Characteristic Response to Water Stress in Young Plants of Vegetable Cultivars Introduced from the Tropical Zone

By TOKUHIRO MOMONOKI* and YOSHIE S. MOMONOKI**

* Okinawa Branch, Tropical Agriculture Research Center
(Maesato, Ishigaki, Okinawa, 907-01 Japan)
** Faculty of Agriculture, Tokyo University of Agriculture
(Sakuragaoka, Setagaya, Tokyo, 156 Japan)

Introduction

As crops in the humid-subtropical region including Okinawa often suffer from rapid water loss due to high temperature combined with strong solar radiation in the long summer period, crop resistance to water stress is one of the important subjects. Recently, by the analysis of vegetation, the authors found that vegetable cultivars introduced from the tropical evergreen lowland rain forest zone (tropical zone) are adaptable to the humid-subtropical region, Okinawa[9]. These cultivars seem to have some properties to overcome water stress.

The present study was designated to know characteristic responses to water stress[4-8,13] in some vegetables introduced from the tropical zones, using simple and practical techniques.

Materials and methods

Vegetable cultivars used in this study are shown in Table 1. The seeds of cultivars except Japanese ones were introduced from Malaysia, Thailand and Taiwan in 1983. Seeds which germinated in Petri dishes with two layers of wet filter paper were transferred to paper pots and placed in a greenhouse. Yard-long bean at the stage of fully expanded trifoliate leaf, cucumber at the fully expanded first leaf stage and radish at the fully expanded second leaf stage were used for seedling experiments.

For seedlings, stress treatments were given artificially. Potted seedlings, which had been well watered, were exposed to a jet of either cool (22°C) or warm (30°C) air for 3 min at the room temperature (22°–23°C). The air jet was generated by a hair-dryer 20 cm distant from the seedlings. Then, stomatal closure, diffusion resistance and water potential were determined immediately.

In addition, responses of plants (with 8–10 leaves) growing in the field to water stress caused by wind or strong sun light were observed to know whether the grown plants show the same mode of responses as shown in the seedling experiment.

Th response of stomata was studied microscopically. To obtain leaf epidermis, a cellophane adhesive tape was fixed on leaf surface, and then the tape was removed. The tape stripped epidermis off the leaf. The leaf epidermis thus obtained was immediately stained with 0.5% safranine and mounted on slide glass. Diffusion resistance was measured by a steady state porometer. Water potential was measured by means of a dew point hygrometer in a Wisco C-52 sample chamber[14].

Table 1. Vegetable cultivars used as experimental materials

Vegetables	Cultivars	Abbreviation
Yard-long bean (*Vigna unguiculata*)	Bertam local (Malaysia)	Bertam
	Commercial cultivar (Malaysia, originally imported from Taiwan)	Malaysia-T
	Kasetsart University b (Thailand)	Kasetsart
	Sanjaku sasage (Japan)	Sanjaku
Cucumber (*Cucumis sativus*)	Siburan local (Malaysia)	Siburan
	MTi-2 (Malaysia)	MTi-2
	Hokushin (Japan)	Hokushin
	Ritsurin (Japan)	Ritsurin
Radish (*Raphanus sativus*)	F_1-lobak (Malaysia)	F_1-lobak
	Sibu local (Malaysia)	Sibu
	Taiwan local (Taiwan)	Taiwan
	Natsumionowase (Japan)	Natsuminowase

The degree of leaf wilting was estimated by measuring the drooping angle (the angle between the slope of leaf surface and an imaginary horizontal line running at the basal end of the leaf) of leaves. The angle above the horizontal line was expressed as plus degrees, while that below the horizontal line as minus degrees. The angles were measured with a protractor and a dial caliper.

Bleeding sap of well-watered plants was collected from cut surface of stems just at the height of 4 cm from the ground level. The cut surface was wrapped with absorbent cotton and covered with a vinyl bag at 10 o'clock in the morning. After 24 hr, the cotton was collected from 7 plants of each cultivar, and soaked in 80% methanol. The methanol solution containing bleeding sap was filtered and evaporated *in vacuo* at 35°C. The residue was washed with acetone and subsequently dissolved in 100 μl of 50% methanol. This solution was used for examining the effect of the bleeding sap on growth of rice seedlings following the microdrop method of Murakami[10].

Vascular system of root was observed after treating root cross sections (4 or 7 cm from a root tip) with phloroglucinol-hydrochloric acid. The ratio of stele diameter/root diameter in a cross section was calculated.

Experimental results

1) Response to water stress in yard-long bean

Stomatal adjustment shown in the seedling experiment and field observation is given in Table 2. The Japanese cultivar showed a large number of closed stomata and increased diffusion resistance against water stress, while cultivars of Malaysia and Thailand increased their diffusion resistance, though the number of closed stomata was less than that of the Japanese cultivar. Changes of leaf wilting after the water stress treatment are shown in Fig. 1. The Malaysian cultivars were distinguished for their low degree of wilting and rapid recovery from wilting.

Effect of methanol extract of bleeding sap of roots on rice seedlings is shown in Table 3. The extract of Thai and Japanese cultivars in yard-long bean inhibited the elongation of the second leaf sheath. Although the inhibitive substance was not identified in this experiment, we assume that ABA may be involved, because ABA is known to be closely related to stomatal response to water stress[1,8,13,14] and drought resistance[2,11].

2) Response to water stress in cucumber

In Table 4, stomatal adjustment was compared between Malaysian cultivars and Japa-

Table 2. Stomatal adjustment of yard-long bean plants in respones to water stress

Growth stage	Treatment	Cultivars			
		Bertam	Malaysia-T	Kasetsart	Sanjaku
Seedling					
No. of stomata (/mm²) n = 100		365	498	623	423
% of closed stomata (/mm²) n = 10	Con.	0.0	0.0	0.0	2.0
	22℃	0.2	0.5	0.4	2.4
	30℃	2.3	1.7	1.0	14.9
Diffusion resistance (sec/cm) n = 10	Con.	1.6	1.4	0.7	0.8
	22℃	3.0	2.2	1.0	1.1
	30℃	8.6	4.3	3.5	7.0
Water potential (-Kp) n = 10	Con.	12	10	10	13
	22℃	13	11	13	13
	30℃	14	12	14	16
Grown plant*					
No. of stomata (/mm²) n = 50		391	473	658	446
% of closed stomata (/mm²) n = 50		1.2	1.5	1.6	10.0
Diffusion resistance (sec/cm) n = 45		1.7	1.7	2.5	4.9

*Five successive expanded leaves counted from the top were used per plant.

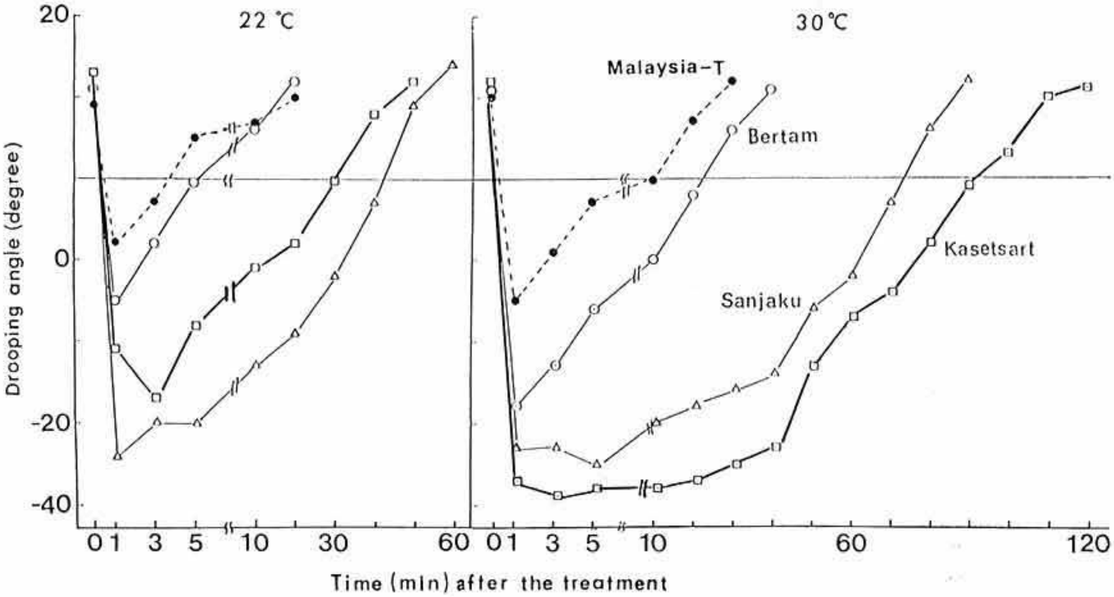

Fig. 1. Leaf wilting of yard-long bean cultivars and its recovery after water stress (air-blow treatment)

nese ones. Apparently, both the number of closed stomata and diffusion resistance increased only in Japanese cultivars, while no such response was observed with Malaysian cultivars. Fig. 2 shows leaf wilting and its recovery. Here again, the Malaysian cultivars showed rapid recovery from the wilting. The recovery proceeded at the rate about four times that of Japanese cultivars. Likewise, grown plants of Malaysian cultivar "MTi-2" in the field showed quick recovery from leaf wilting within 5 to 7 min after shifting the plants from sun to shade.

Table 3. Growth inhibition of rice seedlings* by methanol extracts of bleeding sap from roots of yard-long bean plants

Cultivars	Length of the second sheath (mm)	
	Seedling	Grown plant**
Control	4.9	3.9
Bertam	4.8	4.0
Malaysia-T	4.8	3.9
Kasetsart	4.1	3.2
Sanjaku	4.1	3.1

* n = 30.
** Bleeding sap of grown plants was collected from 7 potted plants with 8-10 expanded leaves.

3) Response to water stress in radish

Stomatal adjustment in response to water stress is shown in Table 5. No response was observed in cultivars from Malaysia and Taiwan. Only Japanese cultivar showed an increased number of closed stomata and increased diffusion resistance. However, changes of water potential were not observed. The same tendency was observed in grown radish plants. As given in Fig. 3, a Japanese cultivar showed severe leaf drooping and late recovery from leaf wilting. On the contrary, the cultivars of Malaysia and Taiwan did not show any sign of leaf wilting. Table 6 and

Table 4. Stomatal adjustment of cucmber plants in response to water stress

Growth stage	Treatment	Cultivars			
		Siburan	Mti-2	Hokushin	Ritsurin
Seedling					
No. of stomata (/mm²) n = 100		550	548	678	687
% of closed stomota (/mm²) n = 10	Con.	0.6	0.6	0.6	0.3
	22℃	0.8	0.6	3.2	0.7
	30℃	0.2	0.0	5.0	3.0
Diffusion resistance (sec/cm) n = 10	Con.	1.6	2.6	2.0	2.3
	22℃	2.4	2.2	10.5	2.6
	30℃	1.6	1.7	10.8	15.1
Water potential (-Kp) n = 10	Con.	8	7	8	8
	22℃	8	8	9	8
	30℃	9	9	9	9
Grown plant*					
No. of stomata (/mm²) n = 50		560	553	667	662
% of closed stomata (/mm²) n = 50		0.5	0.2	6.8	6.0
Diffusion resistance (sec/cm) n = 45		0.5	0.5	2.7	2.6

* See Table 2.

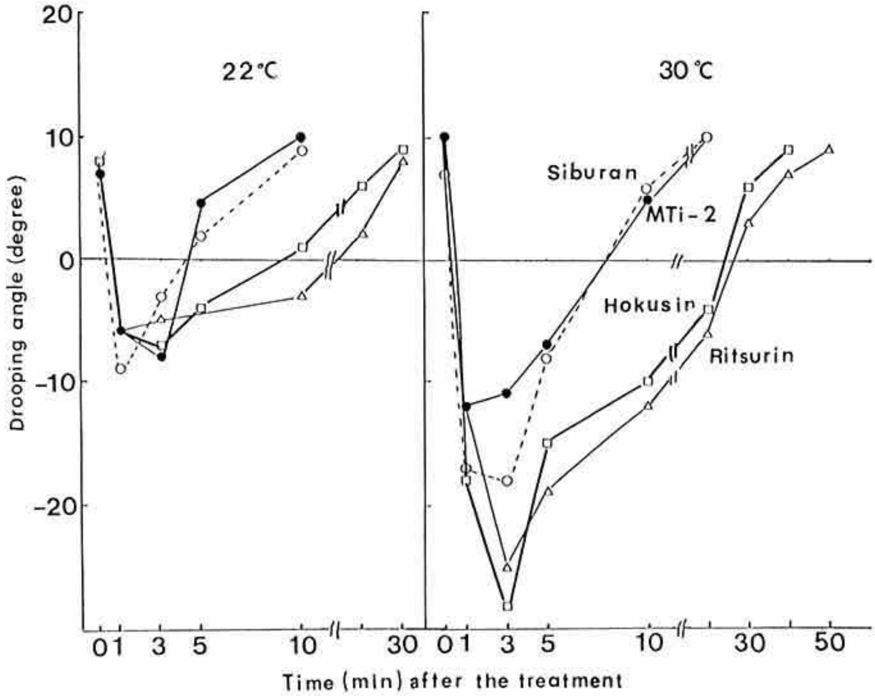

Fig. 2. Leaf wilting of cucumber cultivars and its recovery after water stress (air-blow treatment)

Table 5. Stomatal adjustment of radish plants in response to water stress

Growth stage	Treatment	Cultivars			
		F_1-lobak	Sibu	Taiwan	Natsuminowase
Seedling No. of stomata (/mm²) n = 100		286	305	254	255
% of closed stomata (/mm²) n = 10	Con. 22℃ 30℃	0.0 0.0 0.0	0.6 1.0 0.9	0.0 0.3 2.1	0.2 0.8 4.8
Diffusion resistance (sec/cm) n = 10	Con. 22℃ 30℃	0.6 0.6 0.5	0.7 1.0 0.8	0.8 0.9 0.9	2.7 6.7 7.7
Water potential (-Kp) n = 10	Con. 22℃ 30℃	8 8 9	11 11 11	9 9 10	8 9 9
Grown plant* No. of stomata (/mm²) n = 50		273	300	252	252
% of closed stomata (/mm²) n = 50		0.5	0.3	0.6	3.8
Diffusion resistance (sec/cm) n = 45		0.5	0.5	0.4	1.2

* See Table 2.

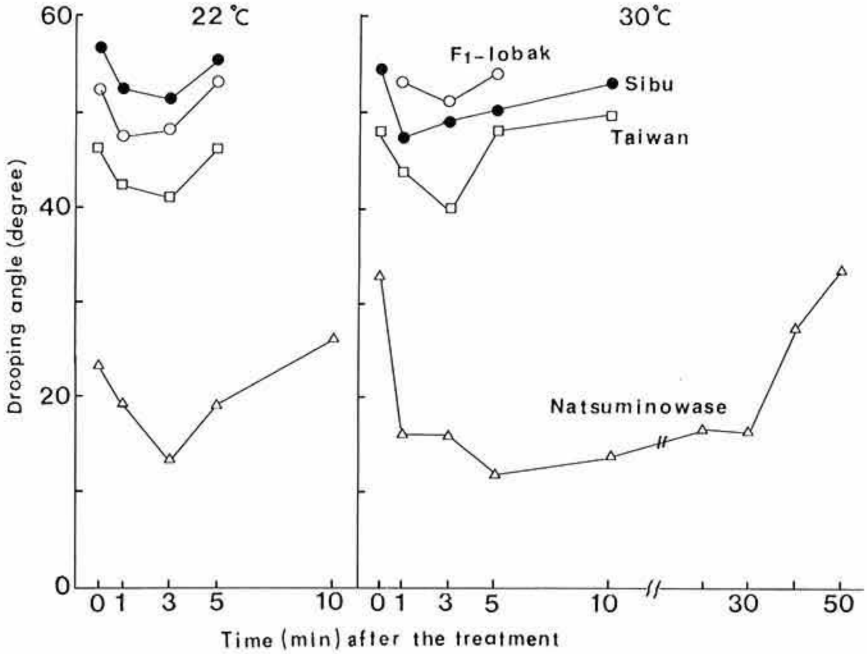

Fig. 3. Leaf wilting of radish cultivars and its recovery after water stress (air-blow treatment)

Table 6. Comparison of stele diameter and vascular system of root among radish cultivars

Cultivars	Ratio (%) of stele diameter*		Density of vascular system	
	Seedling	Grown plant	Seedling	Grown plant
F_1-lobak	78	90	high	high
Sibu	65	81	high	high
Taiwan	64	81	high	high
Natsuminowase	54	75	slightly low	low

*Ratio of stele diameter: stele dimeter/root diameter.

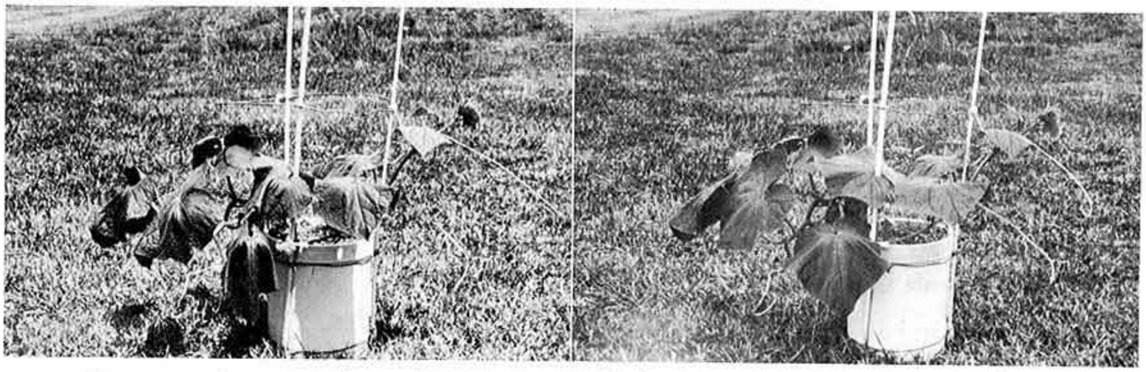

Plate 1. Leaf drooping (wilting) in the hot sun (left), and its recovery in the shade (right)
A cucumber cv. MTi-2 of Malaysia recovered from the leaf drooping in 7 min when placed in the shade.

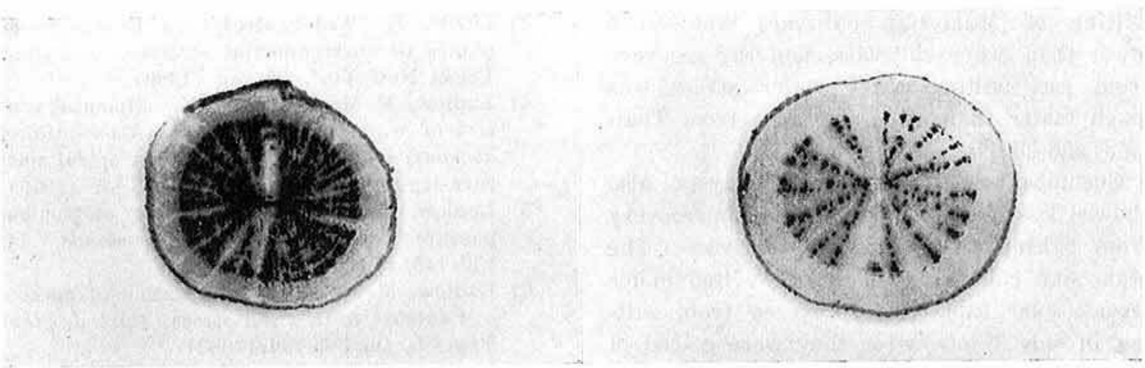

Plate 2. Difference of vascular system in radish roots between Malaysian and Japanese cultivars
Left: Malaysian cv. F_1-lobak, Right: Japanese cv. Natsuminowase.

Plate 2 show the vascular system of radish roots. The vascular system of roots in cultivars from Malaysia and Taiwan is densely arranged and has a large stele diameter compared to that in a Japanese cultivar.

Discussion

When plants are exposed to water stress, plants can resist the stress by avoidance or tolerance[3]. Some plants have poor tolerance but good avoidance, such as sensitive stomatal adjustment[7], small changes in leaf water potential[4,11], leaf movements associated with reduced receipt of solar radiation and deep rooting which related to water capacity[12] etc. for minimizing water loss[4]. On the other hand, the tolerance strategy helps maintain turgor and consequently some metabolic activity under increasing water stress[3,5,6].

In the present study, we tried to find out some definite responses to water stress exhibited by vegetable cultivars introduced from the tropical zone (Malaysia, Thailand, and Taiwan). The Malaysian cultivars of yard-long bean and cucumber exhibited avoidance strategy to water stress, namely good stomatal adjustment and quick recovery from leaf wilting. Particularly, Malaysian cucumber seedlings recovered within 5 to 7 min after water stress: the time to complete recovery was only one-fourth that of Japanese cultivars. Grown plants in the field also recovered within 7 min. Such a quick recovery may induce a quick recovery of photosynthesis suffering from water stress.

On the other hand, radish cultivars introduced from Malaysia and Taiwan showed no change in stomatal closure, no increase of diffusion resistance of stomata and no sign of leaf wilting in response to water stress, whereas Japanese cultivars showed remarkable stomatal closure and severe leaf wilting. The former group of cultivars is characterized by large stele diameters and densely arranged vascular system in roots. This character may favor water uptake from the soil. Thus, it is suggested that the former group has inherited property desirable for drought avoidance.

The results of the present study indicate that cultivars of yard-long bean, cucumber, and radish introduced from the tropical zone, especially from Malaysia are able to overcome water stress during a long summer period in the humid-subtropical region by avoidance strategy in plants.

Summary

Regarding plant responses to water stress, cultivars of *Vigna unguiculata* (yard-long bean), *Cucumis sativus* (cucumber) and *Raphanus sativus* (radish) in the tropical zone were compared with Japanese cultivars. Yard-long bean cultivars of Malaysia and Thailand showed good stomatal adjustment in response to water stress. The degree of

wilting of Malaysian cultivars was much lower than other cultivars, and the recovery from leaf wilting after water stress was much faster than other cultivars from Thailand and Japan.

Cucumber cultivars from Malaysia also showed less leaf wilting and quicker recovery from wilting than Japanese cultivars. The Malaysian cultivars, which had wilted under strong solar radiation, recovered from wilting in only 7 min after they were placed in the shade. Thus, the Malaysian cultivars of *Vigna unguiculata* and *Cucumis sativus* could avoid water stress efficiently.

Radish cultivars from Malaysia and Taiwan showed no change in stomatal aperture and diffusion resistance, and no sign of any leaf drooping against water stress, while Japanese cultivar showed a significant increase of stomatal closure and diffusion resistance and severe leaf wilting. The same phenomenon was also observed with grown plants in the field. Furthermore, the radish cultivars from Malaysia and Taiwan had roots with large stele diameter and densely arranged vascular system compared to the Japanese cultivar.

These results clearly indicate that Malaysian cultivars of yard-long bean, cucumber, and radish hold favorable properties to overcome water stress occurring frequently during a long period of summer in the humid-subtropical zone.

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