COLORADO

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Wheat breeding and genetics.

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The primary goals of the Colorado State University (CSU) Wheat Breeding and Genetics Program are to: a) develop improved hard red winter (HRW) and hard white winter (HWW) wheat cultivars and germplasm adapted for the diverse production conditions in Colorado and the west-central Great Plains, and b) conduct research to improve understanding of genetic and environmental factors that affect wheat yield and end-use quality.

Production conditions, cultivar distribution. Total Colorado winter wheat production in 2014 was estimated at 89.3 x 10^6 bushels, a 101% increase from the 2013 crop and 30% higher than the 10-year average. Average grain yield at 38.0 bushels/acre was the highest since 2010, 40% higher than in 2013, and 20% higher than the 10-year average. The area harvested for grain was estimated at 2.35 x 10^6 acres, up from 1.64 x 10^6 acres in 2013.

Planted acreage estimates for the 2014 crop were as follows: Hatcher – 25.2%; Byrd – 14.8%; TAM 111 – 5.4%; Snowmass – 5.1%; Ripper – 4.4%; Bill Brown – 2.7%; TAM 112 – 2.5%; Brawl CL Plus – 2.3%; Prairie Red – 2.2%; Prowers/Prowers 99 – 2.2%; Settler CL – 2.0%; Danby – 1.4%; Winterhawk – 1.4%; TAM 107 – 1.3%; Jagger – 1.1%; Bond CL – 0.9%; Antero – 0.6%; Goodstreak – 0.6%; Above – 0.5%; Denali – 0.5%; Jagalene – 0.5%; and Other/Unknown – 22.4%.

Breeding program test sites. In summer 2014, we harvested breeding trials at 11 locations in eastern Colorado (Akron, Burlington, Dailey, Fort Collins-irrigated, Julesburg, Lamar, New Raymer, Orchard, Roggen, Walsh, and Yuma). In addition to the Colorado locations, we obtained data from the CSU Elite Trial at two locations in Kansas (Hays and Colby) and one location in Nebraska (Imperial, irrigated). Due to various production problems (i.e., drought or hail), trials at Arapahoe, Genoa, Sheridan Lake, and Healy, KS, were abandoned. Overall, the quality of the data was very good and very useful for line selection decisions in the breeding program.

The CSU Elite Trial at New Raymer was added in 2014 to evaluate resistance to wheat stem sawfly. In autumn 2014, we further expanded the range of our CSU Elite Trial testing with the addition of three trial locations, which makes a total of 21 Elite Trial locations in 2015. One of the new locations is planted under irrigation in collaboration with New Mexico State University at Farmington, NM, in order to help to build a partnership with Navajo Agricultural Products Industry (NAPI), who are interested to have cultivars adapted for their production conditions and end-use markets. The other two trials are planted in Montana (Fort Benton and Hardin, MT) under contract with Northern Seed LLC. In 2015, we adopted a new trial design for the CSU Elite Trial that involves partial-replication (*p-rep* trials) of experimental entries at a given location to allow greater numbers of entries to be included in the trial. Thus, the CSU Elite Trial is now a 100-entry trial instead of a 75-entry trial. Randomization of the *p-rep* trials is being done with codes developed using the *DiGGer* package (http://www.austatgen.org/software/) in the R programming language (codes available upon request).

In autumn 2014, we prepared over 19,000 yield trial plots for planting at 21 total trial locations in Colorado, Kansas, Nebraska, New Mexico, and Montana. At Fort Collins, we also planted over 30,000 F_4 headrows, about 1,000 early-generation (F_2–F_3) populations (including 219 two-gene *Clearfield* populations), and about 1,100 new cross combinations in

autumn 2014 and early spring 2015. We continue to utilize a wide variety of diverse germplasm sources in our crossing program, including elite materials from other regional programs, new sources of leaf and stripe rust resistance, solid-stem cultivars from Montana State for wheat stem sawfly resistance, winter wheat lines introduced from Turkey and other areas of eastern Europe, and germplasm carrying various novel traits from our and other programs. Beginning in autumn 2014, we have started to utilize genomic selection predictions for identifying lines for inclusion in our crossing program.

New cultivar release. In autumn 2014, the hard white winter wheat experimental line CO09W293 (pedigree: KS01HW152-6/HV9W02-267W) was formally released by the Colorado Agricultural Experiment Station. CO09W293 (named **Sunshine**) will be marketed by the Colorado Wheat Research Foundation (CWRF) under the PlainsGold Brand and the CWRF-Ardent Mills Ultragrain Premium Program. In three years of testing in the CSU Elite Trial (28 location-years, 2012-2014), the dryland yield of Sunshine was 101% that of the trial average, compared to 105% for Byrd HRW, 103% for Antero HWW, 99% for Hatcher HRW, and 91% for Snowmass HWW. Sunshine has average test weight and straw strength, is moderately susceptible to stripe rust, and susceptible to leaf rust. End-use quality characteristics of Sunshine are excellent, although the dough-mixing strength of Sunshine is less than that of Snowmass and other lines in our program with Snowmass-type characteristics. Preharvest sprouting tolerance and polyphenol oxidase activity of Sunshine are both similar to those of Hatcher, Snowmass, and Antero.

Foundation and breeder seed increases. In autumn 2014, experimental line **CO11D174** was advanced for Foundation seed production to enable release as a new cultivar in autumn 2015. CO11D174 is a doubled-haploid (DH) HRWW from the cross 'TAM 112/Byrd' made in 2009. CO11D174 is similar to Byrd in many respects, being of medium height and maturity, has a medium-long coleoptile, and average straw strength and test weight. CO11D174 is moderately susceptible to stripe rust, susceptible to leaf rust and stem rust, susceptible to Hessian fly and all biotypes of Russian wheat aphid, and resistant to wheat soilborne mosaic virus and the wheat curl mite. Across 26 site-years in the CSU Elite Trial (2013 and 2014) and UVPT (2014), CO11D174 was the highest yielding entry tested, approximately 7% higher yielding than Byrd. In the 2014 Southern Regional Performance Nursery, CO11D174 also was the highest yielding entry. CO11D174 has shown good overall milling and baking properties in tests conducted in the CSU Wheat Quality Lab. Individual milling and baking properties of CO11D174 are very similar to those of Byrd, although CO11D174 has a slightly greater kernel weight (29.3 vs. 27.7 grams/1,000 kernels) and diameter (2.58 vs. 2.49 mm), a slightly lower flour yield (69.9 vs. 72.0%), and a slightly shorter mixograph mixing time (5.3 vs. 6.6 minutes). Tests conducted on CO11D174 in the 2014 Wheat Quality Council tests also suggest that CO11D174 has a higher water absorption than that of Byrd. A decision on the release of CO11D174 will be made after the 2015 harvest.

In autumn 2014, eight HRW and two HWW lines were advanced for Breeder seed production to enable Foundation seed production and potential release in 2016. All of the lines are being tested in the 2015 CSU Elite Trial and the 2015 CSU Variety Trials (UVPT and IVPT). All of the HRW lines (CO11D446, CO11D1174, CO11D1236, CO11D1298, CO11D1353, CO11D1397, CO11D1539, and CO11D1767) are DH lines lacking the *Clearfield* herbicide-tolerance traits and both of the HWW lines (CO11D1306W and CO11D1316W) are DH lines that lack the *Clearfield* traits and the gluten strength trait in Snowmass and Sunshine. A decision on retention and advancement to Foundation Seed production will be made after harvest in 2015.

Doubled-haploid breeding. Since 2010, the CWRF royalty funds and our partnership with Ardent Mills have provided for a significant expansion of experimental HRW and HWW line development through the use of DH breeding technology. We have used the DH services at Heartland Plant Innovations and Washington State University, and, since 2012, have been working to build our own DH capacity on the CSU campus. In December 2013, we moved into a newly renovated DH lab in the CSU Greenhouse (renovations funded by CSU and CWAC).

In 2014, our advanced yield nurseries consisted of lines developed using both DH and 'conventional' breeding technologies. These trials all were set up as partially replicated trials to allow greater numbers of entries to be included. The trials were divided roughly as follows: HWW DH lines mostly carrying the 'Snowmass trait' (253 lines, six locations), HRW DH lines mostly lacking the 'Snowmass trait' (305 lines, three locations), conventionally-derived and single-seed descent derived HRW and HWW lines mostly lacking the 'Snowmass trait' (248 lines, eight locations), and a group of two-gene, HRW and HWW *Clearfield* lines (37 lines, two locations). From this group of 843 lines, 70 lines (about 8.3%) were selected and advanced to the 2015 CSU Elite Trial. These 70 lines can be categorized roughly as follows: 20 HWW DH lines that carry the 'Snowmass trait', 41 'conventional' HRW, and nine two-gene HRW *Clearfield* lines. Of these two-gene *Clearfield* lines, four are two-gene, Byrd backcross-derived lines.

In 2014, we had approximately 3,314 new DHs at Fort Collins in short-rows for seed increase. The vast majority of these (3,086) had Snowmass as one of the parents of the cross and, thus, had the genetic possibility of carrying the dough strength trait from Snowmass. Based on visual observations of agronomic suitability and DNA marker information, approximately 1,028 of these lines were hand-harvested in July. Following seed processing, we selected 984 of these lines (about 32% of the total) for advancement to yield trials in 2015. These lines can be categorized roughly as follows: HWW DH lines mostly carrying the 'Snowmass trait' (244 lines, seven locations), HRW DH lines mostly lacking the 'Snowmass trait' (244 lines, four locations), and HWW DH lines lacking the 'Snowmass trait' and HRW lines carrying the 'Snowmass trait' (496 lines, one location).

DNA marker implementation. Our current emphasis in marker-assisted selection (MAS) is centered on two main areas. First, we now are conducting routine marker screening for a key set of markers on virtually all experimental lines developed in the breeding program. These include DHs that are under first year seed increase prior to selection and lines that have been selected from F_4 headrows and are in testing in our preliminary yield nurseries (PYNs). Second, we continue to utilize MAS for backcross transfer (introgression) of various traits from non-elite germplasm sources to elite breeding program germplasm. Products of these introgression efforts are then used in 'forward crosses' for transfer of the traits into a DH line development pipeline or the main breeding program pipeline.

In 2014, we conducted DNA marker tests on 5,917 different lines and plant selections. The markers used were for multiple disease/insect resistance, quality-related, herbicide tolerance, and TILLING-derived traits. Most of the markers that we are using are sequence-based (KASP assays), which has dramatically improved our efficiency (lower costs, greater speed) for routine MAS. Overall, the materials tested included 3,236 DH lines under first-year, seed increase and 1,360 lines tested in the 2014 PYNs. Marker screening of plants from which DHs are made can dramatically improve the frequency of desirable DHs obtained. The numbers of markers for all of these materials varied from one to seven and in total we generated over 24,100 data points on these materials to aid in selection.

In 2014, we conducted DNA marker tests for backcross introgression of various traits from non-elite germplasm to elite breeding program germplasm. Across our three most recent crossing cycles, we generated about 4,100 data points from nearly 1,800 individual plants. Target traits for these tests included *Clearfield* herbicide tolerance, low polyphenol oxidase expression, stripe rust resistance, and several different TILLING-derived mutants. Most of the markers we are using for this are KASP markers, especially in the case of TILLING mutants, where we are well positioned to design novel KASP assays in-house.

In addition to the trait-specific ,DNA marker screening described above, we also continue to exploit next generation sequencing technologies (called genotyping by sequencing or GBS) in our breeding program. Our pipeline involves DNA extraction and sample preparation at CSU, outsourcing of the high-throughput DNA sequencing to the core facility at the University of Missouri, and then bioinformatics analysis both at CSU (by Harish Manmathan) and in collaboration with Kansas State University (by Jesse Poland) for generating the marker calls. For each of the GBS assays, we can obtain about 40,000 DNA markers spread across the entire wheat genome, at a cost of about $14/line. Since we began using GBS, we have sequenced about 7,800 breeding/germplasm lines, generating over 300 million total data points (including missing data). We have successfully used the GBS marker data in genomic selection and genome-wide association analysis (GWAS) for preharvest sprouting tolerance and various wheat quality traits (Jessica Cooper Ph.D.) as well as other more complex breeding targets (yield and test weight) for selection and parent identification.

End-use quality evaluation. End-use quality evaluations are done annually on samples from a variety of different field trials and research studies. In 2014, 3,957 different samples went through our quality lab for one or more quality tests. From breeding trials in 2013, we conducted comprehensive milling and baking quality tests on five locations of the CSU Elite Trial and three locations of the Advanced Yield Nursery (including both DHs and conventionally derived lines) and small-scale quality analyses from two locations of the PYN. Our comprehensive testing of advanced generation materials includes the full spectrum of quality tests: NIR protein, single-kernel characterization system, mixographs, polyphenol oxidase, and Quadrumat Senior milling and pup-loaf baking tests. Testing of earlier generation materials generally involves only NIR protein, the single-kernel characterization system, and the mixographs. Our overall strategy in line evaluation is to properly characterize experimental lines in order to inform the line selection and seed increase decision-making processes.

Comprehensive milling and baking quality evaluations are done annually on selected locations of the state dryland (UVPT) and irrigated (IVPT) variety trials. From trials in 2013, grain samples were collected from five dryland

(UVPT) variety trial locations (Akron, Julesburg, Orchard, Roggen, and Yuma) and two irrigated (IVPT) variety trial locations (Fort Collins and Haxtun). Preliminary, small-scale, quality analyses were done to determine suitability of the samples for full-scale analyses. In this process of sample selection, four of the dryland locations (Akron, Julesburg, Roggen, and Yuma) were excluded from analyses beyond protein content with the primary problem being elevated protein values far above the level conductive for meaningful dough mixing and baking quality evaluations. All of the data were reported in the booklet *Making Better Decisions*. The data also are used to develop and update the milling and baking quality scores that are reported in the Variety Characteristics Table in *Making Better Decisions*. Other variety-trial related activities from trials in 2013 include NIR protein on 83 samples from the Collaborative On-Farm Testing program and 74 samples from the western Colorado trials at Hayden and Yellow Jacket.

From trials in 2013, we determined NIR protein content from every plot of six locations (1,800 samples) of our Advanced Doubled-haploid Nursery (AYND). From trials in 2014, we are doing NIR protein on every plot of five locations (750 samples) of the CSU Elite Trial. Our objective is to begin to use 'grain protein deviation' (protein content adjusted for grain yield) as a measure of nitrogen use efficiency (NUE) and develop genomic selection prediction models for NUE selection in the breeding program. This research forms the basis of Sue Latshaw's Ph.D. dissertation, which she hopes to complete in spring 2016.

From trials in 2013, we evaluated 750 samples from a two-year study to evaluate the effect of different glutenin allele combinations on agronomic properties and end-use quality. This research was part of Jessica Cooper's PhD dissertation (successfully completed in November 2014), along with genomic selection model development for preharvest sprouting tolerance and various end-use quality traits.

Russian wheat aphid resistance. Our basic objectives with Russian wheat aphid (RWA) resistance are to transfer different resistance traits to our germplasm and, ultimately, develop resistant cultivars. Due to development of multiple new RWA biotypes, generally lower RWA incidence in recent years, and relatively inexpensive chemical control options, RWA resistance has, over time, become a less critical breeding objective in our program. Because 'nature abhors a vacuum' (according to Aristotle), the wheat stem sawfly now has become a major insect pest in Colorado and a potentially serious cereal aphid called *Sipha maydis* was recently found in New Mexico and western Colorado. Given these realities, we will continue to address all insect pests that affect wheat in Colorado for which there are reasonable opportunities for host–plant resistance. All of these efforts are done in very close collaboration with Frank Peairs and his team.

Over the last few years, we have developed and tested many experimental lines carrying RWA biotype 2 resistance. None of the lines have been yield-competitive with our best cultivars (Byrd HRW or Antero HWW) and, thus, none have been advanced toward release. In order to move the 'RWA yield curve' forward a bit more effectively, we have begun to generate a limited of number of DH lines from crosses with different sources of the *Dn7* resistance gene.

The first group of these DHs was a set that was planted in our Advanced Yield Nurseries in 2014. Among 30 DHs derived from a cross with CO08RWA060 (Yuma-type *Dn7* carrier), 17 were resistant to RWA biotype 2, and 13 were susceptible in greenhouse seedling screening tests. These reactions were confirmed with a DNA marker linked to *Dn7*. Of the resistant lines, only one HRW line (CO12D1380, pedigree: Snowmass/CO08RWA060//CO05W111) was selected and advanced to the 2015 CSU Elite Trial. This line performed very well in 2014, yielding second among 305 DHs in our HRW DH trial. In addition to this line, we had six conventionally derived lines in the 2014 Advanced Yield Nursery and 29 lines in the PYN with RWA resistance in the pedigree. Several of these lines showed good resistance to RWA biotype 2 in greenhouse screening, but none showed adequate merit for advancement to testing in 2015.

In 2015, we have a set of 22 DHs in Advanced Yield Nurseries, also derived from the CO08RWA060 resistance source. Four of these were resistant to RWA biotype 2 and 18 were susceptible in greenhouse screening tests. DNA marker analysis is currently being done on these lines to confirm the presence/absence of *Dn7*. We also have a group of 30 DHs in first year seed increase and 91 PYN lines that carry *Dn7* in the pedigree. We are currently conducting DNA marker screening on these lines to inform selection decisions in summer 2015.

We have completed backcrossing of RWA biotype 2 resistance from CI2401 into Yuma and a modified version of *Dn7* (without the rye secalin proteins) into Bill Brown. These materials already have been used in new crosses and are currently under seed purification to enable release as germplasm in 2015–16. These materials may be useful for future biotype monitoring.

In addition to RWA, we also are aggressively pursuing wheat stem sawfly (WSS) resistance as a breeding objective. With one month of salary support from our program, Terri Randolph (Frank Peairs' team) coordinated solid stem evaluations of segregating populations at Fort Collins. We also have generated a group of 300 DHs from crosses with Byrd and Antero and the solid-stemmed Montana cultivar Bearpaw. We have completed DNA marker analysis for WSS-associated markers in these DHs and are hopeful that some of the DHs will be advanced to yield trials in 2016. In 2014, we added a field site near New Raymer for evaluation of wheat WSS response, and we believe that we have some form of non solid-stem resistance in our germplasm. One DH line (CO11D1397, pedigree: CO050337-2/Byrd) showed very good yield at New Raymer and other locations, low WSS damage, and low larvae counts in the stubble after harvest. This line is in the 2015 UVPT and IVPT and is under Breeder seed increase for potential Foundation seed production and release in 2016.

Personnel updates. CSU wheat breeder Scott Haley completed a six-month sabbatical leave in Europe from December 2013 to May 2014. The focus of the sabbatical was to learn new ideas and new techniques at the interface of crop genomics and wheat breeding. In December 2014, Ph.D. student Sue Latshaw accepted a position in wheat breeding with Bayer CropScience in Lincoln, NE, and will work to complete her Ph.D. in spring 2015. In November 2014, Ph.D. student Jessica Cooper successfully completed her degree program, focusing on genomic selection for end-use quality traits (including preharvest sprouting tolerance), and accepted a position in canola breeding with Cargill in Fort Collins. Our Ph.D. student Craig Beil completed his first year in our program in summer 2014 conducting research to leverage next-generation sequencing technologies (i.e., genotyping by sequencing) to more efficiently exploit winter wheat germplasm from the CIMMYT–ICARDA International Winter Wheat Improvement Program based in Turkey. Craig is currently spending three months with CIMMYT in Mexico (February to May 2015) participating in their international training program. Craig is only the 5th U.S. trainee to participate in this program since the 1960s. In autumn 2014, Ben Conway joined our program (co-advised by Pat Byrne) to work on a Ph.D. focusing on research to improve genomic selection models in wheat using climatological and other covariates. Ben joined us following completion of an M.S. degree in wheat breeding at the University of Maryland.

KANSAS

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Chemical properties of soil with winter cover crops.

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In Kansas, winter cover crops have a new interest with the development of summer crops for biofuel. When a crop is harvested for bioenergy, the residue is removed leaving the soil prone to erosion during the winter fallow period. Winter cover crops may allow maximum biomass harvest by protecting the soil from wind and water erosion. Therefore, the objective of the research reported by Freeman (2014) was to determine the effect of two winter cover crops on the growth of two biofuel crops, corn (*Zea mays* L.) and forage sorghum (*Sorghum bicolor* (L.) Moench) in a corn–forage sorghum rotation. The two rotations, established in 2009, were continuous forage sorghum and corn–forage sorghum. In the corn–forage sorghum rotation, the 2009 plots with corn grew forage sorghum in 2010 and corn in 2011; the 2009 plots with forage sorghum grew corn in 2010 and forage sorghum in 2011. The two cover crops were a legume, Austrian winter pea (*Pisum sativum* var. *arvense* Poir.), and winter wheat. Control plots were fallowed. The experiment was done for two years in Manhattan and Tribune, KS, with harvests of the winter cover crops in the springs of 2011 and 2012. Two levels of nitrogen were added to the soil: 0 and 101 kg/ha N. The main results for both locations were 1) nitrogen increased yield of both corn and sorghum. 2) growth of the winter cover crops did not decrease the yield of the summer crops. and 3) winter wheat produced more biomass than Austrian winter pea. The Austrian pea winter killed in the second year of