ORAL PRESENTATION

Floral development and embryology in *Lomandra longifolia*

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The development of male and female flowers of *Lomandra longifolia* was studied through scanning electron microscopy analysis and cytological observations. Although mature flowers are functionally unisexual, early development is similar in staminate and pistillate flowers. Six stamens are initiated in two alternating whorls of three stamens each, the first opposite the sepals and the second opposite the petals. Lastly, three separate carpels are initiated. The two flower types diverge developmentally when the stamens become bi-lobed. Cytological analysis of the slowly growing abortive pistil of male flowers shows that megasporogenesis does not occur and therefore megagametophyte formation is prevented. Pistil abortion happens before meiosis in the microspore mother cell while the stamens continue to develop until maturity and dehiscence. On the other hand, stamen arrest occurs at an early stage before the onset of meiosis in the microspore mother cells while the pistil continues its development through megasporogenesis and megagametogenesis.

The anther wall in *Lomandra longifolia* comprises four cell layers: an epidermis, an endothecium, one middle layer, and a tapetum. The tapetum layer has uninucleate cells of a glandular type. Meiosis of the microspore mother cell leads to the formation of a tetrahedral tetrad of microspores. The ovule in each carpel is anatropous, crassinucellate, and bitegmic, with the micropyle formed by the inner integument. The archesporial cell divides periclinally to form the primary parietal and primary sporogenous cells. The sporogenous cell functions as the megaspore mother cell while the parietal cell divides to give rise to two parietal layers. The mature megagametophyte is of the *Polygonum* type and has the normal complement of seven cells and eight nuclei. The endosperm development is of the nuclear type. Embryo development is of Graminad type characterized by asymmetric and oblique zygotic division.

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The role of the ubiquitin/ 26S proteasome pathway in plant morphogenesis and embryogenesis

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In recent years, the ubiquitin/26S proteasome pathway was discovered to be a central player for rapid and selective degradation of key short-lived regulatory proteins that play important roles in a variety of cellular processes (Ciechanover et al., 2000; Weissman, 2001). In particular, cell cycle progression and many developmental processes are tightly controlled by ubiquitin-dependent protein degradation. Ubiquitylation is achieved through an enzymatic cascade involving the sequential action of ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin-ligating (E3) enzymes. Among these enzymes, the E3s thought to be the main determinants of substrate recognition and play a central role in the selectivity of ubiquitin-mediated protein degradation. Ubiquitinated proteins become the substrates of the 26S proteasome, which is a protein complex present in all eukaryotes that plays a central role in the degradation of these proteins. The 26S proteasome consists of two multi-subunit protein complexes: the proteolytic core protease (CP) and the regulatory particle (RP), which consists of at least 17 subunits and plays an important role in the selectivity of the ubiquitin/proteasome pathway. The function of most RP subunits is still poorly understood.

To date several classes of E3s have been reported (Jackson et al., 2000). A major type of E3s are the SCF complexes (Deshaies, 1999), which in *Saccharomyces cerevisiae* are composed of four primary subunits: Cdc53 (Cullin1), Rbx1, Skpp1 and an F-box protein. In addition to CUL1, eukaryote genomes encode additional cullins (CUL2, CUL3, CUL4 and CUL5) (Gieffers et al., 2000), which are all believed to form protein complexes with ubiquitin-ligase activity. A series of recent reports has shed light on the molecular composition and function of the CUL3-based E3 ligases (reviewed in Pintard et al., 2004). Cullin-dependent ubiquitin ligases form a class of structurally related multi-subunit enzymes that control the rapid and selective degradation of important regulatory proteins involved in cell cycle progression and development, among others. The CUL3-BTB ligases belong to this class of enzymes and despite recent findings on their molecular composition, our knowledge on their functions and substrates remains still very limited.

In contrast to budding and fission yeast, *CUL3* is an essential gene in metazoans. The model plant *Arabidopsis thaliana* encodes two related *CUL3* genes, called *CUL3A* and *CUL3B*. We recently reported that *cul3a* loss-of-function mutants are viable but exhibit a mild flowering and light sensitivity phenotype (Dieterle et al., 2005). We investigated the spatial and temporal expression of the two *CUL3* genes in reproductive tissues and found that their expression patterns are largely overlapping suggesting possible functional redundancy (Thomann et al., 2005). Thus, we investigated the consequences on plant development of combined *Arabidopsis cul3a cul3b* loss-of-function mutations. Homozygous *cul3b* mutant plants developed normally and were fully fertile. However the disruption of both the *CUL3A* and *CUL3B* genes reduced gametophytic transmission and caused embryo lethality. The observed embryo abortion was found to be under maternal control. Arrest of embryogenesis occurred at multiple stages of embryo development, but predominantly at the heart stage. At the cytological level, *CUL3* loss-of-function mutations affected both embryo pattern formation and endosperm development.

In previous research it had been assumed that much of the selectivity of the ubiquitin/26S proteasome pathway is achieved by ubiqutination reactions. However, recent genetic analyses have also implicated the regulatory particle (RP) subunits of the 26S proteasome as important contributors (Smalle et al., 2002; 2003; Vierstra, 2003). Mutants affecting individual *Arabidopsis* RP subunits display a wide range of phenotypes, consistent with each participating in the destruction of distinct set of targets. In *Arabidopsis*, the RPN1 subunit is encoded by two paralogous genes *RPN1a* and *RPN1b*. Disruption of the *RPN1a* gene caused embryo lethality and seed abortion, while plants lacking *RPN1b* showed no obvious abnormal phenotype. Embryos homozygous for *rpn1a* arrested at the globular stage with defects in the formation of the embryonic root, the protoderm and procambium. Cyclin B1 protein was not degraded in these embryos consistent with the observed defects in cell division. *RPN1a* is mainly expressed in the embryo up to the globular stage where mutant embryos arrest. Double mutant plants (*rpn1a/RPN1a; rpn1b/rpn1b)* produced embryos with a phenotype indistinguishable from that of the *rpn1a* single mutant. Thus, despite a largely overlapping expression pattern, the two isoforms do not share redundant functions during gametogenesis and embryogenesis and may thus be involved in distinct processes. However, complementation of the *rpn1a* mutation with the coding region *RPN1b* expressed under the control of the *RPN1a* promoter indicates that two RPN1 isoforms are functionally equivalent.

Overall, our data indicate that the function of both some of the ubiquitin E3 ligases (CUL3) and some subunits of the 26S proteaesome (RP particles) are essential during embryogenesis, where they might participate in the labelling/recognition of a specific set of protein targets. However phenotype of mutants in majority of E3 ligases as well as of RP particles of the 26S proteasome don't reveal embryo lethality but looks normal or display other abnormalities.

Immunodetection of arabinogalactan proteins (AGPs) in apomictic ovules of *Chondrilla juncea* L.

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Arabinogalactan proteins are a class of proteoglycans playing an important role in various aspects of plant development and tissue differentiation. Recently it was evidenced that special molecules of AGPs are necessary for development of the female gamephyte in Arabidopsis (Acosta-Garcia and Vielle-Calzada, 2004). Investigations of several amphimictic species localized AGPs in the egg apparatus and inside the micropylar canal. It was suggested that these molecules may facilitate pollen tube growth and mark ovule's receptivity (Coimbra and Duarte, 2003; Chudzik et al., 2005).

The aim of the present work was to examine the presence and localization of AGPs epitopes recognized by JIM 8 and JIM 13 Mab in the ovules where fertilization does not occur. *Chondrilla juncea* is an obligate apomictic species with the diplosporous embryo sac of *Taraxacum* type in which the embryo and the endosperm develop autonomously without fertilization. The epitopes recognized by JIM 8 Mab were not detectable at any developmental stage of the ovules. Analysis of immunofluorescence after JIM 13 Mab labelling showed that this epitope was not present in the ovules at the stages of megaspore mother cell and at the early diplodyad. In the older ovules the clear JIM 13 labelling appeared in the cell wall separating the diplodyad cells and in the cell wall surrounding the one-nucleate embryo sac. During the next developmental stages until the cellularization of the embryo sac, the JIM 13 epitopes were still evident inside the cells of the gamethophyte and additionally appeared in the cell walls of the integumental tapetum. Just before the beginning of the embryo development the JIM 13 epitope was completely absent in the embryo sac as well as in the endothelium cells. In the substance filling the micropylar canal the JIM 13 epitopes were not detected at any developmental stage. Lack (absence) of AGPs epitopes recognized by JIM 13 Mab inside the micropylar canal and mature embryo sac of *Ch. juncea* coincides with lack of the fertilization process in the apomictic species.

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Arabinogalactan proteins as molecular markers for generative cell differentiation and development in *Arabidopsis thaliana*

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Arabinogalactan proteins (AGPs) are a class of complex proteoglycans highly glycosylated that are found at the surface of all plant cells. The carbohydrate moiety of AGPs is believed to be responsible for their functions. One can localize AGPs in tissues and cells through the use of specific monoclonal antibodies (MAbs) that detect structurally complex carbohydrate epitopes (Knox, 1997). The use of MAbs has shown that these AGP moieties are differentially expressed during plant development, including during sexual reproduction (Serpe and Nothnagel, 1995).

We have recently shown the expression of AGP genes in *Arabidopsis* pollen grains and pollen tubes and also the presence of AGPs along *Arabidopsis* pollen tube cell surface, and tip region, as opposed to what had been reported earlier. We have also shown that only a subset of AGP genes is expressed in pollen tubes, with prevalence for *Agp6* and *Agp11*, suggesting a specific and defined role for some AGPs in *Arabidopsis* sexual reproduction (Pereira et al., 2005). Other very recent results, (Acosta-Garcia and Vielle-Calzada, 2004) indicate that *Agp18* is essential for the establishment of the female gametophytic phase, also in *Arabidopsis*.

Evaluating the selective labelling obtained with AGP Mabs JIM 8, JIM 13, MAC 207 and LM 2, during *Arabidopsis* pollen development, we postulate that some AGPs are molecular markers for generative cell differentiation and development into male gametes. Furthermore, comparing ecotype *Col-0* with two mutants with reduced levels of AGPs, *mur1* and *reb1-1*, it is possible to conclude that the differences obtained in the labelling patterns don't seem to affect the normal development of the male gametophyte. Being so, the relation between AGPs and microtubules involved in cell morphogenesis, hypothesised for the *reb1-1* phenotype in roots, does not seem to occur during pollen development. To date, *SPOROCYTELESS* is the only gene shown to be required for the initiation of micro and megasporogenesis (Yang et al., 1999). So, in search of finding a precise function for a single AGP, our next step is to study the specific role and localization of *Agp6* using transgenic plants.

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Non-functional embryo sacs in apomictic and sexually reproducing plants

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There are two types of non-functional embryo sacs in Angiosperms, if we exclude degenerations caused by various factors observed in some hybrids or brought about by unfavorable environmental conditions of life, etc. In the first type a non-functional embryo sac has a structure which makes the formation of an embryo and/or endosperm impossible. It is noted sporadically in sexually reproducing plants and more frequently in aposporous and diplosporous ones. The deviation range between minor changes of a normal embryo sac structure and the extremal, non-functional monstrosities. The embryo sacs of the second type have not only an abnormal structure but their development is delayed or retarded in comparison with the mature embryo sac present simultaneously in the ovule. The delayed aposporous embryo sacs which are irregular and non-functional are commonly accepted as the sign of a tendency towards apomixis, broken at an early stage of gametophytogenesis.

The abnormal embryo sacs are poorly examined on account of their random and rare appearence. The genetic and molecular mechanisms of the disturbed development, as well as its retardation in the delayed formation of aposporous initials and embryo sacs are still unknown in spite of their importance to the knowledge of plant embryology and apomixis.

Sporophyte control of petunia (*Petunia hybrida* L.) male gametophyte development

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In petunia self-compatible, self-incompatible, and sterile clones, comparative embryological study revealed some differences in anther development. In both fertile clones, the placentoids (connective exvaginations) growing into the cavity of the microsporangium and determining its "horse-shoe"-like shape are produced in each of the four anther locules. In self-incompatible clone, the placentoids were observed to rather frequently partitioned off the anther locules almost completely. The development of the microsporangium wall took place in the typical way. Fully-developed wall consisted of epidermis, fibrous endothecium, one to two middle layers and the secretory tapetum. Tapetal cells become binucleate by the termination of meiosis and multinucleate at the tetrad stage, containing 4–6 nuclei. Before the beginning of the development of the male gametophyte, the disintegration of the tapetal cell started. Complete degeneration of the tapetum occurred before the formation of binucleate pollen; degeneration of the tapetum, as a layer, took place earlier from the connective side. The tapetum is, as a rule, an unilayer of cells at the outer side and multiseriate at the connective side. The tapetum is of the cellular-secretory type in the self-compatible clones and cellular-secretory with reorganization to amoeboid in the self-incompatible clones. In self-incompatible clones, invaginations and exvaginations of tapetal cells in the anther locule have been observed at the tetrad stage. In both clones, the microsporogenesis is of the simultaneous type. Callose was located in the microsporocyte wall prior to the separation of tetrads of microspores. Mature pollen is bicellular. In the sterile clone, changes were observed in anthers in both tapetal and microsporocytes at the beginning of meiosis with additional defects found at later stages, in both compartments.

In petunia clones, ethylene emission by tissues of the developing anthers and germinating in vitro pollen tubes was measured with a gas chromatography. In the self-compatible and self-incompatible clones, the male gametophyte development in vivo was accompanied with two peaks of ethylene evolution by tissues of developing anthers: the first peak (three-fold increase in the ethylene emission) reveled on the stage of microspore vacuolation, the second peak coincided with the stage of mature pollen before anther dehiscence. Mature pollen was characterized by high ACC content. In sterile clone, microsporocyte destruction was accompanied with a ten-fold increase in the ethylene evolution by anther tissues. In the self-compatible clone, exogenous ethylene (1, 10, 100µl/l) induced the arrest of male gametophyte development on meiotic stage. The first half an hour of pollen germination in vitro was accompanied by ethylene peak possibly is conditioned by pollen tube formation. It is concluded that ethylene is the obligatory factor of male gametophyte growth and development, participating in the intercellular interactions in system of anther-male gametophyte.

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Development of *Arabidopsis* and tobacco seedling shoot apical meristem after *ipt* Transcriptional activation IN pOp/LhG4 system

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A transcription activation system developed for regulated gene expression (Moore et al., 1998) is based on the combination of a synthetic promoter pOp (consisting of two lac operators and a minimal promoter) and the corresponding chimerical transcriptional activator LhG4. This system was used in tobacco and *Arabidopsis* for the study of ipt (a cytokinin biosynthesis gene) expression and its impact on post-embryonic development. Expression of the transgene was achieved by crossing a reporter plant (pOp-*ipt*) with an activator plant expressing the transcription factor *LhG4* under the control of CaMV35S promoter. The aim of this study was to compare shoot apical meristem development in seedlings bearing activated *ipt* with that of controls.

Seedlings of both wild type and all transgenic lines aseptically grown on modified MS medium (Murashige and Skoog, 1962) were collected at several intervals after germination. Paraffin sections were prepared according to classical histological methods. Nuclear Fast red with Alcian blue or Safranin and Fast green were used for double staining. Dehydrated sections were mounted in Eukitt® and documented by Olympus BX–61. Structures on selected median sections were evaluated by means of analySIS® software.

Phenotypic changes typical for cytokinin overproduction were clearly observable on plantlets with activated *ipt* expression after 7 days of culture and they were more pronounced upon further culture. Plantlets with activated *ipt* developed a larger meristem. Enlargement of meristem area was caused in these plants especially by increase of meristem width. Meristems of 15-day-old *Arabidopsis* seedlings were changed to generative ones to various degrees. Evaluation of these changes was performed according to the developmental stages (Smyth et al., 1990). The shift of developmental stages to more progressed flower formation was traced in *ipt*-activated lines.

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Arabidopsis Gene Family Profiler – a new easy-to-use family-oriented gene expression database

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The rapidly increasing volume of publicly available gene expression datasets for Arabidopsis now demands an environment suitable for easy orientation and that enables genome-targeted questions about expression patterns to be answered. We present a currated gene family-oriented gene expression database with a user-friendly graphic interface. Arabidopsis Gene Family Profiler (GFP) gives the user access to normalised Affymetrix ATH1 microarray data collected from NASC within the scope of the AffyWatch Service (Craigon et al., 2004). The database contains transcriptomic data for number of tissues at various developmental stages from wild type plants gathered from nearly 350 gene chips.

The Arabidopsis GFP database has been designed as an easy-to-use tool for users needing an easily accessible resource for expression data of either single genes, predefined gene families or custom user-defined gene sets, with the further possibility of keyword search. The environment enables users to access individual chip experiments and mean data for all appropriate microarrays. Arabidopsis Gene Family Profiler presents a user-friendly web interface using both graphic and text output. Data are stored at the MySQL server and individual queries are created in PHP script. The distinguishing features of Arabidopsis Gene Family Profiler database are 1) presentation of normalised datasets (Affymetrix MAS5 algorithm and calculation of model-based gene-expression values based on the Perfect Match-only model); 2) an intuitive interface; 3) an interactive "virtual plant" visualising the spatial and developmental expression profiles of both gene families and individual genes. Altogether Arabidopsis GFP gives users the possibility to start with simple global questions that can be further refined as highly targeted ones.

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Araceae embryos as tools for ploidy manipulation and somatic fusion

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Somatic embryogenesis has been reported in the family *Araceae*. In *Anthurium scherzerianum* leaf pieces were used as explants; in *Anthurium andreanum* laminas (Hamidah et al., 1997). Somatic embryogenesis on anther filaments of *Spathiphyllum* has first been described by Werbrouck et al. (2000); data are lacking for other *Araceae* genera. Chen and Kuehnle (1999) have described somatic embryogenesis on etiolated *Spathiphyllum* internodes, but only for 1 genotype. We have used *Spathiphyllum wallisii* as a model crop. Polyploidization was accomplished using different methods; regeneration through somatic embryos arisen on anther filaments was most efficient (Eeckhaut et al., 2004).

In the *Araceae* family, intergeneric breeding is strongly hampered by interspecific barriers. Prezygotic barriers can not be overcome; moreover, postzygotic barriers are to be expected even upon (unlikely) embryo formation. For that reason, somatic fusion is considered as an alternative for sexual recombination. However, the creation of a somatic hybrid requires proper protoplast isolation, fusion, selection and regeneration protocols. Based on protocols described by Kuehnle (1997) isolation and preservation of *Spathiphyllum* as well as *Anthurium* protoplasts has yet been accomplished. Somatic embryos were used for protoplast isolation. Moreover, as *Spathiphyllum* somatic embryos (that were induced in the dark) lack chlorophyll, the embryo-derived protoplasts will not be autofluorescent which can be exploited in a selection procedure after fusion with autofluorescent *Anthurium* leaf protoplasts.

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Silencing the [alpha]-Gliadins in wheat

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The gliadins belong to the major seed storage proteins of wheat and determine the viscosity of the dough from wheat flour. The superior baking qualities of wheat are overshadowed by its potential to cause coeliac disease, an inflammatory condition of the gastrointestinal tract. It is thought that the coeliac disease is induced in susceptible patients most likely by the gliadins.

The gliadins are made up of different subtypes (alpha, gamma, omega) each encoded by multiple genes. A genetic approach for reducing the gliadin component to develop wheat varieties usable in the production of gluten free food is also complicated by the hexaploidy of wheat.

The utilisation of a RNAi-silencing approach was chosen to investigate the potential of this technique to silence a larger number of genes in wheat. A successful introduction of the RNAi-techniques into wheat could become a standard method for creating loss-of-function wheat plants.

For our investigations we cloned an alpha gliadin sequence into a RNAi-construct. After stable transformation of wheat the transcription product is a dsRNA. The endosperm proteins of the transgenic plants are analysed by HPLC and PAGE.

Some of our transgenic plants showed a complete lost of alpha gliadin expression in HPLC analysis. For the first time, the application of the RNAi-technology is shown to be successfully used for reducing the expression of a gene family in wheat.

Wheat flour from transgenic, alpha gliadin free wheat was further analysed for the rheological properties. We observed an higher albumin/globulin content in transgenic flour and an 117% higher gluten extensibility resistance in transgenic flour compared to wild-type flour.

Medicago truncatula: A model plant for studying somatic embryogenesis and organogenesis in legumes

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We are investigating the process in which protoplast cells and explant leaves of *Medicago truncatula* (Mt) de-differentiate to establish totipotency and form embryos or roots through proliferation and re-differentiation. The Mt mutant line 2HA has a 500 fold greater capacity to regenerate plants in culture by somatic embryogenesis than wild type Jemalong when auxin (naphthaleneacetic acid) and cytokinin (6-benzylaminopurine) are added to the culturing media (Rose and Nolan, 1995; Nolan et al., 2003). We have used transcriptomic and proteomic analyses which revealed that changes in gene/protein expression correlate with the developmental commitment and growth during somatic embryogenesis and organogenesis (Imin et al., 2004; Imin et al., 2005). We have identified many genes/proteins including several transcription factors that may play critical roles in cellular commitment during embryogenesis.

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Profilin variability in olive (*Olea europaea* L.) pollen cultivars

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Profilins are ubiquitous low molecular weight (12–18 kDa) proteins found in animals, plants, and even viruses. They are structurally well-conserved proteins able to interact with a variety of physiological ligands including cytoskeletal components -actin- and polyphosphoinositides (Goldschmidt-Clermont et al., 1990). Profilins may link the microfilament system with signal transduction pathways. Plant profilins have been shown to be highly crossreactive allergens within a broad range of species and plant tissues. Pollen profilins bind to IgE antibodies of allergic patients, thus triggering the symptoms of type I allergy.

Olive pollen profilin (also named Ole e 2) has been characterized, sequenced and expressed in *E. coli*, showing 86 and 73(identity to birch and grass profilins respectively (Ledesma et al., 1998; Martnez et al., 2002). The protein displays a molecular weight of 15–18 kDa and a high grade of polimorfism in both its nucleotide and amino acid sequences. Three isoforms of the protein have also been described. The present work reports and analyzes the presence of polymorphism in the nucleotide sequences of profilins individually amplified by RT-PCR from the pollen of several olive cultivars. Expression of profilins in the olive pollen cultivars has also been analyzed by Western Blotting and immunofluorescence localization of the protein by confocal microscopy. Localization was mainly performed in pollen tubes grown after in vitro germination of pollen.

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Androgenic switch in barley microspores

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Androgenesis represents one of the most fascinating examples of cell differentiation in plants. In barley, the conversion of stressed uninucleate microspores into embryo-like structures is highly efficient, however little is known about the molecular mechanisms associated with this developmental switch. Another major bottleneck in this process is the successful release of embryo-like structures out of the exine wall of microspores. In the present work, molecular, morphological and biochemical studies were performed during the induction of barley androgenesis and during the transition from multicellular structures to globular embryos. We have employed macroarrays containing 1421 ESTs covering the early stages of barley zygotic embryogenesis to compare the gene expression profiles of stress-induced androgenic microspores with that of uninucleate microspores as they progressed into binucleate stage during pollen development. Principle component analysis defined distinct sets of gene expression profiles that were associated with androgenesis induction and pollen development. Induction of androgenesis by stress was marked by the down-regulation of pollen-related genes, while transcripts involved in sugar and starch hydrolysis, proteolysis, stress response, inhibition of programmed cell death and signaling were up-regulated. Expression analysis of a subset of genes revealed that the induction of *ALCOHOL DEHYDROGENASE 3* and proteolytic genes, such as the metalloprotease *FtsH*, cysteine protease 1 precursor, phytepsin precursor (aspartic protease) and a 26S proteasome regulatory subunit were associated with the androgenic potential of microspores, while the induction of transcripts involved in signaling and cytoprotection were associated with stress responses. Further progress into embryogenic development was only observed in microspores that divided asymmetrically. Independent divisions of the generative and the vegetative nuclei gave rise to heterogeneous multicellular structures, which were composed of two different cellular domains: small cells with condensed chromatin structure and large cells with normal chromatin structure. During exine wall rupture, the small cells died and their death marked the site of exine wall rupture. Cell death in the small cell domain showed typical features of plant programmed cell death. Chromatin condensation and DNA degradation preceded cell detachment and cytoplasm dismantling, a process that was characterized by the formation of vesicles and vacuoles that contained cytoplasmic material. This morphotype of programmed cell death was accompanied by an increase in the activity of caspase–3-like proteases. Taken together, the molecular and cellular changes described in this work represent 'bio-markers' associated with the androgenic switch in microspores, providing a substantial contribution towards understanding stress-induced androgenesis.

Induction, maintenance and preservation of embryogenic competence of *Gentiana cruciata* L. cultures

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Till now, somatic embryogenesis was described only for a few species of *Gentiana* genus. The list includes *G. pneumonanthe* L., *G. kurroo* Royle, *G. punctata* L., *G. pannonica* Scop. and *G. tibetica* King. *G. cruciata* is among the protected species of Polish flora and protocol of its effective vegetative propagation, which could help to protect its natural resources, is highly demand. The aim of our work was to evidence changes which accompany induction of somatic embryogenesis in various type of explants of *Gentiana cruciata*, and present results of protection protocols of embryogenic potential against its loss. Establishment of long-term embryogenic cell suspension and its cryopreservation were developed as alternative procedures for embryogenic potential maintenance.

Structure and ultrastructure changes in primary explants, induction of embryogenesis, developing of somatic embryos and four protocols of cryopreservation of cell suspension of *Gentiana cruciata* L. were describe. The changes that occurred during tissue culture of hypocotyl and cotyledon explant 10-day old seedlings and fragments of leaf explant were studied. Seedling explants were cultured on MS (Murashige and Skoog, 1962) medium supplemented with 1.0 mg/l dicamba + 0.1 mg/l NAA + 2.0 mg/l BAP + 80.0 mg/l adenine sulphate. The hypocotyl callus tissue was initiated by cell divisions of vascular cylinder, but in case of cotyledon only parenchyma cells took a part in callus formation. The explant of leaf blade usually responsed only by proliferation of wounded surface. The effect of auxins (2,4-D, NAA, DIC) and cytokinins (Kin, Zeat, BAP) in various concentration and combinations on leaf explant response was examined. Generally, embryos were formed sporadically on media containing NAA (1.64 % respond explants) or 2,4-D (0.38 %), but they were not produced in the presence of dicamba.

Production of somatic embryos was more effective from suspension culture than agar medium. Liquid culture make possible to carry on embryogenic competence of cell suspension for 5 years. For preservation of proembryogenic masses four protocols of cryopreservation were studied: direct cooling, sorbitol/DMSO treatment, vitrification and encapsulation. Direct cooling and sorbitol/DMSO treatment was unsuccessful. Vitrificated tissue required minimum 3 weeks culture on solid medium for cell proliferation to reach proper fresh weight for manipulation. Alginate beads with PEMs, were directly transferred into liquid medium for post-freezing culture. Vitrification and encapsulation assure the maintenance of high viability of post-freezing PEM, but the encapsulation guaranteed faster restoration of *G. cruciata* cell suspension.

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How pollination affects the egg cell maturation in maize?

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Pollination accelerates egg cell maturation in *Zea mays*, as revealed by LM and TEM analyses (Mól et al., 2000). In order to find factors involved in stigma-to-ovary signalling (a) germination of pollen and pollen tube growth were observed, (b) $[Ca^{2+}]_c$ was monitored in stigma trichomes by CLSM, (c) the electric potential was measured in pistil tissues, and (d) ethylene emission from an ear as well as ACC and IAA levels were determined. Pollen hydration and germination were accompanied with changes of the electric potential in the silk and ovary, and with calcium changes in the stigma trichomes (Mól et al., 2004a). Early $[Ca^{2+}]_c$ increase was related to pollen hydration. Electric potential of the silk showed three series of spikes within 20 min after pollination. Similar electric changes, delayed by 4 min, were found in nucellar tissue. Silks and ovaries were involved in ethylene synthesis but also IAA levels increased in both pistil parts (Mól et al., 2004b). ACC or IAA applied to non-pollinated silks stimulated egg maturation, and the egg response to pollination was cancelled by silk pre-treatment with AVG, AIB or TIBA. Our results indicate that cyto-physiological changes in pistil tissues, detected as early variations of the electric potential, as well as ethylene synthesis and polar auxin transport at the pollination site are involved in stigma-to-ovary signalling in maize after pollination.

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Different categories of cytoplasmic "nucleoloids" in larch microsporocytes involved in pre-rRNA processing and pre-mRNA splicing

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In microsporocytes of many plant species numerous nucleolus-like structures (also called "nucleoloids") appear transiently in the cytoplasm. In general these argyrophilic structures contain Ag-NOR proteins and have recently been shown to include 18S rRNA.These structures are belived to be involved in the transport of ribosomal ribonucleoproteins that enrich the ribosomal pool in the growing microsporocytes. Our study on nucleoloids of microsporocytes of larch (*Larix decidua* Mill.) has used immunodetection and *in situ* hybridization to show that these nucleolus-like structures do not represent just one category. Among argyrophilic cytoplasmic nucleoloids we have distinguished three types of bodies with a different molecular composition and ultrastructure.These are: i) autentic nucleoloids-containing nucleolar proteins and/or 18S rRNA; ii) cytoplasmic splicing bodies-containing Sm proteins and/or TMG snRNA; iii) "nuage"-like splicing structures-containing TMG snRNA. The diverse molecular composition of nucleoloids indicates their diffferent function. These structures are involved in two different metabolic processes. One of them is maturation and transport of ribosomal ribonucleoproteins and other one is pre-mRNA splicing.

Hordeins are present in the gametophytic development and in pollen embryogenesis of *Hordeum vulgare* L.

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Microspore- derived embryos induced by anther or isolated-microspore culture display certain characteristics of zygotic embryos (Reynolds, 1997; Touraev et al., 1997; Pulido et al., 2001). The resemblances between the development of zygotic and microspore-derived embryos suggest that both developmental pathways may have a partially common pattern of gene expression. Consequently, one of the strategies to characterise microspore embryogenesis and molecular markers is to study during microspore embryogenesis the expression of genes which are normally expressed during zygotic embryogenesis (Reynolds, 1997). In this context the expression of hordein genes, encoding the main barley endosperm proteins, was studied using a wide range of methods (cytochemistry, RT-PCR, in situ hybridization, ELISA, Western blotting and immunocytochemistry) to ascertain their presence or absence during the induction and first stages of microspore embryogenesis. Thanks to the very sensitive techniques available we were able to detect and follow the pattern of expression of hordein genes throughout microspore embryogenesis. Surprisingly, hordeins were also detected at different stages of gametophytic development as well as during the very early stages of seed development, when they have not hitherto been detected. The expression and localisation of these proteins and their corresponding transcripts provide new information about barley microspore embryogenesis and its relationship to zygotic embryogenesis. Resemblances and differences in the pattern of accumulation and localisation of these proteins are discussed in relation to the characteristics of these two embryogenic processes.

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Flowering and pollination in *Garcinia indica*

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Garcinia indica Choisy (Clusiaceae) is a self-incompatible, gynodioecious, evergreen tree indigenous to the Western Ghats of India. The tree is known for its nutritive as well as medicinal properties. It bears spherical or globose, dark red berries. The fruit rind has an agreeable flavour and sweetish taste. It is used for imparting acidic flavour to curries and for preparing a cooling syrup given in bilious infections. (-)Hydroxycitric acid, derived from the rind, is indicated as a non-toxic, anti-obesity drug. Seed kernel yields a non-drying edible oil, which is nutritive, demulcent, astringent and emollient, and is applied to ulcerations and fissures. An interesting feature of this species is that there are fifteen types of flowers present on male and female trees. Flowers show variation in length of pedicel, number and arrangement of stamens, presence or absence of rudimentary or perfect pistil, and size and shape of pistil. On an average, 61% of flowers on male tree were perfect male with fertile anthers and no pistil, 28% were imperfect males with fertile anthers and rudimentary pistil, and 11% were bisexual with fertile anthers and a globose ovary bearing ovules. The floral primodia were observed in the last week of October on the trees grown in the College of Agriculture, Dapoli (Ratnagiri District), Maharashtra (India). In male trees first flowering was observed on 25th October in 2003 and on 26th October in 2004. In female trees first flowering was observed on 29th October in 2003 and on 31st October in 2004. Flowering was asynchronous. Flowers opened between 04.00 and 08.00 h, after 34–40 days of bud initiation. Anther dehiscence occurred 15–20 minutes prior to anthesis. Anthers were tetrasporangiate, with 4-layered anther wall. Cytokinesis was simultaneous. Pollen grains were round (14 μm in diameter), 3–4 colporate and with reticulate surface pattern. The number of pollen grains per anther was high (3400 per anther) to compensate for the loss during wind pollination. The atmospheric pollen grains could be trapped as far away as 500m from the male tree. Pollen grains were shed at 2-nucleate stage and 96% pollen grains were viable at the time of dehiscence. Pollen remained viable for four days after dissemination. In in vitro pollen germination experiments, 68% pollen germinated after 3 h in 15% sucrose solution. Stigma was of wet type, sticky and exposed to adhere pollen in air. Each ovary had 6–7 locules with one seed in each compartment. The ovule was anatropous, bitegmic. The embryo sac development was *Polygonum* type. Pollination experiments showed a substantially high fruit set in natural (68%) and controlled (90%) crossings between male and female flowers. In bisexual flowers, fruit set in natural pollination was very low (0.18%) and in controlled pollination experiments 1.3% fruit set was seen following both geitanogmy and xenogamy. In self-incompatible crosses pollen tube growth stopped in the stylar region.

In vitro culture promotes partial autonomous endosperm development in unfertilized ovules of wild-type *Arabidopsis thaliana* var. Columbia

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Autonomous endosperm (AE) is a well-known phenomenon in autonomous apomicts (Koltunow and Grossniklaus, 2003) but is a very rare event in amphimictic plants. In the last decade researches were focused on *FIS1/MEA*, *FIS2* and *FIS3/FIE* genes in *Arabidopsis* which suppress proliferation of the central cell before fertilization. Mutations in this gene complex allow partial development of endosperm and fruit formation without fertilization, indicating that pathways of egg cell and central cell are different, since the mutations do not initiate embryogenesis. Combination of maternal hypomethylation and loss of *FIE* function leads to normally developed endosperm according to the wild-type pattern (Vinkenoog et al., 2000; Vinkenoog and Scott, 2001). It was suggested that part of endosperm-promoting genes that are expected to be imprinted (silent) in ovules of a wild type is lifted in mutant ovules allowing the expression of endosperm-promoting genes. Obtaining AE formation in wild-type of *Arabidopsis* could be very helpful in answering several questions connected with genomic imprinting.

We were successful in induction of partial endosperm development without paternal genome involvement in wild-type cultured in vitro ovaries of *Arabidopsis thaliana*.

Unpollinated pistils were cultured on hormone-free Murashige and Skoog (MS) medium with addition of 6% sucrose and supplemented with: benzylaminopurine (BAP; 2 mg l^{-1}) combined with naphthyl acetic acid (NAA; 0.1 mg l^{-1}), 2,4-dichlorophenoxyacetic acid (2,4-D; explants exposed to 1 h auxin shock 20 or 40 mg l^{-1}, and transferred to hormone-free MS medium). Initiation of autonomous endosperm (AE) development was induced on all media used in 54 ovules from 39 cultured ovaries (26%), with an average frequency of 1.4 ovules/ovary. The highest frequency of partial endosperm formation occurred on media combining the two growth regulators BAP and NAA (59% of ovaries had ovules with AE), although endosperm development was also induced on hormone-free medium (in 20.5% of ovaries). The number of AE nuclei ranged from 2 to ~50, depending on the day of culture and medium; neither cellularization nor differentiation on specific regions typical for endosperm of wild-type *Arabidopsis*, were noted. Fertilization independent endosperm most probably originated from the secondary nucleus, but involvement of the polar nuclei could not be excluded, as indicated by nuclear size and structure. In vitro conditions did not influence egg cell proliferation. Gynogenic embryos were not observed either in the ovules with autonomous endosperm nuclei or in ovules without endosperm induction.

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From wheat to *Arabidopsis*: discovery and analysis of novel egg cell specific genes

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Little is known about the complexity of transcript composition of the female gametes (egg cell and central cell) from seed plants. Moreover, transcriptional changes occurring after fertilization and especially the transition from maternal to embryonic control of seed development are not well characterized. In many angiosperm plants including *Arabidopsis*, the limiting factors are the small size and inaccessibility of female gametes, since the cells are deeply embedded in the maternal tissues of the ovule.

Using a microdissection technique for wheat ovaries (Kumlehn et al., 1998), we are able to isolate female gametes before and after fertilization, as well as defined stages of early embryos. cDNA populations generated from these cells were used to study gene expression profiles after bioinformatical analysis of a few thousand ESTs (Sprunck et al., 2005). These analyses indicate that the mature unfertilized egg cell has a high metabolic activity and protein turnover rate. We also found that transcriptional changes take place considerably and soon after fertilization in wheat, as the transcript composition of the two-celled proembryo is significantly different from the egg cell. Especially genes encoding proteins involved in DNA replication, translation, chromatin remodelling, and other cell cycle-related processes are up-regulated or *de novo* induced after fertilization. Moreover, we were able to identify many novel genes which might have functions specific for the egg cell and/or the two-celled proembryo.

Among the 17.6% "novel" transcripts in the wheat egg cell, several encode proteins with similarity to "hypothetical" proteins from *Arabidopsis* and/or rice. We will report mainly about this approach to identify such "hypothetical" genes in *Arabidopsis* and to study the expression and function of these probably fundamental genes both in *Arabidopsis* and wheat. Three "hypothetical" gene families of *Arabidopsis* were selected for a more detailed analysis. These encode a family of nine predicted membrane-bound proteins, a family of five small and secreted proteins, as well as a subfamily of four members encoding *Armadillo* domain proteins. Functional studies suggest that these are potential key genes involved in cell identity, signaling and/or fertilization of the egg cell.

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Effect of colchicine application on anther culture response in F_1 durum × bread wheat hybrids

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Durum wheat is considered as one of the most recalcitrant to anther culture crops. Based on the assumption that the genes responsible for anther culture response of bread wheat are located on the B genome chromosomes, it was attempted to transfer these genes from bread to durum wheat. This could be achieved via crossing, selfing and backcrossing of the two species, resulting in isolation of durum wheat plants carrying the B genome chromosomes of bread wheat. The present study was conducted to investigate the effect of colchicine on anther culture response of durum × bread wheat F_1 hybrids. For this, three durum wheat cultivars produced at the Cereal Institute of Thessaloniki, Greece (Mexicalli E, Sifnos and Lemnos), exhibiting good yield and pasta quality, were crossed to two bread wheat cultivars (Acheloos and KVZ) exhibiting good response to anther culture (Zamani, 2001). Spikes of the aforementioned genotypes containing anthers in the mid (MU) to late uninucleate (LU) microspore stage were used. Anthers were cultured on two different media (W_{14} and solid potato-2) without and with colchicine during the first threedays in culture. Microspore derived structures were transferred to 190-2 regeneration medium. The plantlets obtained were transferred into Erlemayer flasks containing MS medium without hormones and finally the well-differentiated green plants were transplanted into pots and kept in a growth chamber at 21˚C /19˚C day/night temperature regime and 16 hours illumination.

A negative effect on anther culture response was observed when colchicine was added in the W14 induction medium. A similar effect was observed in two of the hybrids (Sifnos × Acheloos and Lemnos × KVZ) when they were cultured onto potato-2 induction medium. The other two hybrids (Mexicalli × Acheloos and Lemnos × Acheloos) responded positively to colchicine when they were cultured on potato-2, but no significant differences were observed. In both induction media green plant production was influenced negatively after colchicine treatment. The same was observed in albino plant production, confirming previous reports (Barnabas et al., 1991). The results of the present study support the view that anther culture response is genotype depended, as it was previously reported (Navaro-Alvarez et al., 1994). Potato–2 induction medium was the most suitable one for the material studied. Finally, a possible genotype × colchicine × induction medium interaction was detected.

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Protoplast regeneration into plants: do the cell walls play any role?

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Plant protoplasts and cells display an ability to differentiate and form new plants when cultured in vitro under appropriate experimental conditions. This ability varies greatly among the species, which indicates this feature is highly genotype-dependent, and may thus result either in high efficiency of shoot regeneration or in pronounced recalcitrant response.

In the search for biological and molecular factors that might be involved in this phenomenon, we have compared two species presenting totally different behavior in vitro – tobacco (*Nicotiana tabacum*) and sugar beet (*Beta vulgaris*) – in terms of the structure and composition of their cell walls. The material studied included both donor cells used for protoplasts isolation, i.e. guard cells of the leaf epidermis, as well as developing protoplasts in successive stages of wall re-synthesis and callus formation.

The presence, distribution and relative abundance of several antigens have been examined, including those typical for pectins, xyloglucans and arabinogalactan proteins (AGPs). The results of immunolocalization studies clearly showed that protoplasts of both species reform the walls resembling those of the donor cells, but efficiently regenerating tobacco protoplasts differ in several respects from those of sugar beet: (1) tobacco walls contain numerous domains of (1→4)-β-D-galactose characteristic for pectin side-chains, (2) these walls do not show detectable amounts of several domains typifying pectins, xyloglucans and AGPs: α-fucose-(1→2)-galactose, (1→6)-β-D-galactose bearing arabinose residues, and β-glucuronic acid residues.

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