The aim of this study is to investigate both the temporal and spatial expression patterns of the *Ole e 1* gene during male gametophyte development in the olive tree, in order to determine the biological significance of these proteins during pollen ontogeny.

Western blot analysis of anther protein extracts at different stages of development probed with a monoclonal antibody to *Ole e 1* confirms previous immunocytochemical results: *Ole e 1* starts to accumulate at the early stages of microspore development, showing increased levels of the protein during maturation within the anther and reaching the highest concentration in abscission pollen. In situ hybridization studies at LM using PCR generated dig-DNA probes to *Ole e 1*, demonstrated that *Ole e 1* transcripts are located in the microspores and in the tapetum, from the late tetrad stage onwards. At TEM level, the signal was found in association to ER ribosomes in the microspores. Within the mature pollen grains, mRNAs appear in the cytoplasm of both the vegetative and generative cells. This pattern was also confirmed by RT-PCR using specific primers.

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**Structural and molecular aspects of programmed cell death of the anther tapetum**

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Programmed cell death (pcd) which occurs in the normal development of higher plants is recently in the focus of interest of plant cell biologists. Death of the anther tapetum in support of the microspores/pollen grains, described as a cell degeneration is one of the most striking examples of pcd. The species-specific program of tapetal cell differentiation and death show temporal and stage-specific expression. Some alterations in the program including ablation of tapetal cells caused by recombinant DNA techniques result in the male sterility. The secretory tapetum of *Osmithogalum viridens* was the object of our study. Dissolution of tapetal cell walls (which is terminated at the tetrad stage) results in the tapetal cell protoplasts which remain in situ in the loculus periphery. We documented the relation between successive changes of ultrastructure of the tapetal nuclei and non-random cleavage of nuclear DNA (typical for pcd) during differentiation / disintegration of the tapetum cells. We used two methods to detect nDNA damage: single cell gel electrophoresis method - the comet assay and histochemical TUNEL method (which detects DNA breaks by terminal deoxynucleotidyl transferase-mediated *in situ* and labeling). The advantage of the comet assay for examination of tapetum cells lays in the small cell number sufficient to carry out the assay and in a natural condition of the "true proplast" of tapetal cells of *O. viridens*.

To our knowledge these are the first direct data on non-random cleavage of nDNA which starts during tapetum cell differentiation and precedes the cell disintegration.