Role of gibberellins and cytokinins in regulation of germination during development and ripening of *Triticale* caryopses

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(Received: October 5, 1983; Accepted: January 5, 1984)

Abstract

The germination of caryopses of M-T3 *Triticale* generation, which were freshly harvested in different growth and developmental phases has been studied. A significant influence of the abscisic acid (ABA) accumulation on the increment of number of germinating caryopses has been found. Already in the first phase of the embryogenesis considerable stimulating effects of kinetin and gibberellin-A₃ (GA₃) on the germination of embryos which were isolated from freshly collected grains have been shown. When both stimulators were used together marked synergetic reaction occurred. It has been also determined that in the initial period of embryogenesis premature germination occurs, to a higher extent, under the action of cytokinins than gibberellins. Whether in the further phases of the caryopse development, when embryos develop mainly through the cell elongation, mostly gibberellins seem to be responsible for the activation of germination processes. The more mature seeds the quicker germinated whole caryopses and embryos isolated from them at different ripeness, after 3-month storage. The highest stimulation of germination by phytohormones has been found for the most mature caryopses. The action of gibberellic acid has been particular strong.

Key words: gibberellin, cytokinin, development, cereal caryopse, *Triticale*, germination.

INTRODUCTION

In the course of the development and ripening of cereal caryopses contents of auxin-like, gibberellin-like, and cytokinin-like substances vary rapidly. The highest content of growth stimulators occurs in caryopses in the initial period of their development — in milk ripeness (Michael and Seiler-Kelbitsh 1972, Wheeler 1972, Mounla
and Michael 1973, Słomiński et al. 1979). In the further phases of the grain development the level of free phytohormones gradually decreases and in full ripeness it becomes relatively low.

Abscisic acid accumulation begins in the initial period of seed development and increases rapidly with it. Maximum accumulation precedes the phase of full ripeness. It has been proved for ash (Szczepkowska et al. 1978), pea (Euwens and Schwabe 1975), and soybean (Québédeaux et al. 1976). The same is true also for cereal caryopses. The highest ABA content occurs there in milk-wax ripeness (Goldbach and Michael 1975, McWha 1975, King 1976, King et al. 1979).

The physiological role of phenolic compounds, common in plants, has not been yet clarified. It has been determined that, when in low concentrations, these compounds can act as stimulators (Kefeli 1974). The presence of vanillic, ferulic, caffeic and coumaric, as well as other acids in the generative plant organs has been detected many times. The highest global content of these compounds is characteristic of the barley grain in milk ripeness (Kulka 1980).

Endogenous inhibitors can control embryogenesis and the grain ripening, as well as prevent premature germination. Probably, they are also responsible for the starting and maintaining of dormancy. However, their action can be broken by endogenous stimulators. Whereas in non-dormant seeds stimulators can initiate growth of radicula or more rarely hypocotyl.

In the course of caryopse development the presence of mentioned above inhibitors limits continuous embryo growth and prevents germination. If these limitations fail, premature germination occurs. Studies on cotton (Dure 1975) and wheat (King 1976) indicate that ABA controls normal embryo growth. Whereas, according to Black (1980) the total ABA content in a grain does not seem to affect changes in the germination capacity of wheat occurring in the course of its development. Black (1980) thinks ABA to be responsible more for the maintenance of seed dormancy than for its induction. However, much more precise information is needed to clarify the role of ABA in the cereal caryopse development, such as determination of the relationship between contents of free and bounded ABA in all parts of grain and grain germination capacity.

The emphasis should be put on the fact that endogenous phytohormones, which control physiological processes of development and growth, form varied group of inhibitors and stimulators. While, in this paper there have been only studied the effects of gibberellin-A₃ (GA₃), kinetin and their mixture on the germination of embryos and whole caryopses in the course of their ripening and development.
MATERIAL AND METHODS

The investigations were carried out on the caryopses of MT-3 *Triticale* generation. *Triticale* was cultivated in 1982 on the experimental plots of Institute of Plant Biology of the Agricultural-Technical Academy in Olsztyn. Period of the development and ripening of the caryopses lasted 70 days. Plant material for the analyses was collected at first 20 days after flowering and then every five days. In all, 11 samples were collected. Cut down spikes were brought to the laboratory just after the harvest. Then the water contents in caryopses and embryos were determined. Each sample of the harvested spikes was divided into two parts.

The first part of spikes was then surface-sterilized with a compress moistened with ethanol. Directly after harvest grains were collected by hand (in a sterile box) from the middle part of the spike, while 3 extreme bottom and top spikelets were thrown away. The germination was conducted under the sterile conditions, in darkness, at temperature 21-22°C, under constant humidity, during 20 days. The effect of gibberellin-A$_3$ (GA$_3$) and kinetin on the germination was then studied. GA$_3$ came from the Sigma firm (USA) and kinetin from the Loba firm (Austria). The concentration of $1 \times 10^{-5}$ M was used in the case of both compounds. From the same samples of freshly harvested caryopses embryos were isolated, also under the sterile conditions. Next they were subjected to the germination according to Johnston's and Stern's method (1957). Embryos isolated by hand were placed, 50 specimens at a time, on the sterile Petri dishes. Petri dishes contained about 30 cm$^3$ of agar with admixture of 1% glucose, 0.02% streptomycin and appropriate growth stimulators. The germination was conducted in darkness, at temperature 21-22°C, for 8 days. Germinating embryos were observed every 8 hours. Embryos with 2 mm long radicles were considered as germinating.

The second part of spikes was stored at room temperature for 2 weeks. Then caryopses were isolated, dry-stored for 3 months and analyzed. The studies were based on 4 samples which represented main developmental phases: milk, milk-wax, wax and full ripeness. Samples were collected respectively 35, 45, 55 and 70 days after flowering. when water content amounted to 71%, 55%, 45% and 15% respectively. Dry caryopses and embryos isolated from them were subjected to the germination at temperature 21-22°C, such as those freshly harvested. Surface-sterilization of grains with 1% solution of sodium hypochloride and 12 hours grain imbibition at temperature 2-4°C (preimbibition) were additionally done. In those samples where stimulators were needed during germination they were also utilised during preimbibition. After 12-hour preimbibition embryos were isolated in ice, under the sterile conditions. Next they were subjected to germination on agar according to Johnston's and Stern's method (1957).
The germination of caryopses was also studied at temperatures of 10°C and 25°C. All the analyses started on the 30th day after flowering, in case of barley grain (Hordeum sativum L. of Diva variety), and on the 50th day after flowering in case of Triticale grain (Triticale of MT-3 generation). Samples were collected every 5 days. In all, 5 samples of barley grains and 5 of Triticale grains were collected.

RESULTS

The water content of caryopses determined, to a high extent, metabolic activity of the endosperm and embryo tissues. Figure 1 shows that in the initial period of development (milk ripeness) water content in caryopses increased only during the first 40 days after flowering and then decreased rapidly. Whereas, dry mass content increased until the 55th day and in full ripeness decreased insignificantly. The water and dry mass contents in embryos increased gradually during the whole embryogenesis.

![Graph showing water and dry matter content in caryopses and embryos of Triticale](image)

**Fig. 1. Dry mass and water content in embryos and intact caryopses of Triticale in the course of their development and ripening**

Figure 2 presents the results of studies on the germination of immature, freshly harvested caryopses and embryos isolated from them. It was determined that with the grain ripening and development there occurred an increment of the germination capacity of isolated embryos and intact caryopses. In whole caryopses rapid increment of the germination capacity — about 40% — lasted for 35 days after flowering (the
first 4 samples). Then, from the 35th to the 50th day after flowering it became much slower and amounted only to 14%. During the next ten days the germination capacity again rapidly increased attaining 80% and it remained at nearly the same level till the end of ripening.

Fig. 2. Effect of gibberellin-A₃ (GA₃) and kinetin (KIN) as well as their mixture on the germination capacity of developing and ripening Triticale caryopses and embryos isolated from them. Freshly harvested unripe caryopses and embryos isolated from them were subjected to the germination in darkness, at temperature 21-22°C for 20 and 8 days respectively. Grain samples were collected every 5 days between the 20th and 70th day after flowering

30 to 35 days after flowering isolated embryos germinated poorly—germination capacity 5%. During the next 15 days (30-50 days) germination capacity again rapidly increased to over 90% and this high level was maintained till the end of ripening. In full ripeness the value of germination capacity attained 100%.

In whole caryopses a high stimulation of germination by egzogenous phytohormones was noticed in the second part of Triticale development (from the 40th day after flowering). At the same time caryopses started to lose water (Fig. 1). An increment of the germination capacity took place mainly in samples subjected to GA₃ and the mixture of GA₃ and kinetin. The effects of action of GA₃ and its mixture with kinetin were nearly the same. The stimulating effect of kinetin was observed only in the most unripe caryopses and only to the 30th day after flowering (the first 3 samples). During further development kinetin did not affect the germination capacity of grains significantly.

On the contrary, in isolated embryos a high stimulation of their germination capacity by phytohormones was noticed already in the first part of embryogenesis (for 45 days after flowering — Fig. 2). The mixture
of GA₃ and kinetin had the strongest effect, GA₃ action was weaker, and kinetin had the weakest effect. But even the effect of kinetin on the germination of isolated embryos was highly significant in the whole period under study.

Fig. 3. Stimulation of the rate of germination increment of isolated Triticale embryos by GA₃, kinetin (KIN) and their mixture. Embryos were isolated every 5 days between the 30th and 70th day after flowering. Numbers indicate days after flowering, when the samples were collected. An embryo with a radicula 2-mm long was treated as germinating.
Figure 3 illustrates the rate of increment of germination of unripe embryos on agar with glucose. Germination energy as well as germination capacity of isolated embryos increased gradually with their development. Similarly to the changes in germination capacity of isolated embryos also the rate of increment of their germination caused by the action of phytohormones was the most significant in the first half of embryogenesis. Contrarywise to whole caryopses, in all samples of isolated embryos clear synergistic reaction of GA$_3$ and kinetin occurred. Its physiological effect depended on the developmental stage of an embryo.

After the recession of dormancy dry Triticale caryopses harvested at milk, milk-wax, wax and full ripeness were subjected to 12-hour imbibition (at temp. +2°C) and germination in the presence of phytohormones. Bottom part of Fig. 4 presents the results. During the first 24 hours of the caryopse germination GA$_3$ was the most important factor (stimulator). After 32 hours in all samples significant synergistic reaction when GA$_3$ and kinetin acted together was observed. The more ripe were grains the quicker they germinated and also, in the first 24 hours the stronger was stimulation of phytohormones. In the later period (24-56 hours) a high stimulation of the germination by phytohormones was observed also in the least ripe caryopses (milk ripeness), with the lowest rate of increment of germination capacity.

Top part of Fig. 4 illustrates the germination of embryos isolated from unripe caryopses. Embryos were isolated in about 3 months after the harvest. The 12-hour imbibition (at temp. +2°C) in the presence of appropriate phytohormones preceded the isolation. The quicker activation of the germination processes took place in isolated embryos in comparison with whole intact caryopses. In milk ripeness the difference was about 8 hours, in other phases as many as 16 hours. Similarly to whole intact caryopses, the highest germination capacity in the initial period (the first 16 hours) occurred in the samples of embryos isolated from caryopses which were collected at full ripeness (F). After 24 hours of germination embryos which came from caryopses harvested at wax (W), and full (F) ripeness germinated nearly in 100%. Embryos isolated from caryopses collected at milk (M), and milk-wax (M-W) ripeness attained their germination capacity of 95-100% in 40 and 32 hours respectively.

Summing-up, whole caryopses as well as embryos isolated from unripe grains showed a retardation in the initiation of germination processes (Fig. 4). The later was the harvest the quicker germinated whole caryopses and embryos isolated from them. In the same samples embryos germinated quicker than whole, intact caryopses. GA$_3$ was a more active phytohormone both in the germination of embryos and caryopses (especially in the initial period of germination). During the whole period of germination of isolated embryos synergic reaction between GA$_3$ and kinetin occurred. In the initial period of the caryopse germination the
Fig. 4. Effect of GA<sub>3</sub>, kinetin and their mixture on the germination of embryos and whole caryopses of Triticale collected at milk (M), milk-wax (M-W), wax (W) and full (F) ripeness. Studies were carried-out after 3-month dry storage of grains after the harvest.
highest stimulation of germination by phytohormones took place in the caryopses harvested at full ripeness.

Figure 5 presents the results of studies on the effect of temperature on the germination of unripe barley and *Triticale* caryopses. As a rule higher stimulation of the germination by low temperature was noticed in barley grains. For instance in the phase of full ripeness the difference between the germination capacity observed at 10°C and that noticed at 25°C for barley grain amounted to 32%/ and for *Triticale* caryopses— to 14%. In the final period of development (3 last samples) germination capacities of barley and *Triticale* caryopses at 10°C were similar. They amounted to 95%/ and indicated the elimination of dormancy in both cereal species. For *Triticale* caryopses similar high stimulation of germination was caused by the action of GA₃ or its mixture with kinetin (Fig. 2).

![Graph showing germination rates at different temperatures for barley and Triticale](image)

**Fig. 5. Effect of temperature on the germination capacity of freshly harvested, unripe caryopses of barley and Triticale**

**DISCUSSION**

The obtained data on changes in the water and dry mass contents is convergent with that mentioned elsewhere by the authors studying dynamics of the accumulation of organic substances in developing cereal caryopses (Ingle et al. 1965, Lityński 1977, Grzesiuk and Kulka 1981).

The results obtained in the investigations on the germination of freshly harvested unripe *Triticale* caryopses are similar to those got for other cereal species (King 1976, Black 1980, Grzesiuk and Kulka 1981, Weidner 1983). The results of author's studies and the
investigations of others have proved that the increment of germination capacity occurring with the grain development is irregular. The rapid increment of germination capacity of cereal caryopses takes place in the initial period of their development (milk ripeness). It is followed by a retardation in the increment of the caryopse germination capacity. Furthermore in other studied cereal species deep decrease in the germination capacity occurs. The period of lower germination capacity (milk-wax ripeness) is followed by the continuous increment of germination capacity of the caryopses till the phase of full ripeness. King (1976) has proved these tendencies to be true for isolated embryos of two wheat cultivars: WW-15 and Gabo. During the germination of embryos isolated from Triticale caryopses of M-T-3 generation period of the strong inhibition of the germination process (2 first samples — 30 and 35 days after flowering) is followed by a rapid increase in the germination. Probably if embryos were isolated earlier a decrease in the germination capacity, which was characteristic of whole caryopses, could be observed. It confirms, to some extent, the profile of phytohormone stimulation of the germination of isolated embryos.

In the developing cereal caryopses GA₃ and ABA seem to play important role in the control of enzyme synthesis and dormancy (McWha 1975, King 1976, Radley 1976, King et al. 1979). Amen's (1968) conception, so called inhibitor-promotor model, concerning interactions between GA₃ and ABA in the developing and ripening cereal caryopses is still commonly accepted. According to King (1976), a decrease in the germination capacity occurring in milk-wax ripeness is due to the maximum ABA accumulation in a grain. For instance during this period in wheat caryopses ABA concentration increases 60 times. In wheat caryopses high level of this inhibitor has remained unchanged for 25-40 days after flowering. King (1976) has shown a high correlation between an increase in ABA content in caryopses and a decrease in their germination capacity, as well as their ability to synthesize a-amylase.

Characteristic of ripening cereal caryopses is a strong activity of growth stimulators in the initial period of their development (Wheeler 1972, Mounia and Michael 1973, Radley 1976), followed by maximum ABA accumulation. According to author, a rapid increment of the caryopse germination capacity, occurring in the first samples (for the first 35 days after flowering), can result from activity of endogenous growth stimulators.

Results of investigations conducted on isolated embryos (Fig. 2) confirm, to a great extent, data obtained in the studies on whole caryopses. However, germination capacity of embryos is much higher, except 2 first samples. It is probably due to both, mechanical damage of the embryo tissues during isolation and breaking of the endosperm-embryo interactions. Grzelczak and Buchowicz (1977) have
found that in embryos cut-off from endosperm some of the agents which control germination are eliminated. In isolated embryos, in comparison with intact caryopses, it has resulted in a considerable acceleration in the resumption of the transcription process.

In present studies clear synergic reaction between GA$_3$ and kinetin in their stimulation of the germination of isolated embryos has been indicated. In intact freshly collected caryopses in the second part of their development the effect of GA$_3$ and its mixture with kinetin have been similar, whereas kinetin itself has not been active. It proves that in caryopses in the second part of their development GA$_3$ has acted as an agent stimulating germination.

It is well-known that in the initial period of embryogenesis, although increment of embryo mass is small, very intensive cell divisions occur. The divisions last approximately for the first half of the seed development and at that time embryo attains its final cell number, further increment of mass is due to cell elongation (Dure 1975). According to literature, the inhibition of growth and development, which is caused by ABA action can be reversible. It is due to the action of gibberellins or cytokinins, but not necessarily to both of them Aspinall et al. 1967, Khan and Heit 1969, Khan 1971, 1977, Sussex et al. 1975, Walton et al. 1975). Gibberellins affect mainly elongation, whereas cytokinins act mainly on the cell divisions. Therefore, the system that grows mainly through elongation is more sensitive to gibberellins and that which develops mainly through cell divisions is more sensitive to cytokinins. A high stimulation of germination by GA$_3$ in the second part of embryogenesis (embryo grows by elongation and stimulating effect of kinetin only in the earliest period of embryo development (intensive cell divisions) prove this theory.

Therefore in the initial period of grain development premature germination can be more easily promoted by cytokinins, while in the second part of embryogenesis they are mainly gibberellins which can cause germination.

After 3-month storage under air-dried conditions Triticale caryopses in different phases of ripeness lose their dormancy and germinate in 100%$, ripeness independent. To 24 hour of germination in all samples of the caryopses, which have been subjected to preimbibition and germination in the presence of phytohormones, GA$_3$ has caused the highest stimulation of germination. Significant kinetin action and synergic reaction between GA$_3$ and kinetin has taken place only after this period. Varied sensitivity of embryo tissues to individual phytohormones is due to the dominance of elongation process in the initial period of the germination of cereal embryos (6-18 hours), whereas later cell divisions play more important role. The first peak of cell divisions for wheat caryopses occurs only in 30 hours after the start of germination (Yadv and Das
1974). In spite of the fact that in the initial period of germination embryo grows mainly through the cell elongation it is also sensitive to cytokinins (f.e.g. caryopses collected at full ripeness).

Many studies on cereals have shown that dormancy induced by ABA can be terminated by the action of cold, partly by gibberellins and cytokinins and by the prolonged period of dry storage (Khan and Heit 1969, Khan 1971, 1977, Grzesiuk and Kulka 1981).

Significantly higher grain germination capacity at temperature 10°C than that at temperature 25°C has been also noticed in the freshly harvested, unripe Triticale and barley caryopses. In the last samples germination capacity at 10°C has been higher than 90% and similar to each other, irrespective of the depth of dormancy in both cereal species. Interaction of stimulators and inhibitors has been studied in many plants demanding the presence of the cold period. Wilkins (1976) has found that extracts of ash embryos, stored in cool, contain substances stimulating germination. It has been shown that in the cold period the gibberellin level in ash embryos increases (Kentzer 1966). Also in other seeds the cold period results in an increase in the content of endogenous gibberellins (Frankland and Wareing 1962, Jarvis et al. 1968a, b).

It seems that main effect of low temperature lies in an increase in the level of endogenous gibberellins, which can result in the change of inhibitors-stimulators ratio and breaking of dormancy in cereal caryopses.

Acknowledgments

Investigations were supported by Polish Academy of Sciences, under project MR II/7.

REFERENCES


Rola giberelin i cytokinin w regulacji kielkowania rozwijających się i dojrzewających ziarniaków Triticale

Streszczenie

Zbadano kielkowanie świeżo zebranych ziarniaków pszenicy rodu MT-3 w różnych fazach rozwoju i dojrzewania. Stwierdzono istotną zależność pomiędzy przyrostem ilości kielkujących ziarniaków oraz nagromadzeniem kwasu abscyzynowego. Wykazano znaczny wpływ stymulujący kinetyny i GA₃ na kielkowanie izolowanych zarodków ze świeżo zebranego ziarna już w pierwszej fazie embriogenezy. Przy łącznym stosowaniu obu stymulatorów wystąpiła istotna reakcja synergistyczna. Wykazano, że w początkowym okresie embriogenezy, przedwczesne kielkowanie związane jest w większym stopniu z działaniem cytokinin niż giberelin. W dalszych fazach rozwoju ziarniaka, gdy rozwój zarodka odbywa się głównie na drodze elongacji komórek, za uruchomienie procesów kielkowania wydają się być odpowiedzialne głównie gibereliny. Zarodki izolowane z ziarniaków o różnej dojrzalności, po około 3-miesięcznym ich przechowywaniu, oraz całe ziarniaki kielkowały tym szybciej im bardziej dojrzałe było ziarno. Największą stymulację kielkowania pod wpływem fitohormonów stwierdzono w przypadku ziarniaków najbardziej dojrzałych, przy czym szczególnie aktywny był kwas giberelowy.