Phenotypic Expression of Wild-Type Tomato and Three Wilty Mutants in Relation to Abscisic Acid Accumulation in Roots and Leaflets of Reciprocal Grafts

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ABSTRACT
Lycopersicon esculentum Mill. cv Rheinlands Ruhm (RR) and cv Moneymaker and the three wilty mutants flacca (flc), sitiens (sit), and sitiens* (sit*), together with most reciprocal grafts, were grown in pots and in solution culture. Detached leaflets, and control and steam-girdled intact plants, were left turgid or were wilted in air. Detached leaflets and the leaflets and roots of the intact plants were analyzed for their abscisic acid (ABA) content. Turgid RR leaflets contained about 2.9 ng ABA per milligram dry weight. On average, the flc and sit leaflets contained 33 and 11% of this amount, respectively. The lack of ABA approximately correlated with the severity of the mutant phenotype. Mutant roots also contained less ABA than wild-type roots. Wild-type scions on mutant stocks (wild type/mutant) maintained the normal phenotype of ungrafted plants. Mutant scions grafted onto wild-type stocks reverted to a near wild-type phenotype. After the wild-type leaves were excised from solution culture-grown mutant/wild-type plants, the revertive morphology of the mutant scions was maintained, although endogenous ABA levels in the leaflets fell to typical mutant levels and the leaflets became wilted again.

In response to air, detached leaflets lost water and afterwards wilted, whereas mutant leaflets of wild-type characters did not wilt. The wilt phenotype was restored when the wild-type scion was grafted onto the mutant rootstock. The mutant alleles are recessive and map to different gene loci in the tomato genome (16).

Comparison of genotypes with single gene differences, but with different endogenous ABA levels, provides a powerful method for gaining an understanding of the role of ABA in the wild type and of how ABA metabolism alters in response to water stress. Root ABA levels may have an influence on the water economy of the shoot (4). However, there is no information in the literature on the effect of the flc and sit mutations on root ABA content and metabolism.

The role of ABA in the regulation of phenotype, including morphology and wilting behavior, may be clarified by altering the endogenous ABA levels in the mutants by grafting. Additional information on the effect of the mutated genes on ABA metabolism in turgid and stressed plants may be obtained from reciprocal grafts. Thus, in this paper, we present data on the effects of reciprocal grafts on the phenotypes of wild-type and mutant scions and on the accumulation of ABA in response to water stress by roots and leaflets of single genotypes and reciprocal grafts.

MATERIALS AND METHODS
Culture of Plant Material. Lycopersicon esculentum Mill. cv RR and MM and three wilty mutants, namely, flc and sit in the RR background and sit* in the MM background, were grown in a controlled environment chamber (23). Plants were grown either in pots or in solution culture using the continuously draining solution culture apparatus previously described (4).

Experimental Plants. Single genotypes were grown in pots and solution culture, as were various grafts made between the different genotypes. In solution culture, plants were reciprocally grafted when the wild type, flc, sit, and sit* were approximately 5, 6, 7, and 7 weeks old, respectively. The leaves of the stock plants were removed during the next 3 weeks as the scions became established. The grafted plants were left in solution culture for at least 11 d following excision of the last stock leaf to eliminate any pool of endogenous ABA generated by the stock leaves during growth. Plants in pots were grafted at various ages and were allowed to retain their stock leaves.

Experiments were carried out on leaves from the following pot plants, with the number of experimental repeats in parentheses; grafted plants are described as scion/stock: RR (2), MM (1), flc (4), sit (1), sit* (1), RR/MM (1), flc/RR (2), flc/MM (1), sit*/RR (1), sit*/MM (1), fcl/sit* (1), sit*/flc (1). In solution culture, each experiment comprised three controls and three steam-girdled plants, with two of each group stressed and 1 left turgid (details in "Experimental Procedures"). These experiments were carried out on the following plants: RR (2), flc (1), sit* (1), RR/flc (2), flc/RR (2), RR/sit* (2), sit*/RR (2), sit*/flc (1).
flc/sit*(1), sit*/flc(1). Repeated experiments involved separate sets of plants grown several months apart.

**Experimental Procedures.** In experiments on pot plants, leaflets were detached, randomized, and then divided into equal quantities. Each sample contained approximately one leaf's worth of leaflets. Stressed leaflets were dehydrated to a 12% loss of fresh weight by exposure to still air or a stream of warm air. All samples were enclosed in plastic bags and incubated for 6 h at room temperature in darkness.

In other experiments, intact plants were removed from the continuously draining solution culture apparatus; the roots were placed immediately into culture solution, and the shoot of each plant was placed into a plastic bag. The stems of half the plants were steam-girdled by directing a jet of steam for 3 min in a 1 cm band around the stem 0.5 cm above the root system. The steamed portion of the stem became very shriveled. This technique had previously (4) been shown to block phloem translocation completely in both tomato and cocklebur plants. The roots of each plant were blotted to remove excess culture solution and were enclosed in plastic bags. The steam-girdled and control plants were then subdivided into two groups. One group was stressed in a stream of warm air (the root systems still enclosed in their plastic bags to prevent direct drying of the roots) until the intact plants had lost approximately 15% of their fresh weight. The shoots were then replaced into their plastic bags. The turgid and stressed plants were incubated for 6 h at room temperature in darkness. During this time, the stressed shoots drew water from the roots, so stressing them in a natural manner. After this time, the roots (without much secondary thickening) and leaflets (without the petiole, rachis, petiololes, or rachillae of the leaf) were harvested.

All samples of plant material were weighed, frozen in liquid N2, lyophilized, and reweighed; the loss of fresh weight by the leaflets and roots at harvest could then be determined. The dried leaf samples were ground by hand in plastic bags and were thoroughly mixed; a small sample (30–100 mg) was then analyzed for ABA (see “Extraction and Purification Procedures”). The entire root samples were also analyzed for ABA content.

**Extraction and Purification Procedures.** All samples of plant material to be analyzed for their ABA content were homogenized using a Poltron homogenizer (Brinkmann Instr., Westbury, NJ) and extracted with 80% acetone (acetone, 1% acetic acid, 0.01% 2,6-di-tert-butyl-p-cresol) in darkness at 4°C for at least 24 h. The samples were filtered, and the tissue residue was washed with acetone. The acetone was evaporated, and the remaining aqueous fractions were frozen in liquid N2 and lyophilized. The samples were then purified by semi-preparative HPLC as described (2) with some modification (3). The ABA content of the samples was quantified using a Hewlett-Packard 5940A gas chromatograph equipped with a 60Ni-electron capture detector as described (3). Small amounts of (+)-[3H]ABA (16.0–15.0 Ci·mmol−1) were added to the samples to determine losses during the purification procedures. Overall recovery of [3H]ABA added to the samples was between 70 and 95% for both leaflets and roots. All data have been corrected for losses.

**RESULTS**

**Morphology.** Wild-type scions on mutant stocks maintained the normal appearance of ungrafted plants. Mutant scions grafted onto wild-type stocks underwent marked morphological changes: flc scions became vigorous and their leaves expanded to become similar to the wild type; leaves of sit and sit* scions also expanded, although they remained smaller than those of the wild type. The leaves exhibited very little epinasty, and very few aerial roots formed on the stems. These revertive phenotypes were maintained even after the wild-type leaves were excised. Scions of reciprocal grafts of the mutants maintained the shoot morphology of their genotype.

**Wiltiness.** When leaflets were detached from pot plants and stressed, it was observed that mutant leaflets, from flc and sit scions grafted onto wild-type stocks, dried at a rate similar to wild-type leaflets. Leaflets from intact mutant plants wilted considerably faster, about 3 times in flc and 6 times in sit and sit*, as has been observed in other investigations (14, 19). Mutant leaflets from the grafted plants with wild-type stocks (without leaves) grown in solution culture wilted at rates more similar to their normal mutant rates.

**Endogenous ABA Levels.** The endogenous ABA levels in both turgid and stressed leaflets were unaffected by the culture method under which the plants were grown (Fig. 1). This also implies that the leaflets responded similarly whether stressed when detached (leaflets from the pot plants) or when still attached to the intact plant (leaflets from plants grown in solution culture). Turgid RR leaflets contained about 2.9 ng ABA·mg−1 dry weight (Fig. 1). On average, the mutant flc contained 33% of this amount while sit and sit* contained about 11%. These results are in close agreement with other reports (14, 21), and approximately correlate with the severity of the mutant phenotypes. When wilted, the wild-type leaflets accumulated ABA to 2.5 times their basal level, whereas the mutant leaflets were unable to accumulate stress-induced ABA (Fig. 1). The mutants sit and sit* behaved similarly in all comparisons made, as did the two wild types RR and MM in the grafted plants. Hence, all subsequent figures of endogenous ABA levels do not include data from either sit or MM. The steam-girdling procedure did not affect the endogenous

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**FIG. 1.** Leaflet ABA levels ± se. Each pot plant value is the mean of 3. Each value for plants grown in solution culture includes data from both control and steam-girdled plants, as the steam treatment had no effect on the endogenous ABA level; each value for turgid leaflets is the mean of duplicates and for stressed leaflets is the mean of 4.

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ABA level in leaflets of turgid or wilted plants. The root ABA level was also similar in control and steam-girdled plants but, for unequivocal interpretation of the results, only data from stressed steam-girdled plants are included.

When the endogenous ABA levels in the roots were determined, again, only the wild type was capable of producing stress-induced ABA (Fig. 2). Roots of both flc and sit" contained less ABA than did turgid RR roots, although sit" did have a higher level than flc in this experiment (Fig. 2). All the plants contained considerably less ABA in their roots than their turgid leaflets, 2.5, 1.9, and 12.0% in RR, flc, and sit", respectively.

The ABA levels in leaflets from flc and sit" scions grafted onto wild-type stocks were dependent upon the culture method (flcl/RR, Fig. 3; sit"/RR was similar to Fig. 3, so data are not shown). Mutant scions grafted onto RR stocks that still possessed wild-type leaves had endogenous ABA levels comparable to the wild type (Fig. 3). However, when plants with mutant scions grafted onto RR stocks were grown in solution culture, and the wild-type leaves were excised for at least 11 d before the leaflets were harvested and analyzed, the mutant leaflets contained endogenous ABA levels comparable to leaflets of single mutant plants (Fig. 3; cf. Fig. 1).

The endogenous ABA level in leaflets and roots of single genotypes and grafted plants was then investigated in solution culture-grown plants. Leaflet ABA levels were unaffected by the stock genotype and responded to stress as if they were single plants of the leaflet genotype (Table I). The endogenous ABA level in unstressed roots was little affected by the genotype of the scion, even when RR scions were grafted onto mutant stocks. The roots of RR stocks of stressed plants also behaved as if they were part of a wild-type plant regardless of the scion genotype (Table I). However, mutant roots did accumulate stress-induced ABA when they were grown with RR scions, even when the plants were steam-girdled before they were wilted (Fig. 4; Table I). The variation apparent between the different experiments, of ABA accumulated in stressed roots, was found to be largely due to the difficulty in accurately reproducing a particular degree of root stress when dehydrating intact plants. We have shown in other work (4) that the actual amount of stress-induced ABA produced by RR roots is closely related to the severity of the stress, with maximum production at about a 60% loss of fresh weight. In the experiments reported in this paper (Fig. 4), the fresh weight loss by the roots of the stressed intact grafted plants varied from 25 to 55%. The fresh weight loss by the roots correlated very highly with the endogenous ABA level found ($r = 0.93$, $P < 0.001$). Reciprocal grafts between the mutants had no effect on ABA accumulation by the roots (Fig. 4).

**DISCUSSION**

The morphology of the wilted tomato mutants is characterized by leaf epinasty, swelling of the upper stem, and aerial root formation, but the mutant phenotypes revert to near wild-type morphology when ABA is applied exogenously to the leaves (10, 21). Mutant scions grafted onto wild-type stocks exhibited phenotypic reversion both when grown in pots with attached wild type leaves and when grown in solution culture, where the revertant phenotype was maintained even after the endogenous ABA level had dropped to the mutant norm following excision of the wild type leaves (Figs. 3 and 1). This implies that enough ABA must have been supplied to the mutant leaves by the wild type roots to support a normal phenotype. Tomato roots contain considerably less ABA than the leaves (4), even in the mutants (Figs. 1 and 2). However, ABA transported from the roots via the xylem may be more readily available to leaf cell metabolism than the endogenous ABA which, in turgid illuminated tissue, is probably largely compartmentalized in the chloroplasts (5, 8). In contrast, a wild-type endogenous ABA level was necessary for correct stomatal control. Leaves from mutant/wild-type pot plants wilted at a rate similar to wild-type leaves, while leaves from mutant/wild-type plants in solution culture, and from fcl-sit" reciprocal grafts, wilted at rates typical of leaves from nongrafted mutant plants. Thus, the ABA present, whether arising from intrinsic biosynthesis or from imports, the more slowly the leaves wilted. This is consistent with the recent realization (3, 9) that stress-induced ABA plays a minor role in this process: stress-induced ABA does not begin to accumulate until after initiation of stomatal closure. Furthermore, a normal phenotype was generated in the solution culture-grown mutant/wild-type plants, although the leaves retained the rapid wilting characteristic, suggesting that different plant processes require different amounts of ABA.

It is also probable that raising the endogenous ABA level in the mutant leaves by grafting (Fig. 3) or by exogenous application
PHENOTYPIC EXPRESSION OF GRAFTED ABA-DEFICIENT TOMATOES

Table 1. Relative Increases in ABA Content during Stress in Roots and Leaflets from Single Genotypes and Reciprocal Grafts of RR, flc, and sitw

<table>
<thead>
<tr>
<th>Stocks</th>
<th>Scion Roots</th>
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<th>Scion Leaflets</th>
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<tbody>
<tr>
<td>RR</td>
<td>flc</td>
<td>sitw</td>
<td>RR</td>
</tr>
<tr>
<td>RR</td>
<td>6.3*</td>
<td>11.8*</td>
<td>4.1*</td>
</tr>
<tr>
<td>flc</td>
<td>5.4*</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>sitw</td>
<td>3.6*</td>
<td>1.1</td>
<td>1.0</td>
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Fig. 4. ABA levels in the roots of grafted plants grown in solution culture. Values for turgid plants include data from control and steam-girdled plants. The experiments with grafted wild-type scions were repeated twice. Values for stressed plants include data from the steam-girdled plants only. Each value is the mean of duplicates, and the individual data points are plotted.

still does not completely compensate for the mutations in flc and sitw. For example, sitw leaves grown with wild-type leaves never expanded to full size despite having a wild-type endogenous ABA level. Furthermore, we observed that the leaves of flc/RR produced large quantities of anthocyanin, which did not occur in either flc or RR alone. It has been reported that anthocyanin production can be enhanced by ABA and ethylene acting synergistically (15). The excess anthocyanin production by the flc/RR leaves suggests that ABA supplied by the RR roots may act with ethylene released by the flc leaves (13, 20). A fine balance of the two plant growth substances is presumably necessary since sitw/RR leaves produced far less anthocyanin than flc/RR leaves.

Mutant roots from wild-type/mutant grafted plants (Fig. 4) contained the typically low ABA levels previously observed in the roots of flc and sitw tomato plants (Fig. 2) in spite of their vascular attachment to the much larger amount of ABA in the RR leaves (Fig. 1). The low root ABA content presumably reflects either a high catabolic rate or the roots' inability to retain ABA while growing in liquid media. ABA efflux from roots can certainly vary from species to species (4) and may be affected in the mutants.

As mentioned earlier, flc and sitw result from different single-gene mutations (16). The data support the hypothesis that at least some ABA is produced via the same biosynthetic pathway by both turgid and stressed plants because the endogenous level of ABA as well as the ability to produce stress-induced ABA are affected. Now, flc and sitw roots regained the ability to make stress-induced ABA when grafted with wild-type scions before the stress period was imposed (Table 1). Thus, a mobile substance from the leaves must have accumulated in the roots while the plants were turgid that negated the defect in the biosynthetic pathway and allowed the accumulation of stress-induced ABA. This could be a precursor of ABA either at or after the mutated gene product or perhaps a promoter that only becomes effective during water stress. The recovery of the flc and sitw roots supports the hypothesis of Taylor and Tarr (22) that the flc and sit genes may affect a single enzyme, perhaps coding for different polypeptide subunits of the same enzyme. If two separate enzymes were affected it would be predicted that either flc or sitw leaves possess the same transportable substance as the RR leaves and should cause the same recovery of the stress-induced ABA synthesis in the roots of the other mutant. However, the roots of neither reciprocal graft between flc and sitw developed the ability to synthesize stress-induced ABA (Fig. 4). It is also curious that the mutant leaflets remained incapable of stress-induced ABA synthesis even when grown with wild-type leaves (Fig. 3). However, differences in ABA metabolism between roots and leaves have been observed in studies with 18O2 (6, 7). Stress-induced ABA in leaves seems to be derived from a substantial precursor pool (6). The roots apparently have much less of this precursor and more of their stress-induced ABA is probably derived from an earlier precursor in the biosynthetic pathway (7). Nevertheless, perhaps the most likely explanation for the unresponsiveness of the mutant leaflets is that the mobile substance from the RR leaves is only basipetally transported; no experiments were done with RR leaflets grafted apically to mutant leaves. Alternative explanations could include that the roots are a sink for the mobile substance whereas the leaves are not, that the stress-induced biosynthetic pathway in the leaves is in a different compartment and therefore is not accessible to the mobile substance, or there could be enzymatic differences between the roots and leaves.

CONCLUSIONS

Many aspects of plant growth and development require ABA to proceed normally, although, as discussed earlier, different processes seem to require different amounts of ABA. However, the severity of the mutant phenotypes approximately correlated with the scarcity of endogenous ABA. In contrast to the wild type, leaflets and roots of the mutant plants were unable to accumulate stress-induced ABA. However, the notable exception of mutant roots with wild-type scions suggests that these roots accumulated a mobile substance from the turgid leaves that negated the defect in the ABA biosynthetic pathway and allowed the accumulation of stress-induced ABA. The recovery of the flc and sitw roots from wild type/mutant plants, but not from reciprocal grafts of the two mutants, supports the hypothesis that
the "flc" and "sit" genes affect a single enzyme, perhaps coding for different polypeptide subunits.

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LITERATURE CITED