BIOLOGIA PLANTARUM (PRAHA)

31 (1) : 8–18, 1989

Protein and RNA Content and Synthesis in Embryos and Endosperms from Developing *Triticum durum* Seeds

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Abstract. Protein and RNA contents and synthesis were evaluated in the course of wheat grain (*T. durum* cv. Cappelli) development. Embryos and endosperms were considered separately during five phases from the 20th day after anthesis until full ripenes was reached. No clean-cut changes were observed in the pattern of soluble proteins of the embryos. In the endosperms protein synthesis continues till the later phases and appears to be due to the albumin + globulin component. Screening of bands of endosperm proteins from electrophoresis indicates that the gliadins are synthesized early, with the exception of a ω - gliadin. Glutelins with high relative molecular mass also appear to be synthesized when the grain approaches full ripeness.

The RNA content of the embryo and the endosperm is high in the early stages, when high cell proliferation occurs, and declines later on. The synthesis of RNA during *in vitro* imbibition is, however, higher in the later phases of ripening. Most of RNA synthesized in the embryos was ribosomal.

The ontogeny of seeds which begins at fertilization of the ovule continues through three main stages (MUNTZ 1982): i) endosperm and pro-embryo formation; ii) embryo development and storage compound deposition; iii) grain ripening, during which most water is lost.

During these stages many changes occur in the characteristics of the seeds such as the rate of nucleic acid and protein synthesis, hormone content and the composition of free amino-acid pools. Some of these compounds are essential for the developing grain and are mainly utilized during seed germination and the initial growth of the plant.

On this basis, an investigation was carried out into certain biochemical aspects in the course of endosperm and embryo development in *Triticum durum* caryopses, characterized by a "relative dormancy" (MELETTI 1964), and RNA and protein synthesis in the two organs of the caryopsis, which are characterized by different cytological and tissue patterns, was evaluated.

The biochemical events described in the present paper start in caryopses collected

Received February 12, 1988; accepted June 7, 1988

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20 days after anthesis and research on them will improve our knowledge of the control mechanisms of durum wheat dormancy.

MATERIAL AND METHODS

Triticum durum cv. Cappelli caryopses (seeds) were utilized in all the experimental procedures. The seeds were collected from open-field grown plants and the developmental stages considered were 20, 27, 34, 41 and 48 days after anthesis (stages I, II, III, IV, V). All seeds were sterilized with 5% Na-hypochlorite (5 min) and carefully rinsed with distilled water before use. Embryos and endosperms were hand-isolated in sterile conditions by a sharp gauge and stored in sealed glass containers, in the dark at −20 °C.

Extraction and Electrophoretic Analysis of Proteins

The total embryo proteins were extracted at room temperature, twice for 30 min, with a mixture of 0.062 M Tris HCl (pH 6.8), 2% SDS, 5% β-mercaptoethanol (Cole *et al.* 1981). Aliquots of the supernatant were used for SDS-polyacrylamide slab electrophoresis (Lioi *et al.* 1984) by means of a modification of Laemmli's (1970) discontinuous system.

SDS-soluble proteins were extracted, according to the above procedure, from lots of 10 isolated endosperms, ground in a blender and defatted, three times, with cold acetone (15 ml for 15 min).

The protein fractions were extracted from the defatted powder of the endosperms according to the basic procedure devised by Osborne and Mendel (1914) and Rahman *et al.* (1982) and modified as follows: each step was performed three times for 1 h by means of 20 volumes of solvent in stirred centrifuge tubes; the following series of solvents were utilized: 1) 0.5 M NaCl to extract albumins plus globulins; 2) 70% ethanol and 2% β-mercaptoethanol to extract gliadins; 3) borate buffer (pH 10), 1 % β-mercaptoethanol and 1 % SDS to extract glutelins.

The extracts to be utilized for electrophoresis were dialyzed against water overnight and then lyophilized. The samples solubilized by means of Laemmli's (1970) sample buffer were heated at 100 °C for 3 min and 100 μg fractionated in 17% polyacrylamide gel slabs (Payne and Corfield 1979). The tracking dye used was bromophenol blue (0.005%). The gels were stained according to the method devised by Fairbanks *et al.* (1971) for endosperm proteins and modified by Fullington *et al.* (1983), then destained with isopropanol-acetic acid-water (10/10/8, v/v/v).

Protein Quantification and Synthesis *in vivo*

The protein content was evaluated according to Bensadoun and Weinstein (1976). For the evaluation of protein synthesis, isolated embryos and endosperms

were incubated at 23 °C in sterile conditions for 5h, in a water solution of 0.6 MBq ml^{-1} and 0.2 MBq ml^{-1} ^{3}H-leucine (specific activity: 5.4 TBq $mmol^{-1}$). The materials were then carefully rinsed with a sterile solution of 100fold excess cold leucine. Uptake (U) and incorporation (I) were estimated according to the methods devised by SODEK and WILSON (1970) and ROBERTS *et al.* (1973), by utilizing Protosol plus Econofluor solutions (NEN).

RNA Extraction and Fractionation

Total RNA was extracted using the phenol method, modified by AVANZI *et al.* (1972), at pH 7.6. Samples (100 μg) of total RNA were layered on a linear (5%–20%) gradient of sucrose, dissolved in 0.01 M acetate buffer pH 5.2 plus 0.1 M NaCl, with a cushion of 50% (m/v) sucrose, and centrifuged at 55 000 rev. min^{-1} for 3 h at 2 °C. The samples were pumped from the bottom of tubes through an ultraviolet analyzer. Fractions, each of 0.5 ml, were collected and their radioactivity was determined by liquid scintillation.

RESULTS AND DISCUSSION

The seed development sequence, followed by measuring fresh and dry mass, is shown in Fig 1. Starting from stage I, the fresh mass (FM) of the embryo rapidly

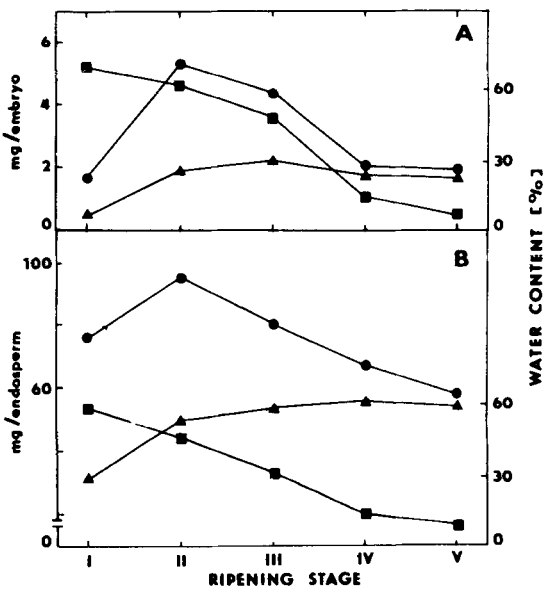

Fig. 1. Seed ripening evaluation: Fresh (●) and dry (▲) mass, water content (■) of embryos (A) or endosperms (B) at various stages. The ripening stages indicated as I, II, III, IV, V were considered at 20, 27, 34, 41 and 48 days from anthesis, respectively.

increases up to stage II; then, a progressive decrease occurs in accordance with the decrease in water content (from a maximum of 67% to 7% at about stage V). The dry mass of the embryo increases up to stage III and remains roughly constant up to full ripeness. The pattern of endosperm development is roughly similar to that of the embryo.

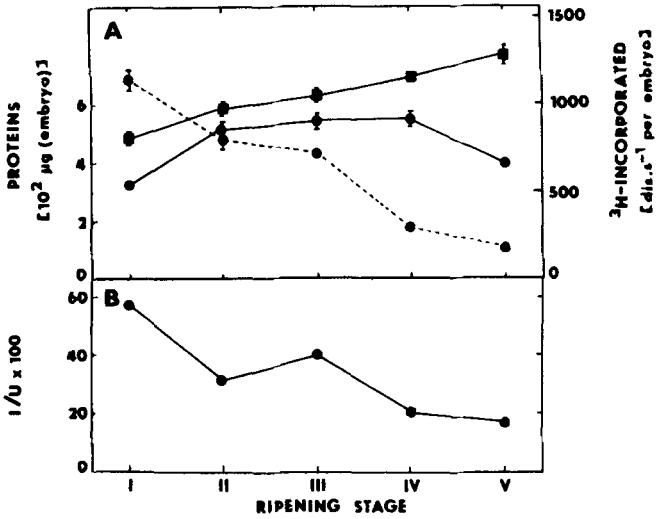

Fig. 2. Protein content and synthesis in developing embryos. Total protein content in dry (■) and imbibed (●) embryos, ^{3}H-leucine incorporation (● + dashed line) (A), and protein synthesis expressed as Incorporation/Uptake ratio (I/U . 100; ●) (B).

The pattern of protein content and synthesis during embryo development shows that the embryos double their protein content while their proteosynthetic activity (I/U . 100) declines (Fig. 2). This is in relation with the increase in dry mass and subsequent decrease in metabolic activity due to the dehydration process. The reduced protein content during embryo imbibition might be due to the rapid turnover or to their moderate leaching (HWANG *et al.* 1979).

During development of the embryo there is no apparent difference between the stages in the pattern of total proteins separated by SDS-PAGE (Fig. 3). This implies that the embryos are capable of early synthesis of most total protein species which are formed until the development is complete.

Developmental changes in total protein content in the endosperm showed a progressive increase mainly between stages III and IV when filling of the endosperm is virtually complete and the dehydration process reaches its maximum value (Fig. 4). This late increase appears to be due mainly to the accumulation of the albumin + globulin component; the increase in other proteins, such as gliadin and glutenin, is rather limited. This pattern, in accordance with the data on Steward

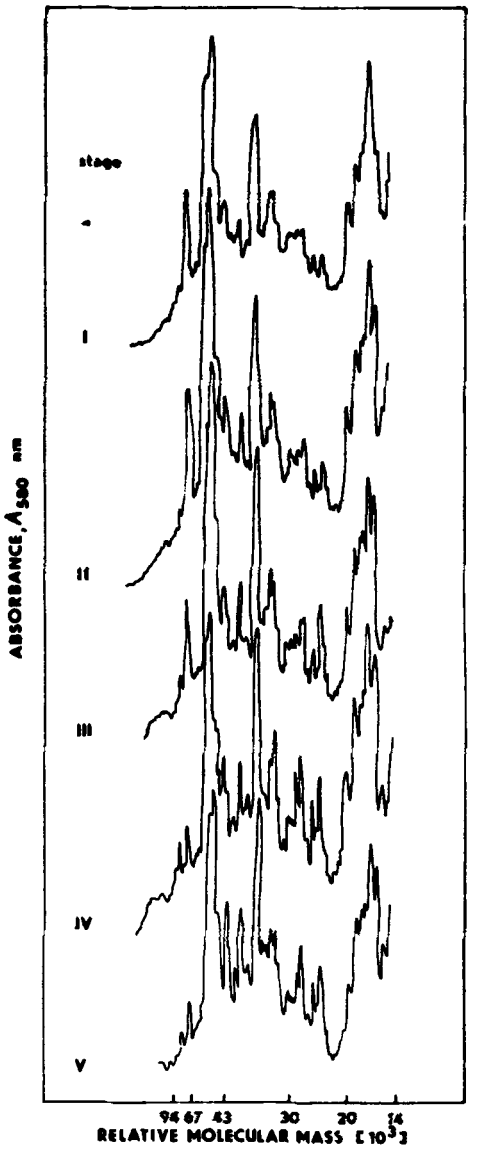

Fig. 3. Electrophoretic pattern of total proteins in developing embryos. Scannings of proteins fractionated by SdS-PAGE.

durum wheat (DEXTER and DRONZEK 1975), might mean that in that phase of their development, together with storage proteins, the wheat endosperms mainly accumulate proteins involved in the enzymatic processes concerning filling or preparation for the future germination process.

The protein-synthesizing activity of developing endosperms as evaluated by means of the incorporation of ^{3}H-leucine into the TCA insoluble fractions and the

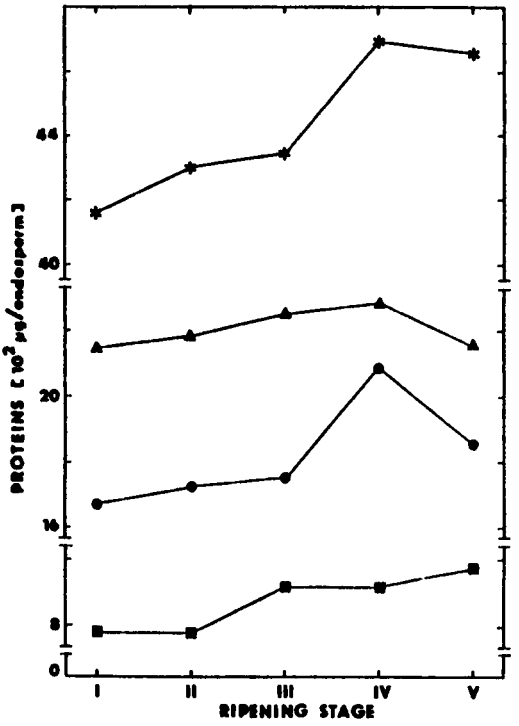

Fig. 4. Protein content in developing endosperms. Total proteins (*), albumins + globulins (●), gliadins (▲), glutenins (■) contents.

I/U . 100 ratio (Fig. 5), confirms the data obtained by quantifying the protein content. The most dramatic increase occurs between stages III and IV for albumin + globulin and, to a lesser extent, for glutenin; gliadin synthesis appears to be practically constant during endosperm development.

This late increase in both protein content and synthesis is indicative of the settlement of specialized proteins (INGLE *et al.* 1965), probably enzymatic proteins, involved in starch synthesis (CAMBELL *et al.* 1981). This process together with protein synthesis continues in *T. aestivum* until physiological maturity is reached (up to 35–40 days, approximately, after anthesis; CAMPBELL *et al.* 1981).

In order to evaluate the changes in the protein subunits from developing endosperms, SDS-acrylamide gel electrophoresis of the proteins was carried out. Figures 6A and 6B contain the electrophoretograms of albumins + globulins and gliadins. The former shows variations when stage II is reached, the latter shows the appearance and increase of a new large gliadin (ωgliadin; BIETZ and WALL 1972) starting from stage II. It accumulates slowly even when synthesis of most proteins in the endosperm is rapidly declining.

These data agree with the findings of TERCÉ-LAFORGUE and PERNOLLET (1982)

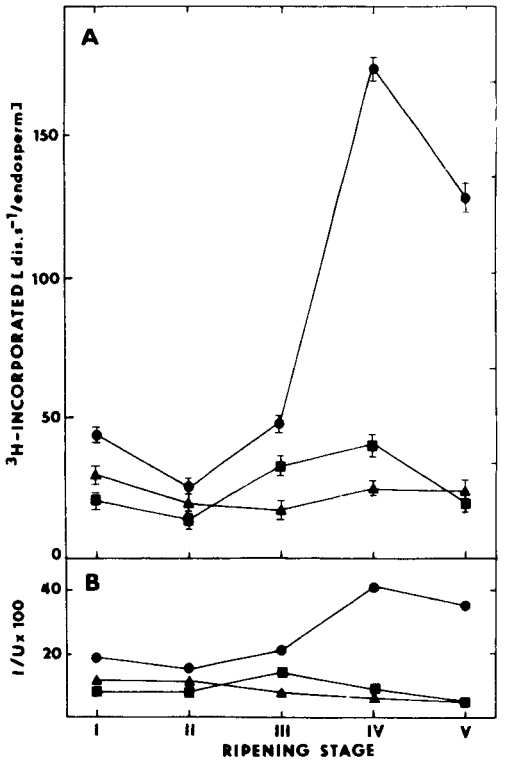

Fig. 5. Protein synthesis in developing endosperms. ^{3}H-leucine incorporation into albumins + globulins (●), gliadins (▲), glutenins. (■) (A). – Protein synthesis is expressed as Incorporation/Uptake ratio (I/U . 100) (B).

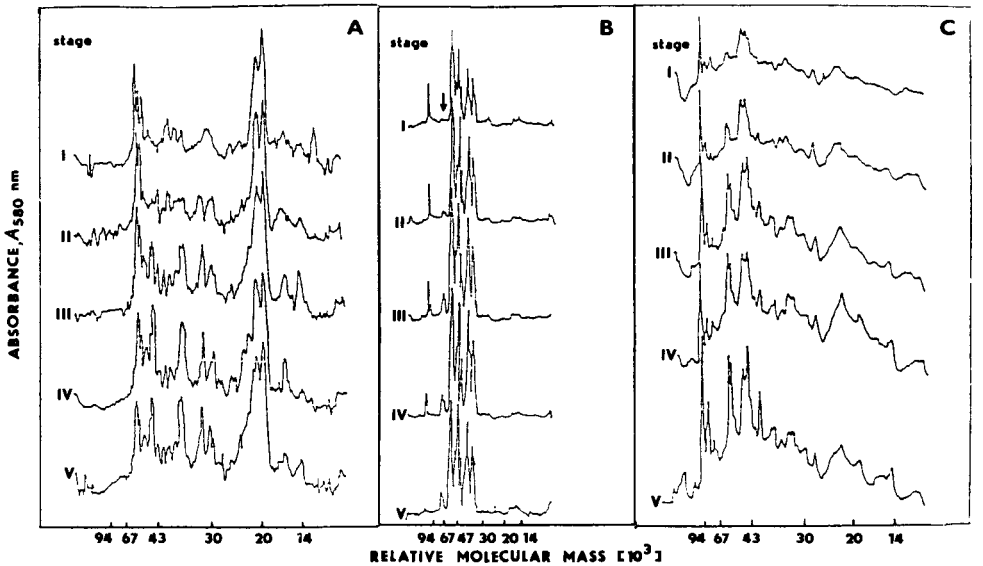

Fig. 6. Densitometric scanning of proteins from developing endosperms. SDS acrylamide gel electrophoretograms of albumins + globulins (A), gliadins (B) and glutenins (C).

who showed that the gliadins are directly synthesized, early (before the 6th day after anthesis) in their mature form, as shown by *in vivo* and *in vitro* experiments (TERCÈ-LAFORGUE *et al.* 1987).

The pattern of glutenins is very similar during the development but the relevant amounts of the M. M. subunits (from 94000 to 30000 M. M. approximately) appeared to increase when the grain approached full ripeness (Fig. 6C).

The assessment of embryo RNA content shows that the highest value is reached at stage II (Fig. 7A, C): this fact must be related to both the rapid increase in fresh mass and the corresponding highest cell proliferation. In 5h-imbibed embryos, the maximum content is reached later; it increases progressively up to stage III and then declines. The data reflect the ^{3}H-uridine incorporation pattern and the evaluation of RNA synthesis efficiency (I/U . 100). It can be hypothesized that the 5h-imbibed embryos utilize their RNA during early development while acquiring the capability of synthesizing new RNA at stage III.

The radioactivity data from the sedimentation profiles (Fig. 8) show that most of RNA synthesized during embryo development is ribosomal and confirms that the highest rate of RNA synthesis *in vitro* is at stage III.

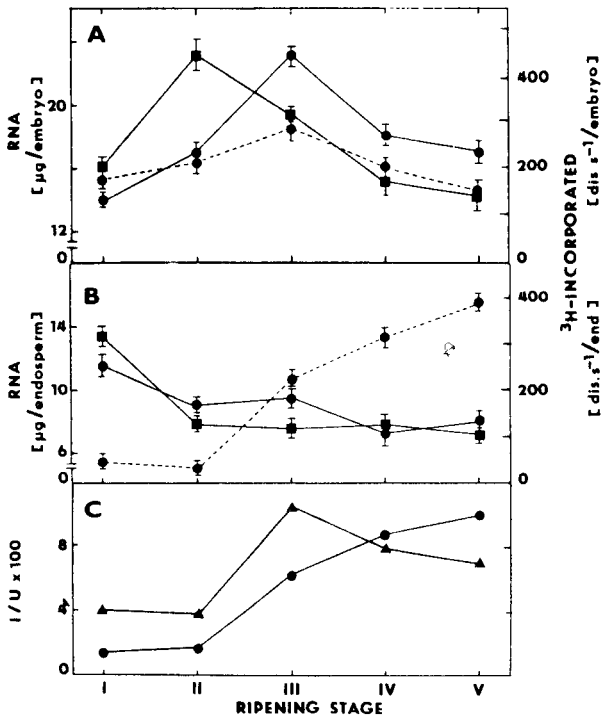

Fig. 7. RNA content and synthesis in developing embryos (A) or endosperm (B). Total RNA content (Oh; ■) (5h imbibition; ●), ^{3}H-uridine incorporation (● + dashed line), and RNA synthesis (C) (I/U . 100; embryos, ▲ ; endosperm ●).

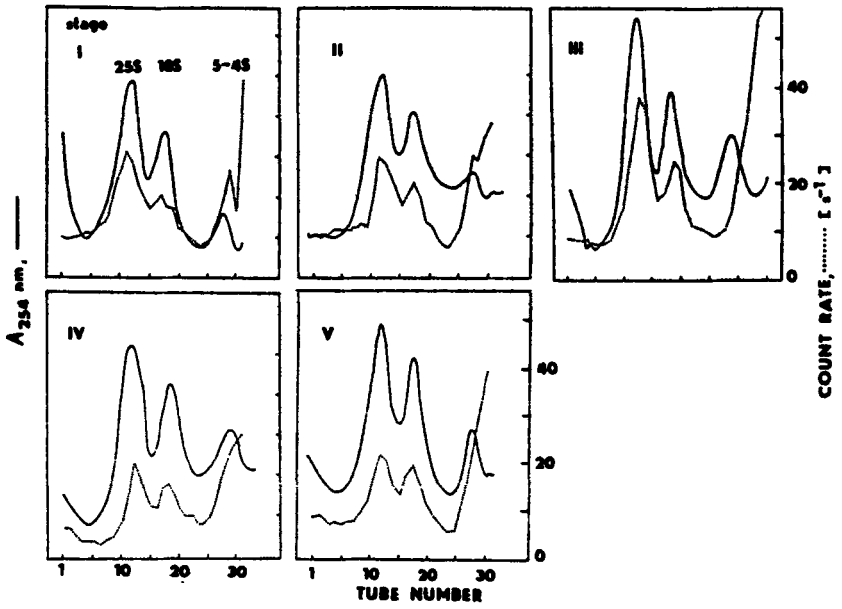

Fig. 8. Sedimentation profiles of total (full line) and radioactive (dotted line) RNA formed during 5h embryo labelling with ^3H uridine.

In the developing endosperms (Fig. 7B, C), the highest content of total RNA occurs at stage I; later, it decreases significantly and remains virtually constant until the grain is fully ripe. These results agree with similar data reported for different cultivars of *T. aestivum* (Kapoor and Heiner 1975, Brunori *et al.* 1977) : they seem to indicate that RNA accumulation occurs very early and is then followed by considerable RNA degradation, probably caused by massive nuclease activity (Matsushita 1959, Weidner and Kulka 1979, Dell'Aquila *et al.* 1982).

The ^3H-uridine incorporation pattern and the evaluation of RNA synthesis efficiency (I/U . 100) show that the RNA synthesis of endosperms, – which is very low and constant in the two early stages –, greatly increases until the completion of grain development.

The ripening period of the wheat grain we have studied begins on the 20th day after anthesis and lasts until the 48th day, and corresponds approximately to the middle and late phases of grain development.

In the wheat endosperms, in the course of the middle ripening phase (stages I and II), protein bodies are formed in the storage tissues (Muntz 1982). In the Cappelli cultivar of *T. durum* the increase in dry mass is accompanied by a moderate accumulation of proteins; moreover, a reduced RNA content and a lower capacity to synthesize RNA occurred, in accordance with the data reported by Ingle *et al.* (1965) In the later phases the RNA synthetic activity greatly increases whereas the RNA content is low and constant.

At this time, when maximum dehydration is reached, a rapid increase in both the content and the synthesis of the albumin + globulin fraction occurs. Since this considerable synthesis of proteins and RNAs is accompanied by increasing amounts of polyamine putrescine, spermine and spermidine (our unpublished data), we conclude that at the final phases of grain development, the endosperms of *T. durum* cv. Cappelli are characterized by the persistence of a marked biosynthetic activity.

In wheat embryos the growth of which is mainly due to cell division and distension, the progress of development, when the increase in dry mass mainly occurs (20–30 days after anthesis) and the differentiation of axis tissues begins (SMART and O'BRIEN 1983), is accompanied by protein accumulation and by an increase in both RNA content and synthesis. Embryo ripening appears to be more negatively affected by the dehydration process. Indeed, when this process reduces the embryo water content by 50 % their metabolism declines considerably and a rapid reduction in protein and RNA synthesis occurs (WALBOT 1972).

Acknowledgements

The authors thank Mr. R. Bertini, Mr. P. Carmignani, Mr. F. Saviozzi and Mr. V. Sbrana for their skilful technical assistance and Mrs. P. Andolfi for her expert typing of the manuscript. They also wish to thank Prof. A. Freer (University of Pisa) for the revision of the text. The research was supported by grants (40 %) from the Italian Ministry of Education and the National Research Council (grant n. 85.006084).

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