International Society for Seed Science (ISSS)
Seed Longevity Workshop
Wernigerode, Germany, July 5 – 8, 2015

Seeds for future generations –
Determinants of longevity

BOOK OF ABSTRACTS

Ulrike Lohwasser & Andreas Börner
(eds.)

Wernigerode 2015

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Patricia Berjak (1939-2015)

Professor Emeritus and Senior Research Associate of the University of KwaZulu Natal (UKZN) South Africa, Patricia Berjak passed away after a short illness in January 2015.

A member of the Academy of Sciences of South Africa (MASSAf), she received numerous other honours, among which were the South African Association of Botanists (SAAB) Silver (2001) and Gold (2006) medals for her excellent contributions to Botany, SA; the Department of Science and Technology's Distinguished Woman Scientist award (2004); the National Order of Mapungubwe Silver (2006); the NRF Lifetime Achievement award (2008) and the eThekwini (the city in which Patricia lived) Living Legends award (2010). Her expertise in seed and plant biology led to her participation in numerous scientific committees including being Chair of the International Seed Testing Association [ISTA] Seed Storage Committee (1995-2001), **President elect** (2005-2008) and **President** (2008-2011) **of the International Society for Seed Science** (ISSS) of whom she was an Executive member from 2005. She was also member of many scientific societies and was extremely active in the editorial work of several journals, including *Cryoletters*, *Journal of Horticultural Science and Biotechnology*, *Seed Science Research* and the *South Africa Journal of Botany*. She organised 6 International Workshops on Desiccation Tolerance and Sensitivity in Seeds and Vegetative Plant Tissues in South Africa.

Her research group is considered among the leaders in the field of seed biology and, particularly, of seed desiccation sensitivity and storage. She has published numerous papers and book chapters in aspects of recalcitrant seed biology relevant to their storage for biodiversity conservation and food security purposes. In this regard, her group was active in developing methods for cryopreservation of such seeds in order to enhance their ultimate storage potential.

Patricia was a mainstay of ISTA and ISSS workshops and other conferences and meetings, and all who met her will remember her group's contributions in the field of recalcitrant seed biology and the extraordinary care she gave to her students. I had the pleasure to welcome her and her husband and collaborator, Prof. Norman Pammenter, in my laboratory in Paris as invited Professors, and I will always remember her love of science, her sense of humour, and her "smiling" blue eyes.

The international scientific community has lost a distinguished specialist in seed biology and botany. Patricia Berjak will be greatly missed by us all, her friends and collaborators, and by seed biologists through-out the world.

Prof. Françoise Corbineau
University Pierre and Marie Curie, Paris
President of ISSS

Program

July 05, 2015

12:00-21:00	Registration	
19:00-22:00	Welcome Reception	

July 06, 2015

Session I : Seed banking – state of the art

08:15	Andreas Börner, Françoise Corbineau	Opening Remarks
08:30	Christina T. Walters (invited speaker) (dedicated to Pat Berjak)	The modern seed bank: Promise, uncertainties and cross-cutting issues
09:15	Jae-Sung Lee	Inter- and intra-species differences in seed longevity: What can be discovered from the germination test data of the CGIAR genebanks?
09:30	Stan Matthews	Quick, convenient measures of seed germination for routine use in seed bank monitoring
09:45	Robert Redden	Seed longevity studies of pea, lentil and chickpea at the Australian Grains Genebank
10:00	Xinxiong Lu	Optimizing seed moisture content based on the risk of power outages at genebanks: A fifteen year study from China
10:15		Coffee Break / Poster Session
11:00	Ola Tveitereid Westengen (invited speaker)	The global back-up

11:45	William John Raupp	The wheat genetics resource center genebank and the rapid curation of germplasm collections using genotyping-by-sequencing
12:00	Bart Panis	Germination and storage behaviour of wild banana seed
12:15	Lydia K. Guja	Comparative longevity and persistence of Australian alpine seed
12:30		Group Photo
12:45		Lunch

Session II : Role of pre- and post-harvest environmental factors on seed longevity

14:00	Fiona Hay (invited speaker)	Why have we seen a decline in the storage potential of rice genebank accessions?
14:45	Kent J. Bradford	Relationships of seed sorption and desorption isotherms to seed longevity
15:00	Steven P.C. Groot	Oxygen is equally important in seed storage experiments as temperature and water activity
15:15	Louise Emma Colville	The influence of storage environment on volatile emission and viability loss during seed artificial ageing
15:30	Katherine Jane Whitehouse	Improved drying protocols to maximise the longevity of rice seed germplasm
15:45		Coffee Break / Poster Session
16:30	Andrea Mondoni (invited speaker)	The ecology of seed longevity
17:15	Lucile Daron	Impact of pre- and post-harvest conditions on leek seed quality

17:30	Hector Eduardo Pérez	Desiccation and cryo-freezing tolerance in seeds of two geographically distant *Uniola paniculata* (Poaceae) populations
17:45	Sttela D. V. F. Rosa	Studies on sensitivity to desiccation of different parts of *Coffea arabica* L. seeds
18:30		Guided Tour Wernigerode
20:00		Dinner

July 07, 2015

Session III - Genetics of inter- and intra-specific variation of seed survival

08:30	Leónie Bentsink (invited speaker)	What do we know about of the role of natural variation in the control of seed longevity?
09:15	Froukje Marije Postma	Seed bank dynamics is affected by genetic and both pre-and post-harvest environmental factors in *Arabidopsis thaliana*
09:30	Mai Abdel-Moez Allam	Genetic studies in oilseed rape (*Brassica napus*) revealed QTL for seed longevity related to seed weight, oil content and nutrition accumulation in seeds
09:45	Mian Abdur Rehman Arif	Genetic architecture of seed longevity in wheat and its relationship with seed dormancy and pre-harvest sprouting
10:00		Coffee Break / Poster Session

10:45	Julia Buitink (invited speaker)	From seed longevity to passive defense against pathogens: Co-evolution of two traits to remain alive in the dry state?
11:30	Julia Zinsmeister	*ABI5* is a major regulator of late maturation in legume seeds, linking longevity, oligosaccharide accumulation and chloroplast dedifferentiation
11:45	Buzi Raviv	The seed coat as a reservoir of growth promoting substances
12:30	Excursion to Companies Alternative Options: 1. MAWEA Aschersleben 2. Agrargenossenschaft Calbe 3. Gartenland Aschersleben 4. Strube Schlanstedt	
17:00	Barbecue IPK Gatersleben (including visits of the genebank, greenhouses, experimental fields, automatic phenotyping)	
22:00/23:00	Transfer to Wernigerode	

July 08, 2015

Session IV - Physiology and biochemistry behind seed ageing – deleterious effects vs. repair mechanisms

08:30	Wanda Waterworth (invited speaker)	Seed longevity and the importance of safeguarding genome integrity
09:15	Françoise Corbineau	Changes in embryo soluble carbohydrates and antioxydant system during ageing of wheat (*Triticum aestivum* L.) grains

09:30	Marc Galland	Genome-wide identification of rice seed vigor and longevity determinants through combined “omics”
09:45	Annette Büttner-Mainik	Quantitative transcriptome, hormone and redox analyses reveal sugar beet seed deterioration mechanisms and their enhancement after priming
10:00	David Riewe	Metabolic marker of seed viability in long-term stored wheat (*Triticum aestivum*)
10:15		Coffee Break / Poster Session
11:00	Ilse Kranner (invited speaker)	Biochemical mechanisms that contribute to seed ageing in the garden pea, *Pisum sativum*
11:45	Sara Mira	Characterization of volatile production during seed storage
12:00	Manuela Nagel	Does controlled deterioration of seeds mimic long-term storage in wheat and barley?
12:15	Gerhard Leubner-Metzger	Tissue-specific hormone metabolism as quality marker for seed technology and storage
12:30	Natanael Vinegra de la Torre	Identification of seed quality markers by activity profiling of proteases
12:45	Marcin Michalak	DNA methylation changes in seeds
13:00	Françoise Corbineau, Andreas Börner	Closing Remarks

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Session I
Seed banking – state of the art

The modern seed bank: Promise, uncertainties and cross-cutting issues

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Seed banks globally are a recognized strategy to safeguard plant genetic resources for crop improvement, conservation, studies in evolutionary biology, forensic science or other purposes. It is critical that the physical seed sample and associated information align, and so the process of seed banking must not affect the viability or genetic identity of the accession. Hence, understanding and promoting seed longevity is a major concern for all seed banks. We know that initial seed quality is important and that processing and storage conditions are critical to seed banking success. However, our understanding about how these factors interact is too incomplete to adequately predict how long seeds survive in seed banks. Primarily, this is because we do not understand the aging process: no single reactant or product correlates with aging kinetics; deterioration occurs over a long period and is initially asymptomatic; information is lacking about many species; individuals within a sample can age at different rates. As a consequence of needing to ensure high quality samples, seed banks often monitor viability. Without knowing what to look for and when, our germination tests ultimately risk consuming the very seeds that we are trying to maintain. Predictive tools and biomarkers of aging will provide technological advances needed to make seed banking more efficient. And, this efficiency is critical to seed bank function because collections get bigger, but budgets do not. Seed banks are handling increasingly diverse materials that do not respond the same way to storage environments or germination conditions as the domesticated samples that provided the original framework for seed banking. This diversity can be expressed at the species, population or individual level. The requirement to standardized treatments contrasts sharply with our expectation that diverse material will, by definition, respond differently. In short, seed banks “buy time” and so it is fitting to convene a workshop on seed longevity, the ultimate response of seeds to time. By seed banking, we also operate in the context of time, forming collaborations with future scientists to anticipate what plant genetic resources will be useful and implementing technologies to ensure high quality, well-documented, ever-available seeds.

Inter- and intra-species differences in seed longevity: What can be discovered from the germination test data of the CGIAR genebanks?

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The eleven genebanks of the CGIAR centers hold over 730,000 accessions, representing the largest and most diverse active ex-situ collection of plant genetic resources for food and agriculture in the world, with nearly 1,000 different species of cereals, legumes, forages, root and tuber crops and trees. Many of these genebanks were set up decades ago and have followed standarized methods for monitoring seed germination. The accumulated germination test data represents a unique (and challenging) opportunity for addressing scientific questions regarding seed collections conserved under genebank conditions. As part of the CGIAR Research Programme for Managing and Sustaining Crop Collections, we have recently started to look at this multi-species data set in a systematic way, with a view to answering questions such as: (i) What are inter- and intra-species differences in seed longevity? (ii) Are there particular groups of accessions with relatively short longevity and others with extraordinary long longevity? (iii) If so, is it appropriate to set uniform intervals for monitoring viability across these groups? (iv) Is seed longevity affected by the origin of the accession and the growing conditions of seed production?

We will present some results of our preliminary analyses and suggestions for ways forward to greater understanding of seed longevity for improving routine operations in genebanks.

Quick, convenient measures of seed germination for routine use in seed bank monitoring

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The need for regeneration of seed bank accessions is assessed by periodic germination tests. Recent work has identified two potential alternative methods that require less time and fewer seeds. The germination percentage (normal seedlings) of 10 lots of radish seed from commercial sources ranged from 77.5 to 100% (4 x 50 seeds at 20 ^{o}C) and also differed in mean germination time (MGT), calculated from frequent counts of radical emergence (RE), which predicted percentage normal (R^2= 0.97). Early counts of RE, determined using automated image analysis, predicted MGT, with $R^2 > 0.90$ for 12 species, including radish at 40h ($R^2 = 0.98$). The 40h RE of the 10 radish lots also predicted MGT ($R^2 = 0.82$) and normal seedlings ($R^2 = 0.79$). Thus an early single count of RE, which could be automated, predicts percentage normal seedlings in radish and possibly many other species. Mean electrical conductivity (EC) of the seed soak water of radish after 5 and 17h at 20 ^{o}C (3 x 100 seeds in 40ml distilled water) predicted both MGT ($R^2 = 0.90$ after 5h; 0.86 after 17h) and percentage normal seedlings (R^2= 0.88 after 5h; 0.79 after 17h). An even quicker EC test after 1, 3, and 5h of just one replicate of 100 seeds also predicted MGT ($R^2 = 0.78$, 0.85 and 0.87 for 1, 3 and 5h respectively) and percentage normal seedlings ($R^2 = 0.87$, 0.93 and 0.94 for 1, 3 and 5h). We suggest two potential ways of quickly evaluating seed germination for use in monitoring seed bank accessions and possibly seed testing generally: 1) a single early count of RE and 2) a 1h measurement of EC on only 100 seeds. When the EC of 20 single seeds of each of the 10 lots of radish was measured after 5h soaking, followed by germination at 10 ^{o}C, high leakage was associated with a later RE. These and other observations support an ageing / repair hypothesis: cellular damage induced by ageing and resulting in higher leakage, is repaired early in germination, resulting in a delay in RE (higher MGT) which increases as damage and therefore leakage increases.

Seed longevity studies of pea, lentil and chickpea at the Australian Grains Genebank

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Genebanks conserve a wide range of plant species, many of which do not have established seed viability decline curves to guide expensive seed viability and regeneration activities. In this study, we investigated the viability decline of two varieties each of pea (*Pisum vulgaris* L.), chickpea (*Cicer arietinum* L.), and for the first time, lentil (*Lens culinaris* Medikus subsp. *culinaris*) under full factorial combination of three temperatures; 40 °C, 20 °C and 2 °C, by three seed moisture levels of 7–7.8% (Low), 7.9–10.3% (High) and 10.9–13.8% (Very High). The six varieties were also tested at Low -18 °C. Eight additional elite lines per crop were tested at Low 20 °C. Germination tests conducted prior to storage (05/02/2003) and periodically provided seed vigour decline curves for each temperature/seed moisture treatment. The initial results over ten years are presented for all seed moisture levels at 40 °C, Very High at 20 °C, and preliminary data for High 20 °C. Decline in seed vigour for all six varieties followed a sigmoid curve of a cumulative normal distribution of negative slope as described by Roberts (1972). Heavy infection of fungi on the seed surface invalidated the chickpea data for the Very High treatments. Significant positive responses in storage time to both a reduction in seed moisture and storage temperature were observed, which confirmed storage behaviour for pea and chickpea, and provided the first report on viability decline in lentil under these treatments. Highly linear regressions of time periods to 85% vigour with seed moisture in the 40 °C treatments were found for two lentils and one pea variety, while one hard seedcoat pea showed greater longevity. Variation in storage behaviour amongst the eight elite lines at Low 20 °C were observed, with a few showing much earlier seed deterioration than the rest. The major storage responses in longevity were to temperature and seed moisture level, with intra-species differences a significant but lesser source of variation. Understanding the seed longevity behaviour of the wide range of plant species conserved in Genebanks is critical to developing effective seed viability testing and regeneration schedules to ensure valuable germplasm is not lost through viability decline.

Optimizing seed moisture content based on the risk of power outages at genebanks: A fifteen year study from China

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FAO standards for genebanking seeds recommend drying them to 10-25% RH at 5-20 °C and then storing them in airtight containers at -18 °C (http://www.fao.org/3/a-i3704e.pdf). The standards provide relatively wide options for preparing seeds for freezer storage, mostly because empirical evidence of optimum moisture treatments to maximize seed longevity at -18 °C will not be known for more than 100 years. Therefore, we approached the question of how to optimize treatments in a different way. Rather than trying to maximize longevity under freezer conditions, we considered the risk of power outages that would cause seeds to warm to ambient, fluctuating temperatures. In 1997, we dried seeds from 7 crop species to 10 moisture levels that represent below, within and above FAO standards, packaged seeds in foil-laminate bags, and stored seeds within buildings at six locations in China having different climates: plateau, sub-temperate continental monsoon, temperate continental monsoon, subtropical monsoon and tropical monsoon. We also stored seeds at a constant 20 °C to serve as a control treatment. Percentage germination and germination speed and vigor were monitored annually to provide a deterioration time course for each moisture treatment at each location. Seed germination was treated like proportion data, allowing us to use the dose.p function from the R statistical package to compare storage times associated with specific levels of deterioration among the different moisture and location treatments. Not surprising, seed longevity was extended by some drying. However, excessive drying to moisture contents less than 4%-6% (depending on crop) tended to reduce seed vigor and longevity. Thus, we observed an optimum moisture content (MC_{opt}) range for storage under ambient temperatures. The range of MC_{opt} varied with location, such that locations with higher mean annual temperature had narrower MC_{opt} ranges. Also, longevity of seeds stored at cooler locations within the MC_{opt} range survived longer than counterparts stored at warmer locations. Our approach and results argue that optimum seed handling procedures before long-term storage should include a risk assessment of power outage factors that would compromise the ability to maintain the -18^{o}C environment. This information, in combination with location-specific ambient temperature fluctuations, should guide drying procedures for genebanks.

The global back-up

O Westengen

Nordic Genetic Resource Centre and the Norwegian University of Life Sciences

Deep inside a mountain on a remote island in the Svalbard archipelago, halfway between mainland Norway and the North Pole, lies the Svalbard Global Seed Vault. The Seed Vault is a global back-up facility for the world's genebanks. It is now the world's largest repository of plant genetic resources for food and agriculture (PGRFA). As of February 2015, 863,969 safety duplicates from 63 public genebanks (figure 1) have been deposited in the Seed Vault.

The Seed Vault consists of three large chambers at the end of a 120 meter long entrance tunnel. This makes the Seed Vault safe from the outside world. It also allows the natural permafrost of the mountain to act as a natural source of cooling. The Seed Vault was also built with climate change in mind, far above the worst-case scenario of sea level rise.

Worldwide, more than 1700 genebanks hold collections of crops for safekeeping. But unfortunately many of these are vulnerable, not only to natural catastrophes and war, but also to simple and avoidable disasters, such as poorly functioning equipment. The Global Plan of Action (GPA) (FAO, 2011) calls for a "rational, efficient, goal-oriented, economically efficient and sustainable system of ex situ conservation". The Seed Vault serves as the back-up storage site in this global system which is now emerging under the framework of the GPA and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). The Genebank Standards, prepared under the guidance of the FAO Commission on Genetic Resources for Food and Agriculture explicitly state that the Seed Vault makes the recommended sort of safety duplication facility available. The international collections at IARCs have the largest share (>2/3) of the safety duplicates in the Seed Vault. The database of the safety duplicate holdings at Svalbard is integrated with Genesys – the global system's accession level database.

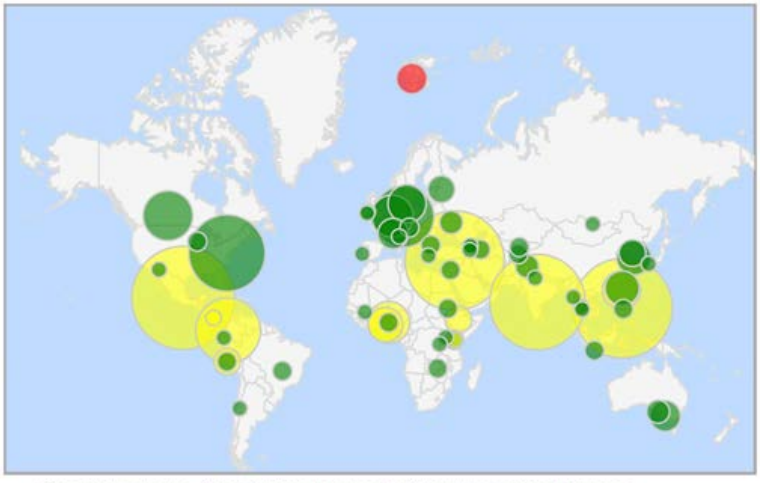

Figure 1 Location of genebanks with safety deposits in the Svalbard Global Seed Vault. The size of the dots reflects the size of the deposits according to 25 size classes. Yellow circles are IARCs and green circles are regional, national or subnational genebanks

The Seed Vault is operated under a three party agreement between the Royal Ministry of Food and Agriculture of Norway, the Global Crop Diversity Trust and the Nordic Genetic resource Center providing for the long term funding, management and operation of the Svalbard Global Seed Vault.

The wheat genetics resource center genebank and the rapid curation of germplasm collections using genotyping-by-sequencing

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The Wheat Genetics Resource Center (WGRC) is located at Kansas State University, in the heart of one of the greatest wheat-growing regions in the world. The main mission of the WGRC, collecting, conserving, and utilizing germplasm in wheat improvement for sustainable production, broadens the crop genetic base assuring future advances in breeding. The WGRC genebank contains passport and evaluation data on ~3,800 wheat species accessions and, in addition, houses ~3,400 cytogenetic stocks.

In wheat, accessions from genebanks and individuals have been widely circulated for the last century. Historically, each genebank has used their own accession identification numbers, often resulting in the loss of globally unique identifiers, cross-referenced collection information, or passport data. Thus, once an accession travels from genebank to genebank, the ability to discern duplicates is confounded. In this context, much effort is given at the WGRC to cross-reference our accessions with those of other wheat gene banks.

Recognizing the importance of identifying duplicity and cross-referencing collections, we used genotyping-by-sequencing (GBS) to ascertain the genetic diversity in our collection of 568 *Aegilops tauschii* accessions and compare it to an undocumented collection. After *de novo* SNP calling using the TASSEL pipeline, removing duplicate tags, and SNP filtering for missing data, 14k SNPs were mapped on wheat D genome. Using allele matching accounting for a ~1% sequencing error (>99% match), we could identify accessions with similar, yet incomplete, passport data as possible duplicates. Of 551 *Ae. tauschii* accessions assayed, 402 were unique, representing a 27% duplication. We also were able to match 118 unidentified accessions from the genebank at Punjab Agricultural University as the same accession represented the WGRC collection. We currently are using this same approach to characterize and curate our collection of over 900 tetraploid wheats.

With a rapid and cost-effective tool to study genetic diversity, giving a consistent characterization of genetic and phenotypic diversity in wheat germplasm GBS will be important in the genetic curation of accessions within and between collection(s). With such information across global collections, it becomes possible identify the truly unique accessions across all of our gene banks, enabling more targeted access to genetic diversity.

Germination and storage behaviour of wild banana seed

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Bananas are the most important fruit crop in the world with a yearly production of 129 million tons and they are cultivated in more than 120 countries in the humid and semi-humid tropics. Because of their sterility edible bananas are vegetatively propagated making seed conservation thus not an option for their long term storage. Until recent, banana collection existed mainly as field and in vitro collections. However, in the year 2003 a cryobank was established at the Bioversity International Transit Centre (ITC), Leuven Belgium.

During the last decades there is more and more interest in the CWRs (Crop Wild Relatives). They are considered an increasingly important resource in breeding for improving agricultural production and for maintaining sustainable agro-ecosystems. For wild bananas, *Musa* spp., standardized protocols for the germination and storage of their seeds are not yet available. In this study, we present some first results on the germination and storage behaviour of seed of *Musa acuminata* and *Musa balbisiana*, the wild ancestors of the edible bananas as well as from other crop wild relatives of *Musa*. In 2014, 48 seed batches were received from 7 providers. Greenhouse germination rates were rather low and erratic and never exceeded 10%. This could partially be improved by applying embryo rescue resulting in an in vitro germination rate between 0 and 96% (average of 30%). The chemical viability test using TTC gave a good estimate on the in vitro regeneration. Subsequently, non-dried (MC between 10.5 and 27.3%) and dried (MC below 10%) seed was subjected to different storage conditions; room temperature, 5 °C, -20 °C and -196 °C for different time periods. We concluded that when sufficiently dried, *Musa* seed can be stored at -20 °C as well as at ultralow temperatures.

Comparative longevity and persistence of Australian alpine seed

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Knowledge of Australian Alpine seed longevity and persistence is lacking, yet is essential for effective *ex situ* conservation and species recovery. Alpine regions of the world are under significant pressure from warming climates. Australia's alpine region is restricted in distribution and elevation, limiting options for range shifts. In particular, Australian alpine peatlands are endangered due to inherently fragmented distribution and small size. Accordingly, seed conservation is needed to safeguard against the loss of Australian alpine and subalpine plants. Our research program 1) compared the longevity of Australian alpine and subalpine seed via an *ex situ* controlled ageing experiment and 2) investigated *in situ* persistence and germination using a seed burial experiment.

1) Comparative longevity. Seeds of 75 Australian alpine species from 21 families were aged under controlled conditions (45 °C and 60% relative humidity) and regularly sampled for germination. Preliminary results for those species with an immediate germination strategy (relatively fast, synchronous germination) indicate that longevity (time to 50% viability loss (t_{50}) ranged from 5 to > 75 days. This may be relatively short compared with the Australian flora in general (p_{50} 3 to 589 days; Merritt *et al*. 2014), but approximately equivalent to European alpine and lowland plants (p_{50} 5 to 95 days; Mondoni *et al*. 2011).

2) *In situ* moderators of longevity. None of the 13 subalpine-peatland species included in the seed burial experiment formed transient soil seed banks; all seeds survived at least one growing season (15 months since dispersal). Although substrate (waterlogged peat versus *Sphagnum* moss) did not affect seed survival, it influenced the proportion of seeds responsive to germination cues, which will likely affect long-term persistence. Seasonal dormancy cycling affected the germination of some species indicating that recruitment potential may significantly fluctuate throughout the year.

The assessment of both comparative longevity and seed persistence is yielding invaluable information for *ex situ* conservation of Australia's alpine flora. However, a dearth of knowledge about the germination requirements of some ecologically important alpine species precludes them from inclusion in comparative longevity assessments and highlights the need for fundamental germination research, particularly in less-studied floras.

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Session II

Role of pre- and post-harvest environmental factors on seed longevity

Why have we seen a decline in the storage potential of rice genebank accessions?

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Seed viability (germination) retest data for genebank accessions regenerated between 1980 and 2003 and stored in the active and base collections of the rice genebank of the T.T. Chang Genetic Resources Center have been downloaded and analysed. There is evidence that, since the early 1990s, the storage potential of the seeds when they are first placed into storage has declined. A number of changes were made to genebank operating procedures in the 1990s, including the drying process, the introduction of manual seed sorting and the packing materials used for the active collection samples. This paper will discuss whether these factors might have caused the increasing failure rate. The role of seed maturity at harvest and the regeneration environment, climate in particular, in determining seed longevity will also be considered, not forgetting how the effect on seed longevity of any of these pre-harvest factors might depend on the genotype of the accession.

Relationships of seed sorption and desorption isotherms to seed longevity

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Seed longevity is highly sensitive to seed moisture content (MC), e.g., a 1% increase in seed MC results in an approximately 50% reduction in seed longevity at a given temperature. The seed MC at a specific relative humidity (RH) is higher when the seed is losing water (desorption isotherm) than when it is absorbing water (sorption isotherm). It is usually not known whether seeds are on their sorption or desorption isotherms when MC is measured or seeds are stored. In addition, it is not clear whether seed ageing rates in storage are more closely related to the water activity (aw) (or RH [aw x 100]) or to the seed MC. If the latter, we could expect seed longevity to be greater for seeds on their sorption curves (lower MC) than on their desorption curves (higher MC), even when both are stored at the same RH. To test this, we characterized the seed sorption and desorption isotherms of five species (lettuce, carrot, radish, pepper and sweet corn). Consistent and reproducible differences in seed MC on the order of 0.5 to 1% MC at the same RH were obtained for seeds of each species depending upon whether they were on their sorption or desorption isotherms. Seeds at a given RH (below ~50%) lost viability more rapidly (by 20 to 50%) during storage at 50 °C when on their desorption isotherm than when on their sorption isotherm. Studies of seed respiration using the Q2 instrument (www.astec-global.com) confirmed that seed aging rates in relation to MC, particularly decreases in germination rates (speed), were closely correlated with decreasing respiration rates of the imbibed seeds. The results indicate that it is MC (or the underlying structural differences associated with water-binding capacity) at a given RH rather than RH or aw per se that determines seed deterioration kinetics. Studies in which seeds were transferred to different RH environments also established the conditions required to move seeds from sorption to desorption isotherms or vice versa. Simply modifying seed drying and storage protocols to assure that seeds are on their sorption isotherms could extend seed longevity significantly.

Supported by the Western Regional Seed Physiology Research Group (WRSPRG) at UC Davis and Coordination for the Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq) in Brazil.

Oxygen is equally important in seed storage experiments as temperature and water activity

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For conservation of desiccation tolerant seeds by gene banks, dry and cool storage conditions are recommended to reduce seed ageing. Maintenance of seed quality during storage is also important for farmers. The seed industry performs fast ageing test by storing the seeds at a relative high humidity and temperature to estimate the shelf life of treated seeds. However, correlations between such controlled deterioration tests and long term storage under dry conditions are frequently poor. One reason is that the physiology of seeds differs with the moisture level, another reason may be neglecting the role of oxygen in the storage tests.

Seed deterioration during ageing is predominantly caused by oxidative processes, which are indeed stimulated at high temperature and seed moisture levels. Since the rate of oxidation is also correlated to the concentration of oxygen in the environment, oxygen levels during storage should also be taken into account. The later, however, has received relative little attention in the seed science and gene bank community. Previously we showed that seed storage at an elevated partial pressure of oxygen stimulates seed aging. In follow-up project we have performed storage experiments with primed celery seeds at ambient pressure with oxygen concentration varying from 1 to 99%. The results show that also at atmospheric pressure there is a clear positive correlation with the oxygen concentration in the storage container and the rate of ageing.

In experiments quantifying the rate of seed ageing in relation to temperature and water activity, it can be a complicating factor that during storage part of the oxygen is ‘bound’ by the seeds due to oxidation of organic matter. As a consequence, the oxygen levels can drop during the experiment, depending on the amount of oxygen included and the rate of binding.

In conclusion, the oxygen concentration during seed storage experiments is an equally important parameter to take into account in seed storage research as is the temperature and water activity of the seeds. Storage under low oxygen levels can provide an addition or possibly an economic alternative to low temperature storage conditions for long term seed conservation and sensitive commercial seeds.

The influence of storage environment on volatile emission and viability loss during seed artificial ageing

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Volatile profiling using gas chromatography-mass spectrometry shows promise as a non-invasive technique for diagnosing seed viability, and also provides an insight into the processes occurring during seed storage. Artificially aged seeds produce a range of volatile compounds including alcohols, aldehydes and ketones, which derive from processes such as alcoholic fermentation, lipid peroxidation and Maillard reactions. The reactions that occur depend upon storage temperature and relative humidity. The effect of relative humidity on volatile profiles was investigated during ageing of seeds of three species: *Lolium perenne* (Poaceae), *Agrostemma githago* (Caryophyllaceae) and *Pisum sativum* (Leguminosae) to gain an understanding of the mechanisms of ageing under different seed moisture conditions. Storage atmosphere also affects volatile emission and seed longevity in storage. The effect of oxygen on seed ageing and volatile emission was investigated by comparing seeds aged under normal atmospheric oxygen conditions with seeds aged in sealed vials containing either oxygen absorbers, oxygen absorbers and silica gel (equilibrated at 60% RH), or silica gel alone. Seeds that were aged in the absence of oxygen maintained higher viability and produced fewer volatiles than seeds aged in air. Comparison of the volatile fingerprint of seeds aged in the absence of oxygen with that of seeds aged in the presence of air enabled volatile compounds that derived from oxygen-dependent processes to be identified. Seeds aged in the presence of silica gel were also longer-lived than those aged without silica, with no effect on seed moisture content or oxygen concentration in the storage containers. Much lower levels of volatiles were accumulated in the presence of silica gel, which acted as a volatile trap. The enhanced survival of seeds aged in the presence of silica gel suggests that silica gel reduced the accumulation of toxic volatile compounds in the headspace of the storage vials, which may otherwise contribute to seed viability loss. These results indicate that the use of inexpensive oxygen absorbers and silica gel could improve seed longevity in storage.

Improved drying protocols to maximise the longevity of rice seed germplasm

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Seeds gradually lose viability in storage and require regeneration in order to maintain genetic integrity. Maximising the regeneration interval by optimising storage longevity will reduce the economic cost of regeneration and limit loss of genetic diversity. The potential storage life of a seed is influenced by post-harvest practices. In this presentation I will focus on the response of seeds in terms of subsequent longevity to different post-harvest drying treatments. Current standards for genebanks recommend that seeds be dried at 5-20 °C and 10-25% RH immediately after harvest. A series of experiments were carried out to test whether these drying conditions are optimal for rice seed germplasm.

An initial experiment was carried out to determine the effect of high temperature drying (45 °C) on seed storage longevity of 20 diverse rice accessions. Seeds exposed to intermittent (8 hr /day) high temperature drying cycles for up to 6 days immediately after harvest showed an improvement in their storage longevity (an increase in p_{50} up to 372.2%) compared with drying in a dryroom at 15 °C/15%. This improvement in longevity increased as seed harvest moisture content increased above 16%, irrespective of harvest maturity. A follow-up study using more controlled drying at specific stages of maturity was conducted, in addition to another set of experiments aiming to assess whether longevity could be improved further by introducing rehydration treatments into the heated-air drying protocol. To provide further evidence of this environmental influence on seed potential storage longevity, harvest moisture content was indirectly manipulated by spanning multiple harvests across the season, coinciding with an increase in ambient temperature. The results will inform decisions on how to handle rice seeds intended for genebank storage immediately after harvest.

The ecology of seed longevity

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The study of ecological factors driving differences in seed longevity across species is crucial for understanding how this seed trait has evolved and may change in the future. Using controlled ageing test (CAT) at high temperature and humidity (e.g. 45 °C, 60% RH) seed longevity has been found to vary considerably across species and the climate at their origin. Species that are long lived under CAT show a longer soil seed bank persistence, suggesting that CAT may have an ecological significance. Changes of seed longevity across plant populations from different climates should therefore be the result of different selection pressures on seed resistance to ageing. For example, plants growing under warmer conditions tend to produce seeds with greater resistance to ageing likely because they have to cope with a more stressful post-dispersal environment. However, whether ageing processes are similar under both conditions is still not well understood. Recent studies have revealed significant transgenerational changes in seed longevity associated with environment-induced effects, indicating that differences between collections could also be driven by plastic responses to the local environment or seasonality. Our observations show that seed longevity has a genetic basis, but it may have strong adaptive responses, which are associated with differential accumulation of mRNA via parental effects. Such ability of maternal environment to affect offspring phenotype through transmission of genetic material, may potentially allow progeny adaptation to habitat conditions experienced by the maternal parent and, therefore, may play a fundamental role in survival and persistence of the species in the face of future environmental challenges, such as climate changes.

Impact of pre- and post-harvest conditions on leek seed quality

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Under commercial production conditions, it is often a challenge to reach a high seed quality in leek (*Allium porrum* L.). Adverse environmental conditions during seed production and/or harvest may be responsible for this fact. In this study, we investigated which production factors affect leek seed quality. In 2012, the average Total Germination (TG) percentage of several cultivars produced in France was exceptionally low (TG=62%). Four batches of a variety named A (2012 average TG=35%) were further studied to understand the origin of this low quality. A good seed quality batch of variety A produced in 2012 in The Netherlands was used as reference. The following characteristics were determined: seed moisture content (SMC), embryo morphology, seed viability and germination percentage. On average, the SMC of the four batches was of 12% before threshing and 9% before shipment. Per batch, two hundred seeds were germinated as BP15, counting at day 14 after sowing. On average, TG=49%, ABN=38% (abnormal seedlings) and NG=13% (non-germinating). Among the NG seeds, 3.1% were empty and 4.6% were pre-germinated. A tetrazolium test was performed to assess the viability of the remaining 5.3% NG seeds. These seeds led to the following results: 32% rotten embryos, 19% dead embryos and 49% viable embryos. In order to test if these results were related to seed aging processes, the reference batch was exposed to 3 weeks of controlled deterioration (30 °C and 75% RH). Germination results of aged seeds were compared to the non-aged reference sample: TG=49% *vs* TG=88%, ABN=43% *vs* ABN=6% and NG=8% *vs* NG=6% (respectively). The four batches studied presented the same characteristics as the aged reference batch: high ABN% and NG%. The precipitations during the flowering period in 2012 were the highest of the past 5 years. The high humidity conditions delayed flowering and seed maturation, postponing the harvest for a month. The umbels were dried in the field during October and threshed at the beginning of November. In conclusion, the environmental conditions (RH% and T) during ripening, drying and shipping appeared to have caused pre- and post-harvest seed aging, mostly due to a prolonged high SMC.

Desiccation and cryo-freezing tolerance in seeds of two geographically distant *Uniola paniculata* (Poaceae) populations

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Seed provenance is an important consideration for germplasm conservation management that has been shown to influence desiccation and freezing tolerance. Here, we focus on potential population effects with respect to desiccation and cryo-freezing tolerance of an important coastal dune grass in need of conservation. We collected seeds from wild populations of *Uniola paniculata* (Poaceae) separated by 465 km, or roughly 4° of latitude, and occurring in climates classified as either equatorial savannah with dry winters (Delnor-Wiggins State Park, DWP population) or warm temperate, fully humid with hot summers (Little Talbot Island State Park, LTI population). Seeds from both populations maintained relatively high initial germination (DWP = 83%, LTI 48%) following post-harvest storage for 3 months within a non-climate controlled shed (ca. 9-28 °C; 59-93% RH). Seeds of DWP and LTI displayed initial moisture contents of 0.14 and 0.16 g g^{-1} which corresponded to water potentials of –94 and –60 MPa, respectively. Seeds of both populations exhibited water sorption isotherms similar to those of desiccation tolerant seeds. Germination was nearly complete (> 84%) and relatively rapid (t_{50} range 2-4 d) for DWP seeds equilibrated to low water potentials (–12.9 to –797.5 MPa). The germination response of LTI seeds equilibrated to the same water potentials was comparatively lower (63-85%) and delayed (t_{50} range 5-14d). The lower germination response for LTI seeds seemed related to fewer viable seeds germinating during the experimental period. Germination was not considerably reduced for seeds of DWP (78-93% germination, t_{50} 2-4 d) or LTI (59-80%, t_{50} 4-20 d) following exposure to any combination of desiccation or cryo-freezing (1, 60, or 1,440 minutes) stress. However, Cox regression detected significant population effects with respect to the differential germination responses among desiccation and cryo-freezing treatments. No other significant explanatory variables were detected. We conclude that seeds of both *U. paniculata* populations are desiccation and cryo-freezing tolerant. Therefore, genebank storage seems feasible for seeds of this species. Furthermore, population differences are most likely due to seeds of LTI possessing shallow dormancy rather than any variability in desiccation or freezing sensitivity that may be related to geography or climate.

Studies on sensitivity to desiccation of different parts of *Coffea arabica* L. seeds

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Coffee seeds are sensitive to desiccation and have low storage potential. They are classified as intermediate seeds. Cooling seed to subzero temperatures is an alternative for long-term seed storage in gene banks for various crop species. However, this technique is still not fully effective for intermediate seed species such as coffee. The aim of this research was to study the physiological changes in *Coffea arabica* seeds cooled to subzero temperatures. The study was carried out at the Seed Analysis Laboratory of the Universidade Federal de Lavras. Seeds from the 2012/2013 crop season of *Coffea arabica* L. Catuaí amarelo IAC 62 were used. Prior to cooling, the seeds were subjected to two types of drying, quick drying in silica gel and slow drying in saturated salt solutions until the seeds reached the predetermined moisture contents of 40, 30, 20, 15, 10, and 5% (wet basis). After drying, the seeds were incubated for temperature equilibrium at 10, -20, and -86 ºC for 24 hours. Physiological quality of the seeds was determined by percentage of normal seedlings, embryo viability in the tetrazolium test, and *in vitro* growth of embryos. At 40 and 10% wb and storage at 10 °C, coffee seeds exhibit greater physiological performance, regardless of drying speed. After exposure to -20 °C, seeds with moisture content between 10 and 30 % wb dried more rapidly and exhibited germination above 70%. However, at the extremes of moisture content (40 and 5%) only the embryos survived exposure to this temperature. Only the seeds subjected to quick drying to a moisture content of 20% survived exposure to the temperature of -86 °C. The low physiological performance of coffee seeds with 5% moisture content after exposure to the temperatures tested is linked to the sensitivity of the endosperm since the embryos were viable at the same moisture content.

Session III

Genetics of inter- and intra-specific variation of seed survival

What do we know about of the role of natural variation in the control of seed longevity?

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Seed longevity, one of the most important seed characteristics, is relevant to both seed resource conservation and crop success. Understanding the mechanisms underlying seed aging, deterioration and vigour loss during dry storage will be beneficial for seed longevity manipulation and improvement. We have been investigating the role of natural variation in the control of seed longevity in the model plant *Arabidopsis thaliana*. We have performed quantitative trait loci (QTL) analyses and identified 10 *GERMINATION ABILITY AFTER STORAGE* (*GAAS*) QTL. The effects of these QTL have been confirmed by the use of near isogenic lines (NILs). The role of the maternal environment on performance of these NILs has been investigated and this revealed that longevity of seeds is strongly determined by the environment in which the mother plant is grown. Furthermore, the NILs have been characterised in several ways in order to understand the mechanisms behind the control of seed longevity, including fine-mapping, transcriptomics and proteomics analyses. In this way several novel genes that affect seed longevity have been identified.

Seed bank dynamics is affected by genetic and both pre- and post-harvest environmental factors in *Arabidopsis thaliana*

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Many plant species form a soil seed bank, which may increase offspring survival in environments where conditions for seedling establishment and survival are unpredictable. However, the extent to which genetic and environmental factors influence seed bank dynamics is not well studied.

We examined genetic, and pre-and post-harvest environmental effects on dormancy cycling and seed mortality with a reciprocal seed burial experiment using two natural populations (from Italy and Sweden) of the annual plant *Arabidopsis thaliana* that are locally adapted and show marked genetic differences in primary dormancy. We buried seeds of the Italian and Swedish genotype at the sites of the two source populations, using experimental seeds produced in both the burial environment and in the greenhouse (six genotype × maternal environment combinations). Seeds were placed in fine-meshed polyester bags, ensuring that the seeds would be exposed to soil particles, water and soil organisms during the burial period, and buried during the natural seed dispersal period. We excavated seed bags at regular intervals the following two years, and recorded the number of healthy and dead seeds. We tested the germinability of healthy seeds, and noted whether dead seeds showed signs of seed predation.

Seeds of all genotype × maternal environment combinations showed annual variation in dormancy. At each experimental site, the germination behavior of the local genotype reflected the natural germination period of the local population, irrespective of the maternal environment of the seeds. Seed mortality stayed low at the Swedish site (<3% of the buried seeds), but increased strongly over time at the Italian site (48% of the buried seeds by the end of the experiment). Seed mortality was affected by genotype and maternal environment in Sweden but not in Italy. In Sweden was more seed predation than in Italy, and a significantly higher proportion of seeds of the Italian genotype produced in the greenhouse was predated compared to other genotype × maternal environment combinations. Overall, our results demonstrate that seed bank dynamics is affected by a combination of genetically and maternal environmentally determined seed properties and by soil environment.

Genetic studies in oilseed rape (*Brassica napus*) revealed QTL for seed longevity related to seed weight, oil content and nutrition accumulation in seeds

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Seed longevity, an important trait for genebanks, is influenced by wide base of genetic background. In recent study, the aim was to define the genetic basis for seed longevity of oilseed rape (*Brassica napus*). Linkage QTL mapping was performed using 475 SSR and AFLP markers and employed to examine seed longevity of Express x V8 mapping population using progenies of three harvests (ExV-5 from 2005, ExV-9 from 2009 and ExV-12 from 2012). The three seed sets were investigated for seed vigour after different years of ambient storage and different periods of controlled deterioration experiments. The germination speed and the proportion of root and shoot abnormal seedlings proved to be the most predictive sub-traits for seed longevity. In total, 294 QTL were detected in nine treatments across three seed sets. QTL detected for three harvests were 107, 127 and 60 QTL for ExV-5, ExV-9 and ExV-12, respectively. Among the detected QTL one or two identical loci were identified for each harvest year across the different treatments. Additionally, one major QTL was detected in five treatments across three years after controlled deterioration tests or ambient storage of ExV-5, ExV-9 and ExV-12 on chromosome C3 (87.8 – 104.2 cM). Major QTL for seed weight, oil content and nutrition accumulation were closely related to seed longevity QTL. The association mapping study of BnASSYST population confirmed some positions and candidate genes related to seed longevity.

Genetic architecture of seed longevity in wheat and its relationship with seed dormancy and pre-harvest sprouting

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Genebanks are established to protect and eliminate danger of extinction of plant genetic resources around the world. They provide an excellent storage facility for the long term *ex-situ* conservation of plant germplasm. Seeds (and in some cases other plant materials) held in these collections provide the raw materials required for breeding new and improved crop varieties. Seed longevity is an important consideration in the context of *ex situ* conservation. It has been proposed that seed dormancy can be an important aspect in the context of seed longevity. Pre-harvest sprouting (PHS) is a trait that has negative relationship with dormancy. Linkage analysis has identified many genetic loci related to seed longevity in a range of plant species (*Arabidopsis*, rice, soybean, barley and tobacco). Association analysis has also emerged as a powerful tool to detect quantitative traits of immense importance. Here, we provide a simultaneous analysis of one linkage mapping bi-parental population and two association mapping panels to decipher the genetic nature of seed longevity, dormancy and PHS in wheat. Bi-parental mapping population analysis revealed one major and nine minor QTLs for seed longevity but one major QTL for seed dormancy. Association mapping populations showed more than one hundred different loci determining seed longevity after various treatments (long term cold storage, accelerated ageing and controlled deterioration) which were distributed all over the wheat genome. The same was observed for seed dormancy and pre-harvest sprouting. Most of seed longevity loci are located at places where biotic and/or abiotic stress responsive loci have already been identified. Seed dormancy and PHS associations do not co-locate with longevity except at few loci giving the indication of separate mechanisms involved in both processes. Among agronomic traits, only height was identified as a potential character influencing seed longevity. Synteny was observed in comparative analysis of seed longevity with barley and rice suggesting the conservation of responsible loci among the three cereals.

From seed longevity to passive defense against pathogens: Co-evolution of two traits to remain alive in the dry state?

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In orthodox seeds, longevity is gradually acquired at the later stages of seed maturation. In our laboratory, we use seeds of *Medicago truncatula*, a model species for legumes, to unravel the mechanisms and regulatory pathways implicated in the acquisition of longevity. In this species, longevity increases progressively over 30-fold after seed filling is terminated and desiccation tolerance is acquired, allowing the separation of genes related to the different developmental processes. In order to further discriminate other developmental programs from those related to longevity, an extensive physiological and molecular analysis was undertaken in developing seeds from plants that were grown under different environmental conditions after seed set. This way, the timing and extent to which longevity is acquired was modulated. Using 104 transcriptomes acquired at different time points during seed maturation for five different parental environments, a network-based approach was applied to isolate a co-expression module related to longevity. Functional analysis in *Arabidopsis* confirmed the predictability and the conserved nature of this module. Interestingly, the longevity module is enriched with genes playing a crucial role in defense against biotic stress. Here, we will present evidence supporting a link between mechanisms implicated in defense against pathogens and survival in the dry state. We will discuss how seeds activate a developmentally regulated defense response during maturation that is also beneficial to long-term survival in the dry state.

ABI5 is a major regulator of late maturation in legume seeds, linking longevity, oligosaccharide accumulation and chloroplast dedifferentiation

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Abscisic acid insensitive 5 (ABI5) is a bZIP transcription factor that activates ABA- and stress-responsive genes in seeds and seedlings. Here, we will present evidence that ABI5 is also an important regulator of late maturation in legumes. Using *Tnt1* insertion mutants of *Medicago truncatula* and EMS mutants of pea, we discovered that seed longevity is severely reduced in these two legume species. Also, in these mutants, the accumulation of raffinose oligosaccharide family (RFO) sugars that takes place at the final steps of seed maturation is impaired. Mature *Mtabi5* and *Psabi5* mutant seeds contain respectively 2-fold less stachyose and verbascose than in wild type seeds. MtABI5 also plays a role in the accumulation of a restricted subset of Late Embryogenesis Abundant (LEA) proteins. A proteomic analysis on the heat stable protein fraction revealed strongly reduced levels of EM1 and several D-34 LEA polypeptides. Transcriptome analysis on developing *Mtabi5* mutants during seed development confirmed that the biochemical phenotypes of RFO accumulation and LEAs were also regulated at the transcriptional level. Surprisingly, this analysis also revealed a strong deregulation of transcripts encoding photosynthesis-associated nuclear genes. This result was confirmed by a transcriptome analysis of hairy roots ectopically over-expressing *MtABI5*. Further biochemical analysis demonstrated elevated chlorophyll and carotenoid levels in mature seeds of the *Psabi5* and *Mtabi5* mutants compared to wild type seeds, suggesting that in these legumes, ABI5 is involved in the dedifferentiation of chloroplasts. These results demonstrate that in legumes, ABI5 links RFO, chloroplasts and longevity. The causal relationship between these factors will be discussed.

The seed coat as a reservoir of growth promoting substances

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The seed coat originates from the integuments surrounding the ovule. In *Arabidopsis thaliana* (Brassicaceae) it is composed of five layers of cells and each of these layers undergoes different path of differentiation during seed development and maturation. Accordingly, in *Arabidopsis* the outer cell layer of the seed coat produces mucilage. When the seed is hydrated, polysaccharide mucilage is expanding through the outer cell layer that surrounds the seed and envelops the imbibed seed. The mucilage layer has been implicated in adherence to soil and in absorbing and storing water for germinating seeds. Our preliminary data suggest that the seed coat of different mucilaginous Brassicaceae species may serve also as a reservoir of organic and inorganic substances that might support seed germination and seedling establishment. Proteome profiling showed that more than 200 proteins are secreted from the seed upon hydration including stress responsive proteins and hydrolases including endonucleases and pectin biosynthetic enzymes. We further confirmed the presence of nucleases/endonucleases in the seed coat by using in gel nuclease assays and by the conversion of supercoiled plasmid DNA into relaxed and linear forms. Analysis of micro and macro elements as well as metabolites revealed secretion of high amount of potassium and nitrate as well as high level of lactic acid – known to function as plant growth promoting factor. Thus, our results explored previously unknown features of the seed coat serving as a reservoir of substances that might play an important role in seed germination and seedling establishment.

Session IV

Physiology and biochemistry behind seed ageing – deleterious effects *vs*. repair mechanisms

Seed longevity and the importance of safeguarding genome integrity

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Deterioration in seed quality occurs during storage, and accumulation of damage to cellular macromolecules including lipids, protein and nucleic acids results in loss in seed vigour and viability. Germination performance and seed longevity correlate with levels of damage to the genome accumulated in the embryo. Mechanisms that mitigate the deleterious effects of DNA damage promote the maintenance of seed vigour and viability. Repair of DNA damage in the early imbibition phase, prior to initiation of cell division, is essential to minimise growth inhibition and mutation of genetic information. Previously we demonstrated that DNA ligase enzymes with specific roles in repair of cytotoxic chromosomal breaks are hypersensitive to accelerated ageing, establishing a direct link between genome integrity and seed quality. In plants, signalling of genome damage is mediated by the PI3 kinase-like kinases ATM and ATR which integrate DNA damage sensing with downstream cellular responses. Here we identify ATM and ATR as important factors which govern seed vigour and viability, and delineate the molecular mechanisms by which DNA damage sensing is integrated with germination. Collectively these studies demonstrate that maintenance of genome integrity is of crucial importance in the seed stage of the plant lifecycle, revealing novel insights into the physiological roles of plant DNA repair and recombination mechanisms. The high levels of conservation of DNA repair and recombination factors across plant species underlines their potential both as central targets for the improvement of crop performance and development as molecular markers for prediction of seed vigour.

Changes in embryo soluble carbohydrates and antioxydant system during ageing of wheat (*Triticum aestivum* L.) grains

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As expected, viability of wheat grains was lost faster at 45 °C and 100% relative humidity (RH) when the embryo water content was 50-60% dry weight than at 30 °C and 75% RH, when the embryo water content was only 17% DW. The half-viability period (P50) was indeed only 6 days at 45 °C and 100% RH against about 3.7 months at 30 °C and 75% RH. Grain susceptibility to accelerated ageing slightly decreased during maturation drying, and sprouting injury before harvest resulted in an increase sensibility to ageing, i.e. in a loss of seed storability. Lipid peroxidation evaluated by H_2O_2 and malondialdehyde (MDA), antioxidant enzyme activities, and soluble sugar contents were measured in the embryo throughout storage under the two conditions to investigate whether grain deterioration was related to lipid peroxidation and to changes in sugar metabolism. Loss of grain viability at 45 °C and 100% RH was associated with an accumulation of H_2O_2 concomitant with a decrease in catalase (CAT) and superoxide dismutase (SOD) activities and an increase in glutathione reductase (GR) activity. In contrast, ageing of grains at 30 °C and 75% RH was not associated with important changes in antioxidant enzyme activities or with an H_2O_2 accumulation. In both conditions, MDA did not accumulate suggesting that ageing of wheat grains was not associated with lipid peroxidation. Change in soluble sugar contents was different during the two types of ageing. Loss of seed viability at 45 °C and 100% RH was concomitant with a marked decrease in sucrose (Su) and a slight increase in raffinose (Ra), and subsequently with an increase in the Ra/Su ratio. In contrast at 30 °C and 75% RH, ageing was associated with an increase in both sugars, but with no strong increase in Ra/Su ratio. Results presented suggest that loss of viability was associated with various mechanisms depending on the conditions of ageing and that accelerated ageing at 45 °C and 100% RH was not the only model to consider in order to understand the mechanisms involved in seed deterioration.

Genome-wide identification of rice seed vigor and longevity determinants through combined "omics"

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In Asia, rice can represent up to 70% of the daily calorie intake. New agricultural practises (*e.g.* direct sowing) and poor storage conditions require high seed vigor and longevity. Yet, to date, the majority of the rice seed quality determinants remain to be identified.

In this study, we performed a two-step analysis to identify the genetic and molecular determinants of rice seed vigor and longevity. First, given that seed germination ability is dependent on mRNA translation, we performed both a genome-wide transcriptomic and a shotgun proteomic study on the germinating rice embryo. Through a Weighted Gene Correlation Network Analysis (WGCNA), 4 transcriptomic clusters significantly correlated with germination process were highlighted. We retrieved the corresponding proteomic data and build the mRNA-protein pairs to identify key biological functions in the seed. Secondly, we aimed to confirm the involvement of these mRNAs and proteins related to germination by studying their profile during rice seed ageing. To do so, seeds were subjected to a Controlled Deterioration Treatment (CDT) during 15 days at either 25, 40 or 45 °C and 85% relative humidity. This CDT had no effect on seed germination at 25 °C, slowed down germination speed at 40 °C or completely blocked germination at 45 °C. To identify the mRNAs that were specifically degraded by CDT, we compared the transcriptome of these samples. Despite considerable phenotypic differences, only 540 and 284 mRNAs were significantly down-accumulated during loss of seed vigor and loss of germinative ability, respectively. CDT also triggers non-enzymatic accumulation of reactive oxygen species (ROS) that predominantly alter proteins by oxidation. Since this mechanism is highly specific and likely to target germination-essential proteins, we identified the oxidized proteins (carbonylation) through immunoprecipitation and LC-MS/MS analysis.

Altogether, this two-step study at two different levels gives high-confidence criteria to pinpoint a small number of genes involved in rice seed vigor and longevity. Future research is underway to cross data with QTL study. In addition, the collaboration with the International Rice Research Institute (IRRI, Philippines) should help to produce rice cultivars with high vigor and longevity.

Quantitative transcriptome, hormone and redox analyses reveal sugar beet seed deterioration mechanisms and their enhancement after priming

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Primed seed are especially prone to ageing, but little is known about the molecular mechanisms underlying deterioration in primed seed. Commercially distributed sugar beet (*Beta vulgaris*) seed is frequently subjected to pretreatments such as priming to ensure rapid and synchronized field emergence. Optimal storage of high-quality seed is mandatory to preserve the improved properties. Distinct ageing and deterioration processes during (suboptimal) seed storage may compromise seed quality, longevity and germination. We aimed to uncover and quantitatively describe the molecular changes that occur upon ageing in pretreated (primed) seeds compared to control (non-primed) seeds. The basis of our studies builds a newly established sugar beet seed ageing model, which details the impact of storage temperature and humidity as stress factors on germination kinetics by means of controlled deterioration. The broad and quantitative physiological data we gained in this approach were complemented with quantitative molecular and biochemical analyses to address the underlying key mechanisms of deterioration. Global analyses of changes in dry-seed transcriptomes using full-genome sugar beet microarrays revealed especially that alterations in redox-related processes are pronounced upon temperature and humidity stress comparing primed seed ageing processes with control (non-primed) seeds. By verification of the microarray results by qRT-PCR, and together with measurements of redox couples, enzyme activities and hormone contents, we provide insight into the diversity of deterioration mechanisms, and we especially show how priming exerts its impact on sugar beet seed ageing.

Metabolic marker of seed viability in long-term stored wheat (*Triticum aestivum*)

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Most crops are propagated, stored and commercialized as orthodox seeds. Seed viability is important for agriculture and conservation of plant genetic resources in genebanks as housed by the IPK Gatersleben.

The biochemical changes coinciding with loss in seed germination were investigated using GC-MS and LC-MS based metabolite profiling. One ambient and cold stored set of 90 wheat seed accessions with high variation in germination (n = 90) and two 40 year coldstored sets of wheat (n = 170) and barley (n = 170) seeds were assayed for germination frequency and sampled for metabolite profiling at the same time point. GC-MS analysis identified a large number of mostly negatively correlated metabolites ranging from R = 0.55 to -0.82 for glycerol and related intermediates in the wheat set with high variation in germination frequency. Consistently, the high correlation between glycerol and germination was confirmed, though to a lower extent, in the 40 year old sets of wheat and barley seeds(R = -0.58 and -0.57). The metabolic signature correlated to seed germination between the two 40 year old sets from wheat and barley was highly similar. Following our interpretation that lipid degradation events might have contributed to an accumulation of glycerol in the seeds with low germination frequency, we investigated the lipidomic composition of the wheat panel with high variation in germination using high-resolution liquid chromatography-mass spectrometry (LC-MS). From 25,500 chromatographic features (m/z), 650 were positively correlated to germination rate (max R = 0.77) and 3,600 were negatively correlated (max R = -0.89). Using a target list for 2000 lipids (Giavalisco *et al*., 2011), we tentatively annotated 1,480 lipid sum formulae based on mass accuracy (2 ppm). 530 features with sum formula annotation displayed significant correlations to the germination frequency. By binning the features into lipid classes, we can show that membrane lipids like galactosyl-lipids and phospholipids are positively correlated with seed germination, while their degradation intermediates like phosphatidic acid, lysophosphatidic acid, diacylglycerols and fatty acids are negatively correlated, linking seed viability to membrane integrity.

Biochemical mechanisms that contribute to seed ageing in the garden pea, *Pisum sativum*

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Seed quality and storability are of paramount significance to agriculture and plant conservation, and considerable economic losses result from sub-optimal seed performance. Here, we will review our work on seed ageing in *Pisum sativum* L. over the last decade, revealing some of the mechanisms that contribute to seed deterioration upon storage and artificial ageing. We first showed that changes in the cellular redox potential correlate with seed viability. These changes were suggested to trigger programmed cell death, which became evident when nucleic acids were degraded by endonucleases into inter-nucleosomal fragments. An analysis of the transcriptome of ageing seeds showed that genes associated with programmed cell death, oxidative stress and protein ubiquitination were altered at the earliest stages of ageing prior to viability loss. Shortly before total germination started to decline, genes associated with the transport and metabolism of lipids, amino acids, inorganic ions, coenzymes and nucleotides were altered. Macromolecules were also degraded by other chemical and biochemical processes. Headspace analysis of ageing seeds by gas chromatography coupled with mass spectrometry revealed that volatile compounds were derived from lipid peroxidation, alcoholic fermentation and Maillard reactions. Besides, evidence was shown for Strecker degradation of amino acids, and for pectin degradation. Using infrared thermography as a non-invasive technique to diagnose viability, we demonstrated that non-viable seeds fail to degrade starch upon germination. In conclusion, seed ageing involves a series of intricately linked processes that include genome reprogramming, programmed and non-programmed cell death.

Characterization of volatile production during seed storage

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This paper contributes to the understanding of factors that regulate seed longevity and mechanisms that cause seed deterioration during storage. The over-all goal is to characterize the types of chemical reactions that occur in dry seeds and to relate these to the properties of the seed glassy matrix and the inevitable decline in seed quality and viability. Oxidation and peroxidation reactions occur in stored seeds and are implicated in chemical degradation. Oxidation of macromolecules gives rise to low molecular weight carbonyl compounds, many of which are volatile and so escape into the airspace of the storage container. The identity of volatile compounds indicates the nature of chemical reactions. Production of volatile compounds from seeds of different species was investigated. Volatile composition produced by seeds differs with the species: species such as *Carum carvi* were detected to produce large amounts of volatile compounds, while others, such as *Eruca sativa,* produced low quantities of only a few compounds. Qualitative and quantitative differences in volatile composition were noted as a function of storage RH: above about 30% RH glycolysis-like reactions increase with increasing RH, and below about 30% RH peroxidation-like reactions increase with decreasing RH. Assessment of volatile production from seeds during storage provides a probe to address questions about the nature and kinetics of chemical reactions that occur in the glassy matrix of stored seeds. This information reveals important information about the glass such as the proximity of reacting ligands and inter- or intra-molecular motions required to effect chemical reactions. Moreover, it could be developed as a non-invasive and early detection method of seed ageing rates.

Acknowledgement

Support by project CGL2006-10536 (MEC, Spain) and S.M. by Jose Castillejo program (MECyD)

Does controlled deterioration of seeds mimic long-term storage in wheat and barley?

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In the last century scientific research into seed longevity revealed that seeds may survive for decades, dependent on species. However, seed longevity is limited and strongly influenced by pre-storage and storage conditions. Methods of artificial ageing under high temperatures and seed moisture contents (MC) have been used to mimic long-term seed deterioration, with the aim to being able to assess seed longevity upon storage. High seed MCs lead to a weakening of the glassy state usually used for commercial or gene bank storage of seeds. Importantly, different MCs may facilitate different (bio)chemical reactions. Seed deterioration caused by reactive oxygen species have been assumed to be involved in viability loss, often assessed through lipid peroxidation or accumulation of hydrogen peroxide.

To enhance our knowledge of (bio)chemical reactions occurring during seed storage we investigated changes in the redox state of the antioxidant glutathione (γ-glutamyl-cysteinyl-glycine) during long-term dry storage at two temperatures (ambient and cold storage in the glassy state) and two regimes of controlled deterioration (CD), one at 45 °C and 13% MC (rubbery state) and the other at 45° and 18% MC (fluid state) in 23 accessions of barley and wheat. The half-cell reduction potentials of glutathione and related thiols were calculated according to the Nernst equation. Viability loss concurred with a shift towards more oxidizing intracellular conditions, although combinations of thiols and disulphides were found to be different during CD at 18% MC compared to storage at ambient temperature or CD at 13% MC. Electron spin resonance (ESR) spectroscopy revealed a negative relationship between the occurrence of radicals and seed viability in material stored in the glassy state at ambient temperature, whereas no correlation was observed for CD treatments. These data suggest that the physical state achieved by the different ageing or storage conditions (glassy, rubbery or fluid) affected (bio)chemical processes leading to seed deterioration in different ways.

Tissue-specific hormone metabolism as quality marker for seed technology and storage

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Hormone metabolism, transport and signalling in crop seeds and fruits are specifically altered by the production, storage, and germination environment. While the application of seed technologies such as priming can enhance speed and uniformity of germination, priming may also have adverse effects on the seed's longevity in storage as it enhances ageing processes. To identify the molecular mechanisms underlying these processes we used tissue-specific hormone profiling and global seed transcriptome analysis to compare how priming effects longevity- and aging-related mechanisms of sugarbeet fruits stored at different temperature and humidity regimes. The hormone profiling and gene expression analysis of hormone-related genes demonstrated that abscisic acid (ABA), gibberellins (GA), ethylene, and auxin (IAA) metabolism and/or transport are spatially and temporally altered by priming and the distinct storage conditions. Several of the hormones exhibited a strikingly similar spatial pattern and quantitative change in endogenous contents suggesting that they may serve as markers for seed quality. Further to this, some of the hormone-related sugarbeet genes identified to exhibit differential expression, are also known from seeds of other species including weeds and vegetables to respond to environmental cues such as altered imbibition temperature during germination. A cross-species comparison will be provided for some of these hormone-related genes and their use as markers for seed-related quality traits will be discussed.

Identification of seed quality markers by activity profiling of proteases

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Fast and uniform germination of a seed lot depends on its quality, which is affected by endogenous factors. These include a wide variety of seed constituents, ranging from those related to seed structure and composition to others participating in reparation and detoxification mechanisms. When repair and protection mechanisms cannot overcome aging damage, seeds lose their germination capacity.

Germination tests are at present the most used method to evaluate seed quality and germination capacity. These test are highly reliable but time consuming and therefore, the development of faster methods to evaluate seed quality is desirable. We are exploring the use of Activity-Based Protein Profiling (ABPP), which relies on the use of small probes that target the active site of certain enzyme subfamilies (such as proteases or hydrolases). These probes react with the active site in a mechanism-dependent manner, consequently binding only enzymes in their active state. In addition, they carry tags that can be used for downstream identification or purification.

In order to identify new seed quality markers, we are carrying out experiments in artificially aged seeds of *Arabidopsis thaliana*. We have identified several protease activities that vary during the aging treatment in two different Arabidopsis accessions. These activities are being further investigated by mass-spectrometry analysis to identify the corresponding proteins. We will discuss the potential of these protease activities as markers for seed quality.

DNA methylation changes in seeds

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Cytosine-specific DNA methylation is an epigenetic factor that plays a significant role in the regulation of plant growth, development and response to stress factors. Notwithstanding, there is a little information about changes in DNA methylation in seeds induced by stress or during storage and the aging process.

In the current study, changes in DNA methylation were analyzed in seed embryos of *Acer platanoides* L., *Quercus robur* L., *Pyrus communis* L. and *Corylus avellana* L. Analysis of the global content of m^5C in seeds was performed with a 2D TLC method. Germination (radicle emergence) and seedling emergence (seedling growth) tests were applied to determine seed viability. In the case of *Pyrus communis* DNA methylation of seedlings were also analyzed.

Our results indicated that desiccation induces sine wave-like alterations in m^5C amount in orthodox seeds. Such dual in nature alternations in response to different water contents may represent a specific response of orthodox seeds to drying and play a relevant role in desiccation tolerance of seeds. Moreover, epigenetic changes were observed not only in severely desiccated seeds but also in 3-month old seedlings obtained from these seeds.

Analysis on recalcitrant seeds showed that these seeds undergo an aging-related decrease in total m^5C during storage. This decline is highly correlated with a decrease in seed viability as reflected by a reduction in germination and seedling emergence. It is likely that the decrease in m^5C level may represent a typical response to aging and senescence in recalcitrant seeds.

Contrary to recalcitrant seeds, after 12 months of cryostorage orthodox seeds at different water contents exhibited a general increase in the level of m^5C. Also conventional storage for 24 months at 3 °C resulted in a significant increase in the amount of m^5C. The observed changes in global methylation did not come together with seed viability measured by germination and seedling emergence. Therefore, the observed changes may show a specific response of orthodox seeds to abiotic stress factors and are reflection of deepening of latent life state. Possible explanations of the mechanisms responsible for changes in genomic DNA methylation are discussed.

Poster Presentations

Session I

Seed banking – state of the art

10 rounds of cabbage accessions reproduction in VIR genebank: Stability of authenticity?

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Russian *Brassica oleracea* L. and vegetable *Brassica rapa* L. worldwide germplasm collection keeping at the N. I. Vavilov Institute of Plant Industry (VIR) from 1923 consists of 3,400 accessions, including landraces, old and advanced cultivars and breeding material from 75 countries. 1,500 accessions of leafy vegetable *brassicas* were collected by N.I. Vavilov himself and his colleagues till 1940, but only 20% accessions were saved from this time because of damage and loss germination ability during 2nd World War. Resumption of VIR collection started from 1945, when loss material was collected from the same locations and bought from the same breeding companies like Vilmorin. From 1950 VIR collecting missions to five continents were resumed and collection enriched very intensively.

The first round of new reproduction and new characterization and evaluation trials for old and new accessions has begun from 1946. Before 2005 *brassicas* accessions were regenerated each four years, so many accessions have more than 10 rounds of reproduction. Regeneration procedure for *brassicas* is complicate: they are cross pollinated moreover insect pollinated crops, some crops are biannual, and the goal of our investigations was study of authenticity of VIR white cabbage collection. Morphological and biological characters of cabbage core collection (9% from all collection; generally old local cultivars/landraces) from description 1946-1979 and 2013 in two ecologo-geographical zones of Russia using the same standardized research methods were compared. According N.I.Vavilov concept of initial material for breeding all accessions have been characterized and evaluated for 30 morphological, biological and agronomic traits.

Stability of all morphological characters was found. Using t-test for dependent simples we determined that only head weight is non significantly increased, that can be explained by improved selection during reproduction process. On the contrary period of vegetation of studied set is decreased significantly in both locations; possibly it is connected with climate change. We conclude that authenticity of VIR cabbage collection is saved successfully.

Long term seed conservation of plant genetic resources in Lithuania

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The collection and studies of the genetic diversity of agricultural crops in Lithuania were initiated by Professor D. Rudzinskas in 1922, after the Plant Breeding Station had been established in Dotnuva.

In 1994 the Baltic-Nordic project for plant genetic resources (PGR) was initiated by the Nordic Genetic Resource Center (the former - Nordic Gene Bank). The main objective of the project was to develop the national plant genetic resources conservation network in Lithuania. In 1997 the long term seed storage was established at the National Plant Genetic Resources Coordinating Centre. The Nordic Gene Bank provided all necessary facilities.

The law on National plant genetic resources was enacted in 2001 by the Parliament of Lithuania and Plant Gene Bank (PGB) was established in 2004. In nowadays 8 institutions in Lithuania are involved in the activity of collection, investigation and conservation of plant genetic resources. The main aim of Plant Gene Bank activity is a long-time preservation of plant genetics resources. Seeds of old landraces and varieties of agricultural crops, advanced varieties and valuable breeding material, as well distinguished populations of wild relatives of cultivated plants and forest trees, ornamental and medicinal plants are stored in the airtight aluminium foil bags at -18 °C temperature in the long-term seed storage.

Today 3,014 accessions of 179 plant species are stored for the long-term conservation, the agricultural crops are represented by the largest number (2176) of accessions. The long-term seed storage is annually supplemented by new accessions.

The investigation of a persistent soil seed bank in submediterranean oak forests of Greece under different grazing regimes

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The soil seed bank in sub-Mediterranean deciduous oak forests and the impacts of different long-term grazing regimes (ruminants, wild boars) on its composition, density, relation with the above-ground vegetation and ecological features were investigated in the frame of the presented doctoral research. Comparisons were made with sporadically grazed forest sites.

The studied forest ecosystems possess 'ecological memory' which reflects their grazing history. The studied soil seed bank is persistent and the seeds' density reaches the levels of 2000 seeds/m2. Especially the long-term boar overgrazing caused the qualitative and quantitative decline of the soil seed bank. The majority of plant taxa appeared in the first 5 cm of soil in the sporadically grazed forest sites. The soil seed bank distribution differed in the overgrazed sites. Species richness in the ruminant grazed seed bank is higher than the species richness in the boar grazed seed bank and lower than the richness in the sporadically grazed seed bank.

Twenty-eight taxa were recorded in the soil seed bank and 83 taxa in the above-ground vegetation. The dominant tree species and many woodland species found in the above-ground vegetation were absent from the soil seed bank. Similarity between the soil seed bank and the above-ground vegetation was low and decreased with grazing. Beta diversity of above-ground vegetation was significantly higher between long-term grazed and sporadically grazed areas. Beta diversity of the soil seed bank declined with grazing.

Regarding the different dispersal and life strategy types of the herb layer taxa, overgrazing reduced both species richness and seed density of typical forest herbs in particular. Plant species richness and seed density of animal-dispersed taxa were reduced by overgrazing while physically-dispersed species were not affected.

Despite its limited restoration potential, the persistent herb seed bank could contribute to restoration if combined with other methods. The features of target species such as the typical forest herbs could be further examined in relation to microhabitat conditions and different climatic treatments. This doctoral research contributed soil seed bank investigation at species pool level and for diversity conservation in this forest type.

Sensitivity of short-lived seeds to desiccation and storage conditions

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Conservation of short-lived seeds (*Salix* spp., *Populus* spp.) is challenging for seed banks. Such seeds lose viability very quickly after harvest at ambient conditions, which shortens the time of their preparation for longer preservation. It is also difficult to classify such seeds as orthodox, suborthodox or recalcitrant. The aim of the research was to determine the effects of desiccation and storage conditions on the germinability of *Populus nigra, P. tremula, Salix alba* and *S. viminalis* seeds (i.e. % of seeds with a radicle and green cotyledons). We hypothesized that the seeds do not have any critical water content and when packed shortly after harvest, they can be safely stored in controlled conditions for a long time. We found that seeds of *Salix* and *Populus* spp. were sensitive to severe desiccation (below water content of 0.04-0.05 $g \cdot g^{-1}$ dry matter; i.e. moisture content 4-5%), therefore we suggest that can be classified as intermediate. We demonstrated that seeds of four investigated species have a safe range of water content at which they could be stored successfully by both conventional and cryogenic techniques, for at least 24 months. This study provides a variety of protocols suitable for the *ex situ* conservation of genetic resources of poplar and willow species in gene banks.

Exploring seed longevity in different kernel types in the CIMMYT maize germplasm collection

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The CIMMYT Maize Germplasm Bank is the largest global seed collection of landrace maize in the world. The original collection dates back to the 1940s, but in its current holdings, the oldest seed date back to the late 1960s-early 1970s. The current monitoring protocol is to test seed germination in accessions in the active collection (storage temperature=0 °C) every five years, and in the base collection (-18 °C) every ten years. In order to gain a better understanding of the longevity of the seed in our vaults, and test for differences among our main kernel types, we collected seed germination data from 232 accessions that had been stored for 3 to 46 years. The three main kernel types are floury, dent and flint, and each was represented by 2-5 landraces with white and/or yellow kernel color. Four replicate samples of 50 seeds each were germinated under controlled conditions and percent germination was recorded. In this poster, we will present our results and use these to make an informed decision about maintaining or changing our current monitoring protocol.

Certification process of small grain cereals BRC in France

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In France, genetic resources are maintained in several Biological Resources Centres (BRC) spread all over the country. Most part of these BRC are involved in a quality management process. The development of quality system becomes a priority in regard of new notions (as collection of reference) and requirement at European and international levels.

In this context, the French small grain cereals BRC engages itself in a certification process since March 2014. The certification's framework applied to principal missions of small grain cereals BRC:

Each year new materials are introduced in collection for different purposes (scientific projects, evaluation networks, ie...). To be able to answer to Nagoya protocol requirement and also to be in agreement with European collection (AEGIS) philosophy, acquisition process has been clarified and standardized.

Regeneration of small grain cereals genetic resources are realized at INRA Clermont-Ferrand with around 10% of the whole collection (≈ 27,000 acc.) characterized per year for primary descriptors. A part of these data is available online on SIReGal database (http://urgi.versailles.inra.fr/siregal).

Active collection is maintained in a 100 m^3 cold room at +4 °C and 30% relative humidity. It is used to regeneration, multiplication, distribution, characterization and assessment. Base collection is maintained at -20 °C in freezer as secure storage. Various tests concerning drying before storage and germination are done to improve conservation conditions.

To improve access to small grain cereals collections, a work on data's curation and submission to SIReGal database has been done and it still in process. Around 4 300 samples have been distributed in 2014, mainly to researchers and breeders but also to farmers and associations. Terms and conditions of BRC have been formalized and a first survey of genetic resources' requesters was launched in autumn 2014 to better understand their expectations and to have a feedback on BRC services (seed quality, available information, delay, documentation …).

Each proceeding has been well documented to clarify and improved our methodology. All documentations are managed by a dedicated softwear AQ_tools (AQ_Tools – Toolbox to manage a QSM © R. Cottin – Cirad 2008-2015 IDDN.FR.001.21032.000; http://golo.cirad.fr/FR/).

Monitoring oilseeds, legumes and cereals in seed storage at Plant Gene Resources of Canada

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Detailed monitoring of seeds in the medium term storage vault at Plant Gene Resources of Canada (PGRC) was conducted from 2006 to 2014 to better understand how the seeds respond to possible fluctuations of temperature and air humidity.

Seed samples of 16 accessions of flax, rapeseed, turnip rape, oriental mustard, sunflower, lentil, pea, barley and oat were placed in both the medium-term storage vault and at ambient conditions in the working area of PGRC in 2006. Seeds were kept in paper envelopes allowing the seeds to stay in equilibrium with the air humidity of the storage environment.

The average temperature and relative air humidity in the storage vault were 3.3 °C (2.5 °C to 6.8 °C) and 36.1% (10.5% to 76.5%), respectively. Under ambient conditions, the average temperature was 20.9 °C (19.1 °C to 23.4 °C) and the relative air humidity was 29.7% (9.3% to 61.8%). A considerable seasonal increase in relative humidity during the summer months was observed in the in the ambient conditions but also in the seed storage vault.

High positive correlations between fluctuating relative air humidity and monthly seed weight measurements suggested seeds absorbed water quickly when relative air humidity increased. The average seed moisture from 2006 to 2014 varied within species and increased in the order: oilseeds, cereals and pulse crops.

Seed samples decreased in germination during storage. Seeds stored under ambient conditions experienced a greater overall decrease in germination. This decrease was particularly high in *B. napus*. Differences between cultivars of a given species were also observed. In sunflower, dormant seed increased in germinability over time.

Conclusions form these observations are:

- For most seed material, the decrease in germination between 2006 and 2014 was negligible in the seed storage vault but considerable under ambient conditions.
- Variation in preserving seed germinability points at great differences among species and cultivars.
- Air humidity and seed moisture content are closely related.
- Variations in equilibrium seed moisture content are due to species and infraspecific differences.
- Seed dormancy is an important issue when assessing seed germinability.
- Close monitoring of the seed storage environment is important.

Are *Momordica charantia* (bitter gourd) seeds truly orthodox?

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Momordica charantia L., called bitter gourd, bitter melon or balsam pear, was likely domesticated in India and southern China and is now found naturalized and widely cultivated throughout the tropics and subtropics for its immature fruit. In Asia, the tender shoot tips are consumed as a healthy leafy vegetable. Bitter gourd is of high nutritional value and has multiple medicinal uses. AVRDC – The World Vegetable Center maintains a diverse bitter gourd collection of 462 accessions. Although bitter gourd seed is categorized as orthodox (able to tolerate desiccation and sub-zero temperature storage), studies at AVRDC revealed bitter gourd seeds are severely damaged during sub-zero temperature storage with germination rates plunging to 0-5%. This has major consequences for safe germplasm conservation and storage.

To determine safe storage conditions for bitter gourd seed, two diverse accessions from Thailand (VI049009) and India (VI049940) were dried to 6% moisture content and then divided into three batches. Seed from batch 1 was left untreated (control) or subjected to different priming treatments to overcome hardseededness. Seeds from batches 2 and 3 were stored for six months at 15 °C and 5 °C, respectively, followed by priming treatments.

Accession VI049009 from Thailand had a much lower germination rate after drying (16%) compared with accession VI049940 from India (70%). Some priming treatments enhanced germination in both accessions. After 6 months of storage, seed germination of VI049009 was enhanced to 25% when stored at 15 °C and to 42% when stored at 5 °C. The germination rate of VI049940 was elevated to 84% and 86%, respectively, under the same storage treatments. Priming of seed following storage further enhanced the germination rate.

In conclusion, genotypes clearly differed in their rates of germination, independent of storage and priming treatment. Seed storage at 5 °C, considered too low by some seed companies, did not reduce seed viability. On the contrary, we observed that the germination rate improved after 6 months of storage, indicating that seed dormancy can be partially overcome in storage. Several priming treatments, which can be easily applied by farmers, enhanced seed germination.

Cooling methodologies of coffee seeds for storage in liquid nitrogen

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Studies contributing to the understanding of the complex physiology of dehydration, cooling and thawing of coffee seeds, aiming to cryopreservation of the species it's a great importance, since the low seed longevity is a limiting factor for the maintenance of germplasm in a long-term. The maintenance of the genebank field is expensive and is under imminent risk of weather events or pathogen attack, endangering the genetic variability. However, preservation in liquid nitrogen (-196 °C) is the only alternative for the storage of species with recalcitrant and intermediate seeds, such as coffee. The cathode water has demonstrated anti-oxidant effects, and then used to minimize the stress related to cryopreservation procedures in plant materials. Since cooling is an important step in cryopreservation, aimed to investigate the effect of some cooling methodologies and immersion in cathodic water in *Coffea arabica* L. seeds, cultivar Catuaí Amarelo 62, before storing them in liquid nitrogen. The methodologies were carried out on seeds samples with: A) 17% humidity, cooled at a rate of -1 °C/min. to a temperature of -40 °C; B) 20% humidity, cooled at a rate of -1 °C/min. to a temperature of -40 °C; C) 17% humidity, cooled at a rate of -1 °C/min. to a temperature of -50 °C; and D) 17% humidity without cooling. All samples were evaluated physiologically by means of the germination test and viability in the tetrazolium test, with/without cathodic water after being removed from the liquid nitrogen. For both tests, the percentage of normal seedlings at 30 days and viable embryos was lower for seeds samples of methodology "A" in the absence of cathodic water when compared to the other treatments.

Long-term storage of plant genetic resources in the Latvian genebank

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The Latvian gene bank of cultivated plants (LGB) preserves seeds of 72 agriculturally important species and their hybrids. Most accessions are of Latvian origin, and information about stored accessions is available in the SESTO and EURISCO databases. The gene bank stores material of Latvian origin collected and bred in Latvia, as well as Latvian accessions repatriated from other gene banks. Most regeneration activities have been concentrated on the repatriated accessions.

The first accessions were put in long-term storage conditions in freezers at -18 ±2 °C in 1999. Germination tests of accessions representing 20 species (cereals, forage grasses, peas and flax) were done after ten years of storage and only small changes were observed. Germination tests were repeated on the same accessions after 15 years of storage. A slight decrease of germination was observed in cereals, peas and most of the grass species accessions (2-10%) depending on species and variety. A significant decline in germination was only found for three grass accessions (more than 15%). Close monitoring of grass accessions is required and in case of further decline of germination, regeneration will be necessary.

In situ and *ex situ* seed longevity of two endangered Brazilian cerrado species (*Dimorphandra,* Leguminosae)

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Seed longevity is dependent of seed features and storage conditions. This investigation was carried out to evaluate the seed longevity of two endangered species of Leguminosae (*Dimorphandra exaltata* and *D. wilsonii*), which seeds exhibit physical dormancy (PY). The longevity was tested *in situ* by burying the seeds in nylon bags at about 5 cm depth in their natural habitat and *ex situ* by storage in cold chamber (5 °C) in bags hermetically closed. Bags were taken at 0, 6, 9 and 12 months, and seed germination was evaluated at 25 °C (optimal temperature) under a 12h photoperiod (30µmol $m^{-2}s^{-1}$) using four replicates of 25 intact or scarified seeds per treatment. Intact seeds were analyzed using Scanning Electron Microscopy immediately after collection and after 12 months stored in the soil and in cold chamber. The germinability of recently collected seeds was of approximately 10% to intact seeds and >85% to scarified seeds. Cold storage for 12 months did not significantly change seed viability and germinability of intact seeds of the both species. After 12 months, less than 50% of the seeds originally buried were recovered, more than 80% of which remained viable. Seeds of *D. exaltata* and *D. wilsonii* gradually overcame the PY during burial; intact seed reached germinability of 32 and 71% respectively after 12 months. The seed coat of both species is widely striate, with shallow fracture lines forming large areolae. After 12 months stored in cold *D. exaltata* seeds did not show clear structural changes. However, *D. wilsonii* showed evident increase in the depth of the fracture lines and in the areolae. Burial promoted deep seed coat changes in both species, more intense in *D. wilsonii*, indicating that temperature and humidity variations throughout the year are among the main factors releasing *Dimorphandra* seeds from PY. We can infer that the seeds of both studied species overcame PY during burial and are able to form small persistent soil seed banks. In addition, the possibility of maintaining the seeds *ex situ* offers viable option for future conservation of these endangered species.

Soil seed bank: The effect of forest cover on the viability of *Araucaria angustifolia* seeds

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After dispersal from the parental plant, the seeds end up in the soil and germination can take place immediately, or be delayed for an indeterminate period of time. The aim of this work was to evaluate the seed viability and seedling establishment of *Araucaria angustifolia* (Brazilian pine) under different environmental conditions. An experimental soil seed bank was installed in a forest fragment (21°20'35.41" S; 44°58'58.58" O) located in Lavras, Minas Gerais State, Brazil, under open (disturbed edge) and closed (conserved area) forest canopies. The seeds were packed in nylon mesh bags (15 x 30 cm) which were placed in a metal cage (120 x 80 x 15 cm) and covered by a thin litter layer. The experiment was initiated in May 2014 and set up as a randomized complete design with four replications of 160 seeds. Seed samples were removed after 30 and 60 days to test viability (germination test), internal damage (x-ray) and water content. Scott-Knott test was used to analyse the interaction between environment and time. The initial values (fresh seeds) of moisture content were 37.5%, radicle protrusion 91%; normal seedlings 85% and damage rate 2.5%. After 30 days in the seed bank, in the open canopy, the values of radicle protrusion and normal seedling had decreased to 73% and 68%, respectively. In the last evaluation, those values declined to 40% (radicle protrusion) and 16% (normal seedlings). Water content dropped to 30.36%. Under the closed canopy, no significant differences were found after 30 and 60 days, in the values of radicle protrusion, normal seedlings and water content. The final damage rate was 10% (open canopy) and 7.5% (closed canopy). Seedling emergence did not occur in the seed bank during this period. Preliminary results demonstrate that the recalcitrant seeds of *A. angustifolia* remain viable for 60 days and the maintenance of forest cover is essential for the seed longevity in the soil seed bank.

Germplasm conservation and seed longevity in the Czech genebank

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Seed genetic resources have been kept in the Genetic Resources Dept., CRI Ruzyně since its foundation in 1951. The initial 6,000 accessions were received from the preceding organisations from the period 1880-1950. The samples in CRI Ruzyně were stored in metallic boxes in the basement and regularly regenerated in 5 year periods. The selected materials from the base collection were stored in commercial cold store since 1965. Their germination ability was regularly counted, but not recorded to the information system EVIGEZ. The new Gene Bank was opened in 1989 and filled subsequently with materials from the commercial cold store and preferable with newly regenerated material. At present there are data on seed longevity available for the period of 23-27 years comparing to the last germination test in 2014. The standard germination ability of the selected 60 oldest wheat accessions did show increase of germination by 2% from the initial 95.3%. Similarly 44 winter barley accessions showed decrease of germination by 12 % from the initial 89.8%, most likely because of lower initial quality. In legumes, 365 bean accessions showed decrease by 1.2% from the mean initial germination 92.1% and 212 peas accessions showed increase by 14.6% from the initial mean germination 74.1%. The oldest 60 accessions of Cruciferae species *Brassica napus*, *B. rapa* and *Sinapis alba* kept the same germination rate as well as triticale. The germination increase in peas is possible to count in favor of dormancy, because dormant seeds were originally not counted to germination rate.

Seed longevity of vegetable seeds from BGHZ-CITA genebank (Zaragoza, Spain) compared to their duplicates from CRF-INIA (Madrid, Spain)

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Spain is one of the richest countries in crop biodiversity in Europe, particularly in vegetable species. Significant efforts have been made in the last decades to collect vegetable landraces to ensure their long-term conservation in *ex situ* collections, thus preventing their irreversible lost. Spanish *ex situ* collections of plant genetic resources for food and agriculture (PGRFA) are organized in a National Network supported by the National Program on Conservation and Utilization of PGRFA. In the group of vegetable species, the BGHZ-CITA in Zaragoza is one of the national active genebanks, although other regional and national institutions maintain important collections. The responsibilities of the Spanish National Centre of Plant Genetic Resources (CRF-INIA) include the conservation of safety duplicates of the seed Spanish collections (base collection) and their documentation in the National Inventory (NI). According to this National Inventory, BGHZ-CITA conserves more than 7,000 accessions belonging to the main vegetable crops. About 75% of these samples are duplicated in the CRF-INIA. The aim of the viability monitoring test is to decide whether regeneration is required.

In the BGHZ-CITA active collection, the seed samples are desiccated with silica gel and stored at -18 ºC. The CRF-INIA base collection is also conserved at -18 °C (-15 °C before 1998) and seed drying is conducted in dehydration chambers (13-15% R.H. and 20 °C), although silica gel was employed on vegetable seeds until the year 2000. In order to monitor the viability of the seeds, both active and base collections perform germination test during storage.

Seed germinability after 20-30 years of storage was studied on 8 vegetable species (onion, cabbage, pepper, melon, watermelon, cucumber, lettuce and tomato), in both genebanks. The accessions analyzed were multiplied in CITA; part of the obtained seeds was stored in this genebank and a duplicate was conserved in CRF some months later. In both collections, seed germination resulted similar and high in most samples, although slightly lower in the CRF materials, with the exception of Cucurbitaceae seeds (long-lived seeds). The longer pre-storage periods of the CRF samples may have played a role in the lower germination of the short/medium-lived seeds.

Martonvásár Cereal Genebank

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The main task of Martonvásár Cereal Genebank is to collect, preserve and maintain wheat species and genetic reserves from related species, and to make detailed investigations on the quality, agronomic value, and biotic and abiotic resistance of the accessions.

The Martonvásár Cereal Genebank could be divided into three main parts: breeder collection (1), genetic stock collection (2) and a set of wheat wild relatives (3).

1. The greatest collection consists of more than 11,500 accessions of breeding stocks, varieties and landraces. Majority of the accessions are *Triticum aestivum* (90%) genotypes, but other cultivated cereals (barley, triticale, durum wheat, etc.) are also represented. The breeder collection is an important part of the cereal breeding programme at Martonvásár.
2. Special attention is given to the high value genetic stocks (1,000 accessions), such as aneuploid materials (nullisomic, monosomic, substitution and addition series), special mutant stocks and amphiploids. Majority of the genetic stocks have been developed at Martonvásár during the last decades (e.g. Rannaya12 monosomic series) and the other part was collected via international material exchanges. The genetic stock collection is used in basic genetic research and pre-breeding activity. The maintenance of genetic stocks often requires cytogenetic control.
3. Third part of our genebank is the set of wild relatives of wheat. This collection consists of about 1,700 accessions, including major species of *Triticum, Aegilops, Secale, Hordeum* and perennial species (*Agropyron, Elymus,* etc.). Many of these accessions possess excellent resistance for biotic and abiotic stresses. They are mostly used in pre-breeding work. Several pre-breeding programmes were started at Martonvásár on the basis of gene bank accessions to transfer the useful traits of wild relatives into hexaploid wheat. The *Triticum monococcum* collection with more than 300 accessions is outstanding among the European collections. The *Aegilops* collection has expanded in the recent years by collecting new accessions (130 accessions) via European expedition.

The whole genebank collection consists of around 14,000 accessions. The long-term *ex situ* maintenance of the accessions takes place in refrigerated storage (-28 °C). Majority of the gene bank accessions are stored for medium term at 4 °C in cold room, at the same time perennial species are also maintained *in situ* in isolated nursery.

The whole database management of the genebank is carried out using a software called *Breeder*, which was developed at Martonvásár for cereal breeding. Characterization of genebank accessions under field conditions is an important part of our activity. All phenotypic and agronomic data collected during the regeneration and conservation process are recorded in the database of *Breeder* which is also used to manage seed production, storage and exchange.

Optimizing seed conservation protocols and cryopreservation at the CRF-INIA genebank to reduce genetic erosion

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The CRF-INIA is the Spanish National Centre for Plant Genetic Resources where a duplicate of all seed active collections belonging to the Spanish Network of the National PGR Program should be deposited. First samples date from 1980's. Nowadays, the long term collection (base collection) comprises approximately 39,000 samples. The CRF-INIA genebank also maintains the largest active collection in Spain, with up to 22.000 accessions, meanly of cereals and grain legumes.

At the CRF-INIA genebank the seed conservation protocol follows the FAO/IPGRI recommendations: seed desiccation at 13-15% RH and 20 °C, and storage at -4 or -18 °C, for the active and base collections, respectively. Seed viability monitoring is performed systematically through germination tests. For most species, the current conservation protocol shows good performance (Martín et al. 2014). However, in some plant groups there is an accumulation of samples with low longevity, for which sample regeneration is costly. For example, after 20 or 30 years of storage, some accessions of Brassica ssp., Lactuca sativa or Secale cereale, have shown low seed viability after storage. In some cases, samples with low germination rates might be not only due to low longevity but also to other reasons, such as problems with dormancy interfiering with the germination test performance and low initial quality of the samples.

The general aim of this project is to find alternative storage protocols to reduce genetic erosion, present and future, of the conserved material at the CRF-INIA seedbank. Cryoconservation of seeds at different water contents are being tested in species with low seed longevity and of problematic regeneration (rye, onion and cabbage). Results would be compared to the conventional storage at -18 °C after medium/long term storage.

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The EcoSeed project – seed performance in a changing climate

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Seed quality is of paramount importance to agriculture, food security and the conservation of wild species. Considerable economic losses result from sub-optimal seed performance, undermining food security and livelihoods. Seed quality is strongly influenced by the environmental stresses experienced by the mother plant. Climate change will further exacerbate economic losses and decrease the predictability of seed yield and quality for the farmer. The looming challenges of climate change and food security require new knowledge of how stress impacts on seed quality, as well as a re-appraisal of optimal storage conditions. EcoSeed addresses these challenges by bringing together a group of distinguished European experts in seed science and converging sciences to characterise seed quality and resilience to perturbation. EcoSeed combines state-of the-art "omics", epigenetics, and post-"omics" approaches, such as nuclear and chromatin compaction, DNA repair, oxidative and post-translational modifications to macromolecules, to define regulatory switchboards that underpin the seed phenotype. Special emphasis is placed on the stress signalling hub that determines seed fate from development, through storage, germination and seedling development, with a particular focus on seed after-ripening, vigour, viability and storability. Translation of new knowledge gained in model to crop and wild species is an integral feature of EcoSeed project design, which will create a step-change in our understanding of the regulatory switchboards that determine seed fate. Novel markers for seed quality and new "omics" information generated in this project will assist plant breeders, advise the seed trade and conservationists alike. In this way, EcoSeed will not only be proactive in finding solutions to problems of ensuring seed quality and storability but also play a leading role in enabling associated industries to better capture current and emerging markets.

Kinetics of seed deterioration in diverse lines of rye, wheat and triticale

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The joint studies were carried on in the framework of USDA-ARS project "Inheritance of seed longevity in *Secale*, *Triticum* and *Triticale*" and the significant differences in cereal seed samples longevity during storage have been observed. Viability assessment has shown variability of seed longevity on species as well as cultivars level. The aim of the presented studies was to verify the results of former experiment, and evaluate the seed longevity of another set of selected rye, wheat and triticale cultivars after accelerated aging. For the experiment 28 accessions of rye, triticale and wheat cultivars have been selected. The seed moisture content has been calibrated on four levels 0.06; 0.08; 0.10 and 0.12 g H_2O/g fw (at 20 °C) after exposure of seed sample to saturated inorganic salt solutions. After moisture content equilibration seeds were vacuum-sealed in airtight aluminum foil bags and stored at constant temperature (35 °C) for a period of 44 weeks. Seed viability as germination ability and radicle length measurements after 96 hours of germination at 25/15 °C day/night temperature were carried out periodically. Seed moisture content at the beginning of experiment and randomly during its progress has been tested as well. Samples of rye cultivars usually exhibited a slightly higher MC than triticale or wheat. Excluding triticale cultivar Cyrkon with highest seed MC at all MC levels, no relationship between seed MC and cultivars has been observed. After 44 weeks of storage at mild ageing environment all but one evaluated cereals seed samples with moisture content 0.08 and 0.10 (g H_2O/g fw) did not show any significant decrease of their viability for the presented period of time. Only triticale cv. Silverado and wheat cv. Nutka did show viability decrease. Seed of both cultivars did not show any specific evidence of physiological and/or morphological differences from the rest of evaluated cereal cultivars as well as did not show higher level of microbiological infestation at germination test. So, the reason of longevity decrease was unclear. At seed moisture content 0.12 and 0.14 (g H_2O/g fw) a very sharp decrease of seed germinability was observed. Germinability data revealed high intraspecies variability of wheat, triticale and rye cultivars longevity. For seed (0.12 MC) samples viability differed from about 90 to 0%; 40 – 0% and 20 – 0% for wheat, triticale and rye seed samples respectively. Similar results were observed for the germination index (GI) data. Seed samples in presented experiment were vacuum sealed in laminated aluminum-foil bags and opened after requested period of time. In formerly reported experiment the same hermetically sealed aluminum-foil bag with seed were opened at every sampling time and resealed after it again. The average value of germinability for each species were shown. After 44 week of storage slightly faster decrease of seed viability were noticed for sample with 0,12 MC. Probably, at such moisture content level seed respiration were increasingly higher and hermetic storage caused lack of necessary oxygen and faster seed aging. Increase of seed moisture content, agree to general rule, resulted in significant decrease of seed storability. In all investigated seed moisture content variants viability of wheat seed samples was maintained on significantly higher level than of triticale and rye.

Seed longevity of cereal seed samples during hermetic *vs.* "open" storage at 350C, and headspace gases effect of hermetically stored samples

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Seed longevity during long-term storage depends substantially on seed moisture content and temperature of storage. Recommended hermetic storage of seed might resulted in accumulation of volatile products of seed aging. It could cause the acceleration of seed ageing. Cereals eg. rye, triticale and wheat seed samples were equilibrated over saturates solution of inorganic salts to different moisture content: about 7, 10 and 13% (fwb). Treated seed samples were sealed in laminated aluminum foil bags and/or were kept over saturated salt solutions. All evaluated seed samples have been stored at 35 °C. During storage seed viability and moisture content were periodically checked. After storage period the head-space gases content of hermetically stored seed were assessed with GC/MS method. Seed longevity was strongly affected by seed species and seed moisture content. The slowest rate of viability decrease was observed for wheat at 7% MC. The highest rate of aging was represented by triticale cultivars. Increase of seed moisture content resulted with decrease of seed longevity despite of evaluated species. At 12% MC all samples lost their viability after couple of weeks. Hermetic or open storage regimes resulted in different seed longevity depending on their moisture content. Seed with 7% MC were stored better as sealed in hermetic container, but seed with higher MC maintained their viability longer in "open" storage. Presented results support recommendations for optimal methods of long-term seed storage in seed bank. Head-space gases assessment let to identify 63 various compounds. The most abundant were: alcohols, organic acids and esters, as well as, hydrocarbons. Volatile aldehydes and/or ketones, putative products of lipid peroxidation were hardly present. There were no specific compounds or group of compounds specific to process of cereal seed aging. The total content of head space gases of hermetically stored cereal seed samples did show different patterns in relationship to seed viability. Increase of amount of total volatile content was observed for wheat and one of triticale cultivar seed sample. In the case of rye seed and the another of triticale cultivar low viable sample showed that less volatile products were accumulated.

Studies on seed germination biology and cryopreservation of endangered Polish archaeophytes

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Archaeophytes are the plant species non-native to region, introduced before 15th century. In Poland these are mainly Mediterranean and Irano-Turanian origin segetal species which are well adapted to traditional methods of cultivation. Currently, huge changes in Polish agriculture, caused the massive disappearance of old segetal weeds.

Seed bank of the Polish Academy of Sciences Botanical Garden in Warsaw was established in 1992 for endangered, rare and protected species of Polish flora. For over 20 years of seed bank activity, seeds of 212 endangered species from 683 natural populations were cryo-preserved in cryogenic seed bank.

Currently, the studies on conservation of endangered archaeophytes are carried on in our seed bank. First research was focused on three species included in "Red List of Vascular Plants in Poland": *Scandix pecten veneris*, *Ranunculus arvensis* and *Kickxia elatine*. Studies on seed germination indicated the requirements for temperature and light for each species. *S. pecten-veneris* and *R. arvensis* showed the highest germinability in the lowest temperature (4 °C), even in total darkness. In higher temperature (15 °C) the germinability was lower, and it was inhibited in the highest (25/15 °C) temperatures. Unlike this two species *Kickxia elatine* produced dormant seeds which germinated in highest temperatures (25/15 °C and 15 °C) and did not germinate in 4 °C. Dormancy for *K. elatine* was effectively broken by wet-cold stratification or by gibberellic acid (GA_3).

In the next step–the tolerance for ultra-low temperatures was checked. Seeds were dried to 3.5-6.5% moisture content. The viability of samples directly immersed in liquid nitrogen and stored in LN_2 for 30 days were compared with un-frozen control samples. Additionally, for *K. elatine*, there were also variants with gradual freezing (0.5 °C/min.). For *S. pecten-veneris* the viability after LN_2 freezing was 84% (for control sample 87%), for *R. arvensis* 75% (control 70%) and for *K. elatine* 68% (control 72%). Also there were no differences in viability of immersed and gradually frozen samples of *K. elatine*. The results showed also that all three species produce seeds with orthodox seed storage behavior that maintain its viability after drying to 3.5-6.5% moisture content.

Stability of seed viability over ten years in the cowpea germplasm collection of IITA

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The Genetic Resources Center (GRC) of the International Institute of Tropical Agriculture (IITA) conserves germplasm of over 33,000 accessions of African staple crops including cowpea, soybean, maize, cassava, yam and banana/plantain. Cowpea (Vigna unguiculata) with more than 15,000 accessions, constitutes about 46% of this collection and comes from 90 different countries across the globe. Seed is regularly regenerated and virus indexed in field and screen house respectively in order to obtain clean and viable seed for conservation and distribution. Harvested seed is processed, packaged and conserved both in medium term storage (MTS) and long term storage (LTS) at temperatures of 5 °C and -20 °C respectively while viability testing is usually carried out on each seed lot after ten years.

An assessment of viability was carried out in 2004 and again in 2014/2015 to determine the viability of 126 accessions of cowpea seed lots conserved both in the MTS and LTS. Viability tests carried out in the laboratory in 2004 showed that 72 accessions in LTS and 107 accessions in MTS had viability above 80% while 49 accessions in LTS and 19 accessions in MTS had viability below 80%. In 2014, 58 accessions in LTS and 86 accessions in MTS had viability above 80% both in the laboratory and the screen house while 30 accessions in LTS and 13 accessions in MTS had viability below 80%. The experiment in the screen house was laid out in RCBD in 3 replicates, ANOVA showed no significant differences between viability in 2004 and 2014/15 for both LTS and MTS and also between the viability in LTS and MTS after ten years.

An improvement of genebank management system in the Czech Republic

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Existing documentation system of genetic resources in the Czech Republic EVIGEZ has not more met the requirements of modern documentation system (documentation of sets of characterization data, including molecular data, image analysis and other aspects) so it is currently moving to a new documentation system GRIN Global.

For proper data migration there was needed at first necessary to analyse the structure of two databases to avoid losing any recorded information. Although both systems contain parts of passport, characterization and inventory data they have a different structure. GRIN Global system allows in most cases more detailed information about genetic resources. This applies for example the recording of germination tests. In EVIGEZ there was recorded only the initial germination and subsequent records of germination test were stored outside the system in Excel tables. Now it is possible to convert these records collectively to database, system components should also report on the necessity of regeneration of material if germination reaches the critical value, contributing more accurate control of the stored material.

The structure of the two systems of characterization and evaluation data did not differ significantly however a big enrichment is the possibility of recording more sets of observation data relating to a single accession.

The greater problem of data migration was in our case, the transfer of taxonomic data. GRIN taxonomy does not wholly correspond with recorder taxonomy in EVIGEZ. GRIN system does not cover all taxa and synonyms and in this system there is not recognised lower taxonomic classification (to variety). But EVIGEZ could be transformed into GRIN Global without losing detailed taxonomy data using taxonomy synonyms.

Part of the GRIN Global system is a website of database that will be used for all information and ordering genetic resources on-line via “shopping cart”. Up to now, it is possible to order genetic resources held in the Czech Republic only through e-mail.

This change of genebank management system will contribute to improving the quality of work the gene bank, and thus improve the quality of service for users of genetic resources.

Monitoring viability of seeds in gene banks: Developing software tools to increase efficiency

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Monitoring the decline of seed viability is essential for effective long term seed storage in *ex situ* collections. Recent FAO Genebank Standards recommend monitoring intervals at one-third the time predicted for viability to fall to 85% of initial viability. This poster outlines the development of software tools to identify accession-specific monitoring intervals. The tools were developed for the long term base collection of the USDA National Plant Germplasm System, located at NCGRP. The base collection consists of 550,000 accessions representing 14.000 species. At NCGRP, seed is dried at 5 °C and 20-25% RH and stored at -18 °C. Initial viability is assessed using AOSA rules. We felt that monitoring intervals should be based on seed longevity estimates characteristic for each species in the collection. Species longevity in storage can be estimated using two approaches: i) The Viability Equations (Ellis and Roberts, 1980) using NCGRP conditions of RH and temperature and species constants found in the Kew Seed Information Database, and ii) NCGRP longevity results obtained at 5^{o}C, fitted to Avrami kinetics and adjusted to -18 °C storage (Walters et al., 2005). We present two software tools that leverage these models to assist making decisions about what accessions are in need of monitor tests. Our approach was to calculate time to 85% initial viability using both methods. The first tool generates a list of genebank accessions from the collection that are in need of monitoring based on a monitoring interval set at 1/3 of the total time needed for germination to decline to 85% viability, which is estimated using both models for a given taxon and initial germination. The second tool is a visualization product which displays viability data against curves generated by the model. The tools can be parameterized based on time, taxonomy, institutional genebank standards and estimated deterioration time courses when needed and are compatible with GRIN-Global. These tools increase genebank efficiency, since resources can be saved once an optimal interval is identified based on species-specific aging kinetics.

Increasing efficiency in evaluating seed viability in genebank materials using Wald's sequential sampling

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Due to the great number and original ecological diversity of materials maintained in genebanks, obtaining large amounts of seeds to perform viability tests as recommended by ISTA norms is a recurrent challenge. CIAT genebank developed a viability test using Wald's Sequential Sampling which gives results using fewer seeds. Sequential sampling does not have a prefixed sample size. It takes samples until a desired precision level is reached. Estimates of very high or very low viability are promptly obtained. Viability estimates near to the limit need more sampling. The objective of this study was to evaluate the performance of this method to meet FAO's Standards for Genebanks of 85% minimum germination.

Four species of *Phaseolus* were evaluated, three species of Rugosi section and one species of Phaseoli section, almost all with unknown seed storage behavior. Seeds exhibiting orthodox behavior are known to remain highly viable after periods of storage involving drying and freezing. Most accessions were stored at -18 °C for 10 years.

The experiment consisted of evaluating 560 seeds for each of eight accessions using germination paper along with supplemental Tetrazolium tests for viability. Following data collection, 1000 sequential sampling simulations were performed, by evaluating batches of 20, 25 and 30 seeds each to determine the total number of seeds required to obtain reliable results about viability of each accession.

The seed tests demonstrated that all evaluated accessions had orthodox seed storage behavior, having viability ranging from 88% and 98.4% after 10 years of storage at -18 °C. Using the real data, the simulations using Wald's Sequential Sampling indicated that in 700 cases two consecutive independent tests using 30 seeds each, rather than 20 or 25, already sufficed to identify accessions with viability $\geq$ 90% (sent to long term storage), and accessions with viability $\leq$ 50% (sent to regeneration). Accessions in between require another similar sequential sampling proposed five years later if any dormancy and meanwhile are kept in medium term storage (+5 °C). Implementation of this new method of seed viability testing can improve the efficiency of seed storage in the genebank and avoid wasting large amounts of seed.

Measuring seed banks´contribution to plant diversity maintenance

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A main question in ecology is how biological diversity is maintained. Competition has been recognised as the major driver of composition and stability of communities. Nevertheless, according to storage effect theory, competing species can coexist across temporal alternation of conditions. In this model, different species have different responses to the environment. In favourable years they store the benefits and are highly regulated by competition, while in adverse periods competition is negligible. Germination biology may determine the coexistence of species by storage effect. If seeds remain dormant during unfavourable years, then they will avoid competition. The opposite will happen when seeds germinate. In this study we ask: Do germination responses in different years vary between species? Are there persistent seed banks? How much can seed banks buffer the competition effects? The study site is a semiarid grassland in Southern Mexico. The study species were the 28 most abundant species of the grassland, from 11 different families. In order to describe the seed banks, we buried 600 seeds of each study species at 3 cm depth. We recovered 200 seeds of each species after 6, 12 and 24 months, and inspected for evidence of germination in the field, predation and decomposition. To assess viability, ungerminated seeds were placed in petri dishes with agar at 1% and then on moist filter paper with ethephon at 0.01 M. We propose a new method to relate seed bank longevity and competition using models of population dynamics. Our results support the application of storage effect models to understand plant diversity maintenance: we have found that germination responses vary between species suggesting species-specific response to the environment. Moreover, 93% of the species had persistent seed banks. In consequence, they can potentially buffer competition during adverse years.

Session II

Role of pre- and post-harvest environmental factors on seed longevity

Changes in reserve compounds of macaw palm (*Acrocomia aculeata*) fruits and seeds stored in different conditions

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Due to the growing concerns about environmental issues and the rising price of fossil fuels, biodiesel has been an interesting alternative, since its performance is similar to traditional fuel, being also biodegradable and recyclable. The macaw palm is an oleaginous palm tree native from Brazilian Cerrado and widely distributed in Americas. Its fruits produce more than 5 tons of oil per hectare per year, making it a species with great potential to be used as a source of raw material for biofuel production. We aimed evaluate changes in the reserve compounds content of macaw palm fruits and seeds stored during one year in three different conditions (room temperature, nursery and cold chamber), in order to verify the involvement of these compounds with seed viability. For the fatty acids composition, there were used samples of embryo, endosperm and mesocarp; and for the total carbohydrates and soluble proteins quantification there were used samples of embryo and endosperm. Fatty acids profile was very similar in all storage conditions and in the structure evaluated over time. The embryo composition was very close to the mesocarp, and the main constituents were oleic, palmitic and linoleic acids. In the endosperm there were found mostly lauric, oleic and myristic acid. The high content of oleic acid especially in the mesocarp (54.86%) confirms that macaw palm can generate a high quality biodiesel with high contents of monounsaturated esters. Protein levels were lower in the endosperm than in the embryo, and in the last one it was observed an increase of these levels after 45 days of storage. On the same way, it was observed a lower amount of carbohydrates in the endosperm; but in the embryos the carbohydrates levels had a significant decrease after 45 days especially in the cold chamber storage, what could be related to a decline in the viability of the embryos stored at this condition. We conclude that the macaw palm oil is well preserved at all storage conditions, but storing the fruits at room temperatures or nursery is better to maintain the reserves contents and the viability of the seeds.

Metabolic responses of immature seeds of *Libidibia ferrea* Mart. under contrasting storage temperatures

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Libidibia ferrea (Mart) is a leguminous tree species that grows in many regions of Brazil. The seeds are composed mainly of lipids and carbohydrates, particularly galactomannan, accumulated in the endosperm as energy source, and possibly preventing protein denaturation during dehydration, due to its high capacity to retain water. The seeds are orthodox showing high natural longevity and tegument dormancy at ripening. However, many of these seeds are naturally dispersed before completion of the maturation cycle, constituting an interesting model for metabolic studies of seeds during storage. In this work, immature and mature seeds collected from trees were dried and stored at -18 °C and 25 °C in the light and in the dark for 1, 2 and 6 months and evaluated for moisture content, germination, water potential, respiration and metabolic profile. At the beginning of the storage period, seeds in both stages showed high O_2 consumption and low CO_2 emission, due possibly to oxidative processes, which were later maintained in immature seeds and reduced in the mature ones during storage. This could explain the higher viability of mature seeds at 25 °C and -18 °C, while the immature ones deteriorate, mainly at 25 °C. The presence or absence of light did not affect substantially the seed physiology during storage. Metabolomic analysis revealed a reduction in some compounds of the tricarboxylic acid cycle such as citric and malic acids during maturation, indicating metabolic shutdown when mature. After artificial drying, immature seeds showed equal proportions of malate and citrate, and high levels of reducing sugars and amino acids, which could indicate an active metabolism. Our results indicate that at seed maturity, the primary metabolism was reduced, similar to what is known to occur in orthodox seeds. Therefore, the degree of maturation at seed dispersal is critical for the maintenance of viability during storage.

Analysis of the sources of variation in quality of cereal seed produced in Scotland

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The Official Seed Testing Station for Scotland tests approximately 3500 seed samples each year, the majority of which are cereals. Testing includes the determination of viability, thousand seed weight, moisture and health status. Sample details such as area of growing and variety are recorded and tests are performed for the purpose of both certification to ensure seed meets certain standards and to provide advice on seed quality to farmers. Seed testing data has been collected since 1998 and is a valuable resource that could be used to inform us about quality of seed crops produced in Scotland.

The impact of adverse weather conditions during seed development and harvest can have serious consequences for the seed industry. Recently the UK has seen an increase in the number of extreme weather events. 2012 for example was a particularly poor year for wheat; a period of cold, wet weather over the summer delayed harvest. Some crops did not complete the final stages of maturation drying, and were harvested before full maturity. This led to increased variation in seed lots and many problems with the wheat crop including lower viability, lower thousand seed weights, increased variability in samples, sprouting, immaturity, high levels of disease, poor storability and poor field emergence.

Further analysis of data collected by the OSTS will aim to determine the degree of effect that year and region of seed production (environmental conditions) and variety (genetics) have on different aspects of seed quality. Such analysis will allow us to determine the extent to which different factors control quality in cereals and whether certain crops may be more resilient to extreme weather conditions and climate change. This information will, together with analysis of future climate predictions, help shape decisions on crop production in Scotland and highlight future research requirements.

Conservation of castor seeds (*Ricinus communis* L.)

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To investigate the effect of different storage conditions on the physiological and health of castor seeds were used seeds of three cultivars, Guarani, IAC-80 and IAC-226, stored in two environments (cold storage and conventional) in three packages (multiwall kraft paper and plastic packaging with and without vacuum to 1 atm), and also under cryopreservation (-196 °C). Seed quality was assessed before storage and after 4, 8 and 12 months by germination test (count 7 and 14 days), emergency, health, water content and oil content. We evaluated the activities of enzymes: esterase (EST), catalase (CAT), malate dehydrogenase (MDH), alcohol dehydrogenase (ADH) and superoxide dismutase (SOD). Was detected seed dormancy, overcome by dipping in liquid nitrogen that caused the rupture of the integument. The maintenance of the physiological quality of seeds of castor bean cultivar IAC-80, IAC-226 and Guarani for 12 months is possible under the following conditions: cryopreservation (-196 °C) as an ideal condition, in plastic or multiwall paper under refrigeration and conventional warehouses, packed in plastic bags under vacuum conditions. Regardless of the storage conditions evaluated, the oil content decreases the incidence of Aspergillus and Penicillium spp. is increased over time. The catalase enzyme stands out as a marker of the deterioration of castor seeds during storage.

Storage of seeds corn under different inoculum potential of *Fusarium verticillioides*

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Seeds are important and efficient vehicles for the dissemination of pathogens. The phytopathogenic fungi may associate to the seeds during their development, after the harvest and during storage. Among the main fungi carried by the corn seeds, *Fusarium verticillioides* stands by the frequency with which it occurs, being mainly responsible for seed rot and reduced stands. Therefore, this study aimed to evaluate the performance of corn seeds under different inoculum potential. There were used cultivar seeds of corn hybrid BM 840 PRO freshly harvested supplied by the company Biomatrix. The seeds were placed in contact with the fungal colonies grown on BDA (potato dextrose agar) + manitol in water potential -2.0MPa. After colony growth, seeds were distributed in the middle, remaining on the substrate for different times of contamination (0, 24, 48, 72 and 96 hours). Soon after exposure in different inoculum potential, seeds were stored under uncontrolled conditions, for a period of seven months. The evaluations were performed before and after seven months of seeds storage. As witness was used treated seed with the fungicide Derosal Plus® (250 mL i.a. 100 kg^{-1} of seeds). The physiological seed quality was assessed by means of the germination test, emergence and cold test, while the sanitary quality was determined by the *Blotter test* with freezing. The increase of potential of the fungi inoculum *F. verticillioides* in the corn seeds generates a decrease in the physiological quality potentials of 72 and 96 hours, mainly in the seeds were stored for seven months under uncontrolled ambient. The storage for seven months reduces the fungi *F. verticillioides* according to the exposure period of the seeds to the pathogen.

How to outlast winter on land and sea: Terrestrial plant seeds and marine dinoflagellate cysts both rely on winter chilling

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Marine algae and land plants were recently discovered to share conserved phytochrome signaling systems. Phytochromes optimize photosynthesis and regulate developmental progression, e.g., seed germination, leaf and stem expansion, reproduction, and seed dispersal. Similarly, we are now beginning to see parallels between the germination of plant seeds and the resting cysts of dinoflagellates, a class of marine alga commonly associated with red tides or harmful algal blooms. Like plant seeds, cyst germination is regulated by the complex interplay of internal and external factors. Newly formed cysts have a mandatory dormancy period during which germination is not possible, but once mature, the resting state will continue if temperatures are unfavorable or oxygen is unavailable. An endogenous annual clock, capable of overriding an otherwise favorable environment for germination, controls seasonal germination of *Alexandrium fundyense* cysts. Indeed the seasonality of the toxic blooms produced by *A. fundyense* is due in part to life cycle alternations between motile, vegetative cells and benthic, resting cysts. Here, we demonstrate that this endogenous annual clock can be synchronized to environmental temperature cycles, specifically winter chilling. Similar to its role in annual and perennial plant seeds, winter chilling accelerates the timing of cyst germination, permitting the onset of growth when environmental conditions are more favorable in the spring. We hypothesize that the mechanism regulating germination arose via the same phytochrome gene transfer from the cyanobacterial endosymbiont that gave rise to photosynthetic chloroplast organelles. Transcriptomic studies are planned to investigate this relationship. The widespread occurrence of dormancy mechanisms in plants and dinoflagellates provides new impetus for investigating adaptation and acclimation of major primary producers to the earth's changing climate.

Hardseededness in subterranean clover (*Trifolium subterraneum* L.) cultivars and mapping populations

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Subterranean clover (*Trifolium subterraneum* L.) is the most widely sown annual pasture legume in Australia. It has a range of cultivars allowing cultivation across most arable regions of southern Australia. “Hardseededness” can be defined as the capacity for a seed to prevent the uptake of water. In the general sense the term hardseededness has been defined to cover both the development and breakdown phases of hard seeds from dormancy under natural conditions. Higher levels of hardseededness, allow the soil seed bank to persist through false breaks (or untimely germinations), so that more viable seeds are present at the beginning of the growth season. The important factor is not the amount of hard seed that is formed, but, rather the residual amount that remain un-germinated throughout the season, giving a seed bank that allows subterranean clover to persist through poor seasons and a one to two years cropping phase. Therefore a high level of hardseededness in subterranean clover has been sought by breeders, especially in lower rainfall environments, to improve seed persistence. Variation in hardseededness in annual clovers is influenced by both genetic and environmental conditions during plant growth, seed development and maturation. In subterranean clover, hardseededness declines over the summer months, in a process known as “seed softening”, at a rate that varies with genotype. Study of the inheritance of seed permeability in subterranean clover has been limited. A temperature fluctuation from freezing point to 100 °C, on subterranean clover seeds, has shown a decrease in hardseededness with higher maximum temperature. A laboratory process designed to simulate field softening over a 12, 16 or 24 week period with exposure to gradual diurnal temperature fluctuation of 15/60 °C was used in this study. This process provides a standard seed softening condition and correlates well to field-based seed softening, particularly in terms of ranking varieties. This study is designed to correlate genetic data from the use of Short Simple Sequence Repeat (SSR) primers and hardseededness in 24 cultivars and also two large segregating F_2 populations of subterranean clover. Hardseededness was assessed and its variation examined, in an attempt to further understand the control of this trait in subterranean clover.

Evaluation of different storage conditions for castor seeds (*Ricinus communis* L.) suitable to family farming in the Brazilian semiarid region

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Castor (*Ricinus communis* L.) is an oilseed species recognized by numerous industrial applications of its oil, easy handling, low production cost, and important alternative for socioeconomic development of family farming in the Brazilian semiarid. Appropriate storage conditions delays aging and deterioration, ensuring better seed longevity and physiological (propagules) and biochemical (commodity) quality. This study investigated the effects of different storage conditions on quality of two varieties of castor seeds (Nordestina and Paraguaçu) packed in cotton bags and tested under four storage conditions during twelve months: 1-(cRHT) controlled relative humidity (RH) and temperature (T); 2-(cRH) controlled RH; 3-(cT) controlled T; 4-(ucRHT) uncontrolled RH and T. Control of RH was conducted by conditioning seed bags in drums with silica gel (3.8% RH), and T control by conditioning seed bags in germination chambers at 15 °C (inside or outside of drums). Germinability, moisture content and electrical conductivity were evaluated monthly. Tetrazolium test, extraction, determination and characterization of total lipids were held quarterly. It was verified that seeds of both varieties have maintained quality when stored at cRHT. However, there was loss of germinanility and vigor as measured by the percentage (Gmax) and speed (T50) of germination and values of the area under the curve (AUC), being verified the worst results in storage at ucRHT for the Northeastern variety, and at cT for Paraguaçu. Induction of secondary dormancy was observed during storage in Paraguaçu variety, which was possibly related to the accumulation of inhibitors of the integument, since viability was confirmed by the tetrazolium test and geminability was recovered by removing the seed coat. From these results we intend to develop a low-cost storage protocol that guarantees seed quality under suitable and useful storage conditions for family farming and its tradition in the cultivation of this oilseed in the Brazilian semiarid.

Influence of metrological conditions and growing methods for the longevity of carrot seeds

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Seed plants of cultivar 'Šatrija BS' were grown in the experimental field and in the greenhouse covered with a polymeric film at the Institute of Horticulture Lithuanian Research Centre for Agriculture and Forestry (IH-LRCAF) in 2011 – 2014. Parameters, which characterize carrots reproductive features, influencing the amount and quality of seeds and meteorological conditions were determined during vegetation. It was observed that genotype and meteorological conditions make influence for the morphological traits of carrots seeds. Seed plants grown under different conditions formed seeds with different weight. Seed plant grown in the greenhouse distinguished with the higher seeds weight (2 g of 1000 seeds) compare with grown in the experimental field (1.8 g of 1000 seeds). According evaluation of viability of carrots seeds during different year of investigations, it was determined that viability of longer-term seeds varies not dependent from the method of growing. Long term investigation showed that the viability seeds from 90 to 94% can be reached that while seed plants were growing in greenhouse and from 76 to 78% while seed plants were growing in experimental field under Lithuanian climatic conditions.

Anatomical and morphological features of honeybush (*Cyclopia* spp.) seed

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Cyclopia is a genus of 23 species endemic to the unique, species-rich and ecologically vulnerable fynbos biome of the Western Cape, South Africa. Both this genus and Aspalathus, as in A. linearis (rooibos tea), belong to the Fabaceae family and carry the typically leguminous traits of pea-like flowers, bean-like seeds produced in pods and nitrogen-fixing root nodulation. Honeybush tea, a popular herbal drink from South Africa, is produced from any of several *Cyclopia* species. More than 70% of material annually harvested for tea production is still taken from wild populations, resulting in ecosystem damage and endangering the survival of *Cyclopia* spp. in their natural habitat. Cultivation and breeding programs have been began in the 1990s to create a sustainable model for the continuation of the honeybush tea industry. As yet, little information about the plants exists and the exciting work of building a knowledge base is still underway. Ongoing research aimed at improving germination percentage of honeybush seed (various species) has identified hardseededness in some *Cyclopia* species and combinational dormancy in others, depending on the species' fire survival strategy: rapid repopulation via seedlings vs. resprouting from undamaged subterranean rootstock. The observation that *Cyclopia* spp. produce colour-dimorphic seed (green and brown mature seed produced on the same individual) excited little curiosity until nurserymen reported a difference in germination response between the seed colours. Recent research has confirmed significant differences in germination response to seed pre-treatments and seed age between the seeds of the two colours but the mechanism of this difference is not yet understood. An introductory study was undertaken through dissection and light microscopy to investigate the anatomy of dimorphic seed of *C. genistoides*, *C. maculata*, and *C. subternata*. Discoveries such as the presence of a hilar valve and a relatively thick cuticle (3 µm) are relevant to discussions on seed dormancy while the presence of a distinct albuminous endosperm is a tantalizing prospect for future research. Surprisingly, no obvious structural differences were found among the three species nor between the green and brown seed, hinting at the existence of biochemical variations between them.

Germination ability of seeds of barley, niger seed, teff and fenugreek stored in the genebank of Ethiopia

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Ethiopia is a primary center of origin and or diversity for many crop plants. Since the establishment of Ethiopian Biodiversity Institute, 68.362 accessions of 52 crop plant species are conserved at e*x situ* seed cold storage which constitutes 90 percent of plant species conserved in gene bank.

Seed deterioration during storage has always been a great problem. For some accessions, seeds had low germination ability even after short storage duration. An even more serious risk is that 50% of the accessions stored in genebanks lose viability or encounter genetic drift after regeneration. The challenge for genebank curators is, therefore, to regenerate seed samples before their viability becomes critically low.

To avoid deterioration of seed, genebank accessions should be monitored for viability during storage. This study was conducted to evaluate viability of barley (*Hordeum vulgare*), teff (*Eragrostis tef*), niger seed (*Guizotia abyssinica*) and fenugreek (*Trigonella foenum-graecum*) after five, ten and twenty years of storage in the long term storage (-10 °C) facility at the Ethiopian Biodiversity Institute and to suggest the relative seed longevity and suitable monitoring intervals. The result revealed that germination test of barley from initial and after five and ten years of storage showed that there is no significant change in viability during storage. The rate of decline in viability varied among the different species and differences were also observed within species tested within similar time intervals. The germination data from initial germination tests (before storage) and after five to thirty years, depending on the species, showed absence of significant loss of viability during storage. Based on the present analysis, ten, fifteen, twenty and thirty year's monitoring intervals could be recommend for barley, fenugreek, teff and niger seed conserved in long term storage.

Oxidative process and speed of deterioration of orthodox and recalcitrant seeds during storage

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The seed bank is one of the most important strategies for the preservation of endangered species, such as brazilwood (*Caesalpinia echinata* Lam), a worldwide important species due to its wood used for violin bow production. The occurrence of this species was markedly reduced in its original geographic distribution. Its seeds are tolerant to desiccation (until 7.6%, wet basis) at shedding and this tolerance increases progressively during maturation. Even the so-called mature seeds (harvested immediately before shedding) show a rapid decrease in viability (three months) when stored in temperatures above 15 °C. However, mature seeds show high values of germination after five years of storage at -18 °C. We analyzed the metabolism of these seeds under different temperatures aiming at understanding the differences on the conservation of the seeds and the germplasm bank formation. Germination and normal seedling development of seeds maintained at low temperatures did not differ during the first year of storage. However, after two years (and until five years) only seeds stored at -18 °C kept high germination percentage and normal seedling development. Changes in O_2 consumption and CO_2 release by seeds incubated at different temperatures demonstrated that the deterioration processes are related to respiration and possibly to other oxidative processes, causing death of embryonic tissues in short periods and loss of seed viability. In order to compare these responses with recalcitrant seeds, we analyzed the embryos of *Inga vera*, which showed contrasting behavior concerning O_2 consumption. Respiration was reduced in both orthodox and recalcitrant embryos with progressive reduction in temperature. However, oxidative process increased with temperature in the orthodox seeds and diminished in the recalcitrant ones. We concluded that oxidative processes are probably involved in the deterioration of both, recalcitrant and orthodox seeds, but in different ways.

The effect of seed priming on differentially developed seeds of aji (*Capsicum baccatum* var. *pendulum* Willd.)

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Capsicum baccatum contains potential antioxidant and anti-inflammatory compounds. The concentrations of free sugars, organic acids and capsaicinoids is sufficiently large and has got unique flavor. *C. baccatum* var. *pendulum* is widely produced and consumed in South America. Species also has got rootstock potential. The influence of priming (KNO_3, *Patula* and *Erecta*) on germination percentages, mean germination time, germination index, seedling emergence, mean emergence time, seedling weight and emergence index of *C. baccatum* var *pendulum* pepper species seeds grown in Antakya and harvested five different development stages (D1, D2, D3, D4 and D5) was investigated. Seeds were harvested at various development stages based on fruit skin color. Results revealed that the physiologically mature seeds (after D3 stage) showed higher seed quality and performance. The greatest advantage of priming was observed at D2 and D3 stages of seeds. Especially, *Patula* treatment at D2 stage had 10% greater germination rates, 4 days faster mean germination time, 15% higher seedling emergence rates, 52 mg heavier seedling weights, and higher emergence index with respect to untreated seeds. Also, priming had minimal effect over lower average emergence time, higher seedling weight and emergence index for seeds found in D4 and D5 stages. Therefore, fruits were harvested in D4 and D5 stage in order to achieve high quality seeds of *C. baccatum* var. *pendulum*. Consequently, if the seed quality is poor in aji, *Patula* priming that is inexpensive, eco-friendly and a simple technique can be proposed.

Natural fecundity and germination characteristics of selected genotypes of *Cyclopia* species

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Cyclopia (honeybush) is a largely unstudied leguminous genus of South African herbal teas. Species occur in the Western and Eastern Cape Provinces within small areas in limited numbers or widespread on the coastal plains and mountainous regions. At present, knowledge of reproductive fitness (fecundity) of cultivated and undomesticated *Cyclopia* genotypes is scant; yet this is critical for evolutionary studies and will assist in conservation of endangered species, management of biodiversity, improvement through plant breeding, and establishment of nurseries and is fundamental for progress in growth, reproduction and maturity. 21 genotypes of two species in trials were evaluated for their potential to produce fruit and seeds in different localities. Fecundity was also determined from two natural populations of each species and compared to that of cultivated material. Seeds were collected and weighed to determine seed mass and evaluated for germination rate and cumulative germination per site. Statistical analysis revealed species differences between and within sites. Compared to *C. genistoides*, *C. subternata* had significantly higher fruit set (12-47% v. 13-27%), seed set (134-495% v. 87-247%), seed number (70-368 v. 19-172) and $1/T_{90}$ germination (9-41 days v. 14-46 days). However, average seed mass of *C. genistoides* was higher than that of *C. subternata* (0.011-0.017g v. 0.0068-0.016g). In *C. subternata*, seed mass/plant was inversely proportional to seed number, whereas, in *C. genistoides*, no such pattern was observed. Generally, cultivated genotypes of *C. genistoides* showed higher fecundity than natural growing ones, whereas in *C. subternata* fecundity varied significantly between cultivated genotypes and natural ones. $1/T_{90}$ germination varied between and within species in all sites, and directly influenced final germination. Our findings show greater fecundity in *C. subternata*, which is a non-sprouter; therefore this species will likely have a higher population, better seed germination and establishment rate than *C. genistoides*, a sprouter, in cultivated and natural environments. In contrast, *C. genistoides* may be expected to withstand hazardous environments better.

Alternative control of microorganisms in rubber tree seeds (*Hevea brasiliensis*) during the storage

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The objective of this work was to evaluate the efficiency of the rosemary vegetal extract compared to treatment with chemical fungicide in the preservation of physiological quality of rubber tree seeds during storage. Seeds were used with initial moisture of 31%. 600 g of seeds were placed in kraft paper bags and plastic bags, where were done 6 holes in each package for gas exchange. The seeds were treated with dried and ground rosemary at doses of 20 g/kg of seeds and the combination of fungicides Tecto 600 (35/100 kg seeds) and Captan 50 (70g/100 kg seeds), and control treatment with 4 replications. Later, these seeds were stored at 10 °C in cold chamber camera. In each 15 days were evaluated germination, IVE and the efficiency of these products in the control of microorganisms. Both treatments, with rosemary and control were more effective in maintaining the germination of seeds up to 75 days after storage. However the rosemary was more effective in maintaining the seeds vigor, since it was superior for the seeds treated with rosemary during 75 days. The chemical fungicides showed low germination probably by the phytotoxicity effect, with germination of 37%, 35% and 3% respectively in 75 days after storage. In seeds treated with rosemary was observed the presence of *Aspergillus* spp., despite the clear reduction in the incidence of *Penicillium* spp. and *Fusarium* spp., being this last one the most harmful to stored seeds. In control there was a higher incidence of *Aspergillu*s spp., *Penicillium* spp., *Fusarim* spp., *Botrytis* spp., and in some cases bacterias. In the chemical treatment was not observed the presence of microorganisms, however the germination was affected with value below 10% in all period evaluated. In this way we conclude that the use of rosemary or possibly other medicinal plants are viable alternatives for the preservation of physiological quality of rubber seeds during storage.

Physiological and biochemical changes during seed deterioration of high oleic sunflower

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High oleic sunflower oil is of interest because of its high oleic acid content (78% on average), which makes it very resistant to oxidation. There is not information about the quality of this type of sunflower seeds comparing with traditional sunflower, regarding possible physiological, biochemical changes during storage. Sunflower seed hybrids with two different characteristics of oil Aguara – 4 (simple hybrid, 45-50% of non- oil oleic type) and OLISUN - 3 (triple hybrid, 45-40% high oleic oil type) were produced under the same environmental conditions, and stored in two types of packing: Kraft paper and plastic vacuum. The seeds were stored at 10 °C, 25 °C and 30 °C. Seed quality was evaluated by the tests: germination, electric conductivity, accelerated aging, seedling emergence index. The tocopherol analysis, as well as changes in enzyme systems, ADH, CAT, SOD, EST, ACP, MDH and ICL were determined. The physiological results were coinciding with the biochemical parameters. It was found that the response to the storage of sunflower in different temperatures varies depending mainly on the packing used. The storage temperature of 10 ° C is more effective in preserving the quality and, in this temperature; storage in paper package is the most suitable. The conservation of sunflower seeds at 25 °C and 30 °C is more efficient on vacuum. Change in the physiological quality of sunflower seeds at different storage conditions are detected by the analysis of enzyme systems of ADH, CAT, SOD, EST, ACP, which is not true for MDH and ICL. It was possible to observe bigger oxidative stability and physiological quality of high oleic seeds, during nine months of storage.

Temperature and environmental conditions of forage peanut storage

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Species of forage legumes are today, very suitable for grazing. Among these species, some peanut cultivars have stood out, due to their good dry matter production and nutritive value, beyond excellent persistence and ability to cover the ground. The objective of this study was to compare different storage times (0, 2, 4, 6 and 8 months) and different storage conditions (cold chamber at 10 °C and environment temperature at 25 °C) of peanut forage seeds. Seeds were treated with fungicide Vitavax-Thiram® in dosage of 300mL of fungicide diluted in 500mL of water for 100 kg of seeds. At the beginning of storage and the periods mentioned, was determined the moisture content, electrical conductivity, germination and seedling emergence in sand. The experimental design was completely randomized design (DIC), arranged in a 5x2 factorial design with five storage periods and two different environments with four replications each. The results were submitted to analysis of variance, and averages were compared by Tukey and F test at 5% probability. Was observed at environment temperature, the peanut forage seeds had their physiological potential decreased over the storage period. After eight months of storage was observed the presence of fungi in this condition. Seeds stored at low temperature (10 °C) for 6 and 8 months maintained physiological quality with high values of germination, emergence and low conductivity, indicating that low temperatures have a positive effect on the preservation of peanut forage seeds.

Effects of collection time and preliminary storage of beech (*Fagus sylvatica* L.) seeds on their germination after dehydration

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Beech seeds, belonging to the suborthodox category, after collection can be dehydrated to a moisture content of 7-9% (fresh weight basis), this making possible their cold storage at -3 °C to -10 °C for a few years. To overcome their dormancy they must be kept at a temperature slightly above 0 °C and at a moisture content around 30% (cool stratification). In the presented experiment we have found that the stratification time of the examined seed lot (typically 14 weeks) could be shortened when after shedding they remained on the ground for 2, 4 or 6 weeks, or when they were preliminarily stored at a temperature similar to natural conditions. It happened even if the moisture content of seeds decreased gradually to 25%. Germinative capacity of seeds treated so (i.e. after preliminary storage or after lying on the ground) decreased from the initial 85% for seeds collected immediately after their fall to 76% after preliminary storage. This decrease was caused mainly by pathogenic fungi. When seeds were dehydrated after 2-6 weeks on the ground or after preliminary storage for 2-6 weeks for both treatment types, the duration of their stratification was shortened by the same periods. During preliminary storage their germinative capacity fell to 47%. We have found that germinative capacity of beech seeds undergoes considerable changes depending on the conditions of treating them before dehydration to a moisture content level permitting their cold storage. Dehydration of seeds whose dormancy was already partially broken, reduced seriously their germinative capacity. Seeds should be dried immediately after seed fall. In a simultaneously conducted experiment we have also found that dehydration of beech seeds affects negatively their germinative capacity if they are dried after breaking their dormancy in controlled conditions.

Ex-situ and *in-situ* longevity of *Swartzia langsdorffii* seeds – a Brazilian rainforest tree

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Swartzia langsdorffii Raddi (Fabaceae-Faboideae), is a tree species native to the Atlantic Forest of Brazil which produces recalcitrant seeds. The aim of this work was to evaluate the ex-situ and in-situ longevity of S. langsdorffii seeds in a seasonally dry habitat. Fruits were collected in Lavras, MG, Brazil and the seeds were manually processed. For in-situ storage, seeds were put on the litter of Semidecidous Rain Forest. For ex-situ storage seeds were placed in perforated plastic bags inside a paper box and incubated on 5, 10, 15, 20, 25 and 30 °C, under constant light. To score germination four replicates of 15 seeds were used per month, placed in plastic trays containing moist sand and incubated in a germination chamber at 25 °C and constant light. To assess the seed water content four replicates of three seeds were weighed per month, before and after drying in an oven at 103 °C for 17 hours. Two-way ANOVA was conducted (7 storage conditions x 4 storage times) and the data submitted to post-hoc comparison of means, at 5% probability. Storage time, storage condition and the interaction between these two factors differed significantly ($p<0.05$), for both germination and seed water content assessments. Until the third month all storage conditions maintained high viability and seed water content, except seeds stored at 5 °C, which started to lose viability after two months with complete loss of viability after three months. Preliminary results of the ongoing storage experiment show that higher viability is maintained during in-situ storage and ex-situ storage at 20 and 25 °C than during ex-situ storage at 5, 10, 15 and 30 °C. Moreover, these recalcitrant seeds can survive the dry season in the soil outside the Atlantic Forest.

Acknowledgements

FAPEMIG, CAPES, CNPq for a scholarship and other financial support.

Improving seed longevity in storage of alpine species

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Alpine ecosystems are particularly sensitive to human threats and natural impacts and several plant species are already at risk of extinction. In this scenario *ex situ* conservation through seed banks will play an important role in safeguarding alpine species and will provide propagating material for *in situ* conservation, habitat restoration and research. However, long term storage of seeds of alpines is a huge challenge. Indeed, current studies have highlighted that most alpine plants produce seeds with a low resistance to ageing, indicating that long-term seed conservation of alpine species using conventional *ex situ* seed banking methods might be problematic. Hence, further knowledge is needed to improve the longevity of alpine seeds and/or to recover the viability loss in storage.

In order to promote and facilitate the use of native seeds in land restoration and reclamation activities, the project NASSTEC (Native Seed Science Technology and Conservation; EU - FP7, Marie Curie Action 2014-2018) is being implemented by a consortium of academic institutions, *ex situ* conservation organizations and seed companies. One of the research targets of the project is to develop seed priming techniques for improving the longevity of alpine seeds in storage and to identify the molecules and genes responsible for regulating seed longevity. Particular attention will be given to species growing in the habitats 6230 and 6170, based on Natura 2000 classification. The data generated will be used to develop seed viability monitoring protocols for seed companies and conservation organizations to enable alpine seed preservation for the protection and restoration of fragile alpine ecosystems.

Session III

Genetics of inter- and intra-specific variation of seed survival

Seed longevity in tobacco (*Nicotiana* spp.) – intraspecific variation and genetic mapping

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With the aim to gather information about seed longevity of the genus *Nicotiana*, the viability of accessions stored at 20 °C, 0 °C and -15 °C/-18 °C for up to 12, 33, and 38 years, respectively, at seed banks in Poland and Germany was investigated. Logistic regression analysis was used to model the proportion of seed lots (accessions) with germination >75%. Considering this threshold, seeds of tobacco can be successfully maintained under controlled ambient conditions (20 °C; paper bags) for up to ten years. At a storage temperature of 0 °C (glass jars) this period is extended to about 30 years whereas after 40 years of storage at a temperature of -15 °C/-18 °C (glass jars) about 60% of the accessions show germination percentages higher than 75%. As in other genera an intraspecific variation was noticeable. Therefore, a genetic study was initiated using an already genotyped mapping population consisting of 122 recombinant inbred lines derived from a cross between the cultivars 'Florida 301' and 'Hicks'. Four germination-related traits were investigated by examining seeds either untreated or after controlled deterioration (CD): total germination (%), normal germination (%), time to reach 50% of total germination (h), and the area under the curve after 200 hours of germination. In total, four genomic regions located on four different linkage groups were identified to be associated with the selected traits. Positive alleles for the individual traits were contributed by both parents. A major quantitative trait locus (QTL) for high percentage total germination located on linkage group 8/18 appeared in both control and deteriorated seeds and was contributed by 'Hicks'. In contrast, 'Florida 301' donated a favorable allele for germination speed on linkage group 7 after CD only. Interestingly, the position of this locus compared well with a QTL detected in the same population, in a former study examining resistance against the black shank disease caused by *Phytophthora nicotianae*. The effects of environmental growing conditions of the mother plants on seed longevity will be discussed.

Optimizing and applying accelerated seed ageing tests to assess flax (*L. usitatissimum*) of different seed colour

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The objectives of this study were (1) to optimize the method for accelerated aging tests for flax (*Linum usitatissimum* L.) and (2) to apply this method to assess seed vigour of flax cultivars with different seed colours.

Seeds of the Canadian linseed cultivar Vimy were artificially aged using the inner chamber method at temperatures of 25, 35, 42 and 45 °C over a period of 24, 48, 72 and 96 hours. Reproducible results with a gradual reduction to about 50% of germinability allowing for differentiation among seed lots were obtained after seeds were exposed to 42 °C over a period of 72 hours. Seed moisture content increased under these conditions from the initial 7.4% to the equilibrium of about 30% within 24 hours. Equilibrium moisture content in the seeds was the highest at the lowest temperature, but equilibrium was reached faster at higher temperatures.

Seeds of 11 flax cultivars with different seed colours were artificially aged at 42 °C, for 48 hours and 72 hours. Depending on the cultivars, the germination reduction ranged from 0 to 72.8% after 48 hours and from 61.5 to 100% after 72 hours. The brown-seeded fibre flax 'Nike' showed the lowest and the yellow-seeded linseed cultivar 'Minerva' the highest germination reductions. The highest germination reductions were found in cultivars with yellow or olive seed colour and the lowest reductions in cultivars with brown seed colour. Some yellow-seeded cultivars showed less germination reduction than brown-seeded cultivars. The 1000-seed weight of the cultivars was not correlated with germination reduction. Germination reduction was negatively correlated ($r = -0.66$ and $r = -0.65$) with initial germination rates. The average seed moisture content increased during accelerated aging from the initial 6.3% to 25.9% after 48 hours and 37.1% after 72 hours. There was a tendency of higher seed moisture contents after accelerated aging to be associated with higher germination reduction ($r = 0.60$ and $r = 0.46$). The results suggest that flax with lighter seed colour needs closer monitoring during seed storage in genebanks than brown seeded material.

Physiological quality and conservation of *Jatropha curcas* L. seeds obtained from fruits at different maturation stages

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Jatropha curcas L. species has been listed as a promising source of raw material for the production of biodiesel and bio-jet fuels. The success of the commercial exploration of that culture depends on obtaining seeds with high physiological quality, which is associated with harvest at the appropriated time. However, the information related to seed physiological quality as a function of the fruit maturation stage during storage is scarce. Thus, the aim of this study was to evaluate the effect of fruit maturation stage in the physiological quality of *J. curcas* seeds during storage. Fruits were collected at different maturity stages based on external color, i.e., yellow, yellow-brown and brown. After extraction, the seeds were dried naturally until achieve moisture content of approximately 8%. The seeds were packed in Kraft paper bag and stored for 18 months at laboratory environment. Initially and every three months, the seeds were evaluated for moisture content, germination, first count of germination, accelerated aging, cold test, electrical conductivity and emergence. There was a reduction in germination of *J. curcas* seeds from nine months of storage, regardless of the maturation stage. However, there was no difference in seed germination between fruit maturation stages throughout the storage period. There was a reduction in seed vigor independently of the fruit maturation stages, mainly from the nine months of storage, as evidenced by the tests of first count of germination, accelerated aging, and cold test for seeds from brown fruits. The reduction in vigor was less intense for seeds extracted from yellow and yellow-brown fruit when compared to those obtained from brown fruits, which was evident by the cold test, electrical conductivity and seedling emergence. Thus, *J. curcas* seeds can be stored for up to nine months without significant loss of germination and vigor, and for up to 12 months maintaining high germination percentage, but with reduction in vigor. The seeds extracted from fruits with external color yellow and yellow-brown are more vigorous and keep for a longer period of time the physiological quality compared to seeds extracted from brown fruits.

Acknowledgments

Fapemig, CNPq, and CAPES.

Bio-economics and Ecosystem Services of Amazonian Native Seed (BESANS)

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Seeds are the natural means of species regeneration, the product of pollinator activity, the basis of local agriculture, a type of non-woody product and a source of essential protein and vegetable fat (seed oil) with many potential uses (industrial oils, biofuels, cosmetics). Consequently they are one of the mainstays of continuing ecosystem services. The Amazon is one of the most biodiverse regions of the world and the forests near Manaus are considered priority conservation areas. Ecological research in the region is fundamentally important to the sustainable and innovative use of species and yet the scientific capacity in seed biology in the Amazon region is extremely limited. Therefore, BESANS is training 20 members of the Amazon Seed Network, 9 institutional staff and around 60 seed/seedling producers in Amazonian species seed biology, and upskilling in conservation biology. The partnership links plant science institutes and aims to understand the seed supply chain (seed development, yield, processing and storage) associated with the nascent seed trade in the Amazonas.

Research on seed biology is critical to accessing species for various development activities (food/energy security, ability to mitigate/adapt to climate change) and the collection and conservation of germplasm. We are characterising the seed storage behaviour of 20 priority species of potential bio-economic value. Target general for which seed biology information is required include: *Bertholletia*, *Carapa*, *Handroanthus*, *Oenocarpus* and *Tabebuia*. Seed quality is being assessed against multiple criteria: 1) co-plots showing the dependence of viability on seed moisture content and drying rate are being used to assign storage categories; 2) data is being generated on seed physical attributes (mass and seed coat ratio) and a probabilistic model of seed desiccation tolerance developed; 3) the morphology of the species is being described and used to identify the most appropriate embryos for cryopreservation research.

Overall, through research and training, BESANS is increasing knowledge of inter-specific variation in seed storage behaviour of tree species and enhancing the work of the Amazon Native Seed Centre in Manaus, so that high quality seeds of native species can be used to support the developing bio-economy in the region.

The seed coat as a reservoir of growth promoting substances

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The seed coat originates from the integuments surrounding the ovule. In *Arabidopsis thaliana* (Brassicaceae) it is composed of five layers of cells and each of these layers undergoes different path of differentiation during seed development and maturation. Accordingly, in *Arabidopsis* the outer cell layer of the seed coat produces mucilage. When the seed is hydrated, polysaccharide mucilage is expanding through the outer cell layer that surrounds the seed and envelops the imbibed seed. The mucilage layer has been implicated in adherence to soil and in absorbing and storing water for germinating seeds. Our preliminary data suggest that the seed coat of different mucilaginous Brassicaceae species may serve also as a reservoir of organic and inorganic substances that might support seed germination and seedling establishment. Proteome profiling showed that more than 200 proteins are secreted from the seed upon hydration including stress responsive proteins and hydrolases including endonucleases and pectin biosynthetic enzymes. We further confirmed the presence of nucleases/endonucleases in the seed coat by using in gel nuclease assays and by the conversion of supercoiled plasmid DNA into relaxed and linear forms. Analysis of micro and macro elements as well as metabolites revealed secretion of high amount of potassium and nitrate as well as high level of lactic acid – known to function as plant growth promoting factor. Thus, our results explored previously unknown features of the seed coat serving as a reservoir of substances that might play an important role in seed germination and seedling establishment.

Long term productivity of seeds can be provided by amelioration

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A seed is a very valuable source which is taken from either a landrace or a cultivar improved by selection with an any breeding method. In elapsed time, its productivity reduces with many uses, genetical and enviromental reasons like foreign pollinations etc. In plants, features belonging to adaptation and yield and quality are related with the protection of values that are already icluded into seed as genetic materials. The long term use and participation to production of these values are vital for humankind. Here, the point of view is environmental aspect rather than endustrial approach, in other words, the prevail subject is necessity of the protection and use of natural varieties. Already, the varieties were increased very much by many succesfull genetical and breeding studies, particularly in crop species all over the world, up to now. So, these genetical sources and/or old cultivars can be ameliorated and used again and again for further generations.

For this, in the results of our basic researches about durum wheat genotypes (landraces or modern cultivars), genes responsible to main morphological characters (adaptation, tillering, spike and spikelet morphologies, quality), didn't be lost, and moreover, some of recessive characters into population such as colour of coleoptile or longer spikelet stem were observed as homozygous in some DH genotypes, because of doubling of the chromosomes (using intergeneric crosses). DH genotypes were more productive, carried higher amount of biomass (harvest index=HI) and more carotenoids in grains than control groups. Total chlorophyll (chlorophyll a and b) was found higher in DH genotypes than parent Kunduru 1149 (*Triticum durum* Desf.) that is a good indicator for increased photosynthetic capacity in plant cells and also for HI and carotenoid amounts at macro level. DH technique didn't be cause of abnormalities in the structures of anatomical and cytological in durum wheat seeds (grains). There were not observed any abnormalities like unregular mitosis or polyteni in epidermal and hipodermal haploid embryonal tissues and counted clearly 14 and 28 chromosomes in haploid and diploid root tips meristematic cells subsequently. The results discussed and published in some of national and/or international symposiums and journals, as examples can be seen below:

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Expression of protein l-isoaspartyl methyltransferase in sunflower seeds as affected by ageing and priming

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In orthodox seeds, non-enzymatic protein damage occurring during dehydration and storage is hypothesized to be a main cause of deterioration. Therefore, for vigorous germination dry seeds should be equipped with protein repair mechanisms. One of these involves protein L-isoaspartyl methyltransferase (PIMT) which counteracts protein misfolding by catalyzing the conversion of abnormal L-isoaspartyl residues to their regular form. PIMT activity is primarily localized in seed tissues during and after maturation desiccation, suggesting its involvement in restoring the functional conformation of the seed proteome. This study was initiated to assess the putative role of PIMT repair pathway on seed vigor in *Helianthus annuus* whose seeds are rather sensitive to deterioration during storage. A sunflower homolog of *Arabidopsis PIMT1* was isolated and characterized, and its expression in seeds was measured by qRT-PCR on a panel of 16 inbred lines subjected to ageing, priming, and priming after-ageing. The wide variability in the physiological response observed among the lines and the considerable interactions between factors produced a rather complex picture. Considered separately, ageing and priming had expected, although only modest, effects on final germination, *i.e.* negative the former (-2.4%) and positive the latter (+4.7%). More marked were the effects on germination rate, with an 8.3% increase in T50 after ageing and a 30.6% reduction after priming. On average PIMT transcript levels were highest in quiescent seeds and reached the minimum after ageing, with a 38% drop; also after priming PIMT mRNA decreased 26%, while in primed after-ageing seeds its abundance was variously affected. Therefore PIMT expression pattern in sunflower resembles that of other protein transcripts with protective role in dry seeds. Correlations between transcript levels and germination percentage and rate were not found in control and aged seeds; on the contrary, correlations were negative and significant when priming was applied, perhaps indicating either an attempted repair response or a reduced degradation in damaged tissues. For the complexity of the responses observed, further studies on gene expression and enzyme activity will be necessary to assess the role of this repair mechanism in maintaining seed vigor in sunflower.

Longevity of elite cultivars is a matter of intense breeding within 'breeder seed' at nil-competition.

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Longevity of elite cultivars is of paramount importance because of time consuming and costly breeding process. Cultivars are assumed fairly homogeneous and only plants which are obviously of incorrect type are removed in maintenance procedure, rendering the technique more roguing rather than selection process. Contrariwise, the molecular tools evidenced that genome undergoes constant remodeling and restructuring, suggesting that it is more flexible and plastic than previously assumed. Latent genetic variation due to relic heterozygosity combined with genetic and epigenetic mechanisms that generate de novo variation may result in considerable intra-cultivar variation. Therefore, cultivars adopted and widely grown by farmers may lose their identity and healthiness in the long-term. Further, contaminating and degrading forces such as out-crossing, volunteer plants, physical admixture, natural selection, mutation and seed-borne diseases, will change the gene pool for the worse. An appropriate non-stop intra-cultivar selection appears to be a viable option aiming beneficial exploitation of the existed and newly developed variation. There is a widespread grasp that a negative relationship between yielding and competitive ability exists. 'Breeder seed' maintenance at normal densities may favour accumulation of strong competitors at the expense of high yielders leading to cultivar deterioration over the years. In order to treat the 'breeder seed' in an effective and sustainable manner, conditions that allow recognition and removal of the undesirable mutations is an imperative need. Ultra-spaced nurseries to exclude plant-to-plant interference for resources (nil-competition) satisfy such a prerequisite for two reasons. They boost the phenotypic expression of variation, and erase the confounding effects of the above relationship. Relevant research in maize, wheat, cotton and soybean is encouraging that intense selection is a useful technique either to upgrade or to avoid gradual degradation of genetic background, to maintain uniformity, and secure optimal quality of 'breeder seed' over longer periods of time. In a lentil landrace there was improvement in seed healthiness through drastic reduction of seed-borne viruses. The final proposal is perpetual selection within 'breeder seed' to ensure its sanitary status, and propagation at dense stand at the following stages to succeed the demanded amounts of certified seed.

Identification and characterization of seed longevity genes in barley (*Hordeum vulgare*)

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Preventing the loss of the genetic variability of cultivated plants and their wild relatives due to changing environmental conditions and cultural practices is a global priority. Worldwide more than six million accessions have been accumulated in *ex situ* genebanks of which more than 90% are stored as seeds. For crop germplasm preservation seed longevity is of particular importance. Our main goal is to identify and characterize seed longevity genes in barley (*Hordeum vulgare*).

To allow accurate mapping of quantitative trait loci (QTLs) controlling seed longevity in barley, near isogenic lines (NILs) were derived from a L94 x 116-5 recombinant inbred line (RIL) population. The L94 x 116-5 RIL population was developed from a backcross of the line 116-5 (F_5), resulting from a cross between the Ethiopian two-rowed barley landrace L94 and the Argentinean six-rowed landrace Cepada Capa, to L94 (BC_1). Backcrossing of one of the resulting RILs, RIL 114 (BC_1F_{12}), to L94 (BC_2) and subsequent selfing and single seed descent led to the formation of the NILs (BC_2F_4). A set of 204 polymorphic single nucleotide polymorphism (SNP) markers from Barley Oligonucleotide Pool Assay 1 (BOPA1) and morphological markers (Blp, Vrs1) were used to genotype the NILs.

Four putative QTLs, identified in the L94 x 116-5 recombinant inbred population, could be confirmed. RNA-Seq analysis of the NILs was employed for mapping of the introgressions and identification of differentially expressed candidate genes and possible downstream targets.

Currently, mapping populations are generated for fine mapping and subsequent identification and cloning of candidate genes. L94 NILs with improved seed quality will be tested employing controlled deterioration tests and ROS related assays in order to identify the processes that are different.

Session IV

Physiology and biochemistry behind seed ageing – deleterious effects *vs*. repair mechanisms

Germination responses of *Ricinus communis* L. seeds to accelerated ageing

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Castor seed (*Ricinus communis* L.) oil has many important industrial uses among which a source of feedstock for biofuel, while considered a major oilseed crop cultivated by family farmers and therefore considered a means to promote social-economic development in the semiarid of the Brazilian northeast region. However, little attention has been given to the processes of seed ageing and loss of viability. Germination tests currently represent the most used method to assess seed viability to measure seed ageing. The aim of the present study was to identify germination parameters associated with seed deterioration caused by accelerated ageing test. Two varieties of castor seeds (Nordestina e Paraguaçu) were submitted to storage under controlled temperature (CT - 15 °C) and non-controlled (NCT – room temperature around 26 °C) for nine months, whereas relative humidity (RH) was around 70% at CT and 40% at NCT. Nine months stored dry seeds were subjected to accelerated ageing (60 hours @ 42 °C and 100% RH). Seed germinability and vigor, electrolyte leakage, moisture content and viability by tetrazolium reduction were determined before and after accelerated ageing. Significant differences were observed in germinability (Gmax, T_{50}, $U_{75\text{-}25}$, AUC) of seeds subjected to storage followed by accelerated ageing, in which Paraguaçu seeds seemed to acquire secondary dormancy during storage, which was broken after accelerated ageing. Tetrazolium test confirmed no loss of seed viability during storage or accelerated ageing. Whereas Nordestina seeds did not show significant differences before and after accelerated ageing. Seed moisture content increased significantly in both varieties after accelerated ageing, while electrolyte leakage became lower indicating that the conditions of accelerated ageing appeared not enough to cause membrane damage, or otherwise were surprisingly enough to promote recovery of eventual membrane damage during seed storage. The accelerated ageing applied to castor seeds induced several differences in germinability for Paraguaçu seeds, but did not cause any differences in Nordestina seeds.

Changes in maize germination ability and antioxidant enzymes activities during seed natural ageing and as a result of priming

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Storage of orthodox seeds for a long period may result in significant reduction of their vigor and growth potential. Seed deterioration is even more evident in case of unsuitable storage conditions such as high moisture content and high temperature, which significantly accelerates seed ageing. The loss of seed germination ability during the natural ageing is due to many factors, including the changes in antioxidant potential of stored seeds. Therefore the main purpose of our research was to investigate the role of antioxidant enzymes during ageing and their involvement in seed viability repair as a result of priming of maize (*Zea maize* L.) seeds.

For this purpose, samples of maize seeds from the Genebank maize collection of Agricultural University of Georgia, which have been naturally aged in the storage facility since 2012 and 2013, and control samples, which is seed of the same varieties harvested in 2014, were tested for activities of antioxidant enzymes – peroxidase and catalase. The effect of priming on germination and antioxidant potential was investigated by soaking seeds in KH_2PO_4 and KNO_3 solutions at different concentrations (0.5, 1 and 2.5%) for 6 hr.

As compare to the control samples, the naturally aged seed samples showed reduction in both antioxidant enzyme activities; this reduction was positively correlated with seeds germination ability. Seed priming significantly enhanced seed germination and early growth. The priming effect was more evident in one-year-aged seeds than in two-year-aged seeds. Priming treatment had no significant influence on germination ability of control seeds (with no aging). Antioxidant activity of aged seeds also increased as a result of seed priming treatment. Activation of antioxidant enzymes and enhancement of seed viability was more noticeable, when seed priming was carried out through KNO_3 than with KH_2PO_4.

Thus, priming treatment with KNO_3 at 1%-concentration showed the best efficiency for the improvement of performance of naturally aged seed and can be recommended for re-storing seed viability of genebank collections.

Vitamin E and hormonal balance during macaw palm seeds germination after storage

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The storage conditions directly influence on seed quality, longevity and, consequently, on germination. Our study bring biochemical information about macaw palm (*Acrocomia aculeata*, Arecaceae) germination in recent recovered and stored seeds in two different conditions, at room temperature (laboratory conditions), and in nursery (exposed to climate variations) for 210 days. Germination experiment: seeds (n=8) were taken out of the fruits and sown in vermiculite at 90% of field capacity during 21 weeks in growth-chambers at 30 °C and 12 h photoperiod. During germination, four physiological phases were chosen to perform biochemical analysis (hormonal, measured by UPLC-MS/MS, and vitamin E, by HPLC): dry seeds (D, before germination experiment), imbibed (I, 12 days after sowing), germinated (G, recent germinated) and non-germinated seeds (NG, end of the experiment after 21 weeks). Embryos were separated from seeds and frozen. Data were analyzed by two-way ANOVA, considering "phase" and "storage treatment" as factors. Vitamin E (α-tocopherol and α-tocotrienol) were detected in embryos of all treatments. D embryos from nursery condition showed higher levels of α-tocopherol and α-tocotrienol. Both vitamin E compounds decreased in G embryos when compared to imbibed phase in all treatments. Bioactive gibberellins (GAs; GA_1+GA_3+GA_4) significantly increased in I embryos from nursery in relation to I embryos from control and laboratory. In G phase, embryos from all treatments showed increase in GAs content in relation to D phase. Abscisic acid (ABA) did not change between treatments after storage.In control, ABA decreased from D to G phase. In nursery and laboratory treatments, ABA showed the same dynamics during germination, with decreases only in G and NG phases. In G embryos, ABA levels were lower than other phases in all treatments. GAs/ABA ratio was higher in control during germination, but GAs/ABA ratio was > 1 in G phase of all treatments. Our results indicate that nursery condition, which fruits were exposed to natural conditions, embryos activate repair mechanisms in response to climate variables (rain/drought) that included increases in Vitamin E as a protective mechanism. In addition, high ABA content during storage could help on keeping viable dormant seeds in macaw palm seed bank.

Role of quinone reductases in germination and in the early development of *Arabidopsis*

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Quinone reductases (QRs) are enzymes that catalyse the two electron reduction of quinones into hydroquinones. This reaction is in competition with other reactions that transfer one electron to form semiquinones. A semiquinone can further react with oxygen to produce superoxide and thus create an oxidant stress in cells. Hence in animals, QRs are considered as detoxifying enzymes.

Schopfer *et al.*, (2008) have shown using isolated plasma membranes from soybean plantlets that subsequently to QR reaction, at alkaline pH, an auto-oxidation takes place leading to the production of semiquinone radicals and thus leading to superoxide formation.

In seeds, reactive oxygen species such as superoxide are necessary for germination. The aim of the project is to show whether mutants affected in QRs have a germination phenotype and to understand the mechanisms that leads to superoxide production via QRs. Mutants seeds of different QR are being characterized : *nqr* (NAD(P)H quinone : oxidoreductase; a membrane-attached enzyme) and *fqr1* (flavodoxin-like quinone oxidoreductase; a soluble protein) and the double mutant *nqrfqr1*.

While germination tests have shown no clear differences between wild type and mutants' seeds, growth of the plantlets was affected. Radicle growth was slower in *nqr* and *fqr1* but not in *nqrfqr1*. Measures of antioxidant enzymes activities were performed. Superoxide (SOD) and guaiacol peroxidase (PRX) activities are 10% to 30% higher in the roots of mutants' plantlets. In the mutants' shoots, there is no difference in PRX activity while SOD activity is decreased in *nqr* and *fqr1* but not in the double mutant. Finally, catalase activity is slightly higher in the shoots of *nqrfqr1* and in the roots of *fqr*. Moreover, spectrophotometrical dosages and spin trapping Electron Paramagnetic Resonance (EPR) measurements will be conducted to detect and characterize ROS production during germination and early plant development.

Physiological behavior of coffee seeds under quick and slow drying

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Coffee seeds are highly sensitivity to desiccation, which has hindered their storage and obtaining seedlings of standard quality. There are several factors that affect the quality of coffee seeds and, among these factors, drying rate and moisture content seem to be highly important, mainly due to the sensitivity of these seeds to desiccation. Slow drying can induce tolerance to desiccation in orthodox seeds, whereas, in sensitive seeds, this results in less tolerance, such that the faster the drying, the lower the moisture content to which seeds can be dried without losing viability. The aim of this study was to investigate physiological changes in coffee seeds dried in saturated salt solutions and silica gel. The study was conducted at the Central Seed Laboratory of the Universidade Federal de Lavras (UFLA). Seeds from the 2012/2013 crop season of *Coffea arabica L.* Catuaí Amarelo IAC 62 were used, which were subjected to different drying rates, quick drying in silica gel and slow drying in saturated salt solutions. Moisture loss during drying was monitored by regularly weighing the seeds until they reached the moisture contents of 40, 30, 20, 15, 10, and 5% (wet basis). Physiological quality of the seeds was determined by the germination test, assessing the percentage of strong normal seedlings, seedlings with expanded cotyledons, and embryo viability in tetrazolium. It may be concluded that drying is detrimental to coffee seeds, regardless of the rate at which they are dried. Moisture content of 5% is highly detrimental to the quality of coffee seeds, and moisture levels close to 30% cause reduced viability and seed vigor. The best physiological seed quality was obtained at 40% moisture slowly dried to 20% moisture, and seeds subjected to rapid drying to 10 and 15% moisture.

Drying speed in the conservation of coffee seeds

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Coffee seeds are sensitive to desiccation and have low storage potential. They are classified as intermediate seeds. Coffee seeds quickly lose viability and do not conserve germinating power for longer than six months. Thus, conservation of coffee seeds has been the subject of many studies and the results obtained so far have been contradictory, making it difficult to provide general information on the most favorable conditions for maintenance of physiological quality for an extended period. The aim of this research was to study the effect of storage on *Coffea arabica L*. seeds dried at different drying rates. The study was carried out at the Central Seed Laboratory of the Universidade Federal de Lavras (UFLA). Seeds from the 2012/2013 crop season of the *Coffea arabica L*. species, Catuaí Amarelo IAC 62 cultivar, were used. We used two types of drying: quick drying in silica gel, and slow drying in saturated salt solutions, until the seeds reached the predetermined moisture contents of 40, 30, 20, 15, 10, and 5% (wet basis). After drying, the seeds were placed in cold storage at controlled moisture (10 °C, 50% RH) for 0 and 4 months. Time zero was characterized by the seed remaining for 24 hours in the predetermined environment. Seed quality was determined by the germination test, assessing the percentage of strong normal seedlings, seedlings with expanded cotyledons, seedling dry matter and embryo viability in tetrazolium. Drying coffee seeds to levels below 10% moisture is detrimental to seed quality, but, after storage, germination and vigor improve. The effects of drying speed on the longevity of coffee seeds vary according to the moisture content after drying. Storage for four months does not affect the germination of coffee seeds, but there is a loss of vigor.

Effects of temperature, moisture content and oxygen on the viability of coffee (*Coffea canephora* var. *robusta* and *C. arabica*) seeds during storage

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The aims of the present work were to determine the effects of the storage conditions (temperature, relative humidity, oxygen) on coffee seed viability evaluated by the ability to germinate at 25 °C. Seeds of various lines of *Coffea canephora* var. *robusta* and *C. arabica* supplied by Nestlé were equilibrated at 10 or 15 °C at various relative humidities (RH) ranging from 20% to 85% in order to modulate the moisture content, and then tested for germination at 25 °C. Dehydration resulted in a progressive loss of viability, and all the seed population died in 40% RH, i.e. when the moisture content fell to about 10-12%. Differences in desiccation sensitivity were apparent among the lines; for example, after equilibration in 50% RH, viability ranged from 13% to 81% depending on the seed batch. All seed batches taken together, a critical moisture content below which 50% of the seed population died has been determined close to 20-22% DW. In order to determine the effects of temperature and moisture content during seed storage on their survival, seeds were placed at 2 temperatures (15 and 25 °C) in hermetic bags with a moisture content at 25-26% DW, or at 12.5-15.5% DW after equilibration in 50% RH. At high moisture content, seeds remained viable for at least 5 months, when seed viability was progressively lost at 25 °C, 50% and 10% of the seed population remaining able to germinate after 1-3 and 4-5 months, respectively. At low moisture content, viability of all the seed batches was lost more rapidly than at high moisture content, and this loss of viability is faster at 25 °C than at 15 °C. Experiments performed with seeds of *C. arabica* placed at 10 °C in 65% RH (i.e. at a water content of 18-19% DW) in air or in anoxia indicated that the absence of oxygen improved seed longevity.

Antioxidants and oxidation contrast process; a factor of diagnosis the longevity and deterioration of some seed medicinal

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Lipids are highly effective in metabolism cycle of seed germination. Breakdown of lipids in the germination process is obvious, but in normal circumstances seed oil oxidation is a sign of seed degradation and reduction of seed longevity. To further examine the problem, an experiment was conducted on two species of medicinal plants, sweet basil (*Ocimum basilicum*) and Balangu (*Lallemantia royleana*) and oxidants and antioxidants activities were studied in fresh seeds and seeds placed in accelerated aging conditions (AAc. Treat,) in time of 24, 48 and 72h at 41 °C and relative humidity of 100%. The results indicated that was a direct negative relationship between antioxidants and pro-oxidant activity of seeds. AAc. Treat, led to intensification of seed oil oxidation and reduced oil content up to 20 percent in addition to changing fatty acid profile. Seed oil oxidation was a two-step process; in the first stage peroxide value activity (24h AAc. Treat,) and in the second stage (48h and 72h AAc. Treat,) anisidine index (one of secondary oxidation products) activity increased. In both seeds tested, the highest amount of α tocopherol was in control which was decreased linearly due to AAc. Treat. However, the greatest amount of polyphenol was obtained in 24h AAc. Treat, and then decreased. It was concluded that in the initial stages of oil oxidation, activity of polyphenol increases to decrease damaging effects of oxidation; however, later it was reduced with increasing oxidation. The results of this experiment showed that antioxidants increase longevity of sweet basil and balangu seeds by controlling oxidation substrate (lipids and oxygen), peroxidants as well as by inactivating free radicals.

Effect of priming treatments on storability of cotton varieties picked at different intervals

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The present study was conducted at Department of Seed Science and Technology, CCS-HARYANA AGRICULTURAL UNIVERSITY, India. Six cotton varieties (*Gossipium hirsutum* H1098, H1117 H 1236 and *Gossipium herbaceum* HD123, HD324 HD432) were sown in the month of April 2012-13. Seeds were picked at different intervals (three pickings), initial data was recorded after delinting, and seeds were stored at 19 °C with 7% moisture content in air tight plastic jars. Different priming treatment (controlled, GA_3, KNO_3, KH_2PO_4, PEG) were given after 15 months of storage and their effect on seed germination, seedling length, seedling weight and electrical conductivity was recorded. Data recorded showed that HD- 432 had maximum mean germination (78.98%) with first picking (79.94%), and interaction between varieties of first picking and priming effect of PEG was found maximum (71.26%). Maximum mean seedling length was observed in variety HD 432 (26.681 cm) treated with PEG at first picking and maximum mean seedling weight was observed in H-1098 (0.325 mg) at first picking which was treated with KH_2PO_4. In terms of different priming effect on electrical conductivity HD 432 was found with minimum (0.269) when treated with PEG. With the above study it was observed that picking first had significant effect on stored seeds in terms of seed quality than second and third pickings.

Physiological and biochemical alterations in rubber tree seeds [*Hevea brasiliensis* (Willd. ex Adr. de Juss.) Müell.-Arg.] during storage

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Recalcitrant seeds, like rubber tree seeds, are damage by dehydration and may also be chilling-sensitive during storage, and generally cannot be stored effectively for useful periods. The objective of this study was to evaluate the biochemical and physiological changes during the storage of rubber tree seeds at different conditions. Mature seeds were harvested and treated or not with chemical fungicides Tecto 600 (35 g.100 Kg^{-1} seeds) in association with Captan (75 g.100 Kg^{-1} seeds) or with ground rosemary (20 g.Kg^{-1} seeds). Then, they were conditioned in paper bags that were placed inside polyethylene bags and stored at 10 °C, 20 °C or 25 °C, in a factorial design 3x3x5 (seed treatments, temperatures and storage periods). Each 15 days, seed moisture content, seedling emergence percentage, seedling growth, emergence speed index, activity of enzymes of the oxidative stress and the reserve compounds content were determined. The seeds of rubber tree stored at 10 ºC show higher moisture content, viability, seedling growth and reserve compounds than those stored at 20 °C and 25 °C. The viability of the seeds stored at 10 ºC was maintained until 75 days. The reduction in the activity of the enzymes of the oxidative stress affected negatively the viability of seeds, especially at higher temperatures. The treatment with chemical fungicides caused phytotoxicity to the rubber tree seeds.

Physiological and cellular changes of stored *Cryptocarya aschersoniana* Mez. seeds

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Various species of the Lauraceae family belong to ecological climax group, with an irregular seed production that are generally sensitive to desiccation, which makes them difficult to store. In this study, we investigated the physiological and cellular characteristics of *Cryptocarya aschersoniana* seeds collected in two years and stored under different conditions for up to 12 months. Seeds were stored at their original moisture content (41 and 47%) and also after pre-drying to 35%. They were placed in a cold chamber (5 °C) at a relative humidity of 40%. After 3, 6, and 12 months of storage, the samples were assessed for moisture content, germination, ultrastructure and histochemical characteristics. Under either storage condition, the seeds remained viable for at least 12 months. The seeds are dispersed in a dormant state, and the dormancy period was extended by the cold storage condition. Histochemical analysis indicated that the stored seeds demonstrated synthesis of phenolic compounds. Seeds stored at their original moisture content partly consumed their reserves during the storage period. As a result, these seeds could become unviable during storage periods exceeding 12 months.

Flow cytometry as a tool for assessing seed viability of the long-term stored seeds

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The aim of this study is to establish a fast and accurate screening method for determining seed viability stored in the genebanks using flow cytometry.

After mitotic division cells undergo the first growth phase (G1), then the phase of DNA synthesis and the second phase of growth (G2). Diploid cells in the G1 phase in nucleus contain DNA 2C and G2 - 4C. Flow cytometry allows determining the quantitative ratio between cells which are in different stages of the cell cycle (G2/G1), which provides information on the physiology of seed, its stage of development, maturity, and advancement of germination. G2/G1 ratio was considered to be a marker of germination, used to track the processes of seed conditioning.

Experiments were carried out with rye kernels subjected to different methods and time of storage wich resulted in different levels of germination of these kernels. The results show that in non-germinating seeds G2/G1 ratio is higher.

Thus, the question is: Is there a possibility to establish a threshold value of G2/G1 which would divide germinating and non-germinating seeds?

Comparative study of fatty acid profile in endosperm and embryos of *Coffea arabica* seed and its correlation with desiccation tolerance

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Coffee seeds are sensitive to desiccation and lose quality after drying, which hinders their storage in the medium and long time. According to recent results, different parts of the seed, embryo, and endosperm respond differently to the drying process, with negative effects on viability and vigor. Structural variations, as well as the proportion of chemical constituents in different parts of seeds, may lead to different behaviors during drying and other post-harvest operations. Therefore, the objective of this study was to determine the lipid content and the drying rate in embryos and endosperms of *Coffea arabica* L. seeds during slow drying in saturated salt solutions and quick drying in silica gel, investigating their relationship to loss of viability as the moisture content of seeds declines from 40% to 5% (wet basis). Fatty acids were extracted from embryos and endosperms and determined in a Gas Chromatograph, Model GC - 17 A. Although change in the fatty acid profile was not observed after slow and fast drying, the values of palmitic and linoleic oils, the most abundant oils, varied significantly between endosperm and embryo. Linoleic fatty acid was predominant in coffee seeds, at concentrations of 0.48 $g.g^{-1}$ in endosperms and 0.41 $g.g^{-1}$ in embryos. The concentration of palmitic acid was 0.35 $g.g^{-1}$ and 0.41 $g.g^{-1}$ in endosperms and embryos, respectively. A composition of more saturated fatty acids contributes to preservation of the functionality of the membranes after desiccation. In contrast, unsaturated fatty acids, such as linoleic acid, are more susceptible to degradation, and products from their peroxidation inactivate membrane-bound proteins, altering their permeability. Thus, the greater predominance of linoleic acid in the endosperm as compared to the embryo supports the hypothesis that this structure may be the main source of damage and of progress of the deterioration process.

Molecular mechanisms of combined priming and aging treatments in sugar beet seeds

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An important process to improve sugar beet seed quality is priming. It leads to accelerated and uniform germination but also to enhanced aging during seed storage, especially in suboptimal storage conditions which cause a drastic reduction in longevity and germination. We analyzed sugar beet seed aging during storage by comparing aging models differing in the three factors: temperature, relative air humidity and duration. The resulting physiology data were used to construct comparative population-based models of seed aging in primed versus unprimed seeds, which delivered storage conditions with defined stress responses. The storage conditions have a distinct impact on the levels of several hormones in dry seeds, but also imbibed seeds, just before the onset of the germination process. Abscisic acid (ABA) contents decrease in imbibed sugar beet seeds with a distinct pattern affected by combined priming and aging treatments. While ABA inhibits sugar beet germination, 1-aminocyclopropane-1-carboxylic acid (ACC), the biosynthetic precursor of ethylene, is known to promote it. Additionally to the measured hormone levels, we analyzed the transcript levels of enzymes that are important in the hormonal pathways. Another important system to visualize negative effects on the seeds due to aging is the biochemical quantification of the relevant reduced and oxidized metabolites. The redox couple glutathione/glutathione-disulfide (GSH/GSSG) is known to be the main antioxidative system of the dry seed and an aging marker. These and other metabolites as well as the transcripts of related enzymes were quantified and connected to the germination kinetics to show the role for the antioxidant system in alleviating seed damage in some combined priming and aging conditions.

Effect of priming on physiological quality of *Handroanthus serratifolius* (Vahl.) S.O. Grove seeds

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Seeds of *H. serratifolius* have short longevity at dry storage. It is known that treatments like seed priming can affect seed quality and change the response of seeds to storage. In order to study the effect of different methods of priming and its influence on storage after controlled deterioration, seeds were osmoconditioned in PEG -1.0 MPa at 10, 15 and 20 °C and hydroprimed at 5, 10 and 15 °C. After priming, germination (radicle protrusion) was assessed daily to determine the final percentage, speed and uniformity of germination. Priming did not affect the final percentage nor uniformity of germination; however, the germination speed was increased after hydropriming at 15 °C and osmoconditioning at 15 °C compared to the control. In the second experiment, in order to evaluate priming effect on seed quality, it was tested hydroprimed (15 °C) and not primed seeds. Conditioned and unconditioned seeds were placed into an incubator (25 °C, dark, 100% RH) until they reach 15% moisture content. After moisture equilibration seeds were placed in a sealed container and incubated at 40 °C for 0, 6, 12, 24, 36 and 48 hours. Samples were taken in each period for determination of viability by germination test. There was a significant effect of conditioning on seed quality regarding germination speed (t_{50}) and tolerance of seeds to controlled deterioration conditions measured by the percentage of germination. The results suggest that priming can increase longevity of *H. serratifolius* seeds.

Generative development and true seeds formation in garlic (*Allium sativum* L.) grown in Lithuania

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Investigations of reproductive organs characteristics and true seeds formation in garlic (*Allium sativum* L.) were carried out in the experimental field of the Institute of Horticulture Lithuanian Research Centre for Agriculture and Forestry (IH-LRCAF) in 2013 – 2014. Eleven garlic accessions, formed stalks were evaluated. Ten garlic cloves were planted of each accession.

Obtained results showed that the most morphological characteristics of reproductive organs were similar within accessions. But variations were observed among accessions. The highest differences were observed in true seeds formation. The rate of seeds was different in an individual clove within accession. 6 accessions from 11 distinguished with the ability of seed formation according to the results of two years investigations. The amount from 1 to 10.6 seeds per clone was obtained in an accession.

Garlic propagation from seeds instead of cloves has a high practical and economical value for breeding purposes, obtaining of virus-free crop. The method would be effective for the maintenance of longevity of valuable propagation material.

Understanding seed longevity of *Aethionema arabicum* dimorphic diaspores

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Aethionema arabicum, an annual plant of the Brassicaceae family, exhibits fruit and seed (diaspores) dimorphism. It is described as an angiosperm shrub and specifically found in specific regions in Mediterranean regions/West Asian regions such as Turkey, Bulgaria and Cyprus. A remarkable phenomenon of this plant is its ability to produce two different types of fruits (large dehiscent and small indehiscent) on the same infructescence. These two types of fruits harbour two different seed types denoted as M+ (mucilaginous) and M- (non-mucilaginous). Further understanding of the differences in the different seed types in terms of their morphology and physiology are currently investigated. The significance of this is related to grasp a more in depth knowledge of how seed dimorphism is a 'bet-hedging' strategy to survive in and adapt to unpredictable environments. This is supported by our finding that the two morphs differ in the key seed properties 'germination', 'dormancy' and 'longevity'. To access seed longevity we compared seed aging of the dimorphic seeds of *Ae. arabicum* with the seeds of other monomorphic species. This was achieved by conducting controlled aging assays at defined 25%, 50% and 75% relative humidity at 25 °C, followed by heat treatment above 40 °C for different period of times. The result presented are discussed in the context of how *Ae. arabicum* seeds may survive in their natural environment. This work is part of ERA-CAPS SeedAdapt consortium project (www.seedadapt.eu) which the aims on establishing *Ae. arabicum* as a dimorphic model species for seed biology research.

Ethanol degradation a simple and sensitive indicator for seed ageing

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Deterioration of seed is initially not noticed until germination slows down and becomes more obvious when the frequency of normal seedlings drops and ultimately total germination. Measuring the ethanol degradation capacity with a breath analyser provides a fast and simple tool to detect an early stage of seed deterioration, which we assume to be related to damage to the mitochondria. The inner mitochondrial membrane is essential for the electron transport chain phase of the aerobic respiration, including ATP and NAD^+ production. The membrane contains a large fraction of phospholipids with poly-unsaturated acyl chains, which are highly prone to oxidation during seed ageing. Oxidation of these phospholipids can result in leakage and disruption of the electron transport chain. Consequently seed ageing is accompanied by a reduction of the aerobic respiration rate upon imbibition and seeds may shift to anaerobic respiration and production of acetaldehyde and ethanol.

Ethanol and acetaldehyde are toxic and the imbibed seed will aim to convert these molecules to the less toxic acetic acid. Both detoxification reactions require the reduction of the coenzyme NAD^+ to NADH. As a functional electron transport chain is essential for a high NAD^+/NADH ratio, damage to mitochondria may result in a reduction of ethanol degradation.

We tested this hypothesis. Chinese cabbage seeds were stored on the lab bench for 19 months. After 0, 3, 9 or 15 months subsamples were takes and placed at -20 °C to reduce ageing. A germination test with these samples showed no decline in total germination nor normal seedlings in the 19 months storage. Head space ethanol levels were analysed with a modified breath analyser (Alcotest 6810 Agri, Dräger Safety AG). Within 1.5 hours there was a clear decline in ethanol levels, while degradation was slower the longer the seeds had been aged on the lab bench. The seeds continues stored at -20 °C degraded all ethanol within 5 hours, whereas the 19 months lab bench stored seeds had degraded only about half of the ethanol in that period. This modified ethanol assay is a very sensitive and simple technique to measure initial stages of seed deterioration during dry seed ageing.

Effects of oilseed rape seed compositions on seed viability after long-term storage

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Oilseed rape mainly produced for vegetable oil, animal feed and bioenergy is with 5.8 Mio tonnes the most important oil crop in Germany and with 72.5 Mio tonnes the fourth important oil crop worldwide. Due to breeders efforts the bitter erucic acid and for animals indigestible glucosinolates ceased causing a change in seed composition over the last century. However, as oil content may affect seed viability a relationship between fatty acid composition and germination after long-term storage is assumed. Therefore, an oilseed rape sub-collection of the Federal *Ex situ* Genebank for Agricultural and Horticultural Crops in Gatersleben, representing the breeding development, was investigated on seed storage compounds and composition. Forty-two oilseed rape accessions (*Brassica napus* ssp. *napus* var. *napus* f. *biennis*) harvested in 1983 were tested on seed viability after 7, 10, 26 and 31y of long-term storage at 7 °C and revealed a half-viability period of 27y. In 2014, fatty acid composition, glucosinolates concentration, oil, protein and water contents were measured. Significant relationships were found for seed oil content, concentration of glucosinolates and seed viability. Most significant negative correlations were found between the polyunsaturated fatty acid eicosadienoic acid and the appearance of normal seedlings. Summarizing, as seed viability and longevity is multi-faceted trait seed oil content, concentration of glucosinolates and eicosadienoic acid are some of the factors which contribute to the final appearance.

Deceleration of seed germination at early aging

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The first feature of aging seeds is the deceleration of germination, which becomes slower and slower. We have shown that the key event at germination initiation is activation of plasmalemma proton ATPase. This activation results from the transition of autoinhibited form to an active one. The enzyme is present in dry seed and translated on long-lived mRNAs. It is activated by fusicoccin and inhibited by ortho-vanadate in vitro, i.e by specific activator and inhibitor of this enzyme.The same effects were obtained in vivo, namely acceleration and inhibition of seed germination. Due to plasmalemma proton ATPase activation, H+ ions are transferred from cytoplasm to cell wall, acidify it and enhance there the activities of hydrolytic enzymes to loose the cell wall structure, a prerequisite for cell elongation beginning. The deceleration of germination of aging seeds is discussed as related to the activity of the enzyme.

Role of chromatin remodeller TRRAP (TRansformation/tRanscription domain-Associated Protein) during seed germination

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In nature, seed production and germination are fundamental phases for plant species propagation and survival. As seed vigor and longevity are important parameters in crop productivity and seed bank storage, studies on seed physiology during conservation and after ripening may contribute for new tools able to increase the seed germination success. Germination is a complex process involving the reactivation of cellular activity thanks to hormonal and chemical signalling (Rajouu et al. Annu. Rev. Plant Biol. 2012, 63:507-33). Dehydration and rehydration during seed development and germination result in accumulation of DNA lesions associated to enhanced oxidative stress. During seed imbibition, DNA repair pathways like Base Excision Repair (BER) are activated and key genes are up-regulated in presence of abiotic stresses (Macovei et al. Plant Physiol Biochem. 2011, 49:1040-50). Both transcriptional reprogramming and DNA repair require chromatin remodelling and local changes of nucleosome structure. Lack of SWR1 chromatin remodelling complex induces accumulation of DNA damage in *A. thaliana* (Rosa et al. Plant Cell. 2013, 25:1990-2001). Studies concerning chromatin remodelling in seeds are strictly related to the nucleosomes condensation that happens during seed maturation (Tang et al. Plant Physiol. 2008, 147:1143-115) and to involvement of the chromatin modifier PICKLE in seed germination (Perruc et al. Plant J. 2007, 52:927-936). TRRAP (TRansformation/tRanscription domain-Associated Protein), which plays an intriguing role as chromatin remodeller in animals, is poorly investigated in plants. TRRAP is a subunit of Histone acetyltransferases (HATs) but it also associates with DNA damage response proteins such as MRN complex and several transcriptional regulators (Murr et al. Oncogene. 2007, 26:5358-5372). TRRAP relaxes the chromatin allowing to reprogram gene transcription and to make the DNA damaged sites more accessible to the DNA repair machinery. In order to find out more information about this function, we are currently exploring the *TRRAP* gene expression profile in the early phases of seed germination in *Petunia hybrida* focusing on its role in response to abiotic stress.

Dynamics of accelerated seed ageing in wheat genetic diversity

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For predicting the storage life of seeds accelerated ageing tests can be applied. The tests subject the seeds to a combination of high temperature and high relative humidity, and this reflects longevity, as seeds with greater longevity deteriorate to a lesser extent than those with poor longevity.

The aim of this work was to investigate intra-specific differences in the ability of wheat seeds for long-term storage using the method of accelerated aging. In experiments 94 accessions of winter wheat from the collection of the Institute of Field and Vegetable Crops (Novi Sad, Serbia) were used. Accessions were phenotypic and genetic diverse material from 21 countries across five continents, divided into two sub-populations. Seeds of all samples were reproduced at the experimental fields of Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben.

Before starting the experiments the initial germination and seed moisture were determined. Then seeds were placed in bottles (100 seeds per each accessions), which were set in an incubator with a temperature of 37 °C and a relative humidity of 100% and were left open for the humidification. Upon reaching the critical seed moisture content (14.5% for wheat) bottles were sealed with corks and were left in the incubator for accelerated ageing. The experiment lasted for six weeks. The germination was determined every week. After four weeks there was a high intra-species difference in the rate of wheat seeds ageing. Seed germination ranged from 0% to 90%. Germination of about 20% accessions was on the level of genetic integrity – 80-90%, and 30% at the level of economic integrity – 50-79%. Thus, about half of all accessions after four weeks of ageing had a germination rate of over 50%. Germination of 75 % accessions after six weeks of ageing decreased to zero.

The rate of accessions ageing did not depend so much on the initial germination, as on each accession's characteristics. A relation between accessions belonging to one or the other sub-populations and the dynamics of their ageing was not found.

Physiological quality maintenance favored by early harvest of *Araucaria angustifolia* seeds

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Araucaria angustifolia (Bert.) O. Kuntze is a native conifer from Brazil and an endangered species. Its seeds have short period of viability, and this is factor that contributes to its vulnerability. This study aimed to evaluate physiological quality during the post-storage period of *A. angustifolia* seeds. Seeds were collected at the pre-maturity, maturity and post-maturity. Thereafter, seeds were stored in a refrigerator for 60 and 180 days and submitted to germination test (four replications of 25 seeds in vermiculite at 25 °C for 80 days), moisture content (four replications of three seeds at 105 °C by 24 hours) and electric conductivity (four replications of 10 embryos in 75 mL distilled water at 25 °C). The moisture content decreased from 55.2% to 46.95% (pre-maturity), from 49.28% to 35.54% (maturity) and from 50.65% to 40.02% (post-maturity) after 180 days of storage. The seeds at the pre-maturity had stability in germination (≈ 84%), even when stored for up to 180 days. However, at the maturity, seeds showed a decrease in viability after 180 days of storage, from 79% to 65% germination. Unlike of the post-maturity, the seeds lost the viability from 93% (fresh seeds) to 86% (60 days of storage) and 11% (180 days of storage). Electrical conductivity was higher for fresh seeds at the pre-maturity (87.28 $\mu S.cm^{-1}.g^{-1}$) than for those stored for 60 days (65.1 $\mu S.cm^{-1}.g^{-1}$). However, at other collect, released leachates content, after 60 days of storage, was higher with the advance of collection period, being 112.68 $\mu S.cm^{-1}.g^{-1}$ (maturity); and 118.93 $\mu S.cm^{-1}.g^{-1}$ (post-maturity). After 180 days of storage, the seeds at the post-maturity showed the highest released leakage content, with 344.77 $\mu S.cm^{-1}.g^{-1}$. Thus, seeds physiological quality was influenced by the development stage in which the seeds were collected. An early collection maintained seeds physiological quality, and may be a strategy to increase the conservation of *A. angustifolia* seeds.

Biochemical changes during storage of *Jatropha curcas* L. seeds from fruits harvested in different maturation stages

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Seeds from *Jatropha curcas* L. are composed of around 38% oil. This species is considered a promising source of raw material for biodiesel production due to the excellent quality of the oil obtained from its seeds. Information relating to the storage potential of *J. curcas* seeds from fruits at different maturation stages and on the relationship between seed viability, lipid peroxidation and activity of antioxidant enzymes during storage are scarce. Thus, the aim of this study was to investigate enzyme activity of the antioxidant defense system and the occurrence of lipid peroxidation during storage of *J. curcas* seeds obtained from fruits at different stages of maturity. Seeds extracted from fruit collected at three stages of maturity were used, based on the outer coloring of the skin, i.e., yellow, brownish-yellow, and brown (dry fruit). The seeds were subsequently stored for 18 months in Kraft paper bags in a laboratory environment. Initially and every three months, the seeds were evaluated in regard to germination, electrical conductivity, lipid peroxidation, protein content, and superoxide dismutase, peroxidase, and ascorbate peroxidase enzyme activity in the seed embryo. The seeds from the three stages of fruit maturity did not differ in regard to germination throughout the entire period of storage, with a reduction in germination being observed as of nine months of storage. Electrical conductivity was greater for the seeds obtained from brown fruit and less for seeds from yellow fruit, and these values increased during storage for all stages of maturity. There was a reduction in protein content and in enzyme activity of the antioxidant defense system in the seed embryo at the three stages of maturity, except for SOD. No relation was observed between reduction in seed viability and lipid peroxidation. Thus, *J. curcas* seeds with high initial quality may be stored under ambient conditions for up to 12 months without significant reduction in germination, when harvested after the yellow stage of fruit maturity. Seed deterioration during storage in ambient conditions occurs without there being lipid peroxidation and is associated with changes in enzyme activity of the antioxidant defense system in the seed embryo.

Acknowledgments

Fapemig, CNPq, and CAPES.

Tocopherols, rather than tocotrienols, protect seeds from lipid peroxidation during germination in *Chamaerops humilis*

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Chamaerops humilis (L.), the only dwarf palm native of continental Europe that is found in the Iberian Peninsula, accumulates tocotrienols rather than tocopherols in quiescent seeds, as it occurs in other monocots. To unravel the protective role of either tocopherols or tocotrienols against lipid peroxidation during seed germination; seed viability, natural and induced germination capacity, seed water content, malondialdehyde levels (as an indicator of the extent of lipid peroxidation) and vitamin E levels (including both tocopherols and tocotrienols) were examined at various germination phases in a simulated, natural seed bank. At the very initial stages of germination (operculum removal), malondialdehyde levels increased 2.8-fold, to decrease later up to 74%, thus indicating a transient lipid peroxidation at initial stages of germination. Tocopherol levels were absent in quiescent seeds and did not increase during operculum removal, but increased later dampening malondialdehyde accumulation. Thereafter, tocopherols continued increasing, while lipid peroxidation levels decreased. By contrast, tocotrienols levels remained constant or even decreased as germination progressed, showing no correlation with lipid peroxidation levels. We conclude that despite having a high amount of tocotrienols, seeds synthesize tocopherols to protect from lipid peroxidation when germination takes place, thus indicating that *de novo* synthesis of tocopherols, rather than tocotrienols, protect seeds from lipid peroxidation events during germination. By contrast, it is suggested that tocotrienols may exert an antioxidant role in quiescent seeds.

Evaluation of seed storage condition on isozyme activity in groundnut

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Seed viability and longevity is an important characteristic in conservation and management of seeds in genebanks. Though the ability to tolerate desiccation and retain viability for a long time is an attribute in orthodox seeds, seeds exposed to unfavorable storage conditions deteriorate and lose their viability. Apart from germination tests which are used to determine seed viability rates in periods of intervals, isozymes can also be used to detect biochemical changes in stored seeds. To evaluate the effect of seed storage conditions, isozyme patterns of seeds of groundnut (*Arachis hypogaea*) stored under short term (ST) storage (+4 °C) for more than ten years and new collection of the same species were compared. Banding patterns of four enzyme systems; Aspartate aminotransferase (AAT), 6-phosphogluconate dehydrogenase (6-PGD), Peroxidase (PER) and Alcohol dehydrogenase (ADH) were assayed. Clear differences were observed in the banding patterns between fresh (new collection) and ST stored samples. The banding patterns in the new collections were well resolved while the ST stored samples showed loss of activity or diffused bands for AAT and PER and lower intensity for ADH and 6-PGD enzyme systems.

Seed repair mechanisms during priming: synergy for a multidisciplinary approach in the PRIMTECH project

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Dehydration and rehydration during seed development and germination are associated with high levels of oxidative stress and DNA damage. Such stress condition also occurs during seed storage, leading to a loss of seed vigor and viability (Rajjou *et al*. 2012, *Annu Rev Plant Biol* **63**: 507-533); hence the key role played by antioxidant system modulation and DNA repair in different aspects of seed physiology, and the need to maintain their functionality for preserving seed vigour. Seed operators use priming to improve seed vigour: seeds undergo controlled rehydration, so as to initiate germination-related processes while preventing radicle emergence. Consequently, primed seeds are equipped with advanced germination and exhibit improved germination rate and uniformity. Moreover, seed priming is often implicated in improving the stress-tolerance of germinating seeds, the so-called 'priming memory' (Chen & Arora 2013, *Environ Exp Bot* **94**: 33-45). A multidisciplinary in-depth study (spanning from agronomy to physiology, biochemistry, and molecular biology) of the molecular mechanisms accompanying the seed response during priming is the main investigation subject of the PRIMTECH project (Advanced PRIMing TECHnologies for the Lombardy Agro-Seed Industry, www.unipv.it/primtech), funded by the Italian Lombardy Region and Cariplo Foundation. The project activity focuses on horticultural crops and bread wheat with the common goal of establishing reliable molecular indicators of the seed response to oxidative stress during early imbibition. Seeds of horticultural crop species (leafy vegetables) are monitored in terms of response to genotoxic stress and antioxidant activity. As far as wheat is concerned, three different functional categories are being monitored: DNA repair, antioxidant systems and methionine cycle. Preliminary results for both species highlight the effectiveness of the target genes as indicators of the seed ability to withstand genotoxic injury resulting from prolonged storage as well as industrial processing. Electron Paramagnetic Resonance-EPR technique is used in parallel investigations to provide qualitative and quantitative profiles of ROS (Reactive Oxygen Species) accumulation in seeds of the target species.

Stimulation of germinability of aged seeds by various physical actions

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Until now, it remained unclear why seeds that were stored for a long time and had lost the germinability, restores it after a weak short-term exposure to the factors of different nature. To answer this question it was necessary to understand why part of aged but living seeds did not germinate.

During storage, the heterogeneity of the seed population increases. The method based on the detection of room temperature phosphorescence (RTP) of dry individual seeds allows us to estimate the changes in seed quality and population heterogeneity. This method allows tosubdivide a lot of aged seeds into three fractions: strong (low RTP level), weak and dead seeds.

It has been shown that during aging some strong seed (fraction I) are transformed to fraction II. This leads to a decrease in germination percentage of seed lot. A characteristic feature of the seed fraction II is twofold greater content of glucose, indicating the activation process and the non-enzymatic hydrolysis of oligosaccharides (Veselova at al., 2015).

We used various seeds of low germination percentage: pea 80 and 38%, wheat 47%, barley 67%, oat 69% and cucumber 82%.

To stimulate the seed germination, the following techniques were applied: γ-irradiation, electro-magnetic field, electric field of the corona discharge, light-impulse irradiation, laser irradiation and sound action.

Measurements of RTP of seeds after any treatment showed that some fraction II seeds returned back to a fraction I. These seeds were cold "improved" because they produced normal seedlings and seed lot germination increase. A specific feature of such “improved” air-dry seeds was the reduction of glucose content in them, indicating the activation of non-enzymatic glycosylation reaction.

Seed treatments causing an "improvement" of seed quality do not repair the damage accumulated during seed aging, but rather favor their further deterioration. The "improved" seeds retain their high quality from several days to weeks or months.

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Galactinol as indicator of seed longevity

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Production of high-quality seed is the main aim of seed companies. However, seed quality is always threatened by seed deterioration. Reduced longevity or storability of seeds is recognized as a major problem contributing to increased costs of crop production. Galactinol is part of the raffinose family oligosaccharides (RFOs) that are known to be involved in stress tolerance defence mechanisms, as osmoprotectants, against abiotic stresses. The RFOs have been proposed to play an important role in conferring desiccation tolerance and storability to seeds. Studying the correlation between primary metabolites and germination phenotypes of Arabidopsis seeds we found a positive correlation among galactinol and seed longevity. This relation is likely conserved over plant species since we could confirm this finding in cabbage and tomato seeds. In order to unravel the role of the galactinol pathway in seed longevity we are currently applying a reverse genetics approach to delineate functions of enzymes (*Galactinol synthase 1* (*GolS1*), *Galactinol synthase 2* (*GolS2*), *Raffinose synthase* (*RS5*), *Stachyose synthase* (*STS*) and *Alpha-Galactosidase* (*AGAL*)) in the galactinol pathway.

Characterization of LEA proteins as potential markers for the prediction of seed longevity

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Seed ageing of oilseed rape has become an important topic over the past years because of the necessity to store seed lots for anticipating the next sowing campaign, especially in winter oilseed rape.

Here, we tested if late embryogenesis abundant (LEA) proteins previously associated to seed longevity in *Medicago truncatula* (Châtelain *et al*., 2012) can be used as potential markers of seed ageing in oilseed rape seeds. Sub-samples of the 15 seed lots from five cultivars provided in three batches each were stored at 30 °C and 75% relative humidity during six months and their germination and seedling growth were monitored monthly, except for the two oldest batches of the line variety which were tested every two weeks. Among five LEA candidates, CapLEA was the most interesting protein because its initial amount in seeds was correlated (R^2 from 0.79 to 0.98) to their germination speed after 110 days of mid-storage, to the germination rate after 145 days of moderate storage and to the germination after a controlled deterioration test (ISTA, 2014).

This work will illustrate how the seed knowledge established on a model species can be translated to crop species and reciprocally, how seed testing can be helpful to distinguish genetic from environmental determinants on seed longevity studies.

Acknowlegments

This work was supported by the Pays de Loire Region through the seed research program Qualisem. We also thank François Poutoire (Maïs Angevin), Chantal de Walsche (Euralis), Frédéric Corre (Syngenta Seeds), Isabelle Pauchet (UFS) for providing seed samples.

Physiological differences between purple and yellow-grained wheat with different anthocyanins characteristics during artificial ageing

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Purple-grained cereals, rich in anthocyanins, obtain increasing interest of not only scientists but also consumers from many different disciplines mainly because their suspected health-promoting properties. Consequently, their corresponding commercial value might grow as well. It is yet unclear if the accumulation of anthocyanins and related polyphenolic compounds negatively affects other valuable traits. Crop yield is still the major consideration for both farmers and food producers. Therefore, it is important to take into account the potential influence of anthocyanins on factors such as seed germination. Right now only two publications mentioned that seeds with pigments showed higher germination percentage than non-pigmented seeds during artificial ageing.

To further investigate the potential effect of anthocyanins on seed germination, seed materials are needed which differ only in the contents of anthocyanins, but not in other metabolic traits. We used wheat mapping populations generated by crossing two pigmented cultivars ‘purple’, ‘purple feed’(‘PF’) and two non-colored cultivars ‘Saratovskaya 29’ (‘S29’) and ‘Novosibirskaya 67’ (‘N67’), i.e. ‘N67’×‘Purple’, ‘N67’×‘PF’, ‘S29’×‘Purple’, ‘S29’×‘PF’, and then equal numbers of yellow and purple offsprings were randomly pooled for bulk propagation. Afterwards, targeted and non-targeted metabolic analyses were performed to demonstrate the metabolic equivalence of the bulks except their anthocyanins contents. We found only slight differences in amino acid contents and elemental composition between different bulks. Eventually, applying artificial aging treatments prior to the assays, germination percentages of four wheat parent lines and corresponding bulks were compared. Differences of germination rates among purple-grained parent lines and yellow-grained parent lines were significant. However, the germination rates of the anthocyanins rich and the anthocyanins-free bulks did not differ. We tentatively conclude that anthocyanins do not have an impact on seed germination in the wheat accessions tested.

Exogenous glutathione pre-treatment alleviates the loss of seed vigour of Siberian wildrye (*Elymus sibiricus* L.) during artificial accelerated ageing

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The objective of our study was to determine the effect of exogenous glutathione (GSH) pre-treatment on seed vigour, membrane lipid peroxidation and ultrastructure of embryo cells of Siberian wildrye (*Elymus sibiricus* L.) seeds under artificial accelerated ageing condition, which was set at 45 °C and 100% relative humidity for up to 72 h. The results showed that seed germination, seedling growth and vigour index in control presented gradually declining trend, and electrical conductivity and malondialdehyde (MDA) content increased with the accelerated ageing duration prolonging. Moreover, ultrastructure of embryo cells was serious damaged in 72 h accelerated ageing duration, presenting that cellular and nuclear membrane were broken, nucleolus was pyknotic and mitochondria swelled. However, GSH pre-treatment could enhance the tolerance of seed to ageing and alleviate the loss of seed germination, vigour index and seedling growth. Membrane integrity was protected including ultrastructure of embryo cells and mitochondria, and correspondingly, electrical conductivity and MDA content were alleviated, particularly at slight damage level. Alleviation effect of glutathione pre-treatment on seed vigour loss maybe due to the protection to membrane integrity of embryo cells and mitochondria, and the mechanisms for further study are required.

Comprehensive mitochondrial metabolic shift during the critical node of seed ageing in rice

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It is already widely recognized that seed vigor may be affected by mitochondrial activity. However, to date, few studies have investigated the mechanism of seed ageing in relation to severely limited mitochondrial metabolism, especially the changes in mitochondrial metabolism during the critical node. The critical node is the transformation from Phase I to Phase II of the seed ageing reverse S-shaped curve, which directly influences seed preservation. Although mitochondrial dysfunction plays a key role in seed ageing, the metabolic shift in the critical node remains poorly understood. Here, we investigated the mitochondrial regulatory mechanisms during the critical node (Phase I–II) of rice seed ageing. Seed was artificially aged at 40 °C and 75% relative humidity for various times (3, 4, 7, 10 and 14 d). The germination percentage significantly dropped from 97% to 92%, 84%, 61%, 22% and 8%, respectively. We chose the seed viability at 4 d aging as the critical node. We showed that during the critical node of seed ageing, the mitochondrial ultrastructure was impaired, causing oxygen consumption to decrease, along with cytochrome *c* oxidase and malate dehydrogenase activity. In addition, the transcript of the alternative pathway of the respiratory chain was significantly induced, whereas the transcript of the cytochrome oxidase pathway was inhibited. These changes were concomitant with the down-regulation of mitochondrial protein levels related to carbon and nitrogen metabolism, ATP synthase complex, TCA cycle, mitochondrial oxidative enzymes, heat shock proteins, and a variety of other proteins. Therefore, while these responses inhibit the production of ATP and its intermediates, signals from mitochondria (such as the decrease of cytochrome *c* and ROS accumulation) may also induce oxidative damage and cell death, triggering the accelerated ageing process in Phase II. These events provide considerable information about the mitochondrial metabolic shifts involved in the progression of seed ageing in the critical node. However, the way in which these events are regulated requires further research.

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