

EVALUATION OF BARLEY AND MALT QUALITY IN THE EASTERN SPRING
BARLEY NURSERY

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ABSTRACT

In the northeastern United States, craft beer is on the rise. With local brewing increasing, the supply of local raw materials becoming an urgent problem in some northeastern states, like Michigan, New York, Ohio, Pennsylvania, and Vermont. The overall goal of the project is to determine which cultivars are best adapted to specific regions in the northeastern United States, and to detect the impact of different environment factors on the barley genotypes. In general, cultivars from Europe had better resistance to pre-harvest sprouting (PHS) and lower beta-glucan levels than two-rowed cultivars developed in North America. The varieties, Explorer, LCS Genie, LCS Odyssey, KWS Fantex, and KWS Beckie are candidates for production in the eastern United States because of their higher levels of resistance to PHS and malt extract, and their lowers levels of beta-glucan.

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1. INTRODUCTION

Although barley (*Hordeum vulgare*) was domesticated more than once, the barley that originated in the Fertile Crescent has contributed the majority of diversity in European and American barley genotypes, as well as those from Central Asia to the Far East (Morrell et al. 2007). Barley has typically ranked fourth in terms of grain quantity produced globally behind maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.). In 2017, approximately, 70% of the world barley crop was used for animal feed, and 21% of the barley crop was consumed for malting, brewing, and distilling (Tricase et al. 2018). As the second use of barley, malting is the process that converts raw barley or other cereal grains into malt.

High quality barley is a prerequisite for the creation of great malt, and barley grain quality ultimately contributes to beer quality. However, the needs of specific brewers differ in terms of desired malt quality, and they are often divided into brewers using cereal adjuncts, and brewers using only malt. According to reports from Brewers Association (2014), when compared to larger commercial brewers, craft brewers tend to seek barley malts with premium flavors and aromas, lower total protein, lower free amino nitrogen (FAN), lower diastatic power (DP), and lower Kolbach index. With the rapid growth of craft beer production in the United States, there has been increasing interest in craft-specific malting barley cultivars, and also locally produced grains and malt. The most notable of regions with increasing local barley demand are in New England (northeast) and the eastern Great Lakes of the USA. The New England states comprise Maine, Vermont, Massachusetts, New Hampshire, Rhode Island, and Connecticut, while the eastern Great Lakes States are Michigan, New York, Pennsylvania, Ohio, and Indiana. Several of these states share a large amount of craft beer production. Statistics from the Brewers Association (2017b), for numbers of craft breweries, ranks Michigan, New York and

Pennsylvania 4th, 5th and 6th, respectively, in the United States. Further, brewers in Pennsylvania produced the most barrels of craft beer in 2017. However, many of these states have had little history of barley breeding or barley production for at least the past 50 years. Seventy-five percent of the barley grown in the United States is produced in Idaho, Montana, North Dakota, Colorado, Wyoming (USDA, 2018). With the rapid development of local malting and brewing, it is difficult for the local barley grain supply to meet the sharply increasing demand for local malting barley. This phenomenon is particularly prominent in some northeastern and Great Lakes states of the USA.

The Eastern States Barley Nursery (ESBN) is a project coordinated by North Dakota State University (NDSU) and partially funded by the Brewers Association. It intended to find barley cultivars that are best adapted to production in these eastern states. Cooperating institutions have included Cornell University, Michigan State University, the Ohio State University, Penn State University, Purdue University, Rutgers University, the University of Maine, and the University of Vermont. Twenty entries of barley were first planted for evaluation in 2015. Twenty-five entries were planted in subsequent years. Some entries were discarded if performance was poor, and then others were added. Individual researchers collect agronomic and disease data, while the NDSU barley program is responsible for evaluation of barley and malt quality of harvested grain. Select entries from three to four locations are malted each year.

2. LITERATURE REVIEW

2.1. Craft Beer

Craft brewing is the most rapidly growing section of the brewing industry in the United States, averaging 21% annualized growth from 2013-17. The Brewers Association (2018b) defines the term craft brewer, and their definition states that a craft brewer should be small and independent. “Small” is defined as a brewery that produces under six million barrels of beer in a year. “Independent” means that less than 25 percent of the brewery is owned by a large non-craft brewery or alcoholic beverage company. “Brewer” requires a craft brewer retain the TTB (Alcohol and Tobacco Tax and Trade Bureau) brewers notice and actively makes beer.

The craft beer market is divided distinctly into four segments: microbreweries, contract brewing companies, brewpubs and local craft breweries. Bart Watson (Brewers Association, 2017b), the chief economist for Brewers Association, investigated these four segments and reported that in 2017, microbreweries and brewpubs were the fastest growing segments, and that about 60% of the craft section’s total growth came from microbreweries. Statistical results from the Brewers Association also showed the craft brewing industry contributed \$76.2 billion to the U.S. economy and more than 500,000 jobs in 2017. During this time the sales of craft beer represented 23% of the U.S beer market. As of the Brewers Association 2018 report, there were 6,266 craft breweries operating in the USA (Brewers Association, 2018c).

Looking geographically at state craft beer sales and production in USA, one can see that the craft brewing industry has had a relatively faster development in some eastern and Great Lakes States (MI, PA, ME, VT, NY) (Table 1). For example, in Vermont, the number of craft breweries increased to 55 in 2017 from only 22 in 2011. In 2017, the barrels of craft beer produced by craft breweries located in Pennsylvania and Michigan ranked 1st and 11th,

respectively on a national scale. Maine ranked third in terms of the number of breweries per capita (per 100,000 21+ adults). There were 329 craft breweries in New York state, and it ranked 5th on a national scale.

Table 1. Barley Acreage, Number of Craft Breweries, and Number of Craft Malthouses in Selected States.

State	Barley Acreage (acres planted) ¹			Number of Craft Breweries (2017) ²	Number of Craft Malthouses (2018) ³
	Highest Production (Year)	2010	2018		
Idaho	1,370,000 (1984)	490,000	550,000	54	2
Montana	2,400,000 (1986)	760,000	790,000	75	3
North Dakota	4,147,000 (1959)	720,000	470,000	12	1
Indiana	130,000 (1942)	--	--	137	2
Maine	28,000 (2001-2003)	16,000	17,000	99	3
Michigan	316,000 (1932)	11,000	20,000	330	14
Massachusetts	--	--	--	129	3
New York	210,000 (1927)	12,000	10,000	329	15
Ohio	318,000 (1928)	--	--	225	9
Pennsylvania	253,000 (1955)	60,000	45,000	282	6
Vermont	6,000 (1936-1940)	--	--	55	2

¹ USDA National Agricultural Statistics Service, 2018.

² Brewers Association, 2018.

³ Personnel communication, Dave Thomas, Craft Maltsters Guild.

In general, most styles of craft beer tend to be more heavily hopped, and most craft brands are prepared from 100% malt. When compared to light lager beers produced by the larger multinational breweries, craft brewers, on average use far more hops and malt in their beers. A Brewers Association survey indicated that craft brewers on average, utilize more than 3.4 times the hops and 2.7 times the malt per barrel than the overall industry (Swersey and Watson, 2015).

According to the statistics at the beginning of 2018, craft brewers consumed almost 40% of the total malt consumed by U.S. brewers, and that scale is continuing to increase (Lake et al. 2008).

With the increase of the craft brewing market, craft brewers need to produce more beer to meet market needs. Demand for hops and the malt will be driven by the increasing craft brewer market. Formulation, and lower efficiency in the brewhouse are primary reasons why craft brewers use significantly more malt/barrel of beer than the large multinational breweries.

2.2. Local Grains and Craft Malt

The growth of craft beer can be explained by many factors, but small size and association with the community are often important drivers. In fact, the Brewer's Association (2018b) proposes that "the majority of Americans live within 10 miles of a craft brewer". This is perhaps reflective of the larger local foods movement, which the Dutch multinational banking and financial services company, Rabobank, called a "permanent and mainstream trend within the food industry" (Zacka, 2014). A 2012 study of consumers in the USA indicated that 52% said local was more important than organic foods, and that they tend to pay more for local. Retailers are paying attention, and as an example, Wal-Mart announced plans in 2013 to more than double its offerings of local produce.

The local food movement can directly connect food producers and consumers in the local region, and is meaningful to promote local economies, health, environmental, community or social impacts (Seyfang et al. 2006). Local grain represents another possibility when compared to the global food model, in which food is often transported long distances before it is finally purchased by the consumer. It means that more dollars are staying local, and family farms are now more viable because they have a new market to sell to and have more diverse crops to grow. As such it should be no surprise that some brewers are also seeking local ingredients.

As will be discussed in a subsequent section, some states are even providing economic incentives to brewers who utilize local ingredients. However, obtaining local can be difficult as the vast majority of barley and hops have traditionally been produced in very centralized regions of the country. A report from the Brewers Association (2018a), states that over 75% of the nation's barley is produced in the states of Idaho, Montana, North Dakota, Colorado and Wyoming. According to United States Department of Agriculture statistical data in 2018, Idaho, Montana and North Dakota still planted the most of the nation's barley (Table 1). However, the overall area planted to barley has been decreasing in the United States for the past 100 years (American Malting Barley Association, 2014).

Almost all of the nation's hops are produced in Idaho, Washington and Oregon (Allen, 2018). Yet, small producers of hops have recently appeared in many states including Michigan, Minnesota, New York and even North Dakota. However, Michigan has shown the greatest growth and now produces hops on 810 acres. Washington, by a comparison has 38,438 acres. Likewise, there has been interest in malting barley production in many non-traditional states, with Alabama, Florida, and North Carolina as examples. In addition, states that have not produced significant amounts of malting barley in recent years are also seeing renewed interest. As example, New York was the second largest producer of barley in the USA in the late 19th century (Schwarz, 2012), but has not been a significant producer since the early 1900's. Similar situations can be seen with states such as California, Illinois, Iowa, Michigan, Ohio, Oklahoma, Nebraska, and Wisconsin. The decline in barley acreage in these states has often been associated with a decline in barley or small grains infrastructure and expertise.

A sharply increasing demand for local malting barley in these new (or renewed) regions creates not only real opportunities for local farmers, but also challenges. For example, when

compared to feed barley, malting barley grain has very strict standards for quality, and the price paid can differ significantly. A higher level of crop management by the farmer is needed to produce quality malt barley. In addition, barley quality is also sensitive to cultivar and environmental conditions, so local farmers need to find best barley cultivars that fit their land and environment. Breeders and seed companies often have not focused on these regions because of limited to no barley production.

Craft malting is a relatively recent phenomenon that has grown from the craft brewing segment and is a direct response to the needs of some brewers. Craft malt is produced from a variety of grains, including barley, wheat, rye (*Secale cereale* L.), millet (*Pennisetum glaucum* L.), oat (*Avena sativa* L.), corn, spelt (*Triticum spelta* L.), and triticale (x Triticale Tschem.-Seys. ex Müntzing). By definition, more than 50% of their malt must be produced from local grain and must be made without the use of gibberellic acid (GA) or other chemical additives during processing. In 2018, the Craft Maltsters Guild counted almost 100 craft malthouses in operation, with a similar number in construction or planning (Thomas, 2018). With the development of craft brewing, the demand for locally grown and produced malt is rapidly increasing. The desire to develop local-to-regional agricultural gave impetus to the development of craft malt industry, and local brewers and distillers need to establish long-term and stable business relationships with local craft malt suppliers. It is a natural extension of the local food movement.

A number of laws and bills have been aimed at promoting craft brewing. On a Federal level the United States Congress approved the Craft Beverage Modernization and Tax Reform Act (CBMTRA) in 2017, with the aim of lowering the Federal excise tax for craft alcoholic beverage producers (Brewers Association, 2019). However, some governmental measures and

policies have been enacted to stimulate local ingredients purchases and local food production in a direct or indirect manner. For example, a proposed bill in Virginia states that farmers who sell hops and grain to craft breweries will not have to pay income tax on those sales (Thomas, 2018). Additionally, the New York State Farm Brewery Law of 2012 required those holding a farm brewery license to purchase 60% of their ingredients for beer from suppliers within New York by 2018 and 90% requirement by 2024. Under the Farm Brewery Law, New York's craft breweries are generating job opportunities, new businesses surrounding the brewing industry, and are supporting NY state's barley and hops growers. The development of craft brewing industry is promoting local tourism and stimulating local consumption (New York State Brewers Association, 2012). Over that past six years, more and more local ingredients have become available for local breweries to use.

2.3. Barley and Malt Quality Considerations for Craft Brewers

2.3.1. Kernel Plumpness

There is a consensus in the malting and brewing industry that increased kernel size and kernel weight positively promote the yield of malt extract due higher levels of starch and a lower proportion of husk (Schwarz and Li, 2011). In addition, plumpness also may predict further malt quality. This is why plumpness is usually a primary consideration when maltsters select the raw materials. The methods (Barley-2c) of the American Society of Brewing Chemists (2009) define % plump as the portion of all kernels in a 100-g sample that are retained above the $6/64 \times 3/4$ -inch openings in a screen. Thin (%) is the proportion of kernels in the same sample that passes through the $5/64 \times 3/4$ -inch screen. Thin kernels have a higher ratio of husk to endosperm and yield lower extract. They also have a greater rate of water uptake in steeping, and their inclusion

can cause uneven malt modification. As a consequence, thins are generally removed and not used in malting.

Generally, six rowed cultivars are less plump than two rowed barley. This is one of the reasons why most of the world's maltsters and brewers prefer two-row barley as the main raw material. However, the % plump of modern North American six-rowed barley, where there have been more breeding efforts, is close to that of two-row (Schwarz et al. 1996).

2.3.2. Protein Parameters

Normal malt specifications consider barley and malt protein, FAN and the ratio of soluble to total protein (Kolbach Index) (Schwarz and Li, 2011). Barley protein is a major factor in malting barley sales.

High protein lead to many issues in malting and brewing processes, including the rate of water absorption and nonuniform modification, lower malt extract, and beer haze problems. Some investigations have shown that a negative relationship exists between protein and starch content (Lake et al. 2008). Although very low protein content (<10.0%) is not common, it can occur. When barley protein is too low, the lower FAN content can limit yeast growth. Yeast activity in the fermentation part of brewing process is very important. Low protein can also result in inadequate enzyme activity, problems in conversion of starch during brewing, as well as, making it difficult to develop malt color in kilning.

In North America, the range for the total protein content of malting barley is 11% to 12.5% (dry basis). The guidelines for barley breeders from the American Malting Barley Association (2019) suggest that two-rowed protein be <13% and <12% for adjunct brewers and all-malt brewers, respectively. Adjunct brewers can tolerate higher levels of protein, as a portion of the malt is replaced with un-malted cereal. Additional enzyme activity and FAN in the malt

are needed. However, high protein can cause some of previously mentioned problems, when used by all-malt brewers. Too rapid of conversion in mashing, and physical stability of the packaged beer are principle concerns.

The ratio of soluble to total protein is call the Kolbach Index, and normal values range from 35-45% (Schwarz and Li, 2011). Soluble protein is that which is measured in wort after laboratory mashing. Kolbach Index is a measure of protein modification during malting, and modern North American malts tend to be well-modified. Malts with values below 35% are considered under-modified. Adjunct brewers generally prefer a high Kolbach Index, as there is often not protein rest stage in mashing. All malt brewers, preparing traditional beers, often want less modified malts.

FAN is largely a measure of amino acids in the laboratory extract. Adequate FAN is needed for proper yeast growth and for color development. However, when in excess, high FAN can cause stability problems like beer haze. The American Malting Barley Association (2019) recommends FAN values of >210 and 140-190 for adjunct brewers and all-malt brewers, respectively.

2.3.3. Germination

Malting is a highly specified and controlled germination process. Cleaned and sorted barley are first soaked in water (steeping) to raise the moisture from around 12 to 42-48%. As discussed previously, uniform plumpness and protein content contribute to uniform water intake speed, and then uniform kernel modification. Germination begins during the steeping process, and some enzymes are activated, while others are synthesized. Some of these enzymes are important in the germination phase, where malt modification occurs. The modifications mainly include the breakdown of protein cell-wall carbohydrates (Schwarz and Li, 2011).

Germination (germinative energy) is determined by placing 100 kernels on a filter paper in a petri dish with 4-mL of water. Germinative energy is the percentage of kernels that have shown visible signs of germination after 72 hrs. The industry standard is that more than 95% of all kernels in a sample are able to germinate (Schwarz and Li, 2011). Kernels that don't germinate, do not produce enzymes and do not modify. As a consequence, extract is lower, and beta-glucans are higher.

2.3.4. Malt Enzymes

Alpha-amylase and DP are the two enzyme activities commonly measured in malt. Both are important in the breakdown of starch during the brewing process (Schwarz and Li, 2011). Alpha-amylase is an endo-enzyme that hydrolyzes the alpha 1-4 linkages in starch. It is important in reducing starch viscosity and creating more substrate for beta-amylase. Diastatic Power is mainly a measure of beta-amylase, which is an exo-enzyme that hydrolyzes maltose units from the non-reducing end of starch chains and dextrans. High levels of these enzymes are required by adjunct brewers, as while adjuncts contain starch, they have no enzymes. All malt brewers desire lower enzyme levels, because if too high, it is difficult to control starch conversion in mashing. The American Malting Barley Association (2019) recommends DP values of 140 minimum and 110-150 (°ASBC) for adjunct brewers and all-malt brewers, respectively. AMBA's guidelines for alpha-amylase are not as definitive, but all -malt brewers generally desire levels below 60 or 70 dextrinizing units (DU).

2.3.5. Beta-Glucan

Beta-glucans are the major component of barley endosperm cell walls, and their degradation is an important component of malt modification. This is because they can cause problems if not adequately degraded (Schwarz and Li, 2011). These issues may include

increased wort and beer viscosity, slow lautering, and filter plugging. The beta-glucan measurement is made on the laboratory extract, as it is the soluble beta-glucans that are problematic. The American Malting Barley Association (2019) recommends values of <100 mg/L for all malts.

2.4. Challenges to Barley Production in Eastern States

The choice of barley cultivars especially suited for local production is important to farmers in several eastern states. There are craft brewers in every state, but they are particularly concentrated in several Northeast and Great Lakes states, like Michigan, New York, Ohio, and Pennsylvania. Yet, the five main states for barley production are Idaho, Montana, North Dakota, Washington and Minnesota, with, 75% of the all barley grown in the US. As such, along with the development of craft brewing, there is an increasing demand for locally grown and produced malt. Many farmers in the eastern states lack malt barley planting experience, and access to information from research institutes with local barley experience. Barley used for malting and brewing must meet specific requirements for nearly 20 different end use quality traits. Because of these exacting needs, malting barley often receives a premium of \$1 per bushel over that of barley for livestock feed (Haag, 2006). Barley grown outside of its area of adaptation often fails to meet the specifications needed for malting and brewing, which include plump kernels >80%, grain protein <12%, germination $\geq 95\%$, and grain free of PHS and deoxynivalenol (DON). Additionally, unadapted cultivars often have lower yields and may be susceptible to local diseases that are may not be present in the area where they were developed.

Fusarium Head Blight (FHB) is a fungal infection caused by several species of *Fusarium*, and occurs mainly on wheat and barley (Wise, 2010). It is also called scab. The main pathogen in much of the United States is *Fusarium graminearum*. The main symptom of the infection in

barley is dark lesions or spots on the developing kernels. Yield losses, low grade and quality, low germination rate and contamination of kernels with mycotoxins are the most common problems caused by FHB. Deoxynivalenol is regarded as the main mycotoxin related to FHB, and is potentially harmful to both humans and livestock. The United States Department of Agriculture (USDA) considers FHB as the worst plant disease in the USA in the past 60 years (Schmale and Bergstrom, 2010). FHB has had widespread occurrence in Midwestern and Eastern regions of the United States over the last several decades (Dill-Macky, 1997, Stack, 2000). Maltsters often screen for DON in regions where FHB occurs, and generally do not accept barley with >0.5 mg/kg (Schwarz and Li, 2011). The main issue is that DON present on the malt can be transferred to beer.

Pre-harvest sprouting is another potential challenge to barley production in the eastern states. PHS is the premature germination of the grain prior to harvest and can occur as a result of rainfall or other high humidity harvest environments (Fang et al. 2008). Although the PHS damaged barley is still alive, it will never perform optimally in the malt house and often compromises malt quality. A rapid loss of seed germination is most problematic. As germination is an essential part of the malting process, PHS damaged barley grain is highly undesirable (Gualano, 2009). Pre-harvest sprouting is often an indirect consequence of low grain dormancy in cereals and genotype is an important factor. The malting industry generally measures PHS using the stirring number method on the Rapid ViscoAnalyzer (Schwarz and Li, 2011). Values below 120 are considered spouted, with values below 60 being severely sprouted.

3. OBJECTIVE

The objectives of the project are to determine which spring barley genotypes are best adapted to specific regions in the northeastern United States, and to detect the impact of different environmental factors on their performance.

4. EXPERIMENT APPROACH

4.1. Materials

Information on barley genotypes tested as part of the Eastern Spring Barley Nursery (ESBN) are shown in Table 2. Both experimental lines and released cultivars were included. Sources were US, Canadian and European breeding programs. The initial selection of genotypes was determined by breeders and maltsters attending the Farmer Brewer Winter Weekend meeting held in Amherst, MA in January 2015. Twenty genotypes were included in 2015, and then 25 in subsequent years. Genotypes were dropped if they performed poorly at multiple locations, and others were added. Decisions on inclusion/exclusion were made by cooperators. Not all cooperators participated in all years. The number of genotypes tested by year and location are shown in Table 3.

Table 2. Barley Genotype, Row-Type, Inventor, Year Released, and Years Tested in the Eastern Spring Barley Nursery.

Variety	Row-Type	Origin	Country	Year Released	Years Tested			
					2015	2016	2017	2018
ND22421	2	NDSU	USA	--	x			
2ND28065	2	NDSU	USA	--		x	x	x
2ND33710	2	NDSU	USA	--			x	
2ND33757	2	NDSU	USA	--			x	x
2ND33760	2	NDSU	USA	--			x	x
2ND33821	2	NDSU	USA	--			x	x
2ND34954	2	NDSU	USA	--				x
2ND34999	2	NDSU	USA	--				x
2ND35001	2	NDSU	USA	--				x
AAC Synergy	2	AAFC ¹	Canada	2012	x	x	x	x
AC Metcalfe	2	AAFC ¹	Canada	1994	x	x		
Accordine	2	Ackermann	Germany	--				x
Acorn	2	CDC ²	Canada	--		x	x	
Bentley	2	Canterra Seeds	Canada	2010	x	x	x	
CDC Copeland	2	CDC ²	Canada	1999	x	x		
CDC Meredith	2	CDC ²	Canada	2010	x	x		
Cerveza	2	AAFC ¹	Canada	2011	x	x		
Conlon	2	NDSU	USA	1996	x	x	x	
Crescendo	2	Nielsen & Smith A/S	Denmark	--				x
Eifel	2	Secobra	France	--				x
Esmá	2	Ackermann	Germany	--			x	x
Explorer	2	Secobra	France	--		x	x	
Expo	2	Secobra	France	--				x
Full Pint	2	OSU ³	USA	--	x			
Harrington	2	CDC ²	Canada	1986	x			
Innovation	6	BARI LLC ⁴	USA	2010	x	x		
Klages	2	UI, ARS, OSU ³	USA	1973	x			
KWS Beckie	2	KWS ⁵	Germany	--		x	x	x
KWS Fantex	2	KWS ⁵	Germany	--		x	x	x
KWS Josie	2	KWS ⁵	Germany	--			x	
KWS Tinka	2	KWS ⁵	Germany	--			x	x
Lacey	6	MAES ⁶	USA	2000	x	x		
LCS Genie	2	LCS ⁷	USA	--		x	x	x
LCS Odyssey	2	LCS ⁷	USA	--		x	x	x

Table 2. Barley Genotype, Row-Type, Inventor, Year Released, and Years Tested in the Eastern Spring Barley Nursery (continued).

Variety	Row-Type	Origin	Country	Year Released	Years Tested			
					2015	2016	2017	2018
Manta	2	Ackermann	Germany	--			x	
ND Genesis	2	NDSU	USA	--	x	x	x	x
Newdale	2	AAFC ¹	Canada	--	x	x	x	x
Pinnacle	2	NDSU	USA	--	x	x	x	x
Pioneer	--	Secobra	France	--		x		
Quest	6	MAES ⁶	USA	--	x	x	x	x
Robust	6	MAES ⁶	USA	1983	x	x		
Sangria	2	Ackermann	Germany	--			x	x
Scarlett	2	ASGC ⁸	Germany	--	x			
Sirish	2	Syngenta	UK	--		x	x	
Steffi	2	ASGC ⁸	Germany	--		x		
Tradition	6	BARI LLC ⁴	USA	2003	x	x	x	x

¹ AAFC: Agriculture and Agri-Food Canada.

² CDC : Crop Development Centre.

³ UI, ARS, OSU : The University of Idaho, ARS, and Oregon State University.

⁴ BARI LLC : Busch Agricultural Resources Inc.

⁵ KWS: <https://www.kws.com/corp/en/>.

⁶ MAES : Minnesota Agricultural Experiment Station.

⁷ LCS : Limagrain Cereal Seeds.

⁸ ASGC : Ackermann Saatzucht GmbH & Co.

Table 3. Eastern Spring Barley Nursery Locations and the Number of Genotypes Planted, 2015-2018

Location	Years/Genotype			
	<u>2015</u>	<u>2016</u>	<u>2017</u>	<u>2018</u>
Indiana (IN), West Lafayette	20	25	25	--
Maine (ME), Orono, ME	20	25	25	25
Maine (ME), Presque Isle, ME	20	25	--	25
Michigan (MI), Posen (Northeast)	20	25	25	25
Michigan (MI), Hickory Corners (Southwest)	--	25	25	25
Michigan (MI), Chatham (Upper Peninsula)	20	25	25	25
Massachusetts (MA), Amherst, MA	--	25	25	25
New Jersey (NJ), Newton	--	--	--	25
New York (NY), Ithaca1	20	--	25	25
New York (NY), Ithaca2	--	--	--	25
North Dakota (ND), Fargo	20	25	25	25
Ohio (OH), Wooster	20	25	25	--
Pennsylvania (PA), University Park	20	25	25	--
Vermont (VT), Alburg	20	25	25	25

4.2. Methods

4.2.1. Barley Quality

4.2.1.1. Test Weight and 1,000 Kernel Weight

Test weight and 1,000 kernel weight were determined according to ASBC methods Barley-2B and Barley-2D (ASBC, 2009). One-thousand kernel weight was determined by counting the number of kernels in a 10 g sample on a Seedburo Model 77 seed counter (Seedburo Corp., Chicago IL) equipped with a Syntron magnetic feeder (FMC Corp., Homer, PA).

4.2.1.2. Barley Moisture

The barley whole grain moisture content was measured by near infrared reflectance (NIR) on a Foss 1241 whole grain analyzer (Foss in North America, Eden Prairie, MN), using the calibration supplied with the instrument.

4.2.1.3. Barley Protein

Percent total protein, calculated on a dry matter basis was determined with a LECO Model FP-428 Nitrogen Determinator System (Leco Crop St Joesph, MD). The method is described in Barley-7 in the Methods of Analysis of the American Society of Brewing Chemists (ASBC,2009).

4.2.1.4. Barley Color

Barley kernel color (brightness) was determined by a modification of ASBC standard method, Barley-9 (2009) using the L value obtained from a HunterLab ColorFlex Model CFLX-45 Spectrocolorimeter (Hunter Associates Laboratory, INC Reston, VA). Higher L-values indicate a dark color.

4.2.1.5. Kernel Assortment

Barley (100 g) kernel assortment was determined by standard ASBC method Barley 2-C (ASBC, 2009) using a Pfeuffer Sortimat (Pfeuffer GmbH, Kitzingen, Germany). Results are shown as the percentage of kernels retained over 7/64" (2.78mm), 6/64" (2.38), and 5/64" (1.98mm) sieves. Percent plump is the percentage of all kernels retained above the 6/64×3/4-inch screen. Percent thin is the percentage of kernels passing through the 5/64×3/4-inch screen.

4.2.1.6. Pre-Harvest Sprouting

Pre-harvest sprouting was determined according to the stirring number test on a Rapid Visco-Analyzer ((Newport Scientific Pty Ltd, Werriewood, New South Wales, Australia) according to AACC method 22-08 (AACC, 2000). Four grams (4 g) of barley was ground to pass a 0.5-mm screen in a UDY mill (Udy Corp., Boulder, CO.) and mixed with 25 g distilled water. The test is conducted at 95°C for 3 min stirring at 160 rpm. Stirring Number (SN) values < 120

are indicative of samples that have damage from pre-harvest sprouting. The lower the number the greater the damage.

4.2.1.7. Deoxynivalenol (DON)

Deoxynivalenol was determined by a modification of the method of Tacke and Casper(1996). Samples were ground on a Perten laboratory mill (model 3600, Perten Instruments, Hägersten, Sweden). The ground material (2.5 g) was weighed into 50 mL conical bottom polypropylene centrifuge tubes. Samples were extracted with the addition of 20 mL of an 84% acetonitrile/water solution and shaken for 1 hr on a horizontal shaker. After shaking samples were allowed to settle, and a 2 mL aliquot of supernatant was then transferred to a SPE column containing 1 g of 50/50% C18/alumina. The gravity filtered (2 mL) supernatant was transferred to tubes, and dried under nitrogen gas. Dried samples were derivatized using TMSI (trimethylsilylamidazole); TMCS (trimethylchlorosilane) 10:1. The derivatized mycotoxins were extracted into 1 mL of isooctane for analysis by gas chromatography with electron capture detection (GC ECD). An Agilent 6890 GC ECD (Santa Clara, CA) was used. Separation was on a 5%phenly methyl siloxane column (30 m × 0.25 mm, 0.25 µm film thickness) (Agilent HP-5). An intermediate polarity deactivated column (1–2 m × 0.53 mm) (Restek, Bellefonte, PA) was used as a guard. Injection volume was 1 µL with a constant head pressure of 1.38 bar helium carrier gas. The initial inlet temperature was 90°C and then increased at a rate of 20°C/min to a final temperature of 300°C. The oven temp was initially 70°C, and then ramped at a rate of 25°C/min to 170°C, and then finally at 5°C/min to 300°C. The detectors were held at 300°C with constant makeup gas of ArCH₄ (argon/methane) at 60 mL/min. Mirex (ULTRA Scientific, Kingstown, RI) was used as an internal standard at 0.5 mg/mL.

4.2.1.8. Barley Germination

Germinative energy was determined by the methods described in the Method Barley-3 of Analysis of the American Society of Brewing Chemists (ASBC, 2009).

4.2.2. Malt Quality

4.2.2.1. Micro-Malting

Micro-malting was conducted only on select samples from locations displaying no to low DON, and no PHS. The methods for micro-malting were previously described by Karababa (et al, 1993). Each barley sample was steeped to 43.7% moisture at 16°C and germinated for four days at 95% relative humidity and 16°C. The germinated green malts were kilned in a forced-air laboratory kiln. The kilning schedule was programmed with a ramp rate between temperature changes of 30 min, except for the last stage which is 40 min. The kilning started at 49°C and was held for 10 hrs. During the second stage, the temperature was raised to 54°C and held for 4hrs. In the third stage temperature was held at 68°C for 2 hrs. In the final stage the temperature was increased to 85°C and held for 3 hrs. Rootlets were removed following kilning by rubbing and screening through a sieve. Malt samples were stored in tightly sealed plastic sample bags at room temperature prior to analysis.

4.2.2.2. Malt Moisture

Moisture is expressed as a percentage of total weight and was determined by a modification of the ASBC standard oven drying method, Malt-3 (ASBC, 2009).

4.2.2.3. Malt Loss

Malt loss was calculated as percentage of dry matter lost in the malting process. Both starting barley and final malt (after removal of rootlets) weights are expressed on a moisture free basis: % malt loss= $100 - [\text{derooted malt weight (db)} \times 100 / \text{barley weight (db)}]$.

4.2.2.4. Fine Grind Malt Extract

Fine grind malt extract (percent dry basis) was determined using ASBC standard method, Malt-4 (2009). The result indicates laboratory extractability of the malt, and in general most modern cultivars are >80%.

4.2.2.5. Wort Soluble Protein

Soluble protein was determined using ASBC standard method, Malt-17 (ASBC, 2009). The method is intended to provide a relatively simple and rapid means of measuring the soluble protein content of unhopped laboratory wort based on the differing UV absorption of protein at 215 nm and 225 nm.

4.2.2.6. Ratio of Wort Soluble Protein to Total Malt Protein (S/T) (Kolbach Index)

The soluble/total ratio was determined by Wort-17, (ASBC, 2009). Soluble protein is divided by total barley protein and then multiplied by 100. The value measures the percent of insoluble barley storage protein hydrolyzed to soluble proteins, peptides and amino acids.

4.2.2.7. Alpha-Amylase (DU)

Malt alpha-amylase is important for dextrinization of starch during mashing. Alpha-amylase activity of the malt samples was determined as described in Technicon Industrial method No.424-76A (Bran and Luebbe, Inc., Tarrytown, NY; (Karababa et. al., 1993). The alpha-amylase activity of the standards was determined by ASBC Malt Method 7 (ASBC, 2009).

4.2.2.8. Diastatic Power (ASBC)

Diastatic power of the malt samples was determined as described in Technicon Industrial method No.424-76A (Bran and Luebbe, Inc., Tarrytown, NY; Karababa et. al., 1993). Diastatic power of the standards was determined by Malt Method- 6 (ASBC, 2009).

4.2.3. Wort Quality

4.2.3.1. Wort Viscosity

Wort viscosity was determined according to method Wort-13 (ASBC, 2009).

4.2.3.2. Wort Color

Wort color was determined spectrophotometrically at 430nm using the method described in method Wort-9 (ASBC, 2009).

4.2.3.3. Wort FAN

Free Amino Nitrogen was determined according to Wort-12 (ASBC, 2009).

4.2.3.4. Wort Beta-Glucan

Wort beta-glucan is expressed in mg/L and was determined by fluorescence using flow injection analysis as described in method Wort-18 (ASBC, 2009). Higher wort beta-glucan levels (>100-200) indicate that the malt may be unsuitable for use by some brewers.

4.3. Experimental Design and Statistical Analysis

There are four years of data for barley quality (2015-2018) and two years of data for malt quality (2015-2017). Analyses were conducted across years using genotypes that were grown in at least two years. All analyses were performed using JMP Pro version 14 (SAS Institute Inc., Cary, NC). Because of the unbalanced nature of the data (i.e. not all genotypes being grown together in all years) analysis of variance was conducted using a mixed model that allowed for calculation of the least square mean (LSMean) values. Barley and malt quality data were determined on composite samples across replicates from each environment. In the mixed model analyses, environments within a year were considered a random effect and lines were considered a fixed effect. *F*-tests were considered significant at $P \leq 0.05$. Because of the expense and time involved in malting lines and collecting the data, it was not possible to malt barley from every

location. Each year, three to four locations were identified that had favorable barley quality for protein, kernel plumpness, stirring number, and DON. Only locations with acceptable values for these traits were malted. Barley from some locations were malted in only one year. An example of this are samples from Ithaca, NY in 2015 and southwest Michigan in 2017. When there is only one year of data from a location, it often occurs that you don't have data for some lines. For example, the cultivar Acorn and the experimental line 2ND28065 were not grown in the Ithaca, NY trial in 2015. When this occurs, it was not possible to calculate an LSMean. LSMean values can only be calculated for barley and malt quality data that are collected on a specific genotype in a specific trial.

To help in classifying and visualizing the data from 23 malted genotypes that had similar barley and malt quality, hierarchical cluster analysis without data standardization was performed using JMP Pro version 14. Results are presented as constellation plots (Figures 1-5). Additionally, the means across genotypes within each cluster were calculated (Tables 27-31). In the clustering analysis, it can group some genotypes were into four categories with hierarchical clustering method. The similarity of genotypes within a cluster (category) is lower than between different clusters.

5. RESULTS AND DISCUSSION

5.1. Statistical Interpretation

The Eastern Spring Barley Nursery project began in 2015 with ten locations in eight states (Table 3). Twenty genotypes were tested in the first year. Twenty-five genotypes were tested in all other years. Some of the initial selections did not perform well at a number of locations and were not tested in subsequent years. For example, the cultivars Full Pint, Harrington, Klages and Scarlett, which all had poor agronomic performance, were all dropped after the first year (Table 2). These cultivars were initially included as they had name recognition among brewers, but the results clearly demonstrated problems with unadapted germplasm. As genotypes were dropped from evaluation, new ones were included. In total, 46 genotypes were tested over the four years. Only six cultivars were tested in all years. These were AAC Synergy, ND Genesis, Newdale, Pinnacle, Quest, and Tradition.

At most, there were 10 cooperating states (Table 2), and cooperators in Michigan, Maine, and New York had two to three test sites per year. Only cooperators in Michigan, Maine, New York, North Dakota, and Vermont participated in all years. Cooperators at Penn State University (PA), and Purdue University (IN) dropped out of the nursery after three years. The cooperator at Purdue University determined that winter barley genotypes might be more successful in their region; thus, they decided to discontinue testing spring barley lines. The cooperator at Penn State retired and there was no one there to continue evaluating the ESNB. Data from New Jersey and Massachusetts were not included in final analyses because of overall poor quality. The ESNB was grown in New Jersey in 2017 and the collaborators were not able to harvest in a timely manner. As a consequence, all samples were sprouted and nearly all contained DON. Samples were tested in Amherst, Massachusetts from 2016-2018. However, over 90% of all samples had

high levels of PHS. The average stirring number value across years in the Massachusetts ESNB was 37.

Analyses of the barley and malt quality results from the ESNB presents some challenges as the data are not balanced. Not all genotypes were tested in all years, nor did all cooperators participate each year. Additionally, some cooperators did not use the same locations for growing the trial each year.

Measurement of harvested barley quality is necessary to determine the suitability of the samples for malting, and in turn the contractual obligation between the grower and maltster brewer. Quality factors always measured include barley protein, % plump kernels, and % germination. In some cases, deleterious factors like PHS and DON are also measured as they can render an otherwise suitable lot of barley unfit for malting. All of the above traits are influenced by the environment, grower practices, and the genotype. In the following sections the least square means across both locations and genotypes are presented. Correlations between environments for the traits are presented to help determine which environments responded similarly.

Due to concerns of the expense and time, only locations with acceptable values for barley quality traits were malted. Grain from six locations (Alburgh, VT, Fargo, ND, Ithaca, NY, Orono, ME, University Park, PA and Upper Peninsula, MI) were malted in 2015, grain from four locations (Fargo, ND, Orono, ME, Northeast, MI and University Park, PA) were malted in 2016 and grain from five locations (Alburgh, VT, Orono, ME, Southwest, MI, University Park, PA and Upper Peninsula, MI) were malted in 2017.

In the analyses of the barley and malt quality traits, samples for each genotype within a location were a composite across replicates. This limited our ability to do a valid statistical test

to determine if there were genotype x environments because replicates within a location were confounded. In the analyses of the study, locations within a year were equivalent to replicates.

5.2. Barley Quality Traits

5.2.1. Barley Protein

Generally, a 9.0-12.0% of protein content in barley is acceptable to craft brewers, but exact specifications will vary between purchasers. Many malt quality problems result from high protein content. For example, high protein content in malt barley may cause over-modification, darker beer color, and beer haze problems. On the other hand, very low protein content in barley may cause problems such as, the lack of beer foam and low enzyme levels.

From the mean data shown in Table 4, it can be seen that barley from most locations was within specification. Only the locations in southwest MI and West Lafayette IN were above 12.0% protein. Differences in protein between locations may be caused by the environment, residual fertility in the soil, and the production practices of the collaborators. The location in Orono, ME had a few samples with protein levels below 9.0%.

Similarly, mean data for the individual samples showed that genotype also had a significant effect on barley protein content. For example, the cultivars AC Metcalfe, Innovation, Lacey, Robust, Conlon, Quest and Tradition all had relatively higher protein contents at most locations. In general, the five six-row barleys in this experiment showed higher protein content. These data together suggest that the both the genotype and row-type should be taken into account by local farmers before making a decision for planting. For instance, it would be better to avoid planting the above-mentioned high protein six-rowed and two-rowed genotypes at locations in southwest MI and northern Indiana. The variety Sangria, which was tested in two years, averaged below 11% protein across environments.

Table 4. Least Squares Mean for Protein Across Years at Each Location

Genotype	Alburg, VT	Fargo, ND	Ithaca, NY	North- east, MI	Orono, ME	Presque Isle, ME	South- west, MI	University Park, PA	Upper Peninsula, MI	West Lafayette, IN	Wooster, OH	<u>Ave</u>
2ND28065	10.5	11.3	9.8	11.5	9.6	9.8	11.8	10.7	11.1	12.3	11.6	10.9
2ND33757	10.6	10.5	9.3	10.5	9.2	9.5	11.4	10.7	10.7	11.4	11.0	10.4
2ND33760	10.7	10.9	9.6	11.4	9.2	9.9	11.8	11.0	10.9	11.3	11.7	10.8
2ND33821	10.1	10.8	9.7	11.1	9.0	10.0	11.6	10.9	11.4	10.8	11.4	10.6
AAC Synergy	10.7	10.9	9.9	11.5	9.0	9.9	12.5	11.1	11.2	12.2	11.7	11.0
AC Metcalfe	11.9	12.0	10.4	12.1	9.6	10.6	14.0	12.0	12.7	14.0	12.1	11.9
Acorn	9.8	10.5	9.8	11.5	8.2	9.5	12.7	10.5	10.8	13.2	11.2	10.7
Bentley	11.4	11.3	9.9	11.9	9.4	10.0	13.0	11.0	11.7	13.0	11.7	11.3
CDC Copeland	10.5	10.7	10.3	12.3	9.3	9.4	13.1	11.2	11.4	12.6	11.9	11.2
CDC Meredith	10.4	11.1	10.1	11.7	9.2	9.6	13.1	11.5	11.4	13.5	11.9	11.2
Cerveza	10.5	11.1	9.7	11.6	9.6	9.5	12.0	11.0	10.9	12.1	11.6	10.9
Conlon	11.7	11.7	10.5	12.4	10.7	11.8	12.4	11.8	12.1	12.9	12.1	11.8
ESMA	10.0	10.8	9.5	11.3	8.8	10.0	11.8	10.2	10.9	13.5	11.1	10.7
Explorer	10.6	11.0	10.2	11.6	8.5	10.3	12.4	11.2	11.3	13.5	11.0	11.1
Innovation	11.4	12.4	10.7	12.9	10.8	12.1	13.3	11.8	12.7	12.7	12.4	12.1
KWS Beckie	10.6	10.9	9.4	11.6	8.3	9.5	12.1	10.6	10.8	12.3	11.3	10.7
KWS Fantex	10.2	10.5	9.5	11.4	8.4	9.9	12.7	10.4	10.8	13.0	11.3	10.7
KWS Tinka	10.0	11.4	9.7	12.1	8.8	9.7	13.7	10.8	10.7	13.1	11.7	11.1
Lacey	11.8	12.7	11.4	12.2	10.5	11.0	13.3	12.1	12.2	12.4	12.2	12.0
LCS Genie	10.1	10.9	10.0	12.5	8.7	9.6	14.0	11.6	10.4	13.4	11.3	11.1
LCS Odyssey	9.8	10.6	9.3	11.3	8.3	9.6	12.0	10.0	10.2	12.6	11.3	10.5
ND Genesis	10.2	10.1	9.7	10.8	9.3	10.0	11.3	10.5	10.6	11.0	11.0	10.4
Newdale	10.8	11.3	9.9	12.0	9.0	10.4	13.4	11.5	11.7	13.0	11.6	11.3
Pinnacle	10.2	9.9	9.9	11.1	9.4	9.3	11.4	10.5	11.1	11.9	11.5	10.6
Quest	11.2	12.5	11.0	12.5	10.5	11.1	12.8	11.5	12.2	12.3	12.5	11.8
Robust	12.3	13.3	11.3	12.8	10.9	11.6	13.0	12.2	12.2	12.4	12.2	12.2
Sangria	10.1	10.9	9.3	11.7	8.6	9.5	12.3	9.8	11.3	13.0	10.4	10.6
Sirish	11.2	11.7	9.6	12.1	8.6	10.2	12.3	10.2	10.9	13.7	11.1	11.1
Tradition	11.6	12.6	10.8	12.2	10.5	11.6	12.4	11.5	12.2	12.8	12.5	11.9
<u>Ave</u>	10.7	11.3	10.0	11.8	9.3	10.2	12.6	11.0	11.3	12.6	11.6	

The correlation coefficients of protein content between different locations are shown in Table 5. Overall, these relatively high correlation coefficients show that genotype has a great influence on the protein content, while environmental factors had less effect. These data also suggest that producers in much of the test region, might be able to use results from other locations to predict protein performance within their area. The exceptions, of course, are West Lafayette, IN and southwest, MI. It is noteworthy that, the correlation coefficients between West Lafayette and other locations were very low. The average protein content of barley from West Lafayette was generally unacceptable to purchasers. Data from Table 6 shows a great variance in protein content of some genotypes in West Lafayette, IN. For example, the protein content of Tradition was 11.4 in 2015, and increased to 14.2 in 2017. In comparison, other locations such as Fargo, ND and Orono, ME did not show such large differences between years. The data strongly suggest that cultivation and environmental factors in West Lafayette may result in a high protein content. In general, high availability of nitrogen and physiological stress caused by drought or high temperature under drought conditions during seed development will increase protein level (Barnabás, 2008). While the protein results from southwest MI show a poor relationship with most other states, they did show a reasonably strong relationship with those of northern MI.

Table 5. Correlations Between Locations for Grain Protein

Locations	Alburgh, VT	Fargo, ND	Ithaca, NY	North-east, MI	Orono, ME	Presque Isle, ME	South west, MI	University Park, PA	Upper Peninsula, MI	West Lafayette, IN	Wooster, OH
Alburgh, VT	1.00	0.84	0.77	0.62	0.78	0.80	0.35	0.75	0.83	0.14	0.69
Fargo, ND	0.84	1.00	0.82	0.79	0.75	0.83	0.49	0.71	0.78	0.24	0.73
Ithaca, NY	0.77	0.82	1.00	0.72	0.83	0.78	0.48	0.85	0.80	0.12	0.81
Northeast , MI	0.62	0.79	0.72	1.00	0.56	0.66	0.75	0.67	0.64	0.48	0.66
Orono, ME	0.78	0.75	0.83	0.56	1.00	-0.81	0.17	0.73	0.79	-0.16	0.81
Presque Isle, ME	0.80	0.83	0.78	0.66	-0.81	1.00	0.29	0.67	0.81	0.11	0.69
Southwest MI	0.35	0.49	0.48	0.75	0.17	0.29	1.00	0.60	0.43	0.64	0.45
University Park, PA	0.75	0.71	0.85	0.67	0.73	0.67	0.60	1.00	0.75	0.11	0.80
Upper Peninsula, MI	0.83	0.78	0.80	0.64	0.79	0.81	0.43	0.75	1.00	0.18	0.74
West Lafayette, IN	0.14	0.24	0.12	0.48	-0.16	0.11	0.64	0.11	0.18	1.00	0.03
Wooster, OH	0.69	0.73	0.81	0.66	0.81	0.69	0.45	0.80	0.74	0.03	1.00

Table 6. Comparison of Protein Content at Three Locations in 2015 and 2017

Genotype	Protein (%)					
	West Lafayette, IN		Fargo, ND		Orono, ME	
	2015	2017	2015	2017	2015	2017
AAC Synergy	11.4	13	10.6	11.1	8.2	7.9
Tradition	11.4	14.2	13.4	13.3	9.6	9.3
ND Genesis	10.4	11.7	9.3	11.0	8.7	8.5
Newdale	11.6	14.4	11.2	11.2	8.7	7.6
Pinnacle	10.9	12.9	10.0	10.6	8.0	9.8
Quest	11.4	13.3	12.9	12.9	9.9	9.7

5.2.2. Barley Kernel Plumpness

The average kernel plumpness at each location was above 80%, which indicates there were no environmental conditions at any of the location that resulted in very poor kernel plumpness (Table 7). The same was true of the genotype means across locations and years. Nonetheless, the mean plumpness data for the different locations and genotypes clearly shows that the both environmental factors and genotypes can affect the plumpness of barley. While, no location had really poor kernel plumpness, there was large variation between different locations.

There were four locations with mean plumpness above 90%, including Alburgh, VT, Orono, ME, and the southwestern and upper peninsula locations of MI. Ithaca, NY and northeast MI had mean plumpness values below 85%. Cultivar selection in these areas would be especially important for farmers to reduce the risk of not meeting plumpness quality specifications.

In terms of genotypes, there were four cultivars with mean kernel plumpness values under 85%. These included CDC Meredith, Cerveza, Newdale and Quest. In fact, these four cultivars exhibited the lowest plumpness in most locations. For example, CDC Meredith had only 69.8 and 71.2% plump kernels in Ithaca, NY, and northeast MI, respectively. Cerveza exhibited only 76.6 and 69.4% plump in Ithaca, NY, and northeast MI. Although ranks changed between locations, the cultivars AAC Synergy, Acorn, Conlon, KWS Fantex and Sirish, were often among the plumpest.

The correlation coefficients of plumpness between different locations are shown in Table 8 . Compared with results for protein, the correlation coefficients for plumpness between locations seem to be very unstable. Almost all correlation coefficients for plumpness were below 0.70. This suggests that environment has a large influence on this trait. The instability makes it difficult to predict the performance of a genotype's plumpness in different locations. It should be noted that even the correlations among three MI locations were not high.

Table 7. Least Squares Mean for Kernel Plumpness Across Years at Each Location

<u>Genotype</u>	<u>Alburg, VT</u>	<u>Fargo, ND</u>	<u>Ithaca, NY</u>	<u>Northeast, MI</u>	<u>Orono, ME</u>	<u>South west, MI</u>	<u>University Park, PA</u>	<u>Upper Peninsula, MI</u>	<u>Ave</u>
2ND28065	90.5	88.3	--	91.1	94.9	77.3	79.3	91.6	87.6
AAC Synergy	95.3	92.9	90.1	91.2	97.8	93.1	93.6	96.9	93.9
AC Metcalfe	90.2	85.5	80.6	78.0	93.3	--	86.2	90.8	86.4
Acorn	94.1	89.4	--	93.2	94.0	98.9	89.0	96.6	93.6
Bentley	93.4	84.4	90.6	76.8	97.1	--	88.7	92.4	89.1
CDC Copeland	90.7	88.0	68.1	83.1	93.8	--	82.4	94.5	85.8
CDC Meredith	92.4	80.3	69.8	71.2	92.2	--	81.3	89.4	82.4
Cerveza	90.0	79.0	76.6	69.4	92.0	--	82.7	92.3	83.1
Conlon	95.6	92.4	94.1	92.5	98.9	95.3	94.5	97.7	95.1
Explorer	92.9	92.8	--	93.8	96.5	95.2	89.2	96.4	93.8
Innovation	93.1	91.2	86.0	80.7	97.6	--	85.5	96.2	90.0
KWS Beckie	88.1	92.7	--	90.4	94.3	98.4	81.0	97.3	91.7
KWS Fantex	98.4	90.0	--	85.3	94.6	96.9	87.1	95.8	92.6
Lacey	93.7	92.3	85.9	76.3	96.5	--	85.5	96.5	89.5
LCS Genie	91.5	87.7	--	90.7	95.3	96.8	90.8	95.7	92.6
LCS Odyssey	93.6	92.2	--	94.6	93.3	96.9	91.3	96.3	94.0
ND Genesis	95.6	88.1	88.3	91.3	94.4	95.5	91.1	94.6	92.4
Newdale	87.0	74.1	72.0	67.2	92.1	83.7	79.9	89.1	80.6
Pinnacle	87.8	87.1	78.5	89.8	82.3	93.2	89.2	89.4	87.2
Quest	88.3	86.1	79.1	78.1	89.4	76.8	81.5	91.6	83.9
Robust	89.8	91.3	86.5	74.3	95.6	--	81.8	93.5	87.5
Sirish	91.4	87.5	--	95.8	94.6	98.7	91.2	97.0	93.7
Tradition	93.9	91.3	88.4	79.6	95.9	71.6	81.2	96.4	87.3
<u>Ave</u>	92.1	88.0	82.3	84.1	94.2	91.2	86.3	94.3	

Table 8. Correlations Between Locations for Kernel Plumpness

<u>Location</u>	Alburg, VT	Fargo, ND	Ithaca, NY	North west, MI	Orono, ME	South west, MI	University Park, PA	Upper Peninsula, MI
Alburg, VT	1.00	0.47	0.68	0.34	0.59	0.30	0.56	0.59
Fargo, ND	0.47	1.00	0.68	0.66	0.42	0.30	0.40	0.78
Ithaca, NY	0.68	0.68	1.00	0.51	0.58	0.31	0.66	0.68
Northeast, MI	0.34	0.66	0.51	1.00	0.13	0.65	0.65	0.60
Orono, ME	0.59	0.42	0.58	0.13	1.00	0.12	0.22	0.65
Southwest, MI	0.30	0.30	0.31	0.65	0.12	1.00	0.71	0.49
University Park, PA	0.56	0.40	0.66	0.65	0.22	0.71	1.00	0.49
Upper Peninsula, MI	0.59	0.78	0.68	0.60	0.65	0.49	0.49	1.00

5.2.3. Stirring Number

Pre-harvest sprouting is a frequent problem for growers of barley and wheat around the world. As some researchers have reported, environmental factors and dormancy related genes together lead to the differential response observed for PHS tolerance (Bailey et al. 1999). When there are concerns, the PHS of barley can be tested using the RVA apparatus, which determines the stirring number. A stirring number value of less than 120 is an indicator of sprout damage in barley. Values below 60 are a sign of severe sprout damage.

Wet and cold weather can easily cause PHS during later grain development through harvest. Delayed harvest can be another factor, and this was likely experienced at some test locations (e.g. Newton, NJ and Amherst, MA). Severe PHS was seen in at least part of the ESNB in all years. The Amherst, MA location had high levels of PHS in all years (2016-18), as did Ithaca, NY in 2017 and 2018, and Presque Isle, ME in 2015 and 2016. High levels of PHS were seen every year in MI: the southwest in 2017 and 2018, upper peninsula in 2016, and the northwest in 2015. The Fargo, ND location experienced severe PHS in 2017.

The mean data for stirring number based on different locations clearly shows that the environment plays an important role in PHS (Table 9). The mean stirring number data for each

location showed great variability. Four of the locations had mean values for stirring number <90. These included Ithaca, NY, Presque Isle, ME, West Lafayette, IN and Wooster, OH. The average PHS in Ithaca, NY was 56, with 15 of 21 cultivars are below 70 and six scored below 50. Only LCS Odyssey and Sirish had stirring numbers greater than 90. In Presque Isle, ME, 17 of 29 cultivars scored below 90, and 14 were below 70. In West Lafayette, IN, 21 of 28 varieties had scores below 90, and 13 were below 70. In Wooster, OH, 15 of 28 scored below 90. In contrast, three of the locations had a mean stirring number of more than 120. These included Alburg, VT, Orono, ME and University Park, PA.

Similarly, the data also suggest that some genotypes are more vulnerable to PHS while others appear to have resistance. When looking at the mean data for each genotype across different the locations, it can be seen that LCS Genie, LCS Odyssey and Sirish all had mean stirring numbers greater than 90, even at the Ithaca, NY and West Lafayette, IN locations. This indicates these three cultivars have a higher level of PHS resistance. However, several cultivars were seen that were especially sensitive to PHS. These included AAC Synergy, AC Metcalfe, CDC Meredith and Cerveza. All had low stirring number values at most locations and should be avoided if PHS is a potential risk in most years. In general, the PHS resistant cultivars were from European programs, while the most susceptible were from Canadian programs.

Therefore, for farmers in the areas with high a risk of PHS, such as Ithaca, NY, Presque Isle ME, Southwest, MI, West Lafayette IN, and Wooster OH, it is recommended that growers choose resistant genotypes. These include European cultivars such as, Sirish, KWS Beckie, KWS Fantex, LCS Genie, and LCS Odyssey; and the six-rowed cultivars Robust, Quest, and Tradition.

Table 9. Least Square Means for Stirring Number Across Years at Each Location

Genotype	Alburg, VT	Fargo, ND	Ithaca, NY	North- east, MI	Orono, ME	Presque Isle, ME	South west, MI	University Park, PA	Upper Peninsula, MI	West Lafayette, IN	Wooster, OH	<u>Ave</u>
2ND28065	166.6	118.7	48.6	107.3	169.7	112.7	74.4	145.8	138.4	72	71.1	111.4
2ND33757	157.8	111	60.4	102.7	170.2	125	128.7	124.7	154.6	80.8	93.5	119.0
2ND33760	113.1	107.5	68.6	93	167.3	115.8	121.4	152.9	161	85	112.1	118.0
2ND33821	106.5	104.9	32.6	99.6	114.1	5.2	108.1	151.4	75.3	48.2	75.6	83.8
AAC Synergy	85.7	71.3	23.7	54.9	105.2	10.8	41.2	84.3	54.8	49.9	45.3	57.0
AC Metcalfe	68.3	67.7	--	110.8	162.9	18.9	14.2	103.8	67.9	60.2	66.3	74.1
Acorn	145.4	144	52.5	159.6	143.1	105.1	144.6	117.2	169.2	94.5	110.3	126.0
Bentley	105.4	76.8	--	109	168.6	37.1	13.8	139.1	74.9	60.3	82.8	86.8
CDC Copeland	89.7	86.2	--	106.1	145.4	27.6	18.2	135.1	92	60.4	60.9	82.2
CDC Meredith	72.4	80.4	--	99.5	103.2	23.3	13.9	107	67.9	60.2	63	69.1
Cerveza	58.2	72.5	--	106.6	100.7	17.1	14.4	87.9	51.3	59.9	44.5	61.3
Conlon	98.2	81.1	13.9	100.6	99.6	46.6	82.2	118.6	116.4	72.5	72.3	82.0
ESMA	103.8	56.1	54.3	64.6	175.6	52.5	96.5	143.3	125.8	--	83.6	95.6
Explorer	156	137	62.1	135.4	126.1	58.4	138.1	151.5	118.2	45.5	76.5	109.5
Innovation	131.6	98.1	--	115.3	165.4	70.5	24.3	175.3	68.8	61.4	102.4	101.3
KWS Beckie	156.6	156.9	57.1	167.6	156.9	139.6	126.7	143.1	180.5	98.8	105.7	135.4
KWS Fantex	167.5	150.5	68.4	161.3	165.9	128.3	163.1	150.1	176	115.5	128.2	143.2
KWS Tinka	97.1	114.9	20.5	79.1	103.8	78.1	89.2	76.6	115.8	8.5	37.3	74.6
Lacey	173.4	106.3	--	132.4	157.1	87.4	48.4	153.7	103.2	63.9	112	113.8
LCS Genie	171.1	130.3	131	146.4	157.3	136.5	159.1	157.3	187.2	108.1	127.3	146.5
LCS Odyssey	144.7	132.9	91.6	153.9	138.1	136.6	159.6	131.6	164.8	93	104.4	131.9
ND Genesis	119.5	107.3	29.2	88.3	141.1	33.1	47.8	127.9	85.2	43.6	71.6	81.3
Newdale	108	85.4	24.4	83.8	153.8	19.6	53.9	136.2	89.5	79.7	63.9	81.7
Pinnacle	157.5	126	88.3	123.7	179	61.9	122.5	173	121.4	87.9	96.8	121.6
Quest	181.9	127.7	54.7	166.3	181.5	142.6	137.3	174.7	150.7	107.8	126	141.0
Robust	175.2	118.6	--	157.4	168.1	147.3	70.2	151	116.6	85.1	144.2	133.4
Sangria	111.3	98.6	61.7	81.1	136.3	69.6	127.5	140	112.8	50	82.8	97.4
Sirish	160.5	143.8	90.5	108.5	158	121	153.4	144.8	160.1	100.9	90	130.1
Tradition	188.2	134.1	47.9	136.2	187.3	133	115.9	164.8	127.7	84.2	125.6	131.4
<u>Ave</u>	130.0	108.5	56.3	115.6	148.3	78.0	90.0	136.6	118.2	72.8	88.8	

The correlation coefficients of stirring number between different locations are presented in Table 10. Compared to the protein correlation coefficients from table 5, the correlation of stirring number between different locations seems to be less stable, and this instability may come from variable weather and growing conditions. During the harvest season, wet and cold weather

during grain fill can promote PHS. Some of the locations have higher correlation values. For example, the correlation values for Alburg, VT with the other locations are ≥ 0.6 , while those for Orono, ME with the other locations are generally ≤ 0.5 .

Table 10. Correlations Between Locations for Stirring Number.

Location	Alburg, VT	Fargo, ND	Ithaca, NY	North-east, MI	Orono, ME	Presque Isle, ME	South west, MI	University Park, PA	Upper Peninsula, MI	West Lafayette, IN	Wooster, OH
Alburg, VT	1.00	0.80	0.59	0.68	0.63	0.83	0.65	0.70	0.70	0.56	0.80
Fargo, ND	0.80	1.00	0.50	0.74	0.30	0.77	0.77	0.43	0.77	0.64	0.61
Ithaca, NY	0.59	0.50	1.00	0.49	0.48	0.61	0.77	0.53	0.69	0.48	0.65
Northeast, MI	0.68	0.74	0.49	1.00	0.38	0.68	0.46	0.46	0.57	0.72	0.75
Orono, ME	0.63	0.30	0.48	0.38	1.00	-0.55	0.26	0.72	0.43	0.34	0.68
Presque Isle, ME	0.83	0.77	0.61	0.68	-0.55	1.00	0.69	0.46	0.86	0.60	0.79
Southwest, MI	0.65	0.77	0.77	0.46	0.26	0.69	1.00	0.39	0.87	0.43	0.55
University Park, PA	0.70	0.43	0.53	0.46	0.72	0.46	0.39	1.00	0.38	0.38	0.73
Upper Peninsula, MI	0.70	0.77	0.69	0.57	0.43	0.86	0.87	0.38	1.00	0.54	0.64
West Lafayette, IN	0.56	0.64	0.48	0.72	0.34	0.60	0.43	0.38	0.54	1.00	0.62
Wooster, OH	0.80	0.61	0.65	0.75	0.68	0.79	0.55	0.73	0.64	0.62	1.00

5.2.4. Barley Kernel Color

Seed color is an important trait for malt barley, in that dark color may indicate weathering and problems associated with microbes. Barley color tends to reflect the local weather conditions around harvest time. In the commercial trading of malt barley, bright and light color barley is desired by barley purchasers.

In the comparison of the average data of seed color based on different locations vs. different genotypes in Table 11, it can be seen that location seems to play a more significant role in determining seed color than genotype. For example, the biggest difference in kernel color was observed between Ithaca, NY (49.5) and northeast MI (54.8). Overall color of genotypes did not change dramatically within a specific location.

Table 11. Least Square Means for Barley Kernel Color Across Years at Each Location

<u>Genotype</u>	Alburgh,	Fargo,	Ithaca,	Northeast,	Orono,	Southwest,	University	Upper	<u>Ave</u>
	VT	ND	NY	MI	ME	MI	Park, PA	Peninsula, MI	
2ND28065	50.7	52.4	--	55.4	52.5	49.7	49.8	51.3	51.7
AAC Synergy	51.1	52.2	49.8	55.6	51.6	50.0	49.5	50.8	51.3
AC Metcalfe	51.5	53.0	50.2	55.5	52.5	--	50.2	51.2	52.0
Acorn	49.8	51.4	--	55.1	50.5	49.3	49.9	50.6	50.9
Bentley	51.5	52.5	50.2	55.7	52.4	--	50.7	51.4	52.1
CDC Copeland	51.3	53.0	49.1	55.9	52.3	--	50.8	51.2	51.9
CDC Meredith	50.1	51.9	48.5	55.5	51.2	--	49.2	50.9	51.0
Cerveza	51.4	52.7	49.9	55.8	52.2	--	50.2	50.9	51.9
Conlon	51.4	52.7	49.6	54.0	52.4	49.7	49.9	51.1	51.4
Explorer	49.8	51.4	--	53.4	50.7	49.7	49.5	50.1	50.7
Innovation	50.8	52.1	49.6	54.5	51.8	--	49.7	50.8	51.3
KWS Beckie	48.3	51.2	--	54.1	50.3	48.5	48.7	49.4	50.1
KWS Fantex	49.9	51.9	--	53.6	50.0	49.7	49.7	50.1	50.7
Lacey	51.1	52.0	50.2	55.0	52.0	--	50.4	51.3	51.7
LCS Genie	50.0	51.0	--	54.2	50.2	49.5	48.6	49.1	50.4
LCS Odyssey	49.0	51.6	--	54.5	50.2	49.2	49.2	49.8	50.5
ND Genesis	50.7	51.5	48.6	53.4	51.3	49.1	49.6	50.0	50.5
Newdale	50.9	52.2	49.4	54.5	51.8	49.8	49.3	50.5	51.1
Pinnacle	50.7	51.0	49.3	54.9	50.5	49.4	49.0	50.2	50.6
Quest	51.0	52.1	49.0	55.4	51.8	49.5	49.9	50.4	51.1
Robust	51.2	52.3	50.3	55.3	52.2	--	50.4	51.2	51.8
Sirish	51.1	52.4	--	55.1	51.3	49.5	49.5	51.0	51.4
Tradition	51.7	52.9	50.1	54.7	52.5	50.0	50.6	51.6	51.8
<u>Ave</u>	50.7	52.1	49.6	54.8	51.5	49.5	49.8	50.6	

The correlation coefficients for barley kernel color between different locations are shown in Table 12. Overall, correlation coefficients between locations were unstable. For example, the correlation value for Fargo, ND with Orono, ME was 0.9, while that for Fargo, ND with Ithaca, NY was 0.5. The correlation values for southwest, MI with Ithaca, NY was 0.9, while that for southwest MI with northeast, MI was only 0.3. This shows that the genotype of barley has little effect on the barley color, while environmental factors have a major influence.

Table 12. Correlations Between Locations for Barley Kernel Color

<u>Location</u>	Alburgh, VT	Fargo, ND	Ithaca, NY	North east MI	Orono, ME	South west, MI	University Park, PA	Upper Peninsula, MI
Alburgh, VT	1.00	0.76	0.75	0.48	0.84	0.74	0.71	0.78
Fargo, ND	0.76	1.00	0.47	0.53	0.86	0.63	0.77	0.83
Ithaca, NY	0.75	0.47	1.00	0.28	0.62	0.93	0.57	0.69
Northeast, MI	0.48	0.53	0.28	1.00	0.54	0.26	0.47	0.61
Orono, ME	0.84	0.86	0.62	0.54	1.00	0.54	0.75	0.85
Southwest, MI	0.74	0.63	0.93	0.26	0.54	1.00	0.54	0.63
University Park, PA	0.71	0.77	0.57	0.47	0.75	0.54	1.00	0.82
Upper Peninsula, MI	0.78	0.83	0.69	0.61	0.85	0.63	0.82	1.00

5.3. Malt Quality Traits

5.3.1. Malt Extract

Malt extract is a major economic concern for brewers because malting barley with higher malt extract can produce a greater amount of beer. Thin and high protein kernels potentially lead to the lower malt extract.

The mean malt extract at all locations, except northeast MI, was above 81% (table 13). The mean malt extract in Northeast, MI was 79.5%. This may be explained by relatively higher barley protein and lower plumpness at this location. Orono, ME had the highest average malt extract, with values up to up to 82.6%. Relatively low protein (9.3%) and plumpness up to 94.2% likely contributed to the greater malt extract.

The mean extract by genotype ranged from 80.3-82.7%. The six-row barley cultivars, Innovation, Lacey, Quest, Robust, and Tradition tended to be slightly lower in malt extract than two-row barleys. As mentioned previously, high malt extract is likely related to relatively lower protein and the high plumpness, and barley cultivars with the highest malt extract actually showed the lowest protein (<11%) and the greatest kernel plumpness (>90%). For example, the malt extract yield of the variety LCS Odyssey was up to 82.2%, while its average protein content

and plumpness were 10.5% and 94.0%. Overall, barley cultivars with the highest malt extract were AAC Synergy, Cerveza, Acorn, KWS Beckie, KWS Fantex, LCS Odyssey and LCS Genie. It is interesting to note that the later four are all European, and the first two are Canadian varieties.

Table 13. Least Square Means for Malt Extract Across Years at Each Location

Genotype	Alburg, VT	Fargo, ND	Ithaca, NY	North- east, MI	Orono, ME	South west, MI	University Park, PA	Upper Peninsula MI	<u>Ave</u>
2ND28065	82.4	81.6	--	80.1	82.5	81.1	81.5	81.4	81.5
AAC Synergy	82.1	82.2	81.9	80.8	83.1	81.2	81.7	81.9	81.9
AC Metcalfe	80.3	80.7	81.2	79.2	82.0	--	80.7	80.8	80.7
Acorn	82.2	82.3	--	81.2	84.3	82.4	82.9	83.4	82.7
Bentley	81.3	81.3	82.3	79.7	82.8	--	81.8	81.3	81.5
CDC Copeland	81.9	81.4	80.6	79.0	82.2	--	80.8	82.5	81.2
CDC Meredith	81.6	80.6	80.5	77.7	81.7	--	80.7	81.2	80.6
Cerveza	82.9	82.0	82.8	81.3	83.2	--	82.5	82.4	82.4
Conlon	80.7	80.9	81.9	78.4	82.2	80.1	81.1	81.0	80.8
Explorer	81.1	80.7	--	79.1	82.4	80.7	80.1	81.4	80.8
Innovation	80.3	80.8	81.4	78.0	82.1	--	81.0	80.2	80.5
KWS Beckie	81.3	82.1	--	80.4	84.1	82.0	81.4	82.2	81.9
KWS Fantex	84.2	80.8	--	80.4	83.9	81.3	81.9	83.0	82.2
Lacey	80.3	80.5	80.9	78.4	81.7	--	80.0	80.5	80.3
LCS Genie	82.5	82.4	--	80.3	83.6	81.0	82.0	82.9	82.1
LCS Odyssey	82.2	82.5	--	79.8	83.5	81.7	82.3	83.2	82.2
ND Genesis	82.3	82.1	82.2	80.4	82.5	81.7	81.9	82.3	81.9
Newdale	81.4	80.5	81.1	78.9	82.4	79.5	80.7	80.5	80.6
Pinnacle	81.0	81.7	81.0	80.7	81.1	81.0	81.1	80.8	81.1
Quest	80.4	80.3	80.1	78.7	81.9	80.2	80.8	80.4	80.4
Robust	79.7	80.5	81.1	78.7	81.2	--	80.0	81.0	80.3
Sirish	80.9	81.8	--	79.5	82.8	81.4	81.9	82.1	81.5
Tradition	80.7	80.2	81.2	78.4	81.5	79.8	81.0	80.4	80.4
<u>Ave</u>	81.5	81.3	81.3	79.5	82.6	81.0	81.3	81.6	

The correlation coefficients for malt extract between different locations are shown in table 14. The results suggest that effect of genotype on malt extract is unstable. The correlation coefficients for Orono, ME and University Park, PA with other locations were all above 0.7.

However, the correlation coefficients between other locations ranged from 0.5 to 0.8. This suggests that predicting malt extract performance across locations may be difficult.

Environmental, genotype, and perhaps, other factors likely impact the level of malt extract.

Table 14. Correlations Between Locations for Malt Extract

Locations	Alburg, VT	Fargo, ND	Ithaca, NY	North-east, MI	Orono, ME	South west MI	University Park, PA	Upper Peninsula MI
Alburg, VT	1.00	0.53	0.51	0.66	0.69	0.47	0.69	0.77
Fargo, ND	0.53	1.00	0.65	0.79	0.67	0.82	0.79	0.77
Ithaca, NY	0.51	0.65	1.00	0.62	0.69	0.50	0.79	0.48
Northeast MI	0.66	0.79	0.62	1.00	0.66	0.82	0.75	0.69
Orono, ME	0.69	0.67	0.69	0.66	1.00	0.69	0.77	0.81
Southwest MI	0.47	0.82	0.50	0.82	0.69	1.00	0.75	0.83
University Park, PA	0.69	0.79	0.79	0.75	0.77	0.75	1.00	0.75
Upper Peninsula MI	0.77	0.77	0.48	0.69	0.81	0.83	0.75	1.00

5.3.2. Malt Protein Parameters

5.3.2.1. Soluble/Total Protein Ration (Kolbach Index)

The ratio of soluble to total protein (S/T) can be an indicator of protein modification, and in turn, malt modification. Malt is traditionally considered as suitably modified when the value for S/T is between 38%-42% (Schwarz & Li, 2011). However, low values for total protein can lead to very high results for S/T and confound the interpretation. From the least square means shown in Table 16, we can see that average S/T values from most locations were over 42%, which is considered as suitably modified malt. There were even four locations with the average S/T values over 55%. This might be considered as very highly modified, but three of these locations had mean total protein below 11%. Only the location in northwest, MI, averaged below 40% (39.5) S/T. The relatively low S/T may indicate inadequate protein modification. Similarly, we showed that mean values for malt extract across years at northeast MI (Table 13) were lower than other locations. Mean total protein at this location was 11.8%

The average S/T values for each genotype showed great variability between different locations. For instance, the S/T of Newdale was 61.9% at Ithaca, NY, 52.5% at University Park, PA, 45.0% at Southwest, MI, and 39.7% at Northwest, MI. This indicates that environment has a strong role on S/T.

Table 15. Least Square Means for Soluble/Total Protein Across Years at Each Location

Genotype	Alburg, VT	Fargo, ND	Ithaca, NY	North-east, MI	Orono, ME	South west, MI	University Park, PA	Upper Peninsula, MI	<u>Ave</u>
2ND28065	62.6	54.5	--	39.1	57.9	44.0	47.9	42.8	49.8
AAC Synergy	60.4	61.2	63.0	43.0	61.8	45.9	54.1	46.1	54.4
AC Metcalfe	55.7	53.8	58.5	40.7	57.5	--	52.2	39.9	51.2
Acorn	56.5	53.6	--	34.3	54.2	37.8	51.5	41.8	47.1
Bentley	57.4	59.8	59.7	43.2	56.8	--	56.4	47.2	54.4
CDC Copeland	64.0	63.2	66.9	47.5	64.3	--	57.7	44.8	58.3
CDC Meredith	62.3	59.8	64.1	43.0	62.3	--	53.0	43.5	55.4
Cerveza	61.6	57.4	62.6	41.8	56.8	--	53.8	42.6	53.8
Conlon	53.2	52.9	50.3	38.7	49.2	40.0	44.4	38.3	45.9
Explorer	53.9	56.3	--	40.5	58.7	42.4	50.5	41.7	49.1
Innovation	49.3	52.9	49.4	40.6	50.8	--	50.7	43.5	48.2
KWS Beckie	57.3	55.4	--	34.6	61.2	37.9	55.2	42.2	49.1
KWS Fantex	61.2	57.1	--	34.8	57.7	39.6	53.7	45.2	49.9
Lacey	48.0	52.4	49.1	39.1	49.3	--	47.0	42.5	46.8
LCS Genie	60.2	60.5	--	39.4	60.7	42.2	52.1	44.8	51.4
LCS Odyssey	63.2	56.0	--	35.0	61.7	41.4	57.8	44.7	51.4
ND Genesis	57.3	58.2	56.2	38.5	55.8	45.1	49.3	42.4	50.4
Newdale	59.1	58.4	61.9	39.7	58.8	45.0	52.5	40.9	52.0
Pinnacle	52.8	56.8	50.1	40.6	49.3	44.6	48.2	42.1	48.1
Quest	52.7	51.6	46.5	37.9	49.1	42.0	49.1	42.5	46.4
Robust	51.0	55.2	48.4	40.3	51.5	44.8	49.5	44.5	48.2
Sirish	53.1	58.6	--	37.2	64.0	--	56.0	40.8	51.6
Tradition	48.0	49.8	45.1	38.2	46.7	42.9	47.1	41.6	44.9
<u>AVE</u>	56.6	56.3	55.5	39.5	56.4	42.4	51.7	42.9	

The calculated correlation coefficients for S/T between different locations are shown in Table 16. Environmental factors may play an important role in malt S/T while genotype has an unstable impact. For example, the correlation value for S/T at Fargo, ND with Ithaca, NY was

0.9, while the correlation value for Fargo, ND with Southwest, MI was 0.4. Correlation coefficients for the three MI locations, with all other locations were very low. The average data for S/T between different locations clearly shows that the environmental factors play an important role in S/T levels.

Table 16. Correlations Between Locations for Soluble/Total Protein

Locations	Alburgh, VT	Fargo, ND	Ithaca, NY	North-east, MI	Orono, ME	South west MI	University Park, PA	Upper Peninsula MI
Alburgh, VT	1.00	0.69	0.95	0.18	0.77	-0.05	0.61	0.40
Fargo, ND	0.69	1.00	0.88	0.53	0.78	0.38	0.66	0.52
Ithaca, NY	0.95	0.88	1.00	0.77	0.97	0.61	0.84	0.33
NE MI	0.18	0.53	0.77	1.00	0.17	0.83	0.14	0.29
Orono, ME	0.77	0.78	0.97	0.17	1.00	-0.04	0.81	0.32
SW MI	-0.05	0.38	0.61	0.83	-0.04	1.00	-0.20	0.23
University Park, PA	0.61	0.66	0.84	0.14	0.81	-0.20	1.00	0.53
UP MI	0.40	0.52	0.33	0.29	0.32	0.23	0.53	1.00

5.3.2.2. Free Amino Nitrogen (FAN)

FAN is largely a measurement of the free amino acids in wort. It is important as the value for soluble protein does not indicate the composition of amino acids in the wort. A minimum 150 mg/L FAN is generally considered necessary for proper yeast growth during fermentation. Values below 150mg/L may lead to poor fermentation and high diacetyl levels (Schwarz and Li, 2011). The American Malting Barley Association (AMBA, 2019) guidelines suggest FAN values of >210 for adjunct brewers and 140-190 for all-malt brewers. From previous experience, the NDSU micro-malting system is known to yield high values for FAN, so means should be viewed in a relative manner.

Free amino nitrogen was affected by both environmental factors and genotype. Fargo, ND had the maximum mean value among all locations (Table 17), while the mean value in Ithaca, NY was the lowest. The calculated correlation coefficients for FAN between different

locations are shown in Table 18. Correlation values are relatively high, suggesting that the genetics also have great influence on FAN.

Table 17. Least Square Means for Free Amino Nitrogen Across Years at Each Location

Genotype	North-			South		University Park, PA	Upper Peninsula MI	Ave	
	Alburg, VT	Fargo, ND	Ithaca, NY	east, MI	Orono, ME				west MI
2ND28065	244.2	272.8	--	186.1	224.3	188.5	208.9	213.7	219.8
AAC Synergy	263.4	276.9	219.4	211.5	226.7	251.5	263.4	224.1	242.1
AC Metcalfe	258.4	283.1	216.4	239.0	233.6	--	264.2	228.4	246.2
Acorn	230.7	255.2	--	177.8	191.9	196.3	222.8	189.0	209.1
Bentley	292.7	294.8	209.5	235.0	223.7	--	265.1	249.3	252.9
CDC Copeland	251.9	281.3	221.3	240.6	241.0	--	260.5	230.5	246.7
CDC Meredith	243.0	273.5	215.2	236.8	218.8	--	245.0	230.5	237.5
Cerveza	243.0	260.8	196.9	200.0	194.1	--	245.5	226.2	223.8
Conlon	251.0	252.9	189.5	185.8	215.4	187.2	215.8	203.6	212.7
Explorer	234.1	290.6	--	208.6	215.2	222.4	236.1	192.9	228.6
Innovation	229.6	314.3	191.2	208.3	227.3	--	250.2	242.3	237.6
KWS Beckie	227.3	280.1	--	184.4	202.4	181.4	232.9	197.2	215.1
KWS Fantex	230.7	258.8	--	175.9	201.5	212.5	225.0	207.3	216.0
Lacey	234.1	293.0	200.6	203.7	214.9	--	242.1	228.0	230.9
LCS Genie	253.0	290.8	--	201.1	226.9	231.8	246.6	206.3	236.6
LCS Odyssey	236.0	256.3	--	176.8	208.7	202.3	239.4	201.9	217.3
ND Genesis	223.6	240.5	174.2	169.6	213.7	196.4	212.6	193.8	203.1
Newdale	237.9	270.6	208.0	200.0	213.6	252.1	246.3	222.3	231.4
Pinnacle	211.4	243.0	166.5	173.1	189.0	191.0	211.7	189.2	196.9
Quest	255.1	291.0	187.8	200.6	225.4	239.4	235.2	232.0	233.3
Robust	206.3	329.6	200.6	204.1	217.5	--	246.7	239.0	234.8
Sirish	243.0	279.8	--	206.2	225.2	227.4	236.4	205.3	231.9
Tradition	220.2	285.9	178.6	197.5	211.2	209.5	227.5	219.8	218.8
Ave	240.0	277.2	198.4	201.0	215.7	212.6	238.3	216.2	

Table 18. Correlations Between Locations for Free Amino Nitrogen (FAN)

Locations	Alburg, VT	Fargo, ND	Ithaca, NY	North-east, MI	Orono, ME	Southwest, MI	University Park, PA	Upper Peninsula, MI
Alburg, VT	1.00	0.07	0.59	0.58	0.53	0.56	0.55	0.42
Fargo, ND	0.07	1.00	0.35	0.54	0.53	0.53	0.56	0.67
Ithaca, NY	0.59	0.35	1.00	0.86	0.65	0.81	0.88	0.63
Northeast, MI	0.58	0.54	0.86	1.00	0.70	0.76	0.82	0.72
Orono, ME	0.53	0.53	0.65	0.70	1.00	0.56	0.56	0.55
Southwest, MI	0.56	0.53	0.81	0.76	0.56	1.00	0.81	0.66
University Park, PA	0.55	0.56	0.88	0.82	0.56	0.81	1.00	0.70
Upper Peninsula, MI	0.42	0.67	0.63	0.72	0.55	0.66	0.70	1.00

5.3.3. Wort Beta-Glucan and Viscosity

High levels of beta-glucan in wort are undesirable to brewers as they can cause difficulties in both lautering and beer filtration by significantly increasing wort viscosity. Values under 100-200 mg/L are often desired for wort beta-glucan, as are wort viscosity values of <1.4-1.5 cP (Schwarz and Li, 2011). From previous experience, the NDSU micro-malting system is known to yield malts with higher beta-glucan levels. As such, relative ranks between genotypes should be compared.

A considerable range in mean beta-glucan values was observed (78.7- 451.0 mg/L; Table 19). However, wort viscosity only ranged from 1.4-1.6 cP. As such, beta-glucan is probably the more sensitive measurement. Results for the mean beta-glucan across years at each location (Table 20), suggest that genotype has a significant effect. For example, the average beta-glucan values across locations for Conlon and Pinnacle were all above 400 mg/L, while Explorer was under 100. This is not surprising, as high beta-glucan levels have been problematic in the NDSU two-row breeding program.

Barley cultivars Explorer and Sirish, with relatively low beta-glucans, had low wort viscosity (Table 21). The beta-glucan of barley cultivars Pinnacle and Tradition were 418.5 and

373.4, respectively, and their wort viscosities were 1.6. This phenomenon suggests beta-glucan and wort viscosity are positively correlated.

The correlation coefficients for beta-glucan from different locations are shown as shown in Table 20. The very high correlation coefficients strongly suggest that genotype has a great influence on the level of beta-glucan, while environmental factors are of less importance. However, there were three locations with high levels of beta-glucan including the northwest and upper peninsula sites of MI and Orono, ME. Barley grain from these locations may have lautering and filtering problems in the brewhouse.

Table 19. Least Square Means for Beta-Glucan Across Years at Each Location

Genotype	Alburg, VT	Fargo, ND	North-		Orono, ME	South		University Park, PA	Upper Peninsula, MI	<u>Ave</u>
			Ithaca, NY	east, MI		west, MI				
2ND28065	171.9	168.1	--	429.9	417.1	143.0	298.8	407.8	290.9	
AAC Synergy	168.2	64.9	129.4	199.8	274.7	115.3	81.6	295.1	166.1	
AC Metcalfe	191.3	78.4	159.2	237.5	272.5	--	90.4	534.8	223.4	
Acorn	117.5	83.8	--	389.3	250.5	135.1	172.7	369.8	217.0	
Bentley	225.7	96.5	147.2	363.2	355.7	--	103.7	355.1	235.3	
CDC Copeland	84.2	50.7	60.1	250.0	170.0	--	98.1	310.4	146.2	
CDC Meredith	109.1	145.2	137.0	417.6	245.1	--	214.5	572.6	263.0	
Cerveza	225.2	146.6	192.7	254.7	475.9	--	120.6	444.2	265.7	
Conlon	298.0	367.1	320.1	667.1	580.9	321.1	452.0	601.7	451.0	
Explorer	40.1	-19.6	--	108.4	146.3	19.2	126.4	129.9	78.7	
Innovation	277.1	218.8	357.8	458.4	409.9	--	335.6	330.9	341.2	
KWS Beckie	70.9	84.0	--	324.0	203.7	76.7	150.8	350.6	180.1	
KWS Fantex	130.3	80.7	--	376.4	277.0	152.7	156.8	516.8	241.5	
Lacey	191.0	162.5	310.5	307.3	314.5	--	234.5	112.0	233.2	
LCS Genie	114.8	23.6	--	233.1	183.4	76.3	159.7	376.0	166.7	
LCS Odyssey	74.2	95.2	--	258.9	273.3	120.9	123.0	361.9	186.8	
ND Genesis	249.1	246.2	280.6	581.7	535.4	205.2	323.4	357.1	347.3	
Newdale	198.9	99.3	112.8	258.9	341.5	86.4	165.0	438.1	212.6	
Pinnacle	383.9	253.4	376.9	642.7	524.6	254.6	404.0	508.0	418.5	
Quest	307.0	304.3	384.4	446.6	449.1	219.2	353.7	301.6	345.7	
Robust	273.8	180.7	351.1	387.3	355.0	--	326.8	318.2	313.3	
Sirish	81.6	17.4	--	258.9	186.3	39.8	107.5	265.2	136.7	
Tradition	343.3	282.7	393.5	515.2	477.8	195.9	450.1	328.7	373.4	
<u>Ave</u>	188.1	140.5	247.6	363.8	335.7	144.1	219.6	373.3		

Table 20. Correlations between Locations for Beta-Glucan

Locations	North-				South		University Park, PA	Upper Peninsula, MI
	Alburg, VT	Fargo, ND	Ithaca, NY	east, MI	Orono, ME	west, MI		
Alburg, VT	1.00	0.85	0.86	0.73	0.88	0.85	0.79	0.25
Fargo, ND	0.85	1.00	0.85	0.88	0.90	0.94	0.91	0.31
Ithaca, NY	0.86	0.85	1.00	0.70	0.70	0.76	0.89	-0.17
NE MI	0.73	0.88	0.70	1.00	0.81	0.92	0.87	0.45
Orono, ME	0.88	0.90	0.70	0.81	1.00	0.92	0.78	0.36
SW MI	0.85	0.94	0.76	0.92	0.92	1.00	0.86	0.68
University Park, PA	0.79	0.91	0.89	0.87	0.78	0.86	1.00	0.19
UP MI	0.25	0.31	-0.17	0.45	0.36	0.68	0.19	1.00

Table 21. Least Square Means for Wort Viscosity Across Years at Each Location

Genotype	North-				South		University Park, PA	Upper Peninsula, MI	<u>Ave</u>
	Alburgh, VT	Fargo, ND	Ithaca, NY	east, MI	Orono, ME	west, MI			
2ND28065	1.44	1.49	--	1.55	1.54	1.45	1.51	1.51	1.50
AAC Synergy	1.44	1.43	1.43	1.49	1.52	1.43	1.51	1.48	1.47
AC Metcalfe	1.48	1.46	1.42	1.51	1.46	--	1.48	1.57	1.48
Acorn	1.44	1.48	--	1.52	1.50	1.50	1.51	1.48	1.49
Bentley	1.50	1.44	1.44	1.50	1.56	--	1.47	1.50	1.49
CDC Copeland	1.40	1.46	1.39	1.49	1.45	--	1.47	1.52	1.45
CDC Meredith	1.46	1.44	1.41	1.54	1.47	--	1.50	1.57	1.48
Cerveza	1.48	1.47	1.44	1.52	1.58	--	1.50	1.52	1.50
Conlon	1.50	1.58	1.42	1.60	1.66	1.45	1.52	1.56	1.54
Explorer	1.42	1.42	--	1.45	1.45	1.42	1.52	1.43	1.45
Innovation	1.46	1.47	1.49	1.60	1.60	--	1.53	1.49	1.52
KWS Beckie	1.41	1.44	--	1.50	1.47	1.44	1.51	1.46	1.46
KWS Fantex	1.46	1.41	--	1.57	1.49	1.49	1.51	1.62	1.51
Lacey	1.43	1.45	1.46	1.54	1.51	--	1.45	1.43	1.47
LCS Genie	1.46	1.42	--	1.47	1.49	1.44	1.50	1.54	1.47
LCS Odyssey	1.43	1.44	--	1.49	1.48	1.42	1.48	1.50	1.46
ND Genesis	1.47	1.49	1.46	1.64	1.61	1.47	1.53	1.52	1.52
Newdale	1.43	1.43	1.41	1.47	1.53	1.44	1.47	1.48	1.46
Pinnacle	1.58	1.49	1.47	1.65	1.59	1.50	1.57	1.60	1.56
Quest	1.46	1.52	1.47	1.59	1.57	1.43	1.54	1.48	1.51
Robust	1.44	1.45	1.46	1.59	1.52	--	1.49	1.54	1.50
Sirish	1.41	1.41	--	1.51	1.45	1.44	1.48	1.43	1.45
Tradition	1.49	1.54	1.52	1.68	1.65	1.48	1.57	1.50	1.56
<u>Ave</u>	1.46	1.46	1.45	1.54	1.53	1.45	1.51	1.51	

Table 22. Correlations Between Locations for Wort Viscosity

Locations	Alburg, VT	Fargo, ND	Ithaca, NY	North- east, MI	Orono, ME	South west, MI	University Park, PA	Upper Peninsula, MI
Alburg, VT	1.00	0.28	0.20	0.55	0.64	0.40	0.50	0.63
Fargo, ND	0.28	1.00	0.35	0.50	0.52	0.40	0.21	0.23
Ithaca, NY	0.20	0.35	1.00	0.71	0.30	0.75	0.42	-0.35
NE MI	0.55	0.50	0.71	1.00	0.63	0.65	0.72	0.35
Orono, ME	0.64	0.52	0.30	0.63	1.00	0.27	0.47	0.32
SW MI	0.40	0.40	0.75	0.65	0.27	1.00	0.48	0.38
University Park, PA	0.50	0.21	0.42	0.72	0.47	0.48	1.00	0.22
UP MI	0.63	0.23	-0.35	0.35	0.32	0.38	0.22	1.00

5.3.4. Malt Enzymes

5.3.4.1. *Alpha-Amylase*

Alpha-amylase indicates the dextrinizing ability of malt, or the ability to breakdown starch to shorter chain dextrans, which allows the beta-amylase to convert them into sugars that yeast can use. Brewers tend to choose barley with the high levels of alpha-amylase for brewing adjunct beers, while lower levels are desired for all-malt brewing.

From the mean data for each genotype (Table 23), it can be seen that there was great variation. Barley cultivars with the high levels of alpha-amylase included AAC Synergy, AC Metcalfe, Cerveza, and Newdale. These barley cultivars are all from Canadian programs and were bred for use in the production of the high adjunct beer. Surprisingly, several of the six-row cultivars (Robust, Lacey and Tradition) had lower alpha-amylase values. European cultivars, which presumably are bred for all-malt production, showed a relatively wide range in alpha-amylase activity.

Location means ranged from 77 dextrinizing units (DU) at Orono, ME to 59.5 in Upper Peninsula, MI. Comparison of location averages for alpha-amylase to barley protein data (Table

4) suggested the relationship is not strong. However, protein data are from all samples, while the alpha-amylase values are for only those samples that were malted.

The correlation coefficients for alpha-amylase between different locations are shown in Table 24. The correlation of southwest, MI with other locations is low, while the correlation of Ithaca, NY with other locations is high. As such, it is hard to say which factor has the largest effect on the level of amylase in malt. In other words, environment factors and genotype seem to both affect the level of alpha-amylase in malt.

Table 23. Least Square Means for Alpha-Amylase Across Years at Each Location

Genotype	Alburg, VT	Fargo, ND	Ithaca, NY	North-east, MI	Orono, ME	South west, MI	University Park, PA	Upper Peninsula, MI	<u>Ave</u>
2ND28065	67.3	73.5	--	75.0	69.8	80.6	55.8	54.4	68.1
AAC Synergy	83.4	82.1	77.1	74.0	87.3	82.9	76.2	76.3	79.9
AC Metcalfe	87.7	78.8	83.8	80.3	87.4	--	76.0	65.7	80.0
Acorn	73.7	76.2	--	64.5	85.7	61.5	66.5	49.2	68.2
Bentley	81.5	81.3	75.3	67.8	75.6	--	80.5	70.3	76.0
CDC Copeland	80.6	74.8	74.5	66.3	80.3	--	74.6	66.3	73.9
CDC Meredith	78.5	76.1	75.1	62.4	77.6	--	71.2	56.7	71.1
Cerveza	81.7	83.8	80.0	73.3	80.1	--	80.3	70.3	78.5
Conlon	81.6	75.5	68.9	61.5	70.3	67.5	67.8	63.4	69.6
Explorer	71.5	92.2	--	61.5	95.6	76.6	69.1	59.9	75.2
Innovation	64.7	75.9	62.9	56.9	83.2	--	63.9	59.3	66.7
KWS Beckie	70.0	73.0	--	63.9	78.3	50.6	61.5	48.9	63.7
KWS Fantex	73.1	67.5	--	80.1	78.2	55.3	63.0	53.5	67.2
Lacey	65.4	69.6	59.7	64.8	70.4	--	60.5	59.5	64.3
LCS Genie	69.8	75.6	--	64.7	75.0	62.1	65.5	50.1	66.1
LCS Odyssey	83.0	78.7	--	66.8	84.9	80.9	67.8	51.6	73.4
ND Genesis	70.8	76.4	67.7	66.1	76.6	69.6	69.3	56.7	69.2
Newdale	90.7	81.7	80.1	77.2	82.4	83.1	80.1	71.8	80.9
Pinnacle	56.9	69.9	53.8	55.8	61.3	79.3	56.8	50.2	60.5
Quest	69.8	74.0	62.2	62.1	71.8	69.3	65.4	64.2	67.4
Robust	63.8	63.3	53.8	48.2	55.5	--	55.2	54.1	56.3
Sirish	81.6	74.6	--	81.6	78.8	61.6	61.7	53.9	70.5
Tradition	63.2	76.4	61.7	59.4	65.5	65.2	61.2	61.1	64.2
<u>Ave</u>	74.4	76.1	69.1	66.7	77.0	69.7	67.4	59.5	

Table 24. Correlations Between Locations for Alpha-Amylase

Locations	Alburg, VT	Fargo, ND	Ithaca, NY	North-east, MI	Orono, ME	South west, MI	University Park, PA	Upper Peninsula, MI
Alburg, VT	1.00	0.51	0.94	0.66	0.59	0.23	0.81	0.57
Fargo, ND	0.51	1.00	0.84	0.26	0.75	0.48	0.70	0.51
Ithaca, NY	0.94	0.84	1.00	0.89	0.84	0.46	0.95	0.76
Northeast, MI	0.66	0.26	0.89	1.00	0.48	0.01	0.42	0.30
Orono, ME	0.59	0.75	0.84	0.48	1.00	0.12	0.60	0.26
Southwest, MI	0.23	0.48	0.46	0.01	0.12	1.00	0.37	0.51
University Park, PA	0.81	0.70	0.95	0.42	0.60	0.37	1.00	0.76
Upper Peninsula, MI	0.57	0.51	0.76	0.30	0.26	0.51	0.76	1.00

5.3.4.2. Diastatic Power (DP)

Diastatic power (DP) is a measurement of the activity of the malt enzymes to convert starch to fermentable sugars, and is believed to mainly reflect beta-amylase activity (Schwarz and Li, 2011). As such, some brewers believe it is an estimate of fermentability. However, high DP in malts, can also be influenced by high alpha-amylase. Levels that are too high or too low may cause problems in the brewhouse. High DP may cause too rapid of conversion, so that malt is difficult to handle for brewers. This is more often the case for all-malt brewers. However, malt with the low DP levels can a long take long time to produce fermentable sugars in mashing.

Examination of the mean genotype data in the table 25, shows that six-row barley obviously has higher levels of DP than two-row barley. The DP level of the five six-row barley cultivars was above 110, while most of two-row barleys were under 100. However, some high DP two-row barleys were found, including AC Metcalfe, Conlon, Lacey, and LCS Genie. When the mean data by location are examined, it can be observed that the average DP level in Northwest, MI was up to 139.1 High protein and the low malt extract were also seen at this location. Overall, the data shows a positive relationship between DP and barley protein content.

The correlation coefficients for DP between different locations are shown in the table 26, Overall, the relatively high correlation coefficients show that the genotype of barley has an effect on the DP level in malt. However, environmental factors also affect the DP level. The correlation coefficient between Northwest, MI and any other locations was very low, while the correlation coefficient between Alburg, VT and other locations were all above 0.7.

Table 25. Least Square Means for Diastatic Power (DP) Across Years at Each Location

Genotype	Alburg, VT	Fargo, ND	North				University Park, PA	Upper Peninsula MI	<u>Ave</u>
			Ithaca, NY	east MI	Orono, ME	Southw est MI			
2ND28065	76.2	121.1	--	124.5	82.9	88.0	90.8	79.7	94.7
AAC Synergy	89.7	102.5	67.8	107.9	78.4	122.0	98.9	85.8	94.1
AC Metcalfe	120.2	122.8	93.2	123.2	105.2	--	119.0	86.5	110.0
Acorn	81.9	122.2	--	114.4	74.7	100.7	95.3	61.9	93.0
Bentley	82.9	105.9	63.5	128.2	78.3	--	106.4	80.5	92.2
CDC Copeland	87.6	100.0	73.6	143.4	85.2	--	121.5	78.3	98.5
CDC Meredith	75.2	104.6	83.5	145.7	82.6	--	120.3	78.8	98.7
Cerveza	83.7	109.0	65.1	158.2	83.3	--	118.3	75.2	99.0
Conlon	98.7	111.2	78.8	181.6	96.8	110.3	128.4	87.1	111.6
Explorer	84.2	121.7	--	111.6	73.0	104.6	94.3	73.1	94.6
Innovation	89.3	153.5	83.4	151.5	129.5	--	131.6	108.2	121.0
KWS Beckie	81.3	122.9	--	122.7	73.6	107.7	91.9	62.5	94.7
KWS Fantex	71.1	113.8	--	118.1	75.5	115.2	90.1	66.4	92.9
Lacey	105.7	148.4	81.3	112.6	114.9	--	129.0	112.6	114.9
LCS Genie	107.2	130.1	--	141.6	90.8	147.2	118.6	74.7	115.7
LCS Odyssey	74.2	113.4	--	151.4	75.9	99.8	88.9	68.8	96.1
ND Genesis	73.2	85.8	61.0	175.3	85.1	91.3	93.5	74.4	92.5
Newdale	89.1	104.7	71.5	121.2	85.3	146.4	119.4	89.4	103.4
Pinnacle	74.4	89.2	43.7	166.7	85.3	86.0	76.8	74.6	87.1
Quest	102.6	150.4	78.1	154.4	111.3	135.0	119.1	116.2	120.9
Robust	125.2	164.4	86.6	167.7	138.7	--	132.4	93.7	129.8
Sirish	84.1	127.6	--	133.4	77.7	113.8	94.4	81.1	101.7
Tradition	124.6	174.3	98.7	143.0	127.9	137.0	131.8	126.9	133.0
<u>Ave</u>	90.5	121.7	75.3	139.1	91.8	113.7	109.2	84.2	

Table 26. Correlations Between Locations for Diastatic Power

Locations	Alburg, VT	Fargo, ND	Ithaca, NY	North-east, MI	Orono, ME	South-west, MI	University Park, PA	Upper Peninsula, MI
Alburg, VT	1.00	0.72	0.78	0.08	0.79	0.74	0.73	0.67
Fargo, ND	0.72	1.00	0.75	-0.04	0.79	0.52	0.56	0.71
Ithaca, NY	0.78	0.75	1.00	-0.19	0.68	0.73	0.85	0.65
Northeast, MI	0.08	-0.04	-0.19	1.00	0.34	-0.18	0.23	0.13
Orono, ME	0.79	0.79	0.68	0.34	1.00	0.49	0.75	0.82
Southwest, MI	0.74	0.52	0.73	-0.18	0.49	1.00	0.77	0.55
University Park, PA	0.73	0.56	0.85	0.23	0.75	0.77	1.00	0.70
Upper Peninsula, MI	0.67	0.71	0.65	0.13	0.82	0.55	0.70	1.00

5.4. Cluster Analysis Within Locations Using Barley and Malt Quality Traits

To help in visualizing the overall malt quality of the 23 malted genotypes at the five locations (Alburgh VT, Fargo ND, Orono ME, University Park PA, Upper Peninsula, MI) that had similar barley and malt quality, hierarchical cluster analysis was performed, and the results are showed as constellation plots (Figures 1-5). Also, the means across genotypes within each cluster are shown in Tables 27-31. The named cultivars in the analysis included nine from Canada, nine from the USA and five from Europe. In the cluster analyses, we grouped the 23 genotypes into four clusters at each location. The similarity of genotypes within the same cluster group is higher than between different clusters.

Malt barley buyers tend to purchase barley with no PHS, high levels of malt extract, and lower levels of beta-glucan because these attributes contribute to the high quality of raw materials for craft beer. Other considerations include carbohydrate degrading enzymes, wort viscosity, and the ratio of soluble protein to total protein (S/T). In the cluster analysis, the relatively best cluster group was selected at each location, and the similarity among barley genotypes in the cluster was analyzed.

5.4.1. Alburg, VT

The four cluster groups for Alburgh, VT are shown in Figure 1. All groups had kernels plumpness over 90%, and malt extract around 81% (Table 27). In order to select the best cluster among the four, cluster three can be excluded due to low resistance to PHS. Clusters one and four had wort beta-glucan levels over 100, and the value of wort viscosity in cluster four was up to 1.5. The FAN and alpha-amylase levels in cluster three, were the highest, and as such these genotypes may be less desirable to craft brewers. Overall, barley genotypes in cluster two showed the best quality for craft brewers. The barley genotypes in cluster two are four European varieties (LCS Odyssey, KWS Beckie, Sirish, and Explorer) and two Canadian varieties (CDC Meredith and CDC Copeland). Compared to other clusters, these genotypes had lower DP and wort β -glucan values.

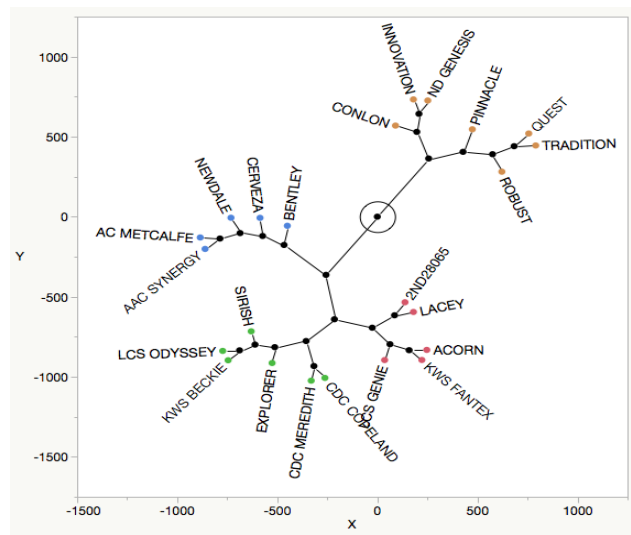


Figure 1. Constellation plot from the hierarchical clustering of barley and malt quality data obtained from 23 barley genotypes (calculated LS means both of barley and malt quality) evaluated for two or more years in Alburg, VT (2015-2017). Four clusters were identified and lines falling within a similar cluster are the same color. Cluster 1 begins in the bottom right hand corners and the numbering of clusters proceeds counterclockwise. Cluster 1 = 2ND28065, Lacey, Acorn, KWS Fantex, LCS Genie; Cluster 2 = Sirish, LCS Odyssey, KWS Beckie, Explorer, CDC Meredith, and CDC Copeland; Cluster 3 = AAC Synergy, AC Metcalfe, Newdale, Cerveza and Bentley; and Cluster 4 = Conlon, Innovation, ND Genesis, Pinnacle, Quest, Tradition and Robust.

Table 27. Mean Barley and Malt Quality Across Barley Lines Within a Cluster in Alburg, VT

Cluster	Count	Plump (%)	Malt protein (%)	Malt extract (%)	Wort protein (%)	S/T (%)	DP (°ASBC)	Alpha-amylase (20o DU)	Beta-glucan (ppm)	Wort viscosity (mPa.s)	FAN (ppm)	TW (lb/bu)	Protein (%)	Stirring number SN§	DON (ppm)
1	5	93.6	9.7	82.3	5.5	57.7	88.4	69.9	145.1	1.4	238.5	47.6	10.5	164.8	0.6
2	6	91.5	9.6	81.5	5.6	59.0	81.1	77.5	76.7	1.4	239.2	45.9	10.5	130.0	0.5
3	5	91.2	10.4	81.6	6.1	58.8	93.1	85.0	201.9	1.5	259.1	47.5	11.1	85.1	0.7
4	7	92.0	10.6	80.7	5.5	52.0	98.3	67.3	304.6	1.5	228.2	46.9	11.2	150.3	1.2

5.4.2. Fargo, ND

Results of cluster analysis for Fargo, ND are presented in Figure 2 and Table 28. The stirring numbers of clusters one and three were under 110, which indicates that barley genotypes in these clusters showed signs of PHS. High beta-glucan content and high wort viscosity seen in genotypes within clusters one and two, which indicates that these genotypes may cause filtration problems during brewing. Genotypes from cluster four likely may be the best choice for craft brewers, due to high kernel plumpness, moderate DP, low wort beta-glucan, and high resistance to PHS. Barley cultivars in cluster four included Explorer, LCS Genie and Sirish, which are from European programs.

LCS Genie and Sirish fell into a sub-cluster within cluster four. These two cultivars had great similarities in barley and malt quality in Fargo, ND. This was also observed at other locations. For instance, Sirish and LCS Genie were in a sub-cluster within cluster four in Orono, ME (Figure 3).

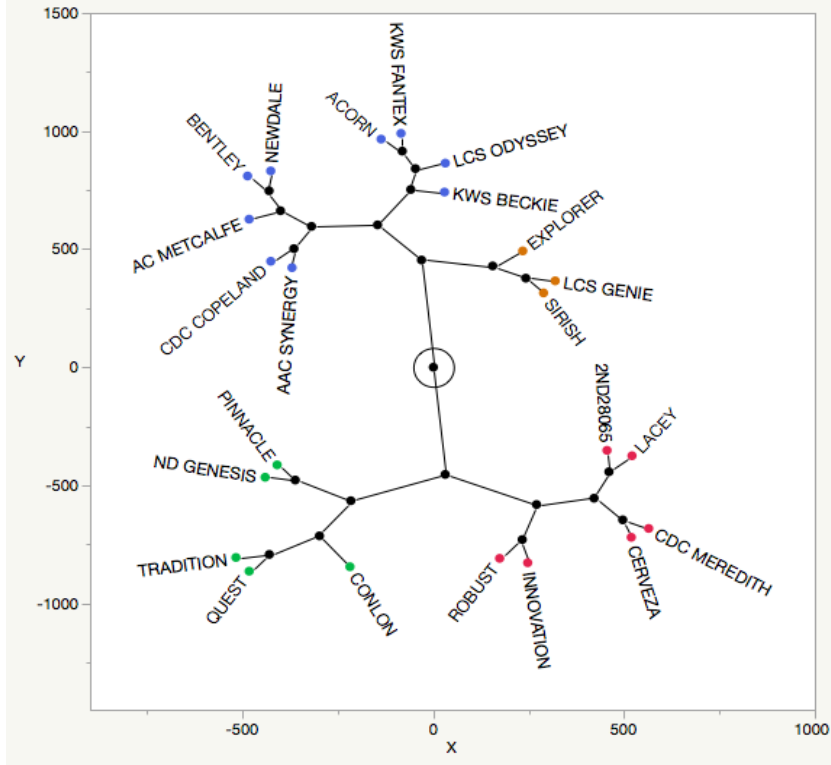


Figure 2. Constellation plot from the hierarchical clustering of barley and malt quality data obtained from 23 barley genotypes (calculated LS means both of barley and malt quality) evaluated for two or more years in Fargo, ND (2015-2017). Four clusters were identified and lines falling within a similar cluster are the same color. Cluster 1 begins in the bottom right hand corners and the numbering of clusters proceeds counterclockwise. Cluster 1 = 2ND28065, Lacey, CDC Meredith, Cerveza, Innovation, and Robust; Cluster 2 = Conlon, Quest, Tradition, ND Genesis and Pinnacle; Cluster 3 = CDC Copeland, AC Metcalfe, Bentley, Newdale, Acorn, KWS Fantex, LCS Odyssey and KWS Beckie; Cluster 4 = Explorer, LCS Genie and Sirish;

Table 28. Mean Barley and Malt Quality Across Barley Lines Within a Cluster in Fargo, ND

Cluster	Count	Plump (%)	Malt protein (%)	Malt extract (%)	Wort protein (%)	S/T (%)	DP (°ASBC)	Alpha-amylase (20o DU)	Beta-glucan (ppm)	Wort viscosity (mPa.s)	FAN (ppm)	TW (lb/bu)	Protein (%)	Stirring number SN§	DON (ppm)
1	6	87.1	12.1	81.0	6.7	55.4	133.5	73.7	170.3	1.5	290.7	51.1	12.0	99.1	0.0
2	5	89.0	11.4	81.0	6.1	53.9	122.2	74.4	290.7	1.5	262.7	50.8	11.4	115.2	0.1
3	9	87.7	11.0	81.5	6.4	57.6	112.0	77.1	81.5	1.4	273.0	50.3	11.0	108.0	0.1
4	3	89.3	11.6	81.6	6.5	58.5	126.5	80.8	7.1	1.4	287.1	51.2	11.2	137.0	0.1

5.4.3. Orono, ME

Results of cluster analysis for Orono, ME are presented in Figure 3 and Table 29. In this location, the values for stirring number in the four clusters were all over 130, suggesting that PHS was not an issue. All four clusters showed beta-glucan contents over 100, and also high wort viscosity. However, beta-glucan content of cluster four was considerably lower than the other three clusters. This observation along with high malt extract and low DP suggest genotypes in this cluster may be best for craft brewers.

Cluster four included four European cultivars, LCS Genie, KWS Beckie, Sirish, and CDC Copeland from Canada. As observed in Fargo, LCS Genie and Sirish fell into a sub-cluster, and these two cultivars had more similarity in barley and malt quality than other cultivars in this cluster.

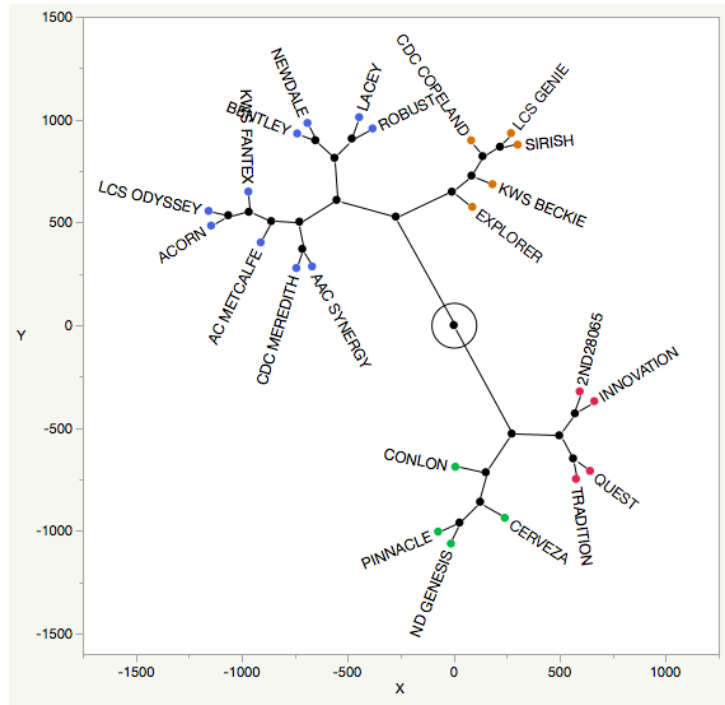


Figure 3. Constellation plot from the hierarchical clustering of barley and malt quality data obtained from 23 barley genotypes (calculated LS means both of barley and malt quality) evaluated for two or more years in Orono, ME (2015-2017). Four clusters were identified and lines falling within a similar cluster are the same color. Cluster 1 begins in the bottom right hand corners and the numbering of clusters proceeds counterclockwise. Cluster 1 = 2ND28065, Innovation, Quest, and Tradition; Cluster 2 = Conlon, Pinnacle, ND Genesis and Cerveza; Cluster 3 = AAC Synergy, CDC Meredith, AC Metcalfe, Acorn, LCS Odyssey, KWS Fantex, Bentley, Newdale, Lacey and Robust; Cluster 4 = CDC Copeland, LCS Genie, Sirish, KWS Beckie and Explorer;

Table 29. Mean Barley and Malt Quality Across Barley Lines Within a Cluster in Orono, ME

Cluster	Count	Plump (%)	Malt protein (%)	Malt extract (%)	Wort protein (%)	S/T (%)	DP (°ASBC)	Alpha-amylase (20o DU)	Beta-glucan (ppm)	Wort viscosity (mPa.s)	FAN (ppm)	TW (lb/bu)	Protein (%)	Stirring number SN§	DON (ppm)
1	4	94.5	10.0	82.0	5.1	51.1	112.9	72.6	438.5	1.6	222.1	50.7	10.4	176.0	0.0
2	4	91.9	9.5	82.3	5.0	52.8	87.6	72.1	529.2	1.6	203.1	51.3	9.8	130.1	0.0
3	10	94.7	9.0	82.7	5.1	57.2	91.0	78.5	296.0	1.5	215.1	50.3	9.3	146.6	0.0
4	5	94.9	8.4	83.0	5.2	61.8	80.1	81.6	177.9	1.5	222.1	49.0	8.7	148.7	0.0

5.4.4. University Park, PA

Results of cluster analysis for University Park, PA are presented in Figure 4 and Table 30. In this location, values of DON (Table 30) in four clusters were significantly above what is considered acceptable (<1ppm). DON was detected in the University Park samples in all three years, and the average value for 2015 was 2.9 mg/Kg. Clearly, the management of FHB is an important issue when producing barley in this region.

Compared to other locations, the level of beta-glucans in all four clusters in University Park were all below 100. Due to high stirring numbers, acceptable malt extract, and moderate levels of DP, genotypes from cluster four would be the best choice among the four cluster groups. All barley genotypes in cluster 4 are two-rowed and from Canada (Bentley, CDC Copeland and Newdale). There was also an interesting phenomenon, in that the beta-glucan of the four cluster was around 70, but the wort viscosities were up to 1.5. The reason for this observation is not clear, but FHB infection can sometimes confound the results of malt analyses.

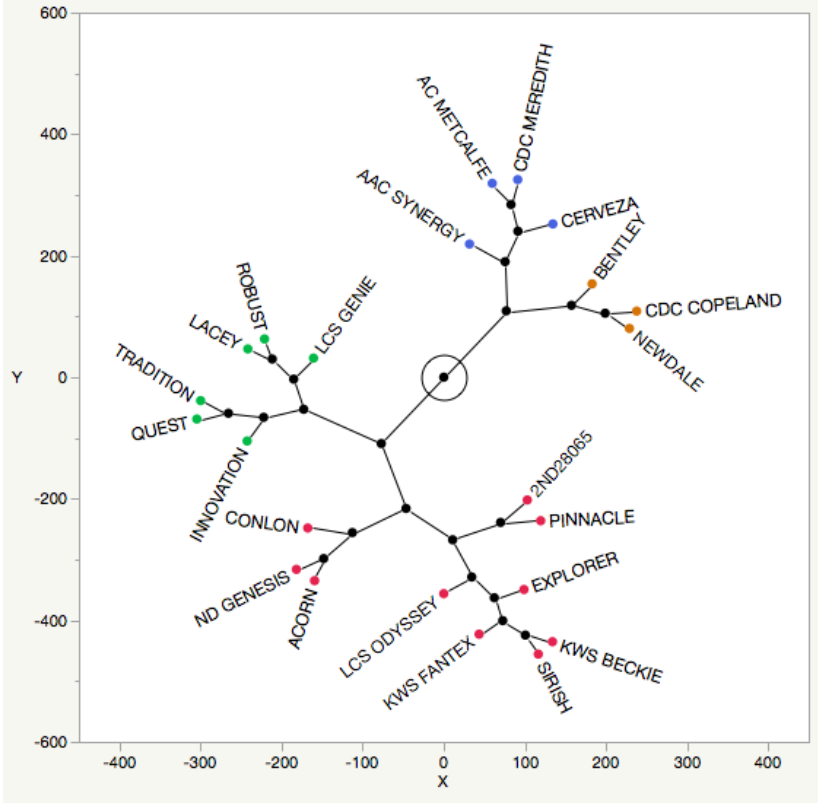


Figure 4. Constellation plot from the hierarchical clustering of barley and malt quality data obtained from 23 barley genotypes (calculated LS means both of barley and malt quality) evaluated for two or more years in University Park, PA (2015-2017). Four clusters were identified and lines falling within a similar cluster are the same color. Cluster 1 begins in the bottom right hand corners and the numbering of clusters proceeds counterclockwise. Cluster 1 = 2ND28065, Pinnacle, Explorer, KWS Beckie, Sirish, KWS Fantex, LCS Odyssey, ND Genesis. Acorn and Conlon; Cluster 2 = Innovation, Quest, Tradition, Lacey, Robust and LCS Genie; Cluster 3 = AAC Synergy, AC Metcalfe, Acorn, CDC Meredith and Cerveza; Cluster 4 = Bentley, CDC Copeland and Newdale;

Table 30. Mean Barley and Malt Quality Across Barley Lines Within a Cluster in University Park, PA.

Cluster	Count	Plump (%)	Malt protein (%)	Malt extract (%)	Wort protein (%)	S/T (%)	DP (°ASBC)	Alpha-amylase (20o DU)	Beta-glucan (ppm)	Wort viscosity (mPa.s)	FAN (ppm)	TW (lb/bu)	Protein (%)	Stirring number SN§	DON (ppm)
1	10	88.3	10.6	81.6	5.4	51.5	94.4	63.9	63.9	1.5	224.2	46.5	10.6	140.4	1.5
2	6	84.4	11.8	80.8	5.8	49.3	127.1	62.0	62.0	1.5	241.4	47.7	11.8	162.8	1.3
3	4	86.0	11.4	81.4	6.1	53.3	114.1	75.9	75.9	1.5	254.5	47.3	11.4	95.8	1.4
4	3	83.7	11.2	81.1	6.2	55.5	115.8	78.4	78.4	1.5	257.3	47.6	11.2	136.8	1.5

5.4.5. Upper Peninsula, MI

Results of cluster analysis for Upper Peninsula, MI are presented in Figure 5 and Table 31. The kernel plumpness in the four cluster for Upper Peninsula, MI ranged from 92.1 to 96.5%, which is very good. However, all four clusters had beta-glucan contents in excess of 200. Genotypes from cluster three may be the best for this location due to relatively lower beta-glucan and wort viscosity. However, genotypes in cluster 3 did show some sign of PHS. This location had severe spout damage in 2016. The two barley genotypes in cluster three were Lacey and Explorer. Explorer is a 2-row from Europe and Lacey a six-row from the USA.

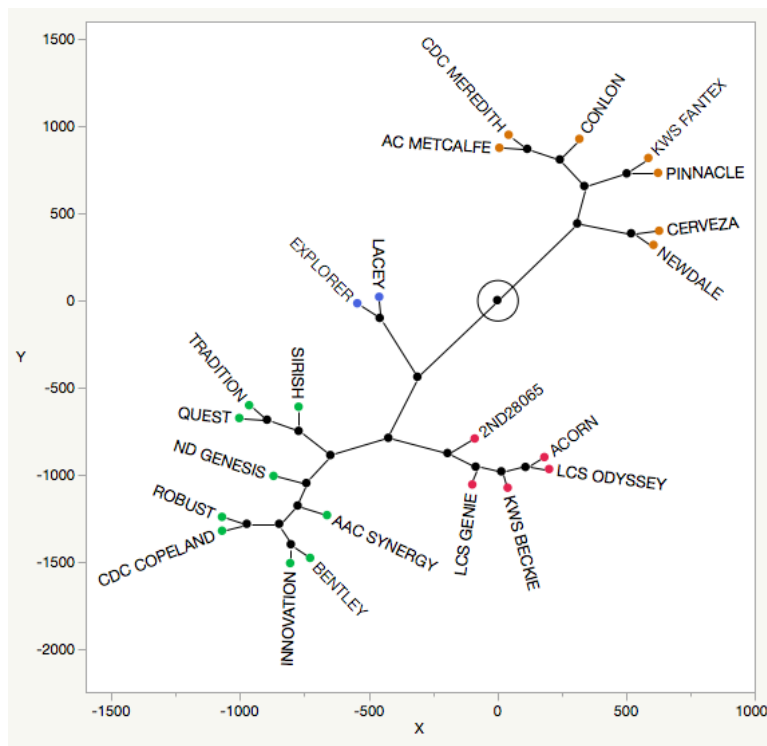


Figure 5. Constellation plot from the hierarchical clustering of barley and malt quality data obtained from 23 barley genotypes (calculated LS means both of barley and malt quality) evaluated for two or more years in Upper Peninsula, MI (2015-2017). Four clusters were identified and lines falling within a similar cluster are the same color. Cluster 1 begins in the bottom right hand corners and the numbering of clusters proceeds counterclockwise. Cluster 1 = 2ND28065, Acorn, KWS Beckie, LCS Odyssey, LCS Genie; Cluster 2 = AAC Synergy, Bentley, Innovation, CDC Copeland, Robust, ND Genesis, Quest, Tradition, Sirish; Cluster 3 = Explorer and Lacey; Cluster 4 = AC Metcalfe, CDC Meredith, Conlon, KWS Fantex, Pinnacle, Cerveza and Newdale;

Table 31. Mean Barley and Malt Quality Across Barley Lines Within a Cluster in Upper Peninsula, MI.

Cluster	Count	Plump (%)	Malt protein (%)	Malt extract (%)	Wort protein (%)	S/T (%)	DP (°ASBC)	Alpha-amylase (20o DU)	Beta-glucan (ppm)	Wort viscosity (mPa.s)	FAN (ppm)	TW (lb/bu)	Protein (%)	Stirring number SN§	DON (ppm)
1	5	95.5	10.0	82.6	4.3	43.3	69.5	50.8	373.2	1.5	201.6	48.0	10.7	168.0	0.1
2	9	94.8	11.1	81.3	4.9	43.7	93.9	62.5	318.0	1.5	226.2	48.8	11.7	103.4	0.0
3	2	96.5	10.8	81.0	4.6	42.1	92.9	59.7	121.0	1.4	210.5	48.9	11.8	110.7	0.0
4	7	92.1	11.0	81.4	4.5	41.8	79.7	61.7	516.6	1.6	215.4	48.2	11.5	98.6	0.0

6. SUMMARY AND CONCLUSIONS

Barley and malt quality are important factors in identifying barley cultivars that are suitable for local production. Of particular importance for the eastern regions is the selection of barley with high resistance to PHS. High kernel plumpness and malt extract and lower wort beta-glucans are also important. Ideally, cultivars meeting these criteria should also have moderate DP and lower levels of FAN. In general, cultivars from Europe had better resistance to PHS and the lower beta-glucan levels when compared to two-rowed cultivars developed by North American programs. While the six-row barley cultivars also had good resistance to PHS, beta-glucan levels were higher and malt extract values were lower. Some barley cultivars with high resistance to PHS included KWS Fantex, LCS Genie, LCS Odyssey, Quest, and Sirish.

We found that genotype had a great influence on the protein and beta-glucan contents, while environmental factors had lesser effects. The protein content of all six-row barley cultivars in this experiment showed undesirable high average protein contents across the eleven locations.

Barley cultivars with higher malt extract were AAC Synergy, Cerveza, Acorn, KWS Beckie, KWS Fantex, LCS Odyssey and LCS Genie. It is interesting to note that the later five are all European, and the first two are Canadian cultivars. However, the Canadian cultivars were, prone to PHS. As such, the cultivars Explorer, LCS Genie, LCS Odyssey, KWS Fantex, and KWS Beckie can be as recommended for many of the ESNB locations because of their good barley and malt quality, and resistance to PHS.

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