ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLVI, nr 1
1977

Effect of gibberellic acid on α-amylase and ribonuclease activities in rye endosperm after various periods of grain storage

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(Received: May 18, 1976)

Abstract

α-Amylase and ribonuclease activities were determined in embryoless rye half-seeds exhibiting different viaibility after various times of storage (2, 5 and 8 years). The half-seeds were incubated for 24, 48, 72 and 96 h with and without GA_3. α-Amylase activity in the endosperm of rye grain stored for 2 and 5 years and incubated without the hormone increased considerably, whereas in 8-year grain, practically incapable of germination, it remained almost unchanged. GA_3 caused a considerable increase of α-amylase activity in all the tested samples. In grain stored for 2 and 5 years the enzyme activity reached its peak under the influence of the hormone earlier than in the control samples. The differences in ribonuclease activity between the particular samples were less pronounced, and the reaction to the hormone much weaker. The results indicate that the reaction of rye endosperm to GA_3 depends on the physiological properties of the grain.

INTRODUCTION

The inductory effect of gibberellic acid on de novo synthesis of at least 3 enzymes — α-amylase, protease and β-1,3-glucanase has been demonstrated in barley endosperm in numerous investigations (Chrispeels and Varner, 1967; Jacobsen and Varner, 1967; Jones, 1971). According to the latest hypotheses, the mechanism of this induction consists in the direct participation of GA in the qualitative and quantitative control of posttranscription processes (cf. Jones, 1973). Neither can it be excluded that GA has a selective influence on the permeability of intracellular membranes, facilitating in this way access to the substrate (Varner and Mense, 1972; Wood and Paleg, 1972).

Most frequently these experiments were run on the aleurone layers of barley, and less frequently on the endosperm of wheat, oats, maize and rice (Paleg et al., 1962; Ingle and Hageman, 1965; Khan et al., 1973; Palmiano and Juliano, 1973). The action of gibberellic acid has not so far been chacked in rye endosperm, the main aim of investigations being not tests with another cereal variety, but rather observation of the reaction with this compound of grain stored for various periods with reduced viability, and ability to enzymatic protein synthesis (Grzesiuk and Kulka, 1971; Kulka 1971; Roberts and Osborne, 1972).

Earlier experiments (Mierzwińska, 1973, 1975) demonstrated that the stimulation of certain enzymes, particularly ribonuclease by GA_3 in ageing lupin seeds depends on their viability. The present paper is a continuation of these investigations. In order to avoid the growth effect of GA the studies were performed on embryoless cereal seeds — a classical material for experiments of this kind.

MATERIAL AND METHODS

Spring tetraploid rye seed breed by dr Z. Tomaszewska (1968) in the Institute of Plant Breeding and Acclimatization was used for study. Grain from various harvests was stored under laboratory conditions. In the present experiments 3 batches were used: (1) grain from the 1966 harvest stored for 8 years, (2) grain from the 1969 harvest stored for 5 years and (3) grain from the 1972 harvest stored for 2 years.

Spring tetraploid rye seed bred by dr Z. Tomaszewska 1968) qualification.

Seed samples from each batch (100 seeds, 4-fold replication) were sterilized for 1 hour in 1 per cent sodium hypochlorite solution, washed repeatedly with redistilled water and the caryopses were cut transversely with a sterilized lancet. The embryonal part of the seeds was discarded and the endosperm was incubated in the presence of GA_3 (experimental samples) and without it (control).

Two methods of incubation were applied: (A) the embryoless half-seeds (100 in each sample) were placed in Petri dishes on filter paper and incubated for 24, 48, 72 and 96 h in darkness at 24°C. The incubation medium contained 200 μg GA_3, 20 μmoles acetate buffer (pH 5.0) and 5 mg streptomycin, the whole made up to a 20 ml volume. The GA_3 concentration was established on the basis of numerous papers reporting that the effect of GA_3 on hydrolytic enzyme activity in cereal endosperm is almost equal within the concentration range 10^{-4}—10^{-8}M (Paleg and Coombe, 1967) Jones and Varner, 1967).

After the lapse of the given time period the whole incubated sample was taken for analysis.

The above described incubation method, although it allows to observe in time the reaction of ageing rye endosperm to GA_3 has the drawback that it does not facilitate intensive penetration of the hormone into the tissues, particularly in the initial period, and makes excretion of hydrolysis products which inhibit enzymic sythesis into the medium difficult (Jones and Armstrang, 1971). Therefore, parallelly another method of incubation (B) was applied, introduced by Varner (1964) and generally adopted in model experiments illustrating the effect of GA_3 on enzyme synthesis in barley endosperm.

(B) Samples prepared as in (A) containing 100 half-seeds were subjected to "preincubation" on Petri dishes in sterile moist sand for 3 days at room temperature. According to Varner (1964), this treatment neutralizes the difference in reaction to GA_3 caused by the non-homogeneity of the seeds. After this period the sample as a whole is washed with redistilled water and transferred to 100-ml Erlenmeyer flasks containing 20 μg GA_3, 20 μmoles acetate buffer (pH-5.0) and 2 μmoles $CaCl_2$, all made up to a 20 ml volume. The flasks with their content were placed for 6 h in a thermostat at 25°C and then shaken for 18 h on a universal type 327 shaker at average amplitude and frequency. The total incubation time for the half-seeds with GA_3 was thus 24 h. After this time enzyme activity was determined separately in the incubation medium and in the half-seeds.

Determination of enzyme activity

A whole incubated sample (100 half-seeds) was homogenized in a mortar cooled with ice with 20 ml 2 mM acetate buffer with 200 μmoles $CaCl_2$ added for 30 min. The homogenate was filtered through cheesecloth and centrifuged for 10 min at 3000 g. The extract obtained was taken for analysis. When incubation (B) was applied, the incubation medium was also analysed, after previous purification by centrifugation.

α-Amylase activity was determined by the starch-iodine method (Jones and Varner, 1967) described in detail by the author in the previous publication (Mierzwińska, 1973).

The results are given in enzymatic units corresponding to the decrease in absorption of the starch-iodine complex at 620 nm by 0.01 during 5 min of incubation of the substrate with the enzyme. According to the control test with a purified α-amylase preparation (Fluka AG) this unit corresponds to 2,85 μg of α-amylase.

Ribonuclease activity was determined by the method of Tuve and Anfinsen in Szarkowski's (1965) modification. As ribonuclease

activity unit was assumed the amount of enzyme increasing extinction in the supernatant as compared with the control sample:

$$\Delta E \frac{1 \text{ cm}}{260 \text{ nm}} = 0.001$$

RESULTS AND DISCUSSION

Grain viability. As seen in Table 1, the examined seed batches differed widely as regards viability. Rye stored for 8 years was practically incapable of germination, whereas 2-year seed germinated in 90 per cent. The intermediate batch stored for 5 years not only exhibited a low viability, but also a large number of abnormal sprouts with deformed stems, reduced root number, no coleoptile etc.

Table 1

Viability of tetraploid rye seeds of different ages treated with GA_3

Age of seeds	Combination	Germination after 4 days		Germination after 7 days	
		Germinating seeds, %	Abnormal sprouts, %	Germinating seeds, %	Abnormal sprouts, %
2 years	Control	87	9	92	10
	GA_3	88	6	92	8
5 years	Control	19	8	24	10
	GA_3	19	6	26	7
8 years	Control	0	0	2	2
	GA_3	1	1	2	2

Treatment of seeds with GA_3 did not change the germination capacity, it reduced, however, the per cent of abnormal sprouts. A similar phenomenon has been observed by the author when ageing lupin seeds were treated with gibberellic acid (Mierzwińska, 1975).

Enzyme activity. The dynamics of amylolytic activity in half-seeds, as shown in Fig. 1 after 96 h of incubation, shows wide differences in this respect both in the control and experimental samples in dependence on the age of the seeds. After 24 h α-amylase activity in the controls (without GA_3) was in all age groups similar and relatively low. As incubation was prolonged, however, the course of the curves changed. In seeds stored for 8 years the enzyme activity remained at almost the same level, whereas in 2-year seeds it steadily intensively incrased, reaching the highest value after 96 h. In seeds stored to 5-years the dynamics of α-amylase activity was similar to

that in 2-years seeds, but the rise in enzyme activity was less intensive, and at the end of the period of investigation it even decreased. These results confirm those of K u l k a (1971) who demonstrated that amylolytic activity in ageing oat and barley seeds drastically falls only after complete loss of viability.

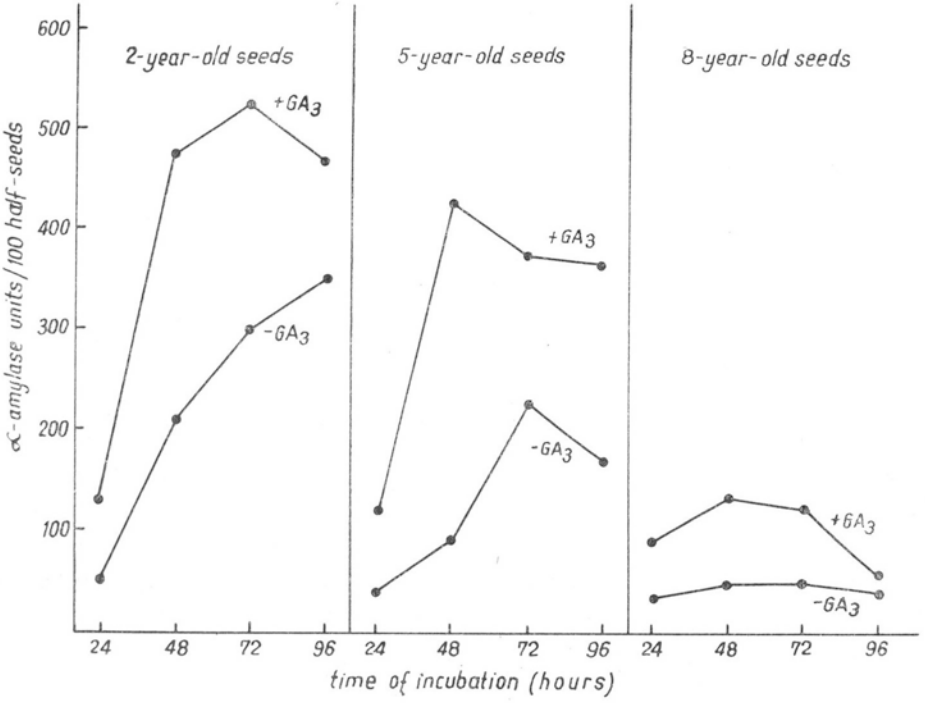

Fig. 1. α-Amylase dynamics in embryoless half-seeds of various-aged rye, incubated with and without GA_3

The reaction of ageing rye endosperm to GA_3 was also different, although a considerable increase of α-amylase activity occurred in all samples after the first 24 h of incubation. This stimulation was relatively highest in the seeds stored 8 years, however, as compared with younger seeds the activity of the enzyme remained low.

Analysis of the curves in Fig. 1 not only indicates an increase of α-amylase activity in the samples, but also its certain acceleration. This is most pronounced in the 5-year-old seeds in which, under the influence of GA_3, the enzyme activity reaches after 48 h almost the same level as in 2-year seeds.

It would seem that a similar acceleration observed also in lupin seeds (M i e r z w i ń s k a, 1973) and in wild oats (C h e n and C h a n g, 1972) may be associated with the increase of hydrolytic enzymes secretion

postulated by numerous authors and caused by the hormone (cf. Jones, 1973).

The results of enzyme activity determination in the (B) combination (Table 2) are rather similar to those obtained in variant (A), although they do not show the reaction to the hormone in a dynamic system.

Table 2

α-amylase activity in different-aged rye endosperm after 24 h of incubation with GA_3 (units/100 endosperms)

Age of seeds	Combination	Tissue extract	Incubation medium	Total	Increase, % in ref. to control
2 years	Control	164	244	408	100
	GA_3	232	378	610	149
5 years	Control	106	196	302	100
	GA_3	152	328	480	158
8 years	Control	30	38	68	100
	GA_3	48	116	164	241

The differences in enzyme activity in dependence on the age of the seeds is in the latter case more distinct since the values obtained by determinations in the tissues and in the incubation medium are much higher. In older seeds the enzyme diffused more readily into the medium, this possibly being connected with the degradation of the protein-lipid membranes with ageing (Koostra and Harrington, 1969).

Similarly as in combination (A), the effect of the hormone was most pronounced in old seeds (241%) which in general exhibited the lowest α-amylase activity.

The ribonuclease dynamics in control rye endosperm of various ages (Fig. 2) and that incubated with GA_3 shows a certain similarity with the course of α-amylase activity. Ageing of the seeds is here also associated with a lowered enzyme activity and its slower increase or even decrease in the seeds stored for the longest time. The differences between the particular batches, are, however smaller, particularly in the first 48 h of incubation.

The rise of enzyme activity due to the action of the hormone was slight, and, in contrast to α-amylase, the weakest stimulation (116%) was noted in the seeds stored for 8 years (Table 3).

In numerous experiments with germinating seeds the action of GA_3 together with the growth effect caused in most cases an intensive increase of ribonucleolytic activity typical for elongation processes (cf. Marcus, 1971). The results, however, obtained in model investiga-

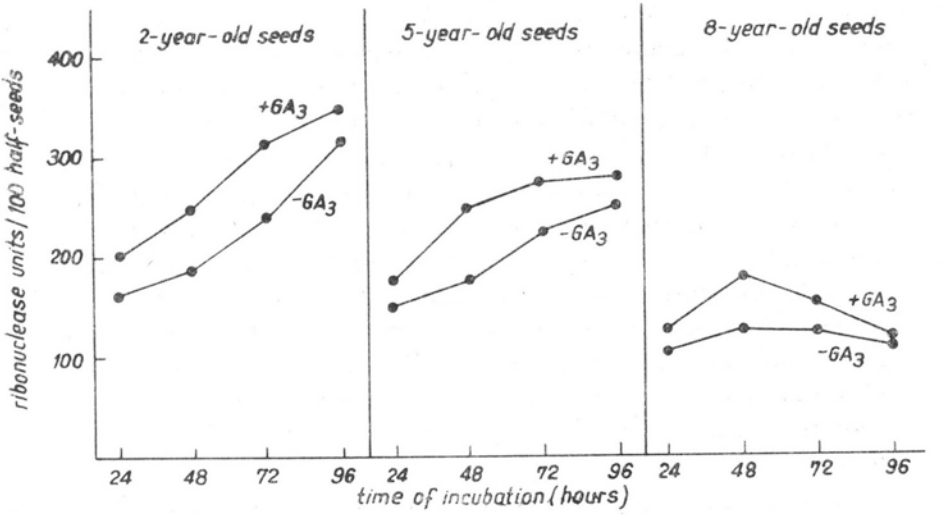

Fig. 2. Ribonuclease dynamics in embryoless half-seeds of various-aged rye, incubated with and without GA_3

Table 3

Ribonuclease activity in various-aged rye endosperm after 24 h of incubation with GA_3 (units/100 endosperms)

Age of seeds	Combination	Tissue extract	Incubation medium	Total	Increase, % in ref. to control
2 years	Control	215	50	265	100
	GA_3	253	85	329	128
5 years	Control	209	41	250	100
	GA_3	246	65	311	124
8 years	Control	115	36	151	100
	GA_3	125	51	176	116

tions on the mechanism of enzymatic induction in the aleurone layers and in embryoless half-seeds were not univocal. Ingle and Hageman (1965) found that treatment with GA_3 of isolated maize endosperm des not produce changes in ribonuclease activity. According to Chrispeels and Varner (1967) ribonuclease activity in barley half-seeds increases during imbibition, but, when isolated aleurone layers are incubated with GA_3, the enzyme activity increases only slightly. Probably in this case the hormone does not so much control synthesis as rather secretion of ribonuclease. In a later publication the preceding conclusion were corrected by the affirmation that at least part of the enzyme is synthesized de novo (Bennet and Chrispeels, 1972).

Of course, on the basis of the present experiments it is not possible to draw conclusions, as to the character of the slight increase of ribo-

nuclease activity observed under the influence of the hormone. In the combination with incubation (B) the intensive release of the enzyme into the medium did not, however, occur which, according to Chrispeels and Varner (1967), accompanies enhanced secretion.

Comparison of enzymatic activity (mainly amylolytic) with the viability of the tested seeds indicates that, when the viability of seeds is greatly lowered, they still may exhibit a high hydrolytic activity and an ability of increase of the latter under the influence of the hormone. These observations are confirmed by the studies of Van Onckelen et al. (1974) who demonstrated that in the process of artificially accelerated ageing of barley seeds, their viability and the hydrolytic activity induced by the embryo descrease much more rapidly than did the ability of the aleurone layers to amylase synthesis under the action of exogenous GA_3.

To sum up the result of these investigations the fact should be-stressed of the steady and intensive rise of α-amylase activity in rye endosperm incubated without GA_3.

A similar increase has not been observed at all in embryoless storage parts of barley (Varner, 1964) and only to a small extent in wheat (Khan et al., 1973) and rice (Palmiano and Juliano, 1973).

It is possible that, similarly as in the cotyledones of papilionaceous plants, this is connected with the presence of an endogenous gibberellin-like substance which may be capable to control enzymatic induction in the first days of imbibition (Dale, 1969). The experiments of Bergmann (1967) demonstrated, however, that in mature rye caryopses endogenous gibberellins are localized in the embryo or the scutellum, whereas in the endosperm these substances can be revealed at earliest after 24-h soaking, owing to diffusion from the embryonal parts. Neither did the trials undertaken by the author to demonstrate the presence of endogenous gibberellins in the storage parts of rye seed by the barley endosperm bioassay give positive results.

It would seem that an explanation of this may be sought in the specific structure of rye storage proteins which, in contrast to other cereal species, consist in a large part of globulins characterized by a relatively high amylolytic activity (Rohrlich, 1969).

The here presented results indicate that: (1) the scheme of hormonal control of α-amylase induction adopted at present on the basis of investigation of the reaction of barley endosperm may require modification according to the seed species and (2) within the same species the degree of induction may change in dependence on the physiological properties of the seeds.

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Wpływ kwasu giberelowego na aktywność α-amylazy oraz rybonukleazy w endospermie żyta różnego wieku

Streszczenie

Badano wpływ GA_3 na aktywność α-amylazy oraz rybonukleazy w ziarnie żyta przechowywanym w ciągu 2, 5 i 8 lat w jednakowych warunkach laboratoryjnych. Zdolność kiełkowania badanego ziarna wynosiła odpowiednio 92, 24 i 2%. Aktywność enzymów oznaczano w pozbawionych zarodków połówkach ziarna, inkubowanych przez 24, 48, 72 oraz 96 godzin z GA_3 i bez. Stwierdzono, że przy znacznym obniżeniu witalności ziarno jeszcze cechuje wysoka aktywność hydrolityczna oraz zdolność jej zwiększania pod wpływem hormonu. W endospermie ziarna 5-letniego z bardzo niską zdolnością kiełkowania aktywność badanych enzymów i ich dynamika zbliżone były do ziarna 2-letniego, kiełkującego w ponad 90%. W endospermie 2-letniego oraz 5-letniego ziarna żyta, inkubowanym bez hormonu, aktywność α-amylazy intensywnie wzrastała, jednak w 8-letnim pozostawała bez zmian. Działanie GA_3 powodowało znaczny wzrost aktywności enzymu (do 241%), która osiągała wartości maksymalne wcześniej niż w próbach kontrolnych. Dynamika rybonukleazy zbliżona była do α-amylazy, jednak różnice pomiędzy oddzielnymi próbami były mniejsze, a reakcja na hormon o wiele słabsza (w ziarnie 2-letnim przyrost 128%). Otrzymane wyniki wskazują, że reakcja endospermu na kwas giberelowy zróżnicowana jest w zależności od rodzaju zbóż oraz od właściwości fizjologicznych ziarna.