Poster session a1

POLLEN ONTOGENY AND DEVELOPMENT

Comparative exine ontogeny in some Zygophyllaceae *sensu lato*

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The exine ontogeny is studied in five taxa among Zygophyllaceae *sensu lato* (*Peganum harmala, Zygophyllum album, Fagonia cretica, Tribulus terrestris* and *Nitraria retusa*). In the beginning the plasmalemma is applied to the callose wall except in *Tribulus*, where it describes "crests" and "hollows". The primexine matrix is fibrillar, bilayered in *Fagonia* and *Tribulus* and one-layered in the three other taxa. The procolumellae are heterogeneous with clear zones and they get compact later. In *Tribulus*, they are built on the "crests". In all cases, a tripartite primordial nexinic lamella (PNL) is set up. The white line is seen at some levels; on its external leaflet the foot layer is observed and on its internal one the endexine with numerous lamellae. This white line disappears often in the mature exine. In *Tribulus terrestris*, the foot layer and the endexine give rise to a nexine coarsely lamellar inside. In the aperture zone, there is an endexinic zone, granulo-lamellar, well developed in *Peganum harmala* where it is three-layered and it is lacking in *Tribulus terrestris*.

Tapetum development in fertile and sterilie anthers of *Actinidia deliciosa*

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In the majority plants primordia for the androecium and the gynoecium are formed, but one arrests its development and degenerates before maturation, originating unisexual flowers.

The kiwifruit, *Actinidia deliciosa* (A. Chev.) C. F. Liang and A. R. Fergunson, is a dioecious species in which staminate vines bear male flowers that contain viable pollen but rudimentary ovaries, and pistillate vines produce female flowers with non-viable pollen and functional ovaries.

In several species, in male sterile lines, the degeneration of pollen grains is invariably related with the malfunctioning of the tapetum. For these reasons, the aim of this study was to follow the ultrastrutural changes in the tapetal cells in the anthers of both male fertile (*A. deliciosa* cv. *Tomuri*) and male sterile plants (*A. deliciosa* cv. *Hayward*). In this study, anthers were observed using light microscopy and transmission or scanning electron microscopy.

In the beginning of the microsporogenesis, the tapetal cells surround the sporogenous tissue and play an important role in pollen development.

In the meiocytes stage no ultrastrutural differences were found between tapetal cells of fertile and sterile anthers. The tapetum, in the anthers of both cultivars, presents orbicules produced in the citoplasm with limiting membrane and are subsequently extruded to the cell surface (facing the locule) where they acquire a sporopolenin coating. Ultrastructural observations of the fertile anthers revealed that the tapetal cells display all characteristics of a secretory tissue.

In the sterile anthers, the microspores released from tetrads start a process of cytoplasmic shrinkage that leads to the degenerescence of all polinic content. Tapetal cells enlarge and became highly vacuolate and its cells undergo citoplasmic desorganization.

Tapetal cells degenerescence in the sterile anthers presents a different pattern of the one observed in fertile anthers. So in the sterile anthers the endoplasmatic reticulum starts showing signs of degradation. The most

salient feature is an unusual elaborate sistem of rough endoplasmatic reticulum, which consists of dilated cisternae containing numerous vesicle-like structures, of varous shapes ans sizes, limited by a single membrane, the inner face of which appears lined with ribossomes. However, as observed under light and scanning electron microscopy, the dehiscence of pollen grains was registered suggesting that the endothecium undergoes a normal developmental pattern in both anther types.

Our results revealed that in male sterile plants the tapetum breakdowns earlier than in fertile anthers, where tapetum disintegration occurs in late microspore stage. There seems to be a relationship between the pollen grain sterility and the tapetal cells degradation. Although some authors describe a correlation between tapetal behaviour and the development of the endothecium, curiously, no ultrastructural changes were observed at the endothecium level.

Keywords: *Actinidia deliciosa*, endothecium, microsporogenesis, tapetum.
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Ultrastructure and germination of *Vitis vinifera* cv. *Loureiro* pollen

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'Loureiro' is one of the most economically important grape cultivars, recommended for almost the totality of the Demarcated Region of Vinhos Verdes. In some vineyards the grape productivity of this cultivar is normal while in others it is extremely low. In this work we study the morphology and germination of freshly collected pollen grains of the *Vitis vinifera* cv. *Loureiro* with high and low productivity. Both cultivars have morphologically perfect flowers.

Tipically *Vitis vinifera* pollen grains present three longitudinal furrows but in the *'Loureiro'* cultivar we found with normal morphology and inaperturated pollen grains. For this reason, is important to investigate if the low productivity is correlated with the presence of acolporated (without furrows or germination pores) pollen grains.

The formation of microspores and pollen grains has been examined by light microscopy and transmission and scanning electron microscopy.

Under scanning electron microscopy, normal pollen grains in polar view appeared subspherical but trilobate, due to the presence of three furrows on its surface, and inaperturated pollen grains are spheroidal. In both pollen types no differences were recorded in the exine sculpturing.

Both pollen types have normally structured generative and vegetative cells with usual aspect and the cytoplasm is dense and rich of organelles. In the inaperturated pollen grains the exine forms a continous layer but the intine is irregulary distributed presenting different thickness around the pollen grain. The ultrastructural differences found in the two pollen types are related with the beginning of the biosinthesis of starch. Mature colporated pollen presented abundant amyloplast filled with numerous starch granules, while in inaperturated pollen these granules were not observed.

The inaperturated pollen grain is viable with a fluorochromatic reaction (FCR) using the fluorescein diacetate, but no germination was recorded both *in vitro* or *in vivo* tests. This led us to suggest that one of the causes for the low productivity of this cultivar is the impossibility to develop a pollen tube. However, a low grape productivity cannot always be ascribed only to the absence of furrows or germination pores, because this can also be found in plants with high productivity. This indicated that in plants with acolporate pollen, productivity seems to depend on others factors.

Further investigations are required to assess if this low productivity in some cv. *Loureiro* may result from genetic sterility or from nutritional, environmental, or pathological conditions unfavorable to pollen germination. These studies may offer considerable help in the research on the evolution and taxonomic classification and agronomic decisions of these cultivars.

Key words: germination, pollen, productivity, viability, *Vitis vinifera*.

Lipid bodies behavior during pollen maturation in *Magnolia* X *soulangeana* Soul.-Bod. (Magnoliaceae)

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This study describes the pollen maturation of *Magnolia* X *soulangeana*, with emphasis on the storage lipid bodies and their relationship with other cell organelles. The young pollen grain contained both numerous amyloplasts and spherical lipid bodies scattered throughout the vegetative cell (VC) cytoplasm. The lipid bodies varied in size and most of them showed high osmiophily. In the generative cell (GC), amyloplasts and abundant lipid bodies were not evident. When this cell began to move away from the intine (see DINIS & MESQUITA 1999), a "collar" of lipid bodies formed in the VC cytoplasm lining the GC periplasm. This "collar" persisted in the intermediate pollen grain, being dispersed later. Although the mature VC still contained a significant amount of lipid bodies, these were generally more reduced in size and osmiophily than in the previous devclopmental stages. In the intermediate and mature pollen, some lipid bodies established an intimate contact with peroxisomes and/or mitochondria, in contrast to the young pollen grain. Following the DAB reaction for catalase, the peroxisomes reacted positively and their abundance and polymorphism became evident. Clearly, the peroxisomes proliferated from the young to the intermediate pollen and their morphology and ultrastructure were modified. These facts point to an increase of activity of these organelles which are presumably of a glyoxysomal type, as reported for other species (e.g., PAIS & FEIJÓ 1987). Therefore, they likely participate in the conversion of lipid reserves to saccharides.

In the intermediate and mature pollen, many lipid bodies also established an intimate association with complex endomembrane compartments identified as protein storage vacuoles/reticulum (see DINIS *et al.* 2000). The latter were filled with electron dense fibrillar material and exhibited a remarkable pleiomorphism, especially in the freeze-fixed and freeze-substituted pollen grains. Frequently, a number of lipid bodies appeared completely surrounded by extremely narrow, ring-like shaped vacuole profiles. Others fitted perfectly into curved, narrow vacuole profiles swelled at their ends. In both cases the lipid bodies were seen generally appressed against the profile membrane. It is uncertain whether these lipid bodies were being protected or, as reported by NOGUCHI (1990), digested.

In the mature pollen there were also many lipid bodies in contact, and/or surrounded by a "collar" of small (c. 0.05 μm in diameter), electron dense vesicles. Interestingly, this association involved the smallest lipid bodies which had generally ill-defined boundaries as they were being decomposed. Although the vesicles had presumably a Golgi origin, it is noteworthy that they reacted positively to both the Thiéry´s test and the PTA staining at low pH, in contrast to the dictyosome cisternae and associated vesicles. The dictyosomes proliferated during pollen maturation and were always very active. Possibly, the intimate association between the lipid bodies and the vesicles represents a functional relationship that may be involved in the synthesis of pollen tube wall precursors.

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Cell wall polysaccharides in differentiating anthers and pistils of *Lolium perenne*

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Anthers and pistils are very complex flower organs that play several important functions in development and differentiation of male and female gametophytes, the events followed by successful pollination and fertilization. In parallel, the sporophytic cells differentiate into well-defined and specialized tissues, which gain specific roles in nutrition of gametophytes, in providing them with structural polysaccharides and proteins, and in protecting against biotic and abiotic factors. Micro- and macrosporogenesis are accompanied by numerous cell divisions, formation of new cell walls and cells expansion, the processes related to synthesis, degradation and a turn-over of some wall constituents. All of these events involve continuous reorganization of cell wall components, contribute further to the changes in their molecular organization and finally lead to the acquirement of cell or tissue identity.

We traced the temporal and spatial distribution of several polysaccharidic epitopes within the cells of differentiating anthers and pistils of *Lolium* - an important grass species. Several monoclonal antibodies were used to detect particular epitopes in non-cellulosic molecules:

a) anti- (1→3, 1→4)-β-glucan that binds to mixed-linkage glucans,
b) anti- (1→4)-β-mannan that recognizes this motif in mannan polysaccharides,
c) JIM7 that binds to highly esterified pectins containing epitopes composed of methyl-esterified residues with adjacent or flanking unesterified residues (CLAUSEN et al. 2003),
d) JIM5 binding to low esterified pectins containing epitopes composed of four or more contiguous unesterified residues adjacent to or flanked by residues with methyl-ester groups,
e) LM5 that detects pectin side chains with four (1→4)-β-D-galactose residues (JONES et al., 1997),
f) LM6 that recognizes pectic side chains with five (1→5)-α-L-arabinose residues (WILLATS et al. 1999).

The results provide new data on the involvement of specific cell wall-domains in the processes of differentiation and maturation of male and female gametophytes in monocot perennial ryegrass.

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Pollen-wall and aperture ontogeny in *Erodium* L'Hér. (Geraniaceae)

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The genera *Erodium* and *Geranium* are the only representatives of the Geraniaceae family native to the flora of the Iberian Peninsula. The family as a whole comprises eleven genera, which are widely distributed throughout the temperate and subtropical zones, although they are not found so commonly in the tropics (CRONQUIST, 1981).

In the Iberian Peninsula there are some thirty species of *Erodium*, including some taxa that present considerable difficulty in their classification. In a recent study (SALINAS *et al.*, 2004) we used S.E.M. to study some parameters of the ornamentation of the pollen wall of species belonging to *Erodium* and T.E.M. to look more closely at the structure of the sporodermis, in the hope that these observations would prove useful in the taxonomy of this genus. This work revealed the need for further studies into the ontogeny of the wall and apertures of the pollen grains.

To clarify the origin, nature and possible functions of the layers and structures we had observed previously, such as the apertural chambers, we undertook a T.E.M. study into the ontogeny of the wall and apertures of the pollen grains of two *Erodium* species: *E. malacoides* (L.) L'Hér., belonging to the Malacoidea Lange section and *E. moschatum* (L.) L'Hér. in Aiton, belonging to the Cicutaria (Lange) Batt section.

For electron microscope studies we selected anthers at various stages in their development, which we determined by examining squashes stained with toluidine blue and acetic carmine under a light microscope. The anther samples were fixed in 3% glutaraldehyde in 0.025 M cacodylate buffer (pH 7.2) for 6 h at 4ºC and then washed in cacodylate buffer. They were then post-fixed with a mixture of 1% osmium tetroxide and 2% potassium ferrocyanide in the same buffer for 2 h at 4ºC. All samples were dehydrated in an ethanol series and embedded in Spurr resin. Ultrathin sections were cut with a Reichert Ultracut E ultramicrotome and stained with uranyl acetate and lead citrate. Electron micrographs were taken with a Zeiss EM 902 microscope.

We compared the ontogenic patterns of both species between each other and also with the *Geranium* species studied by WEBER (1996 a, b). Our results reveal the existence of numerous similarities both between the two species of *Erodium* studied and between these and the *Geranium* species. The origin and function of the apertural chambers also appear to be similar in both genera.

Lastly, we discuss the possible importance of these structures in the evolution of the Geraniaceae family.

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Pollen development in the argan tree (*Argania spinosa* L.)

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The argan (*Argania spinosa* L.) is a Moroccan endemic tree, member of the *Sapotaceae* family, which grows throughout the Southwest of the country (PRENDERGAST & WALKER, 1992). The reproductive biology of this species is practically unknown, with little data available concerning pollen development, pollination, fecundation and the presence of compatibility mechanisms.

The morphological and ultrastructural studies carried out in this work show that the mature pollen of the argan tree is tri-cellular, prolate and 5-colporate, displaying a well developed and structured exine of the rugulate-striate type. These morphological characteristics are substantially different from those displayed by the mature pollen of other *Sapotaceae* species such as *Sideroxylon lanuginosum* Michaux and *Sideroxylon lycioides* L. described as tricolporate and exhibiting psilate ornamentation in their exine (Pollen Reference Collection of the APMRU-US Department of Agriculture), whereas is similar although non-identical to that of *Isonandra montana* which is 3(4)-colporate and rugulate-perforate (PREMATHILAKE & NILLSON, 2001).

In the present study we also report the presence of both lipid and polysaccharide storage materials in the cytoplasm of the vegetative cell at the mature pollen.

Lipid materials mainly appear in the form of individual, spherical lipid bodies of 0.5-0.7 µm in diameter which are densely stained by Sudan Black B. They start to accumulate immediately after the first asymmetric division, reaching its maximun at the tricellular stage, where they begin to change their spherical shape, tending to fuse and collapse due probably to the mobilization of triacilglicerids to provide energy to the maturing pollen grains after the loss of their major nutrient source –the tapetum.

The pollen of the argan also accumulates significant amounts of periodic acid-Schiff (PAS)-positive starch granules, which start to be synthesized inside amyloplasts at the vacuolated/late microspore stage. At the mature tricellular pollen stage, conspicuous roundly-shaped starch granules of 0.7-1.2 µm in diameter fill the cytoplasm of the vegetative cell, together with the abovementioned lipid bodies.

Both lipid and polysaccharide materials are also abundantly detected on the surface of the exine after using the corresponding histochemical techniques.

The relationships between the ultrastructural details observed, the composition of the storage reserves, the exine-associated materials and the type of pollination (80% anemophylous) are widely discussed.

This work was funded by projects CAO99-003 (INIA), BCM2000-1484 and AGL2003-00719 (both from MCyT).

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Sporoderm development of Asteraceae

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We have studied the sporoderm development in some representatives of Asteraceae from different tribes: *Cicerbita macrophylla* (Willd.) Wallr., *Centaurea cyanus* L., *Centaurea jacea* L., *Echinops sphaerocephalos* L., *Calendula officinalis* L., *Dimorphotheca auranthiaca* DC., *Cichorium intybus* L., *Tanacetum vulgare* L. The anthers of different age were treated according to the standard method for electron microscopy, stained with osmium and uranyl-acetate and contrasted with lead according to Reynolds method. Investigations were carried out with transmission electron microscope Jeol 100B.

Pollen grains of Asteraceae have complex and specific structure of ectexine. Some species have cavities in mesocolpia of pollen grains (*Centaurea jacea*, *Calendula officinalis*, *Dimorphotheca auranthiaca*). The others are characterized by the second row of columellae in the place of cavity (*Centaurea cyanus*, *Tanacetum vulgare* and others). As a rule, this row consists of the big columellae.

The process of microspores and pollen grains development can be divided into 2 periods: tetrad and posttetrad (free spores period). At the beginning of tetrad period callose wall (the first wall of microspore) is forming around the microspores. Many small vesicles with electron dense core migrate from microspore cytoplasm and accumulate on the plasma membrane. They deposit around microspore except the areas of future apertures, forming glycocalyx – the "framework" of primexine. The presence of vesicular matrix was revealed earlier for other species of Asteraceae (TAKAHASHI, 1989). In the middle of the tetrad period sporopollenin starts to polymerize on the vesicular glycocalyx. Sporopollenin deposits between groups of vesicles and forms baculate structure of primexine. Future spines become visible like rounded structures on the rows of vesicles. Near the margins of apertures bacula are not developed and ectexine contains only some elements of tectum. At the aperture areas primexine are not formed. At the end of tetrad period sporopollenin does not polymerize mature on some inner rows of vesicles that contact with plasmalemma of the microspore. On this stage of wall development a "stripe" of inner vesicular matrix is visible very clearly. The formation of cavities starts in the area of inner vesicular matrix in the species with cavate pollen grains. Species with non-cavate pollen grains reserve inner matrix that will be replaced by big columellae of the second row at post-tetrad period.

On the early post-tetrad stage the callose wall is destroyed and tetrads are disintegrated. In the post-tetrad period endexine and intine are formed. The development of these inner layers starts in the aperture regions. The same sequence of intine development was appeared for Gerbera jamensonii (SOUTHWORTH, 1983). Besides the ultrastructural elements of sporoderm that are specific for different palinotypes are also developed in post-tetrad period.

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Germination in *Arabidopsis* pollen: polarization and emergence of the pollen tube

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In germinating *Arabidopsis* pollen, a pollen tube emerges from the grain precisely at its point of contact with the stigma, breaching first the exine wall and then the papillar cell wall. This pollen-stigma contact point is distinguished by a lipid- and protein-containing 'foot,' which rapidly provides the pollen cell with a focused source of stigma water within minutes of the grain's arrival. We are investigating the early polarization of the pollen tube relative to this spatial cue, together with the mechanisms necessary for tube exit directly through the exine, in contrast to exit through an aperture. We have found that many pollen tubes emerging from mutant cer6-2 pollen (pollen lacking key lipid and protein coat components, and thus forming a deficient foot are unable to identify the point of contact with the stigma and wander aimlessly, as do tubes emerging from wild-type pollen hydrated from all sides by placement in a high humidity chamber. Several minutes of focused water reception are sufficient to appropriately polarize the tube. To better understand the biomechanical forces necessary for pollen tubes to break through the exine wall after polarization, we are measuring exine material properties with an atomic force microscope, and comparing these properties between wild-type and mutant lap1 pollen grains (pollen with mispatterned, reduced exine walls). Finally, we are gathering evidence for and against focused exine-breaking mechanisms based upon internal turgor pressure, secreted gel swelling, and exine weakening (both from the interior and exterior of the pollen grain).

Poster session a2

SIGNALLING IN POLLEN DEVELOPMENT, STRESS-INDUCED MISROSPORE EMBRYOGENESIS AND POLLEN GERMINATION

Defined cellular rearrangements characterize in vivo and in vitro pollen developmental programmes in *Brassica napus* L.

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Alternative pollen developmental programmes take place under different controlled conditions in *Brassica napus* L. Under stress conditions in vitro, the microspore can be deviated from its developmental gametophytic programme towards an embryogenic pathway to form haploid embryos and plants. The reprogramming of the microspore is only possible at specific developmental stages such as the vacuolate microspore. After induction in vitro, some microspores switch to proliferation and embryogenesis whereas others, not sensitive to induction follow a gametophytic-like pathway in vitro. This reprogramming is accompanied by defined changes affecting various cell activities and structural organization of subcellular compartments which can be considered as markers of the pollen reprogramming process.

In *Brassica napus* L., a dicot species which can be considered as a model for pollen embryogenesis induction, the stress inductive treatment consists on 32ºC for at least 8 hours. If microspore culture is kept at 18ºC, gametophytic development is mimicked in vitro.