ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 50, nr 1—2: 295—301
1981

Further observations on the physico-biochemical and physiological gradients in the ovule during embryogenesis

MARIAN RYCZKOWSKI

Department of Plant Physiology, Institute of Molecular Biology,
Jagiellonian University, ul. Grodzka 53, 31-001 Kraków, Poland

Abstract

In the developing ovule (*Clivia sp.* and *Clivia miniata;* exponential phase of embryo growth) the following gradients were found: of osmotic value, concentration of free amino acids, respiration rate in the endosperm tissue, and osmotic value and respiration rate gradients in the embryo. Generally these gradients (with the exception of respiration rate in the embryo) were directed from the chalaza to the micropyle i.e. in the opposite direction of the embryo elongation (micropyle→chalaza). The obtained results suggest of the direction of some gradients can change during the ovule development.

INTRODUCTION

The previous paper (R y c z k o w s k i, 1980) was a short summary of investigations on the physico-biochemical and physiological gradients in the coat, endosperm tissue and embryo in the developing ovule of *Haemanthus Katharinae*. These investigations were performed during the proembryo and embryo stages. The most important established facts were following: 1) different kinds of gradients were found in the coat, endosperm and embryo during the development of the ovule; 2) the embryo (in the proembryo and embryo stages) is under permanent influence of the chalaza→micropyle gradient of low molecular compounds; 3) generally the direction of different kinds of gradients in the coat; endosperm and partly in the embryo itself is reverse to the direction of the embryo elongation; 4) the directions of gradients can change during the development of the ovule.

It should be stressed that in embryological literature (W a r d l a w, 1955, 1965; P o d d u b n a y a - A r n o l d i, 1964) there are indications concerning the existence of different kinds of gradients in the developing ovule, and their influence on embryogenesis.

The present paper is a short summary of the investigations on different kinds of gradients in the endosperm tissue and embryo in the developing ovule of *Clivia sp.* and *Clivia miniata* (R y c z k o w s k i, 1971, 1976). It was undertaken with the aim to supply further evidence on the existence of gradients in the developing ovule.

MATERIAL AND METHODS

Ovules of *Clivia sp.* were used as the material for experiments on the osmotic gradients and respiration rate (QO_2) gradients; *Clivia miniata* RGL ovules were used for determination of the concentration gradient of free amino acids. The number of days counted from the day the perianth dropped to the day the material was taken for investigations, and dimensions of ovules and embryos were the adopted criteria of the age of ovules, endosperm tissue and embryos. For the sake of brevity the tables contain only the criteria of age in days and dimensions of embryos. The experimental material was collected between 8 and 9 a.m.

The procedure of measuring the dimensions of ovules and embryos was described in previous papers (R y c z k o w s k i, 1960a, 1962).

The technique of endosperm and embryo preparation, their division into chalazal and micropylar parts (halves) was given in previous paper (R y c z k o w s k i, 1967).

Following denotations (abbreviations) were used in this paper: Chpen — chalazal part of the endosperm tissue, Mpen — micropylar part of the endosperm, Chpe — chalazal part of the embryo with cotyledon, Mpe — micropylar part of the embryo with radicle, OV — osmotic value, and QO_2 — respiration rate.

The osmotic value was determined by means of the thermoelectric method (R y c z k o w s k i, 1960a, b), the concentration of free amino acids by use of a Micro Column Automatic Amino Acid Analyser (Technicon Instruments Co Ltd., England; R y c z k o w s k i, 1971), the QO_2 of the endosperm tissue was measured with a Warburg apparatus (R y c z k o w s k i, 1976), and the QO_2 of the embryo by means of the microrespirometric method (R y c z k o w s k i, S z e w c z y k, 1973). The results presented in the tables are the means of 2-3 measurements.

RESULTS

Osmotic value gradients (endosperm tissue and embryo).

1. The osmotic value of the sap from the chalazal part of each of the examined tissues (endosperm and embryo) is always higher than that of the micropylar one of the same tissues (Table 1). 2. During the

exponential phase of the embryo growth there is a distinct chalaza→micropyle osmotic value gradient in the endosperm tissue and embryo itself (Table 1). 3. The elongation of the embryo proceeds in the direction opposite to the osmotic value gradients in the endosperm tissue and embryo itself. 4. The osmotic value of the sap from the embryo is much higher than that of the sap from the endosperm tissue surrounding it (Table 1).

Table 1

Osmotic value (chalaza→micropyle) gradient in the endosperm tissue and embryo of *Clivia sp.* during the exponential phase of embryo growth

Age in days	Dimensions of embryos (mm)	OV of the sap endosperm (M/l) Chpen Mpen	Chpen-Mpen difference	Mean value	OV of the sap embryos (M/l) Chpe Mpe	Chpe-Mpe difference	Mean value
74	1.1×0.9	0.275>0.244	0.031	0.259			
75	2.5×1.3	0.330>0.270	0.060	0.300			
86	3.4×1.5	0.285>0.229	0.056	0.257			
92	4.1×1.8	0.323>0.226	0.097	0.274			
97	6.3×1.9				0.446>0.405	0.041	0.425
97	8.6×2.0				0.401>0.336	0.064	0.368
102	9.3×2.3	0.324>0.266	0.058	0.295	0.379>0.339	0.040	0.359
114	10.1×2.2	0.315>0.279	0.036	0.297	0.389>0.317	0.072	0.353
136	10.8×2.3	0.349>0.225	0.124	0.287	0.354>0.268	0.086	0.311

Abbreviations: Chpen — chalazal part of the endosperm tissue; Mpen — micropylar part of the endosperm; Chpe — chalazal part of the embryo with cotyledon: Mpe — micropylar part of the embryo with radicle: OV — osmotic value: QO_2 — respiration rate.

Table 2

Free amino acids (chalaza→micropyle) concentration gradient in the endosperm tissue of *Clivia miniata* during the exponential phase of the embryo growth

Age in days	Dimensions of embryos (mm)	Total concentration in nmol×g fresh weight^{-1}			
		endosperm tissue			embryo
		Chpen Mpen	Chpen-Mpen difference	mean value	
48	1.0×0.9	6374> 4745	1629	5559	
53	1.1×1.0	8053> 6271	1782	7126	
62	3.6×1.5	14588>10950	3638	12769	
69	4.6×1.6	13069>11542	1527	12305	
53	1.0×0.9	8064> 5609	2455	6837	
70	3.3×1.6	8715> 6642	2073	7679	5274
79	6.4×1.9	11163> 9003	2160	10083	50483
88	7.3×2.6	10707> 8679	2028	9693	55285
104	9.1×2.4	12723> 5139	7584	8931	51098

Abbreviations as in Table 1.

Free amino acid concentration gradient (endosperm tissue).

1. The concentration of free amino acids in the chalazal part of the endosperm tissue is higher than that of the micropylar one of the same tissue (Table 2). 2. During the exponential phase of the embryo growth there is a distinct chalaza→micropyle free amino acid concentration gradient in the endosperm tissue surrounding it (Table 2). 3. The elongation of the embryo proceeds in the direction opposite to the free amino acid concentration gradient in the endosperm tissue. 4. Generally the free amino acid concentration in the embryo is much higher than that in the endosperm tissue (Table 2).

Respiration rate (QO_2) gradient (endosperm tissue and embryo).

1. The QO_2 of the chalazal part of the endosperm tissue is higher than this value established for this tissue of the micropylar part (with the exception of the 69-day old endosperm tissue — Table 3). 2. During the exponential phase of the embryo growth there is a distinct QO_2 chalaza→micropyle gradient in the endosperm tissue (Table 3). 3. The elongation of the embryo proceeds from the micropyle i.e. in a direction reverse to the direction of the QO_2 gradient in the endosperm tissue.

Table 3

Respiration rate (chalaza→micropyle) gradient in the endosperm tissue during the exponential phase of *Clivia sp.* embryo growth

Age in days	Dimensions of embryos (mm)	Respiration rate ($mg\ CO_2 \times h^{-1} \times g$ fresh weight^{-1})		
		Chpen Mpen	Chpen-Mpen difference	mean value
69		0.041 < 0.055	(0.014)	0.048
81	2.8 × 1.4	0.056 > 0.042	0.014	0.049
85	6.5 × 2.0	0.050 > 0.036	0.014	0,043
95	5.4 × 2.0	0.052 > 0.046	0.006	0.049
102	7.1 × 2.2	0.067 > 0.030	0.037	0.049
109	8.7 × 2.3	0.056 > 0.043	0.013	0.050
120	9.7 × 2.2	0.052 > 0.037	0.015	0.045
127	10.0 × 3.4	0.058 > 0.037	0.021	0.048

Abbreviations as in Table 1.

4. The QO_2 of the micropylar part of the embryo (embryos 96-127-day old) is higher than that of the chalazal one (Table 4). 5. In these embryos the QO_2 gradient is directed from the micropyle to the chalaza, and it is inverse to the QO_2 gradient in the endosperm tissue (Table 3 and 4). 6. In 96-127-day old embryos the direction of the QO_2 gradient is consistent with the direction of embryo elongation itself. 7. The QO_2 of the dividing embryo is higher than this value of the intact embryo (Table 4). 8. The QO_2 of the divided and intact embryos is much higher than this value of the endosperm tissue (Table 3 and 4).

Table 4
Respiration rate (micropyle→chalaza) gradient in the *Clivia sp.* embryo during the exponential phase of its growth

Age in days	Dimensions of embryos (mm)	Respiration rate ($mg\ CO_2 \times h^{-1} \times g$ fresh weight^{-1})			
		Chpe Mpe	Chpe-Mpe difference	mean value	intact embryo
86	3.8×1.8	0.842>0.651	(0.191)	0.746	0.557
96	6.4×1.9	0.381<0.451	0.070	0.416	0.345
102	9.1×2.2	0.262<0.317	0.055	0.289	0.220
109	8.7×2.3	0.274<0.321	0.047	0.297	0.238
120	9.7×2.2	0.105<0.283	0.178	0.194	0.243
127	9.0×2.4	0.189<0.227	0.038	0.208	0.180

Abbreviations as in Table 1.

DISCUSSION

Relations between the gradients and developing embryo, general statsments.

1. The following gradients of osmotic value, free amino acids concentration, and respiration rate were found in the endosperm tissue during the exponential phase of the embryo growth i.e. during the differentiation of the proper embryo (Fig. 1A-B). Osmotic value, and respiration rate gradients were also found in the developing embryo (Fig. 1B).

2. The chalaza→micropyle direction of the osmotic value gradient in the endosperm tissue (generally) overlaps the direction of the free amino acid concentration, and respiration rate gradients in this tissue (Fig. 1A).

3. The chalaza→micropyle direction of the osmotic value gradient in the embryo does not superimpose the direction of the respiration rate gradient in it (Fig. 1B). The reverse direction (micropyle→chalaza) of the respiration rate gradient in the embryo is not consistent with the directions of gradients in the endosperm tissue either (Fig. 1A-B).

4. During the embryo differentiation a chalaza→micropyle gradient of low molecular compounds occurs in its environment i.e. in the ovule (endosperm tissue) and embryo itself. The embryo elongation is reverse to the direction of this gradient (Fig. 1A-B).

5. The occurrence of a distinct osmotic value, and free amino acid concentration (chalaza→micropyle) gradients in the endosperm tissue at the begining of the embryo differentiation (dimensions of embryo 1.0 × 0.9 mm; Table 1 and 2) suggests that these gradients exist in the ovule (and endosperm tissue) also during the proembryo stage.

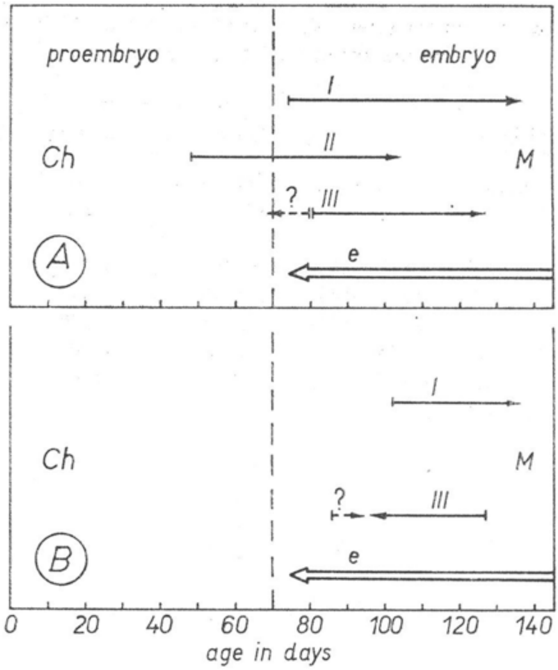

Fig. 1A-B. *Clivia sp.* and *Clivia miniata*

Scheme of the directions of physico-biochemical and physiological gradients in the endosperm tissue (A) and embryo (B) during the ovule development (exponential phase of the embryo growth i.e. differentiation of the embryo proper). Abscissa: age of the ovule, endosperm tissue and embryo in days counted from the day the perianth dropped to the day of sampling; ordinate: left — chalazal end of the ovule (Ch), right — micropylar end of the ovule (M). Arrows: I — direction of the osmotic value gradient, II — direction of free amino acid concentration gradient, III — directions of respiration rate gradients, e — direction of the embryo elongation (micropyle→chalaza).

General characteristics of gradients.

a) The directions of different gradients in various tissues of the ovule or within particular tissues need not be the same. b) The results concerning the respiration rate gradient in the embryo and endosperm tissue (Table 3, 4; Fig. 1A-B) suggest a possibility of a change of the direction of these gradients during the development of the ovule. c) This change may not occur at the same time during the ovule development.

SUPPLEMENT

It should be stressed that the direction of the osmotic value and respiration rate gradients in the endosperm tissue and embryo of the investigated species are the same as the directions of these gradients in these tissues in a *Haemanthus Katharinae* ovule during the exponential phase of the embryo growth (Ryczkowski, 1980). A small difference has been found beween the direction of the free amino acid concentration gradient *(Clivia miniata)* and the direction of the amino-N concentration gradient (*Haemanthus Katharinae;* Ryczkowski, 1977).

Particular gradients and causes of their occurrence in the basic tissues of the developing ovule were discussed in previous papers (Ryczkowski, 1967, 1971, 1976, 1977 and 1978).

The obtained results are in agreement with Wardlaw's (1955, 1965) hypothesis concerning the gradient of low molecular compounds in the developing ovule (embryo-sac, egg cell, zygote) and embryo, and with the investigations of Jensen (1965) on the ultrastructure of the synergids, and with Forman and Jensen's (1965) results on the respiration rate of the developing embryo of *Gossypium hirsutum.*

REFERENCES

Forman M., Jensen W. A., 1965. Plant Physiol. 40: 765-769.

Jensen W. A., 1965. Amer. J. Bot. 52: 238-256.

Poddubnaya-Arnoldi V. A., 1964. General embryology of angiosperms. Moscow (in Russian).

Ryczkowski M., 1960a. Bull. Acad. Polon. Sci., Ser. sci. biol. 8: 143-148.

Ryczkowski M., 1960b. Planta. 55: 343-356.

Ryczkowski M., 1962. Bull. Acad. Polon. Sci., Ser. sci. biol. 10: 375-380.

Ryczkowski M., 1967. Acta Soc. Bot. Pol. 36: 627-638.

Ryczkowski M., 1971. Bull. Acad. Polon. Sci., Ser. sci. biol. 19: 801-806.

Ryczkowski M., 1976. Biochem. Physiol. Pflanzen 170: 1-8.

Ryczkowski M., 1977. Bull. Acad. Polon. Sci., Ser. sci. biol. 1976. 24: 765-769.

Ryczkowski M., 1978. Soc. bot. Fr., Actualites botaniques 125: 285-288.

Ryczkowski M., 1980. Soc. bot. Fr., Actualites botaniques 127: 51-58.

Ryczkowski M., Szewczyk E., 1973. Bull. Acad. Polon. Sci., Ser. sci. biol. 21: 695-699.

Wardlaw C. W., 1955. Embryogenesis in plants. London, New York.

Wardlaw C. W., 1965. In: Encyclopedia of plant physiology. 15/1. Rhuland (ed.). Berlin, Heidelberg, New York.