Effect of Heat Stress Application to Flower and Fruit on Seed Quality of Chili Pepper

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Abstract The effects of elevated flower and pod temperatures (38/22°C, DH; 30/30°C, NH; and 38/30°C, DNH, day and night temperatures, respectively) and whole plant heat stress (38/30°C, WH) after anthesis on fruit development and seed quality of chili pepper (Capsicum annuum L. var. Shishito) were investigated, compared with the control temperature (30/22°C). The highest values of fruit width, length and weight were observed at control conditions. The high temperature treatments significantly decreased fruit growth and development. DH, NH, DNH and WH reduced the growth period of 'Shishito' fruits by 5, 10, 10 and 15 days, respectively. Subjecting the fruit and whole plant to high temperatures adversely affected the seed set and increased the proportion of abnormal seeds per fruit. Partial heat treatments applied to the fruits decreased the carbohydrate content and reduced the germination rates and vigor of the seeds, and the most pronounced adverse effects were observed in the WH treatment. The results indicated that high fruit temperature exerted direct adverse effects on seed formation and sink strength of the seeds in 'Shishito' pepper. Inhibition of the assimilate supply from source organs caused by both the suppression of the sink strength and source productivity may be the major effect of heat stress on the reduction of seed quality.

Key Words: Germination, partial heat stress, vigor

Introduction

High temperatures are a major constraint on crop productivity, especially when high temperatures occur at critical stages of plant growth. Seed growth and development stages are one of the most sensitive stages to heat stress in chili and bell peppers (Aloni et al., 2001; Barker, 1989; Erickson and Markhart, 2002; Pagamas and Nawata, 2007).

Adam et al. (2001) reported that high temperature reduced the rates of fruit growth and development and the mean fruit weight in tomato, with the inhibition of fruit expansion (Pearce et al., 1993). Spears et al. (1997) reported that heat stress reduced the seed size in soybean. Similar results were obtained in rice (Morita et al., 2005), in which high temperature reduced the final grain weight by the decrease in the grain growth rate, due to the inhibition of the expansion of cells between the central point and the surface of the endosperm. These results demonstrated that high temperatures suppress cell enlargement, resulting in a reduction in fruit and seed growth and development.

The reduction in the fruit and seed growth and development is partly due to the suppression of photosynthetic activities, which are known to be inhibited by high temperature. However, the decrease in the photosynthetic rate does not seem to explain the effect of heat damage on fruits and seeds in all the cases. Bhatt and Srinivasa Rao (1993) reported that in pepper the photosynthetic rate and growth level were higher, while flower and fruit numbers were lower at 27/22°C than at 27/17°C. In potato, there was no consistent difference between heat-tolerant and susceptible genotypes in photosynthetic rates under high temperature conditions (38/25°C), although susceptible genotypes showed a lower rate of assimilate transport from leaves than tolerant genotypes (Basu and Minhas, 1991). In bell pepper (Aloni et al., 1991) and tomato (Dinar et al., 1983), high day and night temperatures reduced the export of 14C sucrose from the source leaf and the concentrations of reducing sugars in the reproductive organs. The above reports indicate that heat sensitivity is more related to the availability of the transportable assimilates than the production of assimilates.

In a series of whole plant heat stress experiments conducted in pepper (Aloni et al., 1991) and tomato (Russel and Morris, 1983), it was suggested that flower buds and young leaves compete for assimilates under heat stress conditions, and young leaves appear to be more efficient than the flower buds in importing assimilates. Dinar and Rudich (1985a and b) demonstrated that heat stress caused a reduction in sucrose
uptake by flower buds in tomato. In maize, heat stress limited carbon utilization and partitioning within the endosperm and pedicel, due to the inhibition of the activity of ADP-glucose pyrophosphorylase and starch synthase (Cheikh and Jones, 1995), suggesting that the capacity of sink organs to take up assimilates was inhibited by heat stress.

In our previous study, we demonstrated that poor growth and development of the fruits and seeds of chili pepper under a high temperature regime were caused by the reduction in the accumulation of assimilates in these organs (Pagamas and Nawata, 2007). However, it remains to be determined whether the reduction in seed yield and quality at a high temperature is due to a disturbance of photosynthesis in source organs or the suppression of the sink strength. Direct application of high temperatures to sink organs separately from source organs may affect fruit and seed growth and development directly. The objectives of the present experiment were 1) to compare whole plant and partial fruit heat treatments in their effects on fruit and seed growth and development, and 2) to clarify the mechanisms of the effect of heat stress application after anthesis on seed growth and development.

Materials and Methods

Chili pepper seeds (Capsicum annuum L. var. Shishito) were sown in vermiculite and grown in a phytotron at the Graduate School of Agriculture, Kyoto University, Japan, at temperatures of 30/22ºC (day [06.00-18.00] / night [18.00-06.00] period) under natural sun-light. Ten days after sowing (DAS), the seedlings were transplanted into 3-inch diameter plastic pots filled with vermiculite, and at 23 DAS, the seedlings were re-transplanted into 8-inch pots filled with vermiculite. Plants were 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) and watered twice daily until free drainage occurred from the bottom of a pot to prevent water deficit during the experimental period.

Treatments and experimental design

Ten plants with at least 6 reproductive nodes were subjected to each treatment (Table 1). The flowers that opened on the same day were labeled. Control plants were maintained at 30/22ºC (day / night period) until harvest of the seeds (C). For the whole plant heat treatment, 2 days after anthesis (DAA), pepper plants were moved from control conditions to the heat stress room (38/30ºC) and remained there until harvest of the seeds (WH). For partial heat treatments, pepper plants were grown under control conditions, and only opening flowers were subjected to high day and/or night temperatures (DH: 38/22ºC, NH 30/30ºC and DNH: 38/30ºC), using film heaters (Fig. 1) until harvest of the fruits.

The experiment was carried out using a completely randomized design with 3 replications (each replication with at least 3 samples). Analysis of variance was performed using the SAS statistical analysis package (version 8.1; SAS Institute, Cary, NC). Differences between the treatments were tested by Tukey’s Studentized range test at 1% level of probability.

Fruit and seed growth and development

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

Seed germination and vigor

The fully red fruits from each treatment were harvested and kept at a temperature of 25ºC and a relative humidity of 50% for 1 week to allow slightly immature fruits to ripen fully. Seeds were extracted by hand and air-dried. Seeds were weighed and used for seed quality tests, except for visually abnormal seeds.

The standard germination rate was determined using four 50-seed samples placed on two layers of sterilized filter paper and incubated in darkness at a temperature of 30ºC (8 h) and 20ºC (16 h) for 14 days. Seed vigor was determined based on the accelerated aging germination test using 2 g of seeds aged at a temperature of 41ºC and a relative humidity of nearly
100% for 72 hours, followed by a standard germination test (Department of Horticulture and Crop Science, The Ohio State University, 2004). The germination index (GI) was 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, \( N_i \) is the number of seeds that germinated on day \( i \), and \( S \) is the total number of seeds that germinated by the end of the test. A lower GI value indicated more rapid germination.

Total carbohydrate content

Total carbohydrates were extracted from dry seeds, and the content was determined according to the method recommended by the Association of Official Analytical Chemists (1995). Starch concentration was estimated using D-glucose as the standard.

Results

The fruits from the control plants reached the ripening stage at 55 DAA, whereas the DH, NH, DNH and WH fruits reached it at 50, 45, 45 and 40 DAA, respectively (Table 2). High temperature treatments (WH, DH, NH and DNH) reduced fruit growth of Shishito pepper (Figs. 2 and 3). Fruit width continued to increase and control fruits showed significantly higher values of fruit width than those in the other treatments after 45 DAA (Fig. 2). The increase in the fruit length was significantly different between the control and heat stressed plants (Fig. 3). Fruits from the control plants were longer than those from heat treatments at every stage of growth and development. DH and NH reduced the fruit length and resulted in lower values than those found in control fruits, though significantly higher than those in the fruits from the DNH treatment. The WH fruits displayed the lowest values for fruit length at every stage of development (Fig. 3).

The fruit weight, number of seeds per fruit, percentage of visually abnormal seeds and seed dry weight were all significantly affected by the heat stress treatments (Table 2). Mean fresh weight of individual fruits was reduced by partial heat treatments (DH, NH

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit growth period (DAA)</th>
<th>Fruit weight (g fruit⁻¹)</th>
<th>Seeds per fruit (seeds fruit⁻¹)</th>
<th>Visually abnormal seeds (% fruit⁻¹)</th>
<th>Seed dry weight (g 100 seeds⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>55.0 ± 0.6 a*</td>
<td>7.5 ± 0.3 a</td>
<td>58.0 ± 2.8 a</td>
<td>7.7 ± 1.8 c</td>
<td>0.54 ± 0.01 a</td>
</tr>
<tr>
<td>DH</td>
<td>50.0 ± 0.8 b</td>
<td>2.9 ± 0.2 b</td>
<td>20.1 ± 3.9 b</td>
<td>19.7 ± 4.4 bc</td>
<td>0.59 ± 0.01 b</td>
</tr>
<tr>
<td>NH</td>
<td>45.0 ± 0.6 c</td>
<td>2.8 ± 0.2 b</td>
<td>17.0 ± 2.4 b</td>
<td>23.1 ± 0.1 ab</td>
<td>0.41 ± 0.02 b</td>
</tr>
<tr>
<td>DNH</td>
<td>45.0 ± 0.6 c</td>
<td>2.3 ± 0.2 b</td>
<td>21.1 ± 2.1 b</td>
<td>21.1 ± 4.6 abc</td>
<td>0.59 ± 0.01 b</td>
</tr>
<tr>
<td>WH</td>
<td>40.0 ± 0.6 d</td>
<td>2.4 ± 0.4 b</td>
<td>16.1 ± 2.8 b</td>
<td>24.2 ± 6.8 a</td>
<td>0.29 ± 0.01 c</td>
</tr>
</tbody>
</table>

* Values are means followed by S.E. Different letters indicate a significant difference by the Tukey test at P<0.01 within columns. See text for treatment abbreviations.
and DNH) and by the whole plant heat treatment. Partial and whole plant heat treatments significantly reduced the mean number of seeds per fruit (Table 2). High temperature treatments also significantly increased the percentage of visually abnormal seeds. WH treatment led to the highest percentage of visually abnormal seeds, although the differences were not significant for the fruits in the NH and DNH treatments. The value of the seed dry weight was significantly higher in the control fruits than in the treated ones. Although partial heat treatments reduced the seed dry weight, the value of the seed dry weight in the partial heat treatments was higher than that in the WH treatment.

The effect of heat stress on the total carbohydrate content of visually normal seeds is shown in Table 3. The carbohydrate content was higher in the control seeds than in those from the other treatments. No significant differences in carbohydrate content were observed between seeds among partial heat treatments. Seeds in WH showed the lowest carbohydrate content.

Standard germination and accelerated aging germination rates of visually normal seeds from the control plants were highest among the treatments (Table 3). DH and DNH treatments reduced the seed germination rates and accelerated aging germination rates, whereas the NH treatment led to a smaller decrease in seed quality. The seeds from the whole plant heat stress treatment showed a complete lack of germinability (Table 3). Germination of the seeds from the control plants was faster than that of the seeds from the partial heat stress plants, although NH did not show significant differences from the control.

Discussion

Fruits from the DH, NH, DNH and WH plants reached the ripening stage at 50, 45, 45 and 40 DAA, respectively, faster than those from the control (Table 2). This is in agreement with observations in sweet pepper (Barker, 1989) and tomato (Adam et al., 2001; Peet et al., 1997). High temperature generally accelerates fruit maturation and ripening, and this is considered to be a mechanism for adaptation to stress conditions, enabling the plant to disperse its seeds faster when stressed (Mizrahi, 1982; Walker et al., 1979). In the present study, the NH treatment reduced the duration of the period of fruit growth and development more substantially than the DH treatment, and did not show a significant difference for the DNH treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total carbohydrate content (mg g⁻¹ dry seed)</th>
<th>Germination (%)</th>
<th>Accelerated aging germination (%)</th>
<th>Germination Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>527.0 ± 5.85 a*</td>
<td>100.0 ± 0.00 a</td>
<td>94.0 ± 1.15 a</td>
<td>4.30 ± 0.04 b</td>
</tr>
<tr>
<td>DH</td>
<td>340.5 ± 0.54 b</td>
<td>81.3 ± 1.25 c</td>
<td>60.0 ± 9.13 b</td>
<td>5.87 ± 0.23 a</td>
</tr>
<tr>
<td>NH</td>
<td>361.7 ± 3.80 b</td>
<td>93.0 ± 1.91 b</td>
<td>84.0 ± 2.31 a</td>
<td>4.38 ± 0.02 b</td>
</tr>
<tr>
<td>DNH</td>
<td>340.0 ± 0.54 b</td>
<td>82.0 ± 1.15 c</td>
<td>64.0 ± 1.63 b</td>
<td>5.25 ± 0.18 a</td>
</tr>
<tr>
<td>WH</td>
<td>127.4 ± 1.64 c</td>
<td>0.0</td>
<td>0.0 c</td>
<td>-</td>
</tr>
</tbody>
</table>

* Values are means followed by S.E. Different letters indicate a significant difference by the Tukey test at P<0.01 within columns. See text for treatment abbreviations.
suggesting that high night temperature exerted larger effects on the duration of the period of fruit growth and development than high day temperature (Table 2). However, the activation of fruit growth and development by high temperature was distinctively larger in the whole plant heat treatment than in the fruit heat treatments. This may suggest that subjecting the whole plant to high temperature exerted additional effects on fruit ripening.

In general, following fertilization, cell division is activated in the ovary. After cell division, fruit growth is mostly caused by an increase in the cell volume (Gillaspy et al., 1993). Cheniclet et al. (2005) suggested that the high potential of plant cells for expansion was a strong determinant of fruit size. Exposure of the whole plants or fruits to high temperature throughout the fruit growth and development significantly reduced the width and length of Shishito pepper fruits. The effect of heat stress application to fruit on the fruit length was larger in the DNH treatment than in the DH and NH treatments, in which heat stress was applied to the fruits for only half a day. Similar results were reported in tomato (Peet et al., 1997). Although heat treatments reduced the fruit weight, no differences between the treatments were observed (Table 2). Although detailed mechanisms have not been elucidated, cell division at early stages and cell enlargement at later stages in fruit growth and development may be disturbed by heat stress and be involved in these phenomena.

Increasing the temperature to 30°C or more during the seed filling period reduced the number of seeds per fruit in bell pepper (Barker, 1989) and chili pepper (Pagamas and Nawata, 2007). In our experiments, the whole plant heat treatment (WH) and partial heat treatments (DH, NH, and DNH) reduced the number of seeds per fruit and seed dry weight, and increased the proportion of visually abnormal seeds (Table 2). The presence of growth hormones which has been demonstrated in seeds of many plant species (Copeland and McDonald, 1985) exerts a considerable influence on the growth and development of fruits (Blumenfeld and Gazit, 1970). Ishikawa et al. (2004) showed that the growth of seedless Shishito fruits was suppressed throughout the fruit growth period. A reduction in the number of seeds per fruit may have been partly responsible for the reduction in size of heated fruits in this study. The size of the pepper fruits shows a positive relationship with the seed number (Erickson and Markhart, 2002; Marcelis and Baan Hofman-Eijer, 1997; Rylski, 1973). Our previous study indicated that high temperatures increased the percentage of visually abnormal seeds (Pagamas and Nawata, 2007). The present experiments have revealed that the adverse effects of heat stress on the number of seeds per fruit and the percentage of visually abnormal seeds were not significantly different among the treatments (DH, NH, DNH and WH), demonstrating that high temperature exerts a direct effect on seed formation and seed yield.

High temperature during the grain-filling period limits the assimilation or translocation of photosynthates into seeds (Thomas et al., 2003). Based on the results from our previous study, heat stress (36/27°C) after anthesis is particularly detrimental to subsequent seed growth and development, because of disturbances in the carbohydrate and lipid accumulation, resulting in a reduction in seed weight, standard germination rate and vigor (Pagamas and Nawata, 2007). In the present study, in which the WH temperatures were higher than those in our previous study (Pagamas and Nawata, 2007), we failed to observe germination in the WH seeds and we recorded the lowest carbohydrate content (Table 3). It is considered that the quality and dry weight of seeds in Shishito pepper were strongly reduced by high temperature and this was partially caused by a restriction in the accumulation of storage carbohydrates. Among the partial heat treatments, the DH and DNH treatments appeared to exert larger effects on seed germination and vigor than the NH treatment, as evidenced by low germination and accelerated aging germination rates, and a high value of the germination index (Table 3). Vara Prasad et al. (2000) and Todd Lasseigne et al. (2007) concluded that high day temperature exerted more adverse effects on plant growth and reproductive yield than high night temperature. However, in the present study, no differences in fruit growth and seed size between DH and NH were observed, and seed quality was more substantially influenced by high day temperature than by high night temperature. The cause of this phenomenon has not been elucidated. The adverse effects of partial heat stress on the quality of seeds and carbohydrate content suggest that high fruit temperatures exert a direct effect on the sink ability of seeds, resulting in the lower mobilization of assimilates than in the control and in a reduction in seed quality. However, the whole plant heat treatment led distinctively to larger reductions in the carbohydrate content, seed
dry weight, germination rate and accelerated aging germination rates compared with the partial heat treatments (DH, NH and DNH) (Tables 2 and 3). This suggests that the reduction in seed yield and quality under high temperature conditions is related to both the disturbance of assimilate supply by source organs and the suppression of sink strength in Shishito chili pepper.

References


