Heat Acclimation and De-acclimation for Pod Setting in Heat-tolerant Varieties of Common Bean (Phaseolus vulgaris L.)

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Abstract The effect of exposure to high temperature over a long period of time on pod setting of common bean (Phaseolus vulgaris L.) was investigated for heat-tolerant varieties (Haibushi and Ishigaki No.2) and one heat-sensitive variety (Kentucky Wonder) in an air temperature-controlled phytotron. When the plants were transferred from a moderate daily mean air temperature (DMAT) (23.6°C) to a high DMAT, the pod setting ratio gradually decreased from 7 days after the initiation of the heat treatments. At 29.6°C, the ratio decreased to 0%~15% at 15 days after the transfer and it did not recover thereafter. On the other hand, at around 28°C, the low pod setting ratio recovered again from 15 days after the transfer for the heat-tolerant varieties. At about 30 days after the transfer, the ratio of pod setting increased to the level (around 80%) of that of the plants that were continuously kept at 23.6°C. The recovery was not caused by changes in environmental conditions, that is, day length, light intensity, etc. but by the heat acclimation of flower buds to the air temperature (around 28°C). Heat acclimation appeared also at the same DMAT (around 28°C) but under different combinations of day and night temperatures (32.3°C/23.9°C and 30.4°C/25.7°C). Heat acclimation was not evident in the heat-sensitive variety and it could not be observed even in the heat-tolerant varieties at 29.6°C. When heat-acclimated plants were transferred back to 23.6°C and were left for one month, heat acclimation disappeared.

Key words Acclimation, Flower bud, Flower shedding, High temperature, Reproductive growth

Introduction

Responses of common bean varieties to exposure to a hot spell over a short period of time were reported in our previous paper, showing that heat-sensitive stages of flower buds could be identified for pod setting14). Heat-tolerant varieties (Haibushi and Ishigaki No.2) and one heat-susceptible variety (Kentucky Wonder) showed a different behavior in terms of heat-sensitive stages. On the other hand, their responses to the exposure to heat treatment over a long period of time should be investigated, because in subtropical and tropical areas the hot season lasts for several months and this type of hot spell is a main constraint on common bean production for vegetable and grain use. In the present study, responses to exposure to a prolonged hot spell of about one month were investigated for pod setting using the same heat-tolerant varieties (Haibushi and Ishigaki No.2) and the heat-susceptible variety (Kentucky Wonder) as in the previous paper14). Changes of the pod setting ratio under the prolongation of the period of exposure to hot days were particularly examined. This paper reports the heat acclimation of common bean for pod setting under high temperature and varietal differences in the acclimation.
Materials and Methods

General procedures

Heat-tolerant varieties of common bean (Haibushi and Ishigaki 2) and one heat-sensitive variety of common bean (Kentucky Wonder) were used. They were grown at the daily mean air temperature (DMAT) of 23.6°C, day/night temperatures of 25.6°C/21.6°C (control temperature) during a 12/12-h thermoperiod (7:00 a.m. to 7:00 p.m. /7:00 p.m. to 7:00 a.m.) under natural day length and natural light conditions in the chamber of a phytotron. The DMAT of 23°C had been reported to be optimal for bean growth and yield level\(^{10}\). Plants were grown by the same methods and under the same environmental conditions as those reported in the previous paper\(^{10}\). Air temperature of the chambers was also monitored in the same way as described in the previous paper.

Heat treatment

After flowering, each variety in two pots (one plant or two plants per pot) was subjected to the heat treatment. Tested plants were transferred from the chamber of the control temperature to other chambers at high temperatures in the same phytotron at 10:00 a.m. Air temperature of the chambers for heat treatment were also controlled under the same thermoperiod as that of the control temperature. Flowers at anthesis were marked in the same way as described in the previous paper. For example, in the heat-treatment experiment shown in Figs. 1 to 3, the total number of observed flowers was 203 for Haibushi, 202 for Ishigaki No.2 and 93 for Kentucky Wonder, respectively. On the other hand, in the control experiment (Fig. 4), the number was 224 for Haibushi, 266 for Ishigaki No.2 and 201 for Kentucky Wonder. Flower shedding and pod setting were inspected on the 4th day after anthesis and developing immature pods were removed from the plants, because no immature pods fell after the 4th day. Pod setting ratio, that is, the ratio of the number of developed pods to the number of flowers that opened was determined. Pod setting ratio of the
plants kept at the control temperature was also examined and compared to that of the plants subjected to the heat treatments.

Results

Time course changes in pod setting ratio and heat acclimation

When the plants were transferred to the chamber at a high day temperature (DMAT 28.1 °C, 32.3 °C/23.9 °C), flowers began to fall from about 7 days after the transfer (Figs. 1 to 3). The pod setting ratio decreased to only about 20% within 15 days after the initiation of the heat treatment in all the tested varieties. However, in the two heat-tolerant varieties, Haibushi and Ishigaki No.2, flower abscission stopped. Thereafter the pod setting ratio recovered to about 80% in the two varieties, which corresponded to the value in the control temperature, within about 25 days after the transfer (Figs. 1 and 2). It was assumed that the recovery was due to acclimation induced by the extension of the duration of the period of heat treatment. T test with combined data of the two heat-tolerant varieties showed that the difference in the pod setting ratio for the 6 day period between from 12 to 17 days after the initiation of the heat treatment and from 22 to 27 days was significant at 5% level. On the other hand, in the heat sensitive variety, Kentucky Wonder, recovery of the pod setting ratio was not observed (Fig. 3).

Although the pod setting ratio fluctuated at the control temperature (DMAT 23.6 °C) in the three tested varieties, it did not follow the changes observed under the heat treatment and it was in the range of higher values than 80% (Fig. 4). T test with combined data of all the three varieties showed that the difference in the pod setting ratio between the plants subjected to the high temperature treatment and plants at the control temperature was significant at 5% level from 12 to 17 days after the initiation of the heat treatment. In other experiments whose results are described below, the pod setting ratio at the control temperature showed higher values as indicated in Fig. 4.

Heat acclimation was observed also under a different combination of day/night air temperatures at the same DMAT (28.1 °C). The same recovery of the pod setting ratio was observed in the two heat-tolerant varieties at a higher night temperature (DMAT 28.1 °C, 30.4 °C/25.7 °C) than that indicated in the previous experiment (Figs. 1 and 5).

Critical temperature for the induction of the heat acclimation

An experiment was carried to investigate whether heat acclimation for pod setting could be induced by increasing the temperature by 1.5 °C (DMAT 29.6 °C, 32.5 °C/26.6 °C), compared to the previous experiments. Haibushi and Ishigaki No.2 showed similar changes in the pod setting ratio as those in the previous experiments at the DMAT of 28.1 °C until around 10 days after the initiation of the heat treatment (Fig. 6). However, thereafter, the pod setting ratio continued to decrease more to around 0% at the DMAT of 29.6 °C and the recovery of the pod setting ratio could not be observed even in the heat-tolerant
varieties Haibushi and Ishigaki No.2 (Fig. 6).

**Effect of high temperature before the flowering stage on the induction of heat acclimation**

As it took about 10 to 15 days of heat treatment for the recovery of the pod setting ratio in the previous experiments, experiments were carried out to determine whether heat acclimation occurred in the plants treated from the growing stage before flowering. In the experiments, plants were transferred to the chamber with a DMAT of 27.8°C (32.0°C/23.5°C) from June 1 and they started to open flowers on June 15. All the opened flowers fell until June 17 (only for two days in the high temperature chamber) but thereafter the ability of pod setting recovered gradually (Fig. 7 upper graph). These results suggested that plants at the pre-flowering stage also could be conditioned for heat acclimation and that they required the same period (about 15 days) of heat treatment for the induction of heat acclimation. On the other hand, plants that had already flowered were transferred to the same chamber at a DMAT of 27.8°C on June 22. They showed the same changes in the pod setting ratio as those in the previous experiments (Figs. 1 and 5) and the lowest value of the pod setting ratio appeared on a different day from that of the plants which were subjected to the heat treatment from the pre-flowering stage (Fig. 7 lower graph). In this experiment, although the plants subjected to two different treatments were kept at the same time in the same chamber, the lowest value of the pod setting ratio was observed on different days (Fig. 7 upper and lower graphs). Furthermore, the lowest value of the pod setting ratio was observed on different days from those shown in Figs. 1 and 5 whose data were collected in April and May. These results confirmed that the decrease and recovery of the pod setting ratio at high temperatures were not influenced by the date of onset of the treatment, day length, light intensity, or the fluctuations of the controlled temperature in the chamber.

**Retention of heat tolerance acquired by acclimation**

Experiments were carried out to determine whether the heat tolerance acquired by heat treatment could be maintained by the interception of the heat treatment with a period of exposure to the control air temperature. Plants, which were grown during 36 days at the DMAT of 28.1°C (32.3°C/23.9°C) and were acclimated to a high temperature for pod setting, were transferred again to the chamber at the control temperature (DMAT of 23.6°C, 25.6°C/21.6°C). They were kept there during 28 days, though the cool period was interrupted for 5 hot days at

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**Fig. 6.** Changes in the pod setting ratio of a heat-tolerant variety, Haibushi under a higher temperature (DMAT of 29.6°C) than that shown in Fig. 1.

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**Fig. 7.** Comparison of the pod setting ratio of plants (var. Haibushi) exposed to high temperature at the DMAT of 27.8°C (Day/Night 32.0°C/23.5°C) from the pre-flowering stage (upper graph) and that of plants treated at the same temperature but only from the post-flowering stage (lower graph). Lower graph was shifted to the right direction and the date in X axis of lower graph corresponded to that of upper graph (same calendar days).
the DMAT of 33.3°C (34.2°C/32.3°C) (Fig. 8). After confirmation of the recovery of the high pod setting ratio in the plants at the control temperature, they were transferred again to the chamber at a high temperature, i.e. DMAT of 27.9°C (32.0°C/23.7°C). In these plants the pod setting ratio decreased after re-transfer to the chamber at high temperature in the same way as in those newly treated at high temperature, although they were acclimated again from about 15 days after the transfer. These results suggest that heat acclimation induced by the heat treatment was lost by the interception with a period of exposure to the control temperature.

Discussion

Heat acclimation and cold acclimation are very common in plant species. Heat and cold acclimation enable plants to adapt to extreme temperature conditions. Another term, “hardening” has also been used in place of acclimation. Li et al. reported that the potential for acquiring heat tolerance by acclimation may be the main cause of the genotypic difference between heat-tolerant varieties and heat-sensitive varieties in common bean\(^1\). They evaluated the acclimation through measurements of electrolyte leakage from leaf discs to the incubation water by heat treatment that reflected the thermostability of the cell membrane. The electrolyte leakage could be monitored by measurement of the electric conductivity of the incubation water in which the leaf discs were suspended. They showed that storage of excised trifoliate leaves in a growth chamber at 37°C enabled to minimize the lethal injury of the cell membrane in leaf discs by subsequent treatment with hot water at 50°C. Heat tolerance by acclimation was acquired by pre-treatment at a slightly lower temperature, ranging from 34 to 42°C in other plant species\(^1,2,8,13,20\), than that in subsequent heat treatment. Heat acclimation estimated by the electrolyte leakage measurement with leaf discs was reported in many other plant species\(^5,20,21\). Furthermore, besides the thermostability of the cell membrane, heat acclimation had been detected in foliar damage\(^9\), O\(_2\) evolution and gas exchange in photosynthesis\(^1,6,19\), chlorophyll fluorescence\(^19\) and photosynthetic electron transport of isolated thylakoid membrane\(^17\), TTC test with 2,3,5-triphenyl tetrazolium chloride\(^13,19\), etc. Mechanisms for the acquisition of heat tolerance have not been clarified but some hypotheses were presented. It was suggested that changes in the ratio of unsaturated fatty acids to saturated fatty acids enabled the thermostability of the cell membrane to adjust to high temperature in the same way as in cold acclimation\(^9\). In heat acclimation, activity of catalase and superoxide dismutase increased and the enzymes were expected to repress free radicals that might be a cause of heat injury\(^20\).

In terms of hormonal regulation, the role of abscisic acid in the acquisition of heat tolerance was reported as in the case of cold acclimation\(^1,10\). Synthesis of heat shock proteins may partly be responsible for heat acclimation\(^10\), though some members of the heat shock protein (HSP70s) family and other proteins appeared also in cold acclimation\(^1,7,13\). Therefore, heat acclimation was blocked by protein synthesis inhibitors\(^10\). Although the synthesis of heat shock proteins by heat acclimation was reported in common bean\(^12,18\), physiological and biochemical investigations of the mechanism of heat acclimation have not been carried out in this species.

Duration of the pre-treatment at high temperature that was required for the acquisition of heat tolerance was determined in many studies as follows: 180 minutes in the evaluation of foliar damage in jack pine and white spruce\(^9\). In electrolyte leakage tests, the duration was 2 hours in leaves of \textit{Capsicum annum}\(^19\), 24 hours
in leaves in common bean\textsuperscript{11} and maize\textsuperscript{2}, 10 to 16 hours in leaves and suspension-cultured cells of grape\textsuperscript{9}. The duration ranged from 1 to 5 days for the activity of catalase and superoxide dismutase in wheat leaves\textsuperscript{20}. On the other hand, it took about 30 days for full recovery of the pod setting ratio by heat acclimation in this study. The duration of the pre-treatment at high temperature that was required for the heat acclimation of flower buds was considerably longer than that for the heat acclimation of vegetative organs previously reported. This difference in the duration suggested that different mechanisms were involved between reproductive growth (pod setting) and vegetative growth (thermostability and photosynthesis of leaves, etc.).

Heat acclimation has never been reported in reproductive growth related to fruit (pod) setting, that is, flower bud development, pollination, fertilization and pod development. We confirmed the existence of heat acclimation by repeated experiments related to high temperature treatment, as follows. 1) By heat acclimation, the pod setting ratio of heat-tolerant varieties recovered at the same time after the initiation of the high temperature treatment at a DMAT of 28.1°C. 2) Heat acclimation was observed also at the DMAT of 28.1°C under different combinations of day and night temperatures. 3) Heat acclimation was observed in the plants treated at high temperature from the pre-flowering stage, too. 4) Heat acclimation was observed again when plants de-acclimated at the control temperature were transferred to high temperature conditions. This paper reported for the first time the existence of heat acclimation in reproductive growth in plant species. It is possible that acclimation occurred in pollen development, because pollen cells might be most sensitive to high temperature in the flower bud of common bean\textsuperscript{10}. In common bean, investigations on heat injury to pod setting have been mainly carried out under hot spells over a period of time, namely several days\textsuperscript{11}. On the other hand, around 30 days were required for the heat acclimation in pod setting. Therefore, observation over a long period of time was required to detect the phenomenon, which may account for the fact that heat acclimation had never been detected in the previous studies on heat tolerance in pod setting of common bean and other plant species, too.

In terms of de-acclimation, in this study, when the heat-acclimated plants were transferred to moderate air temperature (23.6°C) and kept there for one month, they lost their heat tolerance. The duration of the period of retention of the heat tolerance in plants under moderate air temperature conditions was investigated in some studies. It lasted for a few days based on electrolyte leakage tests in wheat leaves\textsuperscript{21}. The duration was affected by the duration of the heat treatment for heat acclimation in seedlings of conifers and it ranged from 4 to 14 days in the case of foliar damage in the seedlings acclimated at 38°C for 180 minutes per day for 6 days\textsuperscript{8}. In this study, the duration of de-acclimation was not determined exactly and future studies are required.

Heat acclimation in reproductive growth is a new subject in studies on heat stress physiology. In common bean, this phenomenon should be studied for heat tolerance breeding and to improve cultural practices in warm areas. The present study provided some physiological findings on heat acclimation in reproductive growth. For example, combinations of day and night temperatures did not affect the acquisition of the heat tolerance. Furthermore, it was assumed that day length and light intensity did not influence the acquisition of the heat tolerance by the plants treated at a DMAT of about 28°C, because similar time-course changes in the pod setting ratio were observed under heat treatment irrespective of the date of treatment from early April to late July while the experiments were carried out. The heat tolerance was acquired also by the plants before flowering. This observation suggested that physiological and biochemical processes for heat acclimation occurred not only in flowering plants but also in plants with only developing immature flower buds.

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