ACTA AGROBOTANICA
vol. 37, z. 1 – 1984
47-52

SHORT COMMUNICATION

Influence of IAA, kinetin and ABA on ribonuclease activity in the embryonal roots of two barley (Hordeum vulgare L.) cultivars

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(Received: May 31, 1982)

Abstract

IAA, kinetin and ABA in a concentration of 10^{-7}M did not cause major changes in the activity of ribonuclease in both cultivars. IAA and kinetin in a concentration of 10^{-5}M lowered the activity of the tested enzyme, more in the 'Union' than in the 'Lubuski' cultivar. ABA in a concentration of 10^{-5}M did not change the activity of ribonuclease in both cultivars. It caused, however, the appearance of an extra band with ribonuclease activity on the polyacrylamide gel in both cultivars.

INTRODUCTION

Growth regulators influence the activity of ribonuclease (RNAase) and other nucleases in plant tissues (J a c o b s e n, 1977). In leaf segments of *Rhoeo discolor* kinetin and auxin inhibited the increase of RNAase activity and ABA stimulated it (H o d g e and S a c h e r, 1975). Pea shoots treated with auxins showed a lower RNAase level as compared with control segments (T r u e l s e n, 1967). Data are scarce on the influence of growth regulators on nuclease activity in the roots. S i v ó k et al. (1977) investigated the effect of ABA on nuclease activity in wheat roots. ABA in a 10^{-5}M concentration did not change this activity as compared with the control.

The present paper presents the results of studies on the influence of IAA, kinetin and ABA on RNAase activity in the soluble fraction from embryo roots of two spring barley cultivars 'Union' and 'Lubuski'. These cultivars differed in the size of their root system and RNase activity (S k o c z e k and B o r y s, 1979; 1980) and in the metabolism of the growth regulators (S t r o i ń s k i et al., 1978; K r z y w a ń s k i et al., 1976; P o l i t y c k a et al., 1979).

MATERIAL AND METHODS

TREATMENT OF BARLEY SEEDLINGS WITH GROWTH REGULATORS

Barley (*Hordeum vulgare* L.) seedlings, cultivars 'Union' and 'Lubuski' after 68 h of germination in a thermostat at 26°C without light access in bidistilled water were transferred to Petri dishes about 15 cm in diametre with growth regulator solution. Into each dish 20 ml of IAA, kinetin (6-furfurylaminopurin) and ABA were poured in an appropriate concentration and distilled water as control. The seedlings were lightly dried on filter paper and placed on the dishes, 20 on each. The dishes were put in a thermostat at 26°C for 24 h. Then the roots were cut off with scissors, dried on filter paper, weighed and RNAase was extracted from them.

RNAASE EXTRACTION

Buffer containing 50 mM sodium citrate, pH 6.1 and 0.5 M sucrose was used. The ratio of buffer to fresh weight of the tissue was 2:1. For enzyme extraction equal weighed samples of tissue were taken from both barley cultivars. The comminuted plant material was ground in a mortar at 2°C for 10 min. The homogenate was centrifuged for 30 min at 3500 g and the supernatant was the source of enzyme.

RNAASE DETERMINATION

RNAase activity was tested by the modified method of T u v e and A n f i n s e n (1960). The incubation mixture contained: 0.2/0.1 M phosphate-citrate buffer, pH 6.4, 1.6 mg/ml yeast RNA and enzyme. The mixture of 2.5 ml final volume was incubated at 37°C for 30 min. The reaction was arrested by adding 0.5 ml of 25 per cent perchloric acid containing 0.75 per cent uranyl acetate. The mixture was cooled on an ice bath for 15 min and then centrifuged for 10 min at 10 000 g. The supernatant was 19-fold diluted with distilled water and absorption was determined at 260 nm in an absorption cell 1 cm thick. At the same time a control was run consisting of an incubation mixture of the same components precipitated with 25 per cent perchloric acid with 0.75 per cent uranyl acetate without incubation.

The enzyme activity unit consisted of an increase in the absorption value by 0.1 for the acid-soluble fraction under the conditions of the experiment.

PROTEIN CONTENT DETERMINATION

Protein in the extracts was determined by the method of L o w r y et al. (1951) with the use of bovine albumin (fraction V) as standard.

DISC ELECTROPHORESIS IN POLYACRYLAMIDE GEL

RNAases were separated in polyacrylamide gel after D a v i s (1964) and identified on gel according to the method of W i l s o n (1971). For gel incubation after separation of the enzymatic proteins 0.2/0.1 M phosphate-citrate buffer, pH 6.4 was used.

RESULTS

In the embryo roots of the 'Union' and 'Lubuski' barley cultivars treated with IAA, kinetin and ABA in 10^{-7}M concentrations no significant changes in RNAase activity per 1 mg of protein were found as compared with the control (Table 1). IAA and kinetin in a 10^{-5}M concentration decreased RNAase activity in both barley cultivars (Table 2). Kinetin decreased enzyme activity more than did auxin. Moreover, differences vere observed in the response of the two varieties to IAA and kinetin. In the embryo roots of the 'Union' cultivar auxin and kinetin depressed more RNAase activity as compared with the effect on 'Lubuski' (Table 2). The inhibitory influence of IAA on RNAase activity was noted in the root of lentil (P i l e t and B r a u n, 1970), whereas auxin and kinetin reduced this activity in segments of *Rhoeo discolor* leaves (H o d g e and S a c h e r, 1975).

Table 1

Influence of IAA, kinetin and ABA in 10^{-7}M concentrations on the activity of RNAase in embryo roots of barley cultivars 'Union' and 'Lubuski'

Cultivar	Treatment	RNAase activity	
		units/ml/30 min	units/mg protein
'Union'	H_2O	100.6 ± 2.7	32.5 ± 0.4
	IAA (10^{-7}M)	102.4 ± 6.1	31.7 ± 4.2
	Kinetin (10^{-7}M)	112.3 ± 5.5	32.4 ± 1.9
	ABA (10^{-7}M)	102.5 ± 7.8	32.2 ± 1.3
'Lubuski'	H_2O	83.9 ± 2.4	24.2 ± 0.8
	IAA (10^{-7}M)	86.8 ± 8.1	24.4 ± 3.0
	Kinetin (10^{-7}M)	90.3 ± 8.4	23.5 ± 5.2
	ABA (10^{-7}M)	85.2 ± 1.5	23.6 ± 2.8

The RNAase activity per 1 mg of protein did not change noticeably under the influence of ABA in a 10^{-5}M concentration in either of the barley cultivars (Table 2). Figure 1 shows the amount of RNAase in the soluble fraction of the root homogenate treated with growth regulators in 10^{-5}M concentrations and in the control. In both cultivars seven bands were observed with RNAase activity in the

Table 2

Influence of IAA, kinetin and ABA in 10^{-5}M concentrations on the activity of RNAase in embryo roots of barley cultivars 'Union' and 'Lubuski'

Cultivar	Treatment	RNAase activity			
		Exp. 1		Exp. 2	
		units/mg protein	%	units/mg protein	%
'Union'	H_2O	33.8 ± 2.4	100	32.5 ± 0.4	100
	IAA (10^{-5}M)	29.1 ± 2.1	−13.9	27.4 ± 1.6	−15.7
	Kinetin (19^{-5}M)	26.2 ± 3.3	−22.5	22.7 ± 1.1	−30.2
	ABA (10^{-5}M)	33.9 ± 3.7	+0.3	32.8 ± 3.1	+0.9
'Lubuski'	H_2O	24.2 ± 0.8	100	24.1 ± 2.4	100
	IAA (10^{-5}M)	23.1 ± 1.9	−4.5	22.9 ± 1.6	−5.0
	Kinetin (10^{-5}M)	21.5 ± 1.2	−11.2	20.5 ± 1.8	−14.9
	ABA (10^{-5}M)	24.6 ± 2.5	+1.7	24.3 ± 1.7	+0.8

control and for roots treated with IAA and kinetin. Under the influence of ABA there appeared for the embryonal root homogenate of both barley cultivars an additional band with RNAase activity marked as no. 8 in the figure (Fig. 1). Probably, under the influence of ABA, activation or synthesis of additional RNAase occurs, without being connected with a true increase of the over-all RNAase activity of the soluble fraction of embryo root homogenate (Table 2).

The above presented results allow the affirmation that the growth stimulators, IAA and kinetin, applied exogenously affect RNAase activity in barley root only when applied in high concentrations. The difference between the cultivars 'Union' and 'Lubuski' as regards the influence of the tested growth stimulators on

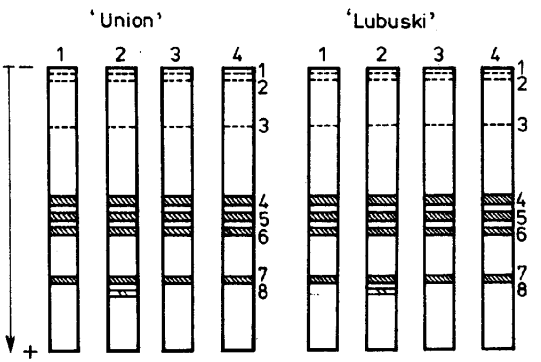

Fig. 1. RNAases in embryo roots of barley plants of the 'Union' and 'Lubuski' cultivars in treatments: 1 – H_2O, 2 – ABA, 3 – IAA, 4 – kinetin in 10^{-5}M concentrations

the RNAase level may be the consequence of the earlier observed differences in growth regulator metabolism between these cultivars (Stroiński et al., 1978; Krzywański et al., 1976; Politycka et al., 1979).

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WPŁYW IAA, KINETYNY I ABA NA AKTYWNOŚĆ RYBONUKLEAZY W KORZENIACH ZARODKOWYCH DWÓCH ODMIAN JĘCZMIENIA (*HORDEUM VULGARE* L.)

Streszczenie

Zbadano wpływ IAA, kinetyny i ABA na aktywność rybonukleazy frakcji rozpuszczalnej w korzeniach zarodkowych na przykładzie dwóch odmian jęczmienia jarego: 'Union' i 'Lubuski'. Odmiany różniły się wielkością systemu korzeniowego, aktywnością rybonukleazy i metabolizmem

regulatorów wzrostu. IAA, kinetyna i ABA w stężeniu 10^{-7}M nie wywołały istotnych zmian w aktywności rybonukleazy u roślin badanych odmian. IAA i kinetyna w stężeniu 10^{-5}M obniżały aktywność badanego enzymu u odmiany 'Union' silniej w porównaniu z odmianą 'Lubuski'. ABA w stężeniu 10^{-5}M nie zmieniał aktywności rybonukleazy u obu odmian, natomiast powodował pojawianie się dodatkowego prążka z aktywnością rybonukleazy na żelu poliakrylamidowym u obu odmian jęczmienia.