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Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: evidence for an interaction with ethylene

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Abstract

To examine whether the reduced shoot growth of abscisic acid (ABA)-deficient mutants of tomato is independent of effects on plant water balance, *flacca* and *notabilis* were grown under controlled-humidity conditions so that their leaf water potentials were equal to or higher than those of well-watered wild-type plants throughout development. Most parameters of shoot growth remained markedly impaired and root growth was also greatly reduced. Additional experiments with *flacca* showed that shoot growth substantially recovered when wild-type levels of ABA were restored by treatment with exogenous ABA, even though improvement in leaf water potential was prevented. The ability of applied ABA to increase growth was greatest for leaf expansion, which was restored by 75%. The ethylene evolution rate of growing leaves was doubled in *flacca* compared to the wild type and treatment with silver thiosulphate to inhibit ethylene action partially restored shoot growth. The results demonstrate that normal levels of endogenous ABA are required to maintain shoot development, particularly leaf expansion, in well-watered tomato plants, independently of effects on plant water balance. The impairment of shoot growth caused by ABA deficiency is at least partly attributable to ethylene.

Key words: Abscisic acid, ABA, ethylene, shoot growth, *flacca*, *notabilis*.

Introduction

Abscisic acid (ABA) is generally regarded as an inhibitor of shoot growth (Trewavas and Jones, 1991; Davies, 1995; Munns and Cramer, 1996). Most of the research underlying this view has involved applications of ABA or correlations of developmental changes to altered endogenous ABA levels. In general, ABA applications have resulted in inhibition of leaf and stem growth; the reduction of shoot growth in plants experiencing water deficits or other adverse environmental conditions often correlates with endogenous ABA accumulation. However, interpretation of these results is complicated by uncertainty that effects of applied ABA are predictive of the role of endogenous ABA (Trewavas and Jones, 1991; Sharp *et al*., 1994).

Paradoxically, ABA-deficient mutants are often shorter and exhibit smaller leaves compared with the corresponding wild types (Quarrie, 1987). Interest has focused on the tomato mutants *flacca* (*flc*), *notabilis* (*not*) and *sitiens* (*sit*), and several authors have reported that leaf and stem growth of these mutants can be substantially restored by applying ABA (Imber and Tal, 1970; Tal and Nevo, 1973; Bradford, 1983; Neill *et al*., 1986; Nagel *et al*., 1994). In addition to reduced growth, ABA-deficient mutants are typically wilty even though the soil is well supplied with water. In the tomato mutants, this results from both high stomatal conductance and decreased plant hydraulic conductance, and these effects can also be prevented by applying ABA (Imber and Tal, 1970; Tal and Nevo, 1973; Bradford, 1983). Accordingly, several authors have attributed the inhibition of leaf and stem growth to shoot water deficits, and the growth-promotive effect of applied ABA in these cases to its ability to restore a favourable water balance (Bradford, 1983; Neill *et al*., 1986; Trewavas and Jones, 1991; Nagel *et al*., 1994; Léon-Kloosterziel *et al*., 1996). Consequently, these findings

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have generally not been regarded as evidence against a direct inhibitory role of ABA in shoot growth, although the observations have led some authors to question this view (Jones *et al*., 1987; Quarrie, 1987; Taylor, 1987; Zeevaart and Creelman, 1988; Abou-Mandour and Hartung, 1992).

The alternative possibility is that endogenous ABA is required to maintain shoot growth independently of its effect on plant water balance. Recent work in this laboratory has shown that an important role of ABA accumulation in the maintenance of root elongation at low water potentials in maize seedlings is to prevent excess ethylene production (Spollen *et al*., 2000), confirming ideas suggested by others (Wright, 1980; Bradford and Hsiao, 1982). Consistent with this finding, it was reported that ethylene production was enhanced in shoots of *flc* (Tal *et al*., 1979) and in whole plants of an ABA-deficient mutant of *Arabidopsis* (Rakitina *et al*., 1994) grown under well-watered conditions. In the case of *flc*, it was also shown that ethylene production could be restored to normal levels with exogenous ABA. (It should be noted, however, that enhanced ethylene production in *flc* was not reproduced in a study by Neill *et al*., 1986.) Further, the ABA-deficient mutants of tomato often exhibit morphological symptoms characteristic of excess ethylene, such as leaf epinasty and adventitious rooting (Tal, 1966; Nagel *et al*., 1994). Despite these early observations and the fact that ethylene is usually inhibitory to shoot growth of terrestrial plants (Abeles *et al*., 1992), the possibility that ethylene is a cause of reduced shoot growth in ABA-deficient mutants has not been assessed.

To distinguish between these possibilities, it is necessary to examine the growth of ABA-deficient mutants in the absence of shoot water deficits. If the reduced shoot growth normally observed is caused by adverse water relations, then under such conditions growth should be restored or even enhanced relative to wild-type plants. On the other hand, if endogenous ABA is required to maintain shoot growth independently of effects on plant water balance, then the mutants should remain smaller. This question has not been definitively addressed. *flc*, *not* and *sit* have been grown under mist and it was observed that stem height became greater than in the wild type, consistent with ABA playing an inhibitory role in stem elongation (Jones *et al*., 1987). In contrast, later formed leaves remained smaller and total leaf biomass remained substantially reduced in the mutants. However, leaf water potentials also remained considerably lower than in the wild type, so interpretation of the role of ABA in leaf growth was not possible. Partial alleviation of shoot growth inhibition in double mutants of *flc*, *not* and *sit* grown at high relative humidity (RH) has been noted (Taylor, 1987), but information on plant water relations was not included so, again, full interpretation was not possible. In other studies in which *flc* was grown at high RH, effects on growth were not reported (Puri and Tal, 1977; Tal *et al*., 1979).

This study assessed whether the reduced shoot growth of *flc* and *not* is attributable to water deficits by growing the plants under controlled-humidity conditions such that the leaf water potentials of the mutants were equal to or higher than those of well-watered wild-type plants throughout development. In addition, the involvement of ethylene in the inhibition of shoot growth in *flc* under these conditions was examined.

Materials and methods

Plant material and growth conditions

Seeds of *Lycopersicon esculentum* Mill. cvs Rheinlands Ruhm (RR) and Ailsa Craig (AC) and the ABA-deficient mutants *flc* in the RR (isogenic) and AC (near isogenic) backgrounds and *not* in the AC background (near isogenic) were obtained from the CM Rick Tomato Genetics Resource Center, University of California, Davis, USA. The *flc* mutant is impaired in the oxidation of ABA aldehyde to ABA (Taylor *et al*., 1988; Marin and Marion-Poll, 1997), and the *not* mutant has a defect in the synthesis of xanthoxin, which is considered a key control step in ABA biosynthesis (Burbidge *et al*., 1999). When indicated with the corresponding wild type, the background of *flc* is not specified.

Seeds were treated with 2.6% sodium hypochlorite solution for 30 min, rinsed for 1 h in flowing tap water, and sown individually in 7 cm diameter, 290 cm^3 plastic pots filled with a 2 : 1 (v/v) mixture of Green Formula Growing Mix (Lambert Peat Moss, Inc., Quebec, Canada) and sand containing 2 g 1000 cm^{-3} of a slow release fertilizer (Osmocote 17-6-12 with micronutrients). The pots were placed in a controlled environment chamber with a day/night temperature of 26/20 °C and a 14 h photoperiod. The photon flux density at plant height was 600 μmol photons m^{-2} s^{-1} photosynthetically active radiation supplied by cool-white fluorescent and incandescent lamps. The day/night RH was 92/95% until day 4 (emergence was defined as day zero), and then varied according to the genotype and treatment as detailed below. Pots were watered via capillary matting, which was irrigated with deionized water four times daily. On day 6, seedlings were selected for uniformity and transplanted (without removal from the soil) into 17 cm diameter, 2500 cm^3 plastic pots containing the same growth medium. After transplanting, supplemental nutrient solution (Peters Professional 20-10-20 with micronutrients, 0.5 $g\,l^{-1}$, Grace-Sierra Horticultural Products Co., Milpitas, California, USA) was supplied twice a week until day 26 and then every 2 d (200 ml per pot).

Applications of ABA and silver thiosulphate

In some experiments, plants of *flc* (RR background) were sprayed with 10 μM (±)-ABA (Sigma). This concentration was used because it had been found that the treatment resulted in substantial recovery of shoot growth (Bradford, 1983). Wild-type plants were not treated because this would have raised the ABA content above normal levels. The effect of spraying with silver thiosulphate (STS), an inhibitor of ethylene action (Beyer, 1976), was examined in both *flc* and RR plants. Preliminary experiments determined that the optimum concentration for restoration of shoot growth in *flc* was 250 μM; higher concentrations resulted in severe toxicity. Solutions of STS were made

as described previously (Cameron *et al.*, 1985). Both solutions contained ethanol and Tween 20 at final concentrations of 0.1% and 0.01% (v/v), respectively. Spray control (sc) plants of *flc* were sprayed with deionized water containing the same concentrations of ethanol and Tween. In all cases, leaves and stems were sprayed to the drip point 30 min before the end of the photoperiod, daily from day 9.

Relative humidity treatments

Plants of *flc*, *flc* + STS, *flc* (sc), and *not* were maintained at a day/night RH of 92/95% throughout the experiments. The other genotypes and treatments were grown under the following RH regimes.

RR: days 4–10, 50/70%; days 11–17, 60/70%; days 18–35, 60/80%.
AC: days 4–10, 50/70%; days 11–20, 70/80%.
RR + STS: days 4–8, 50/70%; days 9–17, 90/90%; days 18–24, 75/80%; days 25–35, 60/80%.
flc + ABA: days 4–8, 92/95%; days 9–35, 70/80%.

Leaf water potentials

In each experiment, leaf water potentials were measured during the light and dark periods at least once a week by isopiestic thermocouple psychrometry (Boyer and Knipling, 1965). From day 21, samples were excised from the youngest fully expanded leaves (expanded leaves were chosen to avoid errors associated with cell wall relaxation after excision). On days 7 and 14, all leaves were expanding, and samples were excised from the oldest leaves. Sampled leaves were unshaded. On day 7, plants were used for single measurements and then discarded. From day 14 onwards, plants were used for multiple measurements, but were not sampled more than once per time point or twice in one day, or on consecutive days.

Growth analysis and leaf ABA content

Three- or five-week-old plants were harvested for measurements of leaf area using a leaf-area meter (Li-Cor, Lincoln, Nebraska, USA), numbers of main stem and side stem leaves, main stem height, and total shoot, leaf and root dry weights. To avoid effects of tissue sampling on plant development, the plants used for growth analysis were not sampled for leaf water potential measurements, but were grown alongside and otherwise treated identically to the plants used for water potential determinations.

On day 21, leaf ABA content was measured 5–8 h into the light period for RR, *flc*, *flc* + ABA, and *flc* (sc) plants. Exposed leaflets of the second and third youngest leaves on the main stem were harvested, weighed, frozen in liquid nitrogen, freeze-dried, and finely ground. The *flc* + ABA samples were briefly (< 5 s) rinsed in deionized water after weighing to remove ABA from the leaf surfaces. The *flc* (sc) samples were treated similarly. The ABA content of the youngest fully expanded leaves was also measured on day 35 for RR, RR + STS, *flc* + STS and *flc* (sc) plants. Samples were extracted in deionized water at 4 °C for 16–20 h, and duplicate measurements of ABA content were made by radioimmunoassay using a monoclonal antibody (AFRC MAC 252; Quarrie *et al.*, 1988). Validation of the assay for tomato leaves by parallel GC-MS was reported previously (Mulholland, 1994).

Leaf ethylene evolution

Ethylene evolution rate was measured from the youngest three leaves of the main stem at weekly intervals for RR, *flc*, *flc* + ABA and *flc* (sc) plants. Two samples per plant were harvested 5–8 h into the light period, each comprising all the leaflets from one side of the petioles, giving a fresh weight of 0.5–1.0 g per sample (measured after ethylene determination). Each sample was immediately placed in a 60 ml syringe, which was then sealed. After approximately 5 min (determined precisely for each measurement), 50 ml of the air in the syringe was injected into a cold trap and the ethylene content measured using a GC equipped with a photoionization detector, as described previously (Spollen *et al.*, 2000). The total amount of ethylene evolved was calculated by correcting for the volume of air remaining in the syringe (sample volume was estimated by placing the leaflets in a glass vial of known volume, and weighing the amount of water required to fill the vial). Preliminary experiments with RR and *flc* plants showed that the rate of ethylene evolution was steady for about 15 min after excision and then increased substantially, presumably due to wounding. Therefore, the reported measurements are considered to be good estimates of the rate of evolution by leaves of intact plants (Jackson and Campbell, 1976).

Statistical analysis

Analyses of variance were performed with means compared using Fischer's least significant difference test at the $P = 0.05$ or 0.1 level.

Results

Leaf water potentials of flc *and RR*

Leaf water potentials of *flc* plants grown for 35 d after emergence at a constant day/night RH regime of 92/95% increased from a minimum of −0.5 MPa during day 7 to a maximum approaching −0.2 MPa during the final days of the experiment (Fig. 1A–E). Measurements made 1 h into the light period were above −0.4 MPa from day 14 (Fig. 1A). Water potentials were not substantially different during the light and dark periods (Fig. 1B-E), consistent with reports that the stomata tend to remain open in darkness and the plant hydraulic conductance decreases late in the day (Tal, 1966; Bradford, 1983). The RH conditions that were necessary to achieve equal or lower leaf water potentials in RR compared to *flc* plants during both the light and dark periods throughout the experimental period were then established. Results obtained from a set of RR plants grown under these conditions are shown in Fig. 1A–E. It should be noted that cell wall relaxation after excision may have caused the measured leaf water potentials in both *flc* and RR plants to be erroneously low prior to day 21, due to the unavoidable sampling of growing leaves.

To confirm that water potentials of growing leaves remained equal or lower in RR than in *flc* later in the experiment, measurements were made on day 26 using a pressure chamber (Boyer, 1967). The upper main stem including the three youngest leaves (as sampled for ethylene measurements; see below) was sampled 7 h into the light period. Results for *flc* and RR were not significantly different at the 0.05 level (means ± standard error [$n = 7$]: *flc*, −0.25 ± 0.04 MPa; RR, −0.34 ± 0.03 MPa).

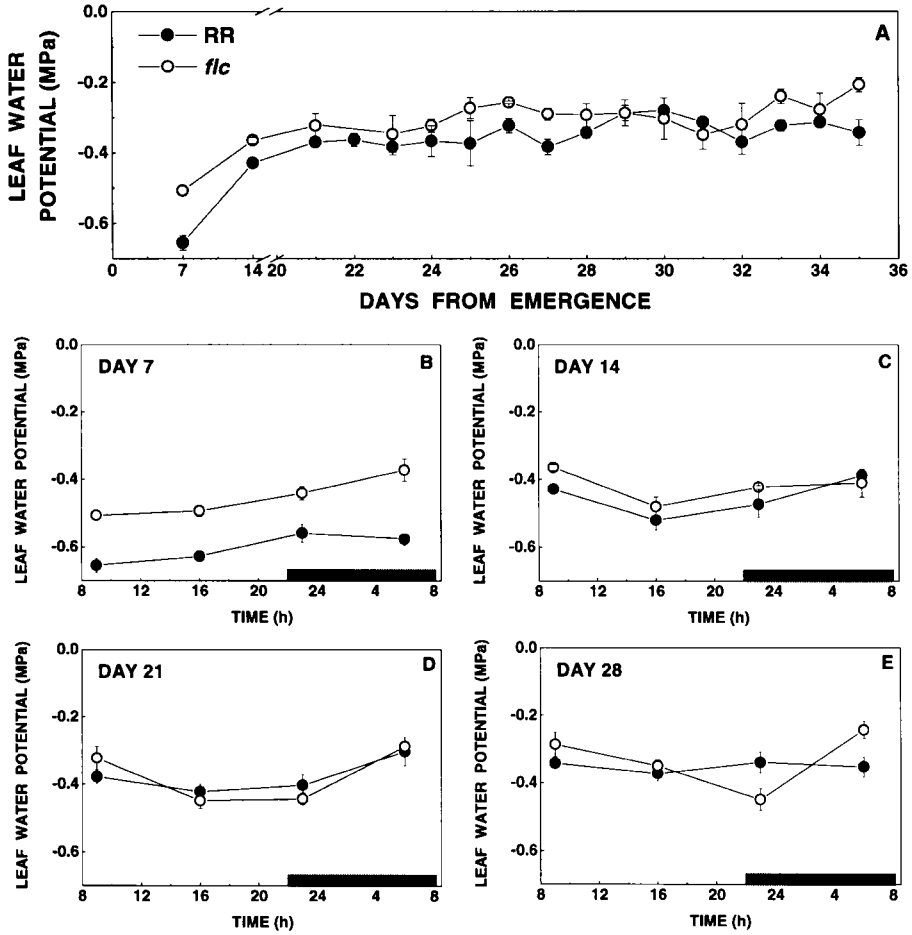

Fig. 1. Leaf water potentials of RR and *flc* plants during 35 d after emergence. Measurements were made 1 h into the light period once a week until day 21 and then every day (A). Measurements were made at additional times during the light and dark periods once a week (B–E). The dark period is represented by the shaded portions of the abscissa. Data are means ± standard error (A, $n=3$; B–E, $n=3$–6).

To examine the effects of spraying *flc* plants with ABA, leaf water potentials were first measured in spray control plants. This treatment caused leaf water potentials to be as much as 0.2 MPa lower than in untreated *flc* plants on days 14 and 21 (Table 1, compare with Fig. 1C–E), possibly due to effects of spraying on cuticular conductance. Equal or lower water potentials in *flc* + ABA compared to *flc* (sc) plants were achieved by changing the RH regime to 70/80% at the onset of the ABA treatment (Table 1).

Growth and leaf ABA content of flc *and RR*

Most parameters of shoot growth were substantially inhibited in *flc* compared to RR plants (Table 2), despite the fact that leaf water potentials were higher in *flc* throughout development. Representative plants at the end of the experiment are illustrated in Fig. 2A–B. Total leaf area was 39% and 48% less in *flc* on days 21 and 35, respectively. This was entirely attributable to reduced leaf expansion, since the number of both main stem and side stem leaves was increased by about 20% at both times

Table 1. *Leaf water potentials of* flc *(sc) and* flc + *ABA plants (RR background) at weekly intervals during 35 d after emergence*

Treatments began on day 9. Measurements were made 8 h into both the light and dark periods (Day and Night, respectively). Data are means ± standard error ($n=3$).

Treatment	Leaf water potential (MPa)							
	Day 14		Day 21		Day 28		Day 35	
	Day	Night	Day	Night	Day	Night	Day	Night
flc (sc)	−0.60 ± 0.02	−0.43 ± 0.04	−0.65 ± 0.03	−0.46 ± 0.03	−0.39 ± 0.03	−0.27 ± 0.04	−0.46 ± 0.07	−0.28 ± 0.01
flc + ABA	−0.67 ± 0.03	−0.52 ± 0.02	−0.60 ± 0.06	−0.52 ± 0.00	−0.38 ± 0.02	−0.41 ± 0.09	−0.52 ± 0.02	−0.45 ± 0.01

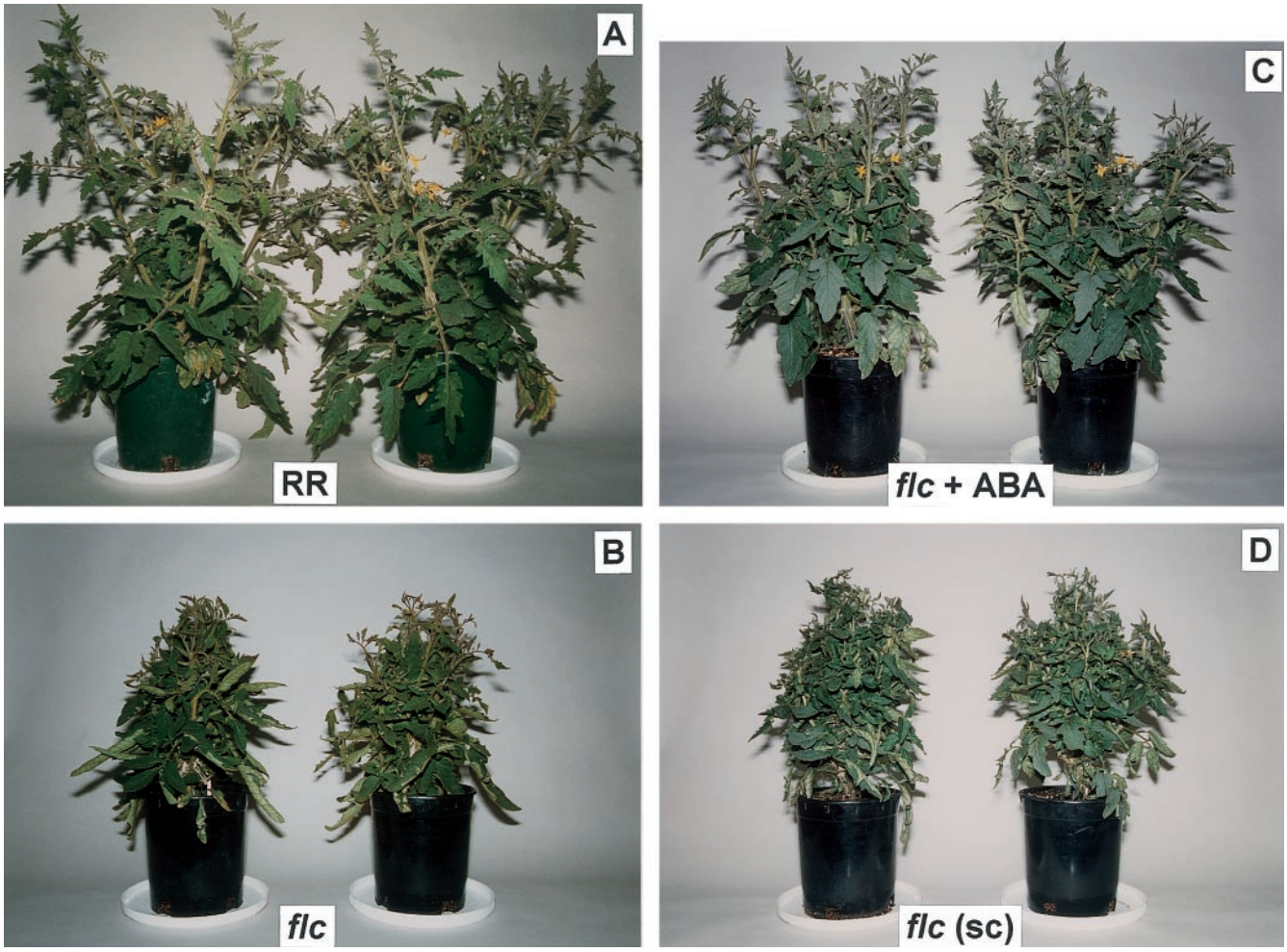

Fig. 2. Representative plants of (A) RR, (B) *flc*, (C) *flc*+ABA, and (D) *flc* (sc) 35 d after emergence. Plants are from the experiments presented in Table 2.

(increases were significant at the 0.05 level except for side stem leaf number on day 21). On day 21, total leaf and whole shoot dry weights were 38% and 28% less, respectively, in *flc* compared to RR, and by day 35 both parameters in *flc* were only approximately 40% of the RR values. In contrast, main stem height was 25% greater in *flc* than in RR plants on day 21. However, this effect was not sustained, and by day 35 stem height was 25% smaller in *flc* compared to RR plants. The ABA content of the growing leaves of *flc* was 33% of the level in RR plants on day 21 (Table 3). The values are similar to those reported earlier (Taylor, 1987) for *flc* (AC background) grown at high RH.

There were no significant differences in shoot growth

Table 2. *Growth analysis of RR*, flc, flc *(sc) and* flc+*ABA plants*

Plants were harvested 21 d and 35 d after emergence in the same experiments as those in which leaf water potentials were measured (Fig. 1, Table 1). Data are means±standard error (n=4–6). For each parameter on each day, values followed by different letters are significantly different at the 0.05 level. The experiments were repeated with similar results.

	Day 21		Day 35			
	RR	*flc*	RR	*flc*	*flc* (sc)	*flc*+ABA
Total leaf area (cm^2)	776±106 a	475±72 b	3832±171 a	1974±133 c	1947±113 c	3355±58 b
No. main stem leaves	13.2±0.2 b	15.4±0.8 a	18.0±0.3 b	22.0±0.0 a	22.2±0.9 a	18.6±0.7 b
No. side stem leaves	30.4±1.9 a	37.2±6.5 a	63.0±3.1 b	79.5±3.5 a	72.8±5.8 ab	67.4±5.3 ab
Total leaf dry wt. (g)	2.4±0.3 a	1.5±0.2 b	21.8±0.9 a	8.3±0.2 c	9.6±0.6 c	14.0±0.4 b
Main stem height (cm)	14.2±0.6 b	17.8±0.8 a	45.8±1.0 a	34.3±0.6 c	28.5±1.4 d	38.0±0.8 b
Total shoot dry wt. (g)	3.5±0.5 a	2.3±0.3 b	38.2±1.7 a	15.3±0.3 c	17.0±0.9 c	23.7±0.8 b
Root dry wt. (g)	0.39±0.07 a	0.24±0.03 b	3.9±0.5 a	1.0±0.1 c	1.1±0.1 bc	1.8±0.2 b

Table 3. *Leaf ABA content of RR,* flc, flc *(sc) and* flc + *ABA plants*

Plants were grown as described in Fig. 1 and Table 1, and measurements were made 21 d after emergence. Data are means ± standard error (n = 5). Values followed by different letters are significantly different at the 0.05 level. The experiments were repeated with similar results. FW, fresh weight.

Treatment	Day 21 ABA content (nmol g^{-1} FW)
RR	0.49 ± 0.05 a
flc	0.16 ± 0.03 b
flc (sc)	0.12 ± 0.02 b
flc + ABA	0.57 ± 0.05 a

parameters of *flc* (sc) compared to untreated *flc* plants, except for main stem height which was slightly decreased (Table 2; Fig. 2D). Also, the ABA content was not significantly different in the *flc* (sc) and *flc* plants (Table 3). The leaf ABA content in *flc* + ABA plants was restored to the value in RR plants, and this treatment resulted in substantial restoration of shoot growth by day 35 (Table 2; Fig. 2C). The improvement in growth occurred despite the fact that leaf water potentials were equal or lower in *flc* + ABA plants than in the other treatments throughout the experiment. The ABA treatment nearly doubled the total leaf area of *flc*; restoration relative to RR plants was 75%. This was due entirely to recovery of leaf expansion, since the numbers of main stem and side stem leaves were significantly decreased and unaffected, respectively. The ABA treatment also restored total leaf and shoot dry weights and stem height by 32–55% from the values of the *flc* (sc) plants.

Root growth was also substantially reduced in *flc* compared to RR plants (Table 2). On day 35, root dry weight was inhibited by 74% compared to the 60% inhibition of shoot dry weight. A greater restriction of root than shoot growth in *flc* has also been reported previously (Bradford, 1983). The ABA treatment slightly increased root dry weight of *flc*, although not significantly when compared to the *flc* (sc) plants.

Leaf water potentials and growth of flc, not *and AC*

Similar experiments were conducted using *flc* in the AC background and *not* (also in the AC background). The second background of *flc* was included to test for consistency of phenotype, and *not* was studied to strengthen the conclusion that the impairment of growth in *flc* is due to ABA deficiency rather than to pleiotropic effects of the mutation. (In *flc*, conversion of ABA aldehyde to ABA is inhibited because synthesis of the molybdenum cofactor required for activity of ABA aldehyde oxidase is impaired by this genetic lesion. Enzymes which are not involved in ABA biosynthesis that depend on the same form of the cofactor, for example, xanthine dehydrogenase, will therefore also be impaired in *flc* [Marin and Marion-Poll, 1997].)

Leaf water potentials of *flc* and *not* ranged between −0.6 and −0.3 MPa throughout the 20 d experiments (Table 4). Water potentials of AC plants were similar to or lower than those of the mutants at all times. Despite this, total leaf area and leaf, shoot and root dry weights were substantially inhibited in both mutants compared to AC plants (Table 5). For all parameters, the inhibition was greater in *flc* than in *not*. For example, leaf area was 58% less in *flc* and 37% less in *not*. This finding is consistent with comparisons of these mutants when grown without control of water balance and correlates with lower ABA contents in *flc* (Taylor and Tarr, 1984; Neill and Horgan, 1985; ABA contents were not measured in the present study). The inhibition of leaf area development was again entirely attributable to reduced leaf expansion, since leaf numbers were not significantly different between the mutants and AC plants.

Stem height was not increased in either *flc* or *not* compared to AC plants at day 20 (Table 5), in contrast to the results on day 21 for *flc* in the RR background (Table 2). However, an additional experiment showed that *flc* plants were significantly taller than AC plants prior to this time; the maximum increase was 27% on day 15. A similar trend was observed in *not*, although in this case the increase was not significant.

Leaf ethylene evolution and effects of STS in flc *and RR*

Plants of *flc* (in both backgrounds) and *not* exhibited substantial adventitious rooting and leaf epinasty, and both symptoms were essentially absent in the *flc* + ABA plants (Figs 2, 3). These characteristics did not occur in wild-type plants under either their normal conditions or the high-RH conditions in which the mutants were grown.

Table 4. *Leaf water potentials of AC,* flc *and* not *plants at weekly intervals during 20 d after emergence*

Measurements were made 8 h into both the light and dark periods (Day and Night, respectively). Data are means ± standard error (n = 3).

Genotype	Leaf water potential (MPa)					
	Day 8		Day 14		Day 20	
	Day	Night	Day	Night	Day	Night
AC	−0.57 ± 0.04	−0.48 ± 0.01	−0.54 ± 0.02	−0.45 ± 0.02	−0.51 ± 0.03	−0.35 ± 0.04
flc	−0.52 ± 0.02	−0.37 ± 0.01	−0.57 ± 0.00	−0.38 ± 0.00	−0.55 ± 0.01	−0.37 ± 0.02
not	−0.57 ± 0.04	−0.35 ± 0.00	−0.52 ± 0.02	−0.41 ± 0.00	−0.50 ± 0.04	−0.33 ± 0.01

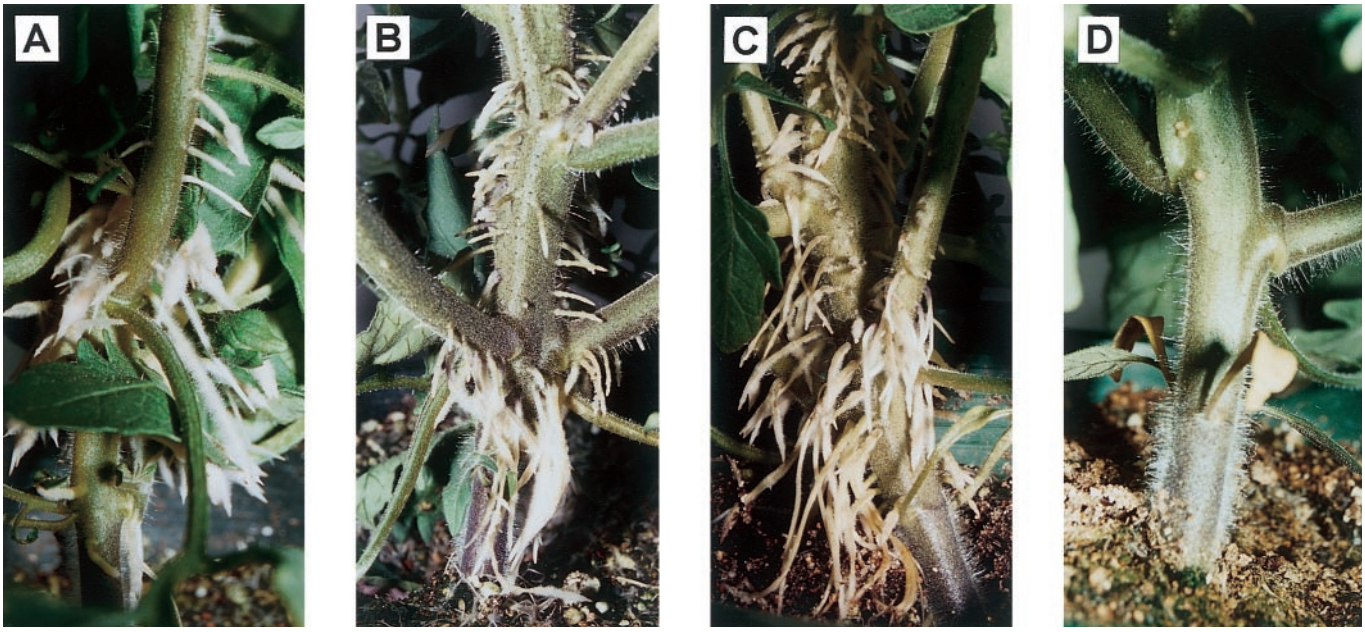

Fig. 3. Basal region of the main stem showing adventitious rooting for (A) *flc* (RR background), (B) *flc* (AC background) and (C) *not* plants 35 d after emergence. Panel (D) shows that adventitious rooting was almost completely absent in *flc* + ABA plants (RR background).

Although these symptoms are characteristic of excess ethylene (Abeles *et al*., 1992), it is uncertain whether the mutants produce more ethylene than wild-type plants, particularly when shoot water deficits are prevented. There are only two studies of ethylene evolution in *flc*, and one of *not*. Tal *et al*. reported that evolution was enhanced in *flc*, but found that this effect was prevented in young (although not older) plants grown at high RH, suggesting that water deficits played an important role (Tal *et al*., 1979). A small increase in evolution in *not* compared to wild-type plants growing in compacted soil has also been reported (Hussain *et al*., 1999*b*). In contrast, Neill *et al*. found no evidence that evolution was increased in *flc* even though the plants were grown in a greenhouse without humidity control (Neill *et al*., 1986). The explanation for the conflicting results in *flc* is unclear, partly because the measurements included unknown contributions from wound-induced ethylene (evolution was measured from excised shoots or leaves over 24 h and 8 h, respectively, in Tal *et al*., 1979, and Neill *et al*., 1986). Therefore, ethylene evolution in *flc* in the absence of shoot water deficits was reassessed.

Pre-wound measurements of growing main stem leaves showed that ethylene evolution rate was doubled in *flc* compared to RR plants at 21 d after emergence, and that this effect was prevented in the plants treated with ABA (Table 6). Ethylene evolution was not significantly different in *flc* and *flc* (sc) plants. Preliminary experiments showed that ethylene evolution was similarly enhanced on day 14, at which time the plants had the same number of leaves as the young plants studied by Tal *et al*. (Tal *et al*., 1979).

To assess whether ethylene was a cause of the inhibition of shoot growth in *flc*, plants of RR and *flc* were sprayed with STS daily from day 9. In the RR + STS treatment, shoot growth parameters on day 35 (not shown) were not

Table 5. *Growth analysis of AC*, flc *and* not *plants*

Plants were harvested 20 d after emergence in the same experiments as those in which leaf water potentials were measured (Table 4). Data are means ± standard error ($n = 5$). For each parameter, values followed by the same letter are significantly different at the 0.05 level. The experiments were repeated with similar results.

	Day 20		
	AC	*flc*	*not*
Total leaf area (cm^2)	752 ± 32 a	319 ± 37 c	471 ± 19 b
No. main stem leaves	13.0 ± 0.3 a	11.4 ± 1.2 a	12.6 ± 0.9 a
No. side stem leaves	23.8 ± 7.5 a	14.2 ± 4.8 a	16.8 ± 4.9 a
Total leaf dry wt. (g)	2.7 ± 0.1 a	1.3 ± 0.1 c	1.8 ± 0.1 b
Main stem height (cm)	13.7 ± 0.5 a	11.8 ± 1.1 a	11.2 ± 0.9 a
Total shoot dry wt. (g)	3.8 ± 0.2 a	2.0 ± 0.2 c	2.6 ± 0.2 b
Root dry wt. (g)	0.32 ± 0.01 a	0.14 ± 0.02 b	0.16 ± 0.01 b

Table 6. *Leaf ethylene evolution rate of RR*, flc, flc *(sc) and* flc + *ABA plants*

Plants were grown as described in Fig. 1 and Table 1, and measurements were made 21 d after emergence. Data are means ± standard error ($n = 5$–18). Values followed by different letters are significantly different at the 0.1 level. The experiments were repeated with similar results. FW, fresh weight.

Treatment	Day 21 ethylene evolution rate (pmol g^{-1} FW h^{-1})
RR	49.3 ± 6.8 b
flc	114.3 ± 26.4 a
flc (sc)	140.6 ± 27.1 a
flc + ABA	75.1 ± 9.1 b

significantly different from the untreated RR plants shown in Table 2, except for total leaf dry weight which was reduced by 16%. Leaf water potentials and ABA content (not shown) were also very similar to those of untreated RR plants. In *flc*, on the other hand, the STS treatment resulted in significant increases in main stem (although not side stem) leaf area and dry weight, stem height and total shoot dry weight compared to *flc* (sc) plants (Table 7). For these parameters, restoration relative to RR + STS plants ranged from 25–50%. The STS treatment also resulted in a 37% restoration of root dry weight. The restoration of main stem leaf area was entirely attributable to recovery of leaf expansion, since the number of main stem leaves was significantly decreased. Leaf water potentials and ABA content (not shown) were very similar to those of *flc* (sc) plants, showing that the restoration of growth did not result from increases in plant water status or ABA levels. The explanation for the lack of effect of STS on side stem leaf area is not known. Preliminary measurements on day 28 showed that these leaves also exhibited enhanced ethylene evolution in *flc*.

Discussion

The results of this study demonstrate that most parameters of shoot growth remained greatly impaired in *flc* and *not* in the absence of shoot water deficits. Root growth was also greatly reduced compared to wild-type plants. Additional experiments with *flc* showed that shoot growth substantially recovered when wild-type levels of ABA were restored by treatment with exogenous ABA, even though improvement in shoot water status was prevented. The ability of applied ABA to restore growth was greatest for leaf expansion. It is concluded that normal levels of endogenous ABA are required to maintain shoot development, particularly leaf expansion, in well-watered tomato plants, independently of effects on plant water balance. Consistent with this conclusion, Mulholland *et al.* reported that leaf growth of barley growing in compacted soil was more inhibited in an ABA-deficient mutant than in the wild type, and the evidence suggested that changes in water relations were not the cause (Mulholland *et al.*, 1996*a*, *b*). It should be noted that, in contrast, it has been shown that ABA-deficiency in maize seedlings at low water potentials was associated with increased shoot growth, indicating that ABA accumulation was causally related to shoot growth inhibition (Saab *et al.*, 1990). This finding is discussed further below.

Table 7. *Growth analysis of* flc + *STS plants (RR background)*

Plants were harvested 35 d after emergence. Data are means ± standard error ($n = 5$). The experiment was repeated with similar results. For parameters that were significantly different from the *flc* (sc) treatment (*0.1 level, **0.05 level; *flc* (sc) data from Table 2), restoration was calculated relative to RR + STS plants. Growth of RR + STS plants is described in the text.

	Day 35	
	flc + STS	Restoration (%)
Main stem leaf area (cm^2)	1207 ± 100*	34.8
Side stem leaf area (cm^2)	782 ± 182	—
No. main stem leaves	19.4 ± 1.1*	60.9
No. side stem leaves	67.0 ± 12.2	—
Main stem leaf dry wt. (g)	6.2 ± 0.3**	42.6
Side stem leaf dry wt. (g)	4.4 ± 1.2	—
Main stem height (cm)	38.3 ± 3.9**	49.5
Total shoot dry wt. (g)	22.4 ± 2.2**	25.2
Root dry wt. (g)	2.5 ± 0.1**	37.3

It is noteworthy that the inhibition of leaf area and shoot dry weight in *flc* compared to RR plants was comparable to that reported previously (Bradford, 1983). In that study, plants were grown in a greenhouse with uncontrolled humidity, and leaf water potentials were substantially lower in *flc* than in the wild type. Similarly, the inhibition of leaf area in *not* was comparable to that reported for greenhouse-grown plants (Taylor and Tarr, 1984). Further, the restoration of leaf area in ABA-treated *flc* plants was considerably greater than that reported earlier (Bradford, 1983), despite the fact that leaf water potentials increased in that study. These comparisons suggest that non-hydraulic effects of ABA-deficiency are the major cause of shoot growth inhibition in *flc* and *not* even when moderate shoot water deficits occur.

There were two exceptions to the trend of decreased shoot growth in the mutants. First, the number of leaves was either unaffected or slightly increased. A small increase in the rate of leaf production has also been reported previously in *flc* (Tal and Imber, 1970) and in *flc*, *not* and *sit* (Jones *et al.*, 1987). These findings indicate that endogenous ABA inhibits leaf initiation, in contrast to its promotive effect on leaf expansion. Second, the mutants initially exhibited a faster rate of stem elongation than wild-type plants. A similar finding that 7-week-old plants of *flc*, *not* and *sit* grown under mist were all taller than the wild type has also been reported (Jones *et al.*, 1987). In the present study, however, this effect was observed only at or prior to 21 d after emergence. In older *flc* plants, the effect of ABA deficiency on stem elongation reversed and the mutant became substantially shorter than RR plants. Similar to our findings, double and triple mutants of *flc*, *not* and *sit* grown at high RH exhibited greater stem heights than the wild type during the first 25 d after sowing, but the effect was reversed by 36 d (Tarr, 1993). In another study, stem height was slightly greater in *flc* only until 40 d from sowing (Bradford, 1983). Thus, endogenous ABA appears to inhibit stem elongation in young tomato plants but to maintain stem growth at later stages of development.

It should be noted that since each leaf is produced at a node, the increased number of main stem leaves in *flc* compared to RR plants would have been associated with an increased number of stem internodes (not measured).

Therefore, the greater stem elongation in *flc* at 21 d after emergence may have been partially (if not wholly) attributable to an increase in internode number rather than individual internode expansion. In older plants, the reduced stem elongation in *flc* despite its greater leaf number indicates that internode expansion was inhibited compared to the wild type.

Involvement of ethylene

The results establish that ABA-deficiency in *flc* causes an increased rate of ethylene production independently of effects on plant water balance. Although enhanced ethylene production has been reported previously in ABA-deficient mutants of tomato (Tal *et al*., 1979; Hussain *et al*., 1999*b*) and *Arabidopsis* (Rakitina *et al*., 1994), the extent to which the increase in those studies was a direct result of ABA deficiency or an indirect result of decreased plant water status was not known. (There are many reports that ethylene production can be increased by plant water deficits [although see Morgan *et al*., 1990].)

Moreover, the partial recovery of shoot growth in STS-treated *flc* shows that the impairment of growth caused by ABA-deficiency is at least partly attributable to ethylene. These conclusions are similar to our recent finding that an important role of ABA accumulation in the maintenance of root elongation at low water potentials in maize seedlings is to limit ethylene production (Spollen *et al*., 2000). The full extent to which ethylene accounts for shoot growth inhibition in *flc* cannot be assessed from these experiments, however, because spraying with STS concentrations higher than 250 μM resulted in the development of severe toxicity symptoms. (In the short-term experiments with ABA-deficient maize seedlings, a STS concentration of 2.5 mM was optimal for root growth restoration [Spollen *et al*., 2000]). Therefore, it is possible that ABA has another function in maintaining shoot growth in addition to prevention of excess ethylene production. It should also be noted that these results do not exclude the possibility that the sensitivity of growth to ethylene was also increased by ABA deficiency.

Restriction of ethylene synthesis or sensitivity also appears to explain the earlier finding from this laboratory, noted above, that ABA deficiency caused increased shoot growth in maize seedlings at low water potentials (Saab *et al*., 1990). Preliminary experiments showed that growth promotion could be prevented by treatment with STS, and that shoot growth could also be increased by applying the ethylene precursor 1-aminocyclopropane-1-carboxylate or ethylene (Feng, 1996). These findings are consistent with reports that ethylene stimulates mesocotyl growth in some species (Suge, 1971; Cornforth and Stevens, 1973). Further, there are a few reports that ethylene can promote stem elongation (Poovaiah and Leopold, 1973; Van Andel and Verkerke, 1978); such an effect may contribute to the small promotion of stem height in the ABA-deficient tomato mutants early in development.

The commonality of these observations suggests that restriction of ethylene production may be a widespread function of ABA. Further, because ethylene is usually inhibitory to shoot growth of terrestrial plants at later stages of development and under various environmental conditions (Abeles *et al*., 1992; Lee and Reid, 1997; Hussain *et al*., 1999*a*), the finding that endogenous ABA maintains rather than inhibits shoot growth in tomato may generally apply.

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