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The formation of polyribosomes during the germination of grains of wheat of different ripeness

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Abstract

The total ribosomal fraction was isolated from whole seedlings from grains of wheat gathered at the milk-, milk-wax and full-ripeness stages. A gradual increase in the amount of the total ribosomal fraction was observed during germination until the 6th day in all of the studied groups. From the 6th to 12th days of germination a decrease in this fraction was found. In all of these groups polysomes constituted 88-90% of the total ribosomal fraction after 48 hrs of germination. From the 2nd to the 12th day, polysomes made up a decreasing percentage of the total ribosomal fraction. The percentage of polysomes contained in this fraction at the begining (0-24 h) as well as at the end (10-12th day) of the studied period of germination of immature grains was lower than in mature ones, which is synonymous with a lower level of protein synthesis in their tissues. When the results were calculated per seedling, it was shown that the less mature the grain, the lower the total ribosome content. These results show that grains gathered at full ripeness are the most vigorous.

INTRODUCTION

In agricultural practice it is very interesting to know at what stage of ripeness are the seeds being used, especially from the point of view of the size and timing of the harvest. The degree of ripeness of the grain can be approximated on the basis of its water content. It is accepted that grains containing more than 50% water are at the milk-ripeness stage, from 50-25% water — at the wax-ripeness stage and less than 25% water, that the full-ripeness stage has been attained.

The subject of germination of seeds of different ripeness has long been of interest to agriculture because unripe seeds are found in almost every batch. This is often the result of harvesting seeds from plants which ripened unequally in the stands or from plants whose seeds

ripened unequally within the ear. In these instances the questions raised most often by farmers deal with the physiological qualities of the seeds and the sowing value of the entire crop.

The most important element determining the biological value of the grain is the efficiency of the protein synthesis system. Earlier studies have shown (Weidner and Zalewski 1982a) that during the development and ripening of seeds, small proteins are incorporated into embryo ribosomes. These changes are both qualitative and quantitative in character. In other studies (Weidner et al. 1980) it has been shown that activation of transcription during germination takes place quicker in fully ripe grains and that early transcription products are detected much later in unripe or stored grains. However, access to the DNA template for endogenous RNA polymerases is several times higher in grains gathered at the milk-ripeness stage than in those gathered at wax or full ripeness (Weidner et al. 1979).

Germination has an effect on all of the RNA fractions of the cell, most notably, an increase in the mono- and polyribosome fractions is observed (Weidner and Zalewski 1982b, c). During the first 30 minutes of imbibition of water by the wheat embryo, abrupt formation of active polysomes is observed which is accompanied by an increase in protein synthesis (Wareing and Philips 1970).

Studies on RNA synthesis and polyribosome formation carried out to date were done most often on isolated embryos and usually dealt with the early stages of germination (Ching 1972, Spiegel et al. 1975, Brooker et al. 1978, Cheung and Shuchadolnik 1978, Cheung et al. 1979). The study undertaken here was carried out over a relatively long period of time (0-12 days) on whole, intact wheat grains. It is known that when the embryo is separated from the endosperm, part of the factors controlling gremination are removed. It should also be mentioned that studies on germination of grains of different ripeness are not very advanced.

MATERIAL AND METHODS

Winter wheat (*Triticum aestivum* L.) variety Grana was grown in 1981 on experimental fields belonging to the Institute of Plant Biology in Olsztyn. Three samples of wheat grain were chosen for the study. These samples differed in their maturity and degree of development. The ears were gathered:

— at the begining of the milk-ripeness stage (20 days after flowering) with the water content of 68.2% in the grains;

— at the milk-wax ripeness stage (30 days after flowering) with a water content of 50.7%;

— at the full-ripeness stage (49 days after flowering) with a water content of 20.3%.

The grains were removed by hand after a two-week period of storage at room temperature. After three months the grains surfaces were sterilized by immersion in 70% ethanol, then placed on rolls of filter paper at 21-22°C and allowed to sprout. Germination was continued over 12 days. During this period, first every 6 hours, later, during the last phase, every 2 days, whole seedlings were isolated and used to study the total ribosomal fraction. Experiments were also carried out on dry embryos isolated from grains of different ripeness according to the method of Johnston and Stern (1957).

Poly- and monoribosomes were isolated from embryos and whole seedlings and assayed in the post-mitochondrial supernatant according to Davies et al. (1972). Due to its high ionic strenght and pH, the homogenation buffer used in this method helped achieve a high yield and prevented degradation of the polysomes. Isolated embryos and wheat seedlings were homogenized in buffer A — 0.2 M Tris HCl, 30 mM $MgCl_2$, 60 mM KCl and 0.2 M sucrose (pH 8.5). The homogenate was centrifuged 10 min. at 5000×g in order to remove fragments of cell walls, nuclei and plastids. The supernatant was centrifuged again for 20 min. at 29 000×g to remove mitochondria and other, lighter, cell organells. Further purification of the total ribosomal fraction was carried out by layering this supernatant over 4 ml of 1.5 M sucrose in buffer B — 40 mM Tris HCl, 10 mM $MgCl_2$, 20 mM KCl (pH 8.5) in tubes from the 65 Ti rotor. Centrifugation was carried out for 90 min. at 95 000×g (38 500 rpm) in a Spinco Model L-3-40 centrifuge. The purified pellets of mono- and polysomes (approx. 1 mg) were suspended in 1 ml of buffer B and layered over a discontinuous sucrose gradient. The gradient was prepared the day before in buffer C — 20 mM Tris HCl, 10 mM $MgCl_2$, 20 mM KCl (pH 8.5) and kept in the cold room. It consisted of four different concentrations of sucrose: 1.8 ml of 500 mg sucrose/ml; 3.8 ml of 375 mg/ml; 3.8 ml of 250 mg/ml and 1.8 ml of 125 mg/ml. This gradient allowed the exact separation of mono- and polysomes.

The separation of ribosomal fractions was carried out in the SW-41 Ti rotor at 122 000 × g (31 500 rpm) for 75 min. The whole process of isolation and fractionation of polysomes was done at 0-4°C. After centrifugation the contents of the tubes (13 ml) were divided into approximately 50 fractions and their extinction was measured at 260 nm. The ribosome content was determined assuming that the extinction at 260 nm of a 1% solution of ribosomes with an optical path of 1 cm is: $E_{1\%}^{1cm} = 133$ (Gualerzi and Cammarano 1969).

All of the results presented in this paper were obtained in 3-5 separate experiments.

RESULTS

Approximately 12% polysomes and 88% monosomes were found in dry embryos in grains of different ripeness. During germination, an increase in the association of monosomes into polysomes was observed (Table 1, Fig. 1). After 6 hrs of imbibition, polyribosomes constituted 50% of the total ribosomal fraction in embryos from ripe grains whereas only about 40% in unripe ones (Table 1). This indicates a certain delay in polysome formation in germinating embryos in grains gathered before having reached full ripeness. A maximum polysome content (88-90%) of the total ribosomal fraction was found in all samples after 2 days of germination. During further growth the polysome content in all groups gradually decreased. The rate at which this took place, however, was largely determined by the stage of development at which the grains had been harvested. After 12 days of germination the percentage of polysomes in the total ribosomal fraction was 43.08% in grains gathered at the milk-ripeness stage, 53.49% at the milk-wax stage and 64.21% at the full-ripeness stage.

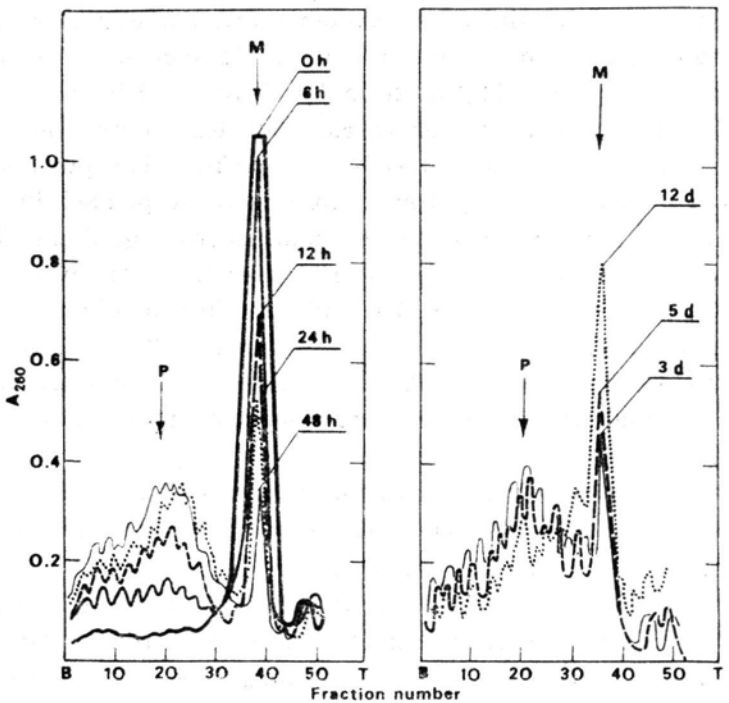

Fig. 1. Sedimentation profiles in a 12.5-50% sucrose gradient of polysomes isolated from dry embryos and whole seedlings from fully ripe grains. The process of polysome formation was studied from 0 hrs (dry embryos) to 12 days (12 d). M — monosome fraction, P — polysome fraction (i.e., material sedimenting faster than monosomes), B — tube bottom, T — tube top

Table 1

Changes in the percentage of monosomes (M), small polysomes (SP) and large polysomes (LP) in the total ribosomal fraction in whole seedlings from grains of different ripeness

Time of germination	Milk-ripeness			Milk-wax ripeness			Full-ripeness		
	LP	SP	M	LP	SP	M	LP	SP	M
6 hrs	24.63	19.17	57.46	21.28	23.29	60.84	27.12	23.32	49.56
12 hrs	36.75	23.19	40.06	24.87	30.57	44.56	44.88	25.55	29.57
24 hrs	31.70	35.86	32.44	29.71	45.71	24.58	40.98	37.85	21.17
2 days	36.90	52.82	10.28	39.58	48.93	11.49	62.54	26.13	11.33
3 days	30.32	54.73	14.95	26.93	58.93	14.14	26.93	57.64	15.43
4 days	25.67	53.81	20.51	24.75	55.85	19.40	14.34	68.79	16.89
6 days	23.72	55.06	21.22	31.84	47.81	20.35	23.38	44.85	31.77
8 days	19.05	50.66	30.29	18.52	52.19	29.29	12.33	55.53	32.14
10 days	16.22	38.09	45.69	19.26	45.32	35.45	11.79	54.26	33.95
12 days	25.17	17.91	56.92	21.60	31.89	46.51	14.59	49.62	35.79

LP — large polysomes — i.e., material sedimenting faster than septamers.
SP — small polysomes — i.e., material sedimenting between monosomes and octamers.

In short, it may be stated that during the first period of germination (up to day 2) a delay in polysome formation takes place in germinating embryos of unripe grains. Similarly, at the end of the studied period of germination, (days 10-12), the percentage of polysomes in the total ribosomal fraction from seedlings of grains gathered before full ripeness is much lower than in those gathered at full morphological ripeness (Table 1).

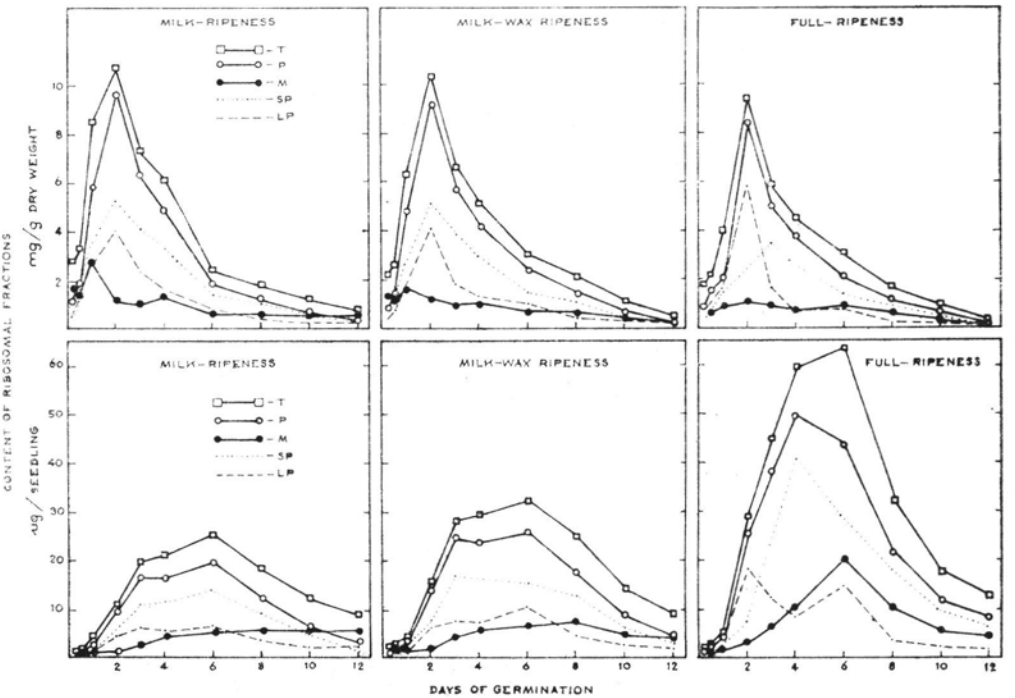

Fig. 2. Changes in the content of the total ribosomal fraction — T (i.e., polysomes plus monosomes plus subunits) in embryos and whole seedlings during germination of grains at milk, milk-wax and full ripeness. Quantitative changes in the monosome (M), large polysome (LP) and small polysome (SP) fractions are also presented. P — signifies the sum of small and large polysomes

Changes in the amount of the total ribosomal fraction and in its subfractions during germination are presented on Fig. 2. It can be seen that the highest content of ribosomes (T) in mg/g dry weight is in 48 hour seedlings, irregardless of the degree of ripeness of the grains from which they came. It should be noted, however, that a slightly higher content of ribosomes in the tissues of seedlings from unripe grains was found. After two days of germination, 10.60 mg ribosomes/g dry weight was found in seedlings from grains gathered at milk-ripeness, 10.35 mg/g at milk—wax ripeness and 9.37 mg ribosomes/g seedlings at full ripeness.

The total ribosome content (T) per seedling was the highest in seedlings at 6 days germination (Fig. 2). A significant increase in the amount of ribosomes was noted along with an increase in the degree of ripeness of the germinating grains. At 6 days of germination (when the ribosomal content was at its maximum) 25 μg ribosomes/seedling were found in seedlings from grains at milk ripeness, 32 μg/seedling at milk-wax ripeness and 63 μg/seedling at full ripeness.

The maximum polysome content (P) per 1 g of dry weight of seedlings was, as in the case of total ribosome content (T), found to occur after 48 hrs of germination. Only the monosome fraction in germinating embryos in unripe grains was found to be at a maximum after 24 hrs germination (Fig. 2).

Insofar as the content of ribosome subfractions in μg per seedling is concerned, the pattern of polysome content (P) was very similar to that of the total ribosome content (T). The monosome content, however, as in the previous calculations was much more variant, especially in seedlings and seedlings from unripe grains.

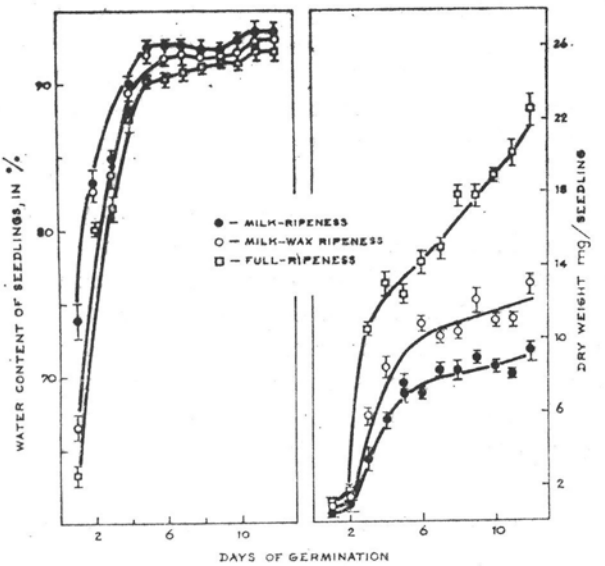

Fig. 3. The water and dry weight content of wheat whole seedlings from grains gathered at milk, milk-wax and full ripeness stages

The dry weight and water content of seedlings from grains of different ripeness were additionally assayed (Fig. 3). An increase in the dry weight of all seedlings was observed during germination up to the 12th day. An exceptionally high rate of increase in dry weight was observed between the 2nd and 6th days. A certain decrease in this rate in seedlings was found after the 6th day which is most evident in both groups of

unripe grains. The water content of seedling in all groups quickly increased to the 4th day of germination and then remained on a similar level (over 90%) until the 12th day (Fig. 3). It was also found that the water content of seedlings from unripe grains (gathered at milk and milk-wax ripeness) was higher each time that in seedlings from fully ripe grains. The greatest differences in the hydration of seedlings were found after the first day of germination.

DISCUSSION

The lowering of the rate of growth of seedlings as well as of the total ribosome content after the 6th day of germination in all of the studied groups is, in the opinion of this author, connected with the depletion of the stored food in the endosperm. A confirmation of this seems to be the changes in the activity of α-amylase in the endosperm of high- and low-protein varieties of wheat (15 and 9% protein in grains). In both varieties the enzyme's maximum activity is found on the 6-7th day of germination (Ching and Rynd 1978).

While studying polyribosome formation during germination of wheat grains, Ching (1972) found that after 1 hour of imbibition, 90% of the total ribosomal fraction was made up of free ribosomes. Similar results were obtained in this study, where 88% monosomes and 12% polysomes were found in dry embryos. Ching (1972) as well as others (Marcus and Feeley 1965, Wareing and Phillips 1970, Weeks and Marcus 1971, Abdu-Baki and Baker 1973, Durre 1975) found that RNAs synthesized during embryogenesis (preformed mRNA, tRNA and ribosomes) are active in protein synthesis during germination and that after only 30 minutes of imbibition, intensive mobilization of ribosomes into polysomes takes place in embryos. Insofar as the preformed mRNA is concerned, however, it has been found that only 25-40% of it is utilized (Brooker et al. 1978, Peumans et al. 1979).

Ching and Rynd (1978) found an increase in the polyribosome content to 50% of the total ribosome fraction after 1 day of germination of wheat seedlings and seedlings of the Yamhill variety. They observed that between the 2nd and 8th days the polysome content stayed level at 80-90% and next that it dropped between the 8th and 11th days. Different results in polyribosome formation in germinating grains were found in this study on the Grana variety (Table 1, Fig. 1). Fourty to fifty percent polysomes were found after only 6 hrs of germination. The highest polysome content (88-90%) was found after 2 days of germination of grains of different ripeness. A gradual decrease in the polysome content of the total ribosomal fraction was observed from the

2nd to the 12th day. Similar results were obtained using different biological material by Wasilewska and Cherry (1974). The polysome content after breaking the dormancy of sugar beet root was 12% of the total ribosomal fraction and rose to 66% after the first 6 hours of development. Similar results were obtained in earlier studies (Weidner and Zalewski 1982c). The polysome content of the total ribosomal fraction gradually increased during germination of rye grains of different ripeness and attained a maximum after 48 hrs (85-87%). After the 2nd day, the polysome content decreased in all of the studied groups.

Woodland (1974) found a positive correlation between the amount of polysomes and the total protein synthesis. It has also been observed that seedlings from high protein varieties of cereals have a higher polysome content than low-protein varieties (Ching and Rynd 1978).

In another report (Weidner and Kulka 1980) it was found that during 48 hrs of germination of wheat grains at milk, wax and full ripeness, the total amount of RNA in the germinating embryos doubled, and that the rate of RNA synthesis was to a great degree correlated with the amount of stored RNA (synthesized during embryogenesis). It was also shown that seedlings from fully ripe grains contained the most RNA. The highest rate of RNA synthesis "de novo" was also observed in these grains.

The slightly greater ribosome content per gram of dry weight in seedlings from unripe grains seems to be integrally tied with their greater water content (Fig. 3). Their higher water content makes them more delicate, which under field conditions may have a very detrimental effect on the plants development.

The results presented in this study as well as in earlier ones show unequivocally that the highest polyribosome content, total RNA and dry weight are found in embryos and seedlings from fully ripe grains. These results prove that the highest biological value is carried by grains gathered at full ripeness.

In conclusion, it should be noted that some authors consider grains gathered during the first half of the third stage of embryogenesis, that is at the wax ripeness stage, to be of the highest biological value (Sójka 1961, Grzesiuk 1961, 1967, 1971, 1972, Grochowski 1975, Górecki and Grzesiuk 1978).

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Formowanie się polisomów podczas kiełkowania ziarniaków pszenicy o różnej dojrzałości

Streszczenie

Z całych siewek wyrosłych z ziarniaków pszenicy zebranych w okresie dojrzałości mlecznej, mleczno-woskowej i pełnej izolowano ogólną frakcję rybosomalną. We wszystkich badanych próbach stwierdzono stopniowy wzrost zawartości omawianej frakcji podczas kiełkowania aż do 6 dnia. W czasie dalszego kiełkowania (od 6 do 12 dnia) obserwowano obniżenie zawartości frakcji rybosomalnej w siewkach. Maksymalną zawartość polisomów w ogólnej frakcji rybosomalnej (88-90%) wykazano po 48 godz. kiełkowania ziarniaków pszenicy o różnej dojrzałości. Od 3 do 12 dnia kiełkowania następował stopniowy spadek procentowego udziału polisomów w ogólnej frakcji rybosomalnej. Zarówno na początku (0-24 godz.), jak i pod koniec (10-12 dzień) badanego okresu kiełkowania, procentowy udział polisomów w ogólnej frakcji rybosomalnej w siewkach wyrosłych z ziarniaków niedojrzałych był niższy niż w dojrzałych, co jest jednoznaczne z niższą syntezą białek w ich tkankach. Również w przeliczeniu na 1 siewkę zawartość ogólnej frakcji rybosomalnej była tym niższa im mniej dojrzałe ziarno poddawano kiełkowaniu. Uzyskane wyniki świadczą o najwyższym wigorze ziarniaków zebranych w dojrzałości pełnej.