Plant Physiol. (1966) 41, 1387–1391

Abnormal Stomatal Behavior in Wilty Mutants of Tomato[1]

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Received May 10, 1966

Summary. An attempt was made to explain the excessive wilting tendency of 3 tomato mutants, notabilis, flacca, and sitiens. The control varieties in which these mutations were induced are Rheinlands Ruhm for flacca and sitiens and Lukullus for notabilis. Although all 3 mutants are alleles of separated loci, they seem to react similarly to water stress. The mutants wilt faster than the control plants when both are subjected to the same water stress. It was demonstrated by measurements of water loss from whole plants that all 3 mutants have much higher rates of transpiration than the control varieties, particularly at night. The extent of cuticular transpiration was compared in both kinds of plants by measuring the rate of water loss from detached drying leaves coated with vaseline on the lower surface. The difference in cuticular transpiration between the mutant and the control plants seems to be negligible. However, various facts point to stomata as the main factor responsible for the higher rates of water loss in the mutant plants. The stomata of the latter tend to open wider and to resist closure in darkness, in wilted leaves, and when treated with phenylmercuric acetate. Stomata of the 2 extreme mutants, sitiens and flacca, remain open even when the guard cells are plasmolyzed. The stomata of the mutants also are more frequent per unit of leaf surface and vary more in their size.

Very few instances of modifications of plant-water relations by single gene mutations have been studied. The only examples analyzed are those of wilty dwarf in tomato (1, 6), wilted in corn (5), and an osmotic mutant of *Arabidopsis thaliana* (3). The wilting of the tomato and the corn mutants result from development of anomalous vessel elements which interfere with water movement. In the first, development of a secondary wall across the end of the vessel elements produces reticulate or scalariform perforation plates (1), whereas in the second mutant differentiation of the 2 large metaxylem vessels of the vascular bundle is delayed (5). The aberrant phenotype of the *Arabidopsis* mutant results from the low osmotic pressure of the cell sap (3).

This paper presents the results of a study of 3 mutants of tomato which tend to wilt rapidly. The mutants are flacca (*flc*), sitiens (*sit*), and notabilis (*not*). All 3 are recessive point mutations produced by x-ray treatment (9, 10, 11). Their chromosomal location, as indicated from tests of allelism and linkage made by Dr. C. M. Rick of the University of California, Davis, is as follows: *not* and *flc* are located in separated loci on chromosome no. 7 and *sit* on chromosome no. 1. The control varieties, designated hereafter as normal plants, in which these mutations were induced are Rheinlands Ruhm for *flc* and *sit* and Lukullus for *not*. Although these mutations are alleles of 3 separated loci, they seem to react similarly to water stress. When normal and mutant plants are subjected to the same water stress, the mutants wilt first. This wilting characteristic is expressed by the mutants in all stages of life. Of the 3, *not* is most vigorous and most nearly resembles the normal plant. The other 2 grow more slowly and differ from the normal plant by swelling of stems and epinasty of leaves, especially on the upper part of the plant. These differences are particularly notable in *sit* which, in addition, frequently develops numerous roots on all parts of the stem.

Experiments and Results

Rate of Transpiration. The first step was to learn whether the rapid wilting of the mutants results from inadequate water supply or from excessive water loss. Preliminary observations on grafted material indicated that stem and root systems in these mutants have, at most, only small effect on water supply and that their characteristic wilting results mainly from excessive water loss. Normal plants grafted on mutant stem or root systems did not wilt prematurely, whereas mutants grafted on normal shoots or roots did wilt pre-

[1] Research supported by NSF grants, GB-82 and GB-3934.

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maturely. More convincing evidence was found by comparing the rates of transpiration of mutant and normal plants. Six plants of each of the mutants and the normal varieties were arranged randomly in a greenhouse where the average light, temperature, and humidity conditions were as follows: noon = 5000 ft-c, 39°, 30 %; night = darkness, 17°, 70 %. The results are presented in table I.

All 3 mutants have much higher rates of transpiration than the normal varieties, the difference being greatest at night. The most rapidly transpiring mutant, *sit*, also is the weakest in growth, whereas the least rapidly transpiring of the 3, *not*, is the most vigorous. The third mutant, *flc*, is intermediate in both respects. It appears clear from these data that all 3 mutants develop unusual water stresses and wilt easily because they transpire excessively.

Table I. *Rate of Water Loss of Mutant and Normal Plants*

Variety	gm Water loss/ gm leaf dry wt/hr		mg Water loss/ cm² leaf area/hr	
	Day	Night	Day	Night
Rheinlands Ruhm (normal)	2.283	0.328	7.75	1.15
flacca	5.057	2.151	15.49	6.53
sitiens	6.570	3.920	18.47	10.89
Lukullus (normal)	2.145	0.350	7.38	1.19
notabilis	4.350	0.918	13.04	2.75

Cuticular Transpiration. Possible differences between mutant and normal plants in respect to cuticular transpiration were investigated by measuring the rate of water loss from detached drying leaves, the lower surface of which was coated with vaseline. The measurements were made under light of sufficiently low intensity to induce closure of the relatively few stomata on the upper leaf surface. The results of these measurements are shown in figure 1.

The results suggest that, if the cuticular transpiration from the upper leaf surface can be taken as an indication of cuticular transpiration of the entire leaf, normal and mutant plants have similar rates of cuticular transpiration. The fact that the rates are somewhat higher in the mutants does not contradict this conclusion. These small differences between mutants, especially *flc* and *sit*, and normal plants may be attributed, at least partly, to transpiration of stomata remaining open on the upper leaf surface in the mutants. This fact and the reason for the coating of the lower surface will be explained later.

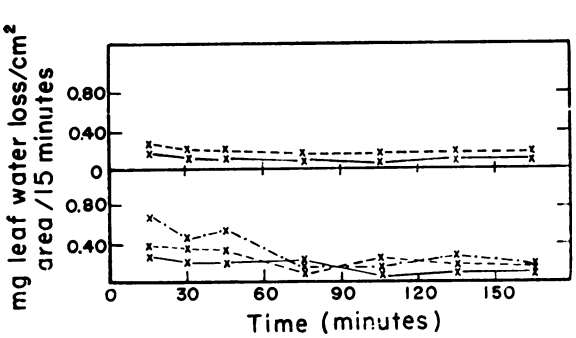

FIG. 1. The rate of cuticular transpiration from detached leaves of mutant and normal plants. Bottom ——— Rheinlands Ruhm (normal); - - - - flacca; -.-.- sitiens. Top ——— Lukullus (normal); - - - - notabilis.

Stomatal Size, Frequency, and Differentiation. Preliminary observations on leaf anatomy indicated that stomata of the mutants are more variable in size, more frequent per unit of leaf area, and open wider under similar light conditions. The size, distribution, and behavior of stomata were determined microscopically by examination of imprints of silicone rubber (7, 13) taken on leaves from the various treatments.

The variation in size of closed stomata, in leaves 2, 6, and 10 weeks of age, was calculated from measurements of 90 stomata chosen randomly from the central region of 6 random leaves at each age. The leaves were floated on water in darkness for 12 hours before impressions were taken in order to induce closure of stomata. The ratio, number of stomata/number of ordinary epidermal cells, and the number of stomata per unit of leaf surface also were calculated from the same leaves. Each value of these 2 ratios is an average of counts of both kinds of cells in 50 random microscope fields (×1000) at each age. The use of the ratio, number of stomata/number of ordinary epidermal cells, eliminates differences in cell size which may result from the conditions of growth and as such it is a good measure of stomatal differentiation (8). The results are represented in table II.

The size of closed stomata varies more in the mutants than in the normal plants, variation being greatest for *sit*. The mutants also have more stomata per unit of leaf surface. This higher frequency of stomata in the mutants may result either from an extensive differentiation of more stomata or simply because of the reduced leaf size in these plants. The two alternatives were checked by calculating the ratio, number of stomata/number of ordinary epidermal cells, in both mutant and normal plants. The ratio was found to be significantly (95 % level) higher in the mutants for leaves of ages of 6 and 10 weeks. This fact suggests that more stomata differentiate in the mutants than in normal plants. The values calculated in leaves of age of 2 weeks are much less accurate than those from older leaves. In young leaves cells are more numerous and smaller than in older leaves, and ordinary epidermal cells may be confused easily with stomatal mother cells.

Stomatal Behavior. The stomata of mutants and normal plants were compared in respect to frequencies of opening and closure in light and dark, respectively, and closure when subjected to

Table II. *The Variation in Size of Closed Stomata, the Ratio of Number of Stomata/Number of Ordinary Epidermal Cells, and the Number of Stomata in Leaves of Different Ages in Mutant and Normal Plants*

Variety	Coefficient of variation of size of closed stomata			No. of stomata/ no. of ordinary epidermal cells			No. of stomata per 0.049 mm² of leaf area		
	2wks	6wks	10wks	2wks	6wks	10wks	2wks	6wks	10wks
Rheinlands									
Ruhm (normal)	24.1	20.1	18.2	0.29	0.26	0.25	22	10	7
flacca	31.3	21.8	25.8	0.31	0.31	0.27	25	10	7
sitiens	37.3	41.7	33.8	0.30	0.31	0.31	25	11	8
Lukullus									
(normal)	24.4	17.4	7.4	0.26	0.25	0.23	24	8	5
notabilis	26.1	22.9	18.7	0.25	0.28	0.28	26	10	7

plasmolysis, treated with phenylmercuric acetate, and during wilting. All 4 tests were made with detached leaves taken from the fifth internode below the top. Leaves were made fully turgid by floating them on water prior to testing. A) The rate of stomatal opening was measured on leaves kept in darkness overnight and then transferred to a light intensity of 3000 ft-c. Stomatal closure was measured on leaves transferred from 2 hours in light intensity of 3000 ft-c to darkness. The frequency of open stomata was calculated by checking about 300 stomata from the central region of the leaf every 15 minutes. Stomata were classified for this purpose as open when the width of the aperture was 2 μ or greater. The results of these observations are shown in figure 2.

The important finding from these observations is that many stomata of all 3 mutants tend to remain open in darkness. Similar frequencies of open stomata were found even after 12 hours in dark. This finding explains the higher rates of transpiration of the mutants at night, shown in table I. The highest frequency of open stomata at night is found in *sit* which also has the highest rate of night transpiration; the lowest is in *not* which has the lowest rate of transpiration at night. It should be noted that the frequency of stomata that remain open at night varies considerably both on a given leaf and among leaves. This variation is not correlated with any obvious features such as size of guard cells or distance from veins. It should be noted also that stomata on the upper leaf surface behave similarly to those on the lower surface in darkness. Open stomata were found, more in *flc* and *sit* and fewer in *not*, mostly close to the main veins.

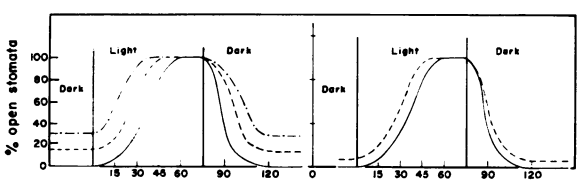

FIG. 2. Rates of stomatal opening and closure in the mutant and the normal plants. Left ——— Rheinlands Ruhm (normal); - - - - flacca; -.-.-. sitiens. Right ——— Lukullus (normal); - - - - - notabilis.

B) The effect of plasmolysis on stomatal closure was studied by removing strips of epidermis from leaves and placing them in a solution of mannitol with an osmotic pressure of 12 bars and examining the strips under the microscope. Many stomata of *sit* and *flc* remained open even when the guard cells were completely plasmolyzed, whereas those checked in *not* and the normal varieties were closed completely.

C) The rates of water loss of mutant and normal plants were measured by weighing detached drying leaves. It was assumed that noticeable differences between mutant and normal plants in respect to this measure would reflect mainly differences in stomatal reaction to increased wilting. This assumption was based on the previous finding of similar rates of cuticular transpiration in mutant and normal plants (figure 1). Prior to the experiment the leaves were floated on water under light of 3000 ft-c in order to make them fully turgid and also to induce maximum opening of stomata. The leaves were then dried carefully on blotting paper and scattered, right side up, on dry petri dishes. Light, temperature, and humidity conditions were as follows: 3000 ft-c, 28°, 30 %. The results are represented in figure 3.

The higher values of the mutants, particularly those of *flc* and *sit*, in the horizontal part of the graphs suggest that some stomata of these plants remain open in drying leaves. This finding was confirmed by checking imprints of wilting leaves dried up to 60 minutes. Open stomata were found on both leaf surfaces, more in *flc* and *sit* and fewer in *not*. The tendency of stomata in all 3 mutants to remain open in dark and in wilted leaves explains why the lower surface of leaves used to check the rate of cuticular transpiration (fig 1) was coated with vaseline. It explains also why the differences between the rates of cuticular transpiration of mutant, especially in *flc* and *sit*, and normal plants were attributed, at least partly, to loss of water through stomata in the first plants.

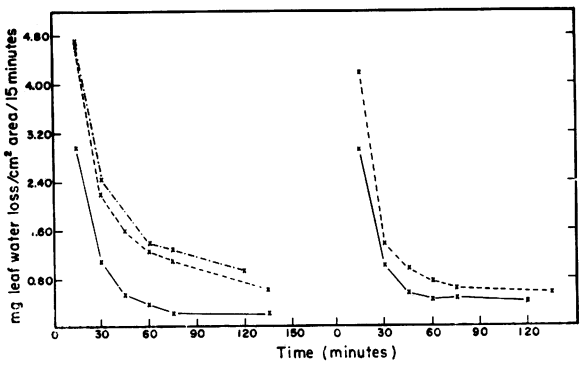

FIG. 3. The rate of water loss from detached drying leaves of mutant and normal plants. Left ——— Rheinlands Ruhm (normal); - - - - flacca; -.-.- sitiens. Right ——— Lukullus (normal); - - - - notabilis.

D) It is well known that phenylmercuric acetate causes closure of stomata (14). The effect of this compound on stomata of both mutant and normal plants was investigated by floating disks of leaves of 1.2 cm diameter on a solution of 1×10^{-4} M phenylmercuric acetate in 0.02% Triton X-100 to improve wetting. Prior to the test the leaves were floated on distilled water under light intensity of 3000 ft-c for 90 minutes in order to induce opening of stomata. Imprints were taken from these disks after floating them for 5 hours on the test solution under the same light conditions. In checking these imprints it was found that many stomata in the mutants remain open, whereas all stomata in the normal varieties were closed. The frequencies of open stomata in the mutants treated with phenylmercuric acetate seem to be similar to those frequencies in darkness (fig 2).

Discussion

The observations reported in the preceding section indicate that the excessive wilting of the mutants results from their higher rates of transpiration. The difference between normal and mutant plants in rates of transpiration are greater at night than during the day time (table I). As indicated in figure 1, mutant and normal plants have similar rates of cuticular transpiration. On the contrary, the extent of wilting as well as the lack of vigor in the mutants is well correlated with the behavior of stomata. The least vigorous mutant, *sit,* has the highest rate of transpiration and its stomata differ most from those of normal plants in their tendency to remain open in dark, in drying leaves, under plasmolysis, and when treated with phenylmercuric acetate. The most vigorous mutant, *not,* differs least from the normal variety in rate of transpiration as well as stomatal behavior. It should be noted also that the mutants have more stomata and fewer hairs per unit of leaf area.

The rate of water loss from leaves is influenced by many internal and external factors. Possible effects of external conditions on the difference between mutant and normal plants were excluded by growing and checking them under similar conditions. The rate of cuticular transpiration was found, as mentioned before, to be similar in both kinds of plants (fig 1). However, these plants differ in size and shape of stomatal aperture and spacing between stomata, all of which may influence the rate of water loss (4, 12). The relative contribution of each of these stomatal features to the higher rates of water loss in the mutants was not estimated. However, the maximum difference between mutant and normal plants is attained under conditions which cause closure of stomata in the latter. This fact is demonstrated most clearly in table I, where the mutants have relatively much higher rates of transpiration at night than during the day time. As mentioned before (fig 2), many stomata of the mutants remain open in darkness, whereas those of normal plants are completely closed. This situation probably also occurs temporarily in day time when high water deficits are built up in the leaves. As mentioned before (section C), some stomata of the mutants remain open in drying leaves, whereas those of normal plants are closed completely.

It is generally assumed that turgor pressure of the guard cells is an important factor in movement of stomata (2). From the data presented in this paper it is clear that turgor pressure alone does not control stomatal opening in these mutants. Stomata in all 3 mutants remain open in dark, in wilting leaves and when treated with phenylmercuric acetate. All these conditions induced closure of stomata in normal plants. Stomata of the 2 extreme mutants, *flc* and *sit,* remained open when the guard cells were plasmolyzed. Further research along this line is in progress in an attempt to explain the difference in stomatal behavior between the mutants and the normal plants.

Acknowledgments

The author is greatly indebted to Dr. P. J. Kramer for his advice during the investigation and in the preparation of this manuscript. Thanks are also extended to Drs. A. W. Naylor and J. R. McWilliam for helpful discussions. Seeds of mutant and normal plants kindly supplied by Dr. C. M. Rick, University of California, Davis.

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