IV International Congress on APOMIXIS

December 3 - 7, 2023 • Rosario, Argentina

Book of Abstracts

Table of Contents

Introduction

The **IV International Congress on Apomixis Research** gave us the opportunity to celebrate 28 years of nonstop progress in this field since our first international meeting, which was held in Texas (USA) in 1995. After that, the apomixis community met in Como (Italy) in 2001, and Wernigerode (Germany) in 2007.

This conference brought together 80 participants coming from 18 different countries. The most represented communities were the argentinian and the italian ones, but there were also eminent professors and scientists from Albany, Australia, Bangladesh, Canada, China, Czechia, France, Germany, India, Mexico, Perú, Portugal, Switzerland, The Nederlands, The United Kingdom and The United States.

We discussed 47 scientific contributions and enjoyed the presentations of 16 invited speakers, 9 session talks selected from the submitted abstracts, 1 round table on scientific policies and a discussion session on perspectives. Finally, we organized an open-to-the-community session in order to share our work with the general public of all ages.

During their stay in Rosario, the attendees had the opportunity to visit some of the iconic places of the city. We hope they found this congress inspiring and went back home with creative new ideas, collaborations and friends, as well as an increased interest in their work.

We would like to thank the institutions and consortiums that provided financial and practical support to the event: the Italian Embassy in Argentina, the Italian General Consulate of Rosario, the Ministry of Foreign Affairs of Italy, the University of Milano, the Government of the Santa Fe Province, the National University of Rosario, the National Agency for the Promotion of Research, Technological Development and Innovation of Argentina, the National Council for Scientific and Technological Research of Argentina (CONICET), the Rosario Board of Trade and the Agricultural Science Foundation (FCA UNR). The congress organization has also received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No [872417], Project MAD and No No [101007438], Project POLYPLOID. We are also grateful for the support received from the Faculty of Agronomy of the National University of Rosario, the Research Institute of Agricultural Sciences of Rosario (IICAR), the Scientific and Technological Centre of CONICET Rosario (CCT Rosario) and the ROSCYTEC Foundation.

Finally, we would like to thank all the members of the Apomixis Argentina Group, for their valuable help during the organization of this event, and specially the people of the IICAR Plant Reproductive Development group.

Dr. Juan Pablo Ortiz
IICAR Director
Local Host

Dra. Silvina Pessino
IV International Congress on Apomixis
President of Organizing Committee

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Sunday 3th December 2023

19:00 - 21:00	**Opening Cocktail** - ***only for invited speakers and organizers*** - Opening talk by **Marco Bocchi**, Consul General of Italy in Rosario. Consulate General of Italy in Rosario (Address: Montevideo 2182, Rosario)

Monday 4th December 2023

08:00 - 09:00	**Registration at the Conference Venue** Rosario Board Trade Convention Center (Address: Paraguay 755, Rosario).
	Morning Session. *Coordinators: Luciana Delgado, Carolina Colono*
09:00 - 10:00	**Opening Plenary Lecture: Prof. Dr. John Carman**, Utah State University, USA. *Apomixis: origins, regulation and speciation implications.*
10:00 - 10:30	Coffee break
10:30 - 11:00	**Recognition Award: the contribution of Camilo Quarin to apomixis research** In charge of Dr. Fulvio Pupilli.
11:00 - 11:30	**Invited speaker: Arp Schnittger**, University of Hamburg, Germany. *A cytological framework of female meiosis in Arabidopsis and maize*
11:30 - 12:00	**Invited Speaker: Viviana Echenique**, CERZOS-CONICET-UNS, Bahía Blanca, Argentina. *New insights into the control of apomixis in Eragrostis curvula.*
12:00 - 13:50	Lunch time
	Afternoon Session. *Coordinators: Maricel Podio, Juan Manuel Vega*
13:50 - 14:10	**Oral presentation: Ramsankar Chandrasekar**, Institute of Biology III, Albert-Ludwigs-University of Freiburg, Germany. *WINDHOSE-RAB GTPASE HOMOLOG A1-dependent membrane localization of the auxin transport protein PINFORMED1 promotes female germline entry in Arabidopsis*
14:10 - 14:30	**Oral presentation: José Carballo**, CERZOS-CONICET-UNS, Bahía Blanca, Argentina. *Unveiling the apomictic allotetraploid genome of Eragrostis curvula.*
14:30 - 14:50	**Oral presentation: Andrés Bellido**, CERZOS-CONICET-UNS, Bahía Blanca, Argentina. *Arabidospis thaliana could provide new insights into the driving forces underlying the switch from sexual to apomictic development*
14:50 - 15:10	**Oral presentation: Xixi Zheng**, University of Regensburg, Germany. *Understanding the Molecular Mechanism of Parthenogenesis in Cereals.*
15:10 - 15:30	**Oral presentation: Marta Mendes**, University of Milano, Italy. *AUXIN RESPONSIVE FACTOR 10 insensitive to miR160 regulation induces apospory-like phenotypes in Arabidopsis.*
15:30 - 16:00	Coffee break
16:00 - 18:00	**Flash presentation of the posters + Poster Session** *(the posters will remain displayed throughout the course of the meeting)*
20:00 - 01:00	**MAD Social gathering at the bar "Silos Davis" on the Paraná River.** Bar "Los Silos (Address: Av. de la Costa Estanislao López 2550).

Tuesday 5th December 2023

Morning Session. *Coordinators: Lorena Siena, Juan Pablo Selva*

09:00 - 09:30	**Invited Speaker: Abed Chaudhury**, Krishan Foundation, Australia. *Apomixis and heterotic perenniality in rice*
09:30 - 10:00	**Invited Speaker: Ueli Grossniklaus**, University of Zürich, Switzerland. *Towards the engineering of apomixis in maize.*
10:00 - 10:30	Coffee break
10:30 - 11:00	**Invited Speaker: Stewart Gillmor**, Langebio, CINVESTAV, Mexico. *Hybrid effects on zygotic genome activation in Arabidopsis thaliana.*
11:00 - 11:20	**Oral presentation: Luciana Delgado**, IICAR-CONICET-UNR, Rosario, Argentina. *3D architecture of the ovule during MMC differentiation in Paspalum rufum.*
11:20 - 11:40	**Oral presentation: Maricel Podio**, IICAR-CONICET-UNR, Rosario, Argentina. *Resolving the gene content of the genomic region associated with apomixis in Paspalum notatum using a diploid genome assembly.*
11:40 - 12:00	**Announcements**
12:00 - 14:00	Lunch time

Afternoon Session. *Coordinators: Viviana Echenique, Juan Pablo Ortiz*

14:00 - 14:30	**Invited Speaker: Olivier Leblanc**, IRD-Montpellier, France. *Functional characterization of apomixis candidate genes in Arabidopsis.*
14:30 - 15:00	**Invited Speaker: Lucia Colombo**, University of Milan, Italy. *Unraveling complex mechanisms in plant reproduction for the crops of the future.*
15:00 - 15:30	**Invited Speaker: Gabriela Pagnussat**, Universidad Nacional de Mar del Plata, Argentina. *A mitochondrial electron shuttle essential for female gametophyte and early embryo development in Arabidopsis.*
15:30 - 16:00	Coffee break
16:00 - 18:00	**Round Table on public scientific policies and strategies**. *Participants:* **Fernando Peirano,** President of the National Agency for the Promotion of Research, Technological Development and Innovation; **Marina Baima**, Secretary of Science and Technology of the Province of Santa Fe; **Roberto Rivarola**, Vice President of Technology Affairs, CONICET; **Sandra Fernández,** Director of CCT CONICET Rosario.
20:00 - 23:00	**Social gathering at Bar "El Cairo"** (Address: Santa Fe 1102)

Wednesday 6th December 2023

Morning Session. *Coordinators: Diego Zappacosta, Andrés Bellido*

09:00 - 09:30	**Invited Speaker: Peggy Ozias-Akins**, University of Georgia, USA. *Insights on asexual reproduction through seeds from Pennisetum/Cenchrus apomictic species.*
09:30 - 10:00	**Invited speaker: Giovanni Gabelli**, DAFNAE, Agripolis, University of Padova, Italy. *Dissecting apomeiosis in alfalfa (Medicago sativa L.): Genomics applied to unreduced gamete mutants and sexually induced polyploids.*
10:00 - 10:30	Coffee break
10:30 - 11:00	**Invited Speaker: Silvina Pessino**, IICAR-CONICET-UNR, Rosario, Argentina. *Integrative use of comparative omics for harnessing apomixis for plant breeding.*
11:00 - 11:30	**Invited Speaker: Thomas Dresselhaus**, University of Regensburg, Germany. *Understanding gene regulatory networks in the egg apparatus to trigger parthenogenesis.*
11:30 - 11:50	**Oral presentation: Tatyana Radoeva**, KeyGene, Wageningen, The Netherlands. *Apomixis: Plant Breeding Technology of the 2020s*
11:50 - 12:10	**Oral presentation: Petra Šarhanová**, Masaryk University, Department of Botany and Zoology, Czech Republic. *A novel method to detect automixis in flowering plants.*
12:10 - 14:00	Lunch time
14:00 - 17:00	Rosario sightseeing Tour | **MAD Project** coordination meeting
20:00 - 01:00	**Gala Dinner at restaurant "Mercurio"** Rosario Board of Trade (Address: Corrientes 796)

Thursday 7th December 2023

Morning Session. *Coordinators: Francisco Espinoza, José Carballo*

09:00 - 09:30	**Invited Speaker: Fulvio Pupilli**, Institute of Biosciences and Bioresources, CNR, Italy. *ORIGIN OF RECOGNITION COMPLEX 3 (PsORC3) is the genetic determinant for the development of unbalanced endosperm in the Paspalum simplex agamic complex (Poaceae).*
09:30 - 10:00	**Invited Speaker: Anna Koltunow**, The University of Queensland, Australia. *Hy-Gain: harnessing apomixis for self-reproducing sorghum and cowpea hybrids for smallholder farmers in sub-Saharan Africa.*
10:00 - 10:30	Coffee break
10:30 - 11:00	**Invited Speaker: Emidio Albertini**, University of Perugia, Italy. *Does APOSTART play a role in apospory?*
11:00 - 12:00	**Discussion session on perspectives. Closing ceremony**
12:00 - 14:00	Lunch time
14:00 - 17:00	**Open session to the public:** Mendel's laws, ADN of humans and plants, Sexual and asexual reproduction, Sexuality and Apomixis in breeding, Women in Science ECU, National University of Rosario (Address: Av. San Martín 750)
20:00 - 01:00	**Barbecue (Asado) at "El Viejo Balcón Puerto Norte" restaurant** (Address: Av. Carballo 198)

List of Posters

Poster	*Title*	Presenting Author	Co-Authors
01	*Incidence of isoforms of the splicing controller BUD13 in apomictic and sexual species*	Draga, S.	Colono, C.; Siena, L.; Gabelli, G.; Podio, M.; Palumbo, F.; Ortiz, J.P.; Barcaccia, G.; Pessino, S.
02	*Developing a Pan-Genome of the diplosporous grass Eragrostis curvula*	Bongiorno, G.; Carballo, J.	Gallo, C.A.; Albertini, E.; Zappacosta, D.; Echenique, V.
03	*Generation of auxin and cytokinin marker lines in Paspalum notatum*	Colono, C.M.	Ortiz, J.P.; Perrone, D.; Permingeat, H.; Orozco, G.; Colombo, L.; Kater, M.; Mendes, M.A.; Pessino, S.C.
04	*Construction of a consensus genetic map of Eragrostis curvula*	Gallardo, J.	Gallo, C.; Sansot Puleston, M.; Echenique, V.; Zappacosta, D.
05	*Studies tending to functionally characterize putative genes to be involved in apomictic pathway/s in Eragrostis curvula*	Díaz, A.R	Selva, J.P.; Carballo, J.; Garbus, I.; Echenique, V,
06	*Correlation between apomixis potential in ovules and fertility in tetraploid individuals of Paspalum almum Chase*	Schneider, J.S.	Hojsgaard, D.; Daviña, J.R.; Honfi A.I.
07	*Embryo sac and fertility analyses in a BIII synthetic Paspalum almum hybrid*	Schneider, J.S.	Escobar, L.M.; Daviña, J.R.; Hojsgaard, D.; Honfi A.I.
08	*Development of KASP markers linked to apomixis in Eragrostis curvula*	Gallardo, J.	Gallo, C.; Rodrigo, J.M.; Echenique, V.; Zappacosta, D.
09	*New assembly and annotation of diploid Bahiagrass (Paspalum notatum Flügge var. saurae) based on ONT long reads.*	Vega, J.M.	Podio, M.; Orjuela, J.; Siena, L.A.; Mariac. C.; Pupilli. F.; Albertini, E.; Pessino, S.C.; Leblanc, O.; Ortiz, J.P.A.
10	*Auxin response repressor IAA16 defective mutants show developmental alterations in female gametophytes and embryos in Arabidopsis thaliana*	Vega, M. Sol	Leblanc, O.; Pessino, S.C.; Ortiz, J.P.A.; Siena, L.A.
11	*Formation of BIII hybrids and effect of ploidy raises on the reproduction of aposporous sunflower (Helianthus annuus L.)*	Ochogavía, A.	Katzaroff, I.; Riviera, L.; Aguilar, G.; Bianchi, M.B.; Bocchini, M.; Marconi, G.; Albertini, E.; Pessino, S.; Nestares, G.
12	*Embryo sac composition and fertility assessment in Paspalum ovale Nees 8x: insights into reproductive mechanisms*	Escobar, L.M.	Schneider, J.S.; Daviña, J.R.; Martínez, E.J.; Honfi A.I. .
13	*Unveil the molecular mechanisms regulating Apomixis in Dandelion*	Cavalleri, A.	Banfi, C.; Cucinotta, M.; Cornaro, L.; Petrella, R.; Van Dijk, P.J.; Rigola, D.; Op den Camp, R.; Colombo, L.
14	*Morphogenetic determinants of plant female germ cell precursors specification and plasticity*	Autran, D.	Ouedraogo, I.; Mosca, G.; Delgado, L.; Leblanc, O.; Lartaud, M.; Conéjéro, G.; Baroux, C.
15	*Functional characterization of AUXIN RESPONSE FACTOR 8 and 18 during ovule development in Oryza sativa*	Perrone, D.	Orozco Arroyo, G.; Colono, C.; Pessino, S.; Kater, M.; Colombo, L.; Mendes, M.
16	*In silico characterization of gene families involved in epigenetic reprogramming associated with the reproductive mode in Paspalum notatum*	Podio, M.	Pessino, S.C.; Ortiz, J.P.A.
17	*Functional characterisation of QGJ, a YODA family member associated with apospory*	Siena, L.A.	Michaud, C.; Ortiz, J.P.A.; Pessino, S.C.; Leblanc, O.
18	*Exploring PLT gene family for insights into parthenogenesis regulation in Eragrostis curvula*	Quevedo, MR.	Suarez, U.; Quevedo, M.R.; Selva, J.P.; Carballo, J.; Zappacosta, D.; Echenique, V.
19	*Expression atlas of Eragrostis curvula reproductive tissues*	Selva, J.P.	Carballo, J.; Percival-Alwyn, L.; Šurbanovski, N.; Zappacosta, D.C.; Cáccamo, M.; Echenique, V.
20	*Genetic systems in new polyploids generated by chromosomal duplication in Paspalum indecorum.*	Novo, P.E	Villalba, A.I.; Carrizo, J.M.; Espinoza, F.
21	*A 3D analysis of the reproductive development of Eragrostis curvula (Schrad.) Ness*	Pasten, M.C.	Carballo, J.; Díaz, A.R.; Mizzoti, C.; Cucinotta, M.; Colombo, L.; Echenique, V.; Mendes M.A.
22	*Resolving the gene content of the genomic region associated with apomixis in Paspalum notatum using a diploid genome assembly.*	Ortiz, J.P.	Vega, J.M.; Podio, M.; Orjuela, J.; Siena, L.A.; Mariac, C.; Pessino, S.C.; Leblanc, O.

The organizing committee of the IV Internationa Congress on Apomixis is pleased to announce the two winners of the **Best Poster Presentation Award**:

P08

Development of KASP markers linked to apomixis in Eragrostis curvula

Gallardo, J. (1,2); Gallo, C. (1); Rodrigo, J.M.(1,2); Echenique, V. (1,2); Zappacosta, D. (1,2)
(1) Centro de recursos naturales renovables de la zona semiárida, CERZOS-CONICET, Argentina.
(2) Depto. Agronomía UNS, Bahía Blanca, Argentina.

P13

Unveil the molecular mechanisms regulating Apomixis in Dandelion

Banfi, C. (1); Cucinotta, M. (1); Cornaro, L. (1); Petrella, R. (1); Cavalleri, A. (1); Van Dijk, P.J. (2); Rigola, D. (2); Op den Camp, R. (2); Colombo, L. (1)
(1) Dipartimento di Bioscienze, Universita degli Studi di Milano, Milan, Italy.
(2) Keygene N.V., Wageningen, Netherlands.

(see Abstracts on Poster section)

IV International Congress on

APOMIXIS

December 3 - 7, 2023 • Rosario, Argentina

Abstracts: Invited Speakers

A cytological framework of female meiosis in Arabidopsis and maize

Balboni, M. (1); Hu, B. (1); Prusicki, M. (1); Hamamura, Y. (1); **Schnittger, A.** (1)
(1) University of Hamburg, Germany.
arp. schnittger@uni-hamburg.de

Meiosis is key for sexual reproduction by reducing chromosome number and generating genetic diversity. Thus, apomixis requires either skipping of meiosis entirely or a modification of the meiotic program to produce unreduced gametes. To engineer apomixis, it is therefore important to understand how meiosis is initiated, until when a meiocycte can return to a mitotic program and how meiosis can be modified to produce unreduced gametes. To this end, we have followed a live cell imaging approach to study meiosis in the model plant Arabidopsis as well as the globally important crop species maize. The focus of this talk will be on the analysis of female meiosis with a first characterization of cellular landmarks of female meiocytes leading to detailed temporal dissection of meiotic progression. Furthermore, we have mapped until when a designated female meiocyte can revert to a mitotic instead of a meiotic program. This work leads to a cytological framework of female meiosis that forms a base for genetic engineering and breeding.

New insights into the control of apomixis in Eragrostis curvula

Echenique, V. (1); Carballo, J. (1); Bellido, A.M. (1); Selva, J.P. (1); Garbus, I. (1); Zappacosta, D. (1); Gallardo, J. (1); Pasten, M.C. (1); Gallo, C.A. (1); Díaz, A. (1); Rodrigo, J.M. (1)
Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS) – Centro Científico Tecnológico CONICET B. Blanca - Dpto. de Agronomía - Universidad Nacional del Sur, 8000 Bahía Blanca, Argentina.
echeniq@cerzos-conicet.gob.ar

Eragrostis curvula, commonly known as weeping lovegrass, is a perennial grass belonging to the *Poaceae* family. It is native to Southern Africa and it has been naturalized in semi-arid regions of Argentina. The species has garnered attention not only for its adaptive qualities but also for its significance as a model organism for the study of apomixis (diplospory). The *E. curvula* complex has a basic chromosome number of × = 10 and includes cytotypes with different ploidy levels (2x – 8x) that may undergo sexual reproduction and facultative or obligate apomixis. Diploid plants are sexual, rare, and do not occur in all forms of E. curvula. Tetraploid plants and plants with higher ploidy levels reproduce by pseudogamous diplosporous apomixis, having a particular type of embryo sac development (Eragrostis-type). Our results indicate that apomeiosis in this species is controlled by a single genomic region (APO-locus) located in one of the two sub-genomes of the species, within a region having an extension of 11,322,729 bp on linkage group 1 of the apomictic Don Walter´s map, delimited by three co-segregating markers. Putative regulatory regions affecting the expressivity of the trait were also mapped. The comparison of the region delimited by these markers between the sexual and apomictic genomes of the species showed a poor level of synteny. The *Eragrostis curvula* APO-locus contains more than 1,400 genes, showing 19% of them orthology with genes in the sexual diploid accession. The region was also found to be enriched in repetitive elements.
Thanks to our improved understanding of weeping lovegrass morphology, we were able to construct an expression atlas of reproductive tissues by sequencing pistils at various developmental stages in both sexual and apomictic plants. This atlas provided us with additional candidate genes to be mapped onto the genome assembly.
By hybridization of an *E. curvula* customized microarray designed based on a reference transcriptome constructed from sexual and apomictic genotypes we have identified several genes involved in apomixis expression. Some of these genes were found exclusively in apomictic cultivars and their orthologs were not identified in any other reported genome. Interestingly, a subgroup of them were located on the APO-locus whereas the others on different genomic positions. In order to functionally characterize these genes a reverse genetic approach was used. To do this heterologous expression systems for Arabidopsis and rice containing candidate genes under the control of constitutive and specific promoters were developed. We have found that such gene expression in the ovule primordium of Arabidposis compromises the cellular identity of the main cells responsible for sexual development. Furthermore, phenotypic and gene expression analyses in A. thaliana mutants, evidenced an extensive re-profiling of the epigenetic context.

Keywords: Eragrostis curvula, Apo-locus, mapping, candidate genes.

Introduction of heterosis in Crop systems via apomixis and heterotic perenniality

Chaudhury, A. (1)
(1) Krishan Foundation, Australia and Kanihati farm, Bangladesh.
kanihati@gmail.com

Based on our earlier work in Arabidopsis we earlier isolated, in collaboration with CSIRO scientists and inactivated the orthologs of FIS Class genes producing autonomous embryo and endosperm in rice. These varieties will now be combined with MIME constructs and second site enhancers will be isolated to achieve full autonomous apomixis in rice. In addition we have developed methods for perennialising rice cultivars as a pathway of fixing heterosis via perennial hybrids. Attempts are being made to achieve these work via "User-led"innovation whereby farmers are organised to do research mimicking the ancient era of crop domestication. Both projects will be continued in "Kanihati"a traditional village in South Asia

Towards the Engineering of Apomixis in Maize

Grossniklaus, U. (1); Chumak, N. (1); Pasquer, F. (1); Brunner, A. (1); She, W. (1); Gaillard, M. (1)
(1) Department of Plant and Microbial Biology, University of Zurich, Switzerland
grossnik@botinst.uzh.ch

Apomixis refers to the asexual reproduction through seed, which generates clones, i.e., plants that are genetically identical to the mother plant. The engineering of apomixis in crop species has tremendous agricultural potential. Among others, it would allow the maintenance of complex genotypes, including those of F1 hybrids. Gametophytic apomixis deviates from sexual reproduction in three major steps: (1) meiosis is circumvented or aborted (apomeiosis), (2) embryogenesis initiates without fertilization (parthenogenesis); and (3) developmental adaptations enable the formation of a functional endosperm.

The aim of our research is to identify mutations that mimic the major components of apomixis in order to combine them to engineer apomixis in crops. In a forward genetic screen in maize, we identified the *non-reduction in female4 (nrf4)* mutant displaying apomeiosis. Homozygous *nrf4* plants produce over 95% unreduced female gametophyte containing functional gametes. Using sequencing-based transposon display, the *Nrf4* gene was identified and found to encode a monocot-specific pioneer protein. The effect of the *nrf4* mutation on meiosis is complex, leading to both first and second division restitution. Nonetheless, depending on the genetic background of the mother plant, up to 35% of the unreduced female gametes were derived from a mitotic division, leaving the maternal chromosome complement unrecombined. Thus, about a third of the *nrf4* mutant meiocytes display mitotic diplospory.

In another forward genetic screen, we searched for mutants displaying parthenogenetic development of an embryo and identified the *parthenogenesis1 (par1)* mutant, which produced up to 6% parthenogenotes. This corresponds to an increase in the spontaneous parthenogenesis rate of about 100-fold. We are currently combining *nrf4* with *par1* to generate synthetic apomixis. Using an alternative strategy, we have already obtained clonal seeds by combining nrf4 with a tetraploid "haploid inducer" (THI) line that we generated. Depending on the particular hybrid combination, we could recover up to 11% clonal progeny. Our findings show that the production of clonal individuals through seed is possible and demonstrates that the engineering of synthetic apomixis is within reach in maize, one of the most important crop plants.

Keywords: apomixis, engineering, genetic screen, maize, non-reduction, parthenogenesis.

Hybrid effects on zygotic genome activation in Arabidopsis thaliana

Alaniz-Fabián, J. (1); Xiang, D. (2); Datla, R. (3); **Gillmor, S** (1)
(1) Langebio, Unidad de Genómica Avanzada, CINVESTAV, Mexico.
(2) National Reseach Council, Saskatchewan, Canada.
(3) Global Institute for Food Security, Saskatchewan, Canada.
stewart.gillmor@cinvestav.mx

After fertilization in animals, maternal mRNAs and proteins regulate development until the onset of zygotic transcription. In plants, the extent of maternal regulation of early embryo development is less clear: hybrid rice zygotes showed an overwhelming maternal transcript bias, while Arabidopsis Columbia (Col)/Cape Verde Islands (Cvi) hybrid embryos had essentially equal maternal and paternal contributions, and Col/Landsberg erecta (Ler) hybrid embryos showed asymmetric activation of maternal and paternal genomes in the zygote.
Using functional assays, we recently demonstrated the Col/Tsu hybrid to be a faithful proxy for understanding parent-of-origin behavior in the reference ecotype Col. Here we analyze transcriptomes of Col/Tsu embryos from zygote-1cell to mature stages, finding a strong maternal transcript bias in zygote-1cell and octant embryos. Quantification of intron reads and comparison of transcript levels in the egg and embryo suggested that the observed maternal bias was due to preferential transcription of maternal alleles in the zygote and early embryo, rather than carryover from the egg cell. Comparison of maternally biased genes with cytosine methylation data revealed a correlation between different degrees of maternal bias and various cytosine methylation contexts.
The transcriptomic data presented here further supports the idea that parent-of-origin contributions to early embryogenesis differ between hybrids of Arabidopsis. In agreement with functional experiments with emb mutants, hybrid Col/Tsu embryos switch from a predominant role of the maternal genome to equal parental genome contributions during the first few days of embryogenesis. By contrast, Col/Ler embryos show variable parental contributions in the zygote and switch to equal contributions in the apical cell, and Col/Cvi embryos show equal parental contributions as early as the 1-2 cell stage. The effects of hybridization on parental contributions to zygotic genome activation will be informative for understanding the mechanistic basis of parental-of-origin regulation of early seed development.

Functional characterization of apomixis candidate genes in Arabidopsis

Leblanc, O. (1); Siena, L. (2)
(1) DIADE, Univ. Montpellier, CIRAD, IRD, Montpellier, France.
(2) IICAR, CONICET-UNR, Zavalla, Argentina.
olivier.leblanc@ird.fr

Paspalum notatum, a subtropical forage grass native to South America, has proved to be a fruitful model for the study of aposporous apomixis in plants. Recently, several genes have been identified through genetic mapping and comparative mRNA analyses, however only a few candidates are currently being analyzed. Using genetic and cellular approaches in the model plant Arabidopsis thaliana, we have undertaken the functional characterization of two of them, *TGS1 (TRIMETHYLGUANOSINE SYNTHASE1)*, a gene involved in the biogenesis of sn(o) RNAs, and; *QGJ (QUI-GON JINN)*, a member of the YODA family. GUS reporter lines indicated that *tgs1* and *qgj* express in opposite ways during ovule development. Depletion of both *QGJ* and *TGS1* led to alterations affecting reproductive female development, in a manner reminiscent of aposporic apomixis. Our results indicate that both gene likely participate in the molecular mechanisms of canalization during female reproductive development in plants.

Understanding gene regulatory networks in the egg apparatus to trigger parthenogenesis

Dresselhaus, T. (1)
(1) Cell Biology and Plant Biochemistry, Institute of Plant Sciences, University of Regensburg, Germany.
thomas.dresselhaus@ur.de

From ultrastructural and cytoskeleton studies the egg cell of flowering plants was described as an unspecialized parenchyma cell being a partial protoplast that is embedded in thousands of ovary cells. It must, however, be a very special and valuable cell as the gene regulatory networks (GRNs) established to restrict its number to a single cell per ovule and to prevent its division until successful fertilization are tremendous, and consist of multiple molecular players and pathways. Defects of the underlying GRNs lead to components of apomixis including apospory and parthenogenesis. We are interested to understand (i) how egg cell specification is determined, whether (ii) the egg cell has similar and/or overlapping functions with its sister cells, the glandular synergid cells – which together form the egg apparatus – and which in some apomictic species are also capable to develop into embryos. We also aim to know (iii) how the egg cell regulates fertilization and (iv) prevents polyspermy, (v) and how it suppresses embryo development until fertilization occurs. We address these and other questions by comparing transcriptomes of sexual and parthenogenetic egg with that of sperm cells, synergid cells and zygotes as well as embryos at different stages using flowering plant species including maize, *Arabidopsis* and *Amborella*. Among others I will report how egg cells aquire their specificity, act in concert with synergids cells in pollen tube reception, sperm cell activation and degradation of 'fertility factors', but also how the embryonic program is activated after gamete fusion and how this knowledge can be applied to engineer parthenogenesis.

A mitochondrial electron shuttle essential for female gametophyte and early embryo development in Arabidopsis

Bellido, A.M. (1); Distéfano, A.M. (1); Setzes, N. (1); Cascallares, M.M. (1); Oklestkova, J. (1); Novak, O. (1); Ramirez, J.A. (1); Zabaleta, E.J. (1); Fiol, D.F. (1); **Pagnussat, G.C.** (1)
(1) IIB-CONICET, Universidad Nacional de Mar del Plata, Argentina.
gpagnussat@mdp.edu.ar

Mitochondrial adrenodoxins (ADXs) are small iron–sulfur proteins with electron transfer properties. In animals, ADXs transfer electrons between an adrenodoxin reductase (ADXR) and mitochondrial P450s, a step essential for steroid biosynthesis. In our lab we found that a mitochondrial ADXR–ADX–P450 shuttle is required for female gametogenesis and early embryogenesis through a maternal effect. The steroid profile of maternal and gametophytic tissues of wild-type (WT) and adxr ovules revealed that homocastasterone is the main steroid present in WT gametophytes and that its levels are reduced in the mutant ovules. The application of exogenous homocastasterone partially rescued adxr and P450 mutant phenotypes, suggesting that gametophytic homocastasterone levels are affected in the mutants and that a deficiency of this hormone causes the phenotypic alterations observed. These findings also suggest not only a remarkable similarity between steroid biosynthetic pathways in plants and animals but also a common function during sexual reproduction.

Insights on asexual reproduction through seeds from Pennisetum/Cenchrus apomictic species

Ozias-Akins, P. (1); Conner, J.A. (1)
(1) Department of Horticulture and Institute of Plant Breeding, Genetics and Genomics, University of Georgia, Tifton GA, USA.
pozias@uga.edu

The sexual grain crop *Cenchrus americanus (Pennisetum glaucum*; pearl millet) has numerous apomictic relatives with which it is interfertile. Interspecific crosses were essential to characterize transmission of the trait from apomictic *C. squamulatus* and revealed that a single dominant "locus" was inherited along with the reproductive trait. The locus was, however, a large part of one chromosome that was shown to be hemizygous and largely non-recombining, suggesting that at least two genes were necessary to encode the different components of apomixis, i.e., apospory and parthenogenesis. Skim sequencing of bacterial artificial chromosome clones containing locus-specific markers led to the discovery of a gene for parthenogenesis, *PsASGRBBML*. This gene has been shown to induce parthenogenesis in pearl millet, rice, maize and tobacco, albeit the latter was at very low frequency. No apomeiosis gene from natural apomicts has yet been cloned and functionally validated. A comparative genomics approach with multiple apomictic *Cenchrus* species is being taken to identify candidate genes for apospory.

Dissecting apomeiosis in alfalfa (Medicago sativa L.): Genomics applied to unreduced gamete mutants and sexually induced polyploids

Gabelli, G. (1); Palumbo, F. (1); Draga, S. (1); Barcaccia, G. (1)
(1) Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), Agripolis, University of Padova, Italy.
giovanni.gabelli@unipd.it

The gene flow mediated by unreduced gametes between diploid and tetraploid plants of the *Medicago sativa-coerulea-falcata* complex is pivotal for cultivated alfalfa breeding. Sexually tetraploidized hybrids are known to represent the best way to exploit progressive heterosis simultaneously derived from gene diversity and heterozygosity as well as polyploidy. Moreover, unreduced gametes combined with parthenogenesis (i.e., apomixis) would enable the cloning of plants through seeds, providing a unique opportunity for the selection of superior alfalfa genotypes with permanently fixed heterosis. Alfalfa meiotic mutants producing unreduced gametes can also be exploited to produce synthetic polyploids by means of unilateral and bilateral sexual polyploidization schemes. The overall achievements reached so far by our research group are presented along with the efforts and strategies we recently made by using genomics and transcriptomics for dissecting reproductive mutants and exploiting neopolyplois. In particular, we investigated the cytological mechanisms and the genetical factors responsible for unreduced gamete formation and the inheritance of this trait in alfalfa, along with transcriptomic profiles and changes associated to sexually induced polyploids. An overall view on strategies suitable to fill the gap between well-established apomeiotic mutants and next-generation genomic resources is also presented and discussed.

Integrative use of comparative omics for harnessing apomixis in plant breeding

Colono, C.M. (1); Marino, L. (1); Altabe, S. (2); Podio, M. (1); Vega, M.S. (1); Vega, J.M. (1); Siena, L. (1); Stein, J. (1); Delgado, L. (1); Ortiz, J.P.A. (1); Balaban, D. (1); Acuña, C. (3); **Pessino, S.C.** (1).
(1) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET-UNR), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Rosario.
(2) Instituto de Biología Molecular y Celular de Rosario, Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Rosario.
(3) Instituto de Botánica del Nordeste, Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional del Nordeste.
pessino@iicar-conicet.gob.ar

The combined use of sexuality and apomixis (i.e., sexual and asexual reproduction via seeds) in breeding programs allows rapid development of a myriad of improved plant varieties at low cost. The genus *Paspalum*, one of the best characterized apomictic complexes, includes more than 370 valuable forage species, which were traditionally used in temperate and subtropical regions to feed livestock. The characterization of the molecular control of apomixis in this genus is oriented to: *i)* the identification of its molecular trigger/s to transfer the trait to major sexual crops, and *ii)* the harnessing of the trait in *Paspalum* breeding. Recently, we sequenced the genome of several *Paspalum notatum* genotypes and identified the genomic region controlling apomixis. Moreover, 454 and Illumina RNAseq analyses were used to spot numerous candidate genes differentially expressed in the reproductive organs of sexual and apomictic plants, some of which map in the genomic region responsible for apomixis. Reverse genetic analyses in model systems revealed that the orthologs of some of these candidates, like *TGS1*, *ARF10* or *IAA30*, can indeed reproduce apomixis components (*i.e.*, apospory and/or parthenogenesis) in a sexual background, confirming they are suitable candidates to transfer apomixis to sexual crops. Besides, the generation of *Paspalum notatum* leaf transcriptomes allowed the rapid development of improved apomictic hybrid genotypes. As an example, we will present the development of genotypes with higher content of polyunsaturated fatty acids (PUFAs) using an apomixis-based breeding scheme assisted by molecular markers. Transcript sequences encoding two key proteins of lipid metabolism, SUGAR-DEPENDENT1 (SDP1) and peroxisomal ABC transporter 1 (PXA1), which have been reported to modulate the 18:3 fatty acid content, were recovered from the *Paspalum* leaf transcriptome. Allele-specific primers were designed and eight representative *Paspalum notatum* genotypes were screened by qPCR to search for the lower SDP1/PXA1 expression levels. Then, fatty acid methyl esters (FAMEs) gas chromatography was used to analyze the PUFAs representation. We selected a 4x sexual genotype with an optimal molecular profile (*i.e.*, low SDP1/PXA1 expression levels, high content of 18:3) and crossed it with an apomictic pollen donor. Three obligate apomictic genotypes with high content of PUFAs were identified in the F1 family, one of them showing 18:3 values 22,5% ± 1% and 64,2% ± 1% higher than the average of the eight original genotypes and the poorest profiles, respectively. These results show that the use of data derived from comparative omics in *Paspalum* allows the development of molecular tools for the transference of apomixis into major crops, as well as the assistance of breeding programs to obtain improved cultivars.

Keywords: apomixis, candidate genes, breeding, marker-assisted selection.

Unraveling complex mechanisms in plant reproduction for the crops of the future

Cucinotta, M. (1); Cornaro, L. (1); Banfi, C. (1); Petrella, R. (1); Balanzà, V. (1); Van Dijk, P.J. (1); Rigola, D. (1); Radoeva, T. (1); van Tunen, A. (1); Op den Camp, R. (1); **Colombo, L.** (1)
(1) Università degli Studi di Milano, Italy.
lucia.colombo@unimi.it

Apomictic reproduction is characterized by the formation of clonal progeny identical to the mother. The introduction of this system in sexually reproducing crops could have a huge impact on the ability to fix valuable and complex traits in plant breeding programs.
Taraxacum officinale L., the common dandelion, is characterized by sexual diploid and apomictic polyploid genotypes. The analysis of a mutant generated by gamma-irradiation has allowed the identification of the locus that might control apomeiosis in dandelions. One of the genes present in the locus is *VACUOLAR PROTEIN SORTING-ASSOCIATED 13 (VPS13)*. The *VPS13* gene family is conserved across all eukaryotes and encodes large proteins involved in the tethering between different organelles to transfer lipids within the cell. These proteins are well studied in humans and yeast, but there is scarce information about *VPS13* in plants. The characterization of AtVPS13S function in Arabidopsis will be presented and discussed.

Keywords: Taraxacum officinale, diplospory.

ORIGIN OF RECOGNITION COMPLEX 3 (PsORC3) is the genetic determinant for the development of unbalanced endosperm in the Paspalum simplex agamic complex (Poaceae)

Bellucci, M. (1); Caceres, M.E. (1); Paolocci, F. (1); Vega, J.M. (2); Ortiz, J.P.A. (2); Ceccarelli, M. (3); De Marchis, F. (1); **Pupilli, F.** (1)
(1) Institute of Biosciences and Bioresources, CNR, Italy.
(2) Instituto de Investigaciones en Ciencias Agrarias de Rosario, CONICET-UNR and Laboratorio de Biología Molecular, Facultad de Ciencias Agrarias, UNR, Argentina.
(3) Department of Chemistry, Biology and Biotechnology, University of Perugia, Italy
fulvio.pupilli@ibbr.cnr.it

Pseudogamous apospory apomixis in *Pasplaum simplex* is controlled by a single dominant locus showing gene degeneration and deregulation, synteny with a conserved chromosome region of grass genomes and block of recombination. The apomixis–linked gene *ORIGIN OF RECOGNITION COMPLEX3 (PsORC3)* exists in three isogenic forms: *PsORC3a* is apomixis-specific and constitutively expressed in developing endosperm whereas *PsORCb* and *PsORCc* are up regulated in late phases of sexual endosperm development in sexual genotypes while are silenced in the same phases and tissue of the apomictic ones. Arabidospsis and rice mutants for the same gene show arrest of endosperm development. This raises the question of whether *PsORC3* may have a role on the development of maternal excess endosperm (4maternal : 2paternal instead of the canonical 2m : 1p) in apomictic *P.simplex*. To test this hypothesis, we down regulated *PsORC3* in sexual tetraploid genotypes and used the transgenic plants as seed parent in interploidy crosses with diploid pollinators to re-create, in the sexual background, the condition of apomictic endosperm development. With this experimental design, we were able to analyze functionally *PsORC3* beyond the apomixis locus. We demonstrate that the *PsORC3b* downregulation in sexual tetraploid plants is sufficient to restore seed fertility in interploidy 4n x 2n crosses and, in turn, that its expression level at the transition from proliferating to endoreduplication endosperm developmental stages dictates the fate of these seeds. Furthermore, we show that only when being maternally inherited, *PsORC3c* can up-regulate *PsORC3b*. Our findings pose the basis for an innovative route - based on the manipulation of *ORC3* - to introgress the apomictic trait into sexual crops and overcome the fertilization barriers in interploidy crosses.

Hy-Gain: harnessing apomixis for self-reproducing sorghum and cowpea hybrids for smallholder farmers in sub-Saharan Africa

Koltunow, A. (1)
(1) Centre for Crop Sciences (QAAFI), The University of Queensland, Brisbane Qld 4072 Australia.
a.koltunow@uq.edu.au

Sorghum and cowpea were domesticated in Africa and continue to be subsistence crops for smallholder farmers in sub-Saharan Africa. Access to high yielding, quality seed is essential for smallholder income. Hybrids provide higher seed yields and Hy-Gain, a project funded in part by the Bill and Melinda Gates Foundation (BMGF) aims to deploy apomixis so that high-yielding self-reproducing hybrid sorghum and cowpea seed can be efficiently produced and economically saved by smallholders. Hy-Gain currently comprises a consortium of five partner agencies. This talk focuses on the progress made to induce synthetic apomixis in sorghum and cowpea.

Does APOSTART play a role in apospory?

Petrella, R. (1); Marconi, G. (1); Cucinotta, M. (1); Gabrieli, F. (1); Colombo, L. (1); **Albertini, E.** (1)
(1) Department of Biosciences, University of Milan, Via Giovanni Celoria, 26, 20133, Milan (Italy). Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX
Giugno 74, 06121 Perugia (Italy).
emidio.albertini@unipg.it

The production of seeds without sexual reproduction (i.e. apomixis) is considered the holy grail of plant biology. The transfer of apomixis to various crop species has the potential to transform plant breeding since it will allow new varieties to retain valuable traits thorough asexual reproduction. Therefore, a greater molecular understanding of apomixis is fundamental. In a previous work we identified a gene, namely *APOSTART*, that seemed to be involved in this asexual mode of reproduction very common in Poa pratensis L. and we showed that *PpAPOSTART* is expressed in reproductive tissues from pre-meiosis to embryo development. Interestingly, it is early expressed in few nucellar cells of apomictic individuals possibly switching somatic to reproductive cell fate as in aposporic apomixis. Moreover, out of 13 APOSTART members, we identified one, *PpAPOSTART_6*, as specifically expressing in flower tissue. *APOSTART_6* also exhibited delayed expression in apomictic genotypes when compared with sexual types. Most importantly, the SCAR derived from the APOSTART_6 sequence, totally co-segregated with apomixis. Arabidopsis genomes present at least ten members. We identified *APOSTART1* (At5g45560) and *EDR2* (renamed APOSTART2, At4g19040) as the closest homologs of *PpAPOSTART_6*. To determine the role of these two genes in plant reproduction, we generated apostart1 apostart2 double mutants by crossing the T-DNA lines for the two genes. Our preliminary results show that mutation of these genes affects crucial steps of female germline progression, suggesting a role of APOSTART in apospory.

Keywords: Apomixis, APOSTART, apospory, plant reproduction.

IV International Congress on

APOMIXIS

December 3 - 7, 2023 • Rosario, Argentina

Abstracts:
Oral Communications

WINDHOSE-RAB GTPASE HOMOLOG A1-dependent membrane localization of the auxin transport protein PINFORMED1 promotes female germline entry in Arabidopsis

Chandrasekar, R. (1); Xu, Y. (1); Peraiso, T. (1); Lora, J. (1); Tucker, M. (4); Friml, J. (3); Weijers, D. (2); Laux, T. (1)
(1) Institute of Biology III, Albert-Ludwigs-University of Freiburg, Schänzlestrasse 1, 79104 Freiburg, Germany.
(2) Laboratory of Biochemistry, Wageningen University, Stippeneng 4, 6708WE Wageningen, The Netherlands.
(3) Institute of Science and Technology Austria (ISTA), Klosterneuburg, 3400, Austria. (4) Waite Research Institute, School of Agriculture, Food and Wine, The University of Adelaide, Urrbrae, SA 5064, Australia.
ramsankar.chandrasekar@biologie.uni-freiburg.de

One of the major factors distinguishing plant development from that of animals is the absence of dedicated germline originating early during embryogenesis. In higher plants, haploid germ cells are ultimately generated from the diploid somatic cells of an adult organism. In the gynoecium of *Arabidopsis thaliana*, a single hypodermal somatic cell in the nucellus of the ovule primordium becomes the megaspore mother cell (MMC), which eventually enters into germline fate and produces haploid megaspores after meiosis. However, the mechanism regulating germline entry and thus MMC specification is largely unknown and is also the aim of our study. Previous studies in our lab had identified WINDHOSE (WIH 1 & 2) genes encoding small peptides downstream of the WUSCHEL (WUS) gene as novel regulators of MMC development. Here we show that WIH proteins are localized to the plasma membrane of the nucellar epidermis, from where they non-cell-autonomously regulate the MMC specification. We have established that WIH proteins affect auxin response patterning in the developing ovules by influencing the PINFORMED1 (PIN1) subcellular localization involving the membrane trafficking proteins RAB GTPASE HOMOLOG A1. We are currently investigating our hypothesis that WIH proteins act as novel tethering factors in delivering the PIN1 cargo transported by RABA-associated vesicles to the plasma membrane. Furthermore, identification of the non-cell autonomous factor downstream of auxin, which defines the germline fate might enable us to engineer apospory in crop plants.

Keywords: MMC, female germline entry, auxin, non-cell-autonomous signaling, intracellular vesicular trafficking, apospory.

Unveiling the apomictic allotetraploid genome of Eragrostis curvula

Carballo, J. (1); Selva, J.P. (1,2); Gallo, C.A.(3); Zappacosta, D. (1,4); Albertini, E. (5); Cáccamo, M. (6); Echenique, V. (1,4)
(1) Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), Universidad Nacional del Sur-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Bahía Blanca, Argentina.
(2) Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.
(3) Departamento de Ciencias de la computación, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.
(4) Departamento de Agronomía, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.
(5) Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università degli Studi di Perugia, Perugia, Italy.
(6) NIAB, Cambridge, United Kingdom
jcarballo@cerzos-conicet.gob.ar

Eragrostis curvula is a C4 grass mainly used as model species for the study of diplosporous apomixis, having a particular type of apomictic embryo sac development, called “Eragrostis type”. In the last few years, we developed several genetic and epigenetic resources in order to disclose the molecular basis of apomixis in E. curvula such as genome assembly of a sexual diploid genotype, a pangenome, small RNAs and mRNAs databases covering different tissues and genotypes and also, we analyse the methylation status of obligate, facultative and sexual genotypes. However, to identify the apomictic region linked to apomixis and the genes involved on this complex trait, a high-quality genome assembly of an apomictic genotype is a fundamental piece. To fill this gap, the apomictic genome of the allotetraploid Don Walter (2n=4x=40) was sequenced and assembled using different techniques. First, ~55X of Oxford nanopore reads were sequenced using super-high accuracy plant model basecalling, obtaining a mean Q value of 24. This quality value allowed us to assembly the reads with flye software using low error rates parameters, getting a N50 value of 1.4 Mb. Then, Omni-C, the latest scaffolding technology, was used to obtain a chromosome scale assembly. These linked reads were mapped against the flye version of the genome and the final assembly was scaffolded using Hi-Rise software. This assembly has an N50 of 28 Mb and a size of 1,891 MB distributed in 11,570 sequences. Even more, 19 complete chromosomes were classified in the A and B sub-genomes containing 38,096 and 35,356 genes, respectively. Synteny analysis between this apomictic genome assembly and the one of Victoria, a sexual diploid, shows two copies of each chromosome in Don Walter except for chromosome 10. In order to identify the region linked to apomixis in the Don Walter assembly, markers linked to apomeiosis obtained from a mapping population were aligned with the genome. Interestingly, the markers aligned to a single region present in the apomictic genome and absent in the sexual one, in which candidate genes previously identified involved in the reproductive development were located. The sequence and assembly of the Don Walter genome is a key tool to continue with the molecular characterisation of candidate genes and to use as a reference for other studies to understand the genetic and epigenetic mechanisms of apomixis in this grass.

Keywords: genome assembly, diplospory, apomixis, Eragrostis curvula.

Arabidospis thaliana could provide new insights into the driving forces underlying the switch from sexual to apomictic development

Bellido, A.M. (1); Carballo, J. (1); Selva, J.P. (1,2); Garbus, I. (1,3); Echenique, V. (1,4).
(1) CERZOS-CONICET, Bahía Blanca, Buenos Aires, Argentina.
(2) Departamento de Biología Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.
(3) Departamento de Ciencias de la Salud, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.
(4) Departamento de Agronomía, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.
andresmbellido@gmail.com

Eragrostis curvula (Schrad.) Nees (weeping lovegrass) is a perennial grass member of the Poaceae family, subfamily Chloridoideae. It is native to Southern Africa and cultivated in semiarid regions of Argentina, where it was naturalized. The *E. curvula* complex has a basic chromosome number of ×=10 and includes cytotypes with different ploidy levels (from 2–8X) that may undergo sexual reproduction, and facultative or obligate apomixis. Diploid (2n=2X=20) plants are sexual, rare, and do not occur in all forms of *E. curvula*. Tetraploid plants (2n=4X=40) and plants with higher ploidy levels reproduce by pseudogamous diplosporous apomixis. By hybridization of a microarray designed based on a reference transcriptome constructed from sexual and apomictic genotypes of *E. curvula* our group have identified several genes involved in apomixis expression. Some of these genes were found exclusively in apomictic cultivars and their orthologs were not discovered in any other genome reported. In order to unravel their possible functions along the definition of the reproductive mode in *E. curvula*, a reverse genetic approach appears as the most powerful tool to face the study. Unfortunately and despite continuous efforts, *E. curvula* remains recalcitrant to the processes of gene transfer. Therefore, in a parallel manner, we have designed a heterologous expression system for *A. thaliana* containing those *E. curvula* apomixis-related genes. We have found that such gene expression in the ovule primordium compromises the cellular identity of the main cells responsible for sexual development. Furthermore, phenotypic and gene expression analyses in *A. thaliana* mutants, evidence an extensive re-profiling of the epigenetic context.

Keywords: Eragrostis curvula, Apo-locus, mapping, candidate genes.

Understanding the Molecular Mechanism of Parthenogenesis in Cereals

Zheng, X. (1); Tornero, M.F. (1); Schwartz, U. (2); Dresselhaus, T. (1)
(1)Cell Biology and Plant Biochemistry, University of Regensburg, 93053 Regensburg, Germany. (2)Computational Core Unit, University of Regensburg, 93053 Regensburg, Germany
xixi.zheng@ur.de

Parthenogenesis, meaning “creation by virgin”, is a key component of apomixis (asexual reproduction through seeds) and describes spontaneous embryogenesis from an unfertilized egg cell and thereby generates offspring genetically identical to the mother plant. Investigating parthenogenesis in crop plants not only has high potentials to immediately fix desired traits including heterosis and thus would create great economic values, but would also help to understand how egg cell fate is determined for embryogenesis initiation. The underlying mechanisms of parthenogenesis remain poorly understood. Here, we use the apomictic grass *Tripsacum dactyloides* to address these questions. As the closest wild relative of maize, Tripsacum is sexually reproducing as a diploid, but all polyploids display apomixis via parthenogenesis. We collected egg cells from diploid and tetraploid Tripsacum lines to compare their gene expression profile by maping RNA-seq reads to the maize B73 reference genome. We observed that parthenogenetic eggs possess relatively specific cell cycle gene expression pattern that confers division potentials. Transcriptional reprogramming considerably contributes via both ON/OFF and differential regulation modes primarily involved in cell differentiation and auxin signaling. Parthenogenesis and zygotic genome activation share similar gene expression alterations associated with RNA metabolisms at both transcriptional (e.g. via ZmBBM1) and post-transcriptional (e.g. via RNase exonuclease) levels. These genes are highly expressed in parthenogenetic eggs but completely silenced in sexual eggs. We are currently characterizing the funtion(s) of candidate genes via creation of ectopic sexual egg cell-expression lines and knock-outs in maize. We found ectopic expression of ZmBBM1 induced haploidy and even twin embryos. ZmBBM1-mEGFP fluorescence are abundantly detected in embryonic pro-vascular and root systems. The ultimate goal of this research is to gain a mechanistic understanding of parthenogenesis and embryogenesis initiation in cereals and to utilize the knowledge generated to contribute and improve the production of haploid maize lines and/or clonal seeds.

Keywords: Parthenogenesis, Tripsacum, maize.

AUXIN RESPONSIVE FACTOR 10 insensitive to miR160 regulation induces apospory-like phenotypes in Arabidopsis

Mendes, M.A. (2); Pessino, S. (1); Cucinotta, M. (2); Colono, C. (1); Costantini, E. (2); Perrone, D. (2); Di Marzo, M. (2); Callizaya Terceros, G. (2); Petrella, R. (2); Azzaro, C. (1); Podio, M. (1); Marconi, G. (4); Albertini, E. (4); Dickinson, H. (3); Colombo, L. (2)

(1) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Rosario (UNR), 2000 Rosario, Argentina.

(2) Dipartimento di Bioscienze, Università degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy.

(3) Department of Biology, University of Oxford, South Parks Road, Oxford OX1 3RB, UK.

(4) Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università degli Studi di Perugia, 06121 Perugia, Italy.

marta.mendes@unimi.it

In *Arabidopsis* and in most Angiosperms, the sexual female germline arises from a pool of somatic cells in the ovule primordium, one of which becomes the megaspore mother cell (MMC) that enters meiotic program and gives rise to four megaspores. Of these, the most chalazal survives- the functional megaspore (FM) – which then undergoes mitosis to form the reduced female gametophyte. Here we show that spatial regulation of *AUXIN RESPONSE FACTOR 10 (ARF10)* is necessary for canonical germline commitment. Expression analysis using GFP-fusion lines revealed that *ARF10* is only active in cells surrounding the MMC in wild type contexts, but is also ectopically expressed throughout the ovule in miR160-insensitive lines. Significantly, these lines develop multiple FMs with differing ploidies, forming putative supernumerary mature gametophytes with altered polarity and unusual cell identity, a phenotype mimicking aposporous apomixis. We also confirm the control of ARF10 expression in the ovule to be complex, being mediated by the combined activity of SEEDSTICK, ARGONAUTE1 and miR160.

Keywords: auxin, ovule, apomixis, plant, reproduction.

3D architecture of the ovule during MMC differentiation in Paspalum rufum

Delgado, L. (1); Ouedraogo, I. (2); Baroux, C. (3); Autran D. (2)
(1) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR), CONICET, University of Rosario, Argentina.
(2) Epigenetics and Seed Development, Research Unit DIADE, CIRAD, IRD, University of Montpellier, France.
(3) Institute of Plant and Microbial Biology, Zürich-Basel Plant Science Center, University of Zürich, Switzerland.
luciana.delgado@conicet.gov.ar

During the early stages of sexual female sporogenesis development, a single sub-epidermal cell from the nucella differentiates into the Megaspore Mother Cell (MMC), marking the somatic to reproductive transition in higher plants. Although the number of MMCs varies by species and genetic background, in most cases, only a single MMC per ovule enters meiosis to form the embryo sac. In Arabidopsis, the restriction to a single MMC per ovule, or MMC singleness, is determined by ovule geometry.
Alternatively, in aposporous apomictic plants, additional enlarged cells, called Aposporous Initials surround the MMC and bypassing meiosis produce unreduced aposporous embryo sacs. *Paspalum rufum* is an aposporic grass organized in agamic complexes, where diploid cytotype reproduce by sexuality and tetraploid by facultative aposporous apomictic. Both reproductive pathways proceed within the same ovule during sporogenesis and megagametogenesis. Moreover, some diploid genotypes are able to produce aposporous embryo sacs in a relative high proportion and more than one reduced embryo sacs were also detected within the same ovule. Despite the potential for apomixis, sexuality prevails in diploids, and apomixis in tetraploids, which is related to heterochrony of ovule growth and reproductive development between both ploidy levels.
The plasticity observed in *Paspalum rufum* in both ovule growth and reproductive developmental pathways offers an interesting model to study the contribution of the growth parameters at cellular level in the somatic to reproductive transition within the ovule primordia.
We generated a collection of three-dimensional images of the ovule primordial spanning MMC differentiation, of diploid and tetraploid sexual and aposporic genotypes of *Paspalum rufum*. Our first analysis allowed us to obtain cellular morphological descriptors of MMC and its neighboring cellular network, suggesting a pool of candidate archesporial cells at early stages, canalized to a single enlarged MMC cell at late stage.
Comparative analysis of the whole ovule cellular architecture throughout development between different reproductive behaviour and ploidy levels will allow us to analyse the MMC singleness in such different contexts, and to understand the sexual to aposporic transitions during the onset of Aposporous Initials.

Apomixis: Plant Breeding Technology of the 2020s

Radoeva, T. (1)
(1) KeyGene, Agro Business Park 90, 6708 PW Wageningen, The Netherlands.
tatyana.radoeva@keygene.com

Apomixis is clonal reproduction through seeds, producing offspring genetically identical to the mother plant. This reproductive system occurs in some 300 wild plant species but not in major crops. Introducing apomixis in crops can yield significant benefits for plant breeding and seed production. The most obvious is the perpetual fixation of heterosis, but in principle, any genetically determined trait can be fixed, regardless of its complexity. In order to introduce apomixis into sexual crops, genes that skip meiosis and fertilization must be identified and brought together.
There are two main approaches to making apomictic crops. The first is the knock-out mutations in known meiotic genes combined with genes causing parthenogenesis (synthetic apomixis). The second is cloning naturally dominant apomixis genes and modifying the sexual orthologs (copy-nature apomixis).
Recently, remarkable progress in apomixis research has been made. Synthetic apomictic rice with high apomixis penetrance has been produced, suggesting that this application may be close to the market. In addition, the first naturally dominant parthenogenesis genes have been isolated (BabyBooMLike in Pennisetum and PARthenogenesis in Taraxacum). Apomixis genes are not completely new but modifications of sexual genes. In the presentation, I will discuss the identification of the PAR gene in Taraxacum and the possible future application of apomixis in plant breeding programs. Concerning the application in breeding, the dominance of apomixis genes and the female specificity of apomeiosis is essential. The recent breakthroughs in apomixis research justify the expectation that apomictic crops will be a reality before the end of this decade.

A novel method to detect automixis in flowering plants

Šarhanová, P. (1); Majeský, Ľ (2); Sochor, M. (3)
(1) Masaryk University, Department of Botany and Zoology, Czech Republic.
(2) Palacký University in Olomouc, Faculty of Science, Department of Botany, Olomouc, Czech Republic.
(3) Centre of the Region Haná for Biotechnological and Agricultural Research, Crop Research Institute, Olomouc, Czech Republic.
sarhanova@gmail.com

Flow Cytometric Seed Screen (FCSS) and genotyping of parents and progeny are commonly employed techniques to discern between apomixis and sexuality in flowering plants. Nevertheless, both methods possess limitations constraining their individual capacity to investigate reproductive modes thoroughly. One of the limitations is the inability to detect automixis. During automixis, meiosis takes place, but instead of regular fertilization, the ploidy of the embryo is reconstituted by the duplication or fusion of two reduced nuclei, where both are the products of a single meiotically dividing cell.
We implemented crossing experiments, FCSS, and genotyping by SSR-sequencing (SSR-seq) in a novel manner to analyze reproduction in apomictic subgenus *Rubus*, where automixis potentially occurs. The significant advantage of this approach lies in its ability to apply both methods to a single seed. Initially, half of the seed is used to determine the ploidy levels of both the embryo and endosperm, facilitating the assignment of the mode of reproduction. Subsequently, the remaining half of the seed is used for genotyping by sequencing highly variable SSR markers. Finally, the results of both methods are compared.
Through the comparative analysis of the seed's genotype with that of the parental individuals, we corroborated the findings obtained through FCSS in most cases. Nevertheless, in about 3 % of seeds, a disparity in results indicated the origin of these seeds through automixis. Thus, we confirmed the presence of automixis in angiosperms for the first time. Furthermore, the analysis of seeds through SSR-seq emerges as an attractive alternative for taxa with deviated reproductive pathways (such as the non-standard fusion of nuclei in the megagametophyte), where FCSS struggles to differentiate between apomictic and sexually derived progeny. An additional advantage of the methodological approach comes from the capacity to distinguish between allogamy and autogamy in both sexually and apomictically originated seeds.

Keywords: Apomixis, Automixis, Autogamy, SSR-seq, FCSS, Rubus.

IV International Congress on

APOMIXIS

December 3 - 7, 2023 • Rosario, Argentina

Abstracts: Posters

P01
Incidence of isoforms of the splicing controller BUD13 in apomictic and sexual species

Draga, S. (1); Colono, C. (2); Siena, L. (2); Gabelli, G. (1); Podio, M. (2); Palumbo, F. (1); Ortiz, J.P. (2); Barcaccia, G. (1); Pessino, S. (2)
(1) Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), Agripolis, University of Padova, Italy.
(2) Institute for Agricultural Science Research or Rosario (IICAR-CONICET-UNR), Argentina.
samela.draga@phd.unipd.it

The down-regulation of a member of the splicing machinery (TRIMETHYLGUANOSINE SYNTHASE 1, TGS1) was recently associated with the emergence of apospory-like phenotypes in *Paspalum* and *Arabidopsis*. A TGS1 interactor named BUD13 displays differential expression in florets of sexual and apomictic plants in at least two distinct aposporous species: *Paspalum notatum* and *Hypericum perforatum*. Interestingly, *Arabidopsis bud13* mutants were associated with defects in early embryo development. Here we report the characterization of the structure and the expression of the *Paspalum notatum* BUD13 floral isoforms and analysed its occurrence across the plant kingdom. Transcripts codifying a long form of the protein (LONG), including ICP4 and BUD13 domains, were found equally represented in sexual and apomictic genotypes. However, a shorter isoform, consisting only of the BUD13 domain, was codified by two different transcripts in apomictic and sexual plants: while the apomictic genotypes overexpressed 18 times (log fold change: 4,2) a 5' UTR-extended transcript (SHORT1), the sexual ones overexpressed 18 times (log fold change: -4,138) a reduced sequence lacking the 5' UTR extension (SHORT2). Both these sequences (SHORT1 and SHORT2) codify an identical short protein, except for two non-conservative aminoacidic replacements. Mapping of the sequences onto the diploid *Paspalum notatum* reference genome revealed that all three transcript variants (LONG, SHORT1 and SHORT2) originated from a unique sub-telomeric locus at Pnchr6. Then, we collected all plant BUD13 protein sequences available at ENTREZ (865 sequences from 245 species), including primitive lineage, gymnosperm, and flowering plants, and carried out an exhaustive BUD13-based phylogenetic analysis. Several plant species (some from primitive lineages) showed only the SHORT forms of the protein (i.e., analogous to the yeast representative BUD13 sequences), whereas others displayed only the LONG one. Finally, a limited list of species exhibited the two forms (SHORT and LONG). Remarkably, the two groups containing the SHORT form revealed a statistically significant prevalence of reported apomicts (19/45; P: 0.4222; 95% confidence interval: 0.2799- 0.5776) with respect to the group containing the LONG form only (13/200; P: 0.065; 95% confidence interval: 0.0365-0.111), suggesting that the BUD13 protein structure could be related with the potential occurrence of asexual reproduction by seeds. Nowadays we are performing *in situ* experiments in *Paspalum* ovules to analyse the spatial distribution of *BUD13* at different developmental stages and developing constructs useful for functional analysis.

Keywords: Apomixis, Paspalum, bud13, splicing machinery, functional analysis.

P02
Developing a Pan-Genome of the diplosporous grass Eragrostis curvula

Bongiorno, G. (1); **Carballo, J.** (2); Gallo, C.A. (2); Albertini, E. (1), Zappacosta, D. (2), Echenique, V. (2)
(1) Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università degli Studi di Perugia, Perugia, Italy.
(2) Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS) Universidad Nacional del Sur - Consejo Nacional de Investigaciones Científicas y Técnicas (UNS-CONICET) and Dpto. de Agronomía (Universidad Nacional del SUR), Bahía Blanca, Argentina.
giacomo.bongiorno@studenti.unipg.it

As large-scale genomic studies have progressed, it has been revealed that a single reference genome pattern cannot represent the genetic diversity present at the species level. The pangenome can complement the missing genetic information based on the analysis of a single reference genome, exhibit hidden genetic variations, and demonstrate the true genetic diversity at the species level. The progress of pangenome research in different species has allowed the identification of large structural variants related to important agronomic traits. Weeping lovegrass (*Eragrostis curvula* [Schrad.] Nees) is a forage grass that reproduces by sexuality and by facultative and obligate apomixis. It presents distinctive variants with different ploidy levels (2x – 8x) and a basic chromosome number of 10. The recent availability of the genome assembly of cv. Victoria has provided a valuable resource for identifying specific genomic regions linked to significant traits, for instance, forage quality. However, it is worth noting that the regions that control apomixis and others related with ploidy are typically hosted by genotypes with higher ploidy levels.
In this work, we focused on constructing a pan-genome of *Eragrostis curvula* to detect genomic variation, establish phylogenetic relationships, and analyze the effects of ploidy in genome evolution and reproductive mode. To do that, we used the genome assembly of cv. Victoria and genomic data, obtained by Illumina reads, of nine genetically diverse accessions of *E. curvula*. The construction of the pan-genome employed an iterative mapping and assembly approach involving the mapping of reads from different genotypes to the reference genome assembly. The mapped reads were used for variant calling, while the unmapped reads were assembled into new genomic fragments to annotate genes absent in the reference genome. These newly assembled sequences were subsequently integrated into the reference genome, and the process was repeated iteratively for other genotypes. When all the accessions were processed, the final pan-genome comprised the reference genome and the newly assembled sequences. This approach proved to be highly efficient for constructing a pan-genome exploiting the reference genome and the assembly of genetically distant genotypes of *E. curvula*. Ultimately, the genomic resources generated were employed to gain a comprehensive understanding of the genetic mechanisms underlying apomixis and related processes.

Keywords: Eragrostis curvula, ploidy, apomixis, pan-genome.

P03
Generation of auxin and cytokinin marker lines in Paspalum notatum

Colono, C.M. (1); Ortiz, J.P. (1); Perrone, D. (2); Permingeat, H. (1); Orozco, G. (2); Colombo, L. (2); Kater, M. (2); Mendes, M.A. (2); Pessino, S.C. (1)
(1) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR), CONICET, Universidad Nacional de Rosario, Campo Experimental Villarino, S2125ZAA Zavalla, Santa Fe, Argentina.
(2) Dipartimento di Bioscienze, Universitá degli Studi di Milano, Milano, Italia.
colono@iicar-conicet.gob.ar

Apomixis is a type of asexual reproduction through seeds leading to the formation of offsprings genetically identical to the mother plant. Sexuality and apomixis are interconnected reproductive routes, possibly behaving as polyphenic traits under the influence of the environment. In previous works we reported heterochronic gene expression in florets of apomixis and sexual *Paspalum notatum* plants. In particular, genes associated with auxin and cytokinin metabolism appeared to be extensively deregulated. Moreover, in parallel experiments we detected that auxin test induces the formation of aposporous-like embryo sacs in sexual genotypes of this species, and increase the expression of parthenogenesis in apomictic ones. The objective of this work was to obtain transgenic lines of *Paspalum notatum* carrying auxin and cytokinin marker, in order to explore the hormones pattern in both reproductive types. Undifferentiated calli were induced from mature seeds of apomictic and sexual individuals and co-transformed using a Biomics gene-gun device and tungsten particles. Transformation vectors carrying a DR5:VENUS (auxin marker construct) or a TCS-GFP (citokinin marker construct) + pUbi-BAR (the latter containing a glufosinate ammonium tolerance selector gene) were used. Two experiments involving 8 plates with 15 calluses each and 4 plates of selection and regeneration controls were performed for both plasmids. Fluorescence microscopy and/or PCR amplification using specific primers revealed 8 and 5 events of stable transformation for the auxin and the cytokinin construction, respectively. The patterns of fluorescent signal are now being analysed in roots using a Zeiss LSM880 confocal microscope. After flowering, we will obtain crosses and identify progeny plants lacking the pUbi-BAR construct, in order to eliminate the selector construct. Then, the hormone distribution pattern will be analysed in ovaries of sexual and apomictic plants. This work will allow to reveal the dynamic of auxin and cytokinin activity during sexual and apomictic reproductive pathways in *Paspalum notatum*.

Keywords: apomixis, Paspalum notatum, markers.

P04
Construction of a consensus genetic map of Eragrostis curvula

Gallardo, J. (1,2); Gallo, C. (1); Sansot Puleston, M. (1); Echenique, V. (1,2); Zappacosta, D. (1,2)
(1) Centro de Recursos Naturales Renovables de la Zona Semiárida, CONICET, Bahía Blanca, Argentina.
(2) Departamento de Agronomía, UNS, Bahía Blanca, Argentina.
jimenagallardo@hotmail.com.ar

Eragrostis curvula is a forage grass used by our group as a model for the study of displosporous apomixis. It includes genotypes with different ploidy levels (from 2X to 8X, with X = 10) and reproductive modes (sexual and facultative or obligate apomixis). Previously, our group built the first highly saturated genetic linkage map of *E. curvula* using a mapping population generated from the cross between two tetraploid genotypes, OTA-S and Don Walter. At that time, the small size of the mapping population (61 individuals) did not allow the generation of a consensus map between the maternal and paternal maps. However, a map was constructed for each parental genotype and the apomeiosis-linked locus (APO-locus) was located in the paternal map. The objective of this work was to expand the mapping population using the same parental lines in order to build a high-density consensus genetic map where to position the APO-locus and to make syntenic analysis. The new mapping population consisted of 109 hybrid individuals coming from the previous population and new ones coming from the new crosses. This population was phenotyped by cytoembryology and through the use of a dominant molecular marker linked to apomeiosis, resulting in 48 sexual offprings and 61 apomictic ones. The genotyping was performed with SNPs using the DArT-Seq technology (SAGA-CIMMYT, Mexico). For the map construction single dose allele markers and biparental SNPs markers were used. Linkage maps for each parent and a consensus map were built. The consensus genetic map contains a total of 1,132 markers, of which 587 were paternal, 514 maternal and 31 biparental. The total length of the linkage map was 4,605 cM and it was made up of 20 linkage group (LG). Synteny analysis was performed using the genome assembly of *E. curvula* (cv. Victoria). In the paternal map, a region delimited by three markers was found that cosegregates with apomeiosis (APO-locus). The APO-locus delimited a region of 11,322,729 bp in the reference genome of cv. Victoria. The construction of this mapping population, added to the of *E. curvula* consensus map are valuable elements for future studies of apomixis.

Keywords: Consensus genetic map, Apo-locus, Mapping population.

P05

Studies tending to functionally characterize putative genes to be involved in apomictic pathway/s in Eragrostis curvula

Díaz, A. R. (1, 2); Selva, J. P (1,2); Carballo, J. (1); Garbus, I. (1,3); Echenique, V. (1,4)
(1) Centro de Recursos Naturales Renovables de la zona Semiárida, CERZOS, Bahía Blanca, Argentina.
(2) Departamento de Biología Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.
(3) Departamento de Ciencias de la Salud, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.
(4) Departamento de Agronomía, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.
ardiaz@criba.edu.ar

Apomixis (asexual seed formation) is a plant ability to bypass sexual reproduction. Without male fertilization, the resulting seed germinates a plant that develops as a maternal clone. This reproductive mode has been documented in many flowering plant species; however main seed crops have not been shown to be capable of apomictic propagation. *Eragrostis curvula* (Schrad.) Nees (weeping lovegrass) is a high photosynthetically-efficient C4 perennial grass, member of the Poaceae family. Weeping lovegrass includes cytotypes with different ploidy levels (from 2X to 8X) and reproductive modes, being mainly an apomictic species. In previous transcriptomic analysis, we have identified several genes displaying differential representation in florets of sexual and apomictic genotypes, suggesting they might be involved in apomictic pathways. Many of these genes do not have an annotation in other species, thereforeit is necessary to functionally characterize them. In an attempt to achieve this characterization, we addressed different approaches: a) RNA *in situ* hybridization technic (RNA-ISH) to determine the expression patterns cell-specific of these genes in reproductive tissues of full apomictic and sexual genotypes; b) expression in rice under different constitutive promoters. In this work, we summarize some results obtained with these techniques on one of the differentially expressed genes. RNA-ISH experiments revealed its differential expression pattern in reproductive tissues, specifically being expressed in ovule tissues of full apomictic plants. Transgenic assays allowed us to obtain different independent lines overexpressing this gene that are currently under phenotypic analysis. These studies will lead to understand the potential role of this gene in apomictic reproduction pathways.

Keywords: Eragrostis curvula, characterization, gene, apomixis, ISH, rice overexpression.

P06
Correlation between apomixis potential in ovules and fertility in tetraploid individuals of Paspalum almum Chase

Schneider, J.S. (1); Hojsgaard, D. (2); Daviña, J.R. (1); Honfi A.I. (1)
(1) Programa de Estudios Florísticos y Genética Vegetal, Instituto de Biología Subtropical (CONICET-UNaM) nodo Posadas, FCEQyN-UNaM, Posadas, Misiones, Argentina.
(2) Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstraße 3, D-06466 Seeland, OT Gatersleben, Germany.
schneider.s.juan@gmail.com

Paspalum almum Chase is a native forage species that occurs spontaneously in natural grasslands of northeastern Argentina. The species has two cytotypes that form a polyploid complex, with sexual self-sterile diploids (2n = 2x = 12) and aposporous self-compatible tetraploids (2n = 4x = 24). The expression of apospory and associated fertility is functional for plant breeding and therefore, here, we evaluated the proportions of developed embryo sacs in mature ovules and the fertility of facultative apomictic tetraploids of *P. almum*. Plant accessions from Santa Fe, Corrientes and Misiones (Argentina) were analyzed. Vouchers are deposited at MNES herbarium. At least 30 cleared pistils per plant were analyzed from thirty plants grown in pots under similar conditions. Embryo sacs were classified in meiotic *Polygonum*-type and aposporous *Paspalum*-type. The apomixis potential (PAO) was estimated as the proportion of aposporous embryo sacs per ovule, i.e., number of aposporic embryo sacs divided by the total number of analyzed ovules. The fertility index (FI) was calculated from seed production under open-pollinated and self-pollinated conditions (FI = Number of seeds per inflorescence / Number of spikelets per inflorescence), averaging data from 3 inflorescences. Both the proportion of aposporic embryo sacs and seed production measurements were carried out on the same plant over a period of one month. Finally, a Pearson-Correlation test was applied between the PAO and FI using the "stats" package in the R environment. The FI varied significantly between pollination types (p-value < 0.001), showing a mean FI of 0.45 ± 0.02 under open-pollination and of 0.29 ± 0.02 under self-pollination. Differences in PAO between pollination conditions were not significant (p-value = 0.49). Under open-pollination conditions, Pearson's test showed a moderate positive correlation between PAO and FI (p-value < 0.001; r = 0.60), while under self-pollination conditions, PAO and FI were not correlated (p-value = 0.99; r = - 0.01). The variation in the expression of apospory at ovule stage and seed production under two alternative pollination types suggests that apomixis may impact ovule abortion and have a differential effect on fertility under contrasting pollination types. Further studies will help us disentangle the reasons of the difference observed between both pollination conditions.

Keywords: Plant, reproduction, fertility, apomixis.

P07
Embryo sac and fertility analyses in a BIII synthetic Paspalum almum hybrid

Schneider, J.S. (1); Escobar, L.M. (1); Daviña, J.R. (1); Hojsgaard D. (2); Honfi A.I. (1)
(1) Programa de Estudios Florísticos y Genética Vegetal, Instituto de Biología Subtropical (CONICET-UNaM) nodo Posadas, FCEQyN-UNaM, Posadas, Misiones, Argentina.
(2) Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstraße 3, D-06466 Seeland, OT Gatersleben, Germany.
schneider.s.juan@gmail.com

Paspalum almum Chase is a native forage species from northeastern Argentina. Only two cytotypes are known grown in nature, diploids (2n = 2x = 12) and tetraploids (2n = 4x = 24). Diploids and tetraploids have different reproductive modes, diploid plants are sexual self-sterile and tetraploid are facultative apomictic self-fertile. A neo-hexaploid P. *almum* hybrid plant was recovered from a tetraploid × tetraploid cross. Here we evaluate the embryo sac anatomy in ovules and fertility in this newly formed hexaploid aiming at assessing its reproductive mode and possible incorporation into current breeding schemes. Three inflorescences were collected at anthesis and 93 cleared pistils were analyzed. Embryo sacs were classified in meiotic *Polygonum*-type and aposporous *Paspalum*-type. Seed set was determined from 10 inflorescences under open-pollination and Fertility Index (FI) was calculated as Number of seeds per inflorescence / Number of spikelets per inflorescence. We found three categories of ovules. Ovules carrying only meiotic embryo sacs (0.54), ovules carrying one meiotic + 1 or more aposporic embryo sacs (0.13 in total, grouped in 0.11 with 1 aposporic embryo sac, 0.01 with 2 aposporic embryo sac; and 0.01 with 3 aposporic embryo sac) and ovules with immature embryo sacs, with clear signs of abortion or no embryo sacs (0.33). FI was 0.03 (82 seeds were obtained from 1062 spikelets). The data indicates that, despite the high proportion of non-viable ovules, much of those viable ones carrying functional embryo sacs failed to develop into a seed. The low fertility of the hexaploid plant is likely responding to the genomic stress caused by the ploidy shift and gene dosage imbalances. Upcoming studies on pollen-pistil compatibility, self-pollinated seed-set, and meiotic and genetic analyses will help us to unravel the cytological and molecular basis of this sterility.

Keywords: neo-hexaploid, embryo sac, fertility.

P08
Development of KASP markers linked to apomixis in Eragrostis curvula

Gallardo, J. (1,2); Gallo, C. (1); Rodrigo, J.M. (1,2); Echenique, V. (1,2); Zappacosta, D. (1,2)
(1) Centro de recursos naturales renovables de la zona semiárida, CERZOS-CONICET, Argentina.
(2) Depto. Agronomía UNS, Bahía Blanca, Argentina
jimenagallardo@hotmail.com.ar

Recently, our working group has built a new linkage map of the diplosporous grass *Eragrostis curvula*. For this purpose, a mapping population composed of 109 individuals was generated from the cross between the tetraploids OTA (sexual) and Don Walter (facultative apomictic pollen donor). This new population was genotyped with SNPs markers obtained through the DArT-seq technology. This technique has multiple advantages over other similar sequencing techniques (eg. GBS), since DArT-seq generates thousands of markers with high efficiency, due to the specific pipeline, the high fidelity restriction enzymes used and the intrinsic system of duplicate samples. Like other techniques, multiple pooled samples can be analyzed, reducing costs and time required, and it is possible to work without a reference genome.
In this new *E. curvula* linkage map it was possible to identify a region associated with apomeiosis (APO-locus) and three closely linked (100%) SNPs markers. Primers designed from the sequence of these SNPs allowed the development of KASP (Kompetitive allele specific PCR) markers able to differentiate between sexual and apomictic *E. curvula* plants (phenotyping).
KASP are codominant markers and the results obtained can be observed by fluorescence at the end of a PCR in a PHERAstar Plus equipment available at the GENeTyC laboratory (CERZOS-CONICET). First, the sequence of each SNP markers linked to the APO-locus was identified (69 bp) and the most appropriate regions for primer design were chosen. The Primer3Plus program was used and 5 sets of KASP primers were designed with specific "tails" for each one of the FAM and HEX alleles for the paternal and maternal alleles, respectively. These 5 sets of KASP markers were tested on the population parental lines and on a set of sexual and apomictic cultivars (20). Based on its efficiency one of these KASPs markers (K277) was selected and used to validate the phenotype of the offspring.
The KASP marker designed, despite being codominant, demonstrated its reliability to perform the phenotypic characterization, distinguishing between sexual and apomictic individuals. Sequences corresponding to SNP markers 100% linked to apomeiosis showed homology with genes, pointing at such genes as potential candidates to be involved in the regulation of the reproductive mode of this grass.

Keywords: KASP markers, phenotypic characterization, APO-locus.

P09

New assembly and annotation of diploid Bahiagrass (Paspalum notatum Flügge var. saurae) based on ONT long reads.

Vega, J.M. (1); Podio, M (1); Orjuela, J. (2); Siena, L.A. (1); Mariac. C. (2); Pupilli. F. (3); Albertini, E. (4); Pessino, S.C. (1); Leblanc, O. (2); Ortiz, J.P.A. (1)
(1) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET-UNR), Zavalla, Argentina.
(2) DIADE, Université de Montpellier, IRD, CIRAD, Montpellier, France.
(3) Institute of Biosciences and Bioresources (IBBR), CNR, Perugia, Italy.
(4) Department Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy.
jvega@iicar-conicet.gob.ar

Paspalum notatum is a subtropical forage grass native to South America. The species consists of sexual self-sterile diploids (var. saurae) and pseudogamous apomictic self-fertile tetraploids (var. common). *P. notatum* is considered one of the models for the study of apomixis in grasses due to the large amount of reproductive biology, genetics, genomic, transcriptomic, and breeding information generated over the last 50 years. Apomixis in the species is under the control of single genomic region (ACR) that exhibits recombination restriction and segregation distortion. This work aimed to produce a high-quality genome assembly and annotation of the diploid cytotype, to be used as a reference for studying the structure of the tetraploid genome and to reveal the gene content of the genomic region responsible for apomixis. A natural diploid biotype (#R1) collected from the center of origin and diversity of the species was used as plant material. Using more than 200x Oxford Nanopore long reads, the reference-based assembly produced a genome of 557.8 Mb distributed across the ten expected chromosome-length scaffolds (N50 = 56.1 Mb), with a high degree of completeness (BUSCO score = 98.73%). The CG content resulted in 45.8% GC and the proportion of unknown bases was 0.05%. Polishing was performed using 100x Illumina short reads. K-mer analysis carried out using GenoScope and short Illumina reads revealed 1.73% of heterozygosity and a 57.86 % of repetitive sequences. Classification of repetitive elements using RepeatExplorer2 showed that Gypsy-like and Ty1-copia retroelements were the most abundant, followed by DNA transposons. The gene annotation performed with MAKER predicted 45,074 gene models, of which 32,101 were classified as high confidence. As expected, a higher gene density of genes was found in distal chromosome regions, whereas a higher frequency of repetitive elements was found in pericentromeric regions. Alignment of the #R1 assembly with the available reference (cv. Crowver) revealed a high degree of sequence conservation. In addition, comparisons with rice, *Sorghum bicolor and Setaria italica* genomes revealed large and small chromosome rearrangements. The characterization of the #R1 genome included the identification of 59 miRNA precursors together with their putative targets and the generation of a set of 4,774 SSRs useful for breeding. The present work provides a comprehensive genomic framework to analyze the structure and evolution of the *P. notatum* agamic complex, to study the determinants of apomixis and to design new molecular breeding strategies.

Keywords: Pasaplum notatum, genome assembly, ONT.

P10

Auxin response repressor IAA16 defective mutants show developmental alterations in female gametophytes and embryos in Arabidopsis thaliana

Vega, M.S. (1); Leblanc, O. (2); Pessino, S.C. (1); Ortiz, J.P.A. (1); Siena, L.A. (1)
(1) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET-UNR), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Rosario.
(2) DIADE, Univ. Montpellier, IRD, CIRAD, 34394, Montpellier, France.
mvega@iicar-conicet.gob.ar

Auxin plays an essential role in many aspects of plant development, including growth and reproduction. Recently, we have found that the auxin response repressor *IAA30* of *Paspalum notatum* is down-regulated in ovules of apomictic genotypes compared to sexual genotypes at the stages of anthesis and early embryogenesis. Furthermore, its expression level is negatively correlated with apospory expressivity in facultative apomicts, and differences in spatio-temporal expression between reproductive mode were revealed by in situ hybridization experiments. These results suggest a contrasting regulation of auxin signalling during sexual and apomictic seed formation in the species. To analyze the biological function of *IAA30* during plant reproduction and investigate its possible association with the apomictic phenotype, we started the functional characterization of *IAA16*, its orthologue in *Arabidopsis thaliana*. RT-PCR experiments showed that *IAA16* is expressed in siliques, leaves, seedlings and roots. Cytoembryological analyses of two *iaa16* mutant lines revealed formation of multiple enlarged cells around the megaspore mother cell, the functional megaspore and the embryo sac, an observation reminiscent of apospory initials. After pollination, both lines showed defects in embryo patterning and occurrence of twin embryos. We crossed iaa16 with a line carrying the pWOX2-CENH3-GFP reporter construct. Analysis of double homozygous F2 plants confirmed the abnormal symmetry patterns detected in the embryos. Only 5-6% of the mutant seeds germinated onto MS germination medium produced normal seedlings, compared to 28% in Col-0. Four mutant seedlings with one cotyledon and two seeds with double shoots (polyembryony) were also detected. The results of this work indicate an essential role of *IAA16* in the specification of the female germline fate and embryogenesis in *Arabidopsis*.

Keywords: IAA16, apomixis, Paspalum notatum, Arabidopsis thaliana.

P11

Formation of BIII hybrids and effect of ploidy raises on the reproduction of aposporous sunflower (Helianthus annuus L.)

Katzaroff, I. (1); Riviera, L. (1); Aguilar, G. (1); Bianchi, M. B. (1); Bocchini, M. (2); Marconi, G. (2); Albertini, E. (2); Pessino, S. (1,3); Nestares, G. (1,3); **Ochogavía, A.C.** (1,3)

(1) Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Campo Experimental J. F. Villarino, Zavalla, Santa Fe, Argentina.

(2) Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università degli Studi di Perugia, Umbria, Italia.

(3) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR, CONICET, UNR), Campo Experimental J. F. Villarino, Zavalla, Santa Fe, Argentina.

anaochogavia@conicet.gov.ar

Soybean and sunflower are main crops that position argentine oilseed production in the global context. The recent transfer of sunflower cultivation to areas of low-quality soil and increased agroecological risks implies a significant decrease in yield and represents an important challenge for breeders. Apomixis is a mode of asexual reproduction through seeds, reported in at least 78 angiosperm families, which represents an unprecedented tool to improve plant breeding strategies. Our research group have identified cultivated sunflower lines that naturally express moderate levels of apospory. The general objective of our work was to explore the formation of triploid (3x) BIII hybrids (2n + n) in the progeny of the aposporous line Rf975 and to determine the effect of ploidy transitions in reproduction. Ten plants of Rf975 (2x, aposporous) and HA89 (2x, sexual control) were cultivated in the field under irrigation and manual weed control. Although at R6 stage both genotypes produced a similar number of young fruits per capitulum (1525 ± 121 and 1583 ± 115, respectively), the Rf975 capitula had a considerable proportion of 3x immature embryos (42.8 %; 95% CI: 26.7-60.4 %, n= 35) in flow-cytometric analyses and produced fewer viable seeds than HA89 (728.4± 100 and 1394± 157, respectively). Moreover, in the F1 of Rf975xHA89 crosses, some 3x embryos progressed until the reproductive stages (13.4 %; 95% CI: 4.54 to 32.1 %, n= 23). Currently, we are analyzing the possible apomictic origin of some F2 individuals by using McSeED-based experiments. Moreover, we applied immature 3x embryo rescue in order to avoid developmental collapse due to endosperm imbalance. From 240 Rf975 rescued embryos, 186 seedlings germinated, 77 reached the V4 vegetative stage, and 48 arrived at the reproductive developmental stage. Eight Rf975 plants from the last group were classified as 3x (14.8%; 95% CI: 6.1-27.6%, n= 54) and 46 as 2x. Under cytoembryological analysis, triploids showed a high proportion of ovules with multiple aposporous embryo sacs (AES) (87.5%; 95% CI: 46.7-99.3%, n= 8), abnormal or scarce pollen grains and no seeds. On the other hand, from 120 cultivated embryos of the control line HA89, 103 seedlings germinated, 31 reached V4 and 22 the reproductive developmental stage. All plantlets were classified as 2x by flow cytometry (100%; 95% CI: 84-100%, n= 26), they showed normal sexual reproduction and produced 7.87 ± 3.2 seeds per capitula after self-pollination. The complete experiment was repeated in two independent seasons (21/22 and 22/23), obtaining similar efficiencies for the embryo rescue assay. Our results indicate that: (i) Rf975 produce BIII hybrids by fertilization of AES; (ii) 13.4% of the viable F1 progeny derived from Rf975xHA89 crosses are BIII hybrids; (iii) embryo rescue allows to reach a similar proportion of viable BIII hybrids after self-pollination (14.8%); and (iv) apospory expression increases in 2x to 3x transitions.

Keywords: sunflower, apospory, BIII hybrids.

P12

Embryo sac composition and fertility assessment in Paspalum ovale Nees 8x: insights into reproductive mechanisms

Escobar, L.M. (1); Schneider, J.S. (1); Daviña, J.R. (1); Martínez, E.J. (2); Honfi A.I. (1).
(1) Programa de Estudios Florísticos y Genética Vegetal, Instituto de Biología Subtropical (CONICET-UNaM) nodo Posadas, FCEQyN-UNaM, Posadas, Misiones, Argentina.
(2) Instituto de Botánica del Nordeste, (CONICET-UNNE), Corrientes, Argentina.
lucasmescobar17@gmail.com

Paspalum ovale Nees ex Steud., a native grass to southern Brazil, Paraguay and northeastern Argentina, exhibits two distinct cytotypes: heptaploids (2n= 7x= 70) and octoploids (2n= 8x= 80). Utilizing Flow Cytometry of Single Seeds studies, 8x was categorized as apomictic. However, comprehensive information regarding the composition of the embryo sac and the potential for apomixis or sexuality during the ovule's development, as well as seed fertility, remains missing. The embryo sac constitution in at least 30 mature ovules and seed fertility in eighteen individuals from three populations from Argentina were analyzed. Seed set was determined in three inflorescences under open-pollination conditions, with the Fertility Index (FI) calculated as number of seeds per inflorescence divided by number of spikelets per inflorescence. At anthesis, ovules had one meiotic embryo sac (MES) at 44.21%, or one MES alongside 1-4 apomictic embryo sac (AES) in 52.62%, while 3.16% remained undeveloped. The MES of the *Polygonum* type consisted of an egg apparatus with one oosphere, 1-2 synergids, a central cell containing 2-3 polar nuclei, and 2-12 antipodals cells. Conversely, the AES displayed characteristics typical of aposporous *Paspalum* types, consisting of one egg-cell, 2-4 polar nuclei in the central cell, and an absence of antipodals. The FI was low, ranging from 2% to 22%. The data strongly suggests that *P. ovale* 8x is facultative apomictic aposporous type. The observed low fertility appears to be associated with pollen limitation, rather than the absence of embryo sacs available for fertilization.

Keywords: Polyploidy, Apomixis, Seed fertility, Embryo sac.

P13
Unveil the molecular mechanisms regulating Apomixis in Dandelion

Banfi, C. (1); Cucinotta, M. (1); Cornaro, L. (1); Petrella, R. (1); **Cavalleri, A.** (1); Van Dijk, P.J. (2); Rigola, D. (2); Op den Camp, R. (2); Colombo, L. (1)
(1) Dipartimento di Bioscienze, Universita degli Studi di Milano, Milan, Italy.
(2) Keygene N.V., Wageningen, Netherlands.
alex.cavalleri@unimi.it

Apomixis is an asexual reproductive mechanism in which the embryo forms without fertilization. The clonal seed propagation allows the fixation of a given genotype through generations, saving costs and efforts that characterize plant breeding. During the last decades, many researchers have been working on this topic, but the molecular mechanism regulating apomixis it still under investigation. *Taraxacum officinale* – the common dandelion – is a natural apomict species with diploid sexual and triploid apomictic populations which reproduces through diplospory. In this type of apomixis, the pairing of chromosomes during meiosis is impaired, leading to the formation of an unreduced megaspore that develops in an unreduced female gametophyte. Eventually this gives rise to an embryo through parthenogenesis. By confocal imaging, I characterized the female germline progression in apomictic and sexual plants at high resolution level, providing a detailed morphological atlas of diplospory. Furthermore, I have demonstrated that in *loss-of-diplospory (lod)* mutant the meiotic process is impaired, causing apomictic-to-sexual transition. I have used different approaches to unveil the genes potentially involved in diplospory and their network. By RNA-seq I have identified several genes deregulated in the apomictic plant respect to the sexual. Further analysis of the experiment results are ongoing.

P14

Morphogenetic determinants of plant female germ cell precursors specification and plasticity

Ouedraogo, I. (1); Mosca, G. (2); Delgado, L. (3); Leblanc, O. (1); Hernandez-Lagana, E. (1); Mendocilla Sato, E. (4); Hamant, O. (5); Boudaoud, A. (6); Godin, C. (5); Grossniklaus, U. (4); Lartaud, M. (6); Conéjéro, G. (6,7); Baroux, C. (4); **Autran, D.** (1)

(1) Epigenetics and Seed Development group, Research Unit DIADE, CIRAD, IRD, University of Montpellier, France.
(2) Center for Plant Molecular Biology (ZMBP), University of Tübingen, Germany.
(3) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR), CONICET, University of Rosario, Argentina.
(4) Institute of Plant and Microbial Biology, Zürich-Basel Plant Science Center, University of Zürich, Switzerland.
(5) Research Unit RDP, INRAE, ENS de Lyon, University of Lyon, France.
(6) LadHyX, Ecole polytechnique CNRS, Institut Polytechnique de Paris, Palaiseau, France
(7) Research Unit AGAP Institute, CIRAD, INRAE, Institut Agro, University of Montpellier, France.
(8) Research Unit IPSIM, CNRS, INRAE, Institut Agro, University of Montpellier, France.
daphne.autran@ird.fr

In higher plants, female gametes formation is a crucial step in the plant reproductive cycle and determines seed formation, hence participating in crop yields. The plant female germline initiates in the ovule primordium, with the specification of the Megaspore Mother Cell (MMC), the only cell which will undergo meiosis to produce gametes. However, germ cell fate in the early ovule appears flexible. Genetic variants and apomictic species show that somatic cells neighboring the MMC can enter the MMC identity program or even directly produce female gametophytes without meiosis. By combining 3D morphometrics, growth modelling, gene markers and genetic analyses, we have shown in Arabidopsis that this developmental plasticity is also part of MMC ontology in wild-type, before channeling toward MMC singleness, a process controlled by ovule tissue growth. Recently, we developed new routes further exploring Arabidopsis MMC growth using time-lapse (3D+t), and establishing a 3D morphometric atlas of ovule primordia in Maize, a sexual grass model, to understand the genericity of MMC formation and its plasticity.

Keywords: ovule, female germ cell fate, megaspore mother cell, morphogenesis, Arabidopsis, Maize.

P15
Functional characterization of AUXIN RESPONSE FACTOR 8 and 18 during ovule development in Oryza sativa

Davide, P. (1); Orozco Arroyo, G. (1); Colono, C. (2); Pessino, S. (2); Kater, M. (1); Colombo, L. (1); Mendes, M.A. (1)
(1) Dipartimento di Bioscienze, Universitá degli Studi di Milano, Milano, Italia.
(2) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET-UNR), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Rosario.
davide.perrone@unimi.it

AUXIN RESPONSIVE FACTORS (ARFs) are a family of genes playing crucial roles in many developmental contexts. They encode transcription factors that regulate the expression of a wide range of other genes, influencing key processes in plant growth and development - among this, the morphology of plant reproductive organs. Recent transcriptomic studies, performed in reproductive organs, suggest that, in ovule developmental program, a deregulation of auxin signalling, and particularly the miR160/ARF10 system are involved in the switch from sexuality to agamic (asexual) reproduction in *Paspalum notatum*. *Oryza sativa*, like *Paspalum notatum*, is a monocot of the Poaceae family, hailing from Asia and widely cultivated worldwide. Hence, for its importance as a major crop and the close phylogenetic relation with *Paspalum*, we focused our studies on characterizing the orthologs of *ARF10* in rice. Phylogenetic analyses strongly suggest that the *ARF10* orthologs in rice are *OsARF8/OsARF18*, based on their phylogenetic relationship and expression during ovule development. We are planning to better characterize their expression by in situ hybridization during *Oryza sativa* ovule development. Furthermore, to functionally characterize these genes we have produced twenty-three (23) independent mutated lines using the Crispr-Cas9 technology, double mutants for both genes were obtained. Preliminary analysis demonstrated reduced seed production in the double mutant lines when compared to the wild type, that could indeed be linked to problems during ovule development. We have started to characterize by confocal and DIC analysis ovule development. Inflorescences from wild type and double mutant plants were collected at stages OVR1 (MMC-stage), OVR2 (megagametogenesis) and OVR3 (mature embryo sacs) in order to evaluate if absence of these genes causes alteration in during female gametophyte formation. In parallel, we are planning to perform an RNA-sequencing to uncover which genes are deregulated in the double mutant, probably targets of *OsARF8/OsARF18* that are involved within this developmental moment.

Keywords: apomixis, ovule development, auxin.

P16

In silico characterization of gene families involved in epigenetic reprogramming associated with the reproductive mode in Paspalum notatum

Podio, M. (1); Pessino, S. (1); Ortiz, J.P. (1)
(1) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET-UNR), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Rosario
podio@iicar-conicet.gob.ar

Paspalum notatum is a rhizomatous perennial grass distributed in tropical and subtropical regions of South America. The tetraploid race of this species reproduces almost exclusively by apomixis of the aposporous type. Apospory (a component of aposporous apomixis) in this species is controlled by a single locus (ACL) that exhibits distorted segregation and recombination suppression. This genomic region contains low and high copy number sequences, coding and non-coding regions, a high abundance of retrotransposons and cytosine methylation. Recently, functional studies have highlighted the role of small interfering RNAs and RNA-directed DNA methylation (RdDM) in the apomictic development. Furthermore, in *Paspalum simplex*, cytosine demethylation had a detrimental effect on parthenogenesis, another of the central components of apomixis. The aim of this work was to identify epigenetic DNA modification pathways active during the sexual and apomictic reproductive development in *P. notatum*. We used the sexual and apomictic transcriptomes of the reproductive development classified by developmental stage, to compare the abundance of transcripts associated with epigenetic modifications. Transcripts were annotated using the BLASTN tool against the Conserved Domains Database (CDD) of the NCBI. In addition, GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) tools were used for transcript classification employing *Arabidopsis thaliana* and *Oryza sativa* databases. All transcripts carrying conserved motifs and molecular functions associated with epigenetics factors were retrieved from the transcriptome.The analysis of their expression levels detected 281 differentially expressed (DE) (Log2FC >|2|, FDR < 0.05), of which 124 were down-regulated and 147 up-regulated in apomictic and sexual genotypes, respectively. Classification of DE transcripts showed members of HDMs histone demethylases, PRC2 histone, DNA methylases and small RNAs-directed transcription. The results presented in this work indicate that several epigenetic pathways are differentially regulated during sexual and apomictic reproductive development in *P. notatum* and may help to efficiently select candidates for further functional characterization analyses.

P17
Functional characterisation of QGJ, a YODA family member associated with apospory

Siena, L.A. (1); Michaud, C. (2); Ortiz, J.P.A. (1); Pessino, S.C. (1); Leblanc, O. (2)
(1) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET-UNR), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Rosario.
(2) DIADE, Univ. Montpellier, IRD, CIRAD, Montpellier, France.
siena@iicar-conicet.gob.ar

Some flowering plants reproduce asexually by seeds while avoiding meiosis and fertilization, but maintaining the alternation of gametophytic and sporophytic generations, a typical feature of sexual plant life cycles. This mode of reproduction, namely gametophytic apomixis, perpetuates the maternal genotype through generations, an appealing outcome for plant breeders. To date, only a handful of candidate genes related with apomixis have been functionally studied. In *P. notatum*, downregulation of *QUI-GON JINN* (*QGJ*, which encodes a MAPK3 protein of the YODA family) in apomictic genotypes was associated with a decrease of the number of unreduced embryo sacs (one of the components of gametophytic apomixis). Moreover, in this species the activity of *TRIMETHYLGUANOSINE SYNTHASE 1 (TGS1)*, whose down-regulation leads to the formation of apomixis-like phenotypes, seems to be antagonistic to that of *QGJ*. The aim of this work was to analyze expression patterns and mutant phenotypes of QGJ in the model species *Arabidopsis thaliana*, to disclose the function of the gene during sexual development. RT-PCR experiments in Col-0 lines revealed higher accumulation of QGJ transcripts in both vegetative and reproductive organs, but mainly in gametophytes and siliques. Next, we monitored protein accumulation by introducing the pAtQGJ:H2B-GUS construct in wild type plants. Reporter expression analysis in somatic tissues showed GUS accumulation in stomas and trichomes. In reproductive organs, GUS staining was observed in the L1 cell layer of the ovule primordium, but not in the placenta. Later, it was expressed in degenerating megaspores after meiosis, while the functional megaspore completely lacked signal. In developing seeds, the endosperm showed GUS accumulation, but the zygote and growing embryos were completely devoid of signal. Furthermore, *qgj* mutants showed alterations during female meiosis, female gametophytic development and early embryo development. These results indicate that QGJ is expressed during megasporogenesis and endosperm development, and suggest that its function is related with megaspore degeneration in the tetrad, gametophyte development and non-cell autonomous control of embryo development.

Keywords: Apomixis, Arabidopsis thaliana, Paspalum notatum, QGJ.

P18

Exploring PLT gene family for insights into parthenogenesis regulation in Eragrostis curvula

Suarez, U. (1); **Quevedo, M.R.** (1); Selva, J.P. (1, 2); Carballo, J. (2); Zappacosta, D. (2, 3); Echenique, V. (2, 3)
(1) Departamento de Biología Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Argentina.
(2) Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS–CCT– CONICET Bahía Blanca), Argentina.
(3) Departamento de Agronomía, Universidad Nacional del Sur (UNS), Argentina
martinqueve1996@gmail.com

Apomixis is an asexual reproduction regulated by three mechanisms that together generate viable seeds genetically identical to the mother. These mechanisms are, 1) apomeiosis (lack of female meiosis), 2) parthenogenesis (embryo development without egg cell fertilization) and 3) pseudogamy (single fertilization of the polar nuclei). Depending on the species, parthenogenesis was found regulated by different genes. In *Taraxacum officinale*, two unlinked dominant loci control diplospory (DIP) and fertilization independent development of an embryo from the egg cell (PAR). In *Pennisetum squamulatum* and *Cenchus ciliaris* BABY BOOM (BBM)-like genes were identified as candidates for parthenogenesis. Ectopic expression of these genes using egg cell specific promoter in rice showed autonomous embryo development. In normal development BBM genes were found expressed during fertilization triggered by the pollen. BBM genes belong to the plethora (PLT) family of transcription factors characterized by two conserved APETALA2 (AP2) binding domains and a bbm-1 domain with functional implications for somatic embryogenesis. *Eragrostis curvula* is a diplosporous apomictic grass used as model for the study of apomixis. Several genetic and epigenetic resources have been developed for this grass; however, the molecular regulation of parthenogenesis was not characterized yet. In order to test if parthenogenesis is also regulated by BBM in *E. curvula* a genome wide identification of PLT family was conducted. In this way the *E. curvula* genome annotation was used to identify genes with AP2 domains. Further phylogenetic analysis showed that the ten PLT genes present in rice are also present in *E. curvula*. PLT5 and PLT6, which are BBM3 and BBM1 respectively, were analyzed in detail to characterize their function in *E. curvula*. Specific PCR primers designed for these genes showed promising results since the expression patterns shows similarities with rice. Moreover, furthermore cloning and sequencing of PLT5 and PLT6 from genotypes contrasting in the reproductive mode showed structural differences both at the genomic and transcript levels. Finally, we are testing if the expression of these genes is triggered by the pollen and can led to the autonomous embryo development in *E. curvula*.

Keywords: parthenogenesis, BABY BOOM, Eragrostis curvula.

P19
Expression atlas of Eragrostis curvula reproductive tissues

Selva, J.P. (1, 2); Carballo, J. (1); Percival-Alwyn, L. (3); Šurbanovski, N. (3); Zappacosta, D.C. (1, 4); Cáccamo, M. (3); Echenique, V. (1, 4)
(1) Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS–CCT– CONICET Bahía Blanca), Argentina.
(2) Departamento de Biología Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Argentina.
(3) NIAB, Cambridge, UK.
(4) Departamento de Agronomía, Universidad Nacional del Sur (UNS), Argentina.
jpselva79@gmail.com

Eragrostis curvula, commonly known as weeping lovegrass, is a perennial grass native to Southern Africa naturalized in semi-arid regions of Argentina. The species has garnered attention not only for its adaptive qualities but also for its significance as a model organism for the study of apomixis (diplospory). Within the context of diplosporous apomixis research our group has developed several genetic and epigenetic approaches aimed at elucidating this particular reproductive mode. In the last few years, small RNAs and mRNAs were sequenced covering reproductive tissues of genotypes with contrasting reproductive modes. Due to the small size of weeping lovegrass flowers and the specific requirements for RNA extraction, in our previous works we used whole spikelet tissue instead of pistils for transcriptomic studies. However, the use of whole spikelet carries certain inherent complications, such as the presence of male sexual processes and other non-target tissues. In this work, a protocol for the identification of female tissue in different reproductive stages as well as the steps to obtain high-quality RNA from pistils samples alone was developed in order to perform comparative transcriptomic studies. Three developmental stages have been chosen from two contrasting genotypes, a fully apomictic (Tanganyika, USDA) and a fully sexual (OTA-S, USDA): Stage 1: Megaspore mother cell (MMC, pre-apomeiotic/pre-meiotic), Stage 2: post-apomeiotic/post-meiotic and Stage 3: mature embryo sac. RNA from three biological replicates (three different plants from each genotype) was extracted using NucleoSpin RNA XS kit and successfully sequence with the Illumina platform (Novogene). As expected, PCA analysis showed that replicates and samples cluster according to the stages. Differential expression analyses showed distinctive gene expression patterns between the fully apomictic and fully sexual *E. curvula* genotypes across the stages 1, 2 and 3. A total of 4986 genes were exclusively expressed in the apomictic genotype, while 5452 genes were exclusive to the sexual one. The strategy used here enables us to capture the intricate interplay of genes throughout the reproductive processes of *E. curvula*. As a result, it elucidates the complex molecular panorama that governs the species reproductive biology, providing a deeper understanding of its mechanisms. Even more, samples from a facultative cultivar will be included to disclose the quantitative regulation of the trait. Finally, the assay will be completed adding post anthesis stages in order to characterize both, meiotic and parthenogenic regulation and disclose the apomictic mechanism in *E. curvula*.

Keywords: female tissue, diplospory, Eragrostis curvula.

P20
Genetic systems in new polyploids generated by chromosomal duplication in Paspalum indecorum.

Novo, P.E. (1,2); Villalba, A.I. (1,2); Carrizo, J.M. (1); Espinoza, F. (1,2)
(1) Universidad Nacional del Nordeste, Facultad de Ciencias Agrarias.
(2) Instituto de Botánica del Nordeste (IBONE-CONICET)
patriciaenovo@gmail.com

Paspalum is one of the most important grass genus due to the high number of its species present in the natural vegetation of northeastern Argentina. Polyploidy is largely widespread in this genus. The most species are considered multiploid, with a sexual self-incompatible diploid cytotype (2n=2x=20) and conspecific apomictic tetraploid (2n=4x=40), pseudogamous, and self-compatible cytotype. The 4x cytotype that reproduce exclusively by sexuality have never been identified in natural populations of 4x apomictic species, despite the existence of 4x genotypes with different degrees of residual sexuality (facultative apomixis). However, doubling the chromosome number of the conspecific 2x cytotype (2xS) is the way to obtain 4x which reproduce by means of sexuality (4xS). New autotetraploids of experimental origin can be used as female parents in hybridization with natural apomictic conspecific 4x (4xA). The objective of this study was to obtain tetraploid sexual plants from 2x of the *P. indecorum* using colchicine. Two botanical collections were the original material for this study, belonging to the 2x cytotype of *P. indecorum*: accession AH1458 and Q4207. Both accessions are reproduced sexually, and are highly self-incompatible but fully cross compatible. A total of 23 seedlings were soaked in colchicine 0.2% and dimethyl sulfoxide 2% during 2 hours. Flow cytometry analysis was performed in 11 surviving plants. Two induced 4x plant was obtained, identified as ind-4x#14 and ind-4x#15. These results was confirmed by chromosome counts in root-tip cells. Embryological analysis of mature ovaries of non-induced plants conducted by interference contrast microscopy showed the typical structure of the meiotic embryo sac (MES). The ind-4x#14 plant showed 77.66% MES, 0% aposporous embryo sacs (AES), 0% mixed ovaries (MES +AES) and the remaining 22.34% were immature or aborted (ES ab/in). These results indicated that this induced plant reproduce by sexuality. In contrast, the other plant (ind-4x#15) showed 85.55% MES, 2.99% MES +AES and 7.46% ES ab/in, indicating that it is facultative apomictic. The meiotic chromosome associations of non-induced 2x plant was regular, presenting only bivalent (II). However, the induced plants presented irregular meiosis, observing univalent (I), bivalents (II), trivalent (III), and quadrivalent (IV). The induced 4x plants less than 1% of spikelets produce in self-pollination, maintaining the low compatibility system of the 2x. However, these plants produced seed after cross-pollinations. The 100% sexual 4x (ind-4x#14) constitutes a novel suitable female parent in crosses with plants of the 4xA cytotypes. These polyploids can be used in the future to perform genetic and epigenetic studies in relation to the reproductive mode in *P. indecorum*.

Keywords: duplication, reproduction, crosses.

P21
A 3D analysis of the reproductive development of Eragrostis curvula (Schrad.) Ness.

Pasten, M.C. (1,2); Carballo, J. (1); Díaz, A.R. (1,3); Mizzoti, C. (4); Cucinotta, M. (4); Colombo, L. (4); Echenique, V. (1,2); Mendes, M.A. (4)
(1) Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), Universidad Nacional del Sur - Consejo Nacional de Investigaciones Científicas y Técnicas (UNS-CONICET), Bahía Blanca, Argentina.
(2) Departamento de Agronomía, Universidad Nacional del Sur, Bahía Blanca, Argentina.
(3) Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca, Argentina.
(4) Dipartimento di Bioscienze, Università Degli Studi di Milano, Milano, Italia.
cielo.pasten@yahoo.com.ar

Apomixis, defined as asexual propagation by seeds, is a type of plant reproduction found in more than 400 angiosperms. This polyphyletic trait has been studied in various species, and it is considered of great importance in the agricultural industry as it would allow the fixation of desired traits and its propagation through generations. The study of ovule development in an apomictic model is necessary to strengthen the knowledge of the mechanisms governing this reproductive mode, and would contribute to improve plant breeding procedures. Weeping lovegrass (*Eragrostis curvula* (Schrad.) Ness) is a perennial grass that has been extensively studied as a model species for diplosporous apomixis. This species, mostly used as forage in semi-arid regions of the world, comprises a polymorphic complex that includes sexual and apomictic cytotypes, where all apomicts are polyploids, ranging from tetraploids (4X) to octoploids (8X). The aim of this work was to provide a thorough description of the developmental stages taking place in the ovule of three tetraploid genotypes of weeping lovegrass: the full apomictic Tanganyika, the facultative apomictic Don Walter, and the sexual OTA, as well as evaluating pollen development, using confocal laser microscopy. Moreover, in order to further understand the mode of reproduction in this species, an *in-situ* hybridization (ISH) was performed using an SPL gene (Squamosa Promoter-binding-Like), found to be differentially expressed between two of the contrasting genotypes (OTA-S and Tanganyika). The present analysis of the female gametophyte aided in increasing the knowledge of the reproductive development in *E. curvula* and allowed the identification of differences between sexual and apomictic development.

P22

Resolving the gene content of the genomic region associated with apomixis in Paspalum notatum using a diploid genome assembly

Vega, J.M. (1); Podio, M. (1); Orjuela, J. (2); Siena, L. (1); Mariac, C. (2); Pessino, S.C. (1); Leblanc, O. (2); **Ortiz, J.P.A.** (1)

(1) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET-UNR), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Rosario.

(2) DIADE, Univ. Montpellier, IRD, CIRAD, 34394, Montpellier, France.

ortiz@iicar-conicet.gob.ar

Several species of the genus *Paspalum* form multiploid complexes in which diploid plants are self-sterile sexual, and polyploids (mostly 4x) are self-fertile pseudogamous apomicts. Apomixis in the tetraploid race is controlled by a single genomic region which, when transmitted to the progeny, confers apospory, parthenogenesis and the ability to form unbalanced (4m:1p) endosperm. Recently, we have generated and annotated a chromosome-level genome assembly of #R1, a natural diploid biotype of *P. notatum* (bahiagrass). To identify the gene content of the genomic region associated with the apomictic phenotype, we used the sequences of 16 markers/genes genetically linked to apomixis in the tetraploid race to query the #R1 assembly. This assay allowed the identification of two regions clustering 4 sequences on chromosome 5 (10Mb) and 12 sequences on chromosome 8 (4Mb). The region of chromosome 5 contains 1,171 gene models, of which 850 have putative homologues in *A. thaliana*. The chromosome 8 region contains 491 gene models, of which of 255 have Arabidopsis homologs. GO Enrichment analyses for both regions revealed the presence of members of the Cellular Component (CC) category associated with the nucleus, plastids, microtubules, cytoskeleton and microbody. In addition, genes related to flowering, shoot development, floral development, rRNA processing, RNA biology and post-embryonic development were identified in the Biological Process (BP) class, and genes involved in methyltransferase activity, transmembrane transport and protein-serin-threonin kinase signalling were detected in the Molecular Function (MF) category. The results presented in this work suggest that, while a single genomic region is associated with apomixis in tetraploid Paspalum genotypes, it is split into two sectors in the diploid genome. Considering their functional annotation, some of the genes in these domains may be key players in the transition from sexual to apomictic reproduction.

Keywords: Paspalum notatum, apomixis, gene content.

IV International Congress on APOMIXIS

December 3 - 7, 2023 • Rosario, Argentina

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Projects funded by the European Union

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