

The role of the embryoless parts of triticale caryopses in inhibiting precocious germination and transcription in the embryo during development and maturation of caryopses

STANISŁAW WEIDNER

Department of Plant Physiology and Biochemistry, Agricultural-Technical Academy, 10-957
Olsztyn-Kortowo bl. 40, Poland

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ABSTRACT

The experiments were conducted on developing and ripening triticale cv. Dagro caryopses. Increasing capability for precocious germination of the caryopses was seen as development and maturation progressed. A significant role of the embryoless parts of the caryopses (testa, pericarp and endosperm) in preventing germination processes was found. Isolated embryos (after 8 days of incubation) germinated by 100% from the 32nd day after flowering, while only 10% of whole caryopses from this sample germinated. Removal of the outer pericarp strongly stimulated germination of unripe caryopses. However, incising the caryopses near the embryo only slightly stimulated this process, which indicates that hypoxia of the embryo is not the cause of triticale embryo dormancy. Another very sensitive indicator of release of dormancy in the caryopses was the increased synthesis of embryo polyribosomal RNA induced by germination. The results of investigations on RNA synthesis in embryos — which undergoes extreme intensification when germination processes are initiated in the caryopses — were in agreement with those of biological studies based on observation of the elongation of the radicle. The lowest inhibition of transcription in the embryo was found when it was completely separated from the testa, pericarp and endosperm. A smaller effect was seen upon removal of the outer pericarp from developing and ripening caryopses, and decidedly the smallest effect still of incising the caryopses near the embryo.

Key words: triticale, dormancy, preharvest sprouting, RNA synthesis

INTRODUCTION

Preharvest sprouting of caryopses depends on the precocious initiation of germination under favorable conditions of humidity, temperature and aeration. Sprouting of grain still in ears reduces its quality and consumption value. Glumeless caryopses such as rye, triticale and wheat are especially prone to

precocious germination. This phenomenon is sometimes the cause of great worldwide losses (Bewley and Black 1985) as well as losses in this country which can reach hundreds of thousands of tons of grain per year (Mazurek 1975, Rzepka 1978). Preharvest sprouting is a very complex process, dependent on many factors (Weidner 1987a).

It was shown in previous papers that precocious germination can occur in cereal caryopses as early as 2-3 weeks after flowering, up until harvest (Weidner 1984a, b, 1987b). In the initial phases of the development of the caryopses when intensive cell division is taking place in the embryo, exogenous cytokinins stimulate sprouting better than gibberellins. In the later phase (from the 35th day after flowering) when embryo growth takes place through elongation, stimulation of germination processes can be achieved mainly by gibberellins (Weidner 1984a, b). Absciscic acid (ABA), present in the developing caryopses (especially in embryos) of such cereals as wheat and triticale, can induce dormancy, but cannot maintain it (King et al. 1979, Walker-Simmons 1987, Weidner 1987b).

The interruption of dormancy of cereal caryopses by growth stimulators (Radley 1979, Paulsen and Heyne 1983, Weidner 1984a, b) and low temperatures (Weidner 1984a), as well as the demonstrated "dormancy" of isolated embryos during development (King 1976, 1982, Radley 1979, Weidner 1987b, c) indicate the chemical regulation of dormancy of cereal caryopses, in which a decisive role may be played by phytohormones. The cited results of studies on the mechanism of dormancy of cereal caryopses show many analogies to the results of investigations on dormancy of buds and other organs (Wereing and Phillips 1985), albeit the presence of the seed coat in the caryopses is an element not found in the dormancy of buds. It is still not clear whether the seed coat creates a physical barrier for gas exchange and if the oxygen deficit inside the caryopses is a factor inducing dormancy, or if the seed coat, and especially the pericarp, are only the sources of germination inhibitors for the embryo, as has been demonstrated many times (Miyamoto et al. 1961, King 1976, McCrate et al. 1982, Paulsen and Heyne 1983, Morris and Paulsen 1988).

The objective of the investigations undertaken in the study was to demonstrate the role played by the embryoless parts of the caryopsis, in particular the pericarp, in inhibiting precocious germination and transcription in the embryo during the development and maturation of triticale caryopses.

MATERIAL AND METHODS

The study was conducted in 1987 on triticale cv. Dagro caryopses cultivated in an experimental field of the Department of Plant Physiology and Biochemistry of the Agricultural-Technical Academy in Olsztyn. The period of

development and maturation of the caryopses lasted about 50 days. The material for study was collected every week, starting on the 18th day after flowering. The caryopses were removed by hand from the middle part of the cut ears discarding three upper and three lower spiklets. The water content in the embryos and whole caryopses was determined in each sample.

Freshly gathered caryopses of different stages of maturity were sterilized in a 1% solution of sodium hypochlorite, then germinated on filter paper in Petri dishes at a temperature of 21–22°C for 8 days. In addition to germination of whole caryopses, germination of caryopses partially incised near the embryo and caryopses with their outer pericarp removed above the embryo was also carried out. Embryos were also isolated from the same samples. The embryos were isolated by hand and, after sterilization in a solution of sodium hypochlorite, placed (50) in sterile Petri dishes with approx. 30 cm³ 0.9% agar containing 1% glucose and chloramphenicol (10 µg cm⁻³). Germination was conducted at a temperature of 21–22°C also for 8 days. An embryo was considered to have been germinated when a 2 mm long radicle had appeared.

Samples of caryopses were taken at other dates during the three main stages of grain development for investigation of the synthesis and formation of polyribosomes. The samples of caryopses were taken at milk ripeness stage 28 days after flowering (with a water content of the grain about 52.6%), at wax ripeness — 37 days after flowering (with a water content of about 45.5%) and at full ripeness — 51 days after flowering (with a water content about 24.2%). Whole, intact caryopses of various degrees of ripeness and those treated as described above were germinated in Petri dishes for 48 hrs. The germination of freshly collected caryopses at milk and wax ripeness and the embryos isolated from them was conducted in the presence of 8-³H-adenosine (0.4 MBq cm⁻³) and chloramphenicol (10 µg cm⁻³). Morphologically ripe caryopses and the embryos isolated from them were germinated additionally in the presence of a ¹⁴C-hydrolysate of amino acids (0.4 MBq cm⁻³). The caryopses were germinated on filter paper in Petri dishes, whereas the embryos isolated from them, on agar with glucose as described. After 48 hrs of germination of embryos or whole caryopses, the germination percentage was calculated, then the embryos were isolated from the caryopses or collected from the surface of the agar medium, the unincorporated precursors were carefully rinsed off, the embryo surfaces dried and the embryos stored in liquid nitrogen until further study.

The total ribosome fraction (polyribosomes + monosomes + ribosome subunits) were isolated from embryos of varying degrees of maturity as previously described (Weidner 1987c). Approximately 2 g of plant material were homogenized in buffer "A" (0.2 M sucrose, 200 mM Tris-HCl (pH 8.5), 30 mM MgCl₂, 60 mM KCl). The homogenate was centrifuged at 29 000 × g in a Janetzki K-70 centrifuge for 20 min. The supernatant was layered on 4 cm³ 1.5 M sucrose in buffer "B" (40 mM Tris-HCl, (pH 8.5), 10 mM MgCl₂,

20 mM KCl) in 65Ti rotor tubes and centrifuged at $95\,000 \times g$ in a Spinco L-3-40 ultracentrifuge for 90 min. The pellet (approx. 1 mg) was suspended in 1 cm³ buffer "B" and layered onto the surface of a sucrose concentration gradient. Fractionation was achieved by centrifugation at $122\,000 \times g$ in a SW-41 rotor for 75 min, using a step gradient in order to obtain distinct separation of the polyribosome and monoribosome fractions. Quantitation of the ribosomes was done assuming that the absorbance of a 1% solution of ribosomes, measured at 260 nm in a cuvette with a 1 cm optical path at 260 nm equals $E_{1\%}^{1\text{cm}} = 135$. (Gualerzi and Cammarano 1969). Radioactivity was assayed using a Beckman LS-1801 liquid scintillation counter by adding 10 cm³ "Tritisol" as the scintillator to 1 cm³ of sample (Fricke 1973). The incorporation of ³H-adenosine into polyribosomal RNA and ¹⁴C-amino acids into ribosomal proteins was determined in the samples from fully ripe grain using the program for dual label counting. All of the results presented in this paper are the mean values of 3-6 separate experiments.

RESULTS

The water content of grain is to a great extent decisive for the metabolic activity of both endosperm and embryo tissues. The changes in the water content of triticale cv. Dagro embryos and whole caryopses were similar to those observed during the development and ripening of a different triticale variety, Lasko (Weidner 1987c). The highest water content in whole caryopses of the Dagro variety (in mg per caryopsis) was found on the 25th day after flowering, whereas in the embryos, on the 39th day.

During the development and ripening of triticale caryopses, a gradual increase in the precocious germination ability of the grain was observed (Fig. 1). The presence of the embryoless part of the caryopsis, composed of the testa, pericarp and endosperm, played a significant role in preventing precocious germination during the whole studied period of development and ripening of the caryopses. Only germination of isolated embryos was found during the initial stage of development (18 days after flowering) (Fig. 1). On the 32nd day after flowering, 100% of isolated embryos germinated (after 8 days of incubation), while only 10% of whole caryopses of this sample did so. Removal of the outer pericarp also strongly stimulated germination of unripe caryopses, from about 30% in the sample of caryopses gathered 25 days after flowering to about 65% (after 8 days of incubation) in the samples of caryopses collected 32 and 46 days after flowering. Incising the caryopses near the embryo had a relatively slight stimulatory effect. This was done to break the physical barrier which can be posed by the seed coat in some seeds to the exchange of gases between the embryo and the atmosphere. A somewhat more distinct effect of incision of the grain on setting off germination processes was

observed only at wax ripeness (39 and 46 days after flowering). The greatest stimulation of precocious germination by incision was observed in the samples of caryopses collected 46 days after flowering; it amounted to about 14% after 8 days of incubation.

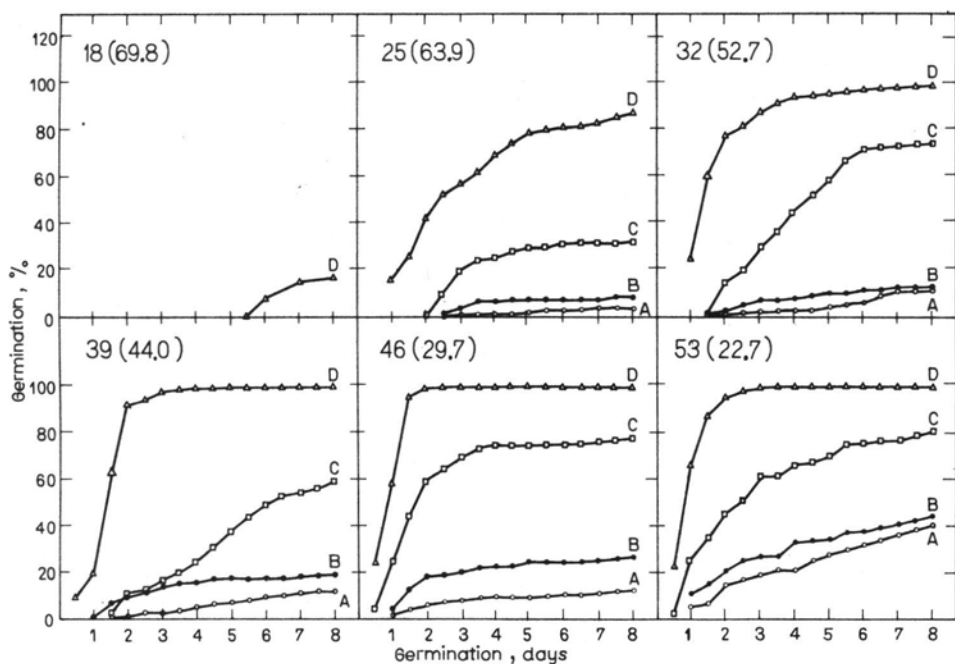


Fig. 1. Germination of freshly collected triticale caryopses and embryos of different ripeness. The numbers 18, 25, 32, 39, 46 and 53 on the figures refer to the number of days after flowering on which the samples of caryopses were taken for study. The water content (per cent) in the different samples of grain is given in brackets. The following samples were germinated: A — intact caryopses, B — caryopses cut partly through near the embryo, C — caryopses after removal of the outer pericarp over the embryo, D — isolated embryos. A caryopsis or embryo was considered to have germinated if its radicle attained a length of 2 mm. The presented values are the means of 3 replicates which differed by less than 10%.

In the second part of the study, the total ribosome fractions isolated from triticale embryos of varying degrees of ripeness were investigated. After 48 hrs of incubation of isolated embryos or whole caryopses under optimal conditions for germination, the proportion of polyribosomes in the total embryo ribosome pool did not differ significantly either in respect to the various degrees of ripeness or different treatment of the caryopses, and ranged from 60 to about 68%.

The previously described results of biological studies based on observation

of the elongation of the radicle (Fig. 1) indicate that along with the development and ripening of caryopses, their ability for precocious germination increases. This is supported by the rise in the synthesis of polyribosomal RNA (induced by germination) in embryos during development and maturation of caryopses subjected to germination (Table 1). A similar

Table 1

The incorporation of ^3H -adenosine and ^{14}C -amino acid hydrolysate into the ribosome fraction of triticale embryos after 48 hrs of germination. The caryopses were collected at milk ripeness (28 days after flowering), wax ripeness (37 days after flowering), and full ripeness (51 days after flowering)

Ripeness stage	Treatment	Bq 1 mg^{-1} total ribosome fraction		Bq per embryo
		^3H	^{14}C	
Milk	A	814	—	4.9
	B	1 017	—	7.3
	C	3 495	—	17.6
	D	133 413	—	372.2
Wax	A	1 185	—	6.5
	B	2 089	—	18.1
	C	4 860	—	46.9
	D	90 755	—	263.3
Full	A	1 226	538	9.1
	B	1 624	619	16.3
	C	17 673	4 972	96.9
	D	97 959	48 837	432.3

The treatment received by samples of caryopses of different degrees of ripeness before germination is denoted by the letters A, B, C, D, — see Fig. 1.

correlation between the results of biological (Fig. 1) and biochemical investigations (Fig. 2, Table 1) was shown by studying the effect of the seed coat on inhibiting precocious germination of triticale caryopses. The strongest stimulatory effect on transcription processes in the embryo, in all samples of caryopses of various degrees of ripeness, was obtained by isolating it completely from the pericarp, testa and endosperm. It should be added that (as was demonstrated on fully ripe caryopses — Fig. 2), in addition to the sharp rise in RNA synthesis in isolated embryos, the synthesis of ribosomal proteins also rose very sharply in comparison with embryos germinating in whole intact caryopses. The removal of the outer pericarp (Table 1) had a rather strong effect on the transcription activity of the embryo in all of the samples of

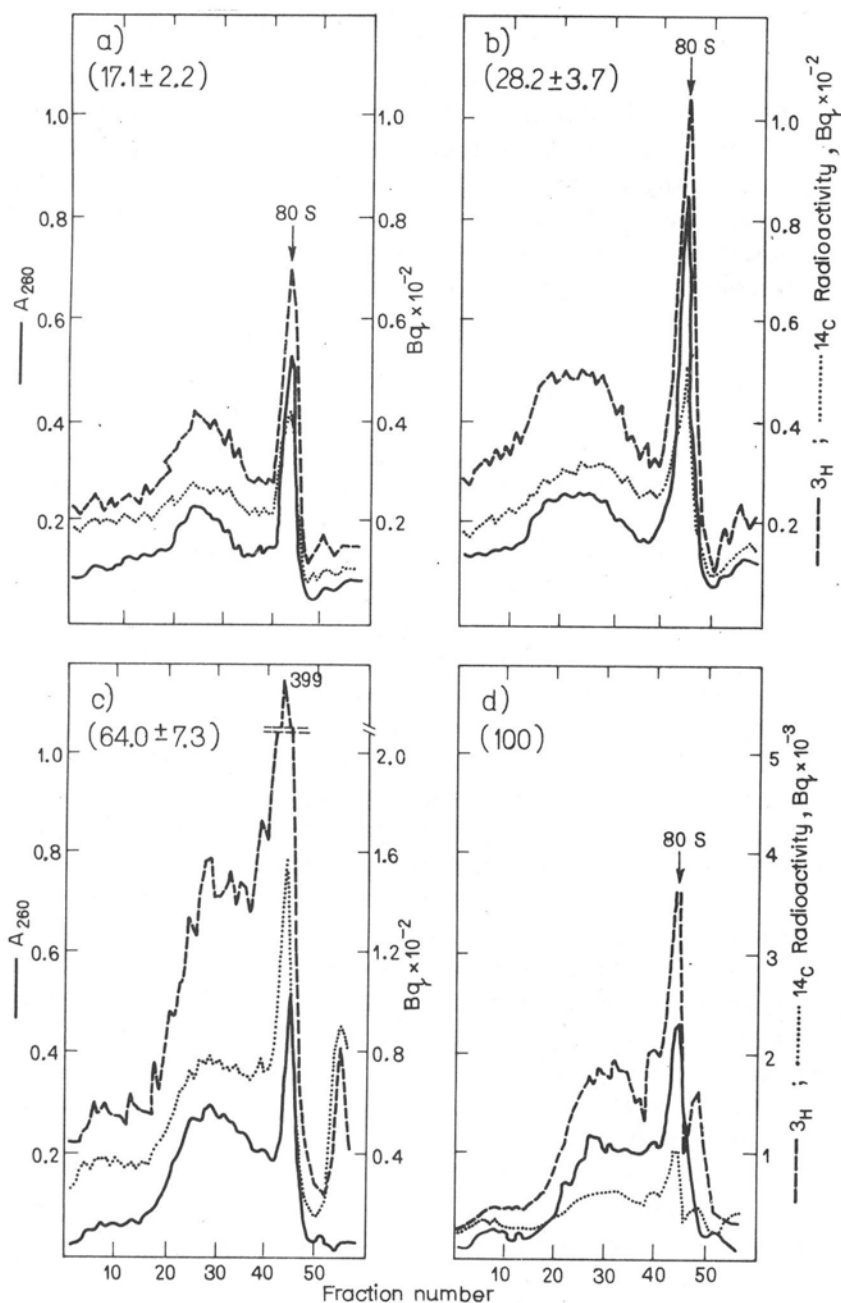


Fig. 2. Sedimentation profiles of polyribosomes (in a 12.5-50% sucrose gradient) isolated from tritcale embryos after 48 hrs of germination. Freshly collected caryopses (at full ripeness) 51 days after flowering were used. The following samples were germinated: a — intact caryopses, b — caryopses cut partly through near the embryo, c — caryopses after removal of the outer pericarp over the embryo, d — isolated embryos. Germination was conducted in the presence of ^{14}C amino acid hydrolysate (0.4 MBq cm^{-3}), $8\text{-}^3\text{H}$ -adenosine (0.4 MBq cm^{-3}) and chloramphenicol ($10 \mu\text{g cm}^{-3}$). The percent of germinated embryos or caryopses after 48 hrs is given in brackets. The monoribosome fraction (80S) is marked by an arrow

caryopses of different degrees of ripeness. This is especially true of RNA synthesis, although it also holds for ribosomal protein synthesis in caryopses collected at full ripeness (Fig. 2, Table 1). Incision of caryopses near the embryo had decidedly the weakest effect on stimulation of transcription in embryos of caryopses of differing degrees of ripeness. Thus, another, very sensitive indicator of intense RNA synthesis in case of initiation of germination processes confirmed the insignificant effect of incising the grain near the embryo on promoting precocious germination.

DISCUSSION

On studying fully ripe wheat caryopses (after cessation of dormancy), Grzelczak and Buchowicz (1977) found that transcription of ribosomal sequences is activated almost immediately in isolated embryos, while it is inhibited for a few hours when the embryo is germinating under natural physiological conditions. The cited authors also conducted detailed investigations on the uptake of a radioactive precursor (^{14}C -uridine) by isolated and not isolated embryos. They demonstrated that the precursor was taken up in comparable amounts. Also in this paper, which describes experiments conducted on unripe and dormant tritcale caryopses, strong inhibition of germination and transcription in embryos by the non-embryo parts of the caryopsis was found. The results presented above show that the presence of the embryoless parts of the caryopsis, e.g., the testa, pericarp and endosperm, inhibits the processes leading to germination not only in dormant, but in non-dormant cereal caryopses as well.

In studies on developing wheat caryopses, Radley (1979) indicated three possible sources of inhibition of germination of embryos. One of them is the presence of the outer pericarp. It could have a purely physical effect, creating a barrier to the exchange of gases between the embryo and the external atmosphere. The inhibitory effect could also be exerted by other tissues surrounding the embryo. In the opinion of the author (Radley 1976), the inner layers of the pericarp, containing chloroplasts, could be the only source of ABA for the embryo. The third form of inhibition, found only in some wheat varieties, is connected with the "dormancy" of the embryo itself. This phenomenon, described by King (1976), is probably related to the balance between endogenous ABA and GA in the embryo during embryogenesis.

Some researchers attribute great significance to the oxygen deficit inside the caryopses, which is supposed to be caused by the presence of the seed coat, and see in it the main cause of dormancy of cereal caryopses (Gordon 1980, Come 1982, Lenoir et al. 1986, Grzesiuk and Kulka 1988). For this reason, additional experiments were conducted in this investigation, entailing

incising triticales caryopses near the embryo, which facilitated gas exchange and interrupted the eventual hypoxia of the embryo. However, it was found that this procedure only minimally enhanced germination processes. Somewhat more pronounced effects were observed only at wax ripeness. It should be added that it is at this stage that triticales caryopses contain high amounts of ABA (King et al. 1979). Therefore, incising the caryopses around the embryos probably increased the removal (washing out) of inhibitors during germination from the tissues surrounding the embryo and the embryo itself. The increased access of oxygen could have caused the oxidation and inactivation of germination inhibitors. It was shown in earlier studies that washing immature embryos in aerated water for an hour caused the greatest enhancement of germination processes in isolated triticales embryos when they were in the mid-phase of their development (Weidner 1987b). In the opinion of this author, the pericarp also plays, to a certain degree, a purely mechanical role since, as was mentioned earlier (Grzelczak and Buchowicz 1977), the pericarp also inhibits transcription involved in germination in caryopses which are not in the state of dormancy. Another indication that the seed coat may be a mechanical obstacle for the growth of the radicle is the fact that many dormant seeds begin to germinate if only the seed coat is removed in the vicinity of the radicle (Wareing and Phillips 1985).

Whatever the effect, it is clear that the seed coat and especially the pericarp of cereal caryopses play a decisive role in preventing precocious germination, which has been demonstrated on the example of triticales caryopses. It seems, however, that the main mechanism of inhibiting germination processes by the pericarp does not involve oxygen deficit inside the seed. Miyamoto et al. (1961) stressed a long time ago that dormancy of wheat caryopses is not caused by physical factors such as restricted water or O_2 uptake, or a tough seed coat, but by a complex of chemical factors, including the catechin-tannin fraction. Also, recently Morris and Paulsen (1988) showed in studies on wheat caryopses that control of germination and preharvest sprouting resides in the embryo and is mediated by inhibitors in the bran. They also found that the endosperm contains only small amounts of active inhibitors.

REFERENCES

- Bewley J. D., Black M., 1985. Seeds, physiology of development and germination. Plenum Press, New York-London.
- Come D., 1982. Germination. In: Croissance et développement. Physiologie végétale II. P. Mazliak (ed.), Hermann, Paris, pp. 129-225.
- Fricke U., 1973. Tritosol: a new scintillation cocktail based on Triton-X-100. Anal. Biochem. 63: 555-558.

- Gordon I. L., 1980. Germinability, dormancy and grain development. *Cereal Res. Comm.* 8: 115-129.
- Grzelczak Z., Buchowicz J., 1977. A comparison of the activation of ribosomal RNA synthesis during germination of isolated and non-isolated embryos of *Triticum aestivum* L. *Planta* 134: 263-265.
- Grzesiuk S., Kulka K., 1988. *Biologia ziarniaków zbóż*. PWN, Warszawa.
- Gualerzi C., Cammarano P., 1969. Comparative electrophoretic studies on the protein of chloroplast and cytoplasmic ribosomes of spinach leaves. *Biochim. Biophys. Acta* 190: 170-186.
- King R. W., 1976. Absciscic acid in developing wheat grains and its relationship to grain growth and maturation. *Planta* 132: 43-51.
- King R. W., 1982. Absciscic acid in seed development. Chapter 7, In: *The physiology and biochemistry of seed development, dormancy and germination*. A.A. Khan (ed.), Elsevier/North-Holland Biomedical Press, Amsterdam. pp. 157-181.
- King R. W., Salminen S. O., Hill R. D., Higgins T. J., 1979. Absciscic acid and gibberellin action in developing kernels of triticale (c.v.6A 190). *Planta* 146: 249-255.
- Lenoir C., Corbineau F., Come D., 1986. Barley (*Hordeum vulgare*) seed dormancy as related to glumella characteristics. *Physiol. Plant.* 68: 301-307.
- Mazurek J., 1975. *Biologia kiełkowania i porostania ziarna odmian zbóż jarych*. IUNG, Puławy.
- McCrack A. J., Nielsen M. T., Paulsen G. M., Heyne E. G., 1982. Relationship between sprouting in wheat and embryo response to endogenous inhibition. *Euphytica* 31: 193-200.
- Miyamoto T., Tolbert N. E., Everson E. H., 1961. Germination inhibitors related to dormancy in wheat seeds. *Plant Physiol.* 36: 739-746.
- Morris C. F., Paulsen G. M., 1988. Localization and physical properties of endogenous germination inhibitors in white wheat grain. *Cereal Chem.* 28/6. (in press).
- Paulsen G. M., Heyne E. G., 1983. Role of embryo response to endogenous inhibitor in preharvest sprouting of wheat. *Proc. 6th Intern. Wheat Genetics Symp. Kyoto, Japan*, pp. 415-418.
- Radley M., 1976. The development of wheat grain in relation to endogenous growth substances. *J. Exp. Bot.* 27: 1009-1021.
- Radley M., 1979. The role of gibberellin, absciscic acid and auxin in regulation of developing wheat grains. *J. Exp. Bot.* 30: 381-389.
- Rzepka D., 1978. Ocena odporności na porastanie i spoczynku późniejszego ziarna odmian żyta di- i tetraploidalnego. Cz.I. Długość spoczynku późniejszego ziarna odmian żyta di- i tetraploidalnego. 133: 59-65. Cz.II. Przydatność metody Hagberga-Pertena w ocenie odporności żyta na porastanie. *Biul. Inst. Hod. Aklim. Rośl.* 133: 67-75.
- Walker-Simmons M., 1987. ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. *Plant Physiol.* 84: 61-66.
- Wareing P. F., Phillips I. D. J., 1985. *Wzrost i różnicowanie się roślin*. PWN, Warszawa.
- Weidner S., 1984a. Role of gibberellins and cytokinins in regulation of germination during development and reaping of triticale caryopses. *Acta Soc. Bot. Pol.* 53: 257-270.
- Weidner S., 1984b. Studies on the mechanism which prevents germination of unripe triticale caryopses. *Acta Soc. Bot. Pol.* 53: 325-337.
- Weidner S., 1987a. Przedsprężne porastanie ziarniaków zbóż i jego regulacja. *Post. Nauk Rol.* 1-2/87: 13-36.
- Weidner S., 1987b. The effect of ABA and AMO-1618 on the preharvest sprouting of triticale caryopses. *Acta Soc. Bot. Pol.* 56: 287-301.
- Weidner S., 1987c. Precocious germination and its regulation in embryos of triticale caryopses. *Acta Soc. Bot. Pol.* 56: 469-483.

Rola bezzarodkowej części ziarniaka w hamowaniu przedwczesnego kiełkowania oraz aktywności transkrypcyjnej w zarodku podczas rozwoju i dojrzewania ziarniaków Triticale

Streszczenie

Badania przeprowadzono na rozwijających się i dojrzewających ziarniakach Triticale odmiany Dagro. Stwierdzono wzrost zdolności ziarniaków do przedwczesnego kiełkowania w miarę postępującego rozwoju i dojrzewania ziarna oraz istotną rolę bezzarodkowej części ziarniaka (okrywa nasienna, owocnia i endosperm) w hamowaniu procesów kiełkowania. Izolowane zarodki (po 8 dniach inkubacji) kiełkowały w 100% od 32 dnia po kwitnieniu, a całe nienaruszone ziarniaki tej próby zaledwie w 10%. Usunięcie zewnętrznego pericarpium silnie stymulowało procesy kiełkowania niedojrzałych ziarniaków, natomiast niewielki wpływ stymulujący wywoływało nacinanie ziarniaków w pobliżu zarodka, co świadczy, że niedotlenienie zarodka nie jest przyczyną spoczynku ziarniaków Triticale. Innym, bardzo czułym wskaźnikiem przerywania spoczynku w ziarniakach była wzmożona synteza polirybosomalnego RNA w zarodkach indukowana kiełkowaniem. Wyniki badań syntezy RNA w zarodkach — ulegającej gwałtownemu wzmożeniu gdy w ziarniakach uruchomione zostają procesy kiełkowania — były zgodne z wynikami badań biologicznych, opartych na obserwacji elongacji korzenia zarodkowego. Najmniejsze hamowanie transkrypcji w zarodku wykazano, gdy całkowicie oddzielono go od okrywy nasiennej, owocni i endospermu. Mniejszy wpływ wywarło usunięcie zewnętrznego pericarpium z rozwijających się i dojrzewających ziarniaków, a zdecydowanie najmniejszy — nacinanie ziarniaków w pobliżu zarodka.