MINUTES OF ANNUAL MEETING
W5168 Multi-state Research Project
Environmental and Genetic Determinants of Seed Quality and Performance
South Dakota State University, Brookings, SD
July 17 – 19, 2025

Summary: In brief, the W5168 meeting took place in Brookings, SD, with a dinner on July 17th, bringing participants together. Over the next day and a half, participants heard 9 state reports, multiple presentations from SDSU seed affiliated researchers, toured 3 facilities including the state of the art teaching facilities for the Department of Agronomy, Horticulture, and Plant Sciences at SDSU and several seed labs in the public and private sector.

Friday, July 18, 2025

The meeting was called to order by the Chair of the group, Tomo Kawashima, at 8:25 AM. He welcomed the participants, and the plans and goals for the meeting were discussed. Welcoming remarks were made by Dr. Blanton, the Director of the South Dakota Agricultural Experiment Station and Dr. Turnispeed, the head of the Agronomy, Horticulture, and Plant Science Department at SDSU.

Dr. Blanton provided a welcome, describing the agricultural environment in South Dakota, remarking on while the population of the state is under 1 million, 40 million of 49 million acres are privately owned and there are unique agriculture rules.

Dr. Turnispeed highlighted SDSU, indicating that there are 12,000 students at SDSU, of which 131 students were in Agronomy, 22 in Horticulture, and 73 in precision agriculture. There are 65-75 Graduate students.

Dr. Xingyou, our SDSU host, provided an overview of the meeting and the logistics for our stay.

Dr. David Clay, Professor at SDSU, gave final opening remarks at 8:50am, discussing SDSU's approach in to precision agriculture research in education as technology integration into precision agriculture. He describe it as, "A management strategy that takes account of temporal and special variability to improve sustainability of agricultural production." 75% of the nitrogen in the plants was coming from the soil and not the fertilizer. Only 25 % of the nitrogen was coming from the fertilizer. The Nitrate adsorbed to the soil despite the negative charges in both, potentially the microbes in the soil account for this retention rather than having the nitrate percolate with the percolating water. Cover crops help reduce greenhouse gas emissions.

- New Precision Farming Textbook by Clay et al. 2017, 32 Chapters, new edition coming.

The first **State report** was provided by Dr. Imtiyaz Khanday University of California (CA): Understanding Seed Dormancy to Improve Germination and Vigor. Khanday reported on understanding the acquisition of vigor traits and dormancy regulation. They investigated seed dormancy in tomatoes, noting a lot of variability in seed development even within the same plant. Had to synchronize the seed development stage. Single seed germination dynamics looking at oxygen use. As soon the seed starts to germinate they use the O2 trapped in the tube. A timeline developed indicated the capacity to complete germination is between 25-28 Days after pollination. Desiccation tolerance was acquired 28-32, and Dormancy alleviation was at 32-38.bRapid completion of germination 45-66 DAP.bUsed a fluorescent Citrine marker for their CRiSPER Cas 9 system to ko NCED genes so they can confidently remove the transgene post NCED gene alteration. Targeting NCED responsible for dormancy but, apparently, not plant stress tolerance/stomatal movement. There were many NCEDs in tomato.

Following Dr. Khanday's state report, **a break and introductions** of individuals present was completed.

Following the break, **a talk was given by Dr. Lan Xu** from Dept of Natural Resources Management, SDSU, entitled, "Strategic grazing and fire on soil seed bank: Impacts of strategic Grazing and fire on soil seed bank heterogeneity in mixed-grass prairie."

Dr. Xu described research on short, tall, and mixed-grass prairie including impacts of drought, fire, and grazing stresses. Despite these stressors, native prairies can maintain the structural diversity of the grasslands, pollinators, and animal populations. The switch from native prairie to grazing land for cattle (repressed fire),homogenous grazer populations, led to diversity decline. Noting heterogeneity: "An area that consists of dissimilar or diverse components or elements; that are not uniform" he argued that all scales are important, course, medium, fine. Management of fire and grazing properly can increase heterogeneity. Months since fire from 0 to 36 shows a large difference in bird species occupancy of the same site. He looked at winter patch grazing as an alternative to fire (i.e. non-pyric strategy) in the Norther Great Plains. Seed bank versus Bud bank. Mixed grass communities are Bud Banks.

- Seed bank preserves genetic diversity: diversity potential. Bud Bank preserves current population persistence.
- Heavily grazed areas may not have the opportunity to produce seeds, over time, no viable seed bank. Difference between above ground visible heterogeneity versus seed bank heterogeneity.

Study: Three pastures (Blocks) Three treatments (Conventional Grazing (CG), Winter Patch Grazing (WPG), Wild Fire (WF)) with enclosures. Had a fire in 2016. Data collection included above ground vegetation sampling and soil seed bank sampling. They identified the species of the seedlings that result from the seed bank grown in the greenhouse. Findings included:

- KBG more abundant in CG, lower in WPG, and lower still after WF.
- Species evenness and richness more similar between CG and WPG compared to WG. WF dominated by 3 species = low diversity.
- WF has negative impact on seed bank richness, diversity and density compared to WPG and CG
- WF appears to reduce perennial graminoid and introduced species and their seed reserve.

All the ranchers are capitalizing on Birder tours on their acreages where Birders will pay to see bird species in a native habitat. This Eco-tourism benefits from increased bird diversity (more species crosses, life-listers) per hour spent scanning.

11:10am – 11:40am – **Tour of the new state-of-the-art building**, student teaching labs including mechanics and hydraulics, open teaching spaces.

Talk by Guilherme Oliverira: SDSU, PhD student. Oat Chromosome rearrangements: SDSU lines Characterization and implications for breeding.

Avena sativa L. Hexaploid A, C, D genomes 42 chromosomes 10.8 Gb. 500,000 years = relatively new. Zea mays, 2.4 Gb 20 chromosomes. Wheat also 6x A, B, D 16 Gb. 3 M years = relatively old. Oat has a Mosaic genome and a lot of chromosomal rearrangements. Olverira studies resistance to crown rust. Amagalon variety. 9,341 SNP markers. KMeans method. PCA to evaluate his germplasm. Areas within the genome within the inversions/translocations that are linked in coupling phase. This is good if this is a beneficial trait, but if it is bad, and no recombinations, there is little to do to break the linkage. Signatures of chromosomal rearrangements on sever chromosomes.

High frequency of crosses with Amagalon=-derived lines in past is affecting the present by causing stratification in the breeding population. Should we prioritize crosses within the same cluster to shuffle the existing variation, or explore biotechnological approaches to induce recombination in these regions? Interestingly, GWAS does not reveal any associations in these regions. There is value in characterizing chromosomal rearrangements to inform breeding efforts. No recombination between clusters in the rearranged regions.

12:20PM LUNCH

2:00 PM **State report,** Dr. Eric Olson, Michigan: MSU Wheat Breeding and Genetics: Preharvest sprouting and Falling Number research highlights.

Soft white wheat commodity. Visual sprouting was formerly the "problem". However, falling number (Alpha Amylase activity) is more discerning. They are moving towards falling number evaluations exclusively. Select varieties that maintains a high FN under experimental conditions, using select varieties with different flowering date and physiological maturity to spread risk. Harvest when conditions are optimal even if this means harvesting at higher % moisture. Consider test weight loss of ~ 0l75 to 1 lb per day.

Visual sprouting and falling number experimental overview: Harvest spikes, after-ripen 5 days in Green house. Mist 3 d in greenhouse, sprout 2 additional days in growth chamber. Found that alpha amylase in red wheat seems to plateau under these conditions while in the white wheats, it continues to climb.

Critical number for FN is 250 and that is what the companies are looking for. Over 250 is acceptable and under this is typical for red wheats. Soft white wheats is another scenario with many falling below 250 by a good margin. Awnless SRW varieties demonstrate a smaller FN due to not leading water to the seed.

SRW can maintain a FN above 250 even after 2-4 days of rain. Misting on multiple days with or without days of dry intervening. SWW FN crashes after only two consecutive days misting. A lot of variability in this though.

At 2:30pm, **State report,** Dr. Ramin Yadegari from Arizona State gave a virtual talk on: "Endosperm gene regulatory networks in maize and sorghum."

Objective I: Understanding how genetic and environmental mechanisms affect the seed germination process. Hypothesized from his work that there are a set of MYB TF genes whose proteins are responsible for (in part) NETL photo assimilate uptake and transit to the endsoperm and endosperm cell types. They can identify gene networks that are regulated by orthologous TF gene proteins that are regulating target genes at the correct time in maize and sorghum that are impactful for endosperm development.

Big Questions

- What gene regulatory networks function in cell types during differentiation and proliferation? How BETL cell differentiation regulated?
- How is endosperm development regulated by environmental queues?
- How is the transition from maternal to zygotic seed nutritive storage regulated?
- Utility of altered metabolite uptake through BETL to engineer desired N/C balance in the endosperm?
- Regulation of metabolite allocation and signaling processes during vegetative plant-ear-kernel axis?
- Metabolic bottlenecks in endosperm development.

End of state reports for the day.

The entire group toured the SDSU Seed Testing Lab led by Kathy Mathiason, the SGS North America Inc. Corporate Headquarters, Brookings, SD, and McCrory Garden.

Dinner concluded the evening.

Saturday, July 19, 2025.

8:30am State report, Dr. Hector Perez, University of Florida: "Why does seed quality vary so much in native species?"

The report focused on Sea oat, (*Uniola paniculata* L.) coastal dune grass and Wire grass. Growers are having difficulty growing out seedlings for out-planting. Strongest predictor of seed production is panicle density. A Goldilocks story, not too many, not too few? OR the more the merrier (seed production wise).

Wire grass carries fire well. Without it, this Longleaf Pine ecosystem would not have the duration or intensity that is required to maintain this ecosystem. Wiregrass is a fire obligate species. Land managers now can request a prescribed burn as early as January. Typically, the ecosystem burns during lightning season which is not January. What are the implications for the switch in timing?

In researching wiregrass, some challenges arise. Wiregrass is notorious for empty seeds and they are also infected frequently. So, awns, and the empty/infected seeds makes seed cleaning a challenge, so typically sowing A LOT of seeds to get a few seedlings. They are asking the question of how does fire timing, and other environmental factors, influence seed quality. Seed quality writ large, Desiccation tolerance and longevity are two aspects of quality that Hector has published on PLOS one.

Take home is that although the seeds can withstand desiccation well, they do not have much longevity in the dry state. So, they are short lived in storage. To the land managers, if you are going to be using these seeds, you must use them quickly post-harvest.

Going beyond just germination, what types of environmental predictors can be useful for seed numbers for *Aristida beyrichiana*? Habitat, Burn month, prefire rainfall and rainfall during development were all highly significant predictors of good seed production. Odds ratio, the odds of filled seeds is 3X greater in mesic habitat. Odds of filled seeds 5X greater chance of having filled seeds after a June burn relative to a March burn. Don't burn early!

Odds of filled seeds for both rainfall parameters were negative, i.e. rain is a negative. BUT, this is probably due to infection due to high humidity. This seems to be Goldilocks in that some rainfall is good, but it plateaus and then becomes detrimental.

State report, Dr. Sabry Elias, also D. Angsumaiee, Dept of crop and soil science, University of Oregon: “Changes in gibberellic acid and abscisic acid contents of red clover during seed development and maturation”.

Red clover, Forage legume, seed production in Oregon. 10-18% yield increase by irrigation and Trinexapec etyl (TE) application. Growth regulator, retards the growth. Probably GA inhibitor because it makes the red clover shorter. Redirects energy into seed development from vegetative growth. Red clover produces hard seeds that, over time, lose this trait and can germinate (take up water) and complete germination. Palisade EC (TE) GA-inhibitor and Irrigation influence both [GA] and [ABA]. Testing from anthesis to harvest. Sampled weekly. Untreated vs TE foliar application irrigated vs non-irrigated plots. Data collected GA3 and ABA quantification using solid phase extraction LC-MS also seed quality, weight moisture. Results No significant difference from week 2 to week 5 (crop harvest). ABA content decline from wk 2 to wk 3 and then more gradual decline thereafter. The ratio of ABA/GA declines over seed development and in pace with the completion of germination. Irrigation also did not influence [GA] or [ABA] over time. So, how does it influence seed germination performance positively? TE application also were not influenced. Physiological dormancy is NOT a substantial concern in red clover seeds.

State report, Dr. Cindy Finneseth, University of Kentucky, Cut flower research project with small scale cut flower growers. Working with 60 native perennial species mainly forbs, one grass. Plugs planted in October/April, variable spacing 12-36”, 3 irrigation regimes in year 1, wood chip mulch. Seed research on these species includes: Plant Mortality, Flowering time/floral production, Pest/disease resistance, Physiological/Harvest maturity, Dormancy/Dormancy alleviation, Synchronization of the completion of germination.

Observations noted: Winter kill**,** Early flowering for some species. Short stems, commercially this is not good. How can we alter this? Supports/Trellising may be required to prevent lodging. Weed control, the wood mulch won’t cut it.

Invitation to Partner with W5168 participants: Seed Collection opportunities with Florida possibly, Lab Exchange programs.

State report, Dr. Bruce Downie, University of Kentucky: “Natural and repair mechanisms in seed and how influences seed longevity”

New toy: nanotemper dianthus – molecular interactions between any two entities – gives you a disassociation. Protein to protein interactions: what do they bind to (protection). Proteins, especially with water, can misalign but there’s ways to fix it. LEAP – physical binding to diverse client proteins in vitro. Problems – LEAP are intrinsically disordered – defined by their client proteins to which they bind. Protein- protein interactions heterodimetrization and homodimetrization (can bind to itself).

Kd4 to self and Kd4 to client – offers variations that can respond to the alterations happening in chemical environment (hypothesis). But how to document this? There's a different between LEAPS recognizing their client – it isn't random. Confusing bit – not only bad times in order to engage, but they are stimulated by non-reducing sugars. No interaction between SMP1 and B122 in the presence of 10:1 mass ratio unless the proteins are also under chemical stress. With mass sucrose, positively correlated with seed longevity. Went from 10:1 to 40:1. The ratio to sucrose matters, for the LEAP to bind to the protein in a stress environment. Protective Take home: leap do not bind proteins equally. Depends on the environment of the LEAP and the client.

Repair – collaboration with scientist in China on Maize. If controls deteriorate, or age the seed – what happens? Poly-A binding protein – couldn't get recombinant protein from maize. Got it from Arabidopsis. Maize PMT recognizes the Arabidopsis protein! With the dianthus – they used un-aged and aged. Used SAM and a "poison" SAH, add the repairing mechanism with the SAM and the poison SAM. Showed that it hurts the protein, As Is. Age + SAH – really doesn't like it. Fresh seeds, germination, all suffer. But dormant seeds resistant to controlled deterioration. When dormancy is alleviated by dry after-ripening, double mutation seeds are susceptible to CD.

9:50am, **talk by** Huayu Xu, Ph. D. candidate, Dr. Xingyou Gu, major advisor, South Dakota State University: "Transcriptional analysis of SLR1 for regulatory roles in seed dormancy and germination in rice." Higher expression level of SLR1 from the male than that from the female gametes!! But does this not contradict what we thought we knew about the battle of the sexes where the male enhancing seed numbers small many, while mom is pushing fewer, larger. Larger less dormant so maybe not a contradiction.

Take homes from Huayu:

- SLR1 is involved in regulation of SD; genic effect of the mutant evaluation.
- SLR1 expressing in germinating embryos
- Genes regulated by SLR1 varied with the embryo genotype
- SLR1 regulation of gene expression is influenced by some epigenetic mechanism
- Genes regulated by SLR1 include those for SD, cell wall metabolism, GA, ABA, BS.
- Interactions of SLR1 and SD and other genes.
- Genetic characterizing SLR1 and SD12 for their relation in dormancy regulation.

Break

State report, Dr. Krista Isaacs, Michigan state University: "Indigenous seed outreach and extension." Isaacs presented on seed research and outreach activities with small scale growers. Background information indicated 72% of the farms in the world are less than 1 Ha, accounting for 473 million smallholder farmers. These farmers use diverse access

points for obtaining seed, including Informal/localized sources based on trust; Intermediary; and formal with Standardized control, certified.

Farmers access seed from different sources depend on the seed type (can it be dried, does it survive ambient storage well, etc.). Isaacs presented on an Indigenous seed sovereignty project, funded by the USDA SARE grant. This project engages with four tribes in Nebraska to a) generate community driven understanding of food and seed sovereignty, b) un(re)cover knowledge about traditional crop varieties, and c)seed production and storage of traditional varieties.

The collaboration is with Indigenous growers to design community-based participatory research on variety selection and maintenance. Objective was to grow varieties in community designed demonstration trials with iterations over the three years of the project. In the initial stages of this work, a survey of growers and seed producers was conducted to identify seeds grown, saved, and what they need access to. Additional questions asked about challenges in acquiring preferred seed and personal goals for saving seeds. After these surveys, workshops were conducted in the spring that focused on communities short- and long-term goals for bringing back, maintaining and conserving Native seeds.

State report, Tomo Kawashima, University of Kentucky, "ACTIN a tug of war: A novel mechanism for determining seed size through endosperm nuclear dynamics in Arabidopsis".

Nuclei are moving to an equidistant position after divisions in the coenocytic endosperm. F-ACTIN generates an aster-shape at each nucleus controlling the movement of the nuclei. Used a dominant negative actin variety no aster-shaped function. The nuclei now act as balls on a table, banging into each other and trying, but failing to find an organized, equidistant pattern. Also, seed size was affected. Smaller than WT. If you Over express WT ACTIN, seed size increases. If you do this in leaves or stems, they become dwarf!

Hypothesis: ACT10 controls nuclear movement towards the chalazal pole and regulates the number of remaining nuclei in the endosperm.

- The number of remaining nuclei in the endosperm dictates the final seed size.
- Endosperm ACTIN isoforms regulate the seed size by balancing nuclear distance and movement.
- ACT8 OE pushes nuclei further away from each other and this increases seed size for act10, more nuclei reside in the coenocytic endosperm and force the seed to expand to accommodate them and seed size increases as well.
- Both DN-actin and ACT10-OE reduce seed size by decreasing the distance/number of nuclei in the coenocytic endosperm.

Additional speakers on Saturday:

Talk by Daniel Lescovar, Texas AM University. Boosting Resilience in Grafted Tomato Transplants.

Drought occurrence and severity are increasing in Texas. Temperature is increasing Globally, Nationally, and Regionally. Grafting is an Alternative tool for managing Biotic and Abiotic Stresses. Had a project on a statewide grafting study productivity increased for the grafted tomato plants. Root traits varied with production environment and the root stock.

Thermotolerant scion genotypes were identified. Chlorophyll fluorescence remained high in her selections when exposed to high heat (either 38/28C 7 days (Stress) or 40C for 7 hours (Shock)).But different scions performed better to heat shock and heat stress. Grafted tomato in organic hydroponic systems. Grafted plants seem to be more resistant to some insect pests. Fruit yield also went up in the grafted plants in the hydroponic system.

Talk by Marya Bibi PhD candidate with Dr. Xingyou Gu, South Dakota State University: "Mitigating transgene flow into weedy rice using CRISPR-Cas9-based multiplex against seed adaptive traits".

Want to reduce transgene flow from cultivated to weedy rice. Grassel 1999 used a Mitigator gene (Dwarfing) in a Herbicide resistant cassette. But this is not neutral for the crop. What they want is Neutral for the crops but negative for the weed when transgene flow occurs. Weedy rice is conspecific to *Oryza sativa*. Weedy rice, although conspecific, has a series of adaptive traits that make it "weedy" undesirable. There are six genes identified as responsible for the adaptive traits and their guide RNAs are targeting each of these the six genes. Some are Bh4, Rc and SD.

Take homes:

- High editing rates for 5 genes detected in the T0 generation
- CC9 induced mutation rate was ~50% in F1 weed/T0 hybrids
- CC9 based multiplex system caused mutation for Bh4, SD7-2 and SD12s resulted in changes in hull color and seed dormancy.
- CC9 based multiplex system did not work for SD702/Rc
- The T0DNA insertion is mapped in chromosome 8.
- How to increase guide RNA to increase nutant rates?
- How many genes are ideal to generated in efficient Transgene Mitigation strategy?

Business meeting

The business meeting concluded the Annual W5168 Meeting. Group members discussed new member recruitment, noting that Cornell University has successfully recruited a seed related individual whom we should reach out to. Everyone was encouraged to recruit

additional individuals working in seed science and introduce them to the group. It was noted that people working in a wide range of seed science – from outreach and engagement with growers to basic science, were welcome.

At the business meeting, the group moved to elect Imtiyaz Khanday as the new chair, Krista Isaacs as the vice-chair, and recruitment of a new secretary would be needed.

It was decided that next year's meeting site would be UC Davis. This motion has passed! The meeting was concluded at 12:30pm on July 18, 2025.