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Influence of Irrigation Level, Growth Stages and Cultivars on Leaf Gas Exchange Characteristics in Snap Bean (*Phaseolus vulgaris*) under Subtropical Environment

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Abstract

This study was conducted to clarify the genotypic differences in diurnal variations in leaf gas exchange characteristics and the effect of irrigation levels, growth stages and cultivars on leaf water status, gas exchange characteristics and seed yields. Associations of leaf water status with photosynthetic rate in five cultivars of snap bean were also determined. Diurnally, leaf gas exchange was highest at 9:30 h, decreased but remained constant between 11:30 to 14:15 h and then decreased up to 16:15 h. Cultivars Haibushi and Ishigaki-2 displayed higher photosynthetic activity throughout the day. The cultivars having higher seed yield showed higher leaf conductance, transpiration rate and leaf vapor pressure deficit at floral bud initiation stage and higher leaf water potential and relative water content in the irrigated plot at flowering stage. The cultivars with slow development of leaf water deficit displayed higher rates of photosynthesis and vice versa. The results showed that midday hours showed large genotypic differences in leaf gas exchange characteristics with constancy of photosynthetically active radiation. Therefore, screening of snap bean cultivars can be made during midday hours for gas exchange characteristics irrespective of growth stages and irrigation levels. Cultivars maintaining higher leaf water content with increasing water deficit can be selected for higher rates of photosynthesis.

Disciplines: Agricultural environment / Crop production
Additional key words: heat tolerance, leaf water content, leaf water potential, photosynthesis, water deficit

Introduction

Tolerance to high temperatures is an important trait for snap bean in the subtropical islands of Japan, where the crop is often subjected to temperatures above 29ºC in the summer season causing significant reduction in yields. Therefore, concerted efforts are needed to evaluate genotypic differences in physiological responses for the identification and development of heat-tolerant cultivars. One approach to searching for physiological markers is to compare genotypes of known heat tolerance. Some progress has been achieved by breeding of Haibushi, a heat-tolerant cultivar[4]. Haibushi maintained higher leaf water content with decreasing water potential than Kentucky Wonder, a heat-sensitive cultivar[3]. Large genotypic differences in leaf water status also exist in snap beans which were related with reproductive responses[5]. Midday drop of leaf water content was associated with the number of pods and seed yield in snap bean[6]. However, these studies did not consider the association of leaf water status with photosynthetic rate, which is one of the most heat-sensitive processes that govern plant growth and yield[1]. Because high temperature stress is usually accompanied by water stress or temporary midday water deficit due to excessive transpiration and it varies with plant growth stages, a consideration of the daytime, irrigation levels and growth stages will be necessary to understand the influences of water status on leaf gas exchange characteristics. The present study was, therefore, conducted to study influence of irrigation level, growth stages and cultivars on leaf gas exchange characteristics and the associ-

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ation of photosynthetic rates with leaf water status and seed yield in snap bean.

Materials and methods

The experiments were conducted during the summer seasons in 2004 and 2005 at Ishigaki Island, Okinawa under natural field conditions. Snap bean cultivars Kentucky Wonder, Haibushi and Kurodane Kinugasa and strains Ishigaki-2 and 92783 were planted in a field on 6 May 2004 and 2 June 2005. The experimental plot was covered with a white cheese cloth layered with polyvinyl sheet on the top during both years. Planting was performed on raised beds in rows 2.5 m in length. Plant to plant distance was 25 cm. The number of replications was four. Plants received water regularly by drip irrigation depending on the requirement. Soil was red-yellow podzolic, highly acidic (pH 4.6) and fine to medium in texture. During 2004, two irrigation treatments, irrigated and unirrigated, were imposed after flowering. The experiment during 2005 was irrigated regularly. Soil water content in the unirrigated treatments decreased to 8.15±0.64 (mean±SD) and 6.70±1.64% at flowering and podding stages, respectively in 2004. Soil water content in irrigated treatments varied between 14 to 17.5% during both years.

Leaf water status and gas exchange characteristics were measured three times in 2004 at floral bud initiation, flowering and podding stages between 10:30 and 11:30 h during floral bud initiation and podding, and between 13:30 and 14:30 h during flowering. Diurnal variation in leaf gas exchange characteristics were measured at floral bud initiation stage in 2005. Three youngest fully expanded leaves (3rd or 4th from the top) were used for recording leaf gas exchange characteristics. A portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebraska, USA), was used to measure the leaf gas exchange characteristics such as photosynthetic rate (P_n), leaf conductance (g_s), transpiration rate (T) and intercellular CO_2 concentration (C_i). Atmospheric CO_2 was supplied to IRGA/chambers through a 6 mm diameter plastic tube fixed at 3 m above ground surface. Photosynthetically active radiation (PAR, 400–700 nm wave length) was recorded with a gallium arsenide phosphide PAR sensor mounted on the leaf chamber of the photosynthesis system. The ambient air temperature was recorded with a temperature humidity sensor (Model SK-L200TH, Sato Keiryoki, Mfg. Co. Ltd., Japan) at 1.5 m height in the experimental plot. The ambient air temperature varied between 35.6 and 42.7ºC during the three occasions in 2004. The sky was cloudless during the measurements at all dates. The same trifoliate leaf was used for measuring leaf gas exchange, leaf water potential (LWP) and leaf relative water content (RWC). After the measurements of leaf gas exchange, the middle lamina was used for LWP and the side lamina for RWC. LWP was measured by the pressure chamber method. RWC was calculated by using the following the equation[2].

$$RWC = \{(M_f - M_d) / (M_s - M_d)\} \times 100$$

Where, M_f, M_d and M_s are the fresh, oven-dry and water-saturated mass of the leaf discs. A sharp cork borer was used to take 8 leaf discs 12 mm in diameter, avoiding the mid-rib and major veins in the leaf. M_s was determined after floating the leaf discs on distilled water for 4 h in the dark. The leaf discs were then dried in an oven at 65ºC for 8 h to record M_d.

On maturity, dry pods per plot were harvested for one month in 2004. Seed yield per plot was recorded to see relationships between water status and gas exchange parameters.

Results

1. Effect of growth stages and irrigation levels on leaf water status and gas exchange characteristics

At floral bud initiation stage, the differences among the cultivars were significant (Table 1). Highest P_n, and T were recorded in Ishigaki-2 while g_s and C_i was highest in Haibushi. VpdL was lowest in Haibushi. T was lowest in Kentucky Wonder. During podding stage, gas exchange parameters varied with irrigation levels in the genotypes. P_n, g_s and T were highest in irrigated plants of 92783 and in unirrigated plants of Haibushi. The least P_n, C_i, g_s, and T were recorded in irrigated plants of Kurodane Kinugasa and unirrigated plants of Kentucky Wonder. During flowering at midday hours (13:30 h), the differences in irrigated and unirrigated treatments were very large. Kurodane Kinugasa showed the highest P_n, g_s and T in irrigated and C_i and VpdL in unirrigated plants. Kentucky Wonder recorded the least P_n, C_i, g_s, and T in irrigated plants.

No significant differences were found in leaf water potential among the cultivars at floral bud initiation stage. Leaf water content showed significant differences, lower in Kurodane Kinugasa than the remaining cultivars. At flowering stage, RWC in irrigated and LWP in unirrigated plants showed no differences among cultivars. In unirrigated plants, RWC was higher in Haibushi, Ishigaki-2 and 92783 than Kurodane Kinugasa and Kentucky Wonder. At podding stage, differences in leaf water status were clearer than floral bud initiation and flowering stages. Within irrigated plants, LWP was highest in Kurodane

Table 1. Leaf water status (leaf water potential, LWP and relative water content, RWC), gas exchange characteristics and leaf vapor pressure deficit (VpdL) at three growth stages in snap bean as affected by irrigation levels and cultivars in a high temperature environment

Genotypes	LWP (MPa)	RWC (%)	Photosynthetic rate (P_n) (μmol CO_2 $m^{-2}s^{-1}$)	Intercellular CO_2 (C_i) (ppm)	Leaf conductance (g_s) (mol $m^{-2}s^{-1}$)	Transpiration rate (T) (mmol H_2O $m^{-2}s^{-1}$)	VpdL (kPa)
Floral bud initiation stage, measured at 10:30 h							
92783	–0.917 a	72.47 a	16.83 ab	288.55 bc	0.859 bc	11.16 b	1.66 a
Haibushi	–0.867 a	72.33 a	15.17 b	300.77 a	1.042 a	11.52 ab	1.45 c
Ishigaki-2	–0.967 a	72.29 a	19.13 a	284.28 c	0.967 ab	11.74 a	1.56 abc
Kurodane Kinugasa	–0.883 a	62.53 b	15.61 b	292.33 bc	0.790 c	10.47 c	1.63 ab
Kentucky Wonder	–0.933 a	69.45 ab	15.83 b	294.11 ab	0.812 c	9.77 d	1.51 bc
Flowering stage, measured at 13:30 h							
Irrigated							
92783	–1.250 b	70.89 a	11.06 bc	300.77 ab	0.687 a	7.23 c	1.30 e
Haibushi	–1.050 a	74.54 a	12.23 b	287.55 abc	0.468 b	7.51 bc	1.88 c
Ishigaki-2	–1.000 a	74.01 a	10.42 bc	310.88 a	0.713 a	8.05 b	1.34 e
Kurodane Kinugasa	–1.133 ab	68.74 a	14.71 a	293.22 ab	0.729 a	9.26 a	1.51 d
Kentucky Wonder	–0.967 a	73.23 a	10.15 c	274.11 bc	0.303 c	4.93 d	1.75 c
Unirrigated							
92783	–1.300 b	66.27 a	1.37 de	203.85 d	0.016 d	0.71 e	4.16 a
Haibushi	–1.367 b	68.12 a	1.04 de	175.80 d	0.011 d	0.43 e	3.86 b
Ishigaki-2	–1.283 b	67.11 a	2.93 d	140.07 e	0.024 d	0.97 e	3.89 b
Kurodane Kinugasa	–1.283 b	60.92 b	0.63 e	264.12 c	0.011 d	0.52 e	4.34 a
Kentucky Wonder	–1.150 b	59.60 b	2.50 de	140.44 e	0.020 d	0.91 e	4.27 a
Podding stage, measured at 10:30 h							
Irrigated							
92783	–1.267 ab	62.91 bcd	16.04 a	282.11 ab	0.807 a	14.44 a	2.20 cd
Haibushi	–1.367 bc	66.96 abc	9.59 cd	293.66 a	0.502 cd	10.61 d	2.40 c
Ishigaki-2	–1.300 b	70.17 a	10.56 bc	290.88 a	0.550 c	13.46 ab	2.78 b
Kurodane Kinugasa	–1.100 a	69.57 a	6.69 e	267.40 c	0.215 fg	7.81 e	4.13 a
Kentucky Wonder	–1.217 ab	68.48 ab	11.97 bc	288.66 a	0.668 b	12.48 bc	2.21 cd
Unirrigated							
92783	–1.483 cd	66.27 abc	8.08 de	290.22 a	0.318 ef	6.83 e	2.38 c
Haibushi	–1.500 cd	68.12 ab	15.52 a	272.22 bc	0.598 bc	11.33 cd	2.27 cd
Ishigaki-2	–2.000 e	67.11 abc	12.22 b	273.22 bc	0.406 de	7.11 e	1.98 d
Kurodane Kinugasa	–1.550 d	60.92 cd	10.97 bc	263.00 c	0.322 ef	7.59 e	2.76 b
Kentucky Wonder	–1.250 ab	59.60 d	5.80 e	248.33 d	0.127 g	2.98 f	2.45 bc

ANOVA for each date was performed separately.
Values followed by different letter (s) in each column are significant at $P<0.05$ (Student t-test).

Kinugasa while RWC was highest in Ishigaki-2. LWP was highest in Kentucky Wonder and RWC was highest in Haibushi in unirrigated plants.

2. Diurnal variation in leaf gas exchange characteristics

The diurnal variation in leaf gas exchange, VpdL and photosynthetically active radiation (PAR) are presented in Fig. 1. PAR was above 1,000 μmol m^{-2} s^{-1} from 9:30 to 14:15 h and ranged from 400 to 800 μmol m^{-2} s^{-1} at 16:30 h. PAR was relatively constant between 11:30 to 14:15 h. No differences were found in PAR recorded during the measurement of leaf gas exchange characteristics. Within day times, P_n, C_i, g_s, and T were higher at 9:30 h than at other times. Differences between 11:30 and 14:15 h were not significant. The values were, however, higher than at 16:15 h. VpdL significantly increased from 9:30 to 16:30 h. The differences within the cultivars were apparent between 11:30 and 14:15 h. Cultivars Haibushi and Ishigaki-2 displayed higher rates of P_n, T, C_i, and g_s than

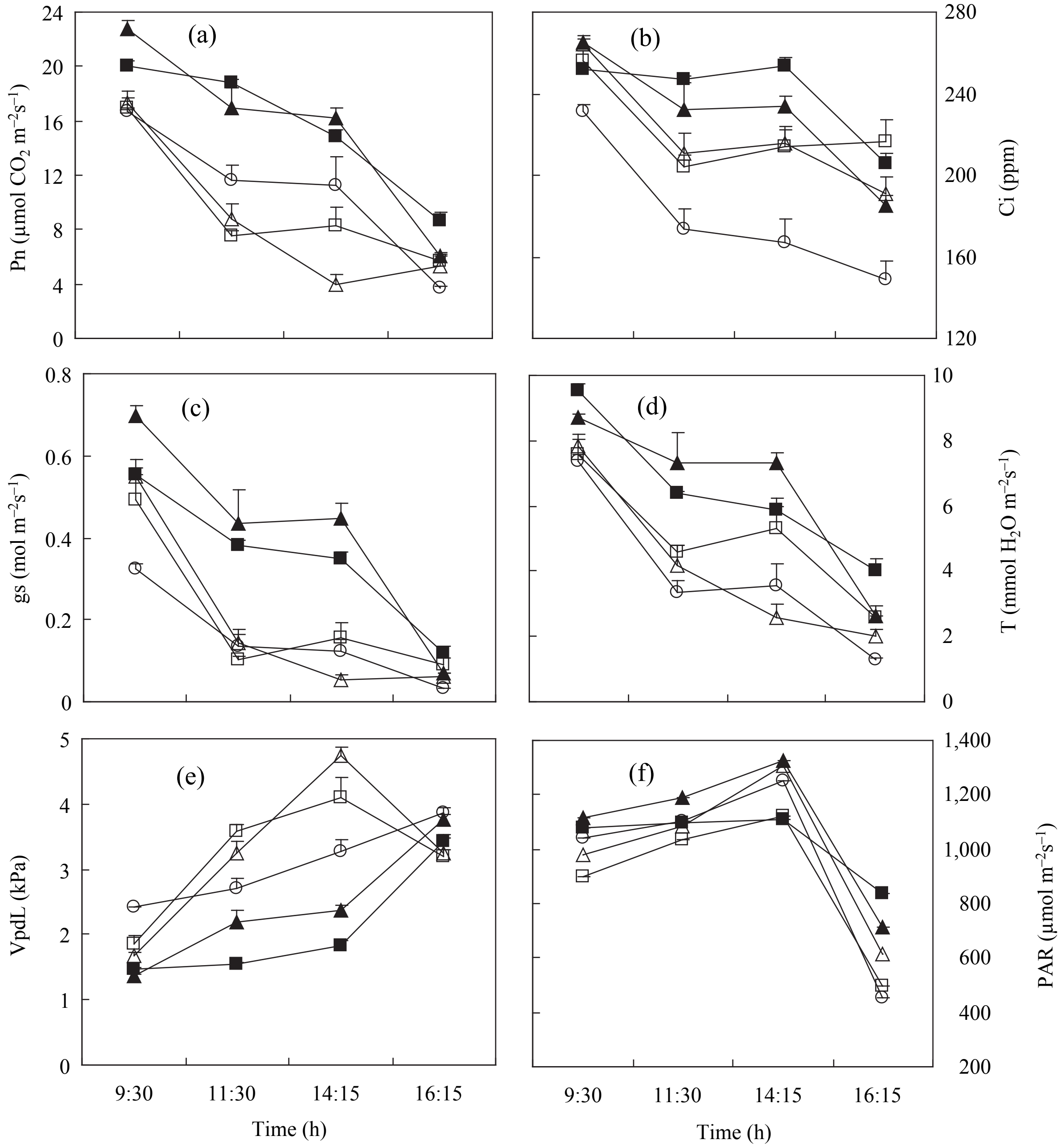

Fig. 1. Diurnal variation in (a) photosynthesis, (b) intercellular CO_2 concentration, (c) leaf conductance, (d) transpiration, (e) leaf vapor pressure deficit and (f) photosynthetically active radiation at floral bud initiation stage in summer 2005

–□– : 92783, –■– : Haibushi, –▲– : Ishigaki-2, –○– : Kurodane Kinugasa, –△– : Kentucky Wonder
Vertical bars represent standard error of mean (n = 9).

the remaining genotypes. Kurodane Kinugasa showed the lowest g_s, C_i and T but higher P_n than 92783 and Kentucky Wonder.

3. Relationships between water status, leaf gas exchange characteristics and seed yield

The coefficients of correlation between water status, gas exchange parameters, VpdL and seed yield at three growth stages are presented in Table 2. At floral bud initiation stage, leaf gas exchange parameters such as g_s, T and VpdL were positively correlated with seed yield while water status was not. At flowering stage, seed yield was positively correlated with LWP and RWC in irrigated and unirrigated plants except with LWP in unirrigated plants. Seed yield was also negatively correlated with g_s and positively with VpdL in unirrigated plants. C_i was positively

Table 2. Coefficients of correlation between water status, gas exchange parameters, VpdL and seed yield at three growth stages

Growth stage	Treatment	LWP	RWC	P_n	C_i	g_s	T	VpdL
Floral bud initiation stage, measured at 10:30 h		–0.268	0.535	0.348	0.120	0.900*	0.837*	0.839*
Flowering stage, measured at 13:30 h	Irrigated	0.904*	0.925**	–0.472	0.174	–0.499	–0.251	0.406
	Unirrigated	0.679	0.837*	–0.176	–0.386	–0.890*	–0.343	0.824*
Podding stage, measured at 10:30 h	Irrigated	–0.725	0.459	–0.218	0.830*	0.006	0.168	–0.339
	Unirrigated	–0.752	0.182	–0.257	0.763	–0.053	–0.086	–0.381

LWP: leaf water potential (MPa), RWC: relative water content (%), P_n: photosynthetic rate (μmol CO_2 $m^{-2}s^{-1}$), C_i: intercellular CO_2 (ppm), g_s: leaf conductance (mol $m^{-2}s^{-1}$), T: transpiration rate (mmol H_2O $m^{-2}s^{-1}$), VpdL: leaf vapor pressure deficit (kPa). The data in all cultivars were used for the calculation of correlation coefficients.
*: P<0.05, **: P<0.01.

correlated with seed yield in irrigated plants at podding stage.

4. Association of leaf water status with leaf water deficit and photosynthetic rate

The leaf water deficit (100-RWC) was negatively related to LWP except for Kurodane Kinugasa, in which no significant relationship was found (Fig. 2a). Leaf water deficit increased with a decrease in LWP. The rate of increase in leaf water deficit with decreasing LWP was significantly higher in 92783 and Kentucky Wonder than Haibushi and Ishigaki-2. The photosynthetic rate (P_n) was positively associated with LWP (Fig. 2b). The increase in P_n was accompanied with increased LWP and vice versa. The decrease in P_n with declining LWP varied in the cultivars, significantly higher in 92783 and Kentucky Wonder than Kurodane Kinugasa and Haibushi. No significant relationship was found in Ishigaki-2.

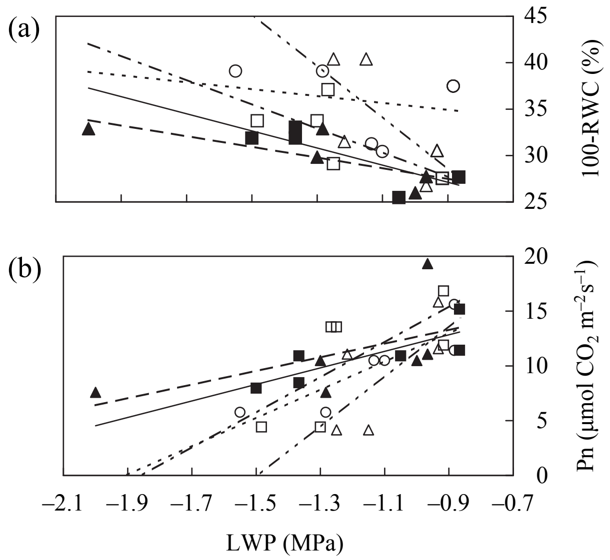

Fig. 2. Relationship between leaf water potential (LWP) and (a) leaf water deficit, 100-RWC and (b) photosynthetic rate (Pn)

Data of 3 growth stages in 2004 were used (n = 9). Regressions are: Y = –9.22x + 18.81, R^2 = 0.70** and Y = 7.54x + 19.62, R^2 = 0.66** for Haibushi, Y= –5.77x + 22.26, R^2 = 0.63* and Y = 6.27x + 18.94, R^2 = 0.33 for Ishigaki-2, Y = –3.73x + 31.54, R^2 = 0.06 and Y = 12.93x + 24.65, R^2 = 0.77** for Kurodane Kinugasa, Y = –13.06x + 15.92, R^2 = 0.56* and Y = 16.12x + 29.94, R^2 = 0.50* for 92783 and Y = –27.82x + 3.45, R^2 = 0.52* and Y =22.86x + 34.22, R^2 = 0.53* for Kentucky Wonder in (a) and (b), respectively.
*: P=0.05, **: P=0.01.

□: 92783, ■: Haibushi, ▲: Ishigaki-2, ○: Kurodane Kinigasa, △: Kentucky Wonder, –·–·–: Linear (92783), ——: Linear (Haibushi), – – – –: Linear (Ishigaki-2), ·······: Linear (Kurodane Kinugasa), –··–·: Linear (Kentucky Wonder)

Discussion

The five snap bean cultivars exhibited differential responses of leaf gas exchange characteristics to high temperature both diurnally and seasonally, which may be related to their heat tolerance. The heat tolerance of the five cultivars can be drawn from the relationship of leaf water deficit with leaf water potential (Fig. 2). Cultivars Haibushi, Ishigaki-2 and Kurodane Kinugasa showed smaller leaf water deficit with decreasing leaf water potential than 92783 and Kentucky Wonder. Therefore, the former were more stress tolerant than the latter. The ranking of tolerance in this study agreed with similar differences in pod setting ratio and seed yield[5]. The cultivar having higher RWC at flowering stage got higher seed yield (Table 2). The differences in leaf water status and gas exchange parameters among the cultivars were not consistent at 10:30 h both at floral bud initiation and podding stage (Table 1). However, cultivar variation was clear at midday hours (13:30 h) during the flowering stage. The decrease in LWP due to water stress was highest in Haibushi while it maintained highest RWC at midday. High yielding cultivars were able to tolerate lower leaf water potentials because low water potential was related with maintenance of high leaf water content[6]. Kurodane Kinugasa displayed the highest P_n in irrigated and the least in unirrigated plants indicating a greater sensitivity to water stress. The cultivars having higher g_s, T and VpdL at floral bud initiation stage got higher seed yield (Table 2). Heat or related internal water stress, however, doesn't develop only by water uptake capacity, which is determined by root volume and root distribution in the soil profile, but also the size of the canopy determined by the leaf area is involved. We didn't measure root volume, root distribution or canopy size of each cultivar in this study. Further study will be needed to clarify the genotypic differences of leaf water status.

Diurnally, peaks of P_n, g_s, C_i and T were attained at 9:30 h while VpdL increased continuously up to 16:30 h (Fig. 1). Leaf gas exchange characteristics showed a slight recovery at 16:30 h. Between 11:30 to 14:30 h, gas exchange decreased compared to 9:30 h but remained constant. This may be due to the fact that variables of ambient air temperature and radiation remained stable between 11:30–16:30 h.

A combination of high temperature and solar radiation in 92783 and Kentucky Wonder resulted in rapid transpiration. It caused a greater leaf water deficit with decreasing leaf water potential as shown in Fig. 2a. Cultivars Haibushi and Ishigaki-2 displayed a smaller leaf water deficit with decreasing leaf water potential as compared to 92783 and Kentucky Wonder. This is because leaf water content is associated with the number of pods per plant and yield in snap bean[6]. Maintenance of high leaf water content is, therefore, beneficial for achieving higher productivity. The relationship of leaf water content with productivity can be explained by the relationship of P_n with leaf water potential (Fig. 2b). P_n decreased at a faster rate with decreasing leaf water potential in 92783 and Kentucky Wonder, which showed faster development of leaf water deficit with leaf water potential than the remaining cultivars. Furthermore, pod setting and retention which make up productivity in snap bean were related negatively to midday drop in leaf water content. This is because every plant process including P_n has a critical water content[7]. The photosynthetic rates are generally influenced by the amount of water present at the site of metabolic activity. Therefore, leaf water content plays an important role in the stress tolerance in snap bean.

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