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Stomatal Response to Abscisic Acid Is a Function of Current Plant Water Status

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ABSTRACT

We investigated, under laboratory and field conditions, the possibility that increasing abscisic acid (ABA) concentrations and decreasing water potentials can interact in their effects on stomata. One experiment was carried out with epidermal pieces of *Commelina communis* incubated in media with a variety of ABA and polyethylene glycol concentrations. In the media without ABA, incubation in solutions with water potentials between −0.3 and −1.5 megapascals had no significant effect on stomatal aperture. Conversely, the sensitivity of stomatal aperture to ABA was trebled in solutions at −1.5 megapascals compared with sensitivity at −0.3 megapascals. The effect of the change in sensitivity was more important than the absolute effect of ABA at the highest water potential. In a field experiment, sensitivity of maize stomatal conductance to the concentration of ABA in the xylem sap varied strongly with the time of the day. We consider that the most likely explanation for this is the influence of a change in leaf or epidermal water potential that accompanies an increase in irradiance and saturation deficit as the day progresses. These observations suggest that epidermal water relations may act as a modulator of the responses of stomata to ABA. We argue that such changes must be taken into account in studies or modeling of plant responses to drought stress.

Experiments with wilty mutants (2, 24) suggest that plants need ABA to maintain a positive water balance. However, it is still not clear whether stomatal control in droughted plants can be entirely explained by modification of ABA relations or whether it is still necessary to invoke some hydraulic regulation. Several groups (5, 16) have suggested that when soil dries stomata can be controlled by chemical signals generated in the root as a function of changing root water status. However, Kramer (12) emphasized that, in the field, dehydration of leaves is a common response to soil drying and that such a change could provide a regulating influence on stomata.

Those espousing the cause of chemical communication between roots and shoots have for the most part been concerned about demonstrating the existence of chemical regulation of stomata. This is most effectively done when high leaf water potential is maintained artificially (6, 31). This is clearly not a situation found in the leaves of many plants under field conditions, where water potential can decrease substantially even if the soil is well charged with water. Many reports reveal that g_s[1] can be reduced by >90% by soil drying in the field (26). When leaf turgor is maintained, quite severe soil drying usually causes a restriction in conductance of only 40 to 50% (6, 31).

Recent investigation of the effects of dehydration treatments on gene expression in germinating seeds (14) or on growth of primary roots (19) has shown that both a modified water status and an elevation in ABA content may be required for regulation. The extent to which this is also the case in the regulation of stomatal behavior of droughted plants has not been investigated. One possibility is that current leaf water status may influence the sensitivity of the shoot responses to any ABA signal (29). The sensitivity concept has been much discussed (1, 23) but comparatively little attention has been given to the importance of sensitivity changes under the conditions generated by drought stress. In this paper we investigate the possibility that increasing [ABA] and decreasing water potentials can interact in their effects on stomata.

MATERIALS AND METHODS

Commelina communis plants were grown in a greenhouse and transferred at the four-leaf stage to a growth cabinet. Abaxial epidermis of the youngest fully expanded leaf was carefully removed and cut into 5- × 5-mm squares (15). Squares were incubated for 3 h in Petri dishes placed in a water bath at 25°C and illuminated from below with a PPFD of 200 μmol m^{-2} s^{-1}. CO_2-free air was supplied to each dish at a rate of 100 mm^3 s^{-1} through a needle dipping into the medium. All dishes contained a solution of PBS plus 0.10 mmol m^{-3} KCl. Either an ABA solution or distilled water was added to the medium to obtain a final [ABA] of 0, 50, 100, 250, or 500 μmol m^{-3}. Water potentials between −0.3 and −1.5 MPa were obtained using PEG 4000 at concentrations of 0, 80, 180, and 300 g L^{-1}. The water potential of the solution was measured after completion of the experiment using a Wescor 5100 C osmometer. After incubation, the epidermal pieces were examined under a projection microscope, and pore widths of individual stomata were measured in the 4- × 4-mm central part of the epidermal piece. Stomata (180–250) belonging to five to six pieces were examined in each treatment. The experiment was carried out three times on different batches of plants, the last experiment being repeated on 2 consecutive d. We define the sensitivity of

[1] Abbreviation: g_s, stomatal conductance.

stomatal aperture to xylem [ABA] as the slope of the response curve of aperture to [ABA].

Maize (*Zea mays* L, F_1 cv LG1) was grown in a field at Grignon near Paris, France in 1990. Variation in concentration of ABA in the xylem sap was obtained by varying soil water status and soil compaction (25, 27). To achieve this, part of the field was irrigated, part was left unwatered, and part was compacted before sowing and left unwatered afterward. Measurements of g_s and xylem [ABA] were carried out for 6 d after silking, from 6:30 to 17:30 (solar time) during periods with PPFD > 800 μmol m^{-2} s^{-1}. Abaxial and adaxial g_s were measured at eight locations on each sampled leaf, using a diffusion porometer (Delta T, Cambridge, U.K.). The calibration of the porometer was performed every 1 h in the field; g_s was fitted on calibration measurements using nonlinear regression on a hyperbola. Leaf water potential was measured using a pressure chamber. A piece of leaf blade, approximately 16 cm^2, was cut and kept in liquid nitrogen for measurements of osmotic potential (using a Wescor 5100 C osmometer). Approximately 150 μL of sap was then extracted with a pressure approximately 0.5 MPa greater than the balancing pressure. Sap was frozen and stored until analysis, carried out using the radioimunoassay method (18). Previous experiments suggested that measured xylem [ABA] were probably closely related to those in the xylem of intact plants (26). Sampled leaves were those inserted on the first or the second node above the ear, receiving perpendicular radiation.

RESULTS

Experiment with *Commelina* Epidermis

In the media without ABA, stomatal aperture ranged from 15 to 22 μm, with an insignificant tendency to increase when water potential decreased from −0.3 to −1.5 MPa (Fig. 1). The response curve of stomatal aperture to ABA had the classical negative-exponential-like shape, but its sensitivity strongly depended on the water potential of the medium. A mean aperture as small as 2.7 μm was observed with 100 μmol m^{-3} [ABA] at −1.5 MPa, whereas it was 11.7 μm at the same [ABA] at −0.3 MPa. The effect of the change in sensitivity of g_s between −0.3 and −1.5 MPa was, therefore, quantitatively more important than the absolute effect of ABA at −0.3 MPa. In the three experiments, the influence of decreasing water potential on restriction in stomatal aperture was approximately the same (Fig. 2).

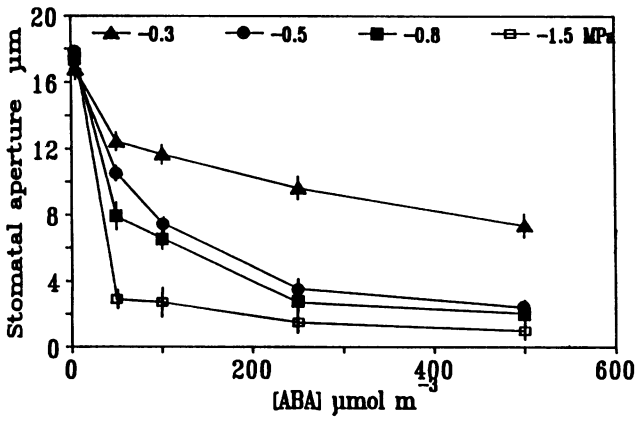

Figure 1. Apertures of *C. communis* stomata in detached epidermis as a function of the [ABA] in the incubating medium, at each of four water potentials. Each point, average of 200 to 300 measurements from five to six epidermal pieces in the third experiment (see text); bars, intervals of confidence (P = 0.05).

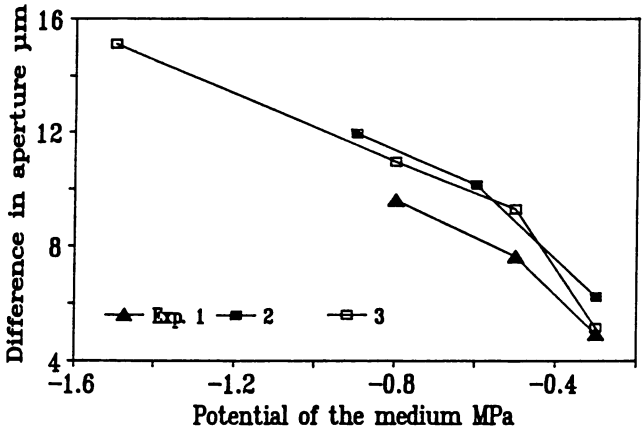

Figure 2. Difference in stomatal aperture between epidermal pieces of *C. communis* placed in media with [ABA] of 0 or 100 μmol m^{-3}, as a function of the water potential of the media. Each line, one experiment.

Field Experiment with *Zea mays*

The meteorological data and the daily time courses of g_s and leaf water status for a typical day during the period under study are shown in Figure 3. Leaf water potential and turgor decreased steadily from 3:00 to 13:00 and remained approximately constant afterward. Although the predawn value of was higher in the irrigated treatment, day time water potential and turgor did not differ significantly between treatments after 6:00. g_s reached its maximum value from 7:30 onward, with a rapid further decline in the nonirrigated and in the compacted treatments.

g_s was related to the xylem [ABA], with curvilinear relationships which depended on the time of the day (Fig. 4). We showed previously (26) that g_s was not related to water potential or to leaf turgor and that common relationships applied between g_s and xylem [ABA] applied for different days and for soils with different mechanical properties or water status. Although the relationship between [ABA] and g_s was less tight from 7:30 to 9:00 than later in the day, the apparent sensitivity of stomata to ABA markedly increased with time of day. This can be deduced from observation of the cloud of points, where high values of g_s could be observed in the morning for [ABA] as high as 180 μmol m^{-3} and from the fitted curves corresponding to different times of the day (Fig. 5). The residuals (difference between fitted and observed values) in the general regression of g_s with xylem [ABA] are an indicator of stomatal sensitivity to xylem [ABA]. They were positively correlated to the leaf water potential but also with the air vapor pressure

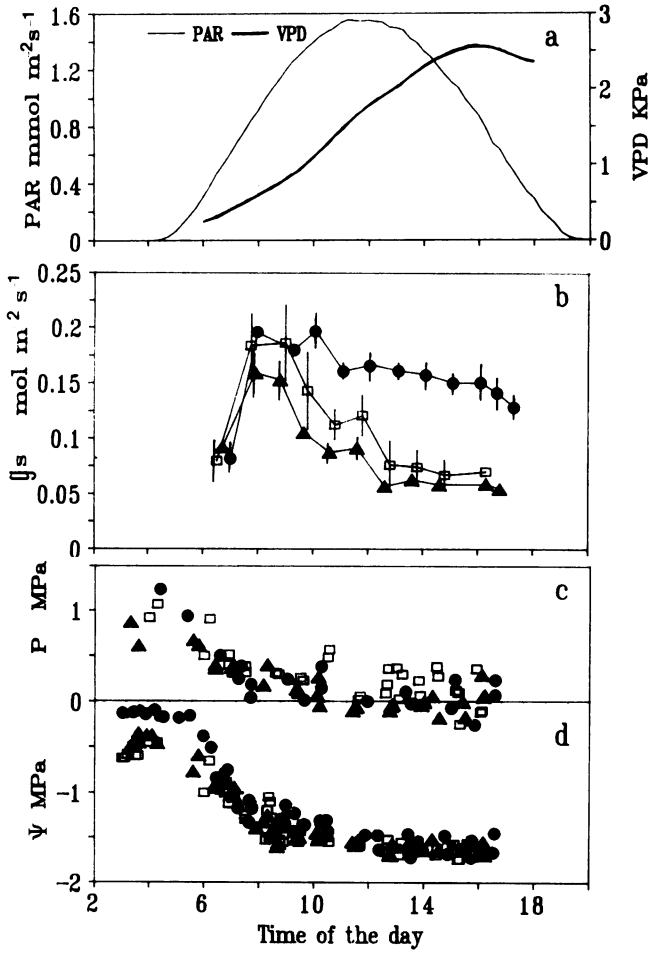

Figure 3. Meteorological data: PAR and vapor pressure deficit (VPD) (a); daily time courses of maize g_s (b); turgor (P) (c); and leaf water potential (Ψ) (d) in the field for a typical day after silking. b: Each point, average of 20 measurements taken on abaxial and adaxial faces of the leaf; bars, intervals of confidence (P = 0.05). c and d: Each point, measurement for one leaf. ●, Irrigated soil; □, nonirrigated soil; ▲, nonirrigated and compacted soil.

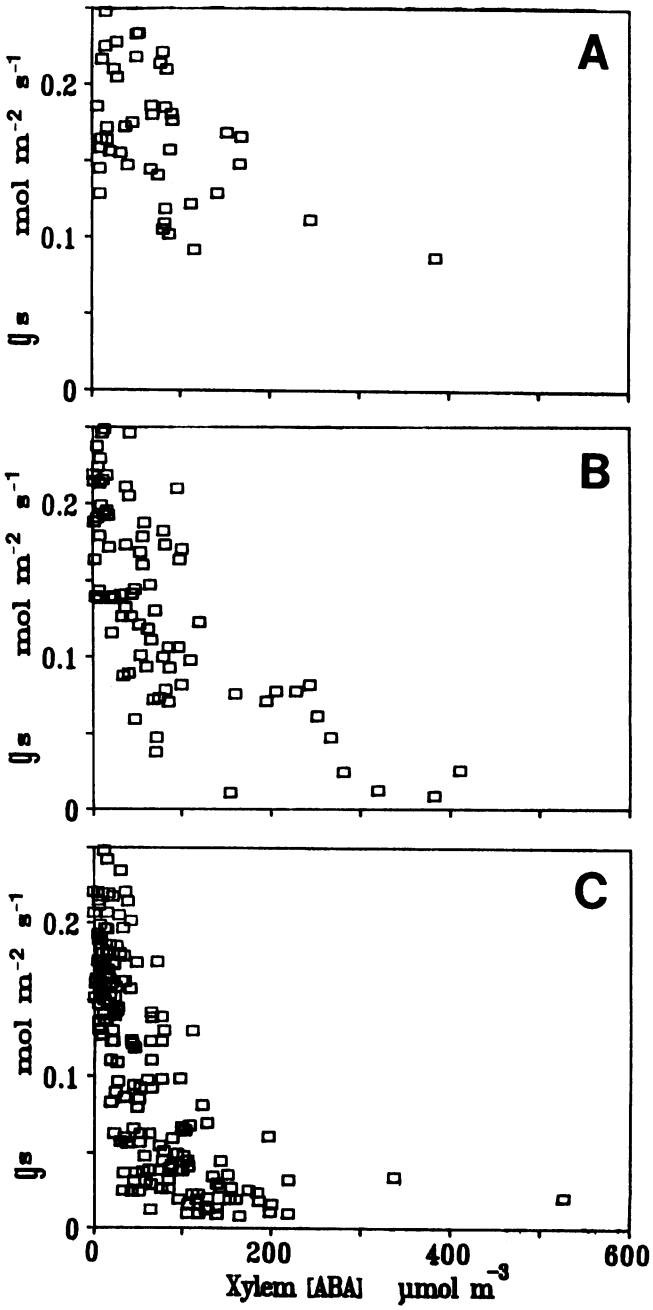

Figure 4. Relationship between maize g_s and [ABA] in the xylem sap at several times of the day. Measurements carried out for 6 d after silking, in periods with PAR 800 μmol m^{-2} s^{-1}. a, 7:30 to 9:00; b, 9:00 to 12:30; c, 12:30 to 16:30. Each point, coupled [ABA] and g_s measurement for one leaf.

deficit and the air temperature. Conversely, they had no relationship with irradiance or with changes in pH or in calcium content of the xylem sap.

DISCUSSION

Shackel and Brinkmann (22) showed that, as stomata open and close in response to environmental stimulus, guard cell and epidermal water contents change rapidly and reversibly. The traditional view (22) is that epidermal water status could integrate a multitude of environmental signals, mediating sensitive control of stomatal behavior. We found, however, that isolated *Commelina* epidermis did not transduce water status into a hydroactive stomatal response in the range of water potentials between −0.3 and −1.5 MPa. Grantz and Schwartz (8) noted the same lack of response of *Commelina* epidermis to water potential but reported that incubation of

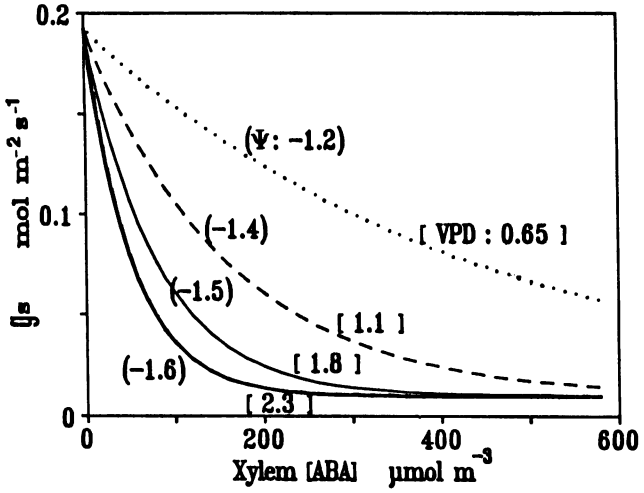

Figure 5. Fitted curves (negative exponentials) on the relationship between g_s and [ABA] at several times of the day. Numbers in parentheses, mean leaf water potential (Ψ) during the period of the day; numbers in brackets, mean vapor pressure deficit (VPD). · · · · ·, 7:30 to 9:00; —, 9:00 to 11:30; ——, 11:30 to 13:00; ___, 13 to 16:30.

leaf discs in the same solutions did cause stomatal closure. They concluded that such responses are mediated by chemical signals that originate from outside the epidermis.

It is now generally accepted that stomatal behavior can be modified by ABA originating from the mesophyll of the leaves or arriving from the roots in the xylem stream. Doubts concerning the importance of such signals have arisen, to some extent, because of the often poor correlation between the intensity of the signal and the stomatal response, particularly in the field (3, 17). As an illustration of this situation, a graph resulting from the superimposition of the three parts of Fig. 4 would suggest a poor correlation between xylem [ABA] and g_s. Our experiments with isolated epidermis from *Commelina* leaves (Figs. 1 and 2) show that the extent of the stomatal response to any ABA signal will depend upon the water status of the epidermis. Although the water potentials used here had no direct effect on stomatal aperture, a reduction in water potential amplified the effect of a given concentration of ABA (Fig. 2).

On the basis of these results, one would predict that, as water potential declines with time of day toward a minimum in middle to late afternoon (Fig. 3), we should see an increase in the sensitivity of the stomatal response to any ABA in the xylem stream. This seems to be the case with stomatal responses of maize plants in the field (Fig. 5), but there are several other possible explanations for a change with time in the apparent stomatal sensitivity to xylem [ABA]. Another explanation for these results could be the increase in ABA flux into the leaf which will result from increasing saturation deficits. Even if xylem [ABA] is constant, increased transpiration rates at higher saturation deficits later in the day will result in increased delivery of ABA from the xylem stream to the stomatal complexes. Grantz (7) suggested that this increase should result in the metabolic adjustment that is responsible for the stomatal response to humidity (13). Our own experiments and calculations of ABA flux made from this data set do not indicate that increased fluxes of given [ABA] result in increasing restriction of stomatal opening (F. Tardieu, J. Zhang, N. Katerji, in preparation). Because other possible explanations such as change of pH or in calcium content in the xylem sap (21) also failed to account for the increase in sensitivity later in the day, the interaction between epidermal leaf water potential and ABA remains the most likely explanation. Decreased epidermal water potentials in dry air will sensitize stomata to ABA arriving in the transpiration stream.

The mechanistic interpretation of the change in apparent stomatal sensitivity cannot be thoroughly discerned from the data presented here. Among other causes, this change may be due to an increased sensitivity of the ABA receptors in the guard cell subjected to low water potential or to a nonlinear relationship between the guard cell turgor and the stomatal aperture which would cause a greater effect of ABA if the turgor is already affected to some extent by the water potential. One other possible cause of an apparently enhanced sensitivity of stomata to ABA could be an increase in the [ABA] near the guard cell sensors, as a result of a redistribution from sites in the plant where ABA can be sequestered (11). Such sites include the the mesophyll chloroplasts and the guard cell cytoplasm (9) which can store large amounts of ABA which will not affect stomatal aperture until it is released to the apoplasm, because the active sites for ABA action are on the outside of the guard cell plasmalemma (10). This redistribution hypothesis cannot explain the deficit-enhanced increase in sensitivity observed in the detached epidermis experiment. If increased response to ABA was a function of ABA release from guard cells, we would expect reduced stomatal opening in epidermal strips incubated at low water potentials without ABA. Such stomatal closure was not observed. This may be because ABA released from the guard cells was rapidly diluted in the incubating solution, but if this is the case, it still cannot be argued that ABA release is the cause of increased sensitivity. We cannot rule out that, in the field experiment, the apparent sensitivity of g_s to xylem [ABA] could be increased by such a mechanism and by the redistribution and extra synthesis of ABA in the mesophyll experiencing low water potential.

The interaction between the ABA signal and the variation in leaf water potential provides an explanation for the commonly observed reduction in leaf conductance shown by droughted plants during the midday and afternoon hours but not in the morning (Fig. 3) (28). The extent of soil drying will provide an ABA signal which may be a relatively constant or even decreasing concentration throughout the day (27) if ABA is supplied by the roots to an increasing flux of water through the transpiration stream. Stomata may open during the early hours of the day because of their relative insensitivity to this signal at this time. Saturation deficit will be comparatively low, and therefore, the efficiency of water use can be high because insolation will also be high. As the day progresses, saturation deficit will increase such that a reduced leaf water potential will increase stomatal sensitivity to the ABA signal. Stomatal closure at higher saturation deficits later in the day may act to optimize water use (4).

These results cast doubt on many calculations of a fixed

amount of ABA required for initiating stomatal closure (30). These calculations are based on experiments performed on highly turgid leaves and as such are probably underestimates of the sensitivity of leaves under most conditions. We suggest a role for xylem ABA as an integrator of edaphic and climatic effects on stomata. Epidermal water relations are involved as a modulator of the xylem ABA response but do not have a controlling influence in their own right as suggested by Schulze (20). It has been argued that a regulatory signal from cells less exposed to the environment than the epidermal cells would provide a stabilizing influence on g_s (8). Observations that g_s is dynamically linked to climatic variation argues for a role for sensitivity changes as a modulating influence on root signals of edaphic responses.

CONCLUSION

The observation that apparent stomatal sensitivity to the ABA signal depends on the epidermal water status somewhat complicates the study of root-to-shoot communication, because a straightforward relationship between ABA supply and g_s cannot entirely explain the responses that we see. Studies or modeling of plant and soil water relations should therefore take into account the leaf or epidermal water potential as well as the ABA supply by roots. These results perhaps go some way toward reconciling the views of those who argue the respective merits of chemical and hydraulic regulation of stomatal behavior (12).

Observations of the kind reported here could not have been made in studies carried out using experimental systems in which leaf water potential is maintained at a high value artificially or because of low irradiance or saturation deficit. These are conditions usually prevailing in controlled environment chambers. In the field, leaf water potential often varies over a wide range and sometimes rapidly with changes in irradiance and saturation deficit. Sensitivity changes reported here may be important for crop physiologists, modelers, and those interested in using variation in ABA production and physiology as a criterion in selections for drought tolerance.

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