

Ribonucleic acids in the embryos of germinating wheat grains of different ripeness

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(Received: April 18, 1980)

Abstract

During 48 h germination of wheat grains of different ripeness the amount of RNA in the germinating embryos doubles, while the rate of synthesis is to a large extent correlated with the level of ribonucleic acids accumulated during the development and ripening of the grain. The highest RNA content was noted in the germs developing from grain harvested at the stage of full ripeness, the lowest — in germs from grain at milk ripeness. Intensity of ^3H -uridine incorporation into the RNA fraction of the germs during 24-h germination also depends on ripeness. Significantly lower RNA synthesis was noted in germinating embryos from wheat grain harvested during milk ripeness as compared to wax or full ripeness.

INTRODUCTION

Process of the development and ripening of wheat grain is divided into three visibly different morphological and physiological stages (Rejowski, 1961; Pawłow, 1967): stage of the endosperm and pragerm formation (stage of green ripeness), stage of the embryo formation and accumulation of nutritive substances (stage of milk ripeness), and stage of grain maturation during which water is lost and the grain passes into the resting stage (stage of wax ripeness and of full morphologic ripeness).

Particular stages of the grain morphogenesis are accompanied by specific biochemical and physiological changes concerning the metabolism of proteins, nucleic acids, carbohydrates, growth regulators and other substances. Hence, physiological state of the grain changes; these changes being also expressed by varying germination ability.

Fresh wheat grains collected during the phase of milk ripeness are usually characterized by minimal germination ability (Grzesiuk,

1967). This grain germinates much better if it is carefully dried in the room temperature to an air-dry state. On the other hand, if unripened wheat grains are left in the ears, which afterwards are carefully dried, they are characterized by full germination ability even if the ears were harvested at the early stage of wax ripeness (Grzesiuk, 1967). Storage of unripe grain inside the ears in the room temperature allows for further development (Pawłow, 1967).

Unripe grains are found in each grain lot. Different ripeness of sowing grain affects also the growth of plants and the yield. The youngest grain (milk ripeness) as a rule gives origin to lower plants, characterized by low fertility (Rejowski, 1961; Grzesiuk, 1967).

The aim of this work was to follow the synthesis and quantitative changes of RNA in the germinating wheat grains of different ripeness. The grain was stored either in the ears, or thrashed. Since there are no such data in the literature, the present studies may explain the relationship between the growth and development of germinating embryos and accumulation of various RNA.

MATERIAL AND METHODS

Studies were carried out in 1978 on winter wheat ("Grana") grains collected in 1978. Wheat was grown upon experimental fields of the Institute of Plant Biology of the Academy of Agriculture and Technology in Olsztyn. Material was collected three times:

1. during milk ripeness — 20 days after blooming,
2. during wax ripeness — 42 days after blooming,
3. during full morphological ripeness — 56 days after blooming.

Ears were divided into two parts: from one part grain was taken out directly after harvest and dried carefully in the room temperature. These grains are denoted as (A) series. The other part of ears was left for two weeks in the room temperature and grain was removed only after this period. These grains are denoted as (B) series. This way six grain samples were obtained. After about 3 months after the last grain sample had been collected the following analyses were performed: germination ability of grains and isolated embryos, increments of coleoptile and embryonic roots, fresh and dry mass of developing embryos, changes of the content of total RNA and its fractions, RNA synthesis, and activity of ribonucleases. Determination of fresh and dry mass, ribonucleic acid content, and activity of ribonucleases in the embryos was made 8 h after the grain preimbibition (initial state) and in 48-h embryos.

ISOLATION AND FRACTIONING OF THE RNA

Grains were sterilized in 2% solution of sodium hypochlorite for 2 min. (Rejman, Buchowicz, 1971). Grains germinated in the solution of (5-³H) uridine (0.01 mCi/ml; 29 Ci/mM) during 24 h in 22°C in a thermostat, upon blotting paper placed in the Petri dishes. Germs were isolated in the ice, washed with non-metabolised precursor and stored in -25°C.

About 300 embryos or germs were homogenized in 15 ml of the buffer "A" (Tanifuji et al., 1970): 0.02 M Tris-HCl (pH 7.4) containing 0.1 M NaCl, 1% bentonit, 2% SDS and 100 µg/l of polyvinyl sulphate. To the homogenate an equal volume of the solution m-cresol-phenol-water (10:70:20 v/v/v) and 8 hydroxyquinoline was added to obtain final concentration of 0.1%. The suspension was shaken, centrifuged (5000×g), and water layer was taken out. The interphase and the sediment were extracted with the mixture of the buffer "A" and chloroform (1:1, v/v) in 65°C for 3 min. (Wasilewska, Kleczkowski, 1974). After rapid cooling samples were centrifuged (5000×g). All water layers (obtained after hot and cold extraction) were deproteinated with a mixture of phenol and chloroform (1:1, v/v), and a mixture of chloroform and isoamylalcohol (20:1, v/v). RNA was precipitated with 2.5 volumes of ethanol, with sodium acetate added to obtain a concentration of 0.2 M. The whole process of extraction and deproteination (with the exception of hot extraction) was carried out in the temperature of 0-4°C. Purified preparation of RNA was dissolved in the buffer "B": 0.025 M Tris-HCl (pH 7.4) containing 5 mM NaCl and 5 mM EDTA. RNA solution was then centrifuged in 5-20% solution of saccharose in buffer "B". Linear gradient of saccharose concentration was prepared automatically (Gradient Former model 570, made by ISCO-USA). In order to carry out sedimentation about 1 mg of RNA (in 1 ml) was spread over the gradient surface. The samples were centrifuged with SW-41 solution for 5 h in 4°C at 40000 rot./min. (196000×g) in an ultracentrifuge Spinco L-3-40. The content of the test tubes (13 ml) was then divided into about 40 fractions, and water was added up to 3 ml. Extinction was measured at 260 nm. Radioactivity was measured in a scintillation counter (Intertechnique-Sd-49) adding 10 ml of tritosol (as a scintillator) into 1 ml of the sample (tritosol efficiency for ³H — 47%) (Fricke, 1973). Per cent of RNA in the solution was calculated from extinction measurements, using a coefficient defined experimentally for non-degraded RNA (highly polymerized RNA of yeast):

$$E \frac{1 \text{ cm, } 1 \text{ mg/lml}}{260 \text{ nm}} = 22$$

Isolation and germination of wheat embryos was made according to the method by Johnston and Stern (1957), at partly changed con-

ditions of embryo isolation. Dry grains were mixed with carbon dioxide powder. After some minutes the mixture was ground in a homogenizer (for 20 sec.). Ground grains were fractioned by sieving.

After repeated flotation isolated embryos were washed with cold distilled water and dried upon blotting paper in the room temperature. The embryos germinated in the Petri dishes containing 30 ml of 0.9 % agar, with an addition of 1 % glucose. Medium surface was sprayed with 0.02 % solution of streptomycyne before about 50 embryos were sown. Germination took place in 22°C for 48 h. Average length of embryonic roots after 12, 24, 36 and 48 h was measured under a binocular NfPK.

MEASUREMENTS OF RIBONUCLEASE ACTIVITY

Wheat embryos or germs were homogenized in a 0.005 M phosphate buffer (pH 7). Homogenates were centrifuged for 10 min. at 2000×g. In the supernatant activity of the ribonucleases was measured with a slightly modified method of Tuve and Anfinson (1960). To 2 ml of 0.2 % RNA solution (from yeast) in 0.1 M of acetate buffer (at pH 5.6), 0.5 ml of the solution containing the enzyme was added. The samples were incubated for 1 h in 37°C. Reaction was interrupted by an addition of 0.5 ml of 0.75 % solution of uranyl acetate in 25 % perchloric acid. The samples were left in the refrigerator for 15 min., and proteins and undecomposed RNA were centrifuged at 6000×g. To 0.2 ml samples water was added to obtain 3 ml. Extinction was measured at 260 nm in relation to the control sample containing the enzyme. The latter sample was incubated without RNA, and then RNA solution was added together with uranyl acetate. A unit of ribonucleic activity was defined as an amount of the enzyme causing an increase of the extinction of $E_{1\text{ cm}}^{260\text{ nm}} = 0.001$. In order to define the optimal pH for ribonuclease activity incubation was performed with the buffer used by Siwecka and Szarkowski (1971): 0.1 M citrate-phosphate buffer with pH range of 3.7-7.0, and 0.2 M buffer Tris-maleic with pH of 7.0-8.0.

RESULTS AND DISCUSSION

COMPARISON OF THE GERMINATION OF GRAIN OF DIFFERENT RIPENESS

Air-dry grain was germinated. Several studies have shown that drying of unripe grain liquidates the resting stage and increases the germination ability (Crocher, Barton, 1953; Grzesiuk, 1967; King, 1976). This is connected with an improvement of trophic conditions and aeration of the embryo, as well as with an inactivation of growth inhibitors.

General characteristic of the germination of wheat grain of different ripeness is presented in Fig. 1 and 2.

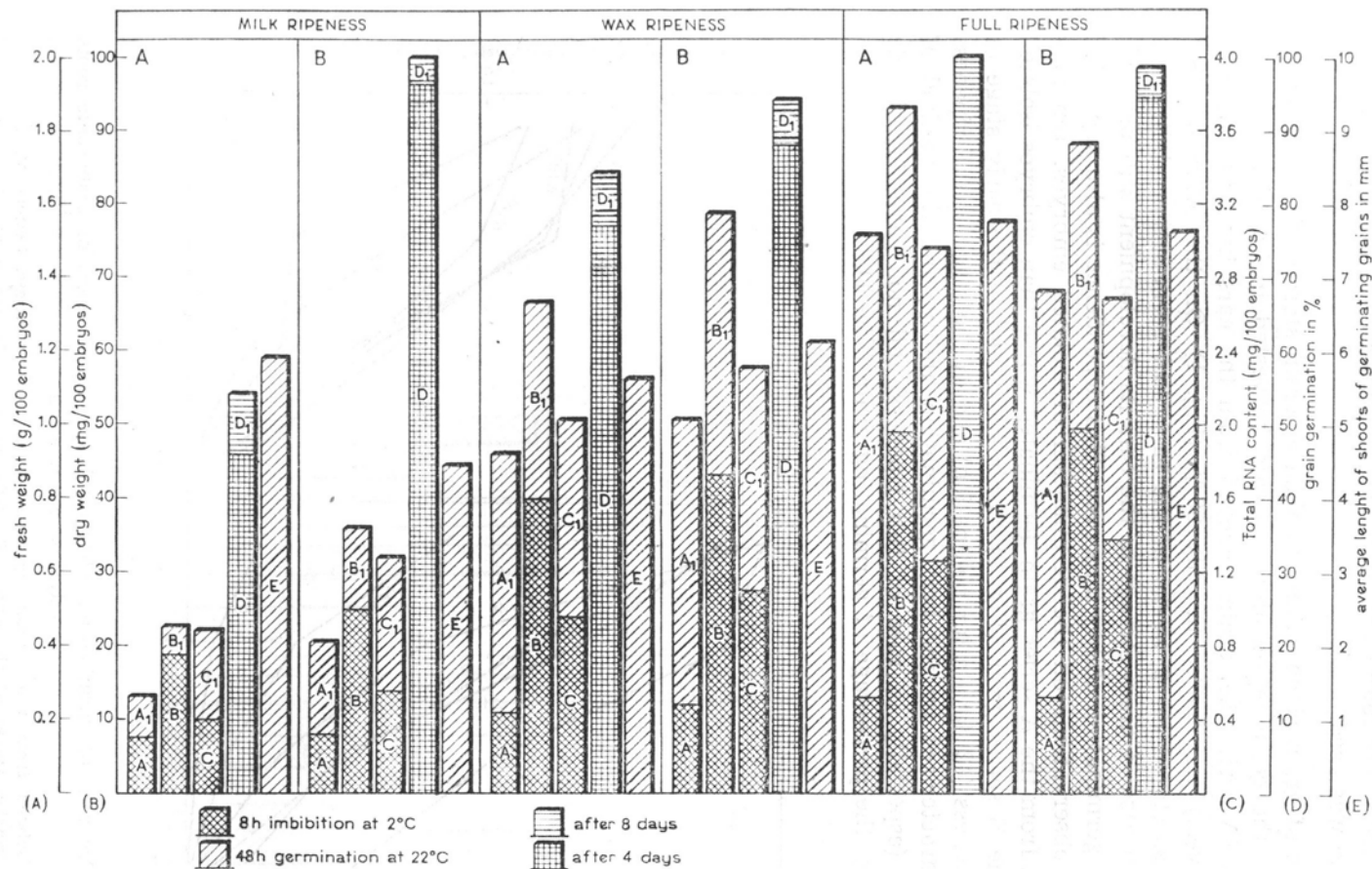


Fig. 1. General characteristic of germinating wheat grain harvested at different stage of ripeness (Germination ability, germination intensity, average length of 48-h coleoptile, fresh and dry mass, and total RNA content in the imbibed embryos and 48-h germs). Letter "A" denotes grain removed from the ears immediately after harvest; letter "B" denotes grain left in the ears (in the room temperature) for 2 weeks after harvest

Maximal activity of ribonucleases in the imbibed embryos and 48-h wheat germs was found for all stages of ripeness when the buffer with pH of 5.6 was used.

Stage of grain ripeness and method of grain storage significantly influenced the germination ability. Grain harvested in the stage of milk ripeness (A series, i.e. grain removed from the ears immediately after the harvest) germinated in only 50 % (Fig. 1). On the other hand, if grain was stored for 2 weeks in the ears, full germination ability was noted. In case of grain storage in the ears its development still proceeds and the germination ability improves. This dependence is also supported by the observations on the germination of isolated embryos. Embryos isolated from the A series grain germinated worse than embryos isolated from the B series grain (Fig. 2). Grain harvested in the early stage of wax ripeness (42 days after pollination) and in the stage of full ripeness was characterized by the best germination ability, and the method of storage (especially in case of full ripeness) did not have any visible effect on the percentage of germinating grain (Fig. 1).

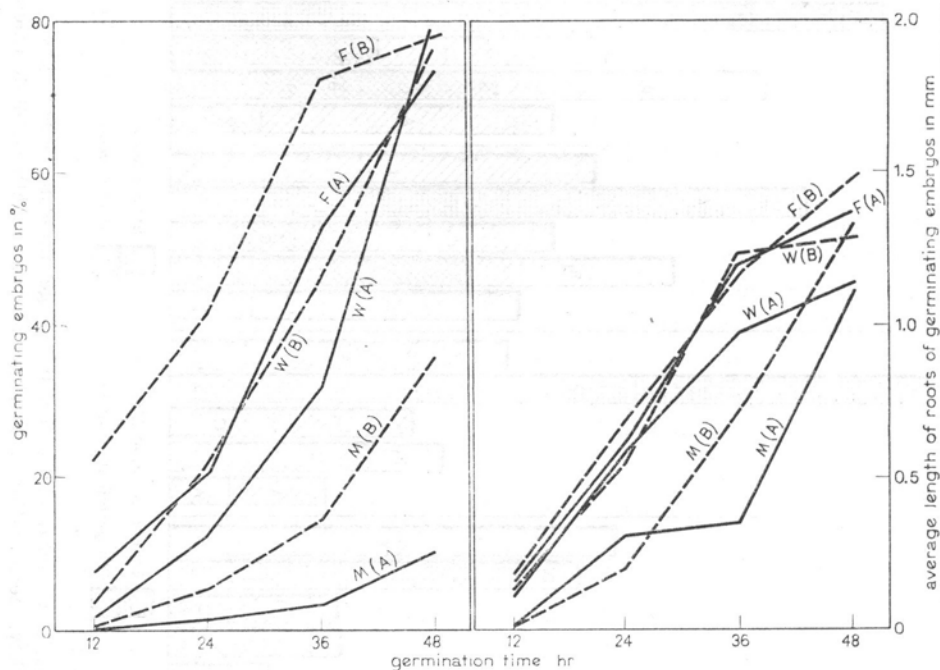


Fig. 2. Per cent of germinating wheat embryos and changes of embryonic roots during germination

Embryos isolated from wheat grain harvested at different ripeness. Letters M, W, and F denote successive stages of ripeness: milk, wax, and full. Letters A and B — see Fig. 1

Fresh and dry mass of growing germs also depends on the stage of grain ripeness and the method of storage. As seen in Fig. 1, the more ripe the grain the higher the fresh and dry mass of the germs, and the average length of coleoptile. Furthermore, the rate of dry and fresh mass growth in the germs depended also on the method of storage, but only in unripe grain (milk and wax ripeness). Growth of the germ mass in the initial stage of germination was more rapid if grain was stored in the ears (Fig. 1). Similarly, elongation growth of germinating roots was more rapid in the embryos isolated from the B series grain (Fig. 2).

RIBONUCLEIC ACIDS

Content of total RNA and of each RNA fraction in the embryos gradually increased, reaching a maximum in the stage of full ripeness (Fig. 1 and 3). This observation is in concordance with the results of other authors (Duffus, Rosie, 1975; Durre, 1975; Kulka et al., 1977).

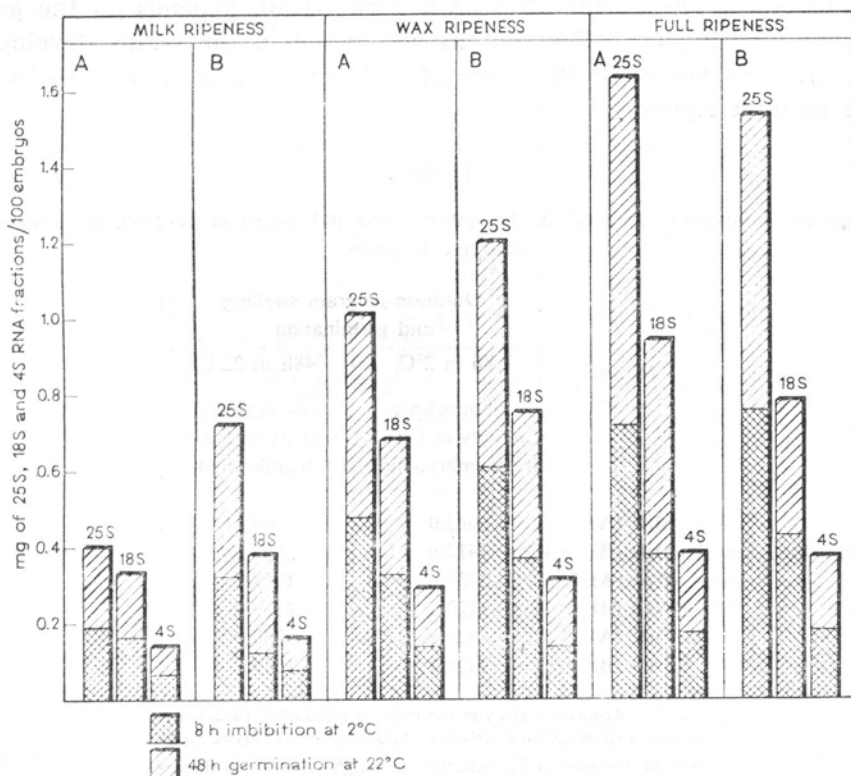


Fig. 3. Content of 25S, 18S, and 4S RNA fractions in the imbibed embryos and in 48-h germs of wheat grain harvested at different ripeness. Total RNA isolated with a phenol-detergent method. Purified preparations fractionated by ultracentrifugation in 5-20% gradient of saccharose concentration.

RNA preparations extracted from the embryos (and germs) of swelling (8 h, 2°C) and germinating (22°C) grain of different ripeness were separated by ultracentrifugation in the saccharose concentration gradient into three fractions differing as regards the sedimentation coefficients: 25S, 18S and 4S RNA (Fig. 3 and 4).

In the second stage of grain development, lasting from 20 to 42nd day after pollination (milk and early wax ripeness), the three RNA fractions accumulated most intensively (Fig. 3). In the third stage of grain development (wax ripeness, 42-56 days) only 25S RNA fraction increased. Storage of unripe grain in the ears also resulted in visible increase of 25S RNA in the embryos (Fig. 3).

During 48 h germination of wheat grain of different ripeness, total content of RNA (Fig. 2) and its fractions (Fig. 3) in the germs doubles. Rate of biosynthesis of particular RNA in the growing (48 h) germs is to a large extent correlated with the amount of ribonucleic acids accumulated previously.

Data presented in Fig. 1 and 3 show that the amount of RNA and its fractions in the germs after 48-h germination depends on the grain ripeness. The highest RNA content was noted in the germs developing from grain harvested in the stage of full ripeness, the lowest — in the stage of milk ripeness.

Table 1

Changes of ribonucleases activity* in the embryos and 48-h wheat germs from grain harvested at different ripeness

Wheat ripeness	Duration of grain swelling and germination	
	8h in 2°C	48h in 22°C
	ribonuclease activity in 1 g of fresh embryo mass	ribonuclease activity in 1 g of fresh embryo mass
Milk (A)	3662.0	3309.2
Milk (B)	1412.9	2694.1
Wax (A)	1723.8	1659.7
Wax (B)	1123.2	1339.6
Full (A)	828.7	730.6
Full (B)	1429.1	1049.7

* — Results are given in the units of ribonucleolytic activity. A unit of ribonucleolytic activity is the amount of the enzyme necessary for the increase of extinction by $E_{260\text{ nm}}^{1\text{ cm}} = 0.001$ in the experimental conditions.

Relatively low content of RNA and its fractions in the germs developing from grain in the stage of milk ripeness (Fig. 1, 3) is probably

caused by only partial formation of the embryo organs, as also by low activity of RNA polymerases. Low rate of RNA accumulation in the germs developing from unripe grain may be also caused by high ribonuclease activity in the tissues (Table 1). In unripe barley grains (early milk ripeness) ribonuclease connected with the ribosomes was found (Luthe, Peterson, 1975).

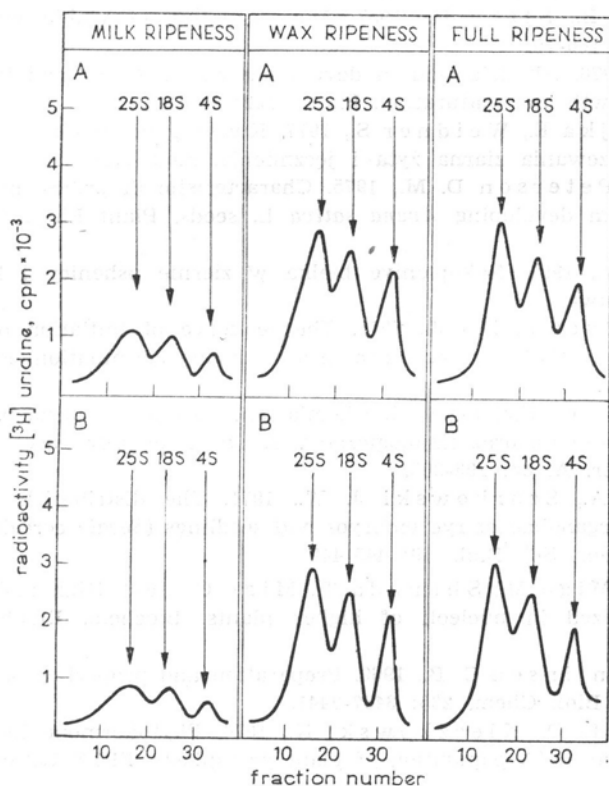


Fig. 4. Sedimentation profiles of radioactive RNA isolated from 24-h germs (developing from grain harvested at different ripeness) 5-20% gradient of saccharose concentration was used. Each time 1 mg of RNA was fractioned

Experiments on the incorporation of uridine into the three RNA fractions showed that the increase of ribonucleic acids content in the developing embryo (and germ) results mainly from the *de novo* synthesis (Fig. 4). It should be, however, added that the incorporation of ^3H -uridine into RNA fraction of 24-h germs developing from grain in the stage of milk ripeness was less intensive than in case of germs developing from grain in the stage of wax and full ripeness.

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Kwasy rybonukleinowe w zarodkach kielkującego ziarna pszenicy o różnej dojrzałości

Streszczenie

Ilość RNA każdej frakcji (25, 18 i 4S) w zarodkach rozwijającego się ziarna systematycznie wzrasta osiągając maksimum w dojrzałości pełnej. Najintensywniejsze gromadzenie wszystkich badanych frakcji RNA zaobserwowano w zarodkach między 20 a 42 dniem po zapyleniu (dojrzałość mleczna i początek dojrzałości woskowej). Podczas 48-godzinnego kiełkowania ziarna pszenicy różnej dojrzałości ilość RNA w kielkujących zarodkach w zasadzie podwaja się a tempo syntezy RNA

w dużej mierze skorelowane jest z ilością nagromadzonych uprzednio kwasów rybonukleinowych w okresie ich rozwoju i dojrzewania. Jedną z przyczyn słabego gromadzenia się RNA podczas kiełkowania w dojrzałości mleczonej może być stwierdzona wysoka aktywność rybonukleaz. Zawartość każdej frakcji RNA w 48-godzinnych kiełkach zależy od fazy rozwoju ziarna. Najwięcej RNA zawierają kiełki wyrosłe z ziarniaków zebranych w dojrzałości pełnej, zaś najmniej kiełki z ziarniaków o dojrzałości mleczonej. Ilościowy wzrost kwasów rybonukleinowych w kiełkujących zarodkach był w znacznej mierze wynikiem syntezy *de novo*, a intensywność włączania ^3H -urydyny do frakcji RNA podczas 24-godzinnego kiełkowania była znacznie niższa w dojrzałości mleczonej niż w dojrzałości woskowej i pełnej.