Assessing Drought Tolerance of Snap Bean (*Phaseolus vulgaris*) from Genotypic Differences in Leaf Water Relations, Shoot Growth and Photosynthetic Parameters

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Abstract: The leaf water relations, photosynthetic parameters and shoot growth of five snap bean cultivars were assessed during the drought period to determine their role in alleviating plant water deficit imposed by withholding irrigation at flowering. Soil water content of irrigated plants was 18-20% while that of unirrigated plants was 6-10% at 60 days after seeding (DAS). Leaf water potential was approximately 0.15MPa lower and relative water content was approximately 5% lower in unirrigated plants than in irrigated plants at 57 DAS. Unirrigated plants had a lower stomatal conductance (gₛ) and intercellular CO₂ concentration (Cᵢ). Reduced leaf water potential and relative water content were associated with a decreased stem elongation rate. Plants with a lower stem elongation rate had a higher specific leaf weight and succulence index (SucI). Significant differences among five cultivars of snap bean were found for all parameters measured. Decreased leaf water potential and stem elongation rate resulting from drought participated in preserving relative water content and improving specific leaf weight and SucI. Maintenance of higher relative water content increased gₛ and Cᵢ. Cultivars that maintained a high relative water content when leaf water potential and stem elongation rate were decreased markedly, were more tolerant to drought than those which a reduced relative water content and the leaf water potential and stem elongation rate were only slightly lowered. Reduced yield (pods per plant and seed biomass) resulting from drought was associated with reduced relative water content.

Key words: Drought tolerance, Leaf water status, Photosynthetic parameters, Shoot growth, Snap bean.

The productivity of kidney bean and snap bean (*Phaseolus vulgaris* L.) is drastically reduced in the summer season on the subtropical islands of Japan. One of the main reasons for the decreased productivity is the decrease in tissue water content due to excessive water loss through rapid transpiration caused by high temperature and water deficit (Omae et al., 2004a, 2005; Kumar et al., 2005). Under water stress conditions, snap bean continues vegetative growth, but only reduced at photosynthetic rates (Suzuki et al., 1987). In many crop species, including snap bean, even small diurnal fluctuations in leaf water status at anthesis can adversely affect the activity of reproduction structures (Saini and Aspinall, 1982; Kuo et al., 1988; Weaver and Timm, 1988; Tsukaguchi et al., 2003). Therefore, lack of photosynthesize supply to reproductive organ may not be the only cause of premature abortion and abscission. However, this issue has not been investigated well in snap bean.

Some plants can maintain photosynthetic rates at low leaf water status by changing in leaf anatomical characteristics and CO₂ conductance (Evans et al., 1994). Recently, Omae et al. (2005) reported that genotypic differences in leaf water status of snap bean correlated with crop productivity under drought conditions. This suggests that differences in leaf water status exist among snap bean cultivars, which may be linked to the drought tolerance mechanisms. However, Omae et al. (2005) did not assess how drought influenced photosynthetic parameters or vegetative growth. In a glasshouse study, water uptake in snap bean under different temperatures conditions related to shoot extension rate (Omae et al., 2004b). Understanding the influence of drought on leaf water relations in relation to photosynthetic parameters and growth under field conditions is crucial for identifying causes of yield reduction and underlying mechanism of drought tolerance in snap bean. The objectives of this study were (1) to evaluate genotypic differences in leaf water relations, photosynthesis, and shoot growth of snap bean under water stress, and (2) to determine which, if any, of the measured parameters could be useful for screening snap bean germplasm for drought tolerance.

Materials and Methods

This study was conducted at the Okinawa Subtropical Station, Japan International Research Center for Agricultural Sciences (JIRCAS), Ishigaki Island, Japan. Snap bean cultivars Kentucky Wonder, Haibushi...
and Kurodane Kinugasa, and strains Ishigaki-2 and 92783 (hereafter referred to as "cultivars") were planted on 29 Nov. 2004 in field beds in a net house (5 m × 20 m, covered with white cheesecloth) under natural conditions. The net house was covered with a polyethylene sheet on the top. There were four rows composed of five plots in each row in the net house. The plot size was 2.5 m² (2.5 m in row length and 1.0 m in width) for all genotypes. The soil was red-yellow podzolic highly acidic soil (pH 4.6) with a fine to medium texture. For unirrigated plots, a fibrous polyethylene sheet (1-mm thick) was buried before seeding in 60 cm soil depth to restrict roots not to grow in deeper soil layers but to allow drainage of excess water. Ten plants of each genotype spaced 25 cm apart were grown in a row in each plot. Two irrigation treatments, irrigated and unirrigated were imposed. Irrigated plants were drip-irrigated regularly while unirrigated plants received no irrigation after the flowering stage (42 days after seeding, hereafter referred to as DAS). Volumetric soil water content was measured with a portable soil water content measurement system (Hydrosense, Campbell Scientific, Inc., N. Logan, UT, USA) connected with a 20 cm probe rod.

1. **Leaf water relations**

Leaf water relations were measured at 57 DAS. After measurement of photosynthetic parameters, the same leaves were used to evaluate leaf water relations between 1100-1200 h. Leaf water potential, relative water content and osmotic potential were measured in the same trifoliate leaf, middle lamina for leaf water potential and side lamina for relative water content and osmotic potential. Leaf water potential was measured by the pressure chamber method (Scholander et al., 1965). For determination of osmotic potential, lamina tissues (without midrib vein) were placed in a 5 ml syringe barrel. The syringe was transferred to −20°C until measurement. Osmotic potential was determined after thawing the frozen samples on ice for 1 h. The sap was expressed, and osmotic potential was measured on 10 µL aliquots placed in an osmometer (Model 5520, Wescor Inc., USA). Relative water content (RWC) was estimated according to the equation

\[
RWC = \frac{(M_f - M_d)}{(M_f - M_s)} \times 100,
\]

where, \(M_f\), \(M_d\) and \(M_s\) are the fresh, oven-dried and water-saturated mass of the leaf discs, respectively. A sharp cork borer was used to collect eight leaf discs 12 mm in diameter, avoiding the mid-rib and major veins. \(M_s\) was determined after the leaf discs were floated on distilled water for more than four hours in darkness. Leaf disc samples were dried in an oven at 65°C for eight hours to record \(M_o\).

2. **Leaf anatomical characteristics**

The data recorded to estimate relative water content were also used to calculate specific leaf weight (SLW) and succulence index (SucI) according to the following equations

\[
SLW = \frac{M_f}{L_\sigma} \quad \text{and} \quad \text{SucI} = \frac{(M_f - M_d)}{L_\sigma},
\]

where, \(L_\sigma\) is total leaf area sampled.

3. **Shoot growth**

The stem elongation rate was recorded between 45 and 60 DAS. Measurements were made about the same time on every occasion. Three main shoots per cultivar per plot were marked with a tag at 4 or 5th internode from the top of the shoot. Measurements of length using a meter rule were made from the base of the tagged internode to the top of the tagged shoot at an interval of three to four days. The length of each internode in the shoot was recorded separately at each time of measurement. Stem elongation rate (SER) was calculated according to the following equation:

\[
SER = \frac{(L_2 - L_1)}{t},
\]

where, \(L_1\) is the sum of the lengths of internodes at the beginning and \(L_2\) is the sum of the length of internodes at the end of a time interval, t.

4. **Photosynthetic parameters**

Photosynthetic parameters were measured at 57 DAS on the youngest fully expanded leaf (4 or 5th

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**Fig. 1.** Changes in soil water content (SWC) in irrigated (solid lines) and unirrigated (dashed lines) treatments from 42–60 DAS. Data represent mean ± S.E.; n=2. Vertical bars indicate standard error.
Table 1. Effect of water stress on leaf water potential (LWP), osmotic potential (OP), relative water content (RWC), specific leaf weight (SLW) and succulence index (SucI). Data were collected 57 days after seeding (DAS) between 1100-1200 h.

| Genotype              | LWP (MPa) | OP (MPa) | RWC (%) | SLW (mg M₂ cm⁻² Lₛ) | SucI (mg H₂O cm⁻² Lₛ) |
|-----------------------|-----------|----------|---------|---------------------|-----------------------|
| **Irrigated**         |           |          |         |                     |                       |
| Haibushii             | -0.767 bc | -0.965 cde | 85.8 ab | 4.03 a              | 16.6 a                |
| Kentucky Wonder       | -0.753 abc| -0.938 cde| 87.2 a  | 1.79 d              | 12.8 bc               |
| 92783                 | -0.753 abc| -0.901 ab | 85.1 ab | 1.53 d              | 13.8 bc               |
| Kurodane Kinugasa     | -0.667 ab | -0.910 abc| 87.0 ab | 1.73 d              | 13.8 bc               |
| Ishigaki-2            | -0.650 a  | -0.865 a  | 88.3 a  | 1.89 cd             | 17.3 a                |
| **Unirrigated**       |           |          |         |                     |                       |
| Haibushii             | -0.917 d  | -1.003 e  | 84.3 ab | 3.08 b              | 17.6 a                |
| Kentucky Wonder       | -0.817 cd | -0.988 de | 83.3 ab | 1.53 d              | 12.4 c                |
| 92783                 | -0.833 cd | -0.943 bcde | 80.3 b  | 1.84 cd             | 14.3 b                |
| Kurodane Kinugasa     | -0.800 c  | -0.952 bcde | 83.9 ab | 1.80 d              | 14.2 b                |
| Ishigaki-2            | -0.767 bc | -0.922 abc| 86.9 ab | 2.34 c              | 17.9 a                |

Means followed by different letter (s) in a column are significant at P < 0.05 (Student t-test, n=3). Mₛ, leaf dry mass; Lₛ, leaf area.

from the top) of the plants in each treatment between 1000-1100 h. A portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebraska, USA) was used to measure the photosynthetic parameters such as net photosynthetic rate (Pₑ), stomatal conductance (gₛ), transpiration rate (E), intercellular CO₂ concentration (Cᵢ) and vapor pressure deficit (VpdL). Photon flux density (PAR, 400-700 nm wave length) was recorded with a gallium arsenide phosphide PAR sensor mounted on the leaf chamber. Transpiration rate was calculated by the following equation:

\[ E = \frac{F(W_s - W_c)}{100S(1000 - W_s)} \]

where, E, F, Wₛ, Wₛ, and S are transpiration rate (mol m⁻²s⁻¹), air flow rate (µmol s⁻¹), sample and reference water mole fractions (nmol H₂O (mol air)⁻¹), and leaf area (cm²), respectively. Ambient air temperature and relative humidity were recorded automatically with a temperature humidity logger (Model SK-L200TH, Sato Keiryoki, Mfg. Co. Ltd., Japan).

5. **Number of pods and seed yield**

All mature pods in each plot were harvested at two weeks to determine the number of pods per plot. Pods in each plot were sun dried for a week and threshed for seed yield. All values of the number of pods and seed yield per plot were converted to per plant by dividing with the number of plants per plot. The number of seeds per pod and seed weight were measured in 20 pods and 20 seeds, respectively in each plot.

6. **Experimental design and statistical analysis**

The experiment was conducted according to two factorial designs, irrigation and cultivar. Two different levels of irrigation, irrigated and unirrigated treatment, were designed with two replications for measurement of soil water content, yield and yield attributes, and without replication for leaf water status, stem elongation rate and photosynthetic parameters. Five cultivars were planted in each irrigated and unirrigated plot. For leaf water status, stem elongation rate and photosynthetic parameters, data were taken from three plants in each plot. The mean values in each plot were statistically compared by Student’s t-test (n=3) using JMP software (Ver.5.0, SAS Instute, Japan) program. The ambient air temperature and relative humidity were recorded every one hr and averaged. The data for soil water content (0-20cm in depth) were taken from 4 spots in each plot, averaged and regarded as one replication. Two replicated data were used for the analysis. A discriminant analysis based on Mahalanobis distance (Duda et al., 2001) was performed to recognize the differential pattern and to classify the five cultivars into closer categories.

**Results**

The average daily ambient air temperature and relative humidity (average ± SD, n=19) were 18.1 ± 2.5°C and 71.2 ± 12.9%, respectively during the treatment period (42-60 DAS). Air temperature during 1000-1200 h was 26.4-28.6°C and relative humidity 47.2-50.6% at 57 DAS. Photon flux density varied from 700-920 µmol PAR m⁻²s⁻¹. Drought or unirrigated treatment resulted in lower soil water content than in irrigated or control treatment (Fig. 1). The differences between the two irrigation levels were significant (P < 0.01, Student’s t-test) except at the beginning, i.e. at 42 DAS. Differences among cultivars were not significant either in the drought treatments or on the days of measurement. On an average, soil...
water content decreased by about 50% relative to irrigated treatment in unirrigated treatment after the flowering at the end of experiment (60 DAS).

1. **Effect on leaf water**

The effect of drought on leaf water status varied with cultivars and parameters measured (Table 1). All parameters of water status including leaf water potential, osmotic water potential and relative water content were lower in unirrigated than in irrigated plants in all cultivars. Cultivar Haibushi showed lowest leaf water potential and osmotic potential in both irrigation treatments. All cultivars showed similar relative water content in each irrigation treatment.

2. **Leaf anatomical characteristics**

The cultivar Haibushi had the highest specific leaf weight in both irrigation treatments (Table 1). SucI of plants was not influenced by irrigation treatment. The cultivars Haibushi and Ishigaki-2 had the greatest SucI in both irrigation treatments.

3. **Effect on photosynthetic parameters**

The effect of drought was significant in Cᵢ, gₛ, E and
VpdL in cultivar Kentucky Wonder and 92783 (Table 2). Both cultivars had higher $C_i$, $g_s$, $E$, and lower VpdL in irrigated than in unirrigated treatment. The cultivar Haibushi showed highest $P_n$, $g_s$, and $E$ among all cultivars in both irrigation treatments. In unirrigated treatment, cultivars Haibushi and Ishigaki-2 were significantly higher in $C_i$ and $g_s$ than Kentucky Wonder and 92783, while Kentucky Wonder and 92783 showed higher VpdL than other cultivars in unirrigated treatment.

4. Effect on shoot growth

Stem elongation rate at all growth stages significantly differed with the cultivar except that at 45-49 DAS in irrigated treatment (Table 3). Unirrigated plants of Kentucky Wonder and 92783 had consistently greater stem elongation rate than Haibushi. In irrigated plants, all cultivars had similar stem elongation rate at 45-49 DAS. Kentucky Wonder and 92783 had greater stem elongation rate than Haibushi at 49-53 DAS and 57-60 DAS. Kentucky Wonder, 92783 and Kurodane Kinugasa had similar stem elongation rate in both irrigation treatments except at 45-49 DAS in unirrigated treatment.

5. Association of leaf water status with photosynthetic parameters and stem elongation rate

Photosynthetic parameters were not significantly correlated with leaf water potential ($R^2=0.01$ and 0.02 in $g_s$ and $C_i$, respectively), but relative water content was positively correlated with $g_s$ and $C_i$ (Fig. 2). Although absolute values of stem elongation rate were not associated with leaf water status, significant
correlation was found between reduction in stem elongation rate with reduction in leaf water potential and relative water content \(100 - \left(\frac{\text{values in unirrigated to irrigated treatments expressed as percentage}}{\text{values in unirrigated to irrigated treatments expressed as percentage}}\right)\), positively with reduction in leaf water potential (Fig. 3a), whereas, negatively with reduction in relative water content (Fig. 3b). Pattern recognition by discriminant analysis classified cultivars Haibushi, Ishigaki-2 and Kurodane Kinugasa in one category and Kentucky Wonder and 92783 in another category.

6. Association of stem elongation rate with leaf anatomical characteristics

Fig. 4 shows the correlation of stem elongation rate with specific leaf weight and Suc1. The stem elongation rate correlated negatively with specific leaf weight (Fig. 4a) and Suc1 (Fig. 4b).

7. Number of pods per plant and seed yield, and their relationship with relative water content

Effect of irrigation was not significant in any cultivars except for the number of pods/plant in 92783 and seed weight in Haibushi (Table 4). The number of pods/plant significantly decreased due to water stress in 92783.

Fig. 5 shows the relationships between reduction in relative water content and reduction in the number of pods and seed yield per plant. The cultivars that exhibited small reduction in relative water content in response to water stress showed a small reduction in pods and seed yield.

**Discussion**

The five snap bean cultivars displayed distinct responses to a prolonged drought. Drought significantly decreased soil water content in the

| Genotype          | Number of pods/plant | Number of seed/pod | Seed weight (mg/seed) | Seed yield (g/plant) |
|-------------------|----------------------|--------------------|-----------------------|----------------------|
| Irrigated         |                      |                    |                       |                      |
| Haibushi          | 26.8 cd              | 7.6 ab             | 268 de                | 25.91 c             |
| Kentucky Wonder   | 25.0 cde             | 8.8 a              | 518 a                 | 44.30 a             |
| 92783             | 27.9 bc              | 8.0 ab             | 376 bc                | 39.06 ab            |
| Kurodane Kinugasa | 40.5 a               | 7.0 b              | 255 e                 | 36.36 abc           |
| Ishigaki-2        | 37.4 a               | 8.0 ab             | 325 cde               | 34.85 abc           |
| Unirrigated       |                      |                    |                       |                      |
| Haibushi          | 24.2 cde             | 7.2 ab             | 297 c                 | 24.81 c             |
| Kentucky Wonder   | 19.2 e               | 8.5 ab             | 459 ab                | 40.75 ab            |
| 92783             | 19.6 de              | 8.3 ab             | 350 cd                | 30.89 bc            |
| Kurodane Kinugasa | 35.4 a               | 7.4 ab             | 285 de                | 31.55 bc            |
| Ishigaki-2        | 35.1 ab              | 7.8 ab             | 265 de                | 33.64 abc           |

Means followed by different letter(s) in a column are significant at P < 0.05 (Student t-test, n=2).
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unirrigated than in the irrigated plots (Fig. 1). Water stress affected leaf water status, photosynthetic parameters and shoot growth in some cultivars. Cultivar Haibushi, Kurodane Kinugasa and Ishigaki-2 showed a larger drop in leaf water potential (Table 1) than the other cultivars. However, there were no differences in relative water content among the cultivars. A steeper leaf water potential gradient from soil to plant may enhance ability of the plants to extract soil water at low soil water content (Coyne et al., 1982). Omae et al. (2005) reported that genotypic differences in pod setting and seed yield in snap bean were associated with the differences in maintenance of midday relative water content. Therefore, the maintenance of relative water content with larger decreases in leaf water potential in some cultivars might relate with their water-absorbing ability and contribute to less reduction in seed yield (Fig. 5).

Reduction in leaf water potential due to water stress was linearly correlated with the reduction in stem elongation rate. A discriminant analysis revealed that the five cultivars displayed two distinct types of responses (Fig. 3a). One group, which included cultivars Haibushi, Ishigaki-2 and Kurodane Kinugasa, showed large reductions (17-20%) in both stem elongation rate and leaf water potential (Fig. 3a), and they less reduced seed yield than other cultivars (Fig. 5). Conversely, Kentucky Wonder and 92783 showed a larger reduction in relative water content compared to cultivars in the other group (Fig. 3b). The reduction in shoot growth due to drought may be related to the water-holding ability under drought such as specific leaf weight and SucI (Fig. 4), which are important leaf anatomical characteristics for restricting leaf water loss through cuticle transpiration under drought conditions. Osmotic adjustment also enables the plants to maintain higher tissue water content, turgor and turgor-related processes during water deficit as reported in many crop species (Morgan et al., 1986; Ritchie et al., 1990; Kumar and Singh, 1998). In this study, there was a small decrease in osmotic potential, $-1.0$ to $-0.87$ MPa with a larger decrease in leaf water potential, $-0.92$ to $-0.65$ MPa (Table 1) indicating limited or no osmotic adjustment. During the reproductive phase, maintenance of higher relative water content with decreasing leaf water potential due to drought plays an important role in terms of higher pod setting, pod retention and seed yield in snap bean (Omae et. al., 2005). It was also demonstrated that Haibushi, a heat-tolerant cultivar maintained higher relative water content with decreasing leaf water potential than Kentucky Wonder, a heat-sensitive cultivar when exposed to different degrees of high temperature (Omae et al., 2005). The results of this study displayed that relative water content was positively correlated with photosynthetic parameters such as $C_i$ and $g_s$ (Fig. 2). Sinclair and Ludlow (1985) also reported that photosynthesis, protein synthesis, $NO_3$ reduction, and leaf senescence are better correlated with changes in tissue water content than with leaf water potential. The reduction in relative water content was strongly and linearly correlated with the reduction in the number of pods per plant and seed yield (Fig. 5a, b), indicating that cultivars with a smaller reduction in relative water content showed a smaller reduction in number of pods and seed yield due to drought stress. Therefore, when we evaluate "the drought tolerance" as less reduction in yield when plants were exposed to drought, cultivars Ishigaki-2 and Haibushi can be recognized to have higher drought tolerance compared to Kentucky Wonder and

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**Fig. 5.** Correlation of reduction in relative water content (RWC) with (a) number of pods per plant and (b) seed yield per plant. Reduction was calculated as the ratio of value of a trait in unirrigated treatment to that in irrigated treatment in percentage. Regressions are (a) $y = 5.415 x - 1.9991, R^2 = 0.92^{**}$ and (b) $y = 5.58827 x - 2.1925, R^2 = 0.76^{*}$. $^{**} P < 0.01, ^{*} P < 0.05$. 

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92783. On the other hand, Kurodane Kinugasa showed unique phenomenon, which was classified as same group with Haibushi and Ishigaki (Fig. 3) according to the relationship between stem elongation rate and water status, but showed intermediate reduction in the number of pods and seed yield among the cultivars (Fig. 5). Further study will be necessary to clarify the physiological mismatch between the classification of the cultivars (Fig. 3) and the results of seed yield (Fig. 5) in Kurodane Kinugasa.

In conclusion, imposition of drought decreased soil water content which adversely affected leaf water status and stem elongation. A smaller reduction in relative water content was displayed by cultivars that showed a larger reduction in stem elongation rate and leaf water potential. Leaf anatomical characteristics, specific leaf weight and SucI were improved by a decrease in stem elongation rate under unirrigated conditions. Relative water content was well correlated with Ci and g:\textsubscript{s}. Most importantly, the cultivars with a smaller reduction in relative water content also displayed a smaller reduction in the number of pods per plant and seed yield.

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