W-4150
Breeding Common Bean (*Phaseolus vulgaris* L.) for Resistance to Abiotic and Biotic Stresses, Sustainable Production, and Enhanced Nutritional

Combined State Reports
Prepared September-October 2021
Hybrid meeting held on August 20, 2021

ARIZONA

University of Arizona (prepared by Judith K. Brown)

Common bean (*Phaseolus vulgaris* L.) and other beans dry beans such as tepary bean (*P. acutifolius*) are susceptible to biotic stress caused by plant pathogens, insect pests, and abiotic stress including drought, producing a viable crop under conditions of 40-170 cm of annual rainfall. Together abiotic and biotic stress reduce yield and quality.

The virome, a subset of the phytomicrobiome, is the subject of our research in the context of outcomes of virus infection of common bean and tepary bean. *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrotic virus* (BCMNV), both seed-transmitted single-stranded, linear RNA viruses, are the causal agents of common bean diseases caused by plant ssRNA viruses. The same five genes control the genetics of resistance to both BCMV and BCMNV in common bean, one dominant (*I*) and four recessive genes (*bc-u*, *bc-1*, *bc-2*, and *bc-3*). The two recessive genes, *bc-1* and *bc-2*, control the systemic movement of both BCMV and BCMNV in common bean plants but often confer partial or incomplete resistance in single infections (Feng et al. 2017, 2018). The genus*, Begomovirus* (Geminiviridae), are important viral pathogens in the Americas where the common bean is widely grown. They are transmitted by the whitefly *Bemisia tabaci* (Genn.) cryptic species, and several are experimentally transmissible by mechanical inoculation. Among them, a core bean-infecting species cluster has been recognized because they were the most prevalent begomoviruses infecting common bean in the neotropics. Their host range is limited to bean and/or one or several leguminous wild hosts, suggesting the long-term cultivation of *Phaselous* spp. within and adjacent to its center of origin they have evolved a restricted host range restricted to these few hosts. This suggests that begomoviruses such as this with a narrow host range could become rare or extinct if bean was not cultivated where the core begomoviruses are endemic, in that their nearly exclusive host-association with common bean, *Macroptilium* spp., *Calopogonium,* and wild *Phaseolus* spp. (Morales and Jones, 2004) is indicative of a narrow host range, with selection unfavorable for expanding to other hosts.

In preliminary (unpublished) studies, BCMV was found to be seed-borne at high frequencies (60-100%) in tepary seed lots of black, tan, and white seeds, all with concomitantly high germination rates. In common bean, BCMV can remain viable for up to 30 years. Thus, under field conditions, seedlings of BCMV-infected common bean can serve as the source for virus spread by aphids in a non-persistent manner. Although in symptomatic bean attributed to BCMV, symptomless BCMV infections can result in >50% yield reduction in certain cultivars, no analogous studies have examined the yield or biomass of tepary bean infected by BCMV. In addition, the potential susceptibility to BCMNV strains, which have become common in the U.S. and elsewhere, infecting common bean.

Tepary bean is a legume native to the southwestern United States, Mexico, and Mesoamerica where it has been grown by the native people since pre-Columbian times. It is more drought-resistant than the common bean and is grown in desert and semi-desert conditions. Although tepary bean as a source of resistance to abiotic and biotic stresses has been poorly studied, until recently, it has been found (by others participating in this project) to harbor resistance to several plant pathogens and drought and may confer resistance to other abiotic stresses. Introgression of multiple resistances from tepary bean into common bean suggests that tepary bean may harbor a pathogenicity island or an equivalent there-of conferred by long-standing evolution in the face of multiple sources of abiotic and biotic stress in its center of diversity, spanning Mexico and the U.S. desert southwest, including southern Arizona where the Native Americans have cultivated tepary bean for more than a millennium. Tepary bean is as nutritious as red and black *P. vulgaris* beans by providing equivalent fiber, protein, fat, and carbohydrates, and cooking prior to eating is essential to combat anti-nutrients that can impede digestion.

The research aims are: (1) Sequence and characterize the virome associated with tepary bean (red, tan, and white seed colors), (2) evaluate the effects of seed-borne BCMV and BCMNV on tepary bean plant performance (greenhouse, initially) under wild type conditions to establish baseline agronomic data for seed production, rate of seed infection, and bean biomass (wet and dry), (3) functional genomics (dual host-pathogen) profiling of BCMV- and BCMNV infected tepary exposed to drought stress, and BCMV/BCMNV-infected/uninfected common bean, positive control) to characterize the host response to infection and the virome population structure. How host-virome interactions play out in the face of abiotic stress is a primary interest, making viral and host genes involved in biotic and abiotic stress scenarios of great interest.

Activities in 2020-2021: (limited due to the SARSCo2 virus outbreak).

1. In 2020-2021, the red, tan, and white tepary bean lines were increased in field plots located at the UA-Maricopa Agriculture Center. The seed was harvested and stored in the seed vault cold room on the main campus, Plant Sciences. Seeds of each color were planted, scored for germination, symptom development, and BCMV infection was analyzed by RT-PCR amplification of a fragment of the coat protein gene and confirmatory sequencing.

2. Seed germination ranged from 95-100%, with virus infection ranging from 58-100%. Based on RT-PCR detection, asymptomatic and symptomatic plants were observed with infection rates of 58- and 100%. This result confirms that BCMV seed transmission (vertically) occurs consistently from generation to generation with similar rates of transmission and percentage of symptomatic and asymptomatic phenotypes.

3. Common bean plants were inoculated with BCMV isolates from red, tan, and white tepary seed and observed for symptom development. Symptomatic plants were positive for BCMV infection. Virus was detected in only symptomatic bean plants, indicating that all infected common bean plants develop symptoms. This contrasts with the results obtained for tepary bean.

Activities in 2021-2022 underway:1. Sequencing (RNAseq) and characterization of the RNA virome associated with tepary bean (red, tan, and white seed colors), with an emphasis on BCMV

and comparative sequence analysis with other BCMV isolates for which genome sequences are available in the GenBank database.
2. Evaluate the effects of seed-borne BCMV and BCMNV on tepary bean plant performance (greenhouse, initially) under wild-type conditions to establish baseline agronomic data for seed production, rate of seed infection, and bean biomass (wet and dry). Experiments will utilize three tepary bean genotypes naturalized to southern Arizona. Tepary bean plants will be grown under contrasting irrigation regimes, well-watered and water-limited conditions in pots in a growth chamber and/or UA campus greenhouse. Water will be applied according to the irrigation frequency such that the water-limited treatment receives approximately half that of the well-watered level.
3. Functional genomics (dual host-pathogen) profiling of BCMV- and BCMNV-infected tepary exposed to drought stress, and BCMV/BCMNV-infected/uninfected common bean, positive control) to characterize the host response to infection and the virome population structure.

CALIFORNIA

University of California, Davis (prepared by Paul Gepts)

After the severe curtailment due to COVID-19 in 2020, field activities returned to a semblance of normality in 2021, at least for experiments conducted on campus. Field plantings of *Phaseolus* beans consisted mainly of lima bean and common bean. For lima bean, advanced lines with emphasis on Large Limas were evaluated in a replicated trial and a diversity panel of lima bean germplasm lines. These trials were conducted with and without insecticide protection to estimate the effect of Lygus (Western Tarnished Plant Bug). An additional experiment was conducted to test a novel sensor technology that can detect Lygus flights continuously during the growing season. For common bean, the Cooperative Dry Bean Nursery was planted in Davis as well. Small plantings were conducted to identify the green cotyledon trait in lima bean. In parallel to the breeding program, a large scale experiment is being conducted with physiologists of the Plant Sciences department at UC Davis, to study the reaction of common bean (and certain tepary bean genotypes) to terminal drought. The plant material has been genotyped at low density for SNPs and are being characterized for photosynthesis parameters. These parameters are obtained by direct measurements of the foliage as well as fixed and mobile (UAV) remote sensing. Given the extended growing season in California's Central Valley, the experiments are being harvested, and analyses will be conducted in the coming months.

DELAWARE

University of Delaware (prepared by Emmalea Ernest)

Snap bean trials were conducted at the University of Delaware's research farm located in Georgetown. These trials continue to identify heat tolerant varieties that are well adapted for processing and fresh market production on the Delmarva Peninsula. Twenty-eight round podded varieties were evaluated in trials planted on June 2 and June 16. Both trials were exposed to heat stress. Bridger (H.M. Clause), PV 857 (Crites Seed) and Jaguar (Crites Seed) produced the highest marketable yield under heat stress. Bridger and PV 857 have performed well in past heat stress trials. Jaguar is new to the trials this year. A trial of 11 flat-podded varieties was planted on May

11. The highest yielding varieties in this trial were BEX175 (Brotherton), Solferina (Seminis), and Tapia (Seminis).

Eighty-seven baby lima inbreds from the University of Delaware lima breeding program were evaluated in replicated yield trials. Heat tolerant breeding lines produce significantly higher yields than standard varieties but do not have the required green seed color. Thirty-nine large-seeded bush "Fordhook" type inbreds from the breeding program were also evaluated. Several breeding lines produced significantly higher yields and matured earlier than the standard variety. Additional experiments were conducted to assess the value of the willow leaf trait for disease and stress avoidance, as described in the impact statement below.

Research Assesses the Usefulness of the Willow Leaf Trait in Lima Bean for Disease and Stress Avoidance *Issue*: Baby lima beans are an important processing vegetable crop for Delaware farmers, but heat stress, drought and diseases cause yield loss each year. A narrow leaf shape (willow leaf) variant exists in lima bean. This trait could be useful in helping plants to avoid stress and disease effects by altering the microclimate of the plant canopy in a way that makes it less conducive to disease development and helps the plant conserve water. However, the effect of the willow leaf trait on lima bean productivity and stress tolerance is unknown.
Response: In 2019 and 2020, field experiments that included Near Isogenic Line (NIL) pairs with and without the willow leaf trait were established at the Newark and Georgetown research farms. The Newark plots were inoculated with *Phytophthora phaseoli* which causes lima bean downy mildew, and rated for disease to determine if the willow leaf trait affected the development of this disease. To evaluate for avoidance of this disease, Georgetown plots were inoculated with *Phytophthora capsici* which causes lima bean pod rot. Additionally, in 2020 and 2021 a plot with four of the NIL pairs was established at Georgetown to compare the yield and other agronomic traits of the willow versus typical leaf shape. Temperature and humidity loggers were placed in these plots to monitor plant canopy conditions throughout the season.
Recognition of Sponsors: This research was supported by a Specialty Crop Block Grant from Delaware Department of Agriculture titled "Development and Testing of Lima Bean Varieties with Improved Plant Architecture and Stress Tolerances" and a CANR Seed Grant "Exploring Effects of Leaf Shape and Plant Architecture on Lima Bean Disease Tolerance and Succulent Seed Quality."

IDAHO

University of Idaho (prepared by Alexander V. Karasev)

Three potyviruses were identified in samples of a free-living rattlepod *Crotalaria micans* collected in Hawaii, three distinct isolates of bean common mosaic virus (BCMV), bean yellow mosaic virus (BYMV), and clover yellow vein virus (ClYVV). Nearly complete genome was assembled for the ClYVV (9,520-nt) and deposited in GenBank under the accession number MT631721; it displayed 95% identity to the Korean ClYVV isolate (KF975894) from soybean. BCMV and ClYVV were biologically separated from this mixed infection in *Nicotiana benthamiana* through a single-lesion selection on *Chenopodium quinoa* with subsequent back inoculations to *N. benthamiana*. These BCMV and ClYVV isolates produced distinct symptoms in *N. benthamiana*, vein clearing for ClYVV and mild mosaic and leaf deformation for BCMV. BYMV was previously reported in Hawaii from vanilla and nasturtium, while BCMV was reported from flowering ginger and lima bean in Hawaii. To the best of our knowledge, this is the first report of ClYVV found in Hawaii, and the first report of BYMV, BCMV, and ClYVV infecting *Crotalaria* spp.

IOWA

Iowa State University (prepared by Donna Winham)

Activities: ISU researchers developed and pilot-tested a pulse cooking class and nutrition education workshop with college students. Information on consumer acceptability and barriers to pulse usage in meals was identified. Students and Training: One undergraduate and one PhD student at Iowa State University.

MARYLAND

USDA-ARS (prepared by Talo Pastor-Corrales)

Objective one: Improving bean yield potential by incorporating resistance/tolerance to major biotic and abiotic stresses, broadening the genetic base, implementing/integrating genomic resources, and coordinating field trial nurseries.

Fine mapping of an anthracnose resistance gene present in Andean common bean landrace Beija Flor BF): This landrace was obtained from the germplasm bank of the State University of Maringa in the state of Parana, Brazil. BF is resistant to multiple Mesoamerican and Andean races of *Colletotrichum lindemuthianum* (CL), one of the most devastating diseases in common bean. The inheritance of resistance study performed using two populations inoculated with four races of CL indicated that the resistance in BF was conferred by a single and dominant gene. The fine mapping the ANT R gene in BF was positioned on a 31.7 Kb genomic region on Pv04, flanked by KASP markers SS333 and SS309. Three candidate genes described in the reference genome of G19833 cultivar, were in the genomic region delimited by markers SS333 and SS309. One of these genes, PHAVU_004G0056001g, contains a leucine-rich repeat domain, with functions related to pathogen resistance.

Validating the KASP marker SS322 tagging the *Ur-11* rust resistance gene present on the Middle American common bean accession PI 181996: *Ur-11* is the most important rust resistance gene; it is the only gene that confers resistance to 89 of 90 races of the highly variable bean rust pathogen (*Uromyces appendiculatus*) maintained at Beltsville. The bean rust threatens dry and snap bean production. Previous fine mapping in our lab, positioned the *Ur-11* gene on a of 9,013 bp genomic region on chromosome Pv11, between KASP markers SS375 and SS322. We then used the SS322 KASP marker to genotype a panel of 201 common bean cultivars that included Andean and Middle American beans, snap and dry beans from different market classes, and with different RRgenes. The SS322 marker was highly accurate (99.5%) in identifying the *Ur-11* locus in the panel of 201 cultivars. Only one false-positive result was observed for SS322. One candidate gene (PHAVU_011G200900g) was discovered in the genomic region delimited by markers SS375 and SS322 present in the reference genome of G19833.

Mapping of the rust resistance gene in Andean snap bean Golden Gate Wax (GGW): A total of 442 F_2 plants from the Pinto 114 (S) x GGW (S) cross were inoculated with races 44, 47, 63, and

73 of the rust pathogens. GGW is resistant to the four races. This phenotypic evaluation indicated that a single and dominant gene in GGW confers rust resistance to the four races. Five susceptible bulks were generated for bulk segregant analysis (BSA) and genotyped with the 12K SNP Beadchip. SNPs associated with the rust resistance gene in GGW were positioned on a 5.3 Mbp genomic region (5.6 to 11.03 Mbp), on chromosome Pv07. KASP markers were developed in the region identified by BSA. A total of eight KASP markers were then used for genotyping the Pinto 114 x GGW F_2 population. Fine mapping positioned the rust resisistance gene in GGW between SS392 (6,748,703 bp) and SS365 (6,949,243 bp) KASP markers in a 200 kb genomic region on Pv07. The gene was linked at 0.0 cM to the KASP markers SS394 and SS364.

Mapping of a second rust resistance on the Andean common bean PI 260418: A gene for rust resistance (temporarily called gene A) in the cultivar PI 260418 was previously mapped on Pv04. A second gene (provisionally called gene B) was identified in PI 260418. This gene, which confers resistance to races 63 and 85, was positioned on chromosome Pv07. Mapping analyses positioned the gene B between KASP markers SS389 (6,694,779 bp) to SS366 (7,240,925 bp) of V.1 of the common bean reference genome G19833. Thus, the genomic region is of 546,146 bp. The phenotypic data indicated that gene A on Pv04 is epistatic to Gene B on Pv07.

Discovering rust resistance genes in the National Cooperative Dry Bean Nurseries (CDBN) 2020: A total of 24 common bean cultivars from a diverse group of bean breeding programs were evaluated in greenhouse for their reaction to races 47, 49, 53 and 67 of the bean rust pathogens (*Uromyces appendiculatus*). Each entry was also genotyped with molecular markers linked to the major rust resistance genes (molecular marker in parenthesis): *Ur-3* (SS68), *Ur-4* (SS240), *Ur-5* (SS183, and *Ur-11* (NDSU_IND_11_48.4598). There were several cultivars with single rust resistance genes: *Ur-3* alone: AC Portage, GN17-7-3, ND Pegasus, NE1-1736, PT11-13-1, La Paz; *Ur-6* alone: Palomino, Othello, *Ur-11* alone: Falcon. Cultivars with two rust resistance genes: with *Ur-3* & *Ur-6*: NE1-17-19, PT16-9; with *Ur-3* & *Ur-11*: NE4-17-10, PT11-13-31, Cultivars with three rust resistance genes: *Ur-3*, *Ur-6*, *Ur-11*: NE2-1737; with four rust resistance genes: *Ur-3*, *Ur-4*, *Ur-6*, *Ur-11*: NE4-17-6

Evaluation of the *Phaseolus acutifolius* (Tepary) F_2 population from TEP 22 x G40173A cross with races of the bean rust pathogen: This study was a collaboration between the bean project at ARS-Beltsville and Dr. Carlos Urrea, University of Nebraska. Dr. C. Urrea developed the population. TEP 22 is a tepary bean cultivar registered by Dr. T. Porch of ARS-Puerto Rico, with immune (resistant) reactions to multiple races of *Uromyces appendiculatus*. The immune *reaction with no visible symptoms exhibited by TEP 22 is unknown in common beans.* The unique resistance in TEP 22 could be of great value for breeding programs aiming to develop rust resistant cultivars. Additionally, TEPP 22 is tolerant to *Xanthomonas axonopodis*, the causing agent of common bacterial blight (CBB) in common bean. We evaluated in the greenhouse a population of 417 F2 plants from the cross TEP 22 (resistant to rust and CBB) x G40173A (Susceptible to rust and CBB). The F2 plants, parents, and check cultivars were inoculated with races 67 and 108 of the rust pathogen and isolate R14 and R19 of the CBB pathogen. The segregation of 417 F_2 plants for rust was: 108 were immune (AA), 204 had TP (tiny pustule) with Aa genotype, and 104 were susceptible (aa). These results indicate an incomplete type of dominance for rust in Tep 22. This type of resistance had been previously reported by Miklas and Stavely (1998). The segregation of

284 F_2 plants for CBB was: 155 plants were resistant (AA, Aa) and 129 were susceptible (aa), indicating that resistance to CBB in Tep 2 is conferred by a single and dominant gene.

Identification of rust resistance gene(s) in promising bean lines 2104-1-1 and 2104-1-2 from Puerto Rico: This was a collaborative endeavor with Drs. J. Beaver and T. Porch. Line 2104-1-1 has the *bgm1*, *I*, SAP6, ALS resistance in PR, and resistance to rust in the presence of a strain that overcame *Ur-3*. Line 2104-1-2 has bgm1, *I*, *bc3*, ALS resistance in PR, and resistance to rust in the presence of a strain that overcame Ur-3. Both lines are individual plant selections of PR 1627-8. Phenotypic evaluation: 2104-1-1 and 2104-1-2 and control cultivars inoculated with races 49, 53, 67, and 108 of *Uromyces appendiculatus*. Both lines and the control cultivar Mexico 309 with *Ur*-5, were resistant to race 53, showing tiny pustules. However, both lines were susceptible to races 49, 67, and 108 indicating that these lines did not have *Ur-3*, *Ur-4*, *Ur-6*, *Ur-7*, and *Ur-11*. These results indicated that, like Mexico 309 (*Ur-5*), the 2104-1-1 and 2104-1-2 lines have only the *Ur-5* rust resistance gene. Genotyping all beans in this study with KASP molecular markers SS68 (Ur-3), SS240 (Ur-4), SS183 (Ur-5), and SS322 (*Ur-11*) also confirmed that the 2104-1-1 and 2104-1-2 lines and Mexico 309 have the Ur-5 RR gene.

Other collaborations during 2021. We conducted the phenotyping in two studies in collaboration with Dr. Miklas, ARS-Prosser, Washington state: In the first study, 10 and 18 cultivars were phenotyped for the presence of the *Ur-7*nd *Ur-11* rust resistance genes, respectively. All 28 cultivars and seven control cultivars with known rust resistance genes were inoculated with three rust pathogen races (53, 67, 108). Of the 18 cultivars evaluated for the presence of *Ur-7* gene: four cultivars had Ur-7, one cultivar had Ur-3 and 12 cultivars were susceptible. Four of 10 cultivars tested for the presence of *Ur-11*, four had Ur-11 alone; 3 had *Ur-3* & *Ur-11* or *Ur-3* & *Ur-7*; 1 had Ur-7 and Ur-11 or Ur-7 & Ur-5. In the second study, we phenotyped a RIL population from the Orion x USPT-WM-12 cross. Orion Cultivar has the *Ur-7* gene for resistance to rust. USPT-WM-12 cultivar has the *Ur-3* gene, but it didn't have Ur-7. The *Ur-3* and *Ur-7* genes are positioned very close to each other on chromosome PV11 of common bean. In the greenhouse, we evaluated 144 lines from the RIL population Orion x USPT-WM-12 for their reaction to races 67 and 108 of the bean rust pathogens. We identified lines with the *Ur-3* gene alone, lines with *Ur-7* gene alone, lines combining *Ur-3* and *Ur-7*, and lines without neither of the two rust resistance genes.

What opportunities for training and professional development has the project provided?
Postdoctoral fellow and one Ph.D. student conducted genetic and genomic research leading to the mapping of rust and anthracnose resistance gene and learning about all phases of using conventional plant pathology, breeding, and genomic tools for dry bean breeding.

How have the results been disseminated to communities of interest?
Results were published in the Bean Improvement Cooperative (BIC) and multiple scientific journals, as shown in the Publications section of this manuscript. (*Journal of Nutrition*, *Journal of Plant Registrations*, and *Phytopathology News*). Results were also presented and discussed during the Nebraska Dry Bean Growers Association bean days in February and August 2020 (reaching about 110 bean growers). A summary of the evaluation of the 2020 CDBN was sent to Dr. Urrea for distribution.

MICHIGAN

Michigan State University and USDA-ARS (prepared by Francisco Gomez and Karen Cichy)

In 2021, dry bean research was conducted by Michigan State University and the USDA-ARS at East Lansing, MI. The MSU dry bean breeding and genetics program conducted 17 yield trials in 2021 in ten market classes and participated in the growing and evaluation of the Cooperative Dry Bean, Midwest Regional Performance, National Drought, and the National Sclerotinia (White Mold) Nurseries in Michigan and winter nursery in Puerto Rico. The nurseries were planted (2 June and 7) and received an average of 13.44" of rain (June - mid Sept). This combined with high heat index and growing degree days led to an unusual earlier than normal harvest. The MSU dry bean breeding program evaluated ~1,800 early generation breeding lines as part of the W4150collaborative winter nursery. Other research by MSU looked at nitrogen fixation in dry beans, anthracnose screening and introgression, and the development of affordable phenotyping platforms using unmanned aerial vehicles (UAS) to estimate important agronomic traits.

The USDA-ARS Dry Bean Genetics Program participated in the following 1) performed breeding trials within the cranberry, kidney, yellow, and black-market classes, and organic beans. 2) Progressed in the development of molecular markers for cooking time. Genomic regions associated with cooking time QTL were identified based on previous QTL and RNAseq studies. The genomes of four bean genotypes with known QTL were sequenced. This genomic information will be used for KASP marker development. 3) Elucidated longevity (shelf life) of fast cooking beans. Seeds from the 2020 field season of select breeding lines were artificially aged in chambers set to mimic tropical and temperate storage conditions, and following the treatment, cooking times were measured. Scanning electron microscopy was also conducted to evaluate how the treatments influenced the cell wall thickness of the beans under the storage conditions. 4) Evaluated beans for use as an ingredient: An industry survey on interest in the use of bean flour was developed and conducted. Milling quality based on particle size and fatty acid profile were evaluated in bean, pea, lentil, and chickpea cultivars. Regular and slow darkening pinto beans from ARS, Prosser and NDSU were evaluated for flour quality.

NEBRASKA

University of Nebraska (prepared by Carlos Urrea)

The 71st annual Cooperative Dry Bean Nursery (CDBN) report was compiled and distributed in March 2021. During the 2020 CDBN, 24 entries were tested in trials in 6 locations in the U.S. and Canada. Final results were compiled and distributed to all project members and made available to the public via the http://cropwatch.unl.edu/varietytest-Drybeans/2020 web page. The 2020 Dry Bean Variety Trial results were posted on the same web page. In 2021 CDBN, 15 entries are being tested in trials in 6 locations in the U.S. and Canada. Dr. Urrea participated in the 2021 Mid-west Regional Performance Nursery (MRPN); 6 Nebraska lines are being tested. The 2021 national Dry Bean Drought Nursery (DBDN) was assembled and distributed with 24 lines from MI, WA, NE, and PR tested in MI, WA, PR, and NE. About 24 F2:3 families from the fourth shuttle breeding cycles between Nebraska and Puerto Rico were tested in Scottsbluff under drought stress and will be tested in Puerto Rico under drought and non-drought stress environments. Two shuttle breeding lines were released as sources of drought tolerance and multiple disease resistance, SB-DT2 (pinto) and SB-DT3 (small red). The national White Mold Monitor Nursery (WMMN) was planted in Scottsbluff in 2021. Studies of bacterial wilt resistance continue. Mapping the bacterial wilt resistance is in progress (G18829/Raven). Dr. Urrea increased breeder to the foundation seed of the upright northern cultivar White Pearl (NE1-17-10) and one slow darkening pinto cultivar Wildcat (NE2-17-18) at the Kimberly Experimental Station Idaho. White Pearl has an upright plant architecture, carries the Ur3 rust resistance genes and the I bean common mosaic virus (BCMV) resistance gene, shows tolerance to common bacterial blight (CBB), and has high yield potential. Wildcat carries the Ur11 rust resistance and the I BCMV resistance genes. Both lines have high yield potential and large seed size. Another great northern, NE1-17-36, and two slow darkening pintos, NE2-17-37 and NE4-17-6 are being increased as a breeder to breeder seed at the Kimberly Experimental Station. Foundation to Foundation seed increases of the two great northern cultivars, Coyne and Panhandle Pride. Dr. Urrea is participating in screening the yellow bean panel led by Dr. Karen Cichy. Dr. Harveson's newest project by my lab, begun in 2021, is a collaboration with several microbiologists in Georgia and Florida. We are characterizing and evaluating the pathogenicity and virulence of bacterial wilt isolates (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*) collected from the stratosphere in the greenhouse this winter. We also continued collaborative work with Carlos Urrea to evaluate Phaseolus breeding lines and germplasm sources to create new regionally adapted and commercially accepted agronomically cultivars with resistance to bacterial diseases, including wilt, brown spot, and fuscous blight. I am also still serving as committee member of a Ph.D. student of Carlos Urrea (Erika Sanchez-Betancourt), studying disease resistance and mapping resistance genes for the bacteria wilt pathogen in Nebraska. We are continuing with testing new copper-alternative chemicals for managing bacterial diseases in Nebraska, while also expanding our targets to include managing other diseases of dry bean, including white mold and bean rust. We have conducted multiple industry projects in 2021 evaluating new chemical fungicidal products and application methods (sprays, seed treatments, etc.) for rust, white mold, and root rot diseases (*Rhizoctonia* and *Fusarium* spp.). Lastly, we have begun studies characterizing root pathogens and diseases (Rhizoctonia and Fusarium root rots) associated with new pulse crops and evaluating them as potential disease problems in dry bean production. This project is in the second phase for being accepted for the Nebraska Dept. of Agriculture's Specialty Crops Block Grant Program for funding, and eventually should be useful for crop rotation systems and dry bean breeding purposes if interest with other pulse crops grown continues in Nebraska.

NEW YORK

Cornell (prepared by Michael Mazourek)

A panel of beans representing key genetics and phenotypic contrasts useful for initiating a bean breeding program focused on sustainability were complied. Germplasm in the panel was informed by previous field surveys, literature reviews, seed catalogs specializing in heirloom seeds and input from members of the W4150 group. Over 100 accessions of the target 144 were received, and many were increased from a single seed to ensure the genetic purity of the stocks. Toward a better understanding of the genetics of this group, we genotyped them with the BIC community 12k SNP chip which separated stocks into Andean and Mesoamerican domestications. This is helpful for selecting parents for populations within domestication to avoid sterility barriers while guiding wide crosses within domestications to maximize diversity. The available accessions were grown in organic conditions as a pilot study before growing the full panel of bean accessions to troubleshoot the experimental design and observe trends in disease resistance, maturity, etc that warrant careful phenotypic data collection.

NORTH DAKOTA

North Dakota State University (prepared by Juan Osorno and Phil McClean)

The 2021 growing season in the region was very unusual given the extreme drought conditions that affected most crops grown. Very mild showers replaced the usually-heavy rainfalls during late May and June in most cases. July, August, and September were drier than normal and few showers now at the end of the season. In spite of this, seed yields at most testing locations has been higher than expected. One entire breeding nursery (and ~15% of another one) were lost due to Dicamba drift. Another location was lost due to compaction plus Fusarium wilt, which is well-known to occur under these conditions. ND. Twilight is a newly released black bean with high seed yield, earliness, intermediate resistance to common bacterial blight, and complete resistance to bean rust. Preliminary data suggests that the resistance may be different from the already known-genes. Regarding the W4150 collaborative trials and nurseries, ~1800 early generation breeding lines were grown in Puerto Rico as part of our collaborative winter nurseries. In North Dakota, some of the CDBN entries were lost due to Dicamba drift/damage. Contrastingly, the MRPN was a very good trial and preliminary results show good seed yields in spite of being a dry/hot year. A manuscript using historical CDBN data was selected as 1 of 3 “editor’s choice” for the Genetics journal. Overall, this study demonstrates that statistical genomics approaches can be used on Multi-Environment Trial (MET) phenotypic data to discover significant genetic effects and to define genomic regions associated with crop improvement. A new pulse pathologist has been hired at NDSU. This is very strategic position since there is no other public university with a pathologist devoted to pulses only. There are new InDel and KASP markers (under validation) for the rust genes *Ur-5* and *Ur-11*. As part of our collaborative work on white mold using a MAGIC population, ~30 resistant lines have been identified along with some known and new genomic regions associated with resistance. Collaborative research has shown that slow darkening pintos offer higher iron bioavailability and faster cooking time than regular darkening pintos. Newly published research has shown that breeding populations can be used with a dual purpose of genetic improvement and mapping. A new atlas of RLK and RLP proteins across 5 legume species is not available. A new reference genome is available in phytozome: black bean line 5-593. Dr. Mark Bassett, University of Florida, developed a large set of backcross introgression lines using the black seeded, purple flower breeding line 5-593 (PI 608674) as the recurrent parent and donor lines with alleles that affect flower and seed coat and color and pattern. 5-593 is dominant allele for all but one of the genes controlling seed coat color and pattern. The 5-593 genotype for these genes is: *T P* [*C r*] *J G B V Rk Gy sal*. This genotype is currently being used to continue understanding the gene interactions among gene controlling seed coat color.

OREGON

Oregon State University (prepared by James Myers)

Green bean acreage remains steady in the state at about 10,000 A. The primary research objective of the OSU snap bean breeding program has been to identify and introgress white mold resistance into elite cultivars. In this regard, we continue developing a MAGIC population, with final 8-way crosses made in 2021. We also conducted the second year of a trial to obtain agronomic data for four nested association mapping (NAM) populations for which WMG904-20-1 is the common parent. This line was derived from an interspecific cross to incorporate white mold resistance from *Phaseolus coccineus* into a snap bean background. These populations have been combined in a NAM analysis for white mold resistance. We participate in the National Sclerotinia Initiative nurseries and screen lines submitted by the private industry for this disease. The OSU vegetable breeding program continues to work on dry beans. We have developed improved -resistant Peruano beans with better seed color than our recent release, ‘Patron’. There continues to be strong interest in western Oregon in growing dry-farmed dry beans. These rely solely on residual moisture from winter rains to produce a crop. In 2021, we evaluated 46 black and red kidney lines originally developed from interspecific crosses to incorporate bruchid resistance from *P. acutifolius*. These were grown in replicated irrigated and unirrigated plots where photosynthetic and leaf temperature parameters and yield and agronomic traits were evaluated.

PUERTO RICO

University of Puerto Rico (prepared by Consuelo Estevez)

The UPR snap bean breeding lines PR2015-9-1A, PR2015-9-1B, PR2015-49-2, PR2015-67-1, PR2015-68-1, PR2015-75-1, and the commercial snap bean cultivars 'Contender' and 'Jade' produced immune reactions when inoculated with the NL3 strain of BCMNV. In collaboration with Dr. Tim Porch, USDA-ARS Research Geneticist at the Tropical Agriculture Research Station, the snap bean breeding lines were screened with KASPar markers for disease resistance genes. PR2015-67-1 and PR2015-68-1 were identified to have the *bgm-1* and *I* genes for virus resistance and the SAP6 QTL for resistance to common bacterial blight, and a SNP marker associated with BGYMV resistance.

Seventy-five bean cultivars and elite breeding lines were screened for web blight resistance. Web blight infection was severe as indicated by a trial mean score of 6.4 and uniform as indicated by a CV = 16.7% for the web blight scores. 'Hermosa', a black bean cultivar recently released in Puerto Rico, combined the lowest mean web blight score of 4.3, which was significantly lower than the mean of the trial and the means of the susceptible checks Morales and Amadeus 77. Hermosa also had the highest seed yield (1,619 kg ha-1) and only 2.3% damaged seed. Although 13IS7910-95, PR1447-8 (sister line of Hermosa), 19F6020 and MST-1 had moderately susceptible web blight scores on the leaf canopy, these lines had mean seed yields > 1,200 kg ha-1 (significantly greater than the mean of the trial) and less than 2% seed damage.

Losses in bean yield due to angular leaf spot (ALS) caused by *Pseudocercospora griseola* (Sacc.) Crous & U. Braun, has been noted by growers in Puerto Rico. ALS9951-101-R1 was derived from the cross ‘Tio Canela 75*2/G6727’. G6727 is an Andean yellow mottled bean landrace from Colombia. The white bean breeding line PR1627-8 was derived from the cross ‘Verano/ALS9951-101-R1’. The mean seed yield of PR1627-8 was 2,087 kg ha^{-1} across eight field trials conducted in Puerto Rico and Haiti. PR1627-8 was resistant to angular leaf spot and had moderately resistant reactions to common bacterial blight in field trials planted at the Isabela Substation. In the field trial planted at the Isabela Substation in February 2021, PR1627-8 was the only line to express rust resistance. Seed from two individual plant selections from PR1627-8 were sent to Dr. Talo Pastor-Corrales, USDA-ARS Research Plant Pathologist at Beltsville, MD, for screening with specific races of the rust pathogen. Results from the evaluation suggest that PR1627-8 has the *Ur-5* rust resistance gene. During the summer of 2021, PR1627-8 was crossed with the white bean breeding line PR0608-81A, which has the *Ur-11* rust resistance gene. Bean lines that combine the *Ur-5* and the *Ur-11* rust resistance genes should have broad and durable resistance. PR1627-8 has the *bgm-1* gene for BGYMV resistance and the *I* gene for resistance to BCMNV. It has expressed resistance to BGYMV in field trials in the Dominican Republic and Haiti (Mainviel, 2019). Based on the superior performance of PR1627-8, it should be considered for release as an improved breeding line or cultivar.

A manuscript has been prepared for the *J. of Agric. of the UPR* describing the release 'Rosalinda' is a multiple disease resistant pink bean cultivar adapted to the humid tropics. 'Rosalinda' was developed and will be released cooperatively by the UPR and the USDA-ARS. 'The pink bean line is resistant to BGYMV, BCMV, BCMNV. Rosalinda, tested as breeding line PR1519-25, was derived from the cross 'Beníquez'/PR0401-259. Rosalinda produced a mean seed yield of 2,649 kg ha^{-1} in seven trials conducted at the Isabela Substation from 2014 to 2019. The mean seed yield of Rosalinda was significantly higher than the check cultivar 'Verano'. Mean seed yields of Rosalinda were acceptable in all trials, ranging from 2,004 to 3,518 kg ha^{-1}. Rosalinda represents the first release of a Mesoamerican race pink bean cultivar. The pink bean cultivar has an indeterminate upright, Type II growth habit. The erect habit of Rosalinda allows pods to avoid touching the soil surface and helps to preserve seed quality.

Bruchid research

The black bean line PR1933-5 and the dark red line PR1933-7 continue to be the best Mesoamerican sources of bruchid resistance in the UPR bean breeding program. These lines also have the *bgm-1* gene and the SW-12 QTL for BGYMV resistance and the *I* and *bc-3* genes for BCMV and BCMNV resistance. In a trial conducted at the Isabela Substation during the summer of 2021, PR1933-5 had an average of 0.5/10 and PR1933-7 had an average of 1.5/10 seed damaged at 30 days after infestation. Badillo averaged 10/10 seed damaged and Verano had 9.5/10 seed damaged at 30 days after infestation. All the lines had a high level of damage at 45 days after infestation, although it should be noted that each pot was initially infested with 10 adult weevils and the warm environment where the pots were stored was favorable for bruchid development. Un-fumigated seed from a field trial planted at the Isabela Substation in February 2021 that included PR1933-5, PR1933-7, and Verano was stored in plastic trays to observe the rates of natural infestation. After three months of storage, susceptible lines were found to have higher levels of natural infestation and seed damage than the resistant lines PR1933-5 and PR1933-7.

Canning quality of UPR bean cultivars

Dr. Karen Cichy, USDA-ARS Research Geneticist at Michigan State University, evaluated canning quality of cultivars and elite bean breeding lines developed by the UPR bean breeding program. A visual 1-5 appeal score (VAS) of the processed beans was determined by a panel of judges trained to distinguish quality traits, where 1 = very undesirable, 3 = neither desirable nor undesirable, and 5 = very desirable. VAS scores were averaged across judges. Canning quality of the white bean cultivar ‘Bella’, the pinto bean line PR1572-26, the black bean cultivar ‘Hermosa’, and the light red kidney cultivar ‘Badillo’ had superior seed appearance after canning. These results demonstrate that bean cultivars released in Puerto Rico could be locally produced and canned.

USDA-ARS-TARS, Mayaguez, PR (prepared by Timothy Porch)

NE and ARS-PR have prepared the release of a small red and a pinto germplasm with drought tolerance that has been developed through the shuttle breeding program between PR and NE. TARS-Tep 23 (*Phaseolus acutifolius*) with broad drought and heat adaptation and resistance to CBB and rust was accepted for release in collaboration with C.A., Honduras, MD, and N.E. Both wild and cultivated tepary genome sequences were published in Nature Communications by a consortium of researchers using cutting-edge sequencing and bioinformatics methods. These genomes show high levels of similarity to each other and to common bean, showing the potential for tepary bean to improve the drought and heat tolerance of common bean. Interspecific tepary/common bean lines are being developed in collaboration with MSU (MI) and some show unique leaf and architecture traits and abiotic stress tolerance. The Intertek SNP platform was tested and used as a marker assisted selection tool, using markers generously shared by different programs in collaboration with PR-UPR, PR-ARS, NE, and the Dominican Republic.

SOUTH CAROLINA

Clemson University (prepared by Sandra Branham)

Snap bean genotypes were evaluated in a field trial in Charleston, SC at the Clemson University Coastal Research and Education Center. The trial had two planting dates, April 19 and May 18 2021, to assess pod production under ideal and heat-stressed field conditions, respectively. A total of 323 accessions were planted, including 266 accessions from the SnAP diversity panel and 57 commercial cultivars. Field layout followed an augmented lattice design consisting of 3 replicate plots divided into 18 blocks per replicate for each planting date. Plots consisted of 20 plants planted into a single row on top of a bed with six-foot centers. Five plants from each plot were harvested when more than 70% of the pods had reached sieve size 4. Harvest for the ideal planting ranged from 50 to 63 days after planting (DAP) and 57 to 63 DAP for the heat-stressed planting. Data collected on a plot basis included the total number of marketable pods (>sieve size 2), the weight of pods, and photos of the collected pods and a representative plant.

Pods per plant produced from the ideal planting date ranged from 28.9 to 36.3 pods, with a mean of 23.4 pods (Figure 1). ANOVA followed by Tukey’s HSD identified fifteen accessions that produced significantly more pods than the growers’ standard cultivar, ‘Caprice’ (mean=25.7 pods). Accession, replicate, and block were all highly significant ($p<2x10^{-16}$). The later planting date induced heat stress during pod production, substantially reducing the number of pods per plant, with mean percentage stability [(pod count in heat/pod count in ideal)*100] of 38.4%. Pods per plant produced under the later, higher temperature planting ranged from 0 to 23.1 pods, with a mean of 8.8 pods. Variation in pod production significantly varied by genotype ($p=9.7x10^{-14}$). Seven accessions had a highly stable pod count (>90% stability) across the two planting dates, with two accessions producing more pods under the heat-stress environment (Figure 1).

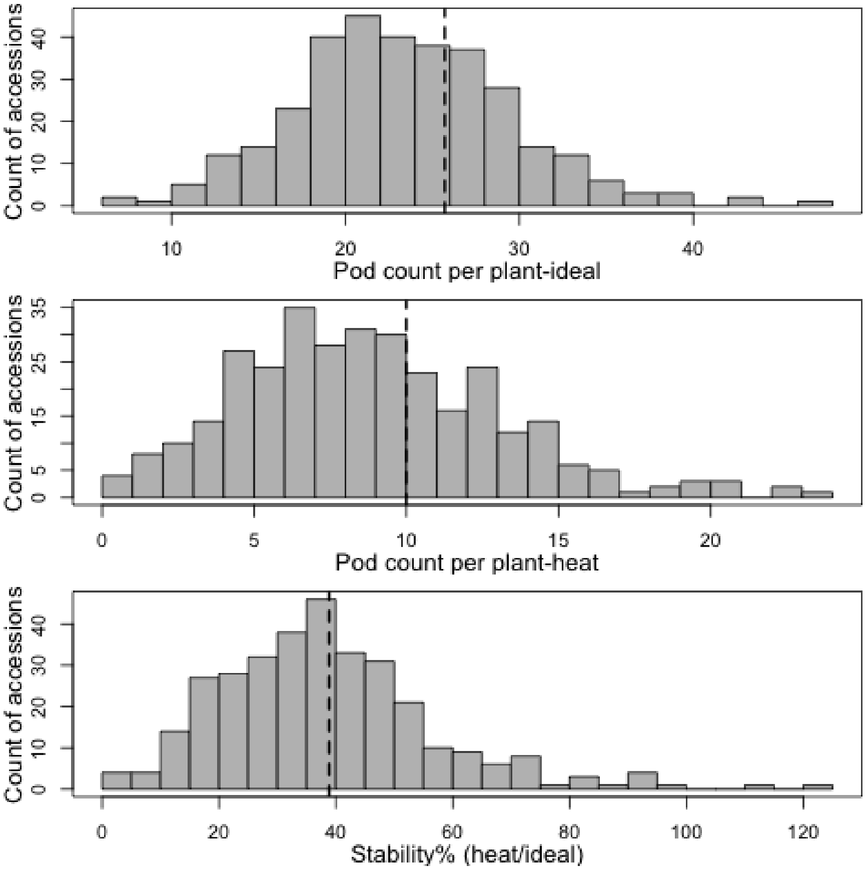

Figure 1. Histograms of pod count per plant under ideal conditions, pod count per plant under heat-stress conditions, and percent stability (heat/ideal) of snap bean accessions evaluated in a field trial in Charleston, SC. The dashed lines mark the mean phenotype of ‘Caprice’, the cultivar most commonly grown by stakeholders in the Southeastern U.S.

WASHINGTON

USDA-ARS, Prosser, WA (prepared by Phil Miklas)

Released USDA-Basin pinto bean and USDA Diamondback slow darkening pinto bean. Both pinto beans possess rust and BCMV resistance and perform well under low inputs (drought and low fertility). Revealed new host-pathogen interactions for resistance to BCMV/BCMNV. Only one recessive resistance allele for the bc-1 locus exhibits a differential interaction with pathogroups based on the presence vs. absence of *bc-u*. Similarly, only one recessive resistance allele for bc-2 exhibits a differential interaction based on whether *bc-u* or bc-4 (newly discovered ‘helper’ gene) is present. *bc-u*, *bc-2*, and *bc-4* were mapped to chromosomes Pv05, Pv11, and Pv05, respectively. Markers for the putative causative mutations for *bc-u*, *bc-2*, and *bc-4* were generated to track the resistance genes for marker-assisted selection. Straw test and field disease screening for white mold reaction in two RIL populations PT9-5-6/USPT-WM-12 and PT12-37/VCP-13, continues to validate WM3.1, WM5.4, and WM7.4 QTL conditioning resistance to white mold.

WYOMING

(Prepared by Jim Heitholt (Powell REC), Mike Moore (Wyo. Seed Cert.), Donna Harris (Sheridan REC))

CDBN

The Cooperative Dry Bean Nursery was grown at Powell by Mike Moore in 2020. The data and discussion were provided to many different individuals, companies, grower meetings, and posted on Wyo. Seed Certification Service website. Results from that 2020 trial were also submitted to Carlos Urrea. The growing season was hot, and there was a killing frost on 8 Sept. PT11-13-31, and PK16-1 ranked first and second in yield. Data collection included days to flowering and maturity.

Breeding: Numerous progeny were advanced to their next generations during the 2020 season, and in some cases, we had enough seed for replicated plots in 2021.

Novelty Trial: A novelty/heirloom trial was conducted in 2020 with multiple nuña-popbean and other heirloom types, 14 lines total. Three popbean lines from Colorado State outyielded our top commercial lines (60 plots, entries were replicated at least three times in six-row plots). We do not have an explanation for why these nuña-popbean lines led in yield. UC-Davis entries (Tiger’s Eye, Southwest Red, Southwest Gold) were also included in this test and those matured in 74, 73, and 76 days after planting, respectively. Poncho, one of the popular early-maturing lines typically grown in the Bighorn Basin matured in 79 days. Although the UC-Davis lines yielded about 15% less than our best commercial pintos, the earliness of the UC-Davis material has convinced us to use these three lines as parents in our breeding program.

Planting Date: A planting-date study with three planting dates (27 May, 10 June, 25 June 2020) and six varieties was conducted. There were two early-maturing types, two mid-maturing types, and two late-maturing types. On 8 Sept 2020, there was a killing frost which greatly compromised the study. Yields of the early-maturing and mid-maturing types were relatively unaffected between the first two planting dates. Yields of the two late-maturing types dropped considerably from the 27 May to the 10 June planting date. All varieties had reduced yield for the 25 June planting.

Planting Configuration: Blackfoot and Windbreaker were grown in 2020 at plant densities ranging from 27K to 84K plants per acre with three different row spacings (7-inch, 15-inch, and 22-inch) and three different irrigation rates. There were 180 plots in total, and at maturity, each plot was split into two, one-half harvested by digging/windrowing/threshing, and the other half was directed harvested (both methods used the same research plot combine). The idea was to quantify the harvest efficiency of varieties differing in morphology among the three management factors. Unfortunately, there was no clear pattern regarding harvest efficiency among the four factors; the average yield loss due to direct harvest was 24%.

Soil Fertility: A study with three levels of preplant fertilizer-P (0, 67, and 135 lb P_2O_5 per acre and two levels of fertilizer-N, 0 and 50 lb/a) were tested on five commercial cultivars (La Paz, Long's Peak, Poncho, Sundance, and Windbreaker) and five experimental lines (three from UW referred to as LPID-3, LPID-7, and LPID-9 and two from ARS-Phil Miklas, PT9-5-6 and PT11-13-1) with 120 total plots. There were no statistical genotype-by-fertilizer interactions on yield; thus, we do not have direct evidence that the five experimental lines are more efficient when grown in lower N and P environments, at least here in the Bighorn Basin of Wyoming. As for yield among the ten genotypes, LPID-3, LPID-9, PT9-5-6, and Windbreaker outyielded the other six entries. LPID-3 was just four days later in maturity than Poncho (the earliest maturing entry) but had poor upright stature (i.e., it tends to lodge). PT9-5-6 had good upright plant architecture. NDVI was collected on all plots on five different dates, but only genotypic differences were observed.

Current 2021 Breeding: During the past year, we have been making crosses among the LPID lines, the USDA-ARS, Prosser (P. Miklas) material, and the UC-Davis material, hoping to find a favorable combination of earliness, good upright stature, and disease resistance. Additionally, we have been making crosses among the late-maturing popbean lines and the early-maturing UC-Davis material, hoping to have an earlier maturing popbean type. Finally, thanks to Phil Miklas, we are using PCR protocols for confirming the presence or absence of three BCMV genes in our experimental progeny.

Current 2021 Research and Screenings: In 2021, we are advancing mid-generation progeny to evaluate the agronomic potential of several bulked lines from crosses made from 2016 to 2018. In a separate project, single-plant selections made in 2020 were sown as plant-to-row plots and are being evaluated in 2021. In 2021, two fertility studies, one with N and K as factors and ten genotypes and a second study with micronutrient fertilizer treatments and nine cultivars, are being conducted. The seeding rate-row spacing-deficit irrigation study is being conducted again in 2021 with four cultivars varying in maturity and upright plant architecture sown at 40K and 80K seed per acre. The 2021 novelty-heirloom trial hosts 23 entries and includes additional nuña-popbean types in collaboration with nutritionists at Univ. Wyoming, a breeder at Oregon State Univ. (Jim Myers), and several scientists at Washington State Univ. The 2021 planting-date study includes four sowing dates and ten genotypes. In addition, Mike Moore is conducting the CDBN at Powell, which was sown in late May 2021.