Higher Stability in Leaf Water Status in Heat-Tolerant Cultivars of Snap Bean (*Phaseolus vulgaris* L.)

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Summary

Snap bean (*Phaseolus vulgaris* L.) is a heat-sensitive crop. A snap bean cultivar Haibushi, which was developed from an accession collected in Southeast Asia, shows higher heat tolerance than other cultivars or lines. For an effective breeding program, elucidation of the mechanism controlling the heat tolerance in Haibushi is necessary. 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. In snap bean cultivars, leaf water status declined at mid-day and there was a cultivar difference in mid-day depression of leaf water status. In heat-tolerant cultivars including Haibushi, mid-day depression in leaf water status was smaller and stomatal conductance was higher than those in heat-sensitive cultivars. Higher stability in leaf water status of Haibushi was due to higher water uptake rate in the morning. Under high temperature conditions leaf temperature of heat tolerant cultivars was lower than that of heat-sensitive cultivars, well reflecting stomatal conductance and growth. However technical hurdles should be cleared to use cool leaf as a selection criterion. We conclude that daily stability in leaf water status could be a reliable characteristic to estimate heat tolerance in *Phaseolus vulgaris*.

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Key words: gas exchange, heat tolerance, *Phaseolus vulgaris* L., water status, water uptake

Snap bean (*Phaseolus vulgaris* L.) is a heat sensitive crop and pod yield is reduced at high temperatures (Nakano *et al*., 1997). Breeding of heat-tolerant snap bean cultivars has been carried out since 1985 in JIRCAS Okinawa station. Among 349 lines and cultivars provided by CIAT and USDA and 37 lines collected in Malaysia and Thailand, heat tolerance was evaluated. One of the lines which showed excellent productivity under high temperature conditions was registered as a new snap bean variety and designated as Haibushi by MAFF in 1995.

Suzuki et al. (2001) evaluated pod yield under field conditions using four cultivars including Haibushi and Kentucky Wonder, which is a relatively heat-tolerant cultivar among commercial varieties cultivated in Japan. While pod yield decreased in all the cultivars when mean air temperature during harvest period exceeded 26 ℃, degree of yield reduction was different among cultivars. Pod yield was the highest in Haibushi and the lowest in Kentucky Wonder. When mean air temperature during the harvest period exceeded 29 ℃, still considerable amount of pods were harvested in Haibushi whereas no pod was harvested in the other cultivars.

Although Haibushi shows higher heat tolerance than other cultivars and lines, market value of Haibushi is not high due to the shape of pods (Egawa *et al*., 2002). Therefore 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 are not clarified.

Leaf water status and gas exchange

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 tomato plants (Bar-Tsur *et al*., 1985). In *Glycine max* Merr. 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.

Daily change in relative water content (RWC) in leaves and gas exchange rate was compared among four cultivars with various heat tolerances under field conditions (Tsukaguchi *et al*., 2003). While stomatal conductance decreased after the peak at 9:00 in all the cultivars, significant difference was observed from 11:00 to 15:00 among cultivars

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(**Fig. 1**). Stomatal conductance was higher in heat-tolerant cultivars (Haibushi and Kurodane-Kinugasa) than in heat-sensitive cultivars (Kentucky Wonder and Oregon). Higher stomatal conductance in the heat-tolerant cultivars at mid-day caused higher photosynthetic rate, which apparently resulted in higher dry matter production under high temperature conditions. Positive correlation between stomatal conductance and RWC was reported in Glycine max (Ito and Kumura, 1986) and *Phaseolus vulgaris* (Mencuccini *et al.*, 2000). However, no relation was observed between stomatal conductance and RWC in this study, which seems inconsistent with the previous studies. There was no difference in RWC between the heat-tolerant and the heat-sensitive cultivars at 12:00 and RWC in the heat-tolerant cultivars was significantly lower than in heat-sensitive cultivars in the morning and evening (**Fig. 2**).

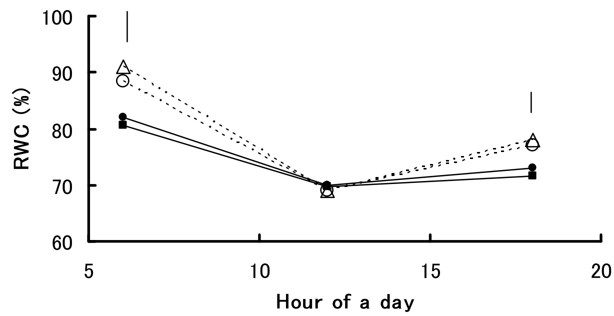

Fig. 2. Diurnal changes in relative water content (RWC) in fully expanded leaves in Haibushi, Kurodane-Kinugasa, Oregon and Kentucky Wonder grown in the field. Symbols are the same as Fig. 1. Vertical bars above symbols represent lsd values at the 5% level.

Stability in leaf water status

Nevertheless, distinct difference in daily RWC change was observed between heat-tolerant and heat-sensitive cultivars. While RWC declined sharply from 90 % to 70 % in the heat-sensitive cultivars, it changed slightly from 80 % to 70% in the heat-tolerant cultivars. In the heat-tolerant cultivars, mid-day depression in leaf water status was small. A negative correlation was observed between mid-day depression in RWC and stomatal conductance in these four cultivars grown under favorable and high temperature conditions (**Fig. 3**). These data imply that daily stability in leaf water status determines productivity under high temperature conditions in *Phaseolus vulgaris*.

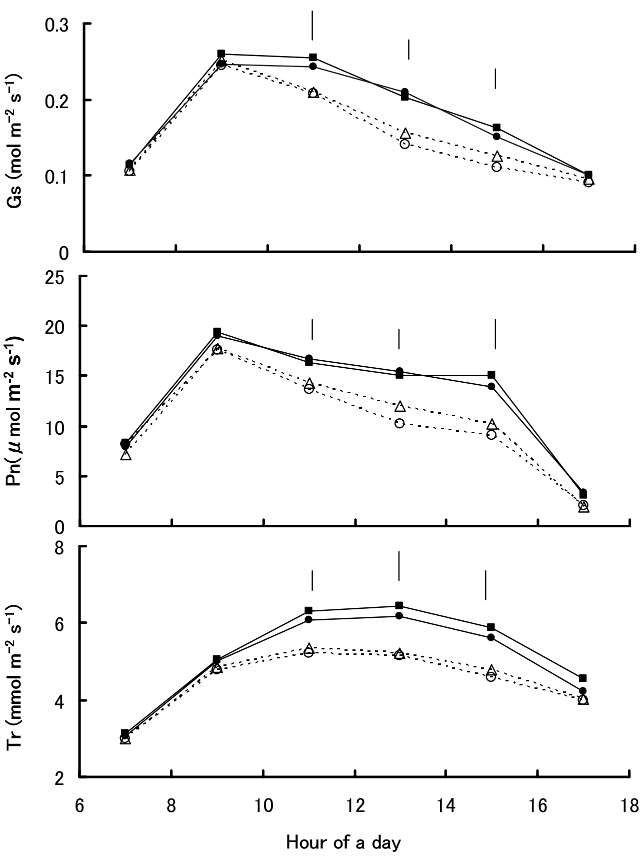

Fig. 1. Diurnal changes in stomatal conductance (Gs), photosynthetic rate (Pn) and transpiration rate (Tr) in Haibushi (solid squares), Kurodane-Kinugasa (solid circles), Oregon (open triangles) and Kentucky Wonder (open circles) grown in the field. Vertical bars above symbols represent lsd values at the 5% level.

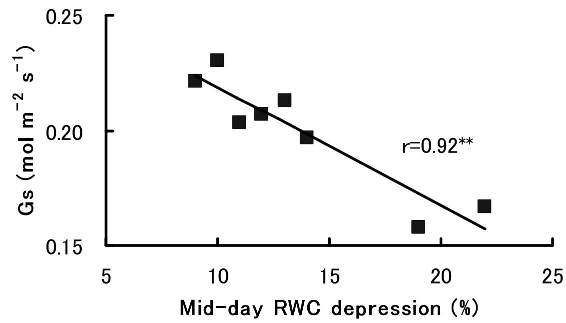

Fig. 3. Relation between mid-day depression in RWC and stomatal conductance (Gs). Mid-day depression in RWC refers to the difference in RWC at 12:00 and at 6:00. Mean values of stomatal conductance from 11:00 to 15:00 are used. ** indicates significance level at 1%.

Using more cultivars and lines with various heat tolerance, Omae et al. (2005a) further investigated the relation between the mid-day depression in leaf water status and heat tolerance. There was a negative correlation between mid-day depression in RWC and seed yield. Stomatal conductance was also associated with mid-day depression in leaf water status. These results suggest that daily stability in leaf water status could be a reliable characteristic to estimate heat tolerance in *Phaseolus vulgaris*.

Then how the daily stability in leaf water status is maintained in heat tolerant cultivars? Omae et al. (2005b) observed that response of plants to desiccation was different between heat-tolerant and heat-sensitive cultivars. In a group of heat-tolerant cultivars including Haibushi, leaf water status was well maintained while vine elongation was severely depressed when xylem pressure potential decreased. In the heat-sensitive group, on the other hand, leaf water status severely decreased while vine elongation was merely affected. Similar phenomenon was observed in *Gossypium barbadense* L. (Lu *et al*., 1994). Heat-tolerant lines tended to have smaller leaf area and higher stomatal conductance under high temperature conditions. Though the mechanism has not been clarified, plant response to desiccation would also be a useful trait for estimation of heat tolerance.

Water uptake

The other factor keeping daily stability in leaf water status is water uptake rate. Water status in plants is a balance between water uptake and water loss through transpiration. Water balance was compared between Haibushi and Kentucky Wonder, contrasting cultivars in heat tolerance (Tsukaguchi *et al*., 2005). Rate of sap flow and transpiration was monitored throughout the day according to the heat balance method by Sakuratani (1981, 1984). Sap flow rate was measured at the base of plant stem and was considered to be water uptake rate to the plant shoot. The sap flow rate was lower than transpiration rate in the morning showing water loss in the plant shoot **(Fig. 4)**. For the comparison of water balance, the cumulative water loss (CWL) in the plant shoot was calculated as CWL=-Σ (Tr-SF), where Tr is transpiration rate per unit leaf area (mg cm^{-2} hr^{-1}) and SF is sap flow rate (mg cm^{-2} hr^{-1}). In Haibushi, CWL increased more gradually and was lower at mid-day than that in Kentucky Wonder. While Haibushi showed a 20 % higher transpiration rate 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 resulted in smaller change of RWC in leaves than in Kentucky Wonder.

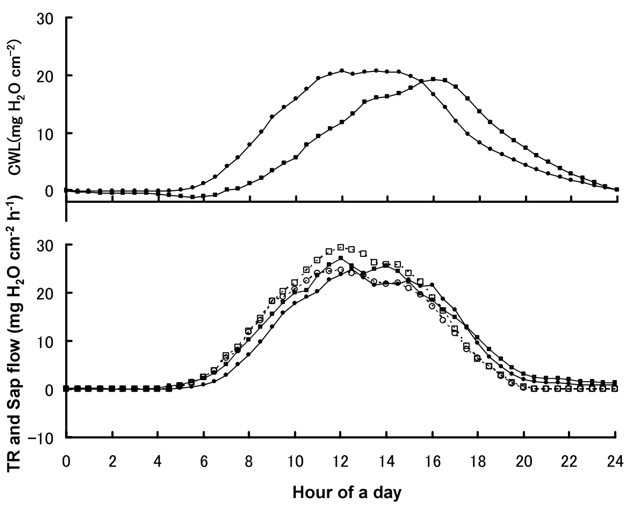

Fig. 4. Diurnal changes in transpiration rate (open symbols with dotted lines), sap flow rate (solid symbols with solid lines) and cumulative water loss (CWL) in Haibushi (squares) and Kentucky Wonder (circles).

Water-uptake is determined by the product of root surface area and root activity. While the shoot root ratio in Haibushi was the same or smaller than that in Kentucky Wonder, specific root length was larger in Haibushi, which suggests that the ratio of fine roots to the total root mass was higher in Haibushi than in Kentucky Wonder (Tsukaguchi *et al*., 2005). 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 (Frensch and Hsiao, 1993; Frensch and Steudle, 1989; Sands *et al*., 1982). Moreover, the anatomical structure of fine roots is profitable for higher water conductivity in the root system (Huang and Eissenstat, 2000). Thus, a larger specific root length in Haibushi would be profitable for water uptake.

Leaf temperature

Higher stomatal conductance is advantageous to plants under high temperature conditions 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). It is unknown whether this leaf cooling positively works for heat avoidance or just a consequence of higher transpiration rate under high temperature condition. Nevertheless, Suzuki *et al*. (2001) reported that a 2℃ rise in mean air temperature over 27℃ 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. Using an infrared radiation thermometer, detection of plants with a low leaf temperature is easy. Heat-tolerant cultivars had a lower leaf temperature than heat-sensitive cultivars **(Fig. 5)** and positive correlation was observed between leaf temperature and growth under high temperature condition **(Fig. 6)** (Tsukaguchi *et al*., 2001). However there turned out to be a difficulty in selecting heat-tolerant plants based on leaf temperature. In the F_3 population between Haibushi and Kentucky Wonder, wide range of leaf movement was observed (Tsukaguchi *et al*., unpublished). Since leaf temperature is determined by radiation load and transpiration rate, leaf temperature does not reflect stomatal conductance if the leaf movement of each plant is different. Therefore selection of heat-tolerant plants by leaf temperature is theoretically possible and promising considering its convenience, there are still hurdles to be cleared.

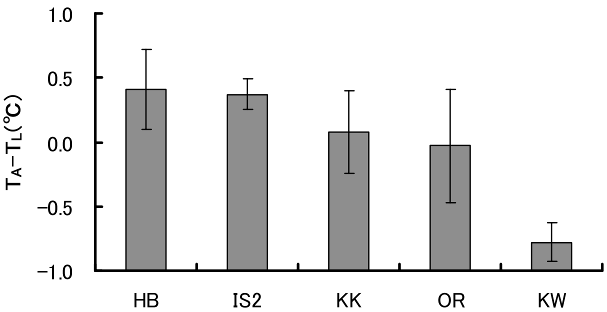

Fig. 5. Air-leaf temperature difference in snap bean cultivars and lines at 14:00. HB, IS2, KK, OR and KW represent Haibushi, Ishigaki 2, Kurodane-Kinugasa, Oregon and Kentucky Wonder, respectively. TA and TL refer to air and leaf temperature. Vertical bars indicate standard error.

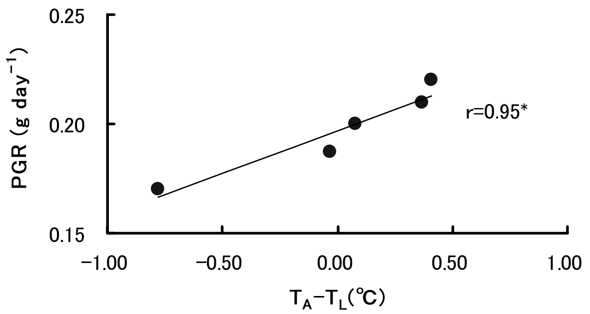

Fig. 6. Relation between air-leaf temperature difference and plant growth rate. * indicates significance level at 5%.

Conclusion

Under high temperature conditions, productivity of a snap bean was restricted by stomatal conductance and thus photosynthetic rate. Stomatal conductance was not determined by leaf water status but by mid-day depression in leaf water status. Therefore keeping daily stability in leaf water status is indispensable for higher productivity under high temperature conditions in snap bean. Furthermore, mid-day depression in leaf water status is a reliable characteristic to estimate heat tolerance of snap bean plants. Higher stability in leaf water status was partly due to higher water uptake rate in Haibushi, a heat tolerant snap bean cultivar.

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インゲンマメ耐暑性品種における日中の高い葉内水分維持特性

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要　約

インゲンマメは高温に弱い作物である。東南アジアで採集された系統から育成されたインゲンマメ品種ハイブシは他の品種あるいは系統よりも高い耐暑性を示す。効率的な育種のためにハイブシの耐暑性の支配機構を明らかにする必要がある。高温はしばしば強日射を伴うが、高温と強日射が相まって植物体の過剰な蒸散を招き、たとえ土壌の水分状態が良好であっても植物体は日中しばしば一時的な水分欠乏状態となる。インゲンマメ品種においても日中の葉の水分状態は低下し、その低下の度合いには品種間差異が認められた。ハイブシを含む耐暑性品種においては感受性品種よりも葉内水分状態の日中の低下は小さく、気孔コンダクタンスも高く保たれた。ハイブシにおいて日中の葉の水状態が安定に保たれたのは午前中の水吸収速度が高かったためと考えられた。高温条件下で成長速度の大きい耐暑性品種では高い気孔コンダクタンスを反映し葉温が低かったが、葉温を耐暑性の評価に用いるには技術的な問題がある。これらのことから、日中の葉の水分維持はインゲンマメの耐暑性を評価する指標となりうると考えられた。

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キーワード：ガス交換、水分吸収、水分状態、耐暑性、*Phaseolus vulgaris* L.

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