained in all tissues of the fruit. With regard to morphological characteristics of a fruit, the haploid produced a small fruit, a small number of locules, and a fine juice sac compared with the 'Banpeiyu' pummelo. The number of seeds per fruit of the 'Banpeiyu' pummelo was approximately 100, whereas the haploid fruit had no seeds. In all probability, the fruit of the haploid obtained in the present study was produced by parthenocarpy.

Key words : 'Banpeiyu' pummelo, Flow cytometry, Parthenocarpy, Seedless

Introduction

In *Citrus* and related genera, some haploid plants have been produced (Froelicher *et al.*, 2007 ; Germana & Chiancone, 2001 ; 2003 ; Hidaka *et al.*, 1979 ; Oiyama & Kobayashi, 1993). However, no flowering and fruiting of haploids has ever been reported because these haploids were very weak, and grew more slowly than the original diploid plants.

Toolapong *et al.* (1996) selected a haploid progeny among small seed-derived seedlings obtained from a cross between 'Banpeiyu' pummelo and 'Ruby Red' grapefruit (*C. paradisi* Macfad.). When this haploid was grafted onto a trifoliate orange (*Poncirus trifoliata* [L.] Raf.) seedling, it showed vigorous growth and flowered seven years after germination. The haploid had small leaves, flowers, and pollen grains compared with 'Banpeiyu' pummelo, and produced slightly fertile pollen grains (Yahata *et al.*,

2005a). On the other hand, in term of the fruit characteristics of the haploid, although it was pollinated with pollen from several diploid citrus cultivars, no fruit set in any of the cross combinations (Yahata *et al.*, 2005b). Therefore, the morphological characteristics of fruits in the haploid pummelo have not yet been determined. Fortunately, three years after achieving the reproductive growth, the haploid bore one fruit for the first time.

In the present study, we reported on the morphological characteristics of fruit in the haploid pummelo produced by Toolapong *et al.* (1996). Furthermore, we analyzed the ploidy level of several tissues in the fruit of the haploid.

Materials and Methods

Plant Materials

A haploid plant obtained from the cross between 'Banpeiyu' pummelo and 'Ruby Red' grapefruit (Toolapong *et al.*, 1996) was used in the present study. The haploid was grafted onto a trifoliolate orange seedling, and maintained for approximately 10 years in the greenhouse of the School of Agriculture, Tokai University. Approximately 10-year-old 'Banpeiyu' pummelo plants grown in the orchard of Mr. Kiyoto Harusaki at Yatsushiro City, Kumamoto Prefecture, were used as controls.

Confirmation of Ploidy Level by Flow Cytometry

Approximately 50 mg segments of juice sac, pulp segment, albedo, and flabedo were collected from one fruit of the haploid pummelo and one fruit of the 'Banpeiyu' pummelo. The samples were chopped with a razor blade and blended for 5 min with 2 mL buffer solution containing 1.0 % (v/v) Triton X-100, 140 mM mercaptoethanol, 50 mM Na₂SO₃, and 50 mM Tris-HCl at pH 7.5, according to the preparation method of Harusaki *et al.* (2000). An aliquot (550 μ L) of each sample was filtered through Miracloth (Merck KGaA, Darmstadt, Germany), and the filtrate was stained with 50 μ L of 0.5 g \cdot L⁻¹ propidium iodide (PI). The relative fluorescence intensity of nuclear DNA was measured with a Flow Cytometry System (EPICS XL; Beckman Coulter, Inc., CA, USA) equipped with an argon laser (488 nm, 15 mW).

Morphological Characteristics of Fruit

The morphological characteristics of the fruit (e.g., the size of the fruit, the number of locules and seeds, and the size of the juice sac) in the haploid were

investigated. As a control, the 'Banpeiyu' pummelo was used, and the characteristics were measured using 10 fruits.

Results and Discussion

A fruit was obtained from an approximately 10-year-old plant of the haploid pummelo grown in the greenhouse of the School of Agriculture, Tokai University (Fig. 1). Flow cytometric analysis revealed that the fluorescence intensity of the nucleus in several tissues of the fruit such as tissues from the juice sac, pulp segment, albedo, and flabedo of the haploid was half those of the 'Banpeiyu' pummelo, indicating that the ploidy level of the haploid was maintained in several tissues of the fruit (Fig. 2). Generally, it is easy to double the number of chromosomes of haploids spontaneously, and therefore the maintenance of haploid cells is extremely difficult (Wenzel *et al.*, 1976). In *Citrus* plants and their relatives, diploid and mixoploid plantlets have been produced from anther cultures (Germana & Chiancone, 2003; Hidaka *et al.*, 1982; Hidaka, 1984). Yamamoto & Tominaga (2004) reported haploid and diploid periclinal chimera arose from the haploid plant of the clementine. In the present study, the haploidy was maintained in all of the tissues of the fruit examined.

The morphological characteristics of the fruit from the haploid plant were compared with those of the 'Banpeiyu' pummelo plant (Fig. 3, Table 1). The fruit weight of the 'Banpeiyu' pummelo was approximately 1800 g, whereas that of the haploid was approximately 200 g, which was much smaller than that of the 'Banpeiyu' pummelo. Furthermore, the haploid



Fig. 1. Fruit of the haploid pummelo. Bar = 5 cm.

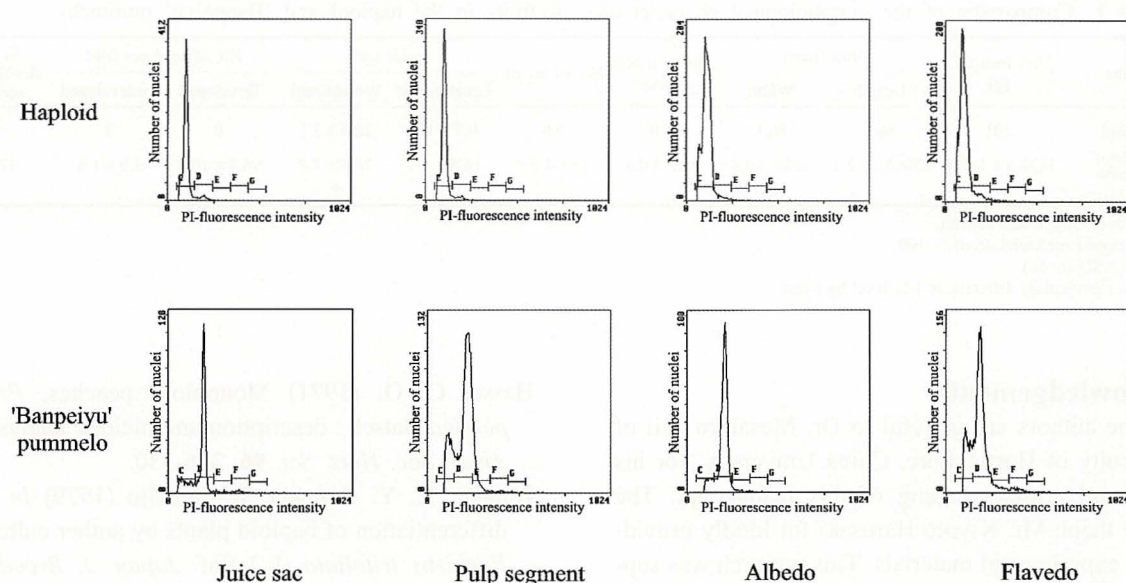


Fig. 2. Flow cytometric analysis of juice sac, pulp segment, albedo, and flavedo in parthenocarpic fruit of the haploid and 'Banpeiyu' pummelo.

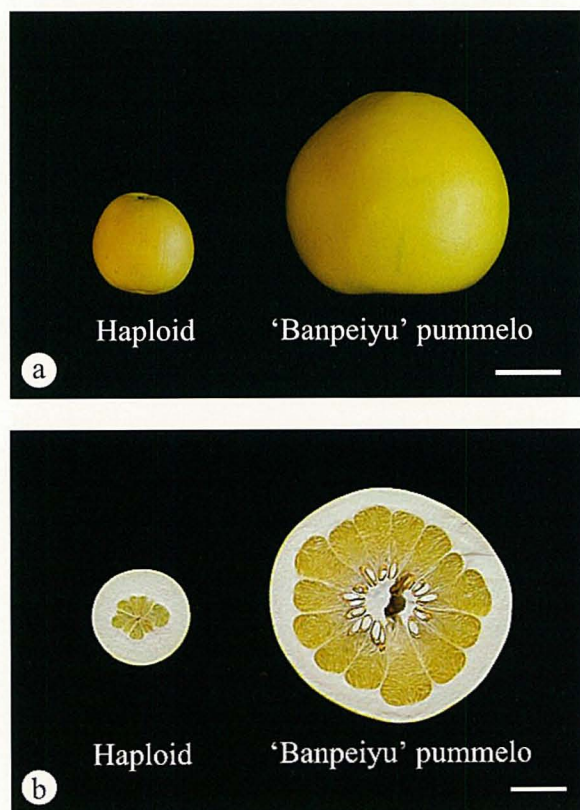


Fig. 3. Morphological characteristics of fruit in the haploid and 'Banpeiyu' pummelo. Bars = 5 cm. (a) Whole fruit. (b) Cross section.

produced a small number of locules and fine juice sacs compared with the 'Banpeiyu' pummelo. The number of seeds per fruit obtained from 'Banpeiyu' pummelo was approximately 100, whereas there were no seeds at all in the fruit of the haploid.

It has been reported that haploids were obtained in fruit crops such as peach, apple, pear, and banana (Assani *et al.*, 2003; Bouvier *et al.*, 2002; Hesse, 1971; Ochatt & Zhang, 1996; Zhang & Lespinasse, 1991). Haploid plants generally show poor growth compared with the original diploid plants. The flowering and fruiting of haploids are rare and are only observed in peaches among fruit crops (Hesse, 1971; Pooler & Scorza, 1995). Hesse (1971) reported that two genotypes of haploid peaches showed very small fruit compared with the original diploid plants. On the other hand, Pooler & Scorza (1995) found that five out of seven genotypes of haploid peach had smaller fruits than that of the original diploid cultivar, whereas the other two genotypes produced large fruits with fertile seeds. In the present study, the haploid pummelo produced small and seedless fruit. Although 'Banpeiyu' pummelo showed a low parthenocarpic habit and rarely produced seedless fruits (Isobe *et al.*, 1982; Iwamasa, 1976), the development of the fruit of the haploid might be caused by parthenocarpic. In the future, we plan to carry out detailed studies on the expression of parthenocarpic in the haploid.

Table 1. Comparison of the morphological characteristics of fruits in the haploid and 'Banpeiyu' pummelo.

Strains	Av. fruit wt. (g)	Fruit (mm)		Shape index of fruit ¹	No. of locule	Juice sac		No. of seed per fruit		% of developed seeds ²
		Length	Width			Length(mm)	Weight(mg)	Developed	Undeveloped	
Haploid	201	86.1	86.1	1.0	9.0	9.7±1.6	18.9±3.1	0	0	—
'Banpeiyu' pummelo	1834.5±147.4 ³	206.6±12.2	165±10.8	1.25±0.3	15.8±0.9	16.5±1.9 [*]	76.5±7.8 [*]	96.8±10.4	3.0±1.8	97.0

¹ Length of fruit/Width of fruit.² (Developed seed/total seed) × 100.³ Mean ± SD (n=10).^{*} Mean significantly different at 1 % level by t-test.

Acknowledgements

The authors are grateful to Dr. Masahiro Mii of the Faculty of Horticulture, Chiba University, for his advice and critical reading of this manuscript. The authors thank Mr. Kiyoto Harusaki for kindly providing the experimental materials. This research was supported by Diet and Cancer Prevention : Exploring Research Technology, Miyazaki Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence, JST, and the Sasakawa Scientific Research Grant from The Japan Science Society.

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半数体ブンタンの果実形質

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要約

‘晩白柚’の実生から得られた半数体ブンタン (*Citrus maxima* (Burm.) Merr.) の果実における様々な組織の倍数性解析と形態調査を行った。半数体の果実における様々な器官、組織の倍数性をフローサイトメーターで解析した結果、半数体の砂じょう、じょうのう、アルベドおよびフラベドともに相対蛍光強度が‘晩白柚’と比較して半数性を示し、この半数体は半数性を維持していることが明らかとなった。半数体ブンタンの果実と砂じょうの大きさは、‘晩白柚’と比べ小さく、室数は著しく少なかった。種子については、‘晩白柚’は1果実あたり100粒程度の完全種子を有していたが、半数体の果実では全く種子が形成されていなかった。おそらく、本研究で得られた半数体の果実は単為結果したものと推察される。

キーワード：‘晩白柚’，フローサイトメトリー，無核，単為結果