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Short Communication

Abscisic Acid-induced Changes in Nucleotide Composition of Rapidly Labeled Ribonucleic Acid Species of Lentil Root

REVERSAL BY KINETIN[1, 2]

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An antagonism between cytokinins and growth inhibitors has been shown in germination (4, 5, 9, 12). Cytokinin-inhibitor antagonism has also been observed at the level of enzyme synthesis (6, 13). Based on a number of studies in which exogenous hormones were used to control germination, it has been hypothesized that in the regulation of germination, gibberellin is the primary stimulus and that the action of inhibitors and cytokinins present in the seed is essentially "preventive" and "permissive," respectively (5, 6, 10). On the basis of this premise gibberellin-induced germinative process(es) would be inhibited by growth inhibitor(s) and reversed by cytokinin(s). Other reports show that an antagonism between cytokinins and growth inhibitors is not limited to germination, but is found in shoot and root growth as well, suggesting the possibility that such hormonal interaction may be an essential feature in the control of plant growth and development (6, 12).

The molecular basis for cytokinin-inhibitor antagonism is not known. Recent work from our laboratories showed that abscisic acid (ABA) induced pronounced changes in the nucleotide composition of rapidly labeled RNA species of pear embryos. This change was largely due to an increase in the UMP content and a decrease in the GMP content (7). This communication will show that similar changes are induced by ABA[3] in rapidly labeled RNA species of excised lentil (*Lens culinaris*) roots, and also that the ABA effect is reversed by kinetin. In addition, it will be shown that in lentil roots ABA promotes ^{32}P labeling of RNA species, whereas kinetin inhibits such labeling. Excised lentil root tips were selected for the present studies because they are extremely sensitive to kinetin treatment (2).

RESULTS AND DISCUSSION

Sucrose density gradient profiles of RNA species of excised lentil roots treated with ABA and kinetin, both singly and in combination, are shown in Figure 1. The absorption profiles show clear separation of two rRNA's (18S and 25S) from sRNA (4S). The radioactive profiles roughly coincide with the absorption profiles only in the region of rRNA. It is well known that the label corresponding to rRNA peaks may represent a mixture of several RNA species, including rapidly labeled heterodisperse

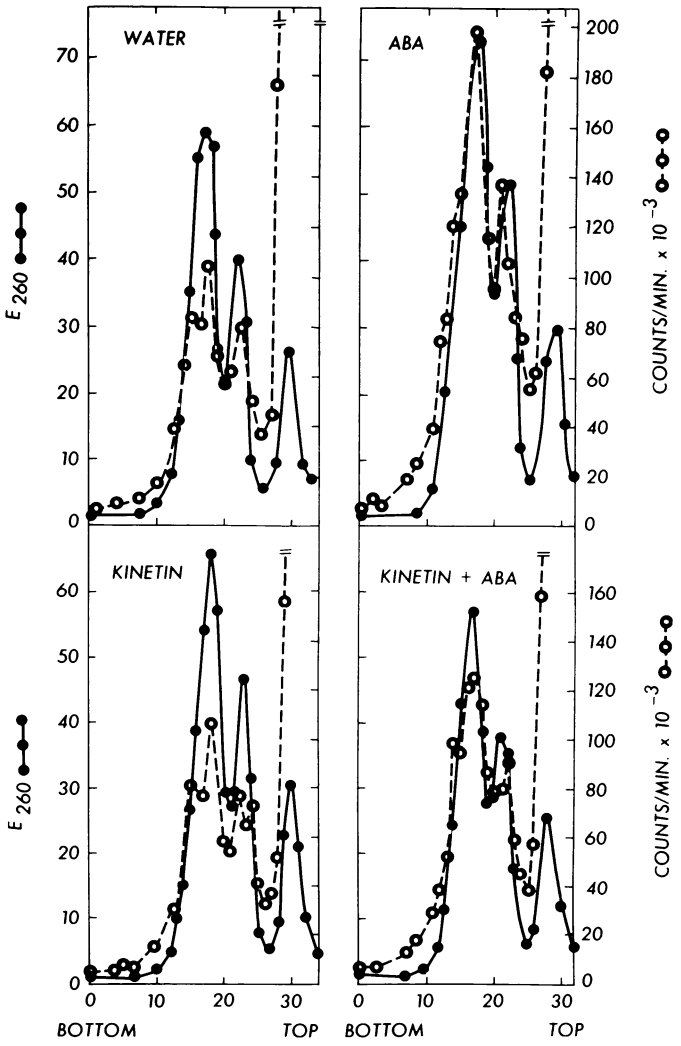

FIG. 1. Sucrose density gradient sedimentation of RNA from excised lentil (*Lens culinaris*) roots. Lentil seeds were sterilized with 1% Clorox solution (sodium hypochlorite) and were grown on 0.75% agar for 3 days at 25 C in dark, after which 1-cm root tips were excised. Totals of 75 root tips, in each treatment, were incubated for 2 hr in hormone solutions, were washed thoroughly, and then were reincubated for 3 hr in the heat-sterilized medium containing 0.03 M sucrose,

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[2] This work was approved by the Director of the New York State Agricultural Experiment Station as Journal Paper 1783.

[3] Abbreviation. ABA: abscisic acid.

Table I. *The Effect of Hormones on ^{32}P Incorporation into Excised Lentil Roots*

See Figure 1 for details.

Treatments	Radioactivity in			
	25S	18S	25S	18S Regions
	cpm × 10^{-3}		*cpm/A* × 10^{-3}	
Water	696	307	245	244
ABA	1202	460	302	297
Kinetin	668	290	198	203
ABA + kinetin	863	314	315	243

Table II. *Effect of ABA and Kinetin on Nucleotide Composition of Rapidly Labeled RNA Species*

Fractions corresponding to 25S and 18S regions (see Fig. 1) were pooled separately. One milligram of yeast RNA was added. RNA was precipitated by making the solution 5% with respect to trichloroacetic acid. The precipitate was centrifuged off after 1 hr at 0 C, and was washed once with 5% trichloroacetic acid and absolute alcohol. RNA was hydrolyzed with 0.5 N KOH for 18 hr at 37 C, and was neutralized with 2.5 M $HClO_4$, and the mononucleotides were fractionated (5-ml fractions) on Dowex 1-formate columns. Radioactivity of each fraction was determined.

Treatments	Total Radioactivity				Nucleotide Ratios	
	CMP	AMP	GMP	UMP	A + U: C + G	U:G
	%					
18S Region						
Water	21.5	33.5	19.1	25.9	1.46	1.36
ABA	19.6	26.4	13.2	40.8	2.05	3.09
Kinetin	24.2	29.4	17.9	28.6	1.37	1.59
ABA + kinetin	21.5	28.7	20.5	29.2	1.37	1.42
25S Region						
Water	22.7	30.3	23.2	23.8	1.18	1.03
ABA	21.4	31.1	19.2	28.3	1.47	1.48
Kinetin	25.4	29.3	19.1	26.4	1.25	1.38
ABA + kinetin	22.0	27.8	22.8	27.4	1.23	1.20

mRNA or DNA-like RNA (1, 3). For the purpose of present experiments designed to show the effect of ABA and kinetin on rapidly labeled RNA species, the labels corresponding to the 18S and 25S regions were only considered.

0.01 M tris-HCl (pH 6.5), 0.01 M $MgCl_2$, 0.06 M KCl, and 40 μc of carrier-free $H_3{}^{32}PO_4$ in 5 ml of solution. Incubation was conducted at 25 C on a metabolic shaker. Extraction and purification of RNA was as described (8), except that the homogenizing mixture contained 5 ml of 0.01 M tris-HCl buffer (pH 7.6), 0.06 M KCl, 0.01 M $MgCl_2$, 0.3 ml (12 mg) of bentonite, 1 ml of 11% sodium dodecyl sulphate, and 5 ml of phenol (washed with tris-HCl buffer). Purified preparation of RNA (approximately 1 ml) was layered on 5 to 20% sucrose gradient in tris-HCl (pH 7.6) buffer containing 0.01 M $MgCl_2$ and was centrifuged at 41,000 rpm for 6 hr in Spinco model SW Ti rotor in an L2-65B ultracentrifuge. Fifteen drop fractions were collected by bottom puncture, and 2 ml of water were added to each fraction, and absorbancy at 260 nm and radioactivity were determined. Concentration of hormones was: ABA, 20 μM; kinetin, 10 μM.

ABA promoted ^{32}P incorporation in the 25S and 18S regions, whereas kinetin inhibited such labeling (Fig. 1, Table I). The inhibitory effect of kinetin was reversed in presence of ABA (Fig. 1, Table I). The effect of hormones on nucleotide composition of rapidly labeled RNA species corresponding to the 18S and 25S regions is shown in Table II. An examination of A + U/C + G or U/G ratios indicates that the label corresponding to the 18S region may be due to DNA-like RNA, whereas those in the 25S region appear to be relatively richer in rRNA. ABA treatment increased markedly the A + U/C + G and U/G ratios of rapidly labeled RNA species in both 18S and 25S regions, the effect on the former, however, was greater than the latter. The ABA effect on DNA-like RNA, as shown previously in the case of pear embryos (8), appeared to be largely due to an increase in the UMP content and a decrease in the GMP content. This change by ABA was completely counteracted by kinetin (Table II).

The qualitative effect of ABA on nucleotide composition appears to be independent of its quantitative effects. The latter appears to vary with different tissues. For example, although ABA promoted labeling of RNA with ^{32}P in the case of root tissues (Fig. 1, Table I), it inhibited such labeling in the case of pear embryos (8). A change in RNA synthesis may be due to a hormone reacting or binding differently with the hormone receptor-chromatin systems from different tissues or organs (11). The observed changes in nucleotide composition by ABA and its reversal by a cytokinin could be due to a direct or an indirect effect of these hormones on sites of RNA transcription. Further work is in progress to elucidate these results.

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