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Ribonuclease Activity of Stressed Tomato Leaflets

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Summary. Homogenates of leaflets of desiccated tomato plants show increased ribonuclease activity compared to homogenates of turgid controls. Much of this increase is independent of changes in translocation to and from the leaflet. Interruption of translocation through living cells by detachment of leaflets or steam damage to the petiolules produces increased ribonuclease activity, but this activity is increased further when excised leaflets are allowed to wilt. Increases in ribonuclease often parallel or precede increases in the soluble nitrogen content. Further increases in activity occur when excised leaves become yellow. Exposure of leaflets to CO_2-free air has little effect on activity at low-light intensity (120 ft-c). These results suggest that water stress directly affected ribonuclease activity at the cellular level.

Increased ribonuclease activity results from various physiological disturbances in leaves. Kessler and co-workers found greater activity in homogenates of leaves stressed by zinc deficiency (6) and dehydration (4) than in homogenates of unstressed controls. Ribonuclease activity of apple leaves increases with leaf age (5). However, barley leaf discs show less ribonuclease activity when they are floated on water (11). These data apparently conflict with the widely-held belief that detachment of leaflets causes an acceleration of normal patterns of senescence (14).

Translocation between leaves and the remainder of the plant body is often reduced during drought (10, 13) and the assimilation of CO_2 is also reduced (1). Changes in ribonuclease activity during drought may therefore be a direct response to dehydration of leaf cells or may result from changes in the immediate cellular environment brought about by factors such as stomatal closure and reduced translocation.

Direct effects of water stress may be separated from translocation effects by comparing the ribonuclease activity of homogenates from (a) wilted attached leaflets, (b) wilted detached leaflets, (c) unwilted attached leaflets, and (d) unwilted detached leaflets. Exposure of turgid leaflets to air low in CO_2 should show if ribonuclease activity responds to changes in amount of CO_2 available under the light intensity used in these experiments. This paper describes the results of these comparisons. Changes in soluble nitrogen content and leaflet color provide additional criteria to aid in assessment of damage with each stress.

Materials and Methods

Marglobe variety tomato plants were grown in a greenhouse in sand with applications of nutrient solution (8) until they were 6 weeks old and then transferred to a laboratory with cool-white fluorescent lights which gave constant illumination of 120 ft-c and a temperature of approximately 21°. Lower leaves of each plant were removed until 5 leaves over 5 cm long remained. Leaflets of the lowermost leaf were sampled when they had reached full size.

The experiments are summarized in table I. Two ml of distilled water maintained a varying number of leaflets from each plant in a turgid condition in centrifuge tubes while a varying number of leaflets were allowed to wilt. Wilted leaflets and unwilted leaflets were harvested from time to time after detachment. The petiolules of other detached leaflets were immersed in water and the leaflets were enclosed for 5 days in centrifuge tubes containing vials of 10 % (W/V) NaOH solution. Changes in these leaflets were compared to changes in detached leaflets exposed to normal air.

In another experiment, the sand was brought to field capacity with distilled water and the plants were allowed to lose water until half of them were wilted. Water deficit, soluble nitrogen content and ribonuclease activity of leaflets attached to wilted plants were sampled and compared to data from unwilted controls.

Petiolules were treated with a thin jet of steam in another experiment and the leaflets were sampled later. Unsteamed leaflets of the same leaf served as controls. All leaflets remained unwilted. Effects of CO_2 deficiency on ribonuclease activity were also studied. Leaflets attached to unwilted plants were enclosed for 5 days in glass flasks above a 10 % (W/V) NaOH solution to reduce the content of CO_2 in the air. Unenclosed leaflets served as controls.

Combined ethanol and water extracts were digested using H_2SO_4 and H_2O_2 (2), followed by treatment with Nessler's reagent (3). Nitrogen content of these extracts was estimated using a Bausch and Lomb

Spectronic 20 colorimeter. To determine water deficit, 1 leaflet was weighed immediately, placed with the petiolule immersed in water for 10 hours, and subsequently blotted and reweighed. The turgid leaflet was dried at 105° and weighed once more. Water deficit was calculated as percent loss relative to total water capacity of the leaflet (12).

Another leaflet of the same leaf was weighed and disrupted in acetate buffer using a glass tissue grinder and centrifuged. The supernatant fluid was assayed for activity at 50°, using yeast RNA as a substrate. The nucleotides released in the reaction were estimated using a Perkin-Elmer Model 139 UV-VIS Spectrophotometer at a wavelength of 250 mμ after unhydrolyzed RNA was precipitated by the stop solution (2). Absorbance due to soluble substances in the RNA solution and each leaf homogenate were determined by appropriate blanks and subtracted from the absorbance of the sample. Optimum activity occurred between pH 5.0 and 5.6. The reaction rate was constant for periods in excess of 30 minutes. Reaction rates for 15 and 30 minute incubation times were periodically compared and the original time course was confirmed for all of the leaf homogenates tested. Samples assayed before and after 3 hours standing at room temperature and 30 minutes at 50° showed insignificant (less than 5 %) loss of activity. This temperature stability is characteristic of plant ribonucleases (9).

Results

Ribonuclease activity of homogenates of unwilted leaflets detached for 2 days was significantly greater than the activity of attached controls (table I). This greater activity persisted throughout the experiment.

Table I. *Relationships between Ribonuclease Activity, Soluble Nitrogen Content and Water Deficit of Leaflets under Various Treatments*

Leaflets taken from fifth leaf over 5 cm long, numbered from stem apex. Means of 6 samples given unless otherwise specified.

Days treated	Treatments	Means and SE Ribonuclease*	Soluble N**	Water deficit***
2	Unwilted attached	0.224 ± 0.005	0.106 ± 0.001	
	Unwilted detached	0.408 ± 0.014	0.087 ± 0.002	
	Wilted-detached		0.094 ± 0.002	
3	First population			
	Unwilted attached	0.269 ± 0.009	0.139 ± 0.006	
	Unwilted detached	0.627 ± 0.015	0.092 ± 0.002	
	Second population			
	Unwilted detached	0.373 ± 0.009		
	Wilted detached	0.835 ± 0.050		53 ± 2
	Wilted attached	0.307 ± 0.031	0.167 ± 0.002	51 ± 2
	Steamed petiolules	0.595 ± 0.006	0.159 ± 0.005	
	Unsteamed petiolules	0.373 ± 0.002	0.139 ± 0.005	
4	Unwilted attached†	0.178 ± 0.004	0.200 ± 0.004	
	Wilted attached†	0.509 ± 0.035	0.320 ± 0.006	65 ± 2
	Steamed petiolules	0.967 ± 0.017		
	Unsteamed petiolules	0.417 ± 0.006		
5	CO_2-free air, attached ††	0.249 ± 0.005		
	Normal air, attached††	0.217 ± 0.007		
	CO_2-free air, detached††	0.618 ± 0.014		
	Normal air, detached††	0.577 ± 0.008		
10	Unwilted attached	0.286 ± 0.006		
	Unwilted detached†††	0.467 ± 0.021		
	Unwilted attached	0.286 ± 0.006		
14	Unwilted attached	0.200 ± 0.012		
	Unwilted detached*†	0.898 ± 0.015		

* Activity expressed as change in absorbance per hr per g fresh or rehydrated weight. 1.5 ml supernatant fluid after 15 min of 100 × *g* centrifugation of leaf grindate incubated 15 min with 0.4 % (W/V) yeast RNA at 50°. 0.1 M Acetate buffer, pH 5.0 used throughout. Reaction stopped with 0.5 ml 0.75 % (W/V) uranyl acetate in 25 % (V/V) $HClO_4$. Absorbance of nucleotides read at 250 mμ.

** Nitrogen extracted using boiling 70 % (V/V) ethanol followed by boiling water expressed as percent fresh or rehydrated leaflet weight. Rehydrated weight estimated from water deficit of another leaflet from same leaf. Soluble nitrogen analysis obtained by H_2SO_4-H_2O_2 wet digestion, followed by treatment with Nessler's Reagent, reading absorbance at 450 mμ.

*** Percent water loss relative to total water capacity. Wilting observed at water deficit of 15 to 20 %.

† Mean of 12 plants.

†† Mean of 3 plants.

††† Leaflets remained green.

*† Leaflets extensively yellowed.

Soluble nitrogen content increased by the next day (day 3). There was no change in leaflet color. Yellowing occurred within 14 days after detachment in another experiment, along with an additional increase in ribonuclease activity.

Detached leaflets showed much greater ribonuclease activity following 3 days without water than did unwilted detached leaflets. Exposure of unwilted detached leaflets to air with a low carbon dioxide content did not bring about an additional increase in ribonuclease activity over the increase shown by unwilted detached leaves exposed to normal air.

Ribonuclease activity increased in lamina of attached leaflets within 3 days after a portion of the petiolules was killed with a jet of steam. Leaflets did not wilt during this experiment. Four days of wilting produced increased activity in attached leaflets. A slight increase in soluble nitrogen also occurred 4 days after wilting. All leaves were dark green when harvested. Exposure of attached unwilted leaves to air having a low carbon dioxide content produced a slight increase in ribonuclease activity.

Leaflets attached to wilted plants lost water more slowly than excised wilted leaflets. Excised leaflets were dehydrated from a water deficit of less than 5 % at the time of excision to approximately 50 % 3 days later. Water deficits of attached wilted leaves increased from 15 or 20 % (incipient wilting) to approximately 50 % during the same interval of time.

Discussion

Increased ribonuclease activity resulting from water stress was not dependent on changes in translocation between leaflets and the remainder of the plant body. Detachment of leaflets or isolation by steaming petiolules also increased ribonuclease activity, but dehydration combined with detachment of leaflets increased the activity further. Exposure of turgid leaflets to air having a low content of carbon dioxide had little effect on ribonuclease activity at this low light intensity. Greater differences in activity might have occurred at higher light intensities as the rate of photosynthesis increased in control leaflets. It appears likely, however, that the major ribonuclease response to water stress is elicited in the leaflet cells in these experiments. Perhaps water stress caused mechanical disruption of the endoplasmic reticulum. Protein was probably degraded during periods of high ribonuclease activity since the content of soluble nitrogen increased in the leaflets to levels exceeding levels which could be accounted for by release of free nucleotides from nucleic acids. Since ribonuclease activity is found primarily in the microsomal and soluble fractions of leaf homogenates (5), leaching of ribonucleases might account for the decline in ribonuclease activity observed by Srivastava and Ware (1). Ribonucleases may have been lost through cut surfaces of their leaf discs as they floated on water.

Ribonuclease responses are unusually sensitive to leaf detachment and water stress. Leaflets remain dark green for several days after ribonuclease activity increases. Ribonucleases are stable enzymes and their assay appears useful as a biochemical indicator of early alterations of leaf metabolism during stress.

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