[Short Report]

Midday Drop of Leaf Water Content Related to Drought Tolerance in Snap Bean (*Phaseolus vulgaris* L.)

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Beans are principally grown in areas where plants are regularly exposed to drought stress under high ambient temperature conditions, and the breeding of drought-tolerant plants is drawing concern. However, progress in the development of tolerant lines is slow due to the lack of simple traits associated with drought tolerance. There is a general consensus that water economy is very critical to plant growth and development. It may therefore be possible to improve the drought tolerance of snap bean by identifying the water relations traits associated with higher productivity. In the subtropical islands of Japan, drought stress in the summer adversely affects bean production both for grain and vegetable use. The stress mainly causes low pod setting ratio, early pod abscission and consequently low productivity (Shen and Webster, 1986; Nakano et al., 1998; Suzuki et al., 2001; Tsukaguchi et al., 1999, 2003). Therefore, it is important to identify the characteristic traits associated with pod setting, the number of pods reaching maturity and finally seed yield. In this report, we clarify the association of the tissue water content of leaves with pod production (the number of pods per plant at harvest), seed size (seed weight) and seed yield, because yield of snap bean for vegetable use is a function of the number of pods per plant, and seed yield is a function of both the number of pods per plant and seed size.

Materials and Methods

The experiment was conducted in a net house covered with a polythene sheet under field conditions. The snap bean (*Phaseolus vulgaris* L.) genotypes used in the study were strains Ishigaki-2, 45817, 86884, 92783 and 3028520 and cultivars Kentucky Wonder, Haibushi, and Kurodane Kinugasa. Details of the origin and phenology of genotypes are described by Omae et al. (2004). On November 26, 2003, ten plants of each genotype were planted at 25 cm spacing in raised beds, 2.5m in length, and split into two groups. One group was irrigated and the other was unirrigated, during the pod formation stage (60 days after sowing, DAS). Irrigated plants received water regularly by drip irrigation, and unirrigated plants received no irrigation until completion of all measurements (up to 135 DAS). The experiment was designed in a split-plot with water stress levels as the main-plots and genotypes as the sub-plots. Soil moisture content (SMC) was determined gravimetrically in each plot. Soil of the field was red-yellow podzolic, highly acidic (pH 4.6) and fine to medium in texture.

Relative water content (RWC) of leaves was measured 27 days after the start of drought stress treatment (87 DAS) at 8:30 and 13:00 hr. At the time of RWC measurement, SMC was 5.38 ± 0.78% (w/w, mean ± SD) at 0 - 15 cm and 6.49 ± 0.43% at 15 - 30 cm soil depth in the unirrigated plots, while it was 7.95 ± 0.83 and 8.41 ± 0.77%, respectively, in irrigated plots. The three youngest fully expanded leaves (3rd or 4th leaves from the top) with three replications were randomly collected from each plot by a split-plot design considering (taking) two water stress levels as
Eight leaf discs, 7 mm in diameter, were cut with a sharp cork borer avoiding the mid-rib and major veins from the leaves in each plot. Then fresh weight (FW) of the leaf discs was measured, and water-saturated weight (SW) was determined after floating the discs on distilled water for 4 hr in the dark. The leaf discs were then dried in an oven at 65 °C for 8 hr to record dry weight (DW). RWC was calculated with the help of the following equation (Kumar et al. 1992).

\[ \text{RWC} \% = \frac{\text{FW} - \text{DW}}{\text{SW} - \text{DW}} \times 100 \]

The ratio of RWC at midday (13:00) to that in the morning (8:30) was also calculated.

All mature pods in each plot were harvested for one month from 10 March to 10 April, and the number of pods and seed yield per plant was recorded. All values were converted to average values per plant. The number of seeds per pod and weight of seed were measured in 20 pods and 20 seeds, respectively, for each genotype.

### Results and Discussion

The number of pods per plant was decreased by drought stress. The rate of the reduction varied with the genotype in the range from 0.9 to 84.5%, and it was lowest in genotype 3028520 followed by Ishigaki-2, Haibushi, 92786, 45817, Kurodane Kinugasa, Kentucky Wonder and 86884 in this order (Table 1). The number of seeds per pod was decreased by drought stress except for Ishigaki-2, and the rate of the reduction varied with the genotype in the range from 3.9 to 25%. Seed size (seed weight) was decreased by the drought stress in all cultivars except for 86884 in which the size was increased by 21.9%, and the rate of the reduction varied from 2.5 to 30.8%. Seed yield was also decreased by drought stress by 1.1 to

| Genotype          | Number of pods/plant | Number of seeds/pod | Seed weight (g) | Yield/plant (g) |
|-------------------|----------------------|---------------------|-----------------|-----------------|
|                   | Irrigated | Unirrigated | Irrigated | Unirrigated | Irrigated | Unirrigated | Irrigated | Unirrigated |
| 86884             | 3.7       | 0.57       | 4.1 (0.42) | 3.1 (0.44) | 0.419 (0.001) | 0.507 (0.012) | 6.20 | 0.87 |
| Kurodane Kinugasa | 53.9      | 38.3       | 6.3 (0.25) | 6.1 (0.21) | 0.142 (0.001) | 0.128 (0.001) | 48.12 | 29.39 |
| Ishigaki-2        | 34.7      | 33.1       | 7.3 (0.52) | 7.3 (0.22) | 0.162 (0.002) | 0.157 (0.002) | 39.67 | 39.23 |
| 45817             | 37.3      | 27.5       | 4.1 (0.22) | 3.8 (0.32) | 0.178 (0.001) | 0.123 (0.001) | 25.26 | 13.87 |
| 3028520           | 20.4      | 20.3       | 5.1 (0.18) | 4.9 (0.22) | 0.215 (0.001) | 0.172 (0.002) | 21.53 | 17.74 |
| Kentucky Wonder   | 9.7       | 3.3        | 6.6 (0.18) | 5.5 (0.14) | 0.408 (0.002) | 0.298 (0.002) | 19.11 | 7.40  |
| 92783             | 18.7      | 14.2       | 7.9 (0.24) | 6.7 (0.41) | 0.204 (0.002) | 0.149 (0.002) | 30.21 | 16.08 |
| Haibushi          | 40.7      | 38.6       | 6.3 (0.16) | 5.5 (0.12) | 0.158 (0.001) | 0.127 (0.002) | 38.42 | 28.52 |

Figures in parentheses represent ± SD of twenty pods and seeds.

| Genotype          | Relative water content (RWC, %) | Midday drop in RWC (%) |
|-------------------|---------------------------------|------------------------|
|                   | At 8:30 hr | At 13:00 hr | Irrigated | Unirrigated | Irrigated | Unirrigated | Irrigated | Unirrigated |
| 86884             | 92.08a     | 86.15ab    | 76.88ab    | 65.57b     | 16.50      | 23.89      |
| Kurodane Kinugasa | 92.02a     | 86.08ab    | 80.91ab    | 77.37ab    | 12.07      | 10.12      |
| Ishigaki-2        | 93.87a     | 84.07ab    | 83.53a     | 78.30ab    | 11.02      | 6.86       |
| 45817             | 85.60ab    | 81.15ab    | 78.86ab    | 68.18ab    | 7.87       | 13.98      |
| 3028520           | 81.71ab    | 85.30ab    | 75.21ab    | 70.56ab    | 7.95       | 15.28      |
| Kentucky Wonder   | 92.38a     | 85.98ab    | 75.93ab    | 73.10ab    | 17.81      | 14.98      |
| 92783             | 85.97ab    | 75.97b     | 74.79ab    | 65.92b     | 13.00      | 17.23      |
| Haibushi          | 89.77ab    | 86.43ab    | 85.57a     | 73.10ab    | 4.68       | 13.42      |

Stress (S) * **
Cultivar (C) ns ns
SxC ns ns
CV (%) 7.12 9.85

*, ** significant at 1 and 0.1% levels of probability (Tuckey test) and ns, not significant.
85.94% varying with the genotype. The seed yield under irrigated condition was the highest in genotype Kurodane Kinugasa followed by Ishigaki-2, Haibushi, 92783, 45817, 3028520, Kentucky Wonder and 86884 in this order. The rate of reduction in the number of pods and seed yield per plant greatly varied with the genotype. Seed yield was significantly and positively correlated with the number of pods per plant \((r = 0.88, P<0.01)\) and the number of seeds per pod \((r = 0.68, P<0.05)\), but negatively with seed weight \((r = 0.67, P<0.05)\). Seed weight was negatively correlated with the number of pods per plant \((r = 0.78, P<0.01)\), i.e., genotypes with lighter seeds produced more pods per plant and vice versa.

There was a significant difference in RWC between the watered and drought-stressed plants. In the morning at 8:30 hr, the drought-stressed plants of 92783 had a lower RWC than the watered plants of 86884, Ishigaki-2, Kurodane Kinugasa and Kentucky Wonder (Table 2). The watered plants of Haibushi and Ishigaki-2 had a significantly higher RWC than the stressed plants of 86884 and 92783 at midday (13:00 hr). However, the difference between watered and drought-stressed plants was clearer in the midday drop of RWC (decrease of RWC at midday relative to that in the morning). In watered plants, the midday drop of RWC was minimal (4.68%) in Haibushi, while in drought-stressed plants it was minimal in Ishigaki-2 (6.86%). The midday drop of RWC in watered and drought-stressed plants was the greatest in Kentucky Wonder (17.81%) and 86884 (23.89%), respectively.

RWC at either 8:30 or 13:00 did not significantly correlate with either the number of pods per plant, seed weight or seed yield per plant. However, the midday drop of RWC strongly correlated with yield-attributes and yield (Fig. 1). In Fig. 1, the relationship was analyzed using the data from both watered and drought stressed plots. The midday drop of RWC negatively correlated with the number of pods per plant (Fig. 1a) and seed yield per plant (Fig. 1c) at a significance level of \(P<0.01\), and positively correlated with seed weight at \(P<0.01\) (Fig. 1b). These results showed that the cultivars with a smaller midday drop of RWC produced a larger number of pods per plant and consequently had higher yield as compared with the plants with a larger midday drop of RWC. The results also showed that there was a large genotypic variation in the midday drop of RWC, which was correlated to the number of pods per plant, seed size and seed yield. It may therefore be possible to use midday drop of RWC as a screening marker for drought tolerance of snap bean plants.

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