INTERNATIONAL SOCIETY FOR SEED SCIENCE
Triennial Conference 2017
Monterey, California USA

FULL TALK & POSTER ABSTRACTS

SUNDAY, September 10, 2017

Welcome Reception
6:30 - 8:00 pm
Dolphins Ballroom and Upper Plaza

Alfred Mayer Plenary Lecture
8:00 - 9:00 pm • **Jill Farrant**
Cypress Ballroom
Sponsored by **MONSANTO**

SEEDS AS MODELS FOR NOVEL APPLICATIONS: PRODUCTION OF EXTREMEOPHYTE CROPS AND CONSERVATION OF THE UNSTORABLE

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Desiccation tolerant (orthodox) seeds, cereals in particular, are the most important contributors to global food security. However, rising temperatures and attendant increased frequency and duration of drought poses a threat to their agronomic production.

The vegetative tissues of cereals are intolerant of extreme water loss, and will die upon loss of between 30 -50% of cellular water. While efforts have been made to improve resistance to water loss, these mechanisms inevitably fail under severe drought conditions. Resurrection plants possess vegetative desiccation tolerance (DT), surviving drying to 5% of cellular water for extended periods without loss of viability. Several lines of evidence, strongly backed by the recent sequencing of the genome of *Xerophyta viscosa* (Poaceae), suggests that this is achieved, at least in part, by adaptation of genetic pathways evolved for DT in seeds. We hypothesise that understanding the mechanisms behind this genetic reprogramming in *X. viscosa* (*inter alia*) will enable the 'unlocking' of mechanisms associated with tolerance of extreme water loss in vegetative tissues of cereals and food crops relevant to food security.

Climate change and deforestation of tropical forests also pose a threat to many species growing in these areas that typically produce desiccation sensitive (DS, or recalcitrant) seeds. Such seeds cannot be stored conventionally, in part due to the fact that they are shed in a hydrated, metabolically active state and are programmed to initiate germination upon or shortly after abscission. We have recently sequenced the genome of an early diverging legume species, *Castanospermum australe*, bearing DS seeds. Using phylogenomic, comparative genomic, transcriptomic and physiological studies, and in comparison with similar data from the model legume *Medicargo truncatula*, our data suggest that seed desiccation sensitivity in this species is the result of a secondary loss of DT. Here we propose that understanding (epi)genetic control of the mechanisms preventing expression of genes and proteins essential for DT, could allow ultimate induction thereof during development of recalcitrant seeds, enabling their storage for purposes of genetic conservation of the species.

Welcome Social
9:00 - 10 pm
Dolphins Ballroom and Upper Plaza

MONDAY, September 11, 2017

SEED DEVELOPMENT SYSTEMS (SDVS)
Chairpersons: **Ramin Yadegari, Julia Buitink**
Cypress Ballroom

Keynote Speakers

8:00 - 8:45 am • **Gwyneth Ingram**
EMBRYONIC SURFACE FORMATION WITHIN THE DEVELOPING SEED: NEW STRUCTURES AND NEW MOLECULAR PLAYERS
Moussu, S.[1], Creff, A.[1], Doll, N.[1], Brocard, L.[2,3], Chamot, S.[1], Fourquin, C.[1], Widiez, T.[1], Nimchuck, Z.[4], Joubès, J.[3], Domergue, F.[3] and **Ingram, G.**[1]
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Arabidopsis seed development involves the concomitant development of two zygotic compartments, the embryo and the endosperm. Post-fertilization the endosperm expands as a coenocyte and then cellularises. Subsequently, the embryo grows invasively through the endosperm, which breaks down. How interactions between the growing embryo and the degenerating endosperm are regulated, and how the physical separation between these two compartments is achieved and maintained, remain poorly understood. Here we investigate signaling pathways involved in this separation process, and in the formation of the embryonic cuticle. We describe a novel structure, the embryo sheath, which forms on the surface of the embryo as it starts to elongate. The sheath is deposited outside the embryonic cuticle, and incorporates endosperm-derived material rich in extensin-like epitopes. Sheath production is dependent upon the activity of ZHOUPI, an endosperm-specific transcription factor necessary for endosperm degradation, embryo growth, embryo-endosperm separation and normal embryo cuticle formation. We show that a cysteine-rich peptide, KERBEROS, whose expression is ZOU dependent, is necessary both for the formation of a normal embryo sheath, and for embryo-endosperm separation. Finally, we investigate the interaction between KERBEROS function and the function of two Receptor-Like Kinases, GSO1 and GSO2, which are also necessary for the formation of a normal embryonic surface. Our results reveal a complex dialogue between the embryo and the endosperm during early seed development.

8:45 - 9:30 am • **Loic Lepiniec**
STRUCTURE AND FUNCTION OF THE CONSERVED *LAFL* GENE REGULATORY NETWORK THAT CONTROLS SEED DEVELOPMENT IN FLOWERING PLANTS
Lepiniec, L., Boulard, C., Barthole, G., Fatihi, A., Kelemen, Z., Marchive, C., Troncoso-Ponce, M.A., Thévenin, J., To, A., Miquel, M., Baud, S. and Dubreucq, B.
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The *LAFL* genes, *LEAFY COTYLEDON 1 (LEC1), LEC2, FUSCA3 (FUS3),* and *ABSCISIC INSENSITIVE 3 (ABI3)* encode a homolog of the *NF-YB* protein and three transcriptional regulators of the "B3-domain" family, respectively. Genetic analyses demonstrated that they have complex pleiotropic, partially synergistic and overlapping function during seed maturation. They are differentially expressed and display some specific activities. For instance, we have shown that LEC2 directly induces the genes coding for seed storage proteins (*e.g.* At2S3) and proteins of the oil bodies (*e.g.* OLEOSIN1), as well as regulatory genes such as *WRINKLED1* (*WRI1*) or two closely related *MYB* genes *(MYB115* and *118)*. WRI1 is a member of the AP2 family that controls glycolytic and fatty acid biosynthetic genes and oil accumulation. MYB115 and 118 repress the maturation program during early endosperm development and induces two desaturase genes (*AAD2* and *AAD3*) involved in omega7 monounsaturated fatty acids.

In order to unravel this complex network, we used the target *OLEOSIN1* (*OLE1*) promoter as model *in*

vitro, in moss protoplasts and *in planta*. We have confirmed that the three AFL-B3 proteins bind the core “RY” DNA elements (5’-CATG-3’), but with different flanking nucleotides. G-box *cis*-elements are also required for the proper activation of *OLE1* promoter *in vivo*, suggesting that other regulatory proteins (e.g. bZIP) are involved. Moreover, LEC2, ABI3 and LEC1 have synergistic effects on the activation of the *OLE1* promoter. Last, LEC1 and LEC2 proteins produced in Arabidopsis protoplasts can form a ternary complex with NF-YC2 *in vitro* in the presence of *OLE1* promoter. Taken together, these results allow drawing a molecular model for the transcriptional regulation of seed genes by the AFL proteins.

Interestingly, the ectopic expression of *LEC1* or *LEC2* in vegetative tissues is sufficient to induce somatic embryogenesis. This developmental activity provides a possible explanation to the establishment of various genetic and epigenetic mechanisms that tightly repress their expression in vegetative tissues. Last, we are investigating the LAFL regulatory network in various plant species demonstrating its conservation in flowering plants. We are exploiting this knowledge to develop biotech strategies aimed at controlling oil and/or protein accumulation in crop seeds.

9:30 - 9:45 am • **1-min Poster Previews**
Poster Program online at
http://isss2017.ucdavis.edu/wp-content/uploads/2017/08/Posters_Cumulative_Final.pdf

9:45 – 10:15 am • **Morning Break**
Monterey Bay Room

Mike Black Founders Lecture
10:15 – 11:00 am • **Oscar Lorenzo**
Cypress Ballroom
Sponsored by **RIJK ZWAAN**

MOLECULAR FRAMEWORK FOR NITRIC OXIDE (NO) SENSING IN SEEDS
Lorenzo, O.
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Seed dormancy and germination are complex traits regulated by the interaction of different signalling molecules such as the phytohormone abscisic acid (ABA) and the gasotransmitter nitric oxide (NO). In order to elucidate their crosstalk, a genetic screening in presence of (+)-S-ABA coupled to NO scavenger (cPTIO) let the identification of two *gap* (*germination in ABA and cPTIO*) mutants, that show ABA and cPTIO-insensitive phenotypes in the transition from dormancy to germination. After characterization and positional cloning of both of them, we found *GAP1* encodes ANAC089 transcription factor and *GAP2* the well-known ABI5 seed master regulator. *gap1* mutants lack the critical transmembrane domain of ANAC089 protein that confers the mutated proteins constitutive nuclear localization. Interestingly, mutants exhibited higher endogenous NO levels avoiding the effect of NO-depletion during seed germination. Furthermore, whole-genome transcriptional profiling indicated the existance of different groups of ABA- and redox-related genes differentially regulated by ANAC089. This transcription factor can specifically bind to the core *cis*-regulatory element GCGTCAGC harbour in the promoters of ANAC089 regulated genes. Consistently, translocation of ANAC089 protein to the nucleus was directed by changes in cell redox status after NO- and redox-related compound treatments. Thus, ANAC089 transcription factor integrates ABA signalling with NO levels to modulate redox homeostasis as a novel master regulator during seed germination in Arabidopsis. Using pharmacological and genetic approaches, we found that ABI5 protein levels are high in NO-deficient mutant backgrounds and low in NO-overaccumulating seeds. *S*-nitrosylation of ABI5 at a specific cysteine residue facilitates its degradation by CUL4-based and the RING-type KEEP ON GOING (KEG) E3 ligases and promotes seed germination. Thus, ABI5 is regulated through the antagonistic action of ABA and NO, as evidenced by the synergistic effect of GSNO on ABI5 destabilization, suggesting an inverse molecular link between NO and ABA hormonal signalling in gene regulation of early seedling development. Currently, the identification of redoxins able to denitrosylate ABI5 at this developmental checkpoint solves one of the biggest challenges in the understanding of the reversible NO role in plant signal transduction networks and establish a molecular framework for NO function during seed dormancy and germination.

SEED MICROBIAL SYSTEMS (SMS)
Chairpersons: **Greg Welbaum, Roberto Benech-Arnold**
Cypress Ballroom

Keynote Speakers

11:00 - 11:45 am • **Susan Meyer**
SEED BANK PATHOGEN COMMUNITY ECOLOGY
Meyer, S.E.[1], Allen, P.S.[2] and Beckstead, J.[3]
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Wildland seed pathogens are a remarkably understudied group of organisms, with most studies using a 'black box' approach. We have used seeds of the winter annual grass *Bromus tectorum* (cheatgrass) as a model system to understand the community of fungal seedbank pathogens associated with cheatgrass seeds and their interactions with host seeds, environmental conditions, and each other, focusing specifically on how they share this common resource through niche partitioning. In this presentation we examine niche differentiation in two ascomycete pathogens common on cheatgrass seeds, *Pyrenophora semeniperda* and *Fusarium* cf. *reticulatum*. SEM studies have shown that the infection hyphae of *P. semeniperda,* which most often attacks dormant seeds, penetrate directly through the caryopsis wall and first grow into the endosperm. *Fusarium* responds to chemical stimuli released early in germination to initiate hyphal growth toward the point of radicle emergence, where it forms an infection cushion and penetrates the embryo of the germinating seed. It thus restricts its attack to nondormant seeds. Because these fungi can actively function at reduced water potentials that prevent radicle emergence, they are at an advantage under conditions of fluctuating water availability, increasing their ability to kill nondormant seeds. We successfully modeled *P. semeniperda* spore germination and mycelial growth using hydrothermal time to determine base temperatures and water potentials for these developmental processes. We also explored whether the wide variation in mycelial growth rate we observed in *P. semeniperda* might be related to strain specialization onto different subsets of the host seed population, a form of intraspecific niche partitioning. We found that slow-growing strains were better able to kill nondormant seeds at high inoculum loads and related this to their increased production of a seed-crippling phytotoxin, cytochalasin B. Fast-growing strains were better able to kill dormant seeds at low inoculum loads. We concluded that mycelial growth rate polymorphism was maintained through temporally varying selection, with slow-growing strains best adapted to attack nondormant seeds in the fall and more common fast-growing strains most successful on dormant seeds in the carry-over seed bank. This somewhat counter-intuitive result highlights the importance of mechanistic studies in a model system for understanding seed-pathogen interactions.

11:45 am - 12:30 pm • **Ron Walcott**
BACTERIAL FRUIT BLOTCH: UNDERSTANDING AND MANAGING A GLOBAL THREAT TO CUCURBIT SEED PRODUCTION
Walcott, R.
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Bacterial fruit blotch (BFB), caused by the Gram-negative bacterium, *Acidovorax citrulli,* threatens the production of cucurbitaceous crop worldwide. The pathogen is seedborne and seed transmitted, and can survive in melon and watermelon seeds for > 30 years. Infested/infected cucurbit seeds facilitate long distance dissemination of the pathogen and are the most important source of primary inoculum for BFB outbreaks. Under warm and humid conditions, BFB can cause 100% crop yield losses; hence, there is zero tolerance for the bacterium in commercial cucurbit seedlots. Additionally, since *A. citrulli* is an A1 regulated pest for the European and Mediterranean Plant Protection Organization, BFB is a major constraint for vegetable seed producers. Unfortunately, there are currently no commercially available BFB resistant cucurbit cultivars, and the efficacy of foliar-applied antimicrobial compounds varies with environmental conditions. As a result, BFB management relies on excluding the pathogen from cucurbit production systems by seed health testing and seed treatments. Despite these efforts, BFB outbreaks continue to occur sporadically, particularly in regions with warm and humid growing seasons. To more effectively control BFB, we have studied the mechanisms of seed infection and the molecular basis of host-pathogen interactions. Interestingly, *A. citrulli* can penetrate female watermelon flowers and localize in the embryos of watermelon seeds without inducing fruit symptoms.

This localization might explain the pathogen's remarkable longevity in seeds. *A. citrulli* populations can be generally divided into two genetic groups, I and II, that differ in their cucurbit host preference. Group I strains are virulent on several cucurbit hosts, while group II strains are highly aggressive on watermelon, but mild on other cucurbit hosts. More recently, we observed that representative group II and I strains differ in their arsenal of putative type III secreted effector proteins, and that effectors are likely responsible for cucurbit host preference. Using a detached immature melon fruit assay, we showed that fruit tissues are more sensitive to differences in virulence between strains of the two *A. citrulli* groups. This fruit assay will allow further elucidation of the mechanisms of virulence and host preference that may lead to effective BFB host resistance.

12:30 – 1:30 pm • **Lunch**
Upper Plaza and Dolphins Ballroom

12:30 – 1:30 pm • **Seed Science Careers Panel**
Carmel Room
A panel of human relations experts or employees from academia, industry, governmental agencies and non-governmental agencies will provide a brief, informal presentation on how they began in their career and provide advice on the types of skills, knowledge, and background they look for when bringing in new employees. The panel is interactive with time built-in for questions and answers. Students will develop an understanding of how to prepare for a career in seed science and what they can expect from different types of careers via a question and answer session with panelists.
Seed Science Career Panel Members
• Corine de Groot, Research Team Lead, Bejo Zaden B.V.
• Heqiang (Alfred) Huo, Assistant Professor, University of Florida
• Manuela Nagel, Head of Cryo- and Stress Biology, IPK Gatersleben
• Steven Penfield, Professor, John Innes Centre
• Wim Soppe, Seed Technology Team Leader, Rijk Zwaan Breeding B.V.

SEED DEVELOPMENT SYSTEMS 1 (SDVS-1)
Seed Development, Seed Filling
Chairpersons: **Ramin Yadegari, Julia Buitink**
Cypress 1&2

1:30 - 1:45 pm • **Sonia Gazzarrini**
SNRK1 PHOSPHORYLATION OF FUSCA3 REGULATES EMBRYO GROWTH RATE, SEED YIELD AND PLANT GROWTH AT HIGH TEMPERATURE IN ARABIDOPSIS
Chan, A.[1,2], Carianopol, C.[1,2], Tsai, A.Y-L.[1,2,3], Varathanajah, K.[1], Chiu, R.S.[1,2] and **Gazzarrini S.**[1,2]
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Contact: Sonia Gazzarrini, gazzarrini@utsc.utoronto.ca
The transcription factor *FUSCA3* (*FUS3*) acts as a major regulator of seed maturation in Arabidopsis. FUS3 is phosphorylated by the SnRK1 catalytic subunit AKIN10/SnRK1α1, which belongs to a conserved eukaryotic kinase complex involved in energy homeostasis (Tsai and Gazzarrini, 2012; Plant J.). Here we show that AKIN10 and FUS3 share overlapping expression patterns during embryogenesis, and that FUS3 is phosphorylated by AKIN10 in embryo cell extracts. To understand the role of FUS3 phosphorylation, we generated *fus3-3* plants carrying FUS3 phosphorylation-null ($FUS3^{S>A}$) and -mimic ($FUS3^{S>D}$) variants. While $FUS3^{S>A}$ and $FUS3^{S>D}$ rescued all the *fus3-3* seed maturation defects, $FUS3^{S>A}$ showed reduced transcriptional activity and enhanced *fus3-3* previously uncharacterized phenotypes. $FUS3^{S>A}$ embryos displayed increased seed abortion due to maternal $FUS3^{S>A}$ and delayed embryo development, which resulted in a strong decrease of seed yield (~50%), suggesting phosphorylation of FUS3 plays an important role during reproductive development and early embryogenesis. Accordingly, the *akin10* and *akin11* mutants displayed a frequency of seed abortion similar to *fus3-3*. $FUS3^{S>D}$ delayed flowering similarly to ML1:FUS3 overexpression. However, no FUS3 expression was detected during vegetative development, suggesting FUS3 phosphorylation may regulate the embryonic expression of genes involved in flowering time. When plants were grown at elevated temperature, most $FUS3^{S>A}$ phenotypes were exaggerated and next generation seedlings overall grew poorly. Collectively, these results suggest that FUS3 phosphorylation by SnRK1 is required for embryo development and integration of environmental cues to ensure the survival of the next generation (Chan

et al., 2017; J. Exp. Bot., accepted). FUS3 and AKIN10 localizations in reproductive organs, as well as genetic interaction between *fus3-3, akin10/11* and *FUS3* phosphomutants will be discussed.

1:45 - 2:00 pm • **Hannetz Roschzttardtz**

PHYLOGENETIC STUDY OF IRON DISTRIBUTION IN PLANT SEEDS

Ibeas, MA.[1], Grant-Grant, S.[1], Vargas-Pérez, J.[1], Coronas, MF.[1], Navarro, N.[1], Vidal, EA.[3], Perez, M.F.[2] and **Roschzttardtz. H.**[1]

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Nutrient reserves in the seed must be sufficient to sustain plant establishment until the root system has developed enough to provide nutrients from the soils. High nutrient content of seeds is particularly important for plants growing in unfavorable nutritional conditions and has been related to higher seed viability and seedling vigor. Besides its impact on plant growth, nutrient levels in seeds are an important consideration for human and/or livestock seed-based nutrition.

Iron is an essential micronutrient for plant growth and development. Despite its importance, the prevalent low iron bioavailability in the soils of main agricultural areas of the world limits plant productivity, fertility, and germination rates. As a consequence, iron contents in seeds is diminished which results in negative impacts in human and animal health, since seeds are a main source of food for humans and animals. In humans, iron deficiency in women and children under two years is a serious and growing public health problem and a major concern for the World Health Organization. Therefore, understanding seed iron distribution and storage at the physiological and molecular level is key to design biotechnological applications to improve iron content of staple seeds.

Our present knowledge on iron distribution in seeds is mostly limited to studies in the model plant *Arabidopsis thaliana*. It has been shown that iron has a particular pattern of accumulation in the vacuoles of the endodermis cell layer during *Arabidopsis thaliana* seed maturation. However, little is known about seed iron distribution in other plants, in particular in plants of agronomic interest. As a first step to unravel the mechanisms underlying iron distribution and storage in seeds, we are using a histochemical method for iron detection in plant tissues (Perls/DAB stain) to characterize seed iron distribution in different plant species. Our results suggest plants use different strategies for iron storage in seeds, which might have an impact on total iron content and seed physiology. To our knowledge, our work represents the first phylogenetic study of seed iron distribution in plants.

2:00 - 2:15 pm • **Leonardo Jo**

TRANSCRIPTIONAL REGULATORY NETWORKS CONTROLLING SOYBEAN SEED MATURATION

Jo, L.[1], Pelletier, J.M.[1], Baden, R.[1], Cagliari, A.[1], Goldberg, R.B.[2] and Harada, J.J.[1]

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Soybean (*Glycine Max*) is the most produced and consumed oilseed in the world. The majority of storage compounds in the soybean seed accumulate in the maturation phase of seed development. Understanding the initiation and establishment of soybean seed maturation may allow us to develop strategies to improve soybean seed quality. We analyzed whole-genome transcriptome datasets to identify two groups of co-expressed genes with spatial and temporal expression patterns that correlate with the maturation program of the soybean seed. In addition, these two groups are highly enriched for genes involved in processes that occur during the maturation of the seed, such as lipid storage and accumulation of storage proteins. Several putative regulators of seed maturation were identified in the clusters, including LEAFY COTYLEDON1 (LEC1), ABSCISIC ACID INSENSITIVE3 (ABI3), BASIC LEUCINE ZIPPER 67 (bZIP67) and ABA-RESPONSIVE ELEMENT BINDING PROTEIN 3 (AREB3). The results suggest that this group of transcription factors play an important role in setting up the maturation program of the soybean seed. In order to identify target genes that are transcriptionally regulated by LEC1, ABI3, bZIP67 and AREB3, we performed chromatin immunoprecipitation and differential gene expression analysis during the maturation stage of soybean seed development. Detailed analysis of target genes showed a complex transcription factor regulatory network in which different combination of transcription factors are involved in distinct biological programs in soybean embryos, such as seed maturation, photosynthesis and embryo morphogenesis. Motif enrichment analysis suggest that a unique set of cis-regulatory elements determine each of these biological programs. A significant enrichment of G-box

(CACGTG) and RY (CATGCA) elements in the promoter region of genes that are part of the maturation program was observed. In addition, we observed a spatial organization of these two motifs in cis-regulatory modules near the transcriptional initiation site in the promoters of maturation genes. Moreover, functional analysis of cis-regulatory modules showed that the regions enriched in regulatory motifs are important to determine transcription factor occupancy and activation of maturation related genes. The results obtained in this study are helping us to elucidate the complex transcriptional network that controls soybean seed maturation.

2:15 - 2:30 pm • **Elwira Sliwinska**

ENDOREDUPLICATION – A MECHANISM TO REGULATE DNA SYNTHESIS DURING SEED DEVELOPMENT AND GERMINATION

Rewers, M. and **Sliwinska, E.**
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Endoreduplication is an alternative form of the cell cycle in somatic tissues, in which repeated rounds (endocycles) of nuclear DNA replication occur without subsequent mitosis. Increase in DNA content by endoreduplication leads to endopolyploidy of some cells (somatic polyploidy; DNA content >4C). It affects cell growth and differentiation as well as transcriptional and translational activity. Endopolyploid cells occur in seed tissues undergoing differentiation and expansion, and in specific cell types, such as those of the endosperm, pericarp, suspensor, and cotyledons, making them polysomatic (i.e. containing cells of different ploidy). Our long-term studies, which cover over 30 species, revealed that the intensity of endoreduplication in developing and germinating seeds is species- and organ-specific and usually correlates negatively with genome size. For example, during development of seeds of *Phaseolus vulgaris* in the axis the only endopolyploid cells are those with an 8C DNA content and their proportion only slightly changes with seed maturation, while in the cotyledons the intensity of endoreduplication greatly increases: from 30% at 20 DAF (8C-32C) to 40% at 60 DAF (8C-128C). In contrast, in developing seeds of *Medicago sativa* in both the embryo axis and cotyledons, endopolyploidy up to 8C occurs and its intensity changes little. Generally, higher endopolyploidy occurs in haustorial and non-persistent cotyledons than in persistent ones. During germination it either does not change or it decreases in the cotyledons, while it increases in the axis. This increase occurs in different regions of the axis, depending on the type of seedling establishment; it is the highest in the transition zone of epigeal species and in the hypocotyl in hypogeal species. In conclusion, in polysomatic species (the majority of angiosperms) endoreduplication seems to be a common strategy during seed development to establish large storage cells in cotyledons, and during Phase II of germination, when the mitosis still does not occur, to drive axis elongation. However, there are also non-polysomatic species, e.g. *Helianthus annuus*, in which endoreduplication does not occur in any organ or developmental stage. Thus cell division may be also a mechanism of development of cotyledon storage tissues and be involved in the completion of germination.

2:30 - 2:45 pm • **Inmaculada Sanchez-Vicente**

NITRIC OXIDE REGULATION OF SEED LIPID ACCUMULATION INVOLVES bZIP REVERSIBLE *S*-NITROSYLATION

Sánchez-Vicente, I.[1], Albertos, P.[2], Mateos, I.[1], Sanz, C.[3] and Lorenzo, O.[1]
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Nitric oxide (NO) is a signalling molecule involved in a plethora of physiological events along the whole plant life, related not only to growth and developmental processes, but also to biotic and abiotic stresses. Our work is focused on early plant development, including seed maturation, dormancy and germination where some members of the basic region/leucine zipper motif (bZIP) transcription factor family are key regulators. We get new insights in the study of these early plant development stages through the NO molecular mechanisms involved in bZIP regulation. NO exerts its function mainly through *S*-nitrosylation, defined as the specific posttranslational modification by which NO is attached covalently to a cysteine residue, modifying the protein properties. We analyzed the repercussion of this important modification in specific bZIP targets involved in seed development and germination.

Our results show a fundamental NO involvement in the regulation of the proper seed maturation, taking part in seed fatty acid accumulation and promotion of seed germination. We find that accumulation of major fatty acid storage compounds is impaired in different NO mutant backgrounds and also in gain- and loss-of-function lines of the bZIPs analyzed.
Additionally, NO function in embryo fatty acid storage regulation involves the specific and reversible *S*-nitrosylation of a basic/leucine zipper transcription factor bZIP, which in turns can activate the expression of the FAD3 desaturase. This bZIP is the closest homolog of ABI5, previously characterize as a key seed germination repressor, whose protein stability is also modified by *S*-nitrosylation. A search of *in planta* protein interactors of these bZIPs led the identification of specific redoxins among other partners. Likewise, this posttranslational modification can also be reversed by the denitrosylation activity of these redoxins, suggesting a novel and reversible mechanism of bZIP regulation. These results led us to understand the NO control of seed fatty acid profile during maturation and germination.

2:45 - 3:00 pm • **Alexandre Marques**
MODELLING SEED DESICCATION SENSITIVITY USING THE ARABIDOPSIS *abi3-6* SEED DEVELOPMENTAL MUTANT
Marques, A.[1], Nogueira, R.[1], Nijveen, H.[2], Kuil, A.[1], Somi, C.[1], Ligterink, W.[1] and Hilhorst, H.[1]
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Seeds are important for plants as means of regeneration. Desiccation tolerance (DT) is an important mechanism that allows them to withstand long periods of dry storage in a quiescent state. In nature, DT is one of the main mechanisms to avoid extinction of plant species and allows their conservation *ex situ*. Desiccation sensitive-seeded species face imminent risk of extinction due to deforestation and climate change whereas *ex situ* conservation is not possible with the currently available methods.
The mechanisms causing seed desiccation sensitivity (DS) are poorly understood. The genetic studies of natural DS species is limited and, thus, we aimed to investigate seed DS through the Arabidopsis mutant *abi3-6*. Seeds of this mutant resemble the phenotype of those from natural DS species. It presents an attractive model as the seeds display DS when shed but are DT at the mid-development. We observed large phenotypical differences between the seeds dried inside or outside the siliques for both mutant and wild type. Desiccation of seeds inside the siliques appeared to be slower, allowing wild type seeds to survive desiccation as early as 11 days after flowering (daf). The *abi3-6* seeds achieved maximum survival when dried inside the siliques at 16 daf after which DT decreased continuously until becoming completely DS at the mature stage.
RNA-seq was performed on both *abi3-6* and wild type seeds at 10, 11, 16 and 18 daf, aiming to study the early and late acquisition of DT in both genotypes. The observed transcriptomic changes may contribute to the understanding of acquisition and loss of DT in seeds. Understanding the mechanisms underlying seed DS may provide a basis to develop methods for conservation of DS-seeded species.

SEED ECOLOGICAL SYSTEMS 1 (SES-1)
Chairpersons: **Bob Geneve, Norman Pammenter, Waheed Arshad**
Cypress 3&4

1:30 - 1:45 pm • **Annisa Satyanti**
ENVIRONMENTAL DRIVERS AND ECOLOGICAL CONSEQUENCES OF WITHIN-SPECIES VARIATION IN GERMINATION STRATEGY
Satyanti, A.[1,2,3], Guja, L.K.[3,4] and Nicotra, A.B.[1]
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Plant establishment and subsequent persistence are strongly influenced by germination strategy, especially in temporally and spatially heterogeneous environments. Germination strategy determines

the plant's ability to synchronise germination timing and seedling emergence to a favourable growing season and thus may be key to persistence under more extreme and variable future climates. However, the determinants of variation in germination strategy are not well resolved. Using a widespread alpine herb *Oreomyrrhis eriopoda* that varies in germination strategy across its range, we examined which environmental factors drive variation in germination strategy and what the consequences of possessing a certain seed germination strategy are at seedling establishment. We found that the germination patterns of *O. eriopoda* populations can be broadly classified into four strategies: immediate, staggered, postponed, postponed-deep. Temperature variability (seasonality) at the site of seed collection was the most important determinant of germination strategy, but those patterns depended on the time scale of climatic assessment. Long-term climate and the conditions during seed development were significant predictors of germination strategy whereas the conditions immediately before seed development were less important. Autumn seedlings from populations with an immediate germination strategy and early-germinating seeds of the staggered strategy exhibited a higher leaf production rate than spring seedlings. This study highlights that seed development conditions and long-term temperature seasonality strongly affect germination strategy and suggests potential for both plastic variation and local adaptation in germination among populations of alpine plants. Selection for germination strategy is also correlated with shifts in early seedling vegetative traits and establishment characteristics. The immediate germination strategy is associated with higher temperature seasonality and higher seedling growth. If such within-species variation in germination strategy is common, either as a result of local adaptation or plasticity, it might help to buffer impacts of climate change. Thus, within-species variation in seed germination and seedling traits, and the drivers thereof, warrant consideration when climate change impacts are assessed across communities.

1:45 - 2:00 pm • **Robert Geneve**

WATER GAP COMPLEXES IN PHYSICALLY DORMANT SEEDS

Geneve, R.L.[1], Baskin, C.C.[2, 3], Baskin, J.M.[3], Jayasuriya, K.M.G.G.[4] and Gama-Arachchige, N.S.[4]
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Physical dormancy occurs in at least 16 angiosperm plant families and is caused by water-impermeable palisade cells in seed coats or fruit coverings. The breaking of physical dormancy involves disruption or dislodgement of water-gap structures causing the seeds/fruits to become water permeable. The water-gap region is a morphologically distinct area of the seed or fruit coat forming a water gap complex. The location, anatomy, morphology and origin of water gaps can differ between and even within families. Water-gap structures sense environmental conditions to allow seeds with physical dormancy to become permeable just prior to the commencement of conditions favorable for germination and plant establishment. There are three basic water gap morphologies characterized by the way the water gap opens. These can be organized as Type I, II, and III water gaps. In Type I water gaps, specific cells pull apart to form a surface opening, while in Type-II water gaps a portion of the surface structure is pulled back from adjacent cells to open the water gap. Type-III water gaps are the least common type and have a circular, plug-like structure that is dislodged to permit water entry. In addition, seeds may have water gap complexes that are either simple or compound depending the whether there is only a single primary water gap structure involved in dormancy release or whether there is an additional secondary water gap structure that opens permitting water entry.

2:00 - 2:15 pm • **Dongfang (Emily) Zhou**

THE PRODUCTION AND FUNCTION OF MUCILAGE BY SWEET BASIL (*OCIMUM BASILICUM* L.) SEEDS

Zhou, D.[1], Barney, J.[2] and Welbaum, G.E.[1]
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Sweet basil (*Ocimum basilicum* L.) seeds produce a thick layer of mucilage around the testa within minutes after hydration. Mucilage is most prevalent among plant species adapted to surviving in arid sandy soils, though its significance in determining the ecological fitness is unclear. The mucilage produced by these seeds is reported to be composed of cell-wall polysaccharides that are deposited in testa cells during development. In this study, sweet basil seeds were examined using light and environmental scanning electron microscopy. The mucilage of basil seeds is held together by columnar structures that unfolded from the pericarp and helped hold and stabilize the mucilage to the

seed surface. The mucilage was removed using diluted hydrochloric acid to compare performance of seeds with and without mucilage. Mucilage removal inhibited laboratory seed germination under ideal conditions and significantly reduced the seed water content four fold. The mucilage anchored seeds and increased their resistance to movement in the environment. Osmometry showed the water potential of fully hydrated seeds to be near zero suggesting that the mucilage provides a pool of loosely bound water to germinating seeds and seedlings in arid environments. Testing in soil with various levels of hydration confirmed intact basil seeds with mucilage germinated to higher percentages and survived longer than seed with mucilage removed.

2:15 - 2:30 pm • **Bin Wen**

HIGH-TEMPERAURE ADAPTATION IN TROPICAL PLANT SEEDS

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The tropics are characterized by high temperatures, which may be an important stressor to tropical plant seeds. Air temperatures have important effects on developing seeds; while after shedding, seeds are mostly affected by soil surface temperature. Taking Xishuangbanna in southwest China, an edge area of tropical Asia, as example, there soil surface maximum temperature often excess 60°C on open ground in sunny days, with a extreme value of 71.4°C documented by weather observation station there. However, active cellular membrane is usually destroyed by high temperature around 60°C, how seeds survive so high temperature in tropics?

Temperature changes greatly depending habitats, meanwhile high temperature tolerance in seeds varies from species to species. It was found that seeds native to tropical rainforest are usually high-temperature sensitivity, including recalcitrant seeds, which can not be dried so neither can tolerate high temperature above 60°C, such as *Hopea hainanensis* and *Baccaurea ramiflora*, and seeds of some rare and endangered species, which also are high-temperature intolerant even if desiccation tolerant, such as *Pellacalyx yunnanensis* and *Tacca chantrieri*. Fortunately, no soil surface maximum temperature more than 30°C has been detected in intact tropical rainforest there. This category of seeds adapts the avoidance strategy to high temperature, obviously. Another category of seeds, including those produced by weeds, invasive plants and pioneer species, adapts the tolerance strategy to high temperature. They exhibit much stronger tolerance to high temperature in air-dried state, such as *Amaranthus spinosus*, *Piper aduncum*, and *Tithonia diversifolia*.

High-temperature tolerance in seeds may contribute to plant distribution in the tropics. Nowadays, globular warming, deforestation, rainforest fragmentation and other human activities are enlarging hot habitats and making hot habitats hotter. This decreased habitats suitable for climax species in tropical rainforests, made them rare and endangered; on the other hand, increased habitats suitable for non-climax species, paved way for plant invasion.

2:30 - 2:45 pm • **Michelle D'Aguillo**

SEED DORMANCY ENABLES A FORM OF TEMPORAL HABITAT SELECTION

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Plants can engage in habitat selection through phenology, by undergoing development transitions in response to specific environmental cues that vary on an annual basis. By restricting germination, seed dormancy has the potential to enable a form of temporal habitat selection through germination phenology. Using dormant and non-dormant genotypes of *Arabidopsis thaliana*, we examined if seed dormancy alters or restricts the conditions to which seedlings and reproductive individuals are exposed. Seeds were matured under warm (25°C) and cool (14°C) environmental conditions and dispersed in a multi-year field experiment in central North Carolina. Soil temperature and moisture were recorded hourly, and germination and reproductive phenology were recorded weekly. We examined soil temperature and moisture experienced by seeds during the seven days preceding germination, the first two weeks of seedling establishment, the second two weeks of seedling establishment, and the reproductive period. Preliminary results indicate that dormant and non-dormant genotypes occupy different areas of environmental space early in the life cycle, and these differences tend to dissipate later in the life cycle. In general, dormant genotypes germinate in response to, and expose seedlings to, significantly higher soil moisture and temperature, and these differences are most pronounced when seeds were matured at cool rather than warm temperatures. These results indicate

that seed dormancy can enable a form of temporal habitat selection and alter the environmental conditions experienced post-germination.

2:45 - 3:00 pm • **Andrea Loayza**
INTERACTIVE EFFECTS OF SEED SIZE AND ENVIRONMENTAL CONDITIONS ON THE OUTCOME OF THE INTERACTION BETWEEN RODENTS AND A THREATENED ATACAMA DESSERT SHRUB)
Loayza, A.P.[1], Luna, C.A.[1] and Calviño-Cancela, M.[2]
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Spatial and temporal variations in the biotic and abiotic conditions of an environment can lead to context dependence in the strength and outcome of species interactions. *Myrcianthes coquimbensis* is a narrow endemic shrub of the Atacama Desert whose seeds are solely consumed by three species of scatter-hoarding rodents. These rodents act both as seed predators and dispersers, depending partly on seed size. Along its distribution range, strong interannual variability in rainfall leads to changes in *M. coquimbensis* crop sizes; wet years are characterized by fruit abundance, whereas in dry years fruits are scarce. In this study we parameterized an individual-based model -using three years of field data, as well as cafeteria experiments- to determine how context-dependence in terms of fruit availability and seed size interact to alter the sign and strength of the interaction between rodents and *M. coquimbensis*. We found that the quality of seed dispersal services provided by the three rodent species were strongly context-dependent, but were also determined by seed size. During both wet and dry years, large seeds manipulated by rodents had lower recruitment probabilities than those that were ignored and left under the parent plant. In contrast, during wet and dry years, small seeds manipulated by all rodent species had higher recruitment probabilities than small seeds that were ignored under the parent plant. Differences in recruitment probabilities were partly explained by the habitats where seeds arrived. In wet years, seeds had a higher recruitment probability under conspecific plants than in rock cavities, whereas the opposite was true in dry years. Finally, we also found an interaction between habitat and seed size on the probability of recruitment; in wet years the probability of recruitment in rock cavities decreased with smaller seeds, in contrast, in dry years this probability increased with larger seeds. In summary, our results provide new evidence that the strength and sign of the interaction between a plant and its frugivores can vary with time, with the identities of the interacting species, and with the interaction of time and seed size.

3:00 – 3:30 pm • **Afternoon Break**
Monterey Bay Room

SEED GERMINATION SYSTEMS 1 (SGS-1)
Chairpersons: **Camille Steber, Steven Penfield, Dominique Ardura**
Cypress 1&2

3:30 - 3:45 pm • **Wenjing Ge**
EXAMINING THE FUNCTION OF GID1-REGULATED GENES
Ge, W.J.[1] and Steber, C.M.[2]
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Plant species survival and agriculture both depend on seed germination occurring in a season and environment conducive to seedling growth and development. Dormant seeds are unable to germinate when first released from the mother, thereby preventing germination out of season (fall vs spring). Dormancy can be lost through a period of dry storage called after-ripening (AR), or exposure to the moist wintry condition of cold imbibitions. The plant hormone gibberellin (GA) stimulates seed germination. The proposed research examines the hypothesis that gibberellin A (GA) hormone receptors regulate dormancy loss via regulation of gene transcription and translation. GA signaling leads to inactivation or destruction of DELLA (Asp-Glu-Leu-Leu-Ala) family repressors of GA responses. In Arabidopsis, GA is perceived by the three homologous receptors, GID1a, GID1b, and GID1c (GA-INSENSITIVE DWARF1, 68-85% amino acid identity) comprised of a GA-binding core connected to a lid domain by an a-loop hinge.

In sly1 (sleepy1) mutants, GA and GID1 can also inactivate DELLA repressors via GID1-GA-DELLA complex formation without DELLA proteolysis. This is seen when GA-insensitive sly1-2 seed dormancy is rescued without DELLA destruction by long after-ripening (2 years AR, 85% germination) and GID1b overexpression (GID1b-OE, 75%). If AR and GID1b-OE reduce sly1-2 seed dormancy via similar mechanisms, then they should result in similar transcriptome changes.
Nelson and Steber identified transcripts showing altered accumulation when germination of the GA-insensitive sly1-2 mutant was rescued by after-ripening or by GID1b-OE. And stimulation of sly1-2 germination by AR and GID1b-OE are associated with partly overlapping transcriptional changes. This suggests that these 26 genes showing differential regulation by GID1b-OE are important regulators of dormancy. Thus, we will determine whether genes regulated in response to GID1b-OE play an important role in seed dormancy.

So far, we have been working on determining if GID1b-OE-regulated transcripts are differentially expressed in gid1 loss of function mutants. The effects of GID1 loss and gain-of-function on the expression of GID1b-OE-regulated genes will be examined in dormant (0 wk AR) and after-ripened (1 mo AR) seeds by RT-qPCR analysis performed at the different imbibition time points used for the microarray analysis.

3:45 - 4:00 pm • **George Bassel**

TEMPERATURE VARIABILITY IS INTEGRATED BY A SPATIALLY-EMBEDDED DECISION-MAKING CENTER TO BREAK DORMANCY IN ARABIDOPSIS SEEDS

Topham, A.T.[1], Taylor, R.E.[1], Yan, D.[2], Nambara, E.[2], Johnston, I.G.[1] and **Bassel, G.W.**[1]
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Plants perceive and integrate information from the environment to time critical transitions in their life cycle. Some mechanisms underlying this quantitative signal processing have been described, while others await discovery. Seeds have evolved a mechanism to integrate environmental information by regulating the abundance of the antagonistically acting hormones ABA and GA. Here we show that hormone metabolic interactions and their feedbacks are sufficient to create a bistable developmental fate switch in *Arabidopsis* seeds. A digital single cell atlas mapping the distribution of hormone metabolic and response components revealed these to be enriched within the embryonic radicle, identifying the presence of a decision-making center within dormant seeds. The responses to both GA and ABA were found to occur within distinct cell types, suggesting crosstalk occurs at the level of hormone transport between these signaling centers. We describe theoretically, and demonstrate experimentally, that this spatial separation within the decision-making center acts to preferentially process variable temperature inputs from the environment to promote the breaking of dormancy. In contrast to other noise-filtering systems including human neurons, the functional role of this spatial embedding is to leverage variability in temperature to transduce a fate-switching signal within this biological system. Fluctuating inputs therefore act as an instructive signal for seeds enhancing the accuracy with which plants are established in ecosystems, and distributed computation within the radicle underlies this signal integration mechanism.

4:00 - 4:15 pm • **Jack Mitchell**

MONITORING THE PRE-GERMINATION GENE EXPRESSION OF INDIVIDUAL *ARABIDOPSIS THALIANA* SEEDS USING A REAL-TIME LUCIFERASE REPORTER SYSTEM TO ADDRESS SEED VARIABILITY

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Variability is present in biology across many scales, from populations, individuals and cells down to the molecular events within cells. Variability within seeds impacts seed behaviour and adaptations to the environment, consequently this can pose many problems to agriculture where crop uniformity is desired. We propose that understanding this variability is critical in enhancing strategies to increase crop uniformity and maximize agricultural output across varying climatic conditions. Individual seeds vary in absolute hormone concentrations as well as hormone sensitivity thresholds, both of which play a role in the developmental mechanisms during seed development. The antagonistic relationship between the hormones absissic acid (ABA) and gibberellin (GA) is known to control the physiological state of developing seeds, ABA induces dormancy and prevents germination, whereas GA stimulates germination and cell expansion. Although this relationship is widely accepted the exact mechanisms and roles that ABA and GA play during seed development are not understood in depth. A major

obstacle to monitoring gene expression patterns during seed development is the destructive nature of sampling and measurement methods which involve the need to kill samples. As a result tracking the dynamics of expression over developmental time is currently not possible. We have developed a method to monitor gene expression in Arabidopsis thaliana seeds in real-time using a luciferase based reporter system. ABA and GA-responsive genes were monitored in real-time by inserting promoters upstream of a luciferase reporter and transformed into the Arabidopsis thaliana transparent testa mutant (tt4-1). When these seeds are exposed to luciferin the gene expression patterns of the selected genes can be monitored non-invasively from initial imbibition to germination providing an insight into the changes taking place during seed development. In the future dual-luciferase reporters are to be developed to enable the monitoring of multiple expression patterns from different combinations of genes to further develop current understanding of seed behavioural changes pre-germination.

4:15 - 4:30 pm • **Mohan Niroula**

CHARACTERIZATION OF A QTL AND CANDIDATE GENE (*LSGA2OX2*) ASSOCIATED WITH DARK INHIBITION OF LETTUCE SEED GERMINATION

Niroula, M.[1], Huo, H.[1,2], Still, D.[4], You, Y.S.[4], Wei, S.[5], Truco, M.[3], Michelmore, R.W.[1,3] and Bradford, K.J.[1]
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Promotion of germination in lettuce and Arabidopsis seeds by light is associated with activation of gibberellin (GA) biosynthesis. We used a Recombinant Inbred Line (RIL) population derived from a lettuce cultivar (*Lactuca sativa* cv. Salinas) that does not require light to germinate and a light-requiring accession of *L. serriola* (US96UC23) to map a quantitative trait locus (QTL) for the inhibition of lettuce seed germination by darkness. The same QTL was mapped to chromosome 7 in two independent mapping experiments using F6 and F9 generations of RIL seeds harvested from different locations (California, USA and Chile, respectively). A GA catabolic gene (*LsGA2ox2*) located within this QTL from both Salinas (Sal) and US96UC23 (UC) contained identical deduced protein sequences, but twelve single-nucleotide polymorphisms (SNPs) were identified in 1.4 kb of *LsGA2ox2* promoter nucleotide sequences from the two lettuce genotypes, and 9 of these SNPs were clustered in a 93 bp region. Expression analyses revealed that *LsGA2ox2* transcripts remained at a high level in non-germinated UC96US23 seeds imbibed for 24 h in the dark, while its expression substantially decreased in Salinas seeds imbibed in the dark and in imbibed seeds of both genotypes in the light. Complementation of an Arabidopsis *atga2ox2* mutant line with *ProUCGA2ox2::UCGA2ox2* and *ProUCGA2ox2::SalGA2ox2* or expression of *ProUCGA2ox2::UCGA2ox2* in Salinas lines resulted in strong inhibition of seed germination in the dark. By contrast, RNAi silencing of *LsGA2ox2* in US96UC23 lines resulted in enhanced seed germination in the dark. Site-directed mutagenesis of three SNPs in the 93 bp window of the UC promoter resulted in failure to complement *atga2ox2* mutants, suggesting that these SNPs are critical for upregulation of *LsGA2ox2* in seeds imbibed in the dark. Furthermore, sequence analysis of *LsGA2ox2* and its promoter region from cv. Grand Rapids (*L. sativa*, light-requiring) and W48 (*L. serriola*, not light-requiring) lines showed that all 9 SNPs in the 93 bp promoter region are conserved among either light-sensitive or light-insensitive genotypes, regardless of species. The combination of genetic mapping, mutant complementation, and conserved SNPs implicates degradation of GA by LsGA2ox2 in the inhibition of germination by darkness.

4:30 - 4:45 pm • **Ruth Finkelstein**

ROLES OF ABI5/ABF BINDING PROTEINS (AFPS) IN MODULATING THE ABSCISIC ACID CORE SIGNALING PATHWAY

Lynch, T.J., Erickson, B.J., Pham, V., Losic, T., Morales, A. and **Finkelstein R.R.**
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Several Abscisic Acid-Insensitive(ABI)5/ABRE Binding Factor(ABF) binding proteins (AFPs) inhibit ABA response, resulting in extreme ABA resistance and failure to complete maturation in seeds of transgenic Arabidopsis overexpression lines. Annotated only as proteins with domains of unknown functions, numerous roles have been proposed for these proteins. These include inhibiting function of

the transcription factors that they bind by promoting their proteasomal degradation and by altering expression of ABA-regulated genes through interactions with chromatin modifiers such as histone deacetylases and the TOPLESS family of co-repressors. We have identified additional interactions with many components of the ABA core signaling pathway and are testing the functional significance of all of these interactions genetically and biochemically. The AFPs are predicted to be intrinsically unstructured proteins and we propose that they act as scaffolds for multiple protein complexes affecting ABA and stress response.

4:45 - 5:00 pm • **Ross Johnson**
SOG1 LINKS THE DNA DAMAGE RESPONSE TO ORGAN REGENERATION
Johnson, R.J.[1], Conklin, P.A.[1], Tjahjadi, M.[1], Shi, F.[1], Missirian, V.[1], Toal, T.[1], Brady, S.M.[1] and Britt, A.B.[1]
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In Arabidopsis, DNA damage-induced programmed cell death is limited to the meristematic stem cell niche. The significance of this cell-type specific programmed cell death is unclear. Here we demonstrate in roots that it is the programmed destruction of the mitotically-compromised stem cell niche that triggers its regeneration, enabling growth recovery. In contrast to wild-type plants, *sog1* plants, which are defective in damage-induced programmed cell death, maintain their cell identities and stereotypical structure of the stem cell niche, but these cells fail to undergo cell division, terminating root growth. We propose DNA damage-induced programmed cell death is employed by plants as a developmental response, contrasting with its role as an anti-carcinogenic response in animals. This role in plants may have evolved to restore embryonic root growth after the accumulation of DNA damage in seeds.

SEED CONSERVATION SYSTEMS 1 (SCS-1)
Maturation/Recalcitrance/Longevity
Chairpersons: **Christina Walters, Andreas Borner**
Cypress 3&4

3:30 - 3:45 pm • **Olivier Leprince**
REVISITING THE IMPLICATION OF RAFFINOSE FAMILY OLIGOSACCHARIDE SUGARS IN SEED LONGEVITY POINTS TO A ROLE DURING MATURATION AND NOT STORAGE
Leprince, O.[1], Vandecasteele, C.[2], Ly Vu, B.[1], Hundertmark, M.[1], Righetti, K.[1], Gallardo, K.[3], Aime, D.[3], Prosperi, J.-M.[4] and Buitink, J.[1]
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Legume seeds are particularly well-endowed with raffinose family oligosaccharide (RFO) sugars, which are anti-nutritional compounds for mono-gastric animals. RFO sugars accumulate during the final stages of seed maturation and are metabolized during seed germination. While genetic evidence suggests that RFOs are involved in seed vigor and seedling establishment, their involvement in seed longevity remains elusive. Taking advantage of the wide genetic diversity of *Medicago truncatula* and its long post-filling phase during which seed longevity is acquired, we examined the relationship between seed longevity and RFO contents. Seed lots of 174 recombinant inbred lines grown in two consecutive years were stored at 60% and 75% RH at 35°C. Viability after storage was not correlated with the contents of total RFO, sucrose/RFO or stachyose, which is the predominant sugar in *M. truncatula*. For 262 accessions from the core collection of this species survival curves were constructed to determine the P50 values (time of storage to reach 50% survival, determined at 60% RH, 35°C). No correlation was found between P50 and contents of galactinol, sucrose, RFO and sucrose/RFO ratio. In a parallel study, we investigated the link between sugar and protein composition during seed maturation and the acquisition of longevity. Using developmental time series from seeds produced under five environmental conditions (drought stress, heat and cold stress, greenhouse, and optimal conditions), we established that the increase in P50 was highly positively correlated with stachyose (r=0.92), verbascose (r=0.807), total RFO (r=0.885) and Suc/RFO (r=0.77). P50 was also highly correlated with legumin b content (r=0.827). These results indicate that RFO can be used as an

indicator of seed maturation and acquisition of longevity. However, they are not the main factor determining the genetic variability of longevity in *M. truncatula*.

3:45 - 4:00 pm • **Jun Liu**

SEED AGING-RELATED LONG NON-CODING RNA (LNCRNA) IN RICE (*ORYZA SATIVA* L.)

Fan, F.[1,2], Zhang, Q.J.[1], Liu, Q.J.[1], Gao, J.D.[1], Fu, H.[3], Cheng G.H.[2] and **Liu J.**[1]
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Long non-coding RNA（lncRNA）modulate gene expression patterns at all levels from epigenetic, transcription to post-translation, and play important roles in a wide range of biological processes. However, how lncRNA regulate seed aging remains unknown. In this study, we performed a whole transcriptome strand specific RNA sequencing (ssRNA-seq) for rice seed and analyzed the differences of lncRNAs expression in rice seed embryo during accelerated aging. Most lncRNAs are down-regulated in aged seeds. According to the KEGG results of lncRNA's co-localization and co-expressed genes, the functional pathways of up-regulated lncRNAs in aged seeds are different froma those down-regulated lncRNAs. The major pathways of up-regulated lncRNA's co-expression and co-localization genes are related to base excision repair, while the down-regulated genes related to plant pathogen interaction, plant hormone signal transduction, energy metabolism, *etc*.

The cDNA sequence of lncRNA *XLOC-037529* was obtained by RACE cloning. *XLOC_037529* showed significant differences before and after aging, but may not function synergistically with miRNAs. Compared to *indica* genome, *XLOC-037529* showed that: (1) it has a total length of 1325 bp with different alternative splicings, (2) the 5' end was conserved, (3) the 3' end was not conserved and a polyA tail in the 3' end.

4:00 - 4:15 pm • **Lynnette M.A. Dirk**

AFFINITY-SELECTION PHAGE DISPLAY AND PAIRED-END PHAGE SEQUENCING IDENTIFY A SMALL REPERTOIRE OF CLIENT PROTEINS COMMON TO ORTHOLOGOUS ARABIDOPSIS AND SOYBEAN DEHYDRIN PROTEINS

Unêda-Trevisoli, S.H.[1], **Dirk, L.M.A.**[2], Hao, G.[3], Barrios, L.L.[3], Chakrabarti, M.[3], Hunt, A.G.[3] and Downie A.B.[2]
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The seed's tolerance to desiccation is the result of synergistic interaction of several mechanisms, many of which involve the hydrophilic Late Embryogenesis Abundant (LEA) proteins. The total complement of LEA proteins has been cataloged, and orthologous pairs identified, for Arabidopsis (*Arabidopsis thaliana*) and soybean (*Glycine max*). Aiming to expand our knowledge of LEA function in protecting cells from lethal desiccation damage, we focused on the seed stored proteome, one seed component requiring protection during maturation desiccation. Our objective is to identify preferred client proteins (CP) should they exist, for LEA orthologs in Arabidopsis and soybean. Recombinant dehydrin orthologs from these species, At2g21490 (Arabidopsis) and Glyma04g01180 (soybean), were used as bait for affinity-selection phage display. A T7 phage cDNA library, normalized for transcripts present in the mature, dehydrated, 12-, 24 , or 36-h imbibed Arabidopsis seeds, was used in triplicate for each immobilized LEA dehydrin, along with triplicate wells containing BSA as a random, unrelated control protein, to assess protein binding preferences. Phage titer increased considerably over four rounds of biopanning in the three replicate wells for both LEA dehydrin proteins and for BSA. Phage was PEG purified from each of the final round sub-libraries. Two limited-round, PCR reactions were performed which attached regions complementary to the Illumina flow cell oligomers, including Illumina sequencing primer sites, and a bar code unique to each microtiter plate well (replication), on the tags, allowing analysis by Paired-end PhageSeq (PEPS). PEPS exhaustively canvassed the recovered CPs, which are being analyzed using a translated tag processing procedure focused on proteins in-frame with the phage coat protein and represented in the Arabidopsis proteome. PEPS greatly increased the depth of CP coverage allowing greater refinement of the LEA-bound CP regions assisting in our understanding of CP motifs aiding binding by the intrinsically disordered LEAs.

4:15 - 4:30 pm • **Wynston Woodenberg**

ZYGOTIC EMBRYO CELL WALL RESPONSES TO DRYING IN THREE GYMNOSPERM SPECIES DIFFERING IN SEED DESICCATION SENSITIVITY

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Plant cell walls (CWs) are dynamic in that they can change conformation during ontogeny and in response to various stresses. Though seeds are the main propagatory units of higher plants, little is known of the conformational responses of zygotic embryo CWs to drying. This study employed cryo-scanning electron microscopy to compare the effects of desiccation on CW morphology of zygotic embryos across three gymnosperm species that were shown to differ in seed desiccation sensitivity: *Podocarpus henkelii* (highly desiccation-sensitive); *Podocarpus falcatus* (moderately desiccation-sensitive); and *Pinus elliottii* (desiccation-tolerant). Hydrated embryos of all three species showed polyhedral cells with regular walls, typical of turgid cells with an intact plasmalemma. Upon desiccation to *c.* 0.05 g g^{-1} (dry mass basis), CWs assumed an undulating conformation, the severity of which seemed to depend on the amount and kind of dry matter accumulated. After desiccation, intercellular spaces between cortical cells in all species were comparably enlarged relative to those of hydrated embryos. After rehydration, embryo CWs of *P. henkelii* and meristematic CWs of *P. falcatus* remained slightly undulated, suggestive of plasmalemma and/or CW damage, while those of *P. elliottii* returned to their original conformation. Cell areas in dried-rehydrated *P. henkelii* meristem and cotyledon were also significantly lower than hydrated cells from undried embryos, suggestive of incomplete recovery even though water contents of the two states were comparable. Electrolyte leakage measurements suggest that the two desiccation-sensitive species incurred significant plasmalemma damage relative to the tolerant species upon desiccation, which may have contributed to the CW abnormalities after rehydration. Immunocytochemistry studies revealed that of the four CW epitopes common to embryos of all three species, an increase in arabinan (LM6) upon desiccation and rehydration in desiccation-tolerant *P. elliottii* was the only significant difference. Seed desiccation sensitivity in species like *P. henkelii* and *P. falcatus* may therefore be partly based on the inability of the plasmalemma and consequently CWs of dried embryos to regain their original conformation following rehydration. Moreover, embryo CWs of the desiccation-sensitive species may lack the ability to produce additional arabinan during dehydration and rehydration, which compromises CW flexibility and prevents its full conformational recovery following rehydration.

4:30 - 4:45 pm • **Érica Leão-Araújo**

RADIOGRAPHIC ANALYSIS OF *Campomanesia adamantium* SEEDS TO EVALUATE THE DESICCATION SENSITIVITY

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Campomanesia adamantium is native to the Brazilian Savannah and presents a high potential of use, e.g., consumption of fresh fruit, medicinal plant, manufacture of sweets, ice cream and alcohol distillates. The seeds of this species are recalcitrant. Recalcitrance is a phenomenon of intolerance to dehydration and affects the seeds physiological quality. The seed desiccation can be monitored by retraction of the tissues from the integument, which is identified by the inspection of internal morphology of the seed. Radiographic images have been recommended to evaluate the internal morphology of seeds allowing to stablish a relationship with their physiological potential. Thus, this work aimed to evaluate internal morphology changes during dehydration of *Campomanesia adamantium* seeds by using radiographic analysis and the effects on the physiological quality. The water content of the seeds was initially determined and adjustments were made to obtain samples with 48%, 36%, 30%, 27%, 24% and 21% of water (web basis). The individual radiographs of the seeds were obtained by a digital X-ray equipment and the images evaluated using the ImageJ® software to determine the free internal space. Germination, first germination count, germination speed index, mean germination time and seedling lenght evaluations were performed. With these data, polymonial

regression graphs were constructed. The X-ray images were used to obtain data of free internal space and percentage of seeds with physical injuries. Data were submitted to analysis of variance and the effect were studied through polynomial regression. The germination and vigor decreased as the seeds were dehydrated. The free internal space was higher as the water content of the seeds was reduced. The percentage of seeds with physical injuries also followed this behavior with a tendency of stabilization in water contents higher than 36%. The damage caused by the reduction of the water content as well as the increase of the retraction of the internal tissues to the seed coat had a negative effect in the seed vigor. The X-ray image analysis is efficient to determine internal free spaces during desiccation of *Campomanesia adamantium* seeds that are associated with reduction in their physiological quality.

4:45 - 5:00 pm • **Nicholas Genna**
SEED MASS AND DETERIORATION: IMPLICATIONS FOR *EX SITU* LONGEVITY
Genna, N.G. and Perez, H.E.
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Seed bank storage is financially challenging and hinges upon the successful maintenance of seed viability in a variety of species over many decades. Accessions must be monitored for viability in a timely manner and replaced or regenerated if significant deterioration is detected. To date, evidence suggests that seed storage behavior is not uniform and longevity varies across species and accessions. Seed mass is a plant functional trait that has often been examined as a potential source of longevity differences. Currently, evidence suggests that interspecific seed mass variation does not influence the rate of seed deterioration. However, little is known about how intraspecific seed mass variation drives deterioration *ex situ*. Here, we assessed mass-based deterioration responses in two *Rudbeckia mollis* (Asteraceae) seed lots differening in age. The first seed lot was stored for one year at room temperature (23–24°C, 30–40% RH) while the second seed lot consisted of relatively fresh seeds stored at 4°C for four months. Air density separation of each seed lot generated light, intermediate, and heavy mass-based classes. All mass classes were subjected to germination testing under alternating simulated seasonal temperatures including winter (22/11°C), spring (27/15°C), fall (29/19°C), and summer (33/24°C). Mass-based germination responses were similar across temperatures and mass classes for the relatively fresh seed lot. Conversely, mass-based germination responses diverged in the one year old seed lot with light seed germination percent being 2.8 times higher than heavy seeds under summer temperatures. Saturated salt accelerated aging (SSAA) was subsequently utilized to promote deterioration in the relatively fresh seed lot to understand if a mass-based germination response would develop. We found that seed mass significantly influenced germination following increasing aging stress. Final germination percent in light class seeds was 1.7 times greater than intermediate or heavy seeds after 20 d of SSAA. Similar germination responses between one year old seeds and relatively fresh seeds subjected to SSAA suggest that mass dependent viability loss may occur in *R. mollis*. Future research will investigate underlying mechanisms that may promote differential deterioration in seeds of different mass of this species.

Poster Session I
5:15 - 7:00 pm
Monterey Bay Room

M001.
SOYBEAN SEED TREAMENT WITH A FORMULATION CONTAINING MANGANESE CARBONATE AS RAW MATERIAL: EFFECTS ON INITIAL PLANT DEVELOPMENT AND MANGANESE UPTAKE BY PLANTS
Dias, M.A.N.[1], Silva, D.B.[1] and **Cicero, S.M.**[1]
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The supply of Mn for soybean crop has gained more attention since the introduction of glyphosate-resistant (GR) cultivars. Evidences suggests direct and indirect effects of glyphosate in reducing the Mn uptake by plants, which demands higher inputs of this nutrient in current GR soybean cropping systems. Seed treatment may consist in a practical and economical method to deliver Mn to soybean crop, however, the formulation containing Mn must not harm the seeds. Water insoluble sources of micronutrients, such as carbonates, are less prone to cause toxicity to plants compared to water soluble sources, especially when used as seed treatment. This study aimed to evaluate the Mn uptake

by soybean plants that received a manganese carbonate formulation (50% wt./vol.) as seed treatment, also associating the results with plants initial growth. The treatments were composed of three GR soybean cultivars (M6210IPRO; NS6700IPRO; RK7214IPRO) and four doses of Mn (0 “untreated”, 1, 2 and 4 mg $Mn.kg^{-1}$ of seeds). The evaluations consisted of germination test, leaf area, leaf chlorophyll concentration, root and shoot dry mass, plant height and Mn concentration and accumulation in plants. Seed germination was negatively affected only by the highest dose of Mn. In general, mean values of leaf area and tissues dry mass increased with the application of Mn formulation compared to the control, except for the highest dose. Chlorophyll concentration tended to decrease with the application of Mn, probably due to the dilution effect caused by leaf area increase. The uptake and accumulation of Mn increased in the leaf tissues of soybean plants as the Mn doses increased in the seed treatment, thus confirming the possibility of supplying Mn to soybean plants using manganese carbonate as raw material.

M002.
SEED HEALTH TEST VALIDATION IN THE REGULATORY MOVEMENT OF SEED INTERNATIONALLY

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Phytosanitary disputes and liability claims due to discrepancies in seed health testing methods can have serious implications for the movement of seed both nationally and internationally. Few plant pathologists are aware of the different procedures followed by the major role players in achieving international standards in seed health testing. This paper aims to highlight the importance of, and describe, the validation systems of the three primary organizations that publish universally accepted, standardized testing methods for global use. These are the International Seed Testing Association (ISTA), the International Seed Health Initiative (ISHI), and the U.S. National Seed Health System (NSHS). EPPO and IPPC systems are also mentioned. The procedures followed by ISTA and ISHI are based on the validation procedure manual published by Sheppard and Cockerell in 2000 and make use of collaborative multi-laboratory tests and elaborate statistical procedures and analyses to validate new seed health test methods. These procedures differ greatly from those followed by the NSHS. ISHI and ISTA methods can be approved as NSHS methods but the reverse is not true unless collaborative multi-laboratory tests are conducted. The procedures followed by the three main role players are described, compared and differences are highlighted providing an overall synopsis of international seed health method validation and testing.

M003.
SOG1 LINKS THE DNA DAMAGE RESPONSE TO ORGAN REGENERATION

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In Arabidopsis, DNA damage-induced programmed cell death is limited to the meristematic stem cell niche. The significance of this cell-type specific programmed cell death is unclear. Here we demonstrate in roots that it is the programmed destruction of the mitotically-compromised stem cell niche that triggers its regeneration, enabling growth recovery. In contrast to wild-type plants, *sog1* plants, which are defective in damage-induced programmed cell death, maintain their cell identities and stereotypical structure of the stem cell niche, but these cells fail to undergo cell division, terminating root growth. We propose DNA damage-induced programmed cell death is employed by plants as a developmental response, contrasting with its role as an anti-carcinogenic response in animals. This role in plants may have evolved to restore embryonic root growth after the accumulation of DNA damage in seeds.

M004.
THE LOW FALLING NUMBER PROBLEM OF WHEAT: APPLYING KNOWLEDGE ABOUT SEED BIOLOGY TO A REAL WORLD ISSUE

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The Hagberg-Perten Falling Number test is used by the wheat industry to measure starch degradation caused by alpha-amylase enzyme activity in flour. Grain with too much alpha-amylase activity must be sold at a severe discount because it results in poor quality baked goods. Problems with an excess of alpha-amylase result from two independent genetic causes, insufficient seed dormancy to resist preharvest sprouting and a developmental defect called late maturing alpha-amylase (LMA). Preharvest sprouting is the germination of physiologically matured grains on the mother plant when rainy, cool conditions break dormancy and induce germination. Alpha-amylase is naturally produced to mobilize stored reserves during sprouting. In susceptible varieties, LMA is the induction of alpha-amylase in response to a high or low temperature shock can during late seed maturation. Over the last 4 years, over 8,000 Falling Number data points have been collected on Washington State University Cereal Variety trials at locations across the state. Falling Number data in years without challenging weather was not predictive of Falling Number in environments with LMA or sprouting. Moreover, ANOVA analysis of the dataset as a whole suggested that genetics accounted for only 15% of the variability for Falling Numbers. However, such analyses fail to take into account that there is more than one cause of the problem. When weather data was used to tease apart which low Falling Numbers events were due to LMA and/or preharvest sprouting, a different picture of heritability emerged. Being able to see the data in terms of separate components will help to making better breeding decisions, and serve as a first step to understanding the genetics of this problem.

M005.
MONITORING THE PRE-GERMINATION GENE EXPRESSION OF INDIVIDUAL *ARABIDOPSIS THALIANA* SEEDS USING A REAL-TIME LUCIFERASE REPORTER SYSTEM TO ADDRESS SEED VARIABILITY

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Variability is present in biology across many scales, from populations, individuals and cells down to the molecular events within cells. Variability within seeds impacts seed behaviour and adaptations to the environment, consequently this can pose many problems to agriculture where crop uniformity is desired. We propose that understanding this variability is critical in enhancing strategies to increase crop uniformity and maximize agricultural output across varying climatic conditions. Individual seeds vary in absolute hormone concentrations as well as hormone sensitivity thresholds, both of which play a role in the developmental mechanisms during seed development. The antagonistic relationship between the hormones absissic acid (ABA) and gibberellin (GA) is known to control the physiological state of developing seeds, ABA induces dormancy and prevents germination, whereas GA stimulates germination and cell expansion. Although this relationship is widely accepted the exact mechanisms and roles that ABA and GA play during seed development are not understood in depth. A major obstacle to monitoring gene expression patterns during seed development is the destructive nature of sampling and measurement methods which involve the need to kill samples. As a result tracking the dynamics of expression over developmental time is currently not possible. We have developed a method to monitor gene expression in Arabidopsis thaliana seeds in real-time using a luciferase based reporter system. ABA and GA-responsive genes were monitored in real-time by inserting promoters upstream of a luciferase reporter and transformed into the Arabidopsis thaliana transparent testa mutant (tt4-1). When these seeds are exposed to luciferin the gene expression patterns of the selected genes can be monitored non-invasively from initial imbibition to germination providing an insight into the changes taking place during seed development. In the future dual-luciferase reporters are to be developed to enable the monitoring of multiple expression patterns from different combinations of genes to further develop current understanding of seed behavioural changes pre-germination.

M006.
ENVIRONMENTAL MATERNAL EFFECTS ON TEMPERATURE-DEPENDENT DORMANCY RELEASE IN *POLYGONUM AVICULARE* SEEDS

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Although after-shedding environmental regulation of seed dormancy has been widely studied and modelled, less work has focused on maternal effects on dormancy behavior and there is almost no

attempt to include these effects on predictive models of seedling emergence. In order to study and model the effects of (i) mother plant flowering-time and (ii) photoperiod during seed-development on primary dormancy level, temperature-dependent dormancy release and field emergence patterns, we performed field and lab essays using *Polygonum aviculare* seeds. To achieve (i), we carried out plantings in contrasting sowing-dates. To accomplish (ii), plants were grown under natural and extended (+4hs) photoperiod conditions. At dispersal-time, groups of collected seeds were destined to measure primary dormancy level (freshly harvested seeds germination was tested at 10, 15, 20, 25 and 10/24°C); to quantify dormancy loss rate (seeds were buried in pots and stored at 1.6, 4.8 and 9.8°C. Periodically, seeds were exhumed and germination was tested at 10, 15, 20, 25 and 10/24°C); and to verify lab results under field conditions (seeds were buried in the field and emerged seedling were counted at regular intervals). Results showed no differences in primary dormancy level at dispersal-time. However, dormancy release rate was significantly affected by seed-maturation conditions. With delay in planting-date and under short-day conditions an increase in dormancy loss rate was observed. In spite of these differences, the temporal pattern of seedling emergence in the field was similar between treatments. This can be explained, at least partially, by the fact that although seeds from the late sowing were dispersed later in the season they lost dormancy faster -showing a similar emergence pattern to that of seeds matured earlier-. Obtained results were verified including maternal effects in a mathematical model and performing simulations. Overall, presented results suggest that maternal environment regulates dormancy loss rate in order to maintain a temporal emergence pattern that maximizes plants fitness.

M007.

M008.

CAUSE OF SEED DORMANCY IN BAMBARA GROUNDNUT (*Vigna subterranea* (L.) Verdc.) AND TREATMENTS TO BREAK THE DORMANCY

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Bambara groundnut is an underutilized crop despite its potential as a future crop. It is suspected that bambara groundnut has after-ripening dormancy. Farmers usually store the harvested seeds for ca. 8 months until being used for next planting season. Therefore, when they plant the seeds, the dormancy has been released. The objectives of this study were to find the cause of dormancy and to get the best treatment for breaking the dormancy. The experiment was arranged in a nested design. Breaking dormancy treatment and after-ripening period were nested on genotypes. Two genotypes were used, 'Sukabumi' landrace and 'Sumedang' landrace. The breaking dormancy treatments were untreated, scarification, soaking in KNO_3 1% for 2 hours, and scarification followed by soaking in KNO_3. After-ripening period were 1, 2, and 3 months after harvesting (MAH). The experiment was carried out using 3 replications. During after-ripening, the seeds were stored without pods in plastic bags in an ambient room. The results showed, at 1 MAH seeds of both landraces soaked in KNO_3 did not reduce the intensity of dormancy. However, the scarification treatment, and the scarification followed by soaking in KNO_3 decreased the intensity of dormancy. This finding proved the impermeability of testa. During the period of 1 to 3 MAH, 'Sukabumi' landrace showed decrease in the intensity of dormancy from 45.3% to 9.3% with increase in germination from 36.0% to 73.3%. 'Sumedang' landrace also experienced a decrease in the intensity of dormancy from 42.7% to 13.3% with increase in germination from 30.7% to 74.7%. This finding proved the existence of after-ripening. 'Sumedang' indicated a faster response to germination inhibition by exogenous ABA and required more exogenous GA_3 to obtain the best germination, as compared to 'Sukabumi'. In addition, 'Sumedang' showed tendency to imbibe slower than 'Sukabumi'. In addition, 'Sumedang' had relatively thicker testa, higher contents of lignin in testa and of tannin in seed than 'Sukabumi'. Although each factor observed did not differ significantly, it was suspected that cumulative factors could have a significant effect. Scarification treatment followed by soaking in KNO_3 was the best treatment for breaking the seed dormancy.ø

M009.

THE PHYSIOLOGAL AND MOLECULAR ASPECTS OF THE *ARABIDOPSIS THALIANA* SEED LIFE SPAN UNDER FIELD CONDITIONS

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Seed dormancy and longevity together determine the seed life span. In nature, seed dormancy ensures correct timing of germination and seed longevity is essential for survival during unfavourable conditions. In agriculture, these two traits are important related to seed quality and, in general, for food security. Although seed dormancy and longevity both have been studied extensively, their molecular basis is still unclear. Moreover, both traits are usually studied during seed dry storage, conditions that are contrasting to the moist environment that seeds encounter under natural conditions.

Here, we study the physiological and molecular aspects of the seed life span under natural conditions by soil seed bank experiments. In this experiment, we have used a series of *Arabidopsis thaliana* genotypes (*DELAY OF GERMINATION* near isogenic lines), which have distinct seed dormancy and longevity phenotypes, as was revealed from earlier dry storage experiments (after-ripening). We show that all genotypes undergo dormancy cycling in the soil (secondary dormancy is induced in autumn and released during spring). We will discuss these results and the effect of the parental environment during seed maturation on dormancy cycling. Furthermore, we have investigated the genome-wide transcriptional changes during dormancy cycling. The first analyses of this RNA-seq experiment will be presented.

M010.

PERFORMANCE OF *RICINUS COMMUNIS* SEEDLINGS UNDER WATER RESTRICTION: POSSIBLE INVOLVEMENT OF OSMOPROTECTIVE MOLECULES, HSPS AND GLUCONEOGENESIS ENZYMES

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Ricinus communis (castor bean) is an oilseed species that stands out in the agricultural and industrial scenario due to the chemical composition, quality and applicability of the oil extracted from its seeds. It is considered an easy-to-handle crop, with low production costs, which can be grown in unfavorable environments due to its wide range of abiotic stress tolerance and environmental adaptation. The present study aimed to evaluate a link between physiology, metabolites and gene expression in seedlings of 2 *Ricinus communis* cultivars (EBDA-MPA34 and PARAGUAÇU) under different water restriction conditions. Besides physiological parameters (root growth), the concentration of osmoprotective molecules was determined as well as the expression of genes of heat shock proteins (HSPs) and genes involved in the regulation of gluconeogenesis (phosphoenolpyruvate carboxykinase - PEPCKase and fructose-1,6-bisphosphatase - FBPase) in roots and cotyledons of the 2 cultivars. Initially, seeds were imbibed in water until radicle protrusion (2.0mm), and subsequently seedlings were kept in water (0.0MPa, control) or transferred to 3 different PEG8000 solutions (-0.2, -0.6 and -1.0MPa) during 5d. Physiological parameters included length (cm), dry matter (mg) and the ratio of dry matter per centimeter (mg/cm) of roots. The concentrations of osmoprotective molecules were determined by HPLC, and the gene-expression for HSP, PEPCKase and FBPase by RT-qPCR. In general, both cultivars responded similarly to the increasing water restriction stress. However, cv. PARAGUAÇU performed better at the most severe water restriction condition that was tested (-1.0MPa). There was a good positive correlation between the level of water restriction and the concentrations of certain osmoprotective molecules (galactinol and trehalose in both the roots and cotyledons) and expression of HSP, PEPCKase and FBPase genes in both organs. Increase of water restriction resulted in a decrease of growth and less accumulation of biomass in the roots of both cultivars. The better performance of cv. PARAGUAÇU at -1.0MPa nicely correlated with higher concentrations of osmoprotective molecules and expression of genes that encode HSP and enzymes involved in the regulation of gluconeogenesis, as compared to cv. EBDA-MPA34 in this same condition.

M011.

HYDROPHILIC SEED COATING TO ENHANCE STAND STABLISHMENT UNDER DROUGHT STRESS

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There is an increase in the use of cover crop seeds as the benefits of cover crops include the improvement of yields by enhancing soil health and nutrient cycling, reduction of soil erosion and conserving soil moisture. Cover crops may be sown during the growing season of the main cash crop, especially in the temperate region due to the short growing season. Cover crops are commonly broadcast several weeks to months after the main crop is sown (interseeding), but the cover crops may encounter environmental stress after sowing, especially drought stress. Seed technology including seed coating with hydrophilic materials in the coating may represent an interesting approach for increasing crop tolerance to soil moisture content fluctuation after broadcast seeding. The current study aimed at developing seed treatments and coatings using a hydrophilic component to hold moisture around the germinating seeds and to act as tackifier to adhere seeds to the soil surface and enhance early seeding establishment of cover crops. Red clover and rye grass seed coatings were produced by Summit Seed Coatings, Caldwell, ID with 1:1 and 4:1 build-up and each coating with and without Hydroloc, an OMRI approved hydrophilic component. Water stress was imposed by sowing seeds on the surface of a water saturated media of Turface in containers in the greenhouse and then allowing to air dry for 2 days and then sealing the containers to simulate a drought stress occurring during the germination period. There was a significant decrease in total germination percentage (> 30% decrease), seedling establishment percentage (> 40% decrease), shoot and root length, fresh and dry weight of seedlings of non-coated seeds compare to all hydrophilic coated seed treatments. Field studies were conducted to investigate the benefit of coated seed technology on field establishment for red clover and rye grass. Selected coated seed treatments had greater stand establishment than the noncoated control from broadcast applications. Coating technology could represent an effective strategy to minimize early season drought stress.

M012.

AFFINITY-SELECTION PHAGE DISPLAY AND PAIRED-END PHAGE SEQUENCING IDENTIFY A SMALL REPERTOIRE OF CLIENT PROTEINS COMMON TO ORTHOLOGOUS ARABIDOPSIS AND SOYBEAN DEHYDRIN PROTEINS

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The seed's tolerance to desiccation is the result of synergistic interaction of several mechanisms, many of which involve the hydrophilic Late Embryogenesis Abundant (LEA) proteins. The total complement of LEA proteins has been cataloged, and orthologous pairs identified, for Arabidopsis (*Arabidopsis thaliana*) and soybean (*Glycine max*). Aiming to expand our knowledge of LEA function in protecting cells from lethal desiccation damage, we focused on the seed stored proteome, one seed component requiring protection during maturation desiccation. Our objective is to identify preferred client proteins (CP) should they exist, for LEA orthologs in Arabidopsis and soybean. Recombinant dehydrin orthologs from these species, At2g21490 (Arabidopsis) and Glyma04g01180 (soybean), were used as bait for affinity-selection phage display. A T7 phage cDNA library, normalized for transcripts present in the mature, dehydrated, 12-, 24-, or 36-h imbibed Arabidopsis seeds, was used in triplicate for each immobilized LEA dehydrin, along with triplicate wells containing BSA as a random, unrelated control protein, to assess protein binding preferences. Phage titer increased considerably over four rounds of biopanning in the three replicate wells for both LEA dehydrin proteins and for BSA. Phage was PEG purified from each of the final round sub-libraries. Two limited-round, PCR reactions were performed which attached regions complementary to the Illumina flow cell oligomers, including Illumina sequencing primer sites, and a bar code unique to each microtiter plate well (replication), on the tags, allowing analysis by Paired-end PhageSeq (PEPS). PEPS exhaustively canvassed the recovered CPs, which are being analyzed using a translated tag processing procedure focused on proteins in-frame with the phage coat protein and represented in the Arabidopsis proteome. PEPS greatly increased the depth of CP coverage allowing greater refinement of the LEA-bound CP regions assisting in our understanding of CP motifs aiding binding by the intrinsically disordered LEAs.

M013.

THE NATURAL PROTECTION AND REPAIR MECHANISM: THE ORCHESTRATION OF SEED VIGOR

Dirk, L.M.A.[1], Zhao, T-Y.[2], Unêda-Trevisoli, S.H.[3] and **Downie, A.B.**[1]
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Orthodox seed are those capable of surviving extreme dehydration (5-10% moisture content; anhydrobiosis) and constitute the majority of human nutrition. Anhydrobiosis permits facile global food transport and storage, forming the basis for classical agriculture by allowing a portion of each harvest to be stored to sow the next crop. An underappreciated role seeds play is in the encapsulation of technology in a transportable, storable, quantifiable entity. This seminar will be for those interested in the fundamental underpinnings of anhydrobiosis as it relates to seed vigor. The natural protection and repair mechanism (NPRM) has long been recognized, in orthodox seeds, as the means by which the cells comprising the propagule prepare for, and survive, extreme water loss. The NPRM is an umbrella term referring to any physical or physiological alteration that renders the cell: 1) more tolerant of water loss and attendant stresses and; 2) better able to repair the damage to macromolecules and cellular components, accrued during the sojourn in the dehydrated state, upon subsequent rehydration (imbibition). The more competent the cells comprising the seed are at performing these tasks, the greater their longevity and the more superior their vigor. Areas to be explored in this seminar will, therefore, include late embryogenesis, when protective mechanisms are deployed to prepare the cells for loss of water and metabolic quiescence. The subsequent existence in the dehydrated state will be analyzed focusing on what is occurring at the level of the cell that may possibly mitigate damage accrued when repair is at least limited by lack of water.

M014.
CRYOPRESERVATION AT IPK GATERSLEBEN –IS SEED CRYOSTORAGE NEEDED FOR ORTHODOX SEEDS?

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The Gatersleben federal *ex situ* gene bank of agricultural and horticultural plants houses more than 300 *in vitro* and 1,500 cryopreserved accessions. The accessions held at ultra-low temperature (-196°C) include back-ups for the vegetatively propagated genetic resources, such as garlic and mint. In addition, with more than 1,400 potato cultivars, the Gatersleben cryo-vault maintains also one of the world's largest potato collections. In general, the vast amount of plant genetic resources are preserved long-term as orthodox seeds after drying to <20% RH, packaging occasionally in anoxic conditions and storage in the cold (< -18°C). Only plants that do not produce heterozygous or any seeds are backed up in cryostorage.

The maintenance of plant genetic resources either in cold or in cryo generate specific costs over long-term periods. Here, we discuss the option of cryopreservation for orthodox seeds. The pros and cons are considered in relation to plant species, pollination and breeding type, multiplication cycle, seed longevity, required working time, equipment and consumables. The spreadsheet tool developed by Keller et al. (2013; GRES 60, 913-926) is applied to find the breakeven point and rational explanation for a specific bio-banking scenario.

M015.
CRYOPRESERVATION OF COFFEE SEEDS

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Despite intense research on coffee seeds in recent decade, few advances have been achieved and their long term conservation is still limited. Storage at temperatures below freezing is used for various species with characteristics similar to coffee, and it may be viable for coffee seeds. The aim of recent studies was to investigate the storage of coffee seeds at below freezing temperatures after different methods of preparation. In a first experiment, it was evaluated the physiological quality of seeds subjected to different cryopreservation protocols (in liquid nitrogen at -196°C), resulting from the combination of different drying speeds, moisture levels of 0.20 and 0.23 g H2O.g-[1]db, and cooling speeds. In the second experiment, the best protocols were tested in coffee seeds from different cultivars to simplify cryopreservation steps to obtain the simplest and fastest method. The first experiment showed coffee seeds with moisture content of 0.20 g H_2O.g-[1]db respond better to

cryopreservation than 0.23 g $H_2O.g^{-1}db$. *Coffea arabica* L. seeds dried to 0.20 g $H_2O.g^{-1}db$ in saturated saline solutions (75 or 68 % RH) and immersed in liquid nitrogen have their quality preserved. The second experiment showed that fast drying in silica gel to 0.20 g $H_2O.g^{-1}db$ and direct immersion in liquid nitrogen makes cryopreservation of coffee seeds possible. Fast drying in silica gel followed by direct immersion in liquid nitrogen and subsequent reheating in a water bath allows cryopreservation of *Coffea arábica* L seeds. Other authors obtained similar results after drying in saturated saline solutions and controlled pre-freezing or drying in saturated saline solutions and direct immersion of seeds in liquid nitrogen. Faster drying in silica gel, associated with direct immersion, is recommended and represents simplification of the methodology for routine use in laboratories.

M016.
IMPLEMENTING THE DRY CHAIN DURING STORAGE REDUCES LOSSES AND MAINTAINS QUALITY OF WHEAT AND MAIZE SEEDS

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Cereals seeds are vulnerable to insect and fugal attack and to loss of germination and vigor during storage. High seed moisture accelerates seed deterioration, so maintenance of seed dryness through hermetic packaging (The Dry Chain) may prevent these storage losses. Seeds of wheat and maize were dried to 8, 10, 12 and 14% seed moisture content (SMC) and stored in traditional porous storage bags (paper, woven polypropylene, jute and cloth) as well as in hermetic plastic bags (Super Bags), and insect and fugal activities and seed deterioration were monitored. At the time of packaging, 30 larvae of Khapra beetle (*Trogoderma granarium)* and 30 adults of lesser grain borer (*Rhizopertha dominica*) were introduced separately into each type of bag. After four months of storage at ~75% relative humidity, little change was observed in moisture contents of seeds stored in Super Bags while a significantly higher change in SMC (Increased with environmental RH) occurred in all other storage bags. Insect populations were lower in both maize and wheat seeds stored in Super Bags at 8 and 10% SMC, while seeds stored in all other storage bags had very high insect populations irrespective of the initial SMC. Storage losses in maize were up to 10% of initial weight while in wheat up to 11% weight losses were observed in traditional bags compared to less than 1% losses for maize and wheat stored in Super Bags. Seed storage in Super Bags at 8 and 10% SMC also maintained seed germination percentages and starch and crude protein contents. Malondialdehydes, aflatoxins B_1 and G_2 were present at higher amounts in seeds at 14% SMC in Super Bags and in all traditional storage bags, while seeds stored in Super Bags at 8 and 10% SMC had lower values of these compounds. In conclusion, insect populations, storage losses and deterioration of seed quality with respect to germination, loss of food reserves and increased aflatoxin contamination were highly related to seed moisture contents. Maintaining the Dry Chain through hermetic storage of seeds at 8 and 10% SMC resulted in better storage of wheat and maize seed under humid conditions.

M017.
A GLASS JAM JAR WITH EQUILIBRATED SILICA GEL TO STUDY THE ROLE OF OXYGEN IN SEED STORAGE

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Maintenance of seed vitality during seed storage is of importance for seeds as propagation material and for gene banks. The effect of humidity and temperature on seed ageing is well recognized. Nowadays also the role of oxygen in seed aging is getting more attention. To quantify the combined effect of oxygen, temperature and humidity on seed longevity a test system is needed which allows these three factors to be set independently. Different methods to create anoxia or low oxygen conditions were evaluated. Glass jam jars provided a good basis as test systems to study longevity. The lid is airtight for many years most likely decades. The headspace can be flushed through a temporally hole in the lid which can be sealed airtight with aluminum tape. The transparent glass wall of the jar allows measurement of oxygen with an oxygen sensor dot attached to the inside. With RH equilibrated silica gel as a buffer different and stable moisture levels can be set. To create anoxia conditions we tested flushing with nitrogen and different oxygen absorbers. Under dry conditions only the iron based absorbers with a moisturizer showed to be effective. An increase in moisture or water

activity due to the moisturizer can be prevented by adding a calculated amount of moisture absorber, e.g. dried silica gel.

M018.

MORPHOLOGICAL DIVERSITY, SEED CHLOROPHYLL FLUORESCENCE AND SEED QUALITY OF EGUSI WATERMELON (*CITRULLUS MUCOSOSPERMUS)* COLLECTION

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Twenty-two Egusi watermelon *(Citrullus mucusospermus)* and three commercial varieties (*C. lanatus*) were evaluated using morphological characters to determine the extent of genetic diversity in Egusi that can be explored for watermelon breeding and to aid in long term conservation. Morphological data was used to calculate the genetic similarity and to construct a dendrogram. Analysis of Variance (ANOVA) was calculated using STAR statistical package and effects declared significant at 5% level. Principal Component (PC) analysis using eleven quantitative variables and accessions were plotted on two dimensions using the first three principal components. Seed chlorophyll fluorescence (CF) at different fruit maturity (35, 40, 45, 50 and 55 days after pollination) was also analyzed to identify the optimal time to harvest for maximum germination and vigor. Germination parameters at different maturity period were calculated and data were correlated with mean CF. Results of cluster analysis and dendrogram revealed seven clusters of Egusi and one cluster of varieties which were separated into two major groups. High morphological diversity was observed between the two groups (81%), among Egusi (66%) and, between varieties and Egusi (73%). ANOVA conducted on quantitative traits (i.e., fruit weight, total soluble solids, fruit length, fruit width, number of seeds per fruit and 100-seed weight) demonstrated highly significant variation among accessions except, pericarp thickness. PC analyses indicated that the first three PCs explained 40%, 25% and 13% (total of 79%) of the total variation. The CF analysis results showed no significant difference at different maturity period however, a significant variation in CF values was detected among accessions. Mean CF of nineteen Egusi accessions significantly varied from 17-72 pA while, no significant difference among the three varieties at 23-27 pA. Germination test results at day14 (final count) indicated significant influence of accessions and maturity period on percent germination. MGT, T_{50} and AUC were best at 55 days fruit maturity for accessions showing linear germination trend. Correlation analyses indicated weak inverse relationship between mean CF and germination parameters. It appears that CF analysis is less appropriate tool to use in predicting seed maturity to maximize germination and vigor of Egusi type watermelon.

M019.

TRANSCRIPTIONAL REGULATORY NETWORKS CONTROLLING SOYBEAN SEED MATURATION

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Soybean (*Glycine Max*) is the most produced and consumed oilseed in the world. The majority of storage compounds in the soybean seed accumulate in the maturation phase of seed development. Understanding the initiation and establishment of soybean seed maturation may allow us to develop strategies to improve soybean seed quality. We analyzed whole-genome transcriptome datasets to identify two groups of co-expressed genes with spatial and temporal expression patterns that correlate with the maturation program of the soybean seed. In addition, these two groups are highly enriched for genes involved in processes that occur during the maturation of the seed, such as lipid storage and accumulation of storage proteins. Several putative regulators of seed maturation were identified in the clusters, including LEAFY COTYLEDON1 (LEC1), ABSCISIC ACID INSENSITIVE3 (ABI3), BASIC LEUCINE ZIPPER 67 (bZIP67) and ABA-RESPONSIVE ELEMENT BINDING PROTEIN 3 (AREB3). The results suggest that this group of transcription factors play an important role in setting up the maturation program of the soybean seed. In order to identify target genes that are transcriptionally regulated by LEC1, ABI3, bZIP67 and AREB3, we performed chromatin immunoprecipitation and differential gene expression analysis during the maturation stage of soybean seed development. Detailed analysis of target genes showed a complex transcription factor regulatory network in which different combination of transcription factors are involved in distinct biological programs in soybean embryos, such as seed maturation, photosynthesis and embryo morphogenesis. Motif enrichment analysis suggest that a unique set of cis-regulatory elements determine each of these biological programs. A significant

enrichment of G-box (CACGTG) and RY (CATGCA) elements in the promoter region of genes that are part of the maturation program was observed. In addition, we observed a spatial organization of these two motifs in cis-regulatory modules near the transcriptional initiation site in the promoters of maturation genes. Moreover, functional analysis of cis-regulatory modules showed that the regions enriched in regulatory motifs are important to determine transcription factor occupancy and activation of maturation related genes. The results obtained in this study are helping us to elucidate the complex transcriptional network that controls soybean seed maturation.

M020.
VARIABILITY IN ARABIDOPSIS SEED GERMINATION TIME

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Variability in the germination time of genetically identical seeds in a common environment may function as a bet hedging strategy in unpredictable environments. However, the mechanisms underlying variability in seed germination time are largely unknown. We are using *Arabidopsis thaliana* as a system to understand the genetic and mechanistic basis for variability in germination time within a season. We have shown that natural accessions of Arabidopsis show variation in the extent of variability in their seed germination time in the lab and that the distributions of seed germination times tend to be reproducible for each genotype. We have used the MAGIC multi-parent RIL population to map a locus underlying the extent of variability and are now testing candidate genes from this region. We have also found that the extent of germination time variability produced by a given genotype may be increased or decreased by modulating the levels of ABA and GA. We now plan to use a combination of computational modelling and further experiments to investigate how varying the levels of GA or ABA that a population of seeds is exposed to could modulate the extent of inter-seed differences in germination time.

M021.
INTERACTIONS OF GENETIC FACTORS AND MATERNAL ENVIRONMENTS DURING SEED DEVELOPMENT INFLUENCE TEMPERATURE SENSITIVITY OF LETTUCE (*LACTUCA SATIVA* L.) SEED GERMINATION

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High temperature-induced inhibition of lettuce (*Lactuca sativa* L.) seed germination (thermoinhibition) is influenced by genetics and maternal environment during seed maturation. The objective of this research was to identify the genetic and physiological bases of this cross-generational plasticity of the lettuce seed thermoinhibition trait. Quantitative trait locus (QTL) mapping studies of the sensitivity of lettuce seed germination to high temperature were conducted on a recombinant inbred line population developed from a cross between *L. sativa* wild accession PI251246 (PI) x *L. sativa* cv. Salinas (Sal) produced in five environments. A major high-temperature germination QTL (*qHTG9.1)* with varying magnitude was consistently identified, while minor QTLs were inconsistent across environments. QTL analysis of genetic by environment (GxE) variance revealed a major plastic high-temperature germination QTL (*qPHTG9.1)* on chromosome 9. This plastic QTL, which explained 33% of the GxE variance, collocated with *qHTG9.1*, suggesting that the main trait allele, which has been attributed to *ETHYLENE RESPONSE FACTOR 1* (*LsERF1*), is sensitive to the maternal environment during seed development and is involved in setting the maximum germination temperature of the seeds during subsequent imbibition. Cross-generational phenotypic plasticity was also associated with the differential expression of abscisic acid (ABA), gibberellin (GA) and ethylene biosynthesis, metabolism and response genes in both developing and imbibed mature seeds. In general, seeds developing at warm temperature had higher expression of GA- and ethylene-related genes, while ABA-related genes were suppressed. In particular, transcript levels of the GA biosynthesis gene *GIBBERELLIN-3-OXIDASE 1* (*LsGA3ox1*) increased with the maturation temperature in PI seeds, while transcripts of the GA degradation gene *GIBBERELLIN-2-OXIDASE 2* (*LsGA2ox2*) decreased with increasing maturation temperature in both genotypes. Imbibed seeds showed gene expression patterns associated with success or failure of germination at warm temperatures. Our results illustrate that seed maturation

temperature affects the expression of genes known to influence dormancy and germination and that there are genotypic differences in these expression profiles.

M022.

PHOTOCHEMICAL ACTIVITY CHANGES ACCOMPANYING THE EMBRYOGENESIS OF *PISUM SATIVUM* L. WITH YELLOW AND GREEN COTYLEDONS

Smolikova, G.N.[1], Shiroglazova, O.V.[1], Vinogradova, G.Y.[2], Leppyanen, I.V.[3], Titova, G.E.[2] and **Medvedev, S.S.[1]**

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The seeds of many angiosperms are able to form chlorophyll-containing embryos, referred to as chloroembryos. The chloroembryos and adjacent seed coats contain photochemically active chloroplasts. But photosynthesis in the embryo essentially differs from the reactions occurring in leaf. In seeds, its main biological role is the production of reserve biopolymers, but not the metabolically available and transportable carbohydrates, characteristic for the leaf primary metabolism. Moreover, the photosynthesis in seeds mainly relies on the maternal plant sucrose as a source of carbon, but not on the atmospheric carbon dioxide, as it is known for leaf chloroplasts. Although the embryonic photosynthesis is known since decades, its mechanisms are not completely understood so far. Thus, it is still unclear, how the light-dependent reactions generally can occur, as the seeds are typically covered with thick and firm tissues of the pericarp and seed coat. The mechanisms of the chlorophyll degradation and termination of photosynthesis at the final stages of the seed maturation are also poorly understood. Here we address the dynamics of photochemical activity in the yellow- and green-seeded pea cultivars by the pulse amplitude modulation (PAM) fluorometric analysis. Despite of their shielding from the light by the pod wall and seed coat, photochemical reactions were registered in the seeds with green embryo. The fast transients of the chlorophyll *a* fluorescence revealed the maximal photochemical activity at the early- and mid-cotyledon stages. However, it declined rapidly at the late cotyledon stage, and was accompanied with the decrease of the water content below 80%. From the other hand, formation of photoheterotrophic plastids in seeds is triggered by sucrose supplied by the maternal tissues. Therefore, it seems to be possible, that the interruption of sucrose flow as well as the start of dehydration triggers the chloroplast transformation, chlorophyll degradation, and termination of photosynthesis. To assign the genes responsible for the termination of photosynthetic activity, a comparative RNAseq analysis of transcriptome profiles has been conducted using the high-throughput sequencing in Illumina HiSeq2000. This work was supported by grant no. 16-16-00026 from the Russian Science Foundation.

M023.

IRON DISTRIBUTION DURING *ARABIDOPSIS THALIANA* AND *BRASSICA NAPUS* DEVELOPMENT SEED.

Ibeas, M.A., Grant-Grant, S. and Roschzttardtz, H.

GMM, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile

Contact: Miguel Ángel Ibeas, maibeas@uc.cl

Iron is an essential micronutrient for most living organisms, including plants. The role of iron in seed yield is an important agronomical trait because iron deficiency affects plant reproduction and crops yield. Plants are the primary food source of humans and its nutritional content is the main importance to human health. According to the World Health Organization, 30% of the population is anemic, and biofortification is a possible alternative to combat iron deficiency, and seeds with higher mineral content may contribute to this goal. One of our main goals is to understand the molecular basis of iron homeostasis during seed development in plants. Using *Arabidopsis thaliana* as a model plant, it has been described that iron accumulates in vacuoles of endodermis cell layer during embryo maturation. Using Perls/DAB staining we were able to find that embryos from related species to Arabidopsis have a different iron distribution pattern. In those embryos, iron accumulates in several cell layers in hypocotyl, including endodermis and at least one layer of cortex. Interestingly, cotyledon of those embryos accumulates iron only in the endodermis cell layer. In addition, we were able to evaluate iron accumulation during seed development and we found that iron accumulates in endosperm nuclei prior to reach the embryo in early stages of development of *Brassica napus* seed. Our results open new

questions about the molecular mechanism controlling iron loading in seeds.

M024.
AUTOMATED GERMINATION ANALYSIS USING TIME_LAPSE IMAGING AND DEDICATED IMAGE ANALYSIS SOFTWARE

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The aim of this project was to develop an imaging system that can replace manual scoring of seed germination in an R&D environment. This imaging system consists of a piece of hardware to allow time-lapse image capture of germinating seeds together with software that allows fast and repeatable image analysis with data output in a csv-file. There are a number of disadvantages of manual scoring, but from an R&D perspective the increase in resolution of the data is probably the most important advantage of automated germination analysis. Now we can follow and analyze germination 24 hours per day and 7 days a week at any desired interval of time-lapse imaging. Here we show the hardware and software that was used in a proof of concept project. The advantages are demonstrated by an experimental example. In this experiment we show that Brassica oleracea var. A12 seeds display a multiphasic rhythm in their germination. This possibly indicates that these Brassica seeds show a germination rhythm that fits a circadian rhythm.

M025.
IDENTIFICATION OF A LOCUS CORRESPONDING TO A PREHAVRVEST SPROUTING TOLERANT MUTANT, *ERA8,* IN WHEAT (*TRITICUM AESTIVUM* L.)

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Preharvest sprouting (PHS) is the germination of mature wheat grain on the mother plant when cool and wet conditions occur before harvest. PHS causes severe losses for wheat growers. Lack of seed dormancy accounts for 60-80% of PHS susceptibility. *ERA8* was created using EMS mutagenesis and the mutation was selected for increased sensitivity to the dormancy hormone ABA, resulting in increased seed dormancy and PHS tolerance. This gene is effective in the soft white spring background, Zak, and represents a new source of PHS tolerance. The goal was to identify *ERA8*-linked molecular markers for genomic selection during breeding. We mapped the *ERA8* gene using both traditional quantitative trait locus (QTL) analysis and using a next-generation sequence-based approach. Using both methods, we localized *ERA8* to a region of chromosome 4A. QTL analysis of the Louise/Zak*ERA8* RIL population was conducted in R/qtl using nucleotide differences identified by genotyping by sequencing (GBS). A total of 4 significant ($p<0.05$) QTL were identified and one of them increased ABA sensitivity due to the *ERA8* allele. *ERA8* was also mapped using bulk segregant analysis and exome capture of the Zak/Zak*ERA8* backcross population, where *ERA8* was the only segregating gene impacting germination on ABA. Over 70 EMS induced single nucleotide polymorphisms (SNPs) between wild type and mutant were identified solely on the 4A chromosome. Additional recombinants from the backcross population were identified and used to fine map the *ERA8* mutation down to a 4.9 cM region. *ERA8* is currently being crossed into wheat breeding lines to increase preharvest sprouting tolerance. The *ERA8* SNP markers identified by this project are currently being used for rapid genomic selection in breeding lines. Identification of candidate genes in the region is currently underway.

TUESDAY, September 12, 2017

SEED CONSERVATION SYSTEMS (SCS)
Chairpersons: **Christina Walters, Andreas Borner**
Cypress Ballroom

Keynote Speakers

8:00 - 8:45 am • **Bruce Downie**
THE NATURAL PROTECTION AND REPAIR MECHANISM: THE ORCHESTRATION OF SEED VIGOR
Dirk, L.M.A.[1], Zhao, T-Y.[2], Unêda-Trevisoli, S.H.[3] and **Downie, A.B.**[1]
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Orthodox seed are those capable of surviving extreme dehydration (5-10% moisture content; anhydrobiosis) and constitute the majority of human nutrition. Anhydrobiosis permits facile global food transport and storage, forming the basis for classical agriculture by allowing a portion of each harvest to be stored to sow the next crop. An underappreciated role seeds play is in the encapsulation of technology in a transportable, storable, quantifiable entity. This seminar will be for those interested in the fundamental underpinnings of anhydrobiosis as it relates to seed vigor. The natural protection and repair mechanism (NPRM) has long been recognized, in orthodox seeds, as the means by which the cells comprising the propagule prepare for, and survive, extreme water loss. The NPRM is an umbrella term referring to any physical or physiological alteration that renders the cell: 1) more tolerant of water loss and attendant stresses and; 2) better able to repair the damage to macromolecules and cellular components, accrued during the sojourn in the dehydrated state, upon subsequent rehydration (imbibition). The more competent the cells comprising the seed are at performing these tasks, the greater their longevity and the more superior their vigor. Areas to be explored in this seminar will, therefore, include late embryogenesis, when protective mechanisms are deployed to prepare the cells for loss of water and metabolic quiescence. The subsequent existence in the dehydrated state will be analyzed focusing on what is occurring at the level of the cell that may possibly mitigate damage accrued when repair is at least limited by lack of water.

8:45 - 9:30 am • **Manuela Nagel**
GERMINATION DECLINE DURING BARLEY DRY STORAGE IS ASSOCIATED WITH REDUCTION OF TOCOCHROMANOLS AND GLUTATHIONE
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Barley (*Hordeum vulgare* L.) is one of the most important fodder crop and used for brewery industry. In world's gene banks more than 460 thousand accessions are stored after drying in cold storage conditions (-18°C). However, its seed longevity is limited and strongly influenced by genetic, pre-storage and storage conditions.
Here, to enhance our knowledge of biochemical reactions occurring during seed storage we investigated changes in tocochromanols and the redox state of the antioxidants glutathione (glutamyl-cysteinyl-glycine). Two long-term dry storage regimes (ambient and cold storage in the glassy state) were compared with two controlled deterioration treatments, one at 45°C and 13% MC (rubbery state) and the other at 45° and 18% MC (fluid state).
No correlation was found between germinations of controlled deterioration treatments and the lipid-soluble tocochromanols. However, significant associations were shown for 23 dry-stored accessions. Physiological germination after ambient and cold storage revealed correlation coefficient of r = 0.51 for ✔tocopherols and of r = 0.41 for ✔tocotrienol. The water-soluble glutathione and related thiols were converted to disulphides. Dependent on the storage/deterioration regime a strong shift was analyzed towards more oxidizing intracellular conditions, especially in seeds subjected to long-term dry storage. These data suggest that the physical state achieved by the different ageing or storage conditions (glassy, rubbery or fluid) affect biochemical processes leading to seed deterioration in different ways.

We conclude that especially the amount of ✔tocopherols and/or ✔tocotrienols after dry storage can be indicative for seed germination.

9:30 - 9:45 am • **1-min Poster Previews**
Poster Program online at
http://isss2017.ucdavis.edu/wp-content/uploads/2017/08/Posters_Cumulative_Final.pdf

9:45 – 10:15 am • **Morning Break**
Monterey Bay Room

Derek Bewley Career Lecture
10:15 - 11:00 am • **Kent Bradford**
Cypress Ballroom
Sponsored by **UNITED GENETICS**

A SEEDCENTRIC VIEW OF BIOLOGY
Bradford, K.J.
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Seeds offer a unique perspective from which to view biology. An individual seed is an autonomous biological entity that must rely on its own resources (and resourcefulness) to persist after dispersal and to time its transition to germination and seedling growth to coincide with environmental opportunities for survival. At the same time, much of what interests us about seed biology is determined by the behaviors of populations of individual seeds. The percentage of seeds in a population that is in a particular state (e.g., dormant, germinated, dead) at a given time is a fundamental metric of seed biology. This duality of individual diversity underlying consistent population-wide behavior patterns has been described using population-based threshold (PBT) models. While conceptually simple, these models can describe a wide diversity of behavior of seed populations in response to temperature, water potential, oxygen, hormones, light, and aging. Recently, this seed behavior has been linked to respiratory rates of individual seeds, indicating that basic metabolic processes within seeds vary among individuals in accordance with PBT principles. As we look more broadly to microbial, plant, and animal biology, examples of cellular diversity in function, hormonal sensitivity, gene expression, and developmental responses abound. Extension of the PBT approach to describe and understand how cells function in tissues could prove beneficial in understanding regulation of developmental patterns. Extending this even further, it is possible to conceive of the interactions of transcription factors and promoters, and even of enzymes and their substrates, as representing populations of molecules with defined means and variances of expression or function that do not represent error or noise, but rather a fundamental mechanism of biological functioning. Projecting this concept to seed and plant ecology, the interactions of individuals and populations with their environments can also be described by PBT models. Examples will be provided to illustrate how concepts and quantitative approaches developed for the analysis of seed populations could be applied across biological scales from molecules to ecosystems. By demonstrating how to understand and celebrate inherent variation, a seedcentric viewpoint could lead to novel experimental approaches and mechanistic insights into the nature of biology itself.

SEED ECOLOGICAL SYSTEMS (SES)
Chairpersons: **Bob Geneve, Norman Pammenter**
Cypress Ballroom

Keynote Speakers
11:00 - 11:45 am • **Jeff Walck**
WHAT IS A GERMINATION NICHE AND HOW DOES IT RELATE TO CLIMATE CHANGE?
Walck, J. and Hidayati, S.
Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee, USA
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Knowing the range of conditions over which germination occurs for a species is vital to understanding species' responses to climate change as well as re-establishing plant populations from ex situ seed banks. From testing seeds over a range of environmental conditions, the width of this range can be determined and various terms have been coined to describe it. Germination niche, germination niche-

breadth, germination tolerance range, germination niche width, and germination envelope are some examples. The range has been described using species diversity indexes or modified versions of them (Shannon equitability index, Pielou's evenness index, Levins' B, inverse Simpson index) or by defining the absolute range between the maximum and minimum conditions of germination. While some research has focused on quantifying the width of this range, other studies have determined the cardinal (or threshold) conditions, particularly the base temperature associated with this range. Data for these studies have been based on field emergence or more commonly on laboratory conditions. Studies on these ranges for germination have been done to examine the correlation between germination width and geographic or ecological range size, with equivocal results, or to predict species' responses to climate change. However, gaps on our knowledge concerning germination niches are plentiful. First, investigations into germination niches usually focus on the germination stage and temperature responses, which make up only one part of the regeneration and establishment of a species. Second, population variation in germination niches over spatio-temporal scales is rarely considered – determination usually being limited to a single population. Third, how these measurements of germination niches can be extrapolated to natural settings remains problematic, especially when normally the range tested does not include extremes that may occur during climate change. And fourth, additional life cycle stages need to be incorporated to better understand the relationships among niche widths of stages over a broad range of species at a community level.

11:45 am- 12:30 pm • **Mark Ooi**
THE (SOMETIMES) FORGOTTEN ROLE OF SEEDS IN ECOLOGY
Ooi, M.K.J.[1]
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The life-history processes surrounding seeds are arguably the most important drivers of population persistence in many natural ecosystems around the world. To fully understand plant population dynamics, we therefore need to develop a clear picture of the contribution seed-related processes make. However, there is still a surprising lack of understanding of many of the fundamental factors surrounding seed ecology – and it appears that the role of seeds are at times forgotten. I will present studies investigating both theoretical and applied ecological questions, which highlight the importance of seeds in determining outcomes. This will include examples ranging from modelling species' extinction risk under future climates, to studies addressing the stress-gradient hypothesis and the role of nurse plants. The importance of ecological understanding to applied science will also be discussed. Here, it is not seeds that have been overlooked, as they are the focus of large-scale conservation efforts particularly in *ex situ* initiatives; but it is often the role of the ecological conditions from where the species have originated from that is the forgotten element. When trying to develop protocols for utilising seeds held in *ex situ* collections, ignoring the role that ecological processes play can have negative impacts on both the practical approach used and the costs associated with conservation.

12:30 – 1:30 pm • **Lunch**
Upper Plaza and Dolphins Ballroom

SEED MICROBIAL SYSTEMS (SMS)
Chairpersons: **Greg Welbaum, Roberto Benech-Arnold, Anne Pollard**
Cypress 1&2

1:30 - 1:45 pm • **Phil Allen**
VARIATION IN GENERAL RESISTANCE TO THE FUNGAL PATHOGEN PYRENOPHORA SEMENIPERDA IS ECOLOGICALLY SIGNIFICANT FOR BROMUS TECTORUM SEED POPULATIONS
Allen, P.S.[1], Meyer, S.E.[2], Beckstead, J.[3] and Clement, S.[2]
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Bromus tectorum is a selfing winter annual grass that is highly invasive in the western United States. The generalist seed bank pathogen Pyrenophora semeniperda can kill B. tectorum seeds in the seed bank, with highest in situ mortality typically observed with dormant seeds and where autumn precipitation is unreliable. Inoculum augmentation in contrasting habitats resulted in near-complete mortality at sites where P. semeniperda was less abundant, but higher seed survival where the

pathogen naturally occurred at high levels. This suggested selection on genetic variation in general resistance to pathogen attack. We tested two hypotheses. First, host genotypes from sites where high seed mortality coincides with occasional stand failure (i.e., necessitating stand recovery from the seed bank) will be more resistant to the fungus. Second, seeds in the carryover seed bank are more likely to belong to resistant lines than seeds produced on current-year plants from the transient seed bank. Bromus tectorum SNP haplotypes from contrasting habitats were subjected to laboratory experiments in which dormant seeds were inoculated with low levels of the fungus. General resistance was greatest in haplotypes from salt desert sites where the pathogen is abundant and lowest in haplotypes from montane or warm desert sites with lower levels of disease. Seeds from current-year plants and from the carry-over seed bank were used to produce 100 lines of each group from two cold desert sites, one with high disease levels and a history of stand failure and one with lower levels of seed bank diseases. These 400 lines were SNP-genotyped to identify host lineages overrepresented in either the current stand or the seed bank. Seeds of eight lines of each lineage were included in laboratory inoculation tests with dormant seeds inoculated at low levels. For the site with low field disease levels, lineages from current-year and seedbank seed populations did not differ in pathogen resistance. However, seedbank lineages from the die-off-prone site were significantly more resistant to the pathogen than lineages from current-year seeds at this site. These results confirm both hypotheses, and indicate that selection on genetic variation in general resistance has significant ecological consequences in this pathosystem.

1:45 - 2:00 pm • **Roberto Benech-Arnold**

MICROFLORA RESPIRATION AND SENSITIVITY TO HYPOXIA ARE INSTRUMENTAL FOR PERICARP-IMPOSED DORMANCY EXPRESSION AT HIGH INCUBATION TEMPERATURE IN SUNFLOWER (*Helianthus annuus*)

Benech-Arnold, R.L.[1,2], Dominguez, C.P.[1,2*], Rodriguez, M.V.[2] and Batlla, D.[2,3]

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Dormancy displayed by sunflower achenes cause serious problems to the seed industry. In this crop, dormancy can be imposed by the pericarp. Pericarp-imposed dormancy in sunflower is expressed at high incubation temperatures (i.e. 30 °C). This embryo-covering tissue is thought to interfere with the entry of oxygen to the embryo (i.e., hypoxia). We used an inbred line and a commercial hybrid, both displaying pericarp-imposed dormancy, to evaluate the effect of different atmospheric oxygen concentrations combined with different incubation temperatures (12 °C and 30 °C) on i) germination of achenes and seeds (i.e. without pericarp) in both genotypes and ii) sensitivity to ABA, in seeds of the inbred line. We also attempted to elucidate the nature of the constraint to oxygen diffusion imposed by the pericarp. Results showed that, in both genotypes, the germination of achenes decreased with increasing hypoxia at both temperatures. Seeds from the inbred line were very sensitive to hypoxia at 30ºC but not so sensitive at 12ºC. Instead, seeds from the commercial hybrid displayed very low sensitivity to hypoxia at any temperature. Seed sensitivity to ABA in the inbred line also increased with hypoxia and incubation temperature. Oxygen consumption by isolated pericarps was enhanced at 30 °C as compared to 12 °C. However, when a phytotherapy treatment (fungicide + antibiotics) was applied to isolated pericarps from both genotypes incubated at 30 °C, oxygen consumption was reduced dramatically showing that it was mostly due to respiration of pericarp-associated microbiota. Moreover, the phytotherapy treatment increased germination of achenes from the commercial hybrid and the inbred line, strongly suggesting that high oxygen consumption at high incubation temperature due to enhanced microbial respiration limits achene germination even in the commercial hybrid where sensitivity to hypoxia is so low. In conclusion, although in both genotypes the nature of the constraint to oxygen diffusion appears to be the same (i.e. enhanced microbial respiration with increased incubation temperature), in the inbred line dormancy expression seems to be driven by an exacerbated sensitivity to hypoxia, whereas in the commercial hybrid, the pericarp arises as a more severe restraint when incubation is performed at high temperature.

2:00 - 2:15 pm • **James Hourston**

WITH OR WITHOUT FUNGI? FRUIT FRACTURE BIOMECHANICS OF LEPIDIUM DIDYMUM GERMINATION

Sperber, K.[1a], Steinbrecher, T.[2a], Graeber, K.[2], Scherer, G.[1], Clausing, S.[1], Wiegand, N.[1], **Hourston, J.E.**[2], Kurre, R.[3], Leubner-Metzger, G.[2b*] and Mummenhoff, K.[1b*]

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Plant/microbe interactions can play a huge role in the success or failure of plants at a crucial and vulnerable life stage. Examples of plants and microbes forming mutualisms which can improve germination are difficult to study and consequently rarely reported. We present evidence of an interaction between species of common fungi and the hard fruits of Lepidium didymum (Brassicaceae) which is fundamental to survival in distinct environments. Dispersal of fruits with hard pericarps (fruit coats) encasing seeds has evolved many times independently within taxa which have seed dispersal as their default strategy. The mechanisms by which the constraint of a hard pericarp determines germination timing in response to the environment are currently unknown. Here we show that the hard pericarp of Lepidium didymum controls germination solely by a biomechanical mechanism which is targeted by the fungi. We demonstrate fungal-mediated erosion of the pericarp tissues in a process guided by plant tissue architecture. Lepidium didymum's hard pericarp acts as a mechanical barrier to delay germination, but it does not restrict water uptake or gas exchange. Mechanical dormancy is conferred by preventing full imbibition of the encased non-dormant seed. The lignified endocarp has biomechanically and morphologically distinct regions which serve as predetermined breaking zones. This pericarp-imposed mechanical dormancy is released by the activity of common fungi which weaken these zones by degrading non-lignified pericarp cells. We propose that the hard pericarp with this biomechanical mechanism contributed to the global distribution of this species in distinct environments and in association with the global distribution of the common fungi. Therefore, the fungal colonisation of fruits leads to a much faster onset and higher maximum germination as it effectively breaks this pericarp-imposed dormancy.

2:30 - 2:45 pm • **Satriyas Ilyas**

NOVEL SEED TREATMENT WITH RHIZOBACTERIA ON HOT PEPPER (*Capsicum annuum* L.): ITS EFFECTS ON RHIZOBACTERIA AND SEED STORABILITY, PLANT GROWTH, AND INCIDENCE OF PHYTOPHTHORA BLIGHT

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Novel seed treatments, biopriming and seed coating, using rhizobacteria have been developed to overcome phytophthora blight in hot pepper. The disease is caused by Phytophthora capsici, a seed- and soil-borne fungal pathogen. Effects of biopriming on controlling phytophthora blight (PB) and improving plant growth in the greenhouse (experiment 1), and effects of biopriming and seed coating in the field (experiment 2) were evaluated. Effects of both treatments were also evaluated on the viability of seed and rhizobacteria during storage (experiment 3). All experiments were carried out on hot pepper seed cv. Laris which is susceptible to P. capsici. Results of experiment 1, bioprimed for 24h with F2B1 or combination of E1+F2B1 isolates improved plant height 14-21 days after transplanting (DAT), and reduced PB from 13.3% in positive control (seeds were soaked in potato dextrose broth without rhizobacteria, and the resulted plants were inoculated with P. capsici inoculum soil) down to 6.1% at 5 days after inoculation (DAI) of P. capsici inoculum soil. Seed treatment with metalaxyl was not effective to control PB. While in experiment 1 all rhizobacteria used were isolated from healthy hot pepper plants among the PB infected ones, in Experiment 2 and 3 only the best (E1+F2B1) was used and rhizobacteria isolates from Piper nigrum were also included. Biopriming with E1+F2B1 increased plant height compared to negative control at 25 DAT. Seed coating plus E1+F2B1 increased leaf number at 18 and 25 DAT compared to negative and positive controls. Seed coating plus ST116B or E1+F2B1 or biopriming with E1+F2B1 reduced disease incidence 20-22.5% compared to metalaxyl (100%) at 17 DAI. Biopriming and seed coating with rhizobacteria maintained seed viability (79 - 89%) for 24 weeks stored at 27–30^{0}C compared to metalaxyl (54.3%). Biopriming with E1+F2B1 isolates resulted the

highest seed vigor index after 24 weeks of storage. Population of rhizobacteria in seed tissue decreased in bioprimed seeds from 105-107 cfu g-1 to 104 cfu g-1 after being stored for 24 weeks. Population of rhizobacteria in coated seeds was only 104 cfu g-1 at start of storage and remained the same at the end of storage period.

2:45 - 3:00 pm • **Anne Pollard**

BIOCHEMICAL AND MOLECULAR REGULATION OF DEFENSE RESPONSES TO A PATHOGENIC SOIL FUNGUS IN DORMANT SEEDS

Pollard, A.T.[1], Okubara, P.[2], James, M.[1] and Fuerst, E.P.[1,3]
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Dormancy and decay resistance of weed seeds in the soil seedbank challenge long-term weed management. Dormant seeds employ various physical and chemical defenses to inhibit seed decay pathogens, but little is known about biochemical seed defenses.

We evaluated activities of the defense enzymes polyphenol oxidase, peroxidase, exochitinase, and oxalate oxidase produced by dormant wild oat (Avena fatua) and wheat (Triticum aestivum) seeds in response to the pathogenic soil fungus Fusarium avenaceum isolate 'F.a.1'. Caryopsis decay was scored with a visual assessment and quantitative real-time PCR was used to evaluate seed defense responses at the transcriptional level. Additionally, quantitative PCR was used to measure the extent of F.a.1 infection in seeds and soil at various time points. Activities of the degradative fungal enzymes protease and xylanase were measured in soil and wild oat caryopses throughout the course of infection. Experiments were conducted both in vitro on agar plates and in soil.

We found that F.a.1 induced the seed defense enzymes polyphenol oxidase, peroxidase, and exochitinase, but inhibited activity of oxalate oxidase. Moreover, wild oat and wheat seed responses to F.a.1 infection were similar qualitatively, but different quantitatively. Preliminary qRT-PCR results indicate that F.a.1 induces polyphenol oxidase, exochitinase, and NADPH oxidase, but not oxalate oxidase, at the transcriptional level in wild oat and/or wheat. Fungal abundance in seeds and soil and activities of F.a.1-derived degradative enzymes increased over time of infection.

Our results suggest that biochemical and molecular defense responses may contribute to the prolonged survival of wild oat and other weed species in the soil seedbank. These results warrant further investigation of fungal-induced seed decay as a potential alternative approach to managing the dormant weed seedbank in the soil.

2:45 - 3:00 pm • **Buzi Raviv**

THE DEAD, MATERNALLY DERIVED ORGANS ENCLOSING EMBRYOS FUNCTION AS LONG TERM STORAGE FOR ACTIVE PROTEINS AND NUTRIENTS WHICH SUPPORT SEEDLING ESTABLISHMENT

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In angiosperms, the embryo is dispersed from the mother plants surrounded by remnants of the mother reproductive organs such as indehiscent fruits and seed coats. For instance, in dicots, the integument layers will form the seed coats; in wild grasses, the dispersal units consist of the caryopsis surrounded by the dead floral bracts. The maternally derived organs (MDO) of grasses are undesired in agriculture but their adaptive value has not been fully explored. We investigated the proposal that the MDO of seeds and dispersal units have been evolved to function more than just means for physical embryo protection. We showed that dead floral bracts of Wild Emmer Wheat (*Triticum turgidum* var dicoccoides) store and release upon hydration active hydrolases including nucleases and chitinases, which maintain activity years after the mother plant dies. Proteome and ICP analysis revealed multiple oxidative and pathogenesis stress related proteins and nutrients that are released upon hydration. Further analysis showed that although germination from the intact dispersal unit of wild emmer wheat was delayed, post germination growth was better than that of separated caryopses. In some of the studied dicot species, seed coats and pericarps exhibited microbial growth control activity of the seed coat extracts, even after years of storage in uncontrolled conditions. Thus, our study show that the dead, MDO enclosing the embryo store active hydrolases and other substances that can engineer the

micro environment of the germinating seed; support seed persistence in the soil, serve as a first line of defense during germination and increase seedling establishment.

SEED DEVELOPMENT SYSTEMS 2 (SDVS-2)
Seed Maturation and Dormancy
Chairpersons: **Ramin Yadegari, Julia Buitink, Alexandre Marques**
Cypress 3&4

1:30 - 1:45 pm • **Xing-You Gu**
EVOLUTION OF EMBRYO DORMANCY INVOLVED LOCAL GENE DUPLICATION, RECOMBINATION HOTSPOT FORMATION, AND ADAPTATION TO CHANGING SEED DEVELOPMENT TEMPERATURES IN *ORYZA*
Gu, X.-Y. and Feng, J.
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Seed dormancy (SD), a key adaptive trait of both ecological and agricultural importance, can be imposed by physiological/development-related factors in the embryo (embryo dormancy), or enforced by the embryo-surrounding tissues. Natural differentiation of SD between conspecific weedy and cultivated rice (*Oryza sativa* L.) was associated with quantitative trait loci (QTL). Some of the QTL alleles isolated from a tropic ecotype of weedy rice are related to specific dormancy types, such as *qSD12* for embryo dormancy. *qSD12* is the largest QTL, regarding to the size of main effect on germinability reported for cultivated, weedy and wild rice (*O. spp.*). This major QTL also interacted with other SD loci and with environmental factors (G-by-E) to influence the degree of primary dormancy. *qSD12* was further dissected into three physically tightly linked loci (*SD12a*, *b* & *c*), with each having an independent effect and two or three together having cumulative effects on the duration of SD. *SD12a* and *c* are annotated as closely related bHLH family transcription factors, while *SD12b* encodes a protein of unknown molecular function. The *SD12*s (*SD12a*, *b* & *c*) were highly expressed in 10-d embryos and influenced transcriptional levels of heat shock protein and other stress-responsive genes. *SD12a* and *c* were involved in G-by-E interactions, resulting in enhanced dormancy in relatively high temperature environments during seed development. Both *SD12a* and *c* have a local duplicate flanked or separated by tandem repeats of transposable elements, but these bHLH duplicates had no phenotypic effect. This multigenic QTL region is equipped with two recombination hotspots, which increased recombination fractions between *SD12*s and between *SD12s* and their flanking regions. Corresponding to the presence of recombination hotspots, the haplotype for functional alleles at all *SD12*s is present only in some tropical ecotypes of wild or weedy rice from Southern Thailand. This research advanced our understanding on the origin, genome organization and functional differentiation of natural genes controlling embryo dormancy, and provided novel genes to manipulate germinability in crop breeding.

1:45 – 2:00 pm • **Jose Maria Barrero**
ARABIDOPSIS *PM19-LIKE 1* GENE IS INVOLVED IN DORMANCY AND GERMINATION AND IS LOCALISED IN COTYLEDON CELLS
Barrero, J.M., Dorr, M., Talbot, M., White, R. and Gubler, F.
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Increasing seed dormancy is a major goal for cereal breeders around the world, because the premature germination of grains before harvest is a serious problem in all wheat growing regions. This problem arises because of the lack of grain dormancy in modern wheat varieties which produce grain prone to germinate on the mother plant following rain. Recently, we have characterised two wheat genes, *TaPM19-A1* and *A2*, finding that their expression positively correlates with grain dormancy. Although a role for these genes in regulating grain dormancy is clear, the function of AWPM19 (ABA-induced wheat plasma membrane) gene family is unknown. The expression of *PM19* genes is under the control of the hormone abscisic acid (ABA), which is a well-known dormancy promoting factor. Previous studies in wheat indicated that *PM19* encodes a protein localised in the plasma membrane, and other indirect studies in Arabidopsis suggested that could also be found in the seed oil bodies. We have taken advantage of the model plant Arabidopsis to study the function of this class of genes. In this work we have identified the Arabidopsis orthologous gene *PM19-Like 1* (*PM19L1*) and demonstrated that knockout mutations alter seed dormancy. Not only primary dormancy but also secondary

dormancy in response to high temperature was increased by the *PM19L1* mutation. We have used transgenic plants to over-express or repress the expression of *PM19L1,* which resulted in a decrease or an increase in seed dormancy, respectively. We have also investigated the function of *PM19L1* by localising the PM19 protein to plasma membranes of cotyledon cells in Arabidopsis seeds. The function of PM19 could be related to the control of dormancy by changes in membrane characteristics in response to temperature.

2:00 - 2:15 pm • **Steven Penfield**

NATURAL VARIATION IN THE MATERNAL CONTROL OF SEED DORMANCY VIA A COMPLEX ENDOSPERM-EXPRESSED PHD FINGER TRANSCRIPTION FACTOR LOCUS

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Dormancy and dispersal are frequently linked characters which enable the mother plant to optimize the behavior of her progeny, spreading their germination over space and time. Given the ecological importance attached to the maternal control of seed dormancy, we optimized a Genome Wide Association Study to capture maternal environmental effects on seed dormancy. In addition to detecting previously known large effect loci at both *Delay Of Germination 1* (*DOG1*) and *DOG6*, we found an additional locus on chromosome 2 specifically associated with regulation of germination after maturation at lower temperatures. This locus contains two PhD-finger transcription factors which are only expressed in the endosperm during early seed development, and conserved haplotypes correlating with primary dormancy implicated both genes in seed dormancy control. Loss of function analysis showed that this locus acts maternally to promote the development of strongly dormant states, and also to prevent high frequency seed abortion as previously observed in the endosperm-specific Polycomb Repressive Complex 2 (PRC2) *medea* mutant. Taken together, our data show that natural variation in the maternal control of germination exists in Arabidopsis, and suggest that a key mechanism is through maternal repression of gene expression in the endosperm.

2:15 - 2:30 pm • **FeiYian Yoong**

GENOTYPIC TOLERANCE OF LETTUCE SEEDS TO THERMOINHIBITION IS ASSOCIATED WITH EARLY CELL WALL MODIFICATION IN THE MICROPYLAR ENDOSPERM AND EXPRESSION OF MULTIPLE MANNANASE GENES

Yoong, F.Y.[1,2]**,** Hill, T.[1], Ma, S.[3], Souza, G. A.[1], Reyes Chin-Wo, S.[4], Huo, H.[1,5], Froenicke, L.[4], Elmore, M.[4], Michelmore, R.W.[1,4] and Bradford, K.J.[1]
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Lettuce (*Lactuca sativa*) seeds (e.g., cv. Salinas) usually exhibit thermoinhibition (failure to complete germination) when imbibed at temperatures above 28°C. Genetic and molecular studies with an accession of *L. serriola* (US96UC23), seeds of which can germinate up to 37°C, showed that *LsNCED4* (*9-cis-EPOXYCAROTENOID DIOXYGENASE 4*) encoding an enzyme in the ABA biosynthetic pathway is a key regulator of thermoinhibition. Silencing of *LsNCED4* in the Salinas background (*LsNCED4*-RNAi) enabled germination at high temperatures. Seeds of a primitive *L. sativa* accession (PI251246) also germinate at temperatures up to 33°C, which mapping and physiological studies attributed to modified regulation of the *LsERF1* (*ETHYLENE RESPONSE FACTOR* 1) gene. Lettuce seeds (achenes) have a two-cell-layered endosperm and a fused testa/pericarp enclosing the embryo, of which the endosperm is the primary restraint on radicle emergence. Examination of developing and mature seeds revealed no anatomical alterations in the tissues enclosing the embryo of PI251246 and *LsNCED4*-RNAi seeds versus Salinas seeds. At permissive temperatures for germination, structural modifications of the micropylar endosperm immediately adjacent to the radicle tip were evident within 6 h of imbibition. Cell walls of this endosperm region stained brightly with calcofluor white (indicating cellulose), while adjacent lateral endosperm cell walls did not stain, indicating that galactomannan storage carbohydrates in the cell walls are degraded by mannanase prior to radicle emergence in the micropylar endosperm. This change in cell wall structure did not occur in Salinas seeds imbibed at a thermoinhibitory temperature. Previous studies had identified *LsMAN1* encoding a mannanase that

appeared only after radicle emergence in the lateral endosperm of lettuce seeds. However, biochemical studies had detected mannanase activity in the micropylar endosperm tightly bound to the cell walls prior to radicle emergence. Analysis of RNASeq data identified *LsMAN1* with delayed expression following imbibition, but also a distinct gene encoding a mannanase (*LsMAN2*) whose expression is already elevated at 6 h of imbibition only in seeds imbibed at permissive temperatures for germination. Therefore, as also occurs in tomato seeds, distinct mannanase genes are expressed in a tissue-specific temporal sequence to regulate weakening of the micropylar endosperm and subsequent mobilization of reserves in the lateral endosperm.

2:30 - 2:45 pm • **Julia Buitink**
A ROLE FOR AUXIN IN THE ACQUISITION OF LONGEVITY DURING SEED DEVELOPMENT
Buitink, J., Pellizzaro, A., Righetti, K., Lalanne, D., Ly, Vu B. and Leprince, O.
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Besides the well-studied regulatory pathways involved in embryogenesis and seed filling, additional pathways are activated during late maturation that confer to the seeds the remarkable capacity to survive almost complete desiccation. This trait is an important factor in the preservation of seed viability and quality during dry storage. Seed longevity is an essential parameter to ensure fast and homogenous seedling establishment to ensure high yield. Using a systems biology approach, integration of phenotypic data on longevity in a gene co-expression network obtained on seed maturation in *Medicago truncatula* led to the isolation of a gene module linked to longevity. Cross-species comparison revealed that genes in this module also show high connectivity in Arabidopsis, and one of the characteristics of this module was the high enrichment in genes related to auxin for both species. Recent progress in the development of auxin-responsive sensors and mutants defective in auxin biosynthesis in Arabidopsis makes it possible to tackle the complex role of auxin in seed maturation with temporal and spatial precision. Here, we will present experimental evidence showing that dynamic changes in auxin responses during seed maturation are genetically linked to seed longevity and ABA signaling controlling the survival in the dry state.

2:45 - 3:00 pm • **TBD**

3:00 – 3:30 pm • **Afternoon Break**
Monterey Bay Room

SEED DORMANCY SYSTEMS 1 (SDRS-1)
Chairpersons: **Camille Steber, Steven Penfield**
Cypress 3&4

3:30 - 3:45 pm • **Wirat Pipatpongpinyo**
COEVOLUTION BETWEEN SEED DORMANCY AND AWN IN WEEDY RICE
Pipatpongpinyo, W., Feng, J., Ye, H. and Gu, X.-Y.
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The seed dormancy (SD) and awn traits are both of adaptive significance in grass species. Awn, a needle-like appendage extended from the lemma of a floret, promotes seed dispersal and movement into soil, while SD regulates the timing of germination so that favorable genotypes could complete the life cycle in local ecosystems. This research aimed to address co-evolutionary relationship between the two adaptive traits using a genetic system developed from conspecific weedy and cultivated rice (*Oryza sativa*). The SD degree was correlated with the awn length (AL) in various ecotypes of weedy rice, with awned plants tended to have stronger dormancy. The correlation was accounted for by a few haplotype blocks, where a linkage disequilibrium (LD) between the SD and AL quantitative trait loci (QTL) could not be broken by >10 generations of phenotypic selection for delayed germination. Fine mapping of the *SD8/AL8* haplotype delimited the LD to a genomic sequence of <1 Mb, which accounted for 27% and 64% of the variances for SD and AL, respectively, in a near-isogenic background. Both *SD8* and *AL8* interacted with the QTL *SD1-2* in similar patterns, resulting in reduced dormancy or awn length. *SD8* was further dissected from *AL8*. *AL8* was collocated with *RAE2/GAD1*, a gene with pleiotropic effects on grain length, grain number and awn development in wild rice (*O.*

rufipogon). However, *AL8* has only the effect on AL, and differs in functional mutations from the *RAE2/GAD1* alleles reported for the wild relative. In summary, this research provided evidence that the SD and awn traits co-evolved and tight linkage is a genetic mechanism underlying the coevolution. The research also suggested that some functional changes occurred to the linked genes during evolution.

3:45 - 4:00 pm • **Françoise Corbineau**

ETHYLENE REGULATES ARABIDOPSIS SEED DORMANCY VIA A CROSSTALK WITH GIBBERELLINS AND ABSCISIC ACID AND THE N-END RULE PATHWAY

Yesbergenova-Cuny, Z., Wang, X., Biniek, C., Bailly, C. and **Corbineau F.**
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Arabidopsis seed dormancy corresponds to an inability to germinate in darkness at temperature higher than 10°C, and its regulation by the hormonal balance between abscisic acid (ABA) and gibberellins (GAs) is well documented. Ethylene (10-100 ppm) also strongly stimulates the germination of dormant seeds placed at 25°C. All the mutants affected in the ethylene signalling pathway (etr1, ein2, ain1, ein4 and erf) are dormant since they do not germinate at 25°C, but easily germinate at 15°C, except etr1, which is more dormant, suggesting the involvement of ethylene in the regulation of dormancy. Three to 4 days of stratification at 4-5°C promote germination at 25°C of all mutants including the ethylene insensitive ones (etr1, ain1, ein2), suggesting that cold can act directly on germination by bypassing the ethylene signalling pathway although it induces an accumulation of ETR1, EIN4, EIN2 and AIN1 transcripts. Two mutants (ain1 and etr1) that do not respond to exogenous ethylene, do not also respond to GA3, but GA3 synergically acts with exogenous ethylene in ain1 mutant. Germination at 15°C is inhibited by ABA, etr1 being the highest sensitive, suggesting a crosstalk between ABA and ethylene. This inhibitory effect of ABA is reversible by exogenous ethylene. Stronger dormancy of etr1 and ain1 mutant seeds, and low responsiveness to ethylene of Cyp 707A1 mutant confirm a crosstalk between ABA and ethylene at the level of ABA catabolism. Seed responsiveness to ethylene is regulated by the N-end rule pathway of the proteolysis, since mutants affected in this pathway (PRT6, ATE1-ATE2) do not respond to this hormone. Using mutants affected in the ERF-VII (Rap2.2, Rap 2.3, Rap2.12, Hre1, and Hre2) potential substrate of the N-end rule pathway, the involvement of this pathway in the regulation of seed dormancy is discussed as related to ethylene signalling.

4:00 - 4:15 pm • **Heqiang Huo**

DOG1* REGULATES BOTH SEED DORMANCY AND FLOWERING THROUGH *MicroRNA156

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The life cycle of annual flowering plants is marked by distinct developmental phases. It initiates with seed germination and progresses through juvenile, adult, flowering, embryogenesis and seed maturation phase transitions. The transitions from seed dormancy to germination and from adult to flowering are coordinately regulated by genetic and environmental factors as they strongly affect the success of establishment, reproduction, colonization and adaptation of plant populations. The *DELAY OF GERMINATION1* (*DOG1*) gene is involved in regulating seed dormancy in response to temperature during seed maturation and has also been associated genetically with pleiotropic flowering phenotypes across diverse *Arabidopsis thaliana* accessions and locations. Here we show that *DOG1* is involved in regulating both seed dormancy and flowering times in lettuce (*Lactuca sativa*) and Arabidopsis through an influence on levels of microRNAs miR156 and miR172. In lettuce, suppression of *LsDOG1* expression enabled seed germination at high temperature and promoted early flowering in association with reduced miR156 and increased miR172 levels. Overexpression of *MIR156* (gene encoding miR156) under the CaMV35S promoter in lettuce caused extremely late flowering, whereas sequestering miR156 resulted in early flowering and loss of lettuce seed dormancy, as evident in preharvest sprouting. In Arabidopsis, higher miR156 levels resulting from overexpression of the *MIR156* gene enhanced seed dormancy and delayed flowering. These phenotypic effects, as well as conversion of *MIR156* transcripts to miR156, were compromised in *DOG1* loss-of-function mutants but were enhanced in *DOG1* gain-of-function mutants. As miR156 suppresses expression of miR172, bypassing this inhibition by overexpression of *MIR172* reduced seed dormancy and promoted early flowering in Arabidopsis, and the effect on flowering required functional DOG1. Transcript levels of several genes associated with miRNA processing were consistently lower in dry seeds of Arabidopsis and lettuce when *DOG1* was mutated or its expression was reduced; in contrast, transcript levels of

these genes were elevated in a *DOG1* gain-of-function mutant. DOG1 apparently mediates environmental influences during seed maturation involved in the resetting of high miR156/low miR172 levels during embryogenesis that subsequently influence both the progression of seeds from dormancy to germination and of plants from adult to flowering phase.

4:15 - 4:30 pm • **Gonda Buijs**

A METHOD TO ACCELERATE DORMANCY RELEASE IN ARABIDOPSIS THALIANA THAT MIMICS DRY STORAGE ON THE GENETIC LEVEL

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The seed life span stretches from when the seeds reach maturity on the mother plant until the seed is not viable enough anymore to germinate. At the start of the seed life span, seeds possess a genotype specific level of dormancy. A dormant seed is viable but will not germinate even though the environment is favourable. The genetic basis for seed dormancy is well defined: Quantitative Trait Loci (QTL) for seed dormancy have been identified (*DELAY OF GERMINATION*) but thus far only for *DOG1* an underlying gene has been identified.

Arabidopsis thaliana is a wide-spread species of which accessions have been collected from very different natural habitats. The level of dormancy can vary tremendously between these different accessions. The accessions often used in research have low dormancy (Colombia-0, Landsberg *erecta*), but some accessions (Cape Verde Islands, Iberian Peninsula populations) can remain dormant for over a year during dry storage (after-ripening, AR). Studying these deep dormant accessions will aid in seed dormancy research, but is limited due to time constrains.

In seed dormancy and longevity research, treatments to artificially accelerate the seed life span are used. These treatments usually comprise storage at high temperatures and high relative humidity or fully imbibed (stratification). However, these artificial treatments do not always mimic dry seed storage (AR) at a genetic level. The alleviation of dormancy during dry seed storage is thought to be mainly caused by the oxidation of seed proteins and mRNAs. Based on the role of oxidation in the dormancy release and ageing process, we use the EPPO (Elevated Partial Pressure of Oxygen) system to accelerate dormancy release. In the EPPO system, seeds are stored under increased ambient air and thus increased oxygen pressure. EPPO has already shown to accelerate seed ageing. Here, we show that also dormancy release can be accelerated and mimicked in a non-invasive manner by the EPPO system. We used a genetics approach to reveal whether EPPO dormancy release occurs by the same mechanisms as seed dry AR.

4:30 - 4:45 pm • **Giles Grainge**

HARNESSING THE POWER OF LIGHTING – A MOLECULAR APPROACH TO UNDERSTANDING HOW GAS PLASMA ACTIVATED WATER (PAW) ALLEVIATES THE PHYSIOLOGICAL DORMANCY OF *ARABIDOPSIS THALIANA*

Grainge, G.[1], Steinbrecher, T.[1], Nakabayashi, K.[1], Iza, F.[2], Sharma, G.[2], Dunford, A.[3], Kennedy, S.[3] and Leubner-Metzger G.[1]
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It is a priority for seed companies to establish uniform seedling emergence within a broad range of abiotic conditions. To achieve this goal, the industry actively pursues the development of innovative seed treatments, and the emergence of utilizing gas plasma is drawing attention. The formation of non-thermal atmospheric gas plasma at a gas-aqueous interface results in the production of many transient reactive species (OH·, NO_2·, NO radicals) and more stable compounds (H_2O_2, NO_3^-, NO_2^-). Several of these chemicals synthesised in gas plasma activated water (PAW) are known to affect seed germination and dormancy, and therefore open an intriguing area of study. Previous reports on PAW have shown significant improvement in germination speed of mung bean and increased tolerance to both salt and osmotic stress, however, no underlying mode of action has been ascertained. This work looks at deciphering which constituent chemicals of the PAW are critical to seed germination performance and whether this is linked with dormancy alleviation of seeds. The stable nitrogen anions,

nitrate and nitrite, act through increasing the GA/ABA ratio via activating the nuclear bound transcription factor nin-like protein 8 (NLP8). Nitric oxide is a known dormancy breaking radical which functions as part of the N-rule pathway regulating ethylene response factors. Hydrogen peroxide is an established intercellular signalling molecule associated with dormancy alleviation, and the more reactive, oxygen species O_2^- and $OH^·$ are critical for endosperm weakening. Here we show that PAW can break the dormancy of *Arabidopsis thaliana* ecotype C24, and describe the effects this has on the expression of genes implicated in the germination regulatory pathways that are influenced by NO_3^-, NO, H_2O_2. Furthermore, mutant studies were conducted to reveal which specific underpinning mechanisms PAW influences. Improving our understanding of this novel technology aids the process of establishing its true potential as a new agri-technology. If the improvements to seed germination performance are optimized and shown to be conserved amongst crops seeds, the environmentally friendly aspect and practically of PAW technology would be an enticing prospect for the seed industry.

4:45 - 5:00 pm • **Guillaume Née**

DELAY OF GERMINATION1 REQUIRES PP2C PHOSPHATASES OF THE ABA SIGNALLING PATHWAY TO CONTROL SEED DORMANCY

Née, G.[1,2], Kramer, K.[3], Kazumi Nakabayashi, N.[1], Yuan, B.[1], Yong Xiang, Y.[1,4], Emma Miatton, E.[1], Iris Finkemeier, I.[2,3] and Soppe, W.J.J.[1,5]
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Seed germination is a major decision point in the life of plants determining future growth and development. This timing is controlled by seed dormancy, which prevents germination under favourable conditions. The plant hormone abscisic acid (ABA) and the protein DELAY OF GERMINATION 1 (DOG1) are essential regulators of dormancy. The function of ABA in dormancy is rather well understood, but the role of DOG1 is still unknown. The connection between ABA pathway and DOG1 pathway has been mentioned as an outstanding question in seed biology. Using affinity purification from functionally complemented *dog1* lines and proteomics based methods, we identified more than 100 proteins that complex with DOG1 in seeds. Among them, we identified four phosphatases from which two belong to clade A of type 2C protein phosphatases: ABA-HYPERSENSITIVE GERMINATION 1 (AHG1) and AHG3. BiFC interaction studies suggest that DOG1 interact specifically with a subset of PP2C including AHG1 that is specifically expressed in seeds and has been shown to be immune to inhibition by ABA and the ABA receptors of the PYR/PYL/RCAR family. Genetic analysis demonstrated that AHG1 and 3 have redundant but essential roles in the control of seed dormancy and that DOG1 works upstream of this phosphatases since the phenotype of the double *ahg1 ahg3* mutant is fully epistatic to the phenotype of *dog1* in a triple *dog1 ahg1 ahg3* mutant. In addition, the genetic analysis indicates that DOG1 negatively influence the action of AHG1 and 3 regarding seed dormancy. We propose that the ABA and DOG1 dormancy pathways converge at clade A of type 2C protein phosphatases.

SEED SCANNING SYSTEMS (SSS)

Chairpersons: **Alan Taylor, Gerhard Leubner, Maor Matzrafi**
Cypress 3&4

3:30 - 3:45 pm • **Sebastian Blunk**

THE EFFECT OF SEED ENHANCEMENT TECHNOLOGIES ON THE GERMINATION OF SUGAR BEET QUANTIFIED BY X-RAY COMPUTED TOMOGRAPHY

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With an increasing demand for food production, there is a need to increase crop yield and efficiency. Seed enhancement technologies (e.g. seed pelleting, seed coating and seed priming) are important for ensuring consistency in crop yield, however, their efficacy has mainly been investigated in laboratory conditions excluding soil or under field conditions evaluating the yield. The application of X-ray Computed Tomography (X-ray CT) allows a non-destructive and temporal quantification of the germination process in soil to understand the interactions between the soil and the surrounding soil matrix.

In this study, we used X-ray CT to quantify the differences between applied seed enhancement technologies (naked, coated, pelleted and pelleted + coated) prior to sowing, checking on the spatial distribution of applied seed coating materials (e.g. wood, clay, pesticides). Furthermore, we analysed the effect of a wide range of treatments on sugar beet seed germination during the early growth stage (e.g. first 4 days). The results indicated coated seeds had a slower initial growth rate in comparison to the other treatments, although the growth rate rapidly increased after emergence so that all treatments were similar by day 4. The pelleting treatment, used to improve sowing accuracy when the seeds, showed a steady increase in root growth whereas the naked treatment expressed a constant rate after day 2. The pre-germination priming treatment accelerated the growth rate of all treatments significantly over a growth period of 14 days compared to the non-primed treatments regardless of physical enhancements applied on the outer surface.

With this study, the suitability of X-ray CT for quantification and visualisation could be verified and the germination process of enhanced sugar beet seeds *in situ* observed. Furthermore, it was possible to quantify physical alterations of seed enhancements and the distribution of materials within coatings and pelleting. This study contributes towards the selection of appropriate seed enhancement technologies to ensure maximum yield and consistency.

Keywords: Sugar beet, germination, seed enhancement, X-ray Computed Tomography

3:45 - 4:00 pm • **Tina Steinbrecher**

NON-DESTRUCTIVE 3D VISUALIZATION OF SEEDS USING SYNCHROTRON RADIATION X-RAY TOMOGRAPHIC MICROSCOPY: NEW INSIGHTS INTO BRASSICACEAE SEED COAT STRUCTURES

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Potent high-resolution scanning technologies are currently being adapted to visualize seeds and reveal their internal structures. These technologies provide new tools to identify seed diseases and to give new insights into the seed's internal structural morphology. The aim of imaging in biology is to localize and analyze structures in their native state. Conventional microscopy often requires fixation, dehydration and thin-sectioning of the samples. In contrast, hard X-rays with an energy of 10–100 keV are highly penetrating and provide non-destructive 3D visualization, high resolution quantitative investigations of biological samples. Synchrotron facilities like the Swiss Light Source (SLS) at the Paul Scherrer Institute bring tremendous advantages compared to traditional X-ray tomography with significantly higher resolution, short acquisition times, a better signal-to-noise ratio and quantitative reconstructions.

The seed coat represents the interface of the seed with the external environment and is an important component in the germination process as well as a key determinant of seed quality. Proanthocyanidins (PAs) are complex flavonoid compounds which confer a brownish color to the seed coat. Alterations of PAs can result in abnormal germination, loss of dormancy and premature sprouting. We used Synchrotron Radiation X-ray Tomographic Microscopy (SRXTM) at the beamline for TOmographic Microscopy and Coherent rAdiology experimentTs (TOMCAT) at the SLS to study the seeds of a unique set of distinct *Lepidium sativum* (Brassicaceae) accessions and the dimorphic seeds of *Aethionema arabicum* (Brassicaceae). The *Lepidium* accessions differ in their seed coat properties and PA contents leading to distinct germination behaviors. The dimorphism in *Ae. arabicum* is associated with several distinct anatomical, physiological and biomechanical differences between two fruit and seed morphs while the fruit ratios depend on the environmental conditions during seed development. The SRXTM provided cutting-edge 3D imaging and revealed physical seed coat properties. This allows investigation into the connection between PAs, biomechanical properties and the structure of the seed coat. The application of new promising technologies will help to reveal seed morphologies, to predict seed germination, and to develop new technologies with the ultimate goal to improve seed quality.

4:00 - 4:15 pm • **Pedro Bello**

SINGLE-SEED RESPIRATION PATTERNS REVEAL MULTIPLE SUBPOPULATIONS IN SEED LOTS

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Germination time courses can be analyzed to quantify seed responses to diverse environmental and physiological conditions such as temperature, water potential, oxygen, hormones, dormancy, aging, and other factors. However, obtaining sufficiently detailed data on germination timing of seed populations requires repeated observations at frequent intervals, which is labor-intensive and often impractical. Respiration is among the earliest metabolic processes initiated following hydration of a dry seed, and respiration rates have been linked with seed quality. Instruments have been developed to monitor oxygen consumption of individual seeds at frequent (hourly) intervals following imbibition, providing complete respiratory time courses for populations of individual seeds in an automated manner. Conversion of these oxygen consumption curves into population oxygen depletion (POD) curves results in time courses that closely resemble germination time courses. Median rates of germination and of respiration were linearly correlated across diverse conditions for lettuce, tomato and radish seeds. In addition, the population-based threshold models that have been used to analyze germination rates can be applied directly to the analysis and quantification of POD time courses as well. Furthermore, having data for every seed in a measured population provides sufficiently detailed information to enable the identification of sub-populations of seeds having distinct respiratory behavior within a single seed lot. Testing of many commercial seed lots of maize, for example, revealed that they are often composed of multiple subpopulations with widely differing respiration characteristics. Subpopulations of seeds in lot can arise from many sources, such as location on the mother plant or in the fruit, maturity at harvest or dispersal, age or storage conditions, and physical mixtures. Modeling these subpopulations separately and summing their individual contributions allows apparently complex germination/respiration time courses to be accurately predicted. The existence of multiple subpopulations can often explain cases in which the assumption of a single normal distribution of seed sensitivity thresholds does not match well to the data. As subpopulations can vary in the medians and variances of their germination behavior, and can respond independently to germination-influencing factors, a multi-population threshold model can describe a wide array of apparently non-normal germination distributions that have been observed.

4:15 - 4:30 pm • **Steven Groot**

VARIATION IN RICE SEED LONGEVITY UNDER DRY STORAGE CONDITIONS WITH ELEVATED OXYGEN LEVELS

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Maintaining seed quality during storage is important for seed industry and farmers. Seed ageing is inevitable during storage and results in loss of vigour and germination. The rate of ageing is influenced by storage conditions and genetic factors. The deterioration is the result of accumulation of damage mainly by oxidation reactions, and the rate is increased by high temperature, moisture and oxygen levels. To estimate longevity in general CD tests are used with high moist and temperature to speed up deterioration. However, the results of these test often show poor correlation with long-term storage under dry conditions. This is mainly due to differences in the physiology of seeds at different water activity under these two ageing conditions. Previous research by our group has shown that for several crops seed ageing under dry conditions can be accelerated by storing at an elevated partial pressure of oxygen (EPPO). Also with rice, seed ageing can be accelerated by storing seeds under high oxygen levels (EPPO). Rice seed lots from different varieties showed variation in their tolerance to 5wk EPPO ageing at water activity of 0.4 and 35°C. EPPO aged rice seeds show both loss of germination over storage time and reduced vigour with slower germination.

Vigour loss during seed storage is often accompanied with a decline in oxidative respiration activity after imbibition due to mitochondrial damage. Poor functioning of the mitochondria will result in ethanol production. Indeed, upon imbibition EPPO aged rice seeds produced more ethanol, compared to seeds stored for the same time under lab conditions, upon imbibition. Rice seed lots with low tolerance to EPPO ageing produced more ethanol in the assay than more tolerant seed lots. This

indicates that EPPO aging results in more mitochondrial damage and that part of the variation between seed lots (and potentially genotypes) is in the tolerance towards the induction of oxidative damage.
In conclusion, dry ageing of rice seeds under EPPO conditions shows the damaging effects of oxygen during storage, making it a potentially better method to study genetic variation for seed longevity in rice under dry storage conditions than a traditional CD test.

4:30 - 4:45 pm • **Maor Matzrafi**

HYPERSPECTRAL TECHNOLOGIES FOR ASSESSING SEED GERMINATION AND HERBICIDE RESPONSE IN *AMARANTHUS PALMERI*

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Weeds are the most important biotic stressor of cropping systems, decreasing productivity by more than 34% worldwide. Treatments with herbicides are by far the most cost-effective means of controlling weeds. The misuse of herbicides has created high selection pressure driving the evolution of herbicide resistance in various weed species. *Amaranthus palmeri* has developed resistance to most of the herbicides targeted for its control. Combined with characters such as high seed fecundity and germination in a wide range of temperatures, *A. palmeri* can be ranked as one of the most noxious weeds in the world. The ability to estimate seed viability and herbicide susceptibility is a key factor in the development of long-term weed management strategies. We developed a toolbox based on hyperspectral technologies and data analyses aimed to predict *A. palmeri* seed germination and response to the herbicide trifloxysulfuron-methyl. Using hyperspectral imaging, we accurately distinguished between germinating and non-germinating seeds. Sensitive and resistant plants were identified with high degrees of accuracy from leaf contact hyperspectral reflectance profiles acquired prior to herbicide application. High correlation between leaf physiological parameters (photosynthetic rate, stomatal conductence and photosystem II efficiency) and herbicide response (sensitivity/resistance) was also found. Our work demonstrates that hyperspectral reflectance analyses can provide reliable information about seed germination and levels of plant susceptibility in *A. palmeri*. The use of reflectance-based analyses has enormous potential in preventing ineffective herbicide applications. It also has potential for use in mapping tempo-spatial population dynamics in agro-ecological landscapes.

4:45 - 5:00 pm • **Xia Xin**

A NON-INVASIVE TECHNIQUE FOR DETECTING SEED VIABILITY BY USING ELECTRONIC NOSE

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Seed viability test is one of the routine work at genebank. Germination and TTC tests were classical methods to detect seed viability, however, both methods consume many time and seeds. Our study aimed to develop a new technique that can detect seed viability in a non-invasive and quick manner. Many reports have demonstrated that seeds volatile components changes with reduction in viability. In this study, we tried to draw the smell fingerprint of seeds volatiles by using electronic nose technique. Wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), and oilseed rape *(Brassica napus* L.) seeds were used as models to represent starch, protein, and oil seeds, respectively. Each crop had three or six varieties, and each variety had four or five different germination rates. Seeds were sealed in vials, and the volatiles signals were collected using a PEN3 electronic nose with oxide sensors array. The results showed that PEN3 electronic nose technique can separate seeds of different viabilities using PCA or LDA. Using BP neural network analysis, PEN3 electronic nose technique could predict viability of unknown seed samples. The BP neural network training accuracy were higher than 96%, meanwhile the predicted accuracy was over 98%, indicating that this method is reliable. The process of this technique was without any treatment, indicating this technique is totally non-invasive. In addition, this technique could save a lot of time and labor, as it only needs about 1 minute to collect the volatile signals. Therefore, PEN3 electronic nose technique could be a non-invasive, reliable, quick and low-cost technique for seed viability detection.

Poster Session II
5:15 - 7:00 pm
Monterey Bay Room

T001.
PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF ROOTS OF *Ricinus communis* L. SEEDLINGS EXPOSED TO COMBINED WATER RESTRICTION AND HIGH TEMPERATURE STRESS

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Drought and extreme temperatures are common in arid and semi-arid regions, and limit agricultural productivity in these regions. Among the species tolerant to these conditions, *Ricinus communis* (castor bean) is an oilseed species that is known for the quality and diversified applications of the oil extracted from its seeds. The present study aimed to evaluate the effect of combined stress (water restriction x high temperature) on root growth in seedlings of 2 *Ricinus communis* cultivars (EBDA-MPA34 and PARAGUAÇU). Additionally, the same seedlings were used for primary metabolite and gene expression analysis for some heat shock proteins (HSP) genes and genes involved in the γ-aminobutyric acid (GABA) shunt (glutamate decarboxylase - GAD; GABA-transaminase - GABA-T and semialdehyde succinic dehydrogenase - SSADH). Initially, seeds were imbibed in water at 25°C until radicle protrusion (2.0mm), after which seedlings were kept in water at 25°C (0.0MPa/25°C, control) or transferred to PEG8000 solutions with osmotic potentials of 0.0, -0.2 and -1.0MPa at 20, 25 and 35°C until 5cm of root. Analyzed physiological parameters included daily root growth (cm/day), root dry weight (mg) after 7 days and the ratio of dry weight per centimeter root (mg/cm). The concentrations of primary metabolites were determined by HPLC and GC-TOF-MS analysis and the expression of the coding genes for HSPs, GAD, GABA-T and SSADH by RT-qPCR analysis. The combination of water restriction and high temperature stress compromised, as might be expected, the development and establishment of castor bean seedlings, reducing the growth rates and dry weight accumulation in roots of both cultivars. Additionally, both cultivars accumulated primary metabolites with osmoprotective functions like galactinol, trehalose, stachyose and GABA and increased the expression of HSPs and enzymes involved in the GABA shunt in response to the combined stresses. PARAGUAÇU showed a higher tolerance to more severe combined stresses (-1.0MPa and 35°C), as compared to EBDA-MPA34. In these conditions PARAGUAÇU also had higher levels of primary metabolites with osmoprotective function as compared to EBDA-MPA34.

T002.
APPLYING KNOWLEDGE ABOUT SEED DORMANCY REGULATION IN *ARABIDOPSIS THALIANA* TO CONTROL *ALOPECURUS MYOSUROIDES*

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In order to feed the world's increasing population, we need to make efficient and effective use of our arable land. Arable weeds are ubiquitous, persistent, and can significantly reduce crop yields. *Alopecurus myosuroides* (blackgrass) is an annual, seed propagated, grass weed that causes considerable problems for arable farmers in Western Europe: 1) it has a germination window that overlaps with winter crop sowing and seed shed occurs before winter crops are harvested, 2) multiple-herbicide resistance is widespread, and 3) seeding results in severe infection of subsequent crops. Therefore current cultural, chemical, and physical control methods are insufficient and new approaches must be investigated. Seed dormancy prevents germination of viable seeds even under favorable conditions. Dormancy levels are controlled by both genetic and environmental factors. Research using the model plant *Arabidopsis thaliana* has provided a good understanding of the mechanisms underlying establishment and breaking of seed dormancy. We aim to use these data to alter the dormancy of blackgrass and thereby provide novel means to control this weed. Controlling blackgrass by increasing dormancy is attractive because its seed bank has an annual decline of ~75%, and ~65%

of emerged seedlings come from seeds that are less than a year old. In this project we will develop blackgrass-specific methods to assess and alter phytohormones, gene expression, and various seed physiology traits. Most importantly, we will develop protocols for transient and/or stable blackgrass transformation which will allow for testing of numerous hypotheses. By sufficiently understanding how to regulate blackgrass seed dormancy in the lab, we can use these data to explore various methods to alter dormancy of seeds that are set in the field.

T003.
A METHOD TO ACCELERATE DORMANCY RELEASE IN *ARABIDOPSIS THALIANA* THAT MIMICS DRY STORAGE ON THE GENETIC LEVEL

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The seed life span stretches from when the seeds reach maturity on the mother plant until the seed is not viable enough anymore to germinate. At the start of the seed life span, seeds possess a genotype specific level of dormancy. A dormant seed is viable but will not germinate even though the environment is favourable. The genetic basis for seed dormancy is well defined: Quantitative Trait Loci (QTL) for seed dormancy have been identified (*DELAY OF GERMINATION*) but thus far only for *DOG1* an underlying gene has been identified.

Arabidopsis thaliana is a wide-spread species of which accessions have been collected from very different natural habitats. The level of dormancy can vary tremendously between these different accessions. The accessions often used in research have low dormancy (Colombia-0, Landsberg *erecta*), but some accessions (Cape Verde Islands, Iberian Peninsula populations) can remain dormant for over a year during dry storage (after-ripening, AR). Studying these deep dormant accessions will aid in seed dormancy research, but is limited due to time constrains.

In seed dormancy and longevity research, treatments to artificially accelerate the seed life span are used. These treatments usually comprise storage at high temperatures and high relative humidity or fully imbibed (stratification). However, these artificial treatments do not always mimic dry seed storage (AR) at a genetic level. The alleviation of dormancy during dry seed storage is thought to be mainly caused by the oxidation of seed proteins and mRNAs. Based on the role of oxidation in the dormancy release and ageing process, we use the EPPO (Elevated Partial Pressure of Oxygen) system to accelerate dormancy release. In the EPPO system, seeds are stored under increased ambient air and thus increased oxygen pressure. EPPO has already shown to accelerate seed ageing. Here, we show that also dormancy release can be accelerated and mimicked in a non-invasive manner by the EPPO system. We used a genetics approach to reveal whether EPPO dormancy release occurs by the same mechanisms as seed dry AR.

T004.
EXAMINING THE FUNCTION OF GID1-REGULATED GENES

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Plant species survival and agriculture both depend on seed germination occurring in a season and environment conducive to seedling growth and development. Dormant seeds are unable to germinate when first released from the mother, thereby preventing germination out of season (fall vs spring). Dormancy can be lost through a period of dry storage called after-ripening (AR), or exposure to the moist wintry condition of cold imbibitions. The plant hormone gibberellin (GA) stimulates seed germination. The proposed research examines the hypothesis that gibberellin A (GA) hormone receptors regulate dormancy loss via regulation of gene transcription and translation. GA signaling leads to inactivation or destruction of DELLA (Asp-Glu-Leu-Leu-Ala) family repressors of GA responses. In Arabidopsis, GA is perceived by the three homologous receptors, GID1a, GID1b, and GID1c (GA-INSENSITIVE DWARF1, 68-85% amino acid identity) comprised of a GA-binding core connected to a lid domain by an a-loop hinge.

In sly1 (sleepy1) mutants, GA and GID1 can also inactivate DELLA repressors via GID1-GA-DELLA complex formation without DELLA proteolysis. This is seen when GA-insensitive sly1-2 seed dormancy is rescued without DELLA destruction by long after-ripening (2 years AR, 85% germination) and GID1b

overexpression (GID1b-OE, 75%). If AR and GID1b-OE reduce sly1-2 seed dormancy via similar mechanisms, then they should result in similar transcriptome changes.
Nelson and Steber identified transcripts showing altered accumulation when germination of the GA-insensitive sly1-2 mutant was rescued by after-ripening or by GID1b-OE. And stimulation of sly1-2 germination by AR and GID1b-OE are associated with partly overlapping transcriptional changes. This suggests that these 26 genes showing differential regulation by GID1b-OE are important regulators of dormancy. Thus, we will determine whether genes regulated in response to GID1b-OE play an important role in seed dormancy.

So far, we have been working on determining if GID1b-OE-regulated transcripts are differentially expressed in gid1 loss of function mutants. The effects of GID1 loss and gain-of-function on the expression of GID1b-OE-regulated genes will be examined in dormant (0 wk AR) and after-ripened (1 mo AR) seeds by RT-qPCR analysis performed at the different imbibition time points used for the microarray analysis.

T005.
IDENTIFICATION OF GENE REGULATORY NETWORKS DRIVING CHANGES IN THE BIOMECHANICAL PROPERTIES OF EMBRYO CELLS AND THE SEED TO SEEDLING TRANSITION

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The seed to seedling transition in Arabidopsis is driven exclusively through changes in cell shape. Changes in the mechanical properties of the embryo cell wall therefore underlie this transition, and cell wall modifying gene expression represents the downstream targets of the germination process. The expansin genes are prime target for cell wall loosening during seed to seedling transition, however, the underlying regulatory network remains unknown. Using data from yeast one-hybrid, we identified transcription factors that directly bind to expansin promoter regions. These transcription factors mediate the penultimate step of the germination process through their regulation of gene expression which directly alters the physical properties of the cell wall. Using data from yeast two-hybrid, we determined which of these TFs bind to the growth and expansin repressor protein DELLA. This led to the generation of a regulatory network directly linking signals from the environment to growth-promoting gene expression. Furthermore, we examined whether these TFs that bind both expansin promoters and DELLA proteins play a role in seed germination. Using inducible overexpression and knockout lines, phenotypes for 66% of the TFs tested were identified. This GRN uncovers hub regulators and their regulatory consequences during seed-to-seedling transition, and potentially provides genetic targets for the enhancement of seed vigour and seedling establishment.

T006.
PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN SUNFLOWER SEEDS UNDER WATER STRESS AND DETERIORATION

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The sunflower is cultivated in different regions of the world and it can be subject to environmental stresses during the field emergence, especially water availability. Furthermore, being an oilseed is more subject to deterioration after harvest. The objective of this study was to evaluate the physiological and biochemical changes in sunflower seeds under water stress and deterioration conditions. Two seed lots of sunflower, cultivar Helio 253, with different vigor levels were used. Initially, the physiological quality of the seed lots was evaluated by germination and vigor tests. Then, they were submitted to water stress in PEG 6000 solutions at 0,0; -0.2; -0.4; -0.6 and -0.8 MPa and evaluated by germination, first count, length and dry weight of seedlings. Biochemical evaluations such as lipids peroxidation and antioxidant enzymes activity (SOD, CAT, POX and APX) were evaluated on the 2nd, 4th and 6th days after sowing (DAS). The same evaluations were performed for the seeds subjected to the deterioration process at 41° C and 100% RH for 0, 48, 72 and 96h. Seed germination reduced with the osmotic potential decrease, regardless of seed vigor level. In general, there was an increase in the SOD and APX activity for higher vigor seed lot and POX activity was lower during germination of the both lots. There was an increase in MDA and reduction of SOD and CAT activity during seed germination for lower vigor lot subject to deterioration.

T007.

HORMONAL CHARACTERIZATION OF DORMANCY CYCLING IN *POLYGONUM AVICULARE* SEEDS

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Polygonum aviculare, an important weed species, forms stable seed banks cycling between dormant and non-dormant states according to seasonal changes. Seeds of this species have been widely studied for the last few years because of its dormancy behavior. Available models developed for seed dormancy in *P. aviculare* allowed us to generate seed populations with different dormancy levels providing an advantage for dormancy study over other species. The objective of this work was to characterize the mechanism(s) involved in the dynamics of primary dormancy release (PD) and secondary dormancy induction (SD) in *P. aviculare* seeds. Quantification of endogenous abscisic acid (ABA), seed incubation at different concentrations of exogenous ABA and qPCR for sequences related to ABA metabolism (*PaNCED* and *PaABA8OHase*) were performed at different times in a cycle from seeds with PD and different stages during the release from primary dormancy to a state of low dormancy (LD) and finally during re-induction to secondary dormancy (SD). We showed that primary dormancy release in *P. aviculare* was characterized by a loss of sensitivity to exogenous ABA and that sensitivity was recovered when re-induced into secondary dormancy. Endogenous ABA and sequences related to ABA metabolism quantification showed a decrease from PD to LD that remained constant during SD induction. We showed that the mentioned decrease in ABA content during primary dormancy release was not associated to dormancy mechanism as this decrease occurred early in incubated PD seeds with zero germination. We concluded that sensitivity to ABA instead of endogenous ABA content would be one of the main mechanisms controlling loss and induction of dormancy in *P. aviculare* seeds.

T008.

SEED ENHANCING TREATMENTS: COMPARATIVE ANALYSIS OF GERMINATION CHARACTERISTICS OF 23 WILD SPECIES

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The response of seeds from 23 wild plant species to a range of seed enhancing treatments was studied in order to assess the potential of treatments to improve germination in restoration projects. These treatments were exposure to the smoke-derived compound karrikinolide (KAR_1), potassium nitrate (nitrate), and to plant growth regulator gibberellic acid; all described as cues of germination. We tested the hypothesis that sensitivity of the 23 species to these compounds is related to their ecological niche. The three ecological niches we considered were open land, open-pioneer and woodland, each with its own characteristics. Hence, the germination of a species will be adapted to different light conditions and other environmental signals related to the niche. As representatives of environmental signals, the effects of KAR_1, nitrate and GA_3 on germination were studied. Two of these signals are associated with a reduced competition (KAR_1 and nitrate). We also investigated the effect of different light regimes (constant light, absence of light and 12h photoperiod) and compounds on germination parameters, which included final germination and germination rate. The results showed a wide variation of responsiveness of the different species to the three compounds, which was also affected by the light conditions. No interaction was found between responsiveness to compounds and ecology group. Additionally, no single treatment increased the germination of all the tested species, indicating that different species require unique treatments to improve germination. However, final germination and germination rate were affected by light conditions which relates to the ecology of the species.

T009.

GENETIC VARIATION IN THE EFFECT OF SEED MATURATION ENVIRONMENT ON SEED PERFORMANCE

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Seed set is a crucial phase in plant's life cycle yet particularly sensitive to stresses. The intrinsic properties of seeds, such as the ability to germinate, are acquired during seed embryogenesis and seed maturation on the mother plant. Prolonged exposure to stress of the mother plant during seed development can alter the innate quality of the seed. Once shed from the mother plant, the ability of the seed to germinate is determined by either favorable or unfavorable germination conditions. However, germination requirements can be alleviated by the effect of the maturation environment. In addition, effects of the maturation environment have been shown to be expressed in a genotype-dependent manner. Therefore, the interaction between the genotype, the maturation and germination environment of the seed (G x E x E) are instrumental in determining seed performance.

To date, little is known about the genetic basis of GxE in regard to seed performance. To address this, we grew an Arabidopsis Bayreuth x Shahdara recombinant inbred line (RIL) population under different mild stress environments from flowering until seed harvest: standard, high temperature, high light and low phosphate conditions. The seeds produced under each environment were used for extensive phenotyping. Traditional linkage analysis (QTL analysis) revealed germination QTLs interacting with both the seed maturation environment and the germination conditions (QTL x E). In order to gain insight into the underlying molecular mechanisms of this variation, transcriptomics and metabolomics data were generated from fresh dry seeds of the different populations. In a generalized genetical genomics approach, regions in the genome responsible for variation in gene expression (eQTLs) and metabolite content (mQTLs) were mapped and compared to phenotypic QTLs. Deeper understanding of the genetic basis of the effect of the production environment on seed performance provides a basis for improvement of seed performance prediction.

T010.
GENE IDENTIFICATION OF THERMOTOLERANT LETTUCE MUTANT BY BULKED SEGREGANT ANALYSIS AND WHOLE GENOME SEQUENCING

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Lettuce (*Lactuca sativa*) seed germination is generally inhibited at warm temperatures (>30°C), resulting in seed thermoinhibition. Using ethyl methanesulfonate (EMS) mutagenesis, we generated two independent thermotolerant mutants, TG01 and TG10, which can germinate well at 35°C. Physiological analysis revealed that both mutants have similar responses to salinity, PEG8000, a gibberellin biosynthethesis inhibitor (paclobutrazol), an ethylene signal transduction inhibitor (STS, silver thiosulfate), and abscisic acid (ABA). Backcrossing to wild type and reciprocal crossing indicated that these two mutants were allelic and recessive. To expedite identification of the causal gene(s), DNA from segregating thermotolerant F2 seeds from backcrosses of each mutant to the parental line was bulked and sequenced for detection of single-nucleotide polymorphisms (SNPs). SNPs were screened to identify those due to the EMS treatment that were present at greater than average frequency. Two independent candidate mutations were identified at different physical positions in the zeaxanthin epoxidase gene (*ABSCISIC ACID DEFICIENT 1/ZEAXANTHIN EPOXIDASE*, or *ABA1/ZEP*) in TG01 and TG10. The mutation in TG01 caused an amino acid replacement, whereas the mutation in TG10 resulted in alternative mRNA splicing. Endogenous ABA contents were reduced in both mutants, and expression of the *ABA1/ZEP* gene from wild-type lettuce under its own promoter fully complemented the TG01 mutant. Conventional genetic mapping confirmed that the causal mutations were located near the *ABA1/ZEP* gene. The bulked segregant whole genome sequencing approach was more efficient than conventional mapping and identified the specific gene responsible for the phenotype.

T011.
THE INFLUENCE OF MATERNAL ENVIRONMENT ON SEED AND SEEDLING QUALITY TRAITS AND METABOLITES PROFILES OF THE SEEDS IN A RECOMBINANT INBRED LINE POPULATION OF

TOMATO

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Many factors are influencing successful germination, seedling establishment and further growth and development in all plants. Among these factors seed quality is one of the most important with a clear effect on successful crop development. High quality and well developed seeds will aid a successful life cycle of crops, from germination to seedling establishment through to fruit and seed production, especially under stressful environmental conditions. It is generally thought that seed quality is affected by many environmental cues such as drought, light and temperature. The maternal environment including climate conditions, is often reported as a profound effective factor influencing the development of seed quality. However, there is little knowledge about the genetic and environmental factors, and their interaction, that influence seed quality and seedling establishment. The aim of this study is to identify the molecular mechanisms that are involved in seed quality and also how these mechanisms are controlled by adverse maternal environmental conditions. For this purpose we used a tomato recombinant inbred line (RIL) population consisting of 101 lines grown under various nutritional environmental conditions, like high phosphate and low nitrate, during seed development. With a combination of genetic, physiological and 'omics' technologies in a so-called generalized genetical genomics (GGG) design, we aim to construct the molecular networks that regulate seed and seedling quality. Further studies on these networks can ultimately help to predict the effect of different maternal environmental conditions on seed quality and this information will be very useful to improve production of high-performance seeds. Extensive phenotyping of the harvested seeds showed strong variation for most of the seed germination traits such as G_{max}, t_{50} and U_{8416} under different germination conditions. This variation, as well as the variation measured in primary metabolite content in the dry mature seeds (mQTLs), was not only dependent on genetic factors, but also on the maternal environment and a strong genetic x environment (G x E) effect. Some phenotypic QTLs co-located with mQTLs, which might indicate a causal relationship between the observed changes in metabolite content and seed and seedling quality traits.

T012.

INTRAESPECIFIC VARIABILITY AND MATERNAL ENVIRONMENT EFFECTS ON SUNFLOWER (*Helianthus annuus*) DORMANCY TRAITS

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Although the presence of dormancy in sunflower fruits at harvest is an usual problem to the seed industry, there is little information about intraspecific variability for this trait and the regulation by the environment during fruit development. The aim of the present work was to define dormancy phenotypes resulting from intraspecific variability, and their interaction with the maternal environment. To achieve these objectives 20 sunflower genotypes were sown under irrigated field conditions in different planting dates during two consecutive years. Genotypes were classified according to i) dormancy expression along the thermal range ii) rate of dormancy release during storage, iii) contribution of the fruit components to dormancy, and iv) embryo sensitivity to GA and ABA. At harvest, and at regular times during storage, achene, seed and embryo germination was evaluated at different temperatures (10-30°C). In addition, in selected genotypes, achenes and embryo germination response to ABA, GAs and their corresponding synthesis inhibitors was also evaluated. Two main groups were obtained: HTED and LTED (High and Low temperature expressed dormancy), the first of which included the wild relatives and two domesticated lines, and the rest belonged to the LTED group. Both groups were subdivided further according to the persistence of embryo dormancy, and coat-imposed dormancy. Interaction with the maternal environment was significant for most genotypes tested. Obtained results showed that fruits exposed to lower temperatures and shorter photoperiods during development presented a lower achene and embryo dormancy level at harvest and a higher achene

dormancy release rate during storage. These differences in dormancy level were mainly expressed at low incubation temperatures (10°C); temperature at which dormancy was expressed more strongly in most of the genotypes. In addition embryos excised from fruits matured under late sowing environments showed a significant lower inhibition of germination by ABA than those matured under earlier sowing date environments, indicating that observed dormancy level regulation by the maternal environment could be partially explained by differences in embryo sensitivity to ABA. Sensitivity of isolated embryos to ABA appears as the main trait related to differences in dormancy level among genotypes, and in response to the maternal environment.

T013.

GENETIC AND ENVIRONMENTAL CONTRIBUTIONS TO THE EXPRESSION OF SEED GERMINATION UNDER STRESS

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The ability of seeds to complete germination under environmental stress is an important attribute contributing to the economic production of agricultural and horticultural crops. Profit margins in the U.S. lettuce market can often be maximized by producing a crop to take advantage of favorable commodity prices caused by geographic shifts between production areas of coastal California and the low desert areas of southern California and southwestern Arizona. Subsequently, lettuce seeds are sewn in fields of the low desert production areas where high temperatures inhibit germination, and increasingly, where water quality may be low and its availability uncertain. Thus a seed lot must germinate quickly and uniformly regardless of environmental conditions and less water must be used to establish the crop. Producing a seed crop with consistent and outstanding vigor is challenging. While it is generally known that environmental factors during seed maturation can affect seed vigor, the magnitude of the effect is generally less appreciated. We have produced seed from a recombinant inbred line of lettuce over multiple seed production environments and evaluated environmental and genetic contributions to seed vigor. We have observed surprisingly large phenotypic plasticity of these seed lots when germinated under environmental stress (e.g., exposure to far-red light, high temperature, reduced water potential) even though germination of these seeds is near 100% under control (20 °C, continuous red light) conditions. Having knowledge of genetic factors affecting trait stability, or its opposite – plasticity, would be valuable information that can be applied to many agronomic traits that are genetically complex. Although environmental conditions during seed development and maturation can have large suppressive effects on vigor, some genetic lines consistently exhibit vigor higher than the population average. This observation, coupled with quantitative trait loci mapping, may provide a path forward to uncover the genetic basis and identity of alleles contributing to increased seed vigor, trait stability and plasticity.

T014.

EFFECTS OF HEAT SHOCK ON GERMINATION, PLANT HORMONES AND GLYCEROPHOSPHOLIPIDS OF *PINUS DENSIFLORA* SEEDS

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Pinus densiflora seeds improved their germination in response to heat shock (HS) at the high humidity and this study was carried out to understand the mechanisms of the HS-induced stimulation. Seeds (>95% viability) were collected from South Korea in October 2015 by the Wild Resource Plants Seed Bank and rapidly dried to 5.0% moisture content. Seeds were pre-equilibrated at 100% relative humidity (RH), 21°C for 1 week and then heat treated at 45°C for 0 (control) and 1 days (HS) at this RH. A thermal gradient plate (TGP) was used to evaluate the germination response to constant and fluctuating temperatures in range of 5 to 35°C, and the effects of exogenous abscisic acid (ABA), gibberellic acid (GA_3) and jasmonic acid (JA) on germination were tested with the different concentrations (0, 10, 50 and 100 µM). Subsequent analyses for plant hormones and glycerophospholipids were made after 0, 2, 6, and 10 d germination of HS and control seeds. Plant

hormones and glycerophospholipids were measured by LC-MS and MALDI-TOF-MS, respectively. HS seeds at 100% RH promoted total germination and T50, time to reach 50% germination, of *P. densiflora* seeds whereas HS at 30% RH did not influence seed germination. TGP results showed that HS improved seeds germination in the wide range of temperature, but germinations of HS and control seeds were not affected by various hormone treatments. ABA contents increased up to 6 d germination, but then quickly decreased at 10 d germination in accordance with seedling protrusion. Levels of ABA were significantly lower in HS seeds after 6 and 10d germination. Salicylic acid content significantly decreased by 6 d germination in HS and control seeds. JA contents were also changed during imbibition and significant differences between HS and control was observed at 2 and 6 d germination. Total phophatidic acids and total phosphoinositoles showed opposite trends to ABA levels during germination in HS and control seeds.

T015.
EVIDENCE FOR ANTAGONISTIC INTERACTIONS BETWEEN FUNGAL PATHOGENS CAUSING SEED DEATH OF INVASIVE AND NATIVE GRASS SPECIES

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*Bromus tectorum (*cheatgrass) is an invasive grass species in western North America. A phenomenon known as “die-off” (stand failure) has affected large areas of *Bromus*-infested wildlands. One notable fungal pathogen from these die-offs is *Fusarium* cf. *acuminatum*. We investigated whether this *Fusarium* was an antagonistic competitor relative to co-occurring fungal seed pathogens from *Bromus* die-offs, specifically *Epicoccum nigrum*. Treatments consisted of seed inoculations with a strain of each species alone or together with a strain of the other species. Two strains of each species were included. Inoculations were applied to *B. tectorum* and to native *Elymus elymoides* host seeds. Each inoculation treatment was replicated five times. For each replicate, twenty-five seeds were inoculated with a liquid suspension of spores and mycelium. Seeds were first incubated at low water potential, then transferred to free water, simulating conditions in the field. Seed mortality caused by each fungus (as indicated by the color of the infection cushion over the floret attachment scar) was then measured at 15 days. We found limited support for additive interactions with only 1 of 16 scenarios showing that the combination treatment increased seed mortality compared with single species inoculation. We also found limited support for the hypothesis of full competitive exclusion, where one pathogen strain completely dominates the other strain and the presence of the co-occurring strain does not alter seed mortality for the dominant pathogen; this was found in 1 of 16 scenarios. Instead we found that the majority of scenarios (11/16) supported reciprocal inhibition, such that seed mortality was reduced in the co-inoculation treatment relative to single inoculations of either species. All four fungal strains in single-species inoculations caused seed mortality of > 50% on *Bromus*, with mortality as high as 83%. Seed mortality was much lower overall on native *Elymus* seeds, perhaps indicating some level of host specialization for these strains. In conclusion, we found that seeds may be more likely to escape pathogen-caused death when there are multiple pathogen species present, indicating that simplistic laboratory experiments do not adequately account for the complex interactions of the seed pathogen community.

T016.
BIOCHEMICAL AND MOLECULAR REGULATION OF DEFENSE RESPONSES TO A PATHOGENIC SOIL FUNGUS IN DORMANT SEEDS

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Dormancy and decay resistance of weed seeds in the soil seedbank challenge long-term weed management. Dormant seeds employ various physical and chemical defenses to inhibit seed decay pathogens, but little is known about biochemical seed defenses.

We evaluated activities of the defense enzymes polyphenol oxidase, peroxidase, exochitinase, and oxalate oxidase produced by dormant wild oat (Avena fatua) and wheat (Triticum aestivum) seeds in response to the pathogenic soil fungus Fusarium avenaceum isolate ‘F.a.1’. Caryopsis decay was scored with a visual assessment and quantitative real-time PCR was used to evaluate seed defense

responses at the transcriptional level. Additionally, quantitative PCR was used to measure the extent of F.a.1 infection in seeds and soil at various time points. Activities of the degradative fungal enzymes protease and xylanase were measured in soil and wild oat caryopses throughout the course of infection. Experiments were conducted both in vitro on agar plates and in soil.

We found that F.a.1 induced the seed defense enzymes polyphenol oxidase, peroxidase, and exochitinase, but inhibited activity of oxalate oxidase. Moreover, wild oat and wheat seed responses to F.a.1 infection were similar qualitatively, but different quantitatively. Preliminary qRT-PCR results indicate that F.a.1 induces polyphenol oxidase, exochitinase, and NADPH oxidase, but not oxalate oxidase, at the transcriptional level in wild oat and/or wheat. Fungal abundance in seeds and soil and activities of F.a.1-derived degradative enzymes increased over time of infection.

Our results suggest that biochemical and molecular defense responses may contribute to the prolonged survival of wild oat and other weed species in the soil seedbank. These results warrant further investigation of fungal-induced seed decay as a potential alternative approach to managing the dormant weed seedbank in the soil.

T017.
HIGH-TEMPERAURE ADAPTATION IN TROPICAL PLANT SEEDS

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The tropics are characterized by high temperatures, which may be an important stressor to tropical plant seeds. Air temperatures have important effects on developing seeds; while after shedding, seeds are mostly affected by soil surface temperature. Taking Xishuangbanna in southwest China, an edge area of tropical Asia, as example, there soil surface maximum temperature often excess 60°C on open ground in sunny days, with a extreme value of 71.4°C documented by weather observation station there. However, active cellular membrane is usually destroyed by high temperature around 60°C, how seeds survive so high temperature in tropics?

Temperature changes greatly depending habitats, meanwhile high temperature tolerance in seeds varies from species to species. It was found that seeds native to tropical rainforest are usually high-temperature sensitivity, including recalcitrant seeds, which can not be dried so neither can tolerate high temperature above 60°C, such as *Hopea hainanensis* and *Baccaurea ramiflora*, and seeds of some rare and endangered species, which also are high-temperature intolerant even if desiccation tolerant, such as *Pellacalyx yunnanensis* and *Tacca chantrieri*. Fortunately, no soil surface maximum temperature more than 30°C has been detected in intact tropical rainforest there. This category of seeds adapts the avoidance strategy to high temperature, obviously. Another category of seeds, including those produced by weeds, invasive plants and pioneer species, adapts the tolerance strategy to high temperature. They exhibit much stronger tolerance to high temperature in air-dried state, such as *Amaranthus spinosus*, *Piper aduncum*, and *Tithonia diversifolia*.

High-temperature tolerance in seeds may contribute to plant distribution in the tropics. Nowadays, globular warming, deforestation, rainforest fragmentation and other human activities are enlarging hot habitats and making hot habitats hotter. This decreased habitats suitable for climax species in tropical rainforests, made them rare and endangered; on the other hand, increased habitats suitable for non-climax species, paved way for plant invasion.

T018.
THE PRODUCTION AND FUNCTION OF MUCILAGE BY SWEET BASIL (*OCIMUM BASILICUM* L.) SEEDS

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Sweet basil (*Ocimum basilicum* L.) seeds produce a thick layer of mucilage around the testa within minutes after hydration. Mucilage is most prevalent among plant species adapted to surviving in arid sandy soils, though its significance in determining the ecological fitness is unclear. The mucilage produced by these seeds is reported to be composed of cell-wall polysaccharides that are deposited in testa cells during development. In this study, sweet basil seeds were examined using light and environmental scanning electron microscopy. The mucilage of basil seeds is held together by columnar structures that unfolded from the pericarp and helped hold and stabilize the mucilage to the seed surface. The mucilage was removed using diluted hydrochloric acid to compare performance of

seeds with and without mucilage. Mucilage removal inhibited laboratory seed germination under ideal conditions and significantly reduced the seed water content four fold. The mucilage anchored seeds and increased their resistance to movement in the environment. Osmometry showed the water potential of fully hydrated seeds to be near zero suggesting that the mucilage provides a pool of loosely bound water to germinating seeds and seedlings in arid environments. Testing in soil with various levels of hydration confirmed intact basil seeds with mucilage germinated to higher percentages and survived longer than seed with mucilage removed.

T019.

EFFECTS OF BORON FERTILIZATION ON PEANUT SEED GERMINATION TESTED IN A LAB FIELD™ TABLE

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Peanut (*Arachis hypogaea* L.) is an important crop for eastern Virginia (VA) and North Carolina (NC) where it thrives in sandy soils. Boron (B) is not retained in these soils, and seeds with <13mg kg^{-1} B may have hollow heart and reduced seed quality. Therefore, B is routinely applied as fertilizer regardless of soil test results to prevent deficiencies in peanut seed crops but may contribute to water pollution. A mixture of two market types and newer and older cultivars of peanuts were fertilized with 0, 0.6, 1.1kg ha^{-1} B at the Tidewater Agriculture Research and Extension Center. Seeds were germinated in sand on a Lab Field™ table to simulate soil conditions in Eastern VA and NC fields. The peanuts were hand planted on the Lab Field™ table maintained at a constant sand temperature of 25°C. Mean time to germination (MTG) and germination percentage were recorded to compare treatments. There were no differences in MTG or germination percentage between fertilized and unfertilized plants, market types, or newer and older commercial cultivars on the Lab Field™ table. Based on this research, B fertilization in the VA and NC production region is not necessary to produce a high quality vigorous peanut seed. The Lab Field™ table was an effective tool for testing germination under simulated field conditions.

T020.

MORPHOPHYSIOLOGICAL SEED DORMANCY IN HEPTACODIUM

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Heptacodium miconiodes is an endangered, monotypic genus in the Caprifoliaceae endemic to China. It was determined that its seeds display nondeep simple morphophysiological dormancy. Seeds had an underdeveloped embryo at the time of fruit dispersal with an embryo that occupied approximately 12% of the seed length. Cold stratification (8-weeks at 5°C) was effective for dormancy release, but embryo growth prior to germination only occurred at warm temperatures (20°C). Gibberellic acid treatment partially substituted for cold stratification. Final seed germination percentage was not different after warm or cold stratification, however seeds initially exposed to cold stratification germinated faster and more uniformly. Cold stratified seeds reached 50% final germination approximately 55 days sooner than warm stratified seeds. Prior to radicle emergence, embryos grew to fill approximately 60% of the seed through an endosperm channel that occupied the center portion of the endosperm. Cells in the endosperm channel had thinner cell walls and fewer storage vesicles compared to other endosperm cells. Channel cells formed a dissolution zone ahead of embryo elongation assumed to be involved with enzymatic hydrolysis of storage reserves.

T021.

VARIATION IN RICE SEED LONGEVITY UNDER DRY STORAGE CONDITIONS WITH ELEVATED OXYGEN LEVELS

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Maintaining seed quality during storage is important for seed industry and farmers. Seed ageing is inevitable during storage and results in loss of vigour and germination. The rate of ageing is influenced by storage conditions and genetic factors. The deterioration is the result of accumulation of damage mainly by oxidation reactions, and the rate is increased by high temperature, moisture and oxygen levels. To estimate longevity in general CD tests are used with high moist and temperature to speed up deterioration. However, the results of these test often show poor correlation with long-term storage under dry conditions. This is mainly due to differences in the physiology of seeds at different water activity under these two ageing conditions. Previous research by our group has shown that for several crops seed ageing under dry conditions can be accelerated by storing at an elevated partial pressure of oxygen (EPPO). Also with rice, seed ageing can be accelerated by storing seeds under high oxygen levels (EPPO). Rice seed lots from different varieties showed variation in their tolerance to 5wk EPPO ageing at water activity of 0.4 and 35°C. EPPO aged rice seeds show both loss of germination over storage time and reduced vigour with slower germination.

Vigour loss during seed storage is often accompanied with a decline in oxidative respiration activity after imbibition due to mitochondrial damage. Poor functioning of the mitochondria will result in ethanol production. Indeed, upon imbibition EPPO aged rice seeds produced more ethanol, compared to seeds stored for the same time under lab conditions, upon imbibition. Rice seed lots with low tolerance to EPPO ageing produced more ethanol in the assay than more tolerant seed lots. This indicates that EPPO aging results in more mitochondrial damage and that part of the variation between seed lots (and potentially genotypes) is in the tolerance towards the induction of oxidative damage.

In conclusion, dry ageing of rice seeds under EPPO conditions shows the damaging effects of oxygen during storage, making it a potentially better method to study genetic variation for seed longevity in rice under dry storage conditions than a traditional CD test.

T022.
THE EFFECTS OF CATHODIC INVIGORATION OF FIELD CURED AND STORED RICE SEEDS ON PLANT ESTABLISHMENT AND GROWTH IN THREE RICE SPECIES

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Seed germinability and vigour are vital for successful plant establishment in direct seeded rice systems. Poor postharvest practices such as delayed field curing of rice among farmers in the tropics, particularly sub-Saharan Africa (including Ghana), may compromise storage longevity and subsequent plant establishment and vigour due to seed exposure to weathering, high temperatures and wetting-drying cycles. Three upland rice species (*Oryza sativa* [O.s], *O. glaberrima* [O.g] and an interspecific hybrid [O.s × O.g]) were grown in Ghana, harvested, and field cured for five weeks. Seed samples were harvested at maturity (control) and after two and five weeks of field curing, subsequently stored at 4℃ for 20 months and then sown in the greenhouse with and without invigoration (CW and W). After storage the seeds were assessed for endosperm integrity and germinability and subsequent seedling emergence and plant growth. Field curing for two or five weeks after harvest (delayed field curing) resulted in damage to the endosperm evidenced by sloughing of the caryopsis coat, exposure and subsequent destruction of aleurone grains, and formation of deep cracks through the endosperm in all the three species. Delayed field curing reduced seedling emergence rate and plant growth (in terms of culm, root, panicle and total biomass) in all three species. Post-storage invigoration with W and CW improved seedling emergence rate and vigour relative to the controls; however, seedlings generated from CW treated seeds were more vigorous than those from W treated seeds. O.s x O. g appeared to be more susceptible to field curing and aging evidenced by pronounced damage to the endosperm and reduced seed germination and emergence compared with O. s and O. g. The response of the species to CW invigoration was similar in terms of seedling emergence and vigour. However, CW invigoration improved total biomass accumulation in O. g compared with O.s and O. s x O. g. The results validate the use of cathodic invigoration for alleviating field curing and/or storage induced loss in seed germinability, seedling emergence rate and vigour, and plant growth in rice species typically grown in the humid tropics.

T023.
IMPACTS OF DELAYED FIELD CURING OF RICE IN A TROPICAL ENVIRONMENT ON RICE SEED QUALITY

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In Sub-Saharan Africa, in common with most developing countries in the tropics, harvesting and threshing of rice are delayed in the field due to lack of mechanization. In Ghana, not only do farmers harvest their rice crop far beyond harvest maturity, they also keep (cure) the harvested panicles in the open field for up to five weeks before threshing due to lack of labour. This practice is believed to compromise the subsequent quality of the seeds as a consequence of being exposed to repeated wetting and drying events. The present study tests the following hypotheses: (1) seeds exposed to wetting and drying (wet cured seeds) during field curing exhibit more fissures and cracks than those that are field cured within a dry environment (dry cured seeds); (2) wet cured seeds exhibit higher levels of physiological deterioration of both embryo and endosperm prior to, and during storage than dry cured seeds. Three upland rice species, viz. *Oryza sativa*, *O. glaberrima* and an *O. sativa* × *O. glaberrima* interspecific hybrid, were grown in Ghana, harvested, and cured in the open (wet) and within ventilated but rainproof containers (dry) in the field for five weeks. Seeds were assessed for physical and physiological quality at weekly intervals for five weeks. The relationships between seed moisture content (MC) and water activity (WA) differed between curing environments. However, within curing environments, relationships between MC and WA were comparable between the species. Delayed field curing caused multiple cracks in the endosperm but the cracks were less frequent in the dry cured seeds. Seed germinability was higher in the dry cured environment. Field curing within a dry environment during prolong field curing could improve seed viability, aid in moisture management and minimize damage to the endosperm.

T024.
THE EFFECT OF SEED ENHANCEMENT TECHNOLOGIES ON THE GERMINATION OF SUGAR BEET QUANTIFIED BY X-RAY COMPUTED TOMOGRAPHY

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With an increasing demand for food production, there is a need to increase crop yield and efficiency. Seed enhancement technologies (e.g. seed pelleting, seed coating and seed priming) are important for ensuring consistency in crop yield, however, their efficacy has mainly been investigated in laboratory conditions excluding soil or under field conditions evaluating the yield. The application of X-ray Computed Tomography (X-ray CT) allows a non-destructive and temporal quantification of the germination process in soil to understand the interactions between the soil and the surrounding soil matrix.

In this study, we used X-ray CT to quantify the differences between applied seed enhancement technologies (naked, coated, pelleted and pelleted + coated) prior to sowing, checking on the spatial distribution of applied seed coating materials (e.g. wood, clay, pesticides). Furthermore, we analysed the effect of a wide range of treatments on sugar beet seed germination during the early growth stage (e.g. first 4 days). The results indicated coated seeds had a slower initial growth rate in comparison to the other treatments, although the growth rate rapidly increased after emergence so that all treatments were similar by day 4. The pelleting treatment, used to improve sowing accuracy when the seeds, showed a steady increase in root growth whereas the naked treatment expressed a constant rate after day 2. The pre-germination priming treatment accelerated the growth rate of all treatments significantly over a growth period of 14 days compared to the non-primed treatments regardless of physical enhancements applied on the outer surface.

With this study, the suitability of X-ray CT for quantification and visualisation could be verified and the germination process of enhanced sugar beet seeds *in situ* observed. Furthermore, it was possible to quantify physical alterations of seed enhancements and the distribution of materials within coatings and pelleting. This study contributes towards the selection of appropriate seed enhancement technologies to ensure maximum yield and consistency.
Keywords: Sugar beet, germination, seed enhancement, X-ray Computed Tomography

T025.
ACTIVITIES OF THE ISTA SEED SCIENCE ADVISORY GROUP TOWARDS SEED TEST DEVELOPMENT

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The ISTA Seed Science Advisory Group (SSAG) has three aims, to 1) provide a link between fundamental research and the use of that research to meet the needs of ISTA members, 2) appraise the evidence for efficacy of techniques/equipment produced for use in seed testing laboratories and 3) **r**espond to requests from within ISTA for advice related to scientific issues. This poster focuses on 1 and 2 and outlines and illustrates the way in which we shall deliver these aims. We encourage people with ideas for development to contact us, both people working in seed research who wish to progress their work towards practical application and seed analysts who may have made observations that could be developed further with support from the SSAG. We recognise that even though both scientists and seed analysts may have ideas that have potential, individuals may not have the opportunity to work on their practical development. We aim to encourage interaction between ISTA and ISSS to help meet our aims in seed test development.

T026.
MAGNETIC RESONANCE IMAGING FOR SOYBEAN SEED EVALUATION

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Magnetic resonance imaging (MRI) is a noninvasive and nondestructive technique that provides morphological, physiological and histological information in living organisms. It is based on the acquisition either of sequential cross-sectional or 3D images of the spatial distribution of 1H nuclei in biological structures. In spite of the MRI efficiency to evaluate metabolic activity and identify internal injuries and abnormalities in living tissues, there is no information about its application for soybean seed assessment. Thus, this research aimed to evaluate a gradient echo FLASH (Fast Low Angle Shot) sequence for MRI of soybean seeds. MRIs were obtained in a 2 T/30 cm horizontal superconducting magnet 85310HR (Oxford Instruments, Abingdon, UK) interfaced to a Bruker Avance AVIII console (Bruker-Biospin, Ettlingen, GE) running Paravision 5.1 software (Bruker, Ettlingen, GE). A solenoidal radiofrequency coil (8 mm in diameter, made of AWG 18 copper wire with two variable capacitors for matching and tuning calibration) was used to evaluate each seed individually (hydrated up to 15% water content in wet basis). A T1-weighted 3D FLASH sequence (repetition time = 100 ms, echo time = 3.3 ms, flip angle = 45°, 8 averages, 20 min/seed) was acquired. A volume of 12 × 12 × 8 mm^3 was covered with 96 × 96 × 16 points, producing voxels with spatial resolution of 125 × 125 × 500 μm^3. The gradient echo FLASH sequence allowed a suitable image acquisition with short echo and repetition time to obtain images weighted mainly by the contrast in T1. MRIs were very useful to identify physiological processes during seed imbibition, characterized by ribbing of hyperintense tonality in the cotyledons, i.e., MRI signal corresponding to the water that was being directed to the embryonic axis. Furthermore, this technique allowed the identification of changes in internal tissues caused by mechanically and seed storage fungi (*Aspergillus* sp and *Penicillium* sp) induced damages as compared to intact and uninoculated seeds.

T027.
IMAGE ANALYSIS AND VIGOR OF TOBACCO SEEDS

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This feasibility study provides some evidence of the potential of image analysis to be used for evaluate the vigor of tobacco seed lots. The research was carried out to verify the efficiency of the GroundEye system in the evaluation of vigor in tobacco seeds. Six lots of the cultivars BAT 2101 (Burley varietal group), CSC 447 and CSC 449 (both from the Virginia varietal group) were used. To characterize and evaluate the quality of these lots, germination, first germination count, germination speed index (IVG), time for 50% germination (T50), accumulated germination and mean germination time (TMG) . In addition to these tests, the image analysis of tobacco seedlings from the emergency test was performed using the GroundEye system, version S120. A completely randomized design (CI) was used

for each cultivar evaluated, with a factorial of 6 x 6 x 2, with six lots, six days of emergency test evaluation (5th, 6th, 7th, 12th, and 16th days after Sowing) and two evaluation methods (automatic and manual). It can be concluded that the GroundEye system is efficient in the evaluation of vigor in tobacco seed lots and offers key advantages over alternative methodologies such as its non-destructive nature and suitability for high throughput analyses. Support: CNPq, Fapemig.

T028.
LIPID PROFILING OF FIVE CONIFER SEEDS USING MALDI TOF MS SPECTROMETRY
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Lipid profiling using MALDI TOF MS is simple, easy and high-throughput methodology. Lipid extracted from three species of Pinaceae; *Pinus densiflora, Pinus koraiensis, Larix kaempferi* and two species of Cupressaceae; *Cryptomeria japonica, Chamaecyparis obtuse* have been employed to set-up the method for seed lipid analysis. Seed lipids were extracted using MTBE method, which was suitable for high-throughtput analysis. Extracted lipids were mixed with 9-aminoacridine matrix solution, and applied over the target. Lipid MALDI spectra were taken in negative ion modes and fragment ion analysis of lipid were performed by MALDI TOF/TOF MS. This procedure allowed the quick, precise and accurate analysis of phospholipids, utilizing only a 100mg of sample. Results showed that various phosphatidic acids, phosphatidylinositols and phosphoinositides were detected in the mass spectra. The unique lipid mass profiles obtained from five species of conifer. Clusters of the lipid mass profiles were consistent with the plant taxonomic relationship.

ISSS Annual Meeting
7:00 - 8:30 pm
Cypress 1&2

WEDNESDAY, September 13, 2017

SEED INDUSTRY SPECIAL EVENTS

TOURS AND EVENTS

Tours *Salinas Valley area*
Check in for tours ½ hour before scheduled tour bus departure time at conference registration desk at Monterey Plaza Hotel, lower lobby.
Tour 1 (Purple): Check in at 7:00 am, bus departs 7:30am
Tour 2 (Red): Check in at 7:00 am, bus departs 7:30am
Tour 3 (Green): Check in at 7:45 am, bus departs 8:15am
Tour 4 (Blue): Check in at 7:15 am, bus departs at 7:45am

11:30 am - 2:00 pm • **Lunch and Museum Tour** (self-guided) *CSUMB@Salinas City Center, Salinas*
2:00 - 4:00 pm • **Trade Show and Career Fair** *CSUMB@Salinas City Center, Salinas*
4:00 - 5:00 pm • **Seed Central Networking** *CSUMB@Salinas City Center, Salinas*

5:30 - 6:30 pm • **Seed Central Panel Discussion** *Fox Theater, Salinas*
"The Future of Research and Practice in Seed Biology, Seed Technology and Seed Ecology"

PANEL MEMBERS
Ramin Yadegari • Seed Biology
Johan Van Asbrouck • Seed Technology
Joe DiTomaso • Seed Ecology

THURSDAY, September 14, 2017

SEED GERMINATION & DORMANCY SYSTEMS (SGS/SDRS)
Chairpersons: **Camille Steber, Steven Penfield**
Cypress Ballroom

Keynote Speakers

8:00 - 8:45 am • **Eiji Nambara**
NIN-LIKE PROTEIN8 IS A MASTER REGULATOR OF NITRATE-PROMOTED SEED GERMINATION IN ARABIDOPSIS
Nambara, E. and Yan, D.
Department of Cell & Systems Biology, University of Toronto, Toronto, Ontario, Canada
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Seeds respond to multiple different environmental stimuli and these environmental factors in turn regulate seed germination. Nitrate is a germination stimulator for many plant species. Despite its importance, it remains unclear as to how seed responds to nitrate in the control of seed germination. Here, we report that the Arabidopsis NIN-LIKE PROTEIN 8 (NLP8) is essential for nitrate-promoted seed germination. Loss-of-function mutants of *NLP8* lacked the ability for nitrate-promoted seed germination. NLP8 functions even in the nitrate reductase-deficient mutant background, and this mechanism is conserved amongst Arabidopsis accessions. NLP8 reduced abscisic acid (ABA) levels in a nitrate-dependent manner through directly binding to the promoter of *CYP707A2* encoding an ABA catabolic enzyme. Genetic analysis indicates that the NLP8-mediated induction of *CYP707A2* is essential for the nitrate-promoted seed germination. Finally, our results showed that NLP8 is localized to nuclei and post-translationally activated by nitrate signaling with a different mechanism from nitrate-dependent nuclear retention of NLP7. This indicates that seeds contain a unique mechanism for nitrate signaling.

8:45 - 9:30 am • **Luis Lopez-Molina**
IDENTIFICATION OF A NON-CANONICAL DE NOVO DNA METHYLATION PATHWAY REGULATING DORMANCY-SPECIFIC IMPRINTING AND COLD-INDUCED DORMANCY
Iwasaki, M., Hyvarinen, L., Piskurewicz, U. and **Lopez-Molina, L.**
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Newly produced seeds exhibit primary dormancy, a trait whereby seed germination is blocked under conditions that would otherwise be favorable for germination. Following a dry after-ripening period seeds gradually acquire the capacity to germinate. The time period of dry after-ripening required to release dormancy is a measure of dormancy levels. Dormancy levels can be maternally regulated and this regulation is further modulated by environmental conditions during seed set and particularly by cold, which increases dormancy levels. The Arabidopsis endosperm plays an essential role to implement dormancy by releasing abscisic acid (ABA) towards the embryo. We recently identified in the endosperm a set of imprinted genes, i.e. genes exhibiting preferential paternal or maternal allele expression. Some of the maternally expressed genes where shown to mediate maternal inheritance of seed dormancy (Piskurewicz et al. 2016). Interestingly the imprinting expression pattern of those genes is observed only when seeds are dormant; furthermore, the expression of most of dormancy-specific imprinted genes is suppressed in the seeds produced by mother plants exposed to cold temperatures. The underlying mechanisms sustaining imprinted gene expression and repression of gene expression by cold are poorly understood. We studied one dormancy-specific imprinted gene and found a transposable element (TE) located upstream of its coding sequences. TEs are often associated with genomic imprinting. We will present results showing that DNA methylation mediated by a non-canonical de novo DNA methylation pathway is targeting this TE and is responsible for preferential maternal allele expression and cold-induced gene expression repression.

9:30 – 10:00 am • **Morning Break**
Monterey Bay Room

SEED SCANNING SYSTEMS (SSS)

Chairpersons: **Alan Taylor, Gerhard Leubner**
Cypress Ballroom

Keynote Speakers

10:00 - 10:45 am • **Laura Bowden**
Sponsored by ISTA
SCANNING SYSTEMS AND SEED QUALITY ASSESSMENT
Bowden, L.H.
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Scanning techniques have great potential for development and use in the areas of seed quality assessment and seed testing. A wide range of scanning techniques have been developed for use with seeds, including machine vision, raman and infra-red spectroscopy, hyper-spectral imaging, x-ray, thermal imaging and chlorophyll fluorescence. Techniques have been developed that assess chemical composition, purity, disease, mechanical damage, insect damage, seed vigour and germinability. Some examples include machine vision to identify different species within a sample, the use of x-rays to show mechanical and insect damage, and chlorophyll fluorescence to identify seeds at different stages of maturity. Scanning techniques are usually non-destructive and are very rapid – these traits are appealing, permitting many tests within a short timescale, as well as multiple tests on the same seed. More traditional seed testing methods are often time-consuming and tend to require highly skilled seed analysts.

New technology is being developed at a rapid rate, and in agriculture farmers are increasingly using applications such as precision agriculture to improve efficiency and outputs. The seed sector needs to keep up with this demand for faster results; novel methods of determining seed quality using scanning techniques are some of the most promising of the technologies that have recently been developed.

This presentation is sponsored by the International Seed Testing Association (ISTA). ISTA's vision is "Uniformity in seed testing worldwide", which it aims to achieve by promoting uniformity in seed testing. This presentation will review the potential of various scanning technologies for seed quality determination and discuss what is required when assessing the potential of a new technique as a seed test. It will also consider the importance of collaboration between seed scientists and those involved in seed testing to ensure that the most appropriate techniques are developed, tested and used.

10:45 - 11:30 am • **Birte Boelt**
MULTISPECTRAL IMAGING – A NEW SEED ANALYSIS TECHNOLOGY?
Boelt, B., Shrestha, S., Imran, M., Salimi, Z., Gislum, R. and Joergensen, J.R
Department of Agroecology, Aarhus University, Slagelse, Denmark
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Seed quality is a multiple component including varietal and analytical purity, germination capacity, vigour, seed health and uniformity. Currently, testing for seed quality relies on physical, chemical as well as visual inspections, which are time consuming, and the visual inspections are subjective and difficult to repeat and reproduce.

The VideometerLab instrument (Videometer A/S, Denmark) is a multispectral imaging system equipped with a camera inside an integrating sphere with light emitting diodes (LED) in 19 wavelengths including near-infrared (NIR) regions. The system is calibrated with respect to colour, geometry and self-illumination, thereby gaining a set of directly comparable images. With the use of advanced vision technology it is possible to describe surface characteristics such as changes in colour, texture and chemical composition with high accuracy and repeatability.

Earlier findings have indicated that multispectral imaging has the potential to identify fungal pathogens on the seed surface and to determine seed germination capacity by sequentially illuminating the seed with light in the visual and near-infrared wavelength regions.

Since September 2012 investigations to apply multispectral imaging to elucidate functional seed quality traits have been carried out in collaboration between seed industry and Aarhus University. Activities have focused on:

- Analysing physiological germination of grass seed, the correlation of the protrusion of the radical and the percentage of normal seedlings
- Identifying fungal pathogens on barley seeds
- Distinguishing weed seeds from vegetable seed.

OECD defines the standards for the certification of seeds in the international trade, and the International Seed Testing Association (ISTA) develops, approves and disseminates methods for harmonized and standardized seed quality analysis. The Association of Official Seed Analysts (AOSA) has a similar function in the USA and Canada.

The procedures of seed quality analysis are consolidated and globally accepted and new technologies need to be thoroughly documented before approval is obtained. However, there is a recognized need for new technologies to improve seed quality analysis and to reduce the cost of these labor-intensive tests.

Our presentation will provide information about the obtained results and an evaluation of the possibilities and challenges replacing visual inspection by the multispectral imaging technology in seed quality assessments.

11:30 - 11:50 am • **Ghassen Trigui**

(Ghassen Trigui's talk will be delivered by Birte Boelt)

INTERNAL SEED MORPHOLOGY USING X-RAYS MICROTOMOGRAPHY IMAGING AND IMAGE PROCESSING

Trigui, G.[1], **Boelt, B.**[2] and Léchappé, J.[1]

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[2]*Aarhus University, Dept. of Agroecology, Slagelse, Danemark*

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X-rays microtomography (XRMT) is a non-destructive imaging technique which uses transmitted X-rays to see inside an object without cutting. It provides high-resolution data of the attenuation of X-rays at each point of a specimen after crossing it through different directions. Thus, anatomy, internal structures with different attenuation coefficients can easily be visualized in 3D or virtually cut in various planes of section in the same way as a very thin histological sectioning. The emergence of several commercial systems makes XRMT more accessible for laboratory testing. Particularly in seed science, this approach can be successfully used to assess numerous seed structures (i.e. embryo, cotyledons, teguments, cavities, etc.) and seed physical quality like the presence of cracks, insect damages or defects, with high accuracy. Visually, seed external appearance does not reflect its physical quality or neither provide any prior information of its germination. In fact, it may seem intact while it is mechanically damaged from the inside; has large crack extent or abnormal embryo development. All of these problematic can be detected and quantified using XRMT. It can also be used for the characterization of varieties with different phenotyping traits or for the quality control of coated/treated seeds. This however requires the development of suitable variety-dependent image processing and analysis tools. Here, capabilities of XRMT coupled with image processing procedures are presented and discussed for different applications on seed science.

11:50 am - 12:10 pm • **Andrew Margenot**

APPLICATION OF FOURIER TRANSFORM INFRARED SPECTROSCOPY FOR DETECTION OF BACTERIAL FRUIT BLOTCH DISEASE

Margenot, A.[1], Parikh, S. J.[2], Zhou, B.[3], Walcott, R. R.[4] and Welbaum, G.E[3]

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[2]*Department of Land, Air and Water Resources, University of California Davis, Davis, CA, USA*

[3]*Virginia Tech Corporate Research Center, Blacksburg, VA, USA*

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Contact: Andrew Margenot, ajmargenot@ucdavis.edu; Greg Welbaum, welbaum@vt.edu

Bacterial fruit blotch (BFB), caused by *Acidovorax citrulli* affects the production of cucurbits worldwide, and causes substantial economic losses. Since cucurbit seeds are the most important source of inoculum for BFB outbreaks, seed health testing is an important component of disease management. Currently, there are no nondestructive assays that are sensitive enough to reliably detect *A. citrulli*-infected seeds. Fourier transform infrared spectroscopy (FTIR) may provide the sensitivity necessary to detect *A. citrulli*-infected seeds. Attenuated total reflectance (ATR) FTIR was evaluated for the detection of watermelon seeds infected with *A. citrulli* by pistil inoculation. *A. citrulli* cells produced a unique signature at a detection limit of approximately 10^5 cfu/mL. Infected, dry watermelon seeds whose embryos were infected with *A. citrulli* produced a different spectral profile compared to non-infected seeds. Spectral subtractions between infected and non-infected seeds suggest the potential for indirect detection of *A. citrulli* by altered ester C-O absorbance bands. Principal component analysis (PCA) of seeds infected with bacterial concentrations ranging from 0.001 – 0.1 OD demonstrated potential for multivariate detection of infected seeds at intermediate contamination levels (0.01 OD)

relative to non-infected seeds. This separation was driven by high loading of ester C-O absorbance at frequencies ranging from 1120 - 1000 cm^{-1}, though absorbances identified in pure *A. citrulli* culture were not observed. These results suggest that FTIR can be used to nondestructively detect seeds infected with moderate levels (10^5 cfu/mL) of *A. citrulli* infection.

12:30 – 1:30 pm • **Lunch**
Upper Plaza and Dolphins Ballroom

SEED CONSERVATION SYSTEMS 2 (SCS-2)
Seed storage/Aging/Longevity (Orthodox Seed)
Chairpersons: **Christina Walters, Nicholas Genna**
Cypress 1&2

1:30 - 1:45 pm • **Andreas Borner**
SEED CONSERVATION OF CROP GERMPLASM *IN EX SITU* GENEBANKS – STATE OF THE ART
Börner, A.[1], Nagel, M.[1], Rehman Arif, M.A.[1,2],Agacka-Mołdoch, M.[1,3], Mohler, V.[4], Börner, M.[1,5], Lohwasser, U.[1], Riewe, D.[1], Wiebach, J.[1] and Altmann, T.[1]
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[3]*Institute of Soil Science and Plant Cultivation, State Research Institute, Puławy, Poland*
[4]*Bavarian State Research Center for Agriculture (LfL), Institute for Crop Science and Plant Breeding, Freising, Germany*
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Plant genetic resources play a major role for global food security. The most significant and widespread mean of preserving plant genetic resources is *ex situ* conservation. Today about 1,750 *ex situ* genebanks world-wide maintain 7.4 million accessions. One of the ten largest *ex situ* collections of our globe is located at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, conserving 150,000 accessions from 3,200 plant species and 780 genera. Since the majority of genebank holdings globally is maintained as seed, seed storability is of exceptional importance for germplasm conservation.

At IPK research on seed longevity was initiated for a range of crops and wild relatives stored over decades. Historical germination data accumulated during 35 years of seed germination monitoring were analysed to predict species specific seed longevities. The study considered 75 species comprising 79,075 accessions and 157,402 observations. Beside interspecific differences variation was also detected within species and genetic analyses were initiated in barley, wheat, oilseed rape and tobacco. Loci responsible for seed longevity were identified investigating bi-parental mapping populations but also by genome wide association mapping analyses.

In addition, mass spectrometry based untargeted metabolite profiling experiments were performed in order to detect biochemical changes coinciding with loss in seed germination. GC-MS analysis of the polar metabolome of wheat and barley identified glycerol and related intermediates as highly correlated to germination rate. Therefore, the lipidomic composition of a wheat panel was investigated using high-resolution liquid chromatography-mass spectrometry (LC-MS). A high proportion of tentative oxidized lipids was detected, suggesting lipid oxidation as the causal trigger for membrane degradation.

1:45 – 2:00 pm • **Jaesung Lee**
DISCOVERY OF SEED LONGEVITY GENES IN *AUS* AND *INDICA* RICE SUBPOPULATIONS THROUGH GENOME-WIDE ASSOCIATION STUDIES
Lee, J.S.[1], Yoon, M.R.[2], **Kwak, J.[2], Lee, J.S.[2]** and Hay, F.R.[1*]
[1]*T.T. Chang Genetic Resources Center, International Rice Research Institute, Los Baños, College, Laguna, 4031, Philippines*
[2]*National Institute of Crop Science, Rural Development Administration, Suwon, Gyunggi-do, Republic of Korea*
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The International Rice Genebank at the International Rice Research Institute conserves over 127,000 accessions of wild and cultivated rice. All the accessions are tested at regular intervals as part of the routine viability monitoring, involving the destructive use of seeds and incurring considerable financial cost. To reduce efforts and resources, we have proposed an innovational strategy, to establish a seed

longevity marker platform. Based on presence/absence of genetic markers associated with seed longevity, viability testing and regeneration intervals will be appropriately adjusted for each accession.

While previous studies on seed longevity markers used a mapping population derived through crossing of *indica* and *temperate japonica* parents, we utilized diverse rice panels comprising 200 *aus* varieties (semi-wild type) and 300 *indica* varieties (cultivated type) collected from 54 countries. The high-density single nucleotide polymorphism (SNP) array composed of one million markers was used for genome-wide association study (GWAS).

Seeds were harvested at three different times to take into account the effect of maturity on seed longevity; the sample with greatest longevity was used for genome-wide association mapping. We also considered the initial physiological quality of the seeds as well as the 'rate' of loss of viability. By testing correlation between seed antioxidants and seed longevity, the identified markers for each trait were compared.

2:00 - 2:15 pm • **Christina Walters**

WHAT DO TEMPERATURE ANOMALIES TELL US ABOUT AGING OF ORTHODOX SEEDS?

Walters, C., Hill, L., Crane, J. and Ballesteros, D.
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Orthodox seeds are defined by the observation that aging is slowed when moisture, temperature or both are reduced. Over the past 25 years, we have come to learn that this pattern describes the process of solidification (also known as 'glass' transition or Tg) in drying cytoplasm, a process in which the cytoplasm stiffens because molecules become very compressed and impede the mobility of neighboring molecules. Interestingly, drying below some moisture limit causes an abrupt change in aging response -- to the extent that moisture no longer strongly regulates aging rate. This moisture anomaly (also known as 'critical water content') occurs well below Tg, at temperature x moisture combinations reminiscent of 20th century sorption theory, possibly reflecting so-called 'fast' motions of molecules within solid matrices. As long-term, multifactorial data sets become increasingly available, anomalous effects of temperature on aging rate are also clear. We can detect considerable activity within lipid domains of the cytoplasm that might explain temperature anomalies near -20C. However, without lipid involvement, we can show limited benefits of reduced temperature, analogous to previous studies manipulating water content. Our long-term data sets ostensibly demonstrate that moisture and temperature have nominal effects on seed aging rates beyond certain empirically-defined limits. This presents a sobering reality that seed longevity is finite no matter how stringently storage conditions are controlled. The next obvious questions become how to recognize seeds with poor shelf-life at the onset of storage and how to boost shelf-life without genetic manipulation.

2:15 - 2:30 pm • **Margaret Fleming**

CHANGES IN RNA INTEGRITY DURING SEED AGING

Fleming, M.B.[1], Patterson, E.L.[2], Gaines, T.A.[2] and Walters, C.[1]
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Can a biological marker indicate the effects of seed aging before a seed lot begins to die? We have explored the relationship between RNA damage and loss of germination potential during storage. Degradation of ribosomal RNA of old (stored more than 20 years at 5C) compared to fresh (stored less than 2 years) seeds is observed as a decrease in the ratio of 25S to 18S rRNA, accompanied by an increase in smaller RNA fragments. This change in RNA integrity can be quantified in terms of changes to a quality metric such as the RNA Integrity Number (RIN). Decrease in average RIN from above 7 to below 5 is strongly associated with lost germination potential of a seed lot in both short- and long-lived orthodox seeds stored at 5C for over 25 years.

In addition to slow changes observed during dry refrigerated storage, we tested the relationship between storage stresses, seed germination, and RNA integrity by challenging soybean seeds with freezing of hydrated seeds, drying of germinating axes, and timed exposure to hot dry or warm humid conditions. Despite lethal treatment, average RIN remained high in freeze- and desiccation-damaged seeds. Treatment with temperatures above 35C or relative humidities above 75% resulted in poor correlations between average RIN and germination percent.

RIN assessments on total RNA primarily measure rRNA, and do not report mRNA quality. To characterize damage that mRNA may incur during storage, poly-A mRNA from embryonic axes of fresh (stored less than 2 years at 5C) and old (stored more than 20 years) soybean seed was sequenced as

unfragmented cDNA molecules using the Oxford Nanopore MinION. As expected, fresh seeds produced full-length transcripts. However, old seeds produced just the 3' ends of transcripts, except for a small group of transcripts that had full-length sequences. These dramatic differences suggest that transcripts accumulate lesions preventing poly-T primed cDNA synthesis, or that mRNAs are truncated in old seeds; either situation would obstruct translation during germination. We plan to analyze select transcripts in seed lots of different ages and germination rates to test for a correlation between lost transcripts and lost germination potential.

2:30 - 2:45 pm • **Mikolaj Wawrzyniak**

FEASIBILITY AND CONSEQUENCES OF CRYOPRESERVATION OF SEEDS OF WILD FRUIT TREES

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Wild fruit trees are a great source of biodiversity in nature. They are valuable species for forest habitats, being a source of food for animals and genetic variation for breeders. However, the rapid loss of natural habitats has resulted in decline of many populations in Europe, so *ex situ* conservation of these species is necessary. Seeds of most of European fruit tree species are regarded as orthodox, which can be safely preserved both in conventional and cryogenic conditions (-196°C; LN) in gene banks. Cryopreservation makes it possible to extend considerably the storage time of viable seeds. This method requires identification of a safe range of water content at which seeds can survive cryogenic conditions without losing their viability. In this study we assessed the feasibility of safe cryopreservation and 2-year storage in liquid nitrogen of 6 wild fruit tree species (*Sorbus aria, S. intermedia, S. aucuparia, Cornus sanguinea*, *Malus sylvestris*, *Prunus padus*, and *P. avium*). Collected seeds were desiccated to different levels of seed moisture content, ranging from 1.5% to 29.2% (fresh weight basis). Desiccated seeds were divided into the control (unfrozen seeds) and seeds exposed to liquid nitrogen for 24 h (3-4 replications of 30-50 seeds each). Additionally, part of the samples remained frozen for 2 years. After storage, germination and seedling emergence were tested. All seeds before testing underwent cold-warm stratification to break dormancy. Some of the developed seedlings were subjected to further examination: analysis of biomass allocation (C, N) and global changes in DNA methylation after storage, in search for unseen consequences of cryopreservation. Except for *S. aria*, all the examined species proved to withstand severe desiccation and maintain high germinability (61-89%). Thus seeds of the tested species can be classified as orthodox. Seed moisture content above 16-18% was lethal for cryopreserved seeds. After 2 years of storage, seeds of all the tested species germinated and seedling emergence ranged from 77% to 91%. The analyzed seedlings showed significant differences in global DNA methylation. Anyway, cryopreservation is considered safe and effective, although it can affect further plant growth by changes at the molecular level.

2:45 - 3:00 pm • **Gerhard Leubner**

ROCKET SCIENCE – MOLECULAR MECHANISMS UNDERPINNING SEED AGING AND VIGOR LOSS DURING SPACE TRAVEL AND DRY STORAGE OF VEGETABLE SEED ON THE INTERNATIONAL SPACE STATION

Khan, S.[1], Ignatz, M.[1], Wilhelmsson, P.K.I.[2], Haas, F.B., Gawthrop, F.[3], Rensing, S.A.[2], Griffiths, A.[4] and **Leubner-Metzger, G.[1]**
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Seeds of many vegetable species are known for their resilience during storage in the low-hydrated ("dry") state. They can even survive exposure to the extreme environmental conditions on earth and in space. Seed viability loss is a late response during dry storage, which is well preceded by seed vigor loss due to aging processes. Knowledge on the molecular mechanisms underpinning this storage resilience and vigor/viability loss is important for food security in the context of climate change on earth, as well as for future manned space missions to other planets. In September 2015, one million tiny rocket seeds (*Eruca sativa*) were launched into space on Soyuz 44S to begin their six month stay on board of the International Space Station (ISS). In this Rocket Science project initiated by the Royal Horticultural Society (RHS) thousands of Schools compared the germination and seedling establishment of these "space" seeds with the corresponding "earth" (control) seeds. In agreement with their results we also found that there was no viability loss, but that the space travel resulted in a

slightly reduced germination speed. To investigate this further we conducted artificial seed aging assays which revealed that the aging sensitivity of the “space” seeds was significantly increased when compared with the “earth” seeds. RT-qPCR analysis of genes known to be involved in seed aging were differentially regulated as result of the space travel. Among them were cell-wall- and cytoskeleton-associated genes, as well as chaperones. In contrast to this, several other genes known to be seed aging markers were not differentially expressed. Results from global transcriptome analyses by RNAseq will be presented which reveal underpinning molecular mechanisms of the rocket seed vigor loss due to the space travel. Together with the observed physiological changes these findings demonstrate that while their viability may remain very robust, space-induced molecular changes related to vigor loss can be detected with very high sensitivity. Specific space environmental factors may cause these molecular patterns. The possible physical causes, molecular pathways and implications of these findings for food security of manned space missions will be discussed.

SEED DORMANCY SYSTEMS 2 (SDRS-2)
Chairpersons: **Camille Steber, Steven Penfield**
Cypress 3&4

1:30 - 1:45 pm • **Nitin Kamble** *(talk cancelled)*
PROTEIN L-ISOASPARTYL METHYLTRANSFERASE (PIMT) IS IMPLICATED IN SEED DESICCATION TOLERANCE AND SEED LONGEVITY IN RICE.
Kamble, N.U., Petla, B.P. and Majee, M.
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PROTEIN L-ISOASPARTYL O-METHYLTRANSFERASE (PIMT) is a protein repairing enzyme and catalyses the conversion of spontaneously modified isoAsp to Asp in proteins. The present study in rice demonstrates that PIMT activity sharply increases at maturation phase retains in dry seed and then rapidly decline upon completion of germination. Likewise, deleterious isoaspartyl accumulation also increases during seed maturation and is highly abundant in dry seed but decreases upon imbibition. Transcript and western blot analyses clearly demonstrated distinct tissue and seed development stage specific accumulation of these PIMT isoforms, indicating their participation and specific contribution in seed desiccation in rice. Immunolocalization studies reveal distinct isoform expression in embryo and aleurone layers. For further analysis, we raised transgenic lines for each isoform and the data reveals the distinct roles of each OsPIMT isoform in restricting deleterious isoasp and age induced ROS accumulation to improve seed vigor and longevity. It also raises the intriguing possibility that PIMT repairs antioxidative enzymes and proteins which restrict ROS accumulation, lipid peroxidation, etc. in seed particularly during aging thus contributing to seed vigor and longevity. Collectively, these data imply that PIMT mediated protein repair mechanism initiates during the course of seed development in rice and each PIMT isoform plays a distinct yet coordinated role in maintaining seed vigor and longevity by restricting deleterious isoAsp and ROS accumulation in tissue and development stage specific manner.

1:45 - 2:00 pm • **Toshiyuki Imaizumi**
CHARACTERIZATION OF THE TRANSCRIPTOME DURING SEED DORMANCY CYCLING IN MONOCHORIA VAGINALIS USING MICROARRAYS AND RNA-SEQ
Imaizumi, T.
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Dormancy cycling is important for seedling establishment by facilitating germination when the environment is favorable. The molecular regulation of dormancy cycling is not clear, leading to the question of whether different dormant states, such as primary and secondary, are fundamentally similar. In this study, we investigated transcriptome profiles during dormancy cycling in a summer annual weed, *Monochoria vaginalis*. *M. vaginalis* is one of the most serious weeds in rice paddy fields in Asia. Microarrays were used for a global transcript analysis of *M. vaginalis* seeds retrieved from a rice paddy environment. Microarrays containing 42,378 probes were designed using Ion PGM next-generation RNA sequencing data. Seeds were buried in February 2009 in a rice paddy environment to simulate seed behavior in the soil seed bank. Seed samples were retrieved every other month from April 2009 to December 2010. We evaluated the degree of dormancy by performing germination assays at 25/15°C with an 8h light/16h dark daily cycle. Similar to the other summer annual weeds, depth of dormancy for *M. vaginalis* declined from late winter, then increased from late summer. Microarray analyses revealed that primary dormant seeds had a different transcriptome profile

compared with secondary dormant seeds. Additionally, secondary dormant seeds in 2009 had a different type of transcriptome profile compared to secondary dormant seeds in 2010. Finally, there were different types of transcriptome profiles during shallow dormant states. These results indicate that different dormant states are likely to be regulated by different mechanisms.

2:00 - 2:15 pm • **Stephanie Sjoberg**

THE LOW FALLING NUMBER PROBLEM OF WHEAT: APPLYING KNOWLEDGE ABOUT SEED BIOLOGY TO A REAL WORLD ISSUE

Sjoberg, S.M.[1], Steber, C.M.[2], Garland-Campbell, K.A.[2] and Carter, A.H[1]
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The Hagberg-Perten Falling Number test is used by the wheat industry to measure starch degradation caused by alpha-amylase enzyme activity in flour. Grain with too much alpha-amylase activity must be sold at a severe discount because it results in poor quality baked goods. Problems with an excess of alpha-amylase result from two independent genetic causes, insufficient seed dormancy to resist preharvest sprouting and a developmental defect called late maturing alpha-amylase (LMA). Preharvest sprouting is the germination of physiologically matured grains on the mother plant when rainy, cool conditions break dormancy and induce germination. Alpha-amylase is naturally produced to mobilize stored reserves during sprouting. In susceptible varieties, LMA is the induction of alpha-amylase in response to a high or low temperature shock can during late seed maturation. Over the last 4 years, over 8,000 Falling Number data points have been collected on Washington State University Cereal Variety trials at locations across the state. Falling Number data in years without challenging weather was not predictive of Falling Number in environments with LMA or sprouting. Moreover, ANOVA analysis of the dataset as a whole suggested that genetics accounted for only 15% of the variability for Falling Numbers. However, such analyses fail to take into account that there is more than one cause of the problem. When weather data was used to tease apart which low Falling Numbers events were due to LMA and/or preharvest sprouting, a different picture of heritability emerged. Being able to see the data in terms of separate components will help to making better breeding decisions, and serve as a first step to understanding the genetics of this problem.

2:15 - 2:30 pm • **Jirui Wang**

CHARACTERIZATION OF PRE-HARVEST SPROUTING RESISTANT GENES IN A LARGE GERMPLASM COLLECTION OF CHINESE WHEAT LANDRACES

Zhou, Y.[1], Tang, H.[1], Cheng, M.-P.[1], Chen, Z,-X.[1], Li, Z.-Y.[1], Gao, S.[1], Liu, Y.-X.[1], Dankwa, K.O.[1], Jiang, Q.-T.[1], Lan, X.-J.[1], Pu, Z.-E.[1], Wei, Y.-M.[1], Zheng, Y.-L.[1], Hickey, L.T.[2], Liu, D.[1] and **Wang, J.-R.**[1*]
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Pre-harvest sprouting (PHS) is mainly caused by the breaking of seed dormancy in high rainfall regions, which leads to huge economic losses in wheat. In this study, we evaluated 717 Chinese wheat landraces for PHS resistance and carried out genome-wide association studies (GWAS) using to 9,740 DArT-seq and 178,803 SNP markers. Landraces were grown across six environments in China and germination testing of harvest-ripe grain was used to calculate the germination rate (GR) for each accession at each site. GR was highly correlated across all environments. A large number of landraces (194) displayed high levels of PHS resistance (i.e. mean GR < 0.20), which included nine white-grained accessions. Overall, white-grained accessions displayed a significantly higher mean GR (42.7 - 79.6%) compared to red-grained accessions (19.1 - 56.0%) across the six environments. Landraces from mesic growing zones in southern China showed higher levels of PHS resistance than those sourced from xeric areas in northern and north-western China. Three main quantitative trait loci (QTL) were detected by GWAS: one on 5D that appeared to be novel and two co-located with the grain color transcription factor Tamyb10 on 3A and 3D (Fig.1). An additional 32 grain color related QTL (GCR-QTL) were detected when the set of red-grained landraces were analyzed separately (Fig.1). GCR-QTL occurred at high frequencies in the red-grained accessions and a strong correlation was observed between the number of GCR-QTL and GR (R2 = 0.62). These additional factors could be critical for maintaining high levels of PHS resistance and represent targets for introgression into white-grained wheat cultivars. Further, investigation of the origin of haplotypes associated with the three main QTL revealed that favorable haplotypes for PHS resistance were more common in accessions from higher rainfall zones in China. Thus, a combination of natural and artificial selection likely resulted in landraces incorporating

PHS resistance in China. Moreover, multiple variations in genes controlling germination showing clear allelic frequency changes corresponding to the dispersion of wheat in China.

2:30 - 2:45 pm • **Shantel Martinez**
IDENTIFICATION OF A LOCUS CORRESPONDING TO A PREHAVRVEST SPROUTING TOLERANT MUTANT, *ERA8,* IN WHEAT (*TRITICUM AESTIVUM* L.)
Martinez, S.A.[1,3], Shorinola, O.[4], Beck, S.R.[5], Skinner, D.[2,3], See, D.[2,3], Garland-Campbell, K.A.[1,2,3], Uauy, C.[4] and Steber, C.M.[1,2,3]
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Preharvest sprouting (PHS) is the germination of mature wheat grain on the mother plant when cool and wet conditions occur before harvest. PHS causes severe losses for wheat growers. Lack of seed dormancy accounts for 60-80% of PHS susceptibility. *ERA8* was created using EMS mutagenesis and the mutation was selected for increased sensitivity to the dormancy hormone ABA, resulting in increased seed dormancy and PHS tolerance. This gene is effective in the soft white spring background, Zak, and represents a new source of PHS tolerance. The goal was to identify *ERA8*-linked molecular markers for genomic selection during breeding. We mapped the *ERA8* gene using both traditional quantitative trait locus (QTL) analysis and using a next-generation sequence-based approach. Using both methods, we localized *ERA8* to a region of chromosome 4A. QTL analysis of the Louise/Zak*ERA8* RIL population was conducted in R/qtl using nucleotide differences identified by genotyping by sequencing (GBS). A total of 4 significant ($p<0.05$) QTL were identified and one of them increased ABA sensitivity due to the *ERA8* allele. *ERA8* was also mapped using bulk segregant analysis and exome capture of the Zak/Zak*ERA8* backcross population, where *ERA8* was the only segregating gene impacting germination on ABA. Over 70 EMS induced single nucleotide polymorphisms (SNPs) between wild type and mutant were identified solely on the 4A chromosome. Additional recombinants from the backcross population were identified and used to fine map the *ERA8* mutation down to a 4.9 cM region. *ERA8* is currently being crossed into wheat breeding lines to increase preharvest sprouting tolerance. The *ERA8* SNP markers identified by this project are currently being used for rapid genomic selection in breeding lines. Identification of candidate genes in the region is currently underway.

2:45 - 3:00 pm • **TBD**

3:00 – 3:30 pm • **Afternoon Break**
Monterey Bay Room

SEED ECOLOGICAL SYSTEMS 2 (SES-2)
Chairpersons: **Bob Geneve, Norman Pammenter**
Cypress 1&2

3:30 - 3:45 pm • **Diego Batlla**
A SIMULATION MODEL FOR PREDICTING THE RISK OF WEED EMERGENCE FROM SOIL SEED-BANKS
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The possibility of predicting seedling emergence from soil seed-banks has long been a goal to both seed ecologists and agronomists. On one hand, timing of emergence can have critical consequences for plant fitness and population dynamics. Therefore, forecasting patterns of emergence under different climatic scenarios is relevant for understanding how plant populations will respond to future climate change. On the other hand, because plants are more vulnerable in the seedling stage, the possibility of predicting seedling emergence patterns is instrumental for improving the efficacy of weed control methods in agricultural systems. In the present work we used available bibliographic information regarding the effect of different environmental and management factors on the regulation

of dormancy and germination of two cosmopolitan weed species (*Polygonum aviculare* and *Amaranthus hybridus*) to develop a simulation model able to predict the risk of emergence of both weeds under different agricultural and environmental scenarios. The model structure is able to simulate (1) changes in the seed-bank dormancy level according to soil temperature and water potential, (2) the response of the seed-bank to dormancy terminating factors (light and alternating temperatures) and (3) the effect of soil temperature and water potential on the germination process. The input variables of the model are: maximum and minimum air temperature, soil temperature and water potential, tillage system, preceding crop and crop yield; while the output of the model is the annual progress of the risk of emergence. Simulations made with the model were tested against weed emergence patterns recorded under field conditions showing a good agreement between observed and simulated data. The model can be used as a tool to assist with decision-making in relation to the control and management of weeds under filed situations. In addition, it can also be used to simulate the risk of emergence of both weeds under different climatic conditions, forecasting how climate change could affect species emergence patterns or testing the likelihood of these weeds to invade a certain geographical region. The current model structure can be easily parametrize using available information for predicting the emergence behaviour of other species of interest.

3:45 - 4:00 pm • **Dana MacGregor**

APPLYING KNOWLEDGE ABOUT SEED DORMANCY REGULATION IN *ARABIDOPSIS THALIANA* TO CONTROL *ALOPECURUS MYOSUROIDES*

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In order to feed the world's increasing population, we need to make efficient and effective use of our arable land. Arable weeds are ubiquitous, persistent, and can significantly reduce crop yields. *Alopecurus myosuroides* (blackgrass) is an annual, seed propagated, grass weed that causes considerable problems for arable farmers in Western Europe: 1) it has a germination window that overlaps with winter crop sowing and seed shed occurs before winter crops are harvested, 2) multiple-herbicide resistance is widespread, and 3) seeding results in severe infection of subsequent crops. Therefore current cultural, chemical, and physical control methods are insufficient and new approaches must be investigated. Seed dormancy prevents germination of viable seeds even under favorable conditions. Dormancy levels are controlled by both genetic and environmental factors. Research using the model plant *Arabidopsis thaliana* has provided a good understanding of the mechanisms underlying establishment and breaking of seed dormancy. We aim to use these data to alter the dormancy of blackgrass and thereby provide novel means to control this weed. Controlling blackgrass by increasing dormancy is attractive because its seed bank has an annual decline of ~75%, and ~65% of emerged seedlings come from seeds that are less than a year old. In this project we will develop blackgrass-specific methods to assess and alter phytohormones, gene expression, and various seed physiology traits. Most importantly, we will develop protocols for transient and/or stable blackgrass transformation which will allow for testing of numerous hypotheses. By sufficiently understanding how to regulate blackgrass seed dormancy in the lab, we can use these data to explore various methods to alter dormancy of seeds that are set in the field.

4:00 - 4:15 pm • **Waheed Arshad**

DIMORPHIC FRUITS AND SEEDS IN *AETHIONEMA ARABICUM*: ADAPTATION MECHANISMS TO ABIOTIC STRESS IN UNPREDICTABLE ENVIRONMENTS

Arshad, W.[1], Steinbrecher, T.[1], Collinson, M.[2], Stuppy, W.[3] and Leubner-Metzger, G.[1]
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Diaspores — here, fruits and seeds — function as higher plant dispersal units, eminently adapted to a highly varied and changeable environment. While most plant species commit themselves to the monomorphic propagation strategy, diaspores may also exhibit heteromorphism, where two or more distinctly different types of fruits or seeds are produced by a single individual plant. One species exhibiting this phenomenon is *Aethionema arabicum*, an annual belonging to the most basal lineage of the Brassicaceae family. It has the remarkable ability to produce two distinct fruits on the same infructescence; the two types of fruits harbour two morphologically and physiologically distinct seed types, denoted as M+ (mucilaginous) and M– (non-mucilaginous). Both diaspores differ markedly in their life history strategies of dispersal, germination, and seedling establishment. Our interdisciplinary

and integrative project utilises the distinct diaspores to explore and elucidate early life history traits that have evolved in annual plant species as adaptations to changing environments. When imbibed, the mature M+ seeds produce mucilage from the outer cell walls of the seed coat epidermal cells, comprising a gel-like pectinaceous layer covered in dome-like structures. In contrast, the mature M– seeds possess a smoother seed surface structure lacking mucilage. A comparative analysis of the two morphs will be presented: as the framework of events which brings about this dimorphism is completely unknown, key morphological and developmental changes associated with *Ae. arabicum* M+ and M– seed coat maturation were characterised. The comparative ecophysiological role of mucilage in M+ and M– seeds was further investigated using mucilage removal experiments, biomechanical tests of adhesion, and germination assays in response to distinct ambient water potentials. The obtained results demonstrate that the diaspore-specific differences in the seed-covering layers could provide for separate roles relating to seed hydration, dispersal ecology, and stress tolerance in semi-arid environments. The work builds on resources and knowledge from the ERA-CAPS SeedAdapt project (*www.seedadapt.eu*), and will establish how diaspore heteromorphic systems function as a “bet-hedging” strategy in variable and unpredictable ecological habitats. Together with recently published results, the new unpublished findings make *Ae. arabicum* an attractive model species for future research on dimorphic diaspore ecology.

4:15 - 4:30 pm • **Jake Chandler**

SEEDADAPT: UNRAVELLING THE MOLECULAR MECHANISMS CONTROLLING GERMINATION AND DORMANCY IN *AETHIONEMA ARABICUM* – A MODEL SPECIES FOR DIASPORE HETEROMORPHISM

Chandler, J.O.[1], Graeber, K.[1], Merai, Z.[2], Mittelsten Scheid, O.[2], Rensing, S.A.[3], Grosche, C.[3], Wilhelmsson, P.K.I.[3], Lenser, T.[4], Theißen, G.[4], Sperber, K.[5], Mummenhoff, K.[5], Nguyen, T.-P.[6], Schranz, M.E.[6], Strnad, M.[7] and Leubner-Metzger, G.[1,7]

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Higher plant dispersal units – diaspores, here: fruits and seeds – ensure the successful dispersal and establishment of their progeny. The SeedAdapt consortium (www.seedadapt.eu) elucidates the molecular mechanisms of fruit, seed and seedling traits that evolved as bet-hedging dormancy strategies to changing and unpredictable environments. A fascinating adaptation to harsh, unpredictable environments is the trait of heterodiaspory – the ability of a single plant to produce diaspores with different morphologies and physiological behaviors. The annual Brassicaceae species *Aethionema arabicum* represents an ideal model plant for the study of the underpinning mechanisms of diaspore heteromorphism. *Ae. arabicum* produces two distinct fruit and seed morphs on the same infructescence: the large fruit morph containing multiple mucilaginous seeds (‘M+’) easily dehisces at maturity which leads to the dispersal of the bare M+ seeds. In contrast, the smaller indehiscent fruit morph contains a single non-mucilaginous seed (‘M–’) which is dispersed encased in its fruit coat via abscission. The two diaspores differ in their germination physiology with the M– seed exhibiting a deeper dormancy that is partly imposed by the fruit coat. *Ae. arabicum* also combines this bet-hedging strategy with phenotypic plasticity: the numbers and ratios of each fruit morph depend on the maternal growth conditions with M+ seed development favored under stressful conditions. Results from a large-scale experiment will be presented, in which we found that germination of the distinct diaspore morphs has a distinct fine-tuned temperature response that depends on growth conditions of the parental plant. We hypothesized that the hormonomes, epigenomes, and transcriptomes of the dimorphic diaspores provide 'syndrome × stress memories' that are dispersed to establish the next generation. By integrating results obtained through RNAseq and hormone profiling, we want to provide a holistic view on fruit/seed stress physiology during dormancy release and germination as relevant to the ecological significance of diaspore dimorphism in *Ae. arabicum*.

4:30 - 4:45 pm • **Héctor Pérez**

DO HABITAT AND GEOGRAPHIC DISTRIBUTION INFLUENCE SEED QUALITY IN REMNANT POPULATIONS OF A KEYSTONE BUNCHGRASS?

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Seed quality is a broad term used to describe seed lots based on properties such as germination ability, vigor, purity and seed health. Conservation and restoration practitioners depend on seed lots of acceptable quality in order to meet seedling establishment goals. However, factors such as resource and pollen limitation, unfavorable environmental conditions, and inbreeding modify seed quality. The wide, fragmented geographic distribution of priority taxa also exacerbates these factors and may necessitate seed harvest over broad areas. Here, I address the influence of maternal plant environment, habitat specificity, and geographic distribution on seed lot quality by assessing production of viable and non-viable seeds from seven remnant wiregrass (*Aristida stricta*) populations occurring in xeric or mesic habitats throughout the Southeastern Coastal Plain (SCP) of the United States. Wiregrass is important for restoration of imperiled pine-grassland ecosystems across the SCP. However, *in* and *ex situ* seedling establishment is chronically problematic due to highly variable germination. Approximately 55 to 90% of seeds were non-viable due to lack of seed fill or fungal contamination. The number of empty seeds always outweighed that of infected seeds. Moreover, the ratio of non-viable to viable seeds ranged from 1.2 to 8.3. Interestingly, stressful maternal plant environments may not necessarily account for decreased seed viability. There was strong evidence (Pearson's $\chi^2_{5,\ 237.8}$, $p < 0.001$, $N = 2{,}819$) that wiregrass population affected seed lot quality. Nonetheless, populations were similar for seed lot quality when grouped by habitat. Alternatively, seed lot quality varied considerably when populations were grouped by geographic region. Greater geographic distance between populations influenced seed lot quality more strongly. Seeds from all populations displayed infection by *Curvularia* and some presented *Sorosporium* infection. Seed viability and health seem associated with population characteristics and geographic distribution rather than habitat type. Practitioners may benefit from assessing seed quality at the population level prior to initiating restoration activities. Moreover, high levels of non-viable wiregrass seeds implies that large quantities of seeds are required in order to meet restoration objectives and practitioners should exercise caution if inter-population seed movement becomes necessary.

4:45 – 5:00 pm • **Malaka Wijayasinghe**

IMPORTANCE OF ASSESSING ACCURATE SEED LONGEVITY AND SEED PRIMING: IMPLICATION FOR *EX-SITU* CONSERVATION IN SEED BANK AND INDUSTRY

Wijayasinghe, M.M.[1], Isanta, M.T.[1], Balestrazzi, A.[2], Colville, L.[3], Pritchard, H.W.[3] and Mondoni, A.M.[1]

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Accurate assessment of longevity and quality of seeds is crucial for effective storage, and enabling their future use. Germination is considered to be the most reliable test of seed quality. In this regard, radicle protrusion as an assessment of germinability may over-estimate the longevity of seeds compared with full emergence (radicle plus cotyledon emergence), although the extent of overestimation across species, and the factors contributing to it are not yet well known. Therefore, longevity of seeds of 35 alpine species was studied by evaluating both radicle and full emergence. Additionally, the effectiveness of seed priming on longevity and quality were investigated for six target species. Seeds were subjected to a controlled ageing test (45 °C and 60 % RH) and their longevity was determined based on both radicle emergence and full emergence. A coefficient of overestimation of seed longevity (OESL) was estimated and its' correlates were identified. Moreover, for six target species, seeds were pre-treated with distilled water, -2.0, -10.0 MPa and 2% KNO_3 and seed longevity was estimated. Seed longevity was significantly higher when radicle instead of full emergence was considered as a proxy of seed viability in half of the species tested, most of this variation was explained by differences in the initial seed viability. OESL was highest for species with shorter-lived (low P_{50}) dwarf seeds growing in basic soil. Seed priming significantly increased both radicle emergence and full emergence percentages and decreased the OESL indicating a high rejuvenating effect on seed longevity and quality of the seeds. We have provided clear evidence that radicle emergence may not always be a reliable indicator of the capacity of seeds to complete the

germination process, leading to overestimation of seed longevity in storage. Therefore, we have developed the coefficient of OESL and identified correlates (seed type, soil type and seed longevity) that may be used to prioritize species' vulnerability to *ex situ* storage and optimize viability testing, thereby reducing labour costs and enabling more effective conservation of seed collections. Moreover, seed priming may have important implications for maintaining high quality *ex situ* alpine seed collections.

SEED GERMINATION SYSTEMS 2 (SGS-2)
Chairpersons: **Camille Steber, Steven Penfield, Wenjing Ge**
Cypress 3&4

3:30 - 3:45 pm • **Irfan Afzal**
HERMETIC STORAGE OF MAIZE SEEDS PRESERVES SEED QUALITY THROUGH MINIMIZING DETERIORATIVE CHANGES ASSOCIATED WITH SEED LONGEVITY
Afzal, I.[1], Bakhtavar, M.A.[1], Basra, S.M.A.[1] and Hameed, A.
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One-third of the total food produced for human consumption is lost after harvest, which adversely affects agricultural productivity and food security for the rising populations. The primary cause is poor storage, especially high moisture contents that promote mold and insect damage. After proper drying, packaging of seeds in hermetic containers for storage can preserve seed quality and prevents infestation throughout distribution. In present study, maize seeds were dried to 8, 10, 12 and 14% seed moisture content (SMC) using zeolite drying beads and were stored in hermetically sealed super bag and traditional bags (paper bag, polypropylene bag, jute bag and cloth bag) for18 months. Samples were taken after every six months interval for evaluating seed longevity during storage. Seed moisture content increased in traditional bags while it remained constant in super bags. Seed viability and vigor tests indicated that seed germination (85% final germination after storage period) and vigor was maximum at 8 and 10% SMC while all other treatments lost the vigor and viability completely. Seed crude protein, starch contents, α-amylase activity, total soluble sugars and total reducing sugars were also retained in seeds stored in super bags at 8% SMC. Higher activities of catalase, super oxide dismutase, peroxidase and ascorbate peroxidase along with lower seed malondialdehyde contents, total oxidant status and electrolyte leakage were observed in seeds stored in super bag at 8% SMC. Poor quality of seeds stored in traditional bags was due to higher insect damage and aflatoxin contamination levels with increasing seed moisture contents while in super bags at 8%SMC the infestation was minimal. Overall, super bags are effective in maintenance of seed quality by preventing moisture and air entry into the stored seeds thus preserved the dryness of maize seed in storage. While seed storage in super bags at higher SMC (12 and 14%) led to loss of viability at a faster rate even compared to traditional porous bags suggesting a more important role of seed moisture in deteriorative process compared to oxygen.

3:45 - 4:00 pm • **Ugur Korkmaz**
SEED DORMANCY WAS ASSOCIATED SEEDBANK LONGEVITY IN A SET OF ISOGENIC LINES OF RICE
Pipatpongpinyo, W., **Korkmaz, U**.*, Kena, A., Wu, H., Feng, J. and Gu, X.-Y.
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**Oral and Poster Presenter*
Contact: Xing-You Gu, Xingyou.gu@sdstate.edu
Seed dormancy (SD) and seedbank longevity (SL) are adaptive traits of both ecological and agricultural importance. To address phenotypic and genotypic relationships between SD and SL, a set of 16 isogenic lines (ILs) was developed by introducing single alleles at the *qSD1-2*, *7-1*, *7-2* & *12* loci from weedy rice into a cultivated rice background. A subset of seed samples from individual plants of the ILs was evaluated for SD by germination percentages at 7, 21 and 35 days of warm dry storage. The other set of the samples was buried in 20-cm-deep soil of a rice field for 7 months (Oct. to the next Apr.), and evaluated for SL by decayed/intact seed rates at the excavation and survivability (germination percentages after 14 days of air-drying). A range of phenotypic variation was observed for both traits. The variation for SD was partitioned into main and epistatic (2-4 order) effects of the four loci. Whereas, the variation for SL was partitioned into main effects of the *SD7-1*, *7-2* and *12* loci, and 2 to 3 orders of epistasis of the four loci. Phenotypic correlations between the two traits were significant, with the

genotypes having a higher germination percentage at maturation tended to be higher in the decayed seed rate (r = 0.45 to 0.53) and lower in seed survivability in soil (-0.29 to -0.35). This research provided unambiguous evidence that natural genes for SD are also involved in the genetic control of SL, and seeds dormant at maturation likely survive longer in local soil seedbanks.

4:00 - 4:15 pm • **Dominique Ardura**

TEMPERATURE AND ABSCISIC ACID INFLUENCE GERMINATION, RESPIRATION, AND METABOLOMIC PROFILES OF LETTUCE (*LACTUCA SATIVA* L.) SEEDS

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Lettuce (*Lactuca sativa* L.) seeds exhibit thermoinhibition, an inability to germinate when imbibed above a threshold upper temperature limit. This is due to up-regulation by elevated temperature of expression of *LsNCED4*, a gene encoding an enzyme in the abscisic acid (ABA) biosynthetic pathway. Silencing *LsNCED4* using RNA-interference (RNAi) technology blocks the increase in ABA content and enables germination at thermoinhibitory temperatures. Using sensitive single-seed measurement methods, respiration rates of Salinas (thermosensitive) and RNAi-*LsNCED4* (not thermosensitive) seeds were assessed over a 36-h germination time course at 25 and 35°C, on agar with or without ABA. Salinas seeds imbibed in germination-permissive conditions (25°C, agar) exhibited increasing respiration rates, while respiration quickly decreased to very low rates in seeds imbibed at inhibitory temperatures or with ABA. Metabolic changes in seeds during these treatments were profiled using mass spectrometry-based metabolomics. Primary metabolism was investigated using GC-TOF MS with BinBase for alignment, integration and annotation. Lipid metabolism in both positive and negative modes was investigated using UPLC-Q-TOF MS and MS/MS with Mass Hunter Quantitative Analysis, MS-DIAL, and LipidBlast for alignment, integration, and annotation. The primary metabolism data set contained 995 bins, with 259 known and 736 unknowns. More than 942 unknown complex lipids were found in the integrated dataset of positive and negative electrospray, with 124 unique annotated lipids of known structure. Distinct differences were found in the primary metabolic profiles of germinating and non-germinating lettuce seeds. Respiratory metabolite levels were high when germination proceeded, while both respiration and mobilization of reserves were greatly reduced in thermoinhibited seeds. When germination occurred at elevated temperature in RNAi-*NCED4* seeds, raffinose, 1-kestose and galactinol contents decreased early following imbibition (12-24 h), as occurred at lower temperatures, whereas sucrose levels declined more slowly. Salinas seeds contained higher levels of most storage lipids than RNAi-*NCED4* seeds, suggesting a role for ABA in promoting storage lipid accumulation during seed maturation. Phospholipid metabolism, as measured through PCs, PEs, PIs and PGs, was also distinct in Salinas and RNAi-*NCED4* seeds, indicating that ABA may affect timing and formation of photosynthetic membranes and phospholipid interconversion during early imbibition and germination.

4:15 - 4:30 pm • **Elise Serin**

GENETIC VARIATION IN THE EFFECT OF SEED MATURATION ENVIRONMENT ON SEED PERFORMANCE

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Seed set is a crucial phase in plant's life cycle yet particularly sensitive to stresses. The intrinsic properties of seeds, such as the ability to germinate, are acquired during seed embryogenesis and seed maturation on the mother plant. Prolonged exposure to stress of the mother plant during seed development can alter the innate quality of the seed. Once shed from the mother plant, the ability of the seed to germinate is determined by either favorable or unfavorable germination conditions. However, germination requirements can be alleviated by the effect of the maturation environment. In addition, effects of the maturation environment have been shown to be expressed in a genotype-

dependent manner. Therefore, the interaction between the genotype, the maturation and germination environment of the seed (G x E x E) are instrumental in determining seed performance.

To date, little is known about the genetic basis of GxE in regard to seed performance. To address this, we grew an Arabidopsis Bayreuth x Shahdara recombinant inbred line (RIL) population under different mild stress environments from flowering until seed harvest: standard, high temperature, high light and low phosphate conditions. The seeds produced under each environment were used for extensive phenotyping. Traditional linkage analysis (QTL analysis) revealed germination QTLs interacting with both the seed maturation environment and the germination conditions (QTL x E). In order to gain insight into the underlying molecular mechanisms of this variation, transcriptomics and metabolomics data were generated from fresh dry seeds of the different populations. In a generalized genetical genomics approach, regions in the genome responsible for variation in gene expression (eQTLs) and metabolite content (mQTLs) were mapped and compared to phenotypic QTLs. Deeper understanding of the genetic basis of the effect of the production environment on seed performance provides a basis for improvement of seed performance prediction.

4:30 - 4:45 pm • **Hao Xu**

IDENTIFICATION OF GENE REGULATORY NETWORKS DRIVING CHANGES IN THE BIOMECHANICAL PROPERTIES OF EMBRYO CELLS AND THE SEED TO SEEDLING TRANSITION

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The seed to seedling transition in Arabidopsis is driven exclusively through changes in cell shape. Changes in the mechanical properties of the embryo cell wall therefore underlie this transition, and cell wall modifying gene expression represents the downstream targets of the germination process. The expansin genes are prime target for cell wall loosening during seed to seedling transition, however, the underlying regulatory network remains unknown. Using data from yeast one-hybrid, we identified transcription factors that directly bind to expansin promoter regions. These transcription factors mediate the penultimate step of the germination process through their regulation of gene expression which directly alters the physical properties of the cell wall. Using data from yeast two-hybrid, we determined which of these TFs bind to the growth and expansin repressor protein DELLA. This led to the generation of a regulatory network directly linking signals from the environment to growth-promoting gene expression. Furthermore, we examined whether these TFs that bind both expansin promoters and DELLA proteins play a role in seed germination. Using inducible overexpression and knockout lines, phenotypes for 66% of the TFs tested were identified. This GRN uncovers hub regulators and their regulatory consequences during seed-to-seedling transition, and potentially provides genetic targets for the enhancement of seed vigour and seedling establishment.

4:45 - 5:00 pm • **Renato De Castro**

GERMINABILITY, EXPRESSION AND ACTIVITY OF SUPEROXIDE DISMUTASE IN OSMOCONDITIONED *Ricinus communis* L. SEEDS

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Ricinus communis is an important oilseed crop predominantly grown by family farmers in the northeastern semiarid region of Brazil, where drought is a major environmental factor which may lead to the generation of reactive oxygen species (ROS) during seed germination and seedling establishment. Increased tolerance of seeds and seedlings to abiotic stresses may be achieved through pre-germination treatments such as osmoconditioning which may lead to protective biochemical adjustments against ROS, such as the activation of antioxidant enzymes. Superoxide dismutase (SOD, EC1.15.1.1) is in the first line of defense against the oxidative stress and is responsible

for the dismutation of O_2• into either H_2O_2 or O_2. The present study aimed to evaluate the physiological, biochemical and molecular profiles resulting from *Ricinus communis* seed osmoconditioning. Seeds of 2 genotypes (EBDA-MPA34 and PARAGUAÇU) were subjected to osmoconditioning for 7d in 3 PEG8000 osmotic potentials (-0.2, -0.6 and -1.0MPa, at 25°C), after which osmoconditioned seeds were washed and further imbibed in water for another 7d along with non-osmoconditioned seeds (0,0MPa control). Evaluations involved seed germinability (germination G_{max}; mean germination time – MGT; uniformity between 84 and 16% - U_{8416}; area under de curve index - AUC) and seedling performance (normal seedlings – PNS; root dry mass/length ratio - RDL), along with expression of SOD isoforms and absolute SOD activity. Non-osmoconditioned seeds presented high initial germination, but with better vigor in EBDA-MPA34 (MGT, AUC, PNS). While PARAGUAÇU presented better germinability and vigor after osmoconditioning (G_{max}, AUC, PNS, RDL) at higher water restriction (-1.0 MPa). The expression levels of *RcSODCuZn1* and *RcSODFe3* were higher according to the imbibition time in water and decreased as the osmotic potentials increased in both genotypes. However, the absolute SOD enzyme activity was higher in dry PARAGUAÇU seeds, without significant variation during imbibition in water or in osmoticum, suggesting that they were better prepared to detoxify ROS under water restriction conditions, thus presenting better performance after osmoconditioning at -1,0MPa. Such profile may be related to the ability of PARAGUAÇU seeds to withstand drought in the soil seed bank while being better adapted to face the harsh water restriction conditions of semiarid regions.

Closing Banquet
6:30 - 10:00 pm
Dolphins Ballroom and Upper Plaza