

Short Communication**Abnormal Stomatal Behavior and Hormonal Imbalance in *Flacca*, a Wilty Mutant of Tomato**III. HORMONAL EFFECTS ON THE WATER STATUS IN THE PLANT¹

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M. TAL AND D. IMBER

Division of Life Sciences, Negev Institute for Arid Zone Research, Beer-Sheva, Israel

Two of the major resistances to water flow which govern water status in the plant are the resistance to water absorption in the root and the resistance to water loss in the leaf (6, 15). The effect of hormones on stomatal movement, which controls the flow of water vapor from the leaf, is well documented. While cytokinins (7, 8, 10) and possibly GA (7) induce opening of stomata, auxins (1, 9, 21) and ABA (11) cause them to close. Little is known, however, about hormonal effects on water absorption by the root. Indoleacetic acid treatment was found to increase root exudation in *Pisum* and *Helianthus* (14) and in tobacco (5), and 10^{-5} M GA increased it in tobacco (20). The rate of exudation was decreased, however, by 10^{-4} M GA (20) in tobacco and by benzyladenine (18) in tomato.

A study on the wilty mutant of tomato, *flacca* (*flc*), indicated that the irregularities of the water status in this plant may be explained by the hormonal changes demonstrated in the plant (2, 17, 18). The tomato mutant wilts faster than the normal variety Rheinlands Ruhm (RR), used as control, because its stomata resist closure (16). This persistence of open stomata, which results in a higher rate of transpiration than in the normal plant, was explained by the higher kinetin-like activity (18) and the lower concentration of ABA-like substances (17) in *flc* compared with RR. In agreement with this suggestion was the simultaneous decrease with age of stomatal resistance to closure and kinetin-like activity and the increase with age of ABA- and auxin-like activity (17, 18).

In contrast with the higher rate of transpiration, the amount of exudate per unit root dry weight from 5-week-old mutant plants was only 0.35 that of the normal plant (18). The osmotic pressure of the mutant sap, however, was about twice as high as that of the normal plant. The rate of exudation was lower also in mutant plants enclosed in plastic bags for 12 hr prior to decapitation to prevent transpiration. This fact suggests that the lower amount and the higher osmotic pressure of root exudate in the mutant do not result from the higher transpiration of that plant. In the older plants, however, the exudation of the two genotypes, mutant and normal, was nearly the same (18). The closer agreement with age between the amount of exudation in the mutant and that of the normal plant suggests that root resistance, like stomatal behavior, depends on the specific hormonal status in the mutant.

This suggestion was tested by examining the effect of cytokinins, ABA, and natural and synthetic auxins on bleeding of wounds left after removal of the upper leaves, and on the

Table I. Amount and Osmotic Pressure of Exudate from Roots of Control and Hormone-treated Mutant (*flc*) and Normal (RR) Plants

The table represents one out of three similar experiments. Each value is the average of 10 plants.

Hormone	<i>flc</i>				RR			
	Amount of exudate	Osmotic pressure			Amount of exudate	Osmotic pressure		
		Root exudate (OP _i)	Medium (OP _e)	OP _i - OP _e (ΔOP)		Root exudate (OP _i)	Medium (OP _e)	OP _i - OP _e (ΔOP)
	ml/100 mg root dry wt. 24 hr	atm			ml/100 mg root dry wt. 24 hr	atm		
O	2.6	0.89	0.32	0.57	5.0	0.73	0.31	0.42
Kinetin	1.6 ¹	0.96	0.36	0.60	1.8 ¹	1.14	0.29	0.85 ¹
Abscisic acid	6.5 ¹	0.62	0.33	0.29 ¹	10.2 ¹	0.74	0.34	0.40
Indoleacetic acid	3.0	0.79	0.30	0.49	6.6 ¹	0.62	0.36	0.26 ¹

¹ Significantly different from the control at 95% level.

amount and osmotic pressure of root exudate in mutant and normal plants.

Mutant and normal plants were grown in half-concentration Hoagland solution in 1-liter plastic bottles up to the 9 to 10 leaf stage. They were then treated with the hormone solutions. Plants which were sprayed seven times a day for 2 days with ABA or 2,4-D (10 mg/liter) exhibited strong bleeding compared with the control. There was no bleeding at all in either control mutant plants or in kinetin- or benzyladenine-treated (10 mg/liter) mutant and normal plants. This phenomenon, which indicated that hormones affect water absorption by the root, was investigated more closely. Abscisic acid and IAA (10 mg/liter) were applied as sprays, five times a day for 3 days. Kinetin (1 mg/liter) was added to the root solution for 2 days. The plants were then decapitated and root exudate was collected for 24 hr. In all treatments the root solution was replaced by fresh half-concentration Hoagland solution 1 day before decapitation. The OP_i² and OP_e were determined cryoscopically with a G-66 Siske Osmometer (Table I).

As found previously (18), the amount of exudate per unit root dry weight was lower in *flc* than in RR, although the difference between OP_i and OP_e (ΔOP) was greater in the mutant.

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² Abbreviations: OP_i, OP_e: osmotic pressures of root exudate and medium, respectively.

In both mutant and normal plants, kinetin decreased the rate of exudation, the decrease being greater in RR. Moreover, ΔOP was unchanged in kinetin-treated mutant plants but increased in the treated normal. The stronger reaction of RR to the externally applied kinetin is in agreement with the lower kinetin-like activity indicated in that plant relative to *flc* (18). Abscisic acid increased the rate of exudation in both plant types, but more so in the mutant. This treatment decreased the ΔOP in the mutant. The stronger reaction of *flc* to externally applied ABA agrees with previous findings (2, 17) demonstrating lower concentration and activity of ABA-like substances in the mutant. Indoleacetic acid, like ABA, increased the rate of exudation in both plant types, but to a lesser extent. The ΔOP was lower than that of the control in both genotypes. The increase of exudation and decrease of ΔOP were greater in RR. The stronger reaction of RR to IAA agrees with the lower auxin-like activity found in that plant (17). The proportion of electrical conductivity to OP of the exudate was identical in all treatments, suggesting that the proportion of ionic solutes to total solutes in the exudate was unchanged by the hormonal treatments. Similar and even more pronounced results were also obtained by adding the hormones (1 mg/liter) to the root medium at the time of decapitation. The root medium was left unchanged for the entire period of exudation of 24 hr. This suggests that the hormones affect root resistance directly, and not through their effect on stomatal behavior nor consequently on the water status of the plant. The effect of kinetin and ABA on the amount of exudate was already noticeable within 2 hr after decapitation.

The above findings may explain the following additional observations. Plants of the control variety, RR, were sprayed with 2 and 20 mg/liter of benzyladenine. At the lower concentration, the rate of transpiration of intact plants and detached leaves increased to 112 and 125% of the control, respectively, while the rate of exudation decreased slightly (80%). However, the higher concentration of benzyladenine decreased the transpiration rate of intact plants to about 85% of the control although in detached leaves of the same plant the rate increased to 148% of the control. This treatment decreased the rate of exudation to 37% of that of the control. It is possible that although benzyladenine at both concentrations induced stomatal opening, this opening did not occur in intact plants treated with the higher concentration because of the increased resistance of the root to water uptake in these plants. The hormonal effects on root resistance to water flow may, therefore, also play a role in the intact plant.

Based on previous and present findings, it is hypothesized that hormonal changes are related to the water status of the intact plant as follows. Cytokinins decrease stomatal resistance by opening stomata (7, 8, 10) and increase the resistance of the root to absorption of water (18). The general effect of the hormone is to reduce plant turgor. As expected, cytokinin concentration is decreased in stressed plants (3). This may result in a protection against additional drying. In contrast, ABA and auxins increase the resistance in the leaf (11, for ABA; 1, 9, 21, for auxin) and decrease the resistance of the root to water flow (14, for auxin only). These hormones thus increase the plant turgor. Their concentrations increase, as anticipated, in stressed plants (12, 13). Rapid changes in the concentrations of both cytokinins and ABA were demonstrated (4, 12) in water-

stressed plants. The concentration of cytokinins in both leaves and root exudate was greatly reduced in tobacco plants subjected to ventilation for 30 min (4). Abscisic acid concentration was increased within 4 hr (12) and even as soon as 1 hr (Mizrahi, personal communication) after application of osmoticum to the roots.

The plant undergoes alternating periods of low turgidity during the day, when absorption lags behind the water loss by transpiration, and high turgidity at night, when transpiration lags behind water absorption (6). The periodic changes of water status may induce periodic fluctuations of hormonal concentrations. Diurnal rhythm of auxin level was suggested in *Kalanchoë blossfeldiana* (19). Such hormonal fluctuations may influence stomatal and root resistances.

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