Diurnal Change in Water Balance of Heat-Tolerant Snap Bean (*Phaseolus vulgaris*) Cultivar and Its Association with Growth under High Temperature

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**Abstract**: A snap bean (*Phaseolus vulgaris* L.) cultivar Haibushi shows high productivity under high-temperature conditions. Together with intensive radiation, high temperature enhances transpiration and causes water deficit in plants even when they are irrigated enough. To characterize daily change in water balance of the heat-tolerant cultivar, we compared parameters of water balance, dry matter production and pod yield among cultivars. Four snap bean cultivars, Haibushi, Kurodane-Kinugasa, Oregon and Kentucky Wonder, were grown under optimal temperature (spring cropping) and high temperature (summer cropping) condition in the field. The daily water balance and gas exchange rate in the heat-tolerant cultivar Haibushi were compared with those in the heat-sensitive cultivar Kentucky Wonder, grown in 0.02 m$^2$ Wagner pots. In the summer cropping in the field, dry matter production, pod yield, stomatal conductance, photosynthetic rate and transpiration rate were higher in Haibushi and Kurodane-Kinugasa than in the other cultivars. In a glasshouse, the sap flow rate was lower than the transpiration rate in the morning when the transpiration rate rapidly increased in both Haibushi and Kentucky Wonder. In spite of the higher transpiration rate, Haibushi showed a higher sap flow rate and smaller cumulative water loss in the morning than Kentucky Wonder. We conclude that better growth of the heat-tolerant snap bean cultivar Haibushi under high temperature was due to higher photosynthetic rate resulting from higher stomatal conductance during the daytime, which had a higher water uptake rate.

**Key words**: Heat tolerance, *Phaseolus vulgaris*, Sap flow, Transpiration.

Pod yield of snap bean (*Phaseolus vulgaris* L.) is severely depressed under a high temperature condition. When mean air temperature exceeds 27˚C, pod yield is detrimentally decreased (Suzuki et al., 2001a). The snap bean cultivar Haibushi, developed by Japan International Research Center for Agricultural Sciences (JIRCAS) Okinawa Station (Nakano et al., 1997), shows considerably higher yield than those of other cultivars under high temperature conditions and intensive radiation environment in the field (Suzuki et al., 2001a). Using Haibushi as a breeding material, development of heat-tolerant snap bean cultivars is now in progress. However, useful and convenient selection criteria for heat tolerance and its physiological basis have not been clarified.

A high temperature often coincides with a high solar radiation and causes excessive transpiration. This excessive transpiration leads to temporal water deficit in plants in the daytime, even when soil moisture content is adequate and plants can take up a sufficient amount of water in the nighttime. In soybean (*Glycine max* Merr.) grown in the soil with adequate water content, grain yield was closely related to the afternoon water deficit (Boyer et al., 1980). In a high-temperature environment, water deficit occurred even in well-watered tomato plants (*Lycopersicon esculentum* Mill.) (Bar-Tsur et al., 1985). *P. vulgaris* cultivars adapted to a hot environment tended to have a higher gas exchange rate (Mencuccini and Comstock, 1999). Decline in water potential of vegetative and reproductive organs was considerably larger under high temperature than that under optimal temperature conditions in snap bean plants (Tsukaguchi et al., 2003). These findings suggest that heat-tolerant snap bean cultivars maintain a good water status in the daytime.

Decline in water potential of vegetative and reproductive organs under a high temperature condition was less in the heat-tolerant cultivar Haibushi than that in heat-sensitive cultivar (Tsukaguchi et al., 2003). However, water balance of plants in the daytime, and its association with gas exchange and dry matter production has not been clarified. We investigated the diurnal change in water balance and its association...
with gas exchange and dry matter production in four snap bean cultivars for the better understanding of traits responsible for heat tolerance in Haibushi.

**Materials and Methods**

**Glass house experiment**

**Plant material.** Climbing snap bean cultivars Haibushi and Kentucky Wonder were grown in 0.02 m² Wagner pots containing four-liter soil (Ultisol “Typic Hapludults”) twice in 2000. Sowing date of the first and second cropping was July 12 and September 13, respectively. Before sowing, three grams of calcium carbonate and slow release fertilizer (N:P:K = 15:12.5:6.5 CDUs555, Chisso Co., Tokyo, Japan) were applied to the soil and three weeks after the sowing three grams of the slow release fertilizer was again added to the soil. One plant was grown in each pot. The plants were grown in an air-conditioned glasshouse under natural sunlight. Mean air temperature was 28.1/23.5˚C (day/night) during the first cropping and 26.3/23.3˚C during the second cropping. Rates of transpiration and sap flow.

**Transpiration and sap flow.** Transpiration and sap flow rates were measured on August 12, 14, 16, October 20, 22 and 24, when plants were at the initial stage of flowering. Sap flow was monitored using one of the five plants in each cultivar on each measurement day. Different plants were used on each measurement day. The transpiration rate was measured using the same plant as that used for sap-flow measurement on August 12, 14, October 20 and 22, but using five plants on August 16 and October 24. At 20:00 on the day before measurement, pot soil was saturated with water. The surface of soil was covered with aluminum foil to avoid evaporation from soil and soil water status was monitored using tensiometer (DIK, Daiki Co. Ltd., Japan) throughout the measurement periods. The transpiration rate was measured by measuring pot weight every 30 min from 5:00 to 21:00 on August 12, 14 and 16 and from 6:00 to 20:00 on October 20, 22 and 24. After measuring pot weight, each pot was irrigated to compensate for the water lost during the last 30 min using a syringe with a scale of ml. Water flow in the stem was monitored using the stem heat balance method (Sakuratani, 1981 and 1984). Three mm sensors were attached to the basal part of the plant stem. Data signals were logged every 60 s and averaged over 30 min using a data logger (10X; Campbell Scientific Inc., USA) throughout the measurement period. Sap flow rate was calibrated to the ratio of total amount of sap flow to total amount of transpiration on the measurement day, and after all the measurement leaf area of the plants was determined and transpiration rate and sap flow rate were shown as the values per unit leaf area. Cumulative water loss (CWL) (mg cm⁻²) was calculated as CWL = E(Tr-SF), where Tr is transpiration rate per unit leaf area (mg hr⁻¹ cm⁻²) and SF is sap flow rate (mg hr⁻¹ cm⁻²).

**Gas exchange rate and relative water content (RWC).** On August 16 and October 24 the gas exchange rate in the terminal leaflet of the youngest fully expanded trifoliate leaf was measured and the relative water content (RWC) of the lateral leaflet of the same trifoliate leaf was determined. Photosynthetic rate, transpiration rate and stomatal conductance were measured using a leaf chamber analyzer (Type LCA-4, Shimadzu, Japan). Water use efficiency (WUE) was calculated as WUE = Pn/Tr, where Pn is photosynthetic rate and Tr is transpiration rate measured with a leaf chamber analyzer. Measurements were conducted every hour from 7:00 to 19:00 on August 16 and every 1.5 h from 7:30 to 18:00 on October 24. RWC was determined every 3 h from 6:00 to 21:00. From lateral leaflets, two leaf discs, each 10 mm in a diameter, were punched out avoiding major veins. Fresh weight (Wf) of the six leaf discs from each plant was then quickly measured and the leaf discs were soaked in distilled water for 24 h at 20˚C under low-intensity light. Weight of turgid leaf discs (Wt) were measured after the water on the surface was removed with paper towel. The leaf discs were then oven-dried at 70˚C for 48 hr and dry weight (Wd) was measured. RWC was calculated as RWC = 100-(Wt-Wf)/(Wt-Wd) × 100 (%).

**Dry weight, leaf area and root length.** On August 17 and October 25, the plants were separated into leaflets, stem and roots. Leaf area was measured using automatic area meter (AAM-8, HAYASHI DENKO Co. Ltd., Japan). Root length was measured according to Newman’s (1966) method improved by Tennant (1975). After soil was washed off, the roots were randomly placed in a gridded vat (1 cm × 1 cm), and the number of intersections between roots and grid lines was counted. Root length (R) was estimated as R = N × G, where N is the number of intersections and G is the grid coefficient (0.786 in this case) (Tennant, 1975). After leaf area and root length were determined, plant organs were oven-dried (70˚C) for 3 days and dry weight was measured.

**Field experiment**

**Cultivation.** Four snap bean cultivars were grown in the field of Japan International Research Center for Agricultural Sciences (JIRCAS) Okinawa Station, located on Ishigaki Island, Okinawa, Japan (lat. 24°20' N, long. 124°E, altitude 20 m), which is under subtropical conditions. Four snap bean cultivars were used in this study; Haibushi, which is heat-tolerant cultivar developed at this station (Nakano et al., 1997), Kentucky Wonder, Oregon and Kurodane-Kinugasa are commercial cultivars mainly cultivated in mainland Japan. Each cultivar was grown twice in
2001. Sowing date of the first and the second cropping was March 22 (spring cropping) and May 28 (summer cropping), respectively. Plants were grown in a three-meter row. Inter-row space was 1 m and plant space in a row was 15 cm. Each cultivar occupied one row, which was considered to be one replication, and three replications were arranged in a randomized complete block design.

**Pod yield and dry weight.** Plant dry weight was measured at the beginning of flowering stage. Five plants were harvested from each plot 40 days after sowing, and after oven dried at 70°C for 3 days, dry weight was measured. Pod yield of other five plants was monitored for three weeks. Harvest period of the spring and summer cropping was from May 10 to June 6 and from July 10 to August 6, respectively. In each plot, pods of 8 to 10 days after flowering were harvested daily and fresh weight was measured.

**Gas exchange rate, leaf temperature and relative water content.** At the beginning of flowering (37 and 35 days after sowing in the spring and summer cropping, respectively) gas exchange rate, leaf temperature and relative water content (RWC) of leaves were measured. Three average-size plants were selected for the measurement from each plot. In each plant, the terminal leaflet of the youngest fully-expanded leaf was used for the measurement of gas exchange rate and a lateral leaflet of the same leaf for the determination of RWC. Rate of gas exchange was measured every two hours from 7:00 to 17:00. At 14:00, temperature of the same leaflets was measured with an infrared radiation thermometer (IT2-01, Keyence Co. Ltd., Japan). Relative water content of leaves was measured at 6:00, 12:00 and 18:00. Measurement procedure was the same as described above.

### Results

**Glasshouse experiment.** The sky was clear all day long on August 16, and it was clear in the morning, started to be overcast after 10:00 and was cloudy after 13:00 on October 24 (Fig. 1). Although sap flow and transpiration rates were measured three times in each cropping using different plants, only the data obtained on August 16 and October 24 are shown (Fig. 2), since similar values were observed in the other four measurements. Transpiration rate increased rapidly as solar radiation increased (Figs. 1 and 2). Haibushi
showed a continuously higher transpiration rate than Kentucky Wonder from 9:00 to 18:00, showing a 20% higher rate from 12:00 to 15:00. In both cultivars, sap flow rate was lower than transpiration rate in the morning when transpiration rate rapidly increased. Sap-flow rate was higher than transpiration rate only in the afternoon. Haibushi showed a higher sap flow rate in the morning and the peak appeared earlier than in Kentucky Wonder.

Cumulative water loss (CWL) refers to water balance in a plant and high CWL indicates that the plant is suffering water deficiency. CWL in Kentucky Wonder increased more rapidly than that in Haibushi and leveled off just before noon (Fig. 2). On the other hand, in Haibushi CWL increased much more gradually and was considerably lower at midday when radiation was high. CWL in Haibushi continued to increase due to higher transpiration, and reached a peak about four hours later than that in Kentucky Wonder on both days.

On August 16, photosynthetic rate increased rapidly until 9:00 and decreased gradually thereafter in both Haibushi and Kentucky Wonder (Fig. 5). At 9:00 no significant difference was observed between them but from 11:00 to 15:00 Haibushi showed a higher photosynthetic rate than Kentucky Wonder. No significant difference was observed in water use efficiency (WUE) between Haibushi and Kentucky Wonder both on August 16 and October 24. On August 16, two peaks of WUE were observed at 9:00 and 17:00, whereas on October 24, it gradually decreased after the peak at 9:00. On October 24, photosynthetic rate rapidly decreased after 12:00 and almost 0 at 16:30 while transpiration rate decreased more gradually in the afternoon.

Relative water content (RWC) of leaves declined in the day time in both cultivars (Fig. 4). In Kentucky Wonder
Higher Water Uptake of Heat-Tolerant Snap Bean Cultivar Wonder, RWC was higher than that in Haibushi at pre-dawn while no difference in RWC was observed between the two cultivars at 12:00. Kentucky Wonder showed a higher RWC than Haibushi in the afternoon. 

No significant difference was observed in shoot/root (S/R) ratio between the two cultivars in the first cropping and Kentucky Wonder showed higher S/R ratio in the second cropping (Table 1). Specific root length, however, was significantly larger in Haibushi than in Kentucky Wonder.

Field Experiment. Mean air temperature during the growth period was 24.8 and 29.1°C in the spring and summer cropping, respectively (Table 2). In the spring cropping, pod yield was low. While no pods were harvested in Oregon and Kentucky Wonder, Kurodane-Kinugasa produced 5% and Haibushi still produced 36% of the spring cropping. No significant difference was observed in plant dry weight at the beginning of the flowering among the four cultivars in the spring cropping (Table 2). In the summer cropping, however, plant dry weight of Kentucky Wonder and Oregon was significantly smaller than that of Haibushi and Kurodane-Kinugasa.

In all the cultivars, stomatal conductance increased from 9:00 to 11:00 and then decreased gradually in the afternoon (Fig. 5). In the spring cropping, mean air temperature during the growth period was 24.8 and 29.1°C in the spring and summer cropping, respectively (Table 2).

### Table 1. Leaf Area (LA), dry weight of shoot (shoot DW) and root (root DW), shoot/root (S/R) ratio and specific root length (SRL) of Haibushi and Kentucky Wonder.

|                  | First cropping | Second cropping |
|------------------|----------------|-----------------|
|                  | Haibushi | Kentucky | Wonder | Haibushi | Kentucky | Wonder |
| Leaf area (cm² plant⁻¹) | 2560 | 2110 | * | 3590 | 3080 | n.s. |
| Shoot DW (g plant⁻¹) | 10.3 | 8.5 | * | 13.7 | 12.3 | n.s. |
| Root DW (g plant⁻¹) | 3.3 | 2.7 | * | 3.5 | 3.8 | n.s. |
| S/R ratio (%) | 312 | 317 | n.s. | 410 | 340 | * |
| SRL (cm g⁻¹) | 160 | 113 | ** | 180 | 117 | ** |
| Mean air temperature (°C) | 28.1 | 23.5 | | 26.3 | 23.3 | |

* Seeding date was July 12 and September 13 in the first cropping and second cropping, respectively.
* , ** and n.s. indicate significant difference at the 5%, 1% and no significant difference at the 5% level, respectively (Student's t test, n=5).

### Table 2. Plant dry weight (DW) and leaf temperature (LT) at the beginning of the flowering, and pod yield in snap bean cultivars grown in the field.

| Cultivar             | Spring cropping | Summer cropping |
|----------------------|-----------------|-----------------|
|                      | Harvest period May 10-June 6 | Harvest period July 10-Aug. 6 |
|                      | Yield (g plant⁻¹) | DW (g plant⁻¹) | LT (°C) | Yield (g plant⁻¹) | DW (g plant⁻¹) | LT (°C) |
| Haibushi             | 403 | 17.4 | 30.4 | 147 | 16.7 | 33.6 |
| Kurodane-Kinugasa    | 447 | 16.7 | 30.3 | 21.3 | 15.5 | 33.7 |
| Oregon               | 467 | 18.4 | 31   | 0   | 10.3 | 34.9 |
| Kentucky Wonder      | 383 | 16.5 | 31.3 | 0   | 9.6  | 35   |
| LSD (p=0.05)         | 56.7 | n.s. * | 0.68 | 32  | 2.8  | 0.41 |
| Mean air temperature(°C) | 24.8 | 26.2 | 29.1 | 29.7 |

* Seeding date was March 22 and May 28 in the spring cropping and summer cropping, respectively.
* Measured at 14:00
* n.s. no significant difference at the 5% level.
n=5.
stomatal conductance in Kentucky Wonder declined more rapidly and was lower than that in Haibushi and Kurodane-Kinugasa at 13:00 (Fig. 5a). The differences in stomatal conductance among cultivars were obvious in the summer cropping (Fig. 5b). In the summer cropping stomatal conductance in Oregon and Kentucky Wonder was lower than that in Haibushi and Kurodane-Kinugasa in the afternoon, and photosynthetic rate of Oregon and Kentucky Wonder was significantly lower than that of Haibushi and Kurodane-Kinugasa from 11:00 to 15:00. The peak in transpiration rate was observed at 13:00 in both the spring cropping and summer cropping. As well as stomatal conductance and photosynthetic rate, transpiration rate in Oregon and Kentucky Wonder was significantly lower than that in Haibushi and Kurodane-Kinugasa in the afternoon. In the summer cropping, leaf temperature of Oregon and Kentucky Wonder at 14:00 (on July 2) was significantly higher than that in Haibushi and Kurodane-Kinugasa (Table 2).

In the summer cropping, the relative water content (RWC) of leaves considerably decreased at noon as was observed in the pot experiment (Fig. 6). Among cultivars, no significant difference was observed in RWC at noon. Oregon and Kentucky Wonder showed a significantly higher RWC than Haibushi and Kurodane-Kinugasa at 6:00 and 18:00.

**Discussion**

In the summer cropping of the field experiment, mean air temperature during growth period was higher than 29°C, which exceeded optimal temperature for snap bean (Suzuki et al., 2001a) and pod yield was
lowered (Table 2). In the summer cropping, Haibushi, the heat-tolerant cultivar (Nakano et al., 1997; Suzuki et al., 2001a) produced 36% pod yield of the spring cropping while no pod was harvested in Kentucky Wonder and Oregon. Dry matter production was drastically depressed in Kentucky Wonder and Oregon, but only slightly in Haibushi and Kurodane-Kinugasa in the summer cropping. Pod yield of snap bean is determined by the number of pods, which is a product of the number of flowers and pod-set-ratio. Since pod-set-ratio of snap bean is strongly affected by pollen fertility under high temperature condition (Suzuki et al., 2001a, b), pod yield deterioration in the summer cropping might be due to the decrease of pollen fertility. The relationship between pollen fertility and dry-matter production has not been clarified. However, the larger decrease of dry matter production in the cultivars that produced no pods implies that dry matter production is associated with pod yield under high temperature conditions.

A high temperature often coincides with intensive solar radiation, which causes excessive transpiration and temporal water deficit in plants in the daytime, even when soil moisture content is adequate. Under high temperature conditions, water deficit occurred in snap bean (Tsukaguchi et al., 2003) and tomato plants (Bar-Tsur et al., 1985). In soybean grown in the soil with adequate water content, cultivars which maintained higher water status in the afternoon had a higher grain yield than other cultivars (Boyer et al., 1980). These previous studies indicate that heat-tolerant snap bean cultivars maintain a preferable water status in the daytime under high temperature conditions. In this study, no significant difference in RWC was observed among the cultivars at 12:00 (Fig. 4, 6). However, RWC declined sharply from 90% to 70% in Kentucky Wonder and Oregon, and it changed slightly from 82% to 70% in Haibushi and Kurodane-Kinugasa. In this study, RWC stayed more stable in the daytime in cultivars which showed higher dry matter production under high temperature conditions.

Water status in plants is the result of the balance between water uptake and water loss through transpiration. In the glasshouse experiment water balance was compared between Haibushi and Kentucky Wonder, contrasting cultivars in heat tolerance. Sap flow rate was monitored at the base of plant stem. The sap flow rate was lower than transpiration rate showing water loss in the plant shoot (Fig. 2). For the comparison of water balance, the cumulative water loss (CWL) in the plant was calculated. In Haibushi, CWL increased more gradually and was lower at midday than that in Kentucky Wonder. While Haibushi showed a 20% higher transpiration in the daytime than Kentucky wonder, the delay in the response of sap flow to transpiration was smaller in Haibushi, which resulted in lower CWL in Haibushi at mid-day than in Kentucky Wonder. This lower CWL in Haibushi apparently caused smaller change of RWC in leaves than in Kentucky Wonder.

Water-uptake ability is determined by the product of root surface area and root activity. Physiological factors relating to root activity were not compared in this study, but an interesting factor relating root surface area was observed. While the S/R ratio in Haibushi was the same or smaller than that in Kentucky Wonder, specific root length was larger in Haibushi (Table 1), which suggests that the ratio of fine roots to the total root mass was higher in Haibushi than in Kentucky Wonder. However, these data were obtained only from pot experiment and should be confirmed in the field condition. Fine roots have a larger root surface area per unit root mass, and thus are profitable for water uptake since axial xylem resistance in the root is much lower than resistance to radial flow (Sands et al., 1982; French and Steudle, 1989; French and Hsiao, 1993). Moreover, the anatomical structure of fine roots is profitable for higher water conductivity in the root system (Huang and Eisenstat, 2000). Thus, a larger specific root length in Haibushi is suggested to be profitable for water uptake.

Haibushi maintained a higher stomatal conductance than Kentucky Wonder in the glass house experiment (Fig. 3). In the field experiment, Haibushi and Kurodane-Kinugasa showed higher stomatal conductance (Fig. 5). The cultivars that showed larger dry matter production under a high temperature condition maintained a higher stomatal conductance at mid-day. This result is in agreement with previous studies, which showed that cultivars adapted to a hot climate tend to have higher stomatal conductance in Phaseolus vulgaris (Mencuccini and Comstock, 1999) and cotton (Lu and Zeiger, 1994; Lu et al., 1994, 1997). However, no difference was observed in RWC between Haibushi and Kentucky Wonder at mid-day and RWC in Haibushi was even lower than that in Kentucky Wonder in the morning and evening. In soybean, decline of stomatal conductance was observed in response to decrease in leaf RWC (Ito and Kumura, 1986). In P. vulgaris, stomatal opening occurs in response to the change in water status in leaves (Mencuccini et al., 2000). Although our results seem inconsistent with these previous studies, Pimentel et al. (1999) observed large genotype difference in stomatal aperture in the response to a decline in leaf water potential. Haibushi and Kurodane-Kinugasa may have this advantageous trait to maintain a higher stomatal conductance at a low leaf water status. Higher stability of RWC in the daytime in these cultivars implies that leaf water status was within the optimal range.

In the summer cropping of the field experiment, clear cultivar difference was observed in photosynthesis rate (Fig. 5). From 11:00 to 15:00, Haibushi and Kurodane-Kinugasa showed significantly higher
photosynthetic rate than Kentucky Wonder and Oregon. Measurement was conducted several times throughout the growth period and a similar trend was observed (data not shown). This result was consistent with that of glasshouse experiment, in which Haibushi showed higher photosynthetic rate in the afternoon (Fig. 3). The higher photosynthetic rate in Haibushi than in Kentucky Wonder was not due to WUE, since no significant difference was observed in WUE. These data suggest that Haibushi produced a larger amount of dry matter than Kentucky Wonder and Oregon due to higher photosynthetic rate in the daytime, which is attributed to higher stomatal conductance.

Higher transpiration rate is advantageous to plants under high temperature condition due to a cooling effect of transpiration, when soil water supply is adequate. In Gossypium barbadense L., heat-tolerant lines had a lower leaf temperature (Lu et al., 1994). Haibushi had a lower leaf temperature than Kentucky Wonder at 14:00 (Table 2). It is unknown whether this leaf cooling positively works for heat avoidance or just a consequence of higher transpiration rate under high temperature condition. However, Suzuki et al. (2001a) reported that a 2°C rise in mean air temperature over 27°C caused a yield decline suggesting that a slight cooling effect plays an important role in determining yield. Moreover, leaf temperature may be a useful criterion for selection of heat-tolerant snap bean plants.

From these findings, we conclude that better growth in the heat-tolerant snap bean cultivar Haibushi than in the heat-sensitive cultivar under high temperature conditions was due to higher photosynthetic rate in the daytime. The higher stomatal conductance in Haibushi was then attributed to higher water uptake rate. The cooler leaf of heat-tolerant cultivars compared with heat-sensitive cultivars suggest that leaf temperature could be a useful criterion for the selection of heat-tolerant snap bean cultivars.

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