Abnormal Stomatal Behavior and Hormonal Imbalance in *flacca*, a Wilty Mutant of Tomato

IV. EFFECT OF ABSCISIC ACID AND WATER CONTENT ON RNase ACTIVITY AND RNA

Received for publication January 30, 1976 and in revised form July 7, 1976

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ABSTRACT

Plants of the wilty tomato (*Lycopersicum esculentum*) mutant, *flacca*, and of the normal cultivar Rheinlands Ruhm growing under either "normal" or high humidity were used in this research. Under normal humidity, RNase activity was much higher in mutant plants in which abscisic acid (ABA) and water content were lower than in the normal plant. The mutant also contained less RNA and protein per cell and less soluble RNA relative to ribosomal RNA as compared with the normal genotype. In ABA-treated mutant plants, RNase activity decreased while RNA, protein, the ratio of soluble to ribosomal RNA and water content increased.

Under high humidity, RNase activity in mutant plants was decreased, but was still somewhat higher than that in the normal plant, although water saturation deficit was equal in both plant types. Abrisic acid increased RNase activity in the mutant plants. The content of RNA and protein per cell was similar in both types, but the ratio of soluble to ribosomal RNA remained lower in the mutant. In ABA-treated mutant plants, although the content of DNA and RNA per fresh weight was similar to that of control mutant plants, the ratio of RNA to DNA decreased significantly. In addition, ABA caused an increase of the soluble to ribosomal RNA ratio toward the normal value in mutant plants.

Contrary to ABA, kinetin increased RNase activity in the mutant under normal humidity and decreased it under high humidity.

A similar incorporation of labeled uridine into RNA in normal, mutant, and ABA-treated mutant plants under normal humidity suggests that the difference between mutant and normal plants in respect to total, soluble, and ribosomal RNA results not from a different rate of RNA synthesis but from a different rate of RNA degradation, i.e. RNase activity.

An increase of RNase activity (1, 6, 7, 9, 12, 35) and a decrease of RNA level (8, 12) was found in tomato and other plants subjected to water stress. The soluble fraction of RNA increased while the ribosomal fraction decreased in sugar beet under water stress (25). Low relative humidity appears to be associated with slow accumulation and reduced incorporation of labeled uracil into RNA in etiolated leaves of Jack bean (3).

An association between RNase activity, RNA level, and hormone balance in the plant was also reported. Srivastava (29) found an increase of RNase activity in detached barley leaves treated with ABA and a decrease of this activity following treatment with kinetin. Similar hormonal effects on RNase activity were reported in other plants (5, 13, 28, 39). A decline of RNA level by ABA was found in *Lemna* (36), *nasturtium* (2, 37), and in *Taraxacum*, radish, and potato (37). An inhibition of 28P incorporation by ABA was found in barley (23) and radish leaf discs (37). A decrease of ribosomal RNA and an increase of soluble RNA content were reported in tobacco plants treated with ABA (14). An increase of RNA level, or prevention of its decrease during senescence by kinetin was found in *Xanthium* (22). Kahn and Heit (11) found that kinetin enhanced incorporation of 28P, mainly into the heavy ribosomal RNA, in germinating pear embryos.

The levels of ABA and cytokinins are influenced by the water content in the plant. An increase of ABA level was demonstrated by Wright and Hiron (38) in detached wilted wheat leaves. Similar change of ABA level was found in stressed tobacco plants (21). Itai and Vaadia (10) found a decrease of kinetin-like substances in sunflower plants subjected to water stress. Abscisic acid and cytokinins are involved in the regulation of water balance in the plant. Livne and Vaadia (16) found an increase of stomatal opening in barley leaves treated with kinetin. An opposite effect of ABA on transpiration and stomatal opening was reported by Little and Eidt (15) in woody species and by Mittelheuser and Van Steveninck (20) in wheat and barley leaves. Hormonal effects on root resistance to water flow were demonstrated by Tal and Imber (31) in tomato plants. They found that this resistance was decreased by ABA and increased by kinetin.

The first attempt to elucidate the interrelations among RNase activity, water content, and hormone balance was made by Arad et al. (1). They found that in barley leaves, in which the water balance was not disturbed, ABA increased and kinetin decreased RNase activity. However, these hormonal effects were inverted in leaves subjected to water shortage i.e. ABA decreased and kinetin increased the activity. They interpreted these results to mean that above a certain optimal leaf water content, the specific hormonal effect on the enzyme is manifested. Below this optimum, the hormonal effect on RNase activity is inverted because it results primarily from the influence of these hormones on the water balance.

The relationships among RNase activity, RNA level, hormone, and water balance were studied here by use of *flacca*, a wilty mutant of tomato. Tal (30) found that *flc*,1 under usual greenhouse conditions, wilted easily because its stomata resist closure. Tal and Imber (31) reported that the mutant root is more resistant to water flow than that of the control normal plant. Excessive stomatal opening and higher root resistance and the consequent lower water content in the mutant were explained by lower content of ABA in this plant (32).

It was asked in the present work how RNA levels are correlated with RNase activity in the mutant, and what is the contribution of low hydration and ABA deficiency to changes in RNA

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1 Abbreviations: *flc*: *flacca*; RR: Rheinlands Ruhm; WSD: water saturation deficit.
in this plant. Differentiation between the effects of these factors can be achieved by comparing mutant and normal plants in respect to RNA under "normal" or high humidity, with and without ABA application. Under normal humidity, mutant and normal plants differ in both ABA (32) and water content (33). Under high humidity, however, these plants were expected to differ only in ABA level. This expectation was confirmed in the present work.

MATERIALS AND METHODS

The mutation flc was obtained from the normal tomato (Lycopersicum esculentum) cultivar Rheinlands Ruhm (RR) which was used as the control plant in the present work. Plants were grown in the greenhouse in aerated half-concentration Hoagland solution up to the stage of about seven leaves. One group was grown under usual greenhouse humidity of 60 to 70% (designated hereafter as normal humidity). The other group was grown for about 7 days in a polyethylene cell under humidity of 90 to 100% (designated as high humidity).

Hormonal Treatments. Plants growing under normal humidity were sprayed daily with either water, ABA, or kinetin (10 mg/l). The solutions contained 0.1% Tween-20. Plants growing under high humidity received the hormone (1 mg/l) through their roots. Mutant plants treated with ABA will be designated as flcABA and those treated with kinetin as flcflckinetin.

Gas-Liquid Chromatography of ABA. The upper third of the shoot was extracted for ABA determination. The extraction and chromatography procedures were described by Tal and Nevo (32).

Water Saturation Deficit (WSD). WSD was calculated by subtracting the relative water content (26) from 100%.

RNase Activity. Young leaves were frozen in liquid N₂ and homogenized with 0.1 M K-phosphate buffer (pH 6). The crude homogenate was filtered through Miracloth and the filtrate was used as the enzyme preparation. RNase activity was assayed according to Tuve and Anfinsen (34).

Content of DNA, RNA, and Protein. Upper leaves (1 g fresh weight) were frozen in liquid N₂ and extracted according to Smillie and Krotkov (27). The extract obtained with trichloroacetic acid from residue C contained nucleic acids and protein. The nucleic acids were separated from the protein by dissolving them in 5% boiled trichloroacetic acid. DNA, RNA, and protein were determined, following Burton (4), Schneider (24), and Lowry et al. (18), respectively.

Ribosomal and Soluble RNA. These RNA fractions were extracted and separated by Methylated Albumin Kiesslingruh (MAK) column following Mandell and Hershey (19) or by polyacrylamide gel electrophoresis according to Loening (17).

Incorporation of [5-³H]Uridine. Leaf discs, 16 mm in diameter, were placed on 5 ml of 0.1 concentrated Hoagland solution containing 1 mM Mg, 0.001 M MES (pH 7), and 10 μg/ml gentamycin. After 1 hr, the discs were infiltrated with 5 ml of solution, to which 100 μCi of [5-³H]uridine (27.67 μCi/mmole) were added, and incubated at 25 C under light of 1200 ft-c for 24 hr. The discs were then washed for 60 min with the same solution except that the labeled uridine was omitted. The RNA was extracted from the discs and separated by polyacrylamide gel electrophoresis following Loening (17). RNA profile was determined under light of 260 nm. The gels were frozen on Dry Ice and sections of 1 mm width were transferred to vials containing 3 ml scintillation liquid and 0.2 ml tissue solubilizer (Eastman Kodak) per vial. The vials were incubated overnight at 30 C in a shaker and radioactivity was determined thereafter.

RESULTS AND DISCUSSION

ABA CONTENT

The content of ABA (Table I) was much lower (about one-fifth) in flc than in RR plants under both normal and high humidity. A similar difference in ABA level between RR and flc plants under usual greenhouse conditions was reported by Tal and Nevo (32). Kinetin treatment of flc plants did not cause any change of ABA content. This finding will be discussed later. The decrease of ABA per unit fresh weight in RR, flc, and flckinetin plants under high humidity might be explained mainly by the increase of water content in these plants.

RIBONUCLEASE ACTIVITY AND WSD

Hormone Effects Under Normal Humidity. Normal plants grown under normal humidity reacted to the hormone treatment as expected. ABA increased RNase activity and decreased water saturation deficit, whereas kinetin decreased the enzyme activity and increased WSD. Similar effects of these hormones on RNase activity were reported in other plants (1, 5, 13, 28, 29, 39).

The effects of ABA and kinetin on RNase activity and WSD were also determined in mutant plants (Table II). Under normal humidity, RNase activity and water saturation deficit per fresh weight, protein, or DNA were much higher in flc than in RR plants. In contrast to the normal plants, ABA decreased and kinetin increased both RNase activity and WSD in the mutant plant. Adar et al. (1) found that hormonal effects on RNase activity in barley plants under drought were inverted, with ABA retarding and kinetin enhancing this activity. They suggested that in plants in which leaf water content is below a certain optimum, cell water content supersedes hormonal regulation in effecting RNase activity. Consequently, under such conditions, the influence of these hormones on the enzyme results primarily from their effects on leaf water, with ABA retarding and kinetin enhancing the increase of water saturation deficit. The higher RNase activity in flc may be interpreted in the same way. Low

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>ABA Concentration (mg/1 Kg fresh wt</th>
<th>% of RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>18.9 **</td>
<td>14.8</td>
</tr>
<tr>
<td>flc</td>
<td>4.0 **</td>
<td>2.8</td>
</tr>
<tr>
<td>flckinetin</td>
<td>4.5 **</td>
<td>3.1</td>
</tr>
</tbody>
</table>

* Significant difference between either flc or flckinetin and RR at 95% level.

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>RNAse activity (AA 260 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>102 5.3 312 9.7</td>
</tr>
<tr>
<td>flc</td>
<td>219 10.9 483 14.9</td>
</tr>
<tr>
<td>flcABA</td>
<td>156 8.0 431 11.2</td>
</tr>
<tr>
<td>flcflckinetin</td>
<td>481 25.6 946 19.1</td>
</tr>
</tbody>
</table>

* Significant difference between flc and RR at 95% level.

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ABA content in the mutant causes a decrease of leaf water content below the optimum under which the effect of cell water supersedes the specific effect of ABA on RNase activity. In accordance with this interpretation, ABA, which improves the water balance in the mutant, decreases RNase activity; and kinetin, which increases WSD, increases the activity of RNase. Arad et al. (1) rejected the possibility that the active agent in RNase promotion by kinetin under dry conditions is actually ABA which may rise due to the WSD increase by kinetin. In agreement with their finding in barley, kinetin did not increase ABA level in the tomato mutant.

**Hormone Effects Under High Humidity.** Under high humidity, water saturation deficit values of mutant and normal plants were closely similar (Table III). The effects of the hormones on RNase activity in this mutant, i.e. enhancement by ABA and retardation by kinetin, suggest that water content in flc plants grown under high humidity was above the optimum mentioned before. Accordingly, lower activity of RNase was expected in the mutant because of the lower ABA content, as compared with the normal plant. Contrary to expectation, the activity of RNase was somewhat higher in the mutant, suggesting that there is a certain minimum below which the activity of RNase cannot decrease by lowering the content of ABA as in flc. Resulting from the lower ABA level in the mutant, there is an excess of kinetin-like activity in this plant (33). Only a further disturbance of the ABA to cytokinin balance in flc by treating it with kinetin decreased RNase activity below the normal level.

**CONTENT OF DNA, RNA, AND PROTEIN**

Under normal humidity, the contents of DNA and RNA per unit fresh weight and WSD were higher in flc than in RR plants (Table IV). The content of protein was similar in both plant types. The ratios of RNA to DNA and of protein to DNA, however, were lower in the mutant. In flcABA plants, the content of DNA and RNA per unit fresh weight and WSD decreased, and the ratios of RNA to DNA and protein to DNA increased toward the normal level. The lower RNA to DNA ratio in flc, in which ABA content is lower, and the increase of this ratio in ABA-treated flc plants, are in contrast to the known effect of ABA on RNA level in normal plants under usual conditions. A decline of RNA level by ABA was reported in various plants (5, 36, 37). However, the difference in RNA between RR, flc, and flcABA plants is in correlation with RNase activity in these plants when growing under normal humidity. The lower RNA to DNA ratio in flc and its increase by ABA may be explained by the higher RNase activity in flc and its decrease by ABA.

Under high humidity, the ratios of RNA to DNA and protein to DNA were, more or less, similar in flc and RR plants. The RNA to DNA ratio decreased in ABA-treated flc plants. This effect of ABA on RNA in the mutant is in accordance with the effect of this hormone on RNA in normal plants under usual conditions (5, 36, 37) and is correlated with the effect of ABA on RNase activity in this plant.

**RNA SPECIES**

Soluble (sRNA) and ribosomal RNA were extracted from RR, flc, and flcABA plants and separated by MAK (Fig. 1, normal humidity) and polyacrylamide gel electrophoresis (Fig. 2, normal humidity and Fig. 3, high humidity). By calculating the areas under the curves in Figures 1, 2, and 3, the ratio of sRNA to rRNA in RR, flc, and flcABA can be compared under normal and high humidity (Tables V and VI).

The ratio of sRNA to rRNA, as determined by the two methods and under the two humidity regimes, was lower in flc than in RR plants. In flcABA plants, the ratio increased toward RR value. An increase in the sRNA to rRNA ratio was reported in water-stressed sugar beet (25), and in tobacco plants treated with ABA (14). It seems as if the change which induces the elevation of the sRNA to rRNA ratio in these plants is the increase in ABA level. An increase in ABA level was reported in different plants subjected to water stress (21). The lower

### Table IV. Concentration of DNA, RNA, and Protein and WSD in RR, flc, and flcABA Plants Growing Under 'Normal' or High Humidity.

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>DNA (µg)</th>
<th>RNA (µg)</th>
<th>Protein (µg)</th>
<th>RNA/DNA</th>
<th>Protein/DNA</th>
<th>WSD (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>327</td>
<td>8.99</td>
<td>19.03</td>
<td>27.5</td>
<td>58.2</td>
<td>9.7</td>
</tr>
<tr>
<td>flc</td>
<td>453*</td>
<td>11.13</td>
<td>20.06</td>
<td>24.5</td>
<td>44.3*</td>
<td>14.9*</td>
</tr>
<tr>
<td>flcABA</td>
<td>363**</td>
<td>9.55</td>
<td>19.62</td>
<td>26.3**</td>
<td>54.0**</td>
<td>11.2**</td>
</tr>
<tr>
<td>High Humidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>0.250</td>
<td>7.30</td>
<td>15.36</td>
<td>29.2</td>
<td>61.4</td>
<td>6.3</td>
</tr>
<tr>
<td>flc</td>
<td>0.270</td>
<td>7.84</td>
<td>16.00</td>
<td>29.0</td>
<td>59.2</td>
<td>7.4</td>
</tr>
<tr>
<td>flcABA</td>
<td>0.282</td>
<td>7.42</td>
<td>15.83</td>
<td>26.3**</td>
<td>56.1</td>
<td>6.2</td>
</tr>
</tbody>
</table>

* Significant difference between flc and RR plants at 95% level.  
** Significant difference between flcABA and flc plants at 95% level.  

### Table III. The Effects of ABA and Kinetin on RNase Activity and WSD in RR, flc, flcABA, and flc kinetin Plants Grown Under High Humidity.

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>RNase Activity (AA 260 nm)</th>
<th>WSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per g fresh</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>62</td>
<td>4.0</td>
</tr>
<tr>
<td>flc</td>
<td>88*</td>
<td>5.5</td>
</tr>
<tr>
<td>flcABA</td>
<td>111**</td>
<td>7.8</td>
</tr>
<tr>
<td>flckinetin</td>
<td>45**</td>
<td>2.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>per mg protein</th>
<th>per mg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>250</td>
<td>6.2</td>
</tr>
<tr>
<td>flc</td>
<td>527*</td>
<td>6.4</td>
</tr>
<tr>
<td>flcABA</td>
<td>403**</td>
<td>6.2</td>
</tr>
<tr>
<td>flckinetin</td>
<td>152*</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* Significant difference between flc and RR at 95% level.  
** Significant difference between flcABA and flc at 95% level.
sRNA to rRNA ratio in the mutant may result from its lower content of ABA. This suggestion is supported by the finding that the ratio was lower in flc than in RR plants under both normal and high humidity regimes. Although ABA is lower in flc than in RR plants under both conditions, water content is lower in flc only under normal humidity, but equal in flc and RR plants under high humidity. Since the difference in the sRNA to rRNA ratio between mutant and normal plant results mainly from the change of rRNA, it is suggested here, in accordance with Leshem and Schwarz (14), that ABA increases the sensitivity of rRNA toward the action of RNase. Based on this suggestion, rRNA is relatively more resistant to RNase action in the mutant, which contains less ABA, than in RR, and consequently the ratio of sRNA to rRNA is lower in the first. The greater difference in the sRNA to rRNA ratio between RR and flc plants under normal as compared to high humidity may result from the difference in RNase activity. RNase activity in the mutant was much greater under normal humidity, and only somewhat higher under high humidity, than that of the normal plant. The increase in the sRNA to rRNA ratio in RR, flc, and flcABA plants under high humidity relative to normal humidity results from the greater increase of sRNA under high humidity.

No significant difference was found between the per cent of incorporation of labeled uridine into total RNA, calculated from total uptake, in RR, flc, and flcABA plants. In addition, the ratio of incorporation of labeled uridine into soluble and ribosomal RNA was similar in RR, flc, and flcABA plants. These findings suggest that the rate of RNA synthesis is similar in mutant and normal plants, and that the difference between these plants in respect to total RNA and the ratio of sRNA to sRNA results, as suggested before, from the difference in degradation of RNA, i.e. RNase activity.

**LITERATURE CITED**