Theor. Appl. Genet. 50, 73-77 (1977)

Protein Accumulation, RNA and Soluble Amino Nitrogen Content in Developing Endosperm of Two Varieties of *Triticum aestivum* with High and Low Protein Seed*

A. Brunori, P. Mannino, G. Ancora and A. Bozzini
Divisione Applicazione delle Radiazioni, C.N.E.N., CSN Casaccia, Roma (Italy)

Summary. The kinetics of protein accumulation, the variation in RNA, the soluble amino nitrogen content of developing endosperm of two varieties of *Triticum aestivum*, with high and low protein content in the mature seed, suggest a possible relation between maintenance of the RNA content and the ability to synthesize protein. A sudden halt in protein accumulation is observed as the RNA starts to decrease. The hypothesis is also advanced that maintenance of the RNA content might, in turn, be dependent on the presence, in the endosperm of developing wheat seed, of a certain level of soluble amino nitrogen which could then play the role of limiting factor for protein synthesis.

Key words: *Triticum aestivum* - Wheat - Endosperm - Protein Synthesis - RNA Level - Amino Acid Translocation

Introduction

A considerable amount of experimental data are available on the variation in nucleic acid and the kinetics of protein accumulation, which take place during the process of development and ripening of seed of both monocotyledons and dicotyledons. The development of the endosperm precedes that of the embryo. In both of the storage tissues, endosperm or cotyledons, the accumulation of nucleic acids precedes that of storage protein (Varner 1965; Dure 1975).

In the endosperm of wheat, as in that of maize (Ingle et al. 1965), once the DNA synthesis is over, the DNA content is quite stable during the following phases of seed development (Jennings and Morton 1963; Brunori et al. 1972). At variance with DNA, the evolution of the RNA content of the endosperm follows a different trend: it rises sharply together with the DNA during the early stages of seed development, stays constant for a while, and, as the final stage of ripening approaches, it clearly decreases (Matsushita 1959; Brunori et al. 1972; Nardi et al. 1975).

The rate of protein accumulation in the endosperm of wheat increases from the beginning of seed development until the RNA content starts to decrease; after that time the process of protein accumulation stops within a few days (Nardi et al. 1975).

A positive relation between the level of RNA in the endosperm and the amount of protein accumulated has been reported in some varieties of rice with varying protein percentages in the mature seed (Cruz et al. 1970). In wheat, the variation in RNA content and the level of soluble amino-nitrogen in relation to the efficiency of protein accumulation is not known.

The present paper reports data on the evolution of the RNA content, the level of soluble amino-nitrogen, and the rate of protein accumulation in the endosperm of developing seed of two varieties of *Triticum aestivum* characterized by low and high protein percentages in the dry seed.

Materials and Methods

Seeds of two varieties of *Triticum aestivum*, 'Irnerio' and 'Atlas 66', with low and high protein content respectively, were collected, from 4 days after anthesis, throughout the process of seed development and ripening. Embryos were removed from the seeds as soon as they were large enough to be handled under the stereomicroscope. The dry weight and the water content were determined for samples of seed containing the embryo. The protein content was established by the method of Lowry et al. (1951). Soluble amino nitrogen was determined by the ninhydrin method of Moore and Stein (1954) modified by Moore (1967); L-Leucine was used as the standard. For the analysis of total RNA content, seeds or endosperms were homogenized with an all-glass tissue grinder in 10 vol-

* Publication No.491 from the Divisione Applicazione delle Radiazioni del C.N.E.N., SCN Casaccia, S.M. di Galeria, Rome, Italy.

umes of cold buffer containing 0.1 M NaCl, 1 mM EDTA, 1% SDS, 0.1 M Na-glycine at pH 9.5. The hard dry seeds were first roughly broken up in a mortar. The homogenate was incubated 10 minutes at 70° C, then centrifuged 10 minutes at 20,000 % g to remove the cell walls and starch. The supernatant was precipitated with two volumes of cold 95% ethanol and the precipitate washed twice with 70% ethanol. The pellet was suspended in 0.3 M KOH and the RNA were precipitated by adding 0.2 ml of 5 M perchloric acid to each milliliter of 0.3 M KOH. Because some protein can not be precipitated after KOH incubation (Cherry 1965), the supernatant containing the nucleotides of the hydrolized RNA was shaken with chloroform-isoamylic alcohol 24:1. The amount of RNA was calculated by multiplying the Optical Density at 260 nm by the factor 31.7.

Results

The variation in total protein and water content in the endosperm of developing seed of two varieties of *Triticum aestivum*, 'Irnerio' and 'Atlas 66', is shown in Fig. 1. During the initial stages of development the endosperm of 'Irnerio' accumulates protein more efficiently, but as the water content falls below 50%, the process of protein accumulation stops abruptly in 'Irnerio', while it goes on in 'Atlas 66' till the water content reaches the very low value of 28%. The final amount of protein accumulated in the endosperm of the dry seed is about 5 and 7 mg in 'Irnerio' and 'Atlas 66', respectively, despite the dry weight of the seed, which is 50 mg for 'Irnerio' and 35 mg for 'Atlas 66'.

The total RNA content and the rate of protein accumulation in the endosperm of developing seed of 'Irnerio' and 'Atlas 66' are shown in Fig. 2. In both varieties there is an early and rapid increase of total RNA content. The highest level of RNA content, once reached, is maintained for some days; later the RNA content significantly decreases as ripeness approaches. The total RNA content shows the same trend of variation in the two varieties and even attains the same maximum value (about 100 µg/endosperm). The only difference is the way the RNA content decreases, with results somewhat delayed in 'Atlas 66'. The amount of protein accumulated per day per endosperm, calculated from the protein accumulation curves of Fig. 1, rises progressively and reaches its highest value at the advanced stages of seed development, when the RNA content begins its rapid decline; the process of protein accumulation then stops completely within a few

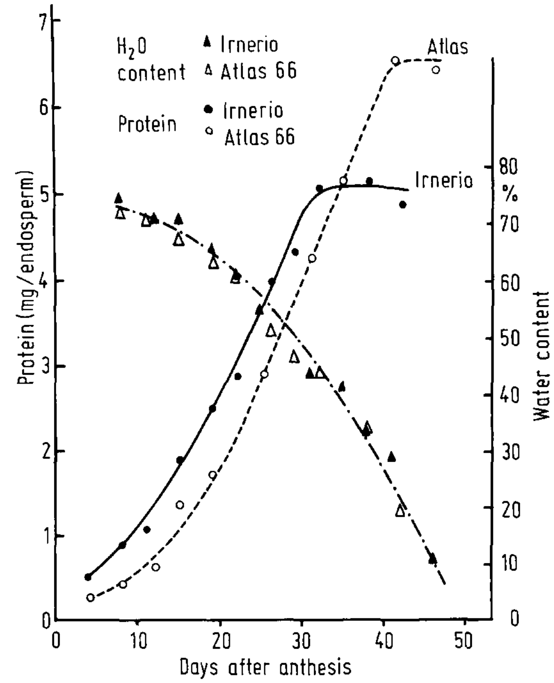

Fig. 1. Variation of protein and water contents in endosperm of two varieties of *T. aestivum* during seed development

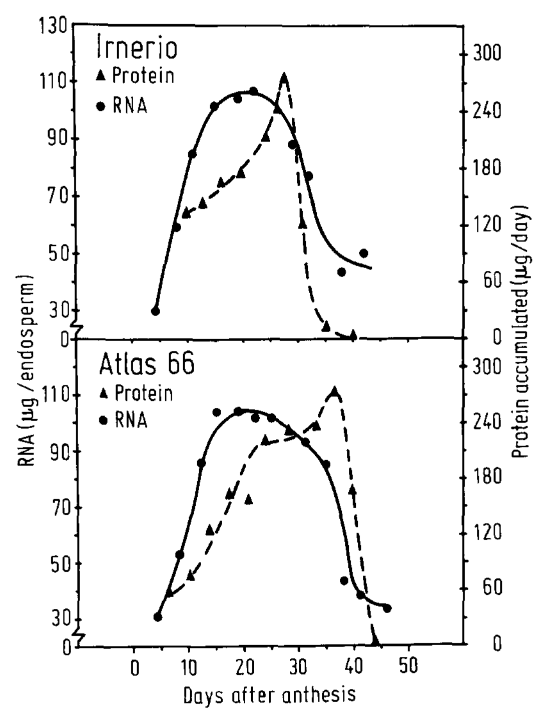

Fig. 2. RNA content in endosperm and protein accumulated per day in two varieties of *T. aestivum* during seed development

days. Although in both varieties the highest value of protein accumulated per day per endosperm is almost the same (about 270 µg), the kinetics of protein ac-

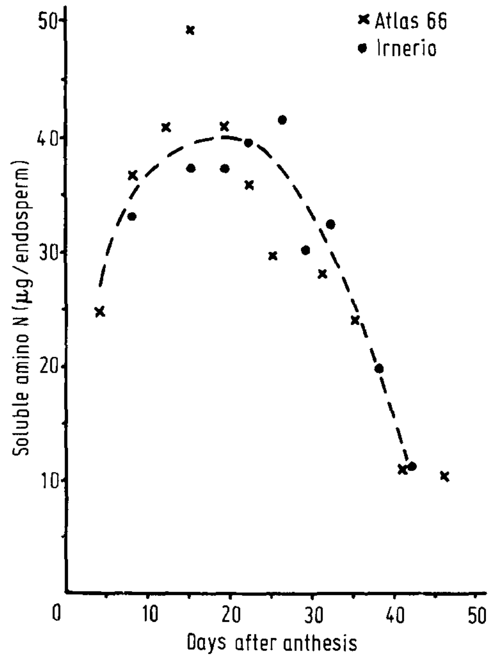

Fig.3. Soluble amino nitrogen content in the endosperm of two varieties of *T.aestivum* during seed development

cumulation differ to some extent, the useful period of protein accumulation in 'Atlas 66' being several days longer than in 'Irnerio'.

In both 'Irnerio' and 'Atlas 66' the soluble amino nitrogen content of the endosperm of developing seed changes in the same manner and no clear differences are evident (Fig.3). The soluble amino nitrogen content, which is high at the beginning of seed development, gradually decreases and reaches very low values in the mature cariopses.

Discussion

The differing protein content in the dry seed of two varieties of *Triticum aestivum*, 'Atlas 66' and 'Irnerio', - 17 and 10 % respectively - is the result of an extended period of protein synthesis rather than the effect of higher rates of protein accumulation. The ability of 'Atlas 66' to accumulate protein until the water content drops below 30 %, in contrast to the apparent cessation of protein accumulation that occurs in 'Irnerio' when the water content is still about 50 % (see Fig.1), tends to reduce the relevance of water content as a critical factor for the synthesis and accumulation of protein in the developing endosperm of wheat seed. During the initial stages of seed development, the endosperm of 'Irnerio' accumulates protein more efficiently. Ten days after flowering, although it has the same content of RNA, 'Irnerio' accumulates twice as much protein as 'Atlas 66'. Since a minor fraction of ribosomes appear as polysomes in devloping wheat seed (our unpublished observation), the lack of relation between the RNA content and the rate of protein accumulation suggests that at least the ribosomal RNA is in large excess. The apparent abundance of ribosomal RNA in developing wheat seed contrasts with the situation in the developing cotyledons of legumes, where more than 80 % of the ribosomes are present as polysomes (Poulson and Beevers 1973). In both varieties the process of protein accumulation suddenly stops as soon as RNA content starts its rapid decline, strongly suggesting that maintenance of the RNA content in the developing endosperm of wheat seed is essential to the process of protein synthesis and accumulation. The possible relation between maintenance of the RNA content and synthesis of protein is reinforced by the situation present in the maturing cotyledon of legumes, where there is no decrease in RNA content and accumulation of protein proceeds, almost until the seeds are dry (Payne and Boulter 1969; Smith 1973; Walbot 1973; Millerd and Spencer 1974). There are however some exceptions to the apparent relation between RNA content and protein synthesis. In maize, protein is accumulated at high rates, while the RNA is decreasing, as can be inferred from the data available (Ingle et al. 1965). In some varieties of rice, when the accumulation of protein stops, the RNA content remains almost constant (Cruz et al. 1970). Altogether, the differing evolution of the RNA content at the end of protein accumulation in wheat and in other species suggests that the cessation of protein synthesis in developing seed might be more complex than a mere problem of maintenance of the RNA content. In wheat the abrupt cessation of protein synthesis, when the RNA content starts to decrease, might result from a selective degradation of the mRNA. Such a possibility, although stimulating and in some way supported by the presence of high levels of ribonuclease activity (Matsushita 1958, 1959), remains to be proven. Results of preliminary experiments underway in our laboratory on the presence of mRNA in the endosperm of developing wheat seed, evaluated as polyA-RNA and on the ability to sustain protein synthesis in a cell free system, indicate an appreciable presence of mRNA even at the advanced stages of seed ripening.

The soluble amino nitrogen content of developing seed is obviously the sum of the synthesis and/or translocation and the polymerization of amino acids into protein. The high level of soluble amino nitrogen in the early stages of seed development, its progressive decrease as the rate of protein accumulation increases, and the very low value of amino acids in the mature seed suggest that the availability of amino acid can play an important role in determining the survival of the ability to synthesize protein in the endosperm of developing wheat seed; and that the lack of amino acids rather than the elimination of the RNA system may be the limiting factor for protein synthesis. Furthermore, a low level of soluble amino acids might trigger the degradation of RNA, as happens in the liver cells of rats starved of protein (Enwonwn and Munro 1970), thus providing an explanation of the fate of the RNA in the maturing wheat seed.

Indirect support for the idea that the maintenance of the RNA level in the endosperm of developing wheat seed may depend on the level of soluble amino nitrogen comes from experiments with nitrogen fertilizers (Nardi et al. 1975), where a large dose of N_2 (400 Kg/ha) does not significantly change the level of RNA while at the same time the total amount of protein accumulated in the mature seed is increased by up to 50 %. The higher protein content is mainly the result of a long term protein synthesis. Surprisingly, the decrease of RNA content in the ripening seed is very low, so that the overall variation of the RNA content resembles that of legumes. This reinforces the concept that RNA stability depends on the presence of a certain level of soluble amino nitrogen. Finally it must be recalled that the high protein content of 'Atlas 66' seed has been related to a better amino acid translocation ability from the plant to the developing seed (Johnson et al. 1968) and this once more points to the availability of amino acids as the limiting factor for protein accumulation in the developing wheat seed.

Literature

Brunori, A.; Avella, R.; Brunetti, N.; Giacomelli, M.: Variation in protein, DNA and RNA during the development and ripening of *Triticum durum* seeds. Informatore Botanico Italiano 4(1) 72 (1972)

Cherry, J.H.: Nucleic acid determination in storage tissue of higher plants. Plant Physiol. 37, 670-678 (1965)

Cruz, L.J.; Cagampang, G.B.; Juliano, B.O.: Biochemical factors affecting protein accumulation in the rice grain. Plant Physiol. 46, 743-747 (1970)

Dure, L.S. III: Seed formation. Ann. Rev. Plant Physiol. 26, 259-278 (1975)

Enwonwn, C.O.; Munro, H.N.: Rate of RNA turnover in rat liver in relation to intake of protein. Arch. Bioch. Biophys. 138, 532-539 (1970)

Ingle, J.; Beitz, D.; Hageman, R.H.: Changes in composition during development and maturation of maize seeds. Plant Physiol. 39, 835-839 (1965)

Jennings, A.C.; Morton, R.K.: Changes in nucleic acid and other phosphorus containing compounds of developing wheat grain. Austral. J. Biol. Sci. 16(2), 332-341 (1963)

Johnson, V.A.; Schmidt, J.W.; Mattern, P.J.: Cereal breeding for better protein impact. Economic Bot. 22, 16-25 (1968)

Lowry, O.H.; Rosebrough, M.J.; Farr, A.L.; Randall, R.J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265-275 (1951)

Matsushita, S.: Studies on the nucleic acids in plants. II. Variation of the RNA contents of wheat and rice grains during ripening processes. Mem. Res. Inst. Food, Sci., Kyoto Univ., Japan 14, 24-29 (1958)

Matsushita, S.: On the protein formation and the changes of the amounts of RNA and ribonuclease activity in the grains during the ripening process of wheat. Mem. Res. Inst. Food Sci., Kyoto Univ., Japan 19, 1-5 (1959)

Millerd, A.; Spencer, D.: Changes in RNA-synthesizing activity in nuclei from cotyledons of developing Pea seeds. Austral. J. Plant Physiol. 1, 331-341 (1974)

Moore, S.: Amino acid analysis: aqueous dimethylsulfoxide as solvent for ninhydrin reaction. J. Biol. Chem. 243, 6281-6283 (1968)

Moore, S.; Stein, W.H.: A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. 211, 907-913 (1954)

Nardi, S.; Brunetti, N.; Brunori, A.; Ferrandi, L.; Giacomelli, M.: Protein accumulation and variation induced by N-fertilizer of some factors relevant to the protein synthesis in durum wheat during seed development. Genetica Agraria 30, 102 (1975)

Payne, P.I.; Boulter, D.: Free and membrane bound ribosomes of the cotyledons of *Vicia faba* (L.). Planta (Berl.) 84, 263-271 (1969)

Poulson, R.; Beevers, L.: RNA metabolism during the development of cotyledons of *Pisum sativum* L. Bioch. Biophys. Acta 308(3), 381-389 (1973)

Smith, D.L.: Nucleic acid, protein and starch synthesis in developing cotyledons of *Pisum arvense* L. Ann. Bot. 37, 795-804 (1973)

Varner, J.E.: Seed development and germination. In 'Plant Biochemistry' (Bonner, J. and Varner, J.E., eds.), pp. 763-792 Acad. Press: New York-London 1965

Walbot, V.: RNA metabolism in developing cotyledons of *Phaseolus vulgaris*. New Phytol. 72, 479-483 (1973)

Received October 21, 1976
Communicated by H.F. Linskens

A. Brunori
P. Mannino
G. Ancora
Divisione Applicazione delle Radiazioni del C.N.E.N.
CSN Casaccia, S.M. di Galeria
Roma (Italia)

A. Bozzini
Crop and Grassland Production Service, Plant Production and Protection Service, FAO
Via delle Terme di Caracalla
I-00100 Roma (Italia)