The role of zeatin and gibberellic acid in breaking of the abscisic acid—induced dormancy in *Triticale* caryopses

STANISŁAW WEIDNER, WŁODZIMIERZ MAKOWSKI, EUGENIUSZ SÓJKA, ANDRZEJ REJOWSKI

Institute of Plant Biology, Agricultural-Technical Academy, Olsztyn-Kortowo, bl. 40, 10-957 Olsztyn, Poland

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Abstract

The investigations were conducted on the germinating embryos and the whole caryopses of *Triticale*. During preimbibition and 24 hours germination caryopses were treated with abscisic acid (ABA), which produced 63% inhibition of embryo growth. Gibberellin-A<sub>3</sub> (GA<sub>3</sub>) reversed the ABA effect in 18%, while zeatin in 22%. The clear synergetic reaction was observed (36%) when both stimulators acted together. There was no significant effect of ABA, ABA and GA<sub>3</sub>, as well as ABA and zeatin on the synthesis of polyribosomal RNA in the initial period of germination of excised embryos. However, during 24 hours germination of whole caryopses ABA caused a two-fold decrease in <sup>3</sup>H-uridine incorporation into the total fraction of embryonic ribosomes. While the incorporation of <sup>14</sup>C-aminoacid mixture into ribosomal proteins was even three-fold lower. Effect of GA<sub>3</sub> and zeatin on breaking of the ABA-induced "dormancy" was studied. It was confirmed that the higher polyribosome contribution to the sum total of ribosomes the more intensive synthesis of ribosomal proteins. No higher <sup>3</sup>H-uridine incorporation into polyribosomal fraction was observed. From the results it may be inferred that in the initial period of germination of *Triticale* caryopses regulation of protein biosynthesis occurs rather at the translation than transcription level.

Key words: germination, abscisic acid, gibberellin, cytokinin, Triticale

INTRODUCTION

There are many concepts and theories on the complex mechanisms of dormancy. According to them dormancy stage results from: 1) the presence of inhibitors in some parts of the seed (Molisch 1922, Wareing 1965); 2) the limited oxygen uptake due to the presence of seed coats, which is followed by higher inside temperature (Thornton 1935, Crocker 1948, Vegis 1964, Roberts 1969); 3) the action of active and inactive forms
of phytochrome (Wesson and Wareing 1969, Kivilaan and Bandurski 1973). However, the majority of research workers believes that the basic mechanism of the dormancy control and induction lies in the action of plant growth regulators varied in time and place (Wareing and Saunders 1971, Khan 1971, 1977, Villiers 1972, Bewley and Black 1982).

According to Amen (1968) seed dormancy results from the particular ratio of the growth inhibitors to the growth stimulators. Also Khan (1971, 1977, 1980) has formed the model of seed germination and dormancy based upon the interaction of phytohormones. Given it, it may be inferred that ABA plays a main role in the induction of seed dormancy, while cytokinins and gibberellins — in the process of dormancy release. ABA also plays an important role in the regulation of growth and development of plant tissues (Addicot and Lyon 1969, Milborrow 1974). There are many papers on the inhibiting effect of ABA on the synthesis of various nucleic acids in plant tissues (Jacobsen 1977, Jacobsen and Higgins 1978a). Whether the effect is direct or indirect is not always clear. The paper presents results of the studies on ABA effect on germination-induced synthesis of the ribosomal fraction in the whole Triticale caryopses and embryos excised from them. The interaction between ABA and growth stimulators has also been analyzed.

MATERIAL AND METHODS

The investigations were conducted on Triticale caryopses of MT-3 generation. The caryopses were harvested at full ripeness in 1982 from the investigation plots of Institute of Plant Biology, Agricultural-Technical Academy, Olsztyn.

Caryopses were dry-stored for 4 months. Then washed with tap water and placed in 1% solution of sodium hypochloride for 3 minutes. Sterilized caryopses were washed with sterile water and their surface dried. Next, sterile material was subjected to 12 hours imbibition at 2°C (preimbibition). In some samples, pointed-out earlier, preimbibition was conducted in the presence of phytohormones. GA₃ and ABA (isomer mixture) were supplied by the Sigma firm, zeatin and 3-indoleacetic acid by the Calbiochem firm. The concentration of every phytohormone solution, used in the analysis, equaled 1×10⁻⁵ M. Hence from many results it could be concluded that during the seed germination phytohormone solutions were the most active at this concentration (Poulson and Beevers 1970, Sussex 1975, Walbot et al. 1975, Takaiawa and Tanifuji 1978).

Preimbibed caryopses were germinated in darkness at 21-22°C at constant humidity and in the presence of chloramphenicol (10 μg·cm⁻³) and phytohormones under the study. 5⁻³H-uridine (radioactivity concentration — 0.4 MBq·cm⁻³ and specific activity — 765 GBq·mM⁻¹) and ¹⁴C-aminoacid mi-
xture (activity — 0.4 MBq·cm\(^{-3}\) and specific activity — 1.295 MBq·mA\(^{14}\)C\(^{-1}\)). Radioactive precursors were supplied by the Chemapol UVVVR firm (Cze-
choslovakia). The embryos were excised from caryopses after 24 hours germination. The process was carried-out in ice. Next, the embryo surface was washed clean of the remainder of unmetabolised precursors and dried. Embryos were stored in the closed dishes at \(-25^\circ\)C, ready for the further analyses.

Embryos were excised also after preimbibition, as well as, preimbition and 16 hours germination of grains in the presence of phytohormones. Next they were germinated for 2 h in the presence of chloramphenicol (10 \(\mu\)g·cm\(^{-3}\)), \(^5\)-\(^3\)H-uridine (0.8 MBq·cm\(^{-3}\)) and phytohormones. After incu-
bation embryos were throughly washed and their germination arrested by sample freezing at \(+25^\circ\)C.

Polyribosomes were isolated from *Triticale* embryos, such as monosomes according to the Davies's method (Davies et al. 1972). Hence, approx. 2 g plant material were homogenized in buffer “A” (0.2 M sucrose, 200 mM Tris-HCl, pH 8.5, 30 mM MgCl\(_2\), 60 mM KCl). The homogenate was centrifuged at 29 000 \(\times\)g in “Janetzkii” centrifuge. Supernatant was placed in the 65-Ti rotor tube on 4 cm\(^3\) layer of 1.5 M sucrose in buffer “B” (40 mM Tris-HCl, pH 8.5, 10 mM MgCl\(_2\), 20 mM KCl) and centrifuged in the Spince L-3-40 ultracentrifuge at 95 000 \(\times\)g for 90 minutes. Polyribosome and monosome sediment (about 1 mg) was suspended in 1 cm\(^3\) of buffer “B” and overlaid on the sucrose linear gradient. Gradient had been prepared in buffer “C” (20 mM Tris-HCl, pH 8.5, 10 mM MgCl\(_2\), 20 mM KCl) a day before and stored in cool. It consisted of four sucrose concentrations: 500 mg·cm\(^{-3}\) — 1.8 cm\(^3\), 375 mg·cm\(^{-3}\) — 3.8 cm\(^3\), 250 mg·cm\(^{-3}\) — 3.8 cm\(^3\), and 125 mg·cm\(^{-3}\) — 1.8 cm\(^3\). Polyribosomes were fractioned by ultracentri-
fugation at 122 000 \(\times\)g in SW-41 Ti rotor for 75 minutes. Polyribosome isolation and fractioning were conducted at 0-4°C. After centrifugation test tube contents (13 cm\(^3\)) were divided by a means of crane and their extinctions were measured at 260 nm. Radioactivity was measured by the Fl-100C scintillation counter, manufactured by the Beckman firm. To each 1 cm\(^3\) of sample 10 cm\(^3\) of “Tritosol” (efficiency for \(^3\)H — 47\%, for \(^{14}\)C — 87\%) were added as a scintillator (Fricke 1973). In aim to assess the amount of ribosomes it was assumed that extinction of 1% solution of ribosomes, measured in cuvette with the optical pathway of 1 cm, at wavelength of 260 nm equaled \(E_{1\%}^{1\text{cm}} = 135\) (Gualerzi and Cammarano 1969).

Inhibiting ABA effect on the embryo growth, as well as 3-indoleacetic acid, GA\(_3\), zeatin, GA\(_3\), and zeatin mixture reversal of this action were also studied during 24 hours grain germination. Samples were collected every 3 h. The fresh mass increment was treated as a measure of the embryo growth. Results were given in terms of average fresh mass of an embryo.
RESULTS

For the first 6 h of the germination the effect of the used phytohormones was small. Only in the samples of caryopses, which were imbibed and germinated in the presence of ABA slightly lower embryo mass was observed (Fig. 1). For the next hours of germination (6-24 h) phytohormone effect was more pronounced and the gradual embryo growth occurred. ABA, present during imbibition and 24 hours germination, caused 63% inhibition of embryo growth. Partial reversal of the ABA inhibiting influence was noticed when

![Graph showing the effect of different phytohormones on embryo growth.](image)

Fig. 1. Zeatin, GA₃, IAA, as well as, GA₃ and zeatin mixture reversal of ABA inhibiting effect on the *Triticale* embryo growth during 24 hours caryopse germination. Germination was preceded by 12 hours imbibition at +2°C in the presence of the same phytohormones

GA₃ and zeatin were added. Gibberelic acid led to approx. 18% and zeatin — to approx. 22% stimulation of embryo growth. When GA₃ and zeatin acted together inverseal of the ABA inhibiting effect in 36% was observed after 24 hours grain germination. In spite of this clear synergic reaction the complete reversal of the ABA inhibiting effect could not be attained. No counteraction of 3-indoleacetic acid (IAA) on the ABA-induced inhibition of embryo growth was observed (Fig. 1).

Figures 2 and 3 show sedimentation profiles of polyribosomes isolated from *Triticale* caryopses after their 2 hours germination and 16 hours of grain germination followed by 2 hours embryo germination respectively. Excised embryos were germinated in the presence of ³H-uridine. Already in the initial period of germination ABA inhibiting effect on the formation of embryonic polyribosomes and its partial reversal by growth stimulators
Fig. 2. Sedimentation profiles of polyribosomes (in 12.5-50% sucrose gradient) isolated from Triticale embryos. Embryos were excised from caryopses after preimbibition in the presence of phytohormones. Next, they were germinated for 2 h in the presence of $^3$H-uridine ($0.8 \text{ MBq} \cdot \text{cm}^{-2}$) and chloramphenicol ($10 \mu\text{g} \cdot \text{cm}^{-2}$) in all samples, as well as, ABA, ABA and GA$_3$, ABA and zeatin in some samples, pointed-out earlier. Arrow indicates monosome fraction (80 S)
Fig. 3. Sedimentation profiles of polyribosomes (in 12.5-50% sucrose gradient) isolated from Triticale embryos. Caryopses were imbibed (12h at +2°C) and germinated (16 h) in water and in the presence of appropriate phytohormones. Next, embryos were excised and germinated (16-18 h) in the presence of $^3$H-uridine (0.8 MBq·cm$^{-3}$) and chloramphenicol (10 µg·cm$^{-3}$), as well as, ABA, ABA and GA$_3$, ABA and zeatin. Arrow indicates monosome fraction (80 S).
Fig. 4. Sedimentation profiles of polyribosomes (in 12.5-50% sucrose gradient) isolated from Triticale embryos. Caryopses were preimbibed and germinated in the presence of ABA, ABA and GA₃, ABA and zeatin, and in water. Next embryos were excised. Caryopses were germinated in the presence of additional radioactive precursors: $^3$H-uridine (0.4 MBq·cm⁻³), $^{14}$C-aminoacid mixture (0.4 MBq·cm⁻³) and chloramphenicol (10 µg·cm⁻³). Broken line separates fractions of large polyribosomes, small polyribosomes and monosomes.
Table 1

Differences in the formation of embryonic polyribosomes in Triticale caryopses after 2, 18, and 24 hours germination of caryopses. Every grain was preimbibed at low temperature. Preimbibition and germination were conducted in the presence of the same phytohormones.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 h</th>
<th>18 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P/T</td>
<td>LP/T</td>
<td>LP/P</td>
</tr>
<tr>
<td>Control</td>
<td>31.6</td>
<td>16.9</td>
<td>53.5</td>
</tr>
<tr>
<td>ABA</td>
<td>21.3</td>
<td>9.3</td>
<td>41.7</td>
</tr>
<tr>
<td>ABA + GA₃</td>
<td>29.1</td>
<td>15.7</td>
<td>53.8</td>
</tr>
<tr>
<td>ABA + zeatin</td>
<td>29.3</td>
<td>15.8</td>
<td>53.8</td>
</tr>
</tbody>
</table>

T — total absorbing material (polyribosomes plus mono-ones plus ribosomal subunits). LP — large polyribosomes (material sedimenting faster than septamers). P — polyribo-ones (material sedimenting faster than mono-ones). Figure 4 illustrates polyribosome division into fractions.
was observed (Table 1). As in the case of embryo growth zeatin was more active than GA₃ in reversal of the ABA inhibiting effect on polyribosome formation. On the other hand, in samples where ³H-uridine was present no significant effect of ABA, ABA and GA₃ mixture, as well as ABA and

<table>
<thead>
<tr>
<th>Treatment</th>
<th>³H incorporation</th>
<th>¹⁴C incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>L.P</td>
</tr>
<tr>
<td>Control</td>
<td>279</td>
<td>209</td>
</tr>
<tr>
<td>ABA</td>
<td>137</td>
<td>40</td>
</tr>
<tr>
<td>ABA + GA₃</td>
<td>136</td>
<td>40</td>
</tr>
<tr>
<td>ABA + zeatin</td>
<td>134</td>
<td>38</td>
</tr>
</tbody>
</table>

M — monosomes; T, L.P, P as in Table 1.

³H-uridine and ¹⁴C-aminoacid mixture incorporation into the polyribosomal fraction. Polyribosomes were isolated from embryos after 24 hours germination of intact caryopses in the presence of phytohormones under study.

zeatin mixture on the synthesis of polyribosomal RNA was found in the first 2 h of germination and from 16 to 18 h. Similar results were obtained for samples with the radioactive precursor during 46 hours grain germination and 2 hours germination of the excised seedlings (data unpublished). In all the studies on polyribosomal RNA synthesis in excised embryos (0-2 h, 16-18 h, 46-48 h) in the control, as well as, in samples treated with ABA and stimulators specific radioactivities (cpm·1 mg⁻¹ RNA) were almost identical after the specified time of germination.

The results were strikingly different when embryos were germinated in intact grains for 24 h in the presence of ³H-uridine, ¹⁴C-aminoacid mixture and phytohormones (Fig. 4, Table 2). ABA caused then nearly two-fold decrease in the polyribosomal RNA synthesis in embryos (on the basis of ³H-uridine incorporation) and nearly three-fold decrease in the ribosomal protein synthesis (on the basis of ¹⁴C-aminoacid mixture incorporation). During germination of the intact caryopses, as in the excised embryos, ABA action also resulted in a significant decrease in the polyribosome contribution to the total ribosomal fraction. After 24 hours grain germination polyribosome contribution to the sum total of embryonic ribosomes was in the presence of ABA — 47.2% and in the control — 67.1%. When the grains were germinated in the presence of inhibitor and GA₃, as well as zeatin an increase in the polyribosome contribution to the total ribosomal fraction amounted to 52.3% and 55.4% respectively. ABA caused also significant changes within small and large polyribosomes (Table 1). From the obtained results it could be also determined that the higher polyribosome contribution to the sum total of ribosomes the higher ribosomal protein synthesis.
Interaction of ABA and GA₃ led to the enhanced ribosomal protein synthesis mainly within monosome fraction. Zeatin reversal was stronger and occurred in all the fractions obtained through ultracentrifugation (Fig. 4, Table 2). However, no enhanced transcriptional activity (on the basis of ³H-uridine incorporation) associated with GA₃ and zeatin reversal of ABA inhibiting effect was observed during germination of Triticale caryopses.

**DISCUSSION**

ABA prevents the starting of the germination process through physiological blocking. Its molecular character is still unknown. It is generally assumed that ABA action lies in the disturbance in mRNA synthesis, posttranscriptional modification, or translation (Dure 1975, Fountain and Bewley 1976, Walton 1977, 1980, Jacobsen and Higgins 1978a,b, Galli et al. 1979). In the samples treated with ABA water uptake in swelling embryos is lower already during preimbibition (Fig. 1). Schopfer et al. (1979) have found that during germination ABA prevents embryo development in a reversible way and its action consists in the inhibition of water uptake, which is associated with embryo growth. According to the authors mentioned above ABA inhibiting action lies more in the regulation of embryonic tissue water uptake, than in the regulation of gene activity. However, differences in water uptake, which were observed in this study during imbibition of embryos, between caryopses which were and were not treated with ABA were relatively small.

It is often assumed that growth stimulator reversal of ABA inhibiting effect indicates that ABA acts upon nucleic acid biosynthesis. The studies on germination of pear embryos and elongation of bean embryonal axes have proved that cytokinin and gibberellin reversal of this inhibition is associated with an increase in the RNA synthesis (Khan and Heit 1969, Sussex et al. 1975). However, in the present studies on intact Triticale caryopses and embryos excised from them such a relation has not been observed. No increase in the polyribosomal RNA synthesis occurs with GA₃ and zeatin release of Triticale caryopses and embryos from the ABA-induced “dormancy”. Moreover, in excised embryos ABA has not caused any decrease in the transcriptional activity. Yet, when intact caryopses were germinated for 24 h approx. two-fold decrease in the synthesis of embryonic polyribosomal RNA took place. As the results obtained are contradictory this phenomenon should be better analyzed. On the other hand, it is generally known that embryo excision stimulates the transcriptional process (Grzelczak and Buchowicz 1977).

A significant correlation between ABA-induced inhibition of the embryo growth and polyribosome formation has been observed. Also a clear in-
crease in the polyribosome contribution to the total fraction of embryonic ribosomes has been noticed during GA₃ and zeatin reversal of ABA inhibiting effect. At that time enhanced synthesis of ribosomal proteins also occurs. The similar increase has been noted in the lettuce embryos, when they have been released from ABA-induced “dormancy” (Fountain and Bewley 1976). There is a close relationship between amount of polysomes and the total protein synthesis (Woodland 1974). It has been proved that the sprouts of high protein cereal varieties contain more polysomes than the sprouts of low protein varieties (Ching and Rynd 1978). All these facts indicate that ABA causes a decrease in the protein biosynthesis already in the earliest hours of germination. The other students have found that GA₃ stimulation of the polyribosome formation and protein biosynthesis takes place already in the initial period of lettuce seed germination, when an increase in RNA synthesis is undetectable (Fountain and Bewley 1976, Bewley and Black 1982). After 24 hours germination of intact Triticale caryopses in the samples treated with ABA ³H-uridine incorporation into polyribosomes was two-fold lower. At the same time ¹⁴C-aminoacid mixture incorporation decreased as many as three times. Hence, direct inhibitor action on the process of transcription is doubtful. From the results obtained it can be inferred that in the initial period of germination of Triticale caryopses phytohormone control of protein biosynthesis takes place more at the translation than transcription level.

Acknowledgment

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REFERENCES


Działanie kwasu giberelowego i zeatyny w procesie znoszenia spoczynku indukowanego działaniem kwasu abscysynowego u ziarniaków Triticale

Streszczenie

Badania przeprowadzono na kielkujących zarodkach i całych ziarniakach Triticale. Traktowanie ziarniaków kwasem abscysynowym (ABA) podczas preimbicji i 24 godz. kielkowania spowodowało 63% inhibicję wzrostu zarodków. Częściowe odwrócenie wpływu ABA powodowała giberelina-A₃ (18%) oraz zeatyna (22%). Przy łącznym zastosowaniu obu stymulatorów, obserwowano wyraźną reakcję synergistyczną (36%). Nie stwierdzono istotnego wpływu ABA, mieszanych ABA i GA₃ oraz mieszanych ABA i zeatyny na syntezę polirybosamalnego RNA w pierwszych okresach kielkowania izolowanych zarodków. Kwas abscysynowy powodował jednak ok. dwukrotnie mniejsze wcielenie ³H-urydyny do ogólnej frakcji rybo- somalnej zarodków, podczas 24 godzinnego kielkowania całych ziarniaków. W tym samym czasie stwierdzono, że wcielenie ¹⁴C-mieszanych aminokwasów do białek rybosomałnych było około trzykrotnie mniejsze. W badaniach nad uwalnianiem ziarniaków z indukowanego kwasek abscysynowym "spoczynku" przez GA₃ i zeatynę, stwierdzono że wraz ze wzrostem udziału polirybosomów w ogólnej puli rybosomów wzrasta również synteza białek rybosomalnych. Nie stwierdzono natomiast wzmożonego wcielenia ³H-urydyny do frakcji polirybosomów. Uzyskane wyniki sugerują, że w początkowym okresie kielkowania ziarniaków Triticale, regulacja biosyntezy białek przez fitohormony odbywa się raczej na poziomie translacji niż transkrypcji.