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Effect of Potassium Levels on the Stomatal Behavior of the Hemi-Parasite *Striga hermonthica*[1]

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ABSTRACT

The hemi-parasite *Striga hermonthica*, exhibits an anomalous pattern of stomatal response, stomata remaining open in darkness and when subjected to water stress. This suggests irregularity in stomatal response due to malfunction of the stomatal mechanism. To test this suggestion guard cells were isolated from the effects of surrounding cells, by incubating epidermal strips at low pH. These stomata responded rapidly to low CO_2 concentrations, darkness, and ABA. Thus, a paradox exists between stomatal behavior observed in whole leaves and that in isolated guard cells. However, when incubated in the presence of high potassium concentrations (>200 millimolar KCl) stomatal responses in epidermal strips resembled those found in whole leaves, with enhanced opening and reduced closing responses. It is suggested that the anomalous behavior of stomata in *Striga* and other leafy hemiparasites can be explained by the modulatory effects of high potassium concentrations which accumulate in the leaves as a consequence of high transpiration rates and the lack of a retranslocation system.

High transpiration rates are characteristic of both root and shoot angiosperm parasites (2, 10, 17, 23). The capture of water and solutes from the host requires a gradient of decreasing water potential toward the parasite, and high transpiration rates are thought to maintain this gradient (19). An anomalous pattern of stomatal behavior has been observed in the root hemi-parasite, *Striga hermonthica,* which showed little change in the transpiration rate in response to light intensity or water stress (17). The lack of response of *Striga* to factors which normally control stomatal behavior result in the stomata remaining open almost continuously. The high transpiration rate has been exploited in the development of antitranspirants as a potential control method (9).

In general, higher plant transpiration rates are modulated via the stomata, through which gas exchange and water loss are controlled. Stomatal aperture is regulated by a series of interdependent feedback loops (11, 12). Intercellular CO_2 concentration is a principle factor controlling gas exchange (5, 6, 11, 12). In light, depletion of intercellular CO_2 levels by the light reactions of photosynthesis stimulates opening. In the dark, respiration increases internal leaf CO_2 concentration and stomata close. Control of water loss is regulated through ABA effects on stomata. As leaf turgor pressure falls to zero ABA synthesis is induced (13, 28). ABA then acts on guard cells causing closure of the stomata by inhibition of solute uptake and increased solute efflux (13).

The behavior of *Striga* stomata could be explained by a lack of response in the guard cells to one or more of the effectors which control stomatal aperture. The purpose of this investigation was to examine the fundamental responses of *Striga* guard cells to changes in their environment.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of *Sorghum bicolor* (l.) Moench cv CSH1 were germinated by placing on moist filter paper and incubating at 30°C for 2 d. The germinated seeds were transferred to 10% Long Ashton Solution for an additional 3 to 4 d. The sorghum seedlings were then potted up in 15 cm diameter pots containing a mixture of John Innes No. 2 compost:sand:peat (7:7:3). The roots of the seedlings were surrounded by a small amount of vermiculite containing seeds of *Striga hermonthica* (collected from Abu Naam, Sudan). The *Striga* seeds were preconditioned on moist filter paper at 30°C for 3 to 4 d before planting. The plants were grown in a glasshouse with day and night temperatures of 38°C and 30°C, respectively. Supplementary lighting was provided for 16 h/d by Thorn 400W MBFI metal halide lamps (Thorn Lighting Ltd., London, UK).

Measurements of Transpiration and Stomatal Conductance in Whole Leaves

Transient exchange rates of CO_2 and H_2O were measured as described previously (10). The internal CO_2 concentrations and the stomatal conductance rates were subsequently calculated from this data using the equations described by von Caemmerer and Farquhar (24), with boundary layer resistances determined according to Parkinson (8). The area of the *Striga* leaf enclosed within the cuvette was determined at the end of the experiment using a Li-Cor portable area meter (LI-3000, Li-Cor Inc. Lincoln, NE). Light was supplied from a Hansatech light source (Hansatech Ltd., King's Lynn, UK) at a level of approximately 1000 W m^{-2} PAR unless otherwise stated. The IRGA was supplied with CO_2 balanced air (300 μL/L; BOC, London, UK). To measure the response to different CO_2 concentrations a gas diluter (GD600, Analytical Development Company, Hoddesdon, UK) was used to give concentrations from 300 to 0 μL/L.

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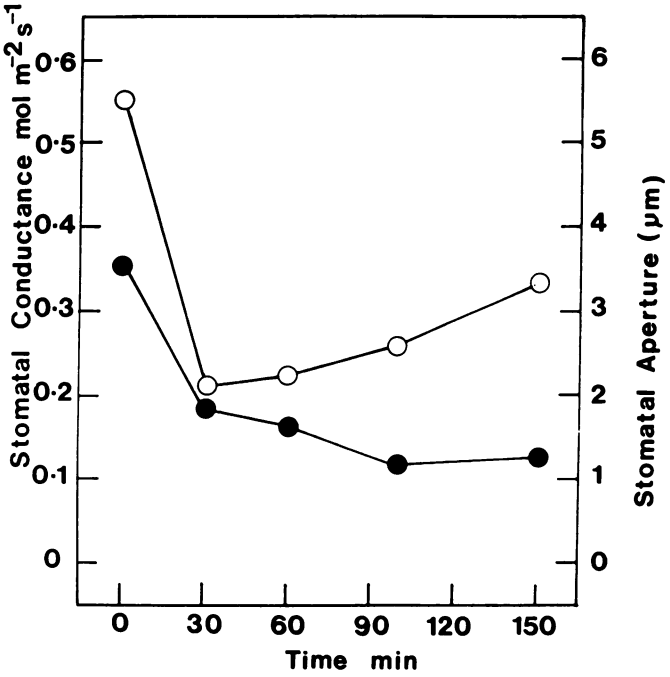

Figure 1. Effect of light on stomatal conductance (○) and stomatal aperture (●) in whole leaves of *S. hermonthica*. The lights were switched off at the start of the time course. The stomatal conductance was measured using an IRGA and the stomatal aperture estimated from Xantopren impressions of the abaxial leaf surface.

To measure the effect of water stress, leaves were removed from plants of *Striga* and from sorghum (uninfected). The leaves were weighed and allowed to dry out under a light source of 20 W m^{-2} PAR, at 25°C with weighing at regular intervals. Measurements of stomatal conductance were taken from the abaxial surface of the leaves using a Delta-T Devices Porometer (Mark II, Delta-T Devices, Cambridge, UK). Stomatal impressions were prepared from the abaxial surfaces using Xantopren Low Viscosity Silicon Impression Fluid (Beyer Dental, Leverkusen, FRG), with correction for bias according to the method of Weyers and Johansen (25).

Preparation of Epidermal Strips

The abaxial epidermis from the youngest fully expanded leaves of *Striga* was used throughout. The experiments were started at the same time each day to avoid the complicating effects of diel rhythms. All leaf material came from well-watered plants. Using a pair of fine forceps the epidermis was peeled from the underlying mesophyll tissue, floated (abaxial side up) on distilled water and trimmed to remove any mesophyll tissue.

'Isolation' of Guard Cells

The guard cells were isolated by incubating the trimmed epidermal strips in 10 mM Mes/Tris buffer (pH 3.8) (18). Viability was measured by staining with a 1 mg L^{-1} solution of Neutral Red Stain. Samples were taken at 15 min intervals and stained for 2 min. After staining the epidermal strips were rinsed with distilled water and examined using a light microscope. The numbers of viable (stained red) and nonviable cells were recorded. The epidermal strips were removed from the isolation medium when epidermal cell viability fell below 10% and the guard cell viability was not below 95%.

Incubation of Epidermal Strips

The basic incubation medium consisted of 350 mM Sorbitol; 10 mM Mes/Tris (pH 6.8) and 50 mM KCl. Gas supplies were regulated at a rate of 10^4 m^3 min^{-1}, and the incubation chambers maintained at a temperature of 25°C. Unless stated otherwise, experiments were carried out in the light (20 W m^{-2} PAR). To determine the response of guard cells to low CO_2 concentrations epidermal strips were incubated both in the presence (300 μL/L) and in the absence (<1 μL/L) of CO_2. All other incubations were carried out in the absence of CO_2. Light-dark responses were tested by incubation of epidermal strips in the dark following 1 h equilibration in light. To measure the response to ABA isolated epidermal strips were first equilibrated for 1 h in the light, and then transferred to incubation medium containing 0.1 mM of ± ABA. For subsequent investigations into the effect of KCl concentrations on stomatal responses, epidermal strips were incubated as described above, in the presence of 0 to 300 mM KCl. In all cases responses were monitored by measuring the change in stomatal aperture using an eyepiece graticule. Each reading

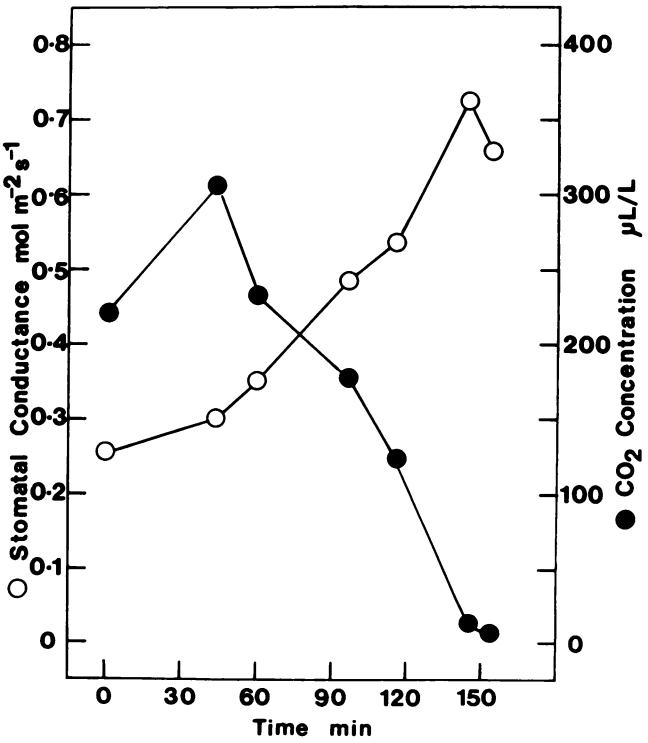

Figure 2. Effect of reducing the CO_2 concentration (●) on the stomatal conductance (○) in whole leaves of *S. hermonthica*. The stomatal conductance was measured using an IRGA connected to an ADC gas diluter which supplied CO_2 of different concentrations from an air supply enriched with CO_2.

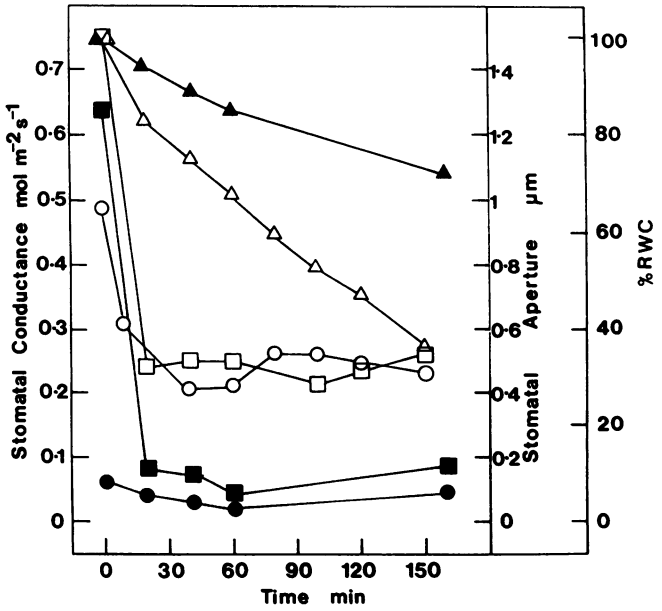

Figure 3. Effect of water stress on detached leaves of *Striga hermonthica* (open symbols) and sorghum (closed symbols). The relative water content (△), the stomatal conductance (□), and the stomatal aperture (○) were monitored throughout the experiment. Stomatal conductance measurements were taken from the abaxial surface of the leaf using a Delta-T Devices porometer. The stomatal aperture was estimated from Xantopren impressions of the lower leaf surface.

is the mean of a minimum of 30 apertures. Each experiment was duplicated a minimum of three times.

RESULTS AND DISCUSSION

Stomatal Behavior in Intact Leaves

Stomatal conductance of intact shoots dropped slightly but the stomata did not fully close when light levels were reduced (Fig. 1). Measurements of stomatal aperture changes from leaf impressions are consistent with the results for the gas exchange measurements, stomatal aperture was only partly reduced. These results confirm earlier findings (10, 17), which showed a lack of response to darkness. The results also imply a lack of stomatal response to CO_2 concentration within the leaf. However, when the CO_2 concentration was decreased, stomatal conductance was observed to increase (Fig. 2). The characteristically high stomatal conductance found in leaves of *Striga* (0.49 mol m^{-2} s^{-1} compared to 0.05 mol m^{-2} s^{-1} in the host) was reflected by the high stomatal aperture (1.81 μm compared with 0.67 μm; Fig. 3) at the start of the experiment.

Following imposition of a water deficit stomatal apertures and stomatal conductances of both *Striga* and sorghum showed an initial fall, those of the host falling somewhat before those of the parasite (Fig. 3). Stomatal conductance showed good correlation with observed stomatal aperture in both parasite and host. The stomata of sorghum were virtually closed within the first 20 min (RWC[2] 94%) but those of *Striga* were still open after 150 min (RWC 35%). The slight rise in stomatal aperture at the end of the experiment may have been due to hydropassive opening, caused by loss of turgor (12).

It would seem that while the stomata of *Striga* can respond to the opening stimulus of reduced CO_2 concentrations, their closing response to water stress and darkening of the leaf is reduced in some way.

Behavior of Isolated Guard Cells

The damped closing responses of *Striga* stomata to darkness and water stress may be the result of their reduced ability to detect, or respond to, stimuli which normally modulate stomatal aperture. To test this, guard cells were isolated within epidermal strips by selective killing of the epidermal cells. This allowed the behavior of guard cells to be studied free from the influence of surrounding cells (18, 26).

'Isolated' guard cells of *Striga* were capable of responding

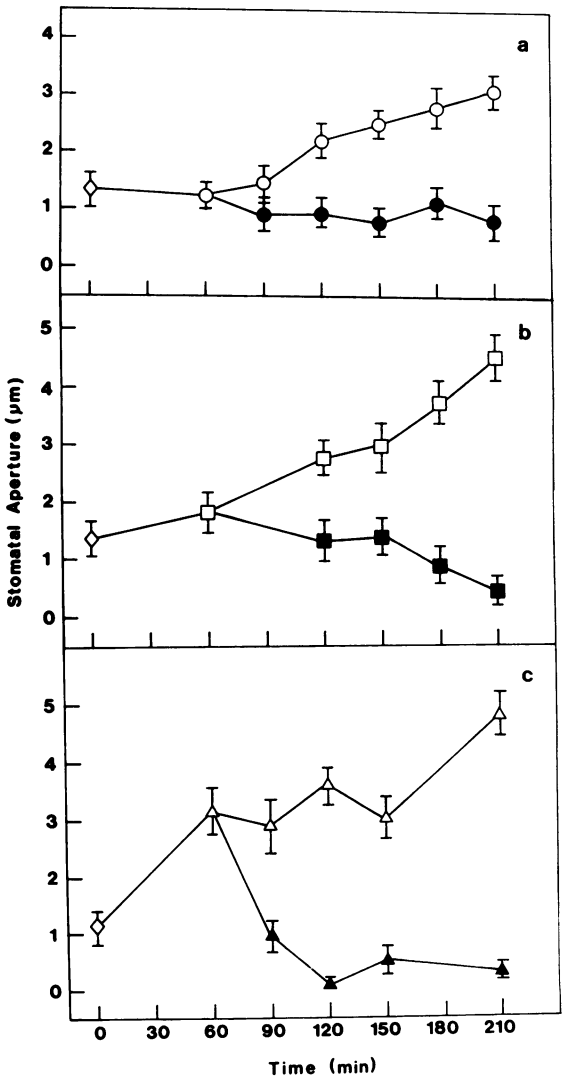

Figure 4. Response of isolated guard cells in epidermal strips of *Striga* hermonthica: a, the effect of the presence (●) and absence of CO_2 (○); b, the effect of light (□) and dark (■), epidermal strips were incubated in low CO_2; c, the effect of −ABA (△) and +ABA (▲).

[2] Abbreviation: RWC, relative water content.

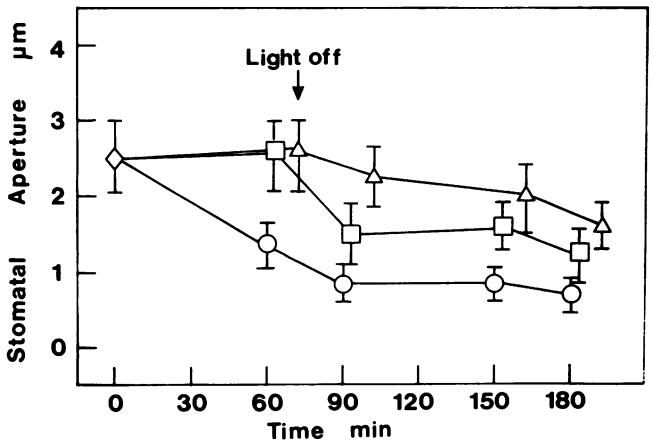

Figure 5. Effect of different KCl concentrations ([○], 0 mM KCl; [□], 50 mM KCl; and [△], 300 mM KCl) on stomatal response to darkness in epidermal strips of *S. hermonthica*.

rapidly to a reduction in CO_2 concentration (Fig. 4a), after 30 min a significant increase in stomatal aperture could be seen at low CO_2 levels. Thus low concentrations of CO_2 stimulated stomatal opening in both isolated epidermal strips (Fig. 4a) and in intact leaves (Fig. 3). Stomatal aperture decreased in the dark (Fig. 4b), even under the opening stimulus of low CO_2 (evident by the large increase in aperture in the light). Application of ABA to epidermal strips had a dramatic closing effect on stomatal aperture even when superimposed on the opening stimulus of low CO_2 concentrations (Fig. 4c). There was an initial rapid closure within the first 30 min followed by a slower decline in stomatal aperture, approaching zero within 1 h of the addition of ABA. In contrast, an exogenous application of ABA to detached shoots of *Striga* had little effect on stomatal conductance (17).

Studies with epidermal strips show that the guard cells of *Striga* respond to the major stimuli which are known to affect stomatal aperture, they respond to changes in CO_2 concentration, and to changes in light intensity which are mediated by the CO_2 response. They will also respond the major control component of the water stress response, ABA. There is then a paradox, in that while the behavior of isolated guard cells is like that of any other plant, an anomalous pattern of stomatal behavior is observed in the whole leaf.

Effect of KCl Concentration on Stomatal Behavior in Isolated Systems

The uncoupling of transpiration from environmental control which is seen with *Striga* may relate to the role played by solutes in stomatal movements. The opening and closing of guard cells is essentially a turgor driven response mediated by changes in solute concentration (4). One of the major solutes involved in stomatal movements is potassium (4, 7, 12). In the leaves of many parasitic plants (14–16, 21), including *Striga* (20), very large amounts of potassium are accumulated (up to 6% of dry weight). This occurs through a combination of high transpiration rates and lack of phloem connections which would allow the retranslocation of excess ions (3).

It is noteworthy that epidermal strips floated on distilled water rapidly lose ions into the medium (11) so that they are dependant on the medium for supplies of potassium ions. Travis and Mansfield (22) observed increased opening of guard cells in response to increased potassium concentrations in epidermal strips of *Vicia faba*. Moreover, Wilson *et al.* (27) found that increased KCl concentrations resulted in increased apertures, even in the presence of ABA. Thus high concentrations of potassium in the vicinity of the guard cells of *Striga* might provide a supply of K^+ for stomatal movements and increase the solute potential of the guard cells and surrounding tissues, maintaining a high stomatal aperture. In order to test the possibility that high potassium concentrations could explain the discrepancies in stomatal behavior between whole

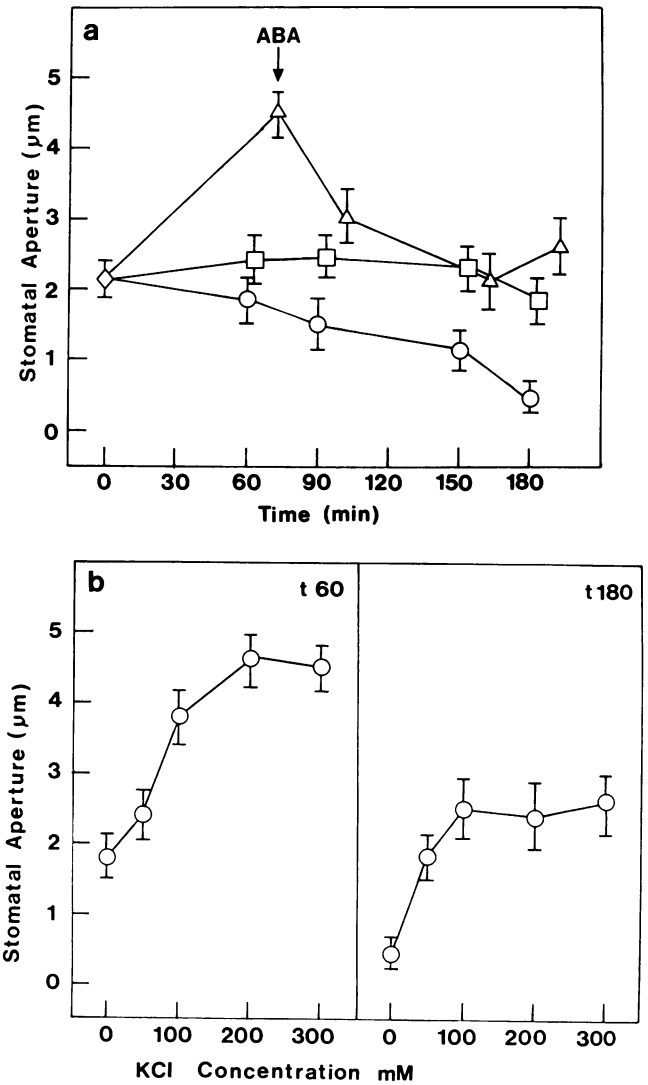

Figure 6. a, Effect of different concentrations of KCl ([○], 0 mM KCl; [□], 50 mM KCl and [△], 300 mM KCl) on stomatal response to low CO_2 and ABA in epidermal strips of *S. hermonthica*; b, Relationship between KCl concentration and stomatal aperture after 60 min incubation in light and low CO_2 followed by 120 min +ABA. The time course is shown in Figure 6a.

leaves and epidermal strips, the effect of exogenous KCl on stomatal aperture in isolated epidermal strips was investigated.

Dark-induced stomatal closure was greatest in the absence of KCl (Fig. 5), after 180 min epidermal strips incubated in 100 mM KCl or greater, had significantly higher stomatal apertures than those incubated in the absence of KCl. High KCl concentrations prevent stomatal closure in darkness. Stomatal aperture opening in response to low CO_2 (Fig. 6a206) was found to increase with increasing KCl concentration, and saturation of the opening response occurred at 200 mM KCl (Fig. 6b). Closure, induced by incubation with ABA (Fig. 6a) was greatly reduced in the presence of high KCl concentrations (Fig. 6b). Stomatal aperture at 0 mM KCl was only 0.46 μm ($\pm$0.21), whereas the epidermal strips incubated in 200 mM KCl had a mean aperture of 2.41 μm ($\pm$0.47).

The present results show that there is a close correlation between solute content of the leaf and sensitivity of the stomatal apparatus to environmental factors which control guard cell movements. During the isolation of guard cells from *Striga* leaves there appears to be a loss of the constraints imposed on them in whole leaves. This would suggest that the underlying cause of the insensitivity of the stomata to periods of water stress, darkness and application of exogenous abscisic acid does not reflect malfunctioning of the stomatal mechanism. The closing behaviour of guard cells of isolated epidermal strips in the presence of high KCl concentrations was similar to the dampened responses of guard cells of intact leaves. This effect of high KCl concentrations appears to provide an explanation of the stomatal responses in whole leaves, and could explain the high stomatal conductances and lack of closing response which have previously been reported in *Striga* (10, 17). The stomatal behavior patterns observed with *Striga* may serve as a model to explain the stomatal behavior in the leaves of other hemi-parasites such as mistletoe. These show similar high transpiration levels (2), reduced sensitivity to water deficits (2, 3, 23) and in some instances nocturnal opening (1), and have a high potassium content (3). We suggest that the accumulation of potassium modifies the response of the stomata to environmental factors, adjusting their response to light and water stress. This will have the effect of acting as a positive feed-forward control, with accumulated K^+ increasing the rate of transpiration, which, in turn, will increase the potassium influx to the leaves.

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