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Gas Exchange, Stomatal Behavior, and $\delta^{13}C$ Values of the *flacca* Tomato Mutant in Relation to Abscisic Acid[1]

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ABSTRACT

The relationship between stomatal conductance and capacity for assimilation was investigated in *flacca*, a mutant of tomato (*Lycopersicon esculentum* Mill.) that has abnormal stomatal behavior and low abscisic acid (ABA) content. The assimilation capacity, determined by measuring assimilation rate as a function of intercellular CO_2 pressure, did not differ in leaves of *flacca* and its parent variety, Rheinlands Ruhm (RR). On the other hand, stomatal conductance of *flacca* leaves was greater than that of RR, and could be phenotypically reverted by spraying with 30 micromolar ABA. Stomatal conductance of *flacca* leaves was also reduced by increasing CO_2 pressure, increasing leaf to air vapor pressure difference, and decreasing quantum flux, irrespective of ABA treatment.

The high conductance of *flacca* leaves resulted in a high intercellular CO_2 pressure. This allowed greater discrimination against $^{13}CO_2$, as evidenced by more negative δ ^{13}C values for *flacca* as compared to RR. The δ ^{13}C values of both *flacca* and RR plants as influenced by ABA treatment were consistent with predictions based on gas exchange measurements, using a recent model of discrimination.

Two important factors influencing the assimilation rates of leaves are stomatal conductance and mesophyll assimilative capacity. The former is determined by stomatal frequency and by reversible movements of the guard cells. The latter is determined by the amounts and activities of enzymes, light harvesting structures, and electron transport components of the chloroplasts. A close relationship has been found between stomatal conductance and photosynthetic capacity under a variety of environmental and nutritional conditions (28, 29), suggesting that these components of leaf CO_2 assimilation are coordinated. The possible mechanisms underlying this coordination are poorly understood (8).

ABA can influence stomatal conductance without affecting photosynthetic capacity (4, 12, 19). Cowan *et al.* (3) have suggested that ABA may tend to adjust stomatal conductance in accordance with current photosynthetic rate. This hypothesis is difficult to test directly, inasmuch as the proposed mechanism involves changes in the distribution of ABA between the mesophyll and the guard cells without requiring variation in total ABA content. Furthermore, there are no known specific inhibitors of ABA synthesis to allow chemical manipulation of endogenous ABA levels. To circumvent these problems, we have utilized the *flc*[4] tomato mutant, which contains only 10 to 20% of the ABA found in its isogenic parent line, Rheinlands Ruhm (26). This deficiency is the apparent cause of the unusually high stomatal conductance of *flc* leaves, as spray applications of ABA completely normalize the mutant (11). This is often cited as evidence that stomatal behavior, particularly in response to water stress, is endogenously regulated by ABA (14). However, stomata respond to many factors in addition to leaf water deficit, such as CO_2, light, and humidity (8, 18), and the extent to which ABA affects these responses is unclear. We have measured stomatal conductance of *flc* plants under a variety of environmental conditions, both with and without supplementary ABA, to investigate the role of ABA in stomatal regulation *in situ*. By simultaneously measuring assimilation rates under the same conditions, we investigated whether ABA might be involved in coordinating stomatal conductance and photosynthetic capacity.

As a result of the correlation between assimilative capacity and stomatal conductance, C_i tends to be conserved. While gas exchange studies give information about C_i at the time of measurement, the model of Farquhar *et al.* (7) can be used to estimate the assimilation-weighted average C_i throughout the plant's life from measurements of the δ ^{13}C value of assimilated carbon. We have compared C_i values of normal and mutant plants, as determined from gas exchange measurements, with those predicted from the δ ^{13}C values of the tissues.

MATERIALS AND METHODS

Plant Material. Seeds of *flc* and its isogenic parent line, *Lycopersicon esculentum* Mill. cv Rheinlands Ruhm, were obtained from Dr. C. M. Rick, Department of Vegetable Crops, University of California, Davis. Seeds were germinated in vermiculite in the greenhouse and seedlings transplanted to 4-L pots of screened soil and sand (2:1) after 14 d. The pots were watered daily with a complete nutrient solution and twice daily with tap water. Conditions in the greenhouse were: 25°C day, 15°C night, 50 to 80% RH (uncontrolled), natural photoperiod (January to March, 1982; average, 14-h day). The greenhouse was shaded so that the maximum quantum flux was 1,000 μE m^{-2} s^{-1} (PAR). Shading was necessary to prevent severe wilting and leaf desiccation in *flc* plants. Under these conditions, the *flc* plants generally maintained turgor, with slight wilting occasionally occurring in the late afternoon (2).

ABA Treatment. Stock solutions were prepared by dissolving

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[4] Abbreviations: *flc*, *flacca*; C_i, calculated intercellular CO_2 partial pressure; RR, Rheinlands Ruhm; g_e, epidermal (stomatal + cuticular) conductance to water vapor; g, total conductance to water vapor; VPD, leaf to air vapor pressure difference; C_a, ambient CO_2 partial pressure; A, net CO_2 assimilation rate; E, transpiration rate; I, quantum flux (PAR).

13.2 or 39.6 mg (±) ABA in 5 ml ethanol and diluting with distilled H_2O to 50 ml. These solutions were then diluted 1:100 to produce the desired concentration (10 or 30 μM). The spray solution contained 0.01% (w/v) Triton X-100 as surfactant. Control plants received sprays containing 0.1% (v/v) ethanol and 0.01% Triton X-100. The stock solutions were stored at 4°C in the dark and renewed every 10 d. Plants of both RR and *flc* were sprayed daily from transplantation (1 to 2 visible leaves) until the end of the experiment.

Gas Exchange. The open gas exchange system utilized a clamp-on chamber which enclosed 2.4 cm^2 of leaf area. CO_2 and water vapor exchange were measured separately for upper and lower surfaces by CO_2- and H_2O-sensing IR gas analyzers (Beckman model 865 and ADC model 225, respectively). Humidity of the ingoing air was regulated by a condenser at 18°C and CO_2 concentration was controlled by injecting CO_2 into the CO_2-free air supply through a mass-flow controller and measured by a third IR gas analyzer (Hartman and Braun URAS 2). Light from a xenon arc lamp passed through 5 cm of water and a glass IR filter before reaching the leaf. Intensity was varied by interposing metal screens between the light source and the leaf. Leaf temperature was controlled by the water-jacketed cuvette, and was measured by a thermocouple appressed for a length of 1.5 cm to the abaxial leaf surface. The air flow rate was 0.7 L min^{-1}, and boundary layer conductances to the diffusion of water vapor, measured with wet filter paper, were 0.98 and 1.33 mol m^{-2} s^{-1} for the upper and lower surfaces, respectively. Gas exchange parameters were calculated according to von Caemmerer and Farquhar (27). The rate of RuBP regeneration was calculated from the model of Farquhar and von Caemmerer (9), using a value for the CO_2 photocompensation point (Γ_*) of 30 μbar (6). All conductance data except those in Figure 4 refer to stomatal plus cuticular conductance (epidermal conductance or g_e). Values given for gas exchange parameters are the total for both sides of the leaf for the projected area enclosed by the chamber.

As the abnormal stomatal behavior of *flc* has been reported to revert to normal as the plants age (25), gas exchange measurements were begun 2 weeks after transplanting, when the plants had 8 to 10 leaves. Measurements were made on the terminal leaflet of the fourth oldest leaf, which was just approaching its final length (2). The gas exchange system was equipped with two chambers, allowing replicate plants to be measured simultaneously. Plants were removed from the greenhouse at dawn and the leaves fitted into the measurement chambers. The quantum flux was gradually increased to 1,000 μE m^{-2} s^{-1} (PAR), which was just saturating for photosynthesis (data not shown). Leaf temperature was maintained at 23.0 ± 0.1°C, VPD at 11.7 ± 0.3 mbar, and C_a at 340 μbar. Approximately 1 h was required under these conditions for transpiration and A to stabilize. An initial measurement was then made which will hereafter be referred to as being under 'standard' conditions. Other parameters remaining constant, C_a was then varied from 50 μbar stepwise to 950 μbar. After altering the CO_2 concentration, 30 min was allowed to reach a new steady state. This was generally sufficient time to allow stomatal movement to cease, although some hysteresis in stomatal responses to CO_2 was observed. The measurement protocol was strictly adhered to so that any hysteresis would affect all treatments in the same sense. Dark respiration was determined at the end of the day with C_a = 340 μbar. In the experiment with 10 μM ABA, two plants from a treatment were measured in the morning and two more from the same treatment in the afternoon. In the experiment with 30 μM ABA, the same two plants were measured at I = 1,000 μE m^{-2} s^{-1} in the morning and at I = 250 μE m^{-2} s^{-1} in the afternoon. After measurements were made on a given treatment, the spraying treatments were reversed and the measurements repeated 4 d later to determine whether short-term reversal of the treatment effects would occur.

Responses to humidity were measured with the plant in a controlled environment of 24°C, 18 mbar VPD, and 650 μE m^{-2} s^{-1} PAR. The terminal leaflet of the fourth leaf was enclosed in an aluminum chamber that was temperature controlled by Peltier heating and cooling. The leaf temperature was measured with a thermocouple probe appressed to the leaf for 2 cm. The chamber air was stirred by a fan to give a boundary layer conductance >2.5 mol m^{-2} s^{-1}. Assimilation was measured by a mass flow controller (Tylan, FC 260) which injected 1% CO_2 to compensate CO_2 uptake. Humidity was regulated by adjusting the flow of dry air through the chamber with additional mass flow controllers. Evaporation rate was calculated by multiplying the total air flow by the mole fraction of water in the air leaving the chamber (measured with a Vaisala Humicap sensor).

Carbon Isotope Composition. Samples derived from whole shoots or roots were analyzed as described previously (5). The assimilation-weighted C_i/C_a was calculated from Farquhar *et al.* (7) using

$$C_i/C_a = \frac{\delta^{13}C_{PDB} + 12.2}{22.6} \quad (1)$$

The predicted transpiration ratio (E/A) was then calculated from

$$E/A = \frac{1.6 \times VPD}{C_a(1-C_i/C_a)} \quad (2)$$

Stomatal Frequency. Counts of stomata per unit area were made on the leaves used for gas exchange measurements. Leaf discs were fixed in 2.5% glutaraldehyde and 1% OsO_4, dehydrated in acetone, critical point dried in liquid CO_2, and sputter-coated with gold for viewing in the scanning electron microscope. Stomatal counts were made from photographs or the microscope screen itself at a magnification × 340.

Statistical Analysis. Data were subjected to analysis of variance and the main effects of variety and ABA and their interaction tested by single degree of freedom comparisons (21).

RESULTS

Gas Exchange. Assimilation rates under standard conditions were not significantly different between control RR and *flc* plants, although those for *flc* tended to be slightly higher (Tables I and II). ABA at 10 μM had a small but nonsignificant effect on A, while 30 μM caused a 15% reduction in both varieties. As expected, g_e of *flc* was more than double that of RR in the absence of ABA (Tables I and II). Conductance of RR plants was unaffected by 10 μM ABA, but the same concentration reduced g_e of *flc* plants by 36% (Table I). The higher concentration of ABA reduced g_e to the same value in both RR and *flc* (Table II). These effects of A and g_e are reflected in the calculated values for C_i. Under standard conditions, C_i of RR leaves was 235 μbar while that of *flc* leaves was 275 μbar (Tables I and II). When ABA reduced g_e, C_i fell as well. At a quantum flux of 250 μE m^{-2} s^{-1}, A was approximately one-half the value at 1,000 μE m^{-2} s^{-1} for all treatments. Conductance declined such that C_i increased only slightly when quantum flux was reduced, the increase being greater in RR than in *flc* (Table II).

The assimilation rates as a function of C_i were identical, within experimental error, for the four treatments at both light intensities (Fig. 1; similar data for I = 1,000 μE m^{-2} s^{-1} from the experiment with 10 μM ABA not shown). The initial slopes of the *A versus* C_i curves at low C_i did not differ significantly among treatments, although they were affected by light intensity (Tables I and II). The apparently lower A in the ABA-treated plants at high C_i and high light was also not statistically significant. The points obtained under the initial standard conditions are indicated by arrows in Figure 1. The variations in C_i described above caused the operating point to move along the *A versus* C_i curve.

Table I. *Effect of 10 μM ABA Spray on Gas Exchange Parameters of RR and flc Tomato Plants*

Means are shown ± SE ($n = 4$). Values in a column followed by the same letter are not significantly different ($P < 0.05$).

Treatment	A[a]	g_e[a]	C_i[a]	dA/dC_i[b]	Dark Respiration
	$\mu mol\ m^{-2}\ s^{-1}$	$mol\ m^{-2}\ s^{-1}$	μbar	$\mu mol\ m^{-2}\ s^{-1}\ \mu bar^{-1}$	$\mu mol\ m^{-2}\ s^{-1}$
RR control	21.2 ± 0.9 a	0.37 ± 0.02 a	232 ± 6 a	0.14 a	1.4 a
RR + ABA	20.9 ± 0.3 a	0.39 ± 0.02 a	239 ± 3 a	0.13 a	1.2 a
flc control	23.8 ± 1.1 a	0.91 ± 0.04 b	277 ± 2 b	0.12 a	1.1 a
flc + ABA	21.0 ± 1.0 a	0.58 ± 0.04 c	264 ± 2 c	0.12 a	1.3 a

[a] Initial measurements: 340 μbar CO_2, 23°C, 11.7 mbar VPD, 1,000 μE $m^{-2}\ s^{-1}$.

[b] Slope of linear regressions for combined data ($C_i < 100$ μbar) of four replicates per treatment.

Table II. *Effect of 30 μM ABA Spray on Gas Exchange Parameters of RR and flc Tomato Plants at Two Light Intensities*

Values within a column followed by the same letter are not significantly different ($P < 0.05$). $I = 250$ and 1,000 μE $m^{-2}\ s^{-1}$.

Treatment	A[a]		g_e[a]		C_i[a]		dA/dC_i[b]		Dark Respiration ($I = 0$)
	1,000	250	1,000	250	1,000	250	1,000	250	
	$\mu mol\ m^{-2}\ s^{-1}$		$mol\ m^{-2}\ s^{-1}$		μbar		$\mu mol\ m^{-2}\ s^{-1}\ \mu bar^{-1}$		$\mu mol\ m^{-2}\ s^{-1}$
RR control	21.6 a	10.4 a	0.40 a	0.25 a	235 a	263 a	0.15 a	0.087 a	1.3 a
RR + ABA	18.4 b	10.4 a	0.28 b	0.16 b	220 b	232 b	0.13 a	0.092 a	1.6 a
flc control	22.8 a	10.4 a	0.81 c	0.32 a	274 c	276 c	0.12 a	0.069 a	1.7 a
flc + ABA	19.1 b	9.4 b	0.27 b	0.14 b	220 b	224 b	0.14 a	0.078 a	2.0 a

[a] Initial measurements: 340 μbar CO_2, 23°C, 11.7 mbar VPD.

[b] Slope of linear regressions for each treatment and light intensity for $C_i < 120$ μbar. No significant difference among treatments; difference between light intensities highly significant ($P < 0.01$).

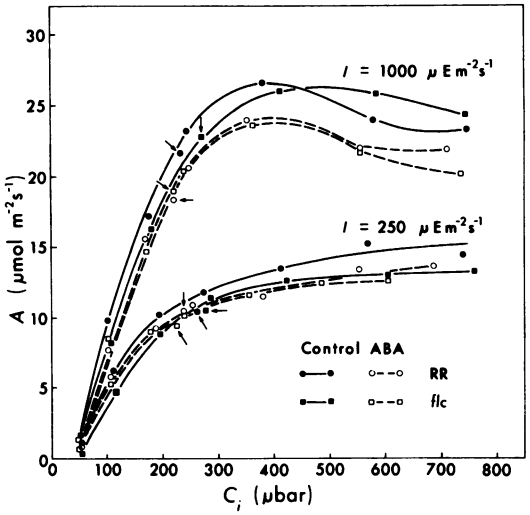

FIG. 1. Assimilation rates of RR and *flc* tomato plants at two quantum fluxes as a function of C_i. The points indicated by arrows were obtained under the initial standard conditions. Data are means of two replicate plants per treatment. For statistical analysis, see Table II. ABA = 30 μM.

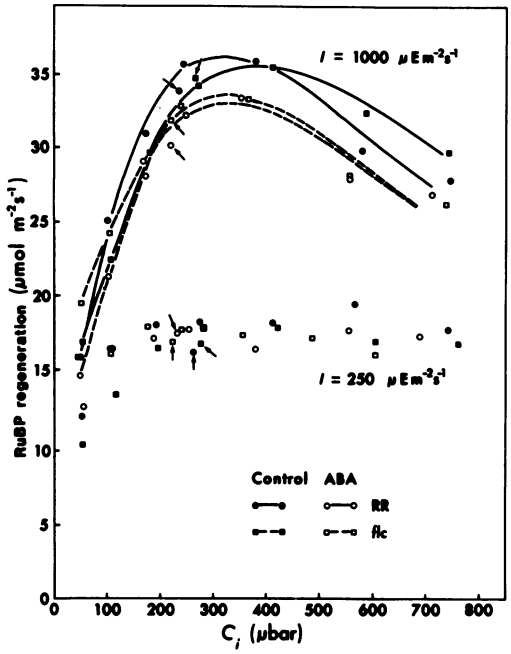

FIG. 2. Calculated rates of RuBP regeneration of RR and *flc* tomato plants at two light intensities as a function of C_i. The points indicated by arrows were obtained under the initial standard conditions. Data are means of two replicate plants per treatment. ABA = 30 μM.

At low light, A increases continuously with C_i, as would be expected because of the substitution of carboxylations for oxygenations (6). The rates of RuBP regeneration required to support the observed A, estimated from A, C_i, and dark respiration rate (9), remained constant at $C_i > 150$ μbar (Fig. 2). A may have been partially limited by RuBP regeneration rate at all C_i, which would explain the light dependence of the initial slopes of the A *versus* C_i curves referred to above. At saturating quantum flux, the rates of RuBP regeneration declined markedly at $C_i > 400$ μbar, suggesting that some unknown factor was limiting A in this region (Fig. 2). We have repeatedly observed this phenomenon in tomato, and others have found it in a variety of species (27; K. C. Woo and S. C. Wong, personal communication). There were no differences

among the treatments in this respect. The operating points when C_a = 340 μbar in both high and low light were near the point where the RuBP regeneration rate was maximal.

The rate of assimilation was sensitive to transpiration rate, as has been observed in other species by Ball (1). In untreated *flc* plants, increasing E from 7 mmol m^{-2} s^{-1} to 15 mmol m^{-2} s^{-1} (by changing ambient humidity) caused the capacity of the mesophyll for photosynthesis to fall by 25% at C_i = 250 μbar. Stomatal conductance of plants sprayed with 30 μM ABA (RR and *flc*) and control RR plants responded so strongly to humidity and CO_2 that E could only be increased from 5 to 8 mmol m^{-2} s^{-1}. In those cases, only a slight effect of evaporation rate on mesophyll capacity for assimilation was observed (data not shown).

Carbon Isotope Composition. Farquhar *et al.* (7) have derived a relationship between the assimilation-weighted average C_i and the $\delta^{13}C$ value of the tissue. From the measured $\delta^{13}C$ values, C_i/C_a was calculated for each treatment (Table III). There was close agreement between the observed and predicted values of C_i/C_a, particularly for *flc* plants. The predicted values of C_i/C_a tended to be greater than the measured values in RR plants. As was noted previously, C_i was less conserved with changes in light intensity in RR than in *flc*, leading to an increase in C_i of RR plants at low I (Table II). The quantum flux densities in the greenhouse were between 500 and 800 μE m^{-2} s^{-1} for most of the day, reaching 1,000 μE m^{-2} s^{-1} only for a short time near midday. It is likely that C_i was greater than that measured at 1,000 μE m^{-2} s^{-1} for much of the day, leading to the increase in isotope discrimination. The ability of *flc* plants to keep C_i constant with varying light intensity (Table II) is supported by the similarity between predicted and observed C_i/C_a values (Table III).

A further prediction of the model (7) is that $\delta^{13}C$ values should be correlated with the ratio of E to A. When measured under standard conditions, E/A varied in the expected manner (Table III). Both $\delta^{13}C$ and E/A were greater in *flc* than in RR, and when ABA decreased g_e, these parameters also fell.

The roots of all plants were enriched in ^{13}C, compared to the shoots, by, on average, 1.1‰, confirming an earlier observation in tomato by Park and Epstein (16). Differences in dark respiration rates were small and showed no consistent relationship among treatments (Table I and II).

Stomatal Behavior. Conductances to water vapor in the dark were 0.15 ± 0.02 mol m^{-2} s^{-1} in *flc* and 0.045 ± 0.004 mol m^{-2} s^{-1} in the remaining three treatments, in agreement with observations that *flc* plants have high transpiration rates at night (2, 22). Dark g_e values of *flc* could be varied between these extremes within 4 d by beginning or discontinuing ABA treatments. Presence or absence of ABA had little effect on g_e values of RR in the dark. These results indicate that cuticular conductance in both varieties was ≤0.045 mol m^{-2} s^{-1}, and that the higher g_e in *flc* plants is due to incomplete stomatal closure (11, 22). Control RR plants in high light and low C_i had a maximum g_e of 0.62 mol m^{-2} s^{-1}, which decreased to 0.38 mol m^{-2} s^{-1} at C_i > 400 μbar (Fig. 3). There was little further closure of stomata at C_i > 400 μbar, as has been reported previously for tomato (15). ABA (30 μM) reduced g_e in RR by about 0.15 mol m^{-2} s^{-1} without substantially altering the shape of the curve. The same pattern, with reduced magnitudes, is evident at low quantum flux (Fig. 3).

Stomata of *flc* also responded to CO_2 at high quantum flux, and the change in g_e was of similar magnitude to that in RR (Fig. 3). However, g_e of *flc* continued to decline as C_i increased. This could be due in part to the slower closing response to increase in CO_2 observed in *flc*. ABA (30 μM) completely normalized the stomatal behavior of *flc*, including the shape of the CO_2 response curve (ABA at 10 μM had an intermediate effect; Table I). The change in g_e upon reducing light intensity was greater in *flc* than in RR. Thus, the responses of *flc* stomata to both light and ABA were considerably greater than those of RR, while the response to CO_2 was approximately the same in the two varieties. The effects of ABA on g_e of both *flc* and RR plants were largely reversible within 4 d (data not shown).

In very humid air (<10 mbar VPD), stomata of RR did not respond to CO_2 (Fig. 4). When the VPD was increased to 18 to 25 mbar, stomata became sensitive to CO_2. ABA (30 μM) caused the conductance to be lower under all conditions, but did not alter the relationships between conductance, CO_2, and humidity. In *flc*, conductance responded very strongly to humidity, dropping almost 50% for a change from 6 to 20 mbar VPD. However, at the higher VPD, stomata failed to close further as CO_2 was elevated. ABA treatment resulted in relationships between conductance, CO_2, and humidity that were similar to those of RR.

The differences in g_e between RR and *flc* may be partly attributable to variation in the areal frequency of stomata. Control *flc* leaves had 78% more stomata per unit area on the adaxial surface and 63% more on the abaxial surface that did RR leaves (Table IV). The very high g_e of *flc* leaves may therefore be due in part to greater stomatal frequency. Treatment with ABA during leaf

Table III. *Carbon Isotope Composition and Predicted and Measured Values of C_i/C_a and E/A for RR and flc Plants as Influenced by ABA Sprays*

Within each experiment, values in a column followed by the same letter are not significantly different ($P < 0.05$). First experiment, n = 4; second, n = 3.

Treatment	$\delta^{13}C$	C_i/C_a		E/A	
		Predicted[b]	Observed[c]	Predicted[b]	Observed[c]
	‰[a]				
RR	−28.94 a	0.741	0.682 a	212	170 a
RR + 10 μM ABA	−28.94 a	0.741	0.703 a	212	183 a
flc	−30.60 b	0.814	0.815 b	296	293 b
flc + 10 μM ABA	−29.86 c	0.781	0.776 c	251	244 c
RR	−29.61 a	0.770	0.691 a	239	178 a
RR + 30 μM ABA	−29.08 b	0.747	0.647 b	218	154 b
flc	−30.54 c	0.812	0.806 c	293	249 c
flc + 30 μM ABA	−29.24 b	0.754	0.647 b	224	153 b

[a] Isotope composition relative to Pee Dee Belimnite.

[b] Predicted values are assimilation-weighted averages over the natural range of quantum flux, calculated according to Eq. 1 and 2 with 11.7 mbar VPD.

[c] Observed values are from spot measurements at 1,000 μE m^{-2} s^{-1}, 11.7 mbar VPD.

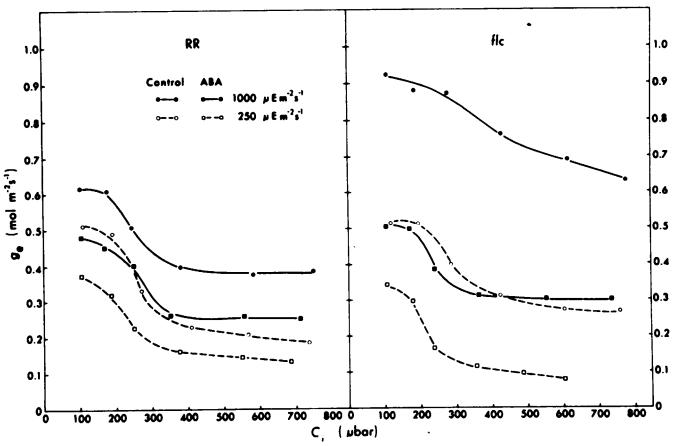

FIG. 3. Conductances of RR (left) and *flc* (right) tomato leaves as influenced by CO_2, quantum flux, and ABA. ABA = 30 μM. Statistical differences between treatments are indicated in Table II.

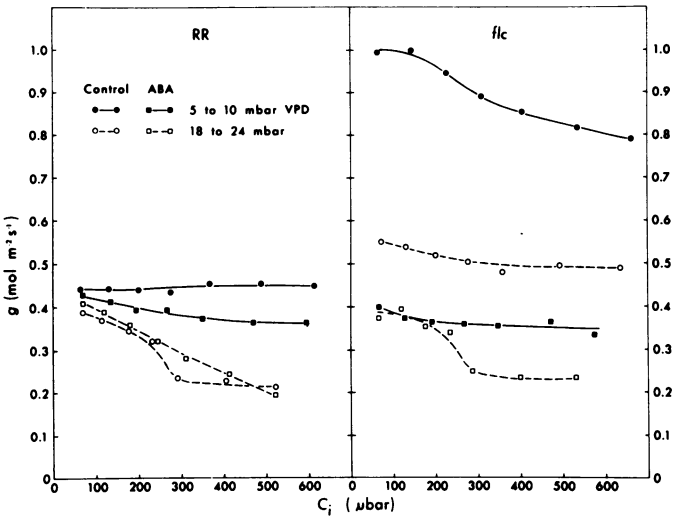

FIG. 4. Conductance of RR (left) and *flc* (right) tomato leaves as influenced by CO_2, VPD, and ABA. ABA = 30 μM.

Table IV. *Stomatal Frequency on Leaves of RR and flc Tomato Plants as Affected by ABA Spray*

Values in a column (± SE) followed by the same letter are not significantly different ($P < 0.05$).

Treatment	10 μM ABA		30 μM ABA	
	Adaxial	Abaxial	Adaxial	Abaxial
		mm^{-2}		
RR control	67 ± 7 a	140 ± 17 a	51 ± 5 a	156 ± 24 a
RR + ABA	68 ± 12 a	159 ± 26 a	47 ± 1 a	201 ± 6 b
flc control	118 ± 8 b	243 ± 23 b	92 ± 3 b	228 ± 6 b
flc + ABA	100 ± 7 b	181 ± 11 ab	54 ± 4 a	187 ± 10 ab

development caused some reduction in stomatal frequencies on both leaf surfaces in *flc*, but actually increased stomatal frequency on the abaxial surface of RR. Nonetheless, g_e of RR plants treated with 30 μM ABA was less than that of control plants, even though stomatal frequencies were greater after ABA treatment. Conductance of mature leaves of *flc* could also be reduced to the normal range by short-term application of ABA. Consequently, there was no consistent relationship between g_e and stomatal frequency.

DISCUSSION

Previous investigations of the relationship between g_e and A have involved variations in leaf age, quantum flux, nutrition, or water status (e.g. 29). These parameters can all influence the mesophyll capacity for photosynthesis (8, 27). Conductance has generally been found to correlate with this capacity. In the present study, the *flc* tomato mutant was utilized to establish different steady-state conductances under identical environmental and nutritional conditions. Although wilty by nature, due to their high g_e (22), the *flc* plants were grown under relatively mild environmental conditions which allowed the maintenance of some turgor (2). Thus, variation in the factors known to affect photosynthetic capacity were minimized to test whether variation in g_e alone would alter mesophyll function. In other words, given the high g_e of *flc* leaves, would mesophyll photosynthetic capacity increase, maintaining a constant C_i, or would photosynthetic capacity remain constant with consequent increase in C_i? The data presented in Tables I and II and Figure 1 support the latter possibility. The A *versus* C_i curves of *flc* and RR plants were indistinguishable, despite the more than 2-fold difference in g_e. When g_e was reduced by ABA treatment, the A *versus* C_i curves were only marginally affected (Fig. 1), but C_i was lower at all values of C_a (data not shown). Previous studies have found no effect of ABA on photosynthetic capacity in short-term feeding experiments (4, 12, 13, 19). The present results extend this conclusion to the case where ABA has been applied throughout leaf ontogeny.

The relationships between g_e and A measured by gas exchange techniques were confirmed by the patterns in carbon isotope discrimination (Table III). The *flc* and RR plants differ in only one genetic locus (22), and the assimilative characteristics of their leaves are identical (Figs. 1 and 2). Thus, differences in $\delta\ ^{13}C$ composition can reasonably be ascribed to the contrasting stomatal behavior. The similarity of ABA effects on g_e and on $\delta\ ^{13}C$ values support this conclusion. These data complement those of Farquhar *et al.* (5) with beans and mangroves in supporting the empirical accuracy of Eq. 1. Furthermore, E/A and $\delta\ ^{13}C$ are correlated in the correct sense, supporting the suggestion (7) that $\delta\ ^{13}C$ composition may be a useful comparative measure of the water use efficiency during growth.

The increase and subsequent decline in calculated RuBP regeneration rates with increasing C_i (Fig. 3) indicates that some unknown factor is limiting photosynthesis at high C_i in these plants. RR plants attain photosynthetic and RuBP regeneration rates 50% greater than those reported here when grown at a light intensity of 2,000 μE m^{-2} s^{-1} (Bradford, unpublished). Similarly, maximum photosynthetic capacity of *Phaseolus vulgaris* plants was greater when grown under high light intensity (27). The fall in RuBP regeneration rate at high C_i did not appear to be related to a diurnal variation in assimilative capacity, or to the amount of carbon previously assimilated, as has been reported in some cases (10).

Tal and coworkers (25) found that *flc* plants contain only 10 to 20% as much ABA as RR plants, even though the *flc* plants are often wilted, a condition that normally stimulates ABA synthesis. They have proposed that the variety of morphological and behavioral consequences of the mutation stem from this deficiency in ABA, inasmuch as the aberrations all revert to normal with ABA treatment (11, 23). Stomata of *flc* remain partially open in darkness and during water stress (22), but close under these conditions when supplied with ABA (12). We have confirmed that g_e of *flc* plants is 3-fold greater than that of RR in the dark, and that this difference is eliminated by ABA. Stomata of *flc* were reported to remain partially open even when the guard cells were plasmolyzed (24), and a similar phenomenon has been observed in an ABA-deficient mutant of *Solanum tuberosum* (17). Abnormal properties of the guard cell walls may therefore contribute to the anomalous stomatal behavior of these mutants, such as their high conductance

in the dark. On the other hand, the response of *flc* stomata to changes in light intensity is even greater than that of RR (Fig. 3). The change in g_e in response to changes in C_i is roughly the same in *flc* and RR, although dg_e/dC_i near the ambient CO_2 operating point is less in *flc*. (Fig. 3). ABA treatment reduced g_e in both RR and *flc* with little effect on the shape of the CO_2 response curves. These data are consistent with the conclusion that stomatal responses to light are not primarily mediated by changes in C_i (20, 28). They also imply that ABA is not required for stomatal responses to light and CO_2, although it does influence them. However, we cannot dismiss the possibility that the small amount of ABA that is present in *flc* leaves is required for stomatal function.

In our experiments, water stress was deliberately minimized, but leaf water potentials were nonetheless lower in *flc* than in RR (2). On one occasion, a *flc* plant wilted as it was being fitted into the gas exchange system. It regained turgor in about 20 min and g_e had fallen only to 0.59 mol m^{-2} s^{-1}. Thus, immediately after an episode of wilting, g_e of *flc* leaves was approximately equal to the maximum g_e of RR leaves. Stomata of *flc* closed markedly when VPD was increased, but again, g_e did not fall below 0.5 mol m^{-2} s^{-1} (Fig. 4), and E increased from 7 mmol m^{-2} s^{-1} to 15 mmol m^{-2} s^{-1}. In the dark, the same leaves can reduce g_e to as low as 0.15 mol m^{-2} s^{-1}. Older *flc* leaves often developed necrotic areas in the interveinal regions. The first evidence of this necrosis, as seen in scanning electron micrographs (not shown), was the appearance of regions of collapsed epidermis surrounding an apparently normal stoma. The implication is that the degree of response of illuminated *flc* stomata to VPD or epidermal water deficit is insufficient to prevent local desiccation and cellular death. With ABA application, *flc* stomata will close sufficiently in the light to maintain turgor (2). Although *flc* stomata exhibit a limited response to increasing VPD or water deficit, additional ABA is required for normal functioning, particularly in the light.

A striking feature of this work is that for a given genotype and ABA treatment, reproducible values for C_i were obtained. The characteristic C_i under standard conditions could be reversibly altered by beginning or discontinuing ABA treatment, irrespective of widely differing stomatal frequencies. Stomatal responses to light and CO_2 were essentially normal in *flc*, contributing to the constancy of C_i, albeit at a higher absolute value than in RR. Stomata of *flc* plants showed only limited responses to increases in VPD or water deficits in the light. ABA may therefore be important in overriding other influences when water deficits threaten, such as enabling stomatal closure to occur despite the strong promotion of stomatal opening by light. Such a mechanism would effectively shift priority toward water conservation at the expense of carbon assimilation, as evidenced by lower C_i values.

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