

Short Communication

Leaf Water Content and Hormone Effects on Ribonuclease Activity

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ABSTRACT

In barley (*Hordeum vulgare*) leaves in which the water balance was not hampered, kinetin and abscisic acid effected the well documented decrease and increase, respectively, in RNase activity. When the plants were exposed to water shortage, leaf-water saturation deficit increased steadily, with kinetin enhancing and abscisic acid retarding the rise. Under drought, the pattern of hormonal effects was inverted, with kinetin enhancing RNase activity over and above the activity assayed in abscisic acid-treated leaves. A very close relationship between RNase activity and water saturation deficit was found and significantly, it was maintained irrespective of the hormonal treatment, which in itself markedly modified leaf-water saturation deficit. The inverted effects of kinetin and of abscisic acid on RNase activity under conditions of water shortage were interpreted as resulting primarily from the effects of these hormones on leaf-water. It is suggested that under conditions of increased water deficiency in the plant, cell-water supersedes hormonal regulation in effecting RNase activity.

Increased RNase activity appears to be associated with a decrease in cellular water content. Dove (2, 3) reported enhanced RNase activity in desiccated tomato leaflets and concluded that water stress directly affected RNase activity at the cellular level. Similarly, Tvorus (15) found that water content of plant tissue declined and RNase activity increased under drought conditions.

It is well established that RNase activity in many plant parts may be modified by cytokinins and ABA. Cytokinins are usually reported as repressing RNase activity and ABA as enhancing it (1, 4, 11, 12, 17). In addition, many reports implicate ABA and cytokinins in the regulation of plant water balance (8, 9, 13). As in their effects on RNase activity in detached leaves, these hormones are known also to have contrary effects on transpiration; cytokinins causing stomata to open and ABA effecting stomatal closure (5, 6, 7, 16). This report examines the interrelations between the effects of kinetin and ABA on leaf water content and their effects on RNase activity.

MATERIALS AND METHODS

Barley (*Hordeum vulgare*) plants were grown in a greenhouse under natural light with a day length of about 13 hr and

diurnal temperature fluctuations of 18 to 35 C. For conditions of optimal water supply, 50 barley seedlings were grown on nylon nets placed on a 2-liter container of half-strength Hoagland solution, which was aerated by gently bubbling compressed air through a capillary tube. For the "dry" treatment, 20 seeds were sown in small plastic containers filled with 1 kg of silt-loam. They were watered once to field capacity at sowing, and since there was no additional watering, soil water content progressively decreased as the seedlings developed.

The first leaf of 10-day-old plants was used in the work on detached leaves. The leaves were placed on nylon frames fixed 5 cm above the floor of a darkened glass container, 20 × 20 × 60 cm, kept at room temperature. Ventilation was provided by keeping the glass cover 1 cm above the container. Humidity was simulated by spraying the leaves with water and placing the nylon frames over a 2-cm layer of water. In addition, the container was lined with moist filter paper. In the dry treatment, water was not added to the container nor applied to the leaves. In the whole plant, 0.1 mM kinetin and 0.4 mM ABA were applied by spraying every 3 days from germination until the end of the experiment. Detached leaves were soaked in either 50 μM kinetin or 40 μM ABA for 30 min. All the hormone solutions and the water controls contained 0.01% Tween-20.

RNase activity in the leaves was determined after freezing the leaves in liquid nitrogen. They were then homogenized with 0.1 M phosphate buffer, pH 6.0, using a chilled pestle and mortar. The crude homogenate was filtered through Miracloth at 2 to 4 C and was used as the enzyme preparation. RNase activity was assayed by modification of the method of Tuve and Anfinsen (14): 1 ml of substrate solution (yeast RNA 4 mg/ml in 0.1 M phosphate buffer, pH 6.0) was placed in tubes; 0.25 ml of enzyme preparation and phosphate buffer was added to give a final volume of 2 ml.

The assay mixture was incubated at 37 C for 1 hr. Undigested RNA was then precipitated with 0.5 ml of chilled 25% perchloric acid containing 0.75% uranyl-acetate. The tubes were chilled and left at 2 to 4 C overnight and then centrifuged at 4000g for 15 min. A 1-ml aliquot of the supernatant was made up to 5.0 ml with water, and the absorption was measured at 260 nm.

Water content of leaves was estimated by measuring the water saturation deficit in 1.5 cm long leaf sections taken 3 cm from the apex of the first leaf of each of five plants. WSD¹ was calculated by subtracting the relative water content from 100%, according to Slatyer (10). In figures, RNase activity

¹ Abbreviation: WSD: water saturation deficit.

is expressed at ΔA_{260} values over the zero time control. Since the leaf water content varied in the different experiments, the fresh weight values of the leaves were corrected to "saturated fresh weight," *i. e.* weight of the leaf tissue with maximal water content, obtained after floating the leaf sections on water for 4 hr at 4 C.

RESULTS AND DISCUSSION

The effect of kinetin and ABA on WSD in leaves of barley is shown in Table I. The WSD of detached leaves in the humid

Table I. Effect of Kinetin and ABA on Water Saturation Deficits in Detached and in Intact Leaves

Detached leaves were kept in either "dry" or "humid" chambers and intact leaves were obtained from plants grown under either optimal water supply (humid) or progressively decreasing soil water content (dry).

Treatment	Humid			Dry		
	Control (without hormones)	Kinetin	ABA	Control (without hormones)	Kinetin	ABA
Detached leaves						
Days after detachment						
0	6.9	6.9	6.9	6.9	6.9	6.9
1	5.0	6.0	4.0	16.7	18.4	9.4
2	3.0	3.0	2.0	21.9	25.0	18.0
3	0.0	1.0	0.0	37.1	45.0	27.7
4	0.0	0.0	0.0	50.0	57.0	43.7
Intact leaves						
Days after seeding						
5				6.0	6.0	6.0
7	5.9	5.9	5.9			
8	6.0	6.2	5.7			
10	6.1	6.5	5.5			
12				9.5	10.5	8.0
15	6.5	7.5	5.5			
19				30.0	37.0	12.0
23				61.0	70.0	18.0

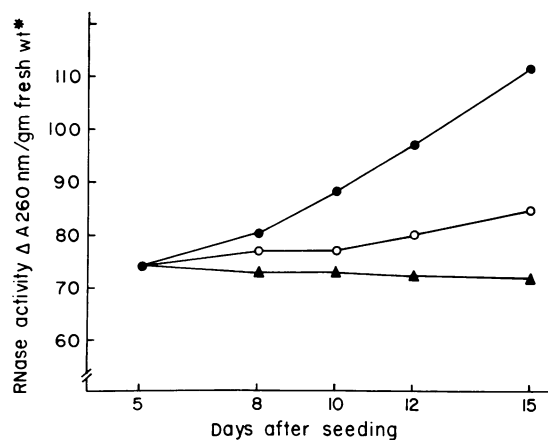


FIG. 1. Effect of ABA and kinetin on RNase activity in leaves of barley seedlings grown under optimal water supply. ●: Leaves sprayed with 4×10^{-4} M ABA; ▲: leaves sprayed with 1×10^{-4} M kinetin; ○: leaves sprayed with water (control); *: saturated fresh wt, obtained after floating the leaf sections on water for 4 hr at 4 C.

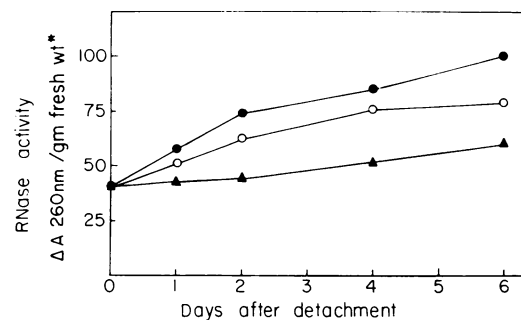


FIG. 2. Effect of ABA and kinetin on RNase activity in detached barley leaves kept humid. ●: Leaves soaked in 4×10^{-5} M ABA; ▲: leaves soaked in 5×10^{-5} M kinetin; ○: leaves soaked in water.

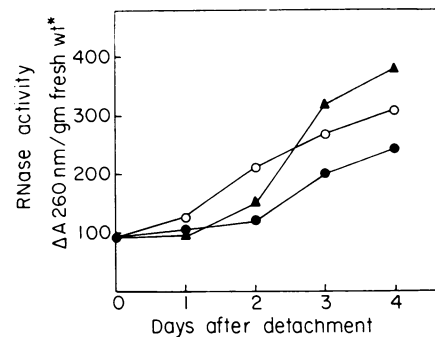


FIG. 3. Effect of ABA and kinetin on RNase activity in detached barley leaves kept in dry atmosphere. Expression of RNase activity and legend as in Fig. 2.

chamber was very low, irrespective of the hormonal treatment. It was also low in leaves of plants growing under sufficient water supply and was not greatly modified by hormone treatments. In contrast kinetin, and particularly ABA, markedly affected leaf-WSD in detached leaves in the dry chamber and in plants exposed to increasing water shortage. As shown in Table I, leaves treated with kinetin dried most rapidly and those treated with ABA least rapidly. Similarly, WSD in leaves of plants growing under conditions of increasing water shortage was lowest after treatments with ABA and highest in the kinetin-treated plants. However, under the dry conditions ABA affected the decreasing WSD values more perceptibly than kinetin affected the increasing WSD values. Nevertheless, in repeated experiments, kinetin consistently effected increase in WSD.

In plants growing under optimal water supply as well as in detached leaves kept in a humid atmosphere, the familiar pattern of hormonal effects on RNase activity was observed; namely, treatment with kinetin effecting a relative decrease in total RNase activity and treatment with ABA effecting increased activity (Figs. 1 and 2). This pattern, however, was inverted essentially in detached leaves exposed to a dry atmosphere as well as in leaves of plants exposed to increasing shortage of water in the soil (Figs. 3 and 4). Initially, when leaf water balance was not seriously hampered, as evidenced for WSD values in Table I, kinetin and ABA effected the well documented decrease and increase respectively in RNase activity. However, as the experiments progressed, the WSD in detached and in intact leaves gradually increased, the familiar pattern of hormonal effects on RNase activity was inverted with kinetin enhancing RNase activity over and above the activity assayed in ABA-treated leaves.

A very close relationship between RNase activity and WSD

becomes evident when RNase activity is plotted against WSD (Fig. 5). Significantly, this close relationship was maintained irrespective of the hormone treatment, which in itself markedly modified leaf WSD.

Wright and Hiron (16) found that a period of wilting greatly increased leaf ABA-content, and we have shown that in the dry conditions, treatment with kinetin effected increased WSD, which is directly related to increased RNase activity. This pointed to the possibility that the enhancing effect of kinetin on RNase activity under dry conditions stemmed from the effect of this hormone in increasing leaf-ABA. Accordingly, the primary results of the kinetin treatment would be enhanced WSD, which in turn would increase ABA content, hence pro-

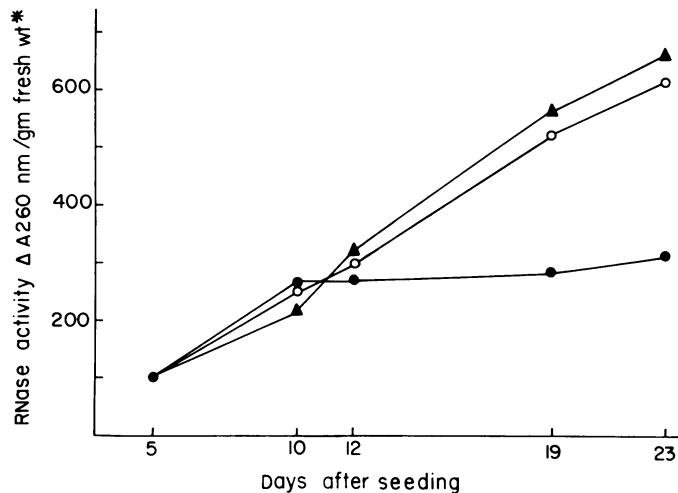


FIG. 4. Effect of ABA and kinetin on RNase activity in leaves of barley seedlings grown under increasing soil water shortage. Legend as in Fig. 1.

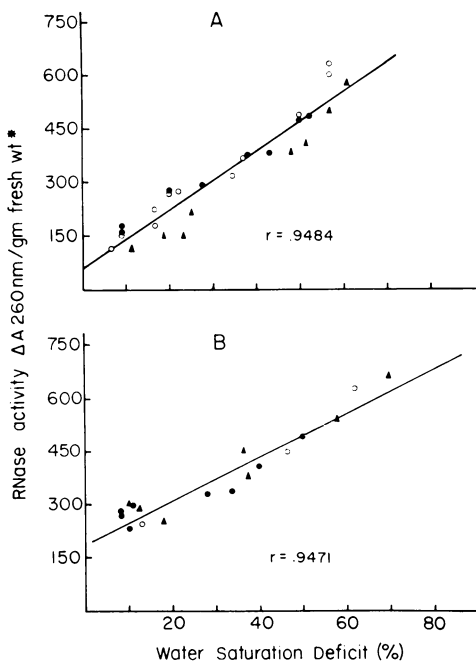


FIG. 5. Correlation between RNase activity and WSD. A: Detached leaves; B: intact leaves. Legend as in Fig. 1. ●: Leaves soaked in 4×10^{-5} M ABA; ▲: leaves soaked in 5×10^{-5} M kinetin; ○: leaves soaked in water.

Table II. Effect of Kinetin on WSD and on Leaf ABA content. Detached leaves were kept in a "dry" chamber for 4 days and were then examined for WSD and extracted for the ABA content.

	ABA	WSD
	pg/mg dry wt	%
Control	132.5	25
Kinetin	82.5	60

moting RNase activity. To test this possibility, the ABA content of kinetin-treated leaves kept in a dry atmosphere, 4 days after detachment was examined (Table II). While kinetin effected an increase of some 35% in WSD over the control, it caused a reduction in the content of leaf ABA below that observed in the control.

Our data indicate that the known effects of kinetin and ABA on RNase activity can be manifested only when the water content of the leaf is not greatly modified. Below a certain optimal leaf-water content, the specific hormonal effects on RNase activity, namely, repression by kinetin and enhancement by ABA, cannot be manifested. We interpret the inverted effects reported here of kinetin and of ABA on RNase activity under conditions of water shortage as resulting primarily from the effects of these hormones on leaf-water. We thus suggest that under conditions of increased water shortage, cell-water content supercedes hormonal regulation in effecting RNase activity.

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