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Sensitive stages of fruit and seed development of chili pepper (*Capsicum annuum* L. var. Shishito) exposed to high-temperature stress

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ABSTRACT

The sensitivity of developmental stages to high temperature was investigated in chili pepper (*Capsicum annuum* L. var. Shishito). Plants were subjected to heat stress (38/30 °C day/night) immediately after anthesis for 5 or 10 days, or from 10 to 30 days after anthesis (DAA), from 30 DAA until harvest of the seeds, or immediately after anthesis until harvest of the seeds. Control plants were grown at 30/22 °C (day/night). Exposure to high temperature (heat stress) during different periods of development after anthesis adversely affected fruit growth, seed yield, and seed quality in chili pepper. Heat stress for the whole period after anthesis, and from 30 DAA until harvest reduced the growth period of chili fruits by 15 and 10 days, respectively. Heat stress from 10 to 30 DAA reduced fruit width and fruit weight. The early stage of seed development from anthesis until 10 DAA was sensitive to high temperature, which affected fruit length, fruit weight and seed set. Applying high temperatures to plants for 10 DAA increased the proportion of abnormal seeds per fruit. High temperatures from 10 DAA until 30 DAA inhibited carbohydrate accumulation and adversely affected seed germinability and vigor. These results suggest that the stage of development at which chili peppers are exposed to high temperatures is an important factor in fruit and seed growth and in seed quality.

1. Introduction

Much attention has been paid to the influence of high temperatures on fruit growth and seed production. High temperatures during fruit development result in reduced fruit and seed yield in many plants, such as tomato (Adam et al., 2001), maize (Monjardino et al., 2005), flax (Cross et al., 2003), summer brassica (Morrison and Stewart, 2002), canola (Young et al., 2004), soybean (Keigley and Mullan, 1986; Spears et al., 1997), and bell pepper (Aloni et al., 2001; Barker, 1989; Erickson and Markhart, 2002). The number of species adversely affected by heat stress suggests that flower development and seed filling stages after anthesis are most sensitive to heat stress, leading to a reduction in fruit and seed yield.

High temperatures frequently occur after anthesis of *Capsicum* species and strongly influence reproductive development and yield. Erickson and Markhart (2001) reported that under high temperature conditions (33 °C), bell pepper plants continued to produce flowers, and these flowers did not abscise upon opening; however, high temperature during post-pollination (33 °C for 2 days) inhibited fruit set (Erickson and Markhart, 2002), suggesting that fertilization is sensitive to high-temperature stress. High temperatures (36/27 °C) during seed development in chili pepper do not induce conspicuous abortion of flowers, but result in a significant reduction in seed production and increase the numbers of abnormal seeds (Pagamas and Nawata, 2007). However, the causes of the increase in the proportion of abnormal seeds induced by heat stress and the sensitivity of seed developmental statges to high temperature conditions have not been elucidated.

The objective of this study was to identify the sensitive stages of fruit and seed development of chili pepper to high temperature after anthesis and clarify the causes of adverse effects of heat on fruit and seed development.

2. Materials and methods

Seeds of chili pepper (*Capsicum annuum* L. var. Shishito) were planted in vermiculite at about 1 cm in depth and grown in a phytotron at the Graduate School of Agriculture, Kyoto University, Japan. Shishito is a sweet chili pepper variety favored in Japanese markets and grown best under the field and greenhouse conditions at temperatures ranging from 21 °C to 33 °C. The seeds were maintained at temperatures of 30/22 °C (day/night) under natural light. Ten days after sowing (DAS), seedlings were transplanted into 3-in. diameter plastic pots filled with vermiculite, and 23 DAS seedlings were transplanted into 8-in. pots. Plants were watered

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twice a day and fertilized weekly with 1 L 0.1% "Hyponex" (60 g/L N, 100 g/L P, 50 g/L K; Hyponex Japan Co. Ltd., Japan).

2.1. Heat treatment

Each treatment consisted of eight plants with at least six reproductive nodes. The flowers that opened on the same day were labeled. For the high-temperature treatment, plants were moved from control conditions to a heat-stress room (38/30 °C, day/night, under natural light) immediately after anthesis for the following periods: 5 days (A-5 DAA), 10 days (A-10 DAA), from 10 DAA to 30 DAA (10–30 DAA), and from 30 DAA until harvesting of the seeds (30 DAA-H). Plants were moved back to control room until harvesting of the seeds after the above period except 30 DAA-H. For the heat stress treatment for all the period of fruit growth (A-H), after anthesis, plants were moved from control conditions to the heat-stress room until harvesting of the seeds. The temperature regime of heat stress treatments in this study (38/30 °C) was determined because in actual seed production such high temperatures frequently occur. There were no visible stress symptoms of whole plants during the heat treatment periods.

2.2. Fruit and seed development

The fruit size (width and length) was determined every 5 days, from 10 DAA until harvest, and the period of fruit growth from anthesis to harvest was recorded. Each fruit was harvested when fully red, and individual fruits were weighed separately. The number of seeds per fruit and the percentage of abnormal (dark brown and flat) seeds were recorded.

2.3. Seed germination and vigor

The fully red fruits from each treatment were harvested and kept at 25 °C and 50% relative humidity (RH) for 1 week to allow any slightly immature fruits to ripen fully. Seeds were extracted by hand and air-dried. Only normal seeds were weighed and used for seed quality tests.

Standard germination rate was determined using four 50-seed samples, except A-5 DAA and A-10 DAA treatments, in which four 25-seed samples were used because of scaresity of harvested seeds, placed on two layers of sterilized filter paper and incubated in darkness at 30 °C (8 h) and 20 °C (16 h) for 14 days. Seed vigor was determined based on the germination index (GI), calculated for each treatment as follows:

$$GI = \frac{\sum T_i N_i}{S},$$

where T_i is the time interval (in days) between seed imbibition and germination, N_i the number of seeds that germinated on day i, and S is the total number of seeds that germinated by the end of the experiment. A low GI value indicated more rapid germination.

2.4. Total carbohydrate

Total carbohydrate was extracted from normal dry seeds. Samples of 0.5 g were boiled with 25 mL H_2O for 3 min and then autoclaved for 1 h at 135 °C. After cooling to 55 °C, 2.5 mL acetate buffer was added to the samples and the mixtures were filled up to 45 ± 1 g by H_2O. After adding 5 mL glucoamylase solution, the samples were hydrolyzed for 2 h at 55 °C, and filtered. The filtrates were used for carbohydrate determination, according to the method recommended by the Association of Official Analytical Chemists (AOAC, 1995). Starch concentration was estimated using D-glucose as the standard.

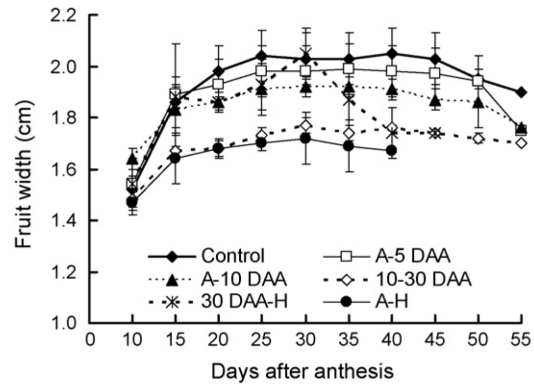

Fig. 1. Effect of the durations of high temperature on fruit width during the development of Shishito pepper fruits. Vertical bars show the standard error.

2.5. Statistical analysis

This experiment was conducted using a completely randomized design. Analysis of variance was carried out using the SAS statistical analysis package (version 8.1; SAS Institute, Cary, NC). Differences between treatments were tested by Tukey's Studentized range test.

3. Results

Shishito fruits were visible within 5 DAA to the naked eye. The development of fruits differed under the various heat treatments (Figs. 1–3). High temperature accelerated fruit growth, resulting in a reduction in the average time from anthesis to maturation to 40 and 45 DAA for the A-H and 30 DAA-H treatments, respectively (Table 1). Fruits in the 30 DAA-H treatment achieved maximum fruit width at 30 DAA, with a subsequent decrease. The high temperature applied from 10 DAA to 30 DAA and throughout the period of seed development reduced fruit width and resulted in significantly lower values than control and other treatments. However, the fruits from the 30 DAA-H treatment at 40 and 45 DAA and the A-5 and A-10 treatments at 55 DAA (fully red stage) showed a reduction in fruit width, but did not differ significantly from the 10–30 DAA treatment (Fig. 1). The increase in fruit length was shown in Fig. 2. Fruits from control plants were longer at every stage of development, except for those fruits from the 30 DAA-H treatment. During the early stages, until 30 DAA, no difference in fruit length was observed between fruits from control and 30 DAA-H-treated plants, in which heat treatment had not been applied until 30 DAA. Heat stress immediately after anthesis, applied until

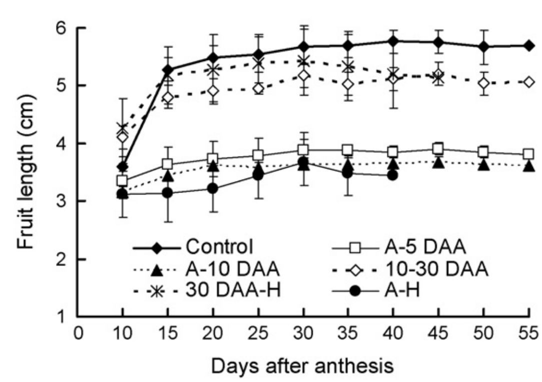

Fig. 2. Effect of the durations of high temperature on fruit length during the development of Shishito pepper fruits. Vertical bars show the standard error.