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CONCENTRATION OF ELEMENTS IN THE CENTRAL VACUOLE SAP AND IN THE DEVELOPING EMBRYO OF AESCULUS GLABRA WILLD.

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SUMMARY

Ovules of *Aesculus glabra* Willd. were used as experimental material. Concentrations of K, Ca, Na, Mg, Fe, Mn, Zn and Cu were determined quantitatively in the central vacuole sap and in the developing embryo.

Generally concentrations of all investigated elements in the central vacuole sap and in the embryo changed during embryogenesis. Total concentration of K + Ca, and Mg + Fe + Mn + Zn + Cu in the sap increased during the beginning of embryo differentiation and subsequently decreased markedly during its further development. A similar change in total concentration of these elements was found in the developing embryo. Concentrations of elements in the embryo (except Mn) were much higher in the embryo than in the central vacuole sap. It is suggested that the elements occurring in the sap were selectively taken up and accumulated in the developing embryo.

1. INTRODUCTION

The present paper is a continuation of quantitative investigations on chemico-physical properties of the central vacuole sap (i.e. sap surrounding the developing embryo) and embryo. Previous papers contained results concerning chemical properties, concentration of reducing and non-reducing sugars (RYCZKOWSKI 1962c, 1964), number and concentration of free amino acids (RYCZKOWSKI et al. 1971, RYCZKOWSKI & RYCZKOWSKA 1973) in the central vacuole sap and the developing embryo. The latest paper (RYCZKOWSKI et al. 1986) contains results of quantitative determinations of K, Ca, Na, Mg, Fe, Mn, Zn and Cu in the central vacuole sap of the developing ovules of *Aesculus hybrida*.

On the other hand it should be stressed that there is a general lack of quantitative investigations on concentration of elements in the ovule during embryogenesis (WARDLAW 1955, ERDELSKA 1983, JOHRI 1984).

This paper contains the results of element determination in the central vacuole sap and in the developing embryo carried out in order to establish concentration changes during embryogenesis, and to compare the concentrations in the central vacuole sap and in the developing embryo.

2. MATERIAL AND METHODS

Ovules of *Aesculus glabra* Willd. collected between 7 and 8 a.m. were used as experimental material. The dimensions of embryos were the adopted criteria

Table 1. *Aesculus glabra*. Element concentrations in the sap surrounding the developing embryo.

Dimensions of embryos mm	K	Ca	ΣK+Ca	Na	Mg	Fe	Mn	Zn	Cu	ΣMg+Fe+Mn+Zn+Cu
	mg/cm³		mg/cm³			μg/cm³				μg/cm³
0.93×0,74	3,00	1,15	4,15		170,0		7,70	3,50	0,16	181,36
1,27×0,87	3,40	1,10	4,50	0,53	172,5	7,10	8,20	2,70	0,21	190,71
4,10×1,80	3,00	1,30	4,30	0,50	212,5	4,96	8,20	3,20	0,16	229,02
5,80×2,60	3,30	1,25	4,55		225,0	4,60	9,07	3,80	0,18	242,65
*14,00×6,90	3,05	1,25	4,30		192.5	4,40	7,80	2,75	0,16	207,61
22,90×8,50	3,05	1,30	4,35	1,10	122,5	3,40	9,60	2,45	0,13	138,08
23,80×9,20	2,95	1,20	4,15	0,85	127,5	2,40	8,00	2,00	0,19	140,09
Sr%	8,15	8,03		21,79	5,34	2,33	9,98	16,52	12,44	

Dimensions of embryos = length of embryo × breadth of cotyledon, * length of the convex side of the embryo.

of their age. The procedure of measuring embryos was described in a previous paper (RYCZKOWSKI 1962b) and the technique of collecting central vacuole sap in RYCZKOWSKI (1962a), and RYCZKOWSKI et al. (1986).

Embryos isolated from ovules after sap collection were washed twice with quartz distilled water and then dried to constant weight in a vacuum dryer at 100 °C, and powdered in an agate mortar. The procedure of single sample digestion was as follows: about 200 mg of powder was transferred into quartz beaker, 3 cm³ of conc. HNO_3 and 3 cm³ of perhydrol in 0.5 cm³ portions were progressively added into the beaker during the whole operation. The sample was heated up to 75 °C for 18 hours. The residue was dilluted in 1:10 water solution of conc. HNO_3.

Determination of elements. A qualitative analysis of six samples of sap and three samples of powdered embryos was performed, using the emission spectrograph PGS-2 (K. Zeiss, Jena) wit graphite arc method. 100 μl samples of the sap, and 100 μg samples of powdered embryos were dosed on graphite electrodes (Ringsdorf Werke, FRG). Excitation conditions for the sap were: direct current arc 8A, time 25.5 s and for embryos: direct current 8A, time 41 s.

A quantitative analysis was made by atomic absorption spectrometry, using the Perkin-Elmer spectrophotometer, Model 503. For further details see RYCZKOWSKI et al. (1986).

3. RESULTS

It was established, that the main inorganic compounds of the sap, and embryo were: K, Ca and Mg. In trace amounts occurred: Al, Ba, B, Cu, Fe, Mn, Na, Ni, P and Zn, but only K, Ca, Na, Mg, Fe, Mn, Zn and Cu were determined quantitatively during embryogenesis.

Central vacuole. Concentration of K and Ca in the sap was within the limits 2.95–3.40 mg/cm³ and 1.10–1.35 mg/cm³ respectively (*table 1*). Concentration

of Na was within the range 0.50–1.10 μg/cm³, and slightly increased during embryo development. Mg concentration in the sap increased markedly during the beginning of embryo development from 170.0 to 225.0 μg/cm³, and during further embryo development this values decreased considerably to 127.0 μg/cm³ (*table 1*). Fe concentration decreased from 7.10 to 2.40 μg/cm³ during embryo development. Concentration of Mn in the sap at the beginning of embryo development increased from 7.70 to 9.07 μg/cm³ (*table 1*), and then slightly dropped in an irregular way. Zn concentration in the sap increased from 3.50 to 3.80 μg/cm³, and then decreased markedly to 2.0 μg/cm³ (*table 1*). Concentration of Cu in the sap was within the range 0.13–0.21 μg/cm³, and practically constant during embryogenesis (*table 1*). Its concentration was the lowest among the investigated elements.

Total concentration of K + Ca and Mg + Fe + Mn + Zn + Cu increased in the sap to a small maximum (dimensions of embryos 5.80 × 2.60 mm), and then decreased during further embryo development (*table 1*).

Embryo. Concentration of K increased in young embryos from 11.20 to 15.84 mg/g fresh wt., and in older ones decreased to 4.94 mg/g fresh wt. (*table 2*). Concentration of Ca changed in an analogical way. That of Na was within the limits 15.11–21.54 μg/g fresh wt. (*table 2*) and dropped during embryo development. Concentration of Mg in young embryos markedly increased from 910.0 to 1210.0 μg, and then in older embryos decreased to 390.0 μg/g fresh wt. (*table 2*). Concentration of Fe was within the range 10.08–15.81 μg/g fresh wt., and seemed to decrease during embryo development (*table 2*). Mn concentration increased from 3.42 to 8.47 μg young embryos, and then decreased to 3.83 μg/g fresh wt. in older ones (*table 2*). Zn concentration in young embryos e increased from 12.08 to 14.46 μg, and during further embryo development dropped to 6.72 μg/g fresh wt (*table 2*). Concentration of Cu was within the range 0.12–0.56 μg/g fresh wt. (*table 2*), tending to increase during embryo development. Cu concentration was the lowest among the elements determined in developing embryo.

Concentration of all elements found in the embryo (except Mn) were much

Table 2. *Aesculus glabra.* Element concentrations in the developing embryo.

Dimensions of embryos mm	K	Ca	ΣK+Ca	Na	Mg	Fe	Mn	Zn	Cu	ΣMg+Fe+Mn+Zn+Cu
	mg/g	f.wt.	mg/g		μg/g	fresh	weight			μg/g
14.0× 6.9	11.20	1.67	12.87	21.54	910.0		3.42	12.08	0.12	925.62
22.9× 8.5	15.84	2.25	18.09		1210.0	15.81	7.23	14.46	0.30	1247.80
23.8× 9.2	12.61	2.08	14.69	20.97	880.0	13.11	8.47	12.65	0.30	914.53
29.2×12.5	9.89	1.52	11.41	19.86	570.0	10.43	5.32	9.42	0.44	595.61
30.7×13.7	8.92	1.24	10.16	15.11	490.0	10.42	5.34	8.94	0.55	515.25
35.8×14.5	7.36	1.08	8.44	18.14	450.0	10.58	4.08	7.55	0.56	472.77
36.8×16.7	4.92	0.92	5.86		390.0	10.08	3.83	6.72	0.48	411.11
Sr%	3.32	4.08		10.72	3.00	20.54	4.33	5.79	7.40	

higher than in the vacuole sap (*table 1* and *2*). Total concentration of K + Ca and Mg + Fe + Mn + Zn + Cu in the developing embryo showed a small maximum (embryo dimensions – 22.9 × 8.5 mm). It occurred after the maximum of element concentration found in the central vacuole sap.

4. DISCUSSION

During the inhibition and first part of the exponential phase of embryo growth (proembryo and embryo proper stages) the rate of water inflow with some elements from the vegetative organs to the fruit and ovule (TAMMES & VAN DIE 1964; HOCKING & PATE 1977; PATE & HOCKING 1978) exceeded the rate of their uptake by the developing embryo and endosperm tissue. The embryo was still of small dimensions (0.93 × 0.74 – 5.80 × 2.60 mm; *table 1*), and endosperm tissue occurred as a thin cytoplasmic bag with nuclei (RYCZKOWSKI et al. 1986). In this period of embryo development concentration of some elements in the sap increased to a small maximum (*table 1*).

A small increase in concentration of some elements in the sap or its absence in others at the beginning of embryo differentiation could be connected with the fact that this increase probably occurred during the proembryo stage, it means that first samples of ovules for sap collection and element determination were taken too late. This suggestion is in good agreement with the results concerning osmotic value, concentration of sugars, and free amino acids changes in the central vacuole sap during embryogenesis (RYCZKOWSKI 1962a, 1962c, 1964, 1969; RYCZKOWSKI et al. 1971).

During the second part of the exponential phase of embryo growth (proper embryo stage) the rate of uptake of elements from the sap by the developing embryo (HOCKING & PATE 1977) exceeded their inflow with water to the ovule, hence a distinct decrease in the concentration of majority of elements in the sap.

During the second part of the exponential phase of embryo growth (dimensions of embryos – 14.0 × 6.9 – 23.8 × 9.2 mm) a small increase in the concentration of elements (except of Fe) was found. This was concomitant with the decrease in concentration of majority of elements in the central vacuole sap (*table 1* and *2*).

During the third part of the exponential phase of embryo growth a marked decrease in concentration of elements was established. It should be stressed that during this period of embryo development the central vacuole was completely occupied by the developing embryo and there was no central vacuole sap, thus the elements were supplied to ovule and embryo from the vegetative plant organs (HOCKING & PATE 1977, PATE & HOCKING 1978).

The uptake of elements by the developing embryo from the sap surrounding it is supported by the following facts: a. the occurrence of the same elements in the sap and in the embryo (*table 1* and *2*), b. the decrease in the concentration of elements in the sap was concomitant with the increase in their concentrations in the developing embryo, c. much higher concentration of all elements (except

of Mn) in the embryo than in the central vacuole sap. It is well known that these elements play essential function in plant tissue metabolism and its growth (GRZESIUK & KULKA 1981, SANDMAN & BÖGER 1983, MOORBY & BESFORD 1983, WYN JONES & POLLARD 1983).

It results from the relation of particular elements K/Na, Ca/Mg, Fe/Mn, Zn/Cu in the central vacuole sap, and in the embryo that some elements were selective taken up by the developing embryo from the sap surrounding it. It resulted in accumulation of a predominant number of elements in the developing embryo as compared with their accumulation in the central vacuole sap. This conclusion is supported by a high accumulation quotient (Ce/Cv-concentration in the embryo/concentration in the sap: Na/Na-21,80; Mg/Mg-6.78; Zn/Zn-5.44; Fe/Fe-4.87; K/K-4.38; Ca/Ca-1.60; Cu/Cu-1.50; and Mn/Mn-0.75) calculated for the determined period of embryo development and central vacuole (dimensions of embryos – 14.0 × 6,9–23.8 × 9,2 mm). It means that a plant controles distribution of elements inside the developing ovule during embryogenesis (HOCKING & PATE 1977).

The inflow of elements into the fruit, ovule, central vacuole, and embryo proceeds through the phloem and xylem tissues in ionic and organic form (TAMMES & VAN DIE 1964, ZIEGLER 1975, PATE & HOCKING 1978). It has been assumed that the investigated elements occurred in the central vacuole sap and in the embryo in ionic and organic form (TULECKE et al. 1961, RYCZKOWSKI 1965, RYCZKOWSKI et al. 1971, WYN JONES & POLLARD 1983). It is suggested that in the embryo the organic form of some elements predominated over the ionic form of elements, and on the other hand in the central vacuole sap this relation is reverse (FLOWERS & LÄUCHLI 1983).

It follows from a comparison of the results obtained by the authors for central vacuole sap and the data found by TAMMES (1959) that concentration of Ca, Mg and Zn in coconut water (one stage of development) is many times lower than that found for the central vacuole sap of *A.glabra* ovules. The concentration of K was more or less the same. TAMMES (1959) did not find Fe and Cu in the coconut water.

These and previous data (RYCZKOWSKI et al. 1986) support the conclusion (RYCZKOWSKI 1962c, 1969) that the medium for embryo and endosperm tissue (protoplasts obtained from them) in vitro cultures should be specific for each species, rich in elements and change during embryogenesis.

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