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Ribonucleic acids of wheat grain and its endosperm during development and ripening

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Abstract

The work present quantitative changes and synthesis of RNA fractions in wheat grain and endosperm during development and ripening. It was found that share of rRNA in total RNA decreases during development both, in the endosperm and in grain. Rapid drop of rRNA content in total RNA takes place in the endosperm since 31st day after blooming, and in grain — since 45th day after blooming. Absolute amount of rRNA, as calculated per 100 grains or endosperms, increases in the first half of grain formation period, and then decreases till the end of development. As a result of degradation of ribosomal RNA in endosperm, low-molecule fraction — 4 S, significantly increased at final stage of development. Decrease of rRNA fraction at final stage of development is not accompanied by an increase of absolute amount of 4 S RNA fraction. Incorporation of ^{3}H-uridine into grain and endosperm RNA is highest at the begining of development. Further on it gradually decreases till the end of development. Synthesis of RNA in grain takes place throughout the whole development. Contrary to this, no incorporation of precursor into rRNA was noted during waxy and full ripeness of wheat grain.

INTRODUCTION

Development process of grain may be divided into three stages (Grzesiuk, 1961, 1972; Sójka, 1961a): stage of endosperm and pre-embryo formation (stage of green ripeness), stage of proper embryo formation and deposition of storage material (stage of milky ripeness), and stage of grain ripening, during which water is lost and grain passes into resting condition (stage of waxy and full physiological ripeness).

Each stage of grain morphogenesis is characterized by specific biochemical and physiological changes. These changes are connected with metabolism of proteins, carbohydrates, nucleic acids, growth regulators,

and other substances. Hence, physiological state of developing grain undergoes changes which are expressed, among others, by varying germination ability. Grain is characterized by the highest biological value in the first half of the second stage of embryogenesis, i.e. et the begining of waxy ripeness. Biological properties of ripening grain affect growth, development, and yield of plants grown from this grain (Grzesiuk, 1961; Johari et al., 1977; Rejowski, 1961a and b; Sójka, 1961b).

Amount of RNA in ripening grain increases rapidly, most frequently reaching a maximum at the begining of waxy ripeness (Kulka, 1966; Grzesiuk, 1972; Johari et al., 1977; Kulka et al., 1977). It should be underlined that high-protein grains, such as wheat, synthetized high amounts of rRNA (calculated per one grain) compared to low-protein ones (Donovan, 1977). Also in endosperm of developing maize cernels, rate of rRNA synthesis is by 30% higher than that in a mutant "opaque — 2" (Jones et al., 1977). It was shown in studies on separated endosperm and embryos, that RNA synthesis in embryos takes place almost till the end of grain ripening (Chang Chong, 1963; Duffus and Rosie, 1975; Durre, 1975; Kulka et al., 1977). With respect to endosperm it is suggested that process of RNA synthesis occurs simultaneously to cell divisions, and usually ends in the stage of milky ripeness (Jennings and Morton, 1963; Donovan, 1977; Jones et al., 1977).

It should be added that studies on ribonucleic acids in developing grains are not too advanced, and usually deal only with general dynamics of changes in RNA content.

The aim of the present work was to make observations on synthesis and quantitative changes of RNA fractions during development and ripening of grain and endosperm of winter wheat. Such studies may throw light upon succession of synthesis and degradation of various forms of RNA during development and ripening of grain, possibly having significant effect upon differences in vigor of grain in various stages of development.

MATERIAL AND METHODS

Studies were carried out in 1977 on winter wheat grain, 'Grana' variety, grown upon experimental plots of the Institute of Plant Biology of the Academy of Agriculture and Technology in Olsztyn. First part of heads was collected on 10th day after blooming (stage of green ripeness), and next ones on 17th, 24th, and 38th day (stage of milky ripeness), and 45th (stage of waxy ripeness) and 55th day (stage of full ripeness).

Grains were divided into two parts immediately after each collection. From one part embryos were removed (in case of grain collected in first stages of development — with a razor blade, in later stages — with preparation needle), so that only endosperm was analysed. In case of second part of seeds whole grains were analysed. This biological material was used for RNA studies.

Wheat grain and endosperm (about 2-4 g) used for isotope studies were isolated in a sterile box, placed in cold-room, counted, weighed, transferred to small crystallizers, and submerged in radioactive solution of RNA precursor. Incubation with 5^3H-uridine of the activity of 0.005 mCi/1 ml (specific activity 29 Ci/mM) was carried out in a thermostat in 25°C for 6 h. The samples were mixed from time to time in order to assure uniform uptake of the precursor by grain and endosperm. After incubation, grains were washed (3-4 times) with re-distilled water and solution of untraced uridine, dried over a blotting paper, and placed in closed weighing bottles in —20°C. Next day RNA was isolated from the samples. All handling and procedures were made in sterile conditions.

Extraction of RNA was carried out with the method of T a n i f u j i et al. (1970). Samples of endosperm or grain were homogenized for 10 min. in a porcelain mortar (in ice) with 0.02 M Tris-HCl buffer (pH 7.4) containing 0.1 M NaCl, 1% bentonite, 2% SDS, and 100 μg/ml of polyvinyl sulphate (buffer "A"). To the homogenous sample thus obtained, equal volume of a mixture of m-cresol-phenol-water (10 : 70 : 20; v/v/v) and 8-hydroxychinoline were added, to obtain final concentration of 0.1%. Suspention was shaken for 10 min in an "Universal Shaker" type 327, and centrifuged at 5000 g for 10 min. Then water was decanted and the rest (middle phenolic layer and precipitate) extracted again with a mixture of buffer "A" and chloroform (1 : 1; v/v) in 65°C for 3 min. (W a s i l e w s k a and K l e c z k o w s k i, 1974). Samples were then rapidly cooled and centrifuged. Both water layers (obtained after hot and cold extraction) were deproteinated with a mixture of phenol and chloroform (20 : 1; v/v). Sodium acetate was added to the solution of purified ribonucleic acids, to obtain concentration of 0.2 M; RNA was precipitated with 2.5 volumes of 96% ethanol. The whole process of RNA extraction and purification (with the exception of hot extraction) was performed in a cool-room, in the temperature of 0-4°C.

Purified RNA preparations (ratio $\frac{E_{280}}{E_{260}} < 0.5$) were dissolved in 2-4 ml of 0.002 M Tris-HCl buffer (pH 7.4), containing 0.05 M NaCl of 0.005 EDTA. Next the RNA solution was centrifuged in 5-20% gradient of saccharose concentration. Linear gradient of saccharose density was prepared with the same Tris-HCl buffer, using Gradient Former model 570, produced by ISCO (USA). In order to obtain sediment, 0,5 mg RNA (in

1 ml of the buffer) was carefully transferred over the surface of saccharose gradient. Samples were centrifuged in 4°C at 40 000 rot/min (196 000 g) in Beckman (model L-3-40) ultracentrifuge, with rotor SW-41. Sample content (13 ml) was divided into about 40 fractions, using glass capillary siphon. Water was added to obtain the volume of 3 ml, and extinction (at 260 nm) and radioactivity were measured. Radioactivity was measured with scintillation counter (Intertechnique-Sd-49), adding 10 ml of tritosol (as scintillator), with the efficiency for ^3H — 47% (F r i c k e, 1973), to 1 ml sample.

Percentage concentration of RNA in solution was calculated from extinction measurements, using a coefficient obtained experimentally for non-degradated RNA:

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

Highly-polymerized RNA from yeast was used in order to obtain this coefficient.

Dry mass was determined in wheat grain and endosperm according to the method by D o r y w a l s k i et al. (1964).

RESULTS AND DISCUSSION

1. Changes of total RNA content in whole wheat grain and in endosperm

As shown in Table 1, RNA content both, in grain and in endosperm (calculated per 100 grains), increases rapidly until 24th day after blooming. Further on, however, it decreases, the process being especially visible in case of endosperm.

Results shown in Table 1 suggest that most of the RNA content is synthetized in grains at the begining of development, and accumulates

Table 1

Total RNA content in wheat grain and endosperm during development and ripening

Days after blooming	Grain			Endosperm		
	RNA content in 100 grains (in mg)	RNA content in 1g of wet mass (in mg)	RNA content in 1 g of dry mass (in mg)	RNA content in 100 endosperms (in mg)	RNA content in 1g of wet mass (in mg)	RNA content in 1g of dry mass (in mg)
10	3.082	1.825	7.154	2.274	1.550	5.797
17	6.704	1.625	5.582	5.621	1.415	4.703
24	7.227	1.373	3.797	6.031	1.240	3.227
31	7.046	1.163	2.469	5.221	0.993	2.038
38	6.346	0.997	1.922	4.999	0.893	1.699
45	5.345	0.803	1.424	3.754	0.590	1.019
55	3.415	0.728	0.896	1.957	0.455	0.558

in the endosperm tissue. According to some authors, intensity of RNA biosynthesis in developing endosperm is roughly proportional to the intensity of cell divisions (Jennings and Morton, 1963; Durre, 1975).

In final stages of grain development intensive degradation of ribonucleic acids takes place in the endosperm (Table 1, Fig. 1). At this time endosperm ribonucleases are characterized by high activity (Ingle et al., 1965; Donovan, 1977; Johari et al., 1977). Hence, it may be assumed that in endosperm cells decomposition of RNA into oligoribonucleotides and ribonucleotides takes place under the effect of RN-ases. It is probable that certain amount of RNA degradation products passes to the embryo, where they become re-synthetized into RNA. Similar conclusion was drawn by Siemienienko (1963) in his studies on RNA synthesis in developing wheat grain. This author stated that synthesis of ribonucleic acids in embryos can take place also at the expense of nucleotides from the dying endosperm tissue.

Basing on these data it may be assumed that aging of endosperm is accompanied by degradation of ribosomes. In fact, starchy part of the endosperm of ripe cereal grains does not contain ribosomes (Abdu-Baki and Baker, 1973).

2. Quantitative changes and biosynthesis of RNA fraction

Studies on quantitative changes in particular RNA fractions in developing grains are quite recent. Johari et al. (1977) have shown that percentage share of rRNA in total RNA of sorghum does not change during grain development.

Preparations of RNA isolated from wheat grains were separated during ultracentrifugation in a gradient of saccharose concentration. Three fractions were obtained, differing with respect to sedimentation coefficients: 25, 18, and 4 S RNA. Relative content of rRNA (18 plus 25 S) in total RNA (expressed in RNA%) decreased systematically along with the development and ripening of grain and endosperm (Fig. 1). Untill 31st day after blooming in case of endosperm, and 45th day in case of grain, percentage share of rRNA gradually decreases. Further decrease of rRNA share is very rapid, and takes place in grain two weeks later than in endosperm. Hence, the results of the present work contradict those by Johari et al. (1977).

rRNA content in developing grain and endosperm, calculated per 100 grains, changes a little differently. In the first part of grain formation period (untill 24th day after blooming) amount of both rRNA fractions (18 and 25 S) increased, and then decreased untill the stage of full ripeness was reached (Fig. 1). As a result of ribosomal RNA

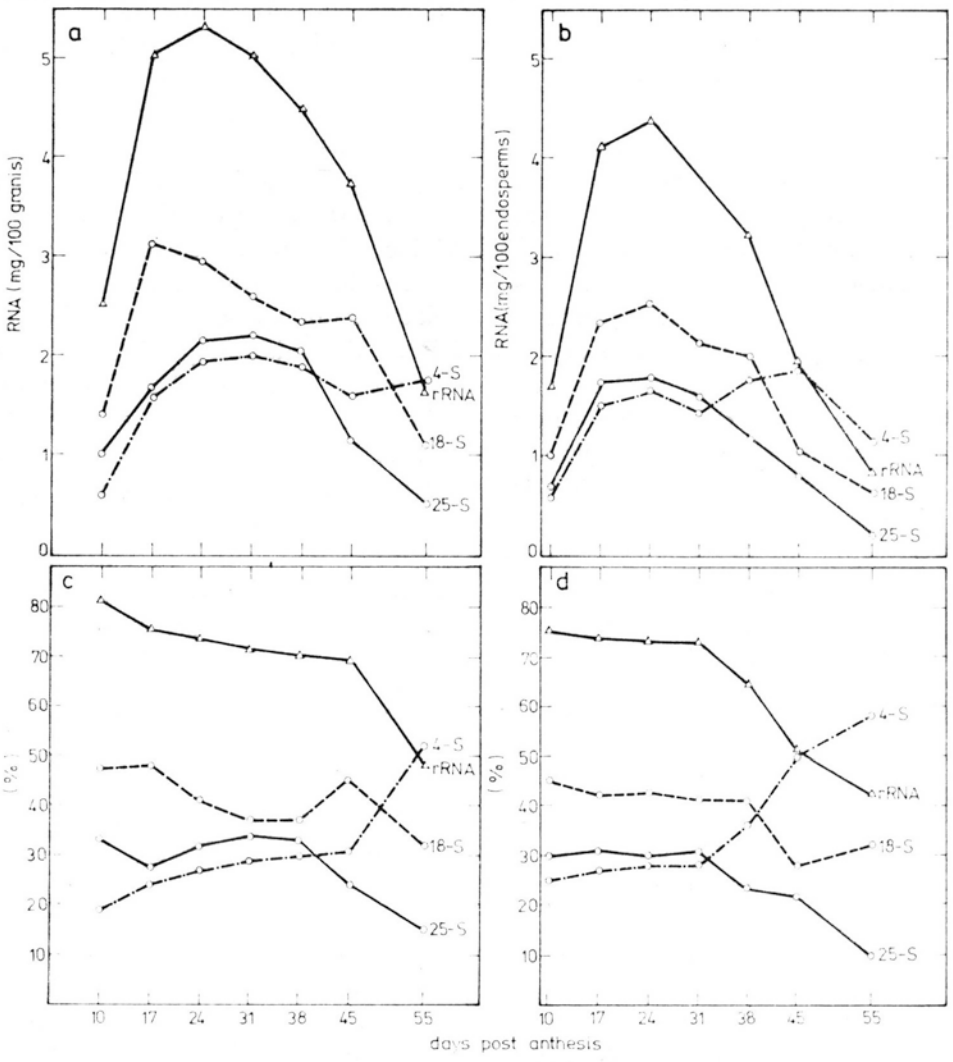

Fig. 1. Changes of total RNA fraction during wheat development and ripening
Remarks: a — grain, c — endosperm — collected 10 days after blooming; b — grain, d — endosperm — collected 55 days after blooming.

degradation (taking place in endosperm), which was most intensive in final stages of wheat grain development, RNA sedimentation profile was predominated by low-molecule 4 S RNA (Fig. 2). It should be added that decrease of both rRNA fractions (per 100 grains or endosperms) was not accompanied by an increase of absolute 4 S RNA content. This fact points to degradation of ribosomal RNA into ribonucleotides or oligoribonucleotides.

On the basis of the above data it may be assumed that during dehydratation process in wheat grain (accompanied by aging of the endo-

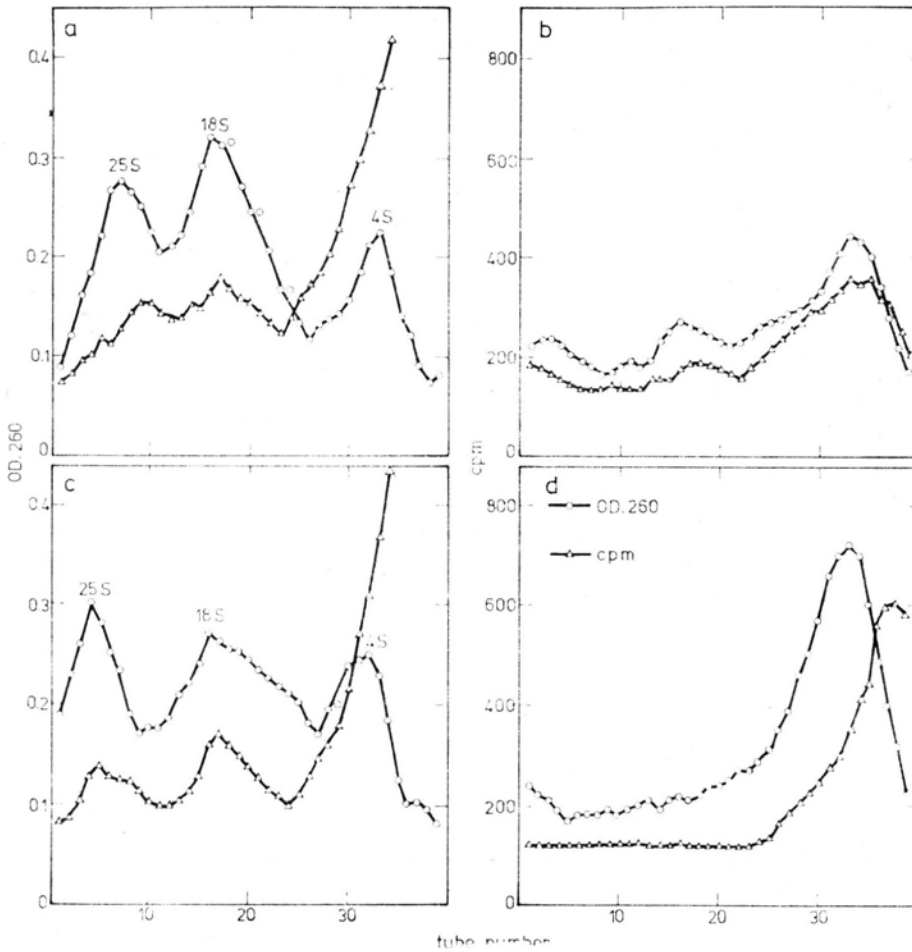

Fig. 2. Profiles of total RNA fraction isolated from wheat grain and endosperm, separated by ultracentrifugation in 5-20% saccharose gradient, and incorporation of radioactive RNA precursor (6 h incubation with ^{3}H-uridine) during wheat development and ripening. See Fig. 1.

The values of cpm from tube 25 and higher should not be taken into account because of contamination with nonmetabolisable radioactive precursor.

sperm) degradation of some ribosomes took place. In fact, starchy part of endosperm of ripe cereal grains does not contain ribonucleoproteid particles, which would resemble ribosomes (Abdu-Baki and Baker, 1973). It should be added that cells of embryo and aleuronic layer of endosperm retain fully active ribosomes of unchanged structure (Abdu-Baki and Baker, 1973; Durre, 1975; Gumilevskaya, 1975).

Results of ^{3}H-uridine incorporation are partly presented in Fig. 2. The highest incorporation of radioactive RNA precursor was observed in rRNA preparations (18 and 25 S) at the begining of formation process,

i.e. during intensive accumulation of rRNA in grain. Along with grain development incorporation of ^{3}H-uridine into rRNA fraction decreased, the lowest values being observed in the stage of full ripeness. Fig. 2 presents profiles of separation and synthesis of RNA only in the most differentiated, extreme moments of development and ripening. Analysis of the results points to the fact that synthesis of RNA in grain takes place till the end of development process. On the other hand, incorporation of radioactive precursor into endosperm did not take place already in the stage of waxy ripeness (and thus also in the stage of full ripeness). It may be stated that incorporation of labelled precursor into ribosomal RNA in the second half of grain development process was connected mainly with the synthesis of these compunds in the embryos. This statement supports the results of Chang Chong (1963) who showed (by autoradiography) that incorporation of ^{32}P into RNA of barley embryos takes place almost till the end of grain ripening. Our studies with ^{3}H uridine incorporation in wheat grain in the first stages of development were almost similar to the results obtained by Johari et al. (1977) for developing sorghum graines.

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Kwasy rybonukleinowe ziarna i bielma pszenicy podczas rozwoju i dojrzewania

Streszczenie

Podczas całego okresu rozwoju i dojrzewania ziarna pszenicy, który trwał 55 dni, pobrano 7 prób. Ziarno i bielmo, bezpośrednio po wyizolowaniu z kłosów, dokarmiano znakowanym prekursorem RNA (^{3}H-urydyną) i ekstrahowano całkowity RNA. Oczyszczony preparat RNA ziarna lub bielma pszenicy frakcjonowano drogą ultrawirowania w gradiencie sacharozowym. Pomiary radioaktywności wykonano w liczniku scyntylacyjnym. Ilość ogólnego RNA (w przeliczeniu na 100 szt.) wzrastała do 24 dnia po kwitnieniu, zarówno w ziarnie jak i w bielmie. W miarę dalszego rozwoju ilość ogólnego RNA spadała, co najwyraźniej zaznaczało się w bielmie. Względna zawartość rRNA w ogólnym RNA (wyrażona w % RNA) malała przez cały okres rozwoju i dojrzewania zarówno w ziarnie, jak i w biel-

mie. Absolutna ilość rRNA, po przeliczeniu na 100 ziaren lub bielm, wzrastała w pierwszej połowie okresu formowania się ziarna, po czym zmniejszała się aż do fazy dojrzałości pełnej. Zmniejszaniu się zawartości obu frakcji rRNA (na 100 ziaren lub bielm) pod koniec dojrzewania nie towarzyszył wzrost absolutnej ilości frakcji 4 S RNA. Świadczy to przypuszczalnie o rozkładzie rybosomalnych RNA do rybonukleotydów bądź oligorybonukleotydów. Inkorporacja ^{3}H urydyny do RNA bielma i ziarna była najwyższa na początku rozwoju i stopniowo malała. Synteza RNA przebiegała w ziarnie do końca dojrzewania, natomiast w bielmie nie zaobserwowano wcielania prekursora do rRNA w okresach dojrzałości woskowej i pełnej.