Nucleic acids in the last stages of maturation of two varieties of winter wheat seeds*

R. GÓRECKI, S. GRZESIUK, A. REJOWSKI

Institute of Plant Biology, Olsztyn, Agricultural and Technical Academy

(Received: September 5, 1977)

Abstract

Using the MAK column chromatography method the process of wheat grain maturation was found to be closely correlated with nucleic acid metabolism. The amount of nucleic acids in the embryos increased until the end of the maturation of the grain. This increase was the result of an intensive synthesis mainly of rRNA fractions and to some extent also of sRNA. The quantitative proportion of various fractions of nucleic acids also changed in the last stages of grain maturation. In the period of wax maturity in wheat embryos a higher content of 4S and 5S RNA was found which partly explains a higher viability of these seeds.

INTRODUCTION

The stages of morphogenesis of a seed are accompanied by appropriate physiological and biochemical changes (Grzesiuk, 1961, 1967). They are related with the metabolism of carbohydrates, proteins, nucleic acids and other substances. Numerous biochemical investigations were performed (Chang Chong, 1963; Fot, 1971; Grzesiuk, 1967; Ingle-Beitz, Hageman, 1965; Jennings, Morton, 1963; Kulka, 1966) and have shown that with the maturation of cereal grains the amount of DNA and RNA calculated per 100 grains (absolute content) increased, reaching a maximum during wax maturity. However, the relative content (in relation to dry mass) decreased with the development of the seeds.

* The work was performed within the problem 09.1.1. coordinated by the Institute for Plant Breeding and Acclimatization.

Similar regularities concerning the dynamics of the changes in nucleic acid content were also observed in developing seeds of other plants eg. leguminous plants (Kulka, Sójka, 1966; Motoshka, Bulko, 1971; Mierzwińska, 1971; Millerd, Whitefeld, 1973; Smith, 1973; Wheeler, Boulter, 1969). In these seeds the greatest amounts of the above mentioned compounds were found when the so called early stage of physiological maturity was reached. Subsequently as full morphological maturity was reached the nucleic acid level decreased.

The further investigations undertaken on the changes of particular nucleic acid fractions in wheat grains were aimed at a partial filling in of this gap.

MATERIALS AND METHODS

The investigations were performed on maturing grains of two varieties of winter wheat Grana and Jana. Samples for analysis were taken during full and wax maturity. The taken ears were divided into two groups: from the first the grains were removed directly after the samples were taken (series A) the second group was stored at room temperature for two weeks and then threshed (group B). Chemical analyses of nucleic acids were performed on embryos always freshly prepared for that purpose. The embryos were isolated by hand using a preparation needle. In order to slow down enzymatic activity this was done at about 1°C. To facilitate the isolation of embryos the seeds were previously swollen for 12 hours in water at a temperature of 1° to 3°C.

In the present work the isolation of nucleic acids was performed by the phenol-detergent method described by Kirby (1966). Frozen embryos were homogenized in a blender (Aid type 309) in the presence of Tris-HCl buffer pH 7,6 with sodium dodecyl sulphate, magnesium chloride, potassium chloride and bentonite. Subsequently freshly distilled phenol was added to the homogenate, mixed and centrifuged at 3 000 x g. The supernatant was collected and the lower phase was reextracted with Tris-HCl buffer pH 7,6. The two aqueous phases were pooled and reextracted with phenol and subsequently with a chloroform: isoamyl alcohol mixture. The nucleic acids were finally precipitated by the addition of 96% ethanol.

The crude nucleic acids preparation was further purified by the method of Ralph and Bellamy (1964) and fractionated on a MAK column according to Chandell and Hershey (1960) modified by Filek (1973). All analyses were performed in tetraplicate.

The nucleic acids content in the examined samples was estimated spectrophotometrically by measuring the absorption of the samples at 260 nm.

The dry weight of the endosperm and embryos was estimated by drying the tissue at 100°C until a constant weight was reached.

RESULTS AND DISCUSSION

Dry weight of endosperm and embryos

The development of the embryo and grain ended at the beginning of wax maturity but the increase in size lasted until the end of full morphological maturity (Table 1). The data presented here show a significant increase in the dry mass of the endosperm and embryo in the last stages of development both in the series A and B.

Table 1

Dry weight (in g) of embryos and endosperm in the last stages of maturation of two varieties of winter wheat

Variety	Maturity	Serie A			Serie B		
		100 embryos	100 endosperms	100 grains	100 embryos	100 endosperms	100 grains
Grana	wax	0.1125	3.4926	3.6051	0.1098	3.1868	3.2966
	full	0.1220	3.6253	3.7473	0.1269	3.4473	3.5742
Jana	wax	0.0951	2.7658	2.8609	0.1160	3.3181	3.4341
	full	0.1074	4.6022	3.7086	0.1256	3.6930	3.8186

However, the obtained results for dry weight are different from those obtained by Grzesiuk (1961), Rejowski (1961) and Sójka (1961). According to these authors the dry weight of developing cereal grain reaches a maximum value in the middle of wax maturity and subsequently decreases slightly. This phenomenon is due to the transformation of simple sugars into polysaccharides within the grain, which is accompanied by the release of previously chemically bound water. A partial decrease in the mass at the end of maturation may also be due to a high intensity of respiration together with a block of the supply of nutrients from the mother plant (Grzesiuk, 1967).

Nucleic acids in embryos

The results of estimations of nucleic acids content in embryos of the investigated of winter wheat presented in Table 2 indicate clearly that more of these compounds are present during the period of full rather than wax maturity. These trends are clearly marked both in respect to

Table 2
Quantitative changes in nucleic acids fractions in embryos of maturing grain of two winter wheat varieties

Variety	Maturity	Serie A				Serie B					
		Total NA content		content of particular NA fractions per 100 embryos		Total NA content		content of particular NA fractions per 100 embryos			
		per 1 g of dry weight	per 100 embryos	s-RNA	DNA—RNA	rRNA + mRNA	per 1 g of dry weight	per 100 embryos	sRNA	DNA—RNA	rRNA + mRNA
Grana	wax full	135	15	3.10	4.18	7.68	127	11	2.19	3.74	5.06
		196	17	3.24	5.84	7.87	139	12	1.92	3.12	6.96
Jana	wax full	129	12	2.21	3.54	6.49	100	12	2.60	3.62	5.77
		145	16	2.36	5.19	8.07	124	15	2.83	4.86	7.30

* Results of NA content is given in OD units, an unit is the amount which gives an OD of 0.01 at E_{260nm}^{1cm} .

Table 3
Percent share of particular nucleic acids fractions in embryos of maturing grain of two winter wheat varieties

Variety	Maturity	Serie A				Serie B			
		sRNA	DNA + RNA	rRNA + mRNA	Total	sRNA	DNA + RNA	rRNA + mRNA	Total
Grana	wax	20.8	28.0	51.2	100	20.0	33.8	46.2	100
	full	19.1	34.4	46.5	100	16.0	26.0	58.0	100
Jana	wax	18.0	28.9	53.1	100	21.7	30.2	48.1	100
	full	15.2	33.1	51.7	100	18.9	32.4	48.7	100

dry weight and in the nucleic acids content per 100 embryos. This was due to their accumulation until the end of maturation which is probably mainly due to intensive RNA biosynthesis (Haward, Weeb, Wareing, 1973). Some workers (Kulka, Rejowski, 1970; Kulka, Sobieraj, 1969) have even demonstrated RNA biosynthesis in embryos of resting grain.

On the other hand in investigations performed earlier (Jennings, Morton, 1963; Kulka, 1966) it was found that in the endosperm ribonucleic acid synthesis stopped during wax maturity and its level decreased during subsequent development of the grain. This phenomenon may be due to the degradation of some RNA fractions to free nucleotides, which may then diffuse into the embryo and be a source of new NA molecules.

It is also worth noting that the way the grains are harvested has some effect on the amount of nucleic acids stored in the embryos.

Fig. 1. Series A. Chromatographic profiles of nucleic acids from embryos of winter wheat grain variety Grana (1) and Jana (2) separated on MAK column from wax (a) and full (b) maturity

I — sRNA; II — DNA—RNA; III — rRNA and mRNA

Embryos from series A had a higher content of these substances than those from series B (Table 2).

The separation of nucleic acids from the embryos on columns of methylated albumin indicated the presence of three basic fractions sRNA (including 4S and 5S RNA), DNA+RNA and rRNA+mRNA (Figs 1 and 2). Within the fraction rRNA+mRNA peaks of 18S and 25S were found and the mRNA peak was not always detectable. According to data from the literature (Filek, 1973; Porollo et al., 1974) a considerable amount of mRNA can be found in the fraction strongly bound to the column.

No. of tube - 5 ml fraction

Fig. 2. Series B. Explanations as in fig. 1

A quantitative presentation (Table 2 and 3) indicates the significant differences in the levels of particular fractions of nucleic acids depending on the level of maturity of the grain and the way it was harvested (series A and B). Considering the dynamics of the changes of low molecular weight RNA (sRNA) content its slight quantitative increase in full maturity should be stressed (Table 2). Its percent share, however, was much higher during wax maturity especially in the Grana

variety (Table 3). A higher participation of a relative amounts of 4S and 5S RNA in wax maturity was perhaps connected with a more intensive protein biosynthesis during this period (Górecki, 1976). This hypothesis seems to be confirmed by an increased accumulation of storage materials (including proteins) during the third stage of the grain ontogenesis that is during wax maturity.

The DNA+RNA fraction was the most numerous both per unit dry weight and per 100 seeds during full maturity. The content of the rRNA+mRNA fractions had also a tendency to increase until the end of the period of maturation which probably indicates an increased synthesis of rRNA. It should be assumed that this fraction is related with the formation of new cytoplasmic structures. This was also observable in an increase of the dry weight of the seed (see Table 1).

Summing up it should again be stressed that the process of maturation of wheat grain was very strictly linked with the increase of the total amount of nucleic acids as well as the increase of particular fractions. The way of harvesting affected to a greater extent the quantities of all nucleic acids than the dynamics of changes of particular types. The results obtained in this work of the analysis of nucleic acids in wheat grain confirm the earlier observations performed by Górecki (1976) on rye and barley grains.

The maturing cereal grain as generally characterized by changing metabolic activity. The highest viability is shown by grains collected in the middle of wax maturity (Grzesiuk, 1961; Rejowski, 1961; Sójka, 1961). The lower level of nucleic acids observed in embryos at the stage of wax maturity did not correspond to the observed viability of the grains in this period which indicates that the viability of the grain is not directly related to the total amount of NA. It seems, that the mechanism of changing of viability of the maturing grain may be strictly related to the dynamics of changes of respective proportions of particular RNA fractions, especially 4S and 5S RNA and rRNA.

The obtained results of the analysis of nucleic acids in the embryos of winter wheat grain do not explain in a satisfactory way the causes of the varied viability of cereal grains in the last stages of maturation when harvesting is already possible. The full explanation of this problem requires more detailed investigations. Protein metabolism is strictly related to nucleic acids and possibly observation of their changes will yield an explanation of the changing viability of cereal grains in the last stages of maturation.

CONCLUSIONS

- 1) The dry weight of the endosperm and the embryos increase until the end of maturation of wheat grains.
- 2) The highest total nucleic acid content is found in grains with full

morphological maturity. The intensive increase in the dry weight of the endosperm is accompanied by a distinct increase of the amount of nucleic acids in the cell.

3) The increase in the total amount of RNA in embryos in the final stages of grain maturation is the result of an intensive synthesis of rRNA and sRNA fractions.

4) The way the grain is harvested (threshed directly after harvesting or after 14 days storage in ears) affects to a greater extent the total amounts of nucleic acids present than the dynamics of changes of particular fractions.

5) The quantitative proportions of various fractions of nucleic acids (sRNA, DNA+RNA, rRNA+mRNA) change during the maturation of the grain. However, the mechanism of changing physiological activity of the grain in the final stages of maturation does not depend on the total RNA content but is rather related to the changes in the respective proportions of the sRNA and rRNA fractions.

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Authors' address

Prof. dr Stanisław Grzesiuk

Dr. Ryszard Górecki and dr.hab. Andrzej Rejowski

Institute of Plant Biology

Agricultural and Technical Academy

10-957 Olsztyn-Kortowo; Poland

Kwasy nukleinowe w końcowych etapach dojrzewania ziarna dwu odmian pszenicy ozimej

Streszczenie

W pracy przedstawiono wyniki badań metabolizmu kwasów nukleinowych dojrzewającego ziarna dwu odmian pszenicy ozimej. Badania przeprowadzono na ziarniakach pszenicy pochodzących z dojrzałości woskowej i pełnej. Za pomocą metody chromatografii kolumnowej MAK stwierdzono, że ilość kwasów nukleinowych (NA) w zarodkach wzrastała wyraźnie do końca dojrzewania ziarna. Było to wynikiem intensywnej syntezy frakcji rRNA i sRNA. Ilościowe proporcje różnych frakcji RNA (sRNA, DNA — RNA, rRNA + mRNA) zmieniały się w końcowym okresie dojrzewania ziarna, co wskazywało na ich różną rolę fizjologiczną w tym procesie. Sposób zbioru ziarna (ziarno młócone bezpośrednio po zbiorze i po 14-dniowym okresie przechowywania) wpływał bardziej na zmiany ilościowe ogólnej sumy kwasów nukleinowych niż na dynamikę zmian poszczególnych frakcji.

Uzyskane wyniki z analiz chemicznych NA nie tłumaczą zadowalająco przyczyn zmiennej żywotności ziarna w końcowych etapach dojrzewania, kiedy to istnieje możliwość jego zbioru.