CHANGES IN CONCENTRATION OF PROTEIN AND NUCLEIC ACIDS IN THE ENDOSPERM DURING OVULE DEVELOPMENT IN *CLIVIA MINIATA*

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ABSTRACT

Changes of protein and nucleic acid concentrations in micropylar and chalazal parts of the endosperm during exponential and stationary phases of embryo growth (*Clivia miniata* Regel) were examined.

The following facts were established: 1. evident two-phase synthesis of protein, DNA and RNA in the endosperm, 2. existence of chalaza-micropyle concentration gradients of analyzed compounds, 3. elongation of the embryo proceedes from the micropylar to the chalazal end of the ovule, i. e. in the direction oposite to that of the chalaza – micropyle protein, DNA and RNA concentration gradients, 4. decrease of concentrations of protein, DNA and RNA in the endosperm after the first maximum of these compounds.

Protein concentration was determined spectrophotometricaly using the method of Lowry, RNA concentration was determined with the orcin method, and concentration of DNA with the diphenylamine method.

KEY WORDS: *Clivia miniata* Regel, gradient of protein, DNA and RNA concentration, endosperm.

INTRODUCTION

Generally, in up-to date literature concerning seed development (endosperm tissue, cotyledons) in monocotyledonous (Ingle et al. 1965) and dicotyledonous plants (Payne et al. 1971; Manteuffel et al. 1976) two or three phases of development are distinguished: 1. phase of intensive mitotic divisions, 2. phase of cells expansion comprising synthesis of storage compounds – starch and proteins, 3. maturation phase – further synthesis of storage compounds, dehydration, beginning of seed dormancy. Sometimes phases 1 and 2 are merged – monocotyledonous plants.

In the authors opinion the above mentioned phases suggest the existence of two phases of proteins synthesis: first – structural and enzymatic proteins and the second – storage proteins.

In previous papers (Ryczkowski 1980) the existence of chalaza → micropyle gradients of osmotic value, concentrations of sugars, free amino acids and metals (Ryczkowski and Reczyński 1988) in the endosperm tissue during embryogenesis was presented.

In few cases (Ingle et al. 1965, Coccuci et al. 1965, Mehta et al. 1972) a decrease of RNA and DNA content (in 2-nd and 3-rd phases) in the endosperm tissue during development was reported.

The present paper contains the results of determination of protein, DNA and RNA concentrations in the endosperm tissue (during the exponential and stationary phases of embryo growth), performed with the aim to establish: 1. the existence of two phases of proteins synthesis in the endosperm tissue; structural and enzymatic (first phase) and reserve (second phase), and relating phases of DNA and RNA synthesis, 2. the existence of chalaza → micropyle concentration gradients of protein, DNA and RNA, and finally 3. whether there occurs a degradation of DNA, RNA (and protein) in the endosperm tissue.

MATERIAL AND METHODS

For research a monocotyledonous plant – *Clivia miniata* Regel, cultivated in a greenhouse was used. Seeds were marked after the perianth dropped. The material was taken twice a week between 8^{00} and 9^{00} a.m.

Ovules preparations, their division into micropylar and chalazal parts and isolation of micropylar (Mpen) and chalazal (Chpen) parts of the endosperm tissue were presented earlier (Ryczkowski 1967). For a single analysis material from 10-15 ovules was used. Ovules of *Clivia miniata* were used due to their fairly big dimensions and long period of development. The dimensions of ovules and embryos as well as the ovule age, counted from the day the perianth dropped till the day of sampling, constituted the adopted developmental criteria.

Determination of proteins concentration.

After initial homogenization, the lipids were removed from the sample with the use of ethanol and diethylene ether mixture (3:1 v/v). Next, proteins were extracted with 0.5 M NaOH solution (2°C) from the tissue residue. After centrifugation (10 min., 10000 x g, 2°C), to the supernatant 10%

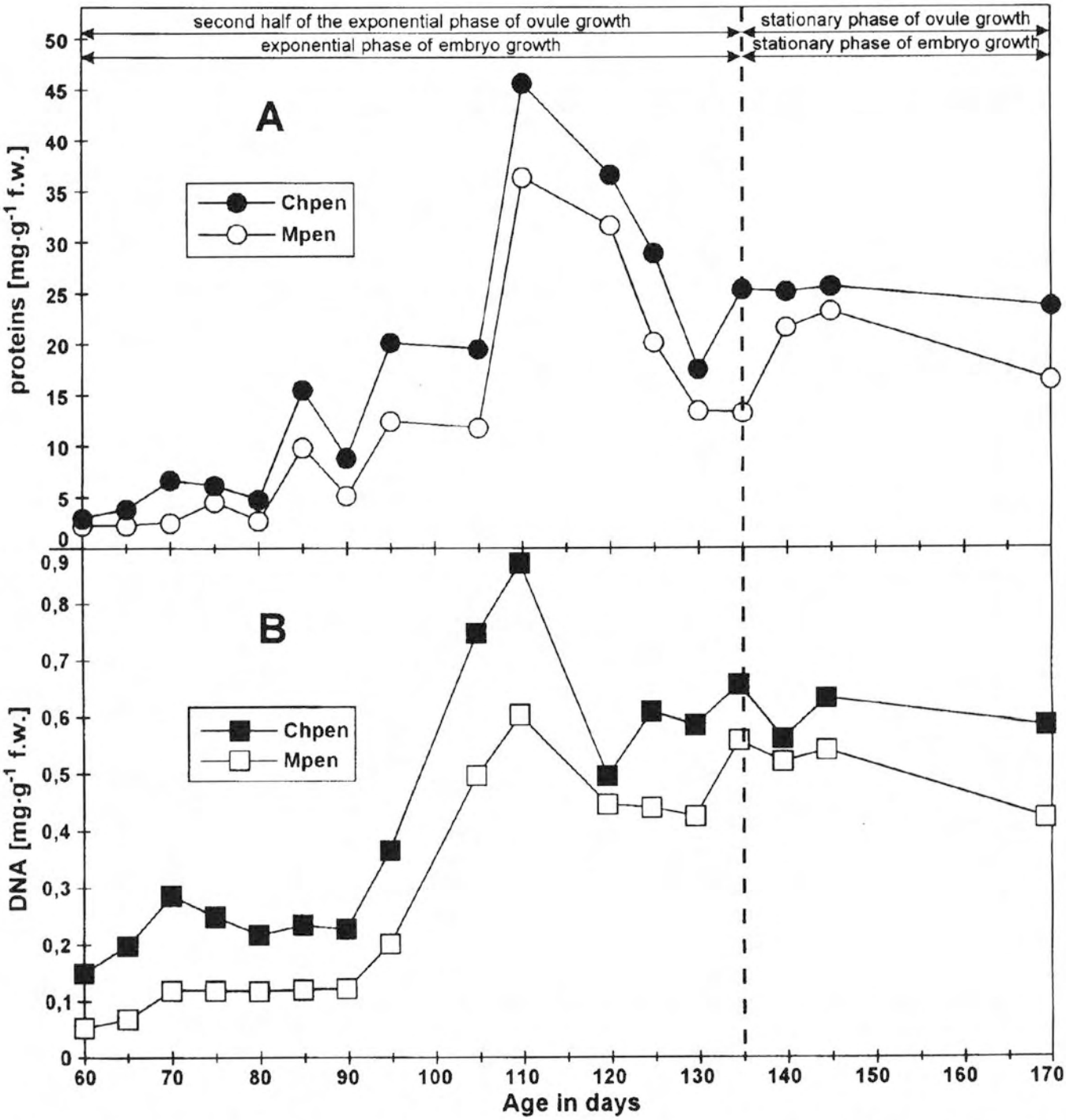

Fig. 1. Changes of protein (A) and DNA (B) concentrations in chalazal (Chpen) and micropylar (Mpen) parts of the endosperm during exponential and stationary phases of embryo growth (*Clivia miniata* Regel).

CCl_3COOH solution was added to precipitate proteins. After centrifugatung, the precipitate was washed with diethyl ether and dissolved with 0.5 M NaOH. The procedure described above was repeated three times. Protein concentration was determined spectrophotometricaly using the method of Lowry. The bovine serum albumin (BSA) was used as a standard (Lowry et al. 1951).

Determination of RNA and DNA concentration.

The endosperm was fractionated according to the modified method of Ogur and Rosen (1950). After homogenization, the lipids were removed from the sample with the use of ethanol and diethylene ether mixture (3:1 v/v). Next, from the tissue residue acid dissolving compounds (free ribo- and desoxyribonucleotides) were extracted with 0.2 M $HClO_4$ solution (2°C). This procedure was repeated three times. After centrifugation (10 min., 10000 x g, 2°C), the precipitate containing acid not dissolving compounds (DNA, RNA) was incubated with addition of 0.9 M $HClO_4$ solution for 20 min. at 90°C and finally centrifuged (10 min., 10000 x g, 25°C). In supernatant, concentration of RNA was determined with the orcin method, and concentration of DNA with the diphenylamine method (Schneider 1957, Ryczkowski and Bochnia 1993).

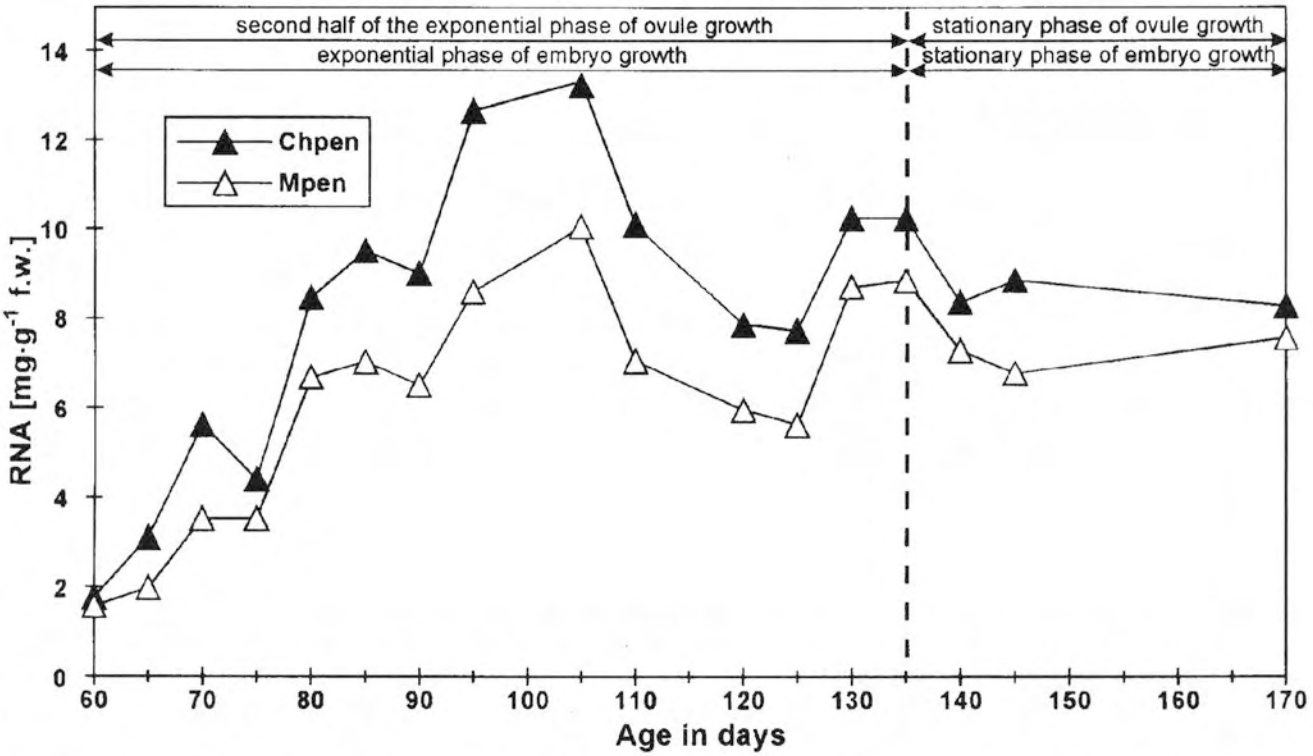

Fig. 2. Changes of RNA concentrations in chalazal (Chpen) and micropylar (Mpen) parts of the endosperm during exponential and stationary phases of embryo growth (*Clivia miniata* Regel).

All obtained values presented in figures are the mean of three separate analyses, and the relative error of determinations is less than 5%.

RESULTS

Protein.

Concentration of protein in 60-105 days old Chpen (chalazal part of endosperm) increased irregularly from 2.94 to 19.52 mg.g^{-1} f.w. (Fig. 1A). In 110 days old Chpen, protein concentration attained its I maximum (45.66 mg.g^{-1} f.w., Fig. 1A), and then rapidly decreased to 17.54 mg.g^{-1} f.w. (Chpen 130 days old, Fig. 1A). Chpen between 135 and 145 days was characterized by another increase of protein concentration up to 25.69 mg.g^{-1} f.w. (Fig. 1A; II maximum). In 170 days old Chpen slight a decrease of protein concentration was found (23.71 mg.g^{-1} f.w., Fig. 1A).

An analogous course of protein concentration changes in Mpen (micropylar part of endosperm, 60-170 days) was found, but in all cases the obtained values of protein in Mpen were lower than in Chpen at the same stages of development (Fig. 1A).

DNA.

The concentration of DNA in Chpen (60-90 days) increased irregularly from 0.15 to 0.23 mg.g^{-1} f.w., Fig. 1B. Subsequently DNA concentration rapidly increased from 0.37 to 0.87 mg.g^{-1} f.w. (Chpen 95-110 days; I maximum, Fig. 1B) and decreased to 0.50 mg.g^{-1} f.w. in 120 days old Chpen. In Chpen between 125 and 135 days, a consecutive increase of DNA concentration took place (from 0.61 to 0.66 mg.g^{-1} f.w., (Fig. 1B, II maximum). In 170 days old Chpen a slight decrease of DNA concentration (to 0.58 mg.g^{-1} f.w.) was recorded (Fig. 1B).

The course of DNA concentration changes in 60-120 days old Mpen (I maximum) was analogous to the changes in Chpen, but concentration in Mpen was always lower than in Chpen at the same stages of development (Fig. 1B).

Changes of DNA concentration in Mpen (125-130 days) differed slightly from the changes in Chpen, but the second maximum in Mpen was also found (Mpen 135 days old, Fig. 1B). In 170 days old Mpen a decrease of DNA concentration to 0.42 mg.g^{-1} f.w. was determined.

RNA.

RNA concentration in Chpen (60-105 days) increased irregularly from 1.77 to 13.32 mg.g^{-1} f.w. up to the first maximum (Fig. 2). Later (110-125 days) in Chpen the decrease of RNA concentration from 10.10 to 7.73 mg.g^{-1} g f.w. (Fig. 2), followed by an increase to 10.25 mg.g^{-1} f.w. (130-135 days) was found (II maximum, Fig. 2). In Chpen 140-170 days old, RNA concentration was lower – 8.27 mg/g f.w., Fig. 2.

The course of RNA concentration changes in Mpen (60-170 days) was similar to the changes in Chpen and in all cases the values obtained for Mpen were lower than for Chpen at particular stages of embryogenesis (Fig. 2).

DISCUSSION

Basing on the obtained data concerning concentration of proteins, DNA and RNA in the endosperm during the exponential and stationary phase of embryo growth, the following

facts were established: 1. evident two-phase synthesis of protein, DNA and RNA, 2. existence of chalaza-micropyle concentration gradients of analyzed compounds, 3. decrease of concentration of protein, DNA and RNA in endosperm after the first maximum (Fig. 1 A-B, Fig. 2).

The authors assumed, that the increase of concentration of proteins, DNA and RNA in endosperm (age: 60-100 days) was generally connected with division and expansion of cells of that tissue (Jennings and Morton 1963, Ingle et al. 1965). It could not be excluded, that the I maximum of concentration was a result of DNA endoreduplication (Nagl 1987). DNA endoreduplication was also reported in cotyledons of dicotyledonous plants (Scharpe and Van Parijs 1973, Smith 1973). It is obvious, that during the discussed period of embryogenesis synthesis of DNA, RNA and structural and enzymatic proteins took place in the endosperm. The first maximum of protein concentration coincided with the decrease of free amino acids concentration (Ryczkowski 1970). Most probably inactivation of synthesis, as well as, partial degradation of enzymes and redundant forms of DNA and RNA, took place by the end of the exponential phase of ovule growth (decrease of concentration Fig. 1 A-B, Fig. 2).

The second maximum of proteins, DNA and RNA concentration (endosperm 130-145 days, Fig. 1 A-B, Fig. 2) was, probably connected with the synthesis of reserve proteins (Jennings and Morton 1963, Ingle et al. 1965, Mauntenffel et al. 1976).

After the second maximum (of protein, DNA and RNA) concentrations mild decrease of their concentrations was found (Fig. 1 A-B, Fig. 2). The decrease of concentrations (contents) of DNA and RNA in the endosperm was established by Ingle et al. 1965; Cocucci and Sturani 1965; Mehta et al. 1972.

The decrease of RNA concentration in endosperm might result from the increase of ribonuclease activity (Ingle et al. 1965, Mehta et al. 1972), inactivation of the process of its synthesis and degradation (Cocucci and Sturani 1965). The decrease of DNA content (concentration) was found by Ingle et al. (1965) in maize endosperm, and in seeds of *Brassica napus* (Ching et al. 1974). In our opinion, the decrease of DNA and RNA concentration (autodegradation) might have been connected with the drastic decrease of respiration intensity (almost to zero; Ryczkowski 1976) and the lack of sufficient energy (Ching et al. 1974) required for these compounds synthesis and their stabilization.

The increase and decrease of protein, DNA and RNA concentrations took place during the decrease of water content in endosperm from 92.5% to 81.3% in relation to f.w.

The chalaza-micropyle gradient of protein, DNA and RNA is consistent with certain suggestions and literature data (Wardlaw 1953; Mikulska et al. 1967; Konopska 1972). The obtained results are in good agreement with data of previous autors concerning chalaza-micropyle gradients of osmotic value, concentrations of sugars, free amino acids, respiration rate, (Ryczkowski 1980) and concentrations of metals (Ryczkowski and Reczyński 1988). The developing embryo elongates in the opposite direction, i.e. from micropyle to chalaza. The issue concerning physico-chemical. gradients in the endosperm during ovule development was widely discussed in the previous papers (Ryczkowski 1967, 1980; Ryczkowski and Reczyński 1988).

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ZMIANY STĘŻENIA BIAŁKA I KWASÓW NUKLEINOWYCH W BIELMIE PODCZAS ROZWOJU ZALĄŻKA U *CLIVIA MINIATA*

STRESZCZENIE

Badania dotyczyły zmian stężeń białka i kwasów nukleinowych w mikropylarnej i chalazalnej części bielma podczas wykładniczej i stacjonarnej fazy wzrostu zarodka (*Clivia miniata* Regel). Stwierdzono następujące fakty: 1. wyraźną dwufazową biosyntezę białka, DNA i RNA w bielmie, 2. gradient stężenia tych związków od chalazy do mikropyle, (zarodek rośnie i wydłuża się od mikropyle do chalazy to jest naprzeciw gradientu chalaza → mikropyle tych związków), 3. spadek stężenia białka, DNA i RNA w bielmie po osiągnięciu przez nie I maksimum (prawdopodobnie związany z inaktywacją procesów syntezy oraz częściowa degradacja białek enzymatycznych i zbędnych form DNA i RNA), 4. po II maksimum (stężenia białka, DNA i RNA), stwierdzono łagodny spadek stężenia tych związków, przypuszczalnie uwarunkowany spadkiem natężenia oddychania i brakiem odpowiedniej puli energii niezbędnej do ich syntezy i stabilizacji.

Stężenie białka oznaczane było metodą Lowry'ego, RNA metodą orcynolową a DNA metodą difenyloaminową.

SŁOWA KLUCZOWE: *Clivia miniata* Regel, gradient stężenia białka, DNA i RNA, bielmo.