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XX International Congress on Sexual Plant Reproduction

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XX International Congress on Sexual Plant Reproduction

Embrapa Recursos Genéticos e Biotecnologia
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FOREWORD OF THE PRESIDENT

The International Association of Sexual Plant Reproduction Research (IASPRR) welcomes you as a participant at this XXth International Congress on Sexual Plant Reproduction (ICSPR) in the capital city of Brasilia. The ICSPR series was originally established to improve communications between plant reproduction researchers of East and West Europe, but we have taken a bolder step in bringing together researchers from around the world, which has brought us here, to Brazil, for our first meeting in South America.

Our local hosts, through careful planning, have prepared an excellent meeting and an enjoyable one. In just a few short years, we as a sexual plant reproduction community have seen exceptional progress in understanding a wide range of topics in developmental biology. The tools of our exploration continue to improve. As the pace of innovation continues to accelerate, we anticipate basic knowledge will emerge on some of the most important and fundamental questions of reproductive development will soon unfold. The challenges in our area will be great, but never has the need been greater. To be able to expand our knowledge of the underlying mechanisms of sexual reproduction will usher in a phase of this work that may allow unparalleled manipulation of crops and plants to meet current and future agriculture and health needs. We hope that this meeting contributes to this process and facilitates the scientific exchange that stimulates creativity and new interest in this area around the world.

Scott D. Russell
President, IASPRR

WELCOME ADDRESS

On behalf of the XX International Congress on Sexual Plant Reproduction Organizing Committee, we are delighted to welcome you to the ICSPR event, held as the 20th congress in this series for the first time in South America, in the beautiful and modern capital city of Brazil, Brasília. The congress is under the patronage of the International Association of Sexual Plant Reproduction Research (IASPRR).

The congress highlights the desire of bringing together experts from a variety of molecular, cellular and functional genomics fields to promote knowledge and research in plant reproduction. A dense scientific program is proposed and as suggested, there is no simultaneous presentation. In addition to more than ten plenary lectures presentations, nine sessions with invited speakers and oral contributions selected from submitted abstracts and more than a hundred posters presentations covering most aspects from gametes to seed development including innovative technical approaches.

With around 170 participants gathered in Brasilia from all continents of the world, the event aims to emphasize the most valuable scientific and technical progress on plant reproduction in recent years. It is our wish to take this new knowledge to the public and private Universities, Research Centers and Institutions, and to be forward thinking in order to contribute to science and agriculture development.

Brasilia is the young capital of the country, inaugurated on April 21st, 1960 with its major buildings designed by the famous modern architect Oscar Niemeyer. It is well-known for its urban planning and architecture, and the designers opted for a style of clean lines and honestly exposed structure. It has an area of 5.789,16 km^2 and its population is 2.2 million. The altitude is 1172m and presents a climate between tropical savannas and temperate rainy, with a dry winter season (May-Set). During this season, days are beautifully sunny and evenings are pleasantly cool and rain precipitation is very low or nil. Brasilia is an excellent site for tourism since it is the only modern city included in the UNESCO Human Cultural Patrimony and the biggest open museum of modern architecture and ideal scenery for any event. The city offers a wide variety of options to the visitor, including modern buildings, nightlife, restaurants, live concerts and a countryside with waterfalls and huge rivers, the Cerrado and areas of historical interest only few or hundred kilometers away from the city.

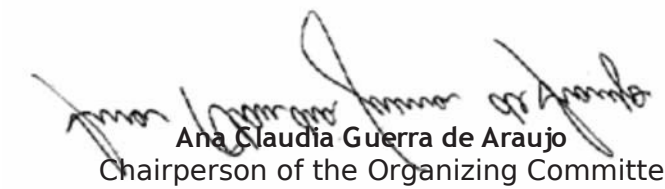
The Congress will take place at the Plaza Convention Brasília, which has a modern and comfortable convention facility and is very experienced

in hosting conventions and other events. The Plaza Convention Brasília is in the Kubitschek Plaza Hotel, in the heart of the city surrounded by tourist monuments.

The Organizing Committee is determined that this conference will be both scientifically rewarding and socially enjoyable. In addition to the Congress Dinner to be held at the regional restaurant Oca da Tribo, where participants will have opportunity to taste the some typical Brazilian food and drink *caipirinha* in an indigenous decorated place, at the cocktail to be held Bamboo Bar at Kubitschek Plaza Hotel after presentations on the first day, participants will listen to Brazilian Choro group.

A visit to Embrapa-Genetic Resources and Biotechnology is also organized. For those who already know the Research Center, a city Tour is offered.

We are certain that you will find the meeting both enriching and enjoyable, and look forward to sharing this experience with you in Brasilia.



Ana Claudia Guerra de Araujo
Chairperson of the Organizing Committee
Researcher at Embrapa Genetic Resources and Biotechnology
Brasilia, DF – Brazil

XX th International Congress on Sexual Plant Reproduction

August 4 - 8 th, 2008
Brasilia, Brazil

WWW.CENARGEN.EMBRAPA.BR/XXICSPR/

The Congress Venue

KUBITSCHEK PLAZA HOTEL
SHN Quadra 2 Bloco E
70702-004 Brasília-DF
Brazil
Phone: 55 61 33193543

Monday, August 4th, 2008

10:00-19:30
Foyer - Congress Secretariat
Ouro Preto Room – Posters Settlement – A

14:00-14:30 - Minas Gerais Auditorium
Opening Ceremony

14:30-15:30 - Minas Gerais Auditorium
Michiel T. M. Willemse, Univ. Wageningen, Netherlands – Plant reproduction: interaction and regulation?
Honour session

15:30-16:15 – Minas Gerais Auditorium
Elliot Meyerowitz, Caltech, USA – Computational Morphodynamics: Live Imaging and Computational Modeling of the *Arabidopsis* Shoot Apical Meristem

16:15-16:45 - Foyer
Coffee Break

16:45-18:45
Session 1- Floral Development
Chairperson: Marcio Alves-Ferreira – Brazil

Lucia Colombo, Dipartimento di Biologia, Università di Milano, Italy – *REM18* and *REM53*: two direct targets of the ovule identity complex in *Arabidopsis*

Ioan Negrutiu, Ecole Normal Superieure de Lyon, Plant Development & Reproduction, Lyon, France – The spatio-temporal control of flower termination in *Arabidopsis*

Marcelo Dornelas, Unicamp, SP, Brazil – Evolution of molecular networks controlling tropical plant reproduction

Marcio Alves-Ferreira, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil – Identification and characterization of novel genes important for stamen development in *Arabidopsis*

Oral Contribution of selected abstract

Romanel, E. and Alves-Ferreira, M., Department of Genetic– Federal University of Rio de Janeiro, RJ - Brazil. Functional analysis of two B3 (REM family) genes expressed at early stages of stamen development in *Arabidopsis thaliana*.

18:45 -19:30 - Minas Gerais Auditorium
Robert B. Goldberg, Department of Molecular Cell and Developmental Biology, University of California, USA – Using Genomics to Dissect Seed Development

19:30- 21:30 - Bamboo Bar, SL level
Welcome Cocktail – Offered by Monsanto, Brazil
Monsanto Brasil offers a Welcome Cocktail

Tuesday, August 5th, 2008

08:00- 08:30
Foyer – Secretary
Ouro Preto Room – Posters Settlement – A

08:30-10:00 - Minas Gerais Auditorium
Session 2 - Male Gametophyte Development
Chairperson: Scott Russell – USA

Anja Geitmann, Université de Montréal, Canada – Challenging the need for a pacemaker – pollen tube growth oscillations explained with a mechanical harmonic oscillator model

Mohan Singh, Plant Molecular Biology and Biotechnology, University of Melbourne, Australia – Transcriptional regulation of male germ cell lineage

Hui Qiao Tian, School of Life Science, Xiamen University, Xiamen 361005, China – Recent Progress in Research of the Fertilization Mechanism in Angiosperms

Oral Contribution of selected abstracts

Coimbra, S., Costa, M, and Pereira, L.G. - Department of Botany Faculty of Sciences, University of Porto, Portugal - Arabinogalactan proteins during *Arabidopsis* male gametophyte development

Suárez C, Alché JD, Castro AJ and Rodríguez-García MI - Departamento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del Zaidín (CSIC), Profesor Albareda 1, E-18008, Granada (Spain) -Localization of arabinogalactan proteins (AGPs) and pectins in the olive (*Olea europaea* L.) pollen grain during *in vitro* germination by CLSM.

10:00-10:30 - Foyer
Coffee Break

10:30-11:00 - Ouro Preto Room
Posters Exhibition - A

11:00-12:30 – Minas Gerais Auditorium
Session 3 - Female Gametophyte Development
Chairperson: Ueli Grossniklaus – Switzerland

Matthew M. S. Evans – Department of Plant Biology, Carnegie Institution, Stanford, CA, USA – Regulation of embryo sac development by *indeterminate gametophyte1*

Gary N. Drews – University of Utah, USA – Identification of Genes Required During Female Gametophyte Development in *Arabidopsis*

Joseph H. Williams, Department of Ecology and Evolutionary Biology, University of Tennessee, USA – Endosperm genetics and the developmental evolution of female gametophyte body plants

Oral Contribution of selected abstract

Ewa Szczuka [1], Aleksandra Seta [1], Marcin Domaciuk [1], Ewa SkórzyDska-Polit [2], Irena GieBwanowska [3] - [1]Department of Plant Anatomy and Cytology, Maria Curie-SkBodowska University, Akademicka 19, 20-033 Lublin, Poland. [2]Department of Plant Physiology, Maria Curie - SkBodowska University, Akademicka 19, 20-033 Lublin, Poland. [3]Department of Plant Physiology and Biotechnology, University of Warmia and Mazury, Oczapowskiego 1A, 10-719 Olsztyn, Poland - Lipoxygenase in the cells of the developing ovule of *Larix kaempferi* (Lamb.) Carr.

12:30-14:00 - Vila Rica Restaurant

Lunch

14:00-14:45 - Minas Gerais Auditorium
Scott Russell- University of Oklahoma, USA – Profiling of the Male Gametophyte of Rice and Other Flowering Plants

14:45-16:15 - Minas Gerais Auditorium
Session 4 - Pollen-Pistil Interaction - Part I
Chairperson: Maria Helena de Souza Goldman, USP, Brazil

Celestina Mariani, Department of Experimental Botany, University of Nijmegen, Netherlands – There is more than a way to make fruit

Tetsuya Higashiyama, Nagoya University Furo-cho, Japan – Behavior and signaling in gametophytic interactions

Jorge Muschietti, Departamento de Fisiologia, Biologia Molecular e Celular, Instituto de Engenharia Genetica e Biologia Molecular, Buenos Aires, Argentina – LePRK2 signal transduction in pollination: hyperphosphorylation and signaling by an unusual style peptide

Oral Contribution of selected abstract

Aurélien Boisson-Dernier*, Tae-Houn Kim, Sabine Frietsch, Marie B. Dizon and Julian I. Schroeder - Division of Biological Sciences, University of California, San Diego, CA, USA - The peroxin loss-of-function mutation abstinence by mutual consent disrupts male-female gametophyte recognition.

16:15-16:45 – Foyer
Coffee Break

16:45-18:15 – Minas Gerais Auditorium
Session 4 - Pollen-Pistil Interaction - Part II
Chairperson: Jorge Muschietti – Argentina

Anja Geitmann, University of Montreal, Canada – High speed delivery to the growing point – quantification of vesicle streaming

in pollen tubes using spatio-temporal correlation spectroscopy (STICS) and fluorescence recovery after photobleaching (FRAP)

Maria Helena de Souza Goldman, Departamento de Biologia FFCLRP/USP, SP, Brazil – Genes important for pistil development and pollen-pistil interaction in *Nicotiana tabacum* L., a wet stigma species

Joseph H. Williams, Department of Ecology and Evolutionary Biology, University of Tennessee, USA – Before pollen-style interactions: the origin and early evolution of the angiosperm fertilization process

Oral Contribution of selected abstract

Kowyama, Y.[1], Tsuchiya, T.[2] and Kakeda, K.[1,-1] Graduate School of Bioresources, Mie University, Tsu, 514-8507, Japan–[2] Life Science Research Center, Mie University, Tsu, 514-8507, Japan–Genomic organization of the sporophytic self-incompatibility locus in *Ipomoea trifida*, a close relative of sweet potato.

18:15-19:00 – Minas Gerais Auditorium
Bruce McClure, Department of Biochemistry, University of Missouri, USA – Pollen endocytosis in S-RNase-based SI

19:00-19:45 – Minas Gerais Auditorium
David Twell, University Leicester, England – Genetic control of male germline development in flowering plants

Wednesday, August 6th, 2008

08:00- 08:30
Foyer – Secretary
Ouro Preto Room – Posters Settlement – A

08:30-09:15 - Minas Gerais Auditorium
Ueli Grossniklaus, University of Zurich, Switzerland – The Molecular Basis of Cell-Cell Communication during Double Fertilization in *Arabidopsis thaliana*

09:15-10:00 - Minas Gerais Auditorium
Thomas Dresselhaus, University of Regensburg, Germany – The Role of Polarity and Cross-Talk for Double Fertilization

10:00-10:30 - Foyer
Coffee Break

10:30-11:00 - Ouro Preto Room
Posters Exhibition - A

11:00-12:30 - Minas Gerais Auditorium
Session 5 - Embryogenesis
Chairperson: Thomas Dresselhaus – Germany

John J. Harada – College of Biological Sciences, UC Davis, USA- Dissection of Arabidopsis Embryo and Seed Development

Wolfgang Werr – University of Cologne, Germany – Cellular decisions in the *Arabidopsis* and maize embryo and the EvoDevo perspective

Oral Contribution of selected abstracts
Stephanie Meyer and Stefan Scholten - Developmental Biology and Biotechnology, Biocenter Klein Flottbek, University of Hamburg, Ohnhorststraße 18, 22609 Hamburg, German – Immediate paternal genome activation and enhanced trans-regulatory interactions in early maize hybrid embryos.

Tomokazu Kawashima[1], Xing-Jun Wang [2], Yuping Bi [2], Koen Weterings[3], and Robert B. Goldberg[1] - Tomokazu Kawashima[1], Xing-Jun Wang[2], Yuping Bi[2], Koen Weterings[3], and Robert B. Goldberg[1] – 1. Department of Molecular, Cell, and Developmental

Biology, University of California, Los Angeles, California 90095 U.S.A. 2. Crop Institute, Shandong Academy of Agricultural Sciences, Shandong, China. 3. Bayer Crop Science N. V. Technologiepark 38 B-9052 Gent, Belgium – *Cis*-Regulatory Sequences Responsible for Suspensor-Specific Transcription

12:30-12:45 - Hall of the Hotel
XXICSPR Congress Photo

12:45-14:00 - Vila Rica Restaurant
Lunch

14:00-18:30
Guided Visits

Thursday, August 7th, 2008

08:00- 08:30
Foyer – Secretary
Ouro Preto Room – Posters Settlement – B

08:30-10:00 - Minas Gerais Auditorium
Session 6 - Reproduction of Tropical Plants
Chairperson: Marcelo Dornelas – Brazil

Jorge Ernesto Mariath, Universidade Federal do Rio Grande do Sul, RS, Brazil – Male gametophyte characterization on Passion flower reproduction

José F. M. Valls, Embrapa, Brazil – Different breeding mechanisms and dispersal strategies in *Arachis*

Paulo Eugênio A. M. de Oliveira – Universidade Federal de Uberlândia, MG, Brazil – Outbreeding and inbreeding in Cerrado plants: ecological consequences and perspectives

Avanci, N.C.[1,2]; Pranchevicius, M.C.S.[1]; Lourenço, E.V.[3]; Quiapim, A.C. [1,2]; Goldman, G.H. [4]; Barkman, T.J [5]; Moraes, L.A.B. [6]; Goldman, M.H.S.[1] [1]Department of Biology, FFCLRP - University of São Paulo (USP), Brazil; [2]PPG Comparative Biology, FFCLRP - University of São Paulo (USP), Brazil; [3]Depart. of Molecular and Cellular Biology, FMRP - University of São Paulo (USP), Brazil; [4]Department of Pharmaceutical Science, FCFRP - University of São Paulo (USP), Brazil; [5]Department of Biological Sciences, Western Michigan University, USA; [6]Chemistry Department, FFCLRP - University of São Paulo (USP), Brazil - A novel pistil-specific methyltransferase gene is capable of producing jasmonate, benzoate and salicylate in vitro and is probably responsible for the jasmonate emission of mature *Nicotiana tabacum* L. flowers.

10:00-10:30 - Foyer
Coffee Break

10:30-11:00 - Ouro Preto Room
Posters Exhibition - B

11:00-12:30 - Minas Gerais Auditorium
Session 7 - Fruits and Seed Development
Chairperson: Rod Scott – England

Fred Berger, Temasek Life Sciences Laboratories, Singapore – Retinoblastoma and its Binding Partner MSI1 Control Imprinting in *Arabidopsis*

Hugh Dickinson – Department of Plant Sciences, University of Oxford, England – Imprinting in the maize endosperm; dissecting the control elements

Rod J. Scott, University of Bath, England – mPC – a novel imprinted gene in Arabidopsis, and how to discover more

Oral Contribution of selected abstract

Rivka Barg[1*], Yehiam Salts[1], Oxana Shaiman[1], Irina Sobolev[1], Tali Eilon[1], Sara Shabtai[1], Erich Grotewold[2]. [1] Department of Plant Genetics, Institute of Field and Garden Crops, The Volcani Center, ARO, P.O.Box 6, Bet Dagan 50250, Israel; [2]Department of Plant Cellular and Molecular Biology and Plant Biotechnology Center, The Ohio State University, 1060 Carmack Road, Columbus, OH 43210, USA - Suppression of cell expansion during the early stages of tomato fruit development is mediated via stage specific expression of the single MYB-like gene *SlFSM1*.

Rodrigues, JCM[1,2], Johnson, SD[2] and Koltunow, AM[2],[1] Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil; [2]CSIRO Plant Industry, Adelaide, Australia – Epigenetic Regulation of autonomous seed formation in *Hieracium*

12:45-14:00 - Vila Rica Restaurant
Lunch

14:00-16:15 - Minas Gerais Auditorium
Session 8 - Apomixis
Chairperson: Vera Carneiro – Brazil

David M. Stelly – Laboratory of Plant Molecular Cytogenetics, Department of Soil and Crop Science, Texas A&M University, College Station, Texas, USA – The *Semigamy* mutant of Cotton (*Gossypium barbadense L.*)

Imran Siddiqi, Centre for Cellular and Molecular Biology (CCMB) Andra Pradesh, India – Gamete formation without meiosis in *Arabidopsis*

John G. Carman – Utah State University, USA – Ovules of apomictic *Boechera* suppress maleness but invest precociously in filial development – typical behaviors for apomictic eukaryotes

Juan Pablo Ortiz, Universidad Nacional de Rosário, Argentina – Genetic and molecular characterization of apospory in *Paspalum sp.*

Vera Carneiro, Embrapa, Brazil – Apomixia em *Brachiaria*

Guimarães, L.A.; Dusi, D.M.A.; Silveira, E.D.; Dornelas, M. C.; Carneiro, V.T.C. Embrapa Recursos Genéticos e Biotecnologia; Universidade de Brasília (UnB); Universidade Federal do Rio de Janeiro, UFRJ; Universidade Estadual de Campinas (UNICAMP). Expression and phylogenetic analysis of two putative MADS-Box like genes of *Brachiaria brizantha* (A. Rich.) Stapf.

Selva JP [1], Cervigni G [1], Ochogavía A[2], Zappacosta D[1], Meier M[1], Pessino S[1] and V. Echenique[1]. [1]Departamento de Agronomía, Universidad Nacional del Sur, CERZOS CONICET, San Andrés 800, Bahía Blanca, Argentina. [2]Laboratorio Central de Investigaciones, Facultad de Ciencias Agrarias de la Universidad Nacional de Rosario, Parque Villarino, Zavalla, Santa Fe, Argentina - Global analysis of the genome, transcriptome and epigenome in the diplosporous grass Eragrostis curvula (Schrad.) Nees.

16:15-16:45 – Foyer
Coffee Break

16:45-17:30 – Minas Gerais Auditorium
Anna Koltunow – CSIRO Plant Industries, Adelaide, Australia – The initiation of apomixis in *Hieracium*

17:30-19:00 – Minas Gerais Auditorium
Session 9 - Applied Biotechnology
Chairperson: Christine Horlow – France

Henry Daniell – University of Central Florida, USA – Containment of transgenes by maternal inheritance or cytoplasmic male sterility engineered via the chloroplast genome

Daniela Aviani - SNPC, MAPA, Brazília, Brazil & Cacilda B. Do Valle - Embrapa, MS, Brazil - Cultivar protection rules for apomitic plants

Christine Horlow, Institut National de la Recherche Agronomique – INRS, Versailles, France – Identification of over expressed and down-regulated genes during Arabidopsis meiosis and microsporogenesis

Oral Contribution of selected abstract

Andrade, R. and Alves-Ferreira, M. - Laboratory of Plant Molecular Genetics; Department of Genetics, Institute of Biology, Federal University of Rio de Janeiro; Rio de Janeiro, Brazil. Identification and functional characterization of cis-elements regulatory of gene involved with desiccation of pollen and seeds of *Arabidopsis thaliana*.

Stephan Nielen, Lucas M. Almeida, Vera T. C. Carneiro, Ana Claudia G. Araujo – Embrapa Recursos Genéticos e Biotecnologia, Brasília-DF, Brazil. Analysis of sexual and apomictic accessions of *Brachiaria brizantha* using fluorescent *in situ* hybridization

Cabral, G. B.Oliveira. L, Carneiro, V.T.C. – Embrapa Recursos Genéticos e Biotecnologia, Faculdades Integradas da Terra de Brasília. Effect of osmotic pressure in *Brachiaria brizantha* cv. Marandu genetic transformation by biolistic

19:30- 22:30
Monsanto Brasil offers the Congress Dinner

Friday, August 8th, 2008

08:00- 08:30
Foyer – Secretary
Ouro Preto Room – Posters Settlement – B

08:30- 09:15
Catherine Albrecht, Univ. Wageningen, Netherlands – The Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE

(SERK) proteins function in brassinosteroid dependent and independent signaling

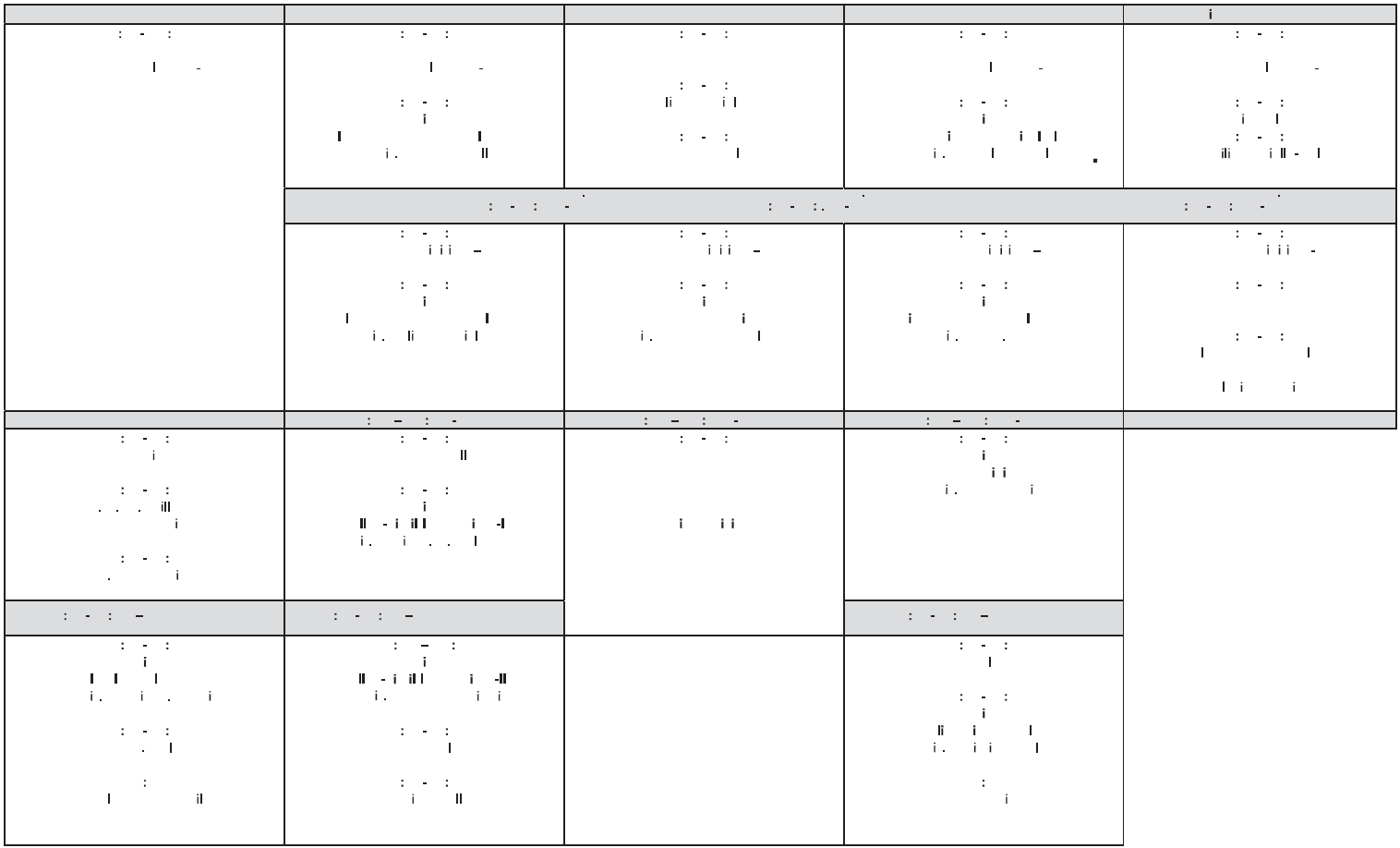
09:15- 10:00
Jean Phillippe Vielle-Calzada, Centro de Investigación y de Estudios Avanzados-Unidad Irapuato, Mexico – Epigenetic regulation of female reproductive development in *Arabidopsis thaliana*

10:00-10:30 - Foyer
Coffee Break

10:30-11:00 - Ouro Preto Room
Posters Exhibition - B

11:00-11:45 – Minas Gerais Auditorium
Simon Hiscock, School of Biological Sciences, University of Bristol, England – The forgotten layer: the stigma pellicle and its role in pollen-stigma interactions

11:45 -12:30 –Minas Gerais Auditorium
IASPRR Assembly
Awards
Closing Ceremony



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SESSION 2 - MALE GAMETOPHYTE DEVELOPMENT

SESSION 3 - FEMALE GAMETOPHYTE DEVELOPMENT

PART III - ABSTRACTS

Session 1 - Floral Development

PART I - PLENARY LECTURES

Plant Reproduction: interaction and regulation?

M.T.M.Willemse
Former Institute: Wageningen University and Research Center. Arboretumlaan 4, 6703BD Wageningen, Netherlands. Present adress: Bennekomseweg 38a, 6717LM Ede Netherlands.

The way of plant reproduction is governed by the changes of the dynamic environment.

Asexual reproduction is marked by mitosis and spore differentiation leading to a mitospore. Plant multiplication and its dispersal are related. Sexual reproduction is realized by meiosis and gamete differentiation and this results in a zygote as unit of dispersal. Plant renewal and dispersal are linked. The differentiation to spore or gamete prepares to their type of dispersal and this includes the interaction with the environment.

In water iso – aniso – and oogamy develop, the stay of the oocyte on the plant leads to the appearance of a special organ to promote the gamete fusion. Due to the formation of a plant from each unicellular stage in the life cycle an extra unit of dispersal, the meiospore, is added.

On land, mosses and ferns inherit alternation of generation, oogamy and dispersal by meiospores. When again the oocyte stays on the plant, as prepared by the heterospory and happens in the seedferns, the unit of dispersal becomes the seed. The result is the gradual change in the gamete fusion, which is independent of water and the intensive cooperation with abiotic and biotic vectors, expressed in flower and fruit. The diploid mother plant realizes both processes: this means a strong internal interaction with the micro- and makroprothallia, with the organization of fertilization and thereafter the developing seed as well as constantly an external interaction with the dynamic environment.

Mainly the early steps during the differentiation to spore or gamete prepare the way of dispersal and gamete fusion. With respect to gene regulation, the differentiation steps should be distinguished to the dispersal and to the sexual reproduction including the recognition, attraction and fusion. Besides most processes are result of the interplay of the mother-plant with the gametophytes, zygote, embryo and endosperm and the environment. Such cooperative aspects need internal as well as external signalling on the right moment. All these complex interactions point to a dynamic cooperation between organisms and environment. A precondition of these interactions could be the existing potency of positive or negative connection between elements present in nature.

Computational Morphodynamics: Live Imaging and Computational Modeling of the *Arabidopsis* Shoot Apical Meristem

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The shoot apical meristems (SAMs) of a flowering plant are the sets of stem cells at the tip of each shoot, which ultimately provide the cells that make the stem, leaves, and flowers. To describe and manipulate the cells of the SAM, we have developed a new set of live-imaging methods for the *Arabidopsis thaliana* shoot apical meristem, so that confocal laser scanning microscope images of the meristem can be acquired repeatedly over several days, and all cell divisions can be observed – both during meristem maintenance, and as new flowers are formed on the meristem flank. We have also developed a set of reporters for gene expression domains, and protein localization domains within cells of the SAM, and a set of methods for changing the patterns of gene expression in the meristem while we are watching. We are in addition able to ablate individual cells, or groups of cells, using a laser attachment to our confocal microscope.

Using these methods, we have studied a number of the functions of the meristem. Two will be discussed – the formation of flowers in a spiral phyllotactic pattern, and the regeneration of shoot meristems from callus tissue in culture. For phyllotactic pattern we have developed a model that accounts for the successive locations of flowers based on a dynamic pattern of auxin transport, controlled by changes in the subcellular location of the auxin efflux carrier PIN1. Computational models of the meristem show that this model fits closely with live imaging observations, and raises some new questions about the mechanisms of communication between neighboring cells in the L1 layer of the shoot. We have also observed, with a number of reporter genes, the earliest stages in the *de novo* formation of new shoot meristems in root-derived callus tissue, which has also led to early-stage models for the interaction of auxin, cytokinin, and the genes that they regulate in allowing an apparently homogeneous and undifferentiated set of cells to spontaneously form highly organized meristems.

Using Genomics to Dissect Seed Development

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During the next 50 years, we will need to produce more food than in the entire history of humankind on an ever decreasing amount of land for agriculture. A major challenge for the 21st century, therefore, is to increase the yields of major crop plants, such as soybean, using state-of-the-art genetic engineering and genomic technologies. One way to accomplish this task is to understand all of the genes required to "make a seed" in order to engineer plants for yield traits such as more seeds, bigger seeds, and seeds with improved nutritional composition. Our laboratory has been investigating gene activity during seed development in order to identify the genes and regulatory networks required to program seed development. In my lecture, I will discuss GeneChip experiments with mRNAs captured using laser capture microdissection (LCM) from *every* soybean and *Arabidopsis* seed compartment (e.g., embryo, endosperm, seed coat), region (embryo proper, suspensor), and tissue (e.g., inner integument, endothelium, seed coat epidermis) throughout all of seed development. In addition, I will discuss complementary experiments using high throughput 454 DNA sequencing technology. Our experiments have estimated the number of genes required to "make a seed." In addition they have uncovered mRNAs that are specific for each seed compartment and region – including those that encode transcription factors. How genes active in different parts of the seed are organized into regulatory networks that program seed development remain to be determined. Nevertheless, the regulatory genes that comprise these networks have been identified in every part of a seed.

Profiling of the Male Gametophyte of Rice and Other Flowering Plants

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In order to determine gene expression of the male gametophyte of rice at the transcriptome level, field grown, disease-free samples of *Oryza sativa* ssp. *japonica*, cultivar Katy plants were collected from fields of Dale Bumpers National Rice Research Center and University of Arkansas Extension Station near Stuttgart, Arkansas, USA. Triplicate biological/technical samples were collected from three fields, frozen and transported for analysis. The Affymetrix Rice 57K GeneChip, an oligonucleotide chip that provides comprehensive coverage of the rice genome, was used to survey expression of transcripts in the male gametophyte. The TIGR Rice Annotation Project (version 5, http://rice.tigr.org/) and International Rice Genome Sequencing Project (IRGSP, http://rgp.dna.affrc.go.jp/IRGSP/ and RAP-DB, http://rapdb.dna.affrc.go.jp/) were used as reference annotation sources. Material for the male gametophyte expressional survey was prepared using the Affymetrix two-cycle cDNA synthesis kit, which allowed linear amplification of isolated RNA. GCOS software was used for analysis and initial normalization, although RMA, GCRMA, PLM, and dChip results were also conducted for comparison. Additional reference controls from the NCBI Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/sites/entrez?db=gds&term=GPL2025%5BAccession%5D&cmd=search) were used to compare other sporophyte tissue sources. All results met quality control criteria and triplicate samples showed high consistency as indicated by Pearson coefficients of correlation exceeding 0.990 for pollen and 0.992 for seedlings (sporophytic control). Of experimental probe sets, 12,228 (21.4%) reported present or marginal in pollen, with 22,565 (39.4%) reporting in seedlings. Of the 24,774 (43.3%) probe sets reporting, 2,209 (8.9%) were found only in pollen, 10,019 (40.4%) in both pollen and seedlings, and 12,546 (50.6%) only in seedlings. Transcriptome data was complemented by data from proteomic (Dai et al 2006a,b, Imin et al. 2004, Kerim et al. 2003) and MPSS (Massively Parallel Signature Sequencing; http://mpss.udel.edu/rice/) studies of rice male gametophyte and reveals a complex and distinctive pattern of

expression. Pearson’s coefficient of correlation revealed r=0.204±0.030 of pollen versus seedlings, reflecting strong divergence in their transcription complements. This reflects common themes with the pollen transcriptome of *Arabidopsis* for which there are multiple studies. The current study represents the first transcriptome of monocot pollen or that of a crop species. Results of other systems will be presented as well.

Pollen endocytosis in S-RNase-based SI

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S-RNase-based self-incompatibility (SI) is found in diverse plant species. It is a single locus gametophytic SI system where pollination specificity is controlled by *S-RNase* genes on the pistil side and *S*-locus F-box genes on the pollen side. Additional proteins such as 120K and HT-B are required for a fully functional SI system. Although the genetic results are clear, it is not as clear how interactions between S-RNase and SLF result in compatibility or incompatibility or how proteins like 120K and HT-B contribute. We demonstrated compartmentalization of S-RNase in the pollen tube endomembrane system and suggested that its sequestration from the cytoplasm accounts for resistance to S-RNase in compatible pollinations. Moreover, the results also suggest that pollen tubes engage in massive uptake and retrograde transport of material, including S-RNase, from the pistil extracellular matrix. Since these processes are likely to be important in SI as well as in compatible pollinations we are working to identify pollen proteins that contribute and to better characterize endocytosis in pollen tubes. In one set of experiments, we used a yeast two-hybrid (Y2H) screen to identify pollen proteins that bind 120K. A pollen C2-domain containing protein (PCCP) binds to the cysteine-rich C-terminal domain of 120K in Y2H and in in vitro pull-down assays. PCCP also binds to phosphatidyl-inositol-3-phosphate containing liposomes. A PCCP-GFP fusion is associated with punctate structures in pollen tubes. In a second set of experiments, we examined uptake and transport of dyes and dye-labeled proteins in pollen tubes growing in media and in planta. The results show that fluid-phase endocytosis plays an important role in uptake.

Genetic control of male germline development in flowering plants

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Pollen grains represent the highly reduced haploid male gametophyte generation in spermatophyte plants. In flowering plants their role is to nurture and deliver a pair of sperm cells to the embryo sac for double fertilisation. Recent advances in our understanding of landmark events in pollen development are largely based on progress achieved using genetic and transcriptomic approaches. Genome-wide analysis has revealed comple patterns of gene expression during male gametophyte development in *Arabidopsis* [1]. There is also evidence for extensive germline gene expression in maize and lily [2,3] and several male germline-specific promoters have been characterized in *Arabidopsis* [4-7]. These new datasets and molecular tools complement progress in genetic analyses that is now beginning to uncover some of the key molecular mechanisms that pattern pollen development.

A central outstanding question in pollen development is how the vegetative cell exits the cell cycle and differentiates to form the pollen tube, while the generative or 'primary male germ cell' divides to form functional twin sperm cells? Recent progress has led to the identification of a non-germline repressor protein, Germline Restrictive Silencing Factor, in lily that suggests the involvement of transcriptional derepression in male germline specification [8]. Moreover, the analysis of a suite of *Arabidopsis* mutants that specifically block cell division in the male germline provides new insight and allows the formulation of molecular models male germline development and pollen patterning. Cyclin Dependent Kinase (CDK) and Chromatin Assembly Factor (CAF1) pathway mutants show that germ cell division can be uncoupled from gamete specification [9,10]. Recent progress involves the identification of an F-box protein that forms an SCF complex in male germ cells that targets the CDK inhibitor proteins for proteasome-dependent degradation. Thus, SCF[TBL1,TBL2] acts as a male germline proliferation factor, but is not involved in cell differentiation. However the R2R3 Myb regulatory protein DUO1 [11], coordinates these processes by activating germline-specific gene expression and its requirement for G2/M-specific expression of Cyclin B1;1 to commit differentiating germ cells to mitosis. DUO1 is therefore a key male germline fate determinant that regulates twin-sperm cell production and the expression of germline specific genes, including AtGCS1/HAP2 [6] that is essential for gamete fusion and double fertilization.

Further progress in understanding the essential switch between germ and non germ cell lineages will continue to benefit from the integration of genetic and genomic technologies, offering new opportunities to build complex functional models of male gametophyte development.

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The Molecular Basis of Cell-Cell Communication during Double Fertilization in *Arabidopsis thaliana*

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Research in our laboratory focuses on the developmental genetics of plant reproduction. We have used genetic and molecular approaches to identify genes controlling the underlying developmental processes using *Arabidopsis thaliana* and *Zea mays* as model systems. Our studies have shown that both genetic and epigenetic mechanisms play a key role in plant reproduction. In this presentation we will focus on cell-cell interactions during double fertilization. We have isolated a female gametophytic mutant, *feronia*, which disrupts double fertilization: in *feronia* mutant embryo sacs the pollen tubes, even if wild-type, are unable to release the sperm cells to effect fertilization. This phenotype suggests that the female gametophyte plays a crucial role in pollen tube reception and, thus, controls the behavior of the male gametophyte (Huck et al., 2003). The *feronia* and *sirene* mutants, which display similar phenotypes (Rotman et al., 2003), define a novel signaling process between the male and female gametophytes during fertilization.

We will report on the molecular characerization of *FERONIA*, which encodes a receptor-like kinase (Escobar-Restrepo et al., 2007) that is expressed at high levels in the synergid cells through which the pollen tube enters to effect double fertilization. The molecular nature and subcellu localization of the FERONIA protein is consistent with its proposed function in a signaling process. I will report on our efforts to identify novel components of this signal transduction pathway using molecular and geneti approaches. Several new components that act in the female gametophyte have been identified and we will report on the progress on their molecular identification. Interestingly, some interspecific crosses result in phenotypes that are very similar to those observed in the *feronia* mutant, suggesting that it may be involved in species-specific interactions. The evolutionary implications of these findings will be discussed.

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The Role of Polarity and Cross-Talk for Double Fertilization

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Double fertilization in flowering seed plants requires intercellular signalling events between many interacting partners as well as the formatic of highly polarized gametic cell structures. The four cell types of the seven celled female gametophyte (embryo sac) communicate with each other to establish polarity and to obtain and maintain their identity. The egg apparatus (egg cell and synergids) secretes signalling molecules to guide the male gametophyte (pollen tube) via tip growth responses. In addition, the degenerating synergid mediates sperm cell discharge and their translocation towards the female gametes (egg and central cell) via reorganisation of actin tracks. After communication and fusion of polar structured gametes (sperm and egg cell as well as sperm and central cell), guidance signals are removed to prevent polyspermy, nuclei migration occurs and embryo as well as endosperm development is activated generating daughter cells of a different fate.

110 years after discovery of the double fertilization process, we are finally beginning to decipher the underlying molecular events after first key players involved in the various processes described above have been identified. I will report about some of these players involved in fertilization mechanisms in Arabidopsis and maize such as, for example, an Armadillo-repeat protein involved in the organisation of actin dynamics in the pollen tube tip and egg apparatus, about small secreted extracellular peptide ligands playing key roles in small range pollen tube and sperm guidance as well as sperm cell discharge. Proteins involved in asymmetric cell divisions include, for example, BTB/POZ-domain proteins.

The initiation of apomixis in *Hieracium*

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Seeds are critical to world food supply. Application of apomixis, or asexual seed formation, to crop plants, where it is currently absent, would produce a second green revolution. Apomixis enables the fixation and perpetuation of a genotype through seed. If developed as a breeding technology, apomixis has the potential to economize hybrid seed production, accelerate the breeding of new crops tailored to new production zones and decrease losses in yield resulting from unfavourable pollination conditions. We have been studying apomixis in the daisy-like plant *Hieracium* which initiates with the mitotic formation of an embryo sac from a somatic cell and then both embryo and endosperm arise without fertilization from cells contained in the unreduced embryo sac. Two independent, dominant loci control the initiation of apomixis and fertilization-independent embryo formation. Here, our current progress towards the isolation of genes found at the locus controlling the initiation of apomixis will be presented together with our analysis of the events and down stream genes involved in the formation of unreduced gametophytes in apomictic *Hieracium*.

The Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK) proteins function in brassinosteroid dependent and independent signaling

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Structurally related Receptor Like Kinase (RLKs) often function in similar signaling pathways. To determine whether this holds true for all 5 members of the Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) family we generated double, triple and quadruple mutants. One member of this family, SERK3, is also known as BAK1, the co-receptor of the brassinolide (BR) perceiving receptor BRI1.

We show that only serk1 but not serk2, serk4 or serk5 mutant alleles enhance the BR insensitivity of serk3-1 mutant roots and hypocotyls. SERK1, together with SERK2 is also essential for male sporogenesis and tapetum formation, a function that is not controlled by BRI1 signaling. Likewise, SERK3 alone controls innate immunity and together with SERK4 can also mediate cell death control in a BR-independent manner. SERK1 also has a unique function, not shared with any of the other members, in the acquisition of embryogenic competence in Arabidopsis explants. This pathway is intimately involved with BRI1-mediated BL signaling and also involves the MADS-box transcriptional regulator AGL15.

This shows that individual SERK proteins serve roles in different and independent signaling pathways, possibly through heterodimerization with different ligand-perceiving receptors and/or recruitment of different target proteins. We will discuss how in plant cells the same receptor protein can serve in different signaling pathways and activate specific downstream targets.

Epigenetic control of ovule development in *Arabidopsis thaliana*

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Our group investigates the genetic and epigenetic basis of female reproductive development in flowering plants. We are interested in understanding the genetic basis and molecular mechanisms that distinguish sexuality form of apomixis, a method of reproduction that bypasses meiosis and fertilization to result in the formation of clonal seeds. To conduct a large-scale transcriptional analysis of the ovule and female gametes in *Arabidopsis thaliana*, we generated large collections of Massively Parallel Signature Sequencing tags (MPSS) corresponding to transcripts present in ovules in which female gametes were either present or absent. By comparing these collections, we identified more than 1,100 genes that are expressed in female gametes but not in the rest of the ovule, and more than 90 new microRNA candidates that are either repressed in wild-type of ovules, or expressed in specific female gametophytic cells. Strikingly, several genes encoding proteins involved in small RNA biosynthesis and function, and not detected in sporophytic tissues, were highly expressed at distinct developmental stages of ovule development. The identification of insertional mutants defective in the specification of female meiotic precursors indicates that as animals, plants require sRNA-interacting proteins to control early gamete formation. Our results indicate that specific epigenetic mechanisms are fundamental to control gamete specification and reproductive destiny in flowering plants.

The forgotten layer: the stigma pellicle and its role in pollen-stigma interactions

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The pollen-stigma interaction consists of: (i) pollen capture, (ii) pollen adhesion, (iii) pollen hydration, (iv) pollen germination, and (v) pollen tube penetration of the stigma. Successful completion of these events normally results in pollen tube growth to the ovary leading to fertilization, unless incompatibility/incongruity factors preclude. The pollen-stigma interaction is thus a necessary pre-zygotic molecular 'courtship' between the haploid male gametophyte and the diploid maternal tissues of the sporophyte. Molecules regulating this interaction reside at the surfaces of pollen and stigma but (self-incompatibility [SI] proteins excluded) our knowledge of their identity remains largely fragmentary.

Stigma surfaces can be classified broadly into two types: 'wet' or 'dry', depending on whether or not they possess a surface secretion. In species with dry stigma, such as members of the Brassicaceae, Poaceae, and Asteraceae, the epidermal cells (papillae) of the stigma are covered by a proteinaceous pellicle. Following the discovery of this layer in the 1970s attention focused on analyzing its protein components in a host of different species. Esterases and glycoproteins were identified as the primary protein components of the pellicle and various hypotheses were formulated as to how these proteins might regulate the early events of pollen-stigma recognition, including SI. Since these pioneering studies however, few studies have sought to pursue the explicit mechanistic role(s) of any pellicle proteins. Here we review what is currently known about the protein constituents of this 'forgotten layer' in, primarily the Brassicaceae and Asteraceae, and discuss recent studies that promise to shed new light on the function(s) of the stigma pellicle in pollen-stigma interactions.

PART II - SESSIONS

Session 1 - Floral Development

REM18 and *REM53*: two direct targets of the ovule identity complex in *Arabidopsis*

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In the model species *Arabidopsis thaliana*, ovule identity is controlled by the action of the MADS-box genes *SEEDSTICK* (*STK*), *SHATTERPROOF1* (*SHP1*), *SHP2* and *AGAMOUS* (*AG*) (Pinyopich et al., 2003, Brambilla, et al., 2007). Among these genes, *STK* is specifically expressed in the ovule while the *SHP* and *AG* genes are also expressed in the developing carpel. Protein interaction experiments demonstrated that the ovule identity factors assemble in protein complexes in presence of the MADS-box proteins SEPALLATA (SEP) (Favaro et al., 2003). In order to better elucidate the molecular mechanisms controlling ovule developmer we are interested in the identification and characterization of genes regulat by the ovule identity MADS-box protein complex.

Arabidopsis ovule primordia were isolated through Laser-Capture Microdissection (LCM), RNA was extracted and used to hybridise Affymetrix microarrays. This analysis led to the identification of a set of transcription factors that are expressed during early stages of ovule development. Subsequently, this gene set was analysed for the presence of multiple MADS-box binding sites in their putative regulatory regions. A subset of genes that were positive in this bioinformatics screen were further analysed by Chromatin Immunoprecipitation (ChIP) experiments using an antibody specific for STK. This allowed us to identify *REM18* (At5g18000)and *REM53* (At3g53310) as direct targets of the ovule identity factor STK. *REM18* and *REM53* belong to REproductive Meristem (REM) family (Franco-Zorrilla et al., 2002), an almost unknown family of transcription factors unique in plants. Expression analysis showed that these genes are developmentally regulated and broadly expressed genes however in situ hybridisation on developing flowers revealed that their expression in wild-

type plants is restricted to the ovule starting from stage 8 of flower development. In the *stk shp1 shp2* triple mutant both *REM18* and *REM 53* are not expressed in the developing ovules. We will present the characterization of the *rem18* and *rem53* mutants which allowed us to understand their roles during ovule development.

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The spatio-temporal control of flower termination in Arabidopsis

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In many non-eudicots and early diverging eudicots, flowers are somewhat indeterminate and display morphological variations such as a variable number of whorls and parts per whorl, an elongated floral axis or spiral phyllotaxy. Variations of floral axis determinacy and compression are considered two major evolutionary innovations that produced compact angiosperm flowers, such as in the eudicot *Arabidopsis thaliana*. Mutants and mutant combinations in genes controlling flower meristem identity, carpel identity, male-female boundary and several more general regulators of development produce a range of flower reversal phenotypes and allowed us to reconstruct at least parts of this evolutionary history. For example, we show that in Arabidopsis, FM termination is initiated at stages 3-4 and needs to be maintained through stage 6 and beyond. We will also show

that the process requires a coordinated control of *AGAMOUS* (*AG*) expression in an inner 4th whorl sub-domain through a combined action of developmental factors acting in both space and time. This combinatorial network of genes controls flower termination and contributes to the more stable and uniform development of flowers, termed floral developmental homeostasis.

Evolution of molecular networks controlling tropical plant reproduction

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Floral transition is one the most drastic changes occurring during the life cycle of a plant. The shoot apical meristem switches from the production of leaves with associated secondary shoot meristems to the production of flower meristems. This transition is abrupt and generally irreversible, suggesting it is regulated by a robust gene regulatory network capable of driving sharp transitions. The moment at which this transition occurs is precisely determined by environmental and endogenous signals. A large number of genes acting within these pathways have been cloned in model herbaceous plants such as *Arabidopsis thaliana*.

The rapid advances made in understanding *Arabidopsis* flowering have allowed researchers to begin similar investigations in annual/perennia crops. This knowledge is greatly accelerating flowering research because, at least in a general sense, the same genes appear to be involved in flower initiation, flower formation, and fruit development in all flowering plants. The information generated in this type of research is important because many of the agricultural products (such as fruits and seeds/grains) are consequence of plant sexual reproduction.

Using the DNA sequence of genes from model plants as a starting point, flowering genes have been successfully isolated from several agriculturally important tropical crops.

We have searched sequence clusters in the SUCEST (sugarcane), FORESTs (*Eucalyptus*), CitEST (*Citrus*) and PASSIOMA (passionfruit) databases and identified more than one hundred sequences that codify putative conserved elements of the autonomous, vernalization, photoperioc response and gibberelic acid-controlled flowering-time pathways. Despite the fact that most of the genes included in these pathways are conserved,

we have observed obvious modifications in the vernalization pathway that might reflect adaptation of tropical plants to warm environments.

We expect that our results will contribute to further studies describing how the flowering-induction pathways function in controlling the flowering process in tropical non-model plants.

Identification and characterization of novel genes important for stamen development in Arabidopsis

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To obtain detailed information about gene expression during stamen development in *Arabidopsis thaliana*, we compared, by microarray analysis, the gene expression profile of wild-type inflorescences to those of the floral mutants *apetala3*, *sporocyteless/nozzle*, and *male sterile1* (*ms1*), in which different aspects of stamen formation are disrupted. These experiments led to the identification of groups of genes with predicted expression at early, intermediate, and late stages of stamen development. Validation experiments using in situ hybridization confirmed the predicted expression patterns. Additional experiments aimed at characterizing gene expression specifically during microspore formation. To this end, we compared the gene expression profiles of wild-type flowers of distinct developmental stages to those of the *ms1* mutant. Computational analysis of the datasets derived from this experiment led to the identification of genes that are likely involved in the control of key developmental processes during microsporogenesis. Among the genes found as expressed in early stages of stamen development, we focus in two member of the REM family of the super-family of B3 genes, REM 22 and 26. Their spatial expression pattern, verified by *in situ* hybridization and GFP/GUS fusion in stable transformants of Arabidopsis, revealed a spatial expression pattern tightly regulated during initiation and tissue differentiation of stamens. Phylogenetic analysis of the B3 super-family in plants showed that the REM family has experienced a fast process of gene evolution when compared with ABI, HIS, RAV and ARF families. *VRN1* is the first and the single member of REM family characterized up to now. VRN1, together

with VERNALIZATION 2 and LIKE HETEROCHROMATIN PROTEIN 1 (LHP; also known as TFL2) are required from maintenance of FLC silencing. Lastly, we applied reverse genetics to characterize several of the genes identified in the microarray experiments and uncovered novel regulators of microsporogenesis, including the transcription factor MYB99 and a putative type II phosphatidylinositol 4-kinase (PI4K). Knock out lines for *MYB99* and *PI4K* genes showed specific defects in microspore formation. *PI4K* is expressed in the tissues affected in the T-DNA insertion line, and the developmental stage at which morphological defects become apparent in the mutant corresponds with the onset of its expression. Electron microscopy characterization of PI4K knock out line tapetum revealed a reduction in lipid accumulation in tapetosomes and elaioplastids. While the function of type II PtdIns 4-kinases in plants is unknown, it has been suggested that in animals, they are involved in the regulation of intracellula trafficking. Thus, the mutant phenotype observed for the At2g40850 insertion line might be caused by a malfunction of lipid trafficking, an essential cellular process for normal tapetum development. The functional characterization of several of the genes that were identified in the microarr experiments yielded novel regulators of microsporogenesis. This result suggests that a systematic study of the identified genes should reveal additional genes that are involved in the control of stamen development and, in particular, pollen formation.

Session 2 – Male Gametophyte Development

Challenging the need for a pacemaker – pollen tube growth oscillations explained with a mechanical harmonic oscillator model

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Pollen tubes grow extremely fast and often in oscillatory manner. This cellular system, therefore, provides a unique opportunity to study plant cell growth at short time scales and while exhibiting dynamical changes over short time intervals. Numerous aspects of this oscillation phenomenon have been investigated in the past and various models have been proposed to explain its mechanism. A frequently cited requirement to explain oscillations is the presence of a pacemaker that dictates the rhythm and provides the initial trigger. The nature of the pacemaker has not been identified, however.

Here, we propose an alternative model, that despite encompassing existing experimental data and not being mutually exclusive with most aspects of previously proposed models distinguishes itself from the latter by the fact that it does not require a pacemaker to explain the onset of oscillation. This model is based on the mechanical relationship between turgor pressure and tensile resistance in the apical cell wall. We propose that the equilibrium between these two forces can be compared to that of a mechanical harmonic oscillator – similar to a weight attached to a spring. It only takes a small disturbance to displace the components of this oscillator from their equilibrium position and cause them to oscillate. Oscillation frequency is determined by numerous mechanical parameters, or in the case of the pollen tube, by cellular feedback processes. However, importantly, the onset of the oscillations in a harmonic oscillator is not triggered by the switching on of a putative pacemaker, but by a mechanical disturbance to the force equilibrium. Consistent with this model we observed that agents causing cell wall hardening (pectin methyl esterase) or softening (auxin) as well as alterations to the turgor pressure are not only able to influence the oscillation profile but also to trigger oscillations in pollen tubes that previously displayed a steady growth rate.

If the mechanical equilibrium between cell wall tension and turgor pressure is involved in controlling the dynamics of the pollen tube growth rate, one should expect the mechanical properties of the cell wall to change during an oscillation cycle. We therefore used micro-indentation to measure cellular stiffness at the growing apex over time. We observed that prior to a rapid growth phase the cellular stiffness was reduced at the apex and it increased during a pulse – consistent with a process of strain hardening. These findings confirm the important role of the mechanical properties of the cell wall for cellular growth processes.

Transcriptional Regulation of Male Germ Line Development in Flowering Plants

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Most of the grains and seeds that form the world's staple food supply are the result of the successful functioning of male and female gametes during fertilization. Despite the importance of plant reproduction, our understanding of the development and differentiation of gamete lineage in higher plants is very limited. How these fundamental developmental processes are controlled at the gene level is almost unknown. Understandin the control of gamete development is a prerequisite for advancing our knowledge of the biology of fertilization in flowering plants. We have shown that in flowering plants, the male germline is initiated by transcriptional control. We have identified a germline-restrictive silencing factor (GRSF) that is ubiquitous in somatic cells, but absent from male germ cells. This silencing factor recognizes silencer sequences in the promoters of genes specific to the germline, stably repressing these genes in cells that are not destined to become germ cells. GRSF is evolutionarily conserved repressor in flowering plants as our experiments with plants from diverse families such have shown the presence of repression mechanism in each of these species. Further research in our laboratory will focus on unravelling the mechanism by which this genetic switch functions to repress somatic genes during plant growth, and what triggers this switch to de-repress the germ line transcriptional program and turn on during the male germ line linage formation.

Recent Progress in Research of the Fertilization Mechanism in Angiosperms

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Fertilization in angiosperms is a complicated process. When the pollen tube enters the degenerated synergid of an embryo sac, two sperm cells are released inside. The two sperm cells are connected in the pollen tube but after their release in the degenerated synergid, they must separate. One of the two sperm cells moves to the egg cell and fuses with it to form a zygote, the other one moves to the central cell to form the endosperm. The process of male and female gamete recognition is a critical interaction but remains poorly understood. In this review, we discuss recent progress in the study of the cell cycle of male and female gametes before fertilization, and the question of synergid degeneration. Herein we analyze the status of research on the movement of the two sperm cells in the degenerated synergid, and the phenomenon of signal reactions between male and female gametes. We also evaluate our progress in understanding the preferential fertilization of sperm cells and egg cell activation.

Keywords: angiosperms; egg cell; fertilization mechanism; sperm cell; zygote

Session 3 - Female Gametophyte Development

Regulation of embryo sac development by *indeterminate gametophyte1*

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Embryo sac development begins with an early free-nuclear proliferatio phase before cellularizing and differentiating into four different cell types. The four cell types occupy stereotypical positions along the micropylar-chalazal axis with the synergids and egg at the micropylar pole, the antipod cells at the chalazal pole, and the central cell in the middle of the embryo sac. Micropylar-chalazal asymmetry is detectable in the mature embryo sac and earlier, before cellularization and even before the first free nuclear division of the functional megaspore. Little is known about the mechanisms regulating polarity of the embryo sac. The maize *indeterminate gametophyte1* (*ig1*) gene and its Arabidopsis ortholog, *ASYMMETRIC LEAVES2* (*AS2*), are expressed in embryo sacs and required for normal abaxial/adaxial polar of lateral organs in the sporophyte. Loss-of-function *indeterminate gametophyte1* (*ig1*) embryo sacs undergo extra rounds of free nuclear divisions and have nuclei in abnormal positions in the syncytial embryo sac. The extra nuclei lead to the production of extra egg cells, extra central cells, and extra polar nuclei as well as other abnormalities. Consequently, *ig1* mutant plants produce several classes of abnormal seed. A putative role for *ig1* in the regulation of micropylar-chalazal polarity is being examined.

Regulatory networks controlling female gametophyte development.

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The female gametophyte is essential for plant reproduction. The *Arabidopsis* female gametophyte is a seven-celled structure composed of one central cell, one egg cell, two synergid cells, and three antipodal cells. How these four cell types acquire their unique features and functions during female gametophyte development is not understood. As a first

step toward dissecting the gene regulatory networks controlling female gametophyte development, we identified a collection of genes expressed in the *Arabidopsis* female gametophyte [Steffen et al. (2007) Plant Journal 51: 281-292]. One of these, *AGL61*, encodes a Type I MADS-domain protein. *AGL61* is expressed in the central cell and during early endosperm development. *agl61* female gametophytes have defects in central cell development. When fertilized with wild-type pollen, *agl61* central cells fail to give rise to endosperm. The expression pattern and mutant phenotype of *AGL61* is similar to that of *AGL80* [Portereiko et al. (2006) Plant Cell 18: 1862-1872], suggesting that AGL61 may function as a heterodimer with AGL80 within the central cell. Consistent with this, AGL61 and AGL80 interact in yeast. To identify genes regulated by *AGL61* and *AGL80*, we tested previously identified central cell-expressed genes for reduced expression in mutant female gametophytes and identified many such genes.

Endosperm genetics and the developmental evolution of female gametophyte body plants.

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The angiosperm reproductive syndrome is notable for its novel double fertilization process that produces an embryo and a unique triploid embryo-nourishing tissue (endosperm) comprised of one paternal and two maternal components. Although triploid endosperm is a feature of the vast majority of angiosperms, there are six other known genetic constructs that have each evolved more than once. These endosperms vary in ploidy level, heterozygosity, maternal to paternal genome ratio, and their relatedness to embryos and sporophyte. Variation in endosperm genetics is the product of the evolution of development of the highly-reduced angiosperm female gametophyte – its central cell (endosperm-precursor) can contain from one to 14 haploid chromosome sets derived from one to four megaspore progenitors. Thus, understanding the constraints and pathways of female gametophyte ontogenetic evolution is the key to appreciating genetic variation in embryo-nourishing tissues. Comparative studies of female gametophyte development, based on strong phylogenetic hypotheses, provide new and perhaps surprising insights into the how and why of nature's often repeated experiments in endosperm biology.

Session 4 - Pollen-Pistil Interaction - Part I

Behavior and Signaling in Gametophytic Interactions

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Gametophytic interactions between the pollen tube and the embryo sac occur deeply inside the pistil of the flower. Due to the inaccessibility, it still remains unknown how gametophytic cells communicate to achieve double fertilization. We developed the in vitro *Torenia* system, whereby pollen tubes growing through a cut style are attracted to a protruding embryo sac and cause double fertilization. By using this system, the synergid cell was shown to emit some diffusible attractant(s) (Higashiyama et al., *Science*, 2001). The attractant molecule was species preferential even in closely relating species, implying that the molecule had rapidly evolved (Higashiyama et al., *Plant Physiol.*, 2006). Thus, genes expressed in the synergid cell might provide insights into the attractant. We are now investigating genes expressed in the synergid cell of *Torenia*, by collecting isolated synergid cells. To examine whether these genes are required for the pollen tube attraction, we developed a laser-assisted thermal-expansion microinjection system. I will discuss in my talk about our recent attempt to down-regulate the genes by injecting morphorino anti-sense origos into the protruding embryo sac. On the other hand, we developed a method for live cell imaging by combining the in vitro system of *Arabidopsis* with a state-of-the-art disk-scan confocal scanning laser microscope equipped with a high-sensitive EM-CCD camera, a high-speed piezo Z-axis drive allowing rapid recording of z-stacks, and a prism to monitor two colors at the same time (Ingouff et al., *Curr. Biol.*, 2007). We could observe the entire process of double fertilization. I will also discuss the mechanism of double fertilization based on the direct observation of fertilization processes

Before pollen-style interactions: the origin and early evolution of the angiosperm fertilization process

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Since the time of Darwin a number of key life history and morphological traits have been proposed as developmental correlates of the extraordinary diversity and ecological success of angiosperms. Many such traits concern their novel and rapid fertilization process in which pollen-style interactions introduce the potential for pre-zygotic gamete selection. Comparative analyses indicate that gymnosperms face an extraordinary limitation in their fertilization biology – pollen reception must be near the egg largely because sperm swim or are transported by very slow growing pollen tubes (rates of less than about 20 µm/h). I will show that among taxa in ancient angiosperm lineages (*Amborella*, *Nuphar*, and *Austrobaileya*) pollen tube growth rates universally exceed those of gymnosperms (80-600 µm/h) but are slower than typical angiosperm rates. Further comparative analyses point to accelerated pollen tube growth rate as a critical innovation that *preceded* the origin of the true closed carpel, long styles, multi-seeded ovaries, and, in monocots and eudicots, much faster pollen tube growth rates. Ancient angiosperm pollen tubes all have callosic walls and callose plugs (in contrast, no gymnosperms have these features). The early association of the callose-walled growth pattern with accelerated pollen tube growth rate underlies a striking pattern of repeated origins of faster and longer-distance pollen tube growth within often solid pathways in phylogenetically derived angiosperms. Pollen tube innovations truly enabled a spectacular diversification of carpel (flower and fruit) form and reproductive cycles in flowering plants.

LePRK2 signal transduction in pollination: hyperphosphorylation and signaling by an unusual style peptide

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In compatible pollination, after pollen grains germinate on the stigma, pollen tubes traverse the style on their way to the ovules. During that journey, pollen tube receptors might perceive style signals, thereby triggeri cytoplasmic events required for tip growth. We characterized two pollen-specific receptor-like kinases, LePRK1 and LePRK2, from tomato mature pollen. Their immunolocalization pattern and the specific LePRK2-dephosphorylation by style extract suggested that at least LePRK2 transduces style signals (Muschietti *et al.*, The Plant Cell 1998, 319-330). We showed in pollen, both LePRK1 and LePRK2 are found in a high molecular weight complex that is dissociated when pollen is germinated *in vitro* in the presence of style extracts. In contrast to the typical manner of receptor kinase activation, we propose this style component transduces the signal by triggering LePRK2 dephosphorylation followed by dissociation of the LePRK complex (Wengier *et al.*, PNAS 2003, 6860-6865). We also demonstrated that LePRK2 is hyperphosphorylated in pollen membrane where some of the phosphorylated residues are important for pollen tube growth. Using a combination of different chromatography systems we purified that style component to homogeneity; it is an extremely stable peptide with a molecular weight of 3,550 Da. that stimulates pollen tube growth through LePRK2. All these findings suggest this style peptide is a key element of pollen-pistil signaling mediated by LePRK2.

Session 4 - Pollen-Pistil Interaction (Self-incompatibility) - Part II

High speed delivery to the growing point - quantification of vesicle streaming in pollen tubes using spatio-temporal correlation spectroscopy (STICS) and fluorescence recovery after photobleaching (FRAP)

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The elongation of pollen tubes within the stigmatic and stylar tissues of the receiving flower is extremely fast and necessitates the continuous synthesis of new cell wall material as well as the secretion of proteins responsible for the softening and digestion of the transmitting tissue. The delivery of cell wall material, membrane and export proteins to growing plant cell surfaces requires the spatial and temporal coordination of secretory vesicle trafficking. Given the small size of vesicles, their dynamics is difficult to quantify. To quantitatively analyze vesicle dynamics in growing pollen tubes labeled with the styryl dye FM 1-43, we applied spatio-temporal correlation spectroscopy (STICS) on time lapse series obtained with high speed confocal laser scanning microscopy recordings. The resulting vector maps revealed that vesicles migrate towards the apex in the cell cortex, they accumulate in an annulus shaped region adjacent to the extreme tip and then turn back to flow rearwards in the center of the tube. Fluorescence recovery after photobleaching (FRAP) confirmed vesicle accumulation in the shoulder of the apex, and it revealed that the extreme apex never recovers full fluorescence intensity. This is consistent with endocytotic activity occurring in this region. FRAP analysis also allowed us to measure the turnover rate of the apical vesicle population which was significantly more rapid than the theoretical rate computed based on requirements for new cell wall material. This may indicate that a significant portion of the vesicles delivered to the apex does not succeed in contacting the plasma membrane for delivery of their contents. We therefore propose that more than one passage into the apex may be needed for many vesicles before they fuse to the plasma membrane and deliver their contents.

Genes important for pistil development and pollen-pistil interaction in Nicotiana tabacum L., a wet stigma species

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The success of the plant reproductive process depends on appropriate pistil development, synchrony of pistil and pollen maturation, as well as compatible pollen-pistil recognition and interactions. These interactions may differ depending on whether the species in which they occur have a wet or dry stigma. Solanaceous species, such as *Nicotiana tabacum*, have wet stigmas, on which a sticky exudate is secreted at maturity. When pollen grains land on a mature stigma, they first come into contact with the exudate that completely covers them. The pollen hydrates, a pollen tube germinates and grows through the exudate that fills the intercellular spaces of the stigmatic secretory zone and stylar transmitting tissue toward the ovules. To establish a large scale survey of tobacco gene expression during pistil development and preparation for pollination, we generated 11,217 high-quality expressed sequence tags (ESTs) derived from stigmas/styles at different flower developmental stages. These sequences were used to create the tobacco ESTs (TOBEST) database and represent 6,177 transcripts/genes. A macroarray analysis of 782 genes has allowed the identification of 38 novel putative pistil-specific genes from which at least 6 encode proteins of unknown functions. From these genes, one for a pectin acetyl esterase (NtPAE1) and one for an unknown protein (TOBR092H06) were chosen for further studies. The NtPAE1 was shown to be pistil-specific and developmentally regulated by real time RT-PCR experiments. Pollinations performed in pistils from the transgenic plants, in which the NtPAE1 has been silenced, revealed a delay in pollen tube growth, indicating the importance of NtPAE1 in establishing the intercellular spaces and facilitating pollen tube penetration. The gene corresponding to TOBR092H06 is specifically expressed at the reproductive organs and is preferential of stigmas/styles, as demonstrated by real time RT-PCR experiments. It encodes a small protein (68 amino acids) that is localized in the nucleus, as shown by GFP fusions. TOBR092H06 RNAi transgenic plants had bigger anthers, pistils and fruits, suggesting that 092H06 protein may be a peptide hormone involved in regulating the growth/development of the reproductive structures.

As an additional approach to identify pistil-specific genes, we have constructed 2 suppression subtractive hybridization libraries (SSH1 and SSH2), which unravel still novel genes. A developmentally regulated pistil-specific gene, encoding methyltransferases (NtPMT1), is alternatively spliced and produces 9 different transcripts. The longer transcript encodes an enzyme capable of producing methyljasmonate, methylbenzoate, and methylsalicylate in vitro. These volatiles may have important roles in pollination biology and/or plant defense. The SSH approach also revealed a stigma/style specific gene encoding a lysine-rich protein of unknown function. GFP fusion experiments have revealed the nuclear localization of the corresponding protein. The RNAi-mediated silencing of this gene resulted in transgenic plants exhibiting remarkably longer styles and an enlarged stigma area. Additionally, the overexpression transgenic plants had stigmas with smaller area. Transversal sections of the mature stigmas/styles clearly showed that this change in size occurs as a consequence of an alteration in cell number that is increased in the specialized tissues of the RNAi plants and decreased in overexpression plants. The results obtained so far suggest that this protein is a negative cell cycle regulator and, for this reason, the gene has been denominated SCI1 (Stigma Cell-cycle Inhibitor 1).The altered pistil development in RNAi plants, in which stigmas are positioned above anthers, may turn self-pollination difficult.

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Session 5 - Embryogenesis

Dissection of Arabidopsis Embryo and Seed Development

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The most common form of embryogenesis is zygotic, but plant cells can undergo several other pathways to make embryos. Differentiated somatic cells can be induced to undergo embryogenesis, usually as a result of hormone treatments. Stress treatments cause microspores to become embryogenic and produce haploid embryos instead of pollen grains. Various cells of the ovule undergo either adventitious or parthenogenic embryogenesis in a suite of processes known as apomixis to give rise to asexual seeds. Although morphological development in all of these different forms of embryogenesis is similar, the cellular processes that cause a cell to change its fate and become embryogenic are not known.

We are using the LEAFY COTYLEDON (LEC) transcription factors to dissect mechanisms controlling embryo development. Ectopic expression of the *LEC* genes causes vegetative and reproductive tissues to acquire embryonic traits and produce somatic embryos. Loss-of-function mutations in the *LEC* genes cause defects in several aspects of zygotic embryogenesis. We discuss the mechanisms by which the LEC transcription factors establish a cellular environment that promotes embryo development.

Cellular decisions in the Arabidopsis and maize embryo and the EvoDevo perspective

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Molecular markers assist in the analysis of embryonic patterning programs and comprise a reliable set for tracing cellular decisions in *Arabidopsis thaliana*. Very informative are members the *WOX* (*WUSCHEL* related homeobox), *KNOX* (*KNOTTED* related homeobox) and *CUC (CUP-SHAPED COTYLEDON)* gene families. In a comparative approach orthologous members of different gene families were isolated from maize and used to visualize cellular decisions during maize embryogenesis and to compare the patterning program between maize and Arabidopsis. The molecular marker data support the conclusion that the embryonic shoot/root axis comprises a discrete adaxial domain in maize, which is already separated from the prospective scutellum in the early proembryo. The complete data set implies that embryonic patterning although involving similar regulatory networks in maize or Arabidopsis has been subject to major adaptations during evolution.

Relative to the specification of the embryonic shoot and root stem cell niches, which are crucial for postembryonic development, phylogenetic analyses of the WOX gene family have been extended to basal angiosperms, gymnosperms, lycophytes and moss. The emergence of the modern angiosperm branch including WUS and WOX5 will be discussed.

Session 6 - Reproduction of Tropical Plants

Male gametophyte characterization on Passion flower reproduction

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Despite the morphological, taxonomical, pharmacologiacal and ecological importance of the *Passiflora* species, we have little knowledge about the reproduction habits and pollen-stigma-style interactions. The pollen is released in bicellular stage and according recent works, certain organelles, can be excluded during the events of gametophyte cell division. The plastids inheritance in angiosperms is mainly maternal. The *Passiflora* genus presents variations in this statement, since it can be maternal, paternal or biparental inheritance. Thus, the present study aims to analyze the pollen development, the pollinations process, and the plastidial inheritance in *Passiflora elegans*. The analysis was performed from flower buds to pre-anthesis flowers, attending the pollen development and the pollination events. The material was fixed in 2.5 % glutaraldehyde and 2.0% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) and postfixed in 2% OsO_4 plus 0.8% $K_3Fe(CN)_6$ in the same buffer, for transmission electron microscopy. The semithin sections were stained with 0.05% Toluidine Blue O, pH 4.4 and the ultrathin sections was contrasted with uranil acetate/lead citrate or potassium permanganate/lead citrate. During pollen differentiation many plastids were observed in all stages. Mitochondria were also found associated with dictyosome groups, distributed at the cytoplasm cortical region of these cells. After meiosis, the plastids are numerous, with starch inside. At the beginning of gametogenesis stage, the nucleus is parietal and small vacuoles fusion in the central region. The cytoplasm is restricted to the cell periphery, concentrating many mitochondria near to the nucleus. A lot of plastids occur around the cytoplasm and, beside vacuolation progress, they migrate near to the nucleus. After the first mitosis, the generative cell inherits a lot

of organelles that are incorporated in its cytoplasm, among then, can be highlighted the presence of many plastids, mitochondria, dictyosomes and oil vesicles. The pollen of *Passiflora elegans* is hexacolpate and spheroidal, the exine is semitectate with bacula inside and contains substances that participate in the pollen recognition. The flowers anthesis occurs near 8:00 am and stay opened one day only. The stigma is dry and has multicellular emergences formed by projections of dermal and subdermal cells, that characterize the receptive surface and guide the pollen tube. The histochemistry tests show proteins and lipids covering the pollen external surface and between the sexine spaces, probably representing primexine or pollenkitt contents. Polysaccharides and pectins are present in the intine, but a cellulosic layer can not detected. The pollen tube germination occurs two hours after the pollination, and its growth in an apoplastic way, with accumulation of pectic compounds in the vacuoles, as well as near the wall of adjacent cells. The experiments show that this species is self-incompatibility of sporophytic type. However, when self-pollination was performed two times, it causes the transposition of this mechanism. The exclusion of plastids does not occur during the stages of pollen formation. So, our data agree with paternal inheritance proposed for the genera.

Different breeding mechanisms and dispersal strategies in *Arachis*

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Although the genus *Arachis* (Fabaceae) encompasses 80 species validly described, focus on reproductive, physiological and agronomic aspects of its species is always biased, due to the consolidated scientific knowledge on the groundnut (*A. hypogaea*), an important crop species in more that 80 countries and a staple food in the semi-arid tropics. For instance, the annual life cycle, so well known to groundnut growers, only occurs in 2/3 of the species of the taxonomic section *Arachis* and in all six species of *Heteranthae*. All species are perennial in the seven remaining taxonomic sections. Groundnut's autogamy is usually taken for granted for *Arachis* species, while there is evidence that several of the most primitive species do require cross-pollination. Another group of species will only produce seed, under insect-free conditions, if flowers are manually tripped, which indicates a dependency on insect visits, with the consequent increase of chances of cross-pollination events. In fact, the groundnut piles up a series of morphological, reproductive and agronomic peculiarities brought in by domestication and continuing selection under agricultural use. The tenacious peg, suppression of the isthmus between pod segments, and ovule proliferation, resulting in more than two seeds per pod in several botanical varieties, are typical changes imposed by domestication. *Arachis villosulicarpa* and *A. stenosperma*, also cultivated for their nutritional grains by Brazilian indian tribes, show much less impact of domestication, although the first is only known from cultivation and the second has cultivated and roadside populations in two widely disjunctive areas, and what seems to be remaining wild populations restricted to Central Brazil. All *Arachis* species develop underground fruits only, and rarely are able to mature seed at more than one meter from the original germination site of the mother plant. That implies a very weak potential for long distance dispersal, a theoretical linear spread of no more than 100 meters per century, but the genus occurs at distances radiating over 2000km from its geographic center. Effective mechanisms for faster propagation, such as stolons or rhizomes, smartly taken advantage of in the modern perennial forage cultivars of *A. pintoi* and *A. glabrata*, as well as seed dispersal by birds, and down-river transportation, including stream piracy promoting changes of river basins, are complemented by a long history of human transportation to sites as distant as the Brazilian Atlantic coast and across the Andes, in Peru. Amphicarpy in section *Heteranthae* and evidences of apomictic seed development triggered by wide interspecific crosses are additional mechanisms to investigate in the genus.

Outbreeding and inbreeding in Cerrado plants: ecological consequences and perspectives

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The Cerrado is the Neotropical savanna region in Central Brazil. It is the second greatest natural biome in Brazil and nowadays its main agricultural frontier. A hotspot for conservation, recognized by its floristic diversity, and threatened by increasing deforestation and human use. The Cerrado woody flora is dominated by forest elements but rich in endemisms at species level, while the herbaceous flora is mostly similar to the flora of other grassland areas elsewhere in the tropics. Seasonal tropical climates, with rains concentrated in the summer and dry winters, are characteristic of the region. Drought, deep nutrient-poor oxisoils and frequent fires were viewed as limiting factors for establishment and seedling survival in these areas. Regeneration and population dynamics would depend on vegetative growth and multiplication. This paradigm of plant formations limited in their productivity and regeneration by abiotic factors dominated the studies of Cerrado plants and discouraged reproductive biology research. But Cerrado and savanna plants as a whole are well adapted to these seasonal conditions and abiotic restrictions. Recursive growth from underground organs and other reproductive strategies allow both woody and herbaceous species to be less affected by the water stress. Deep root systems allow trees to be relatively independent of seasonality, while the herbaceous species, more severely affected by drought, are mostly perennials, persisting by protecting meristems in underground organs. Studies during the last three decades showed that reproductive output of Cerrado species are not more limited by the dry season than species in other tropical plant formations. As for the trees, community level studies showed a diversity of pollination systems and breeding system features which were similar to the observed for other tropical forests. Dominance of obligatory outcrossing, due to dioecy or self-incompatibility, renders Cerrado species dependent on biotic vectors, which work as the most important driving forces for flowering phenology and fruiting success. Breeding biology and genetic markers studies pointed out to large plant populations on a regional scale, which would be result of long distance gene flow either by pollination or seed dispersal. Data for woody species contrast with the breeding biology of herbaceous plants which are mostly self-compatible or autogamous, although mostly zoophylous and non-apomictic. But the turning century

brought some ideas to a different context. Extensive screening of Cerrado and neighboring Caatinga woody plants for the occurrence of polyembryony showed a large percentage of species with extranumerary embryos in their seeds. Occurrence of polyembryony was thought to be the result of apomixis, which would then be much more common and widespread than previously imagined among Cerrado woody species. Studies on otherwise sexual and self-incompatible groups as Bignoniaceae, Bombacaceae and Melastomataceae have really shown sporophytic and gametophytic apomictics, but not all polyembryonic taxa are necessarily apomictic. An extended screening on cerrado plant populations in the Triangulo Mineiro region showed 40.6% of species with polyembryony but in many cases the frequency of polyembryonic seeds was lower than 5%. In these cases, polyembryony may as well be a result of sexual events. In some Vochysiaceae, a typical family in Cerrado areas, extranumerary embryos may be the result of fertilization of multiple embryosacs formed in each ovule. Sexual polyembryony might explain low frequencies observed in many species, but would not explain higher frequencies observed in at least 15% of the studied species. For these species polyembryony is really linked to adventitious embryony and apomixis. It is interesting to notice that in some autonomous apomictics as *Miconia albicans*, seeds are rarely polyembryonic. The survey, thus, confirm the occurrence of apomixis in many Cerrado woody species. Moreover, apomixis was confirmed in species previously described as self-incompatible. In *Tabebuia ochracea*, a common tree in central Brazil, apomictic and sexual populations were recorded for different places. An opposite trend was observed in *Eriotheca pubescens*, a widespread polyembryonic tree able to form mostly clonal populations, but which presented monoembryonic and possibly sexual individuals. These mosaics of breeding systems corroborate the idea that apomixis may function as an alternative reproductive system, providing persistence and facilitating distribution of well adapted genotypes over the cerrado region. It is interesting to notice that apomixis seems to be associated with widely distributed species, while endemic species, with restricted distribution, are mostly sexual. Increasing pressure of disturbance associated with the use of vast Cerrado areas for cash crop plantations, cattle ranching and charcoal production may contribute to the selection of apomictic genotypes among Cerrado woody species.

Session 7 - Fruit and Seed Development

Retinoblastoma and its Binding Partner MSI1 Control Imprinting in *Arabidopsis*

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Parental genomic imprinting causes preferential expression of one of the two parental alleles. In mammals the differential sex-dependent deposition of silencing DNA methylation marks during gametogenesis initiates a new cycle of imprinting. Parental genomic imprinting has been detected in plants and relies on DNA methylation by the methyltransferase MET1. However, in contrast to mammals, plant imprints are created by differential removal of silencing marks during gametogenesis. In the female gamete DNA demethylation is mediated by the DNA glycosylase DEMETER (DME). Based on genetic interactions we show that in addition to DME, the plant homologues of the human Retinoblastoma (Rb) and its binding partner RbAp48 are required for the activation of the imprinted genes *FIS2* and *FWA*. This activation by the Rb pathway is mediated by direct transcriptional repression of MET1 demonstrated by Chromatin immunoprecipitation. Our data identifies a new mechanism required for imprinting, relying on a Retinoblastoma dependent pathway. The potential conservation of this pathway suggests that Rb might also control imprinting in mammals.

Imprinting in the maize endosperm; dissecting the control elements

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A combination of gene dosage and genomic imprinting in the endosperm results in a strong maternal control over early seed developmer in maize. The recently duplicated *Fie1* and *Fie2* sequences, which encode Polycomb Group transcription factors are both expressed solely from the maternal alleles early in development, although the duration of this imprinti controlled monoallelic expression differs between the two genes. Using 'imprinted' reporter constructs (1) we have shown that monoallelic expression is likely to be regulated by differentially methylated regions (DMRs). Surprisingly, while the DMRs of *Fie1* receive their asymmetric methylation in the gametes, differential methylation of these regions in *Fie2* takes place in the primary endosperm cells, after fertilisation (2).

Through functional analysis of these control regions, both in mutant lines of maize and in Arabidopsis, we are endeavouring to unravel the different mechanisms by which one DMR becomes methylated during gametogenesis, and the other after nuclear fusion. Why such recently duplicated and sub-functionalised genes are imprinted by two different processes is also unclear, but may be related to the differing expression pattern of these two sequences throughout the plant life history.

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MPC, a novel imprinted gene in Arabidopsis, encodes the C-terminal domain of a polyadenylate binding protein

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In mammals and flowering plants, a subset of genes are parentally imprinted and expressed only from maternal or paternal alleles. Imprinted genes are often involved in growth control. Imprinting in plants has profound consequences for seed development but only a small number of imprinted genes have been identified. The known imprinted genes in the model species *Arabidopsis thaliana*—FIS1/MEDEA, FIS2, FWA, and PHERES1—are all involved in transcriptional regulation. We identified a novel type of imprinted gene in Arabidopsis, *MPC*, encoding the C-terminal protein-interaction domain of a Poly(A) binding protein (PABP). *MPC* is expressed only from maternally contributed alleles in the endosperm of developing seeds. The MPC protein binds polypeptides that also interact with the C-terminal domain of a full-length PABP, suggesting that MPC and PABPs might compete for partners, with potential effects on mRNA stability and translation. Reduction of *MPC* expression results in seed abnormalities including decreased seed size, and seed abortion. Other maternally expressed imprinted genes in Arabidopsis are regulated by an antagonistic interaction between DNA METHYLTRANSFERASE 1 (MET1), which maintains methylation on cytosines leading to gene silencing, and the DNA glycosylase DEMETER (DME), which removes methylated cytosines. We found that paternal MPC is also silenced by MET1, and we identified a hypermethylated region in the 5' flanking region of the MPC locus and extending into the coding region. However, the maternal MPC allele does not require DME for expression, indicating a new pathway of imprinting regulation in plants.

Session 8 - Apomixis

Cytology, Ontogeny and Genetics of the *Semigamy* Mutant of Cotton (*Gossypium barbadense* L.)

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The *Semigamy* (*Se*) mutant of cotton affords direct opportunities to advance basic biology and/or practical utilization. Here, recent research results will be summarized and briefly contrasted to salient historical interpretations regarding the genetic control of semigamous reproduction in cotton and the effects on the *Se* mutation on fertilization, embryo development and ontogeny. The *Se* mutation was originally detected approximately 45 years ago, when breeder-geneticists recovered a doubled haploid line of tetraploid Pima cotton (*Gossypium barbadense* L.) that produced haploid seedlings from monoembryonic seed at high frequencies (~40%). Subsequent characterization of seedling and plant families from controlled pollinations with genetically marked parents documented bizarre effects on reproduction, including the occurrence of maternal haploids, paternal haploids, and various types of chimeras involving maternal haploid paternal haploid and/or zygotic sectors. Sufficiently proficient cytological methods were not available when early analyses of *Se* were conducted, so the families were characterized based only on plant morphology-fertility syndromes and genetic markers affecting pigmentation or morphology. To enable cytological approaches, we developed stain-clearing methods for efficient video-facilitated cytological analysis of reproduction in semigamous and wild-type lines. Subsequent analyses revealed that the reproductive behavior of *Se* mutants differs markedly from that inferred by non-cytologic means. Comparative analyses of hundreds of ovules collected in timed series relative to pollination reveal *Se* expressivity is much higher than previously inferred, and that all previously noted types of progeny, including haploid and tetraploid sectors, arise from a common cytological state, the developmental fate of which hinge on relative spindle positions and orientations at the first zygotic division. The within-seedling patterns of ploidy levels and parental markers among semigamously derived seedling were studied after crossing genetically marked parents. Differences were pronounced among the cotyledons and seedling leaves in terms of ploidy and, for haploid tissues, for parent- or marker-of-origin. Chimeric sectors in

true leaves were disproportionately derived from the maternal parent. But the lack of over-arching simple patterns indicates that they are not simply controlled, so the full implications are yet to be fully understood. We have tentatively mapped the *Se* locus and are now running independent experiment to confirm the putative location and initiate high-resolution mapping.

Gamet formation without meiosis in Arabidopsis

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Abstract

Apomixis, the formation of asexual seeds in plants, leads to populations that are genetically uniform maternal clones. Transfer of apomixis to crop plants holds great promise in plant breeding for fixation of heterozygosity and hybrid vigour as it would allow propagation of hybrids over successive generations. Apomixis involves production of unreduced (diploid) female gametes that retain the genotype of the parent plant (apomeiosis), followed by parthenogenetic development of the egg cell into an embryo, and functional endosperm formation. The molecular mechanisms underlying apomixis are unknown. We have found that mutation of the Arabidopsis gene *DYAD/SWITCH1* (*SWI1*), a regulator of meiotic chromosome organization, leads to apomeiosis. We found that the majority of fertile ovules in *dyad* plants form seeds that are triploid and which arise from fertilization of an unreduced female gamete by a haploid male gamete. The unreduced female gametes fully retain parental heterozygosity across the genome, characteristic of apomeiosis. Our results demonstrate that alteration of a single gene in a sexual plant can bring about functional apomeiosis, a major component of apomixis.

Ovules of apomictic Boechera suppress maleness but invest precociously in filial development - typical behaviors for apomictic eukaryotes

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Asexual reproduction by way of genetically unreduced spore, gamete or gametophyte formation followed by parthenogenesis (apomixis) is a well established mode of reproduction among eukaryotes. In fact, it occurs in all major phylogenetic clades of eukaryotes where sex predominates. Interestingly, the mechanisms of apomeiosis, like meiosis itself, are conserved across eukaryotes. This suggests apomixis may be as ancient as meiosis, i.e. it may, along with meiosis, be a hallmark of eukaryogenesis. If so, naturally occurring apomixis may be more complicated than previously imagined, i.e. it may well parallel sexual reproduction in complexity with regard to required interdependently functioning gene networks. Such networks are thought to have evolved over hundreds of millions of years of eukaryogenesis at the single cell level. If this is correct, gene networks responsible for apomictic reproduction are likely evolutionarily, genomically and molecularly intertwined with gene networks responsible for sexual reproduction, and these various gene networks may, to a greater or lesser extent, be retained within the genomes of extant eukaryotes.

To examine this possibility, we compared transcriptomes of over 7000 micro-excised ovules from one sexual *Boechera* (Brassicaceae) species with transcriptomes from 14,000 micro-excised ovules from two apomictic *Boechera* species. Ovules were excised and comparisons made at the megasporocyte, megasporogenic and megagametogenic stages. Using ATH1 (*Arabidopsis thaliana*) GeneChip® (Affymetrix) cross species gene profiling, we observed 2750 differentially expressed genes between dissected ovules of apomictic versus sexual *Boechera*. Interestingly, in these female tissues, 37 genes associated with male gametophyte development and function (all of them) were down regulated in apomictic ovules (68-fold higher expression levels, on average, were observed for these genes in ovules from sexual *Boechera*). In contrast, MADS box genes associated with flowering and seed development and other classes of flowering and seed formation genes (gene networks responsible for producing the next filial generation) were precociously expressed in ovules of the two apomictic species. Also, numbers of genes differentiating sexual from apomictic ovules, based on relative expression, increased 5-fold from

the meiosis/apomeiosis stage to the early embryo sac formation stage, which indicates genes responsible for apomixis and sexual programs diverge extensively – both being complicated developmental programs. By the later stage, 2.5 times as many genes were down-regulated in the apomict than in the sexual.

In summary, the entire flower structure of apomictic *Boechera*, not just the germline, appears to be heterochronically reprogrammed to precociously produce the next filial generation but with meiosis, fertilization and other sex-related processes largely abandoned. This is consistent with the hypothesis presented here that apomixis is of ancient origin and that the gene networks responsible for apomixis are evolutionarily, genomically and molecularly intertwined with networks responsible for sexual reproduction and mitosis. The de-emphasis of male function and the precocious formation of the next filial generation are common features of many apomictic eukaryotes. Apomictic aphids are particularly interesting in this respect in that precocious filial development involves up to three generations of live-birth apomictic aphids within a single mother aphid. Similar examples exist among apomictic fungi, jellyfish, flatworms, flukes, roundworms, nematodes, rotifers, snails, worms, brine shrimp, insects, fish, lizards, slime molds, paramecia, diatoms, brown, red and green algae and ferns.

Genetic and molecular characterization of apospory in *Paspalum sp.*

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The genus *Paspalum* comprises numerous grass species that are important forage resources for the tropical and subtropical regions of the Americas. Ploidy levels within the genus range from diploid to 16-ploid, with modes of reproduction from allogamy to apomixis. An important proportion of *Paspalum* species form agamic complexes, where diploid sexual self-incompatible cytotypes, have pseudogamous, self-compatible apomictic polyploid counterparts. Apospory is the more frequent type of gametophytic apomixis in the genus: megagametophytes develop directly

from sporophytic nucelar cells (2n) and show diverse structural organizatior The aposporous embryo sac configuration usually comprise: the egg cell and a large central cell bearing two polar nuclei, and may include one or two synergids but antipodal cell are usually absent. The parthenogenetic development of the embryo from the egg cell, and the formation of the endosperm after the fertilization of the polar nuclei (pseudogamy) complete seed formation. Apospory and meiotic embryo sacs can occur simultaneously in the same plant and even in the same ovule. Gametophyti apomixis tends to occur in polyploids, and then most often at the tetraploid or greater levels.

Over the last 10 years our group has worked on the characterization of apomixis in the genus *Paspalum* mainly focussing on: 1) the mode of inheritance of apospory in tetraploid *P. notatum* and the identification of molecular markers linked to the trait, 2) the generation of a genetic map of the species and the characterization of the linkage group carrying the apospory locus, 3) the detection of genes and pathways involved in the aposporous and meiotic embryo sac development in *P. notatum* and 4) the study of the functionality of apomixis components at the diploid level in *P. rufum*. Inheritance of apospory in *P. notatum* was investigated by using the tetraploid (2n=4x=40) genotypes Q4188 (a fully sexual plant used as pistilate parent) and Q4117 (a natural obligate apomictic plant employed as pollen donor). Genetic analysis of F_1 progenies and several segregating populations derived from it indicated that the trait is controlled by a single dominant locus with a distorted segregation ratio, possibly due to a pleiotropic partial lethal effect, or a partial linkage to a lethal factor. Pollen viability determinations revealed a significantly higher level of non-viable pollen in the aposporous genotype Q4117 than in the sexual Q4188 and analysis of male meiosis of both genotypes showed that Q4117 could present a genomic rearrangement. A full genetic linkage map of the species was developed based on an F_1 family derived from the same parental plants using single dose AFLP markers. In general, polysomic inheritance was observed for most markers, but the linked group carrying apospory showed preferential chromosome pairing and a strong restriction in recombination. Several AFLP markers 100% linked to apospory were detected. Results obtained so far denote apospory in *P. notatum* is controlled by a complex locus spanning a large non-recombinant chromosomal segment, which includes non-coding sequences, retroelement and cytosine methylation. The apospory region in *P. notatum* is related to rice chromosomes 2 and 12. Transcriptome surveys aimed at the identification of genes differentially expressed in aposporous and sexual genotypes of *P. notatum* allowed isolation of 65 candidates, out of which two thirds (45) could be successfully annotated. Several of them were

identical to those related to apospory in *Poa pratensis*. Sequences belong to signal transduction (including several members of an ERK cascade), transcription, proteolysis, cell cycle control, cell wall composition and repetitive elements functional classes. A subgroup of candidates silenced in aposporous plants mapped close to apo-region in *P. notatum*. Curiously, RNA *in situ* tissue hybridization showed that many of the genes are differentially expressed not only in the ovule but also in other tissues like the tapetum of anthers and pollen mother cells. On the other hand, the previous observation of ovules containing an aposporous embryo sac besides the meiotic one in the diploid genotype of *P. rufum* Q3754, suggested that at least some species have the potential for apomictic reproduction at the diploid level. Progeny tests carried out on 2 experimental families derived from a controlled cross (H_1) and the induced self-pollination of Q3754 (S_1) showed that all progenies of H_1 derived from sexual reproduction (n + n), but 5 out of 95 plants from S_1 were of clonal origin (2n + 0). Further experiments, carried out on a third family (H_1) obtained after crossing Q3754 with the tetraploid plant Q3785 showed diploids (75 %), triploids (20 %) and tetraploid (5 %) progenies. Triploids and the tetraploids may have originated from functional aposporous embryo sacs. Likewise, the reconstruction of the developmental route of individual seeds demonstrated that 27.5 % of them derived from fertilized aposporous embryo sacs (2n + n). These results indicate that components of gametophytic apomixis are effectively expressed at the diploid level in *P. rufum* and could be the foundation of a recurrent auto-polyploidization process in the species.

Apomixis in a tropical forage grass - *Brachiaria*

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In Brazil most of the cattle is raised under pasture conditions with around 196 million heads distributed in an area of approximately 259 million hectares, 115 million covered mostly by two apomictic cultivars of *Brachiaria: B. brizantha* cv. Marandu and *B. decumbens* cv. Basilisk. Due

to this low genetic variability, there is an important concern on these cultures mainly because of apomixis. Therefore, studies on the molecular mechanisms involved on the reproductive mode of *Brachiaria* aiming the knowledge of apomixis is conducted by our group. Genetic studies suggested that apomixis is a dominant factor controlled by one Mendelian locus. Apomixis is of the aposporic type, pollen is viable and pseudogamy occurs, i.e. endosperm is a triploid tissue resulting from the fertilization of the central cell while the development of the embryo is autonomous. Comparing to apomictic plants, the seed production of sexual plants of *B. brizantha* is limited by a massive abortion of developing caryopsis, most likely associated with early inbreeding depression. At the molecular level, cDNA sequences from ovaries show differential expression in sexual and apomictic plants and among them, some have evident expression in the synergids, which could be involved with the fertilization and autonomous development of unreduced egg cell. To test the function of candidate genes for apomixis, in vitro regeneration methods using meristem and cell suspension of *Brachiaria* were established allowing the improvement of transformation protocols by biolistics. Advances towards obtaining new intraspecific lines were achieved with evidence of compatibility of *B. brizantha* induced tetraploid sexual and natural tetraploid, apomictic plants. A segregant population of *B.humidicola* for apomixis was recently obtained and RAPD analysis is being performed, to confirm hybrids and genetic mapping is on the way with candidate genes for apomixis of other species and SSR markers.

Containment of transgenes by maternal inheritance or cytoplasmic male sterility engineered via the chloroplast genome

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Maternal inheritance or cytoplasmic male sterility is a promising approach for transgene containment via chloroplast genetic engineering, with added advantages of high levels of transgene expression (up to 47% of total leaf protein), rapid multigene engineering, lack of position effect, gene silencing and pleiotropic effects. Currently, chloroplast genetic engineering has been utilized widely in tobacco, a non-food/feed crop as a bioreactor for production of amino acids, biopharmaceuticals, biopolymers or industrial enzymes. Several other crop chloroplast genomes have been transformed via organogenesis (cauliflower, cabbage, lettuce, oilseed rape, petunia, poplar, potato, tomato) or embryogenesis (carrot, cotton, rice, soybean) and maternal inheritance of transgenes have been observed. Several crop species have stably integrated transgenes conferring agronomic traits including herbicide, insect and disease resistance, drought and salt tolerance, and phytoremediation. Chloroplast transgenic carrot plants withstand salt concentrations that only halophytes could tolerate. Insecticidal proteins engineered via the chloroplast genome killed insects that had developed 40,000 – fold resistance against that protein. Forty sequenced crop chloroplast genomes provide information on plastid genome regulatory sequences and spacer regions for transgene integration.

Chloroplast-derived biopharmaceutical proteins including insulin, interferons and somatotropin have been evaluated by *in vitro* studies. Human interferon alpha 2B transplastomic plants have been evaluated in field studies. Chloroplast-derived vaccine antigens against bacterial (cholera, tetanus, anthrax, plague, Lyme disease), viral (canine parvovirus, rotavirus, etc) and protozoan (amoeba) pathogens have been evaluated by immune responses, neutralizing antibodies and pathogen or toxin challenge in animals. Oral delivery of proinsulin offered protection against development of insulitis (diabetes) in mice; such delivery eliminates expensive fermentation, purification, low temperature storage and transportation. Demonstration of these biotechnology applications bode well for commercial development utilizing this new platform technology.

Evaluation of an apomictic genotype of *Brachiaria brizantha* leading to cultivar release and protection

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The evaluation process leading to cultivar development of a forage grass is a long-term investment, which requires a multidisciplinary team. Forage plants are only valuable when transformed into high quality protein such as milk, meat, leather or hide. Therefore indirect measures of quantity and quality of the forage need to be undertaken starting with plot evaluatio under a cutting regime all the way to animal performance trials, in pastures under grazing. Not before 8-10 years is needed to confirm the usefulness and advantages of the candidate genotype. Apomixis adds an interesting aspect to forage cultivar development for at one hand, it simplifies seed multiplication and yields uniform pastures which are easier to manage, but on the other hand, impairs recombination of useful traits by hybridization if a sexual compatible genotype is not available. Apomixis is never obligate in most useful forage species, therefore there is always the possibility of the residual sexuality yielding some hybrid genotypes in the progeny. *Brachiaria* reproduces predominantly by apomixis of the Panicum type and is pseudogamous, thus the embryo is always a hybrid tissue. The path to cultivar development of BRS Piatã, the first *Brachiaria* cultivar protected by Embrapa, started in 1988 by agronomic trials in plots, established by cuttings since seed was not available and mode of reproduction had not been established at the time. Seed multiplication followed so that regional trial could be carried out between 1994 and 1997. Animal trials to study the effect of the animal on the pasture were started in 1997, in 1000 m paddocks replicated twice. To determine animal performance on BRS Piatã, 2-hectare pastures in two replicates were established and measurements were taken for three years. For over 10 years breeder's seed was multiplied from the most vigorous plants in four different areas, and later, distributed for basic seed production. Twenty years of evaluation and selection were enough to produce differences picked up with molecular markers, between the original plants and those derived from breeder's certified seed. Possible inferences will be discussed at the conference.

Identification of over expressed and down-regulated genes during arabidopsis meiosis and microsporogenesis

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In Eukaryotes, meiosis is a key function of sexual reproduction, a complex and specialized process of cell division that results in haploid cells (e.g; gametes).

In recent years, the availability of Arabidopsis genome sequence, the development of cytological approaches for this species and the combination of forward and reverse genetic approaches have allowed an important progress in the identification of meiotic genes in plants.

Nevertheless identification of a larger number of meiotic genes remains a challenge due to problems of sequence conservation among species and functional redundancy between gene family members.

Hence we have developed a transcriptomic-based approach comparing isolated Arabidopsis male meiocyte mRNAs to different plant organs to enable the identification of a greater range of plant genes involved in meiosis and microsporogenesis.

From transcriptome data, only 2% of total gene number (22144) are significantly expressed and 0.1% are down-regulated during meiosis pathway. Using Arabidopsis T-DNA knock-out mutants, we will confirm the role of candidate genes for meiosis and microsporogenesis in plants.

PART III - ABSTRACTS

Floral development in *Vriesea carinata* Wawra (Bromeliaceae)

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The study of reproductive aspects in different species, including the formation of floral structures, such as anthers and ovules, as well as the developmental events for the formation of the embryo and endosperm can contribute to the understanding of the relations between the several taxa and their evolutionary trends over time. In environments that have been devastated frequently, those approaches can show clues or even evidences of how the species have been adapting to their own survival. In Bromeliacea the presence of two distinct strategies of reproduction is already known – sexual and clonal. The clonal propagation is an important strategy for the spreading of species and, is typical in members of this family. The sexual reproduction allows new genetic recombination, generating more variability in a given population. Because of new approaches and technologies, the morphological characters used to reconstruct phylogenies have been reevaluated and new ones have been introduced. This work has the objectiv of studying the floral development in *Vriesea carinata* Wawra with the aim to contribute with information regarding the reproductive process of this species. The material was fixed in 1% glutaraldehyde and 4% formaldehyde treated with 0.1M sodium phosphate buffer pH 7.2, dehydrated in an ethylic series and included in hydroxyethylmetacrylate. Slices of 2 to 4 ìm of thickness were prepared and stained with 0.05% Toluidine Blue O pH 4.4. The observations and the analogical photomicrographs were made using the Leica DMR – HC and Olympus BX41 microscopes, using Kodak ProImage ASA100 films. The *Vriesea carinata* ovule is originated from a trizonate primordium and anatropous, crassinucellated and bitegmic at maturity. The trizonate character is common for several families of angiosperms (Bouman 1984), however, there is a lack of studies in Bromeliaceae concerning the initial of the ovule development, except for *Dyckia pseudococcinea* L. B. Smith. (Conceição *et al.* 2007). Also, crassinucellates ovules occur in other species of this family (Conceição *et al.* 2007, Lakshmanan 1967, Palací *et al.* 2004 e Rao & Wee 1979). The

megasporogenesis of this species generates a linear tetrad, where only the calazal megaspore is functional. The determination of the functional megaspore involves cytological events as the presence of callose in the cell wall. The female gametophyte is of monosporic origin, and consists of seven cells and eight nuclei. The development of the parietal layers of the anther is of Basic type, according to the classification proposed by Davis (1966). However, in other Bromeliaceae studied before, it was found the monocotiledoneous type (Sajo *et al.* 2005). The sporangia wall, at maturity, is composed of epidermis and endothecium. The microsporogenesis is successive and results in isobilateral and decussate tetrads. This sporogenesis type is not only common in species of Bromeliaceae family, but also in monocotiledoneous (Dahlgren *et al.* 1985). The pollen grain is released in the bicellular stage. When the pollen grains reach the stigma, they hydrate and the pollen tube emerge. The pollen tube grows inside the long empty style and penetrates the ovule through the micropile, characterizing the porogamic fertilization type. The style can be hollow or solid (Van Went & Willemse 1984), the first one usually found in monocotiledons and the last found in eudicotyledons plants. The pollen tube penetrates one of the sinergids while the other remains intact. One of the male gametes fertilizes the middle cell, forming the endosperm primary nucleus, auxiliary generation that originates the xenophytic embryo. The other gamete fertilizes the egg cell, resulting in the zygote that will follow the embryogenesis process to form the sporophytic embryo. These events define the double fertilization, which was determined in the 19th century (Batygina 2006). These results are compatible with a sexual reproduction strategy of *Vriesea carinata*.

Alternative splicing and circadian expression pattern analyses of a pistil-specific methyltransferase gene from *Nicotiana tabacum* L.

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To identify genes specifically or predominantly expressed in tobacco pistils, a differential screening of a *N. tabacum* stigma/style cDNA library was performed. This procedure allowed the isolation of the PA3 cDNA

clone. This clone was sequenced and shown to encode a protein highly similar to benzoic acid/salicylic acid/jasmonic acid methyltransferases. Thes enzymes are responsible for the formation of the corresponding volatile esters that are reported to be involved in plant developmental processes, attraction of pollinators and/or plant defense. Northern blot analysis using the PA3 cDNA as a probe, on membranes containing total RNA extracted from each of the vegetative and reproductive organs, elicited hybridization to four bands of approximately 1.3, 1.1, 0.9 and 0.6 kb exclusively present in stigmas/styles and ovaries. In order to identify these additional transcripts, we searched 3 stigmas/styles cDNA databases constructed in our laboratory (TOBEST, SSH1 and SSH2) and 28 clones were identified, from which two were identical to PA3. Analyses of these clones and further RT-PCR experiments suggested that a precursor mRNA undergoes alternative splicing to produce 9 different transcripts by differential use of splice sites. 5'and 3' RACE experiments demonstrate that all transcripts start with the same exon (exon 1), however they greatly differ in terms of the composition of the other exons. The analyses of all the cDNA clones we have obtained so far suggest that the pistil-specific methyltransferase gene contains 8 putative exons. In an attempt to establish the function of the pistil-specific methyltransferases in the tobacco reproductive biology, we have obtained RNAi transgenic plants. Despite the fact that some of the plants had very low level of methyltransferase transcripts, they were all apparently normal plants that reproduced and had a seed production undistinguishable from the control plants. However, it has to be mentioned that if the floral volatiles have a long distance effect, it would be difficult to observe phenotypes in our experimental greenhouse conditions, in which RNAi and control plants were cultivated in the same environment.

In a parallel approach to study the function of these methyltransferases, we have captured the volatiles emitted by mature *N. tabacum* flowers, at different hours during the day and performed an analysis by GC-MS (Avanci et al., in preparation). The results have demonstrated the emission of methyljasmonate, which peaks in the mornin (at 10 AM). Thus, in order to get a better understanding of the methyltransferase expression pattern during the day/night cycle, we decide to investigate its transcript level at different hours of the day. We have quantified the mRNAs containing only the exons 1, 2, 3 and 4 in stigmas/ styles and ovaries from young (stages 1 and 2) and mature flowers (stages 11 and 12) collected at 10 AM, 4 PM, 10 PM and 4 AM. The transcript level on stigmas/styles was higher at dawn (4 AM) at both young and mature flowers. On the ovary, however, the expression pattern had two peaks per 24 hours: at 10 AM and 10 PM. These day/night variations could be correlated with the time of pollinators visit. However,

N. tabacum is a self-pollinated species that does not depend on pollinators. Thus, it is possible that the pistil-specific methyltransferase gene is a relic trait from the transition of an outcrossing ancestor to a self-fertilization species. On the other hand, the complex process of alternative splicing to produce several pistil-specific methyltransferase transcripts, maintaining the same open reading frame and capable of translating proteins, has not been disrupted during evolution. Therefore, it is expected that these methyltransferases have a current and important function in the *N. tabacum* reproductive biology that will be further investigated.

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Floral antomy of *Croton* L.: source of apomorphies for a giant genus?

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Euphorbiaceae Juss. is widespread in the Brazilian flora. Numerous molecular analyses split the group into five monophyletic families positioned relatively close in Malpighiales. From these, Euphorbiaceae *s.s.* remains one of the largest among the angiosperms, comprising nine different lineages. Infrafamilial studies pointed to a polyphyletic condition in subfamily *Crotonoideae s.l.*, with four lineages, including that of inaperturate crotonoids to which belongs the genus *Croton* L. Studies of the phylogenetic relationships in this group recommended a review of the sectional approach. Meaning to contribute to the knowledge of the relationships between *Croton* species, floral anatomy and development of *C. aff. celtidifolius* Baill, member of the section *Cyclostigma* Griseb., were studied. It is a monoecious tree, up to 10m high, with yellow latex and native of the Atlantic Rain Forest. The inflorescences are terminal racemes of dichasia, the basal ones presenting one pistillate and two staminate flowers.

Flowers are pentamerous and the perianth is differentiated into sepals and petals. Staminate flowers present an androecium with 50 fertile stamens. Pistillate flowers have a gynoecium with multifid styles.. The periphery of the receptacle is differentiated in a slightly lobed nectariferous disc with a starch rich epidermis with stomata and a secretory parenchyma. Collateral bundles derived from the floral pedicel irrigates this parenchyma. Laticiferous ducts are located near the vascular tissue. The pollen grains are large, apolar, spheroidal and inaperturate with exine displaying the

croton pattern of ornamentation. The ovary is syncarpous, tricarpellate, trilocular, with one ovule per locule. Ovules are anatropous, crassinucellate bitegmic, the inner integument being vascularized. The colateral bundle of the funiculus divides at the calaza giving rise to two small ones located in each side of the inner integument almost reaching the micropyle.

The structure of ovules and seeds, especially the vascularization pattern, are one of characters employed to the distinction of Euphorborbiaceae *s.l.* subfamilies. It was also considered in the elevation of these taxa to the category of families. The absence of vasculature in the outer integument was considered a homoplasy to Euphorbiaceae *s.s.* while in the inner one represents a synapomorphy for the crotonoid lineage There are no records on floral anatomy of *Croton* so any synapomorphy that might arise in this context will be a novelty and should contribute to understand the relationships of this group.

Eugenia uniflora reproduction: exception or rule?

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Eugenia s.s. is one of the biggest and most representative of the 130 genera of Myrtaceae, occurring in South and Central America, with ca. 60 african species. There are few data on flower anatomy and palynology regarding the genus and virtually no information related to *Eugenia uniflora* L., a perennial shrub or tree with a furrowed fruit, unusual in the group.

Seven individuals of *E. uniflora* cultivated in the Hortus Botanicus of Museu Nacional/Universidade Federal do Rio de Janeiro were analyzed from the anatomical and palynological point of view. Inflorescences, buds and flowers were fixed in formaldehyde 4% + glutaraldehyde 2.5% in sodium phosphate buffer 0,05M pH 7.2, dehydrated in ethanol series and embedded in Historesin® (Leica). Serial sections 1-3mm thick were obtaine with glass knives in rotatory microtome and stained with Toluidine Blue. Pollen grains were acetolised and measured. The electromicrographs were taken from non acetolised pollen.

The flowers are born in racemose inflorescences whose meristem is protected by several layers of bracts with numerous trichomes and long

coleters. The vasculature of the flower is composed by bundles originated from the fragmentation of the vascular ring from the peduncle that bifurcates in the ovary region. Internal branches from each bundle redirect to the center of the bud, gather on the top of the ovary and then undergo an inversion penetrating in the septum, which has a mixed origin due to style depression and projection of the floral base. The inferous ovary is of receptacular type. The ovules are anacampilotropous, unitegmic and crassinucellated with polygonum embryo sacs. Microsporogenesis is simultaneous and concurrent with megasporogenesis. Pollen grains are parasincolporated, what does not corroborate the sinapomorphy assigned to Vochysiaceae and Myrtaceae.

We have observed variations in sporogenesis and gametogenesis in three individuals. Pollen grains with extra numerical cells as well as diversity in size, shape and content indicated the occurrence *in planta* of a process similar to *in vitro* androgenesis. Vestigial female tetrads and nucelli with two or no embryo sacs were also documented. Embryo development begins 48 hours after pollination. In 72 hours a globular embryo and a nuclear endosperm are observed as well as several cellular masses of nucellar origin. Some of these cells undergo unequal division and further develop in a pluricellular structure with differentiated protoderm, interpreted as an embryo of nucellar origin.

The results are discussed in a functional and phylogenetic perspective.

Subcellular localization of two developmentally regulated pistil-specific methyltransferase sequences of *Nicotiana tabacum* L.

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Different organs have distinct gene expression profiles, including the pistil – the angiosperm female reproductive organ. The differential screening of a *Nicotiana tabacum* stigmas/styles cDNA library has resulted in the isolation of the PA3 cDNA clone and the identification of a new putative pistil-specific gene. The Blast analysis of the PA3 cDNA sequence at the NCBI site showed high similarity to SAM-dependent

methyltransferases. These enzymes are associated with the methylation process of benzoic, salicylic and/or jasmonic acids. The derived methyl-esters of these acids are involved in many important plant processes, as pathogen defense, pollinator attraction, plant development and plant-plant signaling. A northern blot with total RNA from different *N. tabacum* sterile organs (leaves, roots, stem, sepals and petals) and reproductive organs (anthers, stigmas/styles and ovaries) using the PA3 cDNA as probe, has demonstrated the existence of 4 pistil-specific bands. Analysis by northern blot, with total RNA from stigmas/styles and ovaries from the 12 tobacco flower developmental stages, has shown lower expression levels at the beginning of flower development which increase toward anthesis, revealing the developmental regulation of the methyltransferase transcripts. *In situ* hybridization experiments, using the PA3 sequence as a probe, in longitudinal sections of stigma/style have shown the expression in the stigmatic secretory zone and stylar transmitting tissue, the tissues traverse by the pollen tubes during their growth toward the ovary. In ovary transversal sections, the hibridization signals were detected in the vascular tissue and in the ovules, but not in the layer around the megaspore mother cell and embryo sac. Additional experiments performed in our laboratory suggest that the different transcripts detected in the northern blot experiments are the result of an alternative splicing process (Calixto et al., in preparation). A search performed in our 3 stigmas/styles cDNA databases (TOBEST, TOBSH1 and TOBSH2) has allowed the identification of 28 clones encoding the methyltransferase, from which only two were identical to PA3. Among them, there were full-length cDNA clones with different combination of exons and consequently, partially different amino acid sequences. To examine the possibility that the different methyltransferase sequences are targeted to different subcellular localizations, we have constructed chimeric genes in which the GFP was fused to the N-terminal or the C-terminal of two different methyltransferase sequences (PA3 and 46B11). The 4 constructions were introduced separately in SR1 tobacco plants by *Agrobacterium tumefasciens*-mediated leaf disc transformation. The preliminary analysis, through confocal microscopy, performed in leaves and roots of the transgenic plants, indicate a cytosolic localization for both PA3 and 46B11 encoded proteins. The experiments will be completed with their subcellular localization in the stigmas/styles of the transgenic plants, in which it will be interesting to investigate if the interaction with other pistil-specific proteins will altered their final subcellular localization.

Financial Support: FAPESP, CNPq and CAPES.

ORAL CONTRIBUTION

Functional analysis of two B3 (REM family) genes expressed at early stages of stamen development in *Arabidopsis thaliana*

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The ABCE model of floral organ identity proposed for all eudicots has revealed that each whorl is determined by combinatorial floral homeotic proteins. It has been showed that genes from B + C + E classes are responsible for stamen development. Recently, global analysis of gene expression between floral buds of wild-type and *apetala-3* mutant of *Arabidopsis* identified 62 genes expressed exclusively at early steps of stamen development. Among them, two B3 DNA binding genes showed a very specific spatial expression pattern during early stages of stamen development. To study the function of these REM 22 and REM 26 B3 genes, we have conducted different experiments such as over expression, RNAi gene silencing and sub-cellular localization of REM 22 and 26. Moreover we are studying the expression pattern by promoter fusion with GUS and GFP reporters genes. Plants over expressing these two genes do not showed any phenotype at tested growing conditions. Agroinfiltration of *Nicotiana benthamiana* with 35S:REM22:GFP indicated that it is localized in the nucleous. Plant containing RNAi constructs showed abnormal phenotype – they are not able to produce a shoot apical meristem. The confocal analysis of plants containing upstream regulatory region of REM 22 fused with GFP revealed the same expression pattern observed by in situ hybridization, their expression is restricted to cells of stamen primordial at stages 5 to 9 stages of flower development. These data showed, at least, for REM 22 can bind the DNA and can be responsible for the beginning of early stages of stamen development.

Instructions for abstracts and lectures

- Abstract should contain one page not exceeding two; A4 format (21 × 29.7 cm) with margins of 2.5 cm.
- Text should be typed in Times New Roman font and organized as:
  - Title: bold and font size 14. One line blank beneath the title.
  - Authors: plain and font size 12.
  - Filiations: plain and font size 12. Insert numbers for different institutions. One line blank beneath filiations.

- The text is plain and font size 12; 1.5 – spaced and justified. Latin binomials should be in italics.
- Please do not insert figures, tables and/or graphics.
- The abstract should be saved as a Microsoft Word (version 5.x or 6.x).

Characterization of gene encoding a small nuclear peptide specifically expressed in reproductive organs of *Nicotiana tabacum* L.

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The success of plant reproduction depends on the roles of the pistil in receiving and discriminating pollen grains, providing the appropriate conditions for pollen tube growth, as well as producing the female gametophytes. To fulfill these roles the pistil should have an appropriate development that is probably dependent on the proper expression of pistil-preferential or specific genes. To identify genes preferentially expressed in the *Nicotiana tabacum* pistils, we have performed a macroarray analysis with 782 clones of a stigma/style cDNA library (TOBEST project), which revealed 46 putative pistil-preferential genes. One of these genes, corresponding to the clone TOBR092H06, is here characterized. The TOBR092H06 is a full-length clone, encoding a small protein (68 amino acids), with similarity to hypothetical proteins of unknown function. Real time RT-PCR analysis has shown that this gene is specifically expressed in stigmas/styles, ovaries and stamens, with the highest expression level in stigmas/styles. This gene is developmentally regulated in stigma/styles and it is already expressed at very early flower developmental stages, before complete pistil differentiation, as shown by real time RT-PCR experiments. *In situ* hybridization experiments in longitudinal sections of stigmas/styles have shown that the 092H06 gene is expressed in the stigmatic secretory zone and the stylar transmitting tissue. To study the subcellular localization of the 092H06 protein, we have performed N-terminal and C-terminal GFP fusions. The analysis through confocal microscopy in transiently transformed tobacco cells has demonstrated a

clear nuclear localization, with homogenous distribution in the nucleoplasm, for both constructions. In an attempt to reveal the function of the 092H06 protein, transgenic tobacco plants containing RNAi or overexpression 092H06 constructions were obtained. Three out of five RNAi transgenic plants showed stigmas and styles with larger diameters, as well as larger ovaries and longer anthers. The fruits produced by these plants were also larger and bigger. These plants had low level of 092H06 transcripts in stigmas/styles and ovaries. Surprisingly, in one of the RNAi transgenic plants without phenotype the level of 092H06 transcripts in ovaries was higher than in control plants. Higher 092H06 transcript levels were also observed in stamens of four out of five of these RNAi transgenic plants. The RNAi9.3 plant, with the highest 092H06 transcript level in stamens, was male-sterile. The RNAi9.3 pollen grains were incapable of producing fruits after controlled self-pollination or after pollination of SR1 control pistils. Conversely, the RNAi9.3 pistils produced fruits after pollination with SR1 control pollen grains. The RNAi9.3 pollen grains were assayed *in vivo* and *in vitro* and have demonstrated they are unable to geminate and emit pollen tubes. On the other hand, cosuppression was observed in stigmas/styles of all 6 overexpression (Ovex) transgenic plants, in which 092H06 transcript levels were lower than in controls and a different phenotype appeared: stigmas positioned above anthers and petal tips, but with stigmas and styles of approximately normal diameter. In four out of six Ovex plants the 092H06 transcript levels in ovaries were lower than in control plants, but there was no observable ovary phenotype in all of these transgenic plants. In all Ovex transgenic plants, the anther 092H06 transcript levels were higher than in control plants and no phenotype was observed. These apparently contradictory results may be the consequence of a yet unknown feedback mechanism to regulate 092H06 gene expression and function. The small size of this protein, its nuclear localization and the presence of a putative amidation site, characteristic of peptide hormones, suggest that the 092H06 protein may have a role in a signal transduction pathway important in developmental processes. To shed some light in 092H06 function, experiments to express this protein in *E. coli*, to obtain antibodies and to test its interaction with other pistil proteins are in progress.

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A novel stigma/style-specific gene, SCI1, encodes a lysine-rich protein that controls cell division and differentiation

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The success of plant reproduction depends on the appropriate development of the reproductive organs that involve specific regulatory networks. Generally, organ-specific genes are key players of such processes. To better understand the *N. tabacum* pistil development, we have undertaken the characterization of a gene (clone HS1002E06), identified in a stigma/style subtracted cDNA library. This gene is homologou to an *Arabidopsis* gene with unknown function. It encodes a small lysine-rich protein which contains a putative nuclear localization signal, a putative cyclin interaction domain and 15 predicted phosphorylation sites (NetPhos, e”96%). Real time RT-PCR experiments confirmed its stigma/style-specific expression pattern and demonstrated its developmental regulation. The highest transcript levels occur at the very early stages of flower developme in which the stigma/style (S/S) is differentiating. The *in situ* hybridization revealed expression at the specialized tissues of the S/S (stigmatic secretor zone – SSZ – and stylar transmitting tract – STT). The RNAi-mediated silencing of this gene resulted in transgenic plants exhibiting remarkably longer styles and an enlarged stigma area. Additionally, the overexpression transgenic plants had stigmas with smaller area. Transversal sections of the mature S/S clearly showed that this change in size occurs as a consequence of the disparity in cell number that is increased in the speciali tissues of the RNAi plants and decreased in overexpression plants. These results characterize this protein as a negative cell cycle regulator, which we denominated SCI1 (Stigma Cell-cycle Inhibitor 1). Furthermore, in RNAi plants, the larger stigma area was shown to be a consequence of premature cell division in the upper pistil with a parallel premature differentiation of the papillary cells, as observed by scanning electron microscopy (SEM). In overexpression plants, the opposite phenotype was observed, with a delay of cell division and papillary cells differentiation. These results indicate that papillary cells differentiation is coupled to stigma cells division, representing a cross-talk between growth and differentiation, which is intrinsic to pistil development. This observation is consistent with a role of SCI1 in triggering differentiation through cell proliferation

control. Complementarily, the SCI1-GFP fusion protein is targeted to the nucleus and stays compartmentalized in nuclear bodies but not in the nucleolus. Based on the phenotypic similarity between our RNAi transgenic pistils and the *Arabidopsis* pistils treated with an inhibitor of auxin polar transport (NPA), we decided to measure three auxin related genes, ARF8, Aux/IAA13 and Aux/IAA19, in four independent RNAi and overexpression plants using real time RT-PCR. All three genes were significantly altered, up to 5.1 fold for Aux/IAA19, showing that SCI1 influences the transcriptional regulation of some early auxin responsive genes. Thus, we have identified a developmentally regulated stigma/style-specific gene encoding a protein that leads to alterations in cell division and differentiation in a tissue specific manner. The SCI1 protein probably acts as a component of the nuclear signal transduction pathway and may have interconnections with the auxin pathway, providing molecular evidences for a bridge between cell proliferation/differentiation and hormone signaling.

Keywords: pistil development, lysine-rich protein, stigma size, cell division.

Financial support: FAPESP, CNPq, CAPES, FAEPA/FMRP.

Floral development of Brazilian species of *Indigofera* L. (Leguminosae-Papilionoideae)

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Floral and inflorescence development was carried out at *Indigofera lespedezioides*, *I. spicata* and *I. suffruticosa*, legume species that are considered here as models for the tribe Indigofereae. Inflorescences and flower buds of all sizes and ages were collected, dissected and prepared for observations in scanning electron microscope (surface analysis) and photomicroscope (anatomical analysis). All of the species have a convex dome floral apex subtended by one bract. Organogeny: Sepals and stamens are initiated in unidirectional order, from the abaxial side. In *I. suffruticosa*, there is an overlapping in initiation between antesepalous stamens and petals. The carpel primordium and cleft carpel are precocious. Carpel initiates by the time all the sepal primordia have initiated. The ovule initiation takes place before the cleft carpel has closed. The primordia of all organs are

usually initiated by periclinal cell divisions in the second layer (L2) of the floral apex. Exceptions are observed in *I. suffruticosa*, in which antesepalous stamens initiate in deeper layers (L3 and L4), and carpel initiates by periclir and anticlinal cell divisions in L2 and L3. Besides, in *I. suffruticosa* and *I. lespedezioides* the ovule primordia originate in the surface layer (L1). Mid and late stages of development: The five sepals fuse basally, the filaments fuse to form a diadelphous tube and, later, the carpel margin fusion takes place. The petals differentiate in vexillum, wings and keels, the style curves abaxially and the stigma starts to differentiate. The species of *Indigofera* and other Papilionoideae members share many character states from organography and organogeny. However, it is noteworthy that precocious initiation of the carpel in *Indigofera* is an innovation for the subfamily Papilionoideae. Diagnostic characters were found in the late stage of development, confirming the hierarchical-significance hypothesis. Accordin to this hypothesis, specializations can provide helpful data in delimitation of infra-generic taxa. Few studies have been made of floral development in *Indigofera*. So, most data obtained in this study were not reported before for this large genus (FAPESP).

Retinoblastoma and its Binding Partner MSI1 Control Imprinting in Arabidopsis

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ABSTRACT

Parental genomic imprinting causes preferential expression of one of the two parental alleles. In mammals differential sex-dependent deposition of silencing DNA methylation marks during gametogenesis initiates a new cycle of imprinting. Parental genomic imprinting has been detected in plant and relies on DNA methylation by the methyltransferase MET1. However, in contrast to mammals, plant imprints are created by differential removal of silencing marks during gametogenesis. In Arabidopsis, DNA demethylatio is mediated by the DNA glycosylase DEMETER (DME) causing activation of imprinted genes at the end of female gametogenesis. Based on genetic interactions we show that in addition to DME, the plant homologues of the human Retinoblastoma (Rb) and its binding partner RbAp48 are require for the activation of the imprinted genes FIS2 and FWA. This Rb-dependent activation is mediated by direct transcriptional repression of MET1 during

female gametogenesis. We have thus identified a new mechanism required for imprinting establishment, outlining a new role for the Retinoblastoma pathway, which may be conserved in mammals.

Morpho-histological characterization of flower types in pomegranate

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Pomegranate, *Punica granatum*, is characterized by having two types of flowers in the same tree: hermaphroditic flowers with both male and female parts and functionally male flowers which produce a reduced female part and well developed male parts. This condition, defined as functional andromonoecy, can result in decreased yields due to the inability of functionally male flowers to set fruits. When mature flowers are observed in the field, differences can be observed in the morphology of flowers that indicate their sexual characteristics. Morphological and histological analyses of hermaphroditic and functionally male flowers were done through light and scanning electron microscopy to characterize the different flower types observed in pomegranate plants and to better understand their developmental differences. Hermaphroditic flowers had a discoid stigma with elongated papillae, a single elongated style, and numerous stamens inserted on the inner wall of the calyx tube. High numbers of pollen tubes was observed growing through a stylar canal, using both fluorescent staining and histological sections. Ovules were numerous and elliptical. Functionally male flowers showed different ranges of underdeveloped pistils which were reduced to varying degrees. Stigmas had well developed papillae that supported pollen germination. Pollen tubes were observed only in a few styles, suggesting degeneration of styles or lack of adequate conditions for efficient pollen tube growth. Ovules in male flowers were rudimentary and exhibited various stages of degeneration. Pollen germination studies showed that pollen from both types of flowers have similar size, approximately 20µm, and similar rates of germination.

Passiflora elegans Mast. organelles dynamics during pollen development (Passifloraceae)

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The angiosperms plastidial inheritance generally has been transmitted by cytoplasm organelles of the egg cell after fertilization, with less participation of the male gamete organelles during this process. While pollen develops, certain organelles can be excluded during cell division events. Recent works with molecular markers demonstrated that the *Passiflora* genus presents variations in plastidial inheritance, it can be maternal, paternal or biparental. One the other hand, mitochondrial inheritance is always maternal. Thus, the present study aims to analyze the main stages of development during the pollen formation in *Passiflora elegans*. The material was fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) and postfixed in 2% OsO_4 plus 0.8% $K_3Fe(CN)_6$ in the same buffer, for transmission electron microscopy. The semithin sections were stained with 0.05% Toluidine Blue O, pH 4.4 and the ultrathin were contrasted with uranyl acetate/lead citrate or potassium permanganate/lead citrate. The pollen development is analyzed since the prophase I meiotic phase until generativ cell isolation. Many plastids are observed during all analyzed stages. The plastids show variable shape and size, from spherical to elongate, with differentiated arrangement that looks like"Y" and "U" shape. At the beginning of meiosis is observed a great amount of organelles near cytomit channel region. At tetrad with callose stage there is an increase in the number of organelles, mainly in the cytoplasm periphery. Many endoplasmi reticula are distributed in the cytoplasm, and there is an accumulation of lipids vesicles with electron-dense content. Mitochondria are also found associated with dictyosomes groups, distributed at the cytoplasm cortical region of these cells. After meiosis, and consequent microspore release, not many variations were observed. In this phase, the microspore nucleus is conspicuous and of central position; it has active nucleolus with great nucleoli vacuoles. The plastids are numerous and some contain starch inside. At the beginning of gametogenesis stage, the nuclei are parietal and small vacuoles fusion themselves in the central region. The cytoplasm is limited at cell periphery, and concentrate many mitochondria near to the nucleus. A lot of plastids occur around all cytoplasm and, beside vacuolation

progress, they migrate near to the nucleus. Before mitotic division the nucleus is compressed by the central vacuole, against the sporoderm and, surrounded by mitochondria, plastids and dictyosomes groups. After the mitotic division occurs, the generative cell inherit a lot of organelles incorporated in its cytoplasm, among then, can be highlighted the presence of many plastids, mitochondria, dictyosomes and oil vesicles. The plastids inheritance in angiosperms is mainly maternal. However, molecular works on *Passiflora* genus proposed paternal inheritance for the plastids. The exclusion of plastids does not occur during the stages of pollen formation. So, the obtained morphological data agree with paternal inheritance proposed for the genus.

The anther-specific gene encodes a novel exine-related protein with eight conserved repeats in the microspore of lily anthers

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The anther-specific gene, *LLA-1271* has been isolated at the microspore stage from a lily (*Lilium longiflorum* Thunb.) anther subtractive cDNA library. The anther-specific gene is novel and exists in two forms, *LLA-1271a and LLA-1271b*. It is intriguing that the protein encoded by *LLA-1271* contains eight highly conserved sequence repeats. The LLA-1271 protein is heat-stable and heterogeneous having a signal peptide at the N-terminus. The *LLA-1271* gene is temporally expressed at the phase of microspore development. The gene is endo- and exo-genously induced by gibberellin. Studies with the gibberellin biosynthesis inhibitor uniconazole and an inhibitor of ethylene activity, 2,5-norbornadien revealed that the *LLA-1271* is negatively regulated by ethylene and a cross-talk of regulation between gibberellin and ethylene occurs in young anthers. RNA *in situ* hybridization analyses further demonstrated that the gene was expressed both in the tapetum and in the microspore. An immunoblot of separated protein fractions of the anther revealed that the LLA-1271 protein was detected in the wall-released protein fraction of microspore with the treatment of either 0.5% or 2% Triton X-100. The LLA-1271 protein once synthesized in both the tapetum and microspore is secreted and deposited on the surface of microspores. The accumulation of LLA-1271

in the exine before the occurrence of microspore mitosis suggests that the protein may be involved in the early stage of exine development.

Comparative studies of the structural and the soluble proteins in mature and immature pollen grains of *Achillea wilhelmsii*

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Introduction: Pollen grains are male gametophytes of flowerring plants that with self interference in fertilization have origin trace in plants fertilize. *Achillea* plant has medicinal applications that are growing in country differen regions. In this research for acquire scientific informations pertain to this plant pollen grains structural in pollens development stages were accomplished compare study of mature and immature pollen grains and those soluble proteins.

Materials and Methods: *Achillea* plant pollen grains in flowers development different stages were collected from Isfahan city around and samples were studied using light and electronic microscopy(SEM), thus pollen extracts were prepared by incubating pollen grains in phosphate buffered saline PH: 7.4. The electrophoresis of proteins were studied on 12% SDS- polyacrylamide gel.

Results: Images of light and electronic microscopy showed pollens from kind ellipse-spherical, two colpate with echinate exine. In SDS- PAGE protein profiles were seen 6 richly coloured protein band in mature pollens in 14.4 to 66 KD and 5 slightly coloured protein band in immature pollens in 14.4 to 45 KD.

Conclusion: In this research, changes of immature pollens morphological of ellipse form to spherical form in mature pollens, exine surface echins height and accumulation increase, quality and quatity chang of immature and mature pollen grains soluble proteins. Mature pollens have more proteins.

Keyword: *Achillea*, Pollen grains, Soluble protein.

ORAL CONTRIBUTION

Arabinogalactan proteins during *Arabidopsis* male gametophyte development

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Pollen ontogeny is an attractive model to study cell division and differentiation. The progression from proliferating microspores to terminally differentiated pollen is characterized by large-scale repression of early program genes and the activation of a unique late gene-expression program in mature pollen.

Arabinogalactan proteins (AGPs) are a class of structurally complex proteoglycans, concerned in diverse developmental processes. Evidences implicating AGPs in sexual reproduction have been obtained in our group, for several plant species (Coimbra and Salema 1997, Coimbra and Duarte, 2003; Coimbra et al. 2005). Recently, the selective labelling obtained with AGP monoclonal antibodies during *Arabidopsis* pollen and pistil development, suggested that some AGPs can work as markers for gametophytic cell differentiation (Coimbra et al 2007).

Despite the tissue specific carbohydrate epitopes that are present on AGPs, these investigations do not allow to study a single AGP. Antibodies bind to carbohydrate epitopes that are present on many AGPs with different protein backbone, while individual protein backbones can be differentially glycosylated. We have tried to bypass this problem by taking a molecular approach. Most of the AGP genes are always present in several plant organs, but we have recently shown that *AGP6* and *AGP11* are pollen specific (Pereira et al 2006).

So we tried to identify particular phenotypic traits attributable to either *AGP6* or *AGP11*, or both, in a reverse genetics approach. Ds transposon insertion mutant lines for these two genes are available from RIKEN BioResource Center. Both lines were checked in our laboratory for homozigoty, and have been sequenced.

AGP6 and *AGP11* are closely related genes, sharing 68 % of the amino acid sequence, and therefore seemingly constituting a pair of paralog genes, the function of which may be mutually overlapping. Optical and electron microscopy studies revealed that none of the insertion mutants showed obvious defects in morphology and pollen development seemed to occur as in wild type Arabidopsis. To determine whether *AGP6* and

AGP11 function redundantly, we performed crossings to obtain double mutants and study the respective phenotype.

This work was also complemented by gene silencing studies. Two *Arabidopsis* transgenic lines obtained by microRNAi technology, one with the *AGP6* gene silenced and another with both, *AGP6* and *AGP11* silenced were obtained. In both cases the constructions were under the control of the native promoter.

The morphological study of these two lines revealed that the silencing of *AGP6* alone, does not lead to important alterations during pollen development. On the contrary, the knock down of both genes leads to an early abortion of pollen grains, which start by showing the absence of the intine wall formation.

In conclusion, the preliminary results of this study show that *AGP6* and *AGP11* are essential for pollen grain development in *Arabidopsis thaliana*, and that they show a potential functional redundancy in this process.

The disappearance of the intine layer in the aborted pollen grains reveals a specific role for these AGPs during pollen development which needs to be fully characterized.

We are currently producing constructs of *AGP6* and *AGP11* genes, together with the respective endogenous promoter, and with a c-myc epitope tag inserted between the N-terminal signal peptide (which targets the protein to the ER) and the AGP core region. We believe that knock-out plants for each of these two genes transformed with the respective tagged gene, constitute a system in which tagged AGP products can be studied in an isolated way.

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An anther-specific gene encoding *cis*-prenyltransferase in lily *(Lilum longiflorum)* anthers

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A *cis*-prenyltransferase gene, *LLA-66*, was identified from a suppression subtractive cDNA library at the microspore stage of lily anthers. The full-length of *LLA-66* cDNA is 1185 bp long containing an open reading frame that encodes a protein of 308 amino acids with a predicted molecular mass of 35.7 kDa. Sequence alignment revealed that the LLA-66 protein belongs to a family of *cis*-prenyltransferases. Northern blot analysis indicated that the gene was specifically expressed at the microspore stage in the anther. *In situ* hybridization showed that *LLA-66* was located only at the tapetum of anthers walls, indicating that it is tapetum- specific. The promoter of 1084 bp identified by TAIL-PCR contains several pollen-specific and hormone responsive elements. The *LLA-66* gene was cloned into pET-32a, overexpressed in *E. coli* BL21(DE3), and purified using Ni^{2+}-nitrilotriacetic acid agarose. The anti-LLA-66 antiserum was raised against the protein overexpressed in *E. coli* BL21(DE3). To determine the enzyme activity of LLA-66, the overexpressed LLA-66 was subjected to denaturation and renaturation, and the refolded protein in the soluble fraction was successfully obtained. To look insight into the structure of the protein, the soluble form of LLA-66 will be crystallized.

A Rop small GTPase and its target Cdc42/Rac-interactive-binding motif-containing protein involve desiccation during development of lily pollen

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The gene encodes LLP-Rop1, a member of Rop (Rho-like GTPases of plant) GTPases and its target RIC (for Rop-interactive CRIB-containing proteins), LLP12-2, were characterized in lily pollen during development and stress. LLP-Rop1 possesses a fragment of conserved effector domain

similar to Rop GTPases of *Arabidopsis* and shares the highest similarity with Group IV of AtRop family. *LLP-Rop1* is a late gene and its mRNA was detected in roots, stems, mature filaments and anthers. Premature drying and application of various concentrations of abscisic acid (ABA) suggested that dehydration and exogenous ABA significantly decresed the accumulatio of *LLP-Rop1* mRNA in developing pollen while *LLP12-2* mRNA markedly increased. Similar results were observed when pollen germinated with the addition of various concentrations of ABA. Quite a few *LLP-Rop1* remained in germinating pollen, suggesting of a vital role of the gene product during pollen germination. Overexpression of either LLP-Rop1 or LLP12-2 in pollen tubes caused inhibition of tube elongation and formed a swelling tip. The two proteins were localized to the apical and subapical regions of bulbous tip. However, overexpression of LLP-Rop1 dominantly negative (DN) mutant, DN-llp-rop1 caused the inhibition of pollen tube growth, but no appearance of swelling tip occurred. The fluorescence resonance energy transfer (FRET) analysis demonstrated LLP12-2 is a target of active LLP-Rop1. The applications of actin-depolymerizing drug, LatB, or (which blocks PM-localized Ca^{2+} channels) to the LLP12-2-overexpressed pollen tube showed that both of two chemicals suppressed the depolarized growth. Therefore, the LLP12-2 protein may be involved in the regulation of actin dynamics and Ca^{2+} signaling during pollen tube growth.

An uncommon microsporogenesis in *Rhynchospora pubera* L. (Cyperaceae)

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The meiotic division of the pollen mother cells (PMC) originates four nuclei, which three are degenerated and one becomes functional, constituting the pseudomonad. The functional nucleus occupies a predetermined location on the pseudomonad. As the representatives of the Cyperaceae family presents an unusual microsporogenesis, the aim of this study was to describe the ultrastructural features along the male gametogenesis of *Rhynchospora pubera* until the pollen grain development. Samples of *Rhynchospora pubera* collected in Recife (PE), Brazil, were maintained in a green house on the Laboratory of Ecosystems Restorations of State University of Londrina (PR), Brazil. Anthers of several lengths were collected and fixed in a solution of 2.5% glutaraldehyde and 2.5%

paraformaldehyde in 0.1M cacodylate buffer (pH 7.2) for 24 h. The samples were post-fixed in 1% OsO_4, dehydrated in ethanol solutions, and embedded in Araldite resin. Semi-thin sections were stained with toluidine blue or plunged in 1% periodic acid (4h) and maintained in Schiff's reagent (16h). Ultrathin sections were observed with a FEI Tecnai 12 transmission electron microscopy. The PMCs were seen as pyramidal cells surrounded by a thin callose wall, and establishing contact with each other and with the tapetum cells. In initial stages of development, the nucleus of PMC was present in the central region with an evident nucleolus and unpacked chromatin. In more advanced stages chromatin was packed and the nucleus was present in the basal region of the PMC. In pseudomonad the functional nucleus was localized in central region and the degenerating nuclei were confined to the basal region. Each degenerating nucleus was surrounded by a thin PAS-positive wall. In TEM sections, Golgi cisterns containing an electrondense material were seen surrounding degenerating nuclei. The mature gametophyte presented two nuclei with different organization levels: (i) the generative being larger was occupying the apical region containing unpacked chromatin and evident nucleolus and (ii) the vegetative one with compacted chromatin, surrounded by a more electrondense cytoplasm, named as periplasm.

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Analysis of meiotic behavior in selecting potential genitors among artificially induced tetraploid accessions of *Brachiaria ruziziensis* and *B. brizantha* (Poaceae)

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Some African species of *Brachiaria* have been introduced into the Americas and became the most important forage for pastures in the tropics. Accessions of five species – *B. brizantha*, *B. decumbens*, *B. dictyoneura*, *B. humidicola*, and *B. ruziziensis* – have been released as commercial cultivars in different countries, including Brazil. New cultivars can be obtained either from direct selections from the natural existing variability

in the germplasm collections or from interspecific hybridizations. The Brazili *Brachiaria* breeding program underway at Embrapa Beef Cattle Research Center aims at producing new cultivars by intra- and interspecific hybridization. However, *Brachiaria* hybridization is not easy because of apomictic reproduction and differences in the ploidy level among species. In the genus *Brachiaria* diploidy is rare and correlated with sexual reproduction whereas polyploidy and apomixis predominate. To overcome the barrier between sexual diploid and apomictic tetraploid accessions, diploid accessions of *B. ruziziensis* were tetraploidized with colchicine in Belgium in the beginning of 1980s. These accessions continue to be the basis of the interspecific hybridization in Brazil and other countries. In attempt to overcome seed production failure in interspecific hybrids and also to broaden the genetic basis of *Brachiaria* breeding in Brazil, the single diploid accession of *B. brizantha* existing in the Embrapa Beef Cattle germplasm collection was also submitted to chromosome doubling by colchicine treatment applied in plants cultivated *in vitro* at Cenargen/Embrap Microsporogenesis was evaluated in teytraploidized plants by conventional methods. The meiotic behavior in the induced tetraploid plants was very similar with meiotic abnormalities ranging from 5.20% to 54.71% in *B. ruziziensis* and from 39.8% to 63.2% in *B. brizantha*. The most common abnormalities observed in the tetraploidized accessions were those related to irregular chromosome segregation. However, specific abnormalities were recorded in some plants of each species. In one accession of *B. ruziziensis*, irregularities involving chromosome orientation at metaphase plate and chromosome convergence to the poles were recorded, whereas in two plants of *B. brizantha* the chromosomes remained dispersed in the cytoplasm in the first division without forming a metaphase plate in the majority of cells. Several micronuclei of different sizes were formed and, after the occurrence of an irregular first cytokinesis, the meiocytes progressed normally to the second division, generating polyads with unbalanced microspores. Considering that polyploidy is largely correlated to abnormal meiosis in *Brachiaria* and that apomictic plants are pseudogamic, for a tetraploid accession to act as a male genitor, it must have high pollen fertility to fertilize the secondary nucleus of the embryo sac and ensure the correct development of the endosperm, thus producing viable seeds. The current research revealed that only some induced tetraploidized plants of both species can act as female genitors in intraspecific or interspecific crosses.

ORAL CONTRIBUTION

Localization of arabinogalactan proteins (AGPs) and pectins in the olive (*Olea europaea* L.) pollen grain during *in vitro* germination by CLSM

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The cell wall structure of the pollen tube in the olive (*Olea europaea* L.) has been previously studied [1, 2]. The pollen tube wall consists of a unique layer of AGPs and esterified pectins at the tip [3, 4, 5]. In the vicinity of the pollen grain, the pollen tube wall is more complex and consists of an outer layer of non-esterified pectins, a cellulosic middle layer and an inner layer rich in callose and AGPs [3, 4, 5].

With the aim of studying the localization of the different components of the pollen tube wall in the olive during *in vitro* germination, we have used a number of antibodies, namely LM5 LM6, JIM13 and JIM14, which are able to recognized specific epitopes of pectins and arabinogalactan proteins (AGPs). An anti-rat IgG conjugated with FITC was used as secondary antibody and observations were made under a Nikon C1 confocal laser scanning microscope equipped with an Ar-488 laser source.

Our results revealed that AGPs are distributed throughout the pollen tube cell wall, as well as in the cytoplasm and the pollen grain wall. However, we found a differential cell localization depending of the antibody used, since JIM 13 specifically binds to *â*-D-glc*p*A-(1'!3) *á*-D-gal*p*A-(1'!2)-L-Rha residues, whereas JIM14 is able to recognize L-Ara residues in the carbohydrate chains covalently attached to the polypeptides of AGPs. After immunolocalization experiments with JIM13, an intense green fluorescent signal was observed in the outer layer of the pollen tube wall and the plasma membrane throughout the pollen tube. Similar studies using the antibody JIM14 showed that the green fluorescent labeling only located in the apex region of the pollen tube. Similarly, both LM6 and LM5 antibodies gave rise to a different localization of neutral pectins. Thus, the antibody LM5, which specifically binds to [(1'!4) *â*-D-gal*p*]$_4$ residues, showed a preferential fluorescence in the outer region of pollen grain apertures. The antibody LM6, which recognizes [(1'!5) *á*-L ara*f*]$_5$ residues, bound to neutral pectins located in the cell wall throughout the pollen tube, as well as in the apertural regions of the pollen grain. The dynamic of pollen tube wall synthesis in the olive is discussed on the basis of our findings here.

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Air pollution effects on structure, proteins and flavonoids in pollen grains of *Thuja orientalis* L. (Cupressaceae)

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Abstract

Increase in the levels of air pollution due to the increase in industrial and agricultural technology has prompted investigation of mechanisms that contribute to air pollution tolerance in plant. Pollen grains of *Thuja orientalis*. were collected from control (less polluted) and polluted areas (mainly SO_2, NO_2, CO, HC and APM). The pollen grains collected from polluted areas were smaller, shrunken and deformed compared to control ones. SDS–PAGE pattern did not show significant difference in polluted

pollen compared to control ones. HPLC analysis demonstrated that air pollution induces flavonoids accumulation to significantly higher levels in polluted pollen than in control one.

Key words: Intine, Exine, Phenolic fingerprints, Protein profile

Microsporangium development, microsporogenesis and microgametogenesis of *Valeriana scandens* L. (Caprifoliaceae *s.l.*)

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Embryological studies of South-American *Valeriana* species are very scarce. *Valeriana scandens* has the widest geographical distribution of genus in Neotropical and it bears sterile anthers in pistillate flowers. This work aims to describe the microsporangium development, the microsporogenesis and microgametogenesis of fertile anthers from perfect flowers to make a future comparison with sterile ones. Optical microscope analyses were made with buds and flowers collected in an Atlantic Forest fragment at Porto Alegre-RS, Brazil. They were fixed in glutaraldehyde 2% in a sodium phosphate buffer 0.1 M (pH 6.8), included in hydroxyetylmethacrylate, and sectioned at 4-1.5 mm with a Zeiss Mikron microtome. Sections were stained with Toluidine Blue O 0.05% (pH 4.4), or PAS reaction plus Toluidina Blue O 0.05%. Histochemical tests were made with Calcofluor White for cellulose and Aniline Blue for callose wall detection, both in fluorescent light microscopy, Leica DM. Microgametophytes *in vitro* germination were performed using a culture medium (50g/L saccharose; 0.2 g/L boric acid; 0.1 g/L calcium nitrate). Fertile anthers of *V. scandens* are biloculate, diagnostic character of section *Phyllactis*. Epidermis, endothecium, a single middle layer and tapetum are the final differentiation of the anther parietal layers. During the mitosis of sporogenous tissue, tapetum becomes vacuolated, enlarges and causes the suppression of middle layer. The microscope mother cells are isolated by callose wall and starts meiosis I. At this stage, the tapetal cells become tetranucleate and invade the locule. Tapetal cells of *V. scandens* are invasive nonsyncytial type. Caprifoliaceae *s. l.* species were described as periplasmodial or amoeboidal. The cytokinesis is simultaneous and gives rise to a tetrahedrical and, in some cases, isobilateral tetrads. After callose wall dissolution, the sporopolenin is deposited on primexine matrix and

differentiated a tectate microechinate exine. Soon after this stage, tapetum degenerates. The microspores become vacuolated and after mitosis, give rise to a vegetative and a generative cells, the last one with dense cytoplas and conspicuous nucleus. The vegetative cell embraces the generative cell that gives rise to two sperm cells with elliptic format after the second mitosis. At this stage, the vegetative cell contains starch grains formed by two to four sub-units. In mature anther, the endothecium parietal thickness is U-shaped type with base plate. The anther dehiscence occurs before the anthesis and some microgametophytes have their pollen tubes growth precociously, inside the anther locule. Microgametophytes germinate *in vitro* immediately after their deposition in culture medium and they take 20 minutes to achieve the double size of pollen grain. Characteristics like: not proliferate of parietal anther middle layer, simultaneous cytokinesis, microgametophyte dispersed in a three celled form, precocious pollen tube germination inside the anther, and fast pollen tube germination *in vitro* promote an energy efficiency and a reduction of sporophytic and gametophytic generation, optimizing its short annual life cycle.

Megasporogenesis, microsporogenesis and development of gametophytes in the Rare Endangered Plant *Manglietia patungensis* Hu

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Abstract

Manglietia patungensis Hu is an evergreen, broadleaf arbor. It is a species of the Magnoliaceae family, one of the China's unique, endangered plants that have been protected at high priority. *M. patungensis* has a very narrow distribution in western Hubei province, southern Sichuan province and western Hunan province. Due to low sexual reproductivity and other endangered factors, this species faces the increasing risk of extinction. A detailed morphological description of megasporogenesis, microsporogenesis, and development of the male and female gametophytes in the rare endangered plant *M. patungensis* is presented for the first time after three-year's continuous investigation. The ovule is anatropous,

bitegmic, crassinucellate and with a obturator. No irregularities have been found in the process from the differentiation of archesporial cells to the formation of megaspores. The megaspore mother cells underwent miosis and developed into a linear tetrad. The large one at chalazal end is functional megaspore and developed into a polygonum type of embryo sac after the third mitotic division. Each anther of *M. patungensis* with four sacs, a mature anther wall successively comprises an epidermis, endothecium, two-layered middle layer and one or two-layered glandular tapetum. The meioses of microspore mother cell is normal and cytokinesis is modified simultaneous type, and tetrads are tetrahedral, decussate and isoblateralÿoccassionally linear. The pollen grains are monocolpate and two-celled at shedding and the pollen grains germination rate is more than 90%. This results differ from those of species *Manglietia*, such as *M. aromatica* which with a high rate of ovule abortion and a low rate of pollen grain germination, and *M.insignis* which has a higher abortion rate of ovules.These results indicate that the key factors for the low seeding rate was not involved in the development process of male and female gametophytes. The development process of male and female gametophytes was not considered as the key factors responsible for the low seeding rate.

The Role of MATH/BTB Proteins of Wheat and Maize in Asymmetric Divisions during Megagametogenesis and Early Embryogenesis

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By screening egg cell and proembryo cDNAs libraries of wheat and maize we have identified four differentially expressed genes encoding proteins with both a MATH and a BTB domain, designated as TaMAB1-3 and ZmMAB1 (MATH and BTB), respectively. Only one such gene has been functionally characterized in *C. elegans* (*Mel26*) as a key regulator required for the first asymmetric zygote division. While one of the four plant MABs is expressed constitutively during vegetative growth (*TaMAB3*), *TaMAB1* and *ZmMAB1* display an egg cell/early embryo-specific expression pattern. Moreover, *TaMAB2* expression was detected exclusively after fertilisation. Tracking a TaMAB2-GFP fusion during cell division revealed that the protein is present at the nuclear envelope and the preprophase band. In transgenic *Arabidopsis* plants, egg cell derived TaMAB2-GFP is always inherited to the basal cell of the 2-celled proembryo, and thereafter localized in micropylar located suspensors cell. This asymmetric inheritance of TaMAB2-GFP was also observed in transient expression studies of dividing suspension cells. Interaction studies have shown that TaMAB2 may assemble as a homodimer but also interacts with AtCUL3, indicating the involvement of TaMAB2 in ubiquitin mediated proteosomal degradation. Downregulation of *ZmMAB1* leads to an arrest of female gametophyte development. In summary the specific expression pattern.as well as asymmetric and cell-cycle modulated protein localisation suggest a role of MABs as intrinsic polarity factors during female gamete and early embryo formation, and perhaps asymmetric cell divisions in general. Current work is focused on the identification of downstream MAB substrates.

ORAL CONTRIBUTION

Lipoxygenase in the cells of the developing ovule of *Larix kaempferi* (Lamb.) Carr.

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The activity of the enzyme lipoxygenase (LOX; EC 1.13.11.12) was determined spectrophotometrically in the cells of the developing ovule of *Larix kaempferi* (Lamb.) Carr. For precise localization of LOX on the cytological level, the immunogold labelling technique was used. The following parts of the ovule were examined with an electron microscope: the integument, the nucellus, the dividing megaspore mother cell, and the female gametophyte at successive stages of its development.

Investigations with the spectrophotomerical method showed changes in the activity of lipoxygenase in the ovules of *Larix*. The immunogold labelling method demonstrated that LOX occured mainly in the cytosol (most often, the immunogold particles were distributed randomly in the cytoplasm) and vacuoles of the cells of all the investigated ovule parts.

The immunogold particles which revealed the presence of the enzyme were found to be associated with the plasma membrane and were also discovered in the close vicinity of mitochondria and near plastids. LOX was detected near short endoplasmic reticulum cisternae – mainly RER (rough endoplasmic reticulum). Some single immunogold particles were observed at or in the area of the cell walls and nuclei of the ovule cells.

Immunolocalization of lipoxygenase in an electron microscope indicates a functioning “lipoxygenase pathway” in all cells of the investigated ovule parts. The intensity of the immunogold reaction may indirectly indicate differentiated activity of the enzyme in the cells of the particular parts of the *Larix* ovule.

ORAL CONTRIBUTION

Epigenetic Regulation of autonomous seed formation in *Hieracium*

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MULTICOPY SUPPRESSOR OF IRA1 (MSI1) is part of the FIS complex and *msi1* mutants display the typical *fis* phenotype of autonomous central cell proliferation and embryo abortion, but unlike other *fis* mutations, also displays autonomous egg cell proliferation. In apomictic *Hieracium* plants, egg and central cell development are fertilization-independent and seed formation is completely autonomous. This work describes MSI1 function in *Hieracium* seed development by determining if *HMSI1* expression is altered in apomictic plants, particularly in apomictic egg cells. A *Hieracium* MSI1 homologue, *HMSI1*, was isolated and expression analysis determined by RT-PCR and in situ hybridization. Both abundance and spatial distribution of *HMSI1* mRNA is conserved in ovaries of sexual and apomictic plants. Expression analysis in an apomictic mutant which has a deletion in the LOP locus which impairs autonomous seed initiation shows that mRNA levels of *HMSI1* and the *Hieracium* homologue of FERTILIZATION INDEPENDENT ENDOSPERM, *HFIE*, are decreased. However, neither *HMSI1* nor *HFIE* copy number has been altered in the genome of mutant 179, suggesting that these genes are not part of LOP. Furthermore, HMSI1 protein is able to interact with RETINOBLASTOMA (RBR) as previously described for the Arabidopsis proteins. Together, these data suggest that the MSI1-RBR pathway is conserved in apomictic plants and that autonomous seed development is not due to lack of *HMSI1* expression in apomictic egg cells.

New interactions among "old" genes in *Arabidopsis* gynoecium development

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The gynoecium is one of the most complex and multifunctional organs of the plant. Besides enclosing and protecting the ovules, it is involved in the efficient capture of the pollen; in promoting out-breeding

by means of selective mechanisms operating on the pollen, such as self-incompatibility; it participates actively in the delivery of the pollen to the ovule. After fertilization, it originates the fruit (in *Arabidopsis* the silique) which protect the developing seeds and later contributes to their dissemination.

Most of our current knowledge on gynoecium development comes from genetic and molecular research in the model species *Arabidopsis thaliana*. During the last years our knowledge has increased substantially: several genes involved in gynoecium development have been identified and many pathways have been elucidated. Moreover, it is now becoming increasingly clear that gynoecium organogenesis relies upon strong functional connections among these pathways.

Here we present the morphological characterization of the *shp1 shp2 ant* triple mutant. The mature gynoecia of the triple mutant are often open at the apical end and partially split along the medial region, suggesting that these genes cooperate in regulating carpel development. This phenotype suggests that there are still unidentified links between pathways that control different aspects of carpel and fruit development.

Morphological features of *Rhynchospora pubera* L. (Cyperaceae) ovule and embryo sac

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Rhynchospora Vahl has the largest number of Cyperaceae species, comprising about 270 species worldwide. The male gametogenesis in this family is peculiar, since from four nuclei only one is functional. Data of morphological features of gynoecium of this genus is absent. *Rhynchospora pubera* was investigated in order to establish morphological features of the ovule and embryo sac development. Samples of *Rhynchospora pubera* collected in Recife (PE), Brazil, were maintained in a green house in the Laboratory of Ecosystems Restorations of State University of Londrina (PR), Brazil. Ovary from several lengths were collected and fixed by immersion in a solution of 2.5% glutaraldehyde and 2.5% paraformaldehyde in 0.1M cacodylate buffer (pH 7.2) for 24 h at 25°C. The samples were then washed several times in buffer and post-fixed for 2 h in 1% OsO_4 in cacodylate buffer, dehydrated in a graded series of ethanol solutions, and embedded in Araldite resin. Semi-thin sections were dyed with toluidine

blue and analyzed using a light microscope. Ultra-thin sections were observed using a FEI Tecnai 12 transmission electron microscopy. The tricarpelar syncarpous gynoecium is unilocular and contains one anatropous ovule borne in the base. The ovule is crassinucellate, with an archesporial cell cutting off a parietal cell, which undergoes further divisions to form the nucellar tissue. The development of the embryo sac is the *Polygonum* type. The mature embryo sac consists of three persistent antipodals, two polar nuclei that do not fuse before fertilization, and the egg apparatus of which the filliform apparatus in the synergids is persistent. The ovule is surrounded by two integuments, both of them composed of packed cells. The inner integument is composed by two layers of hexagonal cells, whereas the outer integument shows three layers, the two outer are rectangular and the inner has square shape. When megaspore mother cell divides, both integuments appear incomplete. In later stages of the embryo sac development, the innermost cells of the outer integument becomes filled by a blackened-staining substance. The micropyle is only formed at the mature embryo sac stage.

Conifer megagametophytes

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Conifers are an ancient group of seed plants consisting of six or seven extant families. Most families are found predominantly northern hemisphere with a few found predominantly in the southern hemisphere while a few families occur in hemispheres. Megagametophyte development is similar within families but differs among some families The general pattern of development is: the haploid functional megaspore undergoes many free nuclear divisions forming a large sac containing about 2000 nuclei free nuclei; cell wall formation follows creating many thin-walled prothallial cells and one to several archegonial initials; the archegonial initia divide unequally to form a small primary neck cell and a large central cell; the primary neck cell then divides forming usually several neck cells; and, then the central cell divides unequally forming a small ventral canal cell and a large egg cell. There is considerable variation in number and arrangement

of archegonia and in the structure of the egg cells among families. Sporogenous cells surround the early megagametophyte. They degenerate during megagametophyte development and form a “tapetal-like” layer consisting of degenerating cells and their contents plus cellular secretions. At first, these form a complex membranous layer that then differentiates into a complex cell wall similar in structure to the exine and intine of the pollen wall. In the Cupressaceae (which includes the Taxodiaceae) the archegonial initials are not separated by prothallial cells and an archegonial complex forms at the micropylar end of the megagametophyte. Egg cells are complex containing many organelles including plastids and mitochondria. In the Pinaceae, Podocarpaceae, Taxaceae and Cephalotaxaceae, usually several archegonia form at the micropylar end of the megagametophyte. They are separated by prothallial cells and each archegonium has several neck cells. The large central cell divides forming a small ventral canal cell and a large complex egg cell in which plastids engulf egg cytoplasm and form “large inclusions” and mitochondria aggregate around the egg nucleus forming a dense perinuclear zone. In the Araucariaceae, several archegonia form along the sides of the long megagametophyte. Each archegonium has several neck cells and the egg contains a free ventral canal nucleus, a perinuclear zone containing many mitochondria and many large plastids. In conifers, the structure of the egg, including the presence or absence of a perinuclear zone and large inclusions, in combination with the structure of the sperm and contents of the pollen tube cytoplasm that are released into the egg at fertilization, determine the pattern of cytoplasmic inheritance.

The self-incompatibility related HT-protein remains functional in self-compatible *Nicotiana tabacum*: does it have general role in pollination?

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The success of plant reproduction depends on proper pollen-pistil interactions and the specialized tissues of the pistil have the role to recogni and discriminate between the different pollen grains received. Usually wide intergeneric and interspecific crosses are avoided. Intraspecific crosses are successful except when self-incompatibility systems are present. In additior to the recognition function, the specialized tissues of the stigma and style are believed to be responsible for pollen adhesion, nourishment and guidan of the pollen tubes. Identification and characterization of pistil-specific genes are key steps to unraveling these processes. We have recently constructed a suppressed subtractive cDNA library, enriched for stigma/ style transcripts of self-compatible *N. tabacum*. Among the putative pistil-specific clones, we have identified one encoding the HT-protein (NtHT). The NtHT deduced protein sequence exhibited 89.1% identity and 93.9% similarity with the *N. alata* HT-protein (NaHT), a member of the HT-B group of self-incompatibility factors in solanaceous species. The NtHT and NaHT are structurally identical, sharing the same signal peptide for extracellular localization of the protein and the characteristic asparagine-rich domain at the carboxyl-end. To confirm the pistil specificity of the NtHT transcript we performed qRT-PCR experiments using root, stem, leaf, sepal, petal, stamen, stigma/style (S/S), ovary and S/S of STIG1::barnase transgenic plants, in which the stigmatic secretory zone was ablated. The results show that NtHT transcript is pistil-specific. The comparison of the expression levels in wild-type S/S and in STIG1::barnase S/S suggests that NtHT mRNA is more abundant in stigmatic tissues than in stylar tissues. The *in situ* hybridization experiments revealed that the NtHT transcript is specifically present in the stigmatic secretory zone and in stylar transmitting tract cells, tissues traversed by the pollen tube during

its growth toward the ovules. Interestingly, we found a low NtHT transcription level in ovaries and, although the *in situ* hybridization signal is barely detectable at the funiculus, it is relatively strong at the outer integument cells, which interact with the pollen tube. Additionally, the NtHT transcription was examined by qRT-PCR in stigmas/styles at the 12 stages of tobacco flower development. Its mRNA starts to be detected at stage 6 and shows a drastic increase at stage 9, reaching the maximum level at stage 10. At stage 11 the NtHT transcript level is still very high, but lower than at stage 10. At stage 12, which contains pollinated and unpollinated pistils, the NtHT transcript level is much reduced. Western blot experiments, using the antipeptide antibody raised against NtHT sequence, have demonstrated that this antibody is capable of recognizing the NtHT protein. Furthermore, we have showed that the NtHT protein is detectable from stage 9 of flower development and on, in agreement with its transcript level. The amount of NtHT protein gradually rises until stage 12, when anthesis occurs. Interestingly, the NtHT protein is rapidly degraded after pollination, whereas emasculated pistils remain expressing the protein, at least during the subsequent 36 hours. Our results show that the NtHT protein is pistil-specific, and its expression is regulated by pistil development and by pollination, suggesting it has a role in pollen-pistil interactions. Meanwhile, the NaHT highest transcript level occurs at anthesis and the corresponding protein is degraded only after incompatible pollination, being stable after compatible pollinations. Despite their pistil-specific expression and involvement in pollen-pistil interactions, NtHT and NaHt seem to have somewhat different functions. Is NtHT a relic gene/protein from the SI system in the *Nicotiana* ancestor that has been recruited for a compatible pollen-pistil interaction function in *N. tabacum*? The fact that the NtHT protein sequence has been highly conserved during evolution suggests that it has a current and important role in the compatible pollen-pistil interactions that occur at the *N. tabacum* pistils. Future experiments will contribute to elucidate its contribution to the reproduction process.

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PCD and ROS in pollen-pistil interactions in *Olea europaea*

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Programmed cell death (PCD) is a physiological process that occurs during normal development and also in pathological conditions in both animals and plants. Although PCD has been researched for well over a century in animals little is known about its role in plants, especially with regard to their reproductive processes. During fertilization cell death occurs in both pollen and pistil, thus playing an important role in the prevention of self-pollination, a condition known as self-incompatibility. Self-incompatibility has evolved presumably to prevent the deleterious effects of inbreeding. There is increasing evidence that reactive oxygen species (ROS) serve both as direct and indirect mediators of programmed cell death in plant cells. ROS have a dual role in the cell-death process: as a facultative signal during its induction phase and as a contributor to the degradation phase, when alterations that define PCD are evident.

Our approach to this problem has been to study olive (*Olea europaea* L.) pistils with different programmed-cell-death parameters, including DNA laddering and tunnel reaction during the progamic phase. At the same time we have analysed the production of reactive oxygen species, such as H_2O_2, $O_2^{\cdot-}$ and NO, during pollination and fertilisation. We have found evidence of a spatio-temporal relationship between endogenous ROS and PCD, which might suggest that both are involved in self-incompatibility processes in olive species.

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Morphological and physiological flower alterations as consequences of overexpression and silencing of a tobacco pistil-specific pectin acetyl esterase (PAE) gene

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The pistil has a dual function: the production of the female gametophyte and the establishment of pollen-pistil interactions that will determine the fate of the pollen. The objective of our work is to identify genes preferentially expressed in the pistil and to study their function in plant reproduction. To establish a large scale survey of tobacco gene expression during pistil development and preparation for pollination, we generated 11,217 high-quality expressed sequence tags (ESTs) derived from *Nicotiana tabacum* stigmas/styles at different flower developmental stages. These sequences were used to create the tobacco ESTs (TOBEST) database. A macroarray analysis performed with 792 clones has allowed the identification of a putative pistil-specific clone (TOBS004A06) which has high similarity with pectin acetyl esterases (PAE). The clone TOBS004A06 is a full-length cDNA and encodes the complete *Nt*PAE1 protein sequence. The analysis of its amino acid sequence by the SignalP program has indicated the presence of a signal peptide, as expected for a secreted protein involved in cell wall metabolism. Experiments of real time RT-PCR using RNA from roots, stems, leaves, sepals, petals, stamens, stigmas/styles and ovaries have confirmed *Nt*PAE1 pistil-specific expression and preferential expression on stigmas/styles. The analysis of its expression on stigmas/styles at the 12 tobacco flower developmental stages has shown a small expression level at stages 5 and 6, the highest expression level at stage 11 and a drastic decrease at stage 12, when the pistils have been pollinated. The pectin acetyl esterase (PAE) is one of the enzymes in the pectin degradation pathway. It is known that the stigmatic secretory cells have thin walls, mainly composed of pectin and a low amount of cellulose (Cresti et al., 1986). At maturity (stage 12), these cells are loosely arranged and very easily separated, due to presence of large intercellular spaces (Cresti et al., 1986), filled by the exudate through which the pollen tubes grow toward the ovary. Therefore, it is probable

that *Nt*PAE1 has an important role in the loosening of the cells from the stigmatic secretory zone and stylar transmitting tissue. To test this hypothesis, we have produced tobacco transgenic plants overexpressing *Nt*PAE1, as well as RNAi transgenic plants for silencing of *Nt*PAE1. Among the 15 overexpression plants, 2 transgenic plants (PAEOv 8.1 and PAEOv10.1) had thinner pistils. From the 8 RNAi plants obtained, the one with the lowest *Nt*PAE1 expression level (PAERi-16.2) showed tougher and firmer flowers and pistils. Longitudinal sections of stage 11 stigmas/styles of the PAEOv8.1 and PAERi-16.2 transgenic plants were stained with ruthenium red, showing lower and higher pectin content, respectively. The PAERi-16.2 plant was sterile and did not produce fruits after self-pollination. Additionally, the PAERi-16.2 pollen grains were incapable of producing fruits in SR1 control pistils and had an abnormal morphology as observed by scanning electron microscopy. Pollen tube growth on PAERi-16.2 pistils was compared with growth on pistils from SR1 control plants, under fluorescence microscopy. The results showed a delay in pollen tube growth in the PAERi-16.2 pistils, which was more accentuated with PAERi-16.2 pollen than with SR1 control pollen. Our results reveal the importance of the adequate regulation of pectin degradation in pistils for the formation of the intercellular spaces and appropriate pollen tube growth.

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Pollination triggers ovule maturation at early stages of tobacco flower development, which in turn correlates with fruit size and seed viability

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The sexual plant reproduction process consists of pollination and fertilization. During pollination, pollen grains land on the stigma surface and if congruity/compatibility is established, they germinate to produce pollen tubes that penetrate the stigma and grow through the transmitting tissue to fertilize the ovules within the ovary. It is generally accepted that the success of sexual reproduction is dependent on the synchrony of male and female gametophytes development and maturation. In *N. tabacum*, flower development was previously divided in 12 stages (Koltunow et al.,

1990). At stage 1 all floral organs are differentiated and at stage 12 anthers dehiscence, stigmas are covered by exudate, megagametogenesis is being completed and pollination occurs. The timing of pollination is critical since the stigma surface must be receptive and female gametophytes mature. To study whether pollination on pistils before stage 12 would be effective in fruit production, we have studied several parameters at the different developmental stages. Stigma receptivity is characterized by high levels of peroxidase activity and can be evaluated by Peroxtesmo test. Stage 4 stigmas show negative results in the peroxidase activity test and, coherently, mature pollen grains applied on their surface do not hydrate. Stage 5 tobacco stigmas and later stages are positive in this test, indicating receptivity. In agreement with this result, pollen grains hydrate and produce pollen tubes that reach the ovary after pollinations performed at stage 5 stigmas, as observed by fluorescence microscopy. However, pollinations with mature pollen performed at stages 5 and 6 flowers are incapable to form fruits. Successful fruit production was only observed as a result of pollinations performed at late stage 7 stigmas (floral buds e” 34mm) and onward. Nevertheless, as shown by light microscopy, female gametophytes are not yet formed at stages 7 and 8 unpollinated flowers. So, how fruits are formed at these stages? To investigate the development taking place at the ovary in response to pollination we have analyzed unpollinated and hand pollinated pistils at different developmental stages. Twenty-four hours after pollinations performed at stage 7 pistils, structures characteristic of stage 12 ovaries, such as the egg cell and central cells, are present. Our results demonstrate that pollination with mature pollen grains triggers the maturation of the female gametophytes. Additionally, the fruits produced after pollinations at the different developmental stages vary in size and weight. Fruits from stage 7 pollinations were the smallest and fruit size increased correspondingly in later stages pollinations. The weight of fruits from stage 7 pollinations was significantly different (pd” 0.05) from the ones resulting from pollinations at stages 9, 10 and 11. We have also analyzed the germination of seeds from the fruits resulting from pollinations at the different developmental stages. Stage 7 seed germination was significantly lower (pd” 0.05) than the seed germination of fruits from pollinations at stages 8, 9, 10 and 11. Seed germination from stage 8 fruits was also significantly different (pd” 0.05) from the stages 9, 10 and 11 seeds. Taken together, our results suggest that pollination produces signals that stimulate female gametophyte development and that this process is also dependent on the developmental competence of the pistil.

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Pollen viability, stigma receptivity and post-pollination in *Anagyris foetida* (Leguminosae)

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Abstract

Anagyris foetida, a leguminous shrub, deciduous in summer, autumn-winter flowering, with Mediterranean and Irano-Turanian distribution is pollinated by three species of passeriforms (two warblers and a chiffchaff). Given the fragmented nature of its area of distribution and the low density of individuals/population, the study of the reproductive biology of *Anagyris foetida* is an important prerequisite for the potential conservation of the only experimentally documented case of ornithophily in Europe.

We studied the floral biology of the species, trying to discover (1) whether it has floral mechanisms that facilitate or prevent self-pollination and (2) because of its autumn-winter flowering we also investigated the time taken by the pollen tubes to penetrate the ovules in hand-pollinated flowers previously emasculated. Lifetime of the flower was divided in four phases: (I) flower bud with non-dehiscent anthers, (II) flower bud near anthesis and with anthers initiating dehiscence, (III) flower opened (anthesic), and (IV) post-receptive flower (old or postanthesic). In each flower pollen viability was tested by germination "*in vitro*" and stigma receptivity by pollen germination "*in vivo*". The results show that: (1) the stigmatic surface must be scratched through the visit of the pollinators for the pollen to germinate, so it avoids spontaneous self-pollination; (2) the existence of pollen viability and stigma receptivity curves overlapping indicated the no existence of functional dichogamy; (3) ca. 69 % of flowers had at least one penetrated ovule at 26h post-pollination, so the progamic phase can not be considered delayed despite of low temperatures during flowering period.

ORAL CONTRIBUTION

The peroxin loss-of-function mutation abstinence by mutual consent disrupts male-female gametophyte recognition

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In animals and plants, fertilization relies on complex and specialized mechanisms that achieve the precise delivery of the male gamete to the female gamete and their subsequent union. In plants, the male gametophyte or pollen tube carries two sperm cells over a long distance through the maternal tissues to the female gametophyte. During this long assisted journey, a multitude of signal exchanges between the pollen tube, the maternal diploid tissues and the haploid embryo sac take place that culminate in pollen tube reception, the process through which the pollen tube release the sperm cells into the female gametophyte. Here, we report the isolation and characterization of the *Arabidopsis* mutant *abstinence by mutual consent* where pollen tube reception is impaired only when an *amc* pollen tube reaches an *amc* embryo sac leading to pollen tube overgrowth and the absence of sperm release. Moreover, *AMC* is strongly expressed in both male and female gametophytes during fertilization but strongly down-regulated during subsequent embryo development. We further show that YFP-AMC fusion localized to peroxisomes and that AMC functions as a peroxin that mediates protein import into peroxisomes. The identification of *AMC* (Boisson-Dernier et al., 2008) as a gene required for pollen tube reception with essential roles in both male and female gametophytes, points towards a key role for peroxisomes in gamete recognition and successful sperm release.

Boisson-Dernier, A., Frietsch, S., Kim, T.H., Dizon, M.B., and Schroeder, JI. (2008). The Peroxin Loss-of-Function Mutation *abstinence by mutual consent* Disrupts Male-Female Gametophyte Recognition. *Current Biology*.18(1):63-68.

Pollen Tube-Ovule Interaction in Sour Cherry

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The occurrence of an unusual growth of pollen tubes in the ovary of sour cherry has been observed. In the ovary, one or more pollen tubes were either markedly twisted and bifurcated at the entrance of the micropy or they passed round the ovules. Sometimes pollen tubes penetrated to the base of the ovary, or their ends were bending or curling backwards. Besides regular penetration to the nucellus, some tubes were observed to the curl up in a ball in the embryo sac itself, with a tendency of further undirected growth outside it. Sometimes a large ball was noticed on the top of nucelleus before penetration thereof into it. Although the unusual growth of pollen tubes in the micropyle and nucellus of the ovule occurs in small number of pistils, it is associated exclusively with the larger ovules that fluoresced intensely. The penetration to smaller ovules, which always showed intensive fluorescence, was rarely seen, and in such cases, unusual growth of pollen tube was always pronounced. The localisation of insoluble and pectic polysaccharides, i.e. the products of their hydrolysis in the ovule, can be correlated with the guidance of pollen tubes, i.e. the existence of attraction, either in terms of normal stimulation or the inhibitio of growth of pollen tubes by the ovule.

Pollen and stigma morphology related to pollination in Neotropical species of *Indigofera* L. (Leguminosae, Papilionoideae)

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This study aimed to evaluate the morphology (structure, ultrastructure and cytochemistry) of pollen grain and stigma to find characters related to melittophily in seven Neotropical species of *Indigofera* (*I. campestris*, *I. hirsuta*, *I. lespedezioides*, *I. microcarpa*, *I. spicata*, *I. suffruticosa* and *I.*

truxillensis). Stigmas and anthers of the late developmental stages were prepared for light and electron (scanning and transmission) microscopies. Tests to detect oil, polysaccharides and proteins were also performed. The pollen grains were middle size (25-35µm), amb subtriangular to triangular, 3-colporate, mesocolpium perfurated and apocolpium psilate. Plastids containing oil, starch grains and protein bodies occurred in the vegetative cell cytoplasm of all the species analyzed, although in a different quantity in each species. Pollenkit was not observed. Stigma of pre-anthesis flower presented simple trichomes and a thin cuticle covering a round stigmatic surface. The stigmatic surface is formed by secretory cells. During the anthesis, the simple trichomes retracted exposing the stigmatic surface, and secretory cells produced lipophilic and hydrophilic substances. The secreted substances were retained in the subcuticular and intercellular spaces. This type of stigma, made of secretory cells and covered by a cuticle, is considered as semidry. Controlled pollinations (bagged inflorescence) indicated that automatic pollination did not occur; also, visits by stingless bees *Trigona fuscipennis* Friese 1908 were observed. These informations, added of lack of pollenkit and presence of stigmatic secretion, allowed us to conclude that pollen germination depends on pollinator. The flower would be tripped by the stingless bee, which would provoke the cuticle disruption, permiting the contact of pollen with the stigmatic fluid; consequently, the pollen would reidrate and germinate. Melittophilous species usually have oily pollen grain. In *Indigofera* pollen grain contained starch grain and protein bodies besides oil droplets. Then, reserve substances might not be associated with pollinator in this group. It should be mentioned that this is the first report of semidry stigmas in *Indigofera*, the third largest genus in the Leguminosae (FAPESP).

Molecular characterization and polymorphism of superoxide dismutase (SOD) in olive (*Olea europaea* L.) pollen. Putative roles in the interaction pollen-stigma

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Reactive Oxygen and Nitrogen Species (ROS and RNS) offer a dual perspective in physiological systems. Despite the fact that their accumulation may cause important damages to plant tissues, they have been described to play important roles in stress, defence against pathogens and signal

transduction in plants. ROS and particularly NO are recently emerging as signalling molecules in pollen-stigma interactions (Hiscock and Allen, 2008). Therefore, ROS and RNS production, accumulation and transformation into other metabolites have to be tightly controlled. Different enzymes integrate the antioxidant controlling panel of plant tissues. Among these, peroxidases have been shown to be widely present in Angiosperm stigmas. The activity of ther enzymes like NOX (NADPH oxidase) have been described in pollen tubes (Potocký et al., 2007).

The enzyme superoxide dismutase (SOD) catalyzes the disproportionation of superoxide radicals in biological systems. This enzyme family is composed of three major forms depending on their prosthetic group (Mn-SOD, Cu,Zn-SOD y Fe-SOD) (Alché et al., 1998).

In the present study, Cu,Zn-SODs were cloned from mature pollens of a large number of olive cultivars. Over 70 sequences were obtained from either cDNA or gDNA extracted from 11 olive cultivars. Bioinformatic analysis showed that all sequences displayed a high degree of conservation. However, sequence microheterogeneities were frequently observed. They did not affect key amino acids (i.e. Cys residues involved in the formation of intra-molecular bridges, or His residues concerned in the interaction with the Cu atom). Some of the substitutions affected protein motifs liable to post-translational modifications and likely involved in regulation. A deletion of 8 amino acids was detected in a low proportion of the sequences analyzed.

Protein extracts from mature pollen were used to determine SOD activity both by spectroscopic measurement and in native gels. Differences in the level of activity were detected depending on the cultivar analyzed. Four to five reactive bands were observed in the gels, also depending on the cultivar analyzed. The use on specific inhibitors allowed us to determine the presence of a relevant band corresponding to Mn-SOD in a large proportion of the cultivars, and 4-5 bands characterized like Cu, Zn-SOD. Immunoblotting analysis showed several immunoreactive bands in the extract, which may correspond to different isoforms of the enzyme, including the presence of polymeric forms.

Finally, a system to microscopically detect the presence of ROS and particularly SOD activity *in vivo* by generating superoxide radicals was assayed in several samples of olive pollen. SOD activity in the mature pollen was highly dependant on pollen viability. The presence of such a complex antioxidant system in the olive pollen and the occurrence of intercultivar differences points out to a putative involvement of the system in the compatibility behaviour of this species. The olive tree is considered to be preferentially allogamous (this is, preferentially cross-fertilized by pollen from a different olive cultivar). Several models as regard to the

putative role of this antioxidant system in the interaction pollen-pistil in the olive are presented and discussed.

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The involvement of thioredoxins *h* in self-incompatibility in olive trees (*Olea europea* L.)

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Although double fertilization takes place inside the ovary both the stigma and style play a crucial role in fertilization processes. The stigma is the part of the pistil on which pollen grains are received and it is here and in the upper part of the style that the signal for germination is triggered if inter – and intraspecific barriers are successfully crossed.

Among intraspecific barriers, self-incompatibility is an important outbreeding mechanism which is widespread in flowering plants. The molecules involved in the recognition of pollen by the stigma or during the growth of the pollen tube in the style, including thioredoxins *h*, which are involved in the signal cascades of incompatibility in both monocots and dicots, are currently the subject of extensive research. The expression of thioredoxins *h* is related to self-incompatibility in plants displaying either gametophytic or sporophytic self-incompability. In addition, affinity chromatography has revealed an interaction between a thioredoxin *h* and a S-RNase in *Nicotiana tabacum*, highlighting the role of thioredoxin *h* in self-incompatibility reactions.

The self-incompatibility system in olive trees (*Olea europaea* L.) is gametophytic but the molecules involved in pollen-pistil interaction in these species are far from being completely understood.

We describe here the whole cDNA spatial sequence of a thioredoxin *h* and discuss the results of the location of these proteins and their transcripts in the sexual organs.

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Cloning and characterization of Montenegrin *Prunus webbii S*-RNase and "non-*S* RNase" alleles

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Prunus webbii, a wild almond species, displays *S*-RNase based gametophytic self-incompatibility (GSI). *S*-RNases, female component of GSI system, are stylar glycoproteins, included in degradation of self-pollen RNK. Insight into *S-RNase* allele structure of wild almond would be important for evolutive analysis of GSI system as well as for eventual introduction of useful *P. webbii* characteristics into sweet almond cultivars, such as resistance to drought. In order to investigate *S-RNase* allele structure of a *P. webbii* from the Montenegrin region of the Balkans, we have analyzed ten accessions using PCR, sequencing and isoelectric focusing. We detected ten different *S-RNase* allelic variants, obtained nucleotide sequences for six *S-RNase*s and one basic "non *S*-RNase" (first time recorded in *P. webbii*). Two *S*-RNases shared extremely high homology with *S*-RNases of two other *Prunus* species. One *S*-RNase was found to be inactive, but the reson of inactivity was not at the coding sequence level, which should be elucidated in future reserach.

Molecular characterization of self-incompatibility ribonucleases (*S*-RNases) in loquat (*Eriobotrya japonica* Lindl.) cultivars

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Loquat (Eriobotrya japonica Lindl.) is a self-incompatible fruit tree species belonging to the Maloideae subfamily in the Rosaceae. In the Maloideae, self-incompatibility is of the gametophytic type and a stylar ribonuclease (*S*-RNase) governs the female function in the pollen-pistil incompatibility system. This study reports for the first time genomic

characterization of *S*-RNase alleles in loquat cultivars. Primers based on S-RNase sequences from other Maloideae species were used to identify homologue sequences in loquat. Five *S*-RNase alleles were characterised by genomic PCR and sequence analysis, in a group of 30 cultivars. Genomic sequences showed all the structural features of the Maloideae *S*-RNase. Moreover, the good correspondence between results from pollination experiments and the *S* genotypes of cultivars deduced from PCR and sequence analysis confirmed that the genomic regions characterised correspond to S-RNase alleles of loquat. Both inter-incompatibility between cultivars with the same *S* genotype and inter-compatibility between groups of cultivars with different allelic combinations were observed. Six incompatibility groups were defined, combination of the five S-alleles. These results will help in the selection of the most appropriate pollinator cultivars providing an adequate orchard management of this species and also provide information on the *S*-RNases of the Maloideae.

Direct utilization of molecular self-incompatibility analyses in commercial apricot orchards and breeding programmes

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Almost all the European apricot cultivars have been traditionally considered self-compatible; nevertheless more and more exceptions are found. The self-incompatibility trait of apricot is the gametophytic (GSI) type, controlled by the *S*-locus. Since the pistil expressed *S*-RNase gene is better known, several PCR markers were designed for this gene to conduct genotyping studies. One *S*-allele for SC and several alleles for self-incompatibility were described previously in Mediterranean and American cultivars (S_1-S_7) and in Hungarian and Central Asian genotypes (S_8-S_{16}).

In this study a degenerate primer pair designed from *S*-RNase sequences of different *Prunus* species could facilitate the identification of 4 additional alleles (S_{17}–S_{20}) when testing a range of 74 apricot cultivars. Characteristic intron lengths of the new *S*-RNase alleles were determined. Among the tested cultivars, the S_8-allele occurred in 19; the S_2 in 12; the S_1 and S_9 in 4; the S_{13} in 3; the S_{10}, S_{11} and S_{20} in 2 cultivars, while the S_{12}- and $S_{14\text{-}19}$-alleles were only found in 1 cultivar.

cDNA of 7 *S*-RNase alleles, first intron region of 6 alleles and second intron region of 8 alleles were cloned and sequenced. All sequences were

submitted to the NCBI GenBank database. Complete *S*-genotype of 51 economically important apricot cultivars and hybrids of great breeding value was determined. Using molecular analyses and fruit set evaluation, the self-(in)compatibility phenotype of 21 cultivars with previously unknown compatibility properties was clarified; and the full *S*-allele composition of 19 cultivars was determined.

By comparing our results with data published previously, we established a table demonstrating compatibility relationships of apricot cultivars. Sixty seven cultivars were assigned to 3 inter-incompatibility groups and a universal pollen donor group. The information supplied by this table can give direct help for planning orchards with self-incompatible cultivars and selecting parental lines in breeding programs.

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ORAL CONTRIBUTION

Genomic organization of the sporophytic self-incompatibility locus in *Ipomoea trifida*, a close relative of sweet potato

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The genus *Ipomoea*, a member of Convolvulaceae, has a sporophytic self-incompatibility (SSI), which is genetically controlled by a single multiallelic *S* locus. Sequence contigs around the *S* locus (ca. 300 kb) were analyzed on genomic clones from map-based screening of BAC and cosmid libraries. Sequence comparisons of different *S* haplotypes revealed a highly variable region, named *S* haplotype-specific divergent region (SDR). Differences in the size of the SDR were found among *S* haplotypes, and showed that the more dominant the *S* haplotypes tend to exhibit the larger the SDR (S_3<S_{10}<S_1<S_{29}). This suggests that acquisition of sequence complexity by recessive *S* haplotypes is responsible for the differentiation of more dominant *S* haplotypes. From RNA-blot and RT-PCR analyses of the gene transcripts in the *S* locus region, three stigma-specific genes (*SE1*, *SE2* and *SEA*) and an anther-specific gene (*AB2*) were identified, which showed a high level of allelic polymorphism among the *S* haplotypes. Based on cDNA sequence analyses, the predicted SE1, SE2 and SEA proteins share partial sequence similarities with 30-40% identity and are estimated to have several membrane-spanning domains. In database search, the three predicted proteins showed no significant homology to proteins with known function, indicating that the genus

Ipomoea has a novel SSI system. The predicted AB2 proteins show homology to cysteine-rich proteins termed plant defensins, which are members of the g-thionin protein family. However, alignment of the 8 conserved cysteine residues shows that AB2 is structurally different from PCP-A1 and SP11/SCR identified in *Brassica*. Thus, the present study indicates that one of the three stigma-specific genes and the anther-specific *AB2* gene are strong candidates for the *S* determinants in the SSI of *Ipomoea*.

The *S*-locus helps to reveal the evolutionary history of tree fruits: a world beyond the model plants

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The gametophytic self-incompatibility (GSI) system employs stylar ribonuclease enzymes (*S*-RNases) and *S*-haplotype-specific F-box (SFB) proteins as pollen component in *Solanaceae*, *Scrophulariaceae* and *Rosaceae*. The RNase based GSI in various plant families was suggested to have common evolutionary origin; however, several differences were detected between solanaceous and rosaceous GSI. According to the latest model, new *S*-specifities arise by consecutively accumulating mutations in both the pollen and pistil genes.

Apricot (*Prunus armeniaca* L.) S_8-, S_9- and S_C-haplotypes (S_C enables self-compatibility) were analysed using a multi-level approach: fruit set and pollen tube growth analysis, RNase assays and sequencing of the *S-RNase* and *SFB* alleles. Protein evolutionary information was compared to other species inside or outside the *Rosaceae* family.

Self-compatibility (SC) in apricot is due to a pollen-part mutation. SFB_8 was clarified to be the first known progenitor allele of a naturally occurring SC allele in *Prunus*. The first intron of the *S-RNase* represents an autapomorphy among plant I_2-type *RNases* and was clarified to be a phase one intron that lies in a glycine residue. Glycines are often found at the signal-sequence cleavage site favouring intron insertion in phase one in any of the four GGN codons. On the contrary, the *S-RNase* second intron, a phase zero intron, is present in all I_2-type *RNases* including an ancestral form of *S-RNases*, which confirms its more ancient origin.

Sequence analysis revealed that more SNPs accumulated in the S_C *RNase* than in the SFB_C. The S_C-*RNases* of different cultivars differed

more than their corresponding *SFB* sequences. New specificities may arise by accumulating SNPs in both the pollen and pistil genes with *S*-RNases less tolerant of such mutations. However, when pollen carries a loss-of-function mutation, the absence of selection favouring the preservation of SI may permit the increase of mutations in the *S-RNase*. This could be successfully used to deduce the origin and dissemination routs of several apricot cultivars from Asia to Europe.

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New self-incompatibility alleles in Hungarian and Eastern European almond [*Prunus dulcis* (Mill.) D.A. Webb] cultivars

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Almond shows gametophytic self-incompatibility controlled by a single locus with multiple variants, termed *S*-alleles. The *S*-gene product in styles is a ribonuclease enzyme (*S*-RNase). An *S*-allele for self-compatibility allows self-fertilization, whereas alleles for self-incompatibility arrest pollen tube growth when the same allele is present both in the pollen and the style. It has also considerable implications for cultivation and breeding as diploid cultivars sharing both of their *S*-alleles cannot fertilize each other. To date, 30 self-incompatibility (S_1–S_{30}) alleles have been identified in North American and Mediterranean accessions and one allele (S_f) that allows for self-compatibility. Application of DNA-based molecular markers allows for the early selection of the common *S*-alleles.

In this present study, 26 almond cultivars of different geographic origin (Hungary, Ukraine, Moldavia, France and Italy) were analysed by PCR amplification of the first and second intron regions of the *S*-RNase gene. PCR fragments were cloned into a pGEM-T Easy plasmid vector and sequenced.

Altogether, 11 alleles could be detected in the tested 26 almond genotypes. Sequence analysis revealed at least two new *S*-RNase alleles. One of them was found in a Hungarian cultivar, ‘Tétényi bõtermõ’ and was labelled as S_{31}. Since S_{31} is characterized by almost identical intron sizes as S_9, a relatively widespread almond *S*-allele; an allele-specific forward primer was designed to anneal selectively within the second intron of the S_{31}-RNase gene. This allowed for the successful discrimination of S_{31} from

S_9. S_{32}, another previously unidentified almond allele is carried by 4 cultivars Size of its first intron region amplified by the PaConsI-F and EM-PC1consRD primers was 195 bp, while the length of the second intron region amplified by the EM-PC2consFD and EM-PC3consRD primers was 484 bp.

Our results can be applied to assist almond breeding programs with the ability of a reliable *S*-genotype identification of unknown cultivars.

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Cellular and molecular analysis of sporophytic self-incompatibility in yellow passion fruit plants

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Flowering plants have evolved various genetic mechanisms to circumvent the tendency for self-fertilization created by the close proximity of male and female reproductive organs in a bisexual flower. Self-incompatibility (SI) is the most important and widespread mechanism to promote outbreeding and gene flow through pollen in angiosperms. The SI is a genetic barrier used to avoid inbreeding in hermaphrodites that function through recognition and rejection of pollen expressing the same allelic specificity as that expressed in the pistils. In many species, SI is controlled by a single multi-allelic S locus, and genes residing at this S locus are proposed to be responsible for a highly specific recognition event. Rejection of pollen occurs when the S-allele carried by the haploid pollen matches either of the two alleles in the pistil. The genus *Passiflora* has a sporophytic SI. The identification of SI genes and comprehension of cellular events are necessary for understanding sexual reproduction in this species. The purpose of the present research was the identification and observation of cellular and molecular features involved in recognition and rejection of the incompatible pollen in yellow passion fruit (*Passiflora edulis*). The anatomical analysis of pollen-pistil interactions were made one hour after cross – and self-pollination. The incompatibility response in yellow passion fruit induces a reduced growth of pollen tube, accompanied by disorganization of the protoplasm. This disorganization is verified by differe position of the protoplasm structures when compared to compatible tubes and to what is expected for a normal tip growth pattern. In addition, we

have followed PCR and restriction enzyme-based methodology and sequencing analysis for the characterization of genes associate with sporophytic SI in the yellow passion. The results have showed high similarity to genes involved in *Brassica* sporophytic SI. In conclusion, yellow passion fruit incompatible pollen tubes present an early cytological response, also, it is possible that this species presents a S-locus gene similar to a S-gene from other species with sporophytic SI.

The comparison of activity and isoenzyme patterns of some stress enzymes in the style tissue of *Petunia hybrida* following compatible and incompatible pollination

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Petunia hybrida has both gametophytic self-compatible and self-incompatible cultivars. Gametophytic self-incompatibility is a mechanism by which pollen tube growth is inhibited in self-pollination in the upper zone of the stylar tissue. Some stress enzymes are involved in self-incompatibility responses. In this investigation, the activity of some stress enzymes including catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX) were compared between self- and cross-pollinated styles of Bravo cool water mix cultivar (self-compatible) and Bravo purple star cultivar (self-incompatible) of *Petunia hybrida*. Isoenzyme patterns of these enzymes were also investigated in self- and cross-pollinated styles of these cultivars. The highest activity of POX and CAT was found in self-pollinated Bravo purple star styles than the others while the activity of APX did not show any significant differences between self- and cross-pollinated styles in both cultivars. Two CAT isoenzymes and one POX isoenzyme were detected in all samples. Thus there was no variation in either CAT or POX isoenzymes pattern among self- and cross-pollinated styles in both cultivars. Three APX isoenzymes were found in self- and cross-pollinated styles in Bravo purple star cultivar while there were two isoenzymes in self- and cross-pollinated styles in Bravo cool water mix cultivar. Based on our results, it seems that there is a correlation between incompatible pollination and stress enzymes activity in the style of *Petunia hybrida*.

Keywords: Petunia hybrida, self-incompatibility, catalase, peroxidase, ascorbate peroxidase

Pollen Germination in *Arabidopsis thaliana* and across the Brassicaceae

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Pollen grains deliver and release pollen tube cells to the receptive stigma surface of the flower. The long-taught model of focused pollen cell release, or germination, is that stigma fluid enters a desiccated pollen grain through an aperture in the pollen wall, thereby directing the pollen tube to exit the grain through that same aperture. We have found that *Arabidopsis thaliana*'s pollen germination differs from this model; *Arabidopsis* pollen tubes frequently break directly through the exine wall precisely at its point of contact with the stigma surface, regardless of aperture position. We now believe that the focused rupture of the wall biomechanically involves local swelling of a pectin gel beneath the exine, likely together with degradation/weakening of exine structure above the pectin bulge. Using the atomic force microscope, we have measured material properties in various regions of the *A. thaliana* exine wall. We also have begun a survey of pollen germination behaviors across taxa, focusing especially on the Brassicaceae, pollen that land on dry stigmas, and inaperturate pollen (which by definition must rupture the exine wall), and now report that *A. thaliana* pollen is not alone in its germination behavior. Out of over 30 Brassicaceae family members studied, we have found eight species, so far, that germinate by breaking through durable wall, despite nearby apertures. These eight species are distributed across six different tribes. Additionally, we find quantitative differences in aperture use behaviors between: 1) the break-out species (ranging between 12 and 67% break-out), 2) *A. thaliana* ecotypes (ranging between 41 and 67% break-out), and 3) in vivo and in vitro germination conditions (break-out declines by almost half in vitro). We seek both phylogenetic patterns in germination characteristics and ecological sense for the variations we see in these characteristics, across species and among ecotypes.

Embryogenesis in *Dyckia pseudococcinea* L. B. Smith (Bromeliaceae) - a species endemic to the Restingas of Maricá (Rio de Janeiro - Brazil) threatened with extinction

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Dyckia pseudococcinea, an endemic species from Maricá/RJ, is listed as critically endangered according to the List of the Brazilian Flora Threatened with Extinction, principally due to anthropogenic pressure on the region. In order to preserve this species, studies were undertaken to describe its life cycle (with emphasizing the development of its reproductive structures) with the objective of establishing new conservation techniques. In order to generate basic data for these efforts, studies were undertaken to describe the embryo development of *D. pseudococcinea*. Fruits in distinct stages of development were collected in the Ericaceae Restingas of Maricá/RJ, fixed in 2.5% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.2), dehydrated in an ethanol series, embedded in hydroxyethyl methacrylate, sectioned with a Shandon Hypercut rotary microtome, and subsequently stained with toluidine blue O 0.05%. Sectioned material was photographed using an Olympus BX-50 optical microscope coupled to a CoolsnapPro digital camera. Embryogenesis in *D. pseudococcinea* initiates with the establishment of the sporophytic zygote, whose first division is asymmetrical and transversal – giving origin to the apical and basal cells that compose the two celled proembryo. After a number of cell divisions, both cells have contributed to the subsequent developmental stages of the embryo, characteristic of embryogenesis in the Asteraceae – starting with the tree celled proembryo and passing through the four celled, five celled, six celled, quadrant, octant, post-octant, globular embryo, and reniform and cotyledonal phases – until the mature embryo form, this being composed of the cotyledon (which expands laterally over the shoot apex, in the form of a sheath) and suspensor. The present study identified "critical" stages in the embryogenesis of *D. pseudococcinea* and established parallels between the distinct phases of embryogenesis and fruit morphology and size – thus providing a basic understanding of these processes and establishing foundations for future protocols for the *in vitro* culture of the embryos of this species and needed conservation efforts. (Capes/CNPq).

ORAL CONTRIBUTION

Immediate paternal genome activation and enhanced *trans*-regulatory interactions in early maize F_1 hybrid embryos

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Heterosis results from the combination and the expression of genetically-distant genomes after fertilisation. We observed high levels of heterosis in early hybrid maize embryos indicating a major contribution of both parental genomes to this early phase of kernel development. Indeed, transgenic paternal mRNA and protein synthesis coincides with male chromatin decondensation in zygotes 3 to 4 hours after fertilization. To further explore the activation of the paternal genome after fertilization and the contribution of regulatory mechanism to early development we have analysed the allele-specific expression of 25 genes following fertilisation of the egg in maize. Sequence comparisons indicate these genes to be involved in a range of processes, and to be distributed throughout the genome. Our data confirm, in contrast to the situation in other plants and in animals, equivalent parental genomic contribution to the maize zygote. Every gene expressed before the first cell division of the zygote showed paternal transcripts. The maternal effects in zygotes exhibit a mean value below 20% and disappear completely until 6 days after fertilization. Concerning the regulatory mechanisms leading to variation of gene expression in hybrid embryos compared to their parental lines we found large differences at 6 and 8 days after fertilization. Taken the mean of both reciprocal hybrids at 6 days *trans*-regulatory differences are attributabl to the intraspecific variation of gene expression for 82% of the genes analysed whereas at 8 days only 42% of the genes were influenced by *trans*-regulatory interactions.

Our findings confirm that maize evolved a strategy to activate the paternal genome immediately following fertilization providing an explanatio for the early appearance of hybrid vigour in maize embryos. Large-scale change of gene expression regulation may be related to the modulation of the diverse genomes after crossbreeding.

Seed coat, aleurone layer and endothelium in two leguminous species (*Cytisus striatus* and *C. multiflorus*)

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Abstract

The genus *Cytisus* Desf., included in the tribe *Cytiseae* Bercht & J. Presl, consists mostly of shrub-like species occurring in Europe, North Africa, the Canary Islands and Western Asia. *Cytisus multiflorus*, endemic shrub of the western Iberian Peninsula and with white flowers, and *C. striatus*, endemic shrub of the western Iberian Peninsula and north-west Morocco and with yellow flowers, present a monosporic *Polygonum*-type of megagametogenesis and a megasporogenesis characterised by the formation of a triad of cells. Their ovules are ana-campilotropous, bitegmics with both integuments two-layered, and crassinucelates. The seeds present a characteristic aril of funicular origin.

Ovaries from hand cross-pollinated flowers of both species were embedded in resin and cut at 3 µm thick sections. Then, these sections were stained with PAS and hæmatoxylin solution, mounted in Eukitt and studied under a light microscope.

Seed development involves not only the development of the embryo and endosperm, but also the formation of others structures: (a) the endothelium, an ephemeral structure, which it is derived in early phases of seed development from the inner integument of the ovule; (b) the aleurone layer, a not ephemeral structure, differentiated in late phases of seed development from the outermost layer of the cellular endosperm, which stays at the mature seed. Besides that, in mature seed, the embryo is protected by the seed coat which is exclusively formed by the outer integument in both species. This seed coat mainly consists on two layers (palisade and osteosclereids layers). The palisade layer – thick-walled columnar cells – comes directly from the outermost cell layer of the outer integument. The osteosclereids layer – hour-glass cells – comes from the innermost cell layer of the outer integument.

Identifying *cis*-Regulatory Elements for Embryo Region Specific Transcription

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In most higher plants, the asymmetrical division of the zygote produce a small apical cell, which gives rise to most of the embryo proper, and a large basal cell, which generates the suspensor. The embryo proper becomes the next generation plant, whereas the suspensor degenerates by the end of embryogenesis. The Scarlet Runner Bean *G564* gene is expressed specifically in the suspensor during the early stages of seed development; however, *G564* is expressed in the embryo proper during the later stages of seed development. In order to understand cell fate specification in the embryo, it is necessary to identify the *cis*-regulatory elements controlling transcription in the suspensor and embryo proper. I am using mutagenesis to identify suspensor and embryo proper *cis*-regulatory sequences in the upstream region of the *G564* gene. When fused to the *b-glucuronidase* (*GUS*) reporter gene, the -662 to +56 upstream region of *G564* lacks suspensor activity, but is expressed in the later embryo proper. One 10-bp sequence (GAAAAGCGAA) added to *G564* -662 to +56 is able to rescue suspensor activity, but has no effect on transcription in the embryo proper. Therefore, *G564*-662 10bp-1 contains both suspensor and embryo proper *cis*-regulatory sequences. The 10-bp sequence cannot activate suspensor transcription on its own when fused to the Cauliflower Mosaic Virus 35S minimal promoter. Therefore, it may be acting with a sequence in the -662 to +56 region. In order to identify important *cis*-regulatory sequences, the *G564*-662 10bp-1 construct can be mutagenized to reveal both embryo proper and suspensor *cis*-regulatory sequences. Every 45-bp of the *G564* –662 to +56 upstream region was replaced with a mutation sequence that is transcriptionally inactive in the embryo. The mutated upstream regions were fused to the *GUS* reporter gene, and *GUS* enzymatic activity was observed in transgenic tobacco embryos. I am currently analyzing the data to determine which sequences in the *G564* -662 to +56 region are important for suspensor and embryo proper transcription.

ORAL CONTRIBUTION

Cis-Regulatory Sequences Responsible for Suspensor-Specific Transcription

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How cell fates of the early-stage embryo are specified, and what gene regulatory networks control differentiation in the embryo are major unanswered questions. The Scarlet Runner Bean *G564* gene is activated primarily at the transcriptional level in the basal part of the embryo shortly after fertilization. We previously showed by 5'-deletion experiments that there are five repeat sequences present in tandem in the upstream region of the *G564* gene and that suspensor-specific transcription is abolished when all repeat sequences are deleted in transgenic tobacco plants (Weterings et al., Plant Cell, 13, 2001). We carried out gain-of-function experiments and discovered that one repeat sequence is sufficient for transcription in the suspensor. Computational analysis identified a conserved 10-bp motif within the repeat that is also present in the upstream region of other suspensor-active genes. In the repeat sequence, there is also a sequence (10bp-like motif) similar to the 10-bp motif, and mutagenesis experiments showed that both the 10-bp and the 10bp-like motif regions are required for suspensor-specific transcription. Further gain-of-function experiments revealed that a 54-bp fragment containing both motifs is sufficient for suspensor-specific transcription. Interestingly, another gain-of-function experiment revealed that the fifth repeat sequence could not activate transcription in the suspensor. Sequence comparison analyses among suspensor-active repeat sequences and the suspensor-negative fifth repeat sequence discovered suspensor *cis*-regulatory sequence regions in addition to the 10-bp and the 10bp-like motifs. We are performing yeast one-hybrid analysis to obtain factors interacting with the suspensor *cis*-regulatory sequences.

To unravel whether the suspensor gene regulatory network is conserved among plants, the upstream region of the suspensor-active loblolly pine gene *(PtNIP1;1)* was tested and identified to be active in the suspensor of transgenic tobacco embryos. This implies that the machinery regulating suspensor-specific transcription is conserved not only within angiosperms, but also in gymnosperms. Deletion and gain-of-function experiments are being carried out to understand how the pine upstream region regulates suspensor transcription.

Maternal to zygotic transition occurs in zygote stage and the de novo transcripts are essential for triggering embryogenesis in tobacco

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In angiosperm, double fertilization is followed by embryogenesis and endosperm formation. The maternal-to-zygotic transition (MZT) is a major developmental switch during early embryogenesis. In animals, it is clear that there is a delay between fertilization and the maternal-to-zygotic transition and early embryogenesis is largely controlled by maternal transcripts deposited in the egg cell before fertilization. However, in higher plants little is known about the MZT. We combined the large-scale cDNA analysis of egg cells, zygotes and 2-celled proembryos and several egg-cell microculture systems with transcription inhibitor treatment to seek its possible mechanism in tobacco.

We generated cell type-specific cDNA libraries of isolated eggs, zygotes and two-celled proembryos respectively and finally got total of 6469 ESTs. Bioinformatics analysis together with RT-PCR confirmation revealed that some transcripts especially enriched in egg cells soon degrad after fertilization. At the same time, de novo transcripts were gradually appeared in fertilized egg cells. Functional category analysis of the unigene indicates that some of metabolism pathways were activated after fertilizatio Compared with egg cell, zygotes and two-celled proembryos share more similarity in transcriptome, implying a obvious transcript profile reconstruction after fertilization. Our data suggest that MZT already initiate before first zygotic division in tobacco.

In order to further confirm the MZT occurrence after fertilization and reveal the biological impact of the MZT during early embryogenesis, we employed in vitro and semi-in vivo embryogenesis systems to culture zygot at different stages with transcription inhibitor application. Our results show that the maternal transcripts deposited in the egg cell are not enough to trigger embryogenesis. However they do support the zygote development in several important aspects. This suggests an essential role of the de novo transcriptions for zygote directional elongation and the first cell division.

Keywords: *Nicatiana tabocum*, egg, zygote, 2-celled proembryo, cDNA library, Expressed sequence tag (EST), Maternal-to-zygotic transition.

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The role of synergids in the reproductive success of Brazilian *Mucuna* species (Leguminosae, Faboideae)

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In general plants abort their low genetic quality embryos, because they are unable to provide resources to develop all fruits and seeds. In plants which show high fruit and seed abortion rates, anomalies are observed during embryo sac, embryo and endosperm development or changes in the ovular tissues, as for instance callose and lignin deposition in the vascular region. The nutrition of the ovules and the seeds is one of the main factors that leads to fruit and seed abortion. Some haustoria types can absorb and carry maternal resources from adjacent tissues to endosperm and embryo, providing embryo development.

There are few studies about the influence of embryological processes in fruit and seed abortion in Leguminosae, especially in Faboideae subfamily. *Mucuna japira* (Leguminosae, Faboideae) occurs in restricted areas of the Atlantic Forest in southeastern Brazil, while *M. urens* is widely distributed over the country. Fruits and seeds of *M. japira* are aborted in different stages and rarely complete their development, whereas in *M. urens* they reach full development, resulting fertile seeds.

The goal of this study was to compare some embryological data of both *Mucuna* species, in order to verify the occurrence of morphological anomalies in their reproductive processes that causes fruit and seed abortion.

Some features in *M. japira* and *M. urens* were studied in flowers in anthesis and during the first stages of fruit development. Bagged pistils were hand-pollinated with self and cross pollen grain and collected 6, 12, 24, 48, 72 and 96 hours after. Serial sections (12-14 ìm thick) were made from fixed materials (Karnovsky solution for 24 hours), gradually dehydrated through a tertiary butyl alcohol series and embedded in paraffin. Sections were stained in Safranin O and Astra Blue.

A micropilar zygote is formed after 48 hr in cross pollinations in *M. japira* and after 12 hr in *M. urens*. Fruit and seed abortion is probably related to processes that occur after fertilization in both *Mucuna* species. No embryo was observed in any of the *Mucuna* species, but *M. urens* seeds follow their development.

Synergids in *M. urens* do not degenerate after fertilization, a rarely observed feature in legumes. Instead, in *M. urens* synergids become haustoria and penetrate in the inner and outer integuments towards the micropilar region. The function of haustorial synergids seems to be

absorption and transportation of nutrients, which has been described for Acanthaceae, Asteraceae, Crassulaceae, Poaceae and Santalaceae. In *M. japira* haustorial synergids are absent; thus nutrient transportation is deficient, which probably promotes the abortion of their fruits and seeds. This is the first report of haustorial synergids for Leguminosae, a feature worth of more research to support some phylogenetic studies.

Other features that can be related to fruit and seed abortion are:

1) In both *Mucuna* species, the embryo sac is not completely covered by the nucellar region, but in *M. urens* nucellar cells besides having prominer starch grains, they cover the embryo sac in a wider extension than in *M. japira*, indicating that *M. urens* has more efficient mechanisms to acquire nutrients;

2) In *M. japira* many starch grains occur around the micropyle probably associated to phenolic compounds. It is possible that this starch is unavailable for embryo nutrition. In *M. urens* no starch grains were observed around the micropyle, suggesting that this resource has been transported to perform embryo nutrition;

3) In both *Mucuna* species, the nucellar region becomes isolated by callose deposition 96 hours after cross pollinations. Callose can block nutrient transportation resulting in embryo abortion.

The conclusion of this study is that different embryological features of these two *Mucuna* species are related to fruit and seed abortion, and haustorial synergids deserve special attention because it is a rarely observe feature in the Leguminosae family.

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ORAL CONTRIBUTION

A novel pistil-specific methyltransferase gene is capable of producing jasmonate, benzoate and salicylate in vitro and is probably responsible for the jasmonate emission of mature *Nicotiana tabacum* L. flowers

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To have a better understanding of the plant reproductive process, it is necessary to investigate the function of the protein products encoded by pistil-specific genes. Through the differential screening of a *Nicotiana tabacum* stigma/style cDNA library we have identified a pistil-specific cDNA clone (PA3) encoding a protein with high similarity to methyltransferases of the SABATH protein family. This protein family encompasses a group of related methyltransferases (MTs) that catalyze the *S*-adenosyl-L-methionine (SAM)-dependent methylation of natural compounds, like benzoic, salicylic and/or jasmonic acids. The corresponding esters (benzoate, salicylate and jasmonate) have been reported to be involved in important plant processes, as pathogen and herbivore defense, pollinator attraction, plant development and interplant signaling. As shown by TBlastX analysis at the NCBI, the PA3 has the highest similarity with the SAM-dependent benzoic acid/ salicylic acid carboxyl methyltransferase (BSMT) of *Nicotiana suaveolens*. Experiments performed in our laboratory have demonstrated the existence of several pistil-specific methyltransferase transcripts, containing segments of complete identity to PA3 sequence, probably produced by a complex alternative splicing process of 8 exons (Calixto et al., in preparation). According to these results, PA3 represents a cDNA composed of only 3 (exons 1, 6 and 8) of the 8 possible exons. A cDNA, containing the 8 exons, was obtained by RT-PCR from stigmas/styles mRNA and corresponds to the expected complete MT sequence, as previously described in the literature. To investigate the function(s) of the pistil-specific methyltransferase transcripts, we have cloned the cDNAs 46B11 (exons 1, 2, 3 and 4), 134B02 (=PA3) and the complete cDNA in expression vectors of the Gateway (Invitrogen) and/or pET (Novagen) systems. The expression plasmids were introduced into different *E. coli* strains [BL21(DE3); BL21(DE3) Codon Plus-RP and BL21(DE3) Rosetta]. To optimize the protein expression, different conditions were tested, including different temperatures (30ºC, 37ºC, 20ºC and 22ºC) and induction periods (1h, 2h, 3h, 4h, 5h and 20h). We have successfully obtained large amount of all three recombinant proteins. The recombinant proteins from the 46B11 and 134B02 cDNA clones (r46B11 and r134B02) were used to produce polyclonal antibodies in BALB/c mice. The two antibodies have shown high immunoreactivity and distinct specifities. Western blot experiments performed with crude protein extracts from tobacco stigmas/styles and ovaries and the r46B11 antibody were able to expose the presence of the 46B11 native protein in ovary samples, demonstrating that at least this short transcript is translated in plants. This antibody did not recognize the putative protein products of the additional methyltransferase transcripts in the plant extracts. As a preliminary step in determining the substrate(s) of

these pistil-specific methyltransferases, the recombinant protein produced by the full-length cDNA was assayed in the presence of equal amounts of each of the three substrates, separately. Despite the highest similarity with BSMTs, the recombinant protein was shown to efficiently produce methyljasmonate by a GC-MS approach. Methylbenzoate is also produced, but with lower efficiency and methylsalicylate is hardly detected. To our knowledge this is the first MT shown to have activity over these three substrates. The results obtained *in vitro* are in accordance with our results of the in vivo emission of mature *N. tabacum* flowers, in which only methyljasmonate was detected by GC-MS. Additional experiments are in progress to study the role and possible enzymatic activity of the protein products of the other shorter pistil-specific methyltransferase transcripts.

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Mating system and pollen flow in Brazilian urban population of *Tabebuia roseo-alba* (Ridl. Sand. - Bignoniaceae): implications for conservation

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Ipê branco or Guayacan blanco (*Tabebuia roseo-alba*) is a semideciduos tree with explosive white flowering in August-September. Valued as a timber tree, it has been widely planted for both reforestation and ornamentation. Understanding the effects of the spatial isolation on the levels of genetic diversity and gene flow has become a tool of fundamental importance to providing recommendations for *in situ* and *ex situ* conservation of species. Presently, no data about the fine-scale genetic structure of *T. roseo alba* are available. In this study, we used five highly informative microsatellite loci previously transferred from *Tabebuia aurea* aiming to study the parameters of mating system and pollen dispersal of *T. roseo alba* in an urban area located in southest Brazil. For this purpose, three hundred open-pollinated seedlings (twenty individual per family) derived from 15 *T. roseo-alba* seed-trees were sampled in an urban area of Ribeirão Preto (SP) Brazil. The genomic DNA was extracted from leaf tissue using

a cetyltrimethyl ammonium bromide (CTAB) extraction method. Mating system analysis, using mixed-mating model, showed that the studied population is a mixed mating system ($t_m = 0.682$), predominantly alogamous. Significant deviations from random mating were detected for mating among relatives ($t_m - t_s = 0.266$, $P < 0.05$) and correlated mating ($r_{p(m)} = 0.767$, $P < 0.05$), indicating inbreeding in the population. The multilocus outcrossing rate combined with the correlated mating rate has suggested that 52.3 percent of offspring are full-sibs, indicating the possibility of family structure within population. Inbreeding was reflected in the positive and significant fixation index observed in adult trees ($F = 0.259$, $P < 0.05$) and in offspring ($F = 0.39$, $P < 0.05$). These results can reflect the isolation of the trees due to urbanization of the area. Also can be a natural characteristic of its reproduction system, since *T. roseo-alba* flowered massively for approximately three days, limiting the pollen exchange. The effective number of pollen donors mating with each seed-tree was determined to be low ($N_{ep} \sim 3$). The average of pollen flow distance was measured in side of the plot by TWOGENER analysis and showed a pollen dispersal of the 121 m and 137 m for normal and exponential models, respectively. Considering that the studied genotypes were collected from 15 seed-trees and as each family represents the average variance effective size 1.78, it is possible to conclude that the effective population size retained in this urban population is 26.7. Then, to retain in *T. roseo-alba* seed pool samples the effective population size of 100, will be necessary to collect seeds from at least 85 seed-trees.

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Cherimoya dichogamy system (*Annona cherimola* Mill.).

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En chirimoyo, especie cantaridofílica y caso extremo de dependencia de su vector de polinización, la dicogamia protogínica y la ausencia del polinizador original en sus zonas de cultivo limitan fuertemente la producción de fruta. En algunas zonas se observa un cierto cuajado natural que algunoss autores asocian a la presencia esporádica de insectos poco eficientes. Generalmente el ciclo de la flor dicógama se ha descrito bajo criterios morfológicos, poco informativos, sin atender su correspondencia con

cambios fisiológicos en las funciones femenina y masculina. En la flor del chirimoyo el tránsito entre ambas funciones se ha definido por el grado de apertura de los pétalos. En el presente trabajo se caracterizó la dicogamia tanto morfológica como funcionalmente, a nivel de órgano floral, flor, árbol y plantación. La duración de la fase femenina en base al grado de apertura de los pétalos se cifró en 24-26 h, y en 28-32 h cuando se evaluó la funcionalidad de los órganos femeninos. La receptividad de los estigmas determinó la duración de la fase femenina, ya que los óvulos estuvieron maduros y viables a lo largo del ciclo de la flor. La fase masculin se inició con la dehiscencia gradual de las anteras hacia las 13:00 h del segundo día, con una antelación importante a su manifestación morfológica El EPP se cifró en 24 h, siendo la ER el parámetro limitante su duración fue variable y muy dependiente de la HR. En ambientes con HR elevada se observó la prolongación de la receptividad estigmática a la fase masculina, lo que explicaría la adhesión de polen en flores vírgenes y el errático cuajado natural. Ni el viento ni los insectos presentes intervinieron en la polinización del chirimoyo. El periodo de receptividad estigmática coincidió con un incremento variable en la temperatura de la flor, que no se observó nunca durante la fase masculina. El grado de sincronización de las flores de un mismo árbol y entre árboles de la misma parcela fue elevado como cabía esperar por tratarse de una parcela monovarietal. La dicogamia y la sincronización de las flores resultaron en una muy escasa deposición de polen en los estigmas en ausencia de vectores de polinización, no superand los 10 granos de polen por flor al final del ciclo. Se concluye que esta adhesión fue producto de ciertos niveles de autogamia, debido a pequeños solapes entre las funciones femenina y masculina.

Elements of the reproductive biology of Brazilian landraces of sweet potato

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The objective of this work was to study elements of the reproduction of sweet potato (*Ipomoea batatas* L. (Lam.)), *viz*., incompatibility, pollen and flower morphology and pollen viability. Crosses and selfing were performed in order to identify cross- and self-compatibility types. Incompatibility was studied in 13 landraces from the Ribeira Valley, São Paulo State, by measurement of the fruit production and *in situ* observation of the pollen tubes in the pistil of hand-pollinated flowers using

epifluorescence microscopy. A viability test was performed using acetic carmine and tetrazolium chloride in pollen grains collected in the field in four different times along the day (6:00 AM; 8:00 AM; 10:00 AM and 12:00 AM). In order to detect a possible association between incompatibility and flower morphology, several flower characters were measured: corolla length and diameter, calyx length, stigma height and anther height. Pollen morphology was observed with scanning ultramicroscopy.

Fruit production occurred only in cross-pollinated flowers. Among 78 crosses performed between 13 landraces, 46.1% produced fruit. Pollen tubes were observed in the pistils of self-pollinated flowers in 38.5% of the landraces and in 85.9% of the crosses in the cross-pollinated flowers. Therefore, pollen tube penetration in the pistil is not a sufficient condition for crossing success. Part of the reproductive failure is caused by phenomena occurring in the ovule, corroborating the hypothesis that sweet potato has a complex sexual reproductive system. Most landraces had a pollen viability rate over 80% when dyed with carmine, but below 70% when dyed with tetrazolium. However, the variation in pollen viability among the four collecting times was insignificant with either dye. All the flower variables measured showed significant differences at 5% probability, revealing floral polymorphism. Even heteromorphism (within-sporophyte pollen polymorphism), which is rather rare, was found, and 9 pollen morphotypes were detected in the sample. Four well-defined patterns of the relative position of the stigma and the highest anther were observed: stigma well above, slightly above, at the same level as, and below the anther. However, no association was found between floral morphology and incompatibility patterns. Successful pollen tube penetration was found both in crosses between plants with different morphotypes and in crosses between plants with the same morphotype. *(work funded by FAPESP / BIOTA, grant n° 2002/09467-4)*

In vitro germination of *Calophyllum brasiliensis*

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The *Calophyllum brasiliensis* Cambess belongs to the family Clusiaceae and possesses a beautiful and resistant wood whose qualities have been

compared to mahogany. The seeds of this species are recalcitrant and can be storage for only eight months. Its germination may take up to 145 days and the average germination varies from 15 to 95%. In order to turn its propagation in large scale viable, it is important to optimize the germina of this species. In this work seeds were immersed in different concentration of GA_3 (0, 288.68; 577.36; 866.05 and 1154.73 µM) during 24 hours, disinfected and inoculated in solid WPM medium. Seeds were also disinfecte and inoculated in solid WPM medium supplemented with different concentrations of GA_3 (0, 5.77; 11.54; 17.32 and 23.09 µM). Germination was evaluated after 30 days of inoculation. The results indicated that the addition of the 23.09µM GA_3 increased germination from 20 to 73%.

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Keywords: Guanandi, WPM, GA_3.

Head Structure and Sexual Expression in *Lucilia lycopodioides* (Less.) Freire (Asteraceae)

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Representatives of *Lucilia lycopodioides* (Less.) Freire (Asteraceae) present rhizophores (vegetative growing unities) and seeds with low germination rates. These phenomena seem to be associated, and are commonly found in Cerrado species. This work aimed to evaluate the head structure and the sexual expression of *L. lycopodioides*, to improve the knowledge on its fertility. Type, number, distribution and structure of flowers were evaluated in 117 heads, 25 plants and 1818 flowers of three populations: Botucatu (SP), São Roque de Minas (MG) and Ouro Preto (MG). Homogamic heads (a single type of flower – hermaphrodite or pistilate) with 2-24 flowers and heterogamic heads (hermaphrodite and pistilate flowers) with 10-29 flowers were found. Hermaphrodite flowers were more commonly found in the central region of head, and pistilate flowers were found in the head periphery. *L. lycopodioides* seems to be a gynodioecious species: hermaphrodite flowers were male sterile in two plants of Botucatu; and all flowers were morphologically pistilate in one plant of São Roque de Minas and three plants of Ouro Preto. Reduction in the male function was also shown by the low rates of anther-ovule: 1.5:1 in Botucatu; 3.5:1 in São Roque de Minas; and 2.3:1 in Ouro Preto,

considering that the anther and ovule numbers of hermaphrodite flowers in *Lucilia* are generally five and one per flower, respectively. Ginodioecy (coexistence of female and hermaphrodite flowers in different plants) more frequently results of the polymorphic occurrence of male sterile genes in otherwise hermaphrodite populations. It can often form an intermediate stage in the evolution of full dioecy, as already related for Asteraceae. Although gynodioecy is a reproductive mechanism which surely leads to the xenogamy, many abnormal features were observed in *L. lycopodioides* heads, as for instance: pollen grains partly developed and unviable; anthers completely sterile; hermaphrodite flowers with lower or upper numbers of stamens than usually found in *Lucilia* (five); and some male flowers in three plants of São Roque de Minas. These abnormalities and the grouped distribution of representatives, forming small and rare spots in Cerrado drylands, suggest that crosses could be occurring between clones, probably originated from vegetative growing. Thus, populations studied of *L. lycopodioides* must be under inbreeding depression effects (FAPESP).

Floral attractants and rewards in loquat (*Eriobotrya japonica* Lindl.) trees subjected to summer drought

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Loquat (*Eriobotrya japonica* Lindl., Fam *Rosaceae*, subfam *Maloideae*) is a subtropical fruit tree indigenous to southern China, brought to cultivation into in the Mediterranean Basin in the last century. Loquat blooms in November (Northern Hemisphere) forming terminal panicles with more than 200 flowers. The fragrance of loquat flowers attracts a diversity of insects seeking rewards in a date when no many other alternative food sources are available for pollinators. In its native region, loquat blooms heavily after a period of summer rain. In contrast, in Mediterranean climates, naturalized loquats experience recurrent periods of summer drought. With the aim to check how a period of drought may affect loquat attractiveness to insects, we have compared flower size, rewards, attraction to pollinators and reproductive success in fully irrigated trees versus trees in which a summer drought was imposed by withholding irrigation during a period of

8 weeks prior to flower development. Flower number and size, nectar composition and aroma production were chosen as the most significant attractants and rewards to pollinators. Pollen transfer by insects (mainly *Apis mellifera* and *Bombus terrestris*) was measured by pollination level (percentage of flowers visited), uniformity (percentage of stigmas having pollen grains on; each flower has five stigmas), and intensity (pollen load per flower). Reproductive success was compared by fertilization rate 8 days after hand-pollination, fruit seediness and fruit set.

Water stressed loquat (W-S) reached full bloom 21 days before than well-irrigated loquats (Controls) did. Although both treatments produced a similar amount of flowers (223 flowers per panicle versus 229, in controls and W-S, respectively), the panicles of W-S trees were significantly larger and looser. Flowers were, on the contrary, lighter partly due to reduced petals and gynoecia dry weights. Stamens size was not modified by summer drought. However, pollen viability improvement in loquats subjected to water stress (49.9% viability in W-S versus 29.6% in controls) suggests a certain level of reallocation of resources to the male function of the flower. Nectar volume was not measured, but the flowers from well-irrigated trees formed sweeter nectar due to a higher content of glucose, fructose and sucrose. Differences were, nevertheless, non-significant ($p>0.52$) due to the variability found among replications

The main volatiles components of ‘in situ’ head space sampling of loquat aromas were identified as benzene ethanol, P-anysaldehyde and anisinic acid. These three compounds are the main responsible of the distinct loquat flower aroma and accounted for more than 75 % of the area under of the chromatograms of both treatments. Some modifications of flower aroma seem to be imposed by summer drought, probably thru changes in floral organs. The major shift was the high proportion of esters as 2-ethyl 1-hexanol found in the aroma of the flowers of W-S trees. These compounds often referred as ‘green leaf volatiles’, are commonly interpreted as general signals of stress in plants and exert a great attractio to pollinators.

Pollinator activity results suggest that water deficit during summer may indeed increase plant attractiveness to pollinators. The improvement in flower attractiveness in W-S loquats to pollinators reflected in a heavier pollen load per flower (222 pollen grains deposited on control flowers versus 321 pollen grains per flower in W-S trees; $p<0.05$), which led to slight, non-significant, differences in the percentage of fertilized ovules (36% versus 32%), seed per fruit (2.16 versus 1.60), and fruit per panicle (9.7 versus 7.9). The number of flower and stigma visited by insect and which pollen grains deposited barely changed (100% of tagged flowers were pollinated in both treatments and 87% of stigmas in control

and 82% in W-S flowers had pollen on). The extent in which the higher reproductive success in W-S trees was due to phenology modifications and/or due to increased attractiveness will be discussed.

Pollination and breeding system of *Campomanesia pubescens* (DC.) O. Berg (Myrtaceae) in a cerrado área, Mato Grosso do Sul, Brazil

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The reproductive phenology, pollination biology and breeding system of *Campomanesia pubescens* were studied in a population occurring on a cerrado area in Campo Grande, Mato Grosso do Sul, Brazil. *C. pubescens* is a shrub whose plants form groupings and present a flowering peak in September, flowering throughout one or two months. The fruition initiated in October, with peak of matureness in November, extending itself until December. The species possesss actinomorfic flowers, hermaphrodites, with 19,4 mm (± 4 mm) of diameter, "brush" like (opened with great amount of estames), hercogamic or not. They can be fit in the syndrome of the melittophily, therefore they possess diurnal antesis, clear coloration, landing platform, exhale pleasant odor and they are polliniferous. The species does not present agamospermy and is self incompatible, showing a smaller natural conditions (control) frutification rate (18%) than cross-pollination (76%). However the number of seeds in the control treatment was greater that after cross-pollination (7,0±0,9 and 4,6±0,7 seeds respectively). The most frequent visitor was *Apis mellifera* (frequency rate = 0,88), however its visits did not result in fruit-set, while *Bombus* sp., with a low frequency of visits (0,13), is the main pollinator of the species because it presents adequate intrafloral behavior (buzz pollination) and promoted a polen flow among plants, essential characteristic for the formation of fruits in *C. pubescens*.

Reproductive Biology and Hybridization of Two Cerrado *Adenocalymma* Species (Bignoniaceae)

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Late-acting self-incompatibility (LSI) is the most common breeding system in Bignoniaceae, occurring in around 80% species. Self-compatible and apomictic breeding systems are exceptions to the rule in this family. Polyembryony and sporophytic apomixis in Bignoniaceae seems to be related to hybridization and polyploydization as observed in *Tabebuia* and *Anemopaegma*. *Adenocalymma campicola* and *A. peregrina* are sympatric hemixyle shrub species which occurs mainly in disturbed Cerrado areas in the Triângulo Mineiro region, in Central Brazil. They show overlapping flowering period and similar floral morphology which suggest they may hybridize naturally. This work aimed to verify the possibility of hybridization between these species and see if it is associated to apomixis and polyploydy. Although previously circumscribed in the *Memora* genus, recent molecular phylogeny studies placed these species in *Adenocalymma*. The reproductive biology of *Memora* species is unknown, although some studies indicated LSI in *A. bracteatum* e *A. marginatum*. The present study was carried out in Cerrado areas around Uberlândia-MG in 2006 and 2007. We investigated floral and pollination biology, including nectar production, pollen viability and pollen tube growth. Experimental hand pollinations, verification of the number of embryos per seed, and chromosome number in root tips were also performed. The species flowered between January and June. The flower life span of *A. campicola* and *A. peregrina* was around 24 hours with flowers opening late in the morning. Flower morphology was very similar, except for the calyx morphology which showed glands and trichomes in *A. campicola* but was glabrous in *A. peregrina*. The flower visitor spectrum was also similar, with Centridini and Euglossini bees as the main pollinators, and *Trigona spinipes* and *Oxaea flavescens* as the main pollen and nectar robbers. Nectar concentration was higher in *A. campicola* 21% than in *A. peregrina* 12,5%. *A. campicola* produced nectar during all day, and the accumulated volume by the end of the afternoon was 32 ± 17ml (mean ± standard deviation). Nectar volume was difficult to measure in *A. peregrina* because many flowers were injured or produce no nectar at all. This difficulty in nectar

measurements seems to be related to the absence of glands and trichomes in *A. peregrina* calyx, which protect the ovary and the floral nectary from injuries by insects. Pollen viability was higher in *A. campicola* 96% than in *A. peregrina* 77.8%. Both species were mostly self-sterile. In *A. campicola* we obtained 39% fruit-set from hand cross pollination against 1.1% from spontaneous self-pollination and no fruit-set from hand selfing. In *A. peregrina* we found 28.3% fruit-set from cross pollination against 0.97% from hand selfing. *In situ* pollen tube growth analysis in *A. peregrina* and *A. campicola* showed that self- and cross pollen tubes grew down to the ovary at the same speed and penetrated many ovules in selfed pistils, indicating LSI as in most Bignoniaceae studied so far. All seeds analyzed showed just one embryo per seed without polyembryony and no other evidence of apomixis. Experimental hybridization resulted in 54.2% fruit set in *A. peregrina* and 27.3% in *A. campicola*, and mature fruits contained viable seeds. Hybrid seedling morphology was intermediate between both species but apparently closer to *A. peregrina*. Differences in hybrid fruit development between *A. peregrina* and *A. campicola* may be explained by the reduced pollen viability found in *A. peregrina*. Root tip chromosome counting was similar for both species and also for the hybrid seedlings, with chromosome numbers around 2n=40, which is the most common diploid number for the family. In conclusion, although the studied species are mostly self-incompatible, they show similar flowering phenology and floral visitors, and are capable to produce hybrid viable seeds. But hybridization does not yield polyloid and apomitic individuals as in other Bignoniaceae species complexes where hybridization putatively occurs. (CAPES/CNPq)

Study of the lethality in papaya (*Carica papaya* L.).

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The papaya is dioecious and hermaphrodite species and the sex determination in papaya is due to a gene with tree alleles (M_1, M_2 and m). The viable genotypes are M_1M_2 (hermaphrodite plants), M_2m (male plants), and mm (female plants). The literature reports the existence of zygotic lethality in the genotypes M_1M_1, M_2M_2, and M_1M_2. This study was done considering that the lethality can be female gametic or zygotic. Thus, it

was determined the ovule viability by ovary, in two papaya varieties, Golden and Tainung 01. To do the ovule viability, hermaphrodite and female flowers were collected in fixative solution of ethanol and acetic acid (3:1) and submitted to vacuum to guarantee a good fixative solution penetration. After fixation, the ovules/ovary were excised from the ovary wall and cleared in 8N NaOH solution for 24 h, stained overnight in 0,1 % aniline blue in 0,1 M K_3PO_4, mounted in the staining solution and gently squashed under a coverslip. The slides were observed under fluorescence microscopy. The digitalized images were captured by Image Pro-Plus Software (5.1 versions, Media Cybernetics). The callose deposition was used as the indicator of viable and nonviable ovules; it was expected that viable ovules doe not show callose deposition. The results indicated that the lethality observed in homozygote genotypes of papaya can be associate to the viability of ovules since it was observed in hermaphrodite ovaries 41% of viable ovules, on average, and female ovaries showed 75% of viable ovules. On the nonviable ovules the callose deposition was concentrated on the central cell of embryo sac, suggesting that the problem on the components of embryo sac instead of the integuments as it is registered in others species. This is the first time that is registered ovule lethality in papaya, since the lethality due to homozygote genotypes is manifested after double fertilization, during the seed development; so, it is observed abnormal seeds in the fruits. These results suggest that ovule sterility can contribute to this lethality and the *m* allele might restore the M_2 allele function in the hermaphrodite papaya fruits, since the percentage of viable seeds is higher that percentage of viable ovules.

Macairea radula: first report of floral heteromorfism in Melastomataceae

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Herkogamy and heterostyly can be interpreted, in part, as adaptations to reduce self pollination in homoic species. In Melastomataceae, herkogam is considered the principal mean to promote cross-pollination. The separatio between the reproductive elements is given by the difference in height between the stamens and style and anther dehiscence by apical pores. In *Macairea radula* were observed three floral morphs, characterized by differences in size, color and/or morphology of stamens and style. Further data about distribution of the morphs, functionality of the reproductive

elements, visitors and pollination mechanisms were taken from three populations occurring in the Serra da Canastra National Park, Minas Gerais state, Brazil. Measures of the heights of style and stamens from the base of the petals (n = 35) revealed the following floral morphs: in morph **A** the stigma occurs below the anthers level, the antepetalous stamens are yellowish and anthers of the antesepalous stamens are purplish; in morph **B** the stigma occurs above the anthers level and all stamens are yellowish; in morph **C** the reproductive structures of 80% of the flowers show similar lengths and the anthers of antesepalous stamens are purplish and the antepetalous stamens are yellowish. The stigma is receptive until 48 hours after anthesis. Considering the reciprocity between the morphs, morph A can receive pollen from B, morph B can receive pollen from C and morph C can receive pollen from every morph. The differences of pollen viability between the antepetalous and antesepalous stamens, respectively, for each morph, A (61.5 and 88.5%), B (89 and 57%) and C (56 and 68%) is not statistically significant. Besides, the morphs occur in distinct individuals and the population census showed a ratio close to 1:1:1 suggesting that the morphs may present similar reproductive potentials. *Centris* spp., *Bombus morio* and *Oxaea flavescens* are the pollinators of *M. radula* and their visit frequency among the floral morphs is similar. Differential pollen deposition on the bodies of these bee species promotes pollen transfer between the morphs, resulting in cross-pollination in all of the morphs. Studies on the reproductive system are in progress to investigate if the floral heteromorfism of *M. radula* is related to a possible genetic system of self-incompatibility.

Estimation of the rate of selfing using genetic analysis of selfed and open pollinated progenies of *Stylosanthes capitata*

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Stylosanthes capitata is the most important forage legume currently used in Brazilian Savannas. Information from genetic studies of self pollination (S1) or open pollination (OP) of the species are important both for the decision of breeding strategies as well as the composition of future cultivars. Nineteen progenies S1 and OP were evaluated in a random blocks design, with 6 replications and 6 plants per plot, in Campo Grande,

MS, for total dry matter and seed yields. Plants were evaluated on an individual basis. Genetic and phenotypic parameters were obtained using the software Selegen-Reml/Blup. An approximate estimate of the rate of selfing (S) of the species was made using: $(1+S)^2 = \frac{4\sigma^2_{gOP}}{\sigma^2_{gS_1}}$, in which: σ^2_{gOP}, $\sigma^2_{gS_1}$: additive genetic variance among OP and S1 progenies, respectively. Individual narrow sense heritabilities, for the evaluated characteristics in *S. capitata* OP and S1 progenies presented moderate magnitude. The estimated genetic correlation among the characteristics total dry matter and seed yields in OP progenies was 0.84 and in S1 progenies 0.71. The magnitude of the genetic variation coefficient indicates the possibility to obtain gains with selection in future breeding generations. A mean selfing rate of 0.49 was estimated for the characteristics evaluated in *S. capitata*, which shows, therefore, that the species has a mixed reproductive system. Based on this evidence, selection based on an index combining the OP and S1 progeny information was proposed. This index has the objective of improving the population with a simultaneous decrease in endogamic depression and was used in *S. capitata* considering the selected progenies for each type – OP or S1 – and also the index combining both progenies. From the information obtained and pondering the need for short-term selection gains, we recommend the pursuit of synthetic populations, using the five best progenitors identified by the index combinin OP and S1. By this procedure, there should be less inbreeding and the synthetic population should maintain productivity after several generations, which is an advantage both to the pasture system and to the seed producer

The role of glandular staminode in *Jacaranda oxyphylla* pollination

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Abstract

Bignoniaceae presents over 100 genera distributed mainly at Neotropical region and only two of them, *Jacaranda* and *Digomphia*, have a developed staminode. *Jacaranda oxyphylla*, a zoophilous cerrado sub

shrub, possesses flowers with a conspicuous staminode, densely covered with capitate glandular trichomes. The aim of this study was evaluate the role of the staminode and its glandular secretion in *Jacaranda oxyphylla* pollination. Thus, the floral biology was studied focusing on the influence of the staminode on male and female reproductive success and on the composition of the secretion of the staminode glandular trichomes. The floral features of anthesis, pollen viability, stigma receptivity, nectar volume and concentration were determined through field observations and laboratory analysis. Breeding system evaluation was performed and floral visitors were observed and identified. Experiments comparing the effect of staminode presence and absence on pollen removal and pollen deposition efficiency were conducted in open-pollinated flowers. Histochemistry, TLC and GC-FID analyses were performed to determine the main chemical components of the secretion of staminode glandular trichomes. Flower visitors spectrum included small and medium sized bees, and hummingbirds. Anthesis lasted 2 days, and pollination seemed to be effected mainly by medium-sized *Eulaema nigrita* and *Bombus morio* bees and occasionally by hummingbirds and by a small bee, *Exomalopsis fulvofasciata*. Other medium sized bee, *Oxaea flavescens*, was a very common visitor, acting as nectar thief. Small bees, belonging to *Ceratina*, *Augochlora* and *Trigona* genera were frequent visitors, collecting pollen, apparently with a negative impact on the male reproductive success. *J. oxyphylla* showed predominately allogamous and staminode removal resulted in over 200% fewer pollen grains deposited on stigmas but did not affect total pollen removal. The main chemical compounds of glandular trichome secretion, identified histochemically, were phenolics and terpenoids, including essential oils and resins. The monoterpene cineole and diverse triterpenes, including pentacyclic and steroids compounds, were identified by TLC and GC-FID. The secretion is released continually and is available to flower visitors throughout flower life span. In conclusion, we suggest that the staminode of *J. oxyphylla* is multifunctional and influence positively the female reproductive success acting physically as a lever and chemically as a primary and secondary attractive and possibly as defensive agent against herbivores. The presence of substances related to nest building and chemical defenses, to the structural and hormonal development of bees, and to the attraction of Euglossini males, suggests that the secretion of the capitate glandular trichomes of the staminode are involved with complex chemical interactions, providing a variety of substances essential to the biology of pollinator bees of *J. oxyphylla*, including the solitary bees Euglossini, a group closely related to *Jacaranda* genera.

Hummingbird-plant interactions and breeding consequences

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The association between the hummingbirds and its flowers is commonly influenced by the characteristics of floral morphology such as length, diameter and curvature of floral tube. However, other factors may influence this interaction, such as nectar availability and plant distribution patterns. *Gaylussacia brasiliensis*, *Costus spiralis* and *Sinnigia elatior* are trochilophilous species occurring in sympatry on gallery forest edges in the Brazilian Cerrado biome, and which present differences in the mentione floral characteristics. *G. brasiliensis* presents numerous short and narrow tubular flowers, low nectar production and occurs in dense clusters in the environment, characteristics which are generally associates with Trochilinae hummingbirds. On the other hand, *C. spiralis* presents fewer flowers per individual, with longer and slightly arched floral tube, higher nectar production and generally occurs in low densities, characteristics which are associated to pollination by specialized Phaethornithinae hummingbirds. These species do not differ markedly on volume and nectar concentration per flower, but what in fact seems to influence the pollinator preference for each species is the total amount of resource per individual and their distribution pattern on environment. Thus, *G. brasiliensis* which presents lower amounts of nectar and sugar concentration per flower, when analyzed at the level of individual or cluster provides energy values which attract territorial hummingbirds. On the other hand, although *C. spiralis* offers a greater amount of nectar and energy gain per flower, it apparently never provides enough reward to supports this type of behavior by hummingbirds. These two species seem to represent extremes of adaptations to different guilds of hummingbirds. *Sinnigia elatior* does not fit either of these patterns, since it presents flowers with longer and wider corolla and greater nectar production when compared with the others, and can occur either in clusters or more isolated individuals, being visited both by Phaethornithinae and Trochilinae. These three species are good models to compare the different adaptations of the plants and its consequences for pollination and breeding biology. *C. spiralis* flowers attract more specific pollinators and exclude less efficient visitors as short billed and opportunist Trochilinae hummingbir which do not have access to the nectar. *C. spiralis* is an hermaphrodite, self-compatible and non-apomictic species for which trapliner pollinators as the Phaethornithinae hummingbirds promote larger pollen flow. Actually, hand cross pollination experiments failed to produce the same seed set,

germinability and vigor which resulted from natural open pollination by these hummingbirds in C. spiralis, suggesting long distance pollen flow may be essential for the species success. In contrast, *G. brasiliensis* is adapted to attract territorial pollinators that despite providing limited cross pollination and pollen flow, are much more frequent and reliable. *G. brasiliensis* does not present morphologic barriers for any hummingbird guild, but its low nectar availability per flower and attraction of territorial hummingbirds discourages most trapliners visits. Also hermaphrodite and self-compatible, this species must tolerate a higher degree of geitonogamy and selfing resulting from restricted territorial foraging and pollen flow. Somewhere between the extremes, *S. elatior* do not impose morphologic barriers to hummingbird guilds, attracting as many visitors as possible, no matter their foraging strategy or bill morphology. It produces greater caloric reward ensuring visiting and pollination, and its dichogamous flowers probably reduces selfing and increases pollen flow. Thus, these differences in the form these sympatric plants present their flowers to pollinators and the form that they are used by the different guilds of hummingbirds help us to understand the types of adjustments for the functioning of these mutualistic interactions and the consequences of such interactions for population biology and community structure of these plants. (CAPES/ FAPEMIG)

Germination of Qualea grandiflora Mart: Influence of temperature, light and substrate

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Qualea grandiflora Mart. (vochysiaceae), a herbaceous nature specie from brazilian “Cerrado”, with broad commercial utilization, used as ornamental and medicinal, and can also be used in reflorests program. There are necessary some imformations related to germination process this research aimed to evaluate the different temperatures, substrates and light conditions on germination of Qualea grandiflora Mart. There were evaluated alternate temperatures from 15 to 25°C, 20 to 30°C and, constant at 25°C and 30°C; the seeds were evaluated in relation to germinability (%G), speed index of germination (IVG) using as standard the root protrusion at 9,0 mm. There were used the fully randomized

design disposed in factorial scheme 4 x 3 x 2, with 4 replicates of variance analysis and the means were compared by Tuker test ($p<0,05$). For the %G it was observed a significative double interaction among the factors studied, while for the IVG, a triple interaction was observed. Germinated seeds at 25°C and 30°C showed the %G higher in relation to the others. In constant temperatures and the range 20 to 30°C, the seeds were indifferent to luminosity, while from 15 to 25°C, the %G was higher in the absence of luminosity. Considering the seeds germinated in paper roll and sand, %G was higher in the absence of luminosity, and any difference was observed in paper. For sand, the %G was lower in alternate temperatures, did not differing among them. In constant temperatures there were not observed differences in %G in relation to studied substrates, while for the alternate, it was lower only for sand. The temperature at 25°C and 30°C, for the paper roll, the seeds showed higher IVG in Both luminosity conditions.

Keywords: medicinal plant, germination, substrates, temperatures, Qualea grandiflora

Pollination ecology, breeding system and floral glands in *Diplopterys pubipetala*, a Malpighiaceae species from Brazilian cerrado

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Diplopterys pubipetala (A. Juss.) W.R. Anderson & C. Cav. Davis, a Malpighiaceae species widely distributed in Brazil, presents glands located in calyx, corolla and stamens whose structural, functional and ecological aspects are unknown. The purpose of this work was to understand the role and the dynamic of secretion of these floral glands. Phenology, floral biology, visitors behavior, breeding system, floral glands anatomy and ultrastructure of *D. pubipetala* were studied in a population growing in a fragment of cerrado located at Botucatu, Sao Paulo state, Brazil. Buds, flowers and fruits were produced throughout the year, but the flowering peak occurred from August to September (end of dry season) and the fruiting peak was in October. The flowers are hermaphroditic and their zygomorphy is based on the variation in the number of calyx glands, on the difference in form, size, color and glands of the posterior petal, and the disposition of the stamens and stigmas. The conspicuous calyx glands

occur in pairs in the sepal abaxial surface and are constituted by secretory epidermis and specialized parenchyma with vascular supply of xylem and phloem. The secretion, that contains fatty oils, is accumulated in a subcuticular space and is gathering by the specialist bees. The very small glands occur in the fimbriate margins of all the five petals and are constituted by unisseriate secretory epidermis that involves a small group of parenchyma cells vascularized or not. The posterior petal glands are vascularized and more structured than lateral petal glands which are not vascularized. All these glands are terpenes-producing and the secretion also is accumulated in a subcuticular space. In all ten stamens, the connective shows irregular surface constituted by secretory globular epidermal cells and phenolic idioblasts, and the subepidermic region is constituted by endothecium-like cells. The liberation of the viscous secretion, that also contains terpenes, is related to the anther dehiscence mechanism and is collected by *Monoeca* bees. Although the histochemical tests revealed that the secretion in floral glands is predominantly lipophilic, the ultrastructural characteristics indicate the synthesis of both, hidrophilic and lipophilic components. In all floral glands the plastids were the more abundant organelle and presented peculiarities in each glandular type that are related with the lipophilic secretion. The flowers are visited by 11 bee species, *Centris* and *Monoeca* genera being the most frequent. *Monoeca* bees are the most efficient pollinators and they collected oil, pollen and the connective secretion. All the floral glands play important roles as primary and secondary attractive; furthermore, the glandular connective is also related to the increase of pollen transfer efficiency, being essential for maintenance of the interactions with pollen vectors and playing an important role in the reproduction of this *D. pubipetala* population, which is predominantly alogamous. The present study contributes for the knowledge of the diversity of floral glands and their role on the reproduction in Malpighiaceae.

Final pollen development and pollen-pistil interaction in a primitive angiosperm, *Annona cherimola* Mill. (Annonaceae)

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Cherimoya (*Annona cherimola* Mill.) is a subtropical fruit tree species with incipient but promising commercial perspectives, which belongs to the Annonaceae, the largest ancient lineage family in flowering plants.

This species shows protogynous dichogamy, where male and female parts do not mature simultaneously providing an isolation of the sexes and encouraging outbreeding. The objective of this work is to study the final stages of pollen development and the pollen tube pathway in this species. The results obtained show a coexistence of bicellular and tricellular pollen at anther dehiscence, an uncommon fact in angiosperms where generally bicellular pollen is released and tricellular pollen is considered a derived characteristic. This coexistence seems to be due to a late mitosis starting shortly prior to pollen shedding and could be explained by the partially hydrated state of pollen at anther dehiscence that provides a continuous cell activity. The proportion of bi and tricellular pollen was variable and modulated by temperature and provides a bet-hedging strategy with the different behaviour shown for bicellular pollen (slow pollen germination and long viability) and tricellular pollen (fast pollen germination and short viability). The plasticity of pollen during the final stages of pollen development is also shown during the first steps of pollen-pistil interaction on the stigma where stigmatic receptivity is shortened with high temperatures and prolonged with high relative humidity. However, pollen-pistil interaction inside the pistil shows a minor environmental influence. A primitive pistil supports a primitive pollen-pistil interaction. A continuous secretory papillar layer goes from the stigma down through a short semi open style to a primitive obturator in the ovary. The pollen grains germinate in the stigma and compete to reach the short stylar semi open canal and only one to three pollen tubes success to reach the ovule one day after pollination. Thus, restrictions to pollen tube growth seem to be mainly located just at the stigma-style interface. The results are discussed in terms of their implications for the adaptation of the reproductive biology of early angiosperms to changing environmental factors.

Pollen morphology in three species and three commercial hybrids of the Bromeliaceae

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The Bromeliaceae, a neotropical family occurring in almost all Brazilian ecosystems, is comprised of approximately three thousand known species in 54 genera. As part of an ongoing project on bromeliad reproduction and breeding, the present work aims to describe the pollen morphology in

three *Vriesea* species: *Vriesea simplex*, *Vriesea hieroglyphica* and *Vriesea friburguensis* and three commercial hybrids *Vriesea* sp var. *vermelha*, *Vriesea* sp var. *calixto* and *Tillandsia* sp var. *linearis*, which will be used in this breeding program. Pollen grains were collected at anthesis from flowers obtained from plants cultivated in the greenhouse. The pollen grains were acetolysed and observed under the light microscope, for general observations and the scanning and transmission electron microscope for detailed morphological description. Pollen grain morphology is stenopalynous, grain sizes varied from medium to large (larger than 20 µm), monocolpate, elyptical, with bilateral symmetry. In equatorial view the pollen grains are plane on one side and convex on the other side. The exine ornamentation is reticulate, semitectate, with granulated lumina, with lumen size becoming smaller as it approaches the equatorial extremities, close to the colpus. Differences among species and hybrids were detected only after observations of ultrathin sections, under the transmission electron microscope. Pollen grains from all three species showed similar structures, such as a partially discontinuous, thick, slightly undulate tectum, especially where columella was absent, nexine slightly thinner than the tectum, thick intine with various layers of an electrondense material. Morphological characteristics of pollen grains from hybrids differed among each other and also differed from those observed for the three *Vriesea* species. *Tillandsia* sp var. *linearis* presented smaller distances between columella and continuous tectum, while *Vriesea* sp var. *Vermelha* and *Vriesea* sp var. *Calixto* showed higher distances between columella. The most striking differences were observed in the exine and intine, which were thicker in the hybrids, compared to the three *Vriesea* species analyzed.

Reproduction of tropical fabaceae-papilionoideae from Argentina

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Abstract

Many native Papilonoideae form Northwestern Argentina have been mentioned for their foraging value, given their high content of proteins. The study of this group is of great interest because it would constitute an

alternative with regard to the introduced forage species since they are native of the area, and, therefore, they are adapted to local conditions. As a first step in the characterization of 14 simpatric species of Papilionoideae, in this work we determined the preemergent reproductive success (PERS). We estimated Fruit/flower ratio (Fr/Fl) and Seed/ovule ratio (S/O) on entire inflorescences under natural pollination, where PERS = the product of Fr/FL and S/O. Fruit/flower ratio is defined as the proportion of flowers that developed into fruits in each inflorescence. Seed/ovule ratio is defined as the proportion of ovules that developed into matured seeds in all the matured pods within an inflorescence. The highest and lowest values of Fr/Fl were 0.90 in *Cologania ovalifolia* and 0.24 in *Zornia contorta*, respectively. The mean value for the group (0.59 ± 0.05) is below the mean value for self-compatible, hermaphroditic species (0.72). The highest and lowest values of S/O were 0.90 in *Desmodium incanum* and 0.44 in *Galactia latisiliqua*, respectively. The mean value for S/O for the studied species (0.75 ± 0.04), is above the value reported in the literature (0.63). There were no significant differences in PERS values among the studied species (H=13; P=0.45). Considering all the species, PERS averaged 0.44 ± 0.04; this value is below the mean value reported for autogamous species (0.9).

Reproductive success in avocado (*Persea americana* Mill.).

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Avocado (*Persea americana* Mill.) is an evergreen subtropical fruit tree native to Central America and Mexico. It is a member of the Lauraceae a mostly subtropical or tropical family included in the basal angiosperm clade Magnoliidae within the order Laurales. Avocado shows protogynous dichogamy where each flower opens twice: first functionally as female, then the flower closes and reopens the next day functionally as male. Only a very small fraction of the flowers (less than 1%) are able to set fruits. In order to study the causes leading to this low fruit set, we analyzed the progamic phase, from pollination to fertilization under the environmental conditions of Southern Spain in 'Hass', the most important cultivar worldwide. The hand pollinated population of flowers showed higher retention than those left to open pollination although in the first population the fruit set rate was still very low (2%). The response of

stigmatic receptivity to different temperatures and relative humidity was also evaluated both *in vivo* and *in vitro*. The results indicate that the stigma maintains the capacity to offer support for pollen adhesion and pollen germination during the male stage, although the extent of this capacity is highly dependent of temperature and humidity. As a next step, we analyzed the influence of the starch reserves of the flower at anthesis on fruit set. With this purpose, flower buds from different panicles were individually labelled. At anthesis, each flower was collected and histochemically processed to be observed under the microscope after staining with I_2KI for starch reserves. Starch in each flower was quantified with the help of an image analysis system attached to the microscope, showing large differences in starch content among flowers. Finally, we examined the influence of the nutritional status of the flower on fruit set by measuring the starch content in flowers of two populations with different capacity of set fruit. A significant relationship was found between the amount of starch and the capacity of a flower to set a fruit. The results are discussed in terms of the possible implications of the events that take place during the progamic phase and the nutritional status of the flower in reproductive success.

Interspecific crossability of wild *Arachis* species of the taxonomic section *Arachis* associated to the A and B peanut genomes

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Wild *Arachis* species of the taxonomic section *Arachis* are a potential source of genes for peanut breeding. However, use of their variability requires basic information on the reproductive behavior and crossability of such wild relatives. The peanut, *A. hypogaea*, is a tetraploid cultigen with a genome formula AABB. Except for *A. monticola*, also a tetraploid, all other species in the *Arachis* section are diploid, with an A, or B, or other distinct genomes. *Arachis gregoryi*, a Brazilian member of a five diploid species alliance most closely related to the peanut B genome, has been used as the female progenitor in crosses with other 24 species, including representatives of all six botanical varieties of *A. hypogaea*, as well as wild relatives with 2n=2x=18, 2n=2x=20, and 2n=4x=40. An individual female progenitor of the germplasm accession V14957 was used for each crossing combination. Flower buds were emasculated daily,

at dusk, and pollinated and tagged in the next morning. Success in pollinati could be checked some 15 days later, by the elongation of a peg from the base of tagged flowers. Pollen viability was estimated for each male progenitor, using both germination and staining techniques. Only two male progenitors showed low viability, with a germination record below 50% in *A. kempff-mercadoi* (V13250) and the same for both techniques in *A. helodes* (V6325). However this level did not prevent effective pollination and peg formation. Pegs were developed in all crossing combinations. Hybrid seed obtained from crosses with male parents associated to the peanut A genome apparently did not have any dormancy, as they germinated spontaneously, as soon as the pods reached maturity. Such materials are of high interest to the breeding program, as they are interspecific hybrids gathering information linked to both peanut genomes. In the cross of *A. gregoryi* with a peculiar peanut type, of uncertain varietal assignment (the Xingu type, V12549), pods were formed but embryos aborted at early stages. In the only intersectional cross, of *A. gregoryi* with *A. paraguariensis* (V7677, section *Erectoides*), several pegs were formed but only one proceeded to reach full pod development, formin a seed that germinated successfully under laboratory conditions. Successfu intersectional crosses may provide bridging hybrids for a broader gene search considering additional taxonomic sections.

Ontogeny of the fruit in *Dyckia maritima* Baker (Bromeliaceae)

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Dyckia maritima Baker is a rupicolous species with ornamental potential, belonging to the subfamily Pitcairnioideae. Its distribution area includes Rio Grande do Sul and Santa Catarina states (Brazil) and according to a Rio Grande do Sul state decree this species fits the "vulnerable" category, in relation to the extinction threat. This study presents the anatomy and ontogeny of the fruit, in order to contribute with data to the taxonomy of the family and to the ecology of the species. The material was fixed in 1% glutaraldehyde and 4% formaldehyde, washed in 0.1M sodium phosphate buffer, pH 7.2, dehydrated in an ethanol series and finally embedded in hydroxyethylmethacrylate. Semithin sections were performed on a rotary microtome, stained using Toluidine Blue O and examined and registered in light microscopy. The ovary is superior, tricarpellary and trilocular, with the carpels fused only at its ventral regions. The outer and inner epidermal faces contain rectangular cells, in transversal section, elongated in anticlinal and periclinal directions, respectively. The parenchymatous mesophyll is multiseriate, composed of isodiametric cells, and contains three collateral vascular bundles in each carpel. There are six evident dehiscence lines, three of which consisting of fused outer epidermal faces at the ventral side of adjacent carpels, and three consisting of ventral sutures. The initial fruit development is characterized by cell divisions, which are predominantly of the anticlinal type, in all tissues. Subsequently, the pericarp develops by cell enlargement, with stomata and peltate trichomes formation in the epicarp and raphide idioblasts differentiation among mesocarpic cells. The epicarp and the subepidermal layer show dense cell contents of pectic nature, concentrated in cells around the stomata; besides, the outer periclinal walls of epicarp cells are thickened. In the mature fruit, the epicarp becomes strongly lignified and the endocarp undergoes a subtle deposition of cell wall. The differentiation of fibers occurs at the ventral region of each one of the carpels and around the dorsal vascular bundles, constituting bundle sheath and bundle sheath extensions. After maturation, the pericarp suffers a general compression in the mesocarp cells, in consequence of water loss, becoming a dehiscent dry fruit classified as capsule. The dehiscence takes place by tissue rupture at ventral and dorsal regions of carpels, a phenomenon that characterizes the capsule as biscidal – due to the combination of two aperture types, the septicidal and the loculicidal.

Morphology, anatomy, and ontogeny of the pericarp and seed of *Duguetia furfuracea* (A. St.-Hil.) Saff. (Annonaceae)

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Duguetia furfuracea is popularly known as *araticum*, *marolinho-do-cerrado* or *pinha-de-guará* and occurs in varied physiognomies of *cerrado*. Seeds of this species are antiparasitic, particularly against lice when pulverized and diluted in water. Many fruits and seeds of Annonaceae have been analyzed structurally, however these studies are not found for *D. furfuracea*. Thus, the aim of this work is to describe the morphology, anatomy, and the ontogeny of the pericarp and seed of *D. furfuracea*, comparing it with other Annonaceae. The collected material was processed by embedding in methacrylate. The gynoecium consists of numerous distinc carpels. The ovary is superior, one-carpelled, one-loculed, with only one ovule per locule, rarely two. The outer epidermis is single-layered with cuboid, thin-walled cells, and presents multi-layered short-pedunculated stellate scales, in several stages of differentiation, which have strongly thickened and lignified walls, some with phenolic content. The ovary mesophyll shows a hypodermis of cuboid, tannin-containing cells, and many parenchyma layers interspersed by tanniferous idioblasts and pectic cells that are bigger and more frequent in the dorsal and near style regions, several collateral vascular bundles occur immersed in the mesophyll. The inner epidermis is single-layered with similar cells to the outer one. The ovule is ana-campylotropous, bitegmic, crassinucellate, with basal placentation. The funicle is short. At the placenta region occur numerous papillae those produce pectins. The outer integument is four cell layers thick with cuboid, thin-walled, dense cytoplasm, and well evident nuclei. The inner integument consists by two cell layers thick, similar to the cells of the outer integument. The nucellus is formed by three or four cell layers thick with cuboid, thin-walled, denser cytoplasm, and evident nuclei. A hypostasis not much conspicuous can be seen. The fruit is multiple and strobile-like, with carpels tightly packed together but free. The exocarp is single-layered, formed by cuboid, thin-walled and dense cytoplasm cells, some of these with phenolic content; there are stomata and numberless stellate scales. The mesocarp is multi-layered and presents three distinct regions. The outer region is formed by parenchymatous, thin-walled cells,

with several shapes and sizes; a big quantity of tanniferous idioblasts occurs, which is increased during ripening; stone cells occur singly or more often grouped and have strongly thickened walls, some containing tannin, and others showing dense cytoplasm and evident nuclei. The median region consists of a collenchymatous parenchyma formed by large cells, with pectic and irregularly thickened cell walls; at ripening, these cells begin to disintegrate releasing pectins; numerous collateral vascular bundles, which are bigger toward the inside, occur in this region, like as some groups of stone cells, which are surrounded by radially-arranged parenchyma cells. In the axis region of the fruit, the cambium is differentiated in the high caliber bundles, producing secondary xylem and phloem. The inner region is formed by several layers of longitudinally elongated parenchyma cells with pectic walls, evident intercellular spaces and some few tannin-containing idioblasts. The endocarp is single-layered (locally two-layered) and is formed by fibers with slightly thickened walls. The seed is perichalazal and presents evident hypostasis; there is a rudimentary aril and the micropylar plug is not differentiated. The exotesta is single-layered with periclinally elongated cells having thin phenolic walls, some containing tannin. The mesotesta is fibrous, with the outer region formed by longitudinally and obliquely elongated fibers and the inner with transverse fibers; between these, groups of parenchyma cells occur, having intercellular spaces; tannin content is observed in some mesotestal fibers. The endotesta is indistinct of the mesotesta. The exotegmen is single-layered and parenchymatous, while the endotegmen, single-layered too, presents tracheoids with helicoid wall thickening. Ruminations occur on both sides of the seed, and are distributed over its whole length; they are formed by the inner mesotesta, endotesta and tegmen. The endosperm is composed of large cells of several shapes and sizes; these cells have pectic irregularly thickened walls, cytoplasm that stores products and the nuclei are not evident; an increase of the quantity of wall pectins is observed in the median region of the endosperm. The embryo is small and straight; the embryo axis presents two leaf-like cotyledons and shows a little degree of cell differentiation. The present study shows a considerable uniformity of the basic pericarp and seed structure when compared with the results found in the Annonaceae literature. However, important variations occur, such as: stellate scales instead of tufts of trichomes, endocarp composed by fibers, micropylar plug absent, and tegmen with distinct exo- and endotegmen, this one formed by tracheoids with helicoid wall thickening.
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Morphology, anatomy and ontogeny of pericarp and seed of *Mascagnia cordifolia* (A. Juss.) Griseb (Malpighiaceae)

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Mascagnia belongs to Malpighiaceae, family composed of 1,200 species grouped into 66 genera with pantropical distribution. Molecular phylogeny studies have shown that *Mascagnia* is not a monophyletic genus. However, despite fruit characters being traditionally used on group taxonomy, detailed structural studies of reproductive organs of this genus are absent in literature. So this study aim to describe morphology and anatomy of fruits and seeds of *Mascagnia cordifolia* during development. Botanical material was collected, fixed and embedded in methacrylate, according to conventional techniques of plant anatomy. We verified that the ovary of *M. cordifolia* is tricarpellary and trilocular, with one ovule per locule, in axile placentation; these characters are typical of Malpighiaceae. The outer epidermis is uniseriate, with unicellular and multicellular non-glandular trichomes; the ovary mesophyll is formed by approximately six layers of cells in the dorsal region; the innermost cells of the mesophyll initiate transversal elongation; the inner epidermis is uniseriate but divides in periclinal divisions produce two layers. The dorsal region of each carpel presents three projections on the ovarian wall, which will form the wings on mature fruit; the two laterals are bigger than the central one. Ovary presents six ventral bundles that join in pairs forming three bundles; there are six evident lateral bundles and only some procambial strands dorsally. Ovules are suspended, subcampylotropous, bitegmic and crassinucellate; the inner integument (two-four cell layers) is shorter than the outer one (one-five cell layers), funicle is long and thick and the nucellus is ample and projects through the micropyle. Still in floral buds we can observe the formation of the zygote. During fruit development, exocarp maintains uniseriate with the production of new trichomes. The outer mesocarp is composed of about six layers of parenchyma cells, among which we find procambial strands and vascular bundles; in the inner mesocarp, cells are transversally elongated and periclinal divisions produce more than one cell layer in some regions. The endocarp shows one-three cell layers slightly longitudinally elongated. A meristem appears in the distal end of the dorsal projections, making the projections length bigger. The young wings are

formed by uniseriate exocarp and multiseriate mesocarp with parenchyma cells with a loose arrangement. At development, the seed enlarges its size a lot, however the seed coats do not proliferate, only enlarging cell volume; two layers are distinct, the outer has voluminous cells and the inner one has smaller cells, with dense cytoplasm and voluminous nuclei. The consumption of the nucellus starts while are observed the production of nuclear endosperm, restricted to the region around embryo, that goes through globular to heart-shaped stage. The mature fruit of *M. cordifolia* is schizocarpic, with three lateral-winged samaras, each one having two big lateral wings and a poorly developed dorsal wing. The exocarp and outer mesocarp do not undergo many alterations but inner mesocarp cells are slightly transversally elongated and become lignified. The endocarp has up to five layers of longitudinally elongated cells and highly lignified. When the fruit is able to dispersal, exocarp and mesocarp cells collapse and only the inner mesocarp and maintain intact. The wings are formed by uniseriate exocarp with many trichomes and by an aerenchymatous outer mesocarp, where many vascular bundles occur. Mature seed shows collapsed seed coat; the outer layer has anticlinal and inner periclinal thick-wall, that composes the only distinct region on this stage. Both nucellus and endosperm are completely consumed during development. Embryo axis is straight and short, formed by very small cells, with dense cytoplasm and evident nuclei; plumule is undistinguished. Cotyledons are fleshy and well developed, with small protodermal cells and more voluminous ground meristem cells, accompanied by large procambial strands. Reserve of starch and lipids occurs in all the embryo, specially in the cotyledons. The fruits of Malpighiaceae species usually show an inner sclerenchymatous layer, independently of the kind of fruit; in *M. cordifolia* this layer has multiple origin,being formed by inner mesocarp and endocarp. The characters found during seed development and in mature structure are in agreement with literature and are typical of Malpighiaceae. The occurrence of zygote in floral buds may indicate apomixis or self-fecundation, both process find in this family.

Comparative structure of mature embryos and seed germination in some Asia globeflowers

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Though representatives of *Trollius L.* are widely spread all over the Northern hemisphere, their reproduction is scarcely studied yet. Globeflowers

are polycarpy plants which possess only sexual reproduction without cloning. Seeds are known to have long combined true dormancy. Germination demands two-phase stratification for not less than two months

We studied seed germination period and mature degree of embryos at the beginning of dormancy. There were first studied Asian endemic species *Trollius altaicus C.A. Mey., Trollius ledebouri Reischemb., Trollius pumilus D. Don., Trollius lilacinus Bunge. (Hegemone lilacina (Bunge.) Bunge.) and Trollius asiaticus L.*

Mature embryo sac of globeflowers is 8-cellular with small mononuclear synergids and a larger egg cell. Long bubble-like monocellular antipodals form antipodal haustoria. The antipodal apparatus is larger than an ovarian one and functions for a long time, the central antipodal cell is the largest and it simulates an egg cell.

The well-filled seeds of all analyzed *Trollius* species are of small size (1-2 mm). There are revealed the variability in seed dormancy types within one population and essential variability for different species of the genus.

It was first established that *T. ledebouri* seeds do not have dormant period. The sprouts begin to break the seed-covering in 7 days and start growing in 10 days at the warm room conditions without stratification.

An insignificant number of *T. pumilos* sprouts begin breaking the seed-covering in 10 days, and their growth begins in 2 weeks at room conditions. This species starts to sprout in 8 weeks at two-phase stratification.

Seeds of 3 globeflowers species under the study (*T. asiaticus*, *T. altaicus*, *T. lilacinus*) need long dormant period and two-phase stratification. In room conditions (about a 20 ºC) without stratification they do not sprout for not less than a year. At two-phase stratification germinating takes 3 months and more.

Seeds preparation displayed *T. ledebouri* to have a differentiated embryos occupying about 1/3-1/4 of seed lengths. It consists of well-defined rudiment of a root, caulicle, 2 lengthened cotyledons. Such structur is not typical for having earlier been studied species of globeflower. Their winter rest they begin at a stage of a torpedo. The length of cotyledon varies insignificantly for individual seeds and plants.

T. pumilos appeared to have polyvariant embryo development degree in dormancy seeds. Embryo occupies from 1/5 to 1/7 of seed length. Few seeds are in a stage of a torpedo and have well-defined cotyledons, caulicle, a rudiment of a root. However the most embryos stop their development at late-heart stage when cotyledons only start extension, but are not closed and the caulicle is not expressed. Between these extrem cases it is possible to observe all the stages of embryo development.

In *T. altaicus* and *T. asiaticus*, embryos occupy 1/6 – 1/8 length of a seed. They generally develop up to heart stage.

From all the considered species seeds of *T. lilacinus* enter the stage of winter dormancy with the smallest embryos. The embryos occupy 1/8-1/10 of seed length. They stop development on heart stages, though few seeds with embryos on globular stages have been found out as well.

Thus the most advanced embryos were observed in *T. ledebouri* which has the latest period of flowering, while the most underdeveloped ones in *T. lilacinus* which begins flowering before all the other species. It is possible to assume that underdeveloped embryo is the adaptive feature interfering germination of seeds during the short warm period of summer after seeds shedding. It, in turn, rescues plants from entering the dormancy stage when seeds are at the beginning of germination and protects sprouts from destruction during the winter period. That is in *Trollius* the stage of embryo development provides the length of the dormancy period.

ORAL CONTRIBUTION

Suppression of cell expansion during the early stages of tomato fruit development is mediated via stage specific expression of the single MYB-like gene *SlFSM1*

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Very little is known about the mechanisms dictating early tomato development post fertilization to be driven by cell-division, before transition to the cell-expansion growth phase. We have isolated and characterized a novel plant-specific gene, *SlFSM1* (*Fruit SANT/MYB-like1)* harboring a single SANT/MYB-like domain, which expression is specific to the very early stages of tomato fruit development. A low ectopic over-expression of *SlFSM1* (OEX-SlFSM1) results in a severe developmental retardation only during the early stages of seedling development and diminishes towards the transition to the reproductive growth phase (Barg et al 2005, Planta 221:197). Histological analyses demonstrated that this low ectopic over-expression leads to significant reduction in the size of the cells in all the tissues and organs tested including: hypocotyl epidermal cells, epidermis

and palisade cells of fully expanded leaves, and most importantly, in the epidermis and pericarp of mature fruits, compared to WT fruits of similar size and weight. In etiolated seedlings 1mM IAA suppresses hypocotyl elongation in the OEX-SlFSM1 lines to much greater extent than in the WT (15% vs. over 40%), suggesting that it operates down-stream to auxin signaling during early fruit development, apparently to restrict cell expansion during this stage. The Arabidopsis gene At2g21650 encodes for an ortholog of *SlFSM1*. Yeast-2-Hybrid analysis, supported by pull-down experiments of i*n vitro* transcribed and translated proteins, indicated that the partners of At2g21650, are two homologous proteins (At1g10820 and At1g68160) belonging to a novel small plant specific gene family, of unknown function. They contain a single MYB/SANT–like domain which is different from canonical MYB domains. A homolog of these Arabidopsis genes, designated SlFSB1 (Fruit SANT/MYB Binding protein 1) was cloned from our tomato early parthenocarpic fruit cDNA library. According to the tomato EST database and Northern analyses, this gene is non-abundantly expressed throughout fruit development.

Based on the functioning of SlFSM1 as a suppressor of cell expansion, its role in the regulation of tomato early fruit development (phase II), and transition to the cell expansion growth stage (phase III), apparently by complexing with SlFSB1, will be discussed.

Persephone - a sporophytic maternal effect mutant controlling seed development

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The formation of seeds is essential for the life of the flowering plants. Seeds are the product of double fertilization i.e. usually one haploid male gamete fertilizes a haploid egg cell to form a diploid embryo whereas the second haploid male gamete fertilizes a homodiploid central cell giving rise to a triploid endosperm. Since both the embryo and endosperm are enclosed in several layers of maternally derived integuments and seeds remain connected to the mother plant during their growth and maturation, in particular a maternal influence over seed development can be expected. Here we present a newly isolated mutant, designated persephone (per), which exerts a sporophytic maternal effect on seed development. per mutant plants display a seed abortion phenotype that increases over time

and ultimately all seeds in the silique will arrest and die. This reveals a dynamic interaction between a mother plant and a developing seed.

Natural variation in the degree of autonomous endosperm formation reveals independence and constraints of embryo growth during seed development in Arabidopsis thaliana

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Abstract

Seed development in flowering plants is a paradigm for the coordination of different tissues during organ growth. It requires a tight interplay between the two typically sexually produced structures, the embryo, developing from the fertilized egg cell, the endosperm, originating from a fertilized central cell, and the surrounding maternal tissues. Little is known about the presumptive signal transduction pathways administering and coordinating these different tissues during seed growth and development. Recently, a new signal has been identified emanating from the fertilization of the egg cell that triggers central cell proliferation without prior fertilization. Here, we demonstrate that there exists a large natural genetic variation with respect to the outcome of this signaling process in the model plant *Arabidopsis thaliana*. By using a recombinant inbred line population between the two Arabidopsis accessions Bayreuth-0 and Shahdara we have identified two genetic components that influence the development of unfertilized endosperm. Exploiting this natural variation, we could further dissect the interdependence of embryo and endosperm growth during early seed development. Our data show an unexpectedly large degree of independence in embryo growth, but also reveal the embryo's developmental restrictions with respect to endosperm size. This work provides a genetic framework for dissection of the interplay between embryo and endosperm during seed growth in plants.

Biological and bioinformatic analyses of seed-specific promoters from sunflower genes

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In recent years there has been increased interest in studies on the spatial and temporal regulation of transgenes in plants. The identification of promoters that lead to those patterns is crucial for the next generation of transgenic plants. Plant seeds are natural storage organs that accumulat high levels of lipids and proteins during development and constitute ideal targets for expression of recombinant proteins for molecular farming approaches. The aim of this work is to identify new suitable seed-specific promoters capable of leading the expression of transgenes into sunflower seeds. Ha-AP10 is a sunflower LTP (Lipid Transfer Protein) previously described as a protein expressed only in dry seeds. FAD2-1 is a microsomal oleic acid desaturase which catalyze the conversion of oleic acid to linoleic acid in development seeds. These two candidate genes were chosen for promoter region isolation. Expression pattern analyses of Ha-A10 and FAD2-1 genes corresponding to the cDNAs encoding the two proteins isolated from HA89 sunflower line were compared among several tissues. The corresponding transcripts of both genes were detected in mature seed by northern blot analyses. The FAD2-1 transcripts were also found in R6 developmental stage. The 5'- upstream region spanning 1kb was isolated from both genes, cloned, sequenced and "in silico" characterized. Bioinformatics analyses of the upstream sequences were performed using the PLACE and Plant CARE databases. Several cis-elements involved in seed-specific trans-acting factors were identified such as GCN4 motif, E-Boxes, SEF binding sites as well as conserved TATA and CCAT boxes. To determine whether the potential regulatory elements identified in the upstream sequences has any biological significance, the two regions were fused to the âglucuronidase reporter gene and cloned in the AKK 1431 plasmid for particle bombardment. GUS activity was detected in onion cells indicating the functionality of the assayed sequences as promoters. Stable plant transformation as well as a detail functional characterization of regulatory boxes will contribute to evaluate the potentiality of the use of these promoters in biotechnology applications.

Monoembryony versus polyembryony in apomictic *Eriotheca pubescens* (Malvaceae)

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The occurrence of variation in breeding system among plants of the same species is not common, although examples occur in some groups. Yet the occurrence of asexual and sexual reproduction in the same species is frequent in groups as Asteraceae and Poaceae, where apomictic vs. sexual individuals have shown differences in morphology, distribution and biology. Studies in other groups are virtually inexistent. We have studied the occurrence of differences in the reproduction of *Eriotheca pubescens* (Mart. & Zucc.) Schott & Endl. for the last ten years. The species is pseudogamous apomictic and polyembryonic which was confirmed by histological studies showing adventitious embryony of nucellar origin. Polyembryonic individuals are able to produce viable populations which seem to be mostly clonal. But since the beginning of the studies it was possible to identify strictly monoembryonic individuals. By using the occurrence of polyembryony as an indication of asexual reproduction and monoembryony as indication of sexual reproduction we differentiated individuals of *Eriotheca pubescens* in its distribution, fruit and seedling biometry. The study was developed along the BR050 road between Uberlândia and Brasília, in cerrado areas of Central of Brazil. Fruits of individuals of *Eriotheca pubescens* were collected and had the number of embryos per seed, number of monoembryonic vs. polyembryonic seeds and fruit morphological characteristics evaluated. The monoembryonic and polyembrionic seeds were germinated and their seedlings were maintained for nine months in greenhouse to check seedling growth. *E. pubescens* presented predominantly polyembryonic seeds, but the frequency of polyembryony and average number of embryos per seed varied among fruits and individuals. Individuals strictly monoembryonic represented only 13.16% of the observed individuals of *E. pubescens* (n=38) and they showed a more restricted geographical distribution. In the polyembryonic individuals, the frequency of monoembryonic seeds varied from zero to

Functional annotation and expression analysis of novel sequences associated to aposporous development

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Recently, a group of 65 cDNA fragments corresponding to genes differentially expressed in sexual and aposporous *P. notatum* were identified. One-third of the sequences showed no homology to the gene databases, which prevented their functional annotation. These novel sequences probably correspond to genes that remain uncharacterised in plants, or display low levels of conservation. This work was aimed at a progressive investigation of the possible functional role for each one of these novel candidates.

Characterization started with the selection of 5 clones which originated from differential display experiments (N2, N13, N11, N17, N22), based on its particular expression patterns and/or their weak homology to interesting candidates in the data banks. Total RNA was extracted from immature inflorescences of an obligate aposporous *P. notatum* genotype (Q4117). A cDNA amplification library was constructed using the Marathon cDNA Amplification kit (BD Biosciences, Clontech). Two upper and lower nested primers were designed for each one of the sequences, in order to perform 5' and 3' RACE (Rapid Amplification of Complementary DNA Ends) experiments. Flanking regions of clones N13 and N17 were succesfully amplified after two rounds of selective nested amplification, cloned in PGemTeasy (Promega) and double-strand sequence Initial aligment of partial RACE sequences was done with Clustal W2 from the EBI-EMBL website (http://www.ebi.ac.uk/Tools/clustalw2/) and the consensus percentage and full sequence was obtained with Consensus programe (http://structure.bu.edu/cgi-bin/consensus/consensus.cgi). At first, full sequences were BLAST-compared with the general data bases at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), and with specific data bases of rice and green plants at the Gramene website (http://www.gramene.org/multi/blastview). Sequences were translated into the six possible frames (http://us.expasy.org/tool/dna.html) and analysed using some Prosite and ProDom tools (http://ca.expasy.org/tools/scanprosite/) to determine the presence of conserved domains, which were further characterized using the Pfam server (http://pfam.sanger.ac.uk/). Dendrograms representing

genetic distances were constructed with MegAlign (DNAstar). Consensus sequence for N13 presented homology to AJ877257, an *Amborella trichopoda* mRNA for a putative crabs claw transcription factor (CRC gene), a specialized member of the YABBY family, whose expression is restricted to carpels and nectaries. Homology was detected in approximately 50% of the sequence (including the 5´and 3´terminal ends), while the central section was highly variable, as is usual in other members of the crabs claw family. Meanwhile, clone N17 displayed homology to Os10g21160, a retrotransposon protein-like protein.

In situ hybridization experiments were carried out in order to analyze more conclusively if differential expression between the appropriate tissues can be directly associated with the phenotype. Clone 13 hybridized strongly in ovaries, showing differential signals between apomictic and sexual plants.

Results reported here allowed characterization of the structure, possible function and detailed pattern of expression of two novel genes involved in aposporous development in *Paspalum notatum*.

Characterization of the parents of a *Brachiaria humidicola* intraspecific cross that segregates for apomixis, using microsatellites

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The brazilian cattle breeding is essentially based on the use of forage pastures with high nutritional value for the cattle. Grasses of the genus *Brachiaria* has been largely used as forage in Brazil, such as *Brachiaria humidicola*. This is a poplyploid species that reproduces through facultative aposporous apomixis, an asexual reproduction that generate parthenogenetic embryos genetically identical to the mother plant. A better understanding of the molecular mechanism of this mode of reproduction in plants would help opening the possibility of transfering apomixis to sexual plants, enabling cloning of crops through seeds. Twenty three genomic microsatellites were designed from a previously built microsatellite-enriched library for this species. Fourteen of the microsatellites showed polymorphism and were used to access the genetic variability of 58 genotypes of the species germplasm. Twelve of these loci were polimorphic for the parents of an

intraspecific cross, which are the genotypes H031 (sexual) and H016 (apomitic, used as pollen donor). The PIC content of the 14 microsatellites showed a total range of 0,5451 to 0,8506. It was shown by a Simple Matching´s similarity coefficient analysis that the parents are 0.6750 similar, in a total range of 0.6417 to 1,0000, meaning that they are considerably genetically divergent. Novel microsatellites are being develope in order to map the segregation of apomixis in the H031xH016 F1 population.

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ORAL CONTRIBUTION

Effect of osmotic pressure in *Brachiaria brizantha* cv. Marandu genetic transformation by biolistic

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Brachiaria brizantha is a forage grass widely used in Brazil mainly for beef cattle. The Genus *Brachiaria* presents an asexual mode of plant reproduction, apomixis, in which occurs a cloning through seeds. Breeding of these species is very complex since the ploidy level is usually different among them, and also due apomixis be predominant, drastically reducing genetic variability. Genetic transformation has been a powerful tool for plant breeding, and for *Brachiaria*, biolistic has been developed aiming apomixis gene function studies. Mature seeds from *Brachiaria brizantha* cv. Marandu (BRA000591, 2n=4X=32) were used to induce embryogenic calli. From these calli, cell suspensions were obtained in MSCLind liquid medium supplemented with 2,4-D. Two months later from the first step, cell suspensions were plated in MSCLind solid medium with 3 or 12% sucrose in a 6cm plate, 24h prior bombardment. Plasmid pAHC27 or pAHUG containing *gus* gene driven by pUbi1 promoter were used for bombardment (pAHUG also contains *hptII* gene) as well as tungsten particles M10, one shot/plate and 10µg DNA/shot. *Gus* gene histochemical assay was carried out 24h after bombardment showing several blue spots. The cell suspensions left were maintained in MSCLind with higromycin, the ones bombarded with pAHUG only. Two weeks later, the cell suspensions were transferred to MSCLreg solid medium where somatic embryos could be observed in globular and torpedo stages of development in the 12% sucrose treatment when the pAHC27 plasmid was used. All the treatments using pAHUG failed to regenerate somatic embryos, producing only roots.

Plantlets were transferred to MMP medium for elongation and rooting, and some pieces of leaves were used for histochemical assay as well PCR analysis. Gus positive plantlets are being rooted in vitro before being acclimatized in the greenhouse.

ORAL CONTRIBUTION

Global analysis of the genome, transcriptome and epigenome in the diplosporous grass *Eragrostis curvula* (Schrad.) Nees

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The *Eragrostis curvula* complex have a basic chromosome number of x = 10, and include a cytotype series with different ploidy levels (from 2x to 8x) and reproductive modes. The most valuable cultivars as forage are tetraploid and apomictic. This mode of reproduction is strongly related to ploidy. The aim of this work was to identify genetic, epigenetic and transcriptional changes associated to the reproductive mode in leaves and panicles of an euploid series obtained after a tetraploid-dihaploidization procedure followed by chromosome re-doubling with colchicine. Considerable levels of genome polymorphisms were detected between lines, showing markers (31.45% of 1008 RAPD and AFLP) with a revertant behavior (90 % of the polymorphic bands) following the changes of ploidy. This suggests that genome alterations were specific and conferred genetic structures characteristic of a given ploidy level. Polymorphic revertant sequences involved mostly non-coding regions, although some of them displayed sequence homology to known genes. Two per cent of the changes were related to the reproductive mode (apo vs sex). Changes in cytosine methylation patterns were also observed, affecting mainly coding regions (27 % of the 364 markers showed epigenetic variation). A comparative expression analysis based on ESTs showed that from 8,884 unigenes (inflorescence-derived libraries), 112 (1.26%) had significant differential expression in individuals with different ploidy level and/or reproductive mode. Independent comparisons between plants with different reproductive mode (same ploidy) or different ploidy level (same reproductive mode) allowed the identification of genes modulated in response to diplosporous development or polyploidization, respectively. A group of 50 genes were

differencially expressed between the 4x sex and the other plant lines (2x sex, 4x apo) that exhibited highly similar expression patterns. DD analysis (4242 markers from panicles and 7622 from leaves), showed that the 4x apo and 4x sex gene expression profiles were more related and different from the 2x sex one (1.5 % and 9.29% were differential by the reproductive mode and ploidy level, respectively), but confirmed the expression of this particular group of genes (0.64%). As a difference of what happens with the 4x sex plant, the activation/silencing of these genes fail in the 4x diplosporous plant. Diplospory could be the consequence of this failure.

Identification of the mode of reproduction in *Brachiaria humidicola* hybrids

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The *Brachiaria* breeding program at Embrapa Gado de Corte is based on inter- and intraspecific crosses with the objective of increasing the available genetic variability, so as to obtain highly productive, more adapted cultivars. For the crosses to be feasible, the existence of sources of sexuality within the same ploidy level is necessary. The identification of a tetraploid sexual accession of *B. humidicola* in the germplasm, allowed crosses with apomictic tetraploid cv. Tupi to be done. The objective of this work was to determine the reproductive mode of 186 hybrids of this F_1 population, through anatomical analysis of embryo sacs. For this, flowers were collected and the ovaries extracted and clarified using methyl salicilat. At least 40 clarified embryo sacs of each genotype were analyzed with interference contrast microscope. The results of the analysis revealed 102 hybrids with sexual reproductive mode, since they presented only embryo sacs of meiotic origin, and 84 apomictic hybrids, that presented one and/or multiple aposporic embryo sacs. These results are important in the *Brachiaria* breeding program, because the apomictic hybrids may be candidates to new cultivars, whereas the best sexual hybrids may be used as female progenitors in future crosses. Futhermore, by the χ^2 test in the 0.5% significance level, the observed frequencies not differing from the expected in a 1:1 segregation, a simple tetrassomic inheritance and apomixes dominant over sexuality may be suggested.

ORAL CONTRIBUTION

Analysis of sexual and apomictic accessions of *Brachiaria brizantha* using fluorescent *in situ* hybridization

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Brachiaria (Trin.) Griseb. (Poaceae) is one of the most widely cultivated grasses in Brazil and an important forage crop. It is well adapted to poor soils and exhibits tolerance to drought conditions. The genus *Brachiaria* is characterized by ploidy differences among species and the occurrence of sexual and apomictic reproduction. In general, research on the molecular and genetic mechanism conferring apomixis gains in importance since transfer of this trait would allow fixation of hybrids and mass cloning of elite varieties. On the other hand detailed knowledge on the gene or genes involved would open possibilities to break apomixis, thereby allowing recombination through crossings.

The most important species of this genus, *B. brizantha* and *B. decumbens*, are polyploids (2n=4x=36) and their reproductive cycle has been characterized as aposporous apomixes. In general, apomixes is being found in all polyploid *Brachiaria* species, whereas the diploid species (2n=2x=18) are sexual plants. Diploid accessions with sexual reproduction also exist of *B. brizantha* and *B. decumbens*. Up to now there is very little information on the karyotypes in *Brachiaris*. Cytological work mostly concentrated on chromosome counting and analysis of chromosome pairing during meiosis. Cytogenetic studies including the use of FISH (fluorescent *in situ* hybridization) will contribute to the better comprehension of apomixis based on phylogenetic analysis and comparative caryotypes of sexual and apomictic plants. Additionally, physical mapping of genes by FISH is an important tool that potencially allows the localization of specific DNA sequences within chromosomes without the need for genetic polymorphisms and large segregating populations. The presented study on apomictic and sexual accessions of *B. brizantha* introduces the use of FISH in cytogentic analysis of *Brachiaria*. Using heterologous sequences for 5S- and 18S-5.8S-25S-rDNA as probes the number and position of the rDNA sites on metaphase chromosomes of BRA-00274 (diploid sexual) and BRA-00591 (tetraploid apomictic) could be determined. By showing the presence of only one site of 5S-rDNA in the diploid and three sites in the tetraploid accession, these results, together with the morphological

data on chromosome lenght and centromere position, strongly supported the hypothesis of the allopolyploid origin of BRA-00591. It is expected that these initial FISH experiments will facilitate future experiments in *Brachiaria* on physical cytogenetic mapping of DNA sequences associated with apomixis.

Polyploidy and polyembryony in *Anemopaegma* (Bignoniaceae-Bignonieae)

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Polyploidy consists on the presence of more than two chromosome sets within a same organism. It represents one of the most extreme ways through which the genomic architecture of organisms can be changed. Interestingly, the majority of apomictic plants are polyploids suggesting that the expression of apomictic phenotypes is ploidy-dependent. The objective of the present project was to check whether polyembryony is related to polyploydy in selected *Anemopaegma*. For that, we determined chromosome numbers and the number of embryos per seed in five species and two morphs of *Anemopaegma* (*A. acutifolium*, *A. arvense*, *A. glaucum*, *A. scabriusculum*, *A. velutinum*, *Anemopaegma* sp1 and *Anemopaegma* sp2). In order to determine the number of embryos per seed, we used seeds from ripe fruits derived from wild populations as well as seeds generated through artificial and natural pollination. Seeds were imbedded in water, dissected and germinated. During dissection, we quantified the number of embryos present in each seed. During germination, we quantifie the number of seedlings originated from each seed. Chromosome numbers were determined from root meristems imbedded in a solution of 8-hydroxiquinolein 2 mM for 24 hours at 8ºC, and subsequently fixed in Farmer solution (ethanol: acetic acid 3:1). We encountered multiple embryo (2-7) and the emergence of multiple seedlings (2-4) per seed in all studied taxa, except for *A. velutinum*. The majority of the studied taxa presented 2n=4x=80, except for *A. velutinum* that presented 2n=2x=40. In addition, we observed polysomaty in populations of *A. glaucum* that presented both diploidy (2n=40) and tetraploidy (2n=80) within a single root meristem. These results indicate a strong relationship between polyembryony and polyploidy in the studied taxa.

Isolation and Characterization of a *Serk* (*Somatic Embryogenesis Receptor-Like Kinase*) cDNA from the Apomictic *Brachiaria Brizantha*

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Genes that are important in plant transduction signal pathways have been isolated in model plants. The *SERK* (*SOMATIC EMBRYOGENESIS RECEPTOR LIKE-KINASE*) gene is part of a multigenic family of membrane receptors of the type RLK (Receptor-Like Kinase). *SERK* was first isolated from carrot and is expressed at the early stages of somatic and zygotic embryogenesis. Apomixis is a natural process of cloning through seeds in which the embryo development is autonomous. The aim of this work is to study *SERK* expression during the apomictic reproduction in *Brachiaria*. Using degenerated primers, a sequence of *SERK* coding region was amplified by RT-PCR from cDNA of the apomictic *B. brizantha* cv. Marandu ovaries. The amplified sequence (~ 800 pb) was cloned into vector TOPO PCR 2.1. Two clones were isolated and sequenced. Nucleotide sequences were analyzed by PHRED program, grouped by CAP3 program and compared by BLAST with *SERK* sequences deposited at NCBI. Two contigs were obtained that showed high identity with nucleotide sequences of two monocot species. Contig 1 (*BbrizSERK1*) with 919 bp had 88% identity to *Zea mays SERK1* (e-value 5e-175) and 84% to *Oryza sativa SERK1* (e-value 2e-100). Contig 2 (*BbrizSERK2*) with 923 bp showed 98% identity to *Z. mays SERK2* (e-value 0.0) and 96% identity to *O. sativa SERK2* (e-value 0.0). Full length cDNA sequence will be obtained by 5' and 3' RACE. We show the comparison of the deduced amino acid sequence with *SERK* sequences from other species identifying highly conserved structural *SERK* domains. Studies on the expression of *SERK* during zygotic, autonomous and somatic embryo development in apomictic *B. brizantha* will contribute to a better understanding of the reproductive process in this species.

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In situ expression pattern of a putative MAP kinase gene from *Paspalum* during ovary development of *Brachiaria brizantha*

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Plants from the genus *Brachiaria* are widely cultivated and used as forage for cattle production in the tropical areas of Brazil. The genus *Paspalum* is mostly found in subtropical areas in the south of Brazil and Argentina. These genera of grasses have plants that reproduce by both, sexuality and apomixis, more specifically by apospory. Because *Brachiaria* and *Paspalum* show similarities in their ovule development, we are analyzing the expression of some cDNA sequences found to be of interest for *Paspalum* apomictic and sexual development in ovaries of *Brachiaria*. One of them is a cDNA clone N46, sequence corresponding to a MAP kinase (MAP3K) differentially expressed in apomictic and sexual plants of *Paspalum notatum*. In this work, we examined the pattern of this MAP3K *Paspalum* gene expression during ovary development in sexual and apomictic *Brachiaria brizantha* by *in situ* hybridization. For that, 7 µm sections of ovaries of sexual and apomictic *B. brizantha* in stages of megasporogenesis and megagametogenesis were used. RNA preservation was observed staining some sections with acridine orange. The sections were hybridized overnight at 42 C with 600 ng ml⁻¹ of sense or antisense digoxigenin-labelled probe in 100 µL of hybridization solution. After post-hybridization treatments the slides were mounted. Signal of hybridization was observed in ovaries, specially in stage of megasporogenesis (archespore or MMC, coenocyte) of sexual and apomictic plants and less intense or absent in later stages. In agreement with prior observations in *Paspalum*, in the sexual plant the signal was more strong. Unexpectedly, signal was also found when using the sense probe in the same pattern found for the antisense probe. Based on the observed results, we suggest a modulation in gene expression of *Paspalum* clone 46 during *Brachiaria* ovary development.

ORAL PRESENTATION

Expression and phylogenetic analysis of two putative MADS-Box like genes of *Brachiaria brizantha* (A. Rich.) Stapf

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Brachiaria genus has species containing sexual and apomictic plants. In apomictic reproduction, embryo sac differentiates from an unreduced cell and the embryo develops in the absence of egg cell fertilization. MADS-Box genes are transcription factors that are involved in floral organ formation. The characterization of these homeotic genes in *Brachiaria brizantha* can contribute for the understanding of reproductive organs differentiation in apomictic and sexual plants. The objective of the present work was to analyze the MADS-Box genes expression profile, aiming to correlate their activity with apomixis and sexuality. Two MADS-Box sequences, contigs 38 and 119, were identified in cDNA libraries of ovaries in megasporogenesis and megagametogenesis from sexual and apomictic *Brachiaria brizantha*. Phylogenetic analysis showed that the contig 38 deduced aminoacid sequence is close to *AGL6* and contig 119 to *SEPPALATA 2* (*SEP2)* from *Arabidopsis thaliana*. Analysis by qRT-PCR indicated down-regulation of the genes associated to contig 119 in ovaries of apomictic *B. brizantha* during megasporogenesis and down-regulation of the genes associated to contig 38 in ovaries of apomictic *B. brizantha* during megagametogenesis. The expression of genes associated with both contigs was lower in anthers than in ovaries in apomictic and sexual plants of *B. brizantha*. The transcripts of these genes were localized in ovaries, anthers and floral meristems of apomictic and sexual plants in different stages of development. For contig 38 the expression was equivalent in apomictic and sexual plants. The data obtained at this work compared with results from other plants species reported at the literature suggest that the genes associated to contig 38 e 119 are involved with the identity of floral organs and the meristem of *B. brizantha*.

Pollen and anther wall development of male sterile *Miconia albicans* (Sw.) Triana (Melastomataceae)

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Miconia albicans is an obligate apomictic species which presents empty anthers but viable seeds. Have in mind the poverty of reproductive ontogenetic studies in *Miconia* species and the strict association of the anther wall cells with pollen grains production, the goal of this study was to elucidate if the male sterility was related to specific developmental stage of microsporogenesis and anther ontogenesis. Natural populations of *M. albicans* were selected from both Atlantic Forest and Cerrado areas on São Paulo state, southeastern Brazil. Floral buds at several stages of development and flowers were collected from three individuals in each population, from 2005 to 2007. Anthers were dissected and studied under light and scanning electron microscopy. In the early stages of anther development, a protoderm surrounding a mass of fundamental meristem cells and connective cells in the beginning of differentiation were observed. Subsequently, the protodermis gave rise to the epidermis, and the subdermal cell layer formed a primary parietal layer (PPL) and a group of sporogenic cells through discontinuous periclinal divisions. The PPL gave rise to both, outer and inner secondary parietal layers (SPL). Outer SPL apparently gave rise directly to an endothecium with no parietal thickness. Inner SPL, by periclinal divisions, originated two middle layers (ML). Outer tapetum was apparently originated from cells of the peripherical sporogenic tissue. Microspores mother cells (MMC), most of them abnormal, differentiated from sporogenic cells along with the degeneration of the ML. Tapetal cells presented alterations in their morphology and cytoplasmic and/or nuclear contents. Some dyad and tetrad of microspores originated from meiosis on normal microspore mother cells was observed at the end of this stage. Flowers at pre-anthesis stage presented tetrasporangiate anthers with anther wall reduced to a one-layered epidermis and an uncommon endothecium, with no parietal thickness. Most of pollen sacs were empty or presented abnormal cells, resulting in complete male sterility. Events leading to the male sterility were not restricted to one developmental stage, since abnormal cells were observed along all developmental stages of microsporogenesis, and they are probably mostly related to the tapetum. It is worthwhile to emphasize the presence of an uncommon endothecium, which indicates the needy of reassessment the absence of endothecium as a synapomorphy for Melastomataceae.

Identification of the best reference genes for qPCR in sexual and apomictic *B. brizantha*

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Brachiaria is an important Poaceae genera largely used as forage grass for beef cattle in Brazil. In *B. brizantha* most accessions reproduce by apomixis, an asexual mode of reproduction through seeds. The occurrence of both apomictic and sexual reproduction within the same species makes *Brachiaria* an excellent system for understanding the molecular pathways involved in both modes of reproduction. In this work we evaluated eight potencial reference genes obtained from expressed sequence tags (EST) of a cDNA library of *Brachiaria brizantha* for its use in qPCR expression profile analysis. For that, the relative transcription levels these eight potencial reference genes were determined in 20 different samples of sexual and apomictic *Brachiaria brizantha* acessions, including ovaries at the four different developmental stages, megasporogenesis and megagametogenesis, anthers at analogous stages and also in leaves and root tissues. We have used Miner algorithm to evaluate primer efficiency and GeNORM application for selection of the best reference genes for *Brachiaria brizantha*. The primer of the eight genes varied from 0.87 ± 0.012 (87%) to 1.01 ± 0.009 (101%) and the Ct values of the candidate reference genes in all samples were within 13.99 and 33.22 cycles, showing a high range of variation between them. Primer coding for elongation factor one gene (E1F) showed the highest expression levels while primer coding for succinil coA synthetase gene (SUCOA) showed the lowest within the chosen set of samples. Considering the most stable genes according to geNORM, two of them, UBCE (ubiquitin conjugating enzyme) and E1F, showed an M value well-suited for a reference gene. The primers used for these genes showed the M value of 0,47 and 0,79 respectively. These genes have been pointed as suitable reference genes for qPCR in other plants species and also for other experimental techniques and will be used in further gene expression comparative analysis in *Brachiaria brizantha* studies.

ORAL CONTRIBUTION

Identification and functional characterization of cis-elements regulatory of gene involved with desiccation of pollen and seeds of *Arabidopsis thaliana*

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Abstract

The reproductive development in plants is achieved through a comple gene interaction network that involves transcriptional regulation. Most of this regulation is based on binding specificity of transcriptional factors in cis-elements or motifs. Among the events observed reproductive development, we have particular interest in pollen grain desiccation. This process takes place just before the anthesis and it is fundamental to pollen viability. In spite of the differences in the developmental programs of pollen and seeds, recent results have identified genes with expression in both processes, where desiccation is a key event. These results indicated that cis-elements in genes important to osmotic adaptation in pollen and seed might be conserved. Therefore, we aim with this work to identify cis-elements that might be shared by pollen and seed desiccation developmental programs.

The *ms1* (*male sterility1*) mutant fails to form pollen grains because of abnormal tapetal development after microspore releasing from the callos wall. We used data from microarray experiments where gene expression of *Arabidopsis thaliana* wild type and *ms1* plants were compared during flower development. Initially, 228 genes were selected for the analysis based on their expression pattern restricted to the late stages of flower development. These genes were selected because their temporal expressio patterns correspond to the development stage where pollen desiccation takes place. The webtool Athamap (www.athamap.de) was used to identify putative cis-elements in promoter regions of 500 base pairs upstream of the start codon. These genes also were analyzed in Genevestigator (www.genevestigator.ethz.ch) to check their expression pattern in seed and osmotic stress. Based on our analysis, we selected four genes that are highly expressed in seeds and during osmotic stress. These four genes might have in their promoter region cis-elements important to the expressio during osmotic stress in pollen grains and seeds. To evaluate the expressio pattern of these four genes *in vivo*, 500 bp promoter regions were cloned in binary vectors fused to reporter gene GUS. Expression of GUS in

transgenic lines will be inspected by histochemical assays during pollen and seed development. The characterization of cis-element regulators is very important to understand the complex network of gene interactions during osmotic adaptation. Therefore, this work may bring to light common elements crucial to desiccation process observed during pollen and seed development.

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Development of microsatellite DNA markers to study the mode of reproduction of the tropical species *Cariniana estrellensis* (Raddi) Kuntze

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Tropical forests generate the richest biodiversity and most of them are disappearing at an alarming rate. In the last decades, the deforestation and concomitant large-scale fragmentation of the forest has been rapid and extensive. For management and conservation of tree populations in such heterogeneous habitats, it is important to know the reproductive patterns and the distances of effective gene flow mediated by pollen and seeds. *Cariniana estrellensis* (Raddi) Kuntze (Lecythidaceae) is a wide-ranging tropical tree species occurring in Brazil, Bolivia, Paraguay and Peru. Due to high deforestation rates in its native range, *C. estrellensis* is considered an endangered species. Presently, no data about the reproductive system are available. In this work, we report the development of microsatellite (SSR) loci for *C. estrellensis*, in order to evaluate mating patterns and gene flow in a small, isolated fragment of this tropical species in southeast Brazil. Total genomic DNA was extracted from leaf tissue and was digested with RsaI restriction enzyme. A (GA)n and (CA)n microsatellite-enriched library was constructed, using a biotin-labeled microsatellite oligoprobe and streptavidin-coated magnetic beads. A total of 54 positive clones were sequenced and 37 (68.52%) presented microsatellite sequences. Only nineteen SSR were selected for primers design because their sequences presented more than five tandem repeats. A screening of

each primer pair through 13 annealing temperatures (between 45–57°C) was performed with five individuals of *C. estrellensis*. PCR products were denatured and separated on 10% denaturing polyacrylamide gels, stained with silver nitrate and sized by comparison to a ladder standard (Amersham Biosciences). Mendelian analyses were confirmed for each locus, based on analysis of two mother trees and their open-pollinated family. Among the 19 primer pairs tested, only 15 yielded amplification products that were consistent and polymorphic. PCR conditions for these fifteen SSR primers were optimized. Mendelian inheritance was confirmed for all fifteen microsatellite loci, based on the analysis of two mother trees and their open-pollinated family (fifteen individual per family). All sibs displayed at least one of the maternal alleles. Additionally, the 15 primer pairs developed for *C. estrellensis* were tested on genomic DNA of seven individuals of *Cariniana legalis*. Thirteen markers (86.67%) were amplified and ten loci were polymorphic. The results indicated that there is a high potential for transferring microsatellite markers between species of the same genera in the Lecythidaceae family. The development of these new microsatellite markers (SSR) will be a valuable tool to investigate refined questions of mating system, gene flow, family structure and population dynamics of tropical tree species *C. estrellensis*.

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Computational identification of *Nicotiana tabacum* stigmas/styles miRNAs and their targets by the analysis of the TOBEST database

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Our research group has constructed a *Nicotiana tabacum* stigmas/styles cDNA library, sequenced to generate ESTs (Expressed Sequence Tags). The sequences were clustered, resulting in 6,177 assembled sequences, which were organized in a database denominated TOBEST (TOBacco ESTs). We compared the “TOBEST database” with the “miRNA Registry Database” using the BlastN tool to identify the putative microRNAs (miRNA) in the *N. tabacum* stigmas/styles with similarity to known miRNA genes. The TOBEST 051G09 clone revealed significant similarity with the *Arabidopsis thaliana* miR390a. The analysis of the 051G09 sequence through the RNA mFold 2.3 software has shown an RNA hairpin-shaped

secondary structure similar to the Ath-miR390a pre-miRNA fold. In accordance to what is expected for a miRNA structure, we found in the 051G09 sequence the following features: 1) three mismatches between the predicted mature miRNA sequence and its opposite miRNA* sequence, 2) no loop or break in the miRNA or miRNA* sequences, and 3) the predicted secondary structure had high MFEI and negative MFE. Our results show that the 051G09 clone has patterns resembling the previously known *Arabidopsis* mirRNA 390a, a miRNA that plays a role in metabolic processes. A second computational approach was carried out in the TOBEST database to predict new putative miRNAs and their targets. The 6,177 assembled sequences were analyzed by the BlastX program against the nr database (Genbank), showing that 4,625 sequences were similar to previously deposited sequences (hits) while 1,152 showed no significant similarity (cut off e-value of 10^{-5}) to other protein sequences (no hits). Through findMiRNA software we compared TOBEST "hits" versus "no hits" and predicted 4,457 potential alignments between putative miRNAs and their corresponding target sites, from which 2,316 presented MFEI > 0.85. To restrict the list of miRNA candidates, we selected the candidates with no gaps between miRNA:miRNA* at the precursor miRNA and removed from the complete list all candidates with similarity to protein coding sequences identified by TBlastX in the nt database (Genbank). In addition, we also eliminated those candidates with long ORFs (120 aminoacids), as defined by the Expasy's translate tool, in the expectation that those without a long ORF may encode miRNAs. In the end were selected 22 TOBEST clones with high probability of being miRNA clones. Our next aim will be the validation of some predicted miRNA and their targets by experimental methods. Financial support: FAPESP and CAPES.

Use of cryoprotectors in the conservation of *ricinus communis* l. pollen grains

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The castor bean is considered a species of excellent potential for cultivation in Brazil for being an oleaginous plant involved with the

production of biodiesel.. However, the sustainability of a biodiesel program based on this species demands a substantial strengthening of the agricultural technological base. In order to attend the species conservation needs as an important genetic resource a promising methods has been the cryopreservation of biological material, with great relevance for its application in techniques of genetic breeding, as well as a form to avoid the loss of the species genetic integrity. This technique is based on the storage in liquid nitrogen (-196º C) which promotes a reduction on the metabolism to levels so low that all the biochemical processes are significar reduced and the biological deterioration is virtually paralyzed. The objective of this work was to establish the use of a cryoprotector agent to maintain th integrity of the pollen grain of *Ricinus communis* L. during cryopreservation. Floral buds of castor bean were used to evaluate the pollen grain viability through the use of acetic carmim, after treatment in liquid nitrogen (-196º C for one hour using the cryoprotectors, Glycerol and DMSO (dimethylsulphox and methylglycol), in the concentrations of 0.0; 5.0; 10.0 and 15.0%. For thawing, samples were plunged in water bath during 10 minutes at 37º C. The results indicated that the use of 5% DMSO is efficient in the cryopreservation of the pollen grain. It was also verified that were differenc among the treatments used for the pollen conservation. In the analysis of the number of pollen grains burst, cryopreservation in the absence of cryoprotector differed significantly from the tested treatments indicating that the use of cryoprotectors is necessary for the conservation of pollen grains of this species (Project supported by CNPq).

Grant: CNPq e Fapemig

Keywords: Cryopreservation, castor oil plant, glycerol, dimethylsulphoxide and methylglycol.

Callus induction from castor bean anthers

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The castor bean (*Ricinus communis* L.) is an oleaginous plant of social and economic importance. Its oil, extracted by pressing the seeds,

possesses a wide range of industrial applications and recently it has been indicated as an alternative source of energy to the Biodiesel production. Among the techniques of plant breeding, the anthers culture presents as a biotechnological tool of great utility, mainly, for reducing the time necessary to obtain haploid lineages. Therefore, the objective of this work was to induce callus formation from anthers of *Ricinus communis* L. Different concentrations (0.0; 0.5; 1.0 and 1.5 mg L^{-1}) of 2,4-D (2,4-dichlorinephenoxiacetic acid) combined with 0.5 mg L^{-1} kinetin were tested. The floral bud with size varying from 3.0 to 3.9 mm were disinfected, the anthers extracted and inoculated in MS culture medium solidified with 6.0 g L^{-1} agar supplemented with 30 g L^{-1} sucrose and 1.0 g L^{-1} activated charcoal. Callus formation and color after 30 days of cultivation were evaluated. Callus formation from anthers was observed independent of the 2,4-D concentration and its combination with kinetin. The formed callus presented yellowish color with a globular aspect (Project supported by CNPq).

Grant: CNPq e Fapemig

Keywords: *Ricinus communis* L., calogenesis, tissue culture, 2,4-dichlorinephenoxiacetic acid, kinetin.

Pollen ontogeny associated with flower bud and anther size of Castor Bean

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The castor bean is an oleaginous plant of great importance to the energetic source in Brazil and in the world. The objective of this research was to study the pollen grain ontogeny associated with floral bud and anther size, relating the microsporogenesis and microgametogenesis stages in a castor bean (*Ricinus communis* L) population. Floral buds in different stages of development were randomly collected from an experimental field at the Federal University of Lavras, fixed in Carnoy and ethanol : acetic acid (3:1). The material was macerated in 1% acetic carmim for observation and registration of the anther longitudinal size as well as the pollen

ontogenesis phases. The data were analysed and the averages were compared by the Tukey test with 5% significance. The variance analysis demonstrated that there was significative difference between the bud and anther sizes as related with the pollen grain development stage. It was verified that anther and bud sizes are indicated to monitor the different phases of the meiotic division (microsporogenesis) as well as mature pollen grain and micropore stages (microgametogenesis). Pollen grain obtained from floral buds greater than 5 mm, close to anthesis, presented higher pollen viability, statistically superior than the other floral bud size scales (Project supported by CNPq).

Grant: CNPq e Fapemig

Keywords: microsporogenesis; microgametogenesis; *Ricinus communis* L.

Assessment of mating system in *S. capitata* and *S. guianensis* using RAPD markers

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Stylosanthes capitata and *S. guianensis* are forage legume species of high protein level, thus of large interest for use in mixed pastures with tropical grasses. Moreover, they are adapted to low soil fertility and to ecosystems with a well defined dry season. In addition, they constitute a low-cost option to regenerate degraded pastures. The mating system of these species was studied at Embrapa Beef Cattle, Mato Grosso do Sul state, Brazil, using RAPD (Random Polymorphic Amplified DNA) markers, based on a hypothesis of mixed-mating models. For each species, 10 progenies of 20 individuals were analyzed. The following parameters were estimated: multilocus outcrossing rate (t_m); single-locus outcrossing rate (t_s); selfing rate ($s = 1 - t_m$); biparental inbreeding ($t_m - t_s$); inbreeding coefficient of the parents (F); correlation of outcrossing paternity (r_{pm}) and correlation of selfing (r_{ps}). These estimates were calculated using the maximum likelihood method, numerical algorithm Expectation-Maximization (EM). The variance of the evaluated parameters was estimated based on 1000 bootstraps samplings within progenies. The results demonstrated that these species have a mixture of selfing and outcrossing with 68,1% of allogamy in *S. capitata* and 38,6% in *S. guianensis*. The differences

between t_m and t_s revealed that random mating occurs among related parents. The inbreeding coefficients of the maternal genotypes (F) estimated in *S. capitata* (0,643) and *S. guianensis* (0,622) indicate high homozygosis in the parents. The estimated r_{pm} and r_{ps} revealed that in the two populations a considerable number of crosses was biparental (r_{pm} = 0,313 for *S. capitata* and 0,212 for *S. guianensis*) and another, resulted from selfing (r_{ps} = 0,342 in *S. capitata* and 0,102 in *S. guianensis*). These results should assist the breeder in choosing the best breeding method for these species. Besides, the outcrossing rate should guide the germplasm conservation as well as the collecting activities for these species.

Search of RAPD Molecular Markers linked to Apomixis in *Brachiaria humidicola*

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Apomixis has been formally defined as an asexual (agamic) reproduction by seeds. This reproductive event takes place in the ovule of some angiosperms and proceeds, bypassing female meiosis and syngamy, to generate parthenogenetic embryos genetically identical to the mother plant. It occurs in approximately 400 genera from 40 plant families, having evolved multiple times within flowering plants. *Brachiaria* is a forage crop in tribe Paniceae, and apomictic genotypes produce aposporous, 4-nucleate embryo sacs wherein the egg cell can begin to develop into an embryo prior to pollen discharge. Genetical studies indicate that apospory in *Brachiaria* is inherited as a dominant character and is controlled by a single Mendelian factor. Moreover, recent applications of molecular-markers techniques provided strong evidence on single-gene inheritance of the reproductive mode in this species. The identification of a genomic area related to apomixis in *Brachiaria* hybrids has already been reported, but the information so far available includes few markers tightly linked to the apo-gene(s) that could be used for the rapid identification of apomictic progenies. One F1 population with 100 individuals, derived from a *B. humidicola* intraspecific cross between a tetraploid sexual plant (H31) and the cultivar cv. BRS Tupi (tetraploid apomitictic), that segregated 1:1 for

the mode of reproduction (meiotic vs apomeiotic), was used, together with bulk segregant analysis (BSA) for the detection of RAPD genetic markers linked to the gene(s) responsible for apospory. Ten bulks consisting of a mixture of equimolar quantities of DNA (5 apomictic and 5 sexual) with 10 plants each were assembled. Out of the 145 polymorphic primers analyzed in the bulks, two (64 and 78) were present in the apomictic bulk (AB) and not present in the sexual bulk (SB). Markers identified in the male genitor (apomictic) found only in the AB bulk would be an indication of connection to these markers with the characteristic of apomixis. In the future, RAPD reaction with all plants (apomictic and sexual) will be made to determine if these two markers will be significantly linked to apomixes in *B. humidicola*.

Analysis of organ specific expression of *Brachiaria brizantha* cDNA isolated from ovaries

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Brachiaria is an economically important forage grass. Widely used as forage for beef cattle in Brazil, it is cultivated in about 70 million hectares. Its economical value is due to adaptability to poor and acid soils, spittlebug and dry resistance as well as nutritional quality. One limiting factor in *Brachiaria* breeding is apomixis, an asexual reproduction through seeds. Ovary specific promoters from *Brachiaria* are desired for apomixis functional gene expression studies as super-expression or knocking down. The identification of cDNA specific of ovaries may help in the search for their promoters in genomic libraries. The objective of this study is to determine the organ-specificity of three cDNA previously isolated from ovaries. These cDNA (Clones 04, 09 and 21) were isolated from ovaries of apomictic and sexual plants and their expression was detected only in the reproductiv organs (ovaries and anthers) of *Brachiaria brizantha*, by reverse northern. Clones 09 and 21 had been sequenced, and search in public gene banks of the deduced aminoacid sequences had shown similarities with proteins involved with synthesis, processing and regulation of the translation of ribosomal proteins. These sequences were also searched in four EST *B. brizantha* libraries from apomictic (B30) and sexual (B105) ovaries at megasporogenesis and megagametogenesis. Clone 09 showed similarity to contig 39 composed of hits from B30 at megasporogenesis and B105

at megagametogenesis libraries. Clone 21 showed similarity with contigs 34 and 67, from B30 library at megasporogenesis. For clones 09 and 21, RT-PCR experiments for semi-quantitative analysis were conducted using total RNA from ovaries and anthers in megasporogenesis and megagametogenesis, leaves and roots from *B. brizantha* apomictic and sexual plants. Analysis of clone 09 showed differential expression profile. In the apomictic plant the expression was higher in ovaries in megasporogenesis, anthers and roots, while in the sexual plant the expression was higher in ovaries at the late stage of megagametogenesis and roots. Both cDNA sequences showed a lower expression pattern in leaves comparing to the others organs and tissues. The results from clone 21 in apomictic plants showed similar expression in all stages of ovary development, anthers and roots, with lower expression in leaves. For the sexual plant, the expression pattern was similar in all stages of ovary development and tissues.

The search for genes regulating pollen-pistil interactions in *Senecio squalidus* (Asteraceae)

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Complex pollen-pistil interactions are vital for successful fertilisation and reproduction in flowering plants. The tissue of the pistil acts as a physical and chemical interface between the male and female gametophyte beginning at the stigma surface during pollen germination and continuing until successful fertilisation at the ovary. Many different processes occur simultaneously in pistillar tissues, including species recognition, self-incompatibility (SI), pollen hydration, pollen tube growth and pathogen defense. It is clear that a high diversity of both pollen and pistil molecules are required to enable the complex and distinct interactions that occur between these two tissues. The model species *Senecio squalidus* (Oxford Ragwort) is being used to investigate pollen-pistil interactions in the Asteraceae, including a novel sporophytic self-incompatibility (SSI) mechanism, with the aim of identifying potential genes or genetic loci that regulate SSI.

Stigma-specific cDNA libraries have been generated using suppression subtractive hybridization (SSH) and validated by multiple rounds of screening and northern blot analysis. Consequently a set of candidate genes for involvement in pollen-pistil interactions, and in particular self-incompatibility, has been produced. Pistil-specific genes are likely to play important roles in defense, pollen hydration, SI and structural support within the extracellular matrix (ECM). The stigma-specific libraries created using SSH have identified components of all these systems, and may be used to compliment and confirm information from other species. Together the data can be used as a basis for building networks of interacting proteins and molecular pathways both within the pistil and between the female and male reproductive tissues. Additionally, comparisons with pistil-specific datasets from other species, and with extensive *Senecio* EST databases (generated via microarray analysis of mature bud tissue) have identified a number of novel genes in the stigma cDNA libraries. These are likely to correspond to rare transcripts in the stigma transcriptome and highlight the usefulness of SSH as a technique that identifies all genes expressed in the tissue of interest. Potential candidates for the female *S*-gene have

been identified via developmental northern blot and polymorphism analysis. In conclusion, SSH has generated a robust dataset that well represents the stigma-specific transcriptome in *Senecio squalidus*, and will provide a useful resource to study both conserved and novel pollen-pistil interactions in this species.

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