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Deoxyribonucleic Acid Synthesis and Deoxyribonucleic Acid-polymerase Activity during Early Germination of Wheat Embryos at High and Low Viability

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Abstract. ^{3}H-thymidine incorporation and DNA-polymerase activity during early hours of wheat embryo germination at two viability levels have been studied. The patterns of two biosynthetic activities, as well as the dependence of DNA synthesis on protein synthesis, indicated the presence of a delay in the early phase of imbibition of the aged embryos with respect to viable germs.

In seed, ageing induces damages to the membrane and enzyme systems and damages to the genome (VILLIERS 1975). Although genetic damages increase during ageing, these cannot involve directly transcription of genetic material (MARCUS and FEELEY 1964). BERJACK and VILLIERS (1972) reported that aged embryos of *Zea mays* may germinate, after a delay, into normal seedlings. These authors suggested that embryos with damaged macromolecular structures were able to recover some metabolic functions during early germination. During this delicate phase, in which protein, RNA and DNA synthesis occur up to the first cell division (DOBRZANSKA *et al.* 1973), little is known about the onset of DNA synthesis and its relation with early proteins synthetized in aged wheat embryos. In this context, several authors (MORY *et al.* 1972, HOVEMANN and FOLMAN 1979) reported that DNA-polymerase (EC 2.7.7.7.) may represent a typical enzyme connected directly to the onset of DNA synthesis in germinating embryos.

The experiments reported in this paper attempt to correlate DNA synthesis and the development of DNA-polymerase activity, as well as the interdependence of two biosynthetic activities during the early hours of germination of wheat embryos isolated from seeds at high and low viability.

MATERIAL AND METHODS

Two stocks of wheat seeds (*Triticum durum* cv. Appulo) were used: the first, at 87% viability level (87%V embryos), was obtained from seeds harvested in 1978 and the second, at 62% viability level (62%V embryos),

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from the same seeds stored under rapid ageing conditions at 14% moisture content and 38 °C for the required time (ROBERTS and ABDALLA 1968).

The embryos were prepared according to JOHNSTON and STERN (1957).

Germination

20 mg of embryos (50—60 embryos) were germinated at 20 °C in darkness in Petri dishes on two filter papers imbibed with 3 ml germination medium containing 2% (w/v) sucrose and chloramphenicol (10 μg ml^{-1}).

DNA and Protein Synthesis

These activities were measured as labeled thymidine or leucine incorporation into cold trichloroacetic acid (TCA)-insoluble material. At appropriate times the embryos were pulsed for two hours in (6-^{3}H) thymidine (0.5 μCi ml^{-1}; sp. act. 30 Ci $mmol^{-1}$) or L-(U-^{14}C) leucine (0.1 μCi ml^{-1}; sp. act. 324 mCi $mmol^{-1}$). At the end of the pulse, the embryos were washed and homogenized in 2% (w/v) sucrose solution containing 5 mM 2′-desoxythymidine or 12 mM L-leucine (about 100-fold in excess of the corresponding radioactive precursor). Total uptake and incorporation of labeled thymidine and leucine into TCA-insoluble material were assayed essentially according to SEN and OSBORNE (1974). The standard error of determined radioactivity was in the range of 5 per cent or less.

Mitotic Index

Samples of three embryos arising from labeled thymidine incorporation experiments were fixed in Carnoy, stained and squashed by the standard Feulgen method (DARLINGTON 1969). For mitotic activity estimation, at least 1000 cells per slide were examined. The mitotic index was expressed as per cent value of the ratio: observed metaphases/total number of cells scored.

DNA-polymerase Activity

Enzymatic activity was measured as *in vitro* incorporation activity of labeled deoxythymidine triphosphate [(^{3}H)-TTP] by the soluble fraction from homogenate of wheat embryos.

20 mg of wheat germs were homogenized in an ice-cold mortar with 3 ml 50 mM TRIS-HCl, pH 7.6, 25 mM KCl, 1 mM $MgCl_2$, 1 mM 2-mercaptoethanol and 0.25 M sucrose. The homogenate was centrifuged twice at $10000 \times g$ for 15 min and the resulting supernatant was then centrifuged at $100000 \times g$ for two hours. The post-ribosomal supernatant obtained was dialyzed overnight against 50 mM TRIS-HCl, pH 7.6, 10 mM KCl, 1 mM $MgCl_2$, 10% glycerol (w/v) and 1 mM 2-mercaptoethanol. The dialyzed solution was then centrifuged at $10000 \times g$ for 20 min and the clear supernatant, which formed the soluble fraction, was used as the source of enzyme activity.

The reaction mixture to assay DNA-polymerase activity contained in a total volume of 0.25 ml: 25 mM TRIS-HCl, pH 7.6, 4.4 mM $MgCl_2$, 16 mM KCl, 0.1 mM each of dATP, dGTP and dCTP, 1 μCi of (methyl-^{3}H) TTP (sp. act. 30 Ci $mmol^{-1}$), 4% (w/v) glycerol, 0.4 mM 2-mercaptoethanol, 50 μg of activated calf thymus DNA (APOSHIAN and KORNBERG 1962) and 100—150 μg of protein of soluble fraction. After the incubation at 37 °C for

30 min, all the operations were carried out as already described (DELL'AQUILA *et al.* 1978).

Protein content was determined by the method of LOWRY (1951) using crystalline serum albumin as standard.

The values reported in the figures and tables represent the mean of three determinations replicated in two series of experiments.

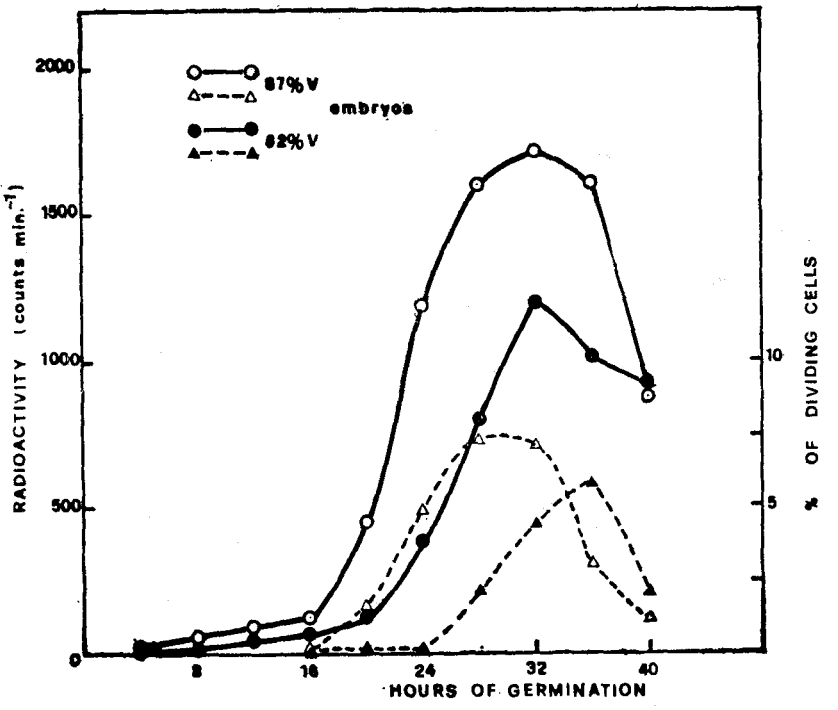

Fig. 1. Time course of ^{3}H-labeled thymidine incorporation into TCA-insoluble material of 20 mg 87%V and 62%V embryos during early hours of germination. Mitotic index was estimated in the same lots of germs.

RESULTS AND DISCUSSION

When wheat embryos germinate in an appropriate medium, the events preceding the initiation of DNA replication (phase S) occur in cells that are in G_1 configuration (CHEN 1973). The time course of ^{3}H-thymidine incorporation into TCA-insoluble material from 87%V germs (Fig. 1) showed that the onset of DNA synthesis began after 4 h. The labeled precursor reached active incorporation between 16—30 h and a peak at 32 h was followed by a sharp drop. In aged germs, the onset of labeled thymidine incorporation was delayed about 8 h but reached a peak at the same time as 87%V germs, even if the incorporation rate was lower than with viable germs. Moreover, the observation of mitotic figures in 87%V embryos indicated that after the first 16 h of germination, in which no mitosis was found, the percentage of dividing cells increased up to a peak at 28—32 h. In 62%V embryos the mitotic activity was observed after the first 24 h, reaching its maximum at 32—36 h.

The onset of DNA synthesis in germinating wheat embryos was reported to be dependent on the *ex novo* synthesis of the same proteins as DNA-polymerase (MORY *et al.* 1972). Our preliminary studies indicated that the post-ribosomal fraction of a homogenate from wheat embryos presented a higher content of DNA-polymerase activity with respect to that of the

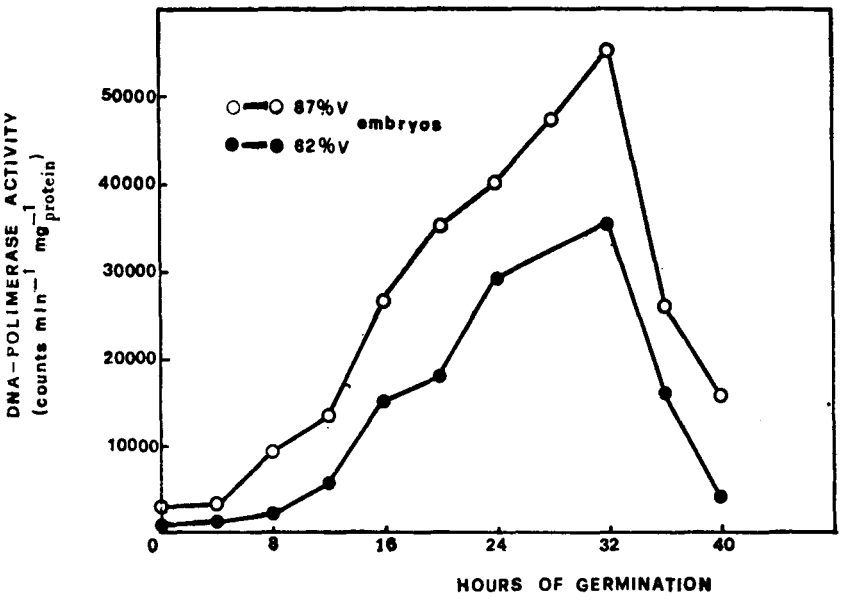

Fig. 2. DNA-polymerase activity during early hours of germination of 20 mg 87%V and 62%V wheat embryos.

corresponding subcellular fractions (data not shown). This soluble DNA-polymerase strictly dependent on added DNA, as template, in the enzymatic reaction test, was reported to be better correlated to the onset of DNA synthesis in several plant embryos (ROBINSON and BRYANT 1975, HOVEMANN and FOLMAN 1979). The time course of ^{3}H-TTP incorporation into DNA (Fig. 2) indicated that the increase of the enzymatic activity preceded the onset of the active synthesis of DNA and presented a slight peak of activity at 32 h of imbibition in both lots of germs. In 62%V embryos, DNA-polymerase activity increased at a lower rate than in 87%V embryos. In both lots of germs ^{3}H-TTP incorporation activity decreased very rapidly after the peak of highest enzymatic activity.

In order to determine the dependence of DNA synthesis on early protein synthesis and on the increase of the DNA-polymerase activity, the protein synthesis inhibitor-cycloheximide was used on germination of both lots of germs. After 0, 12 and 24 h of embryo imbibition the drug was added at the concentration of 20 μg ml^{-1} and, at the end of the first 32 h, ^{14}C-leucine and ^{3}H-thymidine incorporation and DNA-polymerase activity were tested in three series of separate experiments. The inhibiting effect of cycloheximide either on ^{14}C-leucine incorporation or on enzyme activity enhancement in

TABLE 1

Effect of cycloheximide (20 μg ml^{-1}) added, at different times of germination, to 20 mg of 87%V and 62%V wheat embryos on L-(U-^{14}C) leucine incorporation into TCA-insoluble material and on DNA-polymerase activity at the end of the first 32 h of germ imbibition

Time of inhibitor addition [h]	Embryo viability	L-(U-^{14}C) leucine incorp. counts min^{-1}	% inhib.	DNA-polymerase activity counts min^{-1} mg^{-1} protein	% inhib.
Control	87%	57390	—	46869	—
0		558	99	1972	96
12		19290	66	17492	62
24		36476	36	35748	24
Control	62%	20751	—	35861	—
0		207	99	578	98
12		6044	71	5099	86
24		12106	42	27342	24

87%V embryos was higher at 0 time than at 12 and 24 h of drug addition (Table 1). In 62%V embryos there was a more enhanced inhibiting effect on DNA-polymerase increase at 12 h of cycloheximide addition than in viable germs. At 24 h of inhibitor addition the percent decrease of enzymatic activity was similar in both lots of germs. DNA synthesis in 87%V embryos was gradually inhibited when cycloheximide was added at 0 and 24 h, whereas at 24 h the inhibition was negligible (Table 2). On the other hand, in 62%V germs a strong inhibition on ^{3}H-thymidine incorporation was observed both at 0 and 12 h of cycloheximide addition, whereas at 24 h no inhibiting effect of the drug was found.

These data show that the inhibitory effect of cycloheximide on protein synthesis was stronger than that on DNA synthesis and that the drug inhibited DNA synthesis indirectly by inhibiting the protein synthesis necessary for DNA synthesis. This separate effect of cycloheximide on protein

TABLE 2

Effect of cycloheximide (20 μg ml^{-1}) added, at different times of germination, to 20 mg of 87%V and 62%V wheat embryos on (6-^{3}H) thymidine incorporation into TCA-insoluble material at the end of the first 32 h of germ imbibition

Time of inhibitor addition [h]	Embryo viability	Radioactivity Counts min^{-1}	% inhib.
Control	87%	1796	—
0		342	81
12		869	51
24		1690	6
Control	62%	1060	—
0		205	81
12		223	79
24		981	7

and DNA synthesis has already been reported in germinating *Vicia faba* (JAKOB and BOVERY 1969) and in potato tuber slices (WATANABE and IMASEKI 1976). Also MORY *et al.* (1972) demonstrated the dependence of the onset of DNA synthesis on early protein synthesis in germinating wheat embryos, using blasticidine S, a strong inhibitor at the peptide chain elongation level.

In aged embryos, both the delay of the onset of DNA-synthesis and of the mitotic activity and the prolonged effect of cycloheximide on the increase of DNA-polymerase activity and on the labeled thymidine incorporation might be due to a longer phase of activation of some biochemical processes, connected with DNA synthesis, in comparison to that in viable germs. These processes include also removal of inhibitors, synthesis and/or activation of some enzymes directly involved in the polymerization of DNA and availability of an active DNA template (MORY *et al.* 1972). Therefore it is likely that the ageing may induce some changes in regulatory systems, affecting DNA replication and its repair, as already reported in aged wheat embryos by SCHIMPFF *et al.* (1978). On the other hand, a long period of synthesis and turnover of proteins could result in the gradual repair of enzyme lesions, already reducing in this way the macromolecular damages to a minimum during the early hours of aged wheat embryo germination.

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BOOK REVIEW

HAWKES, J. G., LESTER, R. N., SKELDING, A. D. (ed.): THE BIOLOGY AND TAXONOMY OF THE *Solanaceae*. — Academic Press, London 1979. Pp. 738, £ 45.00, US $ 93.25.

This voluminous book appeared as volume 7 of the Linnean Society Symposium Series; it deals with one of the largest families of flowering plants which includes many of economically and medically important species. The volume contains papers presented at the international symposium of the same name, which was organized by the staff of the Department of Plant Biology of the University of Birmingham and the Linnean Society of London on July 13—17, 1976. This symposium was attended by experts in diverse fields of plant sciences united by a common interest in the object of investigation — the *Solanaceae* family.

The contribution are divided into nine sections. The first one "Taxonomy and floristics" (7 papers) presented a general systematic overview of the family and its representations in different parts of the world. The second section "Ethnobotany" (3 papers) introduces the subject of folk uses and ethnobotanical aspects of *Solanaceae*. The next two sections deal with chemical compounds important to mankind "Alkaloids" (7 papers) and "Flavonoids, terpenes and proteins" (5 papers). Anatomical and morphological studies are concentrated in section five "Anatomy and fine structure" (4 papers) and six "Morphology and morphogenesis" (4 papers). The seventh section (16 papers) entitled "Floral biology, incompatibility and haploidy" deals with cytogenesis and reproductive biology. In the comprehensive last two sections "Biosystematics of genera and sections" (9 papers) and "Biosystematics of domesticates" (10 papers) are grouped chapters on wild genera and sections and domesticated taxa with special emphasis on the improvement of cultivated plant taxas by breeding. All chapters are well arranged and illustrated by tables, maps, graphs, pictures and photos. The aim of the twelwe-point resolution of the symposium, published at the end of proceedings, is to improve and set the trend of the future work.

This book is a comprehensive review of the family *Solanaceae* and will serve to taxonomists, botanists, chemists, biochemists, genetists and breeders for many years as a prime source of information and references. It is complemented by Taxonomic and General indexes and is well printed and produced.

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