

LOCALIZATION OF PHENOLIC COMPOUNDS IN COLUMELLA OF CANOLA  
EMBRYO DURING IMBIBITION AND GERMINATION OF SEEDS

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ABSTRACT

Phenolic compounds in the dry embryo inside the seeds are located on the surface of the root cap, and in the space between the cell wall and the plasmalemma. A small number of vesicles which presumably remain from degradation of the endoplasmic reticulum (ER) contain phenolic compounds. The cytoplasm of the dry embryo contains very few ER cisternae, which become restored during imbibition. Imbibition promotes further extrusion of phenolics outside the plasma membrane. After 24 h phenolic compounds are localized in dilated cisternae of shorter and as well longer ER. The new defense compounds in germinating seeds start to be synthesized within 24 h in water.

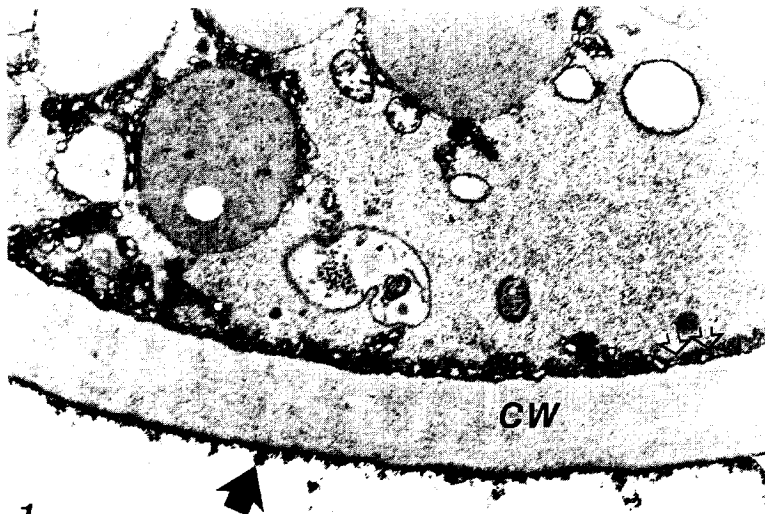
INTRODUCTION

Germination of seeds depends on age and conditions under which they have been kept (Mayer and Poljakoff-Mayber, 1982). Phenolics and other compounds leak from the aging seed (Hill, Taylor and Huang, 1988; Zobel et al., 1991). We found that coumarins can react as autoinhibitors of germination in umbelliferous plants when they are located in the seed coat and on the surface of the embryo (Zobel et al., 1989). The different tissues in the canola embryo are not activated simultaneously (Kuras, 1987), and the localization of phenolic compounds during germination has not been extensively studied. The aim of this paper is to compare localization of phenolic compounds in dry seeds and during seed imbibition.

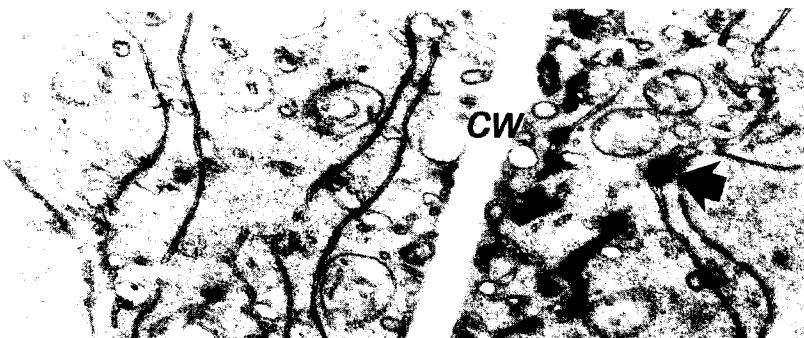
Fig. 1 Localization of phenolic compounds on the surface of columella cells (arrow), and extruded from cytoplasm, thus located between the cell wall (CW) and the plasma membrane (double arrow). Lack of long ER cisternae in the right hand part of the cell.

Fig. 2 After 24 h imbibition the phenolic compounds are located in the first layer of the columella (arrow) in the

(left hand cell).



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## EXPERIMENTAL

Material was fixed with 0.1% caffeine addition to Karnovsky fixative (Zobel, 1986; Zobel et al., 1989).

The ground cytoplasm of the first layer of the columella in the embryo of the dry seed lacked long ER cisternae, and only a few small vesicles contained phenolic compounds. Larger quantities of frothlike phenolic compounds were located on the surface of the columella (Fig. 1) and in the space between the plasma membrane and the cell wall. This area contains numerous deposits of vesicles, most likely from fusion of extruded small vesicles (double arrow). After 6, 9, 12 and 24 h imbibition the membrane structure had been restored, increasing the number of vesicles containing phenolic compounds. After 24 h (Fig. 2) long ER cisternae were observed and parts of them were filled with phenolic compounds of the new, very dense structure, different from the previously observed frothlike structure, suggesting that these are newly formed, different phenolic compounds.

The site of the phenolic compounds may be related to their defense role, forming several barriers on the embryo surface, and within some of their tissues (Zobel et al., 1989; Zobel and Brown, 1991). The high concentrations of phenolic compounds produced *de novo* after 24 h suggest that the germinating canola itself starts to produce protective compounds. As phenylpropanoid biosynthesis is connected with the ER (Hrazdina and Wagner, 1985), the development of long ER with areas containing phenolic compounds may initiate the defense mechanism within the embryo itself.

## REFERENCES

- Hill HJ, Taylor AG, Huang XL. 1988. Seed viability determination in cabbage utilizing sinapine leakage and electrical conductivity measurements. *Journal of Experimental Botany* 39: 1439-1447.
- Hrazdina G, Wagner, GJ. 1985. Metabolic pathways as enzyme complexes: Evidence for the synthesis of phenylpropanoids and flavonoids on membrane associated enzyme complexes. *Arch. Biochem. Biophys.* 237: 88-100.
- Kuras M. 1987. Activation of embryo during rape (*Brassica napus*) seed germination. V. The first zones of ultrastructural changes and their expansion. *Acta Societatis Botanicorum Poloniae* 56: 77-91.
- Mayer AM, Poljakoff-Mayber A. 1982. *The germination of Seeds*. Oxford: Pergamon.
- Zobel AM, Brown SA. 1991. Psoralens on the surface of seeds of Rutaceae and seeds of Umbelliferae and Leguminosae. *Canadian Journal of Botany* 69: 485-488.
- Zobel AM, Kuras M, Tykarska T. 1989. Cytoplasmic and apoplasmic localization of phenolic compounds in the covering tissues of *Brassica napus* radicle between embryogenesis and germination. *Annals of Botany* 64: 149-157.
- Zobel AM, Kuras M, Tykarska T, Heneklaus S, Schnug E. 1991b. Leakage of fluorescent compounds from aging canola seeds as a method for distinguishing dead from living embryos. *Proceedings of International Rapeseed Congress* 5: 1430-1435, Saskatoon.