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Cover

The cover of this volume is the work of Olivier Debré. It is the reduction of the poster which he has designed for ICEM 13, inspired by two electron micrographs. The first of these was taken by Christine Leroux and Annick Loiseau; it is of a Cobalt (40%), Platinum (60%) alloy, and shows two phases obtained after thermal processing at 600°C. The other shows a Kaposi's sarcoma cell, and was taken by Françoise Haguenau.

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Comparative Study of the Immunocytochemical Alkaloid Localization in Different Species of *Lupinus* Seeds

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1. INTRODUCTION

Lupinus seeds are used for human and animal nutrition. The seeds, however, accumulate a high level of toxics alkaloids. Genetic characteristics determine the content of alkaloids in the different species of *Lupinus*, being possible its quantitative modification by hybridation processes and selection of the final varieties with the lowest content in alkaloids, as it is the case with *L. albus* cv Multolupa.

The objective of the present work has been to carry out the immunocytochemical localization of alkaloids in seeds from different species at light and electron microscopy. This is the first time showing immunolocalization of alkaloids in plant tissue. The alkaloids comprising in most of the *Lupinus* species are based on quinolizidine ring structure and include (+)-lupanine and (+)-13-hidroxylupanine as two of the major alkaloids. Quinolizidine alkaloids are synthetized in the leaves and translocated, via phloem, to other plant organs specially the maturing fruits.

2. MATERIAL AND METHODS

Four different species of *Lupinus* have been studied: *L. albus*, *L. angustifolius*, *L. luteus* and *L. mutabilis*. Seeds were soaked for 1 h. in distilled water and inmediatelly fractured in 2-3 mm pieces. The samples were processed for light and electron microscopy by conventional techniques (de Felipe *et al.*, 1986) and also processed by cryotechniques to compare alkaloids localization, since these compounds are soluble in water. Cryotechniques involved cryofixation by rapid inmersion into liquid propane, freeze substitution in acetone between the melting point (-94.4 °C) and -80 °C and embedding in LR White at 60 °C (Quintana, 1994).

1 um sections from tissues processed by both methods were immunolabelled for light microscopy, using silver enhancement (Lucas *et al.*, 1992) after counterstaning with basic fuchsine. Sections were also processed for gold immunolabelling for T.E.M. (de Lorenzo *et al.*, 1993) and high resolution S.E.M.

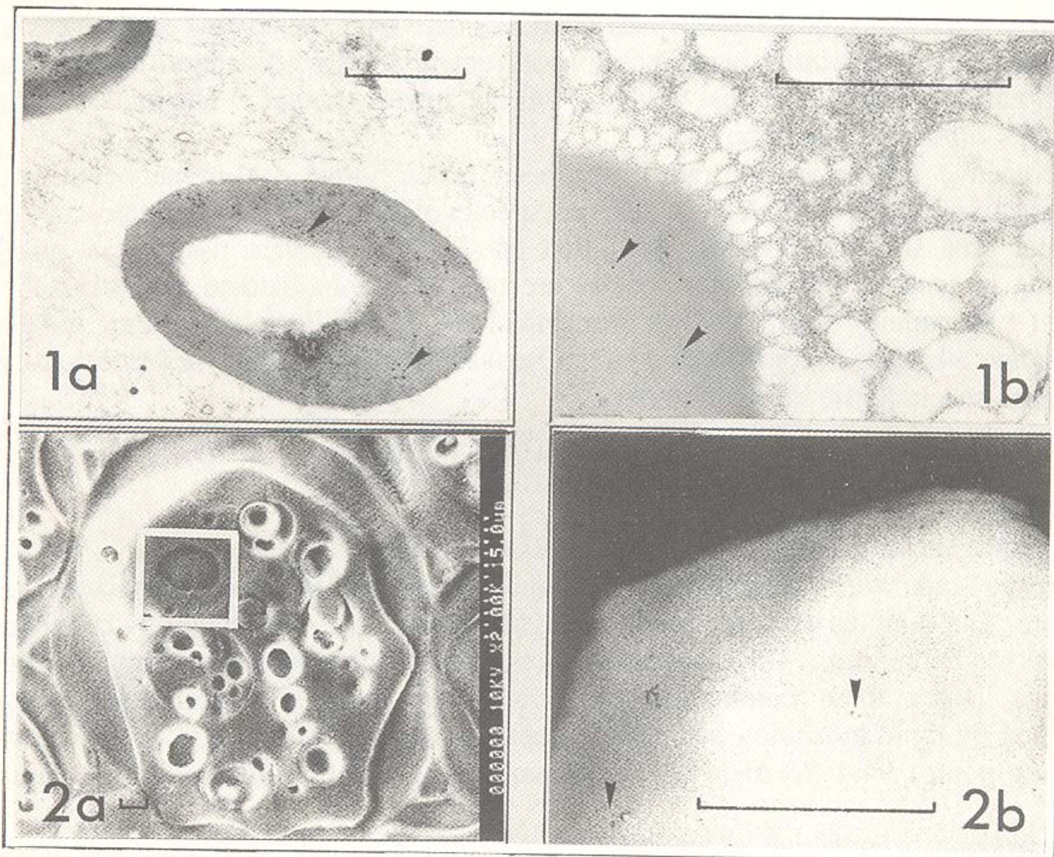
Sheep antiserum against lupanine and 13-hidroxylupanine and preinmune serum were provided by Dr. Greison (Chemistry Centre, Perth, W. Australia). Donkey anti-sheep conjugated to 15 nm colloidal gold was purchased from BioCell Research Laboratories.

3. RESULTS

Immunogold localization of lupanine and 13-hidroxylupanine at T.E.M. was higher in *L. angustifolius* seeds than in other especies, either in chemical- (Fig. 1a) or physical fixed (cryofixed) tissues (Fig. 1b). In the conventionally treated tissues, localization was mainly observed on the matrix of protein bodies, and sometimes also on the membranes. In conventional preparation, protein bodies show internal structure, with empty areas without immunolabelling and dense areas where the gold particles are localized (Fig. 1a, arrowheads). On the contrary, in cryoprocessed tissues, most protein bodies are homogeneous in structure (Fig. 1b). Immunolabelling of lupanine in *L. luteus* (not shown) was much less, although the sites of localization were the same as in *L. angustifolius*. Finally localization in sweet *L. albus* was scarce (not shown).

Preliminary results at S.E.M. (Figs. 2a and 2b, arrowheads) confirmed the sites of localization observed with transmision electron microscopy.

Comparing the convenience of the cryotechniques applied on alkaloid localization, we did not find marked differences on the results with conventional methods. Localization was in the same places of the seed tissues, but the quantitative study of gold labelling by both techniques will be done in a near future.



Figs. 1a and 1b: Alkaloid localization at T.E.M.. Figs. 2a and 2b: Alkaloid localization at S.E.M. Bars= 1 um.

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