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SHORT COMMUNICATION

CMA Banding Patterns of Chromosome of Mid- and Late-maturing Citrus and Acid Citrus Grown in Japan

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Summary

Fluorochrome staining with chromomycin A_3 (CMA) was used to characterize and compare the CMA banding patterns of chromosomes in eight species of citrus that are cultivated in Japan. Chromosomes were classified into five types based on the number and position of CMA positive bands; A: two terminal and one proximal band, B: one terminal and one proximal band, C: two terminal bands, D: one terminal band, E: no band. The CMA banding patterns were 1A+1C+8D+8E in Hassaku, 2A+2C+5D+9E in Hyuganatsu, 1A+2C+7D+8E in Natsudaidai 'Kawano natsudadai', 1A+1B+1C+8D+7E in Iyo 'Miyauchi iyokan', 1A+1B+1C+8D+7E in Tankan 'Tarumizu 1 gou', 3B+2C+5D+8E in Kabosu, 1B+2C+9D+6E in Sudachi and 2B+1C+11D+4E in Yuzu 'Yamane'. A similar CMA banding pattern was observed in related species examined in this study.

Key Words: chromomycin A_3 (CMA), chromosome, citrus, karyotype.

Introduction

Chromosome identification of citrus has progressed rapidly by using fluorescent banding of a base-specific binding fluorochrome, such as chromomycin A_3 (CMA) (Befu et al., 2000, 2001, 2002; Guerra, 1993; Miranda et al., 1997; Yamamoto and Tominaga, 2003, 2004). These studies demonstrated the existence of characteristic CMA banding patterns with a high level of diversity and heterozygosity in citrus chromosomes. The results also revealed CMA banding patterns of important species, such as *Citrus sinensis, C. unshiu, C. paradisi*, and *C. grandis* and similar patterns among related species and cultivars.

In Japan, various citrus species and cultivars have been cultivated. Mid- and late-maturing citrus, such as Iyo (*C. iyo*), Natsudaidai (*C. natsudaidai*), Hassaku (*C. hassaku*), Hyuganatsu (*C. tamurana*) and Tankan (*C. tankan*) and acid citrus, such as Yuzu (*C. junos*), Sudachi (*C. sudachi*) and Kabosu (*C. spaerocarpa*), are very important for the industry. Therefore, in this study, we determined the variability of CMA banding patterns of chromosomes in these citrus, and discuss their phylogenic relationships.

Materials and Methods

Citrus species and cultivars preserved at the Faculty of Agriculture, Kagoshima University were analyzed for CMA banding patterns (Table 1). Young leaves about 3 to 5 mm long were excised from adult trees, immersed in 2 mM 8-hydroxyquinoline at 10℃ for 4 h in the dark, fixed in methanol-acetic acid (3:1), and stored at -20℃. Enzymatic maceration and air drying were performed as described by Yamamoto and Tominaga (2003). The young leaves were washed in distilled water to remove the fixative and macerated in an enzyme mixture of 2% Cellulase Onozuka RS, 1.5% Macerozyme R200, 0.3% Pectolyase Y-23, and 1 mM EDTA, pH4.2, at 37℃ for 60 min. Chromosomes were stained with 0.1 $g \cdot L^{-1}$ CMA and observed under a fluorescence microscope with a BV filter cassette.

Results and Discussion

Depending on their staining patterns, chromosomes were classified into five types based on the number and position of CMA positive bands, as indicated by Miranda et al. (1997) and Befu et al. (2000), i. e., A: two terminal and one proximal band, B: one terminal and one proximal band, C: two terminal bands, D: one terminal band, E: no band. The CMA banding patterns of mid- and late-maturing citrus were 1A+1C+8D+8E in Hassaku, 2A+2C+5D+9E in Hyuganatsu, 1A+2C+7D+8E in Natsudaidai 'Kawano natsudadai', 1A+1B+1C+8D+7E in Iyo 'Miyauchi iyokan', 1A+1B+1C+8D+7E in Tankan 'Tarumizu 1 gou'. The CMA banding patterns of acid citrus were 3B+2C+5D+8E in Kabosu, 1B+2C+9D+6E in Sudachi, and 2B+1C+11D+4E in Yuzu 'Yamane' (Fig. 1 and Table 2).

In chromosome CMA banding patterns, pummelo and its close relatives possessed four to seven types A, B,

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Table 1. List of citrus accessions analyzed in this study.

Common name	Latin name
Mid- and late-maturing citrus	
Hassaku	*Citrus hassaku* hort. ex Tanaka
Hyuganatsu	*C. tamurana* hort. ex Tanaka
Kawano natsudaidai	*C. natsudaidai* Hayata
Miyauchi iyokan	*C. iyo* hort. ex Tanaka
Tarumizu 1 gou	*C. tankan* Hayata
Acid citrus	
Kabosu	*C. spaerocarpa* hort. ex Tanaka
Sudachi	*C. sudachi* hort. ex Shirai
Yamane	*C. junos* Sieb. ex Tanaka

Table 2. CMA banding patterns of somatic chromosomes of *Citrus* species.

Common name	CMA banding pattern[z]
Hassaku	1A +1C+8D+8E
Hyuganatsu	2A +2C+5D+9E
Kawano natsudaidai	1A +2C+7D+8E
Miyauchi iyokan	1A+1B+1C+8D+7E
Tarumizu 1 gou	1A+1B+1C+8D+7E
Kabosu	3B+2C+5D+8E
Sudachi	1B+2C+9D+6E
Yamane	2B+1C+11D+4E

[z] A: two terminal and one proximal CMA (+) regions, B: one terminal and one proximal CMA (+) region, C: two terminal CMA (+) regions, D: one terminal CMA (+) region, E: No CMA (+) region.

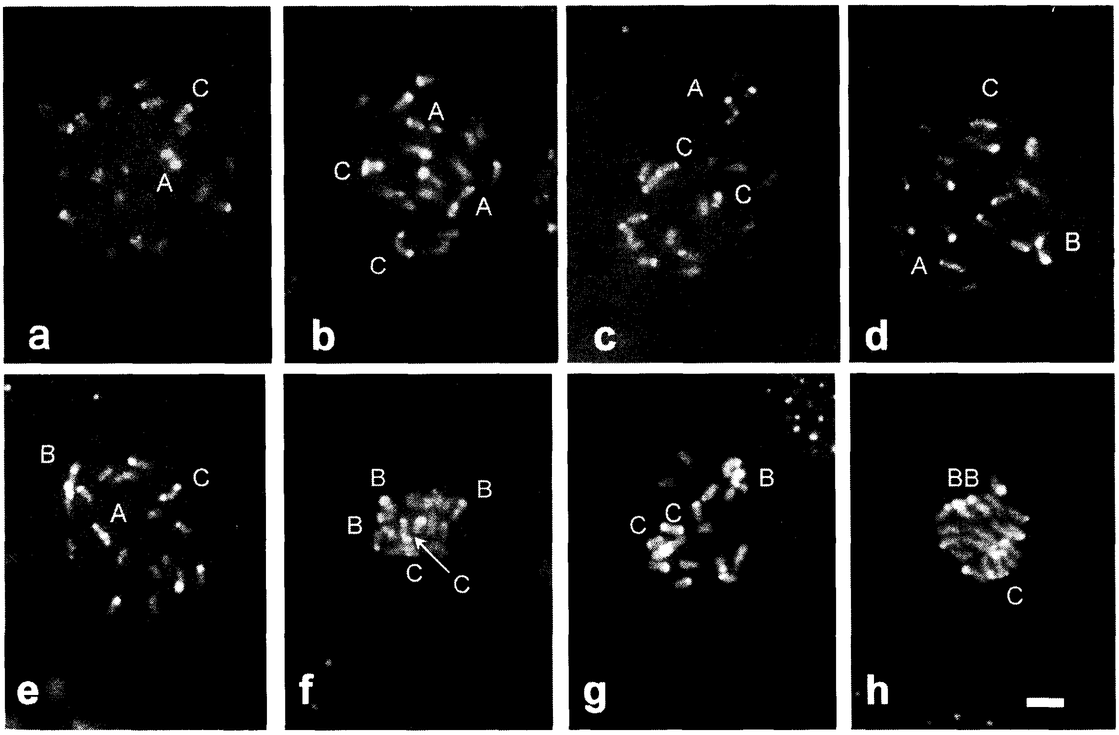

Fig. 1. CMA staining of somatic chromosomes in *Citrus* species. a: Hassaku, b: Hyuganatsu, c: Natsudaidai 'Kawano natsudadai', d: Iyo 'Miyauchi iyokan', e: Tankan 'Tarumizu 1 gou', f: Kabosu, g: Sudachi, h: Yuzu 'Yamane'. A–C: See Table 2. Bar in h represents 5 μm for all figures.

and C chromosomes, but almost all mandarins possessed fewer than three of those types of chromosomes (Befu et al., 2001; Yamamoto and Tominaga, 2003). Hassaku, 'Kawano natsudaidai' and 'Miyauchi iyokan' are believed to be hybrids of pummelo and mandarin (Hirai et al., 1986; Iwamasa, 1999; Yamamoto et al., 1993). Although the origin of Hyuganatsu is unclear, it had genes from pummelo (Yamamoto et al., 1993). There were two types A, B, and C chromosomes in Hassaku, four in Hyuganatsu, three in 'Kawano natsudaidai', and three in 'Miyauchi iyokan'. These numbers are intermediate between those of pummelos and mandarins.

'Tarumizu 1 gou' is considered to be a natural tangor, hybrid of mandarin and sweet orange (Iwamasa, 1999). The CMA banding pattern of the parental mandarin is unknown, but sweet orange has a 2B+2C+7D+7E in chromosome configuration (Befu et al., 2000; Miranda et al., 1997). Although it does not have the type A chromosomes found in 'Tarumizu 1 gou', the resemblance is found in CMA banding patterns of sweet orange and those of 'Tarumizu 1 gou'; a number of types B, C, D, and E chromosomes are similar. However, the reason for identical CMA banding pattern of 'Tarumizu 1 gou' and 'Miyauchi iyokan' that are not close relatives is unclear. The origin of 'Miyauchi iyokan' is unresolved although it seems to be a natural tangelo, hybrid of pummelo and mandarin (Hirai et al., 1986; Iwamasa, 1999). However, there is possibility that it is a natural tangor (Hodgeson, 1967). It may be one explanation for the identical CMA banding pattern found in 'Miyauchi iyokan' and 'Tarumizu 1 gou'.

Yuzu is considered to be a parent of Kabosu and Sudachi (Hirai et al., 1986; Iwamasa, 1999). In our study, types B, C, D, and E chromosomes were observed in Yuzu, Kabosu, and Sudachi. Although Hyuganatsu also seems to be a Yuzu relative because of its fruit characteristics (Iwamasa, 1999), it has two type A chromosomes instead of type B, and CMA banding pattern unlike that of Yuzu. Since *C. icahngensis* is assumed to be closely related to Yuzu (Hirai and Kajiura, 1987), it is very important to determine its CMA banding pattern. Such information would be useful in tracing the phylogeny of Yuzu and related species.

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わが国における主要中晩生および香酸カンキツ染色体のCMA染色

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摘　要

わが国における主要中晩生および香酸カンキツ染色体のクロモマイシン A_3 (CMA)染色を行った．染色体はCMA(+)バンドの有無および位置から5種類に区分できた．すなわち，CMA(+)をA:両端および動原体近傍に有する，B: 一方の端部と動原体近傍に有する，C: 両端に有する，D: 一方の端部に有する，E: CMA(+)がない，である．各種はこれらのうち4，5種類の染色体を有し，独自のCMAバンドパターンを示した．ハッサクでは1A+1C+8D+8E，ヒュウガナツでは2A+2C+5D+9E，'川野なつだいだい'では1A+2C+7D+8E，'宮内伊予柑'では1A+1B+1C+8D+7E，タンカン'垂水1号'では1A+1B+1C+8D+7E，カボスでは3B+2C+5D+8E，スダチでは1B+2C+9D+6Eおよびユズ'山根'では2B+1C+11D+4Eであった．以上の結果，本研究においても近縁の種間では似通ったCMAバンドパターンが観察された．