Meeting Report – W3168

Basic Information

Project No. and Title: W3168 : Environmental and Genetic Determinants of Seed Quality and Performance

Period Covered: 10/01/2014 to 09/30/2015

Date of Report: 03/07/2017

Annual Meeting Dates: 10/10/2014 to 10/11/2014

Participants

Bennett, Mark – The Ohio State University, Bradford, Kent – University of California, Davis (joined by video-link), Geneve, Robert – University of Kentucky, Jourdan, Pablo – The Ohio State University, Leskovar, Daniel – Texas A&M University, McGrath, Mitch – USDA-ARS and Michigan State University, Nonogaki, Hiro – Oregon State University, Perez, Hector – University of Florida, Taylor, Alan – Cornell University, Walters, Christina – USDA-ARS and Colorado State University, Welbaum, Greg – Virginia Tech.

Brief Summary of Minutes of Annual Meeting

Pablo Jourdan welcomed the participants to Ohio State and provided logistical information about the venue. Robert Geneve agreed to chair the meeting. Minutes from the 2013 W2168 meeting were discussed and approved. A general discussion about the new structure for the group and organization of the meeting ensued. Taylor provided some background on the need for the new approach. The overall goal is to foster greater collaboration among the group members. An important element is the identification of funding sources to support the common efforts of the group. Potential new members were identified, and will be contacted by current members explaining the advantages and benefits of participating in the W3168 project. A general consensus was formed regarding the need to keep seed science as an important topic for research and education. There is a perception that there are 'no problems' in seed-related areas and that industry takes care of all seed issues, but this is clearly not the case. There is significant industry involvement in the major row crops, but there is much other seed work that is not being addressed. Of particular concern is the training of future seed scientists. These scientists will come from active research and educational programs in seed science at different institutions. Nonogaki suggested that education is a common core activity of the group. Shared educational activity is an area that the group can develop as part of its future collaborative efforts. Discussion of individual objectives followed, stimulated by presentations from individual participants. A discussion was also held about the group's endorsement of Bradford's offer to host the 2017 International Society for Seed Science meeting in Monterey, CA. An overview of the proposal to the ISSS was given and there was unanimous enthusiasm for assistance with the scientific program of the meeting. State reports were then presented.

Objective 1 - Identifying key factors involved in the enhancement or loss of seed quality. Seed development through post-harvest losses in storage. Specific topics include seed development, desiccation tolerance, and aging in storage.

CO: Walters. Relayed recent work focusing on seed aging kinetics and seed longevity.

FL: Perez. Related activities supporting *Uniola paniculata* (Sea-oats) germplasm collection and preservation. Sea oats is a keystone species of coastal dunes throughout the south-eastern US and Caribbean region.

CA: Bradford. Described research on population-based threshold models that describe seed respiration and germination rates.

OH: Jourdan. Described seed research at the Ornamental Plant Germplasm Center on the campus of The Ohio State University.

Objective 2 - Eliminating seed dormancy as a constraint during seed production and germination in agronomic seed production and ecological/biomass seed establishment. Pre-mature sprouting in cereals and other species and the identification dormancy mechanisms to manipulate germination.

KY: Geneve. Discussed work on seeds of the Asteraceae, and showed stunning images of the internal structure of seeds.

CO: Walters. Discussed elimination of seed dormancy in seeds used for restoration projects by seeking to understand the clock mechanism in dry seeds to increase the success of direct-seeded restoration projects such as the germination of Monterrey pine seeds.

CA: Bradford. Presented video ‘A Dual Role for DOG1 in Regulating Seed Thermo-inhibition and Flowering Time in Lettuce’.

OR: Nonogaki. – Recent work on the manipulation of seed dormancy and germination via a new ‘Spontaneous Hyperdormancy’ system was presented. The Spontaneous Hyperdormancy system can be used to prevent pre-harvest sprouting in cereal crops.

SD: Gu. (excused) Submitted report on recent results involving genetic dissection of tissue-based controls on seed dormancy.

Objective 3 - Enhancing seed vigor and germination in agronomic and other species for improved stand establishment. The emphasis of this objective is on post-harvest technologies.

MI: McGrath. Described progress on understanding and genetically improving sugar beet seedling vigor, emergence, and stand establishment.

NY: Taylor. Summarized recent work on chemical and biological seed treatment and seed coating technologies for conventional and organic systems for high value vegetable seed markets.

TX: Leskovar. Relayed progress in vegetable physiology in the 2^{nd} largest winter garden in Texas focuses on adaptation mechanisms to environmental stresses (heat and drought), and development of integrated sustainable vegetable cropping systems particularly for stand establishment of transplants.

VA: Welbaum. Described his work on thermogradient tables using a gusseted soil chamber to study seed germination under realistic conditions, with other modification adding LED lights for illumination, and micro-irrigation research and a greenhouse cover to increase flexibility in the types and age of plants that can be studied.

FL: Perez. Described research on enhancing seed germination of *Aristida stricta* (wiregrass), a southeastern US native plant in fragmented habitats.

KY: Geneve. Described research on extensive variability of mucilage production and forms from the pericarp of seeds in the Lamiaceae, where it is not clear what critical function such an expensive resource may assume.

Accomplishments

Objective 1 - Identifying key factors involved in the enhancement or loss of seed quality.

CO: Walters. Dynamic mechanical analysis (DMA): measures the tendency of molecules in structures to move, and in peas with 7% water content, there is still motion in seed glasses even at low temperatures. Potential tools to predict longevity and monitor deterioration using small sample sizes have been identified including mechanical analysis, liquid crystallization, volatile assessment, and RNA integrity. In addition, the team is validating predictive tools using historical experiments, such as the Went/Munz experiment where seeds from 93 California native species were placed in sealed ampoules in 1948 and kept at room temperature, then tested for germination in 2014. Some seeds showed remarkable longevity when stored very dry at room temperature; for example, *Clarkia elegans* with germinated to 90% after 2 days. Walters also discussed the ambitious genebanking standards approved in 2013. These emphasize longevity in genebanks to mitigate genetic erosion during regeneration. Research is needed to clarify standards on 'critical' moisture level, the risk of over-drying, optimum drying protocols, optimum harvest time, and viability monitoring frequency.

FL: Perez. Mature seeds of *Uniola paniculata* (Sea-oats) were collected from north and south Florida populations, and data was collected on the relationship between relative humidity and seed water content (i.e. water sorption isotherms), germination ability of seeds following equilibration to a range of relative humidity (i.e. desiccation tolerance), and the germination ability of seeds following factorial treatments of desiccation and duration of storage in liquid nitrogen. Seeds of both populations had similar water sorption isotherms, and the capacity for germination in both populations was retained to high levels (ca. 63-99%) following equilibration to very low (0.5% RH) moisture conditions. Likewise, germination ability remained high (ca. 59-94%) following desiccation and storage in liquid nitrogen for up to the maximum duration tested, i.e. 24 hours.

CA: Bradford. Respiration associated with seed vigor and vitality: The tetrazolium test indirectly indicates actively respiring tissues. Tomato seed respiration and germination responded to both temperature and water potential in two genotypes (T5 and PI341988). At low temperatures and water potentials, respiration continued at slow rates even when germination would be very slow or completely inhibited. Because of limitations to bulk respiration measurements, it would be better to be able to measure the respiration rates of many individual seeds using the Q2 seed respiration instrument. Germination rates are sensitive indicators of seed quality, and respiration rates reflect germination rates. Some important observations: Seed respiration is initiated immediately upon hydration; Respiration rates are closely correlated with germination rates for many seeds and under diverse conditions; Individual seed respiration rates as determined in the Q2 can be converted into "respiration population time courses" that are similar to a germination time course; Analyses and models that are applied to germination time courses can also be applied to these respiration time courses, describing the effects of temperature, water potential, aging and other factors; Automated measurement of seed respiration on a population basis can reveal diverse components of seed quality.

OH: Jourdan. Recent focus has been on developing reliable quantitative germination tests for the very small Begonia seed using an agar substrate and applying this method to compare the germination characteristics of six different species. Other work aims to identify key parameters that affect seed quality in Phlox to generate seeds for germplasm preservation. Also working to enhance Phlox germplasm by interspecific hybridization. Collaborating with Walters (CO) on seed characteristics Begonia and *Magnolia virginiana*, with the goal to determine potential longevity of these seeds in storage, as well as with Perez (FL) on seed internal structure as related to viability using an X-ray procedure, and with Geneve (KY) examining the pattern of germination of Begonia seeds.

Objective 2 - Eliminating seed dormancy as a constraint during seed production and germination in agronomic seed production and ecological/biomass seed establishment.

KY: Geneve. Discussed work on seeds of the Asteraceae, and presented stunning images of the internal structure of selected seeds of this family.

CO: Walters. Discussed elimination of seed dormancy in seeds used for restoration projects by seeking to understand the clock mechanism in dry seeds to increase the success of direct-seeded restoration projects such as the germination of Monterrey pine seeds.

CA: Bradford. DELAY OF GERMINATION 1 (DOG1) was identified in the Cvi accession of Arabidopsis as a QTL conditioning a requirement for long after-ripening times for the alleviation of dormancy. DOG1 expression is also sensitive to the temperature of seed maturation. Higher expression of DOG1 is correlated with stronger dormancy. A DOG1-like gene is expressed during lettuce seed development slightly after expression of NCED4 (encoding an ABA biosynthetic gene) begins. However, silencing NCED4 expression did not appreciably alter expression of DOG1, similar to results in Arabidopsis. DOG1 expression in mature lettuce seeds decreased as the temperature during seed maturation increased. Germination at 34°C of freshly harvested Arabidopsis wild type and *dog*1-1 mutant seeds that were grown at either 10 or 22°C. Regardless of maturation temperature, *dog*1-1 mutant seeds germinated better at high temperature. Overexpression of the lettuce DOG1 from two thermosensitive genotypes (*L. sativa* cv. Salinas and *L. saligna*) and two thermotolerant genotypes (*L. serriola* and *L. sativa* PI251246) can rescue the *dog*1-1 mutant. Silencing of the lettuce DOG1 in the thermosensitive cv. Salinas improved seed germination at high temperature. In summary, Lettuce DOG1-like genes are functional in Arabidopsis and promote seed dormancy and thermoinhibition; DOG1 action lowers maximum germination temperatures; DOG1 action also delays flowering times, as silencing DOG1 in lettuce results in early bolting and flowering; DOG1 may act on both germination and flowering via potentiating the activity of miR156, which has other effects on the plant life cycle; and DOG1 may be involved more generally in sensing environmental conditions (particularly temperature) and modifying life cycle progression (germination, flowering) through influencing the action of microRNAs.

OR: Nonogaki. – For the hormonal regulation of seed germination, the previous studies on the role of abscisic acid (ABA) were expanded. In addition to GeneSwitch technology, a chemically inducible gene expression system which allows induction of seed dormancy by a chemical ligand, a new technology 'Spontaneous Hyperdormancy' was developed. In this system, the nine-cis-epoxycarotenoid dioxygenese (NCED) gene driven by an ABA-inducible promoter causes amplification of NCED expression in a spontaneous manner. The Spontaneous Hyperdormancy system can be applied to preventing pre-harvest sprouting in cereal crops.

SD: Gu. Completed the anlyses of an endosperm genotype-based genetic approach to determine genes regulating seed dormancy through the embryo, endosperm or maternal tissues. Using this new approach, previously identified seed dormancy loci SD12, SD1-2 and SD7-1 were demonstrated to be involved in the regulation of germinability through the embryo, endosperm and maternal tissues, respectively, in rice. Functional analysis of candidate genes cloned from the SD12 seed dormancy QTL in rice were completed, and map-based cloning of the seed dormancy QTL Sd1-2 and SD7-2 was initiated.

Objective 3 - Enhancing seed vigor and germination in agronomic and other species for improved stand establishment.

MI: McGrath. Germination is crucial to developing healthy, vigorous, and productive field populations of sugar beets. Despite planting high-quality, technically-augmented seed for growers with very high germination (>92%), field emergence and persistence continues to hover at ~60% in Michigan. Previous research suggests this difference is the result of stress during germination in the field environment. The East Lansing sugar beet program has focused on stress responses during germination. One way to identify additional genes involved is to examine expression of all genes during germination in different environments. In this case, we generated four transcriptome datasets of a high vigor variety germinated in stressful germination-in-solution laboratory environments, including water, hydrogen peroxide, sodium chloride, and hydrogen peroxide plus sodium chloride. From over 200 million sequences assessed, and in comparison to water, each treatment showed between 75 and 100 statistically significant, differentially expressed genes, with approximately 10% overlap between any pair of treatments, and only seven shared among treatment comparisons. Approximately 50% of these have no descriptors yet found in nucleotide sequence databases, suggesting germination genes are less well represented in comparative genomics resources.

NY: Taylor. Developed rotary pan equipment able to treat as few as 25 grams of seed with novel coatings, and tested it on onion seeds that were pelleted, film coated, and encrusted. Fillers typically used are diatomaceous earth, clays, and calcium carbonate. Pelleting is often done for precision seeding, especially for seed with irregular shapes or elongated seed, but adds 6-7 times the weight of raw seed, thus an application and transportation cost to seed producers. Pellets provide a physical barrier, with some pellets splitting to release the seed, whereas others melt away upon watering. Also developing applications using multiple seed pellets (agglomerates) for situations where stand could be improved with numerous propagules per placement. Developed an alternate method for onion testing, an organic peat medium in rolled paper towels, that is accepted as an AOSA rule. A new AOSA rule has expanded from our onion method called the Cornell method to include all crop seeds and there is a new ISTA rule for the same. Systemic movement of insecticides in treated or pelleted seeds are also of concern since their hydrophobicity determines whether they are absorbed through the seed coat and taken up by the embryo or blocked by seed coat and then taken up by root. In onion seeds (also tomato, pepper, switchgrass, field & sweet corn), non-ionic molecules (assessed by the fluorescent tracer dye coumarin) can move through seed coat but ionic (i.e. rhodamine dye) cannot, and is instead taken up by the root as the result of a selectively-permeable testa. Cucumber and lettuce have an impermeable testa, to both rhodamine and coumarin. Systemic insecticides from seed treatments may last 30 days post-emergence, spread apoplastically and move with transpiration stream, and do not accumulate in the fruit. Biostimulants may also be applied as seed coating, such as proteins where gelatin may enhancement growth, soy flour may lead to promotion of growth of Brassica seedlings, but in all cases, side effects of seed treatment may result in increased proportion of abnormal seedling development, perhaps caused by high loading rates on the seed coats.

Taylor also highlighted recent collaborations with Ohio State (Bennett, Jourdan) on *Taraxacum kok-saghyz* seed coating and germination research, W3168 member Brian Nault on entomological treatment of seeds to control leaf hopper (thiamethoxam) and onion maggot, as well as testing organic-approved treatments of seeds (spinosad), and with Sally Miller on bacterial seed treatments (there are few bacteriocidal seed treatments).

TX: Leskovar. Development of integrated sustainable vegetable cropping systems for Texas emphasizes seed-transplant production and physiology to increase plant survival and enhance stand establishment, plant hormone applications to modulate seedling growth, determining root/shoot developmental responses to water conservation strategies and irrigation technologies, the impact of cropping systems on antioxidants and sensory attributes of vegetable crops, and genotype selection for drought resistance, high yield, quality, and phytochemical content. A recent emphasis has been examination of root genetic traits using electromagnetic scanning systems to breed for specific root attributes.

VA: Welbaum. In addition to the further development of the thermogradient table, research on bacterial fruit blotch in cucurbits was investigated. Nano-chitin seed treatment (during seed priming) was tested to increase disease resistance of plant seedlings with both chitin and nano-chitin seed treatments inducing expression of chitinase in developing and germinating seeds. Welbaum also highlighted recent collaboration with Bradford on the production of a new vegetable production textbook.

FL: Perez. Quantified the proportion of filled, unfilled, and infected seeds of *Aristida stricta* (wiregrass) across seven populations occurring throughout the southeastern US, the germination and presence of contamination and treatment in response to commercial bleach disinfection, and identification of fungal pathogens. Approximately 55-90% of seeds were non-viable due to lack of seed fill (49-77%) or fungal contamination (2-25%). Populations were similar for seed lot quality when grouped by habitat (e.g. xeric vs. mesic). However, populations were different for seed lot quality when grouped by geographic region. Seeds from all populations displayed infection by *Curvularia* and most also showed signs of *Sorosporium* infection, and the use of bleach solutions did not eliminate pathogens on otherwise viable, healthy seeds suggesting that internal tissues may harbor infection.

KY: Geneve. Examined numerous species of the Lamiaceae including chia (*Salvia hispanica*) for mucilage morphology during hydration. Seed mucilage may have a role in adhering to a substrate prior to germination, and may also sequester moisture needed during germination. It was observed that little or no mucilage layer in visible via microscopy in dry seed, but that within a few minutes of hydration, the layer expands to the seed diameter or greater. Further, the unfolding was filmed and each species had it's own characteristic unfolded structures and morphology. Seed mucilage has potential human health benefits, and production of these species is increasing.

Impacts

Seed desiccation is a long-term biological preservation mechanism that allows seed to remain viable in the absence of conducive growth conditions. However, the seed must retain a small amount of moisture, and the fate of this moisture impacts longevity. Identification of seed lots of various species that differ in longevity will allow researchers access to the mechanisms relating seed storage, moisture content, and viability for germplasm preservation.

Native species in threatened habitats require conservation and regeneration of seed for habitat restoration. *Uniol*a paniculata seeds possess significant desiccation and cold tolerance, and it was shown that its seed stored under genebank conditions (5-8% moisture content; -18 or -196°C) was feasible, thus improving the conservation potential of this species.

Viable and non-viable fractions of harvested seed can be highly variable between reduced and fragmented populations of widely distributed taxa. Seed viability appears to be associated with characteristics of individual populations and geography rather than habitat. This knowledge is being used to improve collection strategies in order to preserve the genetic diversity of native threatened species.

Unlike the previous GeneSwitch system, the Spontaneous Hyperdormancy system is designed to respond to the native ABA and is triggered without chemical application. Therefore, the new system is more practical. Since the mechanism of hormonal regulation of seed dormancy is highly conserved among many species, the technology can potentially be applied to various crops. The translational research unexpectedly discovered a positive feedback mechanism in the regulation of ABA biosynthesis and signaling, which is advancing our understanding in basic seed biology.

Germination is crucial to developing healthy, vigorous, and productive field populations of sugar beets. Despite planting high-quality, technically-augmented seed for growers with very high germination (>92%), field emergence and persistence continues to hover at ~60% in Michigan. Two biochemical pathways that influence seed germination and seedling vigor in ways that can improve emergence potential were discovered, and thus can be used as breeding criteria for improve emergence and stand establishment.

Stress germination transcriptomes were obtained, and found to contain many statistically significant differentially expressed genes for which no function can yet be deduced. Stress germination is the experimental measure we use as the definition for seedling vigor, a notoriously difficult quality of germination to measure. Current results will be used to narrow the definition of seedling vigor to specific genes involved in germination under stress.

Respiration is a key predictor of seed quality, and novel instruments were used to rapidly assess respiration is single seed. Such single seed measures now allow respiration to be determined on populations of seeds rather than in bulk as is now currently practiced, and follow the time course of seeds germinating relative to respiration rates. The Q2 instrument has the potential to replace time-consuming and labor-intensive repeated observations required to obtain germination time courses, the most sensitive indicator of seed quality.

Coating technologies can enhance seed performance, but small seed lots are difficult to treat uniformly. A device was constructed that allows relatively small seed lots to be coated with virtually any potential seed enhancing compound for preliminary evaluations. A wide range of compounds is being tested, allowing seed producers to hone in on the most beneficial seed treatments at minimal expense.

Publications

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