ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 54, nr 4: 417-427
1985

The metabolism of aged seeds

The formation of polyribosomes in the embryos of germinating rye grains of different viability

KAZIMIERZ ZALEWSKI

The Chair of Plant Physiology and Biochemistry, Agricultural-Technical Academy, 10-957 Olsztyn-Kortowo, Poland

(Received: March 4, 1985. Revision accepted: June 21, 1985)

Abstract

Changes during seed germination in the cytoplasmic ribosomal fraction, the formation of polysomes and RNA synthesis in rye grain embryos of different age, were studied. Quantitative changes in embryo ribosomes accompanied seed aging. As the viability of the grain decreased, monosomes constituted an increasing proportion of the total ribosome content. The observed fall in RNA synthesis is considered a symptom of the deteriorative processes involved in seed aging.

Key words: seeds, RNA, ribosomes, germination, aging, rye

INTRODUCTION

Studies on the problem of seed aging have shown that the loss of viability is strictly connected with the complex of conditions under which seeds are stored and with the internal factors of the seed itself (Barton 1961, Lityński 1977, Grzesiuk and Kulka 1981).

Under standard storage conditions, grains retain their sowing value for several years. Rye loses its viability the most rapidly (Święcicki 1974), and practically after one year, the seed reserves of this species should be renewed.

In spite of the universal appreciation of the need for good quality of seed grain, the development of seed science and technology of rye has run into serious difficulties. They result from the genetic and physiological

properties of the grain. Rye seed grain has a short post-harvest period of dormancy, a large tendency to overgrow, to age quickly and is characterized by a low suitability for storage. These properties form the characteristic viability and vigor of rye grain and influence its subsequent yield.

The picture of the degenerative processes going on in aging is most clearly seen when it is imbibing or germinating (Abdul-Baki and Anderson 1972, Roberts 1973). The early phase of germination is correlated with the quick formation of polyribosomes from monosomes by association with previously existing or newly synthesized mRNA (Barker and Rieber 1967, Jachymczyk and Cherry 1968). A fall in the percentage of polyribosomes in the ribosome population is accompanied by a fall in the synthesis of protein in the tissue. Such a process is often observed during germination of seeds with a marked decrease in their viability (Osborne et al. 1974, Bray and Dasgupta 1976, Zalewski 1982). Most often, it is emphasized that a drop in the protein synthesis in some seeds is the result of inactivation of the genome (Roberts et al. 1967, Osborne et al. 1974) and of disorders on the level of transcription (Kulka 1971, Roberts and Osborne 1973, Zalewski et al. 1983) and translation (Anderson 1977). Newer data obtained in our laboratory indicate that unfavourable changes within the ribosome itself also influence this process (Zalewski and Weidner 1982a, b).

The biology of pea, soybean and wheat seed aging have now become fairly well understood. The seeds of the remaining cereals, including rye, have, in this respect, been studied only to a slight degree.

The objective of this study was to investigate the processes of RNA synthesis, polyribosome formation and quantitative changes in the ribosome fraction in embryos during the initial 48 hrs of germination of rye grains of differing viability.

MATERIAL AND METHODS

This study was carried out on the winter rye variety, 'Dankowskie Złote', cultivated on experimental plots belonging to the Chair of Plant Physiology and Biochemisty of the Agricultural-Technical Academy in Olsztyn. The grain was always harvested when at the full-ripeness stage. The analyses were run on rye grain harvested in 1976, 1978, 1980 and 1982 (control). The grain was stored uncovered in hygrostats (relative air humidity 50%, temperature 20°C) until the study was conducted in 1983.

Imbibition and germination. Before imbibition and germination, the grain was sterilized in a 2% solution of sodium hypochlorite for 3 min.,

after which it was thoroughly washed with sterile water, its surface dried with sterile gauze and placed in sterile Petri dishes. Imbibition was carried out for 8 hrs in redistilled water at a temperature of +2 C, germination — for 24 or 48 hrs at 22 C. Germination was in the presence of (5-^{3}H)uridine (0.37 MBq·cm^{-3}) in Petri dishes in the dark and constant humidity. In order to check if each stage of the experiment was run under sterile conditions, all of the solutions (including the isotope solutions in which the grain germinated) were innoculated onto full culture mediums used in bacteriology. Dishes with these mediums were then incubated at 37 C for 48 hrs, during which no bacteria cultures were observed growing. After the prescribed period of imbibition or germination, the embryos (sprouts) were isolated by hand from the grains, the excess precursor was washed off and the embryos (sprouts), rinsed well with sterile water, were stored in closed containers at a temperature of −25 C.

Isolation and fractionation of polyribosomes from rye embryos and sprouts were done according to the method of Davies et al. (1972).

Approximately 3-4 g of sprouts were ground in a cooled porcelain mortar, gradually adding buffer "A" of the following composition: 0.25 M sucrose (ribonuclease-free), 200 mM Tris-HCl, pH 8.5, 30 mM $MgCl_2$, 60 mM KCl. The homogenate was centrifuged for 20 min at 29000 × g. Centrifugation was repeated twice. The supernatant was layered over 4 cm^3 1.5 M sucrose in buffer "B" (40 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$, 20 mM KCl) and centrifuged for 90 min in a 65 Ti angle-head rotor at 95000 × g. The ribosome pellet was delicately dispersed in 1 cm^3 of buffer "B" and layered over a cotinuous sucrose concentration gradient of 125-500 mg·cm^3 in buffer "C" (20 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$, 20 mM KCl). The polyribosomes were separated by contrifugation for 75 min at 122000 × g in a SW-41 rotor. The contents of each tube (13 cm^3) were separated into 46-47 fractions, 2 cm^3 distilled water were added to each fraction and the absorption was measured at 260 nm. The ribosome concentration was calculated assuming that:

$$E = \frac{1 \text{ cm} \cdot 1 \text{ mg} \cdot \text{cm}^{-3}}{260 \text{ nm}} = 13.5.$$

The entire procedure of isolating and fractionating the ribosomes was carried out at a temperature of +2-+4 C.

Radioactivity was measured using 10 cm^3 Tritosol per 1 cm^3 sample. This scintillator had an efficiency of 47% for ^{3}H (Fricke 1973). The radioactivity was measured in a Beckman liquid scintillation counter. The quantitative analyses were done in quadruple, the remaining analyses (fractionation in a concentration gradient when ^{3}H-uridine was used), in duplicate.

RESULTS

The experiments conducted in this study showed that rye grain stored under conditions of relatively low humidity aged very quickly. The loss of viability was directly proportional to the length of storage (Table 1).

In all of the analysed grain samples, various ribosome contents in the embryos were found. No relationship between the ribosome content and the viability of the grain was seen (Table 1).

The 24 and 48 hr germination processes caused a rise in the embryo ribosome content. The highest increases were characteristic for fully viable grain (from 1982) and equalled 41.5% after 24 hrs and 172.5% after 48 hrs of germination. These increases were somewhat smaller in grain stored for 2 years (29.9 and 155%, respectively). In the remaining two samples, germination carried out for 0-48 hrs caused only a slight increase in the embryo ribosome content. Here, the grain with lowest viability (3%) was characterized by higher increases compared with grain harvested in 1978, of which 27% still germinated.

During centrifugation in a sucrose concentration gradient, the ribosomes isolated from embryos in grain germinating for 24 hrs were separated into 2 basic fractions: polyribosomes and monoribosomes. In the sedimentation profiles of ribosome fractions obtained from sprouts of grain with high viability (from 1982), ribosomes associated with mRNA — polyribosomes — dominated. The 80S monosome fraction constituted only a small part of the ribosomes applied to the sucrose concentration gradient (18.78%, Table 1, Figs 1 and 2). However, in the sedimentation profiles of ribosomes isolated from sprouts of grain with a lowered (54%) and low viability (27 and 3%), the monosome fraction made up about one-half of the

The ribosome content of embryos and sprouts

Year of harvest	Viability, %	Dry mass of 100 embryos, mg	Imbibition (8h, 2°C)	
			R	
			$mg \cdot g^{-1}$ dry mass of embryos	mg per 100 embryos
1982	94	0.1088	9.529	1.037
1980	54	0.1267	4.753	0.602
1978	27	0.1216	7.159	0.870
1976	3	0.1166	8.233	0.960
LSD P = 1%	5.00	0.0097	0.550	0.079

R — polyribosomes + monoribosomes; P — polyribosomes (Ph + Pl. fractions 1-32); Ph — heavy polyrib

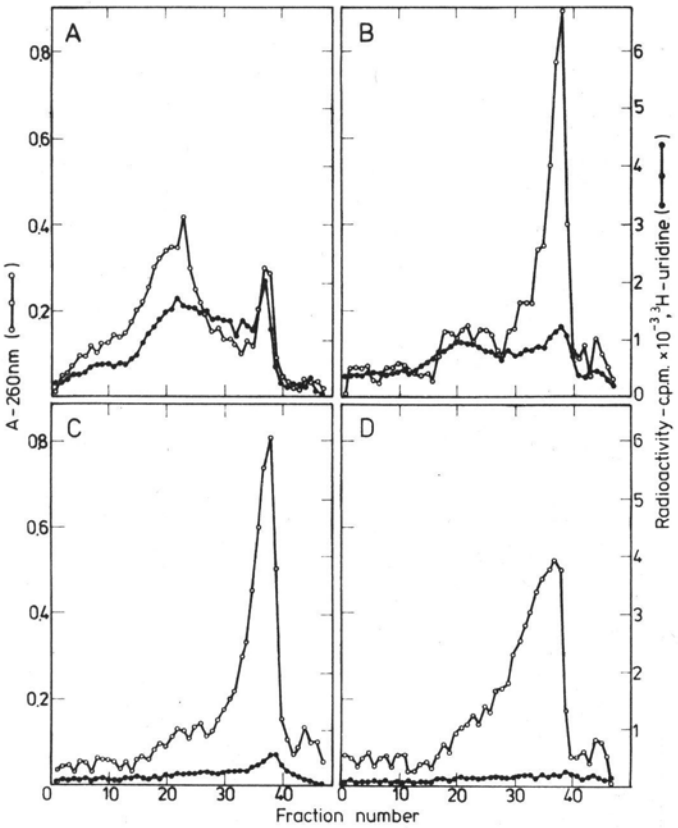

Fig. 1. Incorporation of ^{3}H-uridine into RNA associated with polysomes after 24 hrs of germination of rye grains of different viabilities. The polysomes were separated by ultracentrifugation in a sucrose concentration gradient from 125 to 500 mg·cm^{-3}. The grain was harvested in: A — 1982 (control), B — 1980, C — 1978, D — 1976

Table 1

of rye grains of different age during imbibition and germination

Germination (22°C)									
R		P		composition of fraction R (%)					
mg per 100 sprouts				Ph		Pl		M	
24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
1.468	2.826	1.192	2.340	30.12	25.89	51.10	56.91	18.78	17.20
0.782	1.535	0.375	1.079	14.08	22.08	33.17	48.24	51.56	29.68
0.880	0.965	0.370	0.545	12.70	13.25	29.38	43.23	57.92	43.52
1.133	1.392	0.635	1.069	13.86	20.19	42.03	56.67	44.11	23.14
0.133	0.193	0.102	0.177	1.80	1.43	2.34	2.87	2.65	2.12

osomes (fractions 1-17); Pl — light polyribosomes (fractions 18-32); M — monoribosomes (fractions 33-40).

Table 2

Incorporation of ^{3}H-uridine (0.37 MBq · cm^{-3}) into newly formed polyribosomes in germinating rye grains (24 h, 48 h, 22°C) of different age

Year of harvest	Specific radioactivity							
	R imp. · min^{-1} · mg^{-1} R		Ph imp. · min^{-1} · mg^{-1} Ph		Pl imp. · min^{-1} · mg^{-1} Pl		M imp. · min^{-1} · mg^{-1} M	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
1982	109745	218140	113235	204338	113700	219387	93390	234795
1980	47973	77529	69877	101828	70140	73148	28787	103458
1978	16479	54039	29041	106710	20933	48971	11446	43041
1976	11277	32845	20784	40553	11697	26199	7766	42403
LSD P = 1%	3450	8708						

Symbols: R, Pl, Ph, M — as in Table 1.

Table 3

The absolute radioactivity of individual ribosome fractions isolated from germinating rye grains (24 h, 48 h, 22°C) of different age

Year of harvest	Absolute radioactivity (imp. · min^{-1} per 100 sprouts)							
	R		Ph		Pl		M	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
1982	161106	616465	50068	149504	85292	352834	25746	114127
1980	37515	119007	7693	34512	18193	54165	11607	47134
1978	14501	52147	3245	13644	5412	20429	5834	18075
1976	12742	45720	3263	11397	5570	20666	3881	13658
LSD P = 1%	6976	26183						

Symbols: R, Pl, Ph, M — as in Table 1.

fractionated ribosomes (Table 1). Germination carried on for another 24 hrs caused a decrease in the monosome and an increase in the polysome contents in the sprouts of all of the analysed grain samples.

The different storage times of the grain samples (under identical conditions) which caused an unequal fall in the grain viability, affected the degree of ^{3}H-uridine incorporation into the RNA incorporated next into polysomes

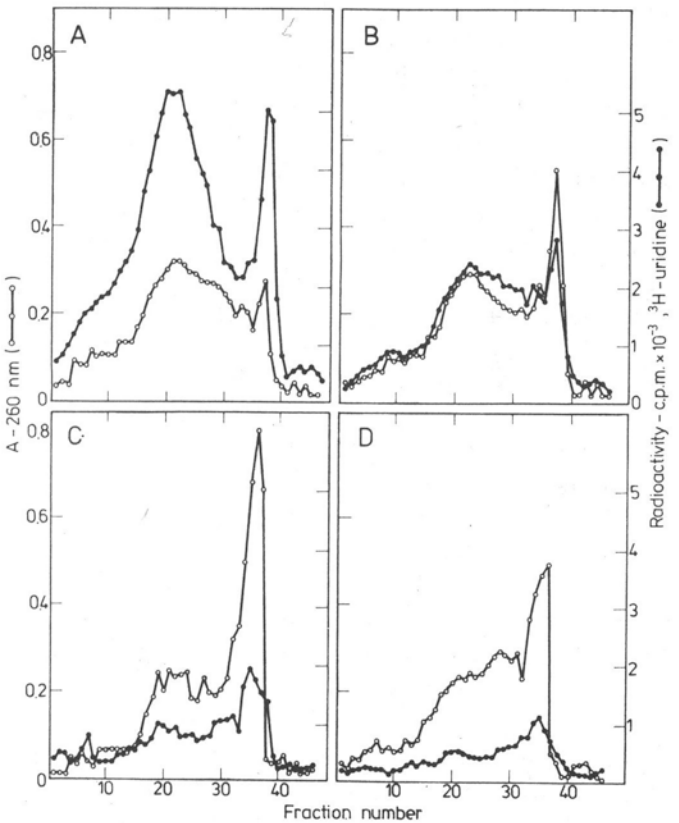

Fig. 2. Incorporation of ^{3}H-uridine into RNA associated with polysomes after 48 hrs of germination of rye grains of different viabilities. Symbols as in Fig. 1

(Tables 2 and 3). The embryos in the control grains were characterized by the greatest intensity of RNA synthesis after 24 hrs of germination as well as by the highest increases in radioactivity after 48 hrs of growth. Incorporation of the marked precursor into RNA in the remaining grain samples took place at a significantly lower rate. It should, however, be emphasized that in spite of the clear-cut differences in the germination ability (54, 27 and 3%), the intensity of RNA synthesis (rRNA + mRNA)

in the grain embryos, measured by the amount of incorporated ^{3}H-uridine, did not drop proportionally to the fall in viability.

DISCUSSION

Relatively much data on the function of plant ribosomes can be found in literature (Golińska and Legocki 1973, Bray and Dasgupta 1976, Siwecka et al. 1977), whereas studies on the structure of the ribosomes of higher plants are only in the initial stages (Chumkina et al. 1975, Filipowicz 1977, Gumilevskaya et al. 1977, Sikorski et al. 1979). In spite of significant progress in studies on transcription and translation of the genetic code, the mechanisms of both processes still are not fully clear. These difficulties will exist until the functions of the individual ribosomal proteins and different rRNAs become precisely understood.

In general, the opinion prevails that the aging of grain is connected with structural and chemical changes in the embryo's ribosomes (Roberts and Osborne 1973, Bray and Chow 1976, b, Bray and Dasgupta 1976, Zalewski and Weidner 1982b). However, it has not yet been shown exactly how these changes affect the formation of polyribosomes during the first stages of grain germination.

The results obtained in this study confirm to a large extent the view about the lowering of the so-called "transcription efficiency" of the embryo's cells during germination of aged rye grains. This is indicated by the smaller increases in the amount of ribosomes in rye embryos from grain stored from 2 to 6 years compared with the grain analysed directly after harvesting in 1982 (Table 1) and by the low specific activities of these samples after 24 and 48 hrs of germination (Table 2).

The data on the grain harvested in 1976 deserve more detailed attention. After 6 years of storage, only 3% germinated. At the same time, the amount of ribosomes in imbibing embryos and the significant increase in this respect after 24 and 48 hrs of germination completely do not fit in with the viability of this sample. The supposition that the discussed increase in the ribosome content takes place through biogenesis from newly synthesized rRNA and ribosome proteins is supported by the fact that there was a significant increase in the absolute (261%) and specific (291%) activities when germination took place for 24 to 48 hrs (Tables 2 and 3). The above data suggest that newly synthesized ribosomes are capable of associating with mRNA. There is no certainty, however, that they take part in translation. Similar studies which have been done on soybean (Anderson 1977) and wheat grains (Zalewski 1982), have shown that even dead embryos and embryo axes are capable of biosynthesis of small amounts of protein. The problem remaining unsolved, however, is the question of the quality

of the proteins synthesized in the aged seeds, since in spite of the fact that small amounts of RNA and protein are synthesized, the embryos are not capable of normal division and growth.

Much data supports the idea that the halting of growth processes in aged grain is connected with a demaged embryo energy system (Ching and Kronstad 1972, Ching 1973, Roberts and Osborne 1973, Van Onckelen et al. 1974, Anderson 1977, Grzesiuk and Tłuczkiewicz 1982). In addition, Roberts and Osborne (1973) found while studying ribosomes by ultracentrifugation in sucrose concentration gradients, that the ribosomes obtained from dead rye grains had a smaller mass than ribosomes isolated from live grains. Further studies showed that, to a large extent fractions 25S and 18S of aged rye grains underwent degradation to low molecular weight forms. Many authors report similar observations (Henri et al. 1974, Bray and Dasgupta 1976, Rejowski et al. 1980, Zalewski et al. 1983). Our knowledge about the other constituent of ribosomes of higher plants, the ribosomal proteins, is rather limited. Even so, it can be stated on the basis of data obtained from studies on aged wheat grain (Zalewski and Weidner 1982a), that the process of aging in grain is also accompanied by numerous quantitative and qualitative changes in ribosomal proteins. These changes can exert an unfavourable effect on the initiation and termination of the protein chain. This view, however, must receive experimental confirmation.

Acknowledgment

This study was financed through IHAR, project PW-09.1-B/01-05.

REFERENCES

Abdul-Baki A. A., Anderson J. D., 1972. Physiological and biochemical deterioration of seeds. In: Seed Biology. T. T. Kozlowski (ed.), vol. II. Academic Press, N. Y., pp. 283-309.

Anderson J. D., 1977. Adenylate metabolism of embryonic axes from deteriorated soybean seeds. Plant Physiol. 59: 610-614.

Barker L. V., Rieber M., 1976. The development of polysomes in the seed of *Pisum arvense*. Biochem. J. 105: 1195-1201.

Barton L. V., 1961. Seed preservation and longevity. Leonard Hill, London.

Bray C. M., Chow T.-Y., 1976a. Lesions in post-ribosomal supernatant fractions associated with loss of viability in pea (*Pisum arvense*) seed. Biochem. Piophys. Acta 442: 1-13.

Bray C. M., Chow T.-Y., 1976b. Lesions in the ribosomes of nonviable pea (*Pisum arvense*) embryonic axes tissue. Biochim. Biophys. Acta 442: 14-22.

Bray C. M., Dasgupta J., 1976. Ribonucleic acid synthesis and loss of viability in pea seed. Planta 132: 103-108.

Ching T. M., 1973. Adenosine triphosphate content and seed vigor. Plant Physiol. 57: 400-402.

Ching T. M., Kronstad W.E., 1972. Varietal difference in growth potential, adenylate energy level and energy charge of wheat. Crop Sci. 12: 785-789.

Chumkina L. W., Gumilevskaya N. A., Kretovich W. L., 1975. Vydielienije i kharakteristika subjedinic 80S-ribosom sukhikh siemian goroha. Biokhimiya 40: 175-185.

Davies E., Larkins B. A., Knight R. H., 1972. Polirybosomes from peas. Plant Physiol. 50: 581-584.

Filipowicz W., 1977. Inicjacja translacji u organizmów eukariotycznych. Post. Bioch. 23: 21-59.

Fricke U., 1973. Tritosol: a new scintillation cocktail based on Triton X-100. Anal. Bioch. 63: 555-558.

Golińska B., Legocki A. B., 1973. Purification and some proporties of elongation factor I from wheat germ. Bioch. Biophys. Acta 324: 156-170.

Grzesiuk S., Kulka K., 1981. Fizjologia i biochemia nasion. PWRiL, Warszawa.

Grzesiuk S., Tłuczkiewicz J., 1982. Viability and vigour of ageing winter wheat grains. Acta Soc. Bot. Pol. 51: 251-262.

Gumilevskaya N. A., Alekhina S. K., Chumikina L. V., Kretovich W. L., 1977. Study of basic proteins of 60S- and 40S-subunits of pea seed ribosomes by two--dimensional polyacrylamide gel electrophoresis. Biokhimiya 42: 1774-1782.

Henri A., Van Onckelen H. A., Verbeck R., Khan A., 1974. Relationship of ribonucleic acid metabolism in embryo and aleurone leyer to α-amylase synthesis in barley. Plant Physiol. 53: 562-568.

Jachymczyk W., Cherry J. H., 1968. Studies on messenger RNA from peanut plants: *in vitro* polyribosome formation and protein synthesis. Biochim. Biophys. Acta 157: 368-377.

Kulka K., 1971. Biochemiczne aspekty starzenia się ziarna owsa i jęczmienia. Zesz. Nauk. WSR Olsztyn 6: 2-89.

Lityński M., 1977. Biologiczne podstawy nasiennictwa. PWN, Warszawa.

Osborne D. J., Roberts B. E., Payne P. I., Sen S., 1974. Protein synthesis and viability in rye embryo. Bulletin 12, Welington: The Royal Society of New Zeland.

Rejowski A., Majkowski K., Kulka K., 1980. Nukleinsäure des alterden Getreidekorns. In: Saatgutvitalität und Pflanzenertrag. Halle, 1: 24-31.

Roberts E. H., 1973. Loss of seed viability: chromosomal and genetical aspects. Seed Sci. Technol. 1: 515-545.

Roberts E. H., Abdala F. H., Owen R. J., 1967. Nuclear damage and the ageing of seeds. Symp. Soc. Exp. Biol. 21: 65-100.

Roberts E. H., Osborne D. J., 1973. Seed ecology. London.

Sikorski M. M., Przybył D., Legocki A. B., Kudlicki W., Gąsior E., Zając J., Borowski T., 1979. The ribosomal proteins of wheat germ. Distribution and phosphorylation *in vitro*. Plant Sci. Lett. 15: 387-397.

Siwecka M. A., Gołaszewski T., Szarkowski W., 1977. Association of ribonucleotic activity with rye embryo robosomes. Bull. Acad. Polon. Sci., Ser. Sci. Biol. 1: 15-19.

Święcicki W., 1974. Przechowywanie materiału siewnego. In: Nasiennictwo. Dominas M. et al. (ed.) PWRiL, Warszawa.

Van Onckelen H. A., Verbeck R., Khan A.A., 1974. Relationship of ribonucleic acid metabolism in embryo and aleurone to layer to α-amylase synthesis in barley. Plant Physiol. 53: 562-568.

Zalewski K., 1982. Formowanie się polirybosomów w zarodkach kiełkującego ziarna pszenicy o różnej żywotności. Hod. Rośl. Aklim. 26: 1-9.

Zalewski K., Weidner S., 1982a. Changes in ribosomal proteins of wheat embryos during accelerated ageing of the grain. Acta Soc. Bot. Pol. 51: 301-308.

Zalewski K., Weidner S., 1982b. Ribonucleic acids and ribosomal proteins synthesis during germination of unripe and aged wheat caryopses. Acta Soc. Bot. Pol. 51: 291-300.
Zalewski K., Weidner S., Kulka K., 1983. Synteza RNA w ziarniakach pszenicy niedojrzałych oraz zestarzałych. Hod. Rośl. Aklim. 32: 1-8.

Metabolizm starych nasion
Formowanie się polirybosomów w zarodkach kiełkującego ziarna żyta o różnej żywotności

Streszczenie

Badano zawartość frakcji rybosomalnej w pęczniejącym i kiłkującym ziarnie żyta w różnym wieku, proces formowania się polirybosomów oraz syntezę RNA włączanego w polisomy podczas pierwszych 48 godzin kiełkowania ziarna. Stwierdzono, że starzenie się ziarna związane jest z zmianami ilościowymi rybosomów w zarodkach. W ogólnej ilości rybosomów udział frakcji monosomów wzrastał w miarę zmniejszania się żywotności ziarna. Symptomem procesów degradacyjnych, towarzyszących procesowi starzenia się ziarna był też obserwowany znaczny spadek natężenia syntezy RNA.