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Effect of dehydration of germinating maize embryos on in situ localization of calcium and magnesium

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RÉSUMÉ

CRÈVECOEUR, M. & R. DELTOUR (1996). Effet de la déshydratation d'embryons de maïs germés sur la localisation in situ du calcium et du magnésium. *Saussurea* 27: 49-57. En anglais, résumés anglais et français

La distribution intracellulaire du calcium et du magnésium a été étudiée dans les cellules radiculaires d' embryons de *Zea mays* germés et soumis à une déshydratation, en utilisant la méthode de précipitation *in situ* de ces cations par le pyroantimonate de potassium. Le contenu relatif du noyau en ces ions a été aussi estimé par microanalyse aux rayons X. Il augmente de la 24e à la 72e h de germination. Pendant cette période l'examen de coupes ultrafines montre que ces cations s'accumulent aussi au niveau du plasmalemme. Dans les cellules d'embryons déshydratés à la 24e heure de germination (résistants à la déshydratation) le seul changement observé consiste en une augmentation du contenu du noyau en calcium et en magnésium. Une accumulation massive de ces cations est observée dans le noyau et le long du plasmalemme, dans les cellules d'embryons déshydratés à la 72e heure de germination (non résistants à la déshydratation). Il est proposé qu'une telle accumulation peut être impliquée dans l'incapacité de ces embryons à survivre à la déshydratation.

ABSTRACT

CRÈVECOEUR, M. & R. DELTOUR (1996). Effect of dehydration of germinating maize embryos on in situ localization of calcium and magnesium. *Saussurea* 27: 49-57. In English, English and French abstracts.

The intracellular distribution of calcium and magnesium was examined in radicle cells of germinated and dehydrated *Zea mays* embryos, by means of *in situ* precipitation of these cations with potassium pyroantimonate. Their relative nuclear content was also estimated by energy dispersive X-ray microanalysis. It was found to increase from the 24th to the 72nd h of germination. During this germination period these cations were also seen to accumulate at the plasmalemma level. In embryos dehydrated after 24 h of germination (dehydration-resistant), the only change observed was an increase of the Ca^{2+} and Mg^{2+} nuclear content. When embryos were dehydrated at the 72nd h of germination (dehydration-unresistant) there was a massive accumulation of these cations in the nucleus and along the plasmalemma. It was proposed that such an accumulation could be involved in the unability of these embryos to resist to dehydration.

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Introduction

Embryos of seeds are known to acquire desiccation tolerance during seed development and to lose this tolerance within hours of germination after periods which vary with the species and the conditions of germination (BEWLEY, 1979; DASGUPTA & al., 1982; LALONDE & BEWLEY, 1985; KOSTER & LEOPOLD, 1988). In *Zea mays* loss of resistance to dehydration occurs at about the 36th h of germination at 16°C (DELTOUR & JACQMARD, 1974; CRÈVECOEUR & al., 1976). To determine why maize embryos become dehydration-sensitive, metabolic and structural changes induced by water loss have been compared in embryos dehydrated at the 24th h and at the 72nd h of germination, at the end of their dehydration and during their regermination (CRÈVECOEUR & al., 1976; 1988; CRÈVECOEUR & DELTOUR, 1985). The first ones are dehydration-resistant and the second ones dehydration-unresistant. During

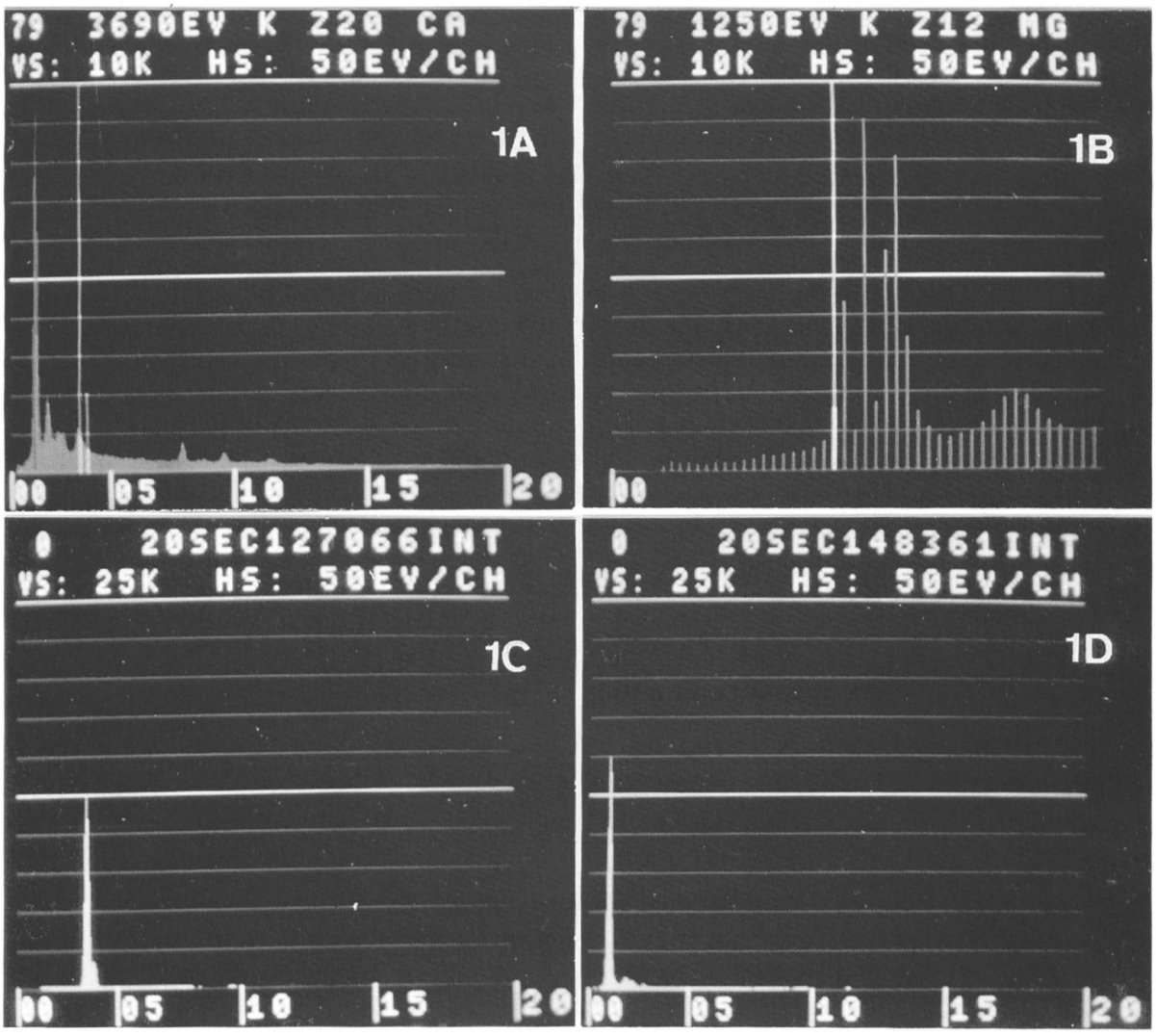

Fig. 1. – Ca^{2+} (A) and Mg^{2+} (B) peaks from a nucleolus X-ray spectrum in a radicle cell fixed with potassium pyroantimonate and peaks of X-ray spectra of standard samples consisting of Ca^{2+} (C) and Mg^{2+} (D).

regermination of the former ultrastructure, transcription and duplication evolve as during germination. In contrast irreversible disorganization of cellular fine structure and inhibition of main nuclear functions were observed, during regermination of dehydration-unresistant embryos. Any of the cellular modifications, described until now during the first 36 h of germination, can be related to the loss of dehydration-resistance of *Zea mays* germinating embryos. Various results led us to suggest the involvement of modifications in the ionic environment of the plasmalemma and the nucleus. Therefore, the present study investigates the intracellular distribution of two cations, Ca^{2+} and Mg^{2+} in *Zea mays* radicle cells, during germination and after a dehydration treatment. The method used is the cytochemical method of *in situ* precipitation of these cations with potassium pyroantimonate. It allows to localize free and loosely bound Ca^{2+} and Mg^{2+} and also to estimate their intracellular content using X-ray microprobes (TANDLER & al., 1970; WICK & HEPLER, 1982; JONES & al, 1982). Such microprobes were used here for identification of ions in pyroantimonate deposits at the ultrastructural level and determination of their relative content in the nucleus.

Material and methods

Germination and dehydration conditions

Kernels of *Zea mays* var CiV2 were germinated at 16°C at darkness for 24 h and 72 h, then dehydrated as previously described (CRÈVECOEUR & al., 1976).

Fixation for localization of Ca^{2+} and Mg^{2+}

Embryos were excised from quiescent, germinated and dehydrated embryos and the coleorhiza discarded. They were immediately immersed in a freshly prepared, saturated solution of potassium pyroantimonate and degazed to allow a fast penetration of this solution into the tissues. After 1 h of incubation at 16°C the samples were fixed in a 1:3 mixture of 20% formaldehyde and saturated solution of potassium pyroantimonate for 18 h, at room temperature. The radicle tips (1mm long) were then excised and rinsed several times in deionized water to take off any excess of potassium pyroantimonate. They were dehydrated with graded series of ethanol and embedded in Epon.

Electron microscopy

Ultrathin cross sections (90 nm thick) were cut through the cortex and examined without staining in a Siemens Elmiskop 101 transmission electron microscope. The Ca^{2+} and Mg^{2+} pyroantimonate precipitates appeared as black deposits of different sizes. For X-ray microanalysis, ultrathin sections were mounted on aluminium grids and examined at 80 KV in a AEI Electron microscope, fitted with a wavelength dispersive analyser. Ca^{2+} and Mg^{2+} diffraction was studied in the plasmalemma, the vacuoles and in different parts of the nucleus e.g. the nuclear envelope, the nucleolus and the nucleoplasm. Sections from five roots were examined and three cells randomly taken in each section for microanalysis in these five intracellular sites .

Energy dispersive X-ray microanalysis (EDAX)

Transverse sections 1 µm thick were cut with a glass knife, mounted on graphite holders and examined under a Stereoscan 600 microscope (Cambridge). This scanning electron microscope was equipped with a 1 µm diameter microprobe and a X-ray energy dispersive spectrometer (EDAX). Peaks of the nucleus X-ray spectrum corresponding to Ca^{2+} and Mg^{2+} were separately photographed with a polaroid camera (Figures 1A & 1B) as well as the heights of energy spectra emitted by standard samples

consisting of either CaO or MgO (Figures 1C & 1D). The heights of the different peaks were then measured on the positives using a stereomicroscope equipped with a calibrated ocular micrometer. The Ca^{2+} and Mg^{2+} nuclear relative content was expressed as % of the ratio of heights obtained for standards and for the nuclear compartment. Three sections from each experimental serie were taken randomly and five nuclei were analysed in each section.

Results

Cytochemical distribution of Ca^{2+} and Mg^{2+}

A fine granular precipitate was found uniformly distributed within cells of radicles fixed in potassium pyroantimonate after 24 h and 72 h of germination (Figures 2 & 4a). In the cytoplasm electron dense precipitates were seen in a few vacuoles after 24 h of germination. Vacuolar precipitates were no more observed at the 72nd h of germination. By this time, pyroantimonate deposits were discerned in mitochondria and proplastids (arrow, Figure 4a). They were also seen at the periphery of the cell in close association with the plasmalemma and portions of this membrane protruding inside the cytoplasm

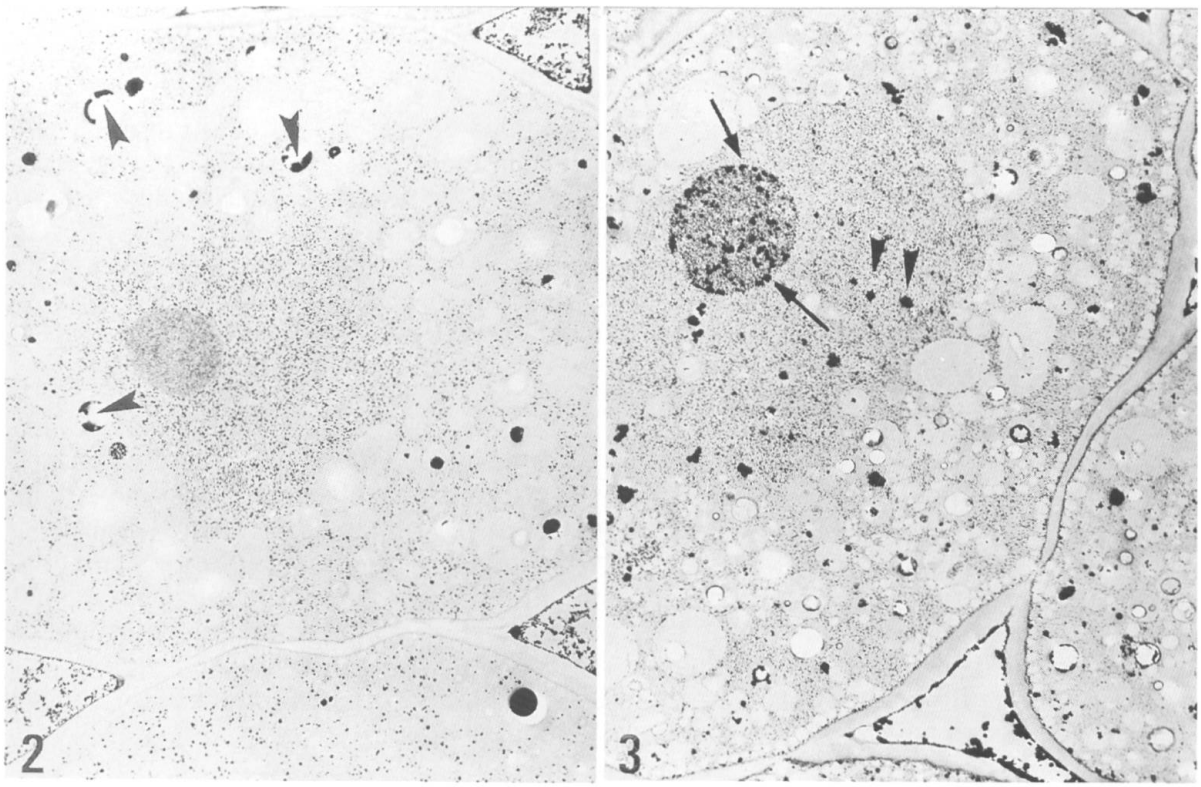

Fig. 2 to 5. – Electron micrographs of maize radicle cells fixed with potassium pyroantimonate.

Fig. 2. – Heavy antimonate deposits in the vacuoles (➤) and fine antimonate precipitates in the nucleus are observed at the 24th h of germination G X: 3420.

Fig. 3. – Electron micrograph of a radicle cell in an embryo dehydrated at the 24 th h of germination. Note the presence of larger antimonate precipitates in the nucleolus (➔) and the nucleoplasm (➤) GX: 3580

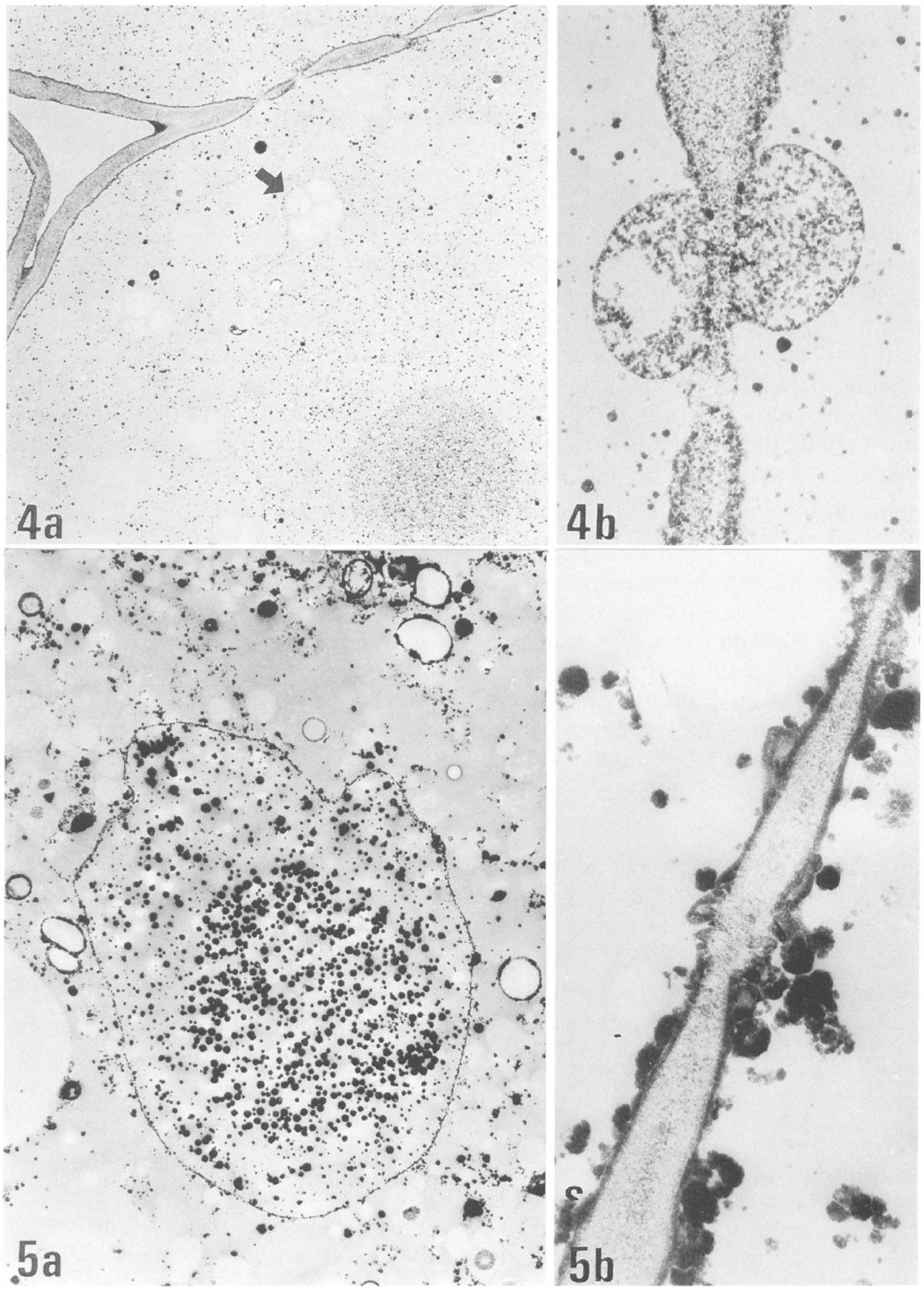

Fig. 4. – Radicle cell of an embryo at the 72nd h of germination. The pyroantimonate deposits are noted in the nucleus and in the plasmalemma. They are also apparent in infoldings of this membrane (4b) GX: 3600 (a); 26700 (b).

Fig. 5. – Portion of a radicle cell of an embryo dehydrated at the 72nd h of germination. Accumulation of large antimonate precipitates is clearly apparent in the nucleus (5a) and along the plasmalemma (5b). GX: 6400 (a); 28650 (b).

(Figure 4). In the nucleus, pyroantimonate deposits were seen in radicle cells in both 24 h and 72 h germinated embryos. Observation of many cells reveal a greater number of nuclear precipitates at the 72nd h of germination.

Dehydration at the 24th h of germination resulted in an increase in the number of pyroantimonate precipitates associated to the nucleolus. In addition the nuclear associated precipitates consisted of two types: a fine type as in 24 h radicle cells and a larger type, 150 nm about in diameter (Figure 3). So larger precipitates were also seen in the extranucleolar region of the nucleus. More striking changes were observed in the intracellular distribution of pyroantimonate deposits, in radicle cells from embryos dehydrated at the 72nd h of germination. The finely granular precipitates were no more observed (compare Figures 4a & 5a) and many large dense precipitates, whose diameter frequently reached 180 nm, were found in the nucleus, particularly in the nucleolus and also associated to the nuclear envelope. In addition, accumulation of large dense deposits was noted along the plasmalemma (Figure 5b). X-ray microanalysis of pyroantimonate deposits, in sections from the different experimental series, reveal that they mainly consisted of Ca^{2+} and Mg^{2+}.

Determination of relative Ca^{2+} and Mg^{2+} nuclear content

Results of Table 1 indicate that the relative Ca^{2+} and Mg^{2+} nuclear content was very low in quiescent embryos. It increased from the 24th to the 72nd h of germination, with some difference between the two cations. In both the nucleolus and the nucleoplasm the Mg^{2+} content showed a much greater increase than Ca^{2+}. In radicle cells from embryos dehydrated at the 24th h of germination the most pronounced rise concerned the Ca^{2+} nucleolar content. A slight increase was also noted for Mg^{2+}, in the nucleoplasm and the nucleolus. More striking increases were observed in radicle cells from embryos dehydrated at the 72th h of germination. This was particularly true for the Mg^{2+} content in the two nuclear compartments.

Table 1. – Relative nuclear content in Ca^{2+} and Mg^{2+} determined by EDAX analysis of semithin sections (1 µm thick) performed in radicle cells

Embryos	*Nucleolus*		*Nucleoplasm*	
	Ca^{2+}	*Mg^{2+}*	*Ca^{2+}*	*Mg^{2+}*
Quiescent	3.16	2.31	1.42	2.18
Germinated 24h	6.19	6.32	5.37	6.97
Germinated 72 h	8.20	11.06	6.33	11.24
Dehydrated at the 24th h	9.35	7.12	5.2	8.67
Dehydrated at the 72nd h	11.92	17.5	7.99	16.12

Discussion

This study firstly demonstrates differences in cytochemical localization of Ca^{2+} and Mg^{2+} ions, in radicle cells from maize embryos germinated 24 h and 72 h. Both cations accumulate in greater amount in the nucleolus, the nucleoplasm and at the plasmalemma at the 72nd h of germination. Cytochemical accumulation of inorganic phosphate ions has been previously described, in the same material and the same cellular sites, after this germination period (DELTOUR & al., 1981). A correlation between this

phosphate inorganic increase and the metabolic reactivation of the nucleus, occurring during germination, namely the transcription, has been proposed. As indicated by hereafter results, a similar relationship may be proposed for the nuclear Ca^{2+} and Mg^{2+} accumulation reported in the present study. Firstly, the preferential localization of antimonate precipitates on the fibrillar components of the nucleolus and the dense fibrillar components surrounding the fibrillar centers, in *Allium cepa* root cells, support this view (RODRIGUEZ-GARCIA & STOCKERT, 1979). Indeed the fibrillar centers of the nucleolus are known to contain the ribosomal genes (THIRY & GOESSENS, 1992). Secondly, it is well known that divalent cations are required for functioning of RNA polymerase (TRES & al., 1972; RISUENO & MEDINA, 1986). Thirdly, the Ca^{2+} and Mg^{2+} nuclear content is very low in quiescent maize embryos in which the nuclear metabolism is almost completely arrested. Finally, the nucleolar accumulation of Ca^{2+} ions in shoot apical meristems of *Sinapis alba* during floral transition (HAVELANGE, 1989), has been related to the increases of rRNA synthesis and of nucleolar size (HAVELANGE & BERNIER, 1974; PRYKE & BERNIER, 1978).

Ca^{2+} and Mg^{2+} also accumulate at the level of plasmalemma and in infoldings of this membrane, at the 72nd h of germination. A similar observation has been previously reported in this material for phosphate inorganic ions (DELTOUR et al., 1981) and for Ca^{2+} and Mg^{2+}, in *Allium cepa* and *Zea mays* root cells (TANDLER & al., 1970, 1973). The ATPase activity detected in plasmalemma invaginations and their increased number in roots which accumulate greater amounts of ions suggest that these infoldings may represent pinocytic vesicles involved in ions uptake (HALL, 1970)

On the other hand our results bring about information on the effect of dehydration of germinating embryos on localization and *in situ* relative content of Ca^{2+} and Mg^{2+}. We show that dehydration at the 24th h of germination does not result in profound changes in distribution of these cations. On the contrary, dehydration at the 72nd h of germination causes their massive accumulation in the nucleus, the nuclear envelope and the plasmalemma. The ionic concentration could reach unphysiological levels, in these cellular sites, that could be responsible for the definitive functional and structural disorganization they undergo (CRÈVECOEUR & al., 1976, 1988). We think in particular to the irreversible condensation of chromatin, the inhibition of main nuclear functions and the alterations in plasmalemma fine structure e.g. ruptures and aggregation of its intramembrane particles (CRÈVECOEUR & al., 1976; CRÈVECOEUR & DELTOUR, 1985). Similar alterations have been reported in the literature, at the membrane and nuclear levels, as result of marked increases in ionic concentration (CHEVAILLIER & PHILIPPE, 1973; COPPS & al., 1976; PINTO DA SILVA, 1972). It has to be pointed out that pyroantimonate precipitates, in dehydration-unresistant embryos, are much more numerous and of a greater size than in quiescent and dehydration-resistant embryos as well as in all other plant cells, examined with this cytochemical assay. This suggests that Ca^{2+} and Mg^{2+} concentrations probably reach a too high level in these cells. Accurate *in situ* determination of mean cations concentration is required to confirm this suggestion.

The loss of desiccation tolerance of seeds during germination has been related in some studies to sugars content namely disappearance of oligosaccharides (KOSTER & LEOPOLD, 1988; LEPRINCE & al., 1992). Biochemical and biophysical data show that carbohydrates can play an active role in desiccation tolerance, by protecting cellular membranes from desiccation-induced injuries (CROWE & al., 1988). Results of the present paper led us to propose that the ionic environment could also play a role in loss of desiccation tolerance. The irreversible structural and functional alterations in dehydration-unresistant maize embryos could partially result from an increased ionic

concentration in the cellular sites that grow richer in ions during germination e.g. the plasmalemma and the nucleus. To test this hypothesis, further biochemical and cytochemical investigations will be made, to get more precise informations on mean Ca^{2+} and Mg^{2+} concentrations, at both the tissue and the cellular levels.

REFERENCES

BEWLEY, J. D. (1979). Physiological aspects of desiccation tolerance. *Annual Rev. Pl. Physiol.* 30: 195-238

CHEVAILLIER, P. & M. PHILIPPE (1973). Aspects structuraux et biochimiques des effets de quelques milieux utilisés pour l'isolement et le fractionnement du noyau de foie de souris. *Exp. Cell Res.* 82: 1-14

COPPS, T. P., W. S. CHELACK & A. PETKAU (1976). Variation in distribution of membrane particles in Acholeplasma laidlawii B with pH. *J. Ultrastruct. Res.* 55: 1-3

CRÈVECOEUR, M., R. DELTOUR & R. BRONCHART (1976). Cytological study on water stress during germination of Zea mays. *Planta* 132: 31-41

CRÈVECOEUR, M. & R. DELTOUR (1985). Effects of dehydration of germinating Zea mays kernels on fracture faces of plasmalemma and nuclear membranes. *Acta Physiol. Pl.* 7: 129-137

CRÈVECOEUR, M., R. DELTOUR & C. VAN DE WALLE (1988). DNA content and nucleic acid synthesis in dehydrated maize embryos. *Pl. Physiol. Biochem.* 26: 65-71

CROWE, J.-H., L. M. CROWE, J. F. CARPENTER, A. S. RUDOLPH, C. AURELL WISTROM, B. J. SPARGO & T. J. ANCHORDOGUY (1988). Interactions of sugars with membranes. *Biochim. Biophys. Acta* 947: 367-384

DASGUPTA J., J. D. BEWLEY & E. C. YEUNG (1982). Desiccation-tolerant and desiccation-intolerant stages during the development and germination of Phaseolus vulgaris seeds. *J. Exp. Bot.* 33: 1045-1057

DELTOUR, R. & A. JACQMARD (1974). A relation between water stress and DNA synthesis during germination of Zea mays L. *Ann. Bot.* 38: 529-534

DELTOUR, R., S. FRANSOLET & R. LOPPES (1981). Inorganic phosphate accumulation and phosphatase activity in the nucleus of maize embryo root cells. *J. Cell Sci.* 47: 77-89

HALL, J. L. (1970). Pinocytic vesicles and ion transport in plant cells. *Nature* 226: 1253-1254

HAVELANGE, A. (1989). Levels and ultrastructural localization of calcium in Sinapis alba during the floral transition. *Pl. Cell Physiol.* 30: 351-358

HAVELANGE, A. & G. BERNIER (1974). Descriptive and quantitative study of ultrastructural changes in the apical meristem of mustard in transition to flowering. I. The cell and nucleus. *J. Cell Sci.* 15: 633-644

JONES, R. G., W. L. DAVIS & S. B. VINSON (1982). A histochemical and X-ray microanalysis study of calcium changes in insect flight muscle degeneration in Solenopsis, the Queen Fire Ant. *J. Histochem. Cytochem.* 30: 293-304

KOSTER, K. L. & A. C. LEOPOLD (1988). Sugars and desiccation tolerance in seeds. *Pl. Physiol* 88: 829-832

LALONDE, L. & J. D. BEWLEY (1985). Desiccation of imbibed and germinating pea axes causes a partial reversal of germination events. *Canad. J. Bot.* 63: 2248-2253

LEPRINCE, O., A. VAN DER WERF, R. DELTOUR & H. LAMBERS (1992). Respiratory pathways in germinating maize radicles correlated with desiccation tolerance and soluble sugars. *Physiol Pl.* 85: 581-588

PINTO DA SILVA, P. (1972). Translational mobility of the membrane intercalated particles of human erythrocyte ghosts: pH-dependent reversible aggregation. *J. Cell Biol.* 53: 777-787

PRYKE, J. A. & G. BERNIER (1978). RNA synthesis in the apex of Sinapis alba in transition to flowering. *J. Exp. Bot.* 29: 953-961

RISUEÑO, M. C. & F. J. MEDINA (1986). The nucleolar structure in plant cells. *Revisiones Sobre Biologia Cellular* 7: 1-140

RODRÍGUEZ-GARCÍA, M. I. & J. C. STOCKERT (1979). Localization of the pyroantimonate-osmium reaction product in plant cell nucleoli. *J. Ultrastruct. Res.* 67: 65-72

TANDLER, C. J., C. M. LIBANATI & C. A. SANCHIS (1970). The intracellular localization of inorganic cations with potassium pyroantimonate. Electron microscope and microprobe analysis. *J. Cell Biol.* 45: 355-366

TANDLER, C. J., M. C. RISUEÑO & M. E. FERNÁNDEZ-GÓMEZ (1973). Inorganic cations in Allium cepa roots. Intracellular localizations. *Protoplasma* 77: 201-210

THIRY, M. & G. GOESSENS (1992). Where, within the nucleolus are the rRNA genes located? *Exp. Cell Res.* 200: 1-4

TRES, L. L., A. L. KIERSZENBAUM & C. J. TANDLER (1972). Inorganic cations in the cell nucleus. Selective accumulation during meiotic prophase in mouse testis. *J. Cell Biol.* 53: 483-493

WICK, S. M. & P. K. HEPLER (1982). Selective localization of intracellular Ca^{2+} with potassium pyroantimonate. *J. Histochem. Cytochem.* 30: 1190-1204

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