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12. We thank Dr. C. A. Thomas for assistance and comments, and Mrs. E. Jenkins for technical help. Supported by NIH grants AI 08937 (to J.I.) and 2 RO1-AI-0818602 (to Dr. C. A. Thomas).

3 March 1970; revised 2 June 1970 ■


# Phenotypic Reversion of Flacca, a Wilty Mutant of Tomato, by Abscisic Acid


Abstract. *The tomato mutant* flacca *wilts rapidly under water deficit because its stomata resist closure. Application of abscisic acid to intact mutant plants changes their morphology toward the phenotype of the control normal variety, Rheinlands Ruhm. The treated mutant plants do not show wilting symptoms, and the resistance to closure of their stomata decreases with hormone treatment.*


Stomatal mechanism is affected by the four groups of plant growth substances. Kinetin, and possibly gibberellin, stimulates stomatal opening (*1*), whereas auxins and abscisic acid induce closure (*2–5*). The concentration of abscisic acid was reported to increase in wilting, detached leaves of wheat (*6*) and in tobacco plants subjected to osmotic root stress (*7*). The authors suggested that endogenous abscisic acid regulates water loss, apparently by reducing stomatal opening.

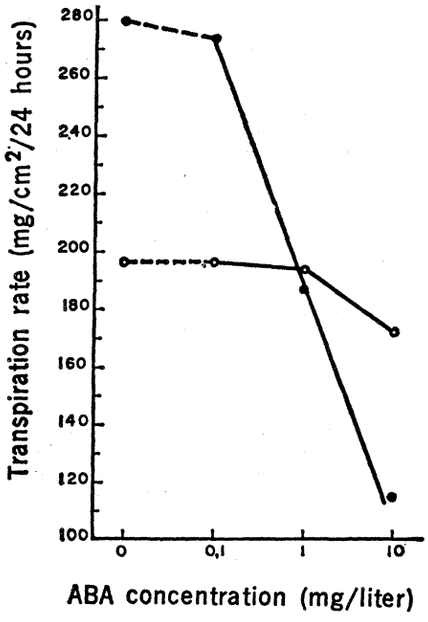


Fig. 1. Water loss from detached leaves from mutant (filled circles) and normal (open circles) plants treated with abscisic acid. Each point represents the mean value of 15 to 20 third leaves from the apex.

We now provide additional support for this suggestion, from results with a wilty mutant of tomato in which the metabolism of abscisic acid appears to be defective (*8*).

The tomato mutant, *flacca* (*flc*), wilts rapidly under water stress due to abnormal stomatal behavior. The stomata of the mutant resist closure under conditions which induce closure in the normal variety, Rheinlands Ruhm (RR), in which the mutation was induced. These conditions include —darkness, wilting, plasmolysis of guard cells, and treatment with phenylmercuric acetate (*9*). The mutant plant is also distinguished from the normal variety by its growth habit; it is much thinner and shorter. In addition, the *flacca* mutant develops, at maturity, symptoms characteristic of auxin excess, namely, strong rooting along the stem, swelling of the upper part of the stem, and epinasty of the leaves (*9*).

When *dl*-abscisic acid (ABA) (*10*) was applied to the mutant seedlings, either by foliage spray (1.0, 10.0 mg/liter) given once a day or in root solution (1.0 mg/liter), a change in the growth habit of the mutant was evident after several days. The mutant plants looked very much like the normal ones in stem thickness and height, and in size, shape, and turgidity of their leaves. In addition, guttation appeared in the morning on the edges of the leaves of the plants treated with ABA, as in normal control plants. Guttation is completely absent in the control mutant plants. Similarly, the symptoms characteristic of an excess of auxin did not appear in those mutant plants sprayed daily with ABA (10.0 mg/liter).

Stomatal opening of mutant and normal plants that were sprayed daily with ABA for 14 days starting from seedling stage was determined indirectly by measuring the transpiration rate of detached leaves (Fig. 1). Cut petioles were inserted in distilled water in sealed beakers, and water loss was determined after 24 hours. Tested *flc* leaves were kept in darkness, since detached mutant leaves of nontreated plants wilt quickly in the light. Those of the normal variety remained in the greenhouse.

The stronger response of *flc* leaves to external ABA as compared with leaves of RR might indicate that the internal concentration of this hormone is much lower than optimum. It should be noted that no full reversion of the mutant phenotype occurred even at 10.0 mg ABA per liter, at least with respect to transpiration rate. Water loss from mutant leaves treated with 10 mg ABA per liter was still higher than that of the control normal leaves when both were put under similar light conditions. When leaves were left in the greenhouse, the mean water loss from ABA-treated *flc* leaves was 256.76 mg/cm²/24 hours, whereas it was 196.76 mg/cm²/24 hours for nontreated RR leaves. Direct observation of stomatal opening (Table 1) with the silicon rubber technique (*3*, *11*) confirmed the above results.

Although the size of stomatal aperture was not measured, it is obvious from the percentages of open stomata that ABA did not change the frequency of open stomata in the light, whereas it induced stomatal closure of *flc* leaves in the dark. It seems, therefore, that ABA in the intact plant ensures normally regulated stomatal behavior.

Three observations indicate that normal stomatal behavior is dependent upon the continuous presence of

Table 1. Percentage of open stomata in leaves of tomato plants. Leaf disks were floated on water for 3 hours either under light (about 33,000 lu/m²) or in the dark before leaf impressions were made.

| Variety | Treatment | | | |
|---|---|---|---|---|
| | Control | | ABA (10 mg/liter) | |
| | Light | Darkness | Light | Darkness |
| *flc* | 100 | 92.48 | 100 | 14.56* |
| RR | 100 | 0.0 | 100 | 2.4* |

* The stomata were open only to a narrow slit.

abscisic acid, and that the hormone does not act as a trigger in stomatal development.

1) Decreased water loss was observed in both old leaves (which completed their development before the treatment with ABA, and young leaves (which developed during the hormonal treatment).

2) Water loss in detached leaves from untreated mutant plants decreased proportionately with the hormone concentration when leaves were inserted in ABA solutions.

3) The treated *flc* plants regained their mutant phenotype very soon after termination of treatment. Wilting symptoms appeared several days after ABA spraying had ceased even in those leaves which had developed during the treatment. This fact might also suggest a rapid turnover of the hormone.

Abscisic acid is a natural inhibitor that interacts with kinetin, auxin and gibberellin in several hormonally regulated plant responses (*12*). The hormone antagonized the kinetin-stimulated transpiration of wheat and tobacco leaves when both were applied externally (*5*, *7*). An excessive kinetin-like activity has been found in the leaves of *flc* plants (*13*). There is also evidence of a lower concentration of substances that inhibit the growth of wheat coleoptiles in the leaves of the mutant, as compared with the leaves of the normal plant. The concentration of endogenous abscisic acid-like substances was ten times lower in *flc* than in normal plants (*8*). These facts and the reversing effect of abscisic acid suggest that the excessive opening of the stomata is caused by an insufficient amount of an internal inhibitor, which is presumably abscisic acid. The closing effect of this substance could be manifested either by affecting the closing mechanism directly, or by antagonizing the opening effect of the internal cytokinins.

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31 March 1970

## Serum Hepatitis Antigen (SH): Rapid Detection by High Voltage Immunoelectroosmophoresis

Abstract. *An immunoelectroosmophoretic technique for rapid detection of the antigen (SH) associated with the serum hepatitis virus has been devised. The technique maintains the specificity characteristic of the Ouchterlony gel-diffusion method, yet detects in 1 to 2 hours one-tenth the amount of antigen required for gel diffusion. The test has immediate application to blood-banking practice since it permits the screening of such labile products as platelets and fresh whole blood, and the detection of antigen in additional serums negative by the Ouchterlony technique.*

A serum hepatitis related antigen (SH), which is identical to the Australia antigen Au(1) (*1*, *2*) and to the "hepatitis antigen" (*3*), has been detected in the serums of patients during the incubation period and early clinical course of hepatitis that follows some transfusions, but not in the serums of patients with the short-incubation type of infectious hepatitis (*2*, *4*). When blood containing the SH antigen is transfused, more than half of the recipients may be expected to develop either clinical or subclinical hepatitis (*5*, *6*), and it therefore is of considerable importance to identify SH antigen carriers in blood donor populations.

The Ouchterlony gel-diffusion technique, most commonly used now for detection of the SH antigen, permits determination of specificity with the use of known standards to obtain fusion of adjacent precipitin lines. This is important since antiserum is derived from multiply transfused patients who have numerous other antibodies. The Ouchterlony technique, however, has two disadvantages in the present application. It is relatively insensitive, detecting only 20 to 30 percent of serum hepatitis carriers in blood donor populations (*7*), and it is a relatively slow test, requiring as long as 1 to 7 days for definitive interpretation. It cannot therefore be used to screen blood products which must be transfused within 1 day of preparation, such as platelets or fresh whole blood.

A complement fixation technique for antigen detection, which has the advantage of a 20- to 100-fold increased sensitivity, has been described (*8*, *9*). This technique also has several disadvantages. Overnight incubation is required to attain a high degree of sensitivity; identification of antigen, particularly when present in small quantities, is difficult and in some cases impossible. Furthermore, since low-titer results must be interpreted with caution, the increased sensitivity afforded by this technique does not appear to be of great practical usefulness for screening.

Most of the immunologic techniques applicable to this problem, such as passive agglutination and radioimmune assays, require either purified monospecific antibody or highly purified antigen if specificity is to be maintained together with an increase in sensitivity.

Table 1. Comparative sensitivity of Ouchterlony gel diffusion (OT) and immunoelectroosmophoresis (IEOP) for detection of serum hepatitis virus specific antigen (SH). Sixteen separate specimens were examined; parentheses indicate the number of assays on each.

| Reciprocal of geometric mean titer | | Sensitivity increase (No. of times) |
|---|---|---|
| OT | IEOP | |
| 26 (4) | 256 (8) | 10 |
| 16 (1) | 256 (1) | 16 |
| 8 (1) | 256 (1) | 32 |
| 4 (1) | 64 (1) | 16 |
| 2 (1) | 64 (1) | 32 |
| 4 (4) | 52 (8) | 13 |
| 4 (1) | 32 (1) | 8 |
| 4 (1) | 32 (1) | 8 |
| 2 (4) | 26 (4) | 13 |
| 4 (1) | 16 (1) | 4 |
| 4 (1) | 16 (1) | 4 |
| 2 (4) | 16 (4) | 8 |
| 2 (1) | 16 (1) | 8 |
| 1.4 (4) | 16 (8) | 10 |
| <1 (4) | 5.6 (8) | >6 |
| Weighted geometric mean: | | >9.9 |