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The concentration of elements in the central vacuole sap and endosperm tissue during embryogenesis (*Clivia miniata* Regel)

MARIAN RYCZKOWSKI, WITOLD RECZYŃSKI

Department of Plant Physiology and Development, Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, 31-120 Kraków, Poland

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Abstract

Changes in the concentration of eight elements (K, Na, Ca, Mg, Fe, Mn, Zn, Cu) in the central vacuole sap and endosperm tissue during development of *Clivia miniata* ovules using atomic absorption spectrometry were determined. Generally, the concentration of most of the elements in the central vacuole sap first increased to a certain maximum and then decreased (proembryo stage). In the endosperm tissue (proper embryo stage) the concentration of elements showed either a tendency to increase (K, Ca, Mg, Fe, Zn, Cu) or no visible change (Na, Mn) during embryogenesis. The concentration of the investigated elements (except Ca) was distinctly higher in the endosperm tissue than in the central vacuole sap. It is suggested that the elements contained in the sap were selectively taken up by the developing endosperm tissue and embryo. The final conclusion is that such selectivity leads to a gradual increase in the concentrations of these elements from the lowest in the central vacuole sap, medium in the endosperm tissue, to the highest concentration in the developing embryo.

Key words: *concentration of elements*, *central vacuole sap*, *endosperm tissue*, *embryogenesis*, *ovule*, *Clivia miniata Regel*

INTRODUCTION

The present paper is a continuation of quantitative investigations on the chemico-physical properties of the ovule (central vacuole sap and endosperm tissue) during embryogenesis (proembryo and proper embryo stages).

Investigations on the occurrence and concentration of particular elements

in the central vacuole sap, endosperm tissue (and embryo) during ovule development were undertaken for two reasons: first is the lack of such data in embryological literature (Wardlaw 1955, Maheshwari 1963, Erdelska 1983, Johri 1984), second is their usefulness for *in vitro* culture of embryo and endosperm tissue, fragments of these tissues or protoplasts obtained from them. The properties of the synthetic medium used for embryo or protoplast culture *in vitro* should be similar to those found for the central vacuole sap or endosperm tissue at a particular stage of embryo development.

In two previous papers (Ryczkowski et al. 1986, Ryczkowski and Reczyński 1986) we reported on the concentration of elements (K, Na, Ca, Mg, Fe, Mn, Zn and Cu) in the central vacuole sap and embryo during the beginning of embryo differentiation and the exponential phase of its growth (dicots).

This paper describes the results of determinations of the concentration of elements in the central vacuole sap and endosperm tissue of developing ovules (monocots), carried out with the purpose of: a) following changes in their concentrations in the central vacuole sap (proembryo stage – inhibition phase of growth), and endosperm tissue (embryo proper stage – exponential phase of growth); b) comparing the obtained results with the data established for the central vacuole and embryo in the developing ovule of dicots.

MATERIAL AND METHODS

The experimental material was the ovules of *Clivia miniata* Regel plants (monocots) cultivated in a greenhouse, collected between 8 and 9 a.m. The age of the embryos was established on the basis of their dimensions. The procedure of measuring embryos was described previously (Ryczkowski 1960b, 1962b). Sap was collected using polyethylene pipets and transferred to polyethylene tubes. The sap-containing tubes were centrifuged for 10 min. at 10000 rpm. The supernatants were transferred into polyethylene containers, tightly covered with parafilm "M" and frozen until determination.

The endosperm tissue without the embryo, integuments and nucellus was placed in a glass vessel and after being weighed (fr. wt.) dried to a constant weight in a vacuum dryer at 100°C ± 2°C. The tissue was then powdered in an agate mortar. The procedure used for digesting the powdered sample was analogical to that described earlier (Ryczkowski and Reczyński 1986), however, this time 2 cm^3 of concentrated HNO_3 and 1 cm^3 of perhydrol (per 100 mg of the sample) were used. After digestion, the dry residue was dissolved in 5 cm^3 of 1:10 aqueous solution of concentrated HNO_3.

Quantitative analysis of K, Na, Ca, Mg, Fe, Mn, Zn and Cu was performed by atomic absorption spectrometry, using a Perkin-Elmer Model 503 spectrophotometer. Concentrations of K, Na, Ca, Mg and Zn were

determined in an air-acetylene flame under standard conditions, while of Fe, Mn and Cu in an electrothermic graphite atomizer HGA-74. Optimal conditions for Fe, Mn and Cu determinations in the central vacuole sap and in the endosperm tissue were as given in Table 1.

Each reported value is the mean of at least two separate determinations.

Standard deviation (S_d) was calculated (8 repetitions of element determination in the same sample) for both series of measurements (central vacuole sap and endosperm tissue). Relative standard deviations (S_r) ($S_r = S_d$/mean value of concentration) are listed in Tables 2 and 3.

Table 1

Experimental conditions for element determination in HGA-74 graphite furnace (cvs – central vacuole sap; et – endosperm tissue)

Element		Drying		Charring		Atomization	
		s	°C	s	°C	s	°C
Fe	cvs	30	100	30	1200	10	2500
	et	25	100	30	1000	7.5	2500
Mn	cvs	30	100	30	1000	10	2600
	et	25	100	30	600	7.5	2500
Cu	cvs	30	100	30	900	10	2550
	et	25	100	30	500	7.5	2500

RESULTS

The concentration of elements in the central vacuole sap (μg cm^{-3}) during the proembryo stage – inhibition phase of its growth (dimensions of embryos – 0.10×0.10-0.49×0.48 mm).

The concentration of K in the sap was within the range of 710 μg cm^{-3}-834 μg cm^{-3} with a slight tendency to decrease during the proembryo stage (Table 2). The Ca concentration in the sap showed a small, irregular increase from 234 μg cm^{-3} to 289 μg cm^{-3}, followed by a decrease of this value to 96 μg cm^{-3} during further embryo growth (Table 2). The concentration of Na in the sap was within the range of 6.8-22.5 μg cm^{-3} with an erratic tendency to increase (younger embryos), followed by a small decrease (older embryos) (Table 2). The concentration of Mg increased markedly from 39.0 μg cm^{-3} to 81.0 μg cm^{-3} (embryos – 0.10×0.10 to 0.20×0.21 mm), and during further embryo growth this value dropped to 36.0 μg cm^{-3} (Table 2). The Fe concentration in the sap increased from 0.43 μg cm^{-3} to 0.71 μg cm^{-3} and then dropped to 0.26 μg cm^{-3} (Table 2) during further embryo growth. The Mn concentration in the sap was characterized by one small maximum – 0.20

$\mu g\ cm^{-3}$ (Table 2). Changes of Zn concentration in the sap were also characterized by one maximum, 2.10 $\mu g\ cm^{-3}$ (Table 2). The concentration of Cu in the sap showed a distinct rising tendency from 0.10 $\mu g\ cm^{-3}$ to 0.20 $\mu g\ cm^{-3}$ during the inhibition phase of embryo growth (Table 2); its concentration was the lowest among the determined elements.

The concentration of elements in the endosperm tissue ($\mu g\ g^{-1}$ fr. wt.) during the embryo proper stage – exponential phase of its growth (dimensions of embryos – 0.49×0.48-9.20×2.00 mm).

Table 2

Clivia miniata. Concentration of elements ($\mu g\ cm^{-3}$) in the central vacuole sap during embryogenesis (proembryo stage)

Dimensions of embryos, mm	K	Ca	Σ K, Ca	Na	Mg	Fe	Mn	Zn	Cu	Σ Mg, Fe, Mn, Zn, Cu
0.10 × 0.10	834	234	1068	8.1	39.0	0.43	0.12	0.60	0.02	40.17
0.11 × 0.11	819	234	1053	6.8	42.0	0.43	0.08	1.13	0.01	43.65
0.16 × 0.16	744	165	909	16.2	54.0	0.52	0.20	1.43	0.05	56.20
0.17 × 0.16	825	207	1032		57.0	0.71	0.14	1.65	0.04	59.54
0.20 × 0.21	710	289	999		81.0	0.49	0.18	1.80	0.06	83.53
0.25 × 0.26	725	220	945	12.3	58.0	0.60	0.20	2.08	0.13	61.01
0.33 × 0.31	770	145	915	11.3	39.0	0.40	0.17	2.10	0.16	41.83
0.35 × 0.35	729	104	833	14.4	39.0	0.38	0.15	1.80	0.10	41.43
0.49 × 0.48	744	96	840	22.5	36.0	0.26	0.11	1.13	0.20	37.70
Sr %	1.9	2.2		7.5	2.9	5.7	5.1	2.3	4.6	

The concentration of K in the endosperm tissue during the embryo proper stage was within the range of 1375-1984 $\mu g\ cm^{-3}$ with an erratic, rising tendency (Table 3). The Ca concentration in the endosperm tissue (embryos –

Table 3

Clivia miniata. Concentration of elements ($\mu g\ g^{-1}$ fr. wt.) in the endosperum tissue during embryogenesis (properembryo stage)

Dimensions of embryos, mm	K	Ca	Σ K, Ca	Na	Mg	Fe	Mn	Zn	Cu	Σ Mg, Fe, Mn, Zn, Cu
0.49 × 0.48	1495	88.7	1584	64.8	60.8	5.15	0.42	4.58	0.60	71.6
0.69 × 0.63	1375	73.4	1448	64.6	60.0	6.00	0.40	3.41	0.72	70.5
1.37 × 1.00	1460	69.8	1530	46.6	63.8	6.43	0.42	4.36	0.85	75.9
3.60 × 1.40	1569	62.1	1631	60.2	67.0	6.12	0.38	4.18	0.59	78.3
3.80 × 1.50	1736	54.8	1791	63.3	69.9	5.45	0.38	4.50	0.69	80.9
6.20 × 1.70	1833	64.1	1897	45.1	68.4	6.77	0.44	5.35	0.98	81.9
7.60 × 1.70	1751	64.6	1816	77.0	71.6	6.42	0.44	6.06	0.82	85.3
8.10 × 1.90	1797	70.2	1867	52.8	76.6	6.70	0.46	5.44	1.11	90.3
8.00 × 1.90	1650	72.7	1723	52.4	71.9	5.89	0.43	5.37	0.90	84.5
9.20 × 2.00	1984	59.1	2043	85.5	81.9	6.69	0.45	6.64	1.09	96.8
Sr %	2.4	13.0		5.5	3.3	16.8	9.5	4.7	9.6	

0.49 × 0.48-3.80 × 1.50 mm) decreased from 88.7 μg cm^{-3} to 54.8 μg cm^{-3}, then distinctly increased to 72.2 μg cm^{-3} during further embryo development (Table 3).

The concentration of Na was within the range of 45.1-85.5 μg g^{-1} fr. wt. (Table 3) without any distinct tendency to change in any direction. The Mg concentration in the endosperm tissue during the embryo proper stage showed an increase from 60.8 μg g^{-1} to 81.9 μg g^{-1}. The Fe concentration was characterized by a small irregular increase from 5.15 to 6.69 μg g^{-1} fr. wt. The concentration of Mn was within the limits of 0.45-0.66 μg g^{-1} (Table 3). The concentration of Cu was within the range of 0.59-1.11μg g^{-1} fr. wt., and changed in an analogical way as the concentration of Zn. Concentrations of Mn and Cu were the lowest among the determined elements (Table 3).

DISCUSSION

Basing upon the results of previous investigations (Ryczkowski 1960a, 1960b, 1962a) it has been assumed that changes of total and specific element concentrations in the central vacuole sap are mainly conditioned by two processes: 1) inflow of water with elements from vegetative organs to the ovule and central vacuole (Tammes and Van Die 1964, Pate and Hocking 1978); 2) withdrawal of particular elements from the central vacuole sap by the developing endosperm tissue (Hocking and Pate 1977).

During the first period of embryogenesis (proembryo stage, embryo dimensions 0.10 × 0.10-0.25 × 0.26 mm) the inflow rate of water with elements to the ovule and central vacuole (Hocking and Pate 1977, Pate and Hocking 1978) exceeded the uptake rate of elements by the developing endosperm tissue. In this period of embryogenesis the concentrations of the majority of elements attained their maximum in the central vacuole sap (Table 2; Ryczkowski 1960a, 1960b). During the second period of embryogenesis (proembryo stage, embryo dimensions 0.25 × 0.26-0.49 × 0.48 mm) the uptake of elements from the central vacuole sap by the developing endosperm tissue (Pate 1975, Hocking and Pate 1977) exceeded the rate of their inflow with water to the central vacuole, hence a distinct decrease in the concentration of the majority of elements in the sap (Table 2; Ryczkowski 1960a, 1960b, 1962a).

It should be stressed that due to the small dimensions of the embryo it was probably without any visible influence on changes in the concentrations of the elements.

The concentrations of elements in the central vacuole sap of the *Clivia miniata* ovule is much lower (except that of Na) than this value established for the central vacuole sap of *Aesculus hybrida* (Ryczkowski et al. 1986), and *Aesculus glabra* ovules (Ryczkowski and Reczyński 1986). Maximal

concentrations in the central vacuole sap of the *Clivia miniata* ovule (monocots) occurred during an earlier period of embryogenesis than the respective maxima in the centrel vacuole sap of *Aesculus* ovules (dicots).

The changes in the concentrations of elements in the central vacuole sap are in agreement with the sap osmotic value and sugar concentration changes in it (Ryczkowski 1960a, 1960b, 1962a).

The uptake of elements by the developing endosperm tissue from the central vacuole sap is supported by the following facts: a) occurrence of the same elements in the endosperm tissue as in the sap (Tables 2 and 3); b) much higher concentration of all elements (except Ca) in the endosperm tissue than in the central vacuole sap; c) the decrease in the concentration of elements in the sap was concomitant with the intensive development of the endosperm tissue, and complete occupation of the central vacuole by it (Ryczkowski 1960b).

Basing upon the above statements and the fact that the central vacuole and endosperm tissue exist concomitantly during the determined period of ovule development (63 days after the perianth dropped; Ryczkowski 1969) it seems justified to consider the withdrawal of some elements from the central vacuole sap by the developing endosperm tissue, and accumulation of these elements in it. These considerations are also based on the fact that the water content in the investigated endosperm tissue was about 83% of fr. wt.

It results from the ratios between particular elements (K/Na; Ca/Mg; Fe/Mn; Zn/Cu) in the central vacuole sap and in the endosperm tissue that some elements were selectively taken up from the central vacuole sap (and probably from the vegetative organs of plant) by the developing endosperm tissue. This resulted in accumulation of a predominant number of elements (except of Ca) in the developing endosperm tissue as compared with their accumulation in the central vacuole sap. This conclusion is supported by a high accumulation quotient (C_{et}/C_{vs} – concentration in the endosperm tissue/concentration in the central vacuole sap; K/K-2.17; Na/Na-4.67; Ca/Ca-0.36; Fe/Fe-13.11; Mn/Mn-2.80; Zn/Zn-3.28; Cu/Cu-9.88) calculated for the determined period of endosperm tissue development (Table 3), and central vacuole sap (Table 2) i.e. during inhibition and the exponential phase of embryo growth.

Taking into account that endosperm tissue (monocots) plays the same physiological-biochemical function as cotyledons (dicots) in relation to the embryo, it is suitable to compare the concentration of elements in both of these tissues. The concentration of all of the elements (except Na and Cu) was much higher in the embryo of *Aesculus glabra* (Ryczkowski and Reczyński 1986) than in the endosperm tissue of *Clivia miniata* ovules (Table 3).

It seems that in the ovules of monocots with a central vacuole, well developed endosperm tissue and embryo, a three-step process of accumulation of elements occurs – the lowest accumulation is in the central vacuole sap

(Table 2), medium in the endosperm tissue (Table 3), and the highest in embryo (preliminary and unpublished data).

In dicots – with ovules having poorly developed endosperm tissue i.e. the ovule of *Aesculus glabra* (Ryczkowski and Reczyński), there is only a two step accumulation of elements – lower in the central vacuole, higher in the embryo.

The inflow of elements into the fruit and ovule proceeds through the phloem and xylem in ionic and organic form (Tammes and Van Die 1964, Ziegler 1975, Pate and Hocking 1978). It has been assumed that the investigated elements occurred in the central vacuole sap and in the endosperm tissue in ionic and organic form (Tulecke et al. 1961, Ryczkowski 1965, Ryczkowski et al. 1971, Wyn Jones and Pollard 1983).

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Stężenie pierwiastków w soku centralnej wakuoli i w bielmie podczas embriogenezy (Clivia miniata Regel)

Streszczenie

Prześledzono zmiany stężenia: K, Ca, Na, Mg, Fe, Mn, Zn i Cu w soku centralnej wakuoli i w bielmie podczas embriogenezy. Stężenia pierwiastków oznaczano za pomocą spektrofotometru absorpcji atomowej (Perkin-Elmer, Model 503). Stwierdzono, że stężenie większości badanych pierwiastów w soku centralnej wakuoli (stadium prozarodka) wzrasta do określonego maksymum, a następnie maleje. W bielmie (stadium zarodka właściwego) stężenie niektórych pierwiastków (K, Ca, Mg, Fe, Zn, Cu) wzrasta, innych (Na, Mn) nie wykazuje wyraźnych zmian. Stężenie oznaczonych pierwiastków (z wyjątkiem Ca) było wyraźnie wyższe w bielmie niż w soku centralnej wakuoli.

Zasugerowano, że pierwiastki występujące w soku centralnej wakuoli były selektywnie pobierane z niej przez rozwijające się bielmo i zarodek. Wysunięto przypuszczenie trzystopniowego gromadzenia się pierwiastków w: soku centralnej wakuoli (stężenie najmniejsze), bielmie (stężenie średnie) i zarodku (stężenie największe).