Water Status of Flower Buds and Leaves as Affected by High Temperature in Heat-Tolerant and Heat-Sensitive Cultivars of Snap Bean (*Phaseolus vulgaris* L.)

Tadashi Tsukaguchi, Yoshinobu Kawamitsu*, Hiroyuki Takeda**, Katsumi Suzuki** and Yoshinobu Egawa**

(Bio-Oriented Technology Research Advancement Institution, JIRCAS Okinawa Subtropical Station, Ishigaki, Okinawa 907-0002, Japan; *Faculty of Agriculture, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan; **JIRCAS Okinawa Subtropical Station, Ishigaki, Okinawa 907-0002, Japan)

Abstract: In snap bean (*Phaseolus vulgaris* L.), flower and pod abscission causes yield reduction under high-temperature conditions. A high temperature enhances transpiration and thus may induce temporal water deficiency in plants in the daytime. The objective of this study was to clarify the effect of a high temperature on the water status of floral organs at their most heat-sensitive stage. We compared the water potential and its components as well as gas exchange between the heat-tolerant cultivar, Haibushi, and heat-sensitive cultivar, Kentucky Wonder, grown under optimal (control) and high-temperature conditions. Haibushi showed higher pollen fertility under high temperature than Kentucky Wonder. Transpiration was enhanced under a high temperature, causing decrease of water potential in leaves and flower buds. The deterioration of water status in floral organs was larger in Kentucky Wonder than in Haibushi. We conclude that temporal deterioration of the water status in flower buds is one of the factors causing pollen damage.

Key words: flower buds, heat tolerance, pollen fertility, Snap bean, transpiration, water potential

Pod production of snap bean (*Phaseolus vulgaris* L.) is severely depressed by high temperatures. The yield depression is partly due to flower and pod abscission. Male organs are more sensitive to a high temperature than female organs (Monterosso and Wien, 1990), as in other legume crops (Hall, 1992). One day and 10 days before anthesis are stages sensitive to high temperatures (Nakano et al., 1998; Suzuki et al., 2001a; Tsukaguchi et al., 1999). Ten days before anthesis corresponds to the stage just after the pollen tetrad stage (Suzuki et al., 1999) and endoplasmic reticulum abnormality occurs in the tapetum at this stage under high temperatures (Suzuki et al., 2001a). Cultivars which had a higher yield under high temperature conditions showed high pollen fertility (Suzuki et al., 2001b). These results suggest that pollen sterility is one of the major factors limiting the yield under high temperature conditions.

High temperatures, often together with intensive radiation, enhances transpiration and thus may induce temporal water deficiency in plants in the daytime even if plants are well watered as reported in tomato (Bar-Tsur et al., 1985). Flower buds at around the tetrad stage are also sensitive to water stress in *Phaseolus vulgaris* L. (Shen and Webster, 1986). There is a possibility that temporal water stress in the daytime adversely affects pollen development solely or together with or without a direct effect of high temperature. It is still unknown whether water stress at this stage damages pollen development through desiccation in floral organs or indirectly through carbohydrate supply or hormonal regulation. However, how water status in flower buds is affected by high temperatures remains unknown. Therefore, a better understanding of the influence of high temperature on the water status in the reproductive organs is necessary for the identification of plant factors which confer tolerance to a high temperature. The objective of this study is to clarify the effect of a high temperature on the water status of floral organs at their most heat-sensitive stage. For this objective, we compared two cultivars, Haibushi, a heat-tolerant cultivar and Kentucky Wonder, a heat-sensitive cultivar.

Materials and Methods

1. Plant materials

Snap bean (*Phaseolus vulgaris* L. cvs. Haibushi and Kentucky Wonder) plants were sown in plastic pots containing four liters of soil on 30 May 2000. Each pot was supplied with 0.3 g each of N, P and K before sowing. One plant was grown in each pot. The plants were grown in a glasshouse under natural light conditions at a high temperature (32/26°C, day/night) and optimal temperature (27/21°C). Relative humidity was maintained at 55±5% under both temperature conditions. Six plants were used for each treatment and each cultivar. Plants were irrigated several times a day, depending on the weather conditions to supply sufficient water to the plants.

2. Determination of pollen fertility

Twenty flower buds were fixed one day before anthesis.
Table 1. Size of flower buds at each developmental stage of pollen in Haibushi and Kentucky Wonder.

| Developmental stage of pollen | Length of flower bud (mm) |
|------------------------------|--------------------------|
| Pollen mother cell           | Haibushi: 2.1>           |
|                              | Kentucky Wonder: 2.1>    |
| Meiosis                      | Haibushi: 2.1-2.3        |
|                              | Kentucky Wonder: 2.1-2.2|
| Pollen tetrad                | Haibushi: 2.3-2.6        |
|                              | Kentucky Wonder: 2.2-2.3|
| Uninucleolar pollen          | Haibushi: 2.4-3.0        |
|                              | Kentucky Wonder: 2.3-3.0|

Fig. 2. Changes in transpiration rate and stomatal conductance of Haibushi (squares) and Kentucky Wonder (triangles) grown under control (open symbols) and high temperature conditions (solid symbols).

With aceto-alcohol (1:3). Anthers of the fixed buds were stained with acetocarmine. Pollen fertility was defined as the ratio of the number of pollen grains well-stained with acetocarmine to the total number of pollen grains examined. Pollen fertility was determined for each flower bud and mean value was calculated for each treatment regarding one bud as a replication.

3. Determination of flower bud size at each stage

Fifty flower buds between 1.8-3.0 mm in length were sampled from each cultivar. After measuring their length, developmental stage of pollen of each flower bud was determined under a light microscope. Flower bud size at each developmental stage of pollen is shown in Table 1. The size of buds at the pollen tetrad stage was 2.3-2.6 mm in Haibushi and 2.2-2.3 mm in Kentucky Wonder.

4. Measurement of gas exchange

At the beginning of flowering, the transpiration rate and stomatal conductance of a fully expanded upper young leaf was measured for each plant using a leaf chamber analyzer (Type LCA-4, Shimadzu). Measurement was done in the morning (9:00—10:30) and in the afternoon (13:00—14:30) on five clear sunny days from the end of June to the beginning of July. Mean photosynthetic active radiation (PAR) during the measurement was 1365 and 1541 µmol m⁻² s⁻¹ in the morning and afternoon, respectively. Measurement of each plant was regarded as one replication.

5. Measurement of water and osmotic potential

Seven flower buds at the pollen-tetrad stage (2.3-2.6 mm in Haibushi and 2.2-2.3 mm in Kentucky Wonder) were quickly and exactly sampled from each cultivar under each temperature condition for the measurement of water potential (Ψ_w) and osmotic potential (Ψ_o). From the same plants, leaf discs of 7 mm in diameter were also sampled from fully expanded young leaves for the measurement of Ψ_w and Ψ_o. The flower buds and leaf discs were placed in a chamber immediately after sampling and left for three hours at 25°C. Water potential of samples was measured with a HR-33T thermocouple psychrometer (Wescor Co. LTD.). Osmotic potential was measured as described by Boyer (1995). Pressure potential (Ψ_p) was determined as

Ψ_p = Ψ_w - Ψ_o.

The flower buds and leaves for the measurement of Ψ_w and Ψ_o were sampled at 5:00 (predawn) and 13:00 (mid-day) on five clear sunny days from the middle of June to the beginning of July. Measurement of each day was regarded as one replication. After all the measurements, the leaf area of each plant was measured on 11 July.

Results

Both cultivars showed almost 100% pollen fertility under optimal temperature (control) conditions (Fig. 1). Under high temperature conditions, however, pollen fertility was 40% and 5% in Haibushi and Kentucky Wonder, respectively.

Under control conditions, stomatal conductance and transpiration rate decreased in the afternoon (Fig. 2). In both cultivars, stomatal conductance was larger in the plants under high temperature conditions than that in plants under control conditions. Under high temperature condition in the afternoon, Haibushi had larger stomatal conductance and thus larger transpiration rate than Kentucky Wonder.

Ψ_w of flower buds decreased at mid-day in both cultivars under both temperature conditions (Fig. 3). In the control plants, Ψ_w decreased in parallel with Ψ_o and thus no significant decline in Ψ_p was observed at

Fig. 1. Pollen fertility of Haibushi and Kentucky Wonder grown under optimal (control) (27/21°C, open bars) and high temperature conditions (32/26°C, dotted bars).
mid-day in both cultivars. Under high temperature conditions, the decrease of \( \psi_w \) at mid-day was larger than that of \( \psi_o \) and therefore, \( \psi_w \) decreased from 0.34 MPa at predawn to 0.24 MPa at mid-day in Haibushi and from 0.37 to 0.21 MPa in Kentucky Wonder.

\( \psi_w \), \( \psi_o \) and \( \psi_p \) of leaves were influenced by high-temperature treatment similarly in both cultivars (Fig. 4). Mid-day decrease of \( \psi_p \) was larger in leaves than in flower buds in both cultivars under both temperature conditions. Leaf \( \psi_p \) decreased from 0.34 MPa at predawn to 0.27 MPa at mid-day in Haibushi and from 0.40 to 0.26 MPa in Kentucky Wonder. The mid-day decrease of \( \psi_p \) in leaves under high-temperature conditions was smaller than that in flower buds in both cultivars.

In flower buds, no significant difference in \( \psi_w \) and \( \psi_p \) was observed between Haibushi and Kentucky Wonder under control temperature conditions. Under high-temperature conditions, although no significant difference in \( \psi_p \) at mid-day was observed between the two cultivars, the rate of decrease in \( \psi_p \) from predawn to mid-day was larger in Kentucky Wonder than in Haibushi. In leaves under high-temperature conditions, the mid-day decrease of \( \psi_w \) and \( \psi_p \) were larger in Kentucky Wonder than in Haibushi, and Haibushi showed a slightly higher leaf \( \psi_p \) at mid-day while no significant difference was observed between the two cultivars. Kentucky Wonder had a considerably higher leaf \( \psi_p \) at predawn than Haibushi. No significant difference was observed in the \( \psi_o \) in flower buds at mid-day between the two cultivars but \( \psi_o \) of leaves at mid-day was lower in Kentucky Wonder than in Haibushi.

Under high temperature conditions, leaf area was smaller than under the control conditions in both cultivars (Table 2), but no significant difference was observed in leaf area between the two cultivars under both temperature conditions.

![Fig. 3. Changes in \( \psi_w \) (triangles), \( \psi_w \) (squares) and \( \psi_o \) (circles) of flower buds in snap bean grown under control (open symbols) and high temperature conditions (solid symbols).](image)

![Fig. 4. Changes in \( \psi_w \), \( \psi_p \) and \( \psi_o \) of leaves in snap bean grown under control and high temperature conditions. Symbols are the same as those shown in Fig. 3.](image)

### Table 2. Leaf area of a whole plant in Haibushi and Kentucky Wonder (cm² plant⁻¹).

| Treatment       | Haibushi | Kentucky Wonder |
|-----------------|----------|-----------------|
| Optimal         | 3677±249 | 3429±371        |
| High temperature| 2886±412 | 2651±206        |

### Discussion

Transpiration is enhanced by high temperature via increased stomatal conductance as shown by Bar-Tsur et al. (1985) in tomato. The rapid transpiration of plants under high-temperature conditions causes a significant decrease in the water potential of leaves at mid-day.

No significant difference was observed in \( \psi_w \), \( \psi_o \) and \( \psi_p \) between the two temperature conditions and between the two cultivars. Since water supply was not limited in this study, water uptake of plants might not be suppressed by high temperatures during the nighttime. Mid-day water status of flower buds at the pollen tetrad stage markedly deteriorated under high temperature conditions (Figs. 2-4). In response to water deficiency, the water potential of reproductive organs declined much less than that of the leaf in wheat (Dorion et al., 1996; Morgan and King, 1984; Saini and Aspinall, 1981). These results suggest that reproductive development is suppressed mainly by a limited assimilate supply as shown by Westgate and Boyer (1985) in maize. This smaller decline in water potential in reproductive organs than that in vegetative organs is possibly because young panicles at the reproductive stage are enclosed in leaf sheaths so that they are protected against desiccation in cereal crops. The diurnal changes in water potential of rice panicles enlarged after their emergence (Tauda and Takami, 1995). Flower buds of snap bean, however, are exposed to the surrounding atmosphere and are directly affected by its temperature. The stage at around the pollen tetrad is one of the most sensitive stages to high temperature in snap bean (Nakano et al., 1998; Suzuki...
et al., 1999; Tsukaguchi et al., 1999). These results suggest that together with the limited assimilate supply the deterioration of water status in flower buds under high temperature conditions caused a drastic deterioration in pollen fertility of snap bean which resulted in yield reduction (Suzuki et al., 2001b).

Water status in leaves was similar to that of flower buds and showed a similar trend (Figs. 3 and 4). However, \( \Psi_P \) of leaves at mid-day was maintained higher than that of flower buds since leaf \( \Psi_0 \) considerably decreased at mid-day presumably due to accumulation of photosynthate. These results show that high temperature deteriorates the water status of the flower buds as well as that of leaves.

The decline of \( \Psi_P \) and \( \Psi_F \) in both the leaf and flower bud from pre-dawn to mid-day was significantly larger in Kentucky Wonder than in Haibushi, in which pollen was less damaged by high temperature. This drastic deterioration of water status in flower buds may have adverse effect on pollen development. High productivity of Haibushi under high temperature conditions (Nakano et al., 1998; Suzuki et al. 2001b) is partly associated with the high performance in maintaining water. Water deficit in plant organs is caused by greater amount of water loss through transpiration than that of water uptake. Haibushi showed a higher transpiration rate than Kentucky Wonder while no significant difference was observed in leaf area between the two cultivars. Haibushi maintained a better water status in leaves and flower buds than Kentucky Wonder in spite of a higher transpiration rate as a whole plant. Therefore, the smaller decline in pressure potential of flower buds in Haibushi than that in Kentucky Wonder under high temperature conditions was possibly due to the higher water conductance.

We conclude that the water status in snap bean deteriorates temporarily at mid-day under high temperature conditions and that flower buds suffer water deficiency which hinders the development of pollen. In Haibushi, a heat-tolerant cultivar, water deficiency of flower buds due to high temperatures was milder than that in Kentucky Wonder, a heat-sensitive cultivar, possibly due to higher water conductance or water uptake ability.

References

Bar-Tsur, A., Rudich, J. and Bravado, B. 1985. Photosynthesis, transpiration and stomatal resistance to gas exchange in tomato plants under high temperatures. J. Hort. Sci. 60 : 405-410.

Boyer, J. S. 1995. Measuring the water status of plants and soils. Academic Press. New York. 79-84.

Dorion, S., Lalonde, S. and Saini, H. S. 1996. Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers. Plant Physiol. 111 : 137-145.

Hall, A.E. 1992. Breeding for heat tolerance. In J. Janick ed., Plant Breeding Reviews, vol. 10. John Willey and Sons, New York. 129-168.

Monterosso, V.A. and Wien, H.C. 1990. Flower and pod abscission due to heat stress in beans. J. Amer. Soc. Hort. Sci. 115 : 631-634.

Morgan, J.M. and King, R.W. 1984. Association between loss of leaf turgor, abscisic acid levels and seed set in two wheat cultivars. Aust. J. Plant Physiol. 11 : 143-150.

Nakano, H., Kobayashi, M. and Terauchi, T. 1998. Sensitive stages to heat stress in pod setting of common bean (Phaseolus vulgaris L.). Jpn. J. Trop. Agr. 42 : 78-84.

Saini, H.S. and Aspinall, D. 1981 Effect of water deficit on sporogenesis in wheat (Triticum aestivum L.). Ann. Bot. 48 : 623-633.

Shen, X.Y. and Webster, B.D. 1986. Effects of water stress on pollen of Phaseolus vulgaris L. J. Amer. Soc. Hort. Sci. 111 : 807-810.

Suzuki, K., Takeda, H., Matsuura, S., Yuo, S. and Egawa, Y. 1999. Morphological study on injury of pollen of snap bean by heat stress. Proc. Int. Symp. "World Food Security", Kyoto : 203-206.

Suzuki, K., Takeda, H., Tsukaguchi, T., Egawa, Y. 2001a. Ultrastructural study on degeneration of tapetum in anther of snap bean (Phaseolus vulgaris L.) under heat stress. Sex. Plant Reprod. 13 : 293-299.

Suzuki, K., Tsukaguchi, T., Takeda, H. and Egawa, Y. 2001b. Decrease of pollen stainability of snap bean at high temperatures and relationship to heat tolerance. J. Amer. Soc. Hort. Sci. 126 : 571-574.

Tsuda, M. and Takami, S. 1993. Changes in water potential in rice panicle under increasing drought stress at various stages. Jpn. J. Crop Sci. 62 : 41-46.

Tsukaguchi, T., Suzuki, K., Takeda, H. and Egawa, Y. 1999. Pod set ratio as affected by pollen fertility under high temperature at reproductive stage in snap bean (Phaseolus vulgaris). Jpn. J. Crop Sci. 68 (extra) : 126-127*.

Westgate, M. E. and Boyer, J. S. 1985. Carbohydrate reserves and reproductive development at low water potentials in maize. Crop Sci. 25 : 762-769.

*In Japanese.