Developing Winter Food Barley for the Pacific Northwest of the US

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Abbreviations:

ABD: Aberdeen, ID; BSR: Barley Stripe Rust; COR: Corvallis, OR; PNW: Pacific Northwest; PUL: Pullman, WA; SRC: Solvent Retention Capacity.

Abstract:

Barley (*Hordeum vulgare* L.) has been cultivated for human consumption for thousands of years. However, most North Americans do not consume barley on a regular basis. In the last decade, there has been a renewed interest in barley production for human consumption. A number of quality traits estimate nutritional value and are useful for food processing. These include β-glucan, grain protein, kernel hardness, solvent retention capacity (SRC), and hull type. The Pacific Northwest (PNW) of the US is a high-yielding region that has a reputation for setting dietary and nutritional trends. However, there are currently no winter food barleys adapted to this area. To determine the potential suitability of winter growth habit for food barley production in the PNW, we developed and tested 14 advanced lines. The germplasm was developed via marker-assisted and phenotypic selection and included hulled lines with waxy starch and hull-less lines with normal starch. Agronomic and food quality traits were measured on samples from three representative environments (dryland, irrigated, and high rainfall) over a two-year period allowing for assessment of performance within and across locations, as well as genotype x environment interaction. Lines with waxy starch had significantly higher levels of β-glucan, harder kernels, and higher water retention capacity. Hull-less lines had, on average, slightly lower yields than hulled lines with the average difference of 105 kg ha^{-1}. Our future food barley variety development will focus exclusively on hull-less types, due to the simplified processing and consumer interest in the nutritional benefits of whole grain.

Introduction:

Barley (*Hordeum vulgare* L.) is one of the oldest domesticated crops and has been cultivated for human consumption for thousands of years. However, most North Americans do not consume barley on a regular basis, and the majority is grown for feed and malt. In 2006, the U.S. Food and Drug Administration approved a health claim for barley based on varieties containing high levels of soluble β-glucan fiber, which has been shown to help reduce post-prandial glucose response, lower blood cholesterol levels, reduce insulin resistance, and reduce abdominal fat (AbuMweis et al., 2010; Bays et al., 2011; Behall et al., 2006; Casiraghi et al., 2006; Kim et al., 2009; Shimizu et al., 2008; Tiwari and Cummins, 2011). The health claim allows "foods containing barley to claim that they reduce the risk of coronary heart disease. Specifically, whole grain barley and dry milled barley products such as flakes, grits, flour, and pearled barley, which provide at least 0.75 grams of soluble fiber per serving" (21 CFR 101.81) (Ames and Rhymer 2008; National Barley Foods Council, 2003). Due to the health claim and reports that most North Americans do not get enough fiber in their diets (Slavin 2005), efforts have increased to breed new food barley varieties, characterize these varieties for food quality traits, and develop food barley products (Baik and Ullrich 2008; Bhatty 1999; Newman and Newman 2008; Sullivan et al., 2013).

Grain β-glucan content is influenced by both environmental and genetic factors. Anker-Nilssen et al. (2008) reported that barley grown in hotter and drier climates tends to have higher β-glucan than barley grown in wetter climates. Chutimanitsakun et al. (2013) also found that β-glucan content was significantly higher with increased daytime temperatures, even under irrigated conditions. There are qualitative and quantitative genetic components to grain β-glucan. In terms of qualitative variation, the recessive allele of the granule-bound starch synthase 1

(*GBSS1*) gene (also termed the "Waxy" (*WX*) locus) has a positive pleiotropic effect on grain β-glucan content (Szczodrak et al., 1992; Xue et al., 1997; Wood et al., 2003). Breeders have, therefore, selected for higher grain β-glucan by targeting the recessive (waxy, high amylopectin) allele (Patron et al., 2002; Islamovic et al., 2013). In terms of quantitative genetic variation, several QTL are reported to participate in the regulation of β-glucan and amylose (Islamovic et al., 2013).

There are a number of other barley grain traits that are important for food uses. These include hull type, grain protein, kernel hardness, and solvent retention capacity (SRC). The hull-less trait (where the lemma and palea do not adhere to the hull) is recessive and determined by allelic variation at the *nud* locus. The causal gene at this locus, is controlled by an ERF family transcription factor, which was cloned by Taketa et al. (2008), For malt barley, the adhering hull serves as a natural filtration device during the brewing process, but for food barley an adhering hull requires additional processing such as pearling. Pearling is the process of physically abrading the grain to remove the outer tissues including the hull, bran, and germ. Once a grain has been pearled, it no longer considered to be whole grain, which is defined as: "the intact, ground, cracked, or flaked caryopsis (kernel or seed), whose principal anatomical components—the starchy endosperm, germ, and bran—are present in the same relative proportions as they exist in the intact caryopsis" (Jones 2010). However, Choo et al. (2001) reported that the hull-less trait is associated with decreased yield, low seed weight, poor emergence, and short plant height compared with hulled types. After assessing food lines with spring growth habit under dryland conditions in the Pacific Northwest, Rey et al. (2009) also concluded that hulled lines had greater yield potential and increased vigor as compared to hull-less lines.

Grain protein is a quantitative trait controlled by QTL on all chromosomes, with the most important on chromosomes 2H, 4H, 5H, and 6H. Candidate genes were identified for two QTLs (*HvNAM1* on 6H and *HvNAM2* on 2H), which are homologs of genes controlling grain protein in wheat (Cai et al., 2013). Environment and growth practices can also have a significant impact on grain protein; increased availability of nitrogen, or heat stress due to drought, can increase protein levels (Zhang et al., 2001). Grain protein is determined principally by the hordein storage proteins found in the endosperm. The role of barley grain protein in human consumption has not been well studied or defined (Baik and Ullrich 2008).

Kernel hardness, defined as the "resistance of the kernel to deformation" (Turnbull and Rahmun 2002), is determined by endosperm texture and has a major effect on processing (milling and pearling) and the end-use of the grain. Harder kernels are more resistant to force and softer kernels are more easily damaged. Hordindolines and grain softness proteins have been mapped to the short arm of chromosome 5H in barley (Nair et al., 2010). This genome position is homeologous with the location of the same genes in wheat. However, unlike wheat, the biochemical basis of kernel hardness is not well understood in barley (Nair et al., 2010). Similar to the market-classes that exist in wheat, barley end-use products may be determined by the softness or hardness of the initial grain input.

Solvent retention capacity (SRC) measures the capacity of a flour to absorb and retain four solvents: water, lactic acid, sodium carbonate, and sucrose (reviewed by Kweon et al., 2011; AACC-International Approved Method 56-11.02, 2009). The basic principle of the test is that compatible solvents can swell polymeric networks. Different solvents emphasize swelling of different polymeric networks because of differences in solvent/polymer compatibility; water swells all polymers in cereal flours (Kweon et al., 2011). SRC is commonly measured on wheat

to assess end-use quality; however, there is little precedence for measuring SRC in barley (Slukova et al., 2012). In wheat, genotype plays a large role in the variation of values, although there is evidence of genotype x environment interactions (Guttieri and Souza, 2003).

There has been a renewed interest in producing barley for human consumption as a high fiber whole grain. The Pacific Northwest (PNW) of the US is a high-yielding region that has a reputation for setting dietary and nutritional trends. Therefore, we see the PNW as a place to re-invigorate a food barley market. However, there are currently no winter food barleys adapted to this area. In the PNW, where winter precipitation patterns prevail, winter and facultative growth habit barley varieties typically have a significant yield advantage over spring growth habit types. Therefore, we have focused our food barley breeding efforts on the former, using both marker assisted- and phenotypic selection (Chutimanitsakun et al., 2013). Advanced lines were tested in the "Oregon Food Barley" (OFOOD) trial over a two-year period under dryland (average annual rainfall between 400 to 600 mm $year^{-1}$ without supplemental irrigation), irrigated (average annual rainfall of less than 400 mm $year^{-1}$ with supplemental irrigation applied), high rainfall (average annual rainfall greater than 800 mm $year^{-1}$), organic (using management practices in compliance with organic regulations), and conventional (using management methods not suitable for organic production) conditions (Western Regional Climate Center). A range of traits – agronomic, abiotic and biotic stress resistance, and grain quality – were measured on these advanced lines. Our objectives were to: (i) assess the agronomic performance of fall-sown barley food germplasm compared to check varieties, (ii) determine if there is a yield penalty associated with the hull-less trait, (iii) characterize food quality attributes, and (iv) assess the stability of agronomic and quality traits across different production environments and years.

Materials and methods:

Thirteen advanced-generation food barley selections, one three-way blend called 'Streaker' (subsequently released as '#STRKR'), and two check varieties were included in the OFOOD trial. The checks were 'Alba': a hulled, non-waxy, winter, six-row, feed variety developed at Oregon State University (OSU) (Graebner et al., 2014) and 'Maja': a hulled, non-waxy, facultative, six-row, malt variety developed at OSU and released in 2006. No hull-less check was used because there were no adapted winter food barleys prior to this trial. The 14 food barley selections were developed using either marker-assisted selection (MAS) or phenotypic selection (PS) as described by Chutimanitsakun et al. (2013). Briefly, the MAS project was designed to develop high β-glucan and winter growth habit germplasm via selection for specific alleles at the *WX* and *VRN-H2* loci. The PS germplasm was selected for the hull-less trait and agronomic performance in target environments. Six of the advanced lines were hull-less and non-waxy, seven were hulled and waxy, and one was hull-less and waxy (Table 1). Eight of the entries (all waxy starch types) were derived by MAS; the remaining non-waxy starch types were derived by PS. All entries were selected for agronomic performance over multiple years and locations prior to inclusion in the OFOOD trial.

The OFOOD trial was grown over a two-year period (2011-2012 and 2012-2013) at three locations. Plot sizes, seeding rates, and management practices varied by location. At COR, seed was prepared by volume, with 90g of seed planted in 9.3 m^2 plots. At PUL, seed was prepared by volume, with 100g of seed planted in 9.3 m^2 plots. At ABD, seed was planted based on thousand kernel weight, with a target of 200 seeds/m^2 in 6.2 m^2 plots. In this report, we present data from the two years and three representative locations for a total of six environments: Corvallis, OR (COR, representing high rainfall conditions); Pullman, WA (PUL, representing dryland

conditions); and Aberdeen, ID (ABD, representing irrigated conditions). In COR 2012 and 2013, PUL 2012 and 2013, and ABD 2013 a randomized complete block (RCB) design with three replications was used. Due to limited seed resources, a RCB design with two replications was used in ABD 2012. Grain yield and plant height were measured for each plot. Heading date was recorded across all locations for ABD 2012 and 2013, PUL 2012 and 2013, and COR 2013; only one replication was recorded in COR 2012. Test weight was measured on grain from all replicates at ABD 2012 and 2013, and PUL 2012 and 2013; it was measured only on the first replicate in COR 2012 and 2013. Food quality traits (grain β-glucan, protein, kernel hardness, and SRC) were measured on grain from a single replicate from each location and in each year. Two technical replications were used for kernel hardness and SRC at each location and year. Winter survival was rated based on the visual assessment of the percentage of surviving plants on a plot basis at PUL and ABD on all replicates over the two years. No differential survival was observed at COR. Resistance to barley stripe rust (incited by *Puccinia striiformis* f. sp. *hordei*) and scald (incited by *Rhynchosporium commune*) was rated by visual assessment of the percentage of leaf area affected by disease, on a plot basis, on all replicates at COR 2012 and on one replicate at COR 2013. These diseases were not observed at PUL and ABD.

For the measurement of grain β-glucan, whole grain samples were ground in a CleanMill 8000 (Newport Scientific, Sydney, Australia). The resulting flour was used to determine the mixed-linkage β-glucan percentage using the Megazyme enzymatic assay procedure (AACC Method 32-23.01; Megazyme International Ireland Ltd., 1999) with the modified protocol developed by Hu and Burton (2008). Grain protein was measured using near infrared reflectance (NIR) spectroscopy (Infratec 1241 Grain Analyzer, Foss, Laurel, MD). Kernel hardness was measured using 300 kernels per sample on a SKCS 4100 (Perten Instruments, Springfield, IL)

single kernel characterization system. Based on the report by Nair et al. (2010) that the hull has little effect on kernel hardness, we removed hulls from hulled selection in order to avoid clogging the SKCS machine. Grain samples from the hulled lines were pearled for 30 seconds using a Strong Scott Pearler (Seedboro Equipment Co., Chicago, IL). The kernel hardness of hull-less lines was measured using whole grain. SRC was measured using the AACC-International Approved Method 56-11.02 (2009). Grain samples were milled on a laboratory hammer mill 3100 (Perten Instruments, Springfield, IL). Hull-less lines were milled from whole-grain and hulled lines were pearled for 30 seconds, as described for kernel hardness assessment, and then milled. SRC is a composite method that uses four "solvents" to create a functionality fingerprint for a flour: water and three aqueous solutions, 50% w/w sucrose, 5% w/w sodium carbonate, and 5% w/w lactic acid. We found that with some barley samples, using the sodium carbonate, lactic acid, and sucrose solutions, a complete pellet did not form after centrifugation. Therefore, we report only results for SRC-water (referred to as SRC-W).

Combined analyses of variance were performed across locations and years for grain yield and height (replicated across all six environments) using the General Linear Models (GLM) procedure in SAS v9.3 (SAS Institute, Cary, NC, 2011). All effects were considered fixed in these analyses. For traits measured on only one or a subset of replicates (heading date, test weight, β-glucan, protein, kernel hardness, and solvent retention capacity), years and/or locations were considered replicates. In order to assess genotype x location and genotype x year interactions, consistency plots and Additive Main effects and Multiplicative Interaction (AMMI) plots (Gauch 1988; Zobel et al., 1988) were created. Mean separation tests were based on F-protected LSD tests. Pearson's correlations were performed using the CORR procedure in SAS v9.3.

Results and Discussion:

Across six environments, significant differences among entries for all traits were observed (Table 2). Grain yield varied from 5701 to 8257 kg ha^{-1}; Alba (hulled, non-waxy) had the highest yield and OBADV10-14 (hull-less, non-waxy) had the lowest yield. On average, hulled lines (excluding checks) yielded 6458 kg ha^{-1}, whereas on average hull-less lines yielded 6353 kg ha^{-1}, a difference of only 105 kg ha^{-1}. Overall, the food barley germplasm was competitive with Maja, a six-row malting barley. Alba, which is a high-yielding feed variety, had a significant yield advantage over all experimental lines (Table 2). Alba is hulled however, and hull-less barley is more attractive for food purposes as it does not require pearling to meet the whole grain standard. On average, the barley hull is reported to account for 11-13% of total grain yield (Rey et al., 2009). When the yield of Alba is adjusted for hull (7266 kg ha^{-1}), the comparison with the average of the hull-less lines (6353 kg ha^{-1}) is more favorable. In a multi-location study comparing differences in yield potential between hulled and hull-less full sib genotypes, Berger et al. (2013) found no significant differences between hulled and hull-less lines when adjustments were made for the hull weight lost during threshing. This supports our conclusions that hull-less lines have the potential to compete with hulled lines when the hull weight is taken into consideration.

These results are contrary to those of Choo et al. (2001) and Rey et al. (2009), who argued that the reduced vigor and lowered grain yield associated with the hull-less trait (even when the weight was adjusted to account for the hull) favors breeding and production of hulled food barley. These data are evidence that this sample of winter hull-less food barley germplasm has promise in terms of agronomic performance across a range of environments.

In the combined ANOVA of grain yield, all main effects (except for replication), two-way and three-way interactions were highly significant (Table 3). Location, year, and the location x year interaction accounted for the greatest portion of the variance. Genotype and the genotype interaction terms, while significant, were not as large. In the consistency plot (Figure 1), the basis of the significant genotype x location interaction is apparent; the median standard deviation of rank is quite high. This indicates that there were changes in rank across locations. Two of the hull-less non-waxy accessions (09OR-86 and 09OR-89) were among the top-ranked entries for yield and had lower standard deviations than the highest yielding entry across locations – Alba (which is hulled). The AMMI plots of grain yield data are shown in Figures 2a and 2b. AMMI1 (Figure 2a), which plots yield performance by the first interaction principal component axis, shows that a number of lines that exhibit a positive interaction perform better under irrigated conditions (including Streaker and 09OR-86), while other lines perform better under dryland and high rainfall conditions. AMMI2 (Figure 2b) plots the interaction first and second principal components, which account for most of the GxE variance. The interaction first and second principal components account for 46% and 29%, respectively, of the total GxE variance for yield and both are significant at $P<0.01$ according to the Gollob test (Gollob, 1968). According to the same test, other interaction principal components are not significant at $P<0.05$. Figure 2b describes more precisely which lines perform best under specific environmental conditions. This plot shows that the dryland (PUL) and high rainfall (COR) locations have a GxE effect in the same direction, though of different magnitude over the two years, but the irrigated location (ABD) tends to favor other lines, although depending on the year the direction of the interaction (positive or negative) is different. This indicates that even though there was a higher

252 yield potential for certain lines under irrigated conditions, there was greater variability under
253 irrigation between years than at the other locations.
254 Heights varied from 79.8 cm (09OR-55: hulled, waxy) to 98.3 cm (Alba: hulled, non-
255 waxy) (Table 2). On average hulled lines were 87.3 cm and hull-less lines were 90.9 cm. Choo et
256 al. (2001) reported that the *nud* allele has a pleiotropic effect causing reduced plant height. In our
257 experiment, hulled and hull-less lines were not significantly different in height. Alba was
258 significantly taller than all but one experimental food line. Maja was not significantly different
259 from eight of the experimental lines and significantly taller than six. In the combined ANOVA of
260 height, all main effects and two-way interactions were highly significant (Table 3). The three-
261 way interaction was not significant. Location, year, and the location x year interaction accounted
262 for a large portion of the variance. Genotype and the genotype interaction terms, while
263 significant, were not as important. Evidence of interactions can be seen in the consistency plot
264 and AMMI plots (Supplementary Figures 1, 2a, and 2b). In the consistency plot, the median
265 standard deviation of rank is high, which indicates that genotypic ranks were very different based
266 on location. In the AMMI plot for height, the interaction first and second principal components
267 account for 54% and 23%, respectively, of the total GxE variance for height and both are the
268 only significant components at $P<0.05$ according to the Gollob test (Gollob 1967).
269 Mean values for heading date ranged between 119 and 142 days after January 1 (Table
270 2). 09OR-55 (hulled, waxy) headed earliest, while 09OR-86 (hull-less, non-waxy) headed latest.
271 Based on the consistency plot (Supplementary Fig. 3), both checks and 09OR-86 consistently
272 flowered the latest. This plot also shows that heading time was more uniform in late lines,
273 whereas early lines had much higher standard deviations of rank.

Mean test weight values ranged from 62.3 kg hL^{-1} (09OR-55: hulled, waxy) to 77.7 kg hL^{-1} (09OR-86: hull-less, non-waxy) (Table 2). On average hulled lines were 65.3 kg hL^{-1}, significantly lower in test weight than hull-less lines, which were 75.5 kg hL^{-1} on average. This difference is expected and can be explained by the absence or presence of the hull, respectively. Supplementary Fig. 4 confirms this: all hull-less lines are ranked higher than the hulled lines.

Grain β-glucan content varied from 3.8% (Maja: hulled, non-waxy) to 6.4% (09OR-27: hulled, waxy) (Table 2). Entries with waxy starch had a significantly higher average β-glucan content (6.0%) compared to entries with normal starch, which were 4.4% on average. This is further evidence for the positive pleiotropic effect of the recessive allele at the *WX* locus on β-glucan. The difference we observed between the waxy and non-waxy classes corresponds with the values reported by Bhatty and Rossnagel (1998) and Fastnaught et al. (1996), where waxy barleys contained 6-8% and non-waxy lines contained 4-6% β-glucan. Based on the FDA health claim, in order to receive the daily recommended soluble fiber, a person needs to consume (per serving) at least 17g of steamed grain or 44g of bread made with 40% barley flour that contained 4.5% β-glucan. A consistency plot of mean ranks of β-glucan content by rank standard deviation showed that 09OR-59, the only hull-less waxy line, had comparable ranks to the other waxy lines, but performed more consistently in percent β-glucan across locations than the other lines (Figure 3). Of the non-waxy lines, Streaker and the checks had low ranks, but were also consistent across locations. This is important for buyers of whole grain products seeking consistency in level of fiber.

Mean values for protein ranged from 11.3% to 13.8% (Table 2). Alba (hulled, non-waxy) had the lowest protein, while 09OR-28 (hulled, waxy) had the highest. These values fall into the middle of the range of typical protein values found in barley (10-17%), but are similar to the

297 values found in hull-less barley by Izydorczyk et al. (2000). A consistency plot showed that the
298 checks and 09OR-86 had consistently low levels of protein across environments (Supplementary
299 Fig. 5).

300 Kernel hardness values varied from 37.8 (OBADV10-14: hull-less, non-waxy) to 67.6
301 (Alba: hulled, non-waxy) SKCS HI units (Table 2). Waxy lines averaged 55.9 SKCS HI units,
302 significantly higher than non-waxy lines, which averaged 47.4 SKCS HI units. Nair et al. (2010)
303 reported a range of 30.1-91.9 SKCS HI units in 959 breeding lines. A subset of the 959 lines
304 were examined for protein, amylose content, and β-glucan, but they found no significant
305 correlations between kernel hardness and any of the other traits. However, Bhatty (1997) and
306 Edney et al. (2002) did find evidence that endosperm texture is firmer as a result of waxy starch,
307 which corresponds with our results. Kernel hardness ranks showed that Alba was the hardest
308 grain and also consistently hard across locations (Figure 4). Streaker and 09OR-86 are both
309 softer and have lower ranks, but Streaker was more consistent across locations. Grain processors
310 who are milling or flaking grain prefer consistently hard or soft grain, respectively that will
311 perform as expected.

312 Mean SRC-W values ranged from 98.2 (09OR-86: hull-less, non-waxy) to 146.9%
313 (09OR-56: hulled, waxy) (Table 2). Lines with waxy starch had a significantly higher average
314 (133.6%), than lines with normal starch, which was 102.2% on average. This is apparent in the
315 consistency plot (Supplementary Fig. 6) where the waxy lines all fall on one side of the median
316 and the non-waxy lines fall on the other. SRC is a test typically run on wheat, and there is only
317 one report for measuring it on barley (Slukova et al., 2012). We found that the lactic acid,
318 sodium carbonate, and sucrose SRC solvents did not give consistent results in this experiment. A
319 compacted hydrated-flour pellet would not form after centrifugation, even with increased speed

and time. There is no evidence in the literature that waxy starch in wheat causes problems with the different solvents. In the literature where SRC is performed on barley, there is also no mention of difficulties with protocol. Slukova et al. (2012) measured SRC on four barley samples (one was only barley bran) and obtained slightly lower percentages from the water test than we did (76-134%). The lines they measured had low protein content (between 6.7-10.9%) and low β-glucan (between 2.5-4.2%), which may be relevant because we found SRC-W to be positively correlated with both β-glucan and protein. More experimentation is required to adjust the protocol for the SRC test, but once all four solvents can be used, this test will be very useful for classifying barley for specific end-use purposes.

Mean barley stripe rust (BSR) percentages ranged from 0% (09OR-56, 09OR-62, and Maja) to 15% (09OR-28) (Table 4). All lines showed high levels of resistance, which is critical in high rainfall areas where BSR is prevalent and can result in yield loss and lowered quality. The intensity of the stripe rust epidemics at Corvallis in 2011 and 2012 is apparent from the stripe rust severity of Thoroughbred, a susceptible feed barley line grown in an adjacent experiment, which was rated an average of 82.5% over the same two years. Mean ratings for scald (on a 1-9 scale) ranged from 1.0 (Alba) to 6.5 (09OR-28) (Table 4). Most lines showed moderate susceptibility to the disease. Scald is not as devastating as BSR, but the necrotic lesions that form on foliar tissue can lead to yield loss and thin kernels (Garvin et al., 1997). Resistance to scald is difficult to achieve as the pathogen rapidly overcomes resistance genes (Brown et al., 1996; Garvin et al., 1997).

Mean winter survival percentages ranged from 72.0 (09OR-59) to 97.4% (Alba) (Table 4). On average, hulled lines had 90.5% survival and hull-less lines had 82.7% survival. Differential winter survival was observed at PUL and ABD; all lines showed 100% survival in

COR both years. In our target environments in the Pacific Northwest, a level of winterhardiness is essential and should be equivalent or greater than the checks. Supplementary Fig. 7 shows that the checks consistently have high levels of winterhardiness, as do some of the experimental lines. Other lines, including 09OR-86, are more inconsistent across locations. However, yield and winter survival were not correlated (r = 0.16, $P = 0.21$), which indicates that lines with intermediate levels of winterhardiness may recover sufficiently to make up for plant mortality over the winter.

Across the six environments, significant positive correlations were observed between β-glucan and kernel hardness (r = 0.40, $P<.0001$), β-glucan and SRC-W (r = 0.73, $P<.0001$), kernel hardness and SRC-W (r = 0.39, $P<.0001$), and protein and SRC-W (r = 0.50, $P<.0001$) (Table 5). These correlations confirm that this germplasm fits the reported association of cereal β-glucans having high viscosity and water-binding capabilities, which allows for greater absorption of water (Izydorczyk and Dexter 2008; Lazaridou and Biliaderis 2007). However, many of the correlations have low r values, which allows for the opportunity to breed for a variety of combinations, including lines with high β-glucan and softer kernels. Significant negative correlations were seen between test weight and β-glucan, kernel hardness, and SRC-W. The negative correlations with test weight were most likely confounded by the fact that all but one of the non-waxy lines were hull-less and all waxy lines were hulled.

Conclusions:

As interest in barley as a crop for human consumption grows, the demand for agronomically sound varieties with good food qualities will increase. The results from this experiment will help to meet needs of farmers, consumers, and processors. The overall grain yields achieved with this winter germplasm are much higher than those reported previously for spring barley germplasm by Rey et al. (2009). Additionally, despite some evidence that the hull-less trait is associated with lower yield and vigor, there were no significant differences between the hulled and hull-less classes in this germplasm (Choo et al. 2001; Rey et al. 2009). Therefore, our future food projects will be focused exclusively on breeding hull-less lines. This is due to an interest in the whole grain benefits and the processing difficulties that arise with pearling. We found that waxy starch played an important role in determining quality traits, including β-glucan, kernel hardness, and SRC-W. Holtekjolen et al. (2008) reports that waxy starch can lead to difficulties in the baking process, if the appropriate amount of water is not used. Therefore, our efforts now focus on hull-less non-waxy types, with modest β-glucan levels. Streaker will be released in 2014, and 09OR-86 is a candidate for release.

Much progress has been made over the last decade in food barley breeding and characterization in different breeding programs around the world; our program will continue to focus on breeding nutritious and delicious barley varieties with good food quality that are adapted for the Pacific Northwest.

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Figure Captions:

Figure 1. Consistency plot of mean rank by standard deviation of rank for grain yield at Corvallis, OR; Pullman, WA; and Aberdeen, ID in 2012 and 2013. Dashed lines indicate median values.

Figure 2a. AMMI1 plot for grain yield at Corvallis, OR (COR); Pullman, WA (PUL); and Aberdeen, ID (ABD) in 2012 and 2013. Squares indicate location, diamonds indicate entry.

Figure 2b. AMMI2 plot for grain yield at Corvallis, OR (COR); Pullman, WA (PUL); and Aberdeen, ID (ABD) in 2012 and 2013. Squares indicate location, diamonds indicate entry.

Figure 3. Consistency plot of mean rank by standard deviation of rank for β-glucan at Corvallis, OR; Pullman, WA; and Aberdeen, ID in 2012 and 2013. Dashed lines indicate median values.

Figure 4. Consistency plot of mean rank by standard deviation of rank for kernel hardness at Corvallis, OR; Pullman, WA; and Aberdeen, ID in 2012 and 2013. Dashed lines indicate median values.

Table 1. Pedigree, row type, hull type, and starch type for all lines in the OFOOD trial grown at Corvallis, OR; Pullman, WA; and Aberdeen, ID in 2012 and 2013.

Line	Pedigree	Row type	Hull type	Starch type
Streaker	Maja/Legacy, F1//Maja///Doyce	Six-row	Hull-less	Normal
OBADV10-13	Strider/Doyce	Six-row	Hull-less	Normal
OBADV10-14	Strider/Doyce	Six-row	Hull-less	Normal
09OR-59	Strider/Merlin, F1//Strider	Six-row	Hull-less	Waxy
09OR-70	Maja/Legacy, F1//Maja///Doyce	Six-row	Hull-less	Normal
09OR-86	Strider/Doyce	Six-row	Hull-less	Normal
09OR-89	Strider/Doyce	Six-row	Hull-less	Normal
09OR-27	Luca/Merlin, F1//Luca	Two-row	Hulled	Waxy
09OR-28	Luca/Merlin, F1//Luca	Two-row	Hulled	Waxy
09OR-31	Luca/Merlin, F1//Luca	Two-row	Hulled	Waxy
09OR-51	Luca/Waxbar, F1//Luca	Two-row	Hulled	Waxy
09OR-55	Strider/Merlin, F1//Strider	Six-row	Hulled	Waxy
09OR-56	Strider/Merlin, F1//Strider	Six-row	Hulled	Waxy
09OR-62	Strider/Merlin, F1//Strider	Six-row	Hulled	Waxy
Alba	Strider/Orca	Six-row	Hulled	Normal
Maja	Strider/88Ab536	Six-row	Hulled	Normal

Table 2. Means of grain yield, height, heading date, test weight, β-glucan, protein, kernel hardness, and solvent retention capacity of water (SRC-W) of the entries in the OFOOD trial grown at Corvallis, OR; Pullman, WA; and Aberdeen, ID in 2012 and 2013.
†Based on field experiment replication. All other traits use environments as replications.

Line	Yield	Height	Heading Date	Test Weight	β-glucan	Protein	Kernel hardness	SRC-W
	kg ha^{-1}	cm	Days after Jan. 1	kg hL^{-1}	% (w/w)	%	SKCS HI units	%
Streaker	6512	91.3	134	74.8	4.2	12.7	44.0	102.5
OBADV10-13	6114	92.3	135	77.4	4.6	11.6	39.3	101.5
OBADV10-14	5701	91.2	137	75.8	4.2	11.7	37.8	98.6
09OR-59	5837	84.9	133	73.1	5.9	13.7	50.7	129.8
09OR-70	6498	87.4	132	72.5	4.8	13.2	57.2	110.4
09OR-86	6758	95.3	142	77.7	4.1	11.4	42.9	98.2
09OR-89	7049	93.7	141	77.0	4.2	11.7	43.8	101.9
09OR-27	6274	93.1	134	66.6	6.4	12.1	58.1	142.9
09OR-28	5804	83.8	131	66.2	6.3	13.8	53.2	130.7
09OR-31	6180	88.1	130	66.2	6.3	13.3	51.6	137.7
09OR-51	6705	84.0	132	67.6	6.0	13.1	47.4	131.9
09OR-55	6411	79.8	119	62.3	5.7	12.1	61.7	123.9
09OR-56	7053	84.3	133	63.7	6.1	12.7	66.0	146.9
09OR-62	6777	83.0	132	64.6	5.5	12.6	58.6	125.0
Alba	8257	98.3	141	66.0	4.4	11.3	67.6	108.4
Maja	6856	91.1	136	63.9	3.8	11.7	46.8	102.1
Mean	6549	88.8	134	69.7	5.2	12.4	51.7	118.3
LSD (P = 0.05)	535†	4.6†	7	2.3	0.6	1.1	5.9	11.4
CV%	12.2	7.6	4.7	2.9	10.3	7.8	9.8	8.4

Table 3. Estimates of variance components for grain yield and height for the entries in the OFOOD trial at Corvallis, OR; Pullman, WA; and Aberdeen, ID in 2012 and 2013.
*Significant at $P < 0.05$.
**Significant at $P < 0.01$.
[†]ns, not significant.

Component of variance	df	Grain yield	Height
Genotype	15	7651024**	449.9**
Rep (Location x Year)	11	598746ns[†]	94.8*
Location	2	230291247**	6805.0**
Year	1	16787710**	2345.2**
Location x Year	2	44361578**	4874.2**
Genotype x Location	30	2806094**	189.4**
Genotype x Year	15	285128*	162.4**
Genotype x Location x Year	30	1812859**	65.4ns
Error	165	622844	45.8

Table 4. Means for barley stripe rust (BSR) and scald ratings for the entries in the OFOOD trial at Corvallis, OR in 2012 and 2013. Means for winter survival are for the trials at Pullman, WA and Aberdeen, ID in 2012 and 2013.

[†]1-9 scale where 1=most resistant and 9=most susceptible

[‡]Based on field experiment replication. Disease notes based on replication by year.

Line	BSR	Scald	Winter survival
	%	1-9 scale[†]	%
Streaker	10.0	6.3	90.5
OBADV10-13	10.5	4.0	79.3
OBADV10-14	10.0	3.5	87.5
09OR-59	5.0	4.5	72.0
09OR-70	10.0	3.8	92.3
09OR-86	5.0	3.0	76.2
09OR-89	5.0	3.0	81.0
09OR-27	10.0	5.5	90.0
09OR-28	15.0	6.5	94.7
09OR-31	12.5	6.0	95.6
09OR-51	12.5	4.5	91.9
09OR-55	10.0	3.5	80.5
09OR-56	0.0	4.5	87.3
09OR-62	0.0	4.0	93.8
Alba	5.0	1.0	97.4
Maja	0.0	4.5	91.4
Mean	7.5	4.3	87.6
Number of env.	2	2	4
LSD (0.05)	13.0	2.0	8.1[‡]
CV%	80.7	22.0	10.9

703 **Table 5.** Pearson's simple correlation coefficients and *P* values for traits measured on all 16
704 entries in the OFOOD trial grown at Corvallis, OR; Pullman, WA; and Aberdeen, ID in 2012 and
705 2013.
706 *Significant at $P < 0.05$.
707 **Significant at $P < 0.01$.
708 †ns, not significant.
709

	Trait						
Trait	**Protein**	**Kernel hardness**	**SRC-W**	**Yield**	**Test weight**	**Height**	**Heading date**
β-glucan	0.35**	0.40**	0.73**	-0.10ns†	-0.42**	-0.23*	-0.19ns
Protein		0.04ns	0.50**	0.09ns	-0.07ns	-0.38**	0.30**
Kernel hardness			0.39**	0.08ns	-0.60**	0.24*	-0.30**
SRC-W				0.15ns	-0.53**	-0.25*	0.07ns
Yield					-0.17ns	0.06ns	0.22*
Test weight						0.11ns	0.15ns
Height							-0.32**

710