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Stomatal Response to Light of *Solanum pennellii*, *Lycopersicon esculentum*, and a Graft-induced Chimera[1]

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ABSTRACT

To learn how species differences in stomatal behavior are regulated, the response of epidermal and leaf diffusive resistance to light was investigated in *Lycopersicon esculentum* Mill., *Solanum pennellii* Corr., and a periclinal chimera having an *S. pennellii* epidermis and an *L. esculentum* mesophyll that was produced from a graft of the two species. *S. pennellii* has about 23% fewer stomata per square millimeter than does *L. esculentum*, and the two species have contrasting stomatal sensitivities to light. The abaxial stomata of *L. esculentum* open in dimmer light and to a greater extent than the adaxial stomata. The abaxial and adaxial stomata of *S. pennellii* respond similarly to light incident on the adaxial epidermis and are less open at all quantum flux densities than comparable stomata of *L. esculentum*. The patterns of response to light of the abaxial and adaxial stomata of the chimera were practically identical to those of *L. esculentum*, and quite unlike those of *S. pennellii*. Thus, the pattern of stomatal light response in the chimera was regulated by the *L. esculentum* mesophyll. The reduction in stomatal frequency of the chimera, which was regulated by the epidermis of *S. pennellii*, contributed to the 40% difference in leaf diffusive resistance between the plants in moderate light.

Reduction of loss of water by plants has been sought to conserve soil moisture (16), to increase survival of plants during drought (9), and to improve plant establishment following transplanting (6). The apertures of the stomata have been modified with chemicals to reduce transpiration (16, 17), and film-forming and reflective leaf coatings have been used as antitranspirants to diminish water loss (9).

Despite an increasing understanding of stomatal physiology, few genetic modifications of plants are known that can be applied to modify stomatal functioning (13). Transpiration of leaves has been experimentally reduced by genetic modification of stomatal frequency or aperture (2, 7). Mutations of tomato that result in altered hormone balance and stomatal functioning are well known (10, 13), but all mutations heretofore reported result in increased, not diminished, water loss.

Ion fluxes and hormonal activities have interdependent roles in modifying stomatal movements (5, 11). The recent report that ABA synthesized in the mesophyll may regulate stomatal response to water stress (3) and the close correlation between stomatal activity and ABA content of unstressed leaves (4) suggests need for further knowledge of the roles of the leaf epidermis and leaf mesophyll in stomatal responses to environment.

We have examined the effect of stomatal frequency on leaf diffusive resistance, and have investigated the roles of the epidermis and mesophyll in the stomatal response to light using graft-produced chimeral plants having the epidermis and stomata of *Solanum pennellii* Corr. and the mesophyll of tomato, *Lycopersicon esculentum* Mill.

MATERIALS AND METHODS

Plant Material. A periclinal chimera (8) composed of *S. pennellii* Corr. and *L. esculentum* Mill. produced earlier (1) and propagated vegetatively was used. *S. pennellii* reputedly is an extremely drought-tolerant relative of tomato from Peru (12), and was used as the scion. The stock of tomato, *L. esculentum*, was homozygous for the white flower and the entire-leafed genes.

The chimera exhibited a leaf shape like that of the entire-leaved tomato stock, which therefore comprised the leaf interior. A photographic comparison of leaf appearance is available (1). There were two types of evidence that the epidermis of the chimera arose from *S. pennellii*. The first was the presence on the epidermis of the chimera of secretory glandular hairs characteristic of *S. pennellii*, but absent on the tomato stock. Because corolla pigmentation in these species is confined to epidermal cells, the color of the flower petals in tomato and *S. pennellii* gave evidence of the epidermal origin. The flowers of the tomato stock were white, and those of *S. pennellii* yellow. The periclinal chimera exhibited the yellow flower color, thus confirming transmission of the epidermis in the draft. The reciprocal graft of the epidermis of *L. esculentum* on the leaf interior of *S. pennellii* was sought but not obtained.

Plants for stomatal investigations were propagated from cuttings, transplanted into potting mix, and cultured in a controlled environment room at 26 ± 1 C, 55 ± 5% RH, in a 16-hr photoperiod with a quantum flux density of 800 μE m^{-2} sec^{-1} (400–700 nm) incident on the uppermost leaves. Several clonal plants of the scion, stock, and chimera were established to provide uniform test material.

Stomatal Measurements. The stomatal frequency was measured at × 430 magnification from negatives of the epidermes made with an acrylic emulsion polymer. The number of stomata in 45 fields, five fields in each of nine negatives obtained from the center leaflet of three leaves on each of three plants was counted to obtain the frequency.

Water loss of detached center leaflets of test plants was measured by periodic weighings on an analytical balance. Leaflets were detached 4 hr after the start of the photoperiod, and the petiole was immediately sealed with silicone rubber. The leaflets were suspended in the turbulent wake of a fan in the illuminated controlled environment room used for plant growth. Leaf areas were measured with a model AAM-5 Hayashi Denko automatic area meter.

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The stomatal diffusive resistances of adaxial and abaxial epidermal surfaces were measured with a ventilated diffusion porometer (15). Individual measurements were completely randomized, and initiated at least 4 hr after onset of the photoperiod. Diurnal patterns of stomatal activity were absent. Opposing adaxial and abaxial surfaces of center leaflets of leaves were sampled at random and at 10-min intervals to allow the unsampled epidermis to recover from the pressure of the porometer clamp. Varying levels of quantum flux density on the leaves were provided by interposing screens between the lights and the plants before the initiation of the photoperiod. Immediately after each measurement of diffusive resistance, the light incident upon the adaxial epidermis was measured with a Lambda quantum meter, model LI-185, held with the sensor parallel to the leaf surface.

Leaf resistance was calculated by assuming that the two leaf epidermes acted as parallel resistors. The hyperbolic response of leaf or epidermal diffusive resistance to quantum flux density was fitted with the linear transformation $Ir = Ir_{min} + I_m r_{min}$ (14), where I is the measured quantum flux density, r the measured leaf or epidermal resistance, r_{min} the minimum leaf or epidermal resistance, and I_m the quantum flux density at $2r_{min}$.

RESULTS

Water Loss by Detached Leaves. Initial rates of water loss in detached, drying leaves were equal in *L. esculentum* and *S. pennellii* (Fig. 1). Following 12 min of exposure, the rate of water loss of *L. esculentum* leaflets exceeded that of *S. pennellii*, a difference that was maintained throughout the experiment. By the end of the experiment (85 min) *L. esculentum* leaflets had lost 24% of their initial fresh weight, but *S. pennellii* leaflets only 14%.

Water loss by excised leaflets of the chimera was initially as rapid as *L. esculentum*, but the pattern of loss became similar to *S. pennellii* within 12 min of excision. After 85 min, leaflets of the chimera had lost 16% of their initial fresh weight.

Stomatal Frequency. *L. esculentum* and *S. pennellii* each showed the usual pattern of having more stomata on the lower (abaxial) than on the upper (adaxial) leaf surface (Table I). Although *L. esculentum* had more stomata than *S. pennellii* on the upper and lower leaf surfaces, both species apportioned about 40% of their total stomata onto the upper epidermis.

Although the leaflet area of the chimera was similar to that of *L. esculentum* and 4-fold greater than the area of *S. pennellii* (Table I), the distribution of stomata was contrary to our expectation that the chimera would show a distribution of stomata like *S. pennellii*, the source of the chimeral epidermis. In contrast with *L. esculentum* and *S. pennellii*, the chimera apportioned its stomata about equally on upper and lower epidermes.

Diffusive Resistance. The responses to light of the epidermal diffusive resistances of stock and scion plants were greatly different (Figs. 2 and 3). Diffusive resistance of *L. esculentum* responded to quantum flux density as in another amphistomatous dicot, to-

Table I. Abaxial and adaxial stomatal frequencies, and leaflet areas, of L. esculentum, S. pennellii, and their periclinal chimera.

	Leaflet Area (cm^2)	No./mm^2 Adaxial epidermis	No./mm^2 Abaxial epidermis
L. esculentum	32.1 ± 2.4[1/]	119 ± 5	166 ± 4
S. pennellii	6.6 ± 0.4	89 ± 2	132 ± 3
Chimera	26.2 ± 2.4	94 ± 3	89 ± 2

[1/] Mean ± standard deviation.

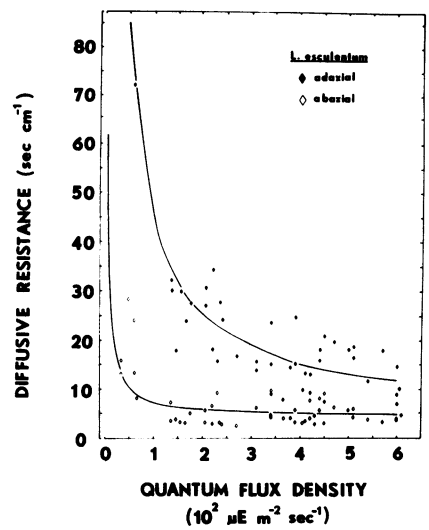

FIG. 2. Response to quantum flux densities incident on adaxial epidermis of diffusive resistance of adaxial and abaxial epidermes of *L. esculentum*. Lines were fitted by linear transformation of a rectangular hyperbola as described in the text.

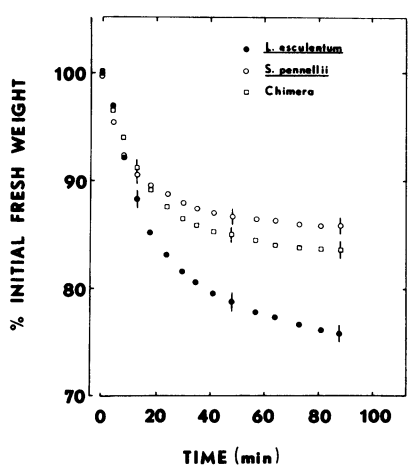

FIG. 1. Rate of water loss from detached drying leaflets of *L. esculentum* (●), *S. pennellii* (○), and periclinal chimera (□). Vertical bar indicates SE of three experiments.

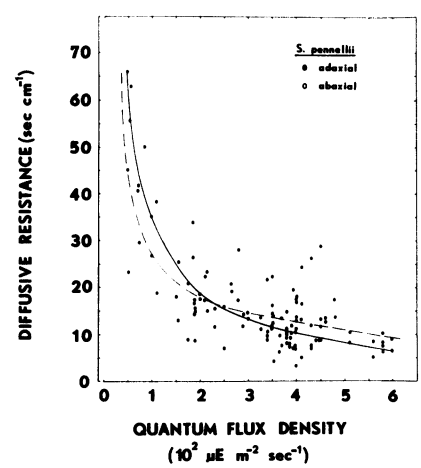

FIG. 3. Response to quantum flux densities incident on adaxial epidermis of diffusive resistance of adaxial and abaxial epidermes of *S. pennellii*. Lines are fitted as in Figure 2.

bacco, for which the upper and lower epidermes exhibited distinctly different behavior (14). The stomata of the lower epidermis opened in dimmer light, and to a greater extent, than those of the upper epidermis. Abaxial diffusive resistance was unresponsive to light greater than 200 μE m^{-2} sec^{-1}, whereas adaxial diffusive resistance was still responsive at the strongest quantum flux density measured, 600 μE m^{-2} sec^{-1}.

In *S. pennellii,* the two epidermal resistances responded similarly to light incident on the adaxial epidermis (Fig. 3), and neither were saturated at a quantum flux density of 600 μE m^{-2} sec^{-1}. Thus, the stomata of both epidermal surfaces of *S. pennellii* respond to light as do the upper surface stomata of *L. esculentum* and tobacco (14).

In the chimera the responses to light of the diffusive resistances of upper and lower epidermes were like those of *L. esculentum,* and quite unlike those of *S. pennellii* (Fig. 4). Thus, in leaflets of the chimera, the mesophyll of the tomato must figure prominently in the light responsiveness of the stomata.

A hyperbolic model fit the responses to light of lower epidermes and leaves better than those of upper epidermes, with coefficients of determination ranging from 0.08 to 0.76 for leaves and from 0.30 to 0.74 for lower epidermes (Table II). The hyperbolae fit the responses of *S. pennellii* more poorly than those of *L. esculentum* and the chimera, which is probably attributable to the unusual light response of *S. pennellii.*

Treating the upper and lower epidermes as parallel conductors allowed comparison of leaf diffusive resistances (Table II). Like their respective epidermes, leaves of *L. esculentum* and *S. pennellii* differed qualitatively in response of diffusive resistance to light, with *L. esculentum* stomata opening more in dim light than those of *S. pennellii.* It is now apparent that the pattern of stomatal light response of the chimera is governed by *L. esculentum,* the donor of the tissue between the epidermes. Compared with *L. esculentum,* the reduction in stomatal frequency of the chimera, which was conditioned by the epidermis derived from *S. pennellii,* contributed to the 34% increase in leaf diffusive resistance at 300 μE m^{-2} sec^{-1} and 48% increase in leaf diffusive resistance at 600 μE m^{-2} sec^{-1} (Table II).

Table II. Minimum resistance (r_{min}), the quantum flux density at $2r_{min}$ (I_m) and the coefficient of determination (R^2) for the regression of leaf or stomatal resistance and quantum flux density. For comparisons among species, values of resistance predicted from the regression $r = I_m r_{min}/(I+r_{min})$ at I = 300 and 600 $\mu E\ m^{-2} sec^{-1}$ are listed in the last two columns.

	r_{min}	I_m	R^2	Resistance ($sec\ cm^{-1}$) at	
				300	600
	$sec\ cm^{-1}$	$\mu E\ m^{-2} sec^{-1}$		$\mu E\ m^{-2} sec^{-1}$	
L. esculentum					
Leaf	2.6	164	0.50	4.1	3.3
Adaxial surf.	5.4	732	0.20	18.7	12.1
Abaxial surf.	4.4	67	0.53	5.3	4.8
S. pennellii					
Leaf	1.3	1450	0.08	7.5	4.5
Adaxial surf.	2.4	1390	0.05	13.2	7.8
Abaxial surf.	7.5	294	0.30	14.5	11.4
Chimera					
Leaf	4.5	65	0.76	5.5	4.9
Adaxial surf.	10.2	296	0.30	20.2	15.2
Abaxial surf.	7.0	33	0.74	7.8	7.4

DISCUSSION

The measures of the water loss of detached, drying leaflets (Fig. 1) substantiate the earlier report (12) that detached leaves of *S. pennellii* desiccate more slowly than leaves of *L. esculentum.* The factors that confer this resistance to desiccation are apparently sexually transmitted (12), but the techniques of vegetative propagation used in this study also suggest some possible physiological controls.

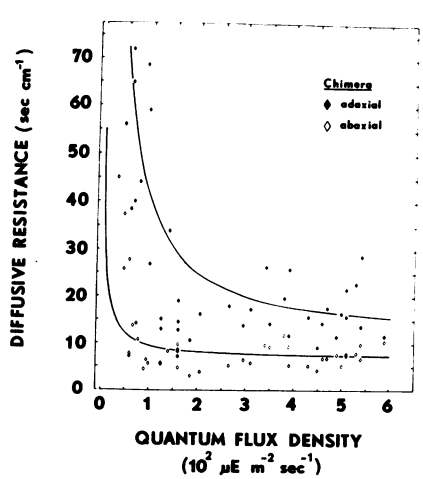

FIG. 4. Response to quantum flux densities incident on adaxial epidermis of diffusive resistance of periclinal chimera having leaf interior of *L. esculentum* bounded by epidermes of *S. pennellii.* Lines fitted as in Figure 2.

Leaflets of a periclinal chimera produced by grafting desiccate more slowly than *L. esculentum* in a pattern reminiscent of *S. pennellii,* the donor of the epidermis (Fig. 1). Part of the desiccation resistance is undoubtedly attributable to the reduced stomatal frequency in the chimera (Table I). Compared with *L. esculentum,* leaflets of the chimera averaged 44% fewer stomata on the lower epidermis and 21% fewer stomata on the upper epidermis.

Interestingly, the chimera also exhibited 22% fewer stomata on the lower epidermis compared with *S. pennellii,* the epidermal donor, although the stomatal frequencies on the upper epidermes were equal (Table I). This was unexpected, since we initially anticipated that the chimera might have stomatal frequencies like *S. pennellii.* The results therefore suggest a different pattern of stomatal differentiation for each epidermis of the chimera; the difference in stomatal frequency was unrelated to the 4-fold difference in leaf area between *S. pennellii* and the chimera.

Epidermal characteristics have been sexually transmitted to modify transpiration (2, 7), but the chimera reveals that some physiological controls reside in the leaf mesophyll. The pattern of stomatal response to light is mediated by the mesophyll. This is amply illustrated by the pattern obtained for a graft of the epidermis of *S. pennellii* with one pattern of stomatal light response onto the leaf interior of *L. esculentum* with a dissimilar stomatal light response. The contrasting patterns of stomatal response might be attributable to different intercellular space CO_2 concentration, or to a differential activity of a hormone mediating stomatal sensitivity to CO_2 or ion flux into guard cells (11).

The latter explanation is plausible because of previous reports of mutants of ABA activity in *L. esculentum* that are conditioned by a single gene (10, 13). Because ABA is reportedly synthesized in the mesophyll (3), and stomata in epidermal strips of tomato mutants deficient in ABA lack qualitative closing responses to darkness and plasmolysis (13), it seems unlikely that a particular pattern of stomatal response to an environmental factor will be consistently genetically transmitted through the epidermis. This further suggests a conservative interpretation for stomatal responses to external stimuli using epidermal tissue devoid of communication with the mesophyll.

Although the contrasting stomatal responses to light may con-

tribute little to the differences in water loss by detached leaves, it is highly probable that the stomata of *L. esculentum, S. pennellii,* and the chimera may be responding differently to leaf turgor. Again, ABA synthesis in the mesophyll and transport to the epidermis (3) may be one of many explanations advanced. Besides the reduced stomatal frequency and unusual stomatal response to light, hormonal changes induced by leaf turgor may be another mechanism conditioning the reported drought tolerance (12) of *S. pennellii.*

Periclinal chimeras may illustrate one way of making vegetatively propagated plants more frugal of water. In our *L. esculentum-S. pennellii* chimera, savings of water would be attributable to lower stomatal frequency rather than to a change in the response of leaf diffusive resistance to light. Experimentally, there are advantages to combining genetically unique leaf interiors with different genetically unique epidermes. This will facilitate inquiries regarding the exchange of water, ions, and growth substances between mesophyll cells and the stomatal apparatus.

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