

b. Field 8. Project Narrative

(ii) Executive summary and table of contents

1. Project title: Developing Multi-use Naked Barley for Organic Farming Systems

2. Project type: Multi-Regional Integrated Project Proposal

3. Legislatively-defined goals:

- (1) Facilitating the development and improvement of organic agriculture production, breeding, and processing methods (30%)
- (2) Evaluating the potential economic benefits of organic agricultural production and methods to producers, processors and rural communities (10%)
- (6) Conducting advanced on-farm research and development that emphasizes observation of, experimentation with, and innovation for working organic farms, including research relating to production, marketing, food safety, socioeconomic conditions, and farm business management (30%)
- (8) Developing new and improved seed varieties that are particularly suited for organic agriculture (30%)

4. Research, Education, and Extension Distribution: 60% Research, 15% Education, 25% Extension

5. Program Staff:

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- Dr. Stephen Jones, Professor, Department of Crop and Soil Sciences, Washington State University (WSU), The Bread Lab, 11768 Westar Lane, Burlington, WA 98233; jones@wsu.edu
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- Dr. Mark Sorrells, Professor, Plant Breeding and Genetics Section, Cornell, 240 Emerson Hall, Ithaca, NY 14853; mes12@cornell.edu

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Regional Stakeholder Advisory Committee:

- Mike Anthony: Chef, Gramercy Tavern
- Mac Ehrhardt: Seedsman, Albert Lea Seed
- Ben Edmunds: Brewer, Breakside Brewing
- Carmen Fernholz: Organic Farmer
- James Henderson: Organic Grains Contracting and Processing, Hummingbird Wholesale
- Scott Garden: Maltster, Great Western Malting
- Micaela Colley: Program Director, Organic Seed Alliance

6. Critical stakeholder needs and project long-term goals

Organic growers need new crops, markets, and rotation options. There is an additional need for high quality organic/non-gmo feed options and demand for culinary and baking grain varieties that are regionally produced and identity preserved. This project will assist in meeting these needs via the release, testing, and development of multi-use (food, feed, and malting) naked (hull-less) barley varieties. A key attribute of these varieties that enables multiple uses is the absence of an adhering hull (p.1).

7. Outreach plan

The outreach plan will include webinars; print and online technical papers; print and online articles in the popular press; presentations at conferences, culinary events, and field days and to targeted advisory committees; and outreach via websites and social media (p.18).

8. Potential economic and/or social benefits

A principal benefit of this research will be rural economic development. This will be achieved by strengthening organic food, feed, and malting-based industries. Organic operations are correlated with counties that have higher than national average farm income and smaller than national average farm size. Multi-use organic barley can provide multiple benefits in an organic farm's rotation and marketing strategy (Marasteanu and Jaenicke, 2016). Measurements will be made of member participation in the value chain and the growth in opportunities to produce and market multi-use organic barley. Consumer preferences and willingness to pay premium prices are expected to translate into opportunities for all involved. Naked barley has the added economic benefits of requiring no specialized equipment for pearling and lower transportation costs per unit of marketable grain (pp.17-18).

9. Stakeholder engagement

Project organizers will be in constant communication with the Regional Stakeholder Advisory Committee and participating stakeholders via personal, electronic, and hard copy media in order to assure full engagement. The broader stakeholder community will be engaged through project updates via direct email from project organizers as well as our outreach efforts (pp.1-2).

(iii) Outcome from previous PI OREI awards:

Award number: 2016-04450

Significant outcomes:

The Planning Grant session - held in Portland, Oregon November 30-December 1 - was attended by 24 scientists and stakeholders. The significant outcomes of the Planning Grant were those proposed: this full proposal, a commitment to preparation of a white paper on the prospects for naked multi-use organic barley, and the development of a national network committed to strengthening organic agriculture through naked multi-use barley.

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(iv) Introduction:

1. Long-term goals, critical needs of stakeholders, supporting outreach objectives:

The **long-term goals** of this multi-region, integrative project are to: (1) provide organic growers, processors, and consumers with a new crop, food, and raw material alternative that will be economically rewarding and sustainable (2) identify and release high-yielding, high-quality, flavorful and nutritious multi-use naked barley varieties for organic systems based on a regional variety testing program anchored in Oregon, Washington, Wisconsin, Minnesota, and New York; (3) characterize key agronomic and food, feed, and malt quality traits in a large, genetically diverse panel of naked barley germplasm grown under organic conditions and maximize the efficiency of selection in this panel via integration of phenotypic and genotypic data; (4) observe, analyze, and report the results of natural selection and artificial selection on an organically grown naked barley composite population – a vehicle for engaging K-12 students and home gardeners in organic grains and foods; (5) understand the economic and environmental benefits of domestic organic naked barley production, and; (6) educate the public on the uses and production value of naked barley using a number of dissemination techniques.

The **primary stakeholders** of this project include organic farmers; barley processors, wholesalers, retailers; food and malt beverage product manufacturers; and consumers. **Critical needs** described by organic farmers at field days, through personal communication, and during the planning grant meeting include: (1) high yielding naked barley varieties with defined multi-use quality profiles; (2) varieties with superior weed competitiveness and resistance to diseases; (3) improved communication between farmers, processors, distributors and end-users. **Critical needs** of food and malt beverage manufacturers include full characterization of flavor, nutrition, quality and functionality. **Critical needs** of barley processors, wholesalers, and retailers focus on the need for reliable sources of domestic, organic, high-quality barley for multiple end-uses. Currently organic barley end-uses and markets are stratified due to the presence of the adhering hull and they could be further stratified if naked food barleys are selected for the highest possible grain β -glucan. Varieties intended for malting/brewing have hulls - due to predominant brewing equipment - and should have low to moderate grain β -glucan. Some brewers could use mash filters and/or blends of malt from both hull adhering and naked barley. However, the hull precludes direct food use and is not an advantage in feeds. Therefore, our approach is to focus on naked (hull-less barley) with moderate grain β -glucan for all three uses.

Research and Extension Objectives:

1. Evaluate multi-use naked barley varieties and breeding lines in diverse, representative organic systems across the United States.
2. Identify and characterize key agronomic and food, feed, and malt quality traits for naked barley lines grown under organic conditions.
3. Understand the genetics of traits important for organically produced multi-use naked barley.
4. Measure the economic and environmental benefits of organic naked barley production and products.
5. Educate the public about the performance of naked barley in various organic production systems, variety attributes, and end-use options.
6. Observe, analyze, and report the results of natural selection and artificial selection on an organically grown naked barley composite population.
7. Investigate, assess, and develop multiple markets for naked barley through engagement with the full spectrum of stakeholders.

2. Stakeholder engagement:

Stakeholders representing growers, food processors, malt processors, and seed producers were engaged from the outset of this proposal. Bakers, chefs, maltsters, brewers, seed producers, growers, and distributors were present at the planning grant meeting that was held as a precursor to this proposal. All gave presentations that have contributed to the writing of this grant and all provided letters of support (see attached). A subset of these stakeholders have agreed to serve on a **Regional Stakeholder Advisory**

Committee (RSAC), which will help guide the direction of this research. RSAC members will join Co-PIs at an annual project meeting held in a different region (e.g. Pacific Northwest, Upper Midwest, and North East) each year. There will be additional digital and in person meetings throughout the year to ensure the research remains on track and relevant.

3. Body of knowledge:

Barley is one of the oldest known domesticated crops. Originally cultivated for human consumption, other end-uses have gained importance over the millennia. Barley is the fourth most important cereal crop in the world (FAO-STAT, 2016) and the second most widely grown organic small grain in the United States. It is a versatile crop with three principal end-uses: feed, food, and malting. Organic barley is produced for all three uses and fetches a significant premium over conventional barley. Each use of barley requires different characteristics, but hull adherence and β -glucan are important for each of the three classes.

Organic breeding: Organic growers need varieties developed specifically for organic conditions (Murphy et al., 2007; Wolfe et al., 2008). Most varieties grown by organic farmers were bred under (and for) non-organic production conditions. As a consequence, these varieties may require improvement for one or more of the following traits: disease resistance, weed competition, input-use efficiency, flavor, and nutritional quality. The wide range of diversity found on organic farms makes targeted regional breeding especially important for organic crops. Crops selected under organic conditions are better suited for weed competition and disease management when grown for large-scale production on organic farms.

Hull adherence and β -glucan: In most barley germplasm, the hull adheres to the grain. In some barley germplasm, however, the hull does not adhere and the grain threshes “clean”, as in wheat. These types are referred to as “hull-less” or “naked”; the latter is the preferred term and is used in this proposal. Adherence of the lemma and palea is determined by alleles at the *nud* locus on chromosome 7HL (Takeda et al, 2008) and the adhering hull accounts for ~ 13% of the weight of harvested barley grain. Breeding programs involved in this planning grant have bred the naked trait into their germplasm. As reviewed by Meints et al. (2015a), β -glucan is a soluble dietary fiber, found in barley and oats. Grain β -glucan content is a quantitative trait, with several QTL (Mohammadi et al., 2015). There is a positive pleiotropic effect of the recessive allele at the Waxy (*WX*) locus with grain β -glucan content. Therefore, many food varieties with high β -glucan also have waxy starch. β -glucanase is the native enzyme that degrades β -glucan: several genes and Quantitative Trait Loci (QTLs) are reported. Grain β -glucan content is routinely measured in the participating food barley breeding programs. In malting barley, the residual β -glucan remaining after malting is measured as “wort” β -glucan.

Feed barley: Key points in a recent review of feed barley by Blake et al. (2011) were (i) approximately 75% of the barley grown in the world is used for animal feed, (ii) although extensive research has documented a broad range of nutritional properties for ruminants, non-ruminants, poultry, and fish, there is usually little or no premium paid for feed barley, and (iii) feed barley prices are often 80-90% that of maize, based on the reported feed values of the two crops. Organic feed barley is a bright spot in this economic landscape, with prices often competitive with conventional malting barley. Hummingbird Wholesale (see letter of support) profitably contracts and markets organic ‘Alba’, a variety with adhering hulls released by Oregon State University. A principal customer for ‘Alba’ is Scratch and Peck Feeds (see letter of support). Both companies would prefer feed barley without hulls.

Most feed barley varieties have adhering hulls, a consequence of the fact that breeding efforts for the past 100 years have largely focused on malting barley germplasm: when a variety did not make malting specifications, it was used for feed. An exception is in the Canadian Prairies where, for the past ~ 30 years, there has been intensive breeding for naked types destined for the swine industry. The hull consists of insoluble dietary fiber, a benefit for ruminants, but of no benefit for non-ruminants, poultry, or fish. When barley is purchased for feed, the buyer recognizes that ~13% of the purchase price is insoluble fiber, which may be of little or no value. In the case of ruminants, where insoluble fiber is necessary, there are other and plentiful sources of fiber besides barley hulls. High β -glucan is a concern in poultry

feeds. In conventional feeds, this can be ameliorated by the addition of exogenous β -glucanase, but this raises costs and may lead to regulatory issues in organic production. For organic production, sprouted grain feeds can help to address the β -glucan issue, as native β -glucanase enzymes are produced during germination. More fundamentally, the modest levels of grain β -glucan we advocate for in food barley (see next section) will make it feasible to have multi-use varieties.

Food barley: Despite its rich cultural and culinary significance in many cultures around the world and its virtues as a fiber-rich and versatile grain, in most modern day societies barley has all but disappeared as a food. Barley foods are on the rebound, and especially with organic consumers, who are more likely to have a heightened awareness of fiber and whole grain nutrition (see letters of support). The USDA received a petition to allow non-organic barley betafiber in 2011 (Kolberg, 2011). The National Organic Standards Board did not recommend adding non-organic barley betafiber to the National List. Therefore, barley betafiber and other barley products used as ingredients in organic food must be organically produced. Because of organic consumers' interest in the health benefits of barley's unique fiber characteristics, the market for organic food barley can expected to grow. Barley has the advantage of providing fiber and other healthful components in a package that has half of the fat content or less compared to oat and greater total dietary fiber content than wheat, oat, or rye (Meints et al., 2015a). Barley β -glucan, at modest levels we propose to target, is effective in reducing the incidence and severity of "metabolic syndrome" through increased satiety, slowed macronutrient absorption, reduced post-prandial glucose response, lowered blood cholesterol levels, reduced insulin resistance, and reduced abdominal fat (see Meints et al., 2015a and references cited therein). The capacity of barley foods to reduce cholesterol was the key factor in approval of the FDA health claim for barley in 2006 and similar claims in Europe (2011) and Canada (2012). As described by Graebner et al. (2015) and references therein, barley also supplies bioactive nutrients such as phenolics, phytate, and tocols.

For human consumption, varieties with adhering hulls must have the hull removed by pearling, a mechanical abrasion process that leads to the grain being ineligible for "whole grain" status. Therefore, naked varieties are preferred for whole grain foods. With the announcement of approved health claims for barley β -glucan, breeding programs have tended to gravitate to one of two routes - whole grain or fractionation - for incorporating the benefits of barley into the diets. In the whole grain route - which is what we are pursuing and believe is most in line with organic production, products, and consumer preference - a modest (~ 4-5%) grain β -glucan is sufficient to meet daily recommended intake of soluble fiber. This amounts to a small side dish of steamed grain or two slices of bread (with 40% barley flour) per day. Focusing on whole grain barley also gives us the opportunity to leverage the health advantages with culinary and sensory attributes. In the fractionation route, the breeding is targeted at achieving as high a β -glucan level as possible so that the β -glucan can be isolated and used as an additive to other foods/food products to enrich their fiber content. Most high β -glucan food varieties available today are waxy starch types, which complicates their use in whole grain foods, brewing, and feeding. We therefore argue that multi-use naked varieties should have normal starch and a modest level of β -glucan. Many organic farms that are looking at small grain crops in rotation do not have dehulling equipment, and would be more likely to include barley as a rotation crop if it was hull-less.

Malting barley: The principal reason for the continued production of barley in most of the world is malting, with most of the malt being destined for brewing and distilling. Barley is the optimum substrate for malting and brewing/distilling because it provides a balance of starch and enzymes for the subsequent nutrition of yeast during fermentation; this simple summary statement is based on extensive research into the genetics, biochemistry, and physiology of the malting process (reviewed by Schwarz and Li, 2011). There is continual progress in utilizing the latest genetics tools to unravel the genetic basis of malting quality parameters (Mohammadi et al., 2015 and references therein). The reason for this vigorous research activity in malting quality is that malting barley fetches a significant premium over feed barley. The recent explosive growth in the US craft brewing and distilling industries is giving further incentive to malting barley research, as these industries use more barley malt per unit of beer than mainstream lagers.

Almost without exception, malting barley research is directed at barley with hulls. In traditional brewing practice, the hulls play a very important role in the brewing step of “lautering” where they provide filtration. Advances in brewing technology, including the use of mash filters, make it possible to achieve higher brewing efficiencies with naked barley. This can also lead to lower carbon and water footprints and an opportunity to explore new horizons in malting and brewing flavor (see letters of support). Because much of the mycotoxin content is located in the hull, naked varieties will generally have lower levels of deoxynivalenol or vomitoxin (Legzdina and Buerstmayer 2004 and references therein). β -glucans are a principal component of endosperm cell walls; therefore, they need to be degraded by native β -glucanases in order to achieve optimum malting quality. Unacceptably high residual β -glucans lead to a number of challenges in brewing. Typical malt barley grain β -glucan levels are in the 4-5% range. As described in the preceding section on feed and food barley, this range of grain β -glucan content is also suitable for feed and whole grain food applications.

Multi-use feed, food, and malting barley: Currently, there is fragmentation in organic (and conventional) barley production due to the presence/absence of hulls and variation in β -glucan level. Farmers seeking the malting price premium grow varieties with hulls because in the US most brewers are not equipped for or familiar with naked barley malts. If their malting barley is not sold for malt, they are forced to sell at lower food or feed prices. Conversely, a food barley grower may not have a sufficient market for naked grain that could otherwise make an excellent malt or feed. If s/he is producing a high β -glucan naked waxy starch variety for food, it will be unacceptable for malting/brewing and feeding. A lack of familiarity with naked barley complicates marketing the crop to the food industry, feeders, and users of malt. Innovative users are willing and keen to learn (see letters of support). This project is directed at discovering paths to create accepted multiple uses for organically-grown barley varieties. This goal will be most readily accomplished by plant breeders developing and releasing naked multi-use varieties with modest β -glucan levels suitable for organic production. Seed companies will make these varieties available (see letters of support). At the same time, food processors, feeders, and maltsters/brewers/distillers need to be familiarized with the newly available naked varieties and their advantages. Given their strong interests in innovation, health, and sustainability, the organic production, processing, and consumer communities stand to benefit directly from the adoption of new multi-use barley varieties.

Weed competition: In organic systems, competition from weeds often reduces barley yields and is a serious impediment for farmers transitioning from conventional to organic production. Surveys of organic farmers consistently show that weed management is a top research priority for them (Jerkins and Ory, 2016). Organic farmers identified small grains in rotation as a key strategy for weed management (Baker and Mohler, 2015). Higher grain yields can be obtained by selecting varieties that can effectively tolerate or suppress weed growth and through breeding barley varieties for increased weed competitive ability (WCA). However, measuring WCA is difficult because of genotype by environment interactions and low heritability. Consequently, it is necessary to use a combination of correlated traits to indirectly assess and select barley genotypes that have superior WCA. WCA describes ecological interactions between weeds and crop plants and can be measured as weed suppression or crop tolerance. Weed suppression is measured as the reduction of weed growth or seed production in comparison to a weed-free control while crop tolerance is measured as the absolute yield or the percent yield loss under weedy versus weed-free conditions.

There have been several studies on both spring and winter barley WCA, but very few have examined the correlation between measured traits and WCA. Also, traits are often measured differently in different studies so they do not readily translate from one study to the next making it difficult to choose which ones to apply in a breeding program. In studies that have compared barley with other small grains, barley has shown relatively better WCA (Beres et al. 2010; Mason et al. 2007; O'Donovan et al. 2005; Lopez-Casteñeda et al. 1995) largely related to early seedling vigor that is characteristic of barley. Three studies have examined traits correlated with weed suppression (Dhima et al. 2010; Christensen 1995; and

Dhima et al. 2000). Dhima et al. (2010) found that barley stem number and barley fresh weight were highly negatively correlated with total weed fresh weight; however, height was not significant. Didon and Boström (2003) and Didon (2002) reported that tillering and leaf area were not correlated with weed suppression in contrast to a report by Lemerle et al. (1996) that, in wheat, tillering capacity and leaf area were correlated with WCA. Crop tolerance to weed competition as measured by yield loss in weedy versus weed-free conditions was reported to be correlated with total weed biomass (Mason et al. 2007), wild oat shoot dry weight and seed yield (O'Donovan et al. 2000). Traits that require directly measuring weed biomass or weed seed yield are too laborious and costly for routine selection in breeding and most testing programs. Mature plant height was generally not correlated with either weed suppression or crop tolerance in barley (Dhima et al 2010; Mason et al. 2007) or wheat (e.g. Lemerle et al. 1996). Consequently, other indirect measures such as early seedling vigor, and rate and timing of stem elongation (early height) (Christensen 1995), and possibly leaf area index (Hansen et al. 2008) are required to predict WCA. Because of the diversity of environments, variety adaptation, cultural practices, and weed populations each breeding program would benefit from a region-specific model that identifies which WCA traits are most effective in their region (Kissing-Kucek, 2017; Worthington and Reberg-Horton 2013).

4. Ongoing activities:

The participating breeding programs have all participated in organic grains research: the most deeply and recently involved is the Cornell University (CU) program, with Mark Sorrells, June Russell, Julie Dawson and Brian Baker. Their OREI-supported project, “Value-Added Grains for Local and Regional Food Systems”, involved assessment of wheat, spelt, emmer, and einkorn in organic management systems. Grain and baking characteristics, including flavor and nutritional quality, were assessed. At the University of Wisconsin- Madison (UW-Madison), the small grains breeder Lucia Gutierrez is collaborating with Julie Dawson to trial small grains, including barley, wheat, and oats under organic conditions. Wisconsin has an innovative brewing industry and interest in novel malting barley (see letter of support from New Glarus Brewing). Organic Valley, headquartered in Wisconsin, has a major interest in high quality organic feeds (see letter of support). At the University of Minnesota (UMN), Kevin Smith's breeding and genetics research has focused on malting/food quality traits and disease resistance, important attributes for organic barley, and he is poised to direct this towards his naked barley program for organic producers (see letters of support from Fernholz and Rahr Malting). At Washington State University (WSU), Stephen Jones and Kevin Murphy have a long history of breeding and promoting organic grains. Currently, their respective labs are working to breed new varieties of malt and food barley under organic conditions focusing on novel color traits, flavor, nutrition, and local adaptation. At Oregon State University (OSU), Pat Hayes has worked closely with local growers and processors to bring organically grown barley-based foods and beverages to grocery shelves and tables (see letters of support from Organic Enterprises of Oregon, Camas Country Mill, Hummingbird Wholesale, Little T Bakery Seastar Bakery, Breakside Brewery, Crux Fermentation project, and Great Western Malting). GrowNYC's Greenmarket program manages a network of farmers markets in New York City, working with over 230 farmers across six states in the Northeast. Greenmarket's mission is to provide all New Yorkers access to fresh, healthy, local food. For the past eight years, Greenmarket's Regional Grains Project has been working with researchers, farmers, millers, and end users to revitalize and sustainably scale up the production of grains in the Northeast. Since the initiative was launched, dozens of farms have integrated small grains into production, new grain processing facilities have been built and new varieties of small grains have been brought to the consumer market, where hundreds of new products from bread to beer, utilizing “local” grains have been developed. Through its educational and promotional initiatives, GrowNYC has the capacity to literally reach hundreds of thousands of consumers, from diverse populations across the city's five boroughs.

All researchers have established extensive networks for developing, growing, processing, and conducting education on organic barley, as evidenced by the letters of support. The U.S. barley research community has a long history of collaboration and exchange of both knowledge and genetic resources. The OSU, UMN, and CU barley researchers participated in two consecutive USDA-NIFA-CAP projects. There are naked barleys in various stages of development in the participating programs. ‘#STRKR’, released by OSU (Meints et al., 2015c), is in commercial production and the variety ‘Buck’ was released by OSU in 2016. There are naked advanced lines in international and national trials, and there is a doubled haploid naked germplasm array suitable for genome wide association studies and genomic selection. A common theme with these varieties and germplasm is a focus on multiple whole grain uses.

5. Preliminary data/information:

Researchers involved in this project have collaborated on multi-use naked barley breeding, production, and end-use. OSU has collaborated with WSU and international cooperators (James Hutton Institute, Scotland; and University of Lleida, Spain) on the INUDFOOD project directed at assessing agronomic and grain quality attributes and their interactions with environment. A publication from this work is in preparation for submission (Meints et al., *in preparation*). OSU, CU, UMN, and Purdue collaborated on a multi-regional naked barley malting trial (2016 harvest) and samples are currently being analyzed for malting, brewing, and sensory attributes at Rahr Malting. The culinary properties of barley have been gaining increasing recognition in events sponsored by individuals, institutions, and supporters involved in this proposal e.g. the Plant Variety Showcase (2016); Barley Day 2016 (featured in the November issue of Edible Portland; <http://edibleportland.com/brewing-with-the-grain/>); and the barley porridge selected as the # 1 dish of the year by the Seattle Times (<http://www.seattletimes.com/life/food-drink/25-best-dishes-of-2016-our-restaurant-critics-favorite-bites/>).

With funding from New York State Craft Beverage Promotion, Greenmarket hosted the Beer and Spirits of New York Pop-Up stand at twenty-eight markets in the fall of 2016. Invited participants were required to produce their products in accordance with New York State Farm Distillery or Farm Brewery license requirements: 75% New York grain for distilleries and 20% for breweries. In 2014, GrowNYC worked with Jimmy Carbone of Jimmy’s 43/Beer Sessions Radio and Valley Malt of Hadley, MA to offer brewers local grains and malt to use in specialty beers brewed for Brewer’s Choice, the premier event of New York City beer week. “SMaSH” beers, (state malt, state hops) are now the standard at this annual event.

With funding from OREI award #2011-51300-30697 Cornell has published four years of organic grains trials grown in Pennsylvania, North Dakota, and two locations in New York, <https://plbrgen.cals.cornell.edu/research-extension/small-grains/cultivar-testing>. The grains evaluated included spring and winter wheat, spring and winter spelt, and spring emmer and einkorn. Three quality and sensory evaluations of multiple varieties were conducted with the participation of professional chefs and bakers in New York. Sensory evaluations of all three were conducted with trained panels for pasta, sourdough baking, and pastries and yeasted breads. Cornell has also published two years of results on growing grains in organic rotations, <http://blogs.cornell.edu/whatscroppingup/2016/09/26/organic-wheat-looked-great-but-yielded-7-5-less-than-conventional-wheat-in-20152016/>.

WSU has been conducting on-farm organic barley trials in Washington and Idaho for the past six years, resulting in the release of two new barley varieties adapted to organic farming systems: 1) ‘Muir’, a stripe rust resistant, two-row, hulled feed barley, and 2) ‘Havener’ a naked food barley with moderate β -glucan (<http://csanr.wsu.edu/grants/beyond-beef-and-barley-soup-development-of-nutritionally-dense-hulless-food-barley-varieties-for-organic-farmers/>; <http://csanr.wsu.edu/grants/organic-wheat-variety-release-in-eastern-washington/>) (Murphy et al., 2015). The WSU-Mount Vernon Small Grains Breeding Program has been growing on-station and on-farm organic barley trials for the past five years in the Skagit Valley. In 2015 and 2016, advanced naked barley spring and winter trials were grown under organic conditions. Data are available at: <http://thebreadlab.wsu.edu/western-washington-variety-trials/>. New naked barley crosses were made in the winter of 2015 and these have been and will be selected and advanced under organic conditions ensuring that they will be adapted to organic conditions.

(v) Rationale and Significance:

The proposed grant is directed at FY 2017 priorities 1, 2, 6, and 8. Organic agriculture, public sector variety development, and barley share a number of challenges and opportunities. Organic producers need new rotation crops in order to help manage weeds and pests and to manage risk through crop diversification. Barley would be ideal in many cases. Public sector variety development programs face funding and variety release challenges; there are significant opportunities for efficiency and productivity via national collaboration.

We see an opportunity to strengthen organic agriculture, public variety release, testing, and development by approaching barley from an entirely new angle. After 10,000 years we believe it is time for barley to join wheat and other grain crops and “go naked”. The organic community is receptive to new foods and developing new markets, making it an ideal launching point for this new version of the world’s oldest crop. For example, the inclusion of sprouted (e.g. partially malted) grain has been of economic benefit in the whole grain bread market. Organic feeds fetch a premium. The craft brewing industry is growing and seeking new markets. Organic consumers are interested in the health benefits that barley’s fiber profile provides. USDA Organic Standards require barley products to be organically produced. Finally, organic producers are in the vanguard of developing new and alternative production systems. In the case of this project we see an opportunity to help develop more robust locally-based organic grain production systems that will be better able to deal with the effects of climate change.

Legislative-defined goals addressed by this project:

- (1) Facilitating the development and improvement of organic agriculture production, breeding, and processing methods.
- (2) Evaluating the potential economic benefits of organic agricultural production and methods to producers, processors and rural communities.
- (6) Conducting advanced on-farm research and development that emphasizes observation of, experimentation with, and innovation for working organic farms, including research relating to production, marketing, food safety, socioeconomic conditions, and farm business management.
- (8) Developing new and improved seed varieties that are particularly suited for organic agriculture.

(vi) Approach:

1. Description of the activities proposed, key personnel, and institutional roles in those and activities and the timeline

We will use four categories of naked barley germplasm in order to reach four categories of target audiences. There is overlap between audiences and connectivity between germplasm groups but for the sake of exposition these are defined separately in Table 1 and discussed in greater detail in the following sections.

Table 1. Germplasm and audiences for the naked multi-use barley for organic systems project.

Germplasm	Primary Audience(s)	Objectives	Connectivity
Naked barley composite.	Students and home gardeners.	Discover genetic diversity, learn plant breeding, prepare and consume own organically grown grain.	The composite consists of 909 pure lines, many of which are represented in the diversity panel.
Diversity panel	Plant breeders.	Understand the genetics of adaptation to organic systems in a broad array of germplasm extensively characterized at the phenotypic and genotypic levels; maximize the efficiency of selection; and facilitate	A subset of selected individual pure lines in the composite are included in the diversity panel; all regional nursery entries are included in the diversity panel; selections from the

		participatory plant breeding.	diversity panel will be advanced to the regional nursery and used as parents for future breeding efforts.
Regional nursery.	Farmers, end-users, outreach, and plant breeders.	Identify new varieties with optimum adaptation, performance, and end use quality in systems contexts; understand genotype × environment × production system interactions.	The regional nursery will include individual selections from the composite and the diversity panel.
Commercial production.	Farmers, end-users, consumers, outreach, plant breeders.	Assess commercial potential of existing varieties for food, feed, and malt.	The commercial varieties are included in the diversity panel and regional nursery.

Experiment 1. Naked Barley Composite

The naked barley composite, which is a mix of 909 different doubled haploids derived from crosses representing a sample of the available naked barley germplasm, will be grown in school gardens in each region in order to teach K-12 students about genetic diversity, plant breeding, how to produce and prepare organic naked barley, and natural vs. artificial selection. Detailed lesson plans that are aligned to the Next Generation Science Standards will be developed based on this germplasm. University collaborators will work with teachers in their respective states. The Naked Composite education/outreach effort will be coordinated by a lead teacher (Johannah Withrow-Robinson, Springfield, Oregon) in collaboration with project personnel. Lead teachers have been identified in participating regions (see letters of support). Student/teacher experiences will be documented and shared via the project website maintained by e-Organic. Oregon Tilth will provide expertise, as needed for schools transitioning to organic or seeking additional expertise for organic production. We have been in contact with the USDA-NIFA National Agriculture in the Classroom Project about this proposal and identified opportunities to work towards developing curricula and sharing resources nationwide.

The Naked Barley Composite will also provide a mechanism for reaching the broad audience of home gardeners, bakers, and brewers. Seed will be available in the High Mowing Organic Seed and Territorial Seed catalogs (see letters of support) along with information on accessing the full project resources via eOrganic.

Experiment 2. Regional Trial

The regional variety trial will generate data from key locations on agronomic and quality profiles of current naked varieties and potential varieties. Trials will be conducted on certified organic farms and certified organic research station land. The resulting data will provide growers and end-users (feed, food, malt, beer) with an objective basis for decision-making. The trial will provide a forum for field days and participatory selection. The trial will be coordinated by Dr. Hayes at OSU. Drs. Murphy and Jones at WSU, Dr. Sorrells at Cornell, Dr. Smith at UMN, and Drs. Gutierrez and Dawson at UW-Madison will be responsible for conducting trials in their respective regions. Seed and data from the trial will also be available to all other interested cooperators (see, for example, letters of support from University of Nebraska, University of Utah, and University of California-Davis).

Experiment 3. Diversity Panel

The diversity panel will provide cooperators with a diverse panel of potential varieties and breeding parents from which to select locally-adapted germplasm and ultimately new varieties. This panel represents a much larger pool of diversity than what is currently available in released varieties. This trial will also test the hypothesis that the most efficient selection method for organic conditions will be phenotype + predicted breeding value. It will provide cooperators with data that can be used, in conjunction with results of participatory breeding, to design crosses and thus create new germplasm. Trials will be conducted on certified organic farms and certified organic research station land. The trial will be coordinated by Dr. Hayes at OSU. Drs. Murphy and Jones at WSU, Dr. Sorrells at Cornell, Dr. Smith at UMN, and Drs. Gutierrez and Dawson at UW-Madison will be responsible for conducting trials in their respective regions. Dr. Gutierrez and graduate student at UW-Madison will coordinate GWAS and genomic predictions. As with the Regional Trial, seed and data of the diversity panel will be available to interested cooperators throughout the US.

Experiment 4. Commercial production

The commercial production dimension of the project is intended to (i) provide growers and end-users with production-scale data and experience with naked barley, (ii) provide end-users with sufficient grain to generate food, feed, malt, and beer, and (iii) provide consumers with sufficient product volume to engage in significant outreach and informal sensory. The knowledge gained in the commercial assessment component will feed back into participatory breeding and selection. Products made from on-farm grain will be featured at workshops and events, such as the Cascadia Grains Conference, the Grain Gathering, Organicology, Organic Seed Growers Conference, MOSES, NOFA-NY, Farm to Flavor dinners, regional grain workshops, OGRAIN field days, Cornercopia UM Student Organic Farm tours, Organic Field Day at UM SW Research and Outreach Center, MN Organic Farming Conference, MN Sustainable Farming Association (SFA) conference and field days, FEAST, Seed to Kitchen dinners. co-op, grocery store, and farmer's market samplings, NY Grains Management Field Day, NY Seed Growers' Field Day, Hudson Valley Farm Hub Field Day, GrowNYC's Union Square Greenmarket (where 400,000 people pass through on a September Saturday), and the Culinary Breeding Network Variety Showcase. At these events, there will be informal blind sensory assessments of baked goods, pastas, grain-based foods (e.g. couscous, polenta), and beverages (e.g. roasted barley tea and beers made from organically-grown grain and malt produced in certified organic facilities). These foods will consist of grain and malted grain. At appropriate events there will also be formal sensory assessment of a subset of selected products to complement the informal assessments and provide quantifiable indications of potential differences between naked barley types. Outreach personnel in each region (Lane Selman in Oregon, Connie Carlson in Minnesota, and June Russell in New York) will facilitate connections between growers and processors in each state and conduct these informal sensory analyses at events and provide educational materials on nutrition and other attributes of different barley varieties.

Timeline

	Year 1	Year 2	Year 3
Naked Barley Composite	X	X	X
Regional Trial	X	X	X
Diversity Panel	X	X	X
Commercial Production	X		X
Proposed new variety release(s)			X
Genotyping	X		
GWAS	X	X	X
Breeding and Selection	X	X	X
Webinars	X	X	X

Extension publications		X	X
Peer-reviewed publications			X
Field days	X	X	X
PD travel to OREI annual PD meeting	X	X	X
Presentations at grower/scientific conferences	X	X	X
Project meetings with RSAC	X	X	X
Weed evaluations	X	X	X
Poultry feed study		X	
Fusarium Head Blight Testing	X	X	

2. Methods to be used in carrying out the proposed project, including the feasibility of the methods and why they were selected

Experiment 1: Naked Barley Composite

In the fall of 2017, 200-gram seed samples (or more if garden space is available) will be provided free of charge to cooperating lead schools in the participating states. The composite seed is multiplied annually at Oregon State University (and samples from every year are archived for future reference). Each lead school will receive a stipend of \$1,000 to cover costs of developing garden space, managing the experiments, organizing field trips to the host breeding program. The seed from control (natural selection) plots at each lead school will be the seed source for cooperators in that state. Students will collect data on the control and selected plots throughout the barley growing season. In addition, lead teachers will post descriptions and images of ongoing activities at the project website. The lead teachers in each state will provide seed, lesson plans that are aligned to the Next Generation Science Standards, and production/processing advice to colleagues in their respective states. At each lead school, outreach, plant breeding, and graduate student personnel will be available for visits throughout the season and each school will be partnered with a chef for a culinary experience involving grain grown, harvested, and processed at the school.

OSU will also be a resource for seed from schools outside the five states. In the fall of 2017, OSU will provide 20 kilos seed stock to High Mowing Organic Seed, Territorial Seed, and other interested seed companies for them to propagate and offer as organic seed in their 2018 and/or 2019 catalogs. The catalog offering will include a brief description of the Naked Composite and its role in the national organic naked barley effort, as well as documentation of how to engage in selection and propagation of individual components within the mixture. Purchasers will be invited to contribute observations and experiences and these will be posted at the project website – an informal “crowd science” approach to organic grains breeding.

This experiment will use an evolutionary-participatory breeding (EPB) model, which emphasizes the contribution of human selection combined with natural selection at site-specific locations (Murphy et al., 2005). An EPB method involves increasing genetic diversity by growing a heterogeneous population that will be better able to deal with pests and disease (*as reviewed by* Murphy et al., 2005). In the case of naked barley, varieties breed true. A key difference between a conventional variety and an EPB-derived variety is that the latter is a mixture of pure lines. Heterogeneity is a positive attribute as it can provide buffering against changes in the environment and changes in both type and strain of crop pests. The only condition is that the crop variety be sufficiently uniform for management and processing purposes. Our project is an excellent candidate for the organic EPB model because it involves breeders and students working together to make selections, and it focuses on a heterogeneous blend of lines that will help bolster the crop against disease and pest pressures.

Experiment 2: Regional Trial

Two trials of twenty entries each (one fall-planted and one-spring planted with 5 common facultative lines), composed of existing varieties, advanced lines, and two covered checks (1 general and 1 local) from the OSU, WSU, and UMN breeding programs will be tested at 18 locations across five states, including Oregon (1 site fall and spring), Washington (3 sites fall and spring), Minnesota (1 site fall and spring), Wisconsin (2 sites fall and spring), New York (2 sites fall and spring). In each state, trials will be conducted on-farm or on-station. At each location, a three replicate Randomized Complete Block will be used. Standard yield trial protocols (e.g. plot size, seeding rate, seeding date) will be used at each location. Soil tests will be conducted at each location and organic fertilizer(s) applied as appropriate.

Each variety will be evaluated for agronomic traits including: heading date, plant height, lodging, brackling, grain yield, test weight, and plump/thin. These traits will be measured using standard units at all locations, following the procedures implemented by the USDA-NIFA Barley and Triticeae CAP projects. Winter trials will be evaluated for winter survival. At each location, cooperators will evaluate locally-occurring diseases using standardized rating scales. Typically, these disease/location combinations are (OSU: scald, stripe rust, leaf rust; WSU: scald, stripe rust, leaf rust, powdery mildew; UMN and UW-Madison Fusarium Head Blight, powdery mildew, stem rust, spot blotch, bacterial leaf streak, net blotch; Cornell: Fusarium Head Blight, powdery mildew, scald). Weed Competitive Ability (WCA) will be measured using the expertise and advice of Dr. Andrew Hulting (OSU; see letter of support) using early vigor scores, early season height, and stand counts. A). Grass and broadleaf weed density will be quantified in each barley variety at several timings including pre-plant, mid-growth cycle, and pre-harvest. As resources allow, participants are encouraged to record additional WCA data due to the critical nature of this phenotype to organic barley production. An example would be NDVI via Greenseeker. Data collection will be ongoing throughout the growing season and similar to that described by Ryan et al. (2009) and Kissing-Kucek (2017). Weed management in these trials will depend on the resources of participants – for example, those with tine-weeders are welcome to use them. At maturity, treatments will be harvested using a small plot combine and measurements of grain yield and test weight, and threshability.

Each variety will be evaluated for food quality traits including: grain protein, starch type, grain β -glucan content (see letter of support), kernel hardness, seed color, and resistance to embryo damage. The phenotypes will be measured following the protocols described in Meints et al. (2015b). Micromalts and malt analysis will be made and performed on all lines by Rahr Malting and Great Western Malting (see letter of support) following the standard procedures of the American Society of Brewing Chemists as described in Mohammadi et al. (2015). Sensory and flavor traits for food, malt, and beer will be measured on a selected subset of entries and environments. Food sensory trials will follow the protocols developed and implemented by Julie Dawson while participating in the Cornell Project. Malt sensory will be conducted following the procedures developed by Briess Malting and described at <http://blog.brewingwithbriess.com/malt-sensory-methods-you-can-perform-in-your-own-home-or-brewery/>. Beer sensory will be conducted as described by Herb et al. (submitted). These abundant data will be used for in-depth analysis of genotype performance, genotype \times environment interaction, and genotype \times production system interactions.

Evaluating the genetic merit of lines and discerning genetic from environment and noise components are key aspects of plant breeding (Allard 1960; Fehr 1984; Hallauer and Miranda Filho 1988; Duvick et al. 2004). Quantitative traits are affected by the environment, making phenotyping crucial in any plant breeding activity. This creates two challenges. First, field trials and experimental designs for large number of genotypes should be carefully chosen to increase heritability by controlling micro-environmental variation, spatial heterogeneity, and experimental error (Cullis et al., 1998). Second, Genotype by Environment Interaction ($G \times E$) is widespread in plants (Cooper and Delacy, 1994; Mathews et al., 2008) and is particularly important for the quantitative traits that are of principal importance for plant breeding (Mathews et al., 2008). Multi-environment trials (MET) are therefore routinely conducted in plant breeding to capture $G \times E$ (Smith et al., 2001). Several strategies have been used to deal with $G \times E$ in breeding programs, but the most effective is to exploit it (Bernardo, 2010) by either developing locally adapted genotypes (Mathews et al., 2008) or to use $G \times E$ to better characterize test genotypes (Cooper et al., 2014). We will improve breeding approaches by evaluating a series of experimental design strategies

to control micro-environmental variation (see Diversity Panel approaches). Additionally, we will characterize G×E across locations to identify locally-adapted materials to be released for each evaluated and potentially new environment. We will use the Regional Trials to characterize G×E in several ways. First, mixed models will be used for estimating variance components and modeling G×E (Piepho 2000; Verbyla et al., 2003; Malosetti et al., 2004; van Eeuwijk et al., 2005; Boer et al., 2007; Mathews et al., 2008). Second, multiplicative models like Additive Main Effects and Multiplicative Interaction (AMMI, Gauch, 1988) and Genotypic Main Effect and G×E (GGE or SREG, Yan et al.; 2000) will be used to graphically represent genotypes and environments in biplots (Gabriel, 1971; Yan and Kang, 2003). The biplots will then be used to identify superior genotypes for specific environments or groups of environments (Yan et al., 2001) and to define Mega-Environments (Braun et al., 1996). Third, factorial regression and partial least squares regression will be used to identify environmental covariates with a strong influence on the G×E (Vargas et al., 1999) and physiological relevant variables. Finally, genotypic stability and adaptation will be studied using Finlay-Wilkinson regression (Finlay and Wilkinson, 1963 and Eberhart and Russell, 1966) and AMMI models. The G×E characterization will give us a very good idea of the G×E and how to exploit it. This characterization will also be used for the Diversity Panel to model G×E (see Diversity Panel approaches).

Experiment 3: Diversity Panel

The diversity panel will consist of 382 naked entries and two covered checks (1 general and 1 local) from the OSU, WSU, UMN, James Hutton (Scotland), and CSIC (Spain) breeding programs as well as commercially available lines and landraces from the NSGC. The lines will include winter, facultative, and spring growth habits, different seed coat colors, waxy and non-waxy starch, and lines with mostly unknown end-use properties. There are over 2,000 doubled haploids and advanced generation lines from which this genetically diverse subset will be selected. The 909 doubled haploids in the Naked Barley Composite are possible donors. All entries, including those in the Composite and other breeding programs trace to exotic (e.g. land race and un-adapted) accessions crossed with more adapted material. This ensures abundant genetic diversity to select for target traits in organic environments, but sufficient disease resistance, straw strength, and resistance to shattering so as to offer the possibility of direct release as a variety or at least as a parent in crosses designed to produce varieties. Selection will be based on pedigree information, observed diversity during seed multiplication, and genotypic data when available. A key consideration is to maximize utility of interconnected small populations based on the work of Riedelsheimer et al. (2013).

The diversity panel will be tested in a total of at least 30 environments (fall- and spring-planting in each of three years in each of five states). Per the letters of support, un-funded collaborators may also elect to grow the panel, providing an even more robust data set upon which to base selections and assess G×E. Each cooperator will maintain their own seed from each planting date, and natural selection is expected in each test environment. This will generate some imbalance but will be advantageous. For example, genotypes requiring vernalization will be eliminated by natural selection from the spring-planted trials and genotypes lacking winter hardiness will be eliminated from the fall-planted trials in some environments. In the future, selections from the panel will be available for genomic selection (GS) because, as pointed out by Lorenz and Smith (2015), GS is most effective in smaller, relevant training populations than in large un-adapted arrays. Our long-term goal (beyond the time frame of this proposal) is to generate a high quality data set from the diversity panel and then develop general or breeding-program training population subsets that are optimized for prediction accuracy.

Trials will be conducted on-station under certified-organic conditions. Each entry will be grown in double head row plots using a Type II augmented design with one replicate of each entry (and repeated checks) at each site. Soil tests will be conducted at each location and organic fertilizer(s) applied as necessary to approximate crop nutrient needs. In spring-sown trials, winter wheat will be planted between double rows to reduce weed competition. In all trials, weed competition assessment will not be possible due to the wide row spacing needed for two-row plot assessments. Manual weed control will be used as necessary. Each entry in the panel at each location will be evaluated for traits described for the regional trial at each location.

Due to the number of samples involved, the minimum quality traits measured at each location will be grain protein, grain β -glucan content (see letter of support from the Idaho USDA-ARS program), kernel hardness, seed color, threshability and resistance to embryo damage. Cooperators may elect to measure additional phenotypes with their own resources.

All entries in the Diversity Panel will be genotyped using the Illumina 50K SNP Chip. DNA extractions and genotyping will be performed at the USDA-ARS Fargo genotyping lab (see letter of support from Dr. Shiaoman Chao). An advantage of using the Illumina 50K SNP chip is the high marker density in the chip from which markers that are determinants of, or associated with, target traits can be identified in the Diversity Panel and related to prior (Illumina 9K) and ongoing (Illumina 50K) research. For future research, relevant marker loci can be targeted for more flexible genotyping assays.

The plant breeding toolkit now includes marker assisted selection (MAS; Tanksley, 1983), and genomic selection (GS; Meuwissen et al. 2001). MAS involves the identification of markers linked to genes or quantitative trait loci (QTL) of relevant traits, and then selecting individuals based on their marker scores (Tanksley, 1993; Hospital and Charcosset, 1997). Genome-wide Association Mapping (GWAS; Jannink et al., 2001) is one of the strategies that has successfully been used in plant breeding populations to identify relevant QTL (Kraakman et al., 2004; 2006; Hayes and Szücs, 2006; Stracke et al., 2009; Waugh et al., 2009; Roy et al., 2010; Bradbury et al., 2011; von Zitzewitz et al., 2011; Gutierrez et al., 2011; Locatelli et al., 2013). GS is another breeding methodology that can increase the rate of genetic gain by reducing the breeding cycle duration or by increasing selection accuracy (Meuwissen et al., 2001; Bernardo and Yu, 2007) and has successfully been applied in plant breeding (Bernardo, 2008; Heffner et al., 2009; Heffner et al., 2010; Rutkoski et al., 2010; Lorenz, 2013; Hayes et al., 2013). The principle of GS consists of using a training population with individuals that have both phenotypic and genotypic observations to train a model; this model is then used to predict breeding values of new individuals based on their genotypes (Heffner et al., 2010). Alternative, if partial phenotypic information is available, the genotypic value of an individual can be predicted based on both the marker and the available phenotypic information (Endelman et al., 2014). The accuracy of this prediction depends on the training population size (Lorenz et al., 2012; Lorenzana and Bernardo, 2009; Asoro et al., 2011), heritability of the trait (Combs and Bernardo, 2013), number of markers (Lorenzana and Bernardo, 2009; Asoro et al., 2011; Heffner et al., 2011), genetic relationship among individuals (Asoro et al., 2011; Crossa et al., 2013; Isidro et al. 2015), and the model used to predict individuals (Solberg et al., 2009; de los Campos et al., 2009; de los Campos et al., 2013; Heslot et al., 2012; Ogutu et al., 2012; Kärkkäinen and Sillanpää 2012). Thus, training populations can be optimized to improve prediction accuracy based on the individuals to be predicted (Isidro et al., 2015; Spindel et al., 2015).

Genomic studies have modeled $G \times E$ either to map quantitative trait loci using mixed models (Mathews et al., 2008; Piepho, 2000; Malosetti et al., 2004; van Eeuwijk et al., 2005; Locatelli et al., 2013; Gutierrez et al., 2015), or to predict breeding values in GS studies (Crossa et al., 2013; Resende et al., 2011; Burgueño et al., 2012; Dawson et al., 2013; Ly et al., 2013; Heslot et al., 2014; Jarquin et al., 2014; Lopez-Cruz et al., 2015; Lado et al., 2016). Therefore, by carefully modeling $G \times E$ we can improve the understanding or prediction of relevant quantitative traits. The main approaches used to model $G \times E$ in GS studies have been either to borrow information from other environments using a covariance matrix (Burgueño et al., 2012), or to use factorial regression with environmental covariates (Heslot et al., 2013). However, evaluating prediction accuracy in the presence of $G \times E$ can be challenging because a choice between using either the same population to train and to evaluate the model (i.e., random cross-validation), or using a population to train the model and a different one to evaluate it (i.e., forward-validation) should be made. Random cross-validation is likely to overestimate the accuracy because individuals are from the same population, while $G \times E$ between training and validation population evaluation may largely drive accuracy in the forward validation approach (Rutkoski et al., 2015b). Therefore, optimization of the training population data set for genotypes and environments can be used to improve prediction accuracy for specific environments (Rutkoski et al., 2015b; Lado et al., 2016).

The Diversity Panel will generate a rich data set with which to investigate key questions about effectively handling genetic diversity for organic systems. The full phenotypic and genotypic characterization of the Diversity Panel over a three-year period will allow for calculation of GEBVs and implementation of crossing decisions in year 3. These data will also allow for (i) calculating the total

genetic merit of the entries using best linear unbiased prediction (BLUP) with a marker-derived relationship matrix (Habier et al. 2007; Endelman et al. 2014) (ii) GWAS to identify genome coordinates of QTLs and candidate genes for all traits within the time-frame of this project and (iii) theoretical assessment of genetic gain and analysis of changes in allele frequencies and linkage disequilibrium resulting from selection beyond the time-frame of this project. The focus of genotyping in this project is on complementing phenotypic assessments rather than replacing them. Results from this portion of our study will fill important research gaps in understanding the application of GS in applied organic plant breeding. While this panel is not ideal for genomic selection, these data can be used to select parents and design training populations for future use in breeding (Rutkoski et al. 2015b). Differential winter survival from fall plantings and vernalization requirement for some entries in spring plantings will result in an unbalanced dataset, however there are statistical approaches for using unbalanced data (Dawson et al. 2013). This will provide important insight into how this nationally coordinated public breeding effort can share and utilize data sets to optimize breeding gains.

The Diversity Panel will allow us to address genotype x environment interaction for productivity, resistance, and grain quality traits. The multi-location/multi-trait assessments (Oregon, Minnesota, New York, Washington, and Wisconsin and with alternative planting dates in each environment) will allow for comparison of alternative selection indices for each location and subsets of locations. This will allow individual breeding programs to tailor their selection in year 3, for their own region in subsequent cycles of selection in the sustained breeding that follows this project. The application of marker assisted selection and selection of parents for crossing will be the focus of sustained and thorough participant discussion and consensus. This will provide important insight into how this nationally coordinated public breeding effort can share and utilize data sets to optimize breeding gains. Finally, selections in the Diversity Panel may be advanced to the regional trial within the timeframe of this project. It is very likely that variety candidates will be identified in this array.

We will explore a wide variety of strategies to combine different training environment data sets modeling G×E to determine their effect on prediction accuracy. We will apply the covariance-among-location method, the use of mega-environments and a simple factorial regression method that incorporates weather-based stress covariates (Heslot et al., 2014), and new strategies to incorporate environmental covariates based on the crop physiology to model G×E. First, treating a trait measured in different locations as multi-trait in a multivariate analysis (Burgueño et al., 2012) allows one to borrow information from other locations by using a covariance matrix among locations. This shows how much information can be shared to maximize prediction accuracy for each location (Piepho and Möhring, 2005) but does not enable prediction for new locations. Second, we will include Mega-Environment information to predict performance for new environments following Lado et al. (2016). Third, we will integrate weather variables that allow predictions for unobserved locations, assuming their expected weather is known (Heslot et al., 2013). Finally, we will explore novel strategies to incorporate environmental covariates based on crop physiology to model G×E for new environments. These methods will provide location-specific predictions enabling us to target putatively superior germplasm to cooperators in new target locations. Finally, we will optimize the Diversity Panel to predict each environment. Lado et al. (2016) found that using all available environments to predict the performance of lines for specific environments was not always the best strategy. Selecting a set of environments to train the model yielded higher prediction accuracies in most situations. We will optimize the selection of sets of environments to train the model for predicting specific environments following Lado et al. (2016).

We will explore a wide variety of strategies to optimize resource allocation in terms of number of genotypes, environments, and replications. We will use resampling strategies and empirical results to evaluate prediction accuracy for a combination of genotypes, replications, and environments. Experimental designs can be optimized for specific objectives (Casler, 2014). However, resource optimization in the presence of G×E is not trivial. Moehring et al. (2014) showed that having large unreplicated experiments in several locations is more efficient than replicating within environments. Furthermore, Endelman et al. (2014) found that it is more efficient to use large training population sizes even when having larger populations was obtained by unbalanced designs. We can think of resource allocation across locations as a gradient between having a small number of genotypes evaluated in replicated experiments in all environments or having non-overlapping unreplicated experiments evaluated

in each environment. We will optimize experimental designs to explicitly account for the existing G×E. We will evaluate a series of designs in the extreme gradient described above. We believe that an optimal design would consist of a series of augmented designs with partial overlap across environments combined with a thorough G×E characterization. The first year of evaluation of the Regional Trials where a complete data set is created with all genotypes evaluated in all locations will be used to characterize genotype by location environment. This information will be used to construct mega-environments. Explicitly modeling for G×E and creating a resolvable unbalanced design within mega-environments will be explored where re-sampling will be used to simulate five experimental design scenarios. The best scenario will be empirically evaluated for the Wisconsin locations in the remaining years to have a proof of concept.

Experiment 4: Commercial production

The on-farm commercial scale trials will be conducted in each of the five states beginning in fall 2017 (for fall-planted varieties) and spring 2018 for spring-planted varieties. There will be commercial production (minimum 1 acre expected to produce at least 2,000 kg of grain) of at least one variety in each of the five states in at least two years. Streaker and Buck are the two winter types and will be grown in OR, WA, and NY (at a minimum). MN and WI collaborators may elect to test these varieties: however, winter hardiness may be an issue for them. The spring varieties will be provided by the Washington State University program (e.g. Havener) and the University of Minnesota program (variety release under consideration). Growers will be provided with certified seed at no charge. The certified seed will be purchased and shipped to growers using OSU core funds from this project. Each grower will receive a stipend of \$500 per year to defray costs. Participating processors/users (e.g. millers, bakers, maltsters, brewers and feeders) will buy the grain at current market prices. Grain will be milled by cooperating regional millers (see letters of support).

Dr. Andrew Ross (OSU) is an experienced artisan baker, noodle maker (Crosbie and Ross, 2004; Crosbie et al., 1999; Ohm et al., 2006; Ross, 2006; Ross and Crosbie, 2010; Ross and Hatcher, 2005; Ross et al., 1997; Ross, 2013) and assessor of cereal quality (Ross and Bettge, 2009). Ross has demonstrated to bakers the use of barley in risen breads and soft pretzels at the “Kneading Conference West” (Ross, 2011). Risen breads made with composite barley/wheat flours will be produced and assessed guided by methods of Rieder et al. (2012), Faergestad et al. (2000), or Londono et al. (2014). Steamed or boiled grains will be processed and assessed using methods developed for rice cooking quality. Barley grain or grits will be cooked using a programmable rice cooker based on the method described by Meullenet et al. (2000). Cooked grains will be assessed for texture using a back-extrusion technique based on the methods described by Leelayuthsoontorn and Thipayarat (2006) and Meullenet et al. (2000). Color of cooked grains will be measured using a tristimulus color meter. If required, cooked grain elongation will be assessed based on methods described by Wang et al. (2007) and Ge et al. (2005). If required, degree of cook measurements will be based on adaptations of the methods of Ferrel and Pence (1964) and Whalen (2007). Tortillas made with composite barley/wheat flours will be produced based on methods described by Guo et al. (2003), Toma et al. (2008), Prasopsunwattana et al. (2009), and Alviola and Awika (2010). Tortillas will be assessed for objective color and textural (mechanical) characteristics based on the methods described for color by Prasopsunwattana et al. (2009), and texture by Alviola and Awika (2010), and Prasopsunwattana et al. (2009).

Since 2005, Lane Selman has been an agricultural researcher at Oregon State University managing multiple participatory research projects involving university researchers and diversified organic vegetable farmers, including the OREI-funded Northern Organic Vegetable Improvement Collaborative (NOVIC) which involves 25 farmers in Oregon. She has experience conducting variety trials on organic farms; organizing outreach events and participatory planning meetings; and developing engagement across various stakeholders. In 2012, she created the [Culinary Breeding Network](#) (CBN) to increase communication and collaboration between plant breeders, seed growers, farmers, produce buyers and chefs to improve quality in vegetables and grains with a focus on organic breeding work. Variety Showcase is an increasingly popular annual CBN event which brings together over 300 attendees to taste existing, unreleased and new vegetable varieties and breeding lines focused on superior culinary quality. The goal is to build community between breeders, farmers and end users, and create a venue for the

exchange of important stakeholder input into breeding projects. Each year, the OREI barley project team will have the opportunity to share their work at this event and gain access to informal flavor and performance evaluation and feedback. Barley grain will be cooked using a programmable rice cooker based on the method described by Meullenet et al. (2000) in addition to utilized in breads and baked goods by collaborating bakers. Evaluators will be asked to provide ratings using a 1-9 scale for appearance, color, flavor and texture.

Pilot malts will be made at the Barley World malthouse at OSU (as budgeted for in phenotyping) and with cooperators at Rahr Malting and Great Western Malting. Malt batch sizes will range from 200 lbs – 1 ton, depending on the participating maltster and will be sufficient to produce pilot brews with cooperating brewers including Breakside Brewing and the Hartwick College Center for Craft Food and Beverage (see letters of support). Larger batch malts will be arranged between growers and commercial maltsters (e.g. Rahr and Great Western Malting) to produce sufficient malt for commercial brews at participating breweries, including Crux Fermentation Project (Oregon) and New Glarus (Wisconsin). Sensory assessments of beers will be organized by Dr. Tom Shellhammer (OSU) to ensure connectivity of the resulting data. Under the auspices of this project, resources permit the following collaboration with Dr. Tom Shellhammer (OSU). The performance of two naked barleys and a traditional covered barley will be compared using lauter tun and mash filtration technologies in the OSU pilot research brewery. The varieties “Buck” and a University of Minnesota experimental variety will be compared against each other and to a standard traditional barley, such as Copeland. The barleys will be malted in the OSU minimalter. Pilot-scale (1.5 – 2.0 hL) brewing trials will be carried out with measurements taken throughout the process and on the finished beer. In one set of experiments, the naked barley malt will be blended with traditional barley malt and/or rice husks to assess performance in a brewhouse fitted with a lauter tun. In a second set of trials we will mash the three malts individually and use a Meura mash filter for wort separation. All wort will be fermented with a standard ale yeast (Wyeast 1056), and following attenuation and diacetyl reduction the beer will be filtered bright, carbonated to 2.6 vol/vol and kegged. The finished beer will be assessed using an Anton Paar Alcoyzer (alcohol, residual extract, fermentability, color, haze, pH), HPLC (iso-alpha acids) and GC (diacetyl). We will use a trained panel to compare sensory attributes of the finished beers using a modified descriptive analysis approach. The finished beers will also be presented to professional brewers for quality and preference ratings at one of the semi-annual MBAA District NW meetings.

Under the auspices of this project, one funded feeding study is feasible. The trial will assess egg and broiler production using Buck barley and will be managed by Dr. Jim Hermes (OSU). Briefly, approximately 270 kg and 550 kg of organically-grown Buck barley will be used for layer and broiler trials, respectively. For the egg trial, there will be three diets: a control, 30% barley with organic wheat and soy, and 60% barley with soy. A fourth ration will be used in the broiler trial: 30% barley with organic corn and soy. The layer trial will involve a total of 90 birds with 30 birds per each of three replications. The egg trial will involve a total of 300 birds with 75 birds per each of three replications. The parameters measured for the egg trial will be mortality, bird body weight (at 6 time points), egg number (daily) and egg quality (0,4, and 8 week assessment of weight, albumen height, yolk color, and shell thickness). In the broiler trial, mortality and body weight (at three and six weeks) will be measured. Dr. Hermes has extensive experience with these types of trials, has all necessary permissions, and has applied for all necessary permissions. On-farm feeding studies will be conducted, as feasible, and organized by Organic Valley (see letter of support).

GrowNYC Greenmarket will conduct outreach to stakeholders and investigate and assess multiple markets for naked barley. GrowNYC will begin to lay the groundwork for the barley market in the Northeast by engaging stakeholders and educating end users on the attributes of varieties that will be developed. Surveys on supply, demand, and sources for high quality organic feed will be conducted in year one. Two culinary events will be held in New York City, targeting taste leaders from the city’s vibrant culinary community that is committed to developing organic agriculture and foods. Funds from the project will also go towards supporting educational staff at GrowNYC’s Grainstand, at the Union

Square Greenmarket, where hundreds of thousands of consumers shop on a weekly basis. Staff will be trained on the nutritional and culinary attributes of barley and will conduct direct-to-consumer sales of varieties that come to commercial scale, thus also providing test marketing for farmers who lack the capacity to direct market.

In conjunction with these commercialization studies, Dr. Brian Baker will conduct an assessment of the feasibility of the commercial adoption of the varieties through a combination of an on-line survey followed by telephone interviews of farmers. The survey will cover the perceived benefits, target markets, barriers to adoption, and the diffusion through seed distribution channels.

3. Expected Results and Outcomes

Naked multi-use barley will offer new production opportunities and rotation options to organic growers. Elimination of the pearling step will enable greater flexibility and lower both processing and transportation costs. Naked barley will be able to compete in a whole grain market the way that adhering-hull and pearled barley cannot. It will provide a new and more accessible product for all users. The net gains will be greater crop diversity, farm income, added value, and consumer satisfaction. The following are concrete deliverables from the project:

1. Webinars on school experiences with naked barley composite and on naked barley variety performance in representative organic systems, product development and assessment.
2. Website featuring lesson plans, images, narratives, recipes, protocols, marketing information.
3. Home gardener experiences and perspectives on plant breeding and natural selection.
4. Popular press and electronic media features of the project.
5. Develop educational and promotional materials.
6. New food products developed, beer & other value-added products such as barley pasta, barley bread
7. Peer reviewed publication(s) on incorporating organic barley genetics resources into experiential learning opportunities, variety trials, G×E, G×S, GWAS for performance in organic production environments, phenotype + genomic predictions for organic breeding, product formulation, sensory assessment and economic impacts.
8. Potential variety release of advanced lines, adoption of currently available varieties in new locations.
9. Selection of lines adapted for local environments. Selection of parents for future breeding efforts.
10. Estimates of the economic and environmental benefits of hull-less barley, including lower handling and energy costs as well as reduced energy use.
11. Assessment of the feasibility of adoption of the varieties developed, including the perceived benefits, target markets, barriers to adoption and potential for technology diffusion through innovative business models.
12. Documentation of the number of farmers who add naked barley to their rotations, consumers and professionals who engage in workshops, stakeholders who gain knowledge about the attributes and benefits of barley, and livestock producers who integrate barley into their feed rotations.

4. Means by which these results and outcomes will be analyzed, assessed, or interpreted

Collaborators on this project are actively engaged in using contemporary analysis tools and developing new tools. For example, the following citations (and references therein) reflect recent work by project members in the areas of GWAS (Graebner et al. 2015; Mohammadi et al., 2014; Juliana et al. 2015; Kulwal et al. 2012), GS (Sallam and Smith, 2016; Rutkoski et al. 2015a; Isidro et al. 2015), and G×E (Meints et al., 2015b; Heslot et al. 2014; Heslot et al. 2013). The following citations describe analysis and interpretation tools for Brewing Science (Sharp et al., 2016), and Poultry Science (Hermes, 2016). The costs of hull removal for small grains is analyzed on eXtension/eOrganic (Baker, 2015). A case study was performed for niche markets in specialty small grains (Baker and Russell, *in preparation*).

5. How results or products will be used

The resulting data, experiences, knowledge and varieties will be used to realize project goals. Beginning with K-12 students and home gardeners, we will stimulate interest in organic grains, genetic diversity and plant breeding. Growers will be provided with participatory breeding opportunities and seed of new varieties that will maximize the productivity, profitability, and sustainability of their operations. Bakers, chefs, maltsters, brewers, and animal feeders will be provided with novel raw material from which to craft superior products with unique flavors, aromas, and textures, and nutritional properties. These products will broaden their impacts and profitability. Consumers will enjoy a broader spectrum of nutritious and flavorful organic barley-based products.

6. Outreach plan

Outcomes and identified priorities from the research will be shared at regional and national conferences - such as the Cascadia Grains Conference, the Organicology Conference, the Organic Seed Growers Conference, MOSES Conference (Midwest), MN Organic Conference, Sustainable Farming Association (MN) and the Cascadia Barley Day - at field days, and with targeted advisory groups. Presentations will be tailored to craft brewing, feed, and food conferences where organic grains are a priority. In years 2-3, direct-to-consumer outreach will be conducted weekly at New York City's largest farmers market. Audiences reached include farmers, seed growers, food processors, maltsters, brewers, bakers, millers, and consumers. For broader information dissemination to the brewing, feed, and food industries we will engage with leading journalists. Project and organizational websites, newsletters, and listservs, as well as social media, will also serve as platforms for outreach to reach thousands of individuals from regional to international audiences.

The RSAC will join researchers at an annual meeting held in one of the regions to discuss progress from the previous year and goals for the next year.

Webinars: Over the course of the project, eOrganic will host four webinars on naked barley. Topics will include production and management, culinary uses of naked and malted barley, and results and outcomes from the trials.

Social Media: Many of the collaborators have Facebook or Instagram accounts for their labs and organizations. These accounts can be used to distribute information about this project. Additionally, an Instagram account will be created specifically for this research in order to visually highlight the many uses of naked barley.

Workshops: Outreach personnel from each region will organize and host workshops for bakers and chefs on the culinary uses and properties of naked barley. Oregon Tilth will help organize and host workshops for growers on production, best management practices, and weed and pest control.

Field Days: All participating institutions will host at least one field day each year of the project. Field days will take place on-station and on-farm during the summer. Presentations will include tours of the regional trial and diversity panel to discuss production, potential varieties, disease resistance, weed management, and rotation options. There will also be presentations on quality, as well as informal sensory analysis on a range of naked barley products from collaborating bakers, chefs, and brewers.

Reports/Publications: Results from the regional trials and diversity panel will be made available to the public through webinars and websites and through newsletter and extension publications. Printed handouts will be created for distribution at field days. We anticipate a total of 6 to 8 research papers will be submitted to peer-reviewed journals by the end of the project that detail our results. When possible, we will publish in journals that are available to the public via open access. We also anticipate a number of popular press articles being written about this project including a feature in 'In Good Tilth'.

Scientific presentations will be made to an international audience at the 13th International Barley Genetics Symposium held in 2020 in Riga, Latvia and at North American Barley Researchers Workshop held in 2020.

Impact measurement: A mail survey will be sent to barley producers in the first and final year of the grant in order to measure the impact of our research on naked barley.

7. Pitfalls that may be encountered

As in all agricultural research, trials can be lost due to a number of biotic and abiotic pressures. In order to understand these potential losses, we will include checks and lines with known disease resistances, winter-hardiness, and weed competitiveness to identify advanced material.

8. Limitations to proposed procedures

Although covered barley is a frequently grown and well-understood crop in the United States, there has been little research conducted on the production traits specific to naked barley. Additionally, there is no market class for naked barley, so there are no specifications for traits. This means that target quality traits and best management practices are unknown for many of the regions where trials will be conducted due to differences in climate and disease pressures.

9. Hazardous materials, potentially invasive species etc.

None that we are aware of.