High β -carotene and Capsaicinoid Contents in Seedless Fruits of ‘Shishitoh’ Pepper

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Abstract. In order to evaluate the advantages of parthenocarpy in the breeding of *Capsicum*, we investigated the percentage of fruit set after emasculation or excision of styles, fruit size, and amounts of β -carotene, capsaicinoids, and ascorbic acids of the seedless fruits of *Capsicum annuum* L. ‘Shishiroh’ no. 562. Seedless fruits were induced from $\approx 80\%$ of flower buds after emasculation or excision of styles. The levels of β -carotene ($44.07 \mu\text{g}\cdot\text{g}^{-1}$), capsaicin ($1.73 \text{ mg}\cdot\text{g}^{-1}$), and dihydrocapsaicin ($1.12 \text{ mg}\cdot\text{g}^{-1}$) of mature seedless fruits were 10 times higher than those of seeded fruits. The amount of ascorbic acid, however, was at the same level ($\approx 230 \text{ mg}/100 \text{ g}$). The length of the seedless fruit was $\approx 50\%$ smaller than that of the seeded fruit at 2 weeks after the flowering and decreased to 44% at mature stage.

Parthenocarpy in plants is defined as the formation of seedless fruits due to the absence of functional pollination. Because suboptimum conditions, such as high or low temperature, often disrupt fruit set, parthenocarpy has been proposed as a possible way to achieve more consistent fruit production.

In pepper, the possibility for introduction of parthenocarpy has been examined, because it is already known that parthenocarpic fruits are abundant when the pepper plants are subjected to cool day temperatures or high night temperatures (Bosland and Votava, 2000). Seedless fruits are small and deformed, and have little commercial value so far (Polowick and Sawhney, 1985; Rylski, 1973). It is reported in recent papers that the size of the seedless fruits would be improved by selection (Shifriss and Eidelman, 1986; Tarchoun and Rezgui, 1999).

In tomato (*Lycopersicon esculentum* Mill.), parthenocarpy has been induced in some commercial cultivars (George et al., 1984). It was shown that the shape of parthenocarpic fruits induced by *pat* genes was normal (George, et.al., 1984), and two genetic parthenocarpic

lines of tomato produced fruits with higher total solids than seeded fruits (Diaz and Hewitt, 1987). To date, biochemical analyses of parthenocarpic fruits for pigment content and other important secondary compounds have not been reported. Recently, it was also reported that parthenocarpy was engineered in two genotypes of tomato by using the *DefH9-iaaM* chimeric gene (Ficcadenti et al., 1999). *DefH9-iaaM* gene consists of coding region of the *iaaM* gene from *Pseudomonas syringae* pv. *savastanoi* under the control of the placental and ovule-specific *DefH9* promoter from *Anthirrhinum majus*. The *iaaM* gene codes for a triptophan monooxidase producing indolacetamide, which is converted to indole-3-acetic acid (IAA).

In this paper, we describe three parameters on seedless fruit set of pepper, which have not been examined in previous reports. First, the percentages of fruit set after emasculation or excision of styles was examined using *C. annuum* ‘Shishitoh’ no. 562, which was confirmed to have high ability to produce seedless fruits in our preliminary experiment using various genotypes. Second, the sizes of the seedless fruits were measured throughout fruit development in order to clarify whether there were key periods when the development of seedless fruit was suppressed. Third, the amounts of three components (β -carotene, ascorbic acid, and

capsaicinoids) of seedless fruits were compared with the seeded ones in order to examine the advantages of the seedless fruits.

Materials and Methods

Plant materials. *Capsicum annuum* ‘Shishitoh’ no. 562 seeds (Japan Horticultural Production Institute and Research Institute, Chiba) were sown in trays in Aug. 1999 in a greenhouse under natural light conditions and minimum soil temperature of 18°C . After 2 weeks, the seedlings were transferred to 600-mL pots and grown for 1 month under the same conditions. Later, the plants were transplanted into the ground and grown for 5 months in a glasshouse, where the ambient temperatures were 30°C maximum/ 18°C minimum, respectively. After 5 months, the plants were $\approx 170 \text{ cm}$ tall, and had nearly 200 flowers and fruits per plant. Five plants were used for the experiments.

The seedless fruits were obtained by emasculation or excision of styles from the flower buds 1 d before flowering. The buds were then covered with paper bags that were removed 2 weeks after flowering. Self-pollinated fruits were used as controls. Self-pollination was induced by covering the flower buds 1 d before flowering and removing the covers 2 weeks after flowering. Flower buds used for production of seedless and self-pollinated fruits were evenly selected among five plants. Treatments were repeated 3 times from Jan. to Mar. 2000.

The length of the fruits (ovaries) was measured from the end of sepal to the bottom of the fruit. The fruit length of the seedless and the self-pollinated fruits was measured at 0 (the day of flowering), 2, 4, 6, 8, and 10 weeks after flowering. Six seeded and six seedless fruits (ovaries) were evenly sampled from the five plants at each stage of development. Three of the seeded and three of the seedless fruits were sampled for ascorbic acid and β -carotene; the other three of each were sampled for capsaicinoids.

Measurement of ascorbic acid, β -carotene and capsaicinoid contents. Ascorbic acid content was measured by 2,4-dinitrophenylhydrazine-method using 10 g (fresh weight) of pericarp tissues, as described by Hamada (1990).

The β -carotene contents were measured according to the methods described by Davies (1976). About 5 g (fresh weight) of pericarp tissue from each of the seedless and the self-pollinated fruits was homogenized in acetone and filtrated. The solutions were filled with acetone up to 100 mL. The β -carotene was extracted with diethylether, washed, and dried over anhydrous Na_2SO_4 . The solvent was evaporated and the residue was dissolved in 100 mL of benzene. The content of β -carotene was calculated using specific extinction coefficient value of 2337 in benzene at 465 nm, using a spectrophotometer (U-Best 30, JASCO Co., Japan).

Capsaicinoids were extracted twice from the placentas of the fruits ($\approx 100 \text{ mg}$) by 80% acetone and the contents of capsaicin and dihydrocapsaicin were measured by HPLC, as described in our previous paper (Sakamoto, 1994).

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Results and Discussion

The percentage of fruit set by self-pollination was 95.1% (Table 1). Emasculation or excision of styles 1 d before flowering resulted in fruit set from $\approx 80\%$ of the treated buds (Table 1). This showed that 'Shishitoh' had high ability to produce seedless fruits either by emasculation or excision of styles. The reduced percentage of fruit set relative to pollinated fruits may have been due to mechanical damage in the process of emasculation or excision of styles. For further experiments, only seedless fruits induced by emasculation were used, because genetic cytoplasmic male sterility is already applied in breeding, and the seedless cultivars will be established by male sterility more easily.

Typical fruits of 'Shishitoh' have lobate shoulders and sunken shape at the apex. The seedless fruits of 'Shishitoh' showed indentation at the middle part of the fruit in addition to the lobate shoulder and sunken apex (Fig. 1).

The length and diameter of 8-week-old seedless fruits were 44% (mean 39.3 mm) and 74% (mean 21.1 mm) of those of the seeded fruits, respectively. These data showed that the suppression of seedless fruit development was obvious in the length of the fruits. The average percentage of the length of seedless to seeded fruits at 2 weeks after flowering was $\approx 50\%$, and then the percentage decreased to 44% according to their maturation (Fig. 2). Therefore, we concluded that the growth of seedless fruits was suppressed throughout the fruit development.

Tarchoun and Rezgui (1999) reported the reduction rate of fruit weight and length in 10 local pepper cultivars varied, and some cultivars produced seedless fruits with a comparable weight and length to those of seeded fruits. Also, the size and shape of cultivated pepper varied from tiny, ovoid Chiltecpin (0.9 cm) to the long, flattened Anaheim (33.02 cm) (Andrews, 1995). Thus, we expect that a seedless pepper with acceptable size and shape would be available after intensive investigation.

Aside from deformation and reduced size, however, the levels of β -carotene and capsaicinoids were 10 times higher in seedless fruits, while ascorbic acid was at the same level in seedless and seeded fruits (Table 2). The increase of β -carotene ($44 \mu\text{g}\cdot\text{g}^{-1}$) is considered to be one of the important advantages of seedless fruits. This value is comparable to β -carotene contents of jalapeño ($26 \mu\text{g}\cdot\text{g}^{-1}$), chili ($28 \mu\text{g}\cdot\text{g}^{-1}$), bell ($14 \mu\text{g}\cdot\text{g}^{-1}$), and yellow

wax type ($29 \mu\text{g}\cdot\text{g}^{-1}$) measured by HPLC (Howard et al. 1994).

However, the increase of capsaicinoids is not desirable for typical 'Shishitoh' usage in Japan. The capsaicinoid contents of 'Shishitoh' fruits are just around or below the limit for human tasting, and 'Shishitoh' fruits are mainly consumed as a nonpungent vegetable. The capsaicinoid contents of seedless 'Shishitoh' exceed the limit, and seedless 'Shishitoh' fruits taste pungent. However, the present result is interesting in considering the relationship between biosynthesis of capsaicinoid and seed

formation. The phenylpropanoid intermediates in capsaicinoid biosynthesis are common with cell wall and lignin formation (Iwai, 1986). It suggests that the phenolic intermediates necessary for lignin formation in seed development were used for biosynthesis of capsaicinoids, resulting in the increase of the capsaicinoid contents in placentas.

Contrary to the increase of β -carotene and capsaicinoid contents, the contents of ascorbic acid did not increase in seedless fruits of 'Shishitoh' (Table 2). We considered that the ascorbic acid content in 'Shishitoh' ($\approx 230 \text{ mg/}$

Fig. 1. Mature fruits obtained by: (A) open-pollination and (B) emasculation in *Capsicum annuum* 'Shishitoh' no. 562. Bars show 5 cm.

Fig. 2. Changes of the lengths of the fruits obtained after self-pollination and emasculation in *Capsicum annuum* 'Shishitoh' no. 562. Vertical bars indicate standard deviations.

Table 1. The fruit set of *Capsicum annuum* 'Shishitoh' no. 562 by self-pollination and parthenocarp induced by emasculation or excision of styles.

Treatment ^a	Total no. of flower buds treated	Fruit set (%) ^b
Self-pollination	53	95.1 $\pm$ 4.9
Emasculation	78	84.4 $\pm$ 8.1
Excision of styles	36	80.3 $\pm$ 5.3

^aExperiments were repeated 3 times from Jan. to Mar. 2000.

^bMean $\pm$ standard error. Nonsignificant ($P > 0.05$) by Student's *t* test.

Table 2. The contents of β -carotene, ascorbic acid, and capsaicinoids in the seeded and seedless fruits of *Capsicum annuum* 'Shishitoh' no. 562.

Fruits	β -carotene ^z ($\mu\text{g}\cdot\text{g}^{-1}$)	Ascorbic acid ^z (mg/100g)	Capsaicin ^y (mg·g ⁻¹)	Dihydrocapsaicin ^y (mg·g ⁻¹)
Self-pollinated	4.41 ± 1.42	225.43 ± 5.65	0.17 ± 0.08	0.12 ± 0.06
Emasculated	44.07 ± 19.84 *	225.73 ± 15.59 NS	1.73 ± 0.22 **	1.12 ± 0.19 **

^zContents in pericarp.

^yContents in placentas.

NS, *, **Nonsignificant at $P > 0.01$; or significant at $P < 0.05$ or 0.01 , respectively (by Student's t test).

Mean ± standard error, $n = 3$.

100 g) was already near its maximum level. It was reported that 'Shishitoh', 'Fushimiamanaga', 'Sapporo Wase', and 'Hirosaki Zairai' contained 2- or 3-fold greater levels of ascorbic acid than 'California Wonder', 'Yolo Wonder', 'Chili Jalapeño', 'Yellow Sweet Long', and 'Sweet Banana' (Saga and Ogawa 1994). For this reason, it is expected that cultivars with low ascorbic acid contents could be further improved with parthenocarp.

Because our results showed that 'Shishitoh' has the ability to set seedless fruits with increased carotenoid and capsaicinoid contents, induction of seedless fruits with acceptable shape would be the next step for practical application of parthenocarp in pepper.

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