A Cytogenetic Study of Three Japanese Cultivars of *Momordica charantia* L.

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Summary Using a base-specific staining method, three Japanese cultivars of *Momordica charantia* were investigated. The present method using enzymatic digestion produced a high level of well-spread metaphase chromosome complements. The cultivars investigated showed the same somatic chromosome number $2n=22$. In condensation behavior from prophase to metaphase, most chromosomes of all cultivars in this study had early condensing segments at the proximal regions. Moreover, at prophase or earlier stage of metaphase, most chromosomes observed had decondensed segments at the distal regions in both arms. Judging by the determination of centromere positions, the chromosome complements at mitotic metaphases in three cultivars consisted of 22 metacentric chromosomes. Moreover, similar sizes and shapes of the chromosomes indicated that the karyotypes of *M. charantia* cultivars were symmetrical. In contrast, fluorescent staining showed different sizes and numbers of chromycin A₃ (CMA) positive and 4',6-diamidino-2-phenylindole (DAPI) negative (CMA⁺DAPI[−]) satellites among cultivars. Four CMA⁺DAPI[−] satellites were observed in *M. charantia* 'Abashi-goya' and *M. charantia* 'Naga-goya,' while two satellites were observed in *M. charantia* 'Shiro-goya.' All satellites were located at one end of sat-chromosomes. In 'Shiro-goya,' a large size difference of satellites was found between two sat-chromosomes, which might be homologous chromosomes with polymorphism. Moreover, faintly CMA⁺DAPI[−] sites were shown at the proximal regions or primary constrictions of most chromosomes in all cultivars.

Key words Somatic chromosome characterization, Base-specific fluorescence staining, *Momordica charantia*, Karyotype.

An economically important genus, *Momordica* L., which belongs to the family Cucurbitaceae, consists of about 60 species (Schaefer and Renner 2010). In this genus, *Momordica charantia* L., which is an annual dioecious climber plant, is one of the most commercially useful species (Zaman and Alam 2009).

Momordica charantia grows in the wild throughout tropical and sub-tropical regions of the world (Zaman and Alam 2009), and the fruits are widely consumed as vegetable foods mainly in South America and South East Asia, including China and Japan. The fruits take different shapes such as long spindle, short spindle, long rugby ball, oblong and olive. Most cultivated strains have important compounds for health, and therefore are also used as a treatment for diabetes, reduction of adiposity, leprosy, jaundice, rheumatism and gout in several cultures (Leatherdale *et al.* 1981, Chen *et al.* 2003, Semiz and Sen 2007). Moreover, because of the

importance of food and medicinal values, many seeds of different cultivars/germplasms have been collected from all over the country by breeding organizations in Asian countries.

Karyological information is useful for planning of plant breeding. There are some studies for *M. charantia* that present karyological information, chromosome measures and karyographs (McKay 1930, Bhaduri and Bose 1947, Lombello and Pinto-Maglio 2007, Zaman and Alam 2009, Bharathi *et al.* 2011, Waminal and Kim 2012, Kausar *et al.* 2014), but not so many. All the studied species presented symmetrical karyomorphology. Only a few reports described the centromere position, although it is quite important for karyomorphological characterization and chromosome classification. Thus, up to now, the cytogenetic study of this species is still poor, basically limited to its chromosome number and karyomorphological data in major cultivars of this species. In particular, there is no information on Japanese cultivars.

According to previous studies, the *Momordica* group

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has quite similar and small-sized chromosomes (Agarwal and Roy 1976, Sinha *et al.* 1996). In this case, chromosome characterization is not sufficient by classical karyotype analysis. Thus, different staining methods such as chromosome banding are helpful for detailed karyomorphological analysis.

In the present study, using base-specific staining methods, three Japanese cultivars of *Momordica charantia* were investigated to clarify centromere positions and natures on all chromosomes, and to characterize the karyotypes of the cultivars.

Materials and methods

Plant materials

Three cultivars, *Momordica charantia* 'Abashi-goya,' 'Naga-goya' and 'Shiro-goya' used in this study were purchased from Tohoku Seed Co., Ltd., and are maintained at the Department of Plant Science, School of Agriculture, Tokai University.

Chromosome preparation

Mitotic chromosome slides were prepared using an enzymatic digestion method. The root tips for chromosome preparation were collected from three-day-old seedlings. The root tips were pretreated in 0.05% colchicine for 1.5 h at 18°C and fixed in 3:1 ethanol–acetic acid and washed using ethanol series (50%, 30%, 10% and distilled water two times, 10 min each). After washing with distilled water, those roots were cut to 3 mm length and treated with 4% Cellulase 'Onozuka' R10 (Serva) and 2% Pectolyase Y-23 (Sigma) at pH 4.8 at 37°C for 1 h. Then, they were washed three times with distilled water for 10 min each, transferred and chopped on glass slides. Suspended cells were mixed with a few drops of the fixative, and then their chromosomes were spread and air-dried for 18 h at room temperature.

Base-specific staining

In fluorescent staining with chromomycin A₃ (CMA), the method of Schweizer (1981) and Plader *et al.* (1998) was followed with slight modifications. The slides were preincubated in McIlvaine's buffer (pH 7.0) for 30 min and rinsed in McIlvaine's buffer supplemented with 5 mM MgSO₄ for 10 min. After being rinsed, they were stained with 0.1 mg/mL CMA in McIlvaine's buffer supplemented with 5 mM MgSO₄ for 10 min in a humid chamber and rinsed in McIlvaine's buffer supplemented with 5 mM MgSO₄ for 10 min. Then, they were mounted with glycerol and stored at 4°C for at least 10 h to prevent fading. These chromosomes stained with CMA were irradiated with BV filter cassette and fluoresced yellow.

In fluorescent staining with 4,6-diamidino-2-phenylindole (DAPI), the fluorescent method of Schweizer (1976) was followed with slight modifications. After CMA staining, the slides were used for sequential DAPI

staining. The slides were destained in 45% acetic acid for 30 min and in 3:1 ethanol–acetic acid for 30 min, and rinsed in distilled water for 5 min. They were dipped in McIlvaine's buffer for 30 min and rinsed in McIlvaine's buffer for 10 min. After being rinsed, they were stained with 0.01 mg/mL DAPI in McIlvaine's buffer for 10 min. These chromosomes stained with DAPI were irradiated with UV filter cassette and fluoresced blue.

Photographs were taken with SenSys (Photometrics) and an Olympus fluorescent microscope (BX51). The chromosome number and number of fluorescent banding segments in each cultivar were confirmed in at least 30 dividing cells. The karyotype formula at mitotic metaphase in each cultivar was based on the data of measurement from the photograph of the well-spread representative chromosome-complement. To make the ideogram, meta- (median-), submeta- (submedian-), subtelo- (subterminal-), and tero- (terminal-) centric classification of chromosome morphology on the basis of the position of centromere was followed (Levan *et al.* 1964).

Results

The results of CMA and DAPI staining in three cultivars, *Momordica charantia* 'Abashi-goya,' 'Naga-goya' and 'Shiro-goya' are shown in Figs. 1–3 and Tables 1 and 2. The present method using enzymatic digestion produced a high level of well-spread metaphase chromosome complements. The cultivars investigated showed the same somatic chromosome number $2n=22$ (Fig. 3 and Table 2). In condensation behaviors from prophase to metaphase, most chromosomes of all cultivars in this study had early condensing segments at the proximal regions (the segment sizes were about 1 μ m). Moreover, at prophase or earlier stage of metaphase, most chromosomes observed had decondensed segments at the distal regions in both arms (Fig. 1). In many cases, these parts were not visible.

'Abashi-goya'

The fluorescent staining result of somatic chromosomes of 'Abashi-goya' is shown in Figs. 1–3 and Table 1. This cultivar showed the somatic chromosome number $2n=22$. In metaphase, the size of the largest chromosome was 2.2 μ m, while that of the smallest chromosome was 1.2 μ m (Table 1). The ratio of the largest chromosome to the smallest was 1.8, and average chromosome length was 1.6 μ m (Table 2). The chromosomes in the metaphase complement decreased gradually in size from the largest to the smallest chromosomes (Fig. 3). Total chromosome length was 35.8 μ m (Table 2). All chromosomes showed clear primary constrictions around the middle parts of the length (Fig. 3). Thus, the chromosome complement at mitotic metaphase consisted of 22 metacentric chromosomes (Table 2). In prophase or prometaphase, two satellites, which were stained

with CMA positive and DAPI negative ($\text{CMA}^+\text{DAPI}^-$), were observed (Fig. 1A and B). In mid-metaphase, four $\text{CMA}^+\text{DAPI}^-$ segments were located at the distal regions of short arms on chromosomes (Fig. 2A and B). Moreover, $\text{CMA}^+\text{DAPI}^-$ sites were shown at the proximal regions or primary constrictions of most chromosomes (Figs. 2A, B and 3).

'Naga-goya'

The fluorescent staining result of somatic chromosomes of 'Naga-goya' is shown in Figs. 1–3 and Table 1. This cultivar showed the somatic chromosome number $2n=22$. In metaphase, the size of the largest chromosome was $2.6\mu\text{m}$, while that of the smallest chromosome

was $1.4\mu\text{m}$ (Table 1). The ratio of the largest chromosome to the smallest was 1.9, and average chromosome length was $1.9\mu\text{m}$ (Table 2). The chromosomes in the metaphase complement decreased gradually in size from the largest to the smallest chromosomes (Fig. 3). Total chromosome length was $42.0\mu\text{m}$ (Table 2). All chromosomes showed clear primary constrictions around the middle parts of the length (Fig. 3). Thus, the chromosome complement at mitotic metaphase consisted of 22 metacentric chromosomes (Table 2). In prophase or pro-

Fig. 1. Fluorochrome staining of prometaphase chromosomes of *M. charantia* 'Abashi-goya' (A. CMA, B. DAPI), 'Naga-goya' (C. CMA, D. DAPI) and 'Shiro-goya' (E. CMA, F. DAPI). Arrowheads indicate $\text{CMA}^+\text{DAPI}^-$ satellites. Bar= $5\mu\text{m}$.

Fig. 2. Fluorochrome staining of mid-metaphase chromosomes of *M. charantia* 'Abashi-goya' (A. CMA, B. DAPI), 'Naga-goya' (C. CMA, D. DAPI) and 'Shiro-goya' (E. CMA, F. DAPI). Arrowheads indicate $\text{CMA}^+\text{DAPI}^-$ segments. Bar= $5\mu\text{m}$.

Fig. 3. Alignments of the chromosome sets at mid-metaphases in somatic cells of *M. charantia* 'Abashi-goya' (A and B), 'Naga-goya' (C and D) and 'Shiro-goya' (E and F) stained with CMA (A, C and E) and DAPI (B, D and F). Arrowheads indicate $\text{CMA}^+\text{DAPI}^-$ segments. Bar= $5\mu\text{m}$.

Table 1. Measurements of somatic mid-metaphase chromosomes of three Japanese cultivars of *M. charantia*.

Chromosome number	Abashi-goya					Naga-goya					Shiro-goya				
	Short arm length (μm)	Long arm length (μm)	Chromosome length (μm)	Relative length	Arm ratio	Short arm length (μm)	Long arm length (μm)	Chromosome length (μm)	Relative length	Arm ratio	Short arm length (μm)	Long arm length (μm)	Chromosome length (μm)	Relative length	Arm ratio
1	1.1	1.1	2.2	6.1	1.0	1.1	1.5	2.6	6.2	1.4	1.4	1.6	3.0	6.3	1.1
2	1.0	1.1	2.1	5.9	1.1	1.1	1.5	2.6	6.2	1.4	1.3	1.6	2.9	6.1	1.2
3	1.0	1.0	2.0	5.6	1.0	1.2	1.4	2.6	6.2	1.2	1.4	1.4	2.8	5.9	1
4	0.9	1.0	1.9	5.3	1.1	1.2	1.3	2.5	6.0	1.1	1.4	1.4	2.8	5.9	1
5	0.9	0.9	1.8	5.0	1.0	1.0	1.2	2.2	5.2	1.2	1.3	1.3	2.6	5.5	1
6	0.8	0.9	1.7	4.7	1.1	1.0	1.2	2.2	5.2	1.2	1.2	1.3	2.5	5.3	1.1
7	0.7	1.0	1.7	4.7	1.4	0.8	1.2	2.0	4.8	1.5	1.2	1.3	2.5	5.3	1.1
8	0.7	1.0	1.7	4.7	1.4	0.8	1.2	2.0	4.8	1.5	0.9	1.3	2.2	4.6	1.4
9	0.8	0.8	1.6	4.5	1.0	0.8	1.1	1.9	4.5	1.4	0.8	1.3	2.1	4.4	1.6
10	0.8	0.8	1.6	4.5	1.0	0.8	1.1	1.9	4.5	1.4	0.8	1.3	2.1	4.4	1.6
11	0.7	0.9	1.6	4.5	1.3	0.9	1.0	1.9	4.5	1.1	1.0	1.1	2.1	4.4	1.1
12	0.7	0.9	1.6	4.5	1.3	0.9	0.9	1.8	4.3	1.0	1.0	1.1	2.1	4.4	1.1
13	0.8	0.8	1.6	4.5	1.0	0.8	0.9	1.7	4.0	1.1	1.0	1.1	2.1	4.4	1.1
14	0.8	0.8	1.6	4.5	1.0	0.8	0.9	1.7	4.0	1.1	1.0	1.1	2.1	4.4	1.1
15	0.6	0.9	1.5	4.2	1.5	0.7	1.0	1.7	4.0	1.4	0.8	1.2	2.0	4.2	1.5
16	0.6	0.9	1.5	4.2	1.5	0.7	0.9	1.6	3.8	1.3	0.8	1.1	1.9	4.0	1.4
17	0.6	0.8	1.4	3.9	1.3	0.7	0.9	1.6	3.8	1.3	0.7	1.1	1.8	3.8	1.6
18	0.6	0.8	1.4	3.9	1.3	0.7	0.9	1.6	3.8	1.3	0.7	1.1	1.8	3.8	1.6
19	0.7	0.8	1.5	4.2	1.1	0.8	0.8	1.6	3.8	1.0	0.7	1.1	1.8	3.8	1.6
20	0.7	0.7	1.4	3.9	1.0	0.7	0.8	1.5	3.6	1.1	0.7	0.8	1.5	3.2	1.1
21	0.6	0.6	1.2	3.4	1.0	0.7	0.7	1.4	3.3	1.0	0.7	0.8	1.5	3.2	1.1
22	0.6	0.6	1.2	3.4	1.0	0.7	0.7	1.4	3.3	1.0	0.7	0.7	1.4	2.9	1

Table 2. Comparison in karyotypes of three Japanese cultivars of *M. charantia*.

Cultivars	$2n$	Total length (μm)	Chromosome length from the largest to the smallest (μm)	Average length of chromosome complement (μm)	Largest chromosome/smallest chromosome	Karyotype formula	Number of CMA ⁺ DAPI ⁻ segments
Abashi-goya	22	35.8	1.2–2.2	1.6	1.8	22m	4
Naga-goya	22	42.0	1.4–2.6	1.9	1.9	22m	4
Shiro-goya	22	47.6	1.4–3.0	2.2	2.1	22m	2

metaphase, two CMA⁺DAPI⁻ satellites were observed (Fig. 1C and D). In mid-metaphase, four CMA⁺DAPI⁻ segments were located at the distal regions of short arms on chromosomes (Fig. 2C and D). Moreover, CMA⁺DAPI⁻ sites were shown at the proximal regions or primary constrictions of most chromosomes (Figs. 2C, D and 3).

'Shiro-goya'

The fluorescent staining result of somatic chromosomes of 'Shiro-goya' is shown in Figs. 1–3 and Table 1. This cultivar showed the somatic chromosome number $2n=22$. In metaphase, the size of the largest chromosome was $3.0\mu\text{m}$, while that of the smallest chromosome was $1.4\mu\text{m}$ (Table 1). The ratio of the largest chromosome to the smallest was 2.1, and average chromosome length was $2.2\mu\text{m}$ (Table 2). The chromosomes in the metaphase complement decreased gradually in size from the largest to the smallest chromosomes (Fig. 3). Total chromosome length was $47.6\mu\text{m}$ (Table 2). All chromosomes showed clear primary constrictions around the middle parts of the length (Fig. 3). Thus, the chromosome complement at mitotic metaphase consisted of 22

metacentric chromosomes (Table 2). In prophase or pro-metaphase, two CMA⁺DAPI⁻ satellites were observed near the ends of chromosomes (Fig. 1E and F). In mid-metaphase, two CMA⁺DAPI⁻ segments were located at the distal regions of short arms on chromosomes (Fig. 2E and F). Moreover, CMA⁺DAPI⁻ sites were shown at the proximal regions or primary constrictions of most chromosomes (Figs. 2E, F and 3).

Discussion

Chromosome size and centromere position

Although the cultivars of *Momordica charantia* had small-sized chromosome of 1 to $3\mu\text{m}$ at metaphase, previous reports presented 0.9 – $1.5\mu\text{m}$ at metaphase, and thus, their chromosome sizes were smaller than those of the present study. The difference of the chromosome size might be due to employed methods of chromosome preparation. Generally, chromosome sizes in air-dry methods are larger than in squash methods (Geber and Schweizer 1988).

The prepared fine metaphase spreads made it possible to determine the positions of primary constrictions

or centromeres for each chromosome in all cultivars studied here. The previous cytogenetic reports noted that the chromosomes of this species were too small to determine the position of the centromere (Zaman and Alam 2009). In a few papers, chromosome analyses with centromere positions have been performed.

Waminal and Kim (2012) analyzed somatic chromosome complements for interspecific comparison and showed a representative karyotype of this species. The complement analyzed by them consisted of all metacentric chromosomes. On the other hand, the latest paper (Kausar *et al.* 2014) reported intraspecific polymorphisms of karyotypes of 30 accessions from Southeast Asian countries. This report showed that all diploidal accession's karyotypes included two to twenty submetacentric chromosomes, and did not show any complement with all metacentric chromosomes.

In contrast, the present study was able to show clear primary constrictions around the middle parts of the length on all chromosomes, and thus we could compare detailed karyomorphological characters among the three Japanese cultivars. Although we observed certain chromosomes with higher arm ratio judging by the determination of centromere positions, the chromosome complements at mitotic metaphases in the three cultivars consisted of 22 metacentric chromosomes (Figs. 2 and 3, Tables 1 and 2). Moreover, similar sizes and shapes of the chromosomes indicated that the karyotypes of *M. charantia* cultivars were symmetrical. The difference of the chromosome reports with centromeric formula might be due to dealing strains or cultivars of this species.

Base-specific fluorescent staining

The fluorescent staining showed different sizes and numbers of CMA⁺DAPI⁻ segments on one end of sat-chromosomes at metaphase stages among the three cultivars. Four CMA⁺DAPI⁻ segments were observed in 'Abashi-goya' and 'Naga-goya,' while two segments were observed in 'Shiro-goya.' In 'Shiro-goya,' a large size difference of satellites was found between two sat-chromosomes, which might be homologous chromosomes with polymorphism. Thus, we suggested that *M. charantia* had a high degree of chromosome polymorphisms among cultivars. Moreover, because of differences in size and chromosome position of CMA⁺DAPI⁻ even in Japanese cultivars, the CMA⁺DAPI⁻ segments might make polymorphisms or chromosome differentiation. Interestingly, faint CMA⁺DAPI⁻ sites were shown at the proximal regions or primary constrictions of most chromosomes in all cultivars.

In the base-specific banding, CMA binds specifically to guanine regions in helical DNA (Ward *et al.* 1965) or heterochromatin (Deumling 1981, Deumling and Greilhuber 1982), while DAPI binds specifically to AT-base pairs in the minor groove of DNA (Portugal and Waring 1988). Thus, it could be concluded that the satellites

of all bitter gourd cultivars in this study were GC-rich. Moreover, the plant chromosome studies using base-specific fluorochromes typically show that rDNA sites or nucleolar organizer regions (NORs) were observed as enhanced bands in GC-specific fluorochromes and quenched bands in AT-specific fluorochromes (Guerra 1988, Kenton 1991, Xu and Earle 1996). Our results of the satellites studied here might therefore have rDNA or NORs.

Further karyomorphological and molecular cytogenetic studies with many cultivars of *Momordica* species are necessary.

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