Calendar Year 2022 - Activity Report

USDA-Agricultural Research Service
Western Regional Plant Introduction Station (WRPIS)
Plant Germplasm Introduction and Testing Research Unit (PGITRU)
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Table of Contents

APPENDIX II

EXECUTIVE SUMMARY

The Western Regional Plant Introduction Station (WRPIS) is one of four regional plant introduction stations in the United States. Activities at WRPIS focus on acquisition, preservation, characterization, evaluation, documentation and distribution of assigned plant genetic resources and their associated information along with conducting mission-related research. This Station includes five curatorial programs and two research programs (alfalfa genetics and genomics for genebank activities). The operation is primarily funded by two CRIS projects [one in National Program (NP) 301 and one in NP 215] managed through the USDA ARS Plant Germplasm Introduction and Testing Research Unit (PGITRU), on the Washington State University (WSU) campus in Pullman, WA. The Temperate-adapted Forage Legume (TFL) Germplasm and the Alfalfa Genetics Research projects are on an ARS worksite in Prosser, WA co-located with the WSU Irrigated Agriculture Research and Extension Center (IAREC). The W6 Regional Multistate Research Project associated with the Station also contributes considerable funding (~14% of the total operating budget) to support its mission. Funds are managed through the State Agricultural Experiment Station (SAES) and originate from the Hatch Multistate Research Fund (MRF) managed by the National Institute for Food and Agriculture (NIFA) and originating from the USDA. The W6 funds cover the salary and fringe benefits for six full-time state employees working for the WRPIS as well as partial land costs, supplies/equipment and farm operations for regenerations, characterization, evaluation, and enhancement research. Goals are achieved through close collaboration among scientists with diverse expertise in fields like agronomy, horticulture, plant pathology, genetics, plant physiology and botany. The W6 project is implemented in close association and collaboration with scientists of SAES, other state, and federal agencies as well as the private sector. The station's scientists also actively collaborate with colleagues at international centers, universities, and non-governmental organizations. Guidance for the complimentary project implementation comes from USDA National Programs and the Pacific West Area Office as well as from the W6 Regional Technical Advisory Committee (RTAC). The global crop plant research community continued to utilize extensively WRPIS germplasm collections. In 2022, 34,861 germplasm samples/order items (e.g., seed packets) were distributed to 525 requestors in 47 domestic states and territories and 38 foreign countries. WRPIS scientists also made poster and/or oral presentations at various regional, national, and international conferences, and participated in 18 peer-reviewed research and book chapter publications.

2022 Highlights

- The new 5-year USDA-ARS Project Plan for the WRPIS in National Program 301 was approved and an overview can be found at: https://www.ars.usda.gov/research/project/?accnNo=443951.
- On December 31, 2022, there were 102,384 accessions belonging to 1,003 genera, 4,616 species (5,270 taxa) in the WRPIS collection. W6 has 17% of the active NPGS accessions.
- We acquired 877 new accessions including 796 native plant accessions collected by the Seeds of Success (SOS) project, 67 expired IPR/CSR accessions from NCGRP, 11 *Phaseolus polystachios* accessions collected by Lyle Wallace, 195 *Medicago truncatula* accessions received from INRA, France, and 3 other misc. accessions.
- We distributed a total of 34,861 packets of seed samples to 525 requestors with addresses in each of the 47 domestic states and territories and 38 foreign countries. 55% (19,319 packets) were

distributed to the U.S. and 46% (15,542 packets) were distributed internationally. A total of 8,767 packets from WRPIS went to the 13 U.S. Western states.

- We uploaded 169 observation data points on 136 accessions into the Germplasm Resources Information Network (GRIN)-Global database. These data points are on 5 established descriptors for 2 different crop species. Our collaborators contributed 88% and WRPIS staff provided 12% of the evaluation data. The database is accessible by researchers worldwide via the internet. A total of 551 images were loaded on 422 accessions, 494 of them were seed images.
- We entered 3,763 seed viability records into GRIN-Global during the reporting period. The USDA ARS National Laboratory for Genetic Resource Preservation (NLGRP) in Fort Collins, CO tested 2,516 accessions and 1245 accessions were tested by the Pullman Germination Technician.
- We shipped 1,632 seed inventories to the National Laboratory for Genetic Resource Preservation (NLGRP), Fort Collins, CO (1,303 accessions were from the SOS collection) and no inventories were sent to the Svalbard Global Seed Vault, Svalbard, Norway for secured backup this year.
- The Cool Season Food Legume program published a collaborative high-throughput spectral reflectance method to select drought tolerant lentil accessions using a multispectral radiometer. Eleven lentil plants with high promise as donors of drought tolerance traits were identified from a total of 49 evaluated in a multi-year trial.
- The Cool Seasons Legumes program and collaborators identified 2 new QTL providing resistance to *Fusarium avenaceum* in lentil, and two PIs (PI299116 and W627760) that were highly resistant. They also identified markers linked to host resistance in lentil to the pea aphid, and two highly resistant lentil accessions, PI 329157 LSP and PI 432085 LSP.
- The Agronomy program generated re-sequencing data for 864 safflower accessions from which SNPs will be extracted to study population structure, relatedness, duplications, and uniqueness, and to create a panel of lines that can be studied via genome-wide association study (GWAS).
- The *Phaseolus* program started a new lima bean project that supports regenerations, phenotyping and genotyping of 700 *P. lunatus* in the collection. They also collected 11 new *P. polystachios* accessions from Florida and Georgia.
- The Horticultural Crops and Beta Program studied 150 accessions of 28 species of Lactuca germplasm from around the world via evaluation and genotyping, for a more accurate characterization of the collection. Several accessions were found to be misidentified and were corrected, resulting in adding 2 new species to the collection and expanding our diversity.
- The alfalfa genetics research program mapped genetic loci associated with verticillium wilt (VW) resistance in alfalfa populations and identified two candidate genes for disease resistance. The gene markers and protocols have been shared with alfalfa seed companies for marker assisted selection. The alfalfa genetics program also created a mapping resource for salt and drought stress and identified 84 SNP markers and putative candidate genes associated with stress resistance, and eight drought resistant lines, which have been shared with seed companies.
- The pathology program worked to develop plant tissue culture procedures using the basal stem disk as the explant for *in vitro* propagation of virus free garlic. The program also worked on optimizing culturing conditions for high yield zoospore production in *Aphanomyces euteiches* strains to be used in future large scale lentil inoculation studies.
- Research into GWAS to identify markers, genes, and pathways associated with traits of interest has yielded new methodologies to expand pathway analysis into self-pollinated crops for the first time, and markers linked to seed protein levels in peas have been identified.

Cover Story:

The Horticultural Crops and Beta curation program is very diverse, consisting of 1,614 species in 270 genera, and a total of 13,757 accessions. Species in this program are of economic value due to their nutritional, medicinal, or aesthetic attributes, and include the ornamental species *Allium stipitatum, Salvia azurea* and *Lomelosia caucasica*. Many of the wild relatives of these crops are also found in the collections, and their phenotypic traits include interesting and beautiful flowering types such as the mountain lettuce (*L. perennis*) image included on the cover of this report. The new acting curator of the Horticultural Crops and Beta Program is Alex Cornwall, who was the previous technician in that program. His expertise in the needs of the diverse crops and wild species will be a great asset to the program.

ADMINISTRATION

Scot Hulbert (Administrative Advisor)
Christian Tobias – (NIFA Representatives)
Peter Bretting (ARS National Program Staff)
Tara McHugh (ARS PWA Area Director)
Marilyn Warburton (ARS Research Leader and Station Coordinator)
Carla Olson (ARS Program Support Assistant)

Calendar year 2022 was a busy year with new curators and technicians, and new projects underway as the unit worked with nearly a full continent of personnel for the first time in a few years. The main offices and laboratories were moved out of Johnson Hall, which has since been demolished, and set up in nearby Clark Hall. Although the space is smaller, we have set it up to the best possible use and are working in genomics and pathology research once again. The WRPIS never stopped operations to deliver on its critical mission of acquiring, conserving, characterizing, and distributing valuable plant genetic resources and their associated information, and we have now begun to expand work in research to make our core mission more efficient, and to increase our genetic characterization and phenotypic evaluation efforts.

PERSONNEL

A few key personnel changes occurred in both the federal and state-funded projects in CY 2022 and the first few months of 2023. New curators were hired for the Agronomy (Dr. Paul Galewski) and Phaseolus (Dr. Sarah Dohle) programs in August of 2022. The Beta biological science technician vacancy in the Horticultural Crops program was filled by David Van Klaveren, who had been the WSU plant technician in seed storage, which created a new vacancy in seed storage. The second plant technician in seed storage was filled in 2022 but not retained past his trial period, leaving a second vacancy in seed storage. The vacancy created in the Unit's IT specialist position after Bo Gao resigned in 2020 was advertised and candidates were interviewed. However, none of the candidates selected by the hiring committee accepted the position, and thus it is still vacant. We will readvertise as soon as allowed at a slightly higher level (GS12). The greenhouse maintenance technician position vacancy from last year was readvertised and Dustin Golembiewski was hired and will begin on August 28th, 2023.

The W6-supported farm and seed cleaning activities have continued to be strained by the lack of a full complement, and changes in the definitions of the positions will be tried for 2023, which may help balance the needs and attributes of each worker in a more effective way.

PROJECTS

For PGITRU, there were two base funded projects entitled "Management of Priority Legume, Oilseeds, Vegetable, Forage Grass, Sugar, Ornamental, and Medicinal Plant Genetic Resources and Associated Information", based in Pullman and "Management of Temperate-adapted Forage Legume Genetic Resources and Associated Information", based in Prosser. Both projects are relevant to USDA ARS, National Program 301- Plant Genetic Resources, Genomics, and Genetic Improvement and are due to begin a new 5-year cycle in 2023. At that time, they will be combined into a single project for logistical and solvency considerations. Work objectives will not change for either of the projects substantially in the new 5-year plan.

The USDA ARS alfalfa genetics research project conducted at the Prosser worksite is entitled “Enhancing Resistance to Biotic and Abiotic Stresses in Alfalfa”. This project is relevant to USDA ARS National Program 215 - Pasture, Forage, and Rangeland Systems. It is in its last year of its current five-year cycle and is due to be renewed in 2024 with the first steps in the drafting process having recently initiated.

The current USDA NIFA W6 Regional Multistate Research Project entitled “Management and Utilization of Plant Genetic Resources and Associated Information” is in its first full year of its 5-year cycle. A renewal proposal for the next 5-year program cycle was submitted, reviewed, and approved with a slated new 5-year project cycle effective date of October 1, 2021.

FUNDING

The total federal USDA ARS budget for the PGITRU was $3,257,476 and includes the budgets for the Pullman-based germplasm ($2,539,778), the Prosser-based temperate-adapted forage legume germplasm ($275,939) and alfalfa genetics research ($441,759) projects. After indirect costs are assessed, net balance was $2,903,622 with $2,563,900 dedicated to salaries and the remainder for supplies, equipment, repairs, and extramural agreements. The discretionary dollar amount per SY (7 in the Unit in 2020), an indicator of the financial health of the Unit, was $36,228; an improvement from last year due to the abolishment of one of the SY positions. The ‘in kind’ support from the W6 Western Regional Multistate Research Project, through WSU, was $477,301.

In 2022, TFL Curator Brian Irish secured in-kind funding (~$20,000) in project support from a collaboration with H. Riday (USDA-ARS) and other forage breeders. This collaboration focuses on characterizing and increasing seed of advanced breeding lines and is to support salaries for temporary technical staff. In addition, the TFL Curator had been leading an inter-agency project between ARS and Bureau of Land Management (BLM) focusing on native plants called the Seeds of Success program. The leadership of this program has transitioned to Agronomy and Safflower Curator, Paul Galewski in 2022. The project is entitled “Management, Evaluation, Acquisition, and Distribution of Native Plant Germplasm for Research and Restoration” with 2022 funding of $150,000 that covers a full-time Support Scientist (Bailey Hallwachs) and temporary employees. The BLM collaboration funding also covers some travel and supplies.

Clarice Coyne is currently collaborating on several soft-funded projects including as Co-PD on USDA AFRI SCRI CAP project “Building a Better Lentil from the Ground Up” $3.1 M, $512,000 to WSU (2018-2023) that covers postdoctoral Research Associate salaries and wages for undergraduate research assistants. She is leading a USDA Pulse Health Crop Initiative project “MP3: More Protein, More Peas, More Profit”, which is an USDA ARS incoming NACA with a total of $469,000 to WSU (2018-2023), that, covers sequencing 400 pea accessions, partial Postdoctoral Research Associate salary, and wages for undergraduate research assistants. C. Coyne is Co-PD on second Pulse Crop Health Initiative project lead by M. Warburton: Lentil 2.0: targeted genomic assisted improvement of seed protein concentration. She is a Co-PD on WA State Department of Agriculture Specialty Crop Block Grant $248,000 to WSU (2020-2023) “Enhancing the competitiveness of Washington peas in a plant-based protein market” that covers one Postdoctoral Research Associate salary (3 years). Co-PD on Foundation for Food and

Agricultural Research project "Increasing total protein content in pea using large-scale phenotyping and targeted breeding with genomic selection" $1.2 M with $343,000 to WSU (2020-2023), covering one Postdoctoral Research Associate (2 years) and undergraduate student research assistant.

Sarah Dohle (PI), Marilyn Warburton (PI), and Lyle Wallace (Collaborator) started a 4-year collaboration (2022-2026) on a $3.3M SCRI lima bean (*P. lunatus*) project lead by UC Davis, Development of Genomic Resources to Improve Lima Bean Breeding for Consumer Quality and Agronomic Traits, which will include genotyping and characterizing the available lima bean collection >700 accessions, among other activities.

Research Geneticist Long-Xi Yu successfully continued to oversee several research grants in 2022 including "Marker Assisted Breeding in Elite Alfalfa Germplasm to Enhance Biomass Productivity During Drought", a grant provided by Alforex Seeds totaling $400,000 with $210,000 to ARS-Prosser (2017-2022); "Bacterial Stem Blight of Alfalfa: Connection with Frost Damage, Development of Resistant Germplasm, and Mapping Resistance Genes" from USDA NIFA Alfalfa Forage Research Program (AFRP) totaling $235,311 with $34,536 to ARS-Prosser (2017-2022) and "Genomics-Enabled Purging Selection to Develop 200 Alfalfa Inbred Lines for High Yield Hybrid Production" from USDA NIFA AFRI totaling $300,000 with $80,000 to ARS-Prosser (2018-2022); "Determining Genetic Factors that Influence Protein Quality and Yield in Alfalfa" from USDA NIFA AFRP totaling $250,000 with $100,000 to ARS Prosser (2020-2022). These grants support two Postdoctoral Research Associates working on bioinformatics, quantitative genetics, and functional genomics for development of markers and improvement of alfalfa with resistance to disease and abiotic stress.

FACILITIES

The WRPIS currently counts on 34,800 square feet of greenhouse facilities (22,375 sq. ft. Federal, 12,425 sq. ft. WSU), many of which have been recently upgraded and refurbished with new covers, electrical wiring and switches; cooling pads, pumps and motors, etc. This activity will continue in 2023. The unit also has access to ~149 acres of farmland (86.2 acres Federal, ~55 acres WSU). New possible leases of nearby farmland to replace the 28 acres of WSU field space lost to the construction of a new airport runway did not work out, so space will be requested from the WSU Palouse Conservation Field Station. This additional space will be needed to allow for rotations between different species and agronomic practices during regenerations. WRPIS staff uses 8 laboratories (5 Federal, 5 WSU), and 20 offices (4 in Federal buildings, 6 in a Federal mobile office building, 10 in WSU buildings). This is a decrease of 2 labs and 2 offices since 2021 due to the move out of Johnson Hall on the WSU campus in order to make space for the new USDA building (see update below). The USDA ARS Alfalfa Research Geneticist and the TFL Curator have assigned office, greenhouse, and laboratory spaces at the Prosser worksite in both Federal and WSU facilities on the IAREC campus, and facilities there remained unchanged.

The construction of the new Plant Biosciences building on the WSU campus will begin in 2023 with an official groundbreaking ceremony on August 8th. This new building will house USDA and WSU Units/Departments, faculty offices and laboratories. It will house all Pullman-based WRPIS scientific and technical staff and include six modern Unit-shared laboratory spaces.

Johnson Hall was demolished in the early part of 2023 and construction scheduled to be completed in late early 2026, after which WRPIS will occupy new facilities.

Greenhouses and screenhouses in all locations (Pullman, Prosser, and Central Ferry) are aging and this presents challenges, especially as there is no permanent Greenhouse Manager to care for them. With help from Wayne Olson, intermittent worker in the unit with many years of experience with our facilities, they have had routine maintenance as needed, and more extensive updates and repairs planned for 2023 and 2024.

In Central Ferry, the backup generator that has been needed for several years has arrived, but installation was delayed due to lack of specific switches and construction of approved foundation; that is moving forward now. New high-throughput phenotyping pipelines are being built with the recent purchase of two drones (one in Pullman, one in Prosser) with multi-spectral cameras, and a ground-based robot capable of capturing thousands of images in many light wavelengths. These images can be converted to phenotypes such as plant heights, biomass, pod counts, chlorophyll content and plant stress, etc., using algorithms trained on actual data, which we are collecting now.

The **Agronomy and Native Plants Program** (Paul Galewski) manages 34,367 accessions of cool season turf and forage grasses, safflower and the native plant accessions collected in recent years by the Bureau of Land Management (BLM) Seeds of Success (SOS) project with day-to-day activities covered by support scientist, Bailey Hallwachs. The **Cool Season Food Legumes Program** (Clarice Coyne) curates a total of 23,110 accessions of pea, chickpea, lentil, faba bean, and other legumes. The ***Phaseolus*/Beans Program** (Sarah Dohle) manages a collection of 17,754 accessions, all belonging to the *Phaseolus* genus. The **Temperate-adapted Forage Legumes Program** (Brian Irish) manages alfalfa, clover, trefoil, and their wild relatives with a total of 13,343 accessions. The **Horticultural Crops Program** (Barbara Hellier) manages 13,810 accessions of sugar beet, lettuce, garlic, and many miscellaneous species that have potential use for ornamental or medicinal purposes.

Acquisition

During the 2022 calendar year, 877 new accessions new accessions were added to the WRPIS collections including 796 native plant accessions collected by the Seeds of Success (SOS) project, 67 expired IPR/CSR accessions from NCGRP, 11 *Phaseolus polystachios* accessions collected by Lyle Wallace, and 3 other misc. accessions were added.

The total number of accessions held at the end of each calendar year over the previous ten years is shown in **Figure 1**. The main changes in numbers of accessions in the collection are due to the addition of SOS native accessions, many of which are then transferred to other NPGS priority sites, and many more incorporated into active WRPIS collections. The value of these agriculturally important additions to the WRPIS collections continues to grow as international access to germplasm is increasingly limited by the changing political environments.

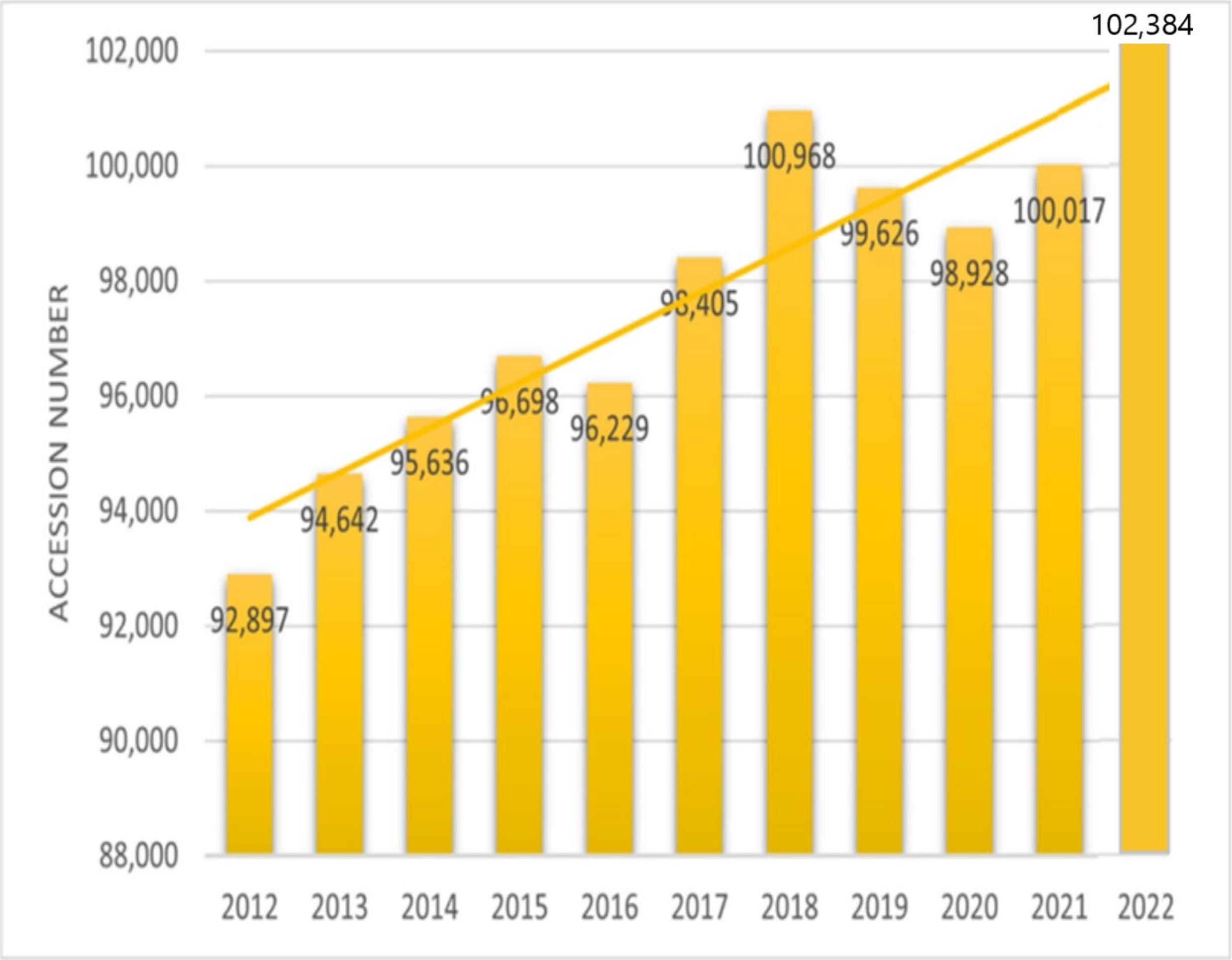

Figure 1. Total number of accessions managed by WRPIS since 2012 (numbers recorded at the end of each calendar year).

Conservation

In 2022, WRPIS was scheduled to regenerate 1,524 accessions by following the established, labor-intensive procedures and protocols for maintaining the genetic integrity and health of all germplasm collections. These included physical isolation, hand planting and transplanting, controlled hand and insect pollination, hand harvesting, cleaning, and packaging for storage and distribution. Additionally, 556 accessions were scheduled to be regenerated for backing up or replenishing back-up inventories at the NLGPR in Fort Collins, CO.

A total of 3,763 seed viability records were entered into the GRIN-Global database. Of these, 1,245 inventories were tested in-house by technician staff. Seed testing personnel with NLGRP in Fort Collins, CO contributed 2,516 tests. For security backup, 1,632 inventories were sent to NLGRP at Fort Collins, CO in 2022 and no inventories were sent to the Svalbard Global Seed Vault, Longyearbyen, Svalbard, Norway (**Figure 2**).

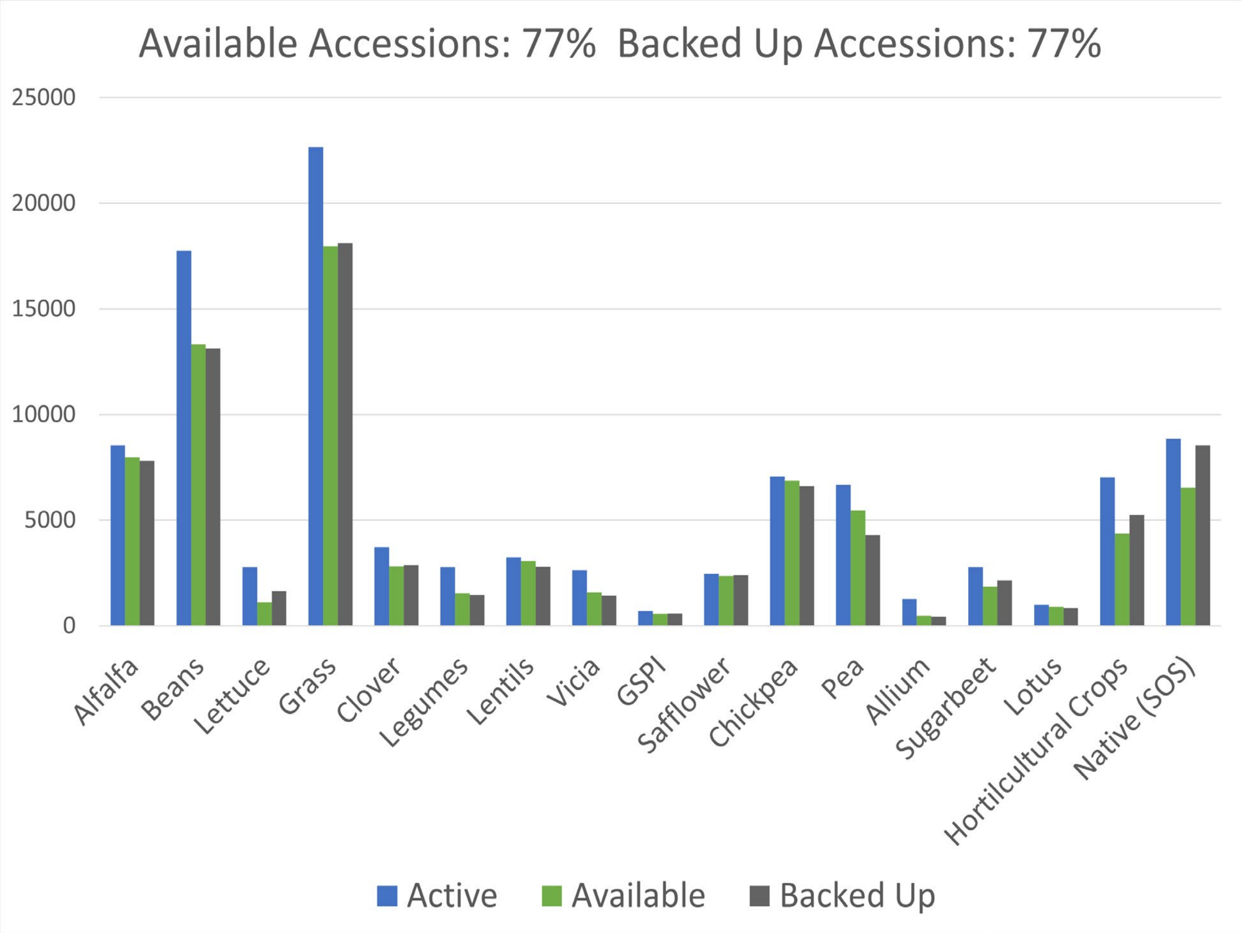

Figure 2: Number of the 102,384 active accessions (93,210 in 5 curatorial programs + 9,174 Seeds of Success) that are available for distribution. 77% of the accessions are backed up in Ft. Collins at the Agricultural Genetic Resources Preservation Research unit, and 20% have been backed up at the Svalbard Global Seed Vault.

Characterization/evaluation

We uploaded 169 observation data points on 136 accessions into the GRIN-Global database. These data points are on 5 established descriptors for 2 different crop species. Our collaborators contributed 88% and WRPIS staff provided 12% of the evaluation data. The database is accessible by researchers worldwide via the online GRIN-Global portal. A total of 551 images were loaded on 422 accessions, 494 of them were seed images.

With funding from USDA NIFA AFRP, and in collaboration with Charles Brummer and others, a multi-site replicated field trial was established spring 2021 in Prosser, WA. A total of 400 individual alfalfa entries were field transplanted in 5-plant plots across two blocks for a total of 4,000 plants. Germplasm being evaluated included wild-collected and landraces plant introduction accessions (383) as well as 17 reference check cultivars. Phenotypic (e.g., growth habit, leaf/stem ratio, etc.) and agronomic performance traits (e.g., yield and quality) were collected during the fall harvest and will continuing throughout 2022. Germplasm being evaluated is to be genotyped with collaborators at Breeding Insight and a GWAS will be conducted to associate markers to important traits. In addition, improved plant selections will be made at the end of the third year and will be combined with collaborators improved germplasm selections of the same original germplasm into pre-bred pools. All summarized data for traits will be incorporated into the GRIN-Global database and associated with accessions.

Cool Season Food Legume Curator Clarice Coyne published a collaborative paper entitled "Multi-trait genomic prediction improves selection accuracy for enhancing seed mineral concentrations in pea" in the journal The Plant Genome (https://doi.org/10.1002/tpg2.20260) This study addresses the time-intensive laborious, and expensive trait evaluation of germplasm collection by taking advantage of high-density genomic data, the costs of have significantly declined relative to phenotyping (trait evaluation). Genotypic data combined with Genomic Selection (GS) is an attractive option as a selection decision tool to evaluate accessions in extensive germplasm collections. The predicted phenotypic values would significantly increase the value of accessions in germplasm collections by giving breeders a means to identify those favorable accessions meriting their attention among the thousands of available accessions in germplasm collections. In this study, C. Coyne and colleagues applied and evaluated genomic selection's potential to a set of 192 pea accessions that were genotyped with 28,832 SNP markers and phenotyped for seed mineral nutrient concentrations to enhance selection of accessions from the USDA Pea Germplasm Collection. Predictive values of traits ranged from a low of 3.19 to 90.34%.

In 2022, Horticultural Crops and Beta Program Curator B. Hellier, met milestones for regeneration and distribution. Agronomy Curator P. Galewski continued the regeneration and distribution work in the grasses and safflower started that year by C. Coyne, and Phaseolus curator S. Dohle continued the regeneration and distribution work in the bean germplasm started by B. Hellier. Although the numbers regenerated in the latter two programs were lower than usual, the new curators, including incoming Horticultural Crops and Beta Program Curator Alex Cornwall, are learning quickly and will be on track to increase numbers in 2023 and beyond. Characterization and Evaluation projects will be added to the work of the new curators after that point.

Figure 3 shows the annual number of orders and order items (e.g., seed packets, garlic bulbs, rhubarb crown pieces, etc.) distributed for each of the past ten years. In 2022, the order number was 645, unique requesters distributed to was 525, and the number of items distributed was high at 34,861. A reduced number of distributions compared to previous years is partly due to delays and restrictions on shipments (especially internationally). Many of the distributions were for large numbers of accessions in a few orders (e.g., 3,782 alfalfa accessions for the Salk Institute for Biological Sciences and 3,458 accessions of Pea for NutriPea in Canada). The increased capabilities for genotyping and high-throughput phenotyping may have also led to larger individual item requests by researchers. Distributing 34,861 order items in 2022 shows significant continued interest in the user community for germplasm held by the WRPIS. Among the distributions, 55% (19,319 order items) were sent to addresses covering 46 U.S. domestic states and Puerto Rico and 45% (15,542 order items) were sent to requesters in 38 countries outside the U.S. Not all distributions were in the form of seed as over 80 order items were distributed as clonal plant material (e.g., garlic and rhubarb).

The most requested plant group at WRPIS were cool season food legumes (12,026 order items). The increase in distributions from this group reflects increases into research on plant-based proteins for human consumption. Other groups with high demand included germplasm distributed from the cool season grasses and safflower (6,530 order items), followed by Phaseolus/bean (6,222 order items), temperate-adapted forage legumes (6,068 order items), horticultural crops program (3,502 order items), and native plants (498 order items).

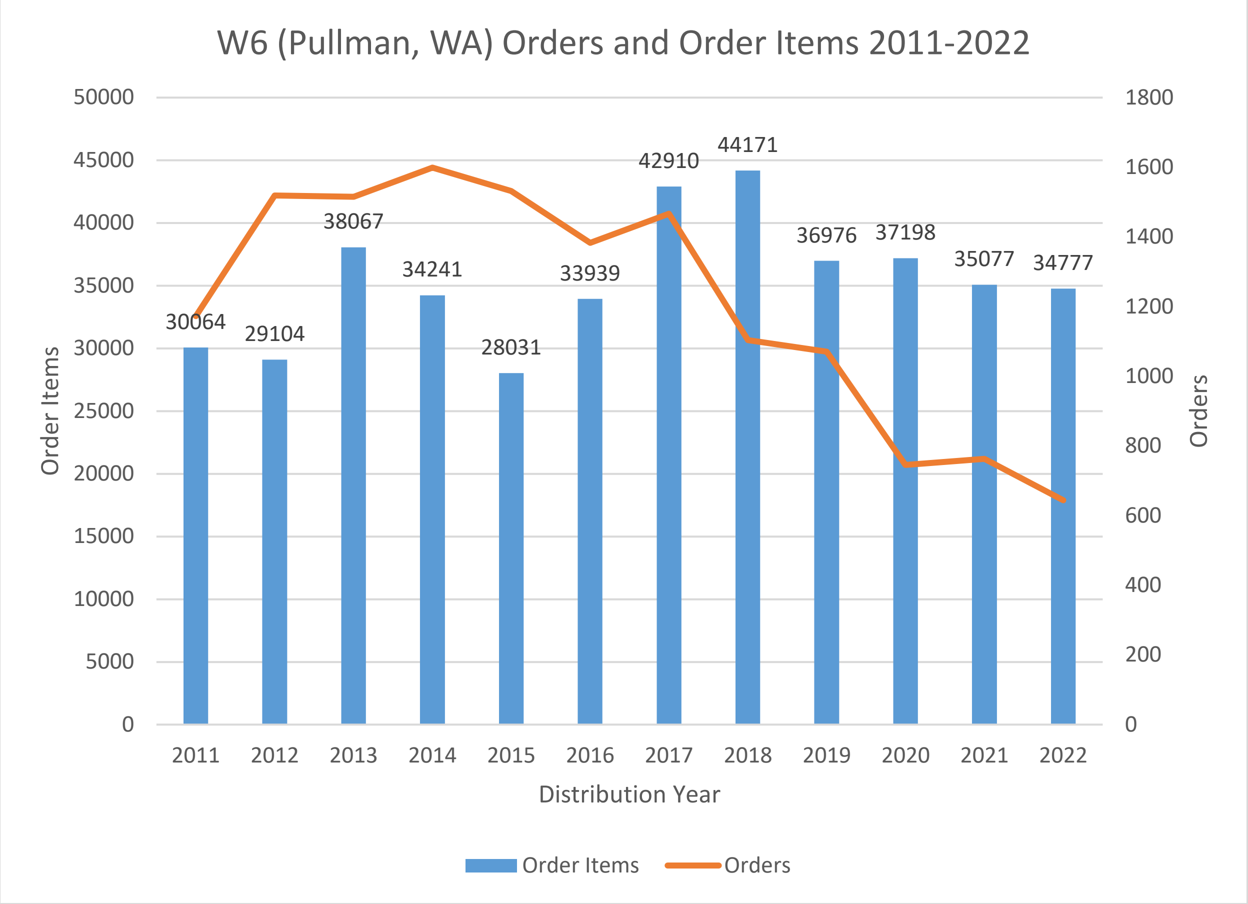

Figure 3. Number of orders and order items distributed annually by WRPIS from 2010 to 2022. *(e.g., - seed packets, garlic cloves, etc.).

Utilization - Western States

Germplasm users in the 13 Western states (AK, AZ, CA, CO, HI, ID, MT, NV, NM, OR, UT, WA, and WY) received 30,994 from all nationwide NPGS genebanks in 2022 (**Table 1**). A total of 7,695 order items were distributed by WRPIS to these western states. Thus, on average ~25% of the samples received by users in these 13 Western states were distributed by WRPIS. These NPGS genetic resources were used for research and education in diverse scientific disciplines and contributed to scholastic and economic activity in this agriculturally important region of the United States. **Table 1** provides the numbers of order items distributed in 2022 to each of the 13 Western states by WRPIS and NPGS, respectively. The number of items received from WRPIS by each state varied from 0 (Hawaii) to 4,908 (California). From the NPGS, the items distributed to the 13 Western states ranged from 175 (Nevada) to 11,644 (California). California, Washington, and Idaho were the top three states in terms of receiving germplasm from WRPIS in 2022.

Table 1. Numbers of plant germplasm order items (e.g., seed, or clonal propagules) distributed in 2022 to each of the 13 Western States by the NPGS and the WRPIS.

State	WRPIS	NPGS	WRPIS %
Alaska	8	1,288	0.6
Arizona	97	2,924	3.3
California	4,908	11,644	42.2
Colorado	67	1,808	3.7
Hawaii	0	232	0.0
Idaho	921	2,742	33.6
Montana	61	9,302	0.7
Nevada	56	175	32.0
New Mexico	59	921	6.4
Oregon	311	1,088	28.6
Utah	155	630	24.6
Washington	2,026	3,788	53.5
Wyoming	98	225	43.6
Total	**7,695**	**30,994**	

CURATORIAL PROGRAM SUMMARIES

1. **Agronomy (**Cool season grasses and Safflower) **Program**
   Curator: Paul Galewski (full-time)
   Technician: Zeke Brazington (full-time)
   Support Horticulturist: Bailey Hallwachs, SOS Project (full-time)

The Agronomy program works on the conservation, preservation, and maintenance of genetic diversity for cool season grasses and safflower. The **Cool season grass** collection consists of 22,842 accessions with 77.6% available for distribution. Similarly, 77.3% of the collection is securely backed up at NLGRP in Fort Collins, CO. A total of 202 accessions were harvested in 2022 and 360 accessions were planted in spring 2023 between two locations plant introduction farms (Pullman, WA and Central Ferry, WA).

The 360 grass accessions selected for regeneration in 2022 were based on the quality, viability, quantity of seed, and isolation requirements (**Figure 1.1**). Grasses are wind pollinated and many grass species are outcrossing which requires isolation by distance (>50 m). at the end of 2022, to choose the accessions to be germinated for regeneration in 2023, we employed a novel approach to field design to address this challenge. Software written in Python and R was able determine field order and isolation by distance by representing the field plots mathematically and assignment of accessions to plots. Each iteration of field maps was scored for isolation criterion and the order retained. The scores were sorted, and the best field order was evaluated. Random assignment was effective to achieve 98% accuracy after 10,000 iterations. In the end, a constraint optimization algorithm was used to achieve a solution and finalize the field order accounting for isolation between accessions and species known to hybridize. More work to understand the initial constraints of accession selection and achieving more efficient solutions will be evaluated next. The benefit to this method for selecting accessions for regeneration over the historical methods is that more priority can be given to accessions which require regeneration based on viability decline rather than fitting a predetermined field design which limits the rate which accessions can be regenerated.

Contamination of field plots by grass weeds is a challenge to maintain purity of seed. Digitization of pure seed samples (standards) is currently underway. Contaminants can be identified and removed in the field by weeding or by identification of contaminated seed prior to germination or during seed cleaning. An organized database which contains digital images of pure seed that is easily accessible is of great utility to the preservation and maintenance of grass accessions.

The safflower collection of 2,454 accessions is in excellent condition. The collection is 97% backed up at NLGRP and 96% of the accessions are available for distribution. No accessions were regenerated this year but to gain experience on how to grow safflower optimally next year is a high priority. The acting curator, Clare Coyne, had started the process to obtain permits for distribution of noxious *Carthamus* spp. (safflower) accessions (USDA APHIS Noxious Weed permit) as well as a permit to receive and distribute the *Brachypodium* T-DNA collection; this will be completed by the new curator.

Figure 1.1. Regeneration activities of the Agronomy program. (A) Establishment and hardening-off seedlings. (B) Planting first year grasses. (C) plots D) inflorescences and E) harvesting.

A. In the Native Plants Program, we continued to collaborate with Bureau of Land Management (BLM) to manage the native plant seeds collected by the SOS program from public lands in the U.S. In 2022 the project dedicated time to quality assessing historical passport data and improvement of queryable passport information included in GRIN-Global. When undertaking this work, significant differences were encountered between historical records in GRIN-Global and BLM SOS database (BG-Base) records for unique identifier names and taxonomic identities. For the more 22,000 SOS NPGS accession records, the team has focused on identifying these discrepancies, determining why they occurred and how to implement fixes. In addition, work continues to improve quantity and quality data for new incoming SOS collections during the process of accessioning. Passport information previously not included in GRIN-Global, like Ecoregion, Slope, Aspect, and Landowner are now being populated into Source Habitant Fields now available in GRIN-Global. This increased information, available to NPGS curatorial staff currently, will aid in germplasm management decisions. The data will eventually be public facing information for stakeholders to aid in selecting germplasm for their research.

B. Genotyping of the safflower collection was greatly advanced with the extraction of DNA from 864 accessions. These were genotyped by sequencing and a total of 965,850 SNPs were identified in these accessions following alignment and SNP calling. Analysis of these data are ongoing in 2023 and data will be uploaded into the GRIN-Global database following publication.

2. **Cool Season Food Legumes Program**
Curator: Clarice Coyne (full-time)
Technician: Britton Bourland (full-time)
Research Assistant Professor: Yu Ma (Specialty Crop Research Initiative - SCRI)
Postdoctoral Research Associate: Lyle Wallace, USDA ARS (with the Phaseolus program)
Postdoctoral Research Associate: Ousseni I. Salia (Foundation for Food and Agriculture)
Postdoctoral Research Associate: Puneet Mangat (WA Specialty Crops Block Grant)

This program manages the germplasm of all cool season food legumes (CSFL) including pea, chickpea, lentil, faba bean, grasspea and numerous *Lathyrus* and *Vicia* legume species with usage other than food, primarily as forage crops. Pea is nested in *Lathyrus,* and lentil is nested in *Vicia* phylogenetically, so these taxa are curated together. Record distributions of pea genetic resources have been seen in the last 5 years due to the interest in the plant protein market. We grew 1,022 accessions for regeneration of low quantity or low germination (to make it available for disctribution) or for security backup. Pea accessions (169) were regenerated on the WSU campus in our greenhouse and insect-proof two-season shadehouses to prevent infection with Pea Seed-borne Mosaic Virus (PSbMV). A total of 25 pea, 41 bitter vetch and 379 chickpeas were regenerated for security back up at Central Ferry farm (**Figure 2.1**). The 33 faba bean accessions regenerated in 2022 were grown with protection from cross-pollination contamination using screened caging. Included in 2022 transplants were 16 perennial *Vicia, Lupinus* and *Lathyrus* accessions and 58 accessions of annual and perennial wild *Cicer* species, wild relatives of cultivated chickpea, some that can be used in breeding, were recently collected, and donated from Turkey. An additional 1,454 plots of chickpea, lentil and bitter vetch were grown in replicated trials for seed protein concentration and agronomic trait studies. Use of drone technologies were introduced into the CSFL in 2021 and applied in 2022 for plant heights and biomass measurement (**Figure 2.1**).

Figure 2.1. Cool season food legume regenerations. Remotely Piloted Aerial Systems (RPAS) photo of 2022 bitter vetch regeneration plots in Central Ferry (left); and Biological Research Technician Britton Bourland controlling the RPAS (right).

A. In collaboration with Lyndon Porter, USDA ARS Prosser, WA, we published a lentil evaluation and mapping study of resistance to Fusarium root rot in a diversity panel of the USDA lentil core collection. Two accessions stand out as good sources of high levels of quantitative resistance, PI 299116 and W6 27760. Two QTL explain 18.4% and 13.1% of the genetic resistance to root rot caused by *Fusarium avenaceum* (**Figure 2.2)**.

B. In collaboration with USDA ARS Plant Pathologist L. Porter, we identified markers linked to host resistance in lentil to the pea aphid, a destructive pest and vector of PSbMV. Dr. Porter's research identified two highly resistant lentil accessions, PI 329157 LSP and PI 432085 LSP, that will be useful to plant breeders looking for good sources of genetic resistance.

C. In collaboration with Professor Nonoy Bandillo of North Dakota State University and USDA ARS Mike Grusak, we deployed genomic selection and machine learning for enhancing pea seed mineral nutrient concentrations using the USDA pea core collection for training and prediction modeling. This demonstrates the most efficient use of historic data from the GRIN-Global database for genetic discoveries in the pea core collection.

D. With collaborators from Argentina and the Czech Republic, we reviewed the adaptive traits from crop wild relatives that could improve the performance of cultivars in marginal environments and breeding strategies to deploy them. Crop wild relatives (CWRs), most often used in breeding as sources of biotic resistance, often also possess traits adapting them to marginal environments. Wild progenitors have been selected over the course of their evolutionary history to maintain their fitness under a diverse range of stresses. Conversely, modern breeding for broad adaptation has reduced genetic diversity and increased genetic vulnerability to biotic and abiotic challenges. There is potential to exploit genetic heterogeneity, as opposed to genetic uniformity, in breeding for the utilization of marginal lands.

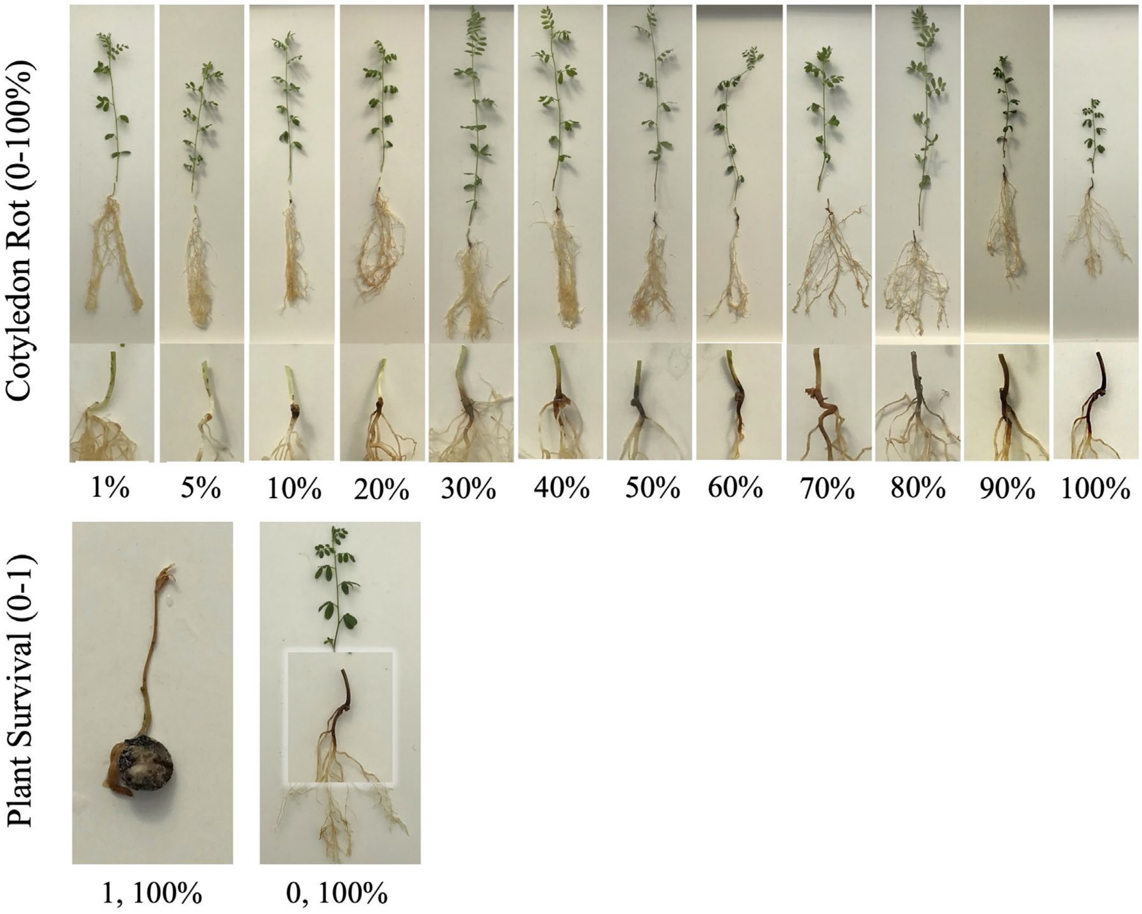

Figure 2.2. The lentil diversity paneled was screened in a replicated and repeated (2X) greenhouse experiment for root reaction to *Fusarium avenaceum,* an important root rot pathogen of lentil. Above are the disease rating scales used in the evaluation.

3. **Temperate-adapted Forage Legumes Program**
   Curator: Brian M. Irish (full-time)
   Technician: Estella Cervantes (full-time)
   WSU Farmer: Jesus ‘Jesse’ Prieto (full-time)
   Collaborator: Elizabeth Martin, Lewis and Clark State College, (part-time)

The Temperate-adapted Forage Legume (TFL) genetic resources program is an important component of the WRPIS in Pullman, WA and operates at the USDA ARS worksite in Prosser, WA. The project focuses on acquiring, maintaining, characterizing, evaluating, and distributing alfalfa, clover, trefoil, and wild relative germplasm accessions as well as database-maintaining all associated documentation.

A. For the 2022 growing season a total of 109 plots were scheduled for regeneration including 58 *Medicago* spp., 42 *Trifolium* spp., 6 *Lotus* spp. and 3 *Acmispon* spp.

accessions. In addition to the germplasm accessions to be increased, the 'Vernal' alfalfa cultivar was used to establish and harvest seed from 8 uncovered sentinel plots. Of the plots being increased in 2022, 31 were carried over from previous plantings due to insufficient seed regenerated in prior years. Phenotypic traits for many accessions regenerated during the year were collected and captured by scanning and generating voucher images for flowers, pods, and seed. All threshed and chalcid-free seed from 2021 increases was submitted to the Pullman farm crew for additional cleaning if needed, and for eventual depositing in seed storage. All passport and associated information and voucher images were provided to technical personnel for loading into the GRIN-Global database. A total of 49 plots to be seed increase were part of collaborations with forage breeders and included *T. pratense* (red clover), *T. incarnatum* (crimson clover) and alfalfa (*M. sativa* subsp. *sativa*) advanced selections/lines.

B. For the 2022 calendar year a total of 6,143 alfalfa, clover, trefoil, and their crop wild relative order items/seed packets were distributed in 82 orders to 69 unique cooperators/recipients. Just over 23% of the order requests, and subsequent shipments, were from/to international cooperators. A few of the requests were for many accessions including one request for the Salk Institute of 3,782 alfalfa accessions (most of the available *M. sativa* accessions) for whole-genome sequencing, phenotyping of root traits and research on carbon sequestration.

C. An annual medic (*Medicago* spp.) germplasm collection was assembled from the Crimean Peninsula of Ukraine in 2008 to fill gaps in geographic coverage in the USDA ARS NPGS forage legumes. A total of 102 accessions across 9 *Medicago* species were acquired. To assess genetic diversity, population structure and to confirm taxonomic identities, the collections were phenotypically and genetically characterized. Phenotyping included the use of 25 descriptor traits while genetic characterization was accomplished using a 3K SNP panel developed for alfalfa (*Medicago sativa* L.) on a Diversity Array Technologies (DArTag) platform. For both field and molecular characterizations, a refence set of 90 corresponding and geographically diverse and representative accessions were also included. Phenotypic descriptors showed consistency among replicate plants within accessions, some variation across accessions within species, and evident distinctions between species. Because the DArTag panel was developed in alfalfa, the transferability of markers to species being evaluated was limited, resulting in an average of ~1500 marker loci detected per species. From these loci, 448 markers present in 95% of the samples were selected for diversity assessment. Principal component and phylogenetic analysis based on the selected markers grouped accessions within same species together and predicted evolutionary relationships among species. Additionally, the markers aided in taxonomic identity of few accessions that were likely mislabeled. The genotyping results also showed that sampling individual plants for these mostly self-pollinating species is sufficient due to high reproducibility between single (n=3 biological) and pooled (n=7 plants) leaf samples (**Figure 3.1**). The phenotyping and the 448 SNP marker set were useful in estimating population structure in the Crimean and reference accessions, highlighting the novel and unique genetic diversity in the Crimean collection. Furthermore, this research showed that the SNP markers could be used in a similar fashion to evaluate other plant genetic resource collections of related *Medicago*. This is a collaborative effort with Breeding Insight colleagues.

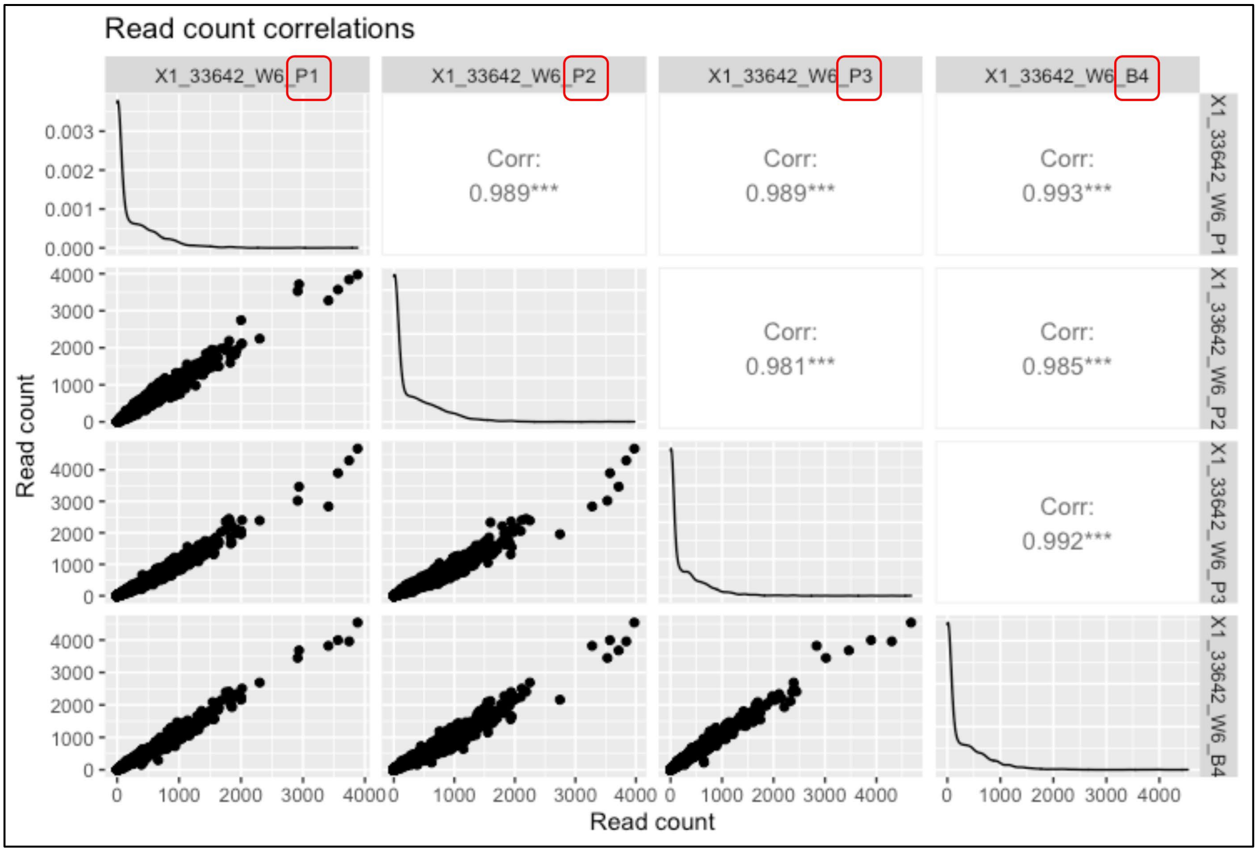

Figure 3.1. For a single accession (e.g., *Medicago arabica* - **W6 33642)** the three individual plant DNA samples (P1, P2, P3) and the bulked DNA sample (B4) showed high correlations with one another in read counts (i.e., they produced similar genotyping results). Therefore, genotyping replicate plant samples within accessions may not be necessary because of the high reproducibility.

D. In the last year of funding from a USDA NIFA AFRP, and in collaboration with Charles Brummer and others, data collection continued for a multi-site replicated field trial that had been established spring 2021 in Prosser, WA. A total of 400 individual alfalfa entries were field transplanted in 5-plant plots across two blocks for a total of 4,000 plants (**Figure 3.2**). Germplasm being evaluated included wild-collected and landraces plant introduction accessions (383) as well as 17 reference check cultivars. Phenotypic (e.g., growth habit, leaf/stem ratio, etc.) and agronomic performance traits (e.g., yield and quality) were collected during the spring and fall harvests with a final harvest scheduled for spring 2023. Pooled leaf samples from 20 individual plants/genotypes for each of the 400 accessions being field evaluated has been DNA extracted. The DNA samples will be genotyped with collaborators at Breeding Insight on Diversity Array Technologies (DArTag) platform with a 3K alfalfa SNP panel. A GWAS will be conducted to associate markers to important traits. Plant selections are planned for early summer 2023, will be clonally propagated combined with collaborators improved germplasm selections of the same original germplasm into pre-bred pools. All summarized data for traits will be incorporated into the GRIN-Global database and associated with accessions.

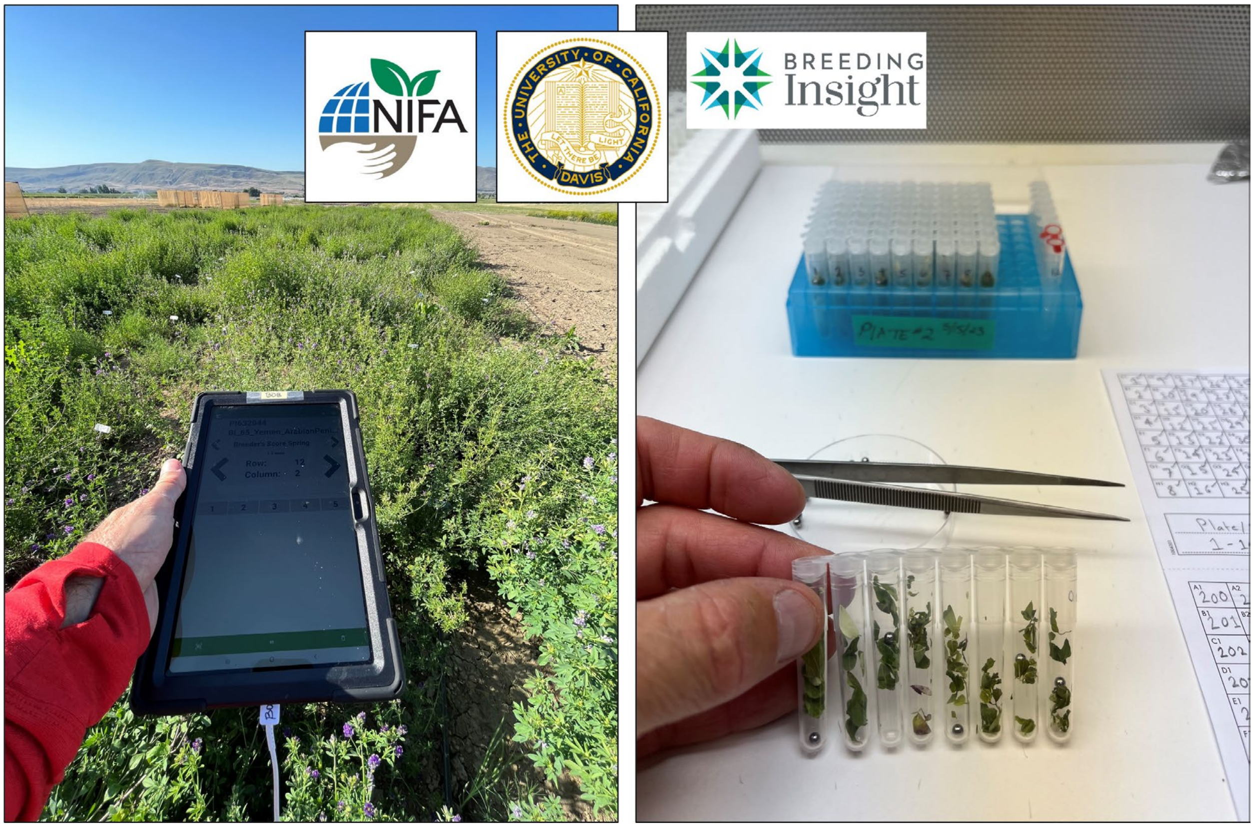

Figure 3.2. Right - Field Book application on Android tablet used to collected phenotype data for 400 alfalfa germplasm entries in a replicated field evaluated trial in Prosser, WA. Left - the same accessions are being genotyped on a DArTag platform using a 3K alfalfa SNP panel.

E. In 2022 we continued to establish working collaborations with public forage breeders (e.g., USDA, academia, non-profit) to evaluate and increase seed of advanced breeding lines. We are working directly with H. Riday, USDA ARS forage breeder in Madison, WI, Zhanyou Xu, USDA ARS research/alfalfa geneticist in St. Paul, MN, and Charles Brummer, research/alfalfa geneticist with the University of California, Davis.

F. The TFL program has also been working closely with Research Molecular Biologist, Lev Nemchinov of the USDA ARS Molecular Plant Pathology Laboratory in Beltsville, MD on characterizing potential diseases in alfalfa germplasm in the NPGS collections and in the Pacific Northwest. The collaboration has mostly focused on providing samples (leaf and seed) of symptomatic alfalfa and using next-generation sequencing to screen samples for previously described or novel organisms that may be causing disease. The research will aid in developing best practices for distribution of germplasm reducing the risk of possible pathogen dissemination.

4. **Horticultural Crops and Beta Program**
Acting Curator: Alex Cornwall (full-time); Barbara Hellier (retired)
Technicians: David Van Klaveren (full-time); Vacant (full-time)

The Horticultural Crops and Beta Program is responsible for 13,757 accessions in four maintenance groups – *Beta* (sugarbeet, table beet, chard and leaf beet, fodder beet and wild Beta and *Patellifolia* species), *Allium* (garlic, leeks, chives and all wild *Allium*), Lettuce (cultivated lettuce and wild *Lactuca* and related genera) and Miscellaneous (minor forage legumes, industrial crops, current and potential ornamental species, medicinal and culinary herbs, rhubarb, and restoration and revegetation species). These collections comprise a significant proportion of the generic diversity, because of the extensive number of genera and species, held at W6 outside of the SOS program.

In 2022 we focused on regeneration and maintenance activities for all taxa in the program. We planted and harvested 320 *A. sativum* and other clonal *Allium* accessions, maintained plots of 60 accessions of rhubarb (*Rheum* spp.) planted and/or harvested 100 inventories of miscellaneous accessions, planted, and harvested 70 inventories of *Lactuca sativa* in the field, 50 inventories of *Lactuca* wild species in the greenhouse, and harvested 15 inventories and planted 10 inventories of *Beta*. For all our regeneration plots we take plant, plot, inflorescence, pod (if appropriate) images (**Figure 4.1**) and we also collect herbarium samples for the miscellaneous and *Allium* accessions.

Figure 4.1. (from left to right) planting seedling accessions in miscellaneous group in Pullman, W6-20282, *Allium caeruleum* umbels, PI 540597, *Beta maritima* plants in the greenhouse, W6-37344, *Astragalus bisulcatus* plant.

In addition to the activities relating to seed-producing germplasm accessions, we maintained the *Allium* and rhubarb clonal collections (363 accessions). We do all the cleaning and preparation work for distribution of these collections. In 2022, we filled 3 garlic orders (7 packets) and 12 orders for rhubarb (70 order items).

A. Alex Cornwall continued work on his dissertation project. He grew out 150 accessions of 28 species of *Lactuca* germplasm from around the world for evaluation and genotyping using Hybrid Exome Capture. DNA from each accession was isolated and sequenced for the purpose of discovering ultra-conserved regions that can be utilized in the taxonomic identification and a more accurate characterization of the *Lactuca* collection. Accessions were also characterized classically, and photographs **(Figure 4.2)** and herbarium samples were taken for voucher specimens. He identified several accessions that were misidentified, resulting in adding 2 new species to the collection and expanding our diversity.

Figure 4.2 From left to right, First row: *L. quercina, L. tatarica*. Second row: *L. georgica, L. undulata, L. floridana*. Third row: *L. graminifolia, L. quercina, L. viminea*

B. In late 2021 we were contacted by a citizen scientist in Alaska who had travelled across the state searching for historically documented rhubarb varieties that had been carried to Alaska over the last century. She wanted them preserved and wanted to donate them to our collection. In 2022, in order to not add duplicate material to the collection, we were able to work in conjunction with a graduate student in Alaska, working on a MS degree in Crop and Soil Science of WSU on beginning a project fingerprinting the heirloom material along with many accessions currently in the collection to determine their novelty.

5. **Phaseolus/Bean Program**
   Curator: Sarah Dohle (full-time)
   Postdoctoral Research Associate: Lyle Wallace, USDA ARS (supervised by C. Coyne)
   Technician: Dawn Tachell (full-time)

The Phaseolus Program manages a collection of 17,653 accessions in 56 taxa, all in the genus *Phaseolus*. It is the largest single genus collection at WRPIS.

In 2022 the Phaseolus Program primarily focused on regeneration and documentation activities (**Figure 5.1**). From Jan. 2022 to December 2022, we filled orders for regenerations of 520 inventories, and placed back in the cold room 379 inventories (a backlog of regenerations from 2019-2021) and added 28 expired PVP accessions.

Figure 5.1. Clockwise starting top left, *P. dumosus* and *P. lunatus* flowers. *P. lunatus* regenerations ready to plant, *P. vulgaris* seed production for protein characterization, Phaseolus greenhouse regenerations; *P. lunatus* in the field.

As usual, all the Phaseolus regenerations are done in the greenhouse to prevent the spread of Bean Common Mosaic Virus (BCMV) within and among the accessions. We continue with regenerations focusing on *Phaseolus vulgaris* but also worked with *P. lunatus, P. coccineus, P.*

dumosus and *P. acutifolius* inventories. We are continuing to improve the IPM program in the Phaseolus regeneration greenhouses.

A. The USDA NIFA SCRI funded lima bean project supported the planting for regenerations and simultaneously collecting leaf tissue for genotyping 100 accessions listed as *P. lunatus* in the collection, nine of which were other *Phaseolus* taxa. In addition, the whole available lima bean collection ~700 was sent to UC Davis, for genotyping and phenotyping. This large, non-replicated planting of an important portion of the collection showed many accessions have poor seed vigor and require scarification and pre-germination for establishment even in greenhouses with soilless media.

B. In support of research efforts to characterize collections and to fulfill the NPGS mission to evaluate crop germplasm, the protein and dietary fiber content of common bean (*Phaseolus vulgaris* L.) has been characterized in a randomized trial of 235 accessions that was phenotyped with a near infrared (NIR) instrument for protein and dietary fiber. This phenotypic data will be combined with published genotyping-by-sequencing (GBS) data to generate marker-trait associations to aid in the nutritional improvement of common bean.

C. In support of the NPGS mission of acquiring crop germplasm, a seed exploration expedition was completed in September of 2021 to collect wild kidney bean (*P. polystachios* L.) in Georgia and Florida to fill a geographical and taxonomic (subspecies) gap in the collections, especially for rarer subspecies sinuatus and smilacifolius. This wild legume is in the tertiary gene pool and may be used for the genetic improvement of domesticated lima bean (*Phaseolus lunatus* L.) via trait introgression. Wild kidney bean may have unique adaptations to disease or climate changes. Eight new accessions of wild kidney bean (two sinuatus subspecies from Georgia and three from Florida; one smilacifolius subspecies from Florida; and two polystachios subspecies from Florida; **Figure 5.2**).

Figure 5.2. A wild kidney bean (*Phaseolus polystachios.* Subspecies smilacifolius, being tagged and processed as an herbarium specimen following collection in Florida.

MISSION-RELATED RESEARCH PROGRAMS

6. **Alfalfa Genetics Program**
   Research Geneticist: Long-Xi Yu (full time)
   Technicians: Martha Rivera (full time)
   Farmer: Jose Luis Godinez

This project focuses on enhancing resistance to biotic and abiotic stresses in alfalfa, including developing molecular markers and germplasm for breeding alfalfa varieties with improved resistance to these stressors.

A. Verticillium wilt (VW) of alfalfa is a devastating disease and causes forage yield reductions of up to 50% in the northern United States and Canada. Current breeding strategies rely greatly on phenotypic recurrent selection that is time consuming and labor costly. In collaboration with commercial alfalfa seed companies, the team led by Long-Xi Yu in Prosser, WA mapped genetic loci associated with VW resistance in alfalfa populations and identified two candidate genes for disease resistance. Functional assessments of the linked genes showed divergent roles on defense against VW disease (**Figure 6.1**). Sequencing of the resistance gene showed variants between resistance and susceptible parents. These variants were then used to develop several DNA markers for VW resistance. Alfalfa seed companies are interested in using these markers to test the VW resistance alleles in their breeding programs. Material transfer agreements have been signed between ARS and two alfalfa Seed Companies (Forage Genetics and S & W Seeds) to deliver these markers to the company for testing VW resistance in their breeding populations. The results have been published in Plant Biotechnology Journal.

B. Drought and high salinity are two abiotic stressors affecting alfalfa production worldwide. Enhancing alfalfa resistance to drought and high salinity is important to meet the challenges of limited water resources and highly saline soils. In collaboration with ARS and university scientists, a team led by Long-Xi Yu in Prosser, WA developed a multifamily mapping population by crossing an elite variety with 32 drought resistance parents selected previously by the team. The F_1 progenies were backcrossed (BC) to the elite variety and the BC population contains 600 individuals that are being evaluated for drought resistance in the field (**Figure 6.2**). The population will be used for mapping genes for drought and salt resistance. The team also identified 84 SNP markers and putative candidate genes associated with five agronomic traits including yield, plant height, dry mater content, maturity and fall dormancy in another breeding population. The team also evaluated 570 alfalfa germplasms accessions and identified drought and salt resistant alfalfa lines. Eight drought resistant lines have been transferred to Alforex and MaxField seed companies, via material transfer agreements (MTA) as breeding materials for use in development of drought resistant alfalfa cultivars.

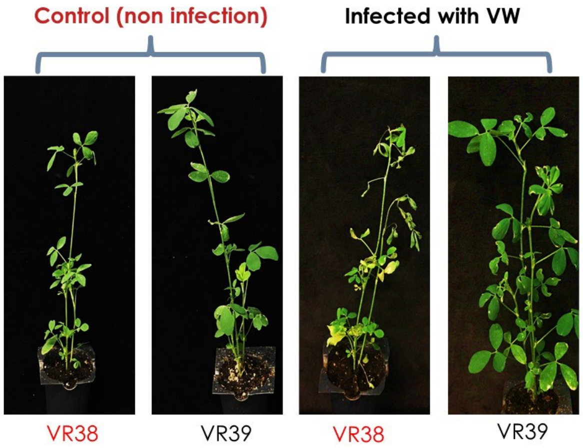

Figure 6.1. Two genes (VR38 and VR39) associated with Verticillium wilt (VW) were identified in alfalfa. Cloning and sequencing analyses revealed that the susceptible genotype carrying VR38 lost resistance due to a single nucleotide deletion in the coding region of the gene, while the genotype carrying the resistant gene VR39 expressed normally resulting resistance to VW.

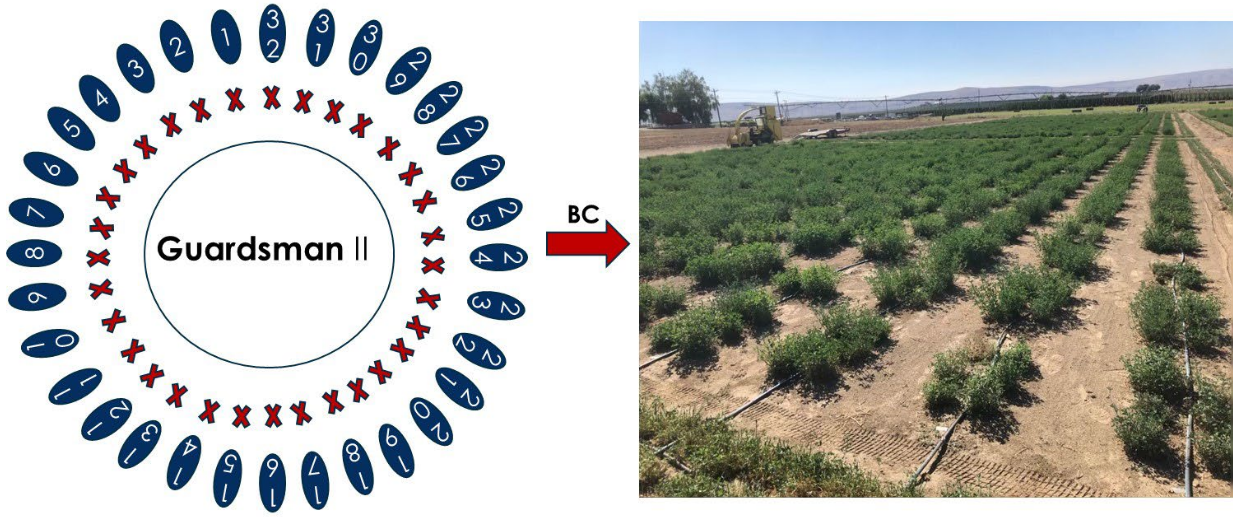

Figure 6.2. Developing multiparent population by crossing 32 drought resistant lines with an elite cultivar Guardsman II with high yield, quality and disease resistance. The F1 progeny then backcrossed (BC) with the elite parent to create F1BC1. The resulting populations are under testing in the field and will be used for gene mapping and genomic selection for drought and salt resistance in alfalfa.

7. **Plant Pathology Program**

Pathology Technician: Shari Lupien (full-time) – prepared report

With the retirement of the pathologist Frank Dugan and abolishment of the position, the Plant Pathology Research Program has reduced in scope. The program is now run by Shari Lupien and encompasses only those aspects of plant pathology pertinent to plant species (native and introduced) in the National Plant Germplasm System (NPGS) that can be done by one technician. This includes some diagnostics expertise, which will be supplemented with colleagues from WSU, and some work on remediation of clonally propagated materials when infected with viruses.

A. During 2022 Shari served as the pathology and genomics lab coordinator responsible for supply and equipment procurement, coordination of equipment maintenance and repair, lab safety orientations, and scheduling and support of laboratory activities. The physical location of our pathology and genomics laboratories were moved from Johnson Hall to Clark Hall in July 2022. Shari coordinated the move, remodel of the Clark Hall labs to accommodate our equipment, unpacked and organized the genomics and pathology labs through the end of December 2022. Shari serves as the PGITRU unit collateral duty laboratory safety officer and SHEM representative for our research unit.

B. Pathology research activities in 2022 included plant disease diagnostic work for PGITRU curators in *Allium* and *Phaeseolus* species, initial work developing plant tissue culture procedures using the basal stem disk as the explant for *in vitro* propagation of garlic. This method can increase the rate of pathogen-free plantlets compared to standard shoot tip culture. The development and evaluation of pathogen removal from PGITRU garlic accessions using garlic tissue culture procedures will continue in 2023. During October through December 2022 Shari worked on optimizing culturing conditions for high yield zoospore production in *Aphanomyces euteiches* strains used in large scale lentil inoculation studies conducted in 2023.

C. Shari was an author on the meeting abstract and poster: "Identification and Pathogenicity of *Fusarium proliferatum* Causing Clove Rot on Garlic", presented at the February 2022 joint National *Allium* Research Conference (NARC), W-3008 (multistate research project on integrated onion pest and disease management), and the 'Stop the Rot' research project team meeting held in Denver, Colorado February 28, 2022.

8. **Genetics Program**
   Supervisory Research Geneticist/Research Leader: Marilyn Warburton (full-time).
   Postdoctoral Research Associate: Renan Uhdre (full-time).

The new Research Geneticist/Leader also has a 100% research commitment and is now working on GWAS in pulses and alfalfa. She is investigating the use of pathway analyses of GWAS results in self-pollinated species, which include many of the species found in the WRPIS genebank. The Postdoctoral Research Associate, Renan Uhdre, hired with funding from the Pulse Crop Health Initiative to work on lentil protein content gene mapping and genomic selection under her supervision has been making excellent progress measuring protein content of lentil seeds from three replicated field trials in 2020, 2021, and 2022, in a population of 183 accessions grown in a randomized complete block design (RCBD) with four replicates. Sub-samples of 0.1 g were analyzed run on a on a LECO FP428 nitrogen analyzer and protein concentration was calculated from the nitrogen data. Renan has also worked to finish GWAS analysis of pea populations in collaboration with C. Coyne. A GWAS using 74,000 thousand high-quality single nucleotide polymorphism (SNP) markers was performed to identify associated variants and neighboring candidate genes with seed protein concentration. Initial results identified several significant SNP-trait associations (**Figure 8.1**), which will be confirmed; chosen SNPs will be converted into user-friendly KASP markers.

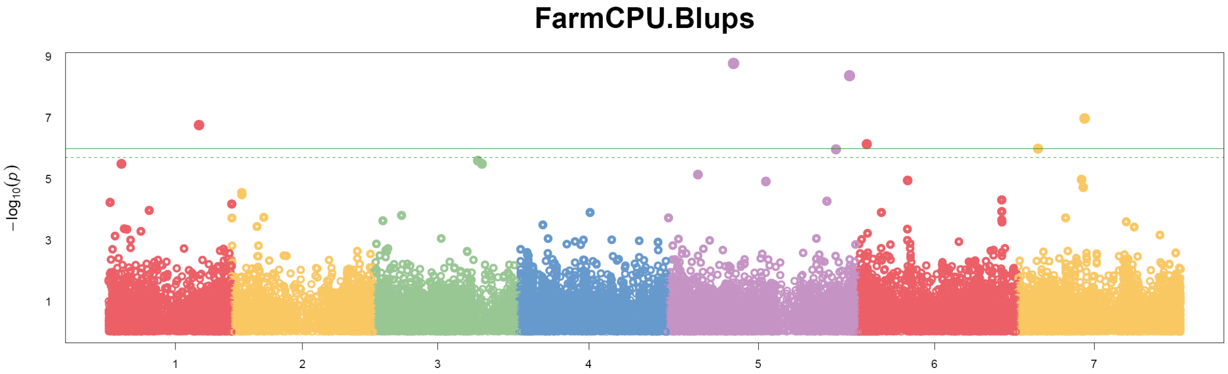

Figure 8.1. Initial results of a genome-wide association analysis of seed protein levels, presented as best linear unbiased predictor values, (BLUPs) run with ~74,000 SNP markers. The Fixed and random model Circulating Probability Unification (FarmCPU) model was used to find associations, and the green horizontal line shows the significance threshold. Colored dots above this line are significant marker-trait associations; colors delineate the 7 chromosomes of pea.

COMMITTEES, SERVICE, PUBLICATIONS

During 2022 WRPIS scientists and curators served as *ex officio* committee members or chairs of their respective national Crop Germplasm Committees (CGC) and other academic or social organizations. Research Leader and Research Geneticist **Marilyn Warburton** was the immediate past president of the Crop Science Society of America and is a fellow of that society. She is a member of the American Association for the Advancement of Science (AAAS) and the Council for Agricultural Science and Technology. She co-supervises one Postdoctoral Research Associate at WSU, and also serves as the WRPIS Station Coordinator. Research Geneticist **Long-Xi Yu** is adjunct faculty with WSU Department of Crop and Soil Sciences. He serves as member of the AOSCA National Alfalfa and Miscellaneous Legumes Variety Review Board and Washington State Hay Growers Association. He also serves as Editor-in-chief of the International Journal of Plant Pathology and Plant Protection, Associate Editor of Frontiers in Plant Science and member of the editorial board of eight peer-reviewed journals. Cool Season Food Legumes Curator **Clarice Coyne** is an elected Fellow of the American Association for the Advancement of Science (AAAS) and a Fellow of the Crop Science Society of America. She supervises one Research Technician and co-supervised three Postdoctoral Research Associates at WSU, one Federal post-doctoral research associate and is serving *ex officio* on three CGCs including *Pisum*, Food Legume, and Forage Legume CGC. She serves on the Scientific Advisory Board for a University of Saskatchewan pea project in Saskatoon, Canada. Besides AAAS, Dr. Coyne is a of member of the American Society of Agronomy, Crop Science Society of America, and the North American Pulse Improvement Association, Pulse Crop Working Group, and an Adjunct Scientist with the Department of Crop and Soil Sciences, Washington State University. She has served on the Crop Science Editorial Board and as Crop Science Society of America (CSSA) Technical Editor, Div. C-1, C-8. She served as a peer-reviewer for the journals Physiological and Molecular Plant Pathology, Legume Science, Frontiers in Plant Science, Crop Science Journal and Agronomy. Temperate-adapted Forage Legume Curator **Brian Irish** is an *ex* *officio* member of the Forage Legume CGC and in 2022 of was the Chair the Plant Germplasm Operations Committee (PGOC) of the NPGS. He is a member of the Federal Implementation Working Group and Research Subgroup on efforts relating to the execution of the National Seed Strategy for Rehabilitation and Restoration. Dr. Irish is actively involved in the Cornell-led and USDA-funded Breeding Insight efforts to leverage recent improvements in genomics and open-source informatics components to accelerate genetic gains in alfalfa. He is an adjunct faculty member with WSU Department of Crop and Soil Sciences and a member of the CSSA and American Phytopathological Society. He served as a peer-reviewer for the journals Crop Breeding and Applied Biology, Journal Plant Registration, Crop Protection, and Plant Disease, and as expert external reviewer for the National Academies of Sciences, Engineering and Medicine on the final report on "An Assessment of Native Seeds Needs and the Capacity to Supply Them". Horticultural Crops Curator **Barbara Hellier** has retired, but during 2022, she was an *ex-officio* member of numerous CGCs including Root and Bulb, Leafy Vegetable, New Crops, Sugarbeet, Forage Legume, and Medicinal and Aromatic Oil and Herbaceous Ornamental Crops. She was also a member of the PGOC Plant Exploration subcommittee. Alex Cornwall will take over these membership roles in 2023. Barbara was a member of the American Society for Horticultural Science and the American Society of Sugarbeet Technologists. Phaseolus curator Sarah Dohle is a member of the WSU Crop and Soils Science Diversity, Equity, and Inclusion committee, the Bean Improvement Cooperative, and CSSA. She served as an advisor for a WSU master's thesis.

In 2022, WRPIS scientists and curators were actively engaged in conducting mission-related research and serving the scientific community. Activities for 2022 were very busy, and personnel participated in technology transfer presenting research as oral and/or poster presentations (some virtual) at either scientific or public meetings and were involved in 18 peer reviewed scientific journal and/or book chapter publications. They were invited to review research proposals by funding agencies such as NIFA and participated as subject matter experts and in many *ad hoc* peer-reviews of manuscripts in scientific journals. Unit members including the Research Leader, 2 new curators and 3 technicians traveled to Ft. Collins to see the facilities of the Agricultural Genetic Resources Preservation Research and meet counterparts for future collaborations and training. Nine groups of students or stakeholders came to tour the farm, seed cleaning, seed storage, laboratory and greenhouse facilities at Pullman, Prosser, and central Ferry during the year (**Appendix II**).

PUBLICATIONS – 2022

Peer reviewed/Book chapter (18)

Arkwazee, H.A., Wallace, L.T., Hart, J.P., Griffiths, P.D. and Myers, J.R., 2022. Genome-Wide Association Study (GWAS) of White Mold Resistance in Snap Bean. Genes, 13(12), p.2297.

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He X, Zhang F, He F, Shen Y, Yu LX, Zhang T, Kang J. Accuracy of genomic selection for alfalfa biomass yield in two full-sib populations. Frontiers in Plant Science. 2022;13.

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Travis A. Parker, Jorge Acosta Gallegos, James Beaver, Mark Brick, Judith K. Brown, Karen Cichy, Daniel G. Debouck, Alfonso Delgado-Salinas, Sarah Dohle, Emmalea Ernest, Consuelo Estevez de Jensen, Francisco Gomez, Barbara Hellier, Alexander V. Karasev, James D. Kelly, Phillip McClean, Phillip Miklas, James R. Myers, Juan M. Osorno, Julie S. Pasche, Marcial A. Pastor-Corrales, Timothy Porch, James R. Steadman, Carlos Urrea, Lyle Wallace, Christine H. Diepenbrock, Paul Gepts. 2022. Genetic resources and breeding priorities in Phaseolus beans: vulnerability, resilience, and future challenges. Plant Breeding Reviews, 46, pp.289-420.

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APPENDIX I – WRPIS Staffing

Current Staffing (June 20, 2023)

Position (Title)	Name	Federal/State	Position type
Pullman			
Research Leader/Station Coordinator	Marilyn Warburton	Federal	PFT
Program Support Assistant	Carla Olson	Federal	PFT
IT Specialist	Vacant (CEC)	Federal	PFT
Biological Science Technician	Renan Uhdre*	Federal	PFT
Biological Science Technician	Shari Lupien	Federal	PFT
Seed Manager/Computer Specialist	Lisa Taylor	Federal	PFT
Seed germination Technician	Melissa Scholten	Federal	PFT
Farm Manager, Central Ferry	Kurt Tetrick	Federal	PFT
Plant Technician (Farmer)	Jennifer Morris	Federal	TFT
Farm Manager, Pullman	Julia Zaring	State/WSU	PFT
Plant Technician	Alec McCall	State/WSU	PFT
Plant Technician	Jason Newell	State/WSU	PFT
Plant Technician	Vacant	State/WSU	PFT
Plant Technician	Vacant	State/WSU	PFT
Agronomy and Safflower Curator	Paul Galewski	Federal	PFT
Biological Science Technician	Ezekiel Brazington	Federal	PFT
Horticulturist	Bailey Hallwachs	Federal	TFT
Cool Season Food Legumes Curator	Clarice Coyne	Federal	PFT
Biological Science Technician	Britton Bourland	Federal	PFT
Horticultural Crops Curator	Barbara Hellier	Federal	PFT
Biological Science Technician (Greenhouse maintenance)	David Van Klaveren	Federal	PFT
Biological Science Technician	Alex Cornwall	Federal	TFT
Biological Science Technician	Vacant	Federal	PFT
Phaseolus Bean Curator	Sarah Dohle	Federal	PFT
Biological Science Technician	Dawn Tachell	Federal	PFT
Postdoctoral Research Associate	Lyle Wallace	Federal	TFT
Prosser			
Temperate Forage Legumes Curator	Brian Irish	Federal	PFT
Biological Science Technician	Estela Cervantes	Federal	PFT
Plant Technician (Farmer)	Jesus Prieto	State/WSU	PFT
Alfalfa Genetics Research Geneticist	Long-Xi Yu	Federal	PFT
Biological Science Technician	Martha Rivera	Federal	PFT

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*Renan Uhdre is a WSU postdoctoral research associate temporarily filling in the position of biological sciences technician.

APPENDIX II – Research, Service and Outreach Activities (Calendar Year 2022)

- February 14-15: **Clarice Coyne** attended and presented virtually a talk "Can genomic prediction play a role in IPM?" to the Pulse Crop Working Group annual meeting.
- February 17: **Marilyn Warburton** attended the American Association for the Advancement of Science (virtually).
- March February 28: **Marilyn Warburton, Barbara Hellier,** and **Alex Cornwall** attended the Root and Bulb GCG CGC meeting. Barbara Hellier presented Allium collection status report.
- February 28-March 1: **Barbara Hellier** and **Alex Cornwall** attended the National Allium Research Conference (virtually).
- March 3: **Brian Irish**, as Chair of the PGOC, organized, invited, and hosted a short workshop/webinar to the NPGS staff on digitization of important, historical documentation.
- March 7-8: **Barbara Hellier, Alex Cornwall, Dawn Tachell,** and **Lisa Taylor** provided tours of unit greenhouses and genebank facilities to WSU Hort. 202 class.
- March 24: **Barbara Hellier** and **Shari Lupien** provided a tour of the genebank facilities and discussed possible collaborations related to virus clean-up in garlic with Jenny Durrin and Shannon Kuhl of the University of Idaho Seed Potato Program.
- May 16: **Clarice Coyne** supervised a summer undergraduate, Griffin Staffenberg, Horticulture-WSU research internship for 10 weeks.
- May 16: **Clarice Coyne** supervised a summer Curricular Practical Training of a M.S. student in statistics student from the University of Idaho.
- June 1: **Clarice Coyne** and **Marilyn Warburton** hosted and mentored visiting scholar Fareeha Riaz from NIAB-College Pakistan Institute of Engineering and Applied Sciences for 3 months on lentil genetics and genomics for nutritional traits.
- June 3: **Clarice Coyne** attended the AAAS Fellow Award Reception and Dinner.
- June 3: **Marilyn Warburton** accompanied aides from Washington state senators on a tour of the facilities and discussions about the new ARS building on the WSU campus.
- June 7: **Barbara Hellier** led a tour of students through the genebank facilities.
- June 7: **Brian Irish** participated in the annual meeting of NE 2210 Multi-State research project on Improving Forage and Bioenergy Crops for Better Adaptation, Resilience and Nutritive Value.
- June 7-9: **Long-Xi Yu** and Brian Irish attended and presented research in the biannual meeting of the North American Alfalfa Improvement conference in Lansing, MI.
- June 14-16: **Marilyn Warburton**, **Brian Irish**, **Clarice Coyne**, **Barbara Hellier**, **Paul Galewski**, **Sarah Dohle**, **Bailey Hallwachs** and **Lisa Taylor** attended the Plant Germplasm Operations Committee and Curator Workshop (virtually). **Brian Irish**, as Chair and along with other PGOC leadership, organized and hosted the meetings.
- June 14-16: **Clarice Coyne** attended and presented a talk "High through-put phenotyping of plant genetic resources" at Plant Germplasm Operating Committee/Curator's Workshop (virtual)
- June 21: **Marilyn Warburton** was the plenary lecturer to the Western Society of Crop Science (virtually).

- July 8: **Marilyn Warburton**, **Brian Irish**, **Clarice Coyne**, **Barbara Hellier**, **Paul Galewski**, **Sarah Dohle**, **Bailey Hallwachs** and **Lisa Taylor** attended the W-6 Regional Technical Advisory Committee meeting (virtually).
- July 15: **Barbara Hellier** and **Alex Cornwall** attended the Herbaceous Ornamental CGC meeting (virtually). Barbara Hellier presented HO collection status report.
- July 25: **Brian Irish** and the TFL program began hosting Columbia Basing College student intern, Araceli Martinez for four weeks.
- August 11: **Brian Irish** and other local SYs hosted Pacific West Area leadership team and gave short TFL overview presentation to Prosser worksite RL/Area Office and other personnel.
- August 16: **Alex Cornwall** and **Barbara Hellier** led a tour of the genebank facilities for Carol Miles, WSU Horticulture professor, and her graduate students.
- August 22: **Barbara Hellier** and **Sarah Dohle** attended the Phaseolus CGC meeting (virtually**).** **Barbara Hellier** presented Phaseolus collection status report.
- August 23-26: **Clarice Coyne** attended and presented a talk "Artificial intelligence in breeding for root rot resistances in pulse crops" at the 8th International Legume Root Diseases workshop (virtual).
- August 29-30: **Marilyn Warburton** and **Renan Uhdre** presented lectures on genome-wide association study to 30 graduate students at the Africa Rice International Center (virtually).
- September 6: **Marilyn Warburton** and **Sarah Dohle** met with the US Dry Pea and Lentil Commission in Moscow, ID.
- September 23: **Barbara Hellier** and **Alex Cornwall** attended the New Crops CGC meeting (virtually). **Barbara Hellier** presented the New Crops collection status report.
- October 5: **Lisa Taylor** led a group of students though the genebank facilities.
- October 6: **Marilyn Warburton** presented a seminar to the WSU Crop and Soil Science Department.
- October 7: **Lisa Taylor** provided a genebank tour to USDA Grain Inspection Personnel.
- October 7: **Brian Irish** served as Technical Expert and Reviewer of the National Academies of Sciences Engineering and Medicine (NASEM) Final Report on "Native Seeds ad An Assessment of Native Seed Needs and the Capacity for Their Supply".
- October 12: **Marilyn Warburton**, **Clarice Coyne**, **Sarah Dohle** and **Lyle Wallace** hosted the USA Dry Pea and Lentil Commission for a stakeholder meeting and tour of the USDA ARS WRPIS genebank facilities.
- October 19: **Marilyn Warburton**, **Brian Irish** and **Long-Xi Yu** participated in an information providing meeting with National Alfalfa Forage Alliance leadership and industry/stakeholder representatives.
- November 6-9: **Marilyn Warburton** attended the Crop Science Society Annual Meeting in her role as President, which included meetings, introducing speaker and award winners, and business meetings.
- November 6-10: **Clarice Coyne** presented research poster on "Progress in unlocking genetic potential in cool season pulses" and co-authored two other presentations "Resequencing of 238 lentil accessions provide insights into diversity, domestication, and genetic basis of important traits" and "Understanding the genetic basis of protein quality in peas and development of genetic resources for breeding high protein pea germplasm" at the ASA-CSSA-SSSA Annual meeting in Baltimore, Maryland.

- November 14-17: **Brian Irish** and **Long-Xi Yu** participated and presented alfalfa genetic and TFL program research at the International Alfalfa World Congress, San Diego, CA.
- December 5: **Clarice Coyne** and **Marilyn Warburton** hosted visiting scholar Dr. Hatice Sari studying nutritional quality of peas on a 12-month Türkiye Fellowship.
- December 5: **Marilyn Warburton** and **Sarah Dohle** attended the Western Pulse Growers Meeting in Moscow, ID.
- December 6: **Marilyn Warburton**, **Barbara Hellier**, **Alex Cornwall**, **Sarah Dohle**, and **Paul Galewski** hosted Anna Murphy, the Executive Vice President of the Beet Sugar Development Foundation, and the American Society of Sugar Beet Technologists, for a stakeholder meeting and tour of the USDA ARS WRPIS genebank facilities.
- December 7: **Marilyn Warburton** and **Clarice Coyne** attended the Western Pulse Growers Association Annual Meeting in Moscow, ID. **Clarice Coyne** presented poster “Progress in unlocking genetic potential in cool season pulses”.