LEAKAGE OF FLUORESCENT COMPOUNDS FROM AGING CANOLA SEEDS AS A METHOD FOR DISTINGUISHING DEAD FROM LIVING EMBRYOS

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INTRODUCTION

Embryos and seedlings of Brassica napus L. contain phenolic compounds (Zobel et al., 1988). Their location changes during embryogenesis and germination (Zobel et al., 1989). The activation of the embryo during seed germination involves reactivation of mitosis, and DNA and starch synthesis (Kuras, 1986). The first zones of ultrastructural changes are in the columella and on the border between the radicle and the hypocotyl (Kuras, 1987). In this paper the membrane organization in these areas has been investigated in seeds harvested in 1978 and 1987, following our preliminary investigations on increased leakage of fluorescent compounds from old seeds. Leakage of sinapine as a fluorescent compound was previously found in dead embryos of Brassica (Taylor et al., 1988).

MATERIAL AND METHODS

Seeds of Brassica napus L. var. oleifera c.v. Gorczanski were obtained in 1978 from the Plant Breeding Institute, Radzikow, Poland and cultivated in the garden of Warsaw University each year until 1987. Preliminary results showed differences in the germination rate and in the increased concentration of leaking fluorescent compounds in aging seeds if fluorescence was measured at 280 and 360 nm. The seeds of the first and last years were used for investigation: analysis of leakage of fluorescent compounds, measurements of total glucosinolates and comparison of membrane structure of cells in the radicula after imbibition for 12 h.

Autofluorescence of compounds in different tissues of a seed was observed in the UV microscope on hand-cut sections, after excitation with 340 nm radiation. Phenolic compounds were precipitated with 0.1% caffeine during fixation for the electron microscope after a 6 h imbibition (Mueller and Greenwood, 1978; Zobel et al., 1989). For observation of the membrane structure the dry and fully imbibed seeds were fixed under optimal conditions: 2 h in 2% glutaraldehyde, pH 7.2, followed by post-fixation for 6 h in 1% OsO_4. Epon 812-embedded thin sections were stained with uranyl acetate and lead citrate. Surface deposition of fluorescent compounds and their leakage from the interior were measured with a UV spectrophotometer at 280 and 360 nm, and the values were compared to those of the fluorescence of sinapine (with an arbitrary unit of 30 micrograms/mL). The concentration of total glucosinolates was evaluated by a recently developed method (Schnug and Haneklaus, 1990a,b). For removal of surface deposits we employed the technique of brief dipping into almost-boiling water developed for furanocoumarins (Zobel and Brown, 1988).

RESULTS

Using autofluorescence we were able to locate the defense barriers for rapeseeds (Fig. 1): surface of the seed (barrier 1), seed coat (barrier 2), surface of the embryo (barrier 3) and seed tissue (barrier 4). The fluorescent compounds gave higher autofluorescence in the seed coat than in the embryo tissue (Fig. 2, arrow). Phenolic compounds precipitated with caffeine were located between the cell wall and the plasmalemma (Fig. 3, arrow). Comparison of membrane structure after full imbibition revealed that it was intact in the 1987 columella cells (Fig. 4) but damaged in the 1978 cells (Fig. 5), from which leakage was sixfold higher. The total glucosinolate concentration in the 1978 seeds was 75.9 micromoles/g (sd. 0.14), and in 1987 21.9 micromoles/g (sd. 0.97).

DISCUSSION

In dry seeds and during the first hours of imbibition, phenolic compounds were located between the cell wall and the plasmalemma, and thus outside the cytoplasm. This would have the advantage of not precipitating proteins and thus not destroying the cells which had synthesized them. Autofluorescing compounds were localized in higher concentrations in the seed coat tissue than in the embryo cells. These compounds were located on the seed coat as the first defense barrier, then in the seed coat cells, on the embryo surface (third barrier), and inside the cells of the embryo. Membranes were degraded in the seeds harvested in 1978. Increased leakage of sinapine from dead seeds observed by Taylor et al., 1988) and from our aging seeds could be explained by such membrane damage and additional massive leakage from the embryo cells.

CONCLUSIONS

(1) Membrane damage in aging seeds and dead embryos can be responsible for leakage of fluorescent compounds.

(2) Four defense barriers composed of fluorescent compounds can be located on the surface of the seed, seed coat tissue, surface of the embryo, and embryo tissue.

(3) Qualitative and quantitative determination of leaking compounds from different areas should be investigated next, as only sinapine was identified in the present work.

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Figure Legends

Fig. 1 Location of four defense barriers containing fluorescent compounds.

Fig. 2 Higher concentration of autofluorescing compounds in seed coat (arrow) than in embryo cells.

Fig. 3 Phenolic compounds precipitated with caffeine during fixation, located between the cell wall (CW) and plasmalemma. 4% paraformaldehyde + 2.5% glutaraldehyde 1% caffeine.

Fig. 4 Easily visible membrane structure in 1987 rape embryo after 12 h imbibition. 2% GA; post-fixation 1% OsO_4.

Fig. 5 Deformed membranes in 1978 rape embryo after 12 h imbibition.

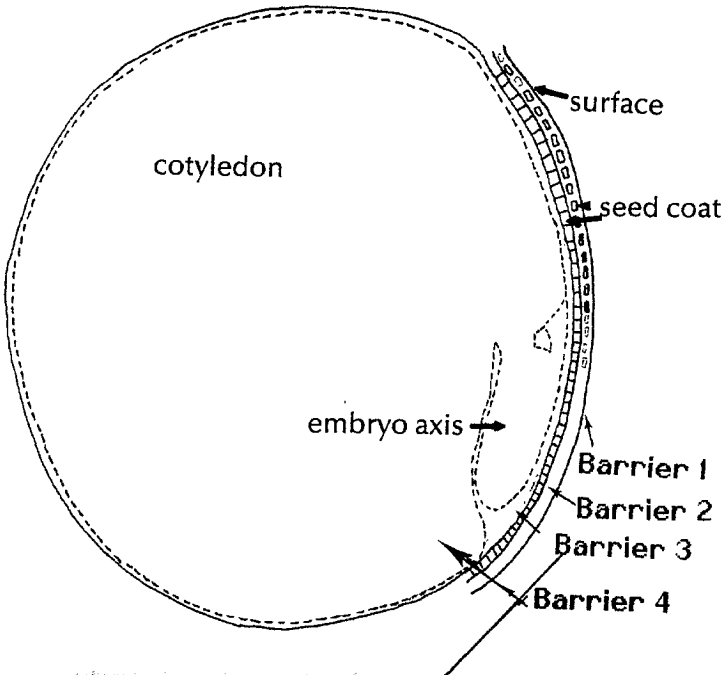
surface
cotyledon
seed coat
embryo axis
Barrier 1
Barrier 2
Barrier 3
Barrier 4

Fig 1

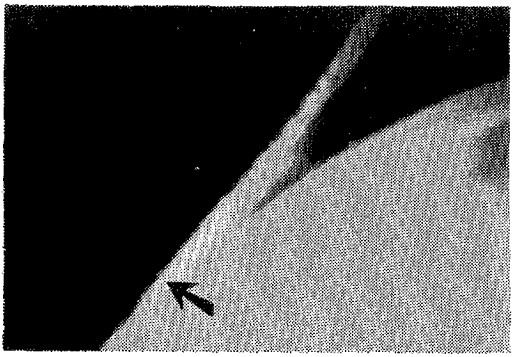

Fig 2

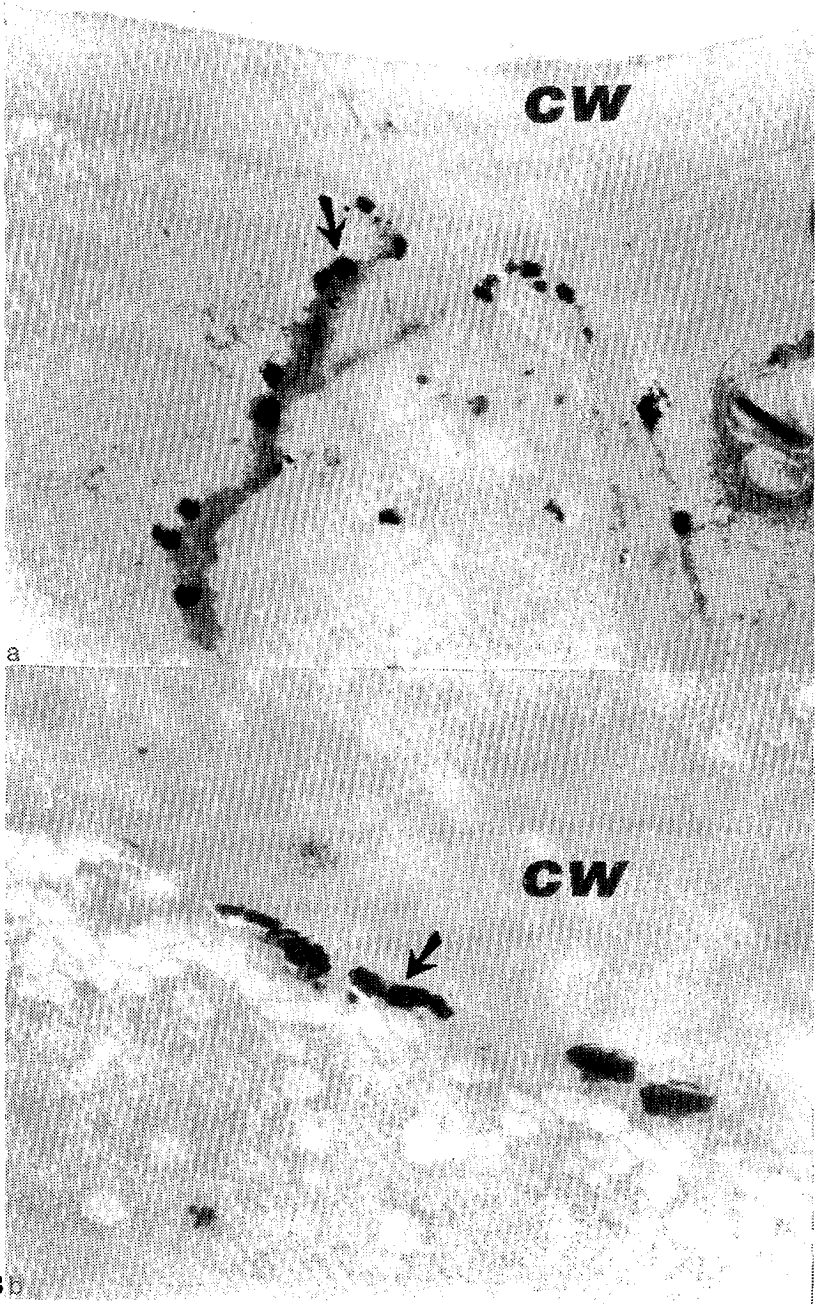

Fig3b

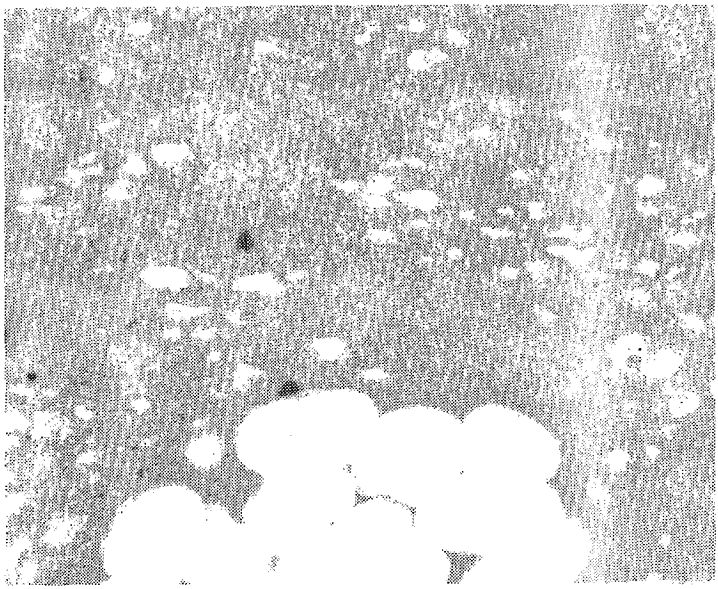

Fig4

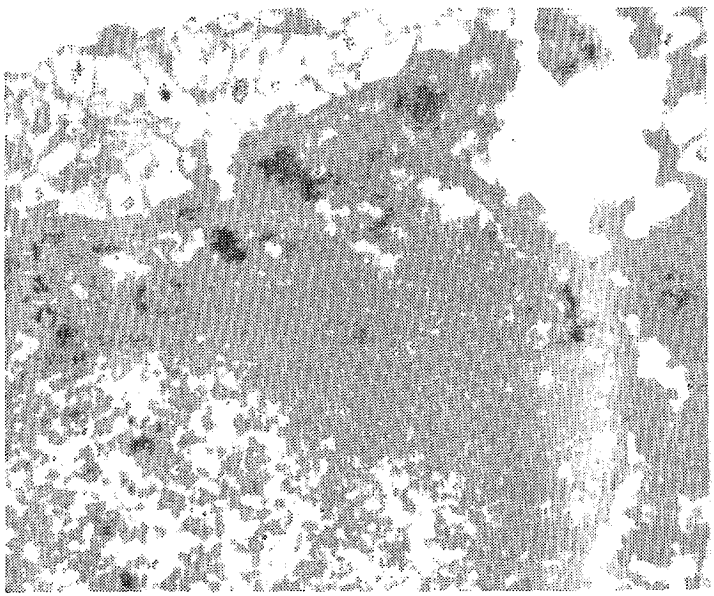

Fig5