

Internal Water Status of Kinetin-treated, Salt-stressed Plants¹

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MARY BETH KIRKHAM,² W. R. GARDNER, AND G. C. GERLOFF

Department of Soil Science and Department of Botany, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

Stomatal resistances and turgor pressures were measured during a 12-day period on leaves of bean (*Phaseolus vulgaris* L. cv. Contender) which were treated with kinetin, were salinized or were treated with kinetin and salinized. Stomatal resistances were highest in salt-stressed plants, and progressively lower in salt-stressed and kinetin-treated plants, control plants, and kinetin-treated plants. Turgor pressures were highest in salt-stressed plants, and progressively lower in control plants, kinetin-treated plants, and salt-stressed and kinetin-treated plants. Stomata appeared to be kept open more widely under kinetin treatment than under control conditions, even when turgor pressures were lower in kinetin-treated plants than in control plants.

Roots of unstressed plants produce cytokinins which are translocated to the leaves through the xylem (8, 20, 22). Reduced amounts of cytokinins in root exudates are observed when plants have undergone a period of soil-water depletion (5), when the root medium has been salinized (4), or when plants have been grown under other unfavorable conditions (6).

Modifications of metabolism in the leaves of water-stressed plants may be, in part, the result of a decreased supply of cytokinins from the root. Shah and Loomis (19) found that changes in leaf RNA and protein caused by wilting could be suppressed by application of cytokinins to the leaf. Ben-Zioni *et al.* (2) showed that cytokinin treatment prior to incubation of water-stressed leaf discs with labeled leucine could partially restore incorporation of the leucine in protein which is decreased by water stress. The use of transpiration rate as a bioassay for cytokinin activity (12) depends on the effect of cytokinins on stomatal aperture. Cytokinins have been shown to cause stomatal opening under unstressed conditions (10, 14, 18).

The experiments cited suggest that reduced cytokinin transport from stressed roots results in disturbed leaf performance and particularly affects stomatal response. Proposals have been made that cytokinins, in association with other hormones such as abscisic acid, may modify the response of plants to stressed conditions by altering stomatal opening and permeability of plant tissues to water (11, 16, 21). Even though the effect of applied cytokinins on the internal water

status of unstressed plants has been studied (refs. 10, 14, and 18 for stomatal resistance; ref. 18 for turgor pressure; refs. 3, 10, 12, 13, 15, and 18 for transpiration; and refs. 10 and 18 for relative water content), their effect on leaf water potentials, turgor pressures, and stomatal resistances of water-stressed plants has not yet been reported. This paper presents these data for leaves from kinetin-treated bean plants growing under a saline condition.

MATERIALS AND METHODS

Forty-eight bean plants (*Phaseolus vulgaris* L. cv. Contender), germinated in sand, were transplanted into an aerated, half-strength Hoagland solution (9), one plant per 2-liter opaque plastic container, when the plants were about 2 weeks old. The plants were placed in a growth chamber (Sherer CEL 37-14) in which day and night temperatures were 25 C and 20 C, respectively. The relative humidity was not controlled; it varied from 60 to 85%. The light quantum flux density at the top of the plants was 45 nanoeinsteins cm⁻² sec⁻¹ from 0600 to 1800 hr. Two fans were operated in the corners of the growth chamber to reduce the boundary layer resistance of plant leaves.

When the experimental period (12-24 June 1972) began, the plants were about 25 days old, and the first and second trifoliolate leaves were expanding. The treatments applied were as follows: 12 plants were controls; 12 plants were stressed with NaCl; 12 plants were sprayed with kinetin and stressed with NaCl; and 12 plants were sprayed with kinetin. The plants were salinized with NaCl in two 2-bar steps at about 1000 hr on 12 June and 14 June. The upper and lower surfaces of the leaves of each plant were sprayed with about 3 ml of 10 µg/ml kinetin (Calbiochem, Los Angeles, Calif.) at the two times salt was added to the nutrient solution, and also on 16, 18, 20, and 22 June at about 1000 hr. Water was sprayed on the leaves that did not receive kinetin.

Water potentials and osmotic potentials were determined about 0930 hr with thermocouple psychrometers (9) and turgor pressures were calculated. Resistances of the lower stomata were measured about 1000 hr with a stomatal diffusion porometer (7), which was calibrated before the experiment began. Leaves were sampled for relative water contents (1) about 1030 hr. Potential, stomatal resistance, and relative water content measurements were made on first trifoliolate leaves on 13, 15, and 17 June, and on second trifoliolate leaves on 19, 21, and 23 June. Potential and relative water contents reported are means of three measurements made on three separate plants growing under the same treatment. Stomatal resistances reported are the means of 10 measurements made on plants growing under the same treatment. The nutrient solution containers were weighed daily about 0900 hr, and distilled water was added to the containers to replace water lost by transpiration.

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² Present address: Advanced Waste Treatment Laboratory, Environmental Protection Agency, Cincinnati, Ohio 45268.

RESULTS AND DISCUSSION

The means of potential, relative water content, stomatal resistance, and transpiration values obtained under the four treatments are presented in Table I. The kinetin-sprayed leaves had a lower stomatal resistance than did control leaves, while the turgor pressure of kinetin-sprayed leaves was lower than that of control leaves. The low stomatal resistances, particularly of the kinetin-sprayed plants, indicate that the stomata were widely open. Stomatal resistances below 0.2 sec cm⁻¹ for the kinetin-sprayed leaves fell below the range in which the porometer gave reliable values and were therefore not included in calculating the means. Nevertheless, the measurements showed that resistances were significantly lower in kinetin-sprayed plants.

The salt-stressed plants sprayed with kinetin also had lower stomatal resistances and lower turgor pressures than did the salt-stressed plants that were not kinetin-treated. Stomatal resistance results for salt-stressed plants did not agree with those of O'Leary and Tarquinio Prisco (17) who reported that kinetin increased the leaf resistance in salt-stressed bean plants. However, they found that the cytokinin, benzyladenine, reduced stomatal resistance in salt-stressed bean plants. On 17 June, 5 days after the first treatment with kinetin and salt, salt-stressed and kinetin-sprayed plants were wilted and had chlorotic spots. Salt-stressed plants remained relatively turgid and green throughout the experiment. There were no visible differences between control and kinetin-treated plants.

The great damage to the kinetin-sprayed and salt-stressed plants probably was the result, in part, of the large amount of water transpired through the open stomata. Transpiration was higher in plants sprayed with kinetin than in control plants until 21 June when there was a decrease in the transpiration rate and stomatal conductance of most plants. It is not known why this decrease occurred. Also, more water was lost from kinetin-sprayed and salt-stressed plants than from salt-stressed plants, until 21 June when the kinetin-sprayed and salt-stressed plants were severely damaged. The relative water contents of control plants and kinetin-sprayed plants were similar. The relative water content of salt-stressed plants was somewhat lower than that of the control and kinetin-sprayed plants. At the end of the experiment, the relative water content of salt-stressed and kinetin-treated plants was very low.

Turgor pressure and stomatal resistance were not coupled in the kinetin-sprayed bean plants. When kinetin was sprayed on the leaves, turgor pressures were reduced, but the stomata remained open. At the beginning of the experiment, salt-stressed and kinetin-sprayed plants tended to have lower turgor pressures, but lower stomatal resistances, than salt-stressed plants. Throughout the experiment, kinetin-sprayed plants tended to have lower turgor pressures and lower stomatal resistances than control plants. These results support the observation (16) that addition of cytokinins to cytokinin-deficient, stressed plants does not mitigate the symptoms associated with water stress, but rather increases them.

Table I. *Effects of Kinetin Treatment and Salt Stress on the Internal Water Status of Bean Plants*

The average coefficients of variation for the measurements are as follows: water and osmotic potentials, 9%; turgor pressure, 14%; relative water content, 3%; stomatal resistance, 18%; and transpiration, 13%.

Date and Treatment	Water Potential	Osmotic Potential	Turgor Pressure	Relative Water Content	Stomatal Resistance	Transpiration	
	bars	bars	bars	%	sec cm ⁻¹	ml day ⁻¹	ml day ⁻¹
13 June						12-13 June	13-14 June
Control	-3.4	-8.0	4.6	84.3	0.6	66	64
+NaCl	-4.3	-10.3	6.0	85.1	1.7	53	34
+NaCl and kinetin	-7.2	-12.9	5.7	87.6	1.2	69	72
+Kinetin	-6.6	-10.1	3.5	87.4	0.4	71	74
15 June						14-15 June	15-16 June
Control	-4.5	-8.9	4.4	90.2	0.6	63	88
+NaCl	-5.6	-12.9	7.3	86.0	4.1	42	30
+NaCl and kinetin	-7.3	-14.0	6.7	83.5	2.1	52	50
+Kinetin	-5.1	-9.1	4.0	88.7	0.3	87	122
17 June						16-17 June	17-18 June
Control	-4.7	-9.6	4.9	86.8	1.5	90	104
+NaCl	-9.9	-17.2	7.3	84.0	18.0	30	16
+NaCl and kinetin	-17.6	-19.2	1.6	81.1	10.7	40	28
+Kinetin	-6.2	-10.7	4.5	85.5	0.5	124	134
19 June						18-19 June	19-20 June
Control	-5.1	-11.1	6.0	86.6	0.7	126	104
+NaCl	-12.3	-20.0	7.7	81.1	9.8	23	12
+NaCl and kinetin	-18.6	-21.8	3.2	80.1	8.8	31	22
+Kinetin	-3.9	-8.2	4.3	85.0	0.3	152	138
21 June						20-21 June	21-22 June
Control	-10.5	-14.4	3.9	88.2	1.3	161	148
+NaCl	-16.2	-20.8	4.6	78.3	17.6	18	25
+NaCl and kinetin	< -48	< -48	0.0	47.8	20.6	8	10
+Kinetin	-9.1	-11.3	2.2	85.1	0.6	114	125
23 June						22-23 June	23-24 June
Control	-10.7	-14.1	3.4	86.0	1.9	187	129
+NaCl	-25.9	-29.6	3.7	81.5	20.0	17	15
+NaCl and kinetin
+Kinetin	-8.6	-11.3	2.7	85.6	0.9	189	197

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