

Studies on Leaf Orientation Movements in Kidney Beans (*Phaseolus vulgaris* L.)

IV. Effect of water spray on leaflet inclination*

Hajime SATO

(Hokkaido University of Education/Sapporo, Kita-ku, Sapporo 002, Japan)

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Abstract : Leaflet movements in response to a reduction of leaf temperature by water spray was examined under severe plant water stress at fine midday, using the lateral leaflets of the third trifoliate leaves in potted plants of two kidney bean (*Phaseolus vulgaris* L.) cultivars : Hime-tebo and Taisho-kintoki. All lateral leaflets not sprayed with water (the control) largely inclined and oriented themselves to their terminal leaflet. The water spray (the spray treatment) rapidly decreased the twisting angles of the leaflets, but did not change the vertical angles. In the control, stomata closed completely (except for abaxial surface of cv. Taisho-kintoki) and water saturation deficit was much higher. However, in the spray treatment, although not significant, the stomata of leaflets slightly opened and the water saturation deficit rose rather slightly. The decrease of the twisting angle synchronized with a reduction of leaf temperature. The change in the twisting angle of the lateral leaflets seemed to be associated with leaf temperatures, not with leaf water potentials. Further, this response was discussed to be caused directly by the change in temperature of the pulvinule rather than the leaf blade.

Key words : Leaf orientations, *Phaseolus vulgaris*, Pulvinule, Sun-avoiding response, Temperatures, Twisting angle, Water spray, Water stress.

インゲンマメの葉の調位運動に関する研究 第4報 水の葉面散布が葉面傾斜に及ぼす影響 : 佐藤 肇
(北海道教育大学札幌校)

要 旨 : ポット栽培したインゲンマメの2品種(姫手亡と大正金時)を材料として、水の葉面散布による葉温の変化に対する側小葉の傾斜反応を、強い土壤水分ストレス状態において検討した。水を散布しなかった小葉は大きく傾斜し、著しい水分ストレス状態にあることを示した。また、すべての側小葉が頂小葉の方向に傾斜することが認められた。この状態における水の散布処理は、小葉の横向き傾斜角を5分間で約20度急激に減少させたが、上下傾斜角にはほとんど影響しなかった。この反応は2つの品種で同様に認められた。また、水を散布しなかった状態で気温より高かった葉温は、散布処理直後から5℃以上急激に低下した。一方、有意ではなかったが、水の散布処理により小葉の気孔がわずかに開き、葉の水ポテンシャルもわずかに低下することが認められた。以上の結果から、側小葉の光忌避反応が、水分ストレスではなく温度の変化と関係し、内生的方向性を示す横向き傾斜の変化によって起こることが明らかになった。この水の散布処理による横向き傾斜角の減少は、小葉の葉身よりも葉枕の温度低下が直接の原因であることが推察された。

キーワード : インゲンマメ、温度、水分ストレス、調位運動、光忌避反応、水の葉面散布、葉枕、横向き傾斜角。

Sun-avoiding (paraheliotropic) movements in response to low plant water potentials or droughts have been observed for many species^{5,7,13,14}). Species with these movements have been described to orient their leaves obliquely to solar radiation under severe water stress, and in that way maintain effective leaf temperature and transpiration^{1,6,18}). In contrast, other observations have shown that the sun-avoiding movement occurred even under well-watered conditions^{2,14}), and that some plants inverted their leaves under severe water

stress^{12,13}). These facts show that the sun-avoiding movements themselves, the relationship between these and environmental factors and their importance to physiological actions of plants are not yet understood sufficiently.

My previous paper¹⁷) suggested that a functional differentiation may exist in leaflet movements of kidney beans ; the vertical response (sun-tracking) to stimulus of light and the twisting response (sun-avoiding) to that of temperature. Because environmental factors are always dependent on each other, it is difficult to distinguish the effects of the different stimuli on their responses.

The objective of this study was to determine

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the effect of leaf temperatures and leaf water potentials on the twisting response in leaflet movements of kidney beans. In order to accomplish this objective, the lateral leaflets were chosen, because responses to a given condition seem to differ in terminal (central) and lateral (side) leaflets in the trifoliolate leaf²²⁾. Further, a control of the leaf temperature by water spray on the leaves was conducted under severe soil water stress, and the experimental period was limited to fine mid-day to remove an effect of directional light.

Materials and Methods

1. Plant materials

Seeds of two kidney bean (*Phaseolus vulgaris* L.) cultivars, Hime-tebo (Vertical type) and Taisho-kintoki (Twisting type), were planted in seedling beds. When the first trifoliolate leaves fully expanded, two plants of the same cultivar were transplanted in a 5.5 liter plastic pot which contained a mixture of 60% sandy loam soil, 20% vermiculite and 20% barnyard manure and then the terminal (central) leaflets of the trifoliolate leaves were set toward a similar direction. These plants were grown in a greenhouse.

When the fifth trifoliolate leaves were expanding, the third trifoliolate leaves were used for the experiment. The plants were kept well watered until one week before the experiment and then irrigation was stopped in order to obtain severe plant water stress. One day before the experiment, the pots were set so that the terminal leaflets of the third trifoliolate leaves in each pot were oriented northward.

2. Experiment methods

All experiments were conducted in a greenhouse and limited to fine midday (11:00 to 13:00) to remove the effect of directional solar radiation. To determine the effect of leaf temperature on leaflet movements, distilled water kept at 23°C was sprayed on both surfaces of the third trifoliolate leaves. The sprayed trifoliolate leaf of one plant (the spray treatment) in each pot was compared with the trifoliolate leaf of the other (the control) which was not sprayed with water and continued to be under severe plant water stress. The vertical and twisting angles of the lateral (side) leaflets¹⁵⁾ in these trifoliolate leaves were measured just before spraying and

at 10 minute intervals from 5 to 35 minutes after spraying.

In order to investigate the change of leaf temperature caused by the spray and non-spray treatments, temperatures on the adaxial (upper) and abaxial (lower) surfaces of lateral leaflets for cv. Hime-tebo were measured with copperconstantan thermocouples (0.12 mm of diameter, Omega Engineering, Inc. USA) and recorded with datalogger (7V13, Nippon Electric-Sanei Co., Japan). The thermocouple was fixed at a point of 1 cm from the base of the leaf blade with gum arabic. Air temperature was simultaneously measured with the thermocouple at a shaded space on a level with the leaves. Solar radiation was measured with a hand-made photosensor set horizontally on a level of the leaves. The photosensor utilized a photodiode (S1133, Hamamatsu Photonics, Japan), which is sensitive to 400 to 700 nm of wave length and had been calibrated with a radiation sensor (LI-109SEB, LI-Cor Instruments, USA). The mean solar radiation during the experiments was approximately $130 \text{ W} \cdot \text{m}^{-2}$ but there was a somewhat large variation.

To determine stomatal response and change of leaf water stress by the spray treatment, stomatal apertures of the adaxial and abaxial surfaces of the lateral leaflets for both cultivars were tested with the infiltration method¹⁰⁾, and the water saturation deficit was also tested on the leaflets of cv. Hime-tebo with the method described by Slavik¹⁹⁾ and Turner²⁰⁾. These tests were conducted 30 minutes after the spray treatment.

Results

Figure 1 shows the effects of the spray treatment and the control of the third trifoliolate leaves after they have been subjected to severe soil water stress. The trifoliolate leaf of cv. Hime-tebo, which was more or less horizontal, opened largely after the spray treatment, but that of the control continued to be closed (Fig. 1a). The trifoliolate leaves of cv. Taisho-kintoki remarkably were closed in both treatments as compared to those of cv. Hime-tebo. The leaf inclination of the spray treatment was, however, different from that of the control (Fig. 1b). The abaxial surfaces of the leaflets were observed in the control, but not in those of the spray treatment. These

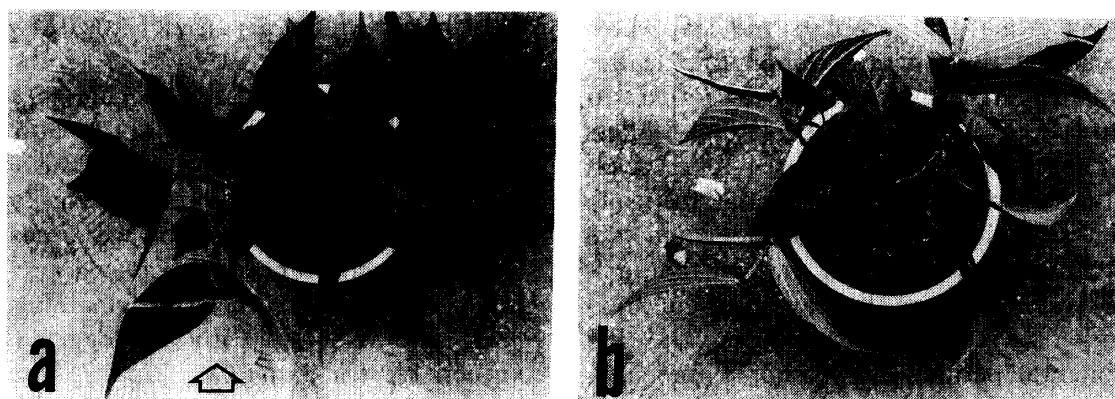


Fig. 1 Responses of the 3rd trifoliolate leaf to water spray for two kidney bean cultivars; Hime-tebo (a) and Taisho-kintoki (b). Symbols \uparrow and \uparrow show the leaves sprayed with water and the control, respectively. 2 plants of each cultivar were grown in the same pot, and each terminal leaflet of the tested leaves was set northward (lower side in photograph). Photographs were taken at a fine midday.

results showed that water sprayed on the leaf affects leaf movements, although a difference of leaf inclination between two cultivars tested was recognized in both treatments. Further all lateral leaflets in the control oriented to their terminal leaflets.

Figure 2 shows the time courses in the vertical and twisting angles of lateral leaflets in response to the spray treatment. Although the vertical angles of leaflets were almost unchanged, the twisting angles rapidly decreased just after the spray treatment (20 degrees within 5 minutes) and then they continued the same status during the next 30 minutes. Such a response was similar in both cultivars tested.

Figure 3 shows the time courses in the lateral leaflet temperature of cv. Hime-tebo in both treatments and the air temperature. Temperatures of the adaxial and abaxial surfaces in the control showed almost the same change during the experiment and were 2.6 to 9.3°C higher than air temperature. In the spray treatment, temperatures of both surfaces rapidly decreased more than 5°C just after the water spraying and then continued at a lower status than those of the control for about 20 minutes. While the temperature of the abaxial surface, thereafter, recovered to that of the control, the abaxial surface maintained a lower temperature than the control for the rest of the testing period.

Table 1 shows infiltration scores tested for the lateral leaflets of both cultivars and water saturation deficit for that of cv. Hime-tebo.

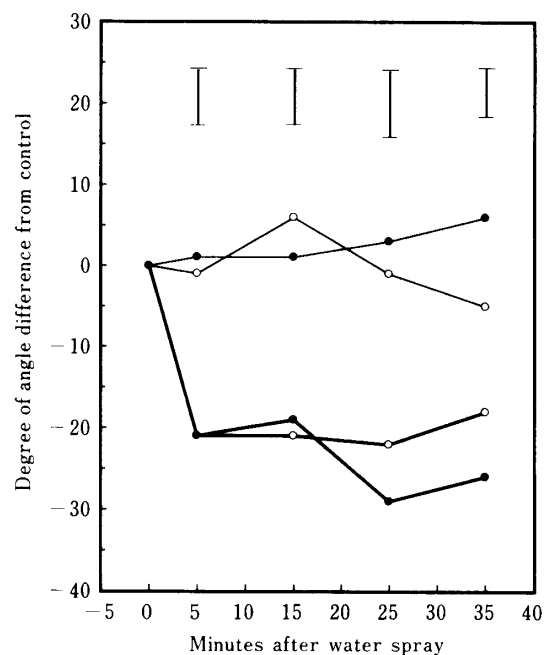


Fig. 2 Changes of vertical (—) and twisting (—) responses of lateral leaflets to water spray for cv. Hime-tebo (●) and cv. Taisho-kintoki (○). Degrees of angle show differences in angle resulting from the spray treatment as compared to the control at each measurement time. Vertical bars show LSD.

While the abaxial surfaces of cv. Taisho-kintoki showed remarkably large infiltration scores in both treatments, the scores of the others were small and especially those of the control were zeros. The scores of the spray treatment in both cultivars were generally larger than those of the control, although not

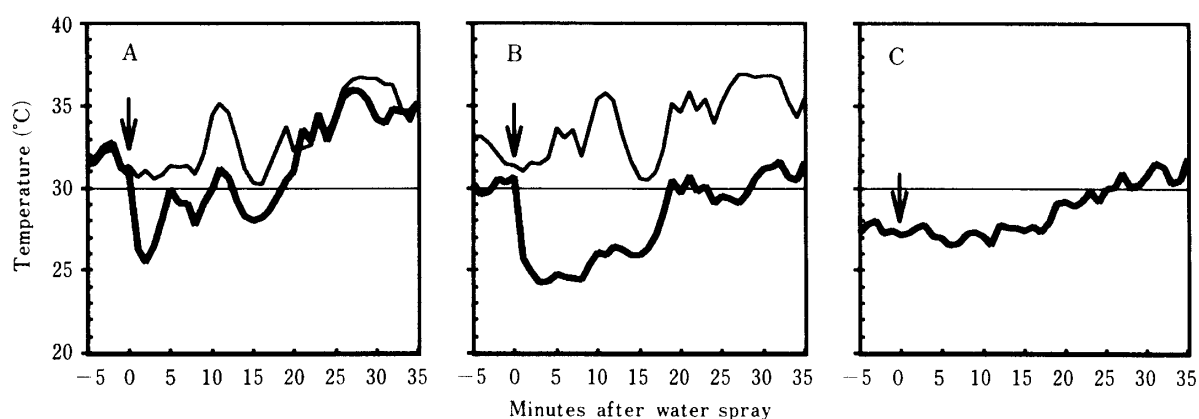


Fig. 3 Changes of temperatures on cv. Hime-tebo at the adaxial (A) and abaxial (B) surfaces of the leaflets treated with water spray (—) and the control (---), and air temperature (C). Arrows show time of water spray.

Table 1. Stomatal responses and change of water saturation deficit of lateral leaflet by water spray.

Treatment	Infiltration score ¹⁾				Water saturation deficit (%) ²⁾	
	Hime-tobe		Taisho-kintoki		Hime-tebo	Taisho-kintoki
	adaxial	abaxial ³⁾	adaxial	abaxial		
Water Spray	0.8	1.0	0.1	5.0	29.0	—
Control	0.0	0.0	0.0	4.7	27.4	—
Significance ⁴⁾	ns	ns	ns	ns	ns	—

1) Mean scores of 3 points on 2 leaflets.

2) Mean values of 4 parts on 2 leaflets of 2 trifoliolate leaves.

3) Surfaces of each leaflet.

4) ns; not significant at the 5% level.

significant. Water saturation deficit of cv. Hime-tebo was slightly larger in the spray treatment than in the control.

Discussion

Trifoliolate leaves of kidney beans inclined largely in the control (Fig. 1), indicating that the leaves were under severe water stress. This was similar to the result for soybean leaves observed by Meyer and Walker¹²⁾. Further, the response of the leaves investigated in this study showed a regularity that all the lateral (side) leaflets oriented to the terminal (central) leaflets. This regular orientation was similar to that seen in the previous study¹⁷⁾, which showed mainly a twisting response (changes of the twisting angle) of the leaflets and was understood as a sun-avoiding (paraheliotropic) movement. As plants undergo

environmental stresses such as droughts, intense radiation or high temperatures, the twisting response of lateral leaflets in kidney beans appears to occur regardless of the direction of light source. This regular response may be associated with internal factors of the pulvinule at the base of the leaflet, but it is doubtful whether it is related to morphological factors, because the lateral leaflets when exposed to a dim light, orient to the light source²¹⁾.

Before the spray treatment, the status of the leaflets that underwent the spray treatment was identical to that described for the control. Although not significant, the spray treatment of leaflets of cv. Hime-tebo led to a slight stomatal opening and to a slight rise of water saturation deficit (Table 1), indicating a slight reduction of the leaf water potential. Because,

although the reason is not known, stomatal apertures of the abaxial (lower) surface on the leaflet of cv. Taisho-kintoki were remarkably large in both treatments, values of the water saturation deficit are considered to have been higher than those of cv. Hime-tebo. Nevertheless the spray treatment caused opening responses of trifoliolate leaves (Fig. 1), although a difference of leaf inclination remained between both cultivars. The opening responses of lateral leaflets in the leaves were due to a decrease in the twisting angle of the leaflets, not to a change of the vertical angle (Fig. 2). The effect of low leaf water potentials on sun-avoiding (paraheliotropic) movements have been previously reported in several species^{1,3,6,13,18}. On the other hand, observations for the leaf of Alfalfa¹⁴ and the unifoliolate leaf of kidney beans² showed that there is no clear relationship between sun-avoiding movements and plant water potentials. It is unlikely that sun-avoiding movements of the lateral leaflet in kidney beans relates directly to the leaf water potential.

Fu and Ehleringer⁸ reported that, at air temperature above 29°C, paraheliotropic leaf movements resulted in lower leaf temperatures than air temperatures. On lateral leaflets of the control in cv. Hime-tebo, however, the leaf temperatures of both surfaces were remarkably higher than the air temperature (Fig. 3), although the leaflets showed marked twisting angles (Fig. 1a). Because the stomata were closed completely in the control (Table 1), the high leaf temperatures observed in this study might result from the inhibition of leaf transpiration³. This inhibition might further control the reduction of transpiration flows at the vascular bundle in the pulvinule. Groot⁹ has observed that, hairs covering the surface of a pulvinule are denser on the upper side than on the lower side, and a more active guttation occurs at hydathodes of the hairs on the upper side. That guttation thus appears to modulate the pulvinule temperature. In conditions of inhibited transpiration by the stomatal closure and exposed pulvinule to the solar radiation, the pulvinule of the leaflet is presumed to be under a strong heat stress.

The spray treatment caused the rapid decrease in leaf temperatures (Fig. 3), although the leaf water potential remained low (Table 1). This fact can be explained by the

cooling effect of evaporation of the sprayed water. Because pulvinules as well as leaf blades were subjected to the spray treatment, the temperature of the pulvinule is thought to decrease similarly to that of the leaf blade. The rapid decrease in the twisting angle of lateral leaflets (Fig. 2) was found to synchronize with the reduction of leaf temperature (Fig. 3). In leaflets of kidney beans, there was no twisting response when only the leaf blade was exposed to light, but there was a much greater twisting response when just the pulvinule was exposed¹⁵. The twisting response might be directly influenced by the change of temperature at the pulvinule itself. Fu and Ehleringer⁸ stated that paraheliotropic movements of the trifoliolate central (terminal) leaflet were not affected by the lamina (leaf blade) temperature but instead by the pulvinule temperature. There is no doubt that directional light induces leaf movements¹¹ and a prominent receptor in the leguminous species is located at the pulvinule^{4,15,21}. The pulvinule of lateral leaflets in kidney beans seems to be the sensor site of both light and heat (or temperature), where the vertical (sun-tracking) and twisting (sun-avoiding) responses are modulated.

Cultival differences in leaf movements have been found in several leguminous species^{14,21,22}. In the leaflet inclination of the two cultivars used in this study, the difference before the water spraying (the control) did not change after the spray treatment (Fig. 1). This cultivar difference was similar to that observed in the well-watered condition, which has shown that the twisting response of cv. Taisho-kintoki has been much greater than that of cv. Hime-tebo when they have been exposed to intense radiation and high air temperature¹⁷. Further, the leaf transpiration rate of cv. Hime-tebo was higher under intense solar radiation than that of cv. Taisho-kintoki¹⁶. A difference of cultivar leaflet movements may be explained by different responses to heat stress of the pulvinule modulated by the transpiration flows.

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