Investigation of winter wheat sowing date management and genetic architecture of winter malting quality in barley and milling/baking performance in soft red winter wheat

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ACADEMIC ABSTRACT

Wheat (*Triticum aestivum*, L) and barley (*Hordeum vulgare*) are widely grown as winter annual grains in a double crop rotation with soybean (*Glycine max,* L. Merr.) in much of the U.S. Improved management strategies and the development cultivars that meet the quality requirements of higher value end-use markets is important to increase production and profitability of winter annual grains and the double crop rotation in the Eastern U.S. In Chapter I, fifteen commercially relevant winter wheat genotypes ranging in maturity were sown in a split-plot design (sowing date=main plot, genotype=subplot) at three different sowing dates (considered to be 'very early' (20-28 days before recommended), 'early (6-11 days before recommended)', or 'recommended') and replicated three times at eight environments (site-year) from 2015-2018 in VA and KY. Grain yield, tiller estimation, heading date, protein, and 1000-kernel weight were assessed for each yield plot. At all environments, sowing earlier in the fall achieved an earlier (P<0.05) heading date, while grain yields varied depending on environment and genotype. Genotype by sowing date interactions were non-significant (P<0.05) at five site-years and significant (P<0.05) at three site-years.

Molecular markers can be associated with phenotypic traits via quantitative trait loci (QTL) mapping, these markers can be used by breeders in marker assisted selection (MAS) to indirectly select phenotypic traits that are difficult or expensive to measure. In Chapter II, the genetic architecture of end-use quality is investigated in two soft red winter wheat bi-parental (Pioneer '25R47' / 'Jamestown' and Pioneer '26R46' / 'Tribute'). Both populations were genotyped with a public 90,000 wheat iSelect SNP-Array, grown over two crop seasons at two Virginia sites, evaluated for quality traits at the USDA-ARS Soft Wheat Quality Lab (SWQL), and analyzed with QTL mapping. This chapter describes a total of 24 putative QTL that were identified on 13 different chromosomes and associated with grain characteristics, milling, and/or baking performance along with phenotypic data for both populations, other putative QTL, and transgressive progeny with exceptional flour yield and cookie diameters. A region on 3A (Qfy.vt.3A.Jtwn) is a strong candidate to be utilized for MAS in soft red winter wheat breeding programs as it explained 6.9-10.3% (Pioneer 25R47 / Jamestown) and 4.6-17.0% (Pioneer 26R46 / Tribute) of the phenotypic variation for flour yield. In Chapter III, malt quality genetic structure was investigated in two winter 'malt x feed' doubled haploid barley breeding populations. Both populations were genotyped with the iSelect InfiniumTM SNP assay consisting of 50,000 barley SNPs, grown in two to three Virginia environments (Blacksburg and Warsaw) during 2017 - 2019, and characterized for 11 phenotypic traits associated with malting quality. QTL mapping validated six previously reported regions (Mohammadi, et al., 2015, GrainGenes 3.0, 2019) that are strongly associated (LOD > 3.0) with relevant malt quality traits. Phenotypic variation for malt quality was largely and consistently explained by QTL on chromosomes 1H, 5H, and 7H in the Endeavor / VA09B-34 population and by two separate QTL on 1H in the Violetta / VA09B-34 population. A region on 4H corresponding with QDp.DiMo-4H, explained between 12.1 - 42.2% (Endeavor / VA09B-34) and 30.0 - 55.7% (Violetta / VA09B-34) of the phenotypic variation for diastatic power (DU). These QTL are recommended for MAS in order to aid breeding strategies that aim to select for improved malting characteristics in Eastern U.S. malt barley breeding material.

GENERAL AUDIENCE ABSTRACT

Wheat (*Triticum aestivum*, L) and barley (*Hordeum vulgare*) are staple crops throughout the world, and are the third and fourth most produced cereals crop according to the FAO. Primarily grown for human consumption, wheat and barley provide a significant percentage of the nutritional requirements for the human populations. According to the United Nations, wheat contributes 20% of all calories consumed by humans. Barley is the primary ingredient used to make beer. Increased productivity of all cropping and livestock systems is required in order to feed a growing human population while also restoring and preserving natural ecosystems. This can be accomplished through breeding and improved cropping systems management. Planting of existing cropland more frequently is fundamental to the improvement of cropping system productivity. In much of the U.S. (southern two-thirds of the lower 48), annual winter grains such as wheat and barley can be grown over the winter and spring in between the typical corn (*Zea mays subsp. mays*) and soybean (*Glycine max,* L. Merr.) growing seasons. Therefore, producing three crops in two years, as opposed to only two. Only between 6 and 11 million acres are double cropped in the US annually, for perspective, in 2018, 89 million acres of both corn and soybeans, which can only grow in summer, were planted. Over half of the soybean (~45 million) acres in Midwestern and Southeastern states could support double cropping. This is a major opportunity to maximize output per unit area, freeing up less productive land to be restored as natural ecosystems, potentially increasing carbon sequestration and species biodiversity. Winter annual grains have a very similar composition (high carbohydrate, low protein and oil) to corn, and could fill similar end-use markets currently dominated by corn (i.e. ethanol or livestock feed). For double cropping to be more widely deployed, it must be more profitable. Increased profitability of growing three crops in two years as opposed to two must outweigh the added cost of planting, managing, harvesting, and marketing the additional winter crop. Therefore, it is important to investigate management strategies that could increase production per unit area and develop new winter annual cultivars with improved end-use characteristics in order to make the winter annual more desirable to the end-users. Chapter I investigates sowing winter wheat earlier in the fall (i.e. 1st week of Oct. or last week of Sept.) in order to achieve an earlier harvest in the spring and earlier soybean planting (yield decreases 0.5 to 1 bu/ac per day that sowing is delayed), while also offering other benefits such as better-established root systems going into winter, which improves water infiltration and reduces erosion. At all environments, sowing earlier in the fall achieved an earlier heading date, while grain yields varied depending on environment and genotype. Genotype by sowing date interactions were non-significant at five site-years and significant at three site-years. Chapters II and III investigate the genetic architecture of winter wheat and winter barley breeding populations for end-use quality traits (milling/baking and malting). This was done in order to identify molecular markers that could be used to screen breeding material for improved end-use quality. The markers could then be used to assist breeders in developing soft red winter wheat cultivars with greater flour yields/improved baking performance and winter malt barley cultivars that can be grown in the Eastern U.S. and are suitable for the craft beer market. Chapter II describes 24 genomic regions that influences milling/baking performance in two soft red winter wheat breeding populations. Chapter III describes 6 genomic regions that influence malting performance in two winter barley breeding populations.

DEDICATION

This dissertation is dedicated to my wife Elizabeth, parents Dave and Patty, siblings Adam and Emily, and stepmom Sheila who supported me through the entire process, and my grandparents Lowell, Ralph, Rose, and Mary who taught me to love agriculture, and always encouraged me to continue my education. I am forever grateful to my PhD adviser, Dr. Griffey, who supported me through the entire program with immense patience and care, providing invaluable guidance, and Wynse Brooks, who is always a reliable friend and source of wisdom and comfort.

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ATTRIBUTIONS

This section describes the contributions of the collaborators, who were involved in experiment design, data collection, trial management, data analysis, and manuscript review for each chapter.

Chapter I: Early sowing influences winter wheat performance in the Mid-Atlantic

Carl Griffey, PhD, is the W.G. Wysor professor of crop breeding and genetics in the School of Plant and Environmental Sciences at Virginia Tech. Dr. Griffey was a co-PI on this project. He was involved in developing the concept for this project, designing the experiment, and reviewing the manuscript and results.

Wade Thomason, PhD, is a Professor and Extension small grain specialist in the School of Plant and Environmental Sciences at Virginia Tech. Dr. Thomason was a co-PI on this project. He was involved in developing the concept for this project, designing the experiment, and reviewing the manuscript and results.

David Holshouser, PhD, is an Associate Professor and Extension Agronomist in the School of Plant and Environmental Sciences at Virginia Tech, based at the Tidewater AREC in Holland, VA. Dr. Holshouser was a co-PI on this project. He was involved in developing the concept for this project, designing the experiment, and reviewing the manuscript and results.

Robert Pitman, MS, is a retired superintendent of the Eastern Virginia AREC in Warsaw, VA. He was involved in planting, managing, and collecting data at the Warsaw location.

Paul Davis, MS, is a retired Virginia Extension Agent and current farmer in New Kent, VA. He was involved in planting, managing, and collecting data at the New Kent location.

Dave Van Sanford, PhD, is Professor of Small Grains breeding and genetics at the University of Kentucky. He was involved in planting, managing, and collecting data at the Lexington, KY location.

Joseph Oakes, PhD, is the current superintendent of the Eastern Virginia AREC in Warsaw, VA. He was involved in planting, managing, and collecting data at the Warsaw location.

Steve Gulick is a retired superintendent of the Northern Piedmont AREC in Orange, VA. He was involved in planting, managing, and collecting data at the Orange location.

Brad Lael is a former superintendent of the Northern Piedmont AREC in Orange, VA. He was involved in planting, managing, and collecting data at the Orange location.

William Taylor is a research specialist at the Tidewater AREC in Holland, VA. He was involved in planting, managing, and collecting data at the Holland location.

Mark Vaughn is a research specialist at the Eastern Virginia AREC in Warsaw, VA. He was involved in planting, managing, and collecting data at the Warsaw location.

Brian Ward, PhD, is Postdoctoral Research Associate at the USDA-ARS at North Carolina State in Raleigh, NC. He helped with data collection at all locations in 2015.

Kyle Brasier, PhD, is Postdoctoral Research Associate at California State University, Chico in Chico, CA. He helped with data collection at all locations in 2015 and 2016.

Neal Carpenter, PhD, is a hybrid design scientist at Bayer Crop Science in Chesterfield, MO. He helped with data collection at all locations in 2015 and 2016.

Sandy Swanson, is a research specialist in the small grains breeding and genetics program at the University of Kentucky. She was involved in planting, managing, and collecting data at the Lexington, KY location.

Chapter II: Quantitative Trait Loci Associated with Improved End-Use Quality in Soft Red Winter Wheat

Carl Griffey, PhD, is the W.G. Wysor professor of crop breeding and genetics in the School of Plant and Environmental Sciences at Virginia Tech. Dr. Griffey was a co-PI on this project. He was involved in developing the concept for this project, designing the experiment, developing both mapping populations, and reviewing the manuscript and results.

Neal Carpenter, PhD, is a hybrid design scientist at Bayer Crop Science in Chesterfield, MO. He helped with scoring the 90k SNP chip and linkage map curation for the Pioneer 25R47 x Jamestown population.

Subas Malla, PhD, is an Assistant Professor at the Texas A&M AgriLife Research Center in Uvalde, TX. He helped with scoring the 90k SNP chip the Pioneer 26R46 x Jamestown Tribute.

Byung-Kee Baik, PhD, is the Researcher Leader of Corn, Soybean and Wheat Quality Research and Director of the Soft Wheat Quality Lab at the USDA-ARS in Wooster, OH. He help facilitate the phenotyping and was involved in reviewing the manuscript and results.

Shiaoman Chao, PhD, is a retired Research Molecular Geneticist, USDA-ARS Cereal Crops Research Unit in Fargo, ND. She ran the 90k SNP chip on both populations.

J. Paul Murphy, PhD, is a Professor of small grains breeding and genetics at North Carolina State University in Raleigh, NC. He oversaw the doubled haploid development of the Pioneer 26R46 x Tribute population.

Gina Brown-Guedira, PhD, is a USDA Professor of Plant Genetics and oversees the USDA Eastern Regional Small Grains Genotyping Lab at North Carolina State University in Raleigh, NC. Her lab performed the data extractions for the 90k SNP chip on both populations.

Chapter III: Comparison of QTL associated with malt quality traits in two 'Malt x Feed' Winter Barley Doubled Haploid Populations

Carl Griffey, PhD, is the W.G. Wysor professor of crop breeding and genetics in School of Plant and Environmental Sciences at Virginia Tech. Dr. Griffey was a co-PI on this project. He was involved in developing the concept for this project, designing the experiment, and reviewing the manuscript and results.

Wynse Brooks, MS, is a Research Specialist in the small grains breeding program in the School of Plant and Environmental Sciences at Virginia Tech. He was involved in the development (determined and made cross) of both doubled haploid populations, field management, developing the concept for this project, designing the experiment, and reviewing the manuscript and results.

Patrick Hayes, PhD, is a Professor of Barley Breeding and Genetics at Oregon State University in Corvallis, OR. His lab development of both doubled haploid populations.

Mary Osenga, PhD, is a Research Specialist at the USDA-ARS Cereal Crops Research Unit in Fargo, ND. She ran the 50k SNP chip on both populations.

Christopher Martens, is Biological Science Lab Technician at the Cereal Crops Research Unit in Madison, WI. He performed the malt quality analysis for both populations in 2017, 2018, and 2019.

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CHAPTER I:

Early sowing influences winter wheat performance in the Mid-Atlantic

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Abbreviations: SRWW: soft red winter wheat, GGE (genotype plus genotype-by-environment) biplot, H17: Holland, VA 2016-17, H18: Holland, VA 2017-18, L17: Lexington, KY 2016-17, O16: Orange, VA 2015-16, O17: Orange, VA 2016-17, W16: Warsaw, VA 2015-16, W18: Warsaw, VA 2017-18

Abstract

An earlier winter wheat (*Triticum aestivum*, L.) maturity can be achieved by sowing earlier in the fall, increasing the likelihood for an earlier harvest and subsequent soybean (*Glycine max,* L. Merr.) sowing date in double crop systems. Soybeans sown earlier in the spring tend to yield more, increasing the potential profitability of double cropping. Information for sowing winter wheat earlier than currently recommended is limited for the Mid-Atlantic region. The objective of the current study is to investigate winter wheat performance (maturity and grain yield/stability) when sown earlier than historically recommended across multi-environment trials. Fifteen commercially relevant winter wheat genotypes ranging in maturity were sown in a split-plot design (sowing date=main plot, genotype=subplot) at three different sowing dates (considered to be 'very early' (20-28 days before recommended), 'early (6-11 days before recommended)', or 'recommended') and replicated three times at eight environments (site-year) from 2015-2018 in Virginia and Kentucky. Two environments Orange 2015-16 (late spring frost) and Orange 2016-17 (fall drought) experienced major weather events. Grain yield, tiller estimation, heading date, protein, and 1000-kernel weight were assessed for each yield plot. At all environments, sowing earlier in the fall achieved an earlier (P<0.05) heading date, while grain yields varied depending on environment and genotype. Genotype by sowing date interactions were non-significant (P<0.05) at five site-years and significant (P<0.05) at three site-years (very early and early were greater than recommended for a majority of genotypes at Warsaw 2015-16 and Holland 2017-18, and very early was lower than early and recommended) for grain yield. Genotype plus genotype-by-environment (GGE) biplot analysis was conducted to investigate grain yield and stability, which identified Hilliard, L11550, Pioneer 25R32 and Pioneer 26R10 in the late maturity group, as strong candidates for earlier sowings.

Introduction

Winter wheat (*Triticum aestivum*, L.) and soybean (*Glycine max,* L. Merr.) double crop rotations are widely used in the Mid-Atlantic USA (Holshouser, 2015). In this region, winter wheat is sown between mid-October and early November, then harvested in June and early July. Recommended sowing dates in Virginia are based on a historical 50% probability of fall freeze (Thomason, et al., 2004). Studies that investigate sowing winter wheat prior to the recommended dates remain limited in the Mid-Atlantic regions, however there are several reports relevant to the Southeast. One study (Morgan, et al., 2011), compared five sowing dates from early September until late November over three years in central Texas, and reported a decrease in grain yield and tiller density for sowing dates after mid-November. Early October sowing did not result in different (P<0.05) grain yields when compared to late October or early November. The late September sowing was not different (P<0.05) for grain yield when compared to early and mid-October. A report from North Carolina advises sowing winter wheat 10-14 days earlier than historically recommended (seven days before the 30-year average local freeze date), if five specifically outlined steps are followed. The report recommends not sowing more than two weeks before the recommended dates, use late heading cultivars sown at a reduced seeding rate, an insecticidal seed treatment, and restrict no-tillage to the Piedmont (Weisz, 2013).

Early sowing can increase fall tiller density, which occurs in response to the warmer fall temperatures, and is associated with increased grain yield (Morgan, et al., 2011, Tapley, et al., 2012, Thiry, 2002). Previous research in Virginia and North Carolina has shown that up to 85% of the yield in any given wheat field is made up by grain heads formed on tillers that developed in the warmer fall weather (Weisz, 2013). This is especially beneficial in no-till systems, which wheat often grows more slowly than in conventionally tilled seedbeds (Thomason, et al., 2004,

Weisz, 2013). Longer established winter wheat crops have greater stress tolerance, better protect the soil, resulting in less erosion, less runoff, and greater water infiltration (Winter and Musick, 1993). Seedling emergence and establishment is quicker with warmer fall temperatures, reducing the likelihood of cooler temperatures delaying vegetative and tiller development, which can be exacerbated under dry conditions (Musick and Dusek, 1980). Earlier seeded wheat allows for a longer grain fill period and reduces exposure to high temperature fluctuations pre and post anthesis, which can result in grain sterility (Hunt, et al., 1991, Tapley, et al., 2013). Winter wheat maturation occurs sooner in the spring as fall sowing is shifted earlier, increasing the likelihood of an earlier harvest and subsequent soybean sowing (Kelly, et al., 2001).

Soybean yields decline as sowing is delayed after early June in the Eastern and Midwestern United States (De Bruin and Pedersen, 2008, Hu and Wiatrak, 2012, Oplinger and Philbrook, 1992). A regional analysis of soybean yields to sowing date for maturity groups (00-8) reported a rapid decline as sowing was delayed after 7 June in the Upper South (-1.1% in yield per day), 27 May in the Deep South (-1.2% in yield per day), and 30 May in the Midwest (-0.7% in yield per day). The authors noted that response to sowing date across maturity groups was remarkably consistent (Egli and Cornelius, 2009). Soybean sowing date trials in Suffolk, VA (maturity groups 4 and 5) showed average yield declines of 0%, 12%, and 33% as sowing was delayed into early June, late June, and July, respectively. By mid-June soybean yield potential is already in decline. The author estimates that average yields decline by roughly 33 kg ha^{-1} per day after mid-June, reinforcing the importance of winter wheat cultivar selection and sowing date in order to lengthen soybean growing season, increasing yield potential (Holshouser, 2015) .

An earlier sown winter wheat crop is not without challenges. Excess tiller growth more rapidly depletes soil moisture, or results in overly thick stands that may lodge before the end of winter

(Thiry, 2002). However, reduced seeding rate decreases competition, and seed input cost (Kelley, 2001, Weisz, 2013, Spink, et al., 2000) and pre-plant N can suppress excess tiller growth and over competition. (Knapp, 1978, Weisz, 2004). Earlier fall sowing extends the duration of exposure to agronomic pests, most importantly Hessian fly (*Mayetiola destructor*) (Thomason, et al., 2004). Seed treatment insecticides and cultivars with race specific genes and horizontal resistance (Yu, et al., 2010) are available to provide protection prior to initial frost, extending the sowing window (Weisz, 2013). The other primary concern is that early sowing increases risk of spring freeze damage to developing wheat heads as this results in lower grain weights. Freeze damage to developing wheat heads can occur after as temperatures drop below -4°C after jointing, but is most severe during boot at -2°C , heading-1°C , and flowering at 0°C (Holman, et al., 2011, Warwick and Miller, 1999). Growing degree day accumulation, photoperiod sensitivity, and vernalization requirements are the primary factors controlling plant development (Beales, et al., 2007, Guedira, et al., 2016, Hu, et al., 2005, Mohler, et al., 2004, Yang, et al., 2009).

If potential risks are properly managed (i.e. excess tiller production, Hessian fly, and late frost damage), sowing winter wheat earlier in the fall is a potential management strategy that could be implemented in the Mid-Atlantic region to improve soybean-winter wheat double crop rotation performance, potentially increasing profitability and sustainability of this system. Therefore, it is necessary to investigate winter wheat performance of commercially relevant genotypes with a range of maturities when sown earlier than historically recommended with optimal management across multi-environment trials in the Mid-Atlantic region.

Material and Methods

This experiment included 14 soft red winter wheat (SRWW) genotypes and 1 experimental hard white (VA11HWW-113) winter wheat genotype. VA11HWW-113 was included in this test because it has the negative allele at all three homeologous *Ppd* genes (photoperiod sensitivity) (Law, et al., 1978), therefore it is expected to be highly photoperiod sensitive. Besides VA11HWW-113 all other genotypes are high yielding, SRWWs that are commercially available or elite experimental lines (designated by VA##W-###). Genotypes range in maturity with the earliest heading 10 days prior to the latest variety, on average, as well as having various combinations of photoperiod alleles (Table 1). Each genotype was sown at three different dates in each environment (Table 2), and considered very early (20-28 days), early (6-11 days), and recommended (Figure 1) based on current extension recommendations (Thomason, et al., 2004). Each genotype was sown in a split-plot design with sowing date as main plot and genotype as sub-plot repeated three times per environment (site-year). The experiment was replicated seven times across Virginia at four different sites, representing much of the state's major small grain growing environments (Table 2). An eighth site-year was grown in Lexington, KY in 2016-17. There is no very early sowing at two sites (New Kent 2016-17 and Lexington 2016-17) due to wet or dry soil conditions during the desired sowing very early sowing window. Therefore, shifting the actual sowing dates in these two site-years (1st sowing is equivalent to early, the 2nd sowing is equivalent to recommended, and the 3rd sowing was about two weeks later than the current recommendation).

Data Collection

Heading date (Julian) was recorded as the date that 50% of spikes were completely emerged from the boot. Grain yield was calculated from total mass of grain harvested from the plot area, and was adjusted to uniform moisture of 13.5%. Moisture (%) and grain volume weight (kg hl^{-1})

of each plot were determined on subsamples using a DICKEY-john® GAC 2500 machine (DICKEY-john, Minneapolis, MN). 1000-kernel weight (g) was calculated by weighing 1000 cleaned seeds. Grain protein (%) was estimated with the FOSS (Hillerød, Denmark) XDS^{TM} near infrared analyzer. Normalized difference vegetation index (NDVI) measurements were collected at growth stage 25 (Zadoks, et al., 1974) using the Trimble Ag (Sunnyvale, CA) GreenSeeker handheld crop sensor to estimate tiller growth (m^{-2}) as previously described (Phillips, Keahey, et al., 2004). Visual freeze damage ratings were estimated at the Orange 2015-16 environment on a scale of 0-100%.

Agronomic Practices

The nearest town and year of harvest is used to describe each environment (site-year) (S. Table 1). All seeds were coated with Gaucho®-XT (imidacloprid, Bayer Crop Science) insecticide and Raxil® MD (Tebuconazole and Metalaxyl, Bayer Crop Science) fungicide. All environments were seeded with a grain drill in seven rows with 15 cm spacing. Harvested area at Lexington, Orange, and Warsaw sites was 1.5 x 2.7 m, and 1.5 x 4.9 m at New Kent and Holland sites. The targeted seeding rate was 275 seeds m^{-2} for the site with conventional tillage and 300 seeds m^{-2} for the sites with no-till following maize (*Zea mays subsp. Mays*) for the very early (20-28 days) sowing date. Seeding rate was increased 15% for the early (6-11 days) sowing date and 25% for the recommended (Figure 1) sowing date, to account for reduced tiller growth in accordance with previous research (Kelley, 2001). Increased seeding rate as sowing date is delayed is the current recommendation for growers (Weisz, 2013). Fall nutrient management, spring nitrogen (N) applications and general crop management practices at each site was based on standard local recommendations from the Virginia Cooperative Extension Service (Brann et al. 2009).

Fungicides and insecticides were applied as needed to achieve near complete disease control (S. Table 2).

Growing Conditions

Adequate and consistent rainfall occurred across all environments, with the exception of Orange 2016-17 (S. Table 3). Recorded rainfall for Orange 2016-17 was 2 mm during October. Orange 2016-17 experienced only one measurable precipitation event between 30 September and 4 November. The historical average for October at Orange is between 80-90 mm. The Orange site is characterized as having heavy clay soils, therefore hydrophobic properties dramatically reduced water availability (S. Table 1). This greatly affected germination and overall stand establishment of the recommended sowing, which explains the dramatic decrease in yield. A single significant spring freeze event occurred at the Orange 2015-16 (-4°C) and to a lesser extent at the Warsaw 2015-16 (0°C) on 10 April 2016. This was approximately 15 to 21 days before heading, depending site, genotype, and sowing date. The first frost date for the 2015-16 growing season occurred on either 18 or 19 October for all Virginia sites, which is consistent with the historical averages, which inform the 50% probability. During 2016-17 and 2017-18, the initial frost occurred between 5 and 12 November for all Virginia sites, nearly a month later than the current estimated date for 50% probability of freeze (Thomason, et al., 2004).

Statistical Analyses

Analysis of variance (ANOVA) for linear mixed-effect models was conducted for split-plot designs over environments for grain yield (kg ha^{-1}), 1000-kernel weight (g), protein (%), and tiller estimation (m^{-2}) with the lme4 package (Bates, et al., 2007) in the R statistical environment (R Core Team, 2016, R Studio, 2014).

$$\boldsymbol{Y_{ijkl}} = \mu + \boldsymbol{E_l(S_k)} + \boldsymbol{D_i} + \boldsymbol{\delta_{ik}} + \boldsymbol{G_j} + \boldsymbol{DG_{ij}} + \boldsymbol{DE(S_k)_{il}} + \boldsymbol{GE(S_k)_{jl}} + \boldsymbol{DGE(S_k)_{ijl}} + \boldsymbol{\varepsilon_{ijkl}}$$

Trait response (Y_{ijk}) is a function of the overall mean (μ), the random effect of the *k*th block (S_k) nested within the *l*th environment (site-year) (E_l), the fixed effect of the *i*th sowing date (main plot treatment) (D_i), the main plot random error effect (δ_{ik}), the fixed effect of the *j*th genotype (sub-plot treatment) (G_j), the interaction of the *i*th sowing date with the *j*th genotype (DG_{ij}), the interaction of the *j*th genotype (G_i) with *k*th block (S_k) nested within the *l*th environment ($GE(S_k)_{jl}$), the interaction of the *i*th sowing date (D_i) with *k*th block (S_k) nested within the *l*th environment ($DE(S_k)_{il}$), the interaction of the *j*th genotype (G_j) *k*th block (S_k) nested within the *l*th environment, the full interaction of the *i*th sowing date with *j*th genotype (G_i) and the *k*th block (S_k) nested within the *l*th environment ($GDE(S_k)_{ijl}$), the with the and the interaction of the and the residual error (ε_{ijk}), the including the [block nested within environment x sowing date] and [block nested within environment x sowing date x genotype] interactions.

Due to significant environment effect a second ANOVA was conducted by environment to further investigate the effect of genotype by sowing date interaction.

$$\boldsymbol{Y_{ijk}} = \mu + \boldsymbol{S_k} + \boldsymbol{D_i} + \boldsymbol{\delta_{ik}} + \boldsymbol{G_j} + \boldsymbol{DG_{ij}} + \boldsymbol{\varepsilon_{ijk}}$$

Trait response (Y_{ijk}) is a function of the overall mean (μ), the random effect of the *k*th block (S_k), the fixed effect of the *i*th sowing date (main plot treatment) (D_i), the main plot random error effect (δ_{ik}), the fixed effect of the *j*th genotype (sub-plot treatment) (G_j), the interaction of the *i*th sowing date with the *j*th genotype (DG_{ij}), and the residual error (ε_{ijk}), including the [block x sowing] and [block x sowing date x genotype] interactions.

Freeze damage rating from Orange 2015-16 were added to the trait response in the by-environment ANOVA as well. Pairwise post-hoc analyses were conducted using the Tukey's

HSD ($\alpha=0.05$) method to compare means between sowing dates for each environment with a significant sowing date by genotype interaction. Furthermore, using the *cor* function in the R statistical environment bivariate (Pearson) estimation of the correlation coefficient of grain yield to tiller estimation, heading date, 1000-kernel weight, and protein for each site-year were generated (R Core Team, 2016, R Studio, 2014).

Yield stability was analyzed by GGE (genotype plus genotype-by-environment) biplot (Yan and Tinker, 2006) in the R statistics environment (R Core Team, 2016, R Studio, 2014) with the interactive GGEBiplotGUI package (Frutos and Galindo, 2016). Results of the GGE biplot analyses are presented with the average-environment coordination (AEC) view showing 'mean performance and stability', also called the mean vs. stability view, and the which-won-where view based on genotype-focused singular value partitioning (SVP=2) (Yan and Tinker, 2006).

Results and Discussion

Maturity

Heading date was influenced significantly ($P<0.001$) by genotype (G), sowing date (D), environment (E), and the interaction (GDE) between these three effects. Sowing date (D) and environment (E) had the largest effect, accounting for most of the treatment variation (Table 3). When evaluated by environment (Table 4.) mean heading date was consistently earlier ($P<0.05$) for the very early and early sowings compared to the recommended sowing (Table 5). The mean heading date for very early and early sowings was reached 2.7–8.1 and 1.5-5.2 days sooner than the recommended sowing, depending on environment. This corresponds with McLeod, et al., (1992), who reports a delay of 10 to 11 days in maturation as winter wheat planting is delayed from early September to late November in Saskatchewan. A study from the Northern China

plain reports zero to two day delay in heading for every five days that winter wheat planting is delayed in the fall (Sun, et al., 2007). Depending on environment, the earliest-heading genotype at the very early sowing had a mean heading date that was 6.3-23.6 days sooner than the latest-heading genotype at the recommended sowing, reinforcing the importance of selecting the appropriate winter wheat genotype and sowing date in order to maximize the overall winter wheat-soybean double crop yield potential. Assuming an average decline of roughly 33 kg ha^{-1} in soybean yield per each day delay in sowing after mid-June (Holshouser, 2015), the potential for influencing soybean yield through the selection of appropriate genotype and sowing date is significant. However, it is necessary to weigh the potential gain in soybean yield with increased risk of the winter wheat crop experiencing a late spring freeze event, which could have a negative impact on grain yield. The earliest genotypes are most susceptible because these break dormancy sooner in response to rising temperatures (Holman, et al., 2011, Kelley, 2001).

Grain Yield

Grain yield was also influenced significantly (P<0.001) by genotype (G), sowing date (D), environment (E), and the interaction (GDE) between these three effects (Table 3). Due to the large environment effect, a second ANOVA was conducted in order to better evaluate the sowing date (D) by genotype (G) interaction (Table 4). When tested by environment the genotype (G) and sowing date (D) interaction was significant (P<0.001) for grain yield at three site-years (W16, H17, H18) (Table 4). Comparison of the sowing date (D) (main plot treatment) and genotype (G) (sub-plot treatment) interaction unreliable as the interaction between the two treatments was non-significant in five environments.

Additional posthoc analysis (Tukey's HSD pairwise comparison) were conducted at the three environments with significant sowing date by genotype interactions (Warsaw 2015-16, Holland

2016-17, and Holland 2017-18), to analyze effect of sowing date on grain yield by genotype (Table 5). Average grain yields were also included for the five environments with non-significant interactions. At the Warsaw 2015-16 environment, there was a clear trend, in that the mid and late maturity genotypes had a negative response for grain yield as sowing was delayed after the very early (24-Sept.) sowing. This trend is similar to the results from Weisz (2013) in North Carolina, where planting late maturing cultivars ten to fourteen days earlier than recommended resulted in grain yields similar to the suggested planting date at a reduced seeding rate when optimally managed. The opposite trend was observed in the four earliest genotypes (Jamestown, Progeny 117, VA13W-177, VA13W-38), having a positive response for grain yield as sowing was delayed (Table 5). At the Holland 2016-17 environment all genotypes, except Progeny 117 had a positive response for grain yield as sowing was delayed after the very early (27-Sept.) sowing, however while largely non-significant grain yields of the early sowing tended to be greater than at the recommended sowing. Conversely, at the early (14-Oct.) sowing 10 genotypes had greater (P<0.05) grain yields than at the very early sowing. Three genotypes (Featherstone 73, SS 8870, USG 3251) had greater (P<0.05) and the other 12 had similar (P<0.05) grain yields at the early (14-Oct.) sowing compared to the recommended (27-Oct.) sowing. Eight genotypes at the recommended (27-Oct.) sowing had greater and seven had similar grain yields compared to the very early (27-Sept.) sowing. This suggests that the very early (27-Sept.) sowing at H17 was likely too early, while the early (14-Oct.) sowing resulted in either a moderately increased or similar grain yield. This is a favorable result as sowing 14-October instead or 27-October would result in an earlier maturity, without a reduction in yield. At the Holland 2017-18 environment, the negative response for grain yield as sowing was delayed after the very early sowing (2-Oct.)

is similar to the Warsaw 2015-16 environment with the exception that all genotypes followed this trend regardless of maturity (Table 5).

In order to compare genotypes at different sowing dates for grain yield performance and stability, a heatmap (Figure 2) and GGE biplots (S. Figures 1-7) were generated as visualization tools to aid in discriminating between genotype performances at each environment (Figure 2). Separate biplots were used for different maturity groups (early, medium, late) as consideration for planting earlier maturing cultivars often originates out of desire for a more timely harvest, rather late maturing cultivars are selected in favor of greater grain yields. The 'mean vs. stability' view of GGE biplot explained 71-99% of genotypic and genotype x environment (site-year and sowing date) variation. The arrow shown on the 'mean vs. stability' view abscissa points to the direction of higher yield performance of genotypes and ranks the genotypes by yield performance. The more stable genotypes are located closer to the 'mean vs. stability' abscissa (horizontal axis) (Yan and Tinker, 2006). The Orange environments were analyzed separately due to lack of relatedness with the other environments (S. Table 1). The heatmap and biplots revealed Pioneer 25R32, Pioneer 26R10, Featherstone 73 and L11550 as genotypes that perform well for grain yield across a majority of the environments and sowing dates, indicating good yield stability, making these genotypes promising candidates for early sowings. The earliest genotypes (VA13W-177, Jamestown, VA13W-38) had the lowest yields across most sowing dates and environments, as expected. VA13W-177 and VA13W-38 consistently performed better than Jamestown.

Tiller estimates

Genotype (G), sowing date (D), and environment (E) significantly (P<0.001) influenced tiller estimates, however the interaction (GDE) was not significant overall environments (Table 3).

However, the interaction between sowing date (D) and environment (E) was significant (P<0.001) (Table 3). Pearson correlation coefficients were generated to better understand how grain yield would be influenced by tiller production. Increased fall tiller density is associated with increased grain yield, greater stress tolerance, less erosion, great water infiltration, and quicker establishment (Morgan, et al., 2011, Tapley, et al., 2012, Thiry, 2002, Winter and Musick, 1993). Weisz (2013) estimates that up to 85% of the yield in any given wheat field is made up by grain heads formed on tillers that developed in the warmer fall weather. Increased tiller density was positively correlated (P<0.05) (r=0.25-0.76) with increased grain yield in six environments (Table 7). Furthermore, tiller estimation (m^{-2}) was positively correlated (P<0.05) (r=0.26-0.89) with sowing date at seven of the eight environment (Table 8). Regardless, of whether grain yield increases with tiller density, improves water infiltration and reduced erosion are positive soil health and environmental outcomes (Winter and Musick, 1993).

Grain quality

Genotype (G), sowing date (D), environment (E), and interaction (GDE) between the three factors was significant (P<0.001) for 1000-kernel weight (Table 3). Grain protein had a similar response, except the interaction was not significant. Grain yield correlations for 1000-kernel weight and protein results were as expected (Simmonds, 1995, Kibite and Evans, 1984). 1000-kernel weight increased and protein decreased as grain yield increased (Table 6). Reduced protein in SRWW to an extent is a favorable outcome as soft wheat products such as cakes, cookie, and other pastries that require weaker gluten. Lower protein is also related with increased flour yield, which benefits the miller (Gwirtz, et al., 2007). However, this would likely be an unfavorable outcome for hard wheat and durum (*Triticum turgidum subsp. durum*), which require much higher protein levels.

Freeze Damage

It is important to discuss the Orange 2015-16 environment as freeze damage is an important consideration when selecting sowing date and genotype (Figure 3). Late spring frosts were not important factors influencing grain yield at seven of the site-years. Weisz (2013) alludes to the risk of freeze damage by suggesting later maturities when an earlier sowing is considered, which would certainly have been the appropriate choice at Orange 2015-16. As expected, at the Orange 2015-16 environment freeze damage (-4°C of 10 April 2016) was most noticeable in the very early sowing in the earliest genotypes. The difference in grain yields is most striking in the earliest genotypes (VA13W-177, Jamestown, VA13W-38, Progeny 117), this tapered off slightly as maturity increased (Figure 3, panel: a). Freeze damage ratings were similar (P<0.05) between the very early and early sowings. For the recommended sowing, rating were lower than very early and early (Figure 3, panel: b). Furthermore, the correlation between freeze damage with grain yield (r=-0.391) and protein (r=0.385) was significant (Figure 3, panel: c and d).

Conclusions

Genotype, environment, and sowing date interactions are significant (P<0.001) for grain yield and maturity and this interaction should be central to management decisions and recommendations for newly released cultivars. Routine trialing of elite breeding material at earlier and later planting dates compared to current norms could be highly informative in terms of production management decisions in order to maximize double crop profitability. The results of this study suggest that positive outcomes such as earlier maturity (earlier soybean sowing), increased or similar grain yields, increased tiller density correlates (improves water infiltration and reduces erosion), and lower seed costs could be realized under favorable growing conditions by extending the recommended sowing dates prior (~7-10 days earlier) to 15 October (central

VA) or 25 October (southeast VA) for mid and late maturing genotypes (i.e. as late or late than Hilliard). Extending the sowing window earlier in the fall can also offer growers more opportunities for a timely sowing, which is beneficial in high rainfall areas such as Virginia. Still, caution should be exercised when sowing the earliest maturing genotypes (i.e. as early as or earlier than Progeny 117), and when growing SRWW in environments with increase frost risks, such as the Orange, VA site.

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Supplemental Material

Supplemental table 1 describes each environment (site-year). Supplemental table 2 lists the complete management applied to each environment. Supplemental table 3 is the average monthly precipitation (mm) at each environment. Supplemental table 1 displays the relationship among environment, which was used to determine how the GGE biplots was conducted. Supplemental table 2 shows the AEC 'mean vs. stability' and which won where/what views of the GGE biplot for the three earliest genotypes (VA13W-177, Jamestown, VA13W-38) at the Warsaw, Holland, and New Kent, VA environments. Supplemental table 3 shows the AEC 'mean vs. stability' and which won where/what views of the GGE biplot for the three earliest genotypes (VA13W-177, Jamestown, VA13W-38) at the Orange environment. Supplemental table 4 shows the AEC 'mean vs. stability' and which won where/what views of the GGE biplot for the five medium

genotypes (Progeny 117, Featherstone 73, Hilliard, Shirley, L11550) at the Warsaw, Holland, and New Kent, VA environments. Supplemental table 5 shows the AEC 'mean vs. stability' and which won where/what views of the GGE biplot for the five medium genotypes (Progeny 117, Featherstone 73, Hilliard, Shirley, L11550) at the Orange environment. Supplemental table 6 shows the AEC 'mean vs. stability' and which won where/what views of the GGE biplot for the seven late genotypes (USG 3251, USG 3315, Pioneer 25R32, Pioneer 26R10, SY 474, SS8870, VA11HWWW-113) at the Warsaw, Holland, and New Kent, VA environments. Supplemental table 7 shows the AEC 'mean vs. stability' and which won where/what views of the GGE biplot for the seven late genotypes (USG 3251, USG 3315, Pioneer 25R32, Pioneer 26R10, SY 474, SS8870, VA11HWWW-113) at the Orange environment.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Figure 1. Virginia Extension recommended sowing dates and location of Virginia testing sites (https://pubs.ext.vt.edu/424/424-005/424-005.html).

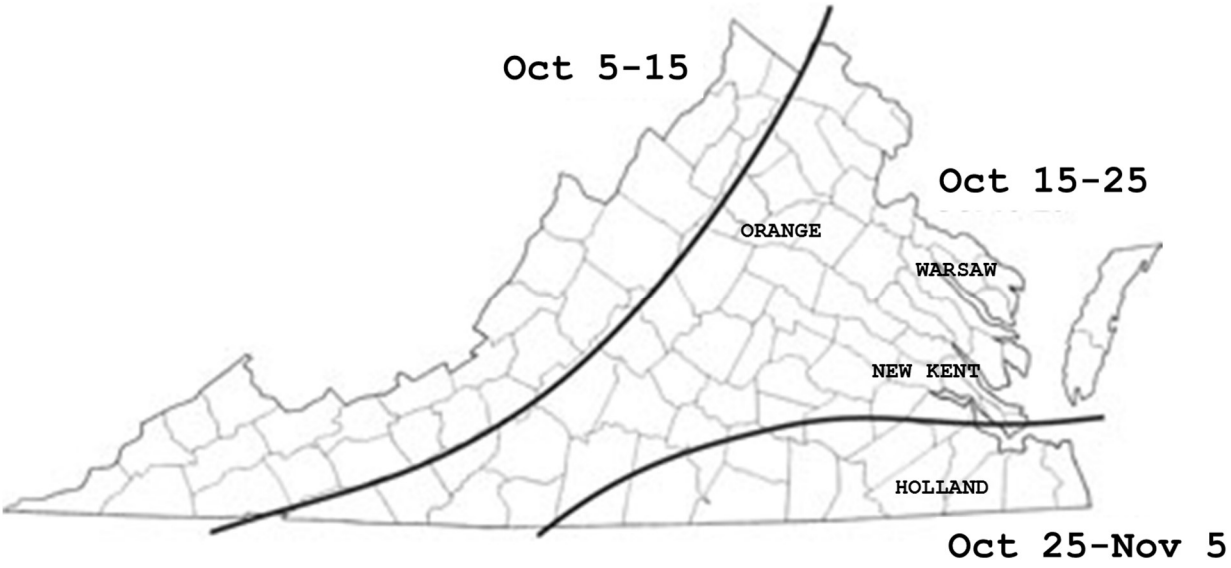
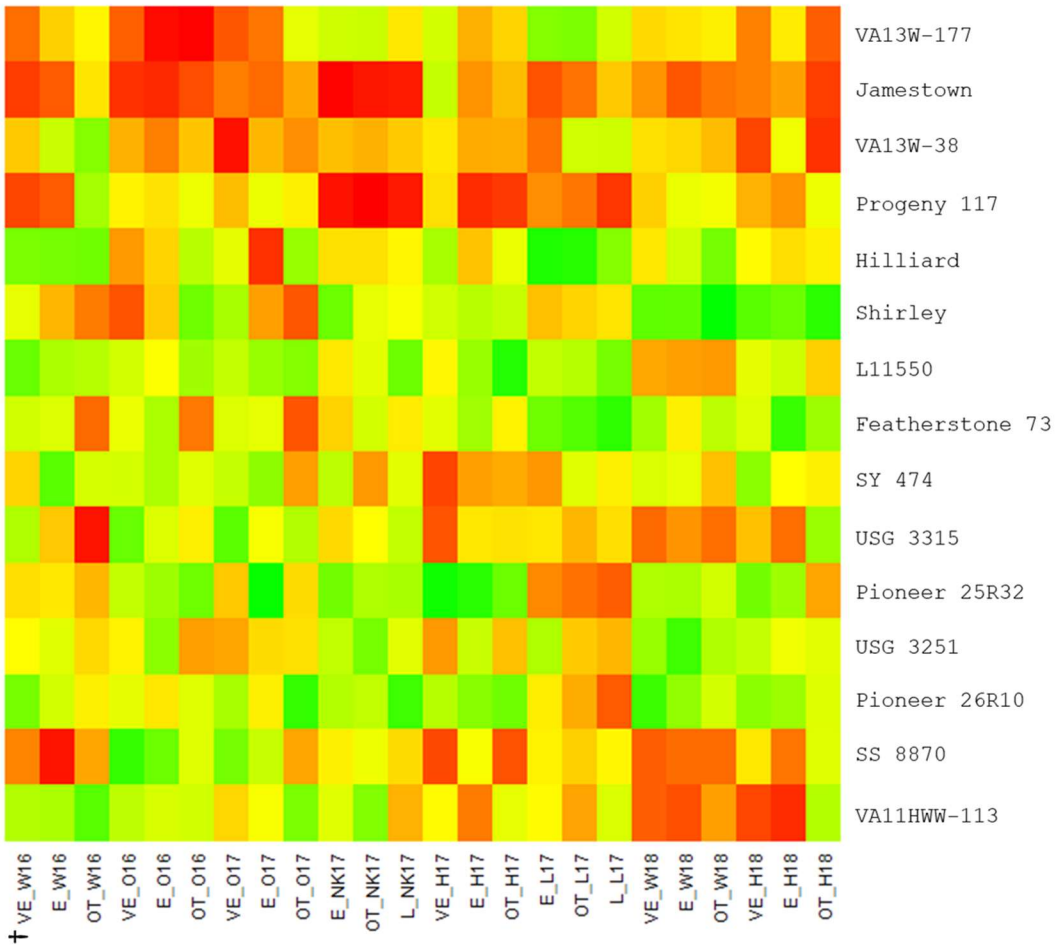
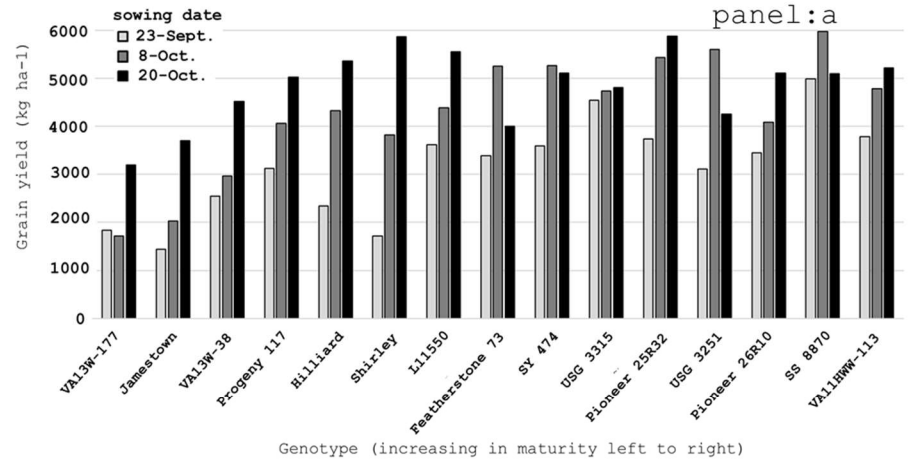


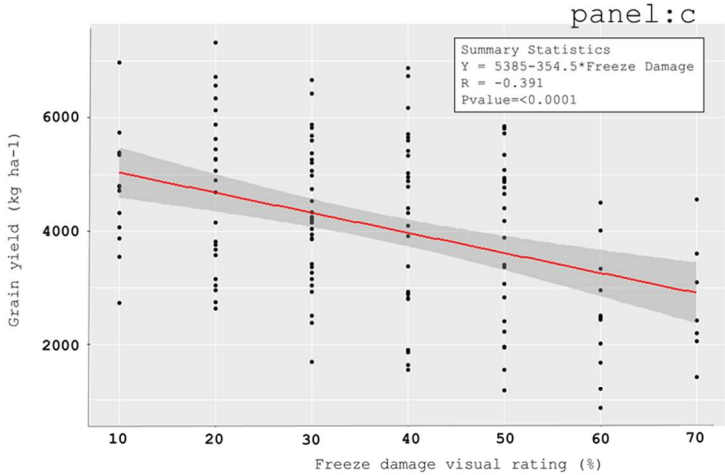
Figure 2. Heatmap representing winter wheat grain yields (kg ha^{-1}) (darker green=high yields, darker red=low yields) at each of the eight site-years and sowing date for each genotype (ordered earliest to latest maturity).

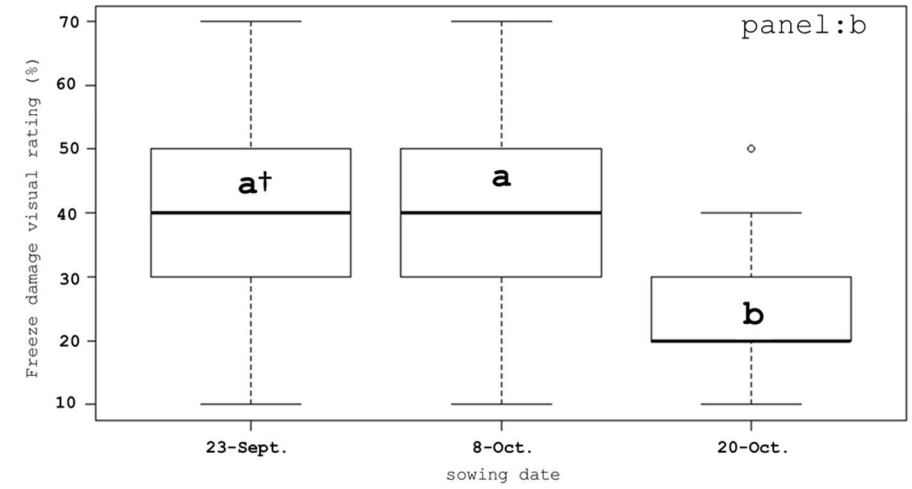


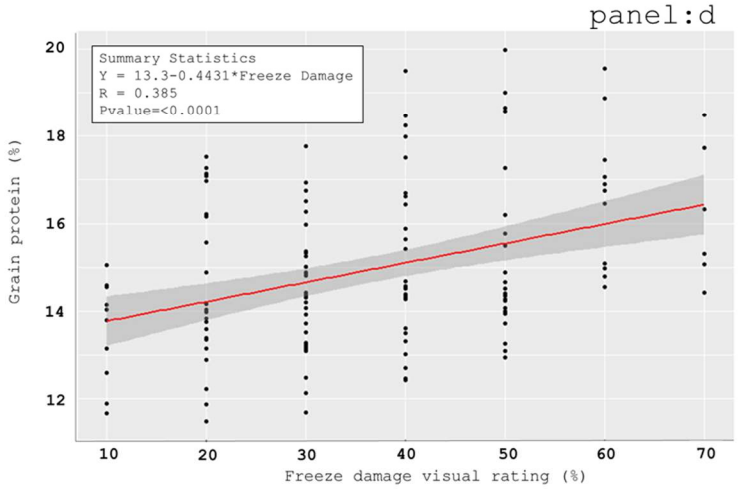
†Indicates site (W=Warsaw, VA, O=Orange, VA, H=Holland, VA, NK=New Kent, VA, L=Lexington, KY)-year (16=2016 harvest year, etc….), sowing date (VE=very early, E=early, OT=recommended, L=late) combination

Figure 3. Winter wheat freeze damage rating analysis from the Orange 2016 environment. Panel a represents winter wheat grain yield (kg ha^{-1}) by sowing date, panel b represents Tukey's HSD ($\alpha<0.05$) means comparison for freeze damage rating (%), panel c is a linear regression (grain yield by freeze damage rating (%)), panel d is a linear regression (grain protein (%) by freeze damage rating (%)).









† Means within environment connected by the same letter are not different (α=0.05)

Table 1.Winter wheat lines with corresponding photoperiod sensitivity alleles and overall average heading date.

Genotype	Photoperiod alleles (*Ppd*)			Heading date
	D1a†	A1a†	B1a†	Average‡ overall
VA13W-177	-	-	+	106.9
Jamestown	+	+	+	107.4
VA13W-38	-	-	+	109.0
Progeny 117	-	-	+	111.2
Hilliard	+	+	+	111.5
Shirley	+	+	+	111.5
L11550	+	-	+	112.0
Featherstone 73	-	+	+	112.4
SY 474	-	+	+	113.2
USG 3315	-	-	+	113.6
Pioneer 25R32	+	-	-	113.7
USG 3251	+	-	+	113.9
Pioneer 26R10	+	-	+	114.1
SS 8870	-	-	+	114.5
VA11HWW-113	-	-	-	116.6

†(+) Has photoperiod insensitive allele, (-) has photoperiod sensitive allele

‡ Average overall site-years and planting dates

Table 2. Planting and harvest dates of winter wheat lines by environment.

Test Location	Abbr.	Year	Very Early	Early	Recommended	Harvest	Extension Recommended Dates
Warsaw, VA	W16	2015-16	24-Sep	9-Oct	22-Oct	20-Jun	15-25 Oct‡
Warsaw, VA	W18	2017-18	25-Sep	6-Oct	23-Oct	21-Jun	15-25 Oct‡
Orange, VA	O16	2015-16	23-Sep	8-Oct	20-Oct	26-Jun	15-25 Oct‡
Orange, VA	O17	2016-17	23-Sep	5-Oct	18-Oct	28-Jun	15-25 Oct‡
Holland, VA	H17	2016-17	27-Sep	14-Oct	27-Oct	13-Jun	25 Oct- 5 Nov‡
Holland, VA	H18	2017-18	2-Oct	18-Oct	4-Nov	14-Jun	25 Oct- 5 Nov‡
New Kent, VA†	NK17	2016-17	4-Oct	18-Oct	30-Oct	25-Jun	15-25 Oct‡
Lexington, KY†	L17	2016-17	10-Oct	30-Oct	14-Nov	28-Jun	10-30 Oct§

†Planting was delayed (very early = early, early = recommended, recommended = late)
‡(Thomason, et al., 2004)
§(Lee, et al., 2007)

Table 3. Analysis of variance for split-plot design with *F*-values for winter wheat grain yield (kg ha^{-1}), tiller estimation (m^{-2}), heading date (Jullian), 1000-kernel weight (mg), and protein (%).

		Grain yield		**Tiller estimation**		**Heading date**		**1000-kernel weight**		**Protein**	
Effect	df	*F*-value		*F*-value		*F*-value		*F*-value		*F*-value	
Genotype (G)	14	15.01	***	7.70	***	105.05	***	63.22	***	25.73	***
Sowing date (D)	2	16.43	***	78.46	***	203.63	***	16.07	***	18.89	***
Environment (E)	7	4.39	***	426.42	***	5.36	***	4.47	***	2.75	**
G*D	28	2.59	**	1.77	**	1.54	***	0.60	NS	1.01	NS
G*E	98	4.51	***	1.86	***	12.79	***	8.18	***	2.31	***
D*E	14	18.15	***	16.39	***	8.72	***	8.66	***	13.10	***
G*D*E	196	1.37	**	1.04	NS	1.92	**	1.54	***	1.01	NS

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

*** Significant at the 0.001 probability level

† NS, non-significant

Table 4. Analysis of variance for each of the eight site-years with *F*-values for winter wheat grain yield (kg ha^{-1}), tiller estimation (m^{-2}), heading date (Julian), 1000-kernel weight (mg), and protein (%).

		Grain yield		Tiller estimation		Heading date		1000 kernel weight		Protein	
Warsaw 2015-16											
Effect	df	*F*-value		*F*-value		*F*-value		*F*-value		*F*-value	
Genotype (G)	14	3.73	***	3.08	**	288.93	***	47.15	***	8.41	***
Sowing date (D)	2	5.56	**	24.33	***	410.80	***	31.32	***	0.85	NS
G*D	28	1.84	*	1.52	NS	5.65	***	1.28	NS	1.16	NS
Warsaw 2017-18											
Effect	df	*F*-value		*F*-value		*F*-value		*F*-value		*F*-value	
Genotype (G)	14	18.74	***	2.96	**	127.65	***	20.24	***	13.62	***
Sowing date (D)	2	8.37	**	2.32	NS	379.22	***	15.29	***	1.02	NS
G*D	28	1.20	NS	0.72	NS	1.89	*	1.26	NS	0.91	NS
Orange 2015-16											
Effect	df	*F*-value		*F*-value		*F*-value		*F*-value		*F*-value	
Genotype (G)	14	7.08	***	3.63	***	29.65	***	11.05	***	4.93	***
Sowing date (D)	2	34.64	***	52.80	***	476.57	***	20.38	***	45.30	***
G*D	28	1.45	NS	0.53	NS	3.21	***	1.35	NS	0.86	NS
Orange 2016-17											
Effect	df	*F*-value		*F*-value		*F*-value		*F*-value		*F*-value	
Genotype (G)	14	1.58	NS	1.14	NS	20.00	***	12.56	***	3.13	**
Sowing date (D)	2	102.81	***	52.76	***	223.46	***	11.81	***	10.08	***
G*D	28	1.07	NS	0.82	NS	4.81	***	1.29	NS	1.19	NS
Holland 2016-17											
Effect	df	*F*-value		*F*-value		*F*-value		*F*-value		*F*-value	
Genotype (G)	14	11.10	***	14.79	***	56.89	***	16.34	***	12.2	***
Sowing date (D)	2	68.36	***	58.72	***	330.97	***	33.77	***	40.55	***
G*D	28	2.40	**	6.79	***	2.00	**	1.89	*	2.18	**
Holland 2017-18											
Effect	df	*F*-value		*F*-value		*F*-value		*F*-value		*F*-value	
Genotype (G)	14	15.40	***	3.93	***	166.70	***				
Sowing date (D)	2	196.61	***	38.45	***	431.38	***				
G*D	28	4.74	***	2.50	**	2.36	***				
New Kent 2016-17											
Effect	df	*F*-value		*F*-value		*F*-value		*F*-value		*F*-value	
Genotype (G)	14	7.43	***	1.77	NS	69.84	***	24.32	***	6.56	***
Sowing date (D)	2	27.91	***	4.91	*	215.18	***	4.68	*	16.38	***

G*D	28	0.77	NS	0.71	NS	3.25	***	1.07	NS	0.44	NS
Lexington 2016-17											
Effect	df	*F*-value		*F*-value		*F*-value		*F*-value		*F*-value	
Genotype (G)	14	11.50	***	1.71	NS	166.70	***	10.78	***	14.71	***
Sowing date (D)	2	34.87	***	270.67	***	431.38	***	0.64	NS	0.83	NS
G*D	28	1.25	NS	1.11	NS	2.36	***	1.64	NS	2.00	***

* Significant at the 0.05 probability level
** Significant at the 0.01 probability level
*** Significant at the 0.001 probability level
† NS, non-significant

Table 5. Winter wheat heading date least-square means by environment of the sowing date (D) by genotype (G) interactions for each of the fifteen genotypes and grouped by similar maturity.

	Warsaw 2015-16						Orange 2015-16						Orange 2016-17					
Genotype	24-Sep		9-Oct		22-Oct		23-Sep		8-Oct		20-Oct		23-Sep		5-Oct		18-Oct	
VA13W-177	93.7	k†	97.0	jk	104.0	i	115.0	kl	115.7	i-l	118.0	c-g	111.7	klm	116.3	f-k	118.0	c-i
Jamestown	94.0	k	99.7	j	105.0	hi	114.7	l	115.7	i-l	116.3	g-l	110.7	lm	112.7	j-m	122.3	a-e
VA13W-38	99.7	j	105.0	hi	108.0	gh	115.3	jkl	116.7	f-k	117.0	e-j	110.3	m	113.7	h-m	118.3	c-i
Progeny 117	100.3	j	104.3	i	109.7	fg	115.3	jkl	116.0	h-l	118.7	b-e	113.0	i-m	116.0	f-l	118.7	b-h
Hilliard	104.3	i	110.7	efg	115.0	a-d	116.0	h-l	117.3	e-i	119.3	a-d	112.7	j-m	116.7	f-k	123.7	abc
Shirley	111.0	efg	115.3	a-d	117.0	ab	115.7	i-l	116.7	f-k	119.7	abc	111.7	klm	117.3	e-j	124.0	abc
L11550	109.0	g	114.7	bcd	116.7	abc	115.3	jkl	117.0	e-j	119.7	abc	111.7	klm	113.3	h-m	118.3	c-i
Featherstone 73	110.0	efg	113.3	def	114.7	bcd	115.0	kl	116.7	f-k	119.3	a-d	115.0	g-m	116.3	f-k	118.3	c-i
SY 474	109.3	g	113.3	def	114.7	bcd	117.3	e-i	117.7	d-h	120.3	ab	116.7	f-k	120.3	a-g	124.7	a
USG 3315	113.3	cde	116.7	abc	117.7	ab	117.0	e-j	117.7	d-h	119.7	abc	115.3	f-m	116.7	f-k	120.7	a-f
Pioneer 25R32	115.3	a-d	117.0	ab	118.3	a	117.7	d-h	118.7	b-e	120.3	ab	116.7	f-k	118.3	c-i	122.3	a-e
USG 3251	113.0	def	116.0	a-d	117.0	ab	115.3	jkl	117.7	d-h	120.3	ab	117.3	e-j	120.7	a-f	123.0	a-d
Pioneer 26R10	110.0	efg	115.3	a-d	116.7	abc	115.7	i-l	117.0	e-j	119.7	abc	118.0	d-j	120.0	a-g	123.7	abc
SS 8870	113.3	cde	116.3	a-d	117.3	ab	115.7	i-l	117.0	e-j	119.7	abc	116.3	f-k	118.7	b-h	124.0	ab
VA11HWW-113	114.7	bcd	117.0	ab	118.0	ab	117.3	e-i	118.3	c-f	121.0	a	118.0	d-j	124.0	ab	125.3	a

	New Kent 2016-17						Holland 2016-17						Lexington 2016-17					
Genotype	4-Oct		18-Oct		30-Oct		27-Sep		14-Oct		27-Oct		10-Oct		30-Oct		14-Nov	
VA13W-177	106.3	o	107.3	o	111.3	klm	101.0	m†	102.0	lm	104.0	g-l	106.3	o	107.3	o	111.3	klm
Jamestown	108.3	no	108.3	no	112.0	jkl	101.0	m	103.7	h-l	105.0	e-j	108.3	no	108.3	no	112.0	jkl
VA13W-38	109.0	mno	110.7	lmn	114.7	g-j	102.3	klm	104.0	g-l	107.3	b-e	109.0	mno	110.7	lmn	114.7	g-j
Progeny 117	111.0	k-n	111.3	klm	116.3	f-i	102.3	klm	105.3	e-i	108.0	a-d	111.0	k-n	111.3	klm	116.3	f-i
Hilliard	112.0	jkl	113.7	ijk	117.3	d-g	102.3	klm	104.3	f-l	106.3	c-g	112.0	jkl	113.7	ijk	117.3	d-g
Shirley	114.3	hij	116.7	fgh	119.7	b-e	101.0	m	103.3	h-m	106.3	c-g	114.3	hij	116.7	fgh	119.7	b-e
L11550	114.7	g-j	117.3	d-g	120.7	ab	101.0	m	102.7	j-m	104.0	g-l	114.7	g-j	117.3	d-g	120.7	ab
Featherstone 73	113.7	ijk	117.0	e-h	120.7	ab	103.0	i-m	105.0	e-j	106.3	c-g	113.7	ijk	117.0	e-h	120.7	ab
SY 474	114.7	g-j	116.7	fgh	119.7	b-e	104.0	g-l	107.3	b-e	108.3	abc	114.7	g-j	116.7	fgh	119.7	b-e
USG 3315	115.3	f-i	116.3	f-i	119.7	b-e	104.0	g-l	106.3	c-g	108.7	abc	115.3	f-i	116.3	f-i	119.7	b-e

Pioneer 25R32	116.0	f-i	117.7	c-f	120.3	abc	104.0	g-l	105.3	e-i	108.0	a-d	116.0	f-i	117.7	c-f	120.3	abc
USG 3251	114.7	g-j	116.3	f-i	120.0	a-d	104.0	g-l	105.7	d-h	108.0	a-d	114.7	g-j	116.3	f-i	120.0	a-d
Pioneer 26R10	113.7	ijk	116.7	fgh	120.0	a-d	105.3	e-i	106.7	b-f	108.0	a-d	113.7	ijk	116.7	fgh	120.0	a-d
SS 8870	115.0	f-i	117.0	e-h	120.7	ab	104.7	f-k	107.3	b-e	108.7	abc	115.0	f-i	117.0	e-h	120.7	ab
VA11HWW-113	121.0	ab	122.0	ab	122.7	a	108.0	a-d	109.0	ab	110.0	a	121.0	ab	122.0	ab	122.7	a

	Warsaw 2017-18						**Holland 2017-18**					
Genotype	25-Sep		6-Oct		23-Oct		2-Oct		18-Oct		4-Nov	
VA13W-177	117.0	op	118.0	m-p	120.0	h-k	106.0	h	106.0	h	107.7	gh
Jamestown	116.7	p	117.7	nop	120.0	h-k	106.0	h	106.0	h	109.3	fgh
VA13W-38	118.0	m-p	118.7	k-n	121.0	ghi	106.0	h	106.0	h	107.7	gh
Progeny 117	118.3	l-o	119.3	j-m	121.0	ghi	106.0	h	106.0	h	107.7	gh
Hilliard	119.7	i-l	121.0	ghi	122.0	efg	106.0	h	106.0	h	111.0	e-h
Shirley	119.7	i-l	121.3	fgh	122.7	c-f	107.7	gh	106.0	h	116.0	b-f
L11550	121.0	ghi	122.3	d-g	123.0	cde	107.7	gh	107.7	gh	120.0	abc
Featherstone 73	121.0	ghi	122.3	d-g	123.3	cde	106.0	h	111.0	e-h	120.0	abc
SY 474	121.0	ghi	122.0	efg	123.0	cde	107.7	gh	109.3	fgh	118.0	a-e
USG 3315	119.7	i-l	122.0	efg	123.7	bcd	111.0	e-h	116.0	b-f	119.3	a-d
Pioneer 25R32	120.3	hij	121.0	ghi	123.0	cde	106.0	h	109.3	fgh	120.0	abc
USG 3251	120.3	hij	121.3	fgh	122.7	c-f	111.0	e-h	112.7	d-h	123.7	a
Pioneer 26R10	120.0	h-k	121.0	ghi	122.3	d-g	111.0	e-h	114.3	c-g	120.0	abc
SS 8870	121.0	ghi	122.7	c-f	124.0	bc	112.7	d-h	116.0	b-f	123.7	a
VA11HWW-113	123.7	bcd	125.0	ab	125.7	a	114.3	c-g	120.0	abc	122.0	ab

† Means within site-year connected by the same letter are not different (α=0.05)

Table 6. Average winter wheat grain yield (kg ha^{-1}) for each genotype by environment and least-square means for environments with significant (P<0.05) of the sowing date (D) by genotype (G) interactions for each of the fifteen genotypes and grouped by similar maturity.

	Warsaw 2015-16						Orange 2015-16			Orange 2016-17		
Genotype	24-Sep		9-Oct		22-Oct		23-Sep	8-Oct	20-Oct	23-Sep	5-Oct	18-Oct
VA13W-177	4199	g-m†	4443	c-m	4416	c-m	1840	1713	3191	6198	5652	3628
Jamestown	3950	j-n	3861	k-n	4308	e-m	1443	2024	3695	6483	5544	2786
VA13W-38	4665	a-l	4930	a-k	5232	a-g	2546	2972	4521	5706	6232	2589
Progeny 117	3994	i-n	3860	k-n	5041	a-j	3124	4056	5018	6933	7016	3327
Hilliard	5624	ab	5346	a-f	5357	a-e	2345	3894	5361	7579	5017	4241
Shirley	5058	a-j	4313	e-m	3652	lmn	3594	5264	5102	7802	7851	2704
L11550	5710	a	5086	a-i	4927	a-k	3610	4387	5555	7811	7772	4390
Featherstone 73	5178	a-h	4844	a-k	3540	mn	3392	5251	3996	7651	7063	2139
SY 474	4719	a-l	5483	a-d	4730	a-l	4981	5968	5098	8364	7342	2756
USG 3315	5345	a-f	4395	d-m	3001	n	3113	5592	4255	6763	6549	3217
Pioneer 25R32	4770	a-k	4548	b-m	4024	i-n	3451	4088	5110	8011	6728	5007
USG 3251	4925	a-k	4814	a-k	4236	f-m	4544	4732	4803	8548	6911	4034
Pioneer 26R10	5632	ab	4887	a-k	4376	e-m	3739	5424	5878	7015	9085	3183
SS 8870	4312	e-m	3517	mn	3920	klmn	1723	3823	5862	8017	6008	2154
VA11HWW-113	5332	a-f	4071	h-n	5516	abc	3789	4785	5209	7118	6902	4456

	New Kent 2016-17				Holland 2016-17				Lexington 2016-17		
Genotype	4-Oct	18-Oct	30-Oct	27-Sep		14-Oct		27-Oct		10-Oct	30-Oct	14-Nov
VA13W-177	6065	6983	6423	3897	i-o†	4447	e-k	4225	g-n	7304	6787	5905
Jamestown	4540	5403	5427	3992	hi-o	4293	f-m	4084	g-o	5575	5252	5279
VA13W-38	5489	6268	6286	3437	nop	4413	e-l	3989	h-o	5762	6318	5931
Progeny 117	4614	5271	5413	3389	nop	3749	k-p	3277	opq	5920	5267	4349
Hilliard	5662	6525	6498	4202	g-n	4540	d-k	4602	c-j	7892	7296	6385
Shirley	6145	6142	6674	3932	i-o	5215	a-e	4807	b-h	5983	6227	5522
L11550	5702	6846	7253	3557	m-p	5393	abc	5806	a	6941	6466	6485
Featherstone 73	5584	6934	6445	3794	j-o	5342	a-d	4404	e-m	7421	7028	6936
SY 474	5742	6779	6376	2338	r	4360	f-m	3970	h-o	6508	5774	5560
USG 3315	6135	7444	6673	2447	qr	4731	b-i	4309	f-m	7037	5752	5165

Pioneer 25R32	6195	7052	7482	5232	a-e	5974	a	5372	a-d	6484	5578	4575
USG 3251	5629	6700	6825	2911	pqr	5134	a-f	4101	g-o	6445	5634	5408
Pioneer 26R10	6513	7147	6962	4089	g-o	5477	ab	5336	a-d	5896	5240	4616
SS 8870	6560	6826	6571	2371	r	4884	b-g	3430	nop	6223	5802	5460
VA11HWW-113	5961	7390	6173	3584	lm-p	4173	g-n	4621	c-j	6566	5522	5856

	Warsaw 2017-18					**Holland 2017-18**			
Genotype	25-Sep	6-Oct	23-Oct	2-Oct		18-Oct		4-Nov	
VA13W-177	5300	5108	5184	6399	g-k	6319	h-l	4996	rst
Jamestown	5005	4523	4755	6393	g-k	5883	j-o	4872	st
VA13W-38	5322	5052	5002	6100	i-n	6505	f-i	4832	t
Progeny 117	5249	5297	5275	6646	e-i	5795	l-p	5637	n-q
Hilliard	5350	5414	5726	7004	def	6231	h-m	5523	o-r
Shirley	5613	5316	5020	7850	a	7294	a-d	6357	g-k
L11550	5094	4828	4883	7149	cde	6723	e-h	5405	o-s
Featherstone 73	5844	5158	5472	7197	b-e	7595	abc	5938	j-o
SY 474	4779	4612	4713	7585	abc	6426	g-j	5531	o-r
USG 3315	5886	6018	5522	6709	e-h	5571	n-q	5948	j-o
Pioneer 25R32	6273	5670	5392	7715	ab	7008	def	5251	p-t
USG 3251	4823	4778	4724	7303	a-d	6507	f-i	5681	l-q
Pioneer 26R10	5793	5558	5395	7588	abc	7006	def	5698	m-q
SS 8870	6124	5859	6153	6913	d-g	5614	n-q	5696	m-q
VA11HWW-113	4779	4490	4903	6108	i-n	5178	q-t	5857	k-o

† Means within site-year connected by the same letter are not different (α=0.05)

Table 7. Pearson correlation coefficients of winter wheat grain yield with tiller estimation (m^{-2}), heading date (Julian), 1000-kernel weight (mg), and protein (%).

Environment	Tiller estimation	Heading date	1000-kernel weight	Protein
		Warsaw		
2015-16	0.46***	0.04	0.21*	-0.18*
2017-18	0.03	-0.18*	0.34***	-0.25**
		Orange		
2015-16	0.04	0.36***	0.67***	-0.84***
2016-17	0.76***	-0.22**	-0.01	-0.27**
		Holland		
2016-17	0.55***	-0.08	0.55***	-0.40***
2017-18	0.58***	-0.40***		
		New Kent		
2016-17	0.25**	-0.34***	0.08	-0.10
		Lexington		
2016-17	0.39***	-0.21*	0.38***	0.04

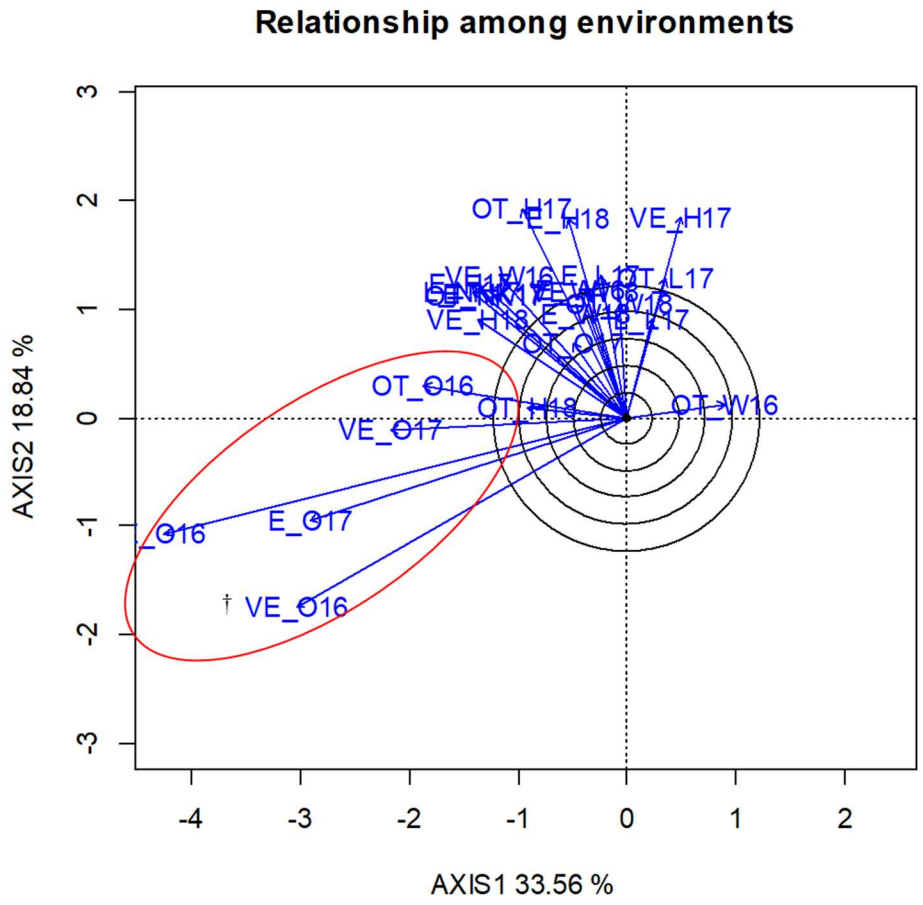
* Significant at the 0.05 probability level
** Significant at the 0.01 probability level
*** Significant at the 0.001 probability level

Table 8. Pearson correlation coefficients of winter wheat grain yield with tiller estimation (m^{-2}) with sowing date.

Environment	Tiller estimation
Warsaw	
2015-16	0.45***
2017-18	0.17
Orange	
2015-16	0.63***
2016-17	0.67***
Holland	
2016-17	0.44***
2017-18	0.51***
New Kent	
2016-17	0.26*
Lexington	
2016-17	0.89***

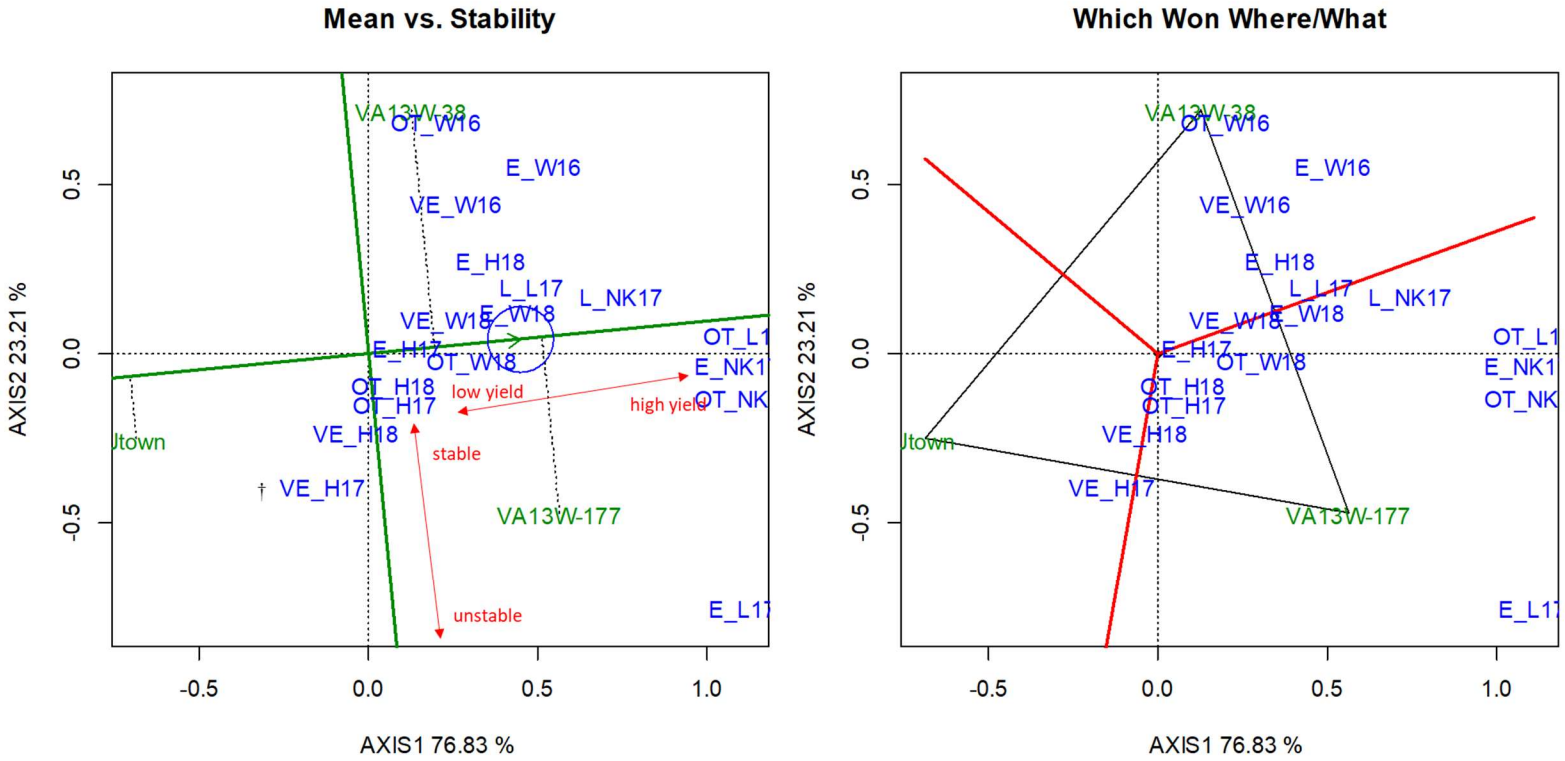
* Significant at the 0.05 probability level
** Significant at the 0.01 probability level
*** Significant at the 0.001 probability level

Supplemental Figure 1. Relationship among environments (site-year and sowing date) (GGE (genotype plus genotype-by-environment) biplot), indicating similarity of winter wheat grain yield performance between site-years.



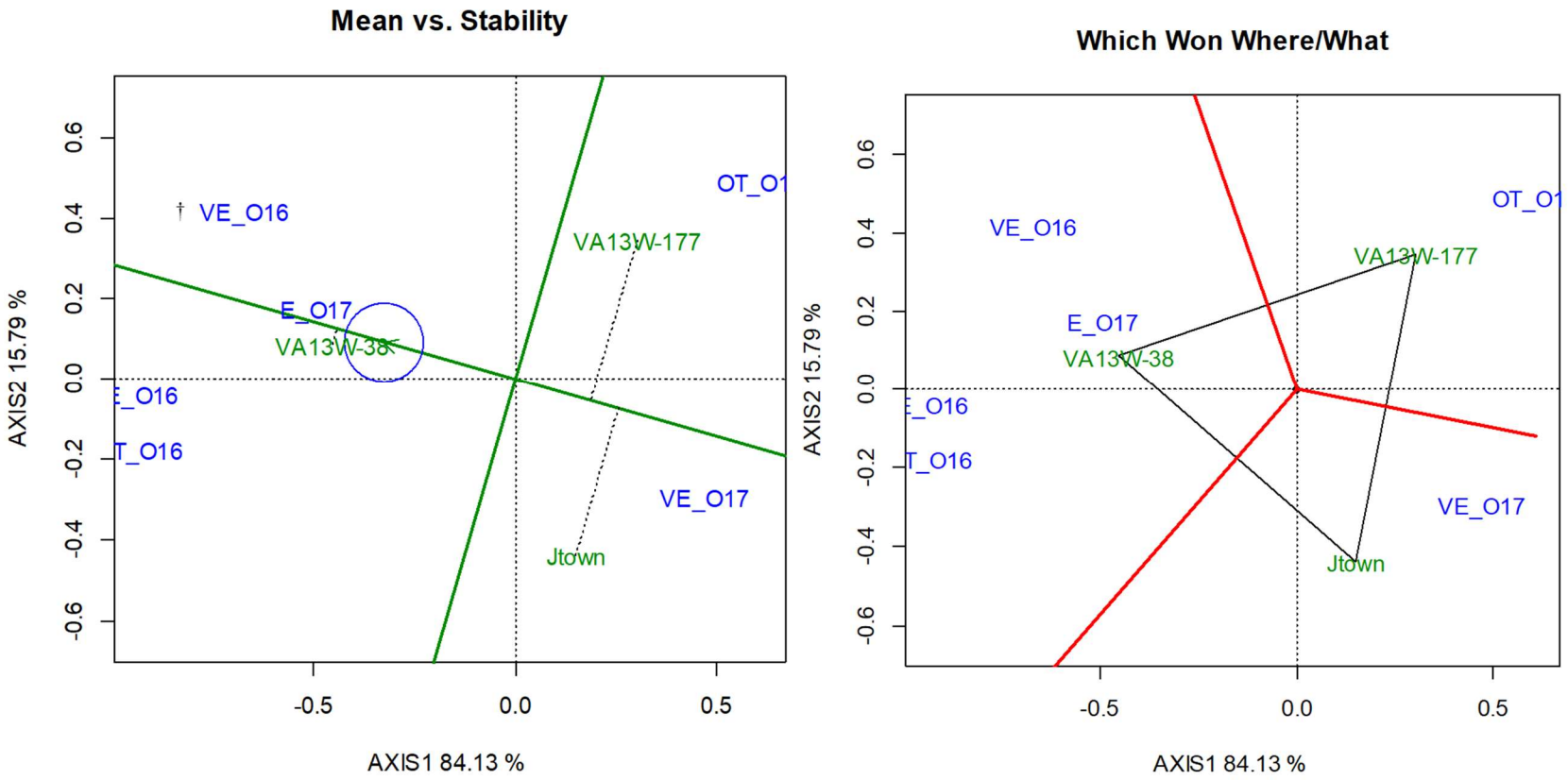
†Indicates site (W=Warsaw, VA, O=Orange, VA, H=Holland, VA, NK=New Kent, VA, L=Lexington, KY)-year (16=2016 harvest year, etc….), sowing date (VE=very early, E=early, OT=recommended, L=late) combination

Supplemental Figure 2. The GGE (genotype plus genotype-by-environment) biplot display of average-environment coordination (AEC or 'mean vs. stability') view to show mean performance and stability and Which Won Where/What view to of the three earliest winter wheat genotypes (VA13W-177, Jtown=Jamestown, and VA13W-38) from Holland, Warsaw, New Kent, VA and Lexington, KY environments (site-years and sowing date). Biplots are based on Scaling = 0, Centering = 0, and SVP=2.



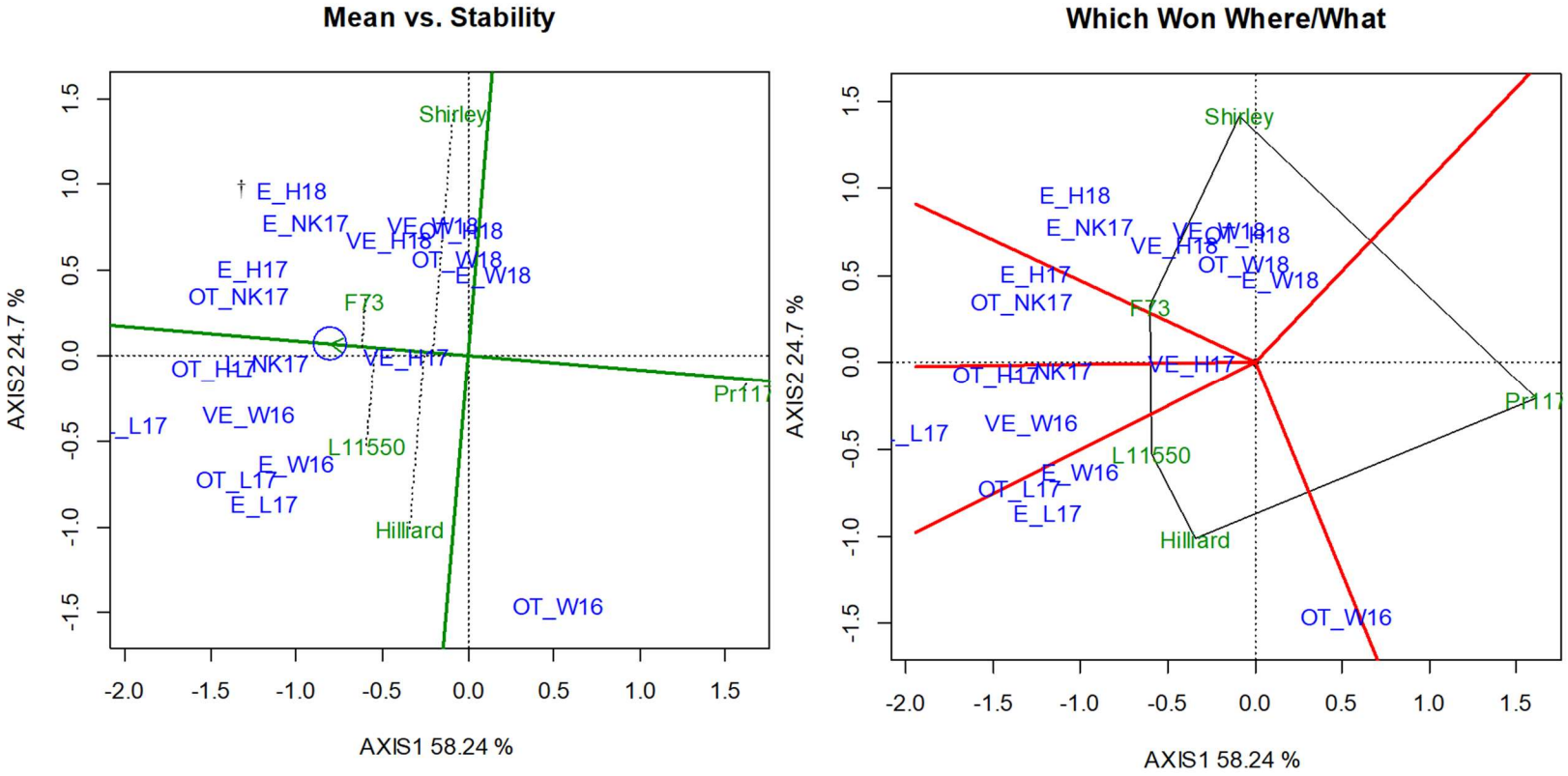
†Indicates site (W=Warsaw, VA, O=Orange, VA, H=Holland, VA, NK=New Kent, VA, L=Lexington, KY)-year (16=2016 harvest year, etc….), sowing date (VE=very early, E=early, OT=recommended, L=late) combination

Supplemental Figure 3. The GGE (genotype plus genotype-by-environment) biplot display of average-environment coordination (AEC or 'mean vs. stability') view to show mean performance and stability and Which Won Where/What view to indicate the three earliest winter wheat genotypes (VA13W-177, Jtown = Jamestown, and VA13W-38) from the Orange, VA environments (site-years and sowing date). Biplots are based on Scaling = 0, Centering = 0, and SVP=2.



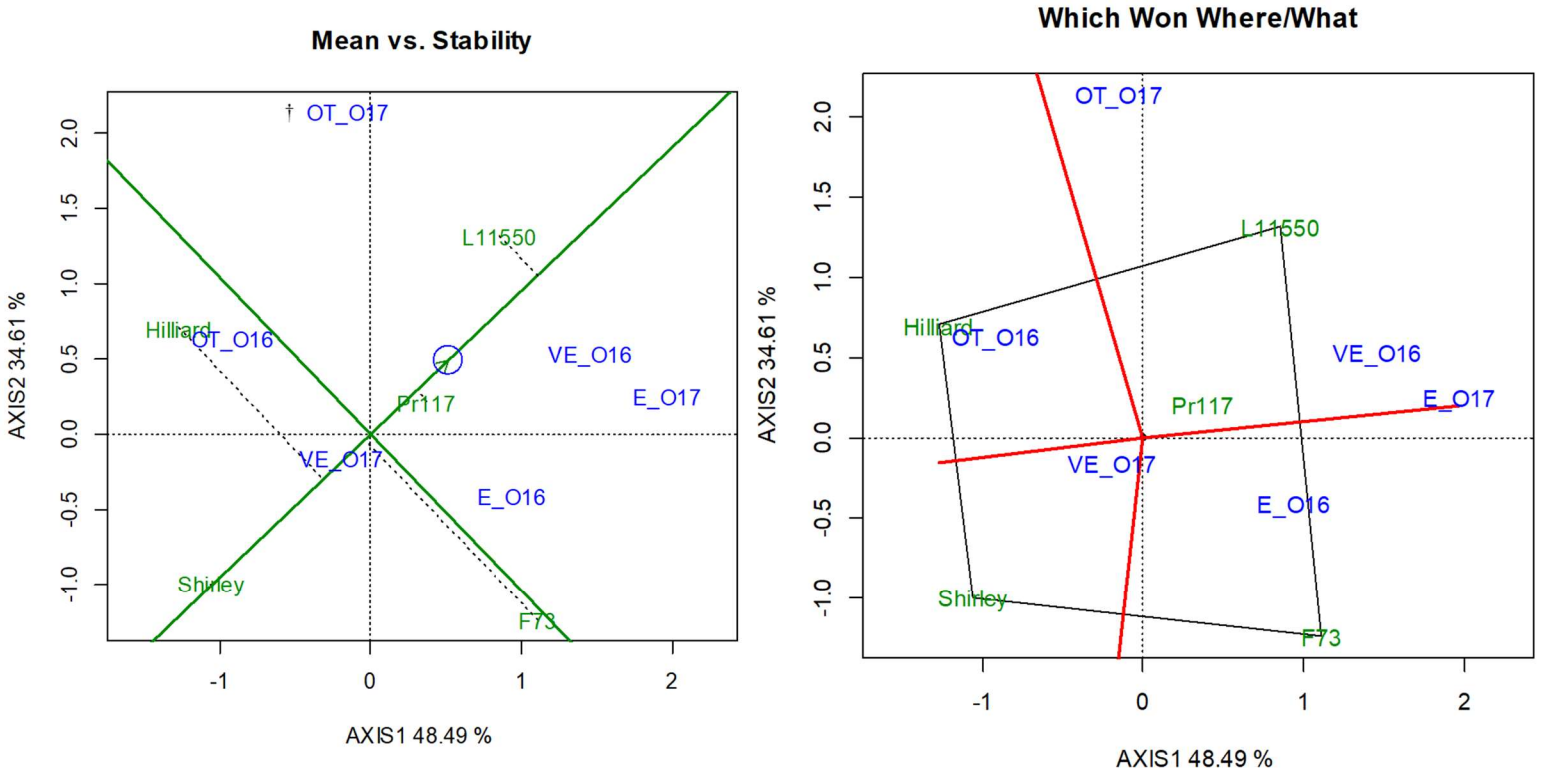
†Indicates site (O=Orange)-year (16=2016 harvest year, etc….), sowing date (VE=very early, E=early, OT=recommended) combination

Supplemental Figure 4. The GGE (genotype plus genotype-by-environment) biplot display of average-environment coordination (AEC or 'mean vs. stability') view to show mean performance and stability and Which Won Where/What view to indicate the five medium maturity winter wheat genotypes (Pr117=Progeny 117, F73=Featherstone 73, Hilliard, Shirley, L11550) from Holland, Warsaw, New Kent, VA and Lexington, environments (site-years and sowing date). Biplots are based on Scaling = 0, Centering = 0, and SVP=2.



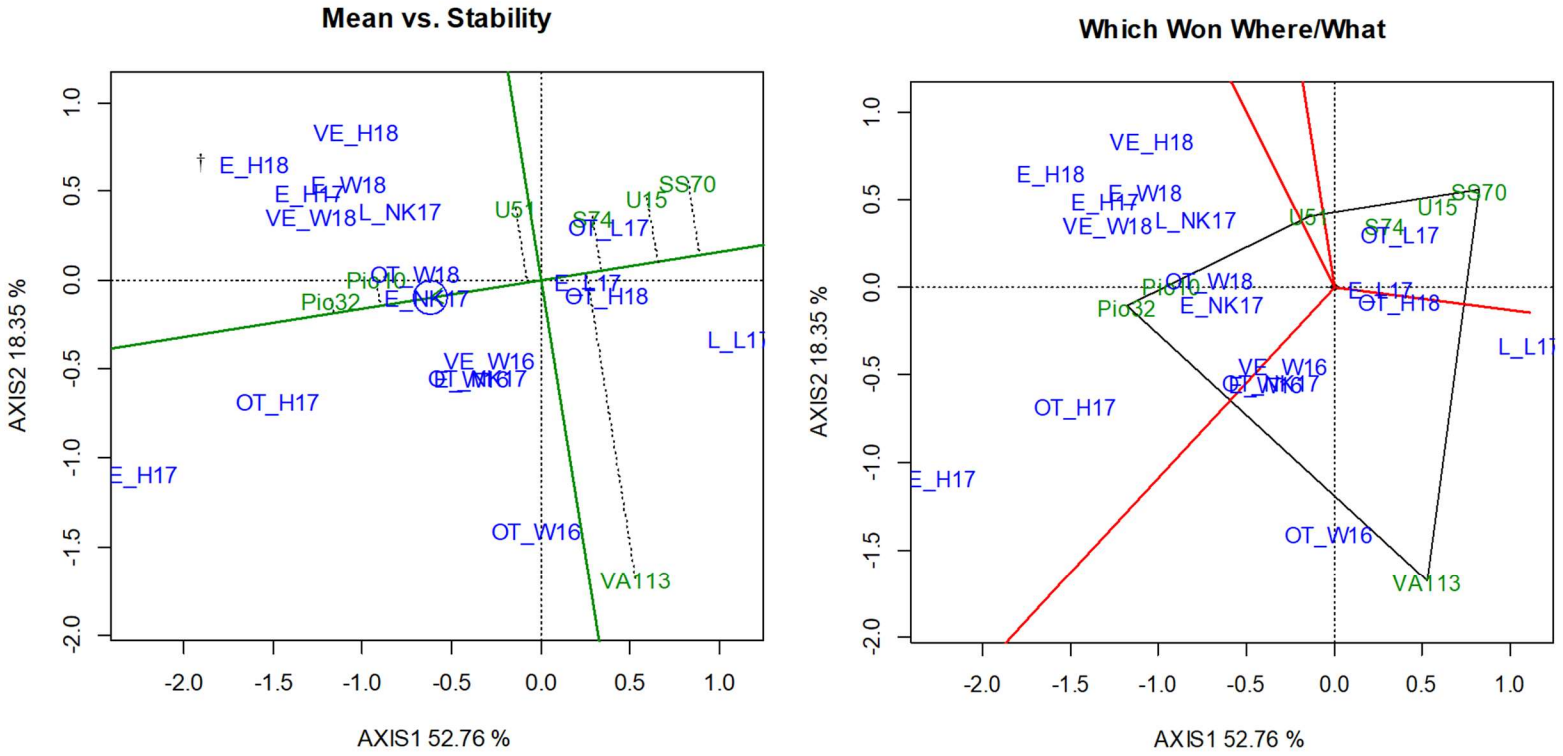
†Indicates site (W=Warsaw, VA, O=Orange, VA, H=Holland, VA, NK=New Kent, VA, L=Lexington, KY)-year (16=2016 harvest year, etc….), sowing date (VE=very early, E=early, OT=recommended, L=late) combination

Supplemental Figure 5. The GGE (genotype plus genotype-by-environment) biplot display of average-environment coordination (AEC or 'mean vs. stability') view to show mean performance and stability and Which Won Where/What view to indicate the five medium maturity winter wheat genotypes (Pr117=Progeny 117, F73=Featherstone 73, Hilliard, Shirley, L11550) from the Orange, VA environments (site-years and sowing date). Biplots are based on Scaling = 0, Centering = 0, and SVP=2.



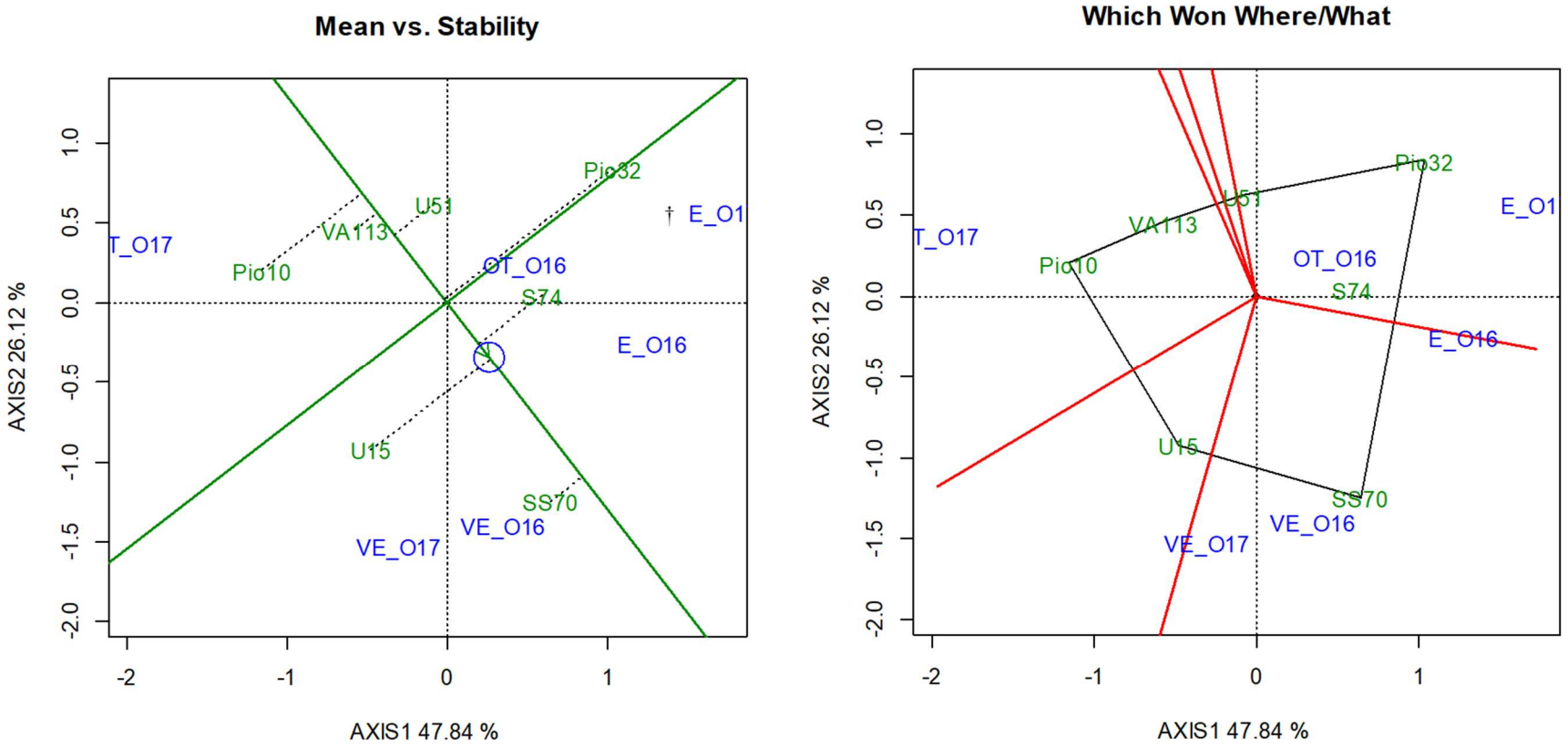
†Indicates site (O=Orange)-year (16=2016 harvest year, etc….), sowing date (VE=very early, E=early, OT=recommended) combination

Supplemental Figure 6. The GGE (genotype plus genotype-by-environment) biplot display of average-environment coordination (AEC or 'mean vs. stability') view to show mean performance and stability and Which Won Where/What view to indicate the seven late maturity winter wheat genotypes (U51=USG 3251, U15- USG 3315, Pio32=Pioneer 25R32, Pio10=Pioneer 26R10, S74=SY 474, SS70=SS8870, VA113=VA11HWWW-113) from the Holland, Warsaw, New Kent, VA and Lexington, KY environments (site-years and sowing date). Biplots are based on Scaling = 0, Centering = 0, and SVP=2.



†Indicates site (W=Warsaw, VA, O=Orange, VA, H=Holland, VA, NK=New Kent, VA, L=Lexington, KY)-year (16=2016 harvest year, etc….), sowing date (VE=very early, E=early, OT=recommended, L=late) combination

Supplemental Figure 7. The GGE (genotype plus genotype-by-environment) biplot display of average-environment coordination (AEC or 'mean vs. stability') view to show mean performance and stability and Which Won Where/What view to indicate the seven medium maturity winter wheat genotypes (U51=USG 3251, U15- USG 3315, Pio32=Pioneer 25R32, Pio10=Pioneer 26R10, S74=SY 474, SS70=SS8870, VA113=VA11HWWW-113) from the Orange, VA environments (site-years and sowing date). Biplots are based on Scaling = 0, Centering = 0, and SVP=2.



†Indicates site (O=Orange)-year (16=2016 harvest year, etc….), sowing date (VE=very early, E=early, OT=recommended) combination

Supplemental Table 1. Soil type, elevation, and tillage type for each site-year.

Season	Location	Soil type	Elevation	Tillage
2015-16	Warsaw, VA	Kempsville Sandy Loam	36 m	conventional
2017-18	Warsaw, VA	Kempsville Sandy Loam	40 m	conventional
2016-17	Holland, VA	Lynchburg fine sandy loam	23 m	no till (following maize)
2017-18	Holland, VA	Rains fine sandy loam	24 m	no till (following maize)
2015-16	Orange, VA	Davidson Clay	146 m	conventional
2016-17	Orange, VA	Davidson Clay	158 m	conventional
2016-17	New Kent, VA	Pamunkey fine loam	4 m	no till (following maize)
2016-17	Lexington, KY	Lowell-Bluegrass silt loam	300 m	conventional

Supplemental Table 2. Management practices by site-year including fertilizer, active ingredient (A.I.) growth regulator, and application dates.

Season	Location	Harvest area	Product	Application rate	Application date
2015-16	Warsaw, VA	1.5 x 2.7 m	Pre-plant fertilizer	36-80-80-5(S)	9/23/2015
			Strane™	0.21 kg A.I. ha-1	12/7/2015
			UAN*	33.6 kg ha-1	12/14/2015
			Tilt®	0.07 A.I. kg ha-1	12/16/2015
			UAN	33.6 kg ha-1	2/12/2016
			Prosaro®	0.14 kg A.I. ha-1	5/3/2016
2017-18	Warsaw, VA	1.5 x 2.7 m	Pre-plant fertilizer	30-60-60	9/20/2017
			Harmony Extra SG®	0.02 kg A.I. ha-1	11/30/2017
			UAN	33.6 kg ha-1	12/7/2017
			UAN	33.6 kg ha-1	2/9/2018
			Tilt®	0.07 A.I. kg ha-1	3/17/2018
			Palisade EC®	0.14 kg A.I. ha-1	3/17/2018
			Fitness®	0.07 kg A.I. ha -1	4/11/2018
			Prosaro®	0.14 kg A.I. ha-1	5/1/2018
2016-17	Holland, VA	1.5 x 4.9 m	Pre-plant fertilizer	9-15-31	9/26/2016
			24-0-03 dry fertiilizer	68.0 kg ha-1	2/28/2017
			Palisade EC®	0.14 kg A.I. ha-1	3/17/2017
			Axel®	0.30 kg A.I. ha-1	3/17/2017
			Fitness®	0.07 kg A.I. ha -1	4/26/2017
2017-18	Holland, VA	1.5 x 4.9 m	Pre-plant	10-17-29	9/27/2017
			Osprey®	0.09 kg A.I. ha-1	1/26/2018
			Harmony Extra SG®	0.02 kg A.I. ha-1	1/26/2018
			24-0-03 dry fertiilizer	68.0 kg ha-1	2/27/2018
			Tilt®	0.14 kg A.I. ha-1	3/17/2018
			Palisade EC®	0.14 kg A.I. ha-1	3/23/2017
			24-0-03 dry fertiilizer	33.6 kg ha-1	4/2/2018
2015-16	Orange, VA	1.5 x 2.7 m	Pre-plant	30-80-60	9/23/2015
			UAN	68.0 kg ha-1	2/21/2016
			Harmony Extra SG®	0.02 kg A.I. ha-1	2/21/2016
2016-17	Orange, VA	1.5 x 2.7 m	Pre-plant	30-80-60	9/23/2015
			UAN	68.0 kg ha-1	2/26/2017
			Harmony Extra SG®	0.02 kg A.I. ha-1	3/5/2017
2016-17	New Kent, VA	1.5 x 2.7 m	Pre-plant	variable	10/3/2016
			UAN	33.6 kg ha-1	10/10/2016
			UAN	33.6 kg ha-1	3/3/2017
			Palisade EC®	0.14 kg A.I. ha-1	3/15/2017
			UAN	68.0 kg ha-1	3/18/2017

			Quilt Xcel®	0.21 A.I. kg ha-1	4/13/2017
2016-17	Lexington, KY	1.5 x 2.7 m	UAN	33.6 kg ha-1	3/1/2017
			Tilt®	0.06 A.I. kg ha-1	3/1/2017
			Harmony Extra SG®	0.02 kg A.I. ha-1	3/5/2017
			UAN	60.0 kg ha-1	3/15/2017

*UAN=urea and ammonium nitrate

Supplemental Table 3. Average precipitation (mm) by month over each growing season by site-year.

Location	Warsaw		Orange		Holland		New Kent	Lexington
Year	**15-16**	**16-17**	**15-16**	**16-17**	**16-17**	**17-18**	**16-17**	**16-17**
Sept.	107	33	166	82	361	80	135	42
Oct.	103	108	86	2	291	69	146	21
Nov.	123	37	59	40	31	33	25	34
Dec.	132	74	84	53	44	29	73	157
Jan.	31	59	40	65	83	30	111	120
Feb.	129	76	75	17	15	39	36	86
March	19	98	42	69	138	80	107	83
April	62	100	67	92	108	89	95	47
May	230	172	88	173	139	104	159	143
June	95	244	118	45	84	41	57	147

CHAPTER II:

Quantitative Trait Loci Associated with Improved End-Use Quality in Soft Red Winter Wheat

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Abbreviations: SRWW, soft red winter wheat; QTL, quantitative trait loci; LOD, logarithm of odds; KASP, kompetative allele specific polymerase chain reaction; SNP, single nucleotide polymorphism; Pioneer 25R47 / Jamestown, PxJ; Pioneer 26R46 / Tribute, PxT; DArT, Diversity Array Technology; SSR, single sequence repeat; GBS, genotyping-by-sequencing; RFLP, restriction fragment length polymorphism; AFLP, amplified fragment length polymorphism; SWQL, USDA-ARS Soft Wheat Quality Lab; TW, test weight (kg hL^{-1}); KP, kernel protein (%); KD, kernel diameter (mm); KH, kernel hardness (0-100); FY, adjusted flour yield (%); SE, softness equivalence (%); FP, flour protein; SRC, solvent retention capacity; LA, solvent retention capacity for lactic acid (%); WA, solvent retention capacity for water (%); SC, solvent retention capacity for sodium carbonate (%); SU, solvent retention capacity for sucrose (%); CD, cookie diameter; TG, cookie top grade.

ABSTRACT

Marker assisted selection (MAS) is a practical tool used by breeders to select phenotypic traits that are difficult or expensive to measure. Genetic studies investigating quantitative trait loci (QTL) for end-use quality (milling and baking) are limited in soft red winter wheat (SRWW) (*Triticum aestivum*). QTL associated with improved end-use quality can be identified and validated with bi-parental mapping populations and DNA marker assays. Two SRWW bi-parental mapping populations (Pioneer '25R47' / 'Jamestown' and Pioneer '26R46' / 'Tribute') were genotyped with a public 90K iSelect SNP-Array, grown over two crop seasons at two Virginia sites, evaluated for quality traits at the USDA-ARS Soft Wheat Quality Lab (SWQL), and analyzed with QTL mapping. A total of 24 putative QTL were identified on 13 different chromosomes and associated with grain characteristics, milling, and/or baking performance. However, only a single QTL, on chromosome 3A (*Qfy.vt.3A.Jtwn*) was consistently detected in both populations for all site-years. *Qfy.vt.3A.Jtwn* is a strong candidate to be utilized for MAS in SRWW breeding programs. The genetics associated with end-use quality in parents of neither population have been investigated previously. One novel QTL (*Qfy.vt.3A.Jtwn*) associated with flour yield is reported herein, along with phenotypic data for both populations, other putative QTL, and transgressive progeny with exceptional flour yield and cookie diameters.

INTRODUCTION

It is estimated that 25% of flour quality is determined by the milling process and 75% is determined by the quality of the wheat. Genotype is a major contributor to overall milling quality (Posner and Hibbs, 2005). It is important for soft red winter wheat (SRWW) breeders to develop cultivars with high flour extraction and superior baking performance, along with appropriate disease resistance and agronomic performance to ensure production of sound grain that meets the needs of millers and bakers. Kernel texture (hard or soft) and protein (content and quality) determine the primary classifications for the majority of winter wheat (*Triticum aestivum L.*) grown in North America. Classes of wheat define the basic parameters for desired end-uses (Bushuk, 1997, Shewry, 2009). Soft wheat grown in the eastern third of the United States has historically been characterized as having soft texture, weak gluten, less starch damage during milling, and low water absorption. SRWW is the dominant class grown in the Southeastern, mid-Atlantic, and southern Midwest regions of the United States (Gwirtz, et al., 2007). It is typically double cropped with soybeans (*Glycine max*, L Merr) in the Southeast and mid-Atlantic regions (Borchers, et al., 2014). SRWW flour is well-suited for a range of products (Souza, et al., 2012), such as cakes, cookies, crackers, biscuits, flat breads, other pastries, and is often blended with hard wheat to produce all-purpose baking flour (Gwirtz, et al., 2007, Kweon, et al., 2011). Physical and compositional characteristics of the flour determines functionality and suitability for specific products. For example, cookies require weaker gluten strength and lower solvent retention capacities compared to crackers (Kweon, et al., 2011).

Souza et al., (2012) detailed the basis for selecting wheat for end-use quality in a comprehensive report that evaluated 1747 experimental units of grain samples from 187 soft winter wheat cultivars released from 1801 to 2005. The report indicates that soft wheat breeders should focus

on milling yield (FY), flour softness equivalence (SE), and sucrose (SU) solvent retention capacity (SRC), because these traits predict long-flow flour milling performance and have value for commercial milling and baking. Variety selection is highly effective due to large genetic variation and relatively small genotype x environmental interactions for such quality traits. The quality traits FY, SE, LA (lactic acid SRC), SU, and SC (sodium carbonate SRC) have medium to high heritability (Guttieri, et al., 2001, Souza, et al., 2012). Guttieri and Souza (2003) reported that genotype contributed 38 to 95% of total variation in flour yield and 61 to 81% of break-flour yield in three soft spring wheat RIL (recombinant inbred line) populations grown over two locations. While, Smith et al. (2011) reported heritability ranging from 0.91 to 0.94 (FY), 0.89 to 0.93 (SE), 0.93 to 0.96 (LA), and 0.87 to 0.92 (SO) in a Pioneer '25R46' / 'Foster' (Van Sanford, et al., 1997) RIL population.

End-use quality characteristics are influenced by growing environment and are under quantitative genetic control; therefore, phenotypic selection is not always possible early in the breeding cycle. MAS for predictive QTL can facilitate selection earlier in the breeding cycle, reducing the amount of field or greenhouse space needed to grow experimental lines. Hundreds of QTL for end-use quality have been reported in wheat, yet most have never been implemented with MAS (Kiszonas and Morris, 2017). Historically, a majority of the end-use quality QTL and DNA markers have been identified in populations derived from either hard by hard or hard by soft crosses, which are generally not informative or lack polymorphism in elite SRWW breeding populations. Kiszonas and Morris (2017) provide a comprehensive list of mapped end-use quality QTL, which primarily were genotyped with SSR, RFLP, AFLP, or DArT markers. QTL reported in soft x soft mapping populations are significantly fewer (Breseghello and Sorrells, 2006, Cabrera, et al., 2015, Gaire, et al., 2019, Ishikawa, et al., 2014, Jiang, et al., 2017, Reif, et

al., 2011, Smith, et al., 2011), and currently only three reports include QTL linked with high-throughput SNP markers associated with milling or baking quality (Table 1).

Despite QTL associated with end-use quality being reported in SRWW, practical use of MAS to improve quality remains limited in variety development programs. Further validation, implementation, and development of more high-throughput markers associated with identified QTL is needed to increase practical application of MAS for improved milling and baking quality traits. Markers associated with *Glu-B1*, *TaSus2-2B*, and the rye (*Secale cereale*) translocations have been clearly documented, validated, and have utility in breeding applications (Cabrera, et al., 2015, Smith, et al., 2011). Investigation of QTL in populations not segregating for these genomic regions are needed to determine significant effects of QTL on other chromosomes, additivity of favorable alleles, and allowing for improved end-use quality in varieties where the rye translocations are desirable. Corroborations of other significant regions are needed to improve breeder confidence in order to further increase deployment of predictive markers and to facilitate improvement of end-use quality in SRWW.

MATERIALS AND METHODS

Plant materials

A primary mapping population (PxJ) comprised of 186 recombinant inbred lines (RILs) was derived from the cross Pioneer '25R47' (PI 631473) (Lively, et al., 2004) by 'Jamestown' (PI 653731) (Griffey, et al., 2010), and previously described by (Carpenter, et al., 2017). The cultivar Jamestown was derived from the cross 'Roane' / Pioneer '2691' and developed at Virginia Tech. Pioneer Hi-Bred International developed cultivar 25R47 from the cross WBE-

2190-B-1 (‘Frankenmuth’ / Pioneer ‘2555’ sib // Pioneer ‘2551’ sib) / WBA-416-H-2 (‘Houser’ / MO-9545 // W-4034D / ‘Augusta’) // Pioneer ‘2552’. Both parents lack rye translocations (*1RS.1BL* and *1AL.1RS*), *Glu-B1*, and *TaSus2-2B*. Both have the same high molecular weight glutenin subunits for *Glu-A1* (Ax2*) and *Glu-D1* (2+12). Jamestown has the photoperiod insensitive allele (*D1a*) at the *Ppd-D1* locus, while Pioneer 25R47 has the sensitive (*D1b*) allele (Table 2). Over all site-years, Jamestown was on average 3 cm shorter in plant height, headed five days earlier, and had average grain yields that were 15.6% lower than Pioneer 25R47 (Table 3).

A validation population (PxT) comprised of 113 doubled haploid (DH) lines was derived from the cross Pioneer ‘26R46’ (PI 612154) by ‘Tribute’ (PI 632689) (Griffey, et al., 2005). The cultivar Tribute was derived from the cross VA92-51-39 / AL870365. Pioneer 26R46 was derived from the cross FL7927-G14 // Pioneer '2555'*3 / 'Coker 80-28'. Both Pioneer 26R46 and Tribute are photoperiod insensitive at *Ppd-D1*, lack *Glu-B1* and *TaSus2-2B*, and have the *Glu-D1* (5+10) and *Glu-A1* (Ax2*) glutenin subunits. Tribute possesses the *1RS:1AL* rye (secalin) translocation (Table 2). On average grain yields of Tribute were 9.4% higher, it was 3 cm taller, and reached maturity two days earlier than Pioneer 26R46 (Table 3)

Agronomic considerations

Complete management information is included in Table 4. Each line from both populations, the parents, and VA adapted checks were grown in yield plots comprised of 7 rows spaced 15 cm apart with a total harvested area of 1.5 m × 2.7 m. Seed samples were coated with Gaucho®-XT (imidacloprid, Bayer Crop Science) insecticide and Raxil® MD (Tebuconazole and Metalaxyl, Bayer Crop Science) fungicide. Fall nutrient management and spring Nitrogen (N) applications were based on standard local management practices (Brann, et al., 2009) and recommendations

from the Virginia Cooperative Extension Soil Testing Laboratory. Plots were managed for optimum quality including application of fungicides to control foliar and spike diseases. Test replicates (across environments) were conducted at Kentland Research Farm (37°11'45"N 80°34'28"W) near Blacksburg, VA and in fields near the Eastern Virginia Agriculture Research and Extension Center (37°59'16"N 76°46'48"W) in Warsaw, VA, during the 2015-2016 and 2016-2017 growing seasons. The validation population (PxT) was grown and managed under the same conditions as the primary population, except that it was not grown at Blacksburg during 2015-2016, due to insufficient seed supply.

Phenotyping

Phenotypic evaluations of twelve quality parameters were performed at the USDA-ARS Soft Wheat Quality Lab (SWQL) in Wooster, Ohio, during the summers of 2016 and 2017. All analyses were conducted by the lead author with USDA-ARS staff or under close supervision, to ensure accurate data collection. Procedures for all traits measured can be found on the SWQL website under Materials and Methods (SWQL, 2017).

All grain samples were thoroughly air-aspirated prior to testing to remove any dust or chaff. Data on TW (kg hL^{-1}) was obtained using a modified version of the AACC method 55-10.01 to assess grain size, condition, and packing efficiency of 150 g from each sample. Assessments of KW (kernel weight), KH (kernel hardness), and KD (kernel diameter) were generated by averaging data on 300 kernels measured on the Perten (Springfield, IL) Single Kernel Characterization System (SKCS) using AACC Method 55-31.01. KP (kernel protein) and moisture contents were measured using the DA Perten NIR 7200. Wheat grain was tempered to 15% moisture for at least 24 hours and milled using a modified Quadrumat Senior Milling System. FY, as the fraction of milled products that comprises the break and reduction flour after milling, was calculated as

((150 g of grain – bran) / 150 g) x 100. SE was calculated as (break flour/total flour) x 100 (Finney and Andrews, 1986). FP (flour protein) was determined using the Unity Spectra-Star (Millford, MA) NIR. Flour SRCs for LA and SO were measured on a single gram of flour using AACC Method 55-11.02 (Kweon, et al., 2011). LA predicts gluten strength, while SC estimates starch damage. Finally, sugar-snap cookies were baked according to AACC Method 10-52.02, *Baking Quality of Cookie Flour – Micro Method.* The combined diameter (cm) of two cookies per samples were measured with the Mitutoyo Absolute Digimatic Caliper (Takatsu-ku, Kawasaki, Kanagawa Prefecture, Japan) to generate CD (cookie diameter). TG (cookie top grade) for both cookies was graded visually for surface appearance, from worst to best on a scale of 1 to 10. The SWQL has a grading system for FY and CD based on samples measured between 2009 and 2016, which is discussed in the phenotypic results of this report. Grades for FY (%) samples are; A (>70.78), B (69.64-70.78), C (68.15-69.63), D (66.75-68.14), F (<66.75) and CD (cm) samples are; A >19.24), B (18.82-19.24), C (18.32-18.82), D (17.87-18.32), F (<17.87).

DNA Extraction

All DNA extraction and SNP Array genotyping was previously described (Carpenter, et al., 2017). Tissue of each DH or RIL was collected when seedlings reached the three-leaf stage and placed into 2-ml test tubes, each containing two stainless steel beads for tissue grinding. Tissue samples were frozen in an ultra-low-temperature (-80°C) freezer and then subsequently ground using a Spex CertiPrep 2000 Geno-Grinder for 15s or until finely ground. The DNA extraction was then implemented using a modified cetrimonium bromide (CTAB) method (Saghai-Maroof, et al., 1984).

Single-Nucleotide Polymorphism Array

The iSelect Infinium™ SNP genotyping assay with 90K wheat SNPs (Illumina, San Diego, CA, USA) was performed on both populations at the USDA-ARS Cereal Crops Research Unit in Fargo, ND. Genome Studio 2.0 software (Illumina, 2018) was used to analyze the SNPs according to genotype. The SNP calling algorithm automatically called most SNPs; however, manual curation was performed on both populations in order to ensure quality. Monomorphic markers and markers with ≥10% missing data, or ≤5% minor allele frequency, or did not meet a 1:1 segregation ratio (chi-square P-value ≥ 0.01) were removed (Wang, et al., 2014).

Linkage Analysis and QTL Mapping

Linkage analysis was performed in JoinMap 4.0 (Van Ooijen, 2006) with the Kosambi mapping function (Kosambi, 1943) to estimate map distance for markers that were assigned to linkage groups based on a popular 90,000 SNP consensus (Wang, et al., 2014). Composite interval (ICIM-ADD) mapping with the BIP functionality to identify QTL in IciMapping 4.1.0 (Wang, et al., 2012). A significant QTL (P=0.05) is based on 1000 permutations and a critical LOD value of 3.0 (Doerge and Churchill, 1996). Linkage maps were drawn using MapChart 2.2 (Voorrips, 2002).

Other statistics

ANOVA with genotype and site-year as fixed effects was conducted using the aov() function across site-years comparing transgressive segregates, checks, and parents for FY, CD, and grain yield (kg ha^{-1}) in the R statistical environment (R CoreTeam, 2016, R Studio, 2014). Each site-year is one replication and was treated as a randomized block due to lack of seed resources and the high heritability of milling and baking traits (Guttieri, et al., 2001, Souza, et al., 2012). Least-squares means and Fisher's LSD (P<0.05) were also generated to determine the significance

between the groups in the R statistical environment (R CoreTeam, 2016, R Studio, 2014). A second ANOVA model was used to investigate interactions of different QTL (fixed effect) combinations within each site-year.

RESULTS

Linkage maps

In both populations, markers from the 90K iselect SNP-Array were placed on all 21 chromosomes. After marker curation, the PxJ population linkage map consisted of 21 linkage groups with 1,969 polymorphic markers with an average marker placement of 1.33 cM, while the PxT population linkage map consisted of 22 linkage groups with 2,212 polymorphic markers with an average marker placement of 1.48 cM. As expected, D chromosome marker coverage was very low due to low polymorphism in this genome.

Phenotypic

FY and CD are primary quality assessments considered by breeders and end-users and serve as a proxy when considering overall milling and pastry baking quality of soft wheat (Souza et al., 2012)**.** Both Pioneer cultivars consistently perform above average. Jamestown and Tribute have below average FY and CD. Pioneer 26R46's average FY and CD across site-years was 72.2% and 19.0 cmPioneer 25R47's average FY was 70.7% and CD was 19.1 cm. In contrast, Jamestown and Tribute had average FY's of 67.3% and 68.4% and CD of 17.7 cm and 17.8 cm (Table 3). In both populations, progeny distribution was normal and had similar averages for FY (69.5 and 69.9%) and CD (18.6 and 18.4 cm). The average range for FY was 65.7 to 72.9% and 65.2 to 73.8%, and for CD it was 16.2 to 19.3 cm and 17.2 to 19.7 cm for the PxJ and PxT population, respectively (Table 3). Lines with the best overall quality were derived from the PxT

population. Two lines consistently had exceptional quality with average grades of A for both FY and CD. Line VA11DH-P46xTrib-103 had an average FY of 73.8% and CD of 19.7 cm. Line VA11DH-P46xTrib-99 had an average FY of 73.5% and CD of 19.5 cm (Table 3).

Correlations among traits were similar with previously reported studies (Cabrera, et al., 2015, Knott, et al., 2009, Smith, et al., 2011, Souza, et al., 2012, Yamazaki and Donelson, 1983). Average correlation (Pearson's) across site-years indicated that FY was positively correlated with CD in both the PxJ (r=0.36, P<0.001) and PxT (r=0.66, P<0.001) populations. In contrast, FY was negatively correlated with FP (r=-0.32, P<0.001), LA (r=-0.34, P<0.001), and SO (r=-0.48, P<0.001) in the PxJ population (Table 5). Similar correlations were observed in the PxT population, FY negatively correlated with FP (r=-0.41, P<0.001), LA (r=-0.58, P<0.001), and SO (r=-0.71, P<0.001) (Table 6).

QTL analysis

Composite interval mapping detected nine putative QTL located on eight different chromosomes in the PxJ population (Table 7) and sixteen QTL on eleven different chromosomes in the PxT population (Table 8). Due to the quantitative nature of milling and baking performance, it is not surprising that many QTL were detected, which is consistent with other reports (Table 1). However, inconsistent QTL lacking validation within multiple populations are unlikely to be applied in MAS, due to lack of reliability, effect, and breeder confidence. Therefore, the 3A QTL (*Qfy.vt.3A.Jtwn*) is the only QTL identified in the current study recommended for MAS, due to its significant and consistent effect in both populations for all site-years.

In the PxJ population LOD scores for *Qfy.vt.3A.Jtwn* ranged from 6.9 to 10.3, explaining between 12.3 to 18.3% of the phenotypic variation for FY at all four site-years. FP is also

influenced by this QTL, with LOD scores that ranged from 5.1 to 7.3, and explaining 9.7 to 17.1% of the phenotypic variation at both Warsaw site-years and the Blacksburg 2016 site-year. In the PxT population LOD scores for *Qfy.vt.3A.Jtwn* ranged from 4.6 to 17.0, explaining 9.2 to 27.1% of the phenotypic variation for all three site-years. The best marker for *Qfy.vt.3A.Jtwn* is IWB47146 (left marker), as it is present in both populations (Figure 1). Location of IWB38639 is identical to IWB47146 (116.975 cM) in the PxT population. IWB32527 is the right marker in the PxJ population and 0.1 cM from IWB47146 (Figure 1). IWB50720 is the right marker in the PxT and is 0.4 cM from IWB47146 (Figure 1). Surprisingly, it is the Jamestown allele that is associated with improved quality.

DISCUSSION

Phenotypic

Improving SRWW milling and baking performance is difficult, and as more QTL are reported, it becomes increasingly unclear which markers to deploy for MAS. Therefore, it is important to apply strict criteria for selecting candidate QTL for MAS, as well as having the appropriate breeding stocks for introgression. The six DHs or RILs (VA11DH-P46xTrib-103, VA11DH-P46xTrib-99, VA11DH-P46xTrib-28, VA11DH-P46xTrib-22, VA11DH-P46xTrib-25, and VAP47xJtwn-126) identified as having better or similar (P<0.05) quality to P26R46 (Table 3) are top candidates for use as elite SRWW breeding lines. All six of these lines carry the Jamestown allele version of *Qfy.vt.3A.Jtwn*. Application of markers associated with this QTL and use of the superior-quality lines identified in this study offers a potential strategy to effectively increase genetic gains for FY. This is especially promising as this QTL region is associated with the lesser quality parents Jamestown and Tribute. Meaning that a backcross

strategy to introgress this region from Jamestown or Tribute into a line such as P26R46 could result in progeny with even greater FY and baking performance.

Qfy.vt.3A.Jtwn was predominately associated with FY, however due to strong correlation with FP, SE, LA, SC, CD, and TG (Tables 5 and 6) selecting for this QTL will also likely improve baking performance. This strong correlation between milling and baking performance (Tables 5 and 6) is very similar to previous reports. Cookie baking quality was reported to increase as FY increases (Guttieri and Souza, 2003, Knott, et al., 2009, Souza, et al., 2012). Souza et al. (2012) reported a correlation of (r=0.55) between FY and CD, (r=0.23) between FY and SE, and (r=0.54) between SE and CD. Knott et al. (2009) reported a negative correlation between SRC (SO) with FY (r=-0.40) and CD (r=-0.33). Smith et al. (2011) offered an explanation for these observations. The endosperm is released more easily from the bran during milling in wheat varieties with higher flour yield potential. Another factor is that softer kernels are less resistant to deformation, and require less force to crack, resulting in finer particle sizes, less starch damage, and lower water absorption (Anjum and Walker, 1991). LA is a measure of gluten strength, which is a function of protein quality and to some extent KH. LA increases with corresponding increases in FP. FP and KH are negatively correlated with FY and CD and positively correlated with SRC traits. (Cabrera, et al., 2015, Souza, et al., 2012, SWQL, 2017, Yamazaki and Donelson, 1983). Therefore, a potential strategy to improve pastry baking performance is to select for high flour extraction.

Milling, baking quality, and kernel morphology QTL

Selecting for increased FY early in the breeding process is not feasible without predictive DNA markers. In order to increase selection pressure earlier in the breeding cycle and reduce necessary field and lab resources, it is important to have high confidence QTL to screen breeding

material and select parental lines for crossing. Sixteen of the QTL identified in the current study directly influenced milling and baking performance (Tables 7 and 8). Several of these putative QTL are likely appropriate for MAS after further validation; however, *Qfy.vt.3A.Jtwn* was the only QTL detected in both populations and has the largest effect on FY. Therefore, making it the most relevant region to consider deploying in an MAS program in order to maximize genetic gain for FY. The other QTL lack consistency, therefore achieving significant genetic gains by selecting any of these QTL is unlikely. However, the significant QTL reported in single populations could have utility in genomic prediction in an effort to estimate breeding values and capture more genetic gain from these minor, inconsistent QTL.

Qfy.vt.3A.Jtwn appears novel as no other published reports indicate that these SNPs are associated with FY. However, Gaire et al. (2019) reported a significant (-log(P) value of 5.03) SNP (IWB29612) associated with SRC (sucrose) that mapped within 20 cM of *Qfy.vt.3A.Jtwn* in the PxT population, which implies partial linkage (Figure 1). However, considering the size of the wheat genome and complexity of quantitative traits it is unlikely that this is the same QTL. Single marker-trait pairwise comparison was conducted using the common marker IWB47146 to demonstrate efficacy of selecting for *Qfy.vt.3A.Jtwn* in these populations and the small genotype x environment interaction (Figure 2 and 3).

The Jamestown allelic group consistently outperformed (P<0.05) the P26R46 and P25R47 allelic groups in both populations at all site-years. Furthermore, single-marker pairwise trait comparisons in the PxT population with the *1RS.1AL* rye translocation as a factor proved highly predictive of FY (Table 9). A large amount of phenotypic variation (10.6 – 11.4% for FY and 36.0 – 36.5% for CD) in the PxT population is attributed to the *1RS.1AL* rye secalin translocation (De Froidmont, 1998). The *1RS.1AL* translocation is reported to reduce end-use quality more

than *1RS.1BL* in soft wheat (McKendry, et al., 2001). The progeny group lacking the *1RS.1AL* translocation with the Jamestown allele for *Qfy.vt.3A.Jtwn* (n=25) had an average FY that was 2.4% greater than the progeny group with the *1RS.1AL* translocation and the Pioneer allele for *Qfy.vt.3A.Jtwn* (n=32). The progeny lacking *1RS.1AL* with the Pioneer allele for *Qfy.vt.3A.Jtwn* (n=20) and the progeny with the translocation and the Jamestown allele (n=35) fell in between the previous groups demonstrating the additive effects of these two regions in regards to improving FY.

Several other QTL are worth noting, regions on 1D (*Qfy.vt.1D.P47*) and 5A (*Qfy.vt.5A.P47*) with P25R47 contributing the positive alleles, consistently influenced FY across all four site-years in the PxJ population (Table 7). Another region on 7A (*Qfy.vt.7A.P47*) with P25R47 also contributing the positive allele consistently influenced FP, KP, KH, and SE. Additionally, the SNP marker IWB13913 associated with the 7A QTL in the PxJ population was previously reported to be a flanking marker associated with *TaTKW-7AL* (Su, et al., 2016) in the 'Clark' (Ohm, et al., 1988) background. The current study corroborates results from Su, et al., 2016 and suggests that this is an important region to target with MAS when the objective is to select for increased grain weight and/or yield. However, neither KW nor KD were correlated with FY in the PxJ (r=0.03, r=0.06) (Table 5) or the PxT (r=-0.01, r=-0.05) (Table 6) populations. The trend was similar for CD, except that KD was negatively correlated with CD (r=-0.31) in the PxJ population (Table 5). This suggests that selecting for the markers associated with increased KD/KW will not significantly influence milling or baking quality, which corroborates previous research that suggests smaller kernels do not have diminished flour yield potential compared to larger kernels (Gaines, et al., 1997). However, kernel size is of interest to breeders as it is a

major component of grain yield (Gegas, et al., 2010). KW and grain yield were significantly correlated (r=0.41, P<0.001) in the PxJ population (Table 5).

CONCLUSIONS

Milling and baking performance traits are quantitatively controlled, yet moderately to highly heritable. Wheat grain from the progeny, parents, and checks in this report with high FY potential tend to have relatively low SRC and FP, and high CD. Selection for the Jamestown allele of *Qfy.vt.3A.Jtwn* could be valuable for making incremental improvements in milling and baking performance in SRWW breeding programs. High performing transgressive segregates (VA11DH-P46xTrib-103, VA11DH-P46xTrib-99, VA11DH-P46xTrib-28, VA11DH-P46xTrib-22, VA11DH-P46xTrib-25, and VAP47xJtwn-126) (Table 3) should also be considered as elite breeding material that could be used to develop high FY/CD breeding populations. Small amounts (5 g) of all six lines are available through the Virginia Tech Small Grains Breeding and Genetics program by request.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplementary Table 1 contains sequences for KASP marker design of predictive markers closely linked to important QTL and the closest gene from the reference sequence (Appels, et al., 2018).

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Figure 1. Partial linkage maps of chromosome 3A displaying *Qfy.vt.3A.Jtwn* in the P25R47 / Jamestown and P26R46 / Tribute populations.

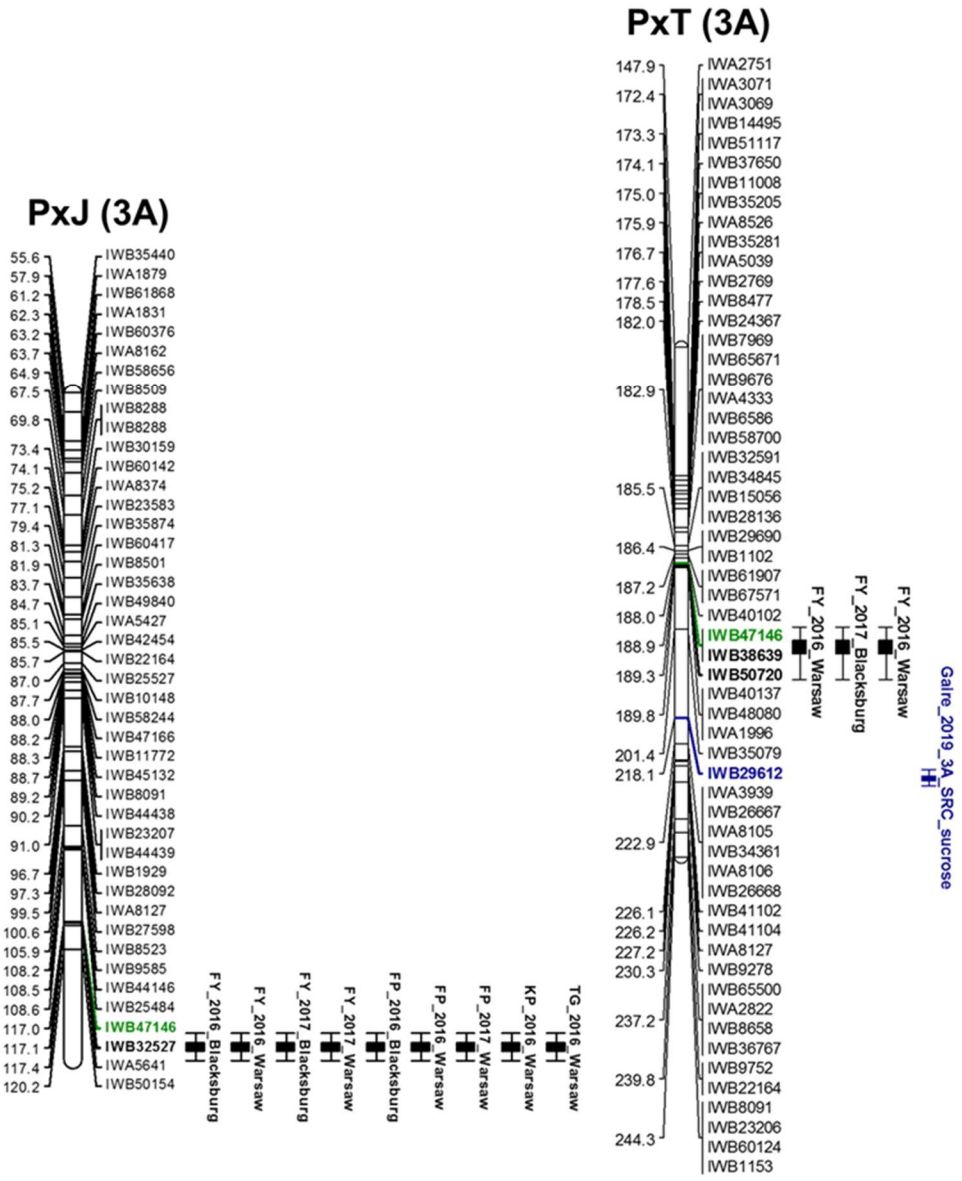

Figure 2. Boxplot representing single marker trait pairwise comparison of least-squares means (Fisher's LSD, $P<0.05$) between progeny with the P25R47 allele and the Jamestown allele for *Qfy.vt.3A.Jtwn* in the P25R47 / Jamestown population at all four site-years.

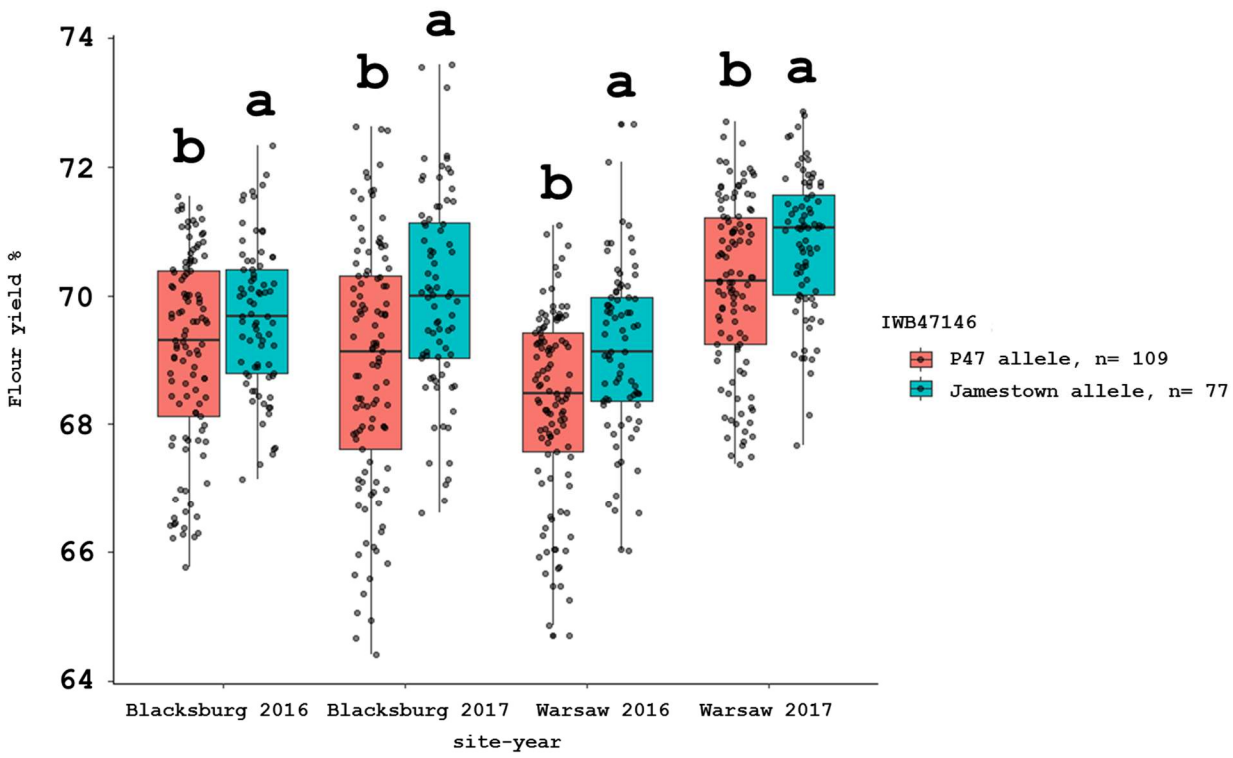

Figure 3. Boxplot representing single marker trait pairwise comparison of least-square means (Fisher's LSD, $P<0.05$) between progeny with the P26R46 allele and the Tribute (same as Jamestown) allele for *Qfy.vt.3A.Jtwn* in the P26R46 / Tribute population at all three site-years.

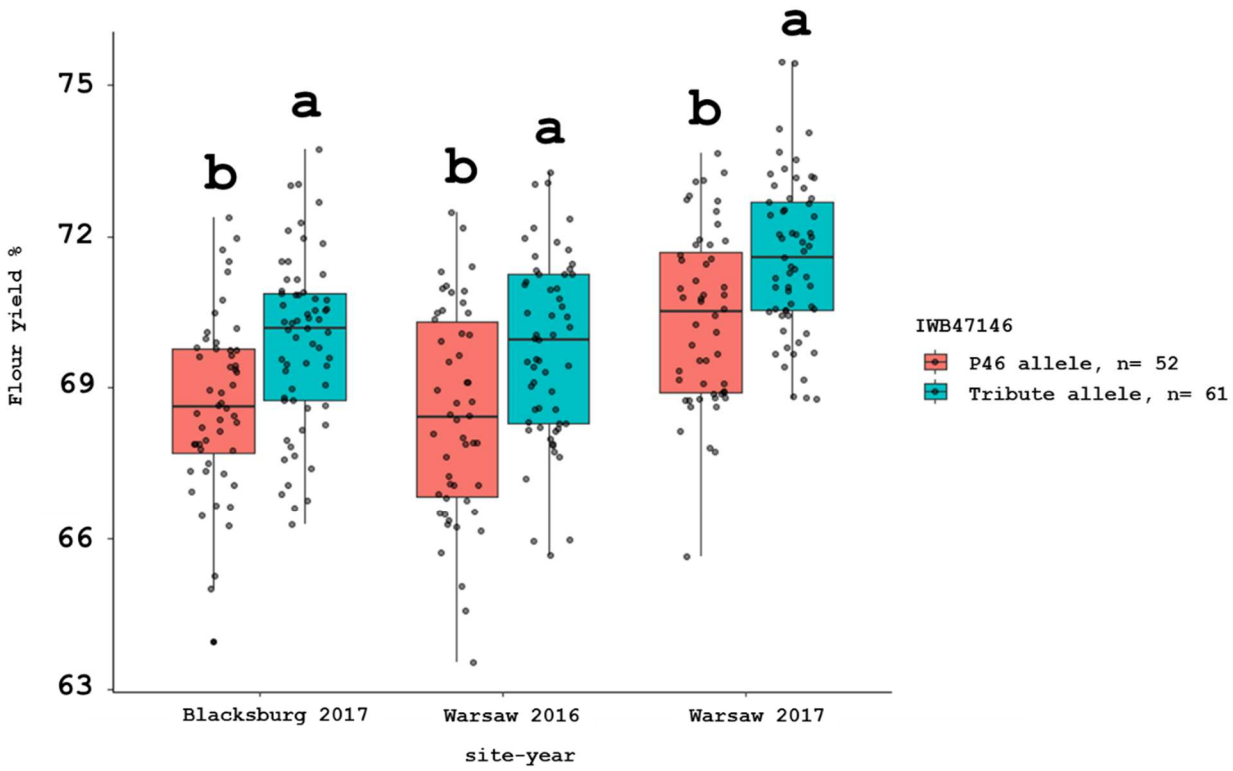

Table 1. Previously reported QTL for end-use quality in soft winter wheat.

Trait	Author (year)	Marker type	Chromosome(s)
Flour yield	Breseghello and Sorrels (2006)	SSR	2D, 5A, 5B
	Smith et al. (2011)	SSR	1B (*1BL.1RS* and *Glu-B1*), 2A, 2B (*TaSus2*), 2D, 3B
	Ishikawa et al., (2014)	DArT & SNP	1B, 2A, 2B, 3B, 4A, 4B, 4D, 5A, 5B, 5D, 6B, 6D, 7A
	Cabrera et al. (2015)	SSR & DArT	1B, 2A, 2B, 3B, 6A, 7A, 7B
	Hoffstetter et al. (2016)	SNP	2B
	Gaire et al. (2019)	SNP	1A, 2A, 2B
Flour protein	Smith et al. (2011)	SSR	2A, 2B, 7D
	Reif et al. (2011)	SSR	1B, 2D, 3A, 5D
	Cabrera et al. (2015)	SSR & DArT	1A, 2A, 2B, 3B, 4A, 4B, 4D, 5A, 6B
	Gaire et al. (2019)	SNP	5A, 6A, 7A
Softness equivalence	Smith et al. (2011)	SSR	2B, 4D, 7D
	Cabrera et al. (2015)	SSR & DArT	1B, 2A, 2B, 4D, 5A, 5B, 6A, 7B
	Hoffstetter et al. (2016)	SNP	2A, 2D, 5A, 5B
	Gaire et al. (2019)	SNP	4A, 4B
Solvent retention capacity (lactic acid)	Smith et al. (2011)	SSR	1B, 1D, 2B, 4D, 7D
	Cabrera et al. (2015)	SSR & DArT	1B, 1D, 2B, 3B, 4A, 4D, 6A
	Jiang et al. (2017)	SSR	3B
	Gaire et al. (2019)	SNP	4B, 5B
Solvent retention capacity (sodium carbonate)	Smith et al. (2011)	SSR	1B, 2B, 3B
	Cabrera et al. (2015)	SSR & DArT	1A, 1B, 2B, 3A, 3B, 6A
	Jiang et al. (2017)	SSR	1B, 1D, 2A, 2B, 2D, 3B, 4A, 6A, 6B, 7A, 7D
	Gaire et al. (2019)	SNP	1B, 7D
Solvent retention capacity (sucrose)	Smith et al. (2011)	SSR	1B, 2B, 3B
	Cabrera et al. (2015)	SSR & DArT	1B, 2A, 2B, 5A, 6A
	Jiang et al. (2017)	SSR	1D, 2D, 3B
	Gaire et al. (2019)	SNP	1B, 2A, 3A
Solvent retention capacity (water)	Smith et al. (2011)	SSR & DArT	1B, 2B, 4A, 5B, 6B, 7B
	Cabrera et al. (2015)	SSR	1B, 2B, 2D, 3B, 7B
	Jiang et al. (2017)	SSR	4A, 4D, 7B, 7D
	Gaire et al. (2019)	SNP	1B, 3A

Table 2. Parental genotypes for major genes associated with end-use quality in soft red winter wheat.

Parent	1RS rye translocations	*Glu-B1*	*Glu-D1*	*Glu-A1*	*Ppd-D1*	*TaSus2-2B*
Pioneer 25R47	non-1RS	no	2+12	Ax2*	insensitive	no
Jamestown	non-1RS	no	2+12	Ax2*	sensitive	no
Pioneer 26R46	non-1RS	no	5+10	Ax2*	insensitive	no
Tribute	*1RS:1AL*	no	5+10	Ax2*	insensitive	no

Table 3. Summary of agronomic performance, average flour yield and cookie diameter of parents, bi-parental populations, highest quality transgressive segregates, and long-term cultivar check Shirley across site-years.

Parents, Progeny, Population	Flour Yield*	Cookie Diameter*	Heading date	Plant height	Grain yield
	%	cm	Julian	cm	kg ha^{-1}
VA11DH-P46xTrib-103	73.8a	19.7a	121	77	4557def
VA11DH-P46xTrib-99	73.5ab	19.5ab	121	78	4298f
VA11DH-P46xTrib-28	73.4ab	19.1abc	119	79	5863a-f
VAP47xJtwn-126	72.9abc	19.1abc	114	74	5758a-f
VA11DH-P46xTrib-22	72.8abc	18.9bcd	111	82	4524def
VA11DH-P46xTrib-25	72.4abc	18.9bcd	117	86	5343a-f
VAP47xJtwn-108	72.4bc	16.3f	116	79	5501a-f
Pioneer 26R46	72.2c	19.0d	110	77	4906a-f
VAP47xJtwn-88	72.0c	18.4d	116	79	6000a-e
VAP47xJtwn-118	71.7cd	19.2abc	116	79	6313ab
VAP47xJtwn-123	71.7cd	19.1abc	114	79	6077abc
Pioneer 25R47	70.7d	19.1abc	114	72	5867a-e
Shirley (check)	69.6e	18.8cd	116	69	6291ab
Tribute	68.4f	17.8e	112	80	5367b-f
Jamestown	67.3g	17.7e	109	69	5077c-f
PxJ Population	69.5	18.6	114	77	5788
PxT Population	69.9	18.4	112	72	4839

*least-square means (Fisher's LSD, $p<0.05$) across site-year, values connected by same letter are not significantly different

Table 4. Winter wheat trial management by site-year.

Locations	Warsaw, VA	Warsaw, VA	Blacksburg, VA	Blacksburg, VA
Coordinates	(37°59'54"N 76°46'23"W)	(37°59'33"N 76°46'24"W)	(37°11'45"N 80°34'28"W)	(36°40'59"N 76°45'38"W)
Year	2015-2016	2016-2017	2015-2016	2016-2017
Soil type	Kempsville Sandy Loam	Kempsville Sandy Loam	Hayter loam	Hayter loam
Elevation	36 m	40 m	518 m	518 m
Area harvested	1.5 x 2.7 m	1.5 x 2.7 m	1.5 x 2.7 m	1.5 x 2.7 m
Fertility	36-80-80 -5(S)dry fertilizer on 9/23 33.6 kg ha^{-1} N (12001.5) on 12/14 33.6 kg ha^{-1} N (12001.5) on 2/12	30-60-60 dry fertilizer on 9/20 33.6 kg ha^{-1} N (12001.5) on 12/7 33.6 kg ha^{-1} N (12001.5) on 2/9	30-60-80-S/8-b1.5 (9/24) 33.6 kg ha^{-1} UAN 30-0-0 (3/8) 56.0 kg ha^{-1} UAN 30-0-0 (4/6) Smart® Zn and Monniplex boron (2586.2 ml ha^{-1}) (4/16)	15-40-60-S/8-B/3 (9/26) 28.0 kg ha^{-1} UAN 30-0-0 (2/17) 67.3 kg ha^{-1} UAN 30-0-0 (3/24)
Pesticides	Strane® (80.8 ml ha^{-1}) on 12/7 Tilt® (47.9 ml ha^{-1}) on 12/16 Prosaro® (95.7 ml ha^{-1}) on 5/3	Tilt® (47.9 ml ha^{-1}) on 3/17 Harmony Extra SG® (9.0 ml ha^{-1}) on 11/30 Prosaro® (95.7 ml ha^{-1}) on 5/1 Fitness® (47.9 ml ha^{-1}) on 4/11	Harmony Extra SG® (7.2 ml ha^{-1}) (11/16) Harmony Extra SG® (7.2 ml ha^{-1}) (4/16) Tilt® (23.9 ml ha^{-1}) on 3/31 Tilt® (23.9 ml ha^{-1}) on 5/26 Prosaro® (80.8 ml ha^{-1}) on 5/12	Harmony Extra SG® (9.0 ml ha^{-1}) (11/15) Harmony Extra SG® (9.0 ml ha^{-1}) (3/24) Tilt® (47.9 ml ha^{-1}) on 3/27 Tilt® (47.9 ml ha^{-1}) on 5/30
Growth regulator	Palisade EC® (95.7 ml ha^{-1}) on 3/14	Palisade EC® (95.7 ml ha^{-1}) on 3/17	Palisade EC® (95.7 ml ha^{-1}) on 3/31	Palisade EC® (95.7 ml ha^{-1}) on 3/27
Tillage	Conventional	Conventional	Conventional	Conventional
Planting date	22 Oct. 2015	23 Oct. 2016	15 Oct 2015	11Oct. 2016
Harvest date	20 June 2016	21 June 2017	26 June 2016	22 June 2017

*UAN=urea and ammonium nitrate

Table 5: Pearson correlation of P25R47 / Jamestown averages of all four site-years among all twelve quality traits, yield (kg ha^{-1}), and heading date (Julian) based on least square means of each winter wheat genotype.

Trait[a]	TW	KP	KH	KD	KW	FY	SE	FP	LA	SC	CD	TG	Y	HD
TW	1	0.31*	0.18	0.14	0.22	0.16	-0.27	0.21	0.07	0.06	-0.07	-0.05	0.05	-0.17
KP		1	0.40*	0.37*	0.35*	-0.22	-0.56*	0.90*	0.08	0.12	-0.40*	-0.33*	-0.26	-0.23
KH			1	0.29*	0.07	-0.15	-0.83*	0.49*	-0.13	0.51*	-0.77*	-0.29*	-0.21	-0.15
KD				1	0.77*	0.06	-0.52*	0.37*	-0.13	0.04	-0.31*	-0.05	0.02	-0.04
KW					1	0.03	-0.42*	0.25	-0.03	-0.06	-0.13	0.04	0.12	-0.14
FY						1	0.18	-0.32*	-0.34*	-0.48*	0.36*	0.58*	0.18	0.17
SE							1	-0.63*	0.04	-0.33*	0.71*	0.29*	0.16	0.27
FP								1	0.15	0.26	-0.52*	-0.51*	-0.32*	-0.21
LA									1	0.29*	-0.17	-0.41*	-0.27	-0.22
SC										1	-0.75*	-0.60*	-0.35*	-0.28*
CD											1	0.56*	0.35*	0.32*
TG												1	0.27	0.19
Y													1	0.64*
HD														1

* Indicates a significant (P<0.001) Pearson's trait correlation using R with Bonferroni multiple comparison adjustment

[a] TW test weight (kg hL^{-1}), KP kernel protein (%), KH kernel hardness (0-100), KD kernel diameter (mm), KW kernel weight (mg), FY flour yield (%), SE softness equivalence (%), FP flour protein (%), LA solvent retention capacity of lactic acid (%), SC solvent retention capacity of sodium carbonate (%), CD cookie diameter (cm), TG cookie top grade (1-9), Y yield (kg ha^{-1}), HD heading date (Julian)

Table 6: Pearson correlation of P26R46 / Tribute average of all three location/years among all twelve quality traits, yield (kg ha^{-1}), and heading date (Julian) based on least-squares means of each genotype.

Traits[a]	TW	KP	KH	KD	KW	FY	SE	FP	LA	SC	CD	TG	Y	HD
TW	1	0.23	0.17	-0.04	-0.08	-0.09	-0.25	0.2	0.1	0.12	-0.25	-0.19	0.00	-0.31*
KP		1	0.17	-0.01	-0.15	-0.44*	-0.17	0.95*	0.57*	0.26	-0.47*	-0.66*	-0.63*	-0.37*
KH			1	-0.09	0.01	-0.33*	-0.85*	0.23	-0.18	0.52*	-0.72*	-0.15	0.03	-0.18
KD				1	0.84*	-0.05	-0.03	-0.09	0.05	0.18	-0.07	-0.05	0.20	-0.1
KW					1	-0.01	-0.12	-0.18	-0.1	0.29*	-0.16	0.00	0.41*	-0.06
FY						1	0.34*	-0.41*	-0.58*	-0.71*	0.66*	0.64*	0.29*	0.47*
SE							1	-0.24	0.16	-0.44*	0.68*	0.12	0.03	0.40*
FP								1	0.53*	0.27	-0.49*	-0.63*	-0.60*	-0.37*
LA									1	0.36*	-0.38*	-0.69*	-0.40*	-0.46*
SC										1	-0.81*	-0.57*	-0.02	-0.46*
CD											1	0.65*	0.1	0.56*
TG												1	0.36*	0.52*
Y													1	0.36*
HD														1

* Indicates a significant (P<0.001) Pearson's trait correlation using R with Bonferroni multiple comparison adjustment

[a] TW test weight (kg hL^{-1}), KP kernel protein (%), KH kernel hardness (0-100), KD kernel diameter (mm), KW kernel weight (mg), FY flour yield (%), SE softness equivalence (%), FP flour protein (%), LA solvent retention capacity of lactic acid (%), SC solvent retention capacity of sodium carbonate (%), CD cookie diameter (cm), TG cookie top grade (1-9), Y yield (kg ha^{-1}), HD heading date (Julian)

Table 7. All QTL identified in the P25R47 / Jamestown RIL population.

QTL Name	Trait[a]	Chr[b]	Position Range	Left Marker	Right Marker	Peak Marker	LOD[c]	PVE[d] %	Add[e]	Positive Parent
Qfy.vt.1D.P47	16B-FY	1D	0.0-3.4	IWB18376	IWB65070	IWB26984	8.3	14.6	0.6	P25R47
	16W-FY	1D	1.6-4.7	IWB26984	IWB27847	IWB65070	5.2	6.8	0.4	P25R47
	17B-FY	1D	1.6-4.7	IWB26984	IWB27847	IWB65070	4.5	6.2	0.5	P25R47
	17W-FY	1D	1.6-4.7	IWB26984	IWB27847	IWB65070	5.8	7.4	0.4	P25R47
	16W-TG	1D	0.0-1.6	IWB18376	IWB26984	IWB26984	4.2	7.9	0.3	P25R47
Qkw.vt.2D.P47	16B-KD	2D	0.7-3.3	IWB26502	IWB22170	IWB2986	4.1	8.9	-0.02	P25R47
	17W-KD	2D	0.7-3.3	IWB26502	IWB22170	IWB2986	3.0	6.4	0.0	P25R47
	16B-KW	2D	0.7-3.3	IWB26502	IWB22170	IWB2986	7.4	9.2	-0.8	P25R47
	16W-KP	2D	0.7-3.3	IWB26502	IWB22170	IWB2986	3.6	6.2	-0.2	P25R47
Qfy.vt.3A.Jtwn*	16B-FY	3A	117.0-117.1	IWB47146	IWB32527	IWB47146	10.3	18.3	-1.7	Jamestown
	16W-FY	3A	117.0-117.1	IWB47146	IWB32527	IWB47146	8.4	14.6	-1.1	Jamestown
	17B-FY	3A	117.0-117.1	IWB47146	IWB32527	IWB47146	6.9	12.3	-0.7	Jamestown
	17W-FY	3A	117.0-117.1	IWB47146	IWB32527	IWB47146	7.6	12.8	-0.9	Jamestown
	16B-FP	3A	117.0-117.1	IWB47146	IWB32527	IWB47146	7.3	17.1	0.2	Jamestown
	16W-FP	3A	117.0-117.1	IWB47146	IWB32527	IWB47146	7.2	16.3	0.2	Jamestown
	16W-KP	3A	117.0-117.1	IWB47146	IWB32527	IWB47146	5.4	11.6	0.2	Jamestown
	16W-TG	3A	117.0-117.1	IWB47146	IWB32527	IWB47146	3.2	7.7	-0.3	Jamestown
	17W-FP	3A	117.0-117.1	IWB47146	IWB32527	IWB47146	5.1	9.7	0.1	Jamestown
Qfy.vt.3B.Jtwn	16W-FY	3B	62.-64.4	IWA2622	IWB69288	IWB69288	18.4	29.9	-0.82	Jamestown
	17B-FY	3B	64.9-65.1	IWB24225	IWB23272	IWB23272	12.1	18.3	-0.81	Jamestown
Qkw.vt.3D.Jtwn	16W-KW	3D	16.3-23.8	IWB52061	IWB34348	IWB52061	8.4	12.3	-1.1	Jamestown
	17W-KW	3D	16.3-23.8	IWB52061	IWB34348	IWB52061	3.4	6.6	-0.7	Jamestown
	16W-LA	3D	16.3-23.8	IWB52061	IWB34348	IWB52061	3.5	6.4	-2.5	Jamestown
Qfy.vt.5A.P47	16B-FY	5A	44.5-44.7	IWB36130	IWB563	IWB5443	4.6	7.7	0.4	P25R47
	16W-FY	5A	44.5-44.7	IWB36130	IWB563	IWB5443	4.5	9.0	0.9	P25R47
	17B-FY	5A	44.5-44.7	IWB36130	IWB563	IWB5443	5.5	10.8	1.0	P25R47
	17W-FY	5A	44.5-44.7	IWB36130	IWB563	IWB5443	6.0	11.0	1.0	P25R47
	16B-SE	5A	44.5-44.7	IWB36130	IWB563	IWB5443	3.8	6.9	0.4	P25R47
	17B-SE	5A	44.5-44.7	IWB36130	IWB563	IWB5443	6.0	10.4	1.0	P25R47
Qtw.vt.6B.Jtwn	16W-KD	6B	63.7-64.3	IWB43059	IWB25091	IWB43059	6.8	12.5	-0.03	Jamestown
	17W-KH	6B	63.7-64.3	IWB43059	IWB25091	IWB43059	4.5	5.6	-0.57	Jamestown
	16B-KW	6B	63.7-64.3	IWB43059	IWB25091	IWB43059	4.2	5.3	-0.56	Jamestown
	16B-TW	6B	63.7-64.3	IWB43059	IWB25091	IWB43059	8.4	13.9	-0.48	Jamestown
	17B-TW	6B	63.7-64.3	IWB43059	IWB25091	IWB43059	6.3	12.5	-0.52	Jamestown
TaTKW-7AL	16B-KD	7A	29.1-30.7	IWB13913	IWB58299	IWB38737	3.1	6.6	-0.02	Jamestown
	16W-KD	7A	29.1-30.7	IWB13913	IWB58299	IWB38737	6.8	13.0	-0.03	Jamestown
	17W-KD	7A	29.1-30.7	IWB13913	IWB58299	IWB38737	4.6	10.0	-0.03	Jamestown
	16B-TW	7A	27.9-29.1	IWB36250	IWB13913	IWB36250	4.4	6.5	-0.3	Jamestown
Qfy.vt.7A.P47	16B-CD	7A	62.5-67.2	IWB38303	IWB3129	IWB62609	4.9	10.9	0.1	P25R47
	16B-FP	7A	69.8-71.2	IWB62609	IWB45866	IWB11241	5.9	10.5	-0.1	P25R47
	16W-FP	7A	59.5-67.2	IWB40616	IWB38303	IWB3129	8.0	14.8	-0.2	P25R47
	17W-FP	7A	71.2-75.2	IWB11241	IWB6849	IWB64354	4.1	7.2	-0.1	P25R47
	17W-FY	7A	70.6-72.6	IWB45866	IWB11241	IWB6849	4.7	6.1	0.3	P25R47
	16B-KH	7A	62.5-69.8	IWB38303	IWB3129	IWB62609	9.1	20.1	-2.9	P25R47
	16W-KH	7A	62.5-67.2	IWB38303	IWB3129	IWB38303	2.9	4.4	-1.7	P25R47
	16B-KP	7A	62.5-67.2	IWB38303	IWB3129	IWB38303	3.4	6.4	-0.1	P25R47
	17B-KP	7A	71.2-75.2	IWB11241	IWB6849	IWB64354	3.2	7.3	-0.2	P25R47
	16B-SE	7A	62.5-69.8	IWB38303	IWB3129	IWB62609	10.7	17.0	1.3	P25R47
	16W-SE	7A	62.5-69.8	IWB38303	IWB3129	IWB62609	5.7	11.7	1.5	P25R47
	17B-SE	7A	62.5-69.8	IWB38303	IWB3129	IWB62609	6.9	12.2	1.1	P25R47
	17W-SE	7A	62.5-69.8	IWB38303	IWB3129	IWB62609	6.5	10.0	0.9	P25R47

*indicates QTL common between both population

[a]numbers indicate year (2016 or 2017), first letter indicates location (Blacksburg or Warsaw), the last two letters indicate trait ([a] TW test weight (kg hL^{-1}), KP kernel protein (%), KH kernel hardness (0-100), KD kernel diameter (mm), KW kernel weight (mg), FY flour yield (%), SE softness equivalence (%), FP flour protein (%), LA solvent retention capacity of lactic acid (%), SC solvent retention capacity of sodium carbonate (%), CD cookie diameter (cm), TG cookie top grade (1-9))

[b]chromosome

[c]logarithm of odds

[d]percent phenotypic variation explained

[e]level of additivity

Table 8. All QTL identified in the P26R46 / Tribute DH soft red winter wheat population.

QTL Name	Trait[a]	Chr[b]	Position Range	Left Marker	Right Marker	Peak Marker	LOD[c]	PVE[d] %	Add[c]	Positive Parent
Qfy.vt.1B.Trib	17B-FY	1B	27.6-29.9	IWB31695	IWB3140	IWB31695	9.4	20.4	-0.8	Tribute
	17W-FY	1B	27.6-29.9	IWB31694	IWB3140	IWB31694	10.8	14.5	-0.7	Tribute
Qcd.vt.1B.Trib	16W-CD	1B	146.6-173.5	IWA131	IWB72780	IWA2998	2.8	6.1	-0.2	Tribute
	17W-TG	1B	146.6-173.5	IWA131	IWB72780	IWA2998	2.9	10.4	-0.3	Tribute
Qla.vt.1B.Trib	17B-CD	1B	175.3-182.3	IWB10908	IWB22081	IWB63002	2.2	3.4	-0.1	Tribute
	16W-LA	1B	178.8-182.3	IWB14010	IWB22081	IWB7600	4.1	13.3	4.8	Tribute
	17B-LA	1B	178.8-182.3	IWB14010	IWB22081	IWB7600	2.5	7.3	2.9	Tribute
	17W-LA	1B	178.8-182.3	IWB14010	IWB22081	IWB7600	4.4	13.3	3.8	Tribute
	17B-TW	1B	179.9-182.2	IWB7600	IWB22081	IWB7600	2.7	9.1	-0.4	Tribute
	17W-TW	1B	177.0-178.7	IWB63002	IWB9566	IWB63002	3.8	11.4	-0.4	Tribute
Qkw.vt.1D.Trib	17B-KW	1D	2.1-21.7	IWB6155	IWB24131	IWB19497	7.1	21.8	-1.6	Tribute
	17W-KW	1D	2.1-21.7	IWB6155	IWB24131	IWB19497	2.9	7.0	-0.9	Tribute
Qkw.vt.1D.P46	17B-KD	1D	130.9-142.3	IWA7797	IWB55047	IWB44725	2.6	7.5	0.0	P26R46
	17B-KW	1D	130.9-142.3	IWA7797	IWB55047	IWB44725	5.1	12.6	1.1	P26R46
	17W-KW	1D	130.9-142.3	IWA7797	IWB55047	IWB44725	2.3	5.0	0.7	P26R46
	17B-LA	1D	130.9-142.3	IWA7797	IWB55047	IWB44725	3.1	10.3	-3.4	P26R46
Qkw.vt.2A.P46	16W-KW	2A	79.9-87.2	IWB12570	IWA5068	IWB12570	2.1	5.3	0.9	P26R46
	17B-KW	2A	79.9-87.2	IWB12570	IWA5068	IWB12570	9.5	24.3	1.5	P26R46
Qkh.vt.2B.Trib	17B-KH	2B	395.6-400.0	IWB50803	IWB32028	IWB28849	2.3	6.9	2.6	Tribute
	17W-KH	2B	396.5-400.0	IWB28849	IWB32028	IWB28849	2.6	6.6	2.6	Tribute
Qse.vt.2D.Trib	17B-SC	2D	3.2-6.2	IWB44589	IWB9726	IWB2986	3.2	3.7	-0.8	Tribute
	16W-SE	2D	6.2-9.0	IWB9726	IWB35594	IWB2986	4.1	8.6	-1.4	Tribute
	17B-SE	2D	4.6-6.2	IWB2986	IWB9726	IWB2986	4.6	9.6	-1.0	Tribute
Qfy.vt.3A.Jtwn*	16W-FY	3A	188.9-189.3	IWB47146	IWB50720	IWB38639	6.3	14.0	-0.8	Tribute
	17B-FY	3A	188.9-189.3	IWB47146	IWB50720	IWB38639	4.6	9.2	-0.6	Tribute
	17W-FY	3A	188.9-189.3	IWB47146	IWB50720	IWB38639	17.0	27.1	-0.9	Tribute
Qfy.vt.3B.Trib	17B-FY	3B	189.4-192.9	IWB633	IWB11875	IWB633	4.5	9.2	-0.6	Tribute
	17W-FY	3B	189.4-192.9	IWB633	IWB11875	IWB633	5.4	6.8	-0.5	Tribute
Qkp.vt.4A.P46	17W-FP	4A	0.0-0.03	IWB10047	IWB42074	IWB10047	4.4	9.7	-0.2	P26R46
	16W-KP	4A	0.0-0.03	IWB10047	IWB42074	IWB10047	2.2	8.0	-0.2	P26R46
	17W-KP	4A	0.0-0.03	IWB10047	IWB42074	IWB10047	4.6	11.2	-0.2	P26R46
Qfy.vt.4A.P46	17B-FY	4A	123.8-128.3	IWB12427	IWB61077	IWB12427	2.2	3.8	0.4	P26R46
	17W-FY	4A	123.8-128.3	IWB12427	IWB61077	IWB12427	6.6	8.2	0.5	P26R46
Qse.vt.5A.Trib	16W-KD	5A	50.2-51.9	IWA5884	IWB9612	IWA5884	4.1	11.1	0.0	Tribute
	17B-KD	5A	48.2-51.9	IWB54141	IWB9612	IWA5884	2.9	8.2	0.0	Tribute
	16W-KH	5A	48.2-51.9	IWB54141	IWB9612	IWA5884	2.1	3.4	1.8	Tribute
	17B-KH	5A	50.2-51.9	IWA5884	IWB9612	IWA5884	2.0	5.8	1.9	Tribute
	16W-KW	5A	48.2-51.9	IWB54141	IWB9612	IWA5884	3.7	10.2	-1.2	Tribute
	17W-KW	5A	48.2-50.2	IWB54141	IWA5884	IWB54141	3.9	9.4	-1.0	Tribute
	16W-SE	5A	48.2-50.2	IWB54141	IWA5884	IWB54141	3.7	7.7	-1.3	Tribute
	17B-SE	5A	48.2-51.9	IWB54141	IWB9612	IWA5884	6.6	14.3	-1.3	Tribute
	17W-SE	5A	48.2-51.9	IWB54141	IWB9612	IWA5884	4.0	9.3	-1.1	Tribute
Qse.vt.6A.P46	17W-CD	6A	31.4-35.7	IWB57726	IWB10911	IWB31589	3.6	6.4	0.1	P26R46
	16W-FY	6A	31.4-35.7	IWB57726	IWB10911	IWB31589	2.0	3.7	0.4	P26R46
	17B-SC	6A	31.4-35.7	IWB57726	IWB10911	IWB31589	4.9	5.9	-1.0	P26R46
	17W-SC	6A	31.4-35.7	IWB57726	IWB10911	IWB31589	6.0	6.8	-0.9	P26R46
Qcd.vt.6B.P46	17W-CD	6B	112.1-131.8	IWB72677	IWA2212	IWB72677	7.1	16.8	0.2	P26R46
	16W-SC	6B	112.1-131.8	IWB72677	IWA2212	IWB72677	3.3	7.8	-1.5	P26R46
	16W-TG	6B	112.1-131.8	IWB72677	IWA2212	IWB72677	2.5	8.4	0.3	P26R46
Qfy.vt.6B.P46	17B-CD	6B	136.4-138.9	IWB3121	IWA3224	IWB26186	5.1	9.1	0.2	P26R46
	17W-CD	6B	131.8-133.7	IWA2212	IWB10245	IWA2212	6.9	13.3	0.2	P26R46
	16W-FY	6B	136.4-138.9	IWB3121	IWA3224	IWB26186	6.4	13.9	0.8	P26R46
	17B-FY	6B	133.7-134.6	IWB48050	IWB35852	IWB48050	4.0	7.9	0.5	P26R46
	16W-SC	6B	131.8-133.7	IWA2212	IWB10245	IWA2212	3.2	6.9	-1.4	P26R46
	17B-SC	6B	135.7-137.2	IWB26626	IWB26186	IWB3121	9.1	11.9	-1.4	P26R46
	17W-SC	6B	137.2-138.9	IWB26186	IWA3224	IWB26186	5.1	5.8	-0.9	P26R46
	17W-SE	6B	131.8-134.6	IWA2212	IWB35852	IWB48050	2.1	4.2	0.7	P26R46
	16W-TG	6B	131.8-133.7	IWA2212	IWB10245	IWA2212	2.4	7.9	0.3	P26R46

*indicates QTL common between both population

[a]numbers indicate year (2016 or 2017), first letter indicates location (Blacksburg or Warsaw), the last two letters indicate trait ([a] TW test weight (kg hL^{-1}), KP kernel protein (%), KH kernel hardness (0-100), KD kernel diameter (mm), KW kernel weight (mg), FY flour yield (%), SE softness equivalence (%), FP flour protein (%), LA solvent retention capacity of lactic acid (%), SC solvent retention capacity of sodium carbonate (%), CD cookie diameter (cm), TG cookie top grade (1-9))

[b]chromosome

[c]logarithm of odds (LOD)

[d]percent phenotypic variation explained
[e]level of additivity

Table 9. Single marker-trait pairwise comparisons of least-squares means between progeny with the different combinations of rye translocation (*1RS.1AL*) and *Qfy.vt.3A.Jtwn* (IWB47146) for Flour Yield (%) in the PxT population.

Pioneer 26R46 / Tribute					
1RS.1AL[b]	*Qfy.vt.3A.Jtwn*	number of individuals	Flour Yield (%)		
			Warsaw 2016	Warsaw 2017	Blacksburg 2017
no	**yes**	25	70.3a[a]	72.2a	70.6a
no	no	20	69.4a	71.2b	69.3b
yes	**yes**	35	69.4a	71.2b	69.4b
yes	no	32	67.9b	69.8c	68.2c

[a]least-squares means (Fisher's LSD, $P<0.05$) by values connected by same letter are not significantly different

[b]DeFroidmont, 1998

Supplemental Table 1. Sequences for KASP marker of best markers associated with important QTL for wheat quality traits.

	chromosome	best marker	synonym name	closest gene to marker	sequence
Qfy.vt.1D.P47	1D	IWB26984	Excalibur_c4876_832	TraesCS1D01G026000	TTGTATCTTGGTGAGCAATGCCTTCAGCAAATCAACTACAGCATACACCC[A/G]TGACACCACAATCCAAGCATGAGCATCAGGGAAGTTGGCTTTCTCCCGAT
	1D	IWB18376	D_GBF1XID01C7T2Q_63	TraesCS1D01G026100	TATCATCAGATAATTCCAAAAGAGAGCGTGCATGCTTGCTTCGTGATCCCTTTCCCATAGGAACATTTCTTTCGCTCATCTGACCAACAACCTCATTACGCAGGAGACCTGACAAGTTTCCCTTT[C/T]GCTTCGATGTGCTCCGAGATGAAGTATAACTTGACGTGTGAAGGCCTTCTCTTATCTGTATT
	1D	IWB65070	RFL_Contig5639_1168	n/a	TACGAGTACATATTCATCTGGGATGAAGACCTTGGGGTGGATCATTTCAA[C/T]GCAGAGGAGTACATCAAACTTGTTAAGAAAAATGGTCTGGATATCTCCCA
	1D	IWB27847	Excalibur_c5892_1129	TraesCS1D01G026000	CCACCAATGCACGGAATGGCGCCCTCCTCAATCTCCAGTTGCTTTACATT[A/G]GGCATGCGCCTCAAGACAAGCGTCTTCAACTGACAGAAGCACCCTGCAGA
Qfy.vt.3A.Jtwn	3A	IWB47146	Kukri_c64268	TraesCS3A01G073400	GACCGGACTTCTGAAGTTCTGCGGCAAACAACCATCAGCTTTTATACATC[C/T]GATTAAGTGCATCGTTTTGAGGAACAATGCCCAAAGGATCATGTGATGTG
	3A	IWB32527	GENE-1533_226	TraesCS3A02G073400	CAAAAGTCCACTCTGCTATTTGATTCTTCTTCAGGTAATAATCCTTGACT[T/C]GACAATGGTGATTCTCTTTTCTGTAGTAATTTGTCAACATAAAGTACCTT
	3A	IWB50720	Ra_c1002_1670	TraesCS3A02G072400	GGATCCATTCCTTGCACAAGTAACAACAGCTTAACCCATGAACTTGTCAG[A/G]TCAGGCCTCTCAGAAGCAACATACATGGAAACCTCCTCATGTCTCAGGAC
	3A	IWB38639	Ku_c18096_552	TraesCS3A02G073400	AGAGTGGACTTTTGGAATCCGAATCCCGGCACAGAGAGCAAACTGGTCGT[A/G]TCTCCTCAGAGGCAACGGCAGTTGAACTGGATGCATCTGGCTCAAATGTT
Qfy.vt.5A.P47	5A	IWB36130	IACX6241	TraesCS5A01G261900	ATTCTCAAGGAACTCGTCTCTCAGAGGAACAGCAATGCCATTCCTCTCTTCGACGCGCTT[C/T]CCCAGGAAAGGATCATTCAGTATAACCTGCTCGCACTCGACGAGCTTCTGCTGAGCGCC
	5A	IWB563	BobWhite_c13900_53	TraesCS5A02G261900	GTCGTTAAACGTCTTTTTGCAGTATTAATTCATAGGACGATAAACTTGAG[T/G]AAAAGGAGTACAAAGCATTCAAAACATCAGCATAAGCTGTAGCAATGTGA
	5A	IWB5443	BobWhite_rep_c64197_143	TraesCS5A01G261200	CAAATAATATCGTACCACCGTGTTGCCGAAAGTAGGCCAGTTTCATATTT[C/T]GTAGCTTTCTTCTCTCTTGGATCAAGCACGCACAAGCTACAACAATCACA
TaTKW-7AL	7A	IWB13913	CAP7_c2350_105	TraesCS7A01G479600	TAGTAAGCTCTTCAACGAGGATGGATGTTGTGTAATTTGGACAAGTGCGA[C/T]GTATGTCACATCTTTTTTTTAATGATCCTAATCTATGATCGAAGTTCGTT
	7A	IWB36250	IACX7848	TraesCS7A02G487100	TCACTTGAAAACGCAACAACCAACTGAGCACCTCGTCCATGGCTTCCCATTTTCAATCAGCAGAACATTCATCAACTAGGAGTTGCGAGCCGTAGGCTGG[A/C]CAGCAGTGGCACATATGGGAAATATTGCTCATGTGCGCAGTAGGTACGCTCAATGCGGAGTACAGCTGTACTTTCATGATGATCCAAATCATATGCCAGC

	7A	IWB58299	RAC875_c46980_148	TraesCS7A02G484200	TCCATTCAGCCTGTGAAGCTGCACCAGAGGCTGCCATTGTTCCTGGGGAG[A/C]ATGGAGGACATGCTTGAGCTAGAAAGCTACGGAGATGTCCAGCAGAAGGT
	7A	IWB38737	Ku_c20410_424	TraesCS7A02G484500	TGCATCGTTAGATGCAAGCTGACGCTGGATTTCAACACCCTCTAATGTTA[T/C]GAGTCTTGGCTTAGGATGCACGATCCATTTCCTCTCAAGGTTTAACATCG
Qfy.vt.7A.P47	7A	IWB38303	Ku_c12139_1714	TraesCS7A02G173500	CTTTATTTGAGTGAAGAGAGATGTGGCATTATGGTTTATTGTCACTGCGG[T/C]GTTTAGATTCAGGGTTTTGCAAAGCAACTTCCCTATGAAGTCGAGACCGC
	7A	IWB62609	RAC875_rep_c69766_246	TraesCS7A02G168300	CCACCTATTACAGGAGACATTATATCCATTCCAGCATTGCCTGGAGTCAT[T/C]GGTTGACCACCAGGTGTACCAGGAAGGTACGCAGCAGAATTTGGTGTCAT
	7A	IWB45866	Kukri_c484_1704	TraesCS7A02G167300	AGGTGGAGAGGCTGCGGCGTGACAATGCAGTTCTGAAGTCCGAGCTTGAA[T/C]GGTTACAAAGGGAGAATGCAGAGCTGAAAGCCAAGTTAGTGAAATCCGAT
	7A	IWB3129	wsnp_Ex_c27914_37074773	TraesCS7A02G210100	GAATAACTTTCCCAGTATCTGTTGATCCAGTGAATGCAATCTTGTCAACATCCATGTGGCTAGCAAGAGCAGCCCCAGCAGTAGGACCGAAACCAGATAC[A/G]ATGTTGAGGACACCTTCGGGTAGTCCAGCCTCATGCAACAGCTTAGAAACATAGAGGGCAGATAGAGGAGTTTGCTCAGCAGTCTTGAGAACGATGGTGT
	7A	IWB11241	BS00082180_51	TraesCS7A02G163700	AAAGGGCGATGTTGTATTGTTGTAACACACACACATGGGAGATGCGCATC[T/G]GCCTGCTGTGCAAGGCGCCTCCGTCCCCATTTGGATGGAGCTCTTGCCTT

Registration of Soft Red Winter Wheat VA11DH-P46xTrib-28, VA11DH-P46xTrib-99, and VA11DH-P46xTrib-103 Germplasm Lines with Exceptional Milling and Cookie Baking Performance

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Abbreviations: BLUP, best linear unbiased predictor; DH, doubled haploids; SRC = solvent retention capacity, SRWW, soft red winter wheat; QTL, quantitative trait loci; NIR, near-infrared; SKCS, single kernel characterization system; AACCI, American Association of Cereal Chemists International.

ABSTRACT

The release of soft red winter wheat (*Triticum aestivum*, L.) germplasm lines VA11DH-P46xTrib-28, VA11DH-P46xTrib-99, and VA11DH-P46xTrib-103 is intended to provide breeders with genetic material having exceptional milling and baking quality performance. The quantitative nature of milling and baking performance makes improvement and early generation selection difficult. Marker assisted and genomic selection breeding schemes can be improved by introducing breeding material with superior end-use quality and use of known predictive DNA markers. The three lines described in this registration have acceptable agronomic performance with grain yields (4605-5733 kg ha^{-1}) similar to or higher than those of Pioneer '26R46' (4568 kg ha^{-1}). The lines have exceptional milling and baking performance with mean flour yields (73.3-73.6%), softness equivalence (55.0-57.3%), flour protein (8.9-9.4 %), solvent retention capacity (SRC) for lactic acid (116.2-118.9 %) and sodium carbonate (67.2-69.7%), and cookie diameters (19.1-19.5 cm) that are better or similar (P<0.05) to those of Pioneer 26R46 (72.1%, 53.1%, 9.3%, 122%, 70.3%, and 18.9 cm).

INTRODUCTION

End-use quality of soft red winter wheat (SRWW, *Triticum aestivum*, L.) germplasm is generally only considered by breeders once pure lines have been selected and tested in yield trials and sufficient grain is available for quality testing. Other than choosing parents and crosses to make, SRWW breeders focus most of their initial efforts on line selection and evaluation of pure lines for grain yield, test weight, and disease resistance, particularly Fusarium Head Blight (*Fusarium graminearum* Schwabe), as these traits are the most economically relevant to growers and are routinely assessed by breeders in the field. End-use quality, comprised of multiple grain physical and compositional traits, is difficult to measure, controlled qualitatively and quantitatively, and strongly influenced by both genetics and environment conditions (Posner and Hibbs, 2005, Souza, et al., 2012). Flour yield and cookie diameter, the most critical soft wheat milling and baking quality parameters, are correlated traits and are moderately to highly heritable. Cookie diameter tends to increase with flour yield, while it decreases with increasing solvent retention capacity, kernel hardness, and flour protein. This is predicable, as the pressure required to crush the wheat kernel increases so does starch damage, which negatively influences cookie diameter. Kernels containing higher levels of protein are generally harder in texture and require more pressure to crush during milling, thus resulting in lower break flour extraction and small cookie diameters (Smith, et al., 2011).

Breeding for end-use quality per se, solely on the basis of phenotypic data for multiple quality traits, is difficult due to the quantitative nature of these traits and lack of reliable high-throughput markers to aid in selection of parents and progeny on the basis of quality. QTL for wheat milling and baking performance have been reported on all twenty-one chromosomes (Breseghello and Sorrells, 2006, Cabrera, et al., 2015, Li, et al., 2009, Souza, et al., 2012). However, markers

associated with most of these QTL are not routinely deployed in breeding programs and, thus, genetic gains for improved quality remain stagnant (Kiszonas and Morris, 2017). A complementary approach to marker assisted or genomic selection is to introduce superior end-use quality transgressive segregates from breeding populations with the aid of predictive genetic markers to help facilitate genetic improvement of traits that influence milling and baking performance. The release of VA11DH-P46xTrib-28, VA11DH-P46xTrib-99, and VA11DH-P46xTrib-103 germplasm lines is intended to provide SRWW breeders with breeding material that has superior end-use quality in an effort to help improve milling and baking performance in future SRWW cultivars.

MATERIAL AND METHODS

Development of Lines

These SRWW germplasm lines were identified as transgressive segregates in a doubled haploid (DH) population designed to map QTL associated with milling and baking quality performance (manuscript in preparation). The doubled haploid lines were derived from the cross Pioneer '26R46' (PI 612154) by 'Tribute' (PI 632689) (Griffey, et al., 2005). Doubled haploid production was conducted in collaboration with J. Paul Murphy at North Carolina State University in Raleigh, NC. A total of 112 full sib DH lines were developed from this cross and screened for milling and baking quality performance. The cultivar Tribute was derived from the cross VA92-51-39/AL870365. Pioneer 26R46 was derived from the cross FL7927-G14//Pioneer '2555'*3/'Coker 80-28'. Both Pioneer 26R46 and Tribute are photoperiod insensitive (*Ppd-D1a*) and lack the Bx7 over-expression glutenin allele at *Glu-B1* and *TaSus2-2B*, while both have the 5+10 glutenin subunits (*Glu-D1*). Tribute possesses the *1RS:1AL* rye (*Secale cereale*) translocation (Table 1). On average across three site-years, Tribute had grain yields that were 9%

higher, it is 3 cm taller in plant height, and reached maturity two days later than Pioneer 26R46 (Table 2).

Field Test Evaluations

Complete management information is included in Table 3. Each DH line, the parents, and adapted checks were grown in yield plots comprised of 7 rows spaced 15 cm apart with a total harvested area of 1.5×2.7 m. All seeds were coated with Gaucho®-XT (Imidacloprid, Bayer Crop Science) insecticide and Raxil® MD (Tebuconazole and Metalaxyl, Bayer Crop Science) fungicide seed treatments. Fall nutrient management and spring Nitrogen (N) applications were based on standard local management practices (Brann, et al., 2009) and recommendations from the Virginia Cooperative Extension Soil Testing Service. Plots were managed for optimum quality in consideration of local conditions, including foliar fungicides applications (Table 3). Tests were grown at Kentland Research Farm (37°11'45"N 80°34'28"W) near Blacksburg, VA during the 2016-17 growing season and in fields at the Eastern Virginia Agriculture Research and Extension Center (37°59'16"N 76°46'48"W) near Warsaw, VA during the 2015-16 and 2016-17 growing seasons.

End-use Quality Evaluations

All grain samples were thoroughly air-aspirated prior to testing to remove any dust or chaff. Test weight (kg hL^{-1}) of wheat grain was determined according to the Approved Method 55-10.01 (AACCI, 1999). Kernel weight (mg), hardness, and diameter (mm) were determined using Single Kernel Characterization System (SKCS, Perten Instruments, Springfield, IL) according to Approved Method 55-31.01 (AACCI 1999) as the averages of 300 kernels. Wheat grain protein (%) and moisture (%) for tempering were estimated using a DA 7200 NIR analyzer (Perten Instruments, Springfield, IL). Wheat grain was tempered to 15% moisture for 24 hours and

milled using a modified Quadrumat Senior milling system to determine milling quality and to obtain flour. Flour yield (%) was the fraction of milled products that comprises the break and reduction flour after milling and calculated as ((grain weight – Bran weight) / grain weight) x 100. Softness equivalence (%) was calculated as (break flour/total flour) x 100 (Finney and Andrews, 1986). Flour protein was estimated using a NIR analyzer (Unity Spectra-Star, Millford, MA). Lactic acid and sodium carbonate solvent retention capacities of flour were determined according to Approved Method 55-11.02 (AACCI 1999) with a modification of flour weight to 1 g as described by Kweon, et al. (2011). Lactic acid SRC predicts gluten strength, while sodium carbonate estimates damaged starch content. Sugar-snap cookie baking test of flour was performed according to Approved Method 10-52.02 (AACCI 1999). The diameter (cm) of two cookies per samples were measured with the Mitutoyo Absolute Digimatic Caliper (Takatsu-ku, Kawasaki, Kanagawa Prefecture, Japan) to generate mean cookie diameter (cm). Cookie top grade for both cookies was graded visually for surface appearance, from worst to best on a scale of 1 to 10.

Statistical Analysis

An augmented single replicate test design was used with eight checks including both parents replicated throughout the test at least twice, each site-year was considered a block. This was done because of limited seed sources, cost of measuring end-use quality, and because milling and baking traits are highly heritable with consistent results between site-years (Guttieri, et al., 2001, Guttieri and Souza, 2003, Souza, et al., 2012). Therefore, numerous replicates are not needed to determine significant differences. The Augmented Complete Block Design (ACBD-R) with R for Windows Version 4.0 was used to analyze the three site-years individually (Table 4) and as multi-year environments (Table 2) (Rodriquez, et al., 2018). Best linear unbiased predictors

(BLUP) of each DH line and checks were generated along with Least Significant Differences (α=0.05) for use in line comparisons.

CHARACTERISTICS

Pioneer 26R46 is considered to have very good milling and baking quality. It is consistently the top performing check/parent in this test. An individual line is considered to have exceptional milling and baking quality if it consistently performs similar to or better than Pioneer 26R46.

Kernel Morphology

Kernel weight (mg) for VA11DH-P46xTrib-103 and VA11DH-P46-Trib-99 was lower ($P<0.05$) than Pioneer 26R46, while VA11DH-P46xTrib-28 had a similar kernel weight in the multi-environment comparison. All three DH lines had kernel diameters (mm) lower ($P<0.05$) than Pioneer 26R46, and test weight (kg hL^{-1}), kernel hardness, and protein (%) were similar to Pioneer 26R46 **(Table 2)**.

Milling and Baking Performance

Flour yield (%) and cookie diameter (cm) are two primary quality parameters considered and used routinely by breeders in identification of breeding lines with better end-use quality. Cookie diameter and flour yield are positively correlated, while both are negatively correlated with flour protein (%) and solvent retention capacity traits (Knott, et al., 2009, Souza, et al., 2012). This is due in part to the endosperm being released more easily form the bran during milling in wheat varieties that have higher flour extraction. Starch granules suffer less damage during milling if the endosperm is more easily disintegrated into fine flour particles as observed in wheat grain of low kernel hardness. Wheat varieties having lower break flour extraction exhibit greater starch damage due to the extra shearing and crushing of endosperm particles during milling (Smith, et al., 2011). Sodium carbonate solvent retention capacity (%) is a measure of damaged starch and

lactic acid solvent retention capacity (%) is a measure of gluten strength, both of which tend to increase as kernel hardness and protein increase. SRWW varieties having high flour yield and lower flour protein tend to have good cookie baking performance, which is what flour millers and bakers typically desire for pastry products (Gwirtz, et al., 2007).

By site-year BLUP analysis indicates that all three DH lines had higher (P<0.05) flour yields at Blacksburg 2017 and Warsaw 2017 site-years than Pioneer 26R46, and were similar at Warsaw 2016. Lines VA11DH-P46xTrib-103 and VA11DH-P46xTrib-99 had higher (P<0.05) cookie diameters at Warsaw 2016 and Blacksburg 2017 compared to Pioneer 26R46, and were similar at Warsaw 2017. Line VA11DH-P46xTrib-28 had similar cookie diameters as Pioneer 26R46 at all site-years (Table 4).

The multi-environment combined BLUP results indicate that all three DH lines are similar to Pioneer 26R46 for flour protein and both solvent retention capacities (lactic acid and sodium carbonate). Line VA11DH-P46xTrib-103 was higher (P<0.05) than Pioneer 26R46 for flour yield and cookie diameter. Line VA11DH-P46xTrib-103 was higher (P<0.05) for softness equivalence and cookie diameter compared to Pioneer 26R46.

Grain Yield and Plant Characteristics

Line VA11DH-P46xTrib-28 had higher (P<0.05) grain yields than Pioneer 26R46. Lines VA11DH-P46xTrib-103 and VA11DH-P46xTrib-99 had grain yields similar to Pioneer 26R46. All three DH lines headed 8-9 days later than Pioneer 26R46 and were 5.6 cm taller. Spikes of VA11DH-P46xTrib-28 have short tip awns, while spikes of VA11DH-P46xTrib-99 and VA11DH-P46xTrib-103 are awned.

Fusarium Head Blight

Data for reaction to Fusarium Head Blight was previously collected on the three doubled haploid lines and both parents over five locations during 2013 and 2014: AR (Fayetteville and Newport), KY (Lexington), NC (Kinston), and VA (Blacksburg). All three lines appear to fit within the normal distribution of progeny, being more susceptible than Tribute and less than or as susceptible as Pioneer 26R46 for FHB severity, FDK, and DON. The average percent FHB severity, FDK, and DON concentration are represented in Table 5. All three doubled haploid lines had lower (P<0.05) FHB severity than Pioneer 26R46. Lines VA11DH-P46xTrib-28 and VA11DH-P46xTrib-99 had fewer (P<0.05) Fusarium damage kernels than Pioneer 26R46. Line VA11DH-P46xTrib-103 was similar to Pioneer 26R46 for Fusarium damage kernels. Only line VA11DH-P46xTrib-28 had lower (P<0.05) DON content compared to Pioneer 26R46, while the other two DH lines were similar to Pioneer 26R46 for DON.

Seed Purification and Increase

Sixty-four individual plants of each DH line were planted, vernalized for 7 weeks, transplanted into larger pots, grown to maturity in a greenhouse, and harvested to produce a pure seed source. Variant or off type plants were discarded prior to harvest, and seed from the remaining plants deemed to be uniform and true to type was bulked to form the breeder seed.

Availability

Small seed quantities of all three lines will be made available upon request for breeding purposes and can be obtained from the Small Grains Breeding and Genetics group at Virginia Tech. Seed of lines VA11DH-P46xTrib-28, VA11DH-P46xTrib-99, and VA11DH-P46xTrib-103 has been deposited with the USDA National Plant Germplasm System, where it will be available five years after publication of this article.

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Table 1. Parental and doubled haploid line genotypes for major genes and QTL associated with end-use quality in SRW wheat.

Parents/DH Line	1RS rye translocations	Glu-B1	Glu-D1	Ppd-D1	TaSus2-2B
Pioneer 26R46	non-1RS	no	5+10	insensitive	no
Tribute	*1RS:1AL*	no	5+10	insensitive	no
VA11DH-P46xTrib-103	non-1RS	no	5+10	insensitive	no
VA11DH-P46xTrib-99	non-1RS	no	5+10	insensitive	no
VA11DH-P46xTrib-28	non-1RS	no	5+10	insensitive	no

Table 2. Multi-environment combined best linear unbiased predictor comparisons of long term soft red winter wheat checks and doubled haploid lines for 11 end-use quality traits, grain yield, heading date, and plant height over three site-years.

Line	Test Weight	Kernel Weight	Kernel Protein	Kernel Hardness	Kernel Diameter	Flour Protein	SRC Lactic Acid	SRC Sodium Carbonate	Flour Yield	Softness Equivalence	Cookie Diameter	Grain Yield	Heading Date	Plant Height	Spike Type
	(kg hL^{-1})	mg	%	0-100	mm	%	%	%	%	%	cm	(kg ha^{-1})	Julian	cm	
VA11DH-P46xTrib-103	74.2	36.5	11.1	16.1	2.8	9.3	116.2	67.2	73.6	56.4	19.5	4792.1	119.3	78.6	awned
VA11DH-P46xTrib-99	74.1	36.0	11.3	16.7	2.8	9.4	116.6	67.9	73.3	57.3	19.4	4605.4	119.3	78.6	awned
VA11DH-P46xTrib-28	74.6	39.8	10.7	17.4	2.8	8.9	118.6	69.7	73.3	55.0	19.1	5733.2	118.2	78.6	tip awn
Pioneer 26R46	75.9	40.9	11.1	13.6	2.9	9.3	122.1	70.3	72.1	53.1	18.9	4567.8	110.0	73.0	awned
Dyna-Gro 9223 (ck)	75.1	35.1	9.7	19.2	2.7	8.0	115.2	69.6	71.8	61.4	19.1	5552.6	114.0	78.7	tip awn
AgriMAXX 415 (ck)	76.6	36.2	10.6	24.5	2.7	8.6	114.1	72.0	70.9	55.5	18.9	5971.2	113.7	75.4	tip awn
USG 3120 (ck)	76.9	40.8	11.0	23.5	2.8	8.8	107.3	75.9	70.3	54.1	18.1	5095.7	107.0	77.0	awned
Shirley (ck)	73.6	36.8	11.0	18.0	2.7	8.7	97.8	74.7	69.8	54.1	18.7	5828.6	112.5	69.9	awnlet
Branson (ck)	74.1	34.3	10.9	17.4	2.6	8.7	123.5	73.4	69.1	58.2	18.6	6296.5	111.3	72.8	awnlet
Tribute	78.1	35.6	11.5	35.6	2.8	9.4	123.5	78.6	68.5	52.5	17.9	4984.8	112.0	70.1	tip awn
Jamestown (ck)	77.3	32.6	11.3	27.2	2.7	9.0	125.9	82.7	67.2	52.5	17.3	4979.3	107.0	67.7	awned
Mean (n=11)	75.5	36.8	10.9	20.8	2.8	8.9	116.4	72.9	70.9	55.5	18.7	5309.8	113.1	74.6	n/a
Least significant difference (P<0.05)	2.0	2.7	0.7	6.1	0.1	0.6	7.5	3.6	1.2	3.0	0.4	854.2	4.81	8.5	n/a

Table 3. Winter wheat trial management by site-year.

	Warsaw, VA	**Warsaw, VA**	**Blacksburg, VA**
Coordinates	(37°59'54"N 76°46'23"W)	(37°59'33"N 76°46'24"W)	(36°40'59"N 76°45'38"W)
Year	2015-16	2016-17	2016-17
Soil Type	Kempsville Sandy Loam	Kempsville Sandy Loam	Hayter loam
Elevation	36 m	40 m	518 m
Area harvested	1.5 x 2.7 m	1.5 x 2.7 m	1.5 x 2.7 m
Fertility	36-80-80 - 5(S)dry fertilizer on 9/23 33.6 kg ha^{-1} N (12001.5) on 12/14 33.6 kg ha^{-1} N (12001.5) on 2/12	30-60-60 dry fertilizer on 9/20 33.6 kg ha^{-1} N (12001.5) on 12/7 33.6 kg ha^{-1} N (12001.5) on 2/9	15-40-60-S/8-B/3 (9/26) 28.0 kg ha^{-1} UAN 30-0-0 (2/17) 67.3 kg ha^{-1} UAN 30-0-0 (3/24)
Pesticides	Strane® (80.8 ml ha^{-1}) on 12/7 Tilt® (47.9 ml ha^{-1}) on 12/16 Prosaro® (95.7 ml ha^{-1}) on 5/3	Tilt® (47.9 ml ha^{-1}) on 3/17 Harmony Extra SG® (9.0 ml ha^{-1}) on 11/30 Prosaro® (95.7 ml ha^{-1}) on 5/1 Fitness® (47.9 ml ha^{-1}) on 4/11	Harmony Extra SG® (9.0 ml ha^{-1}) (11/15) Harmony Extra SG® (9.0 ml ha^{-1}) (3/24) Tilt® (47.9 ml ha^{-1}) on 3/27 Tilt® (47.9 ml ha^{-1}) on 5/30
Growth regulator	Palisade EC® (95.7 ml ha^{-1}) on 3/14	Palisade EC® (95.7 ml ha^{-1}) on 3/17	Palisade EC® (95.7 ml ha^{-1}) on 3/27
Tillage	Conventional	Conventional	Conventional
Planting date	22 Oct. 2015	23 Oct. 2016	11 Oct. 2016
Harvest date	20-Jun-16	21-Jun-17	22-Jun-17

*UAN=urea and ammonium nitrate

Table 4. Least significant differences from best linear unbiased predictor of single replicated tests by site-year for comparison of soft red winter wheat checks (ck) and doubled haploid lines for flour yield and cookie diameter.

	Flour Yield (%)			Cookie Diameter (cm)		
Line (n=11)	**Warsaw 2016**	**Warsaw 2017**	**Blacksburg 2017**	**Warsaw 2016**	**Warsaw 2017**	**Blacksburg 2017**
VA11DH-P46xTrib-103	72.4	75.4	73.7	19.4	19.5	20.0
VA11DH-P46xTrib-99	72.0	75.5	73.0	19.0	19.6	19.9
VA11DH-P46xTrib-28	73.3	74.1	73.0	18.9	19.3	19.1
Pioneer 26R46	72.6	72.7	71.5	18.2	19.2	19.2
Dyna-Gro 9223 (ck)	70.5	72.8	72.0	18.3	19.2	19.8
AgriMAXX 415 (ck)	70.3	71.2	71.3	18.8	18.9	19.0
USG 3120 (ck)	69.5	71.4	69.8	17.3	18.6	18.3
Shirley (ck)	68.7	70.6	70.0	18.3	18.9	19.1
Branson (ck)	67.9	70.4	69.0	18.3	18.9	18.5
Tribute	67.6	69.4	68.3	17.2	18.1	18.3
Jamestown (ck)	66.3	67.2	68.1	16.6	17.3	18.1
Mean (n=11)	70.1	71.9	70.9	18.2	18.9	19.0
Least significant difference (P<0.05)	1.1	0.8	0.8	0.6	0.4	0.4

Table 5. Fusarium head blight (FHB) severity, Fusarium Damaged Kernels (FDK) and Deoxynivalenol (DON) content in selected doubled haploid lines and parents evaluated across AR (Fayetteville and Newport), KY (Lexington), NC (Kinston) and VA (Blacksburg) in 2013 and 2014 harvests.

Line	FHB Severity	FDK	DON
	%	%	ppm
VA11DH-P46xTrib-103	57.7	63.1	24.8
VA11DH-P46xTrib-28	41.5	47.5	18.4
VA11DH-P46xTrib-99	41.6	45.1	23.5
Pioneer 26R46	68.3	60.4	24.9
Tribute	27.3	31.0	9.2
Mean (N=5)	46.9	48.9	19.9
Least significant difference (P<0.05)	6.1	9.9	3.6

Chapter III:

Comparison of QTL associated with malt quality traits in two 'Malt x Feed' Winter Barley Doubled Haploid Populations

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Abbreviations: PVE: phenotypic variation, ME: malt extract, BG: beta-glucan, AA: alpha-amylase, DP: diastatic power, MAS: marker assisted selection, QTL: quantitative trait loci, SNP: single nucleotide polymorphism, BP: barley protein, WP: wort protein V/V34: Violetta x VA09B-34, E/V34: Endeavor x VA09B-34, S/T: soluble over total nitrogen, FAN: free amino nitrogen, AMBA: American Malting Barley Association

Abstract

Marker assisted selection (MAS) is an important tool that allows breeders to select traits using predictive DNA markers that are difficult or expensive to measure, such as end-use quality. Predictive, high throughput markers, used along with the favorable parental genotypes can be an effective tool to introgress favorable quantitative trait loci. In order to effectively deploy MAS in Eastern U.S. malt barley breeding programs, it is important to investigate genetic structure within representative breeding populations. In this study two winter 'malt x feed' doubled haploid barley breeding populations were genotyped with the iSelect Infinium™ SNP assay consisting of 50,000 barley SNPs, grown in two to three Virginia environments (Blacksburg and Warsaw) during 2017 - 2019, and characterized for 11 phenotypic traits associated with malting quality. QTL mapping validated six previously reported regions that are strongly associated (LOD > 3.0) with relevant malt quality traits. Phenotypic variation for malt quality was largely and consistently explained by QTL on chromosomes 1H, 5H, and 7H in the Endeavor / VA09B-34 population and by two separate QTL on 1H in the Violetta / VA09B-34 population. A region on 4H corresponding with *QDp.DiMo-4H,* explained between 12.1 - 42.2% (Endeavor / VA09B-34) and 30.0 - 55.7% (Violetta / VA09B-34) of the phenotypic variation for diastatic power (DU). These QTL are recommended for MAS in order to aid breeding strategies that aim to select for improved malting characteristics in Eastern U.S. malt barley breeding material.

Introduction

Barley (*Hordeum vulgare* L.) is a member of the *Triticeae* family, and is grown on roughly 2.5 million acres in the United States (T-CAP, 2015). In terms of quantity, it is the world's fourth most produced cereal crop (FAOSTAT, 2009). Barley is primarily grown for livestock feed and the malting industry, the latter being used mostly by brewers, distillers, and other food manufactures. Malt for brewing is the primary value-added end-use (AMBA, American Malting Barley Association http://ambainc.org). According to AMBA, 55.6 % of the barley produced in the U.S. goes for the production of beer and 1.7 % for whiskey production. In Europe, roughly one-third of the barley produced becomes malt and two-thirds is used for feed (Dráb, et al., 2014). AMBA defines the "Ideal Commercial Malt Criteria" for U.S. barley breeders based on acceptable values for 18 quality traits for 6-row, 2-row, and adjunct 2-row types (barley plus another non-malted grain e.g. corn or rice) of barley. Producers that grow industry-endorsed cultivars will normally receive a premium price over feed type barley.

Demand for malting quality barley cultivars adapted to the Eastern U.S. has increased significantly in the past decade. The Mid-Atlantic and Southeastern U.S. lack true malting cultivars developed under local conditions. Craft malt houses are operating in many of the eastern states from Georgia to Maine (https://craftmalting.com/craft-malt-finder/). Without malting cultivars appropriate for the eastern U.S., maltsters and brewers have to use non-adapted European and Western U.S. cultivars or Virginia bred feed types such as Thoroughbred (PI 634933) (Brooks, et al., 2005). European and Western U.S. varieties tend to be late maturity, experience dormancy or pre-harvest sprouting problems, and are susceptible to diseases endemic to the Eastern U.S. Virginia Tech winter 6-row feed type cultivars Thoroughbred, Secretariat (PI 673931) (Griffey, et al., 2016), Nomini (PI 566929) (Starling, et al., 1994), and Atlantic (PI

665041) (Brooks, et al., 2014) are very popular, high yielding, and well-adapted to the Mid-Atlantic, Southeastern, Mid-South, and Southern Midwest states. However, these varieties lack appropriate malting performance necessary to meet the needs of craft maltsters. Therefore, it is important to identify genomic regions associated with the malting characteristics in European and Western U.S. breeding lines and to introgress these regions into the backgrounds of feed type breeding lines that have more appropriate agronomic performance for Eastern U.S. growing conditions.

Numerous reports have investigated genetic architecture of malting traits (GrainGenes 3.0). Fang, et al., (2019) summarized 25 bi-parental or association panels that identified QTL on all seven barley chromosomes associated with malt extract, diastatic power, Kolbach Index, viscosity, β-glucan, FAN, and protein content. Hundreds of other unpublished markers associated with malt quality are also reported in GrainGenes 3.0. Selecting for QTL identified in other non-related breeding populations is unlikely to be effective unless they are validated in relevant breeding material. The most recent and relevant report to Eastern U.S. malting barley cultivar development is Mohammadi, et al. (2015). The authors performed a genome-wide association for malting quality across eight U.S. barley breeding programs, and identified 108 and 107 marker-trait associations specific to 6-row and 2-row breeding programs. The most significant region being on the telomeric region of chromosome 5H (*QAa.HaMo-5H, QAa.StMo-5H.2*, and *QS/T.HaMo-5H*). The major challenge for breeders working to improve malting quality, is that many malting quality traits are governed by the same QTL, (i.e. pleiotropic gene effects or gene clusters) (Zale, et al., 2000). Often these polygenic traits can have positive or negative relationships (Matthies, et al., 2014). To further complicate malt barley breeding, craft maltsters desire unique combinations of malting quality traits that differ from those of AMBA to

meet their needs (Brewers Association, 2014). However, breeders should focus on maximizing malt extract percent while limiting negative impacts on important agronomic traits such as yield, pre-harvest sprout damage, and disease resistance. The brewing and distilling industry primarily desires malts that maximize the extraction of soluble sugars, which is determined by a combination of enzyme activity, β-glucan content, and size of the protein fraction (Cu, et al., 2016).

Thousands of segregating populations and tens of thousands of progeny lines have been developed by the Virginia Tech barley breeding program since 2011. Identification of lines with superior malting performance remains a primary challenge, and screening for malting performance in early generations is not viable via phenotypic methods. Furthermore, phenotypic assessment of advance breeding material is difficult to accomplish due to the high costs associated with micro malting and lack of available capacity. Implementation of marker assisted selection to routinely screen parents and early generation progeny to identity lines having acceptable malting quality is a logical strategy. QTL mapping in populations comprised of Eastern U.S. winter feed by malt barley breeding lines for malt quality characteristics has not been investigated previously. In order to make significant improvements in malting quality of Eastern U.S. barley cultivars, it is necessary to saturate barley breeding programs with elite breeding lines that have acceptable agronomic and malting performance. Genetic recombination is the only way to achieve this objective, breeders can use MAS to select against progeny from doubled haploid or recombinant breeding populations that possess too many unfavorable (feed type) alleles. This will allow breeders to direct resources towards breeding material with a greater likelihood of having acceptable malting performance. In order to address this challenge, the initial step is to investigate genetic structure of winter barley breeding populations relevant to

Virginia Tech and the eastern U.S. and to identify genomic regions/predictive markers to target with MAS.

Material and Methods

Populations

Two doubled haploid populations, both consisting of a commercially available winter 2-row malt barley cultivar crossed to VA09B-34, an experimental Virginia winter 6-row feed type were evaluated in the study. The first population consisted of 130 individuals each derived from the cross 'Endeavor' (PI 654824) (Obert, et al., 2009) / VA09B-34 (E/V34). Endeavor was developed by the USDA-ARS in Aberdeen, Idaho and is derived from the cross ORWM8406 / 'Harrington' (spring type growth habit). The second population consisted of 141 individuals each derived from the cross 'Violetta' / VA09B-34 (V/V34). Violetta was developed by Limagrain Cereal Seeds' partners. The common parent between both populations, VA09B-34, was derived from the cross VA00B-279 // VA00B-259 / Thoroughbred (Brooks, et al., 2005), which are all winter 6-row feed types.

Agronomic practices

Test site descriptions are included in S. Table 1. Fall nutrient management and spring Nitrogen (N) applications standard to local management practices (Brann, et al., 2009) and recommendations from the Virginia Cooperative Extension Soil Testing Service, along with pesticide and growth regulator applications, which were applied as needed are included in S. Table 2. All doubled haploids, parents, and checks were planted in plots of 7 rows spaced 15 cm apart with a total harvested area of 1.5 x 2.7 m. Seed samples were coated with Gaucho®-XT (imidacloprid, Bayer Crop Science) insecticide and Raxil® MD (Tebuconazole and Metalaxyl, Bayer Crop Science). The E/V34 population was grown at Warsaw during the 2016-17 and at

Warsaw and Blacksburg during the 2017-18 seasons. The V/V34 population was grown at Warsaw and Blacksburg during the 2018-19 growing season.

Genotyping and DNA extraction

DNA extraction was performed by personnel at the USDA-ARS North Central Small Grain Genotyping Lab in Fargo, ND used a sodium dodecyl sulfate (SDS) extraction protocol modified for robot and block format. The iSelect Infinium^{TM} SNP genotyping assay with 50,000 barley SNPs (Illumina, San Diego, CA, USA) (Bayer, et al., 2017), was performed on both populations at the USDA-ARS North Central Small Grains Genotyping Lab in Fargo, ND. Genome Studio 2.0 software (Illumina, 2018) was used to analyze the SNPs according to genotype. The SNP calling algorithm automatically called most SNPs. Manual curation was performed on both populations in order to ensure marker quality. Monomorphic markers, and markers with >10% missing data or markers that did not meet a 1:1 segregation ratio (chi-square *P*-value $\geq$ 0.01) were removed.

Linkage maps and QTL analysis

Linkage maps were constructed using JoinMap 4.0 (Van Ooijen, 2006) with the Kosambi mapping function (Kosambi, 1943) to estimate map distance for markers that were assigned to linkage groups based on a 50,000 iSelect SNP consensus (Bayer, et al., 2017). The E/V34 map consisted of 1309 SNP markers, 112-251 SNP markers spaced an average of 1.09 cM were place on each of the 7 barley chromosomes. The V/V34 map consisted of 1478 SNP markers, 153-273 SNP markers spaced an average of 1.16 cM were place on each of the 7 barley chromosomes. The Barley Morex International Barley Sequencing Consortium 2017 genome assembly found on GrainGene 3.0, was used to compare the genomic regions identified in this region with other association studies.

Phenotyping

Barley samples were malted and analyzed by the USDA-ARS Cereal Crops Research Unit in Madison, Wisconsin. Dry basis (170 g) samples were malted with steeping (16°C; 4 hours(h) x 4h air x 4h wet, etc.) from 24 to 48h total, dependent on average kernel weight, to a moisture of 45% at Steep-out. Germination was at 17°C for 120h, with 3 minutes of turning every 30 minutes. Grain moisture was checked/adjusted to 45% once during germination. Samples were then kilned for 24h, starting at 49°C (for 10h), and ramping up through stages to a final 3h at 85°C. After kilning, rootlets were cleaned from the malts. Barley testing included American Society of Brewing Chemists (ASBC) Method 2-C, "Assortment", used to determine kernel plumpness as percentage of barley retained on a 6/64" slotted screen, and ASBC Method 2-D, "1000-kernel weight", which yielded an average kernel weight. A Foss Nova NIR (Transmittance mode) was used to predict barley moisture and protein percentages. Quality analyses of the malts included ASBC Malt-4, "Extract" and ASBC Malt-5, "wort analyses", with color (ASBC), soluble protein (%), β-glucan (ppm) and FAN (ppm) determined using a Skalar Segmented Flow Analyzer. Total malt protein (%) was assessed with a Leco FP528 Nitrogen Analyzer, and ASBC Malt-3, "malt moisture" was determined with a drying oven. Amylolytic enzyme levels (diastatic power and α-amylase) were calculated using the extraction procedures described in ASBC Malt-6 and -7, and automated flow analysis with a Skalar Segmented Flow Analyzer.

QTL analysis

Composite interval mapping of additive (ICIM-ADD) effects was performed with the BIP functionality in ICIMapping (Wang, et al., 2012). The whole genome was scanned with a walking speed of 1.0 cM and a probability of 0.001 in stepwise regression, a significant QTL is based on 1000 permutations and a critical LOD value of 3.0 (Doerge and Churchill, 1996).

Results and Discussion

Pearson's correlation was conducted using the cor() function in R (RCoreTeam, 2016, RStudio, 2014) in order to investigate trait relationships. In the E/V34 population, ME was correlated ($P<0.001$) with BP (-0.56, BG (-0.54), AA (0.43). In the V/V34 population, ME was correlated with BP (-0.64), BG (-0.30), and DP (-0.38) (Tables 1 and 2).

A total of six QTL consistently influenced malt quality. QTL for ME, AA, and BG were identified in different regions between the two populations (Table 3). A region on 5H explained the largest portion of (phenotypic variation) PVE (AA: 18.3-59.0%, BG: 32.2-54.2%, FAN: 53.3-77.6%, ME: 19.3-42.3%, S/T: 51.3-66.9%, and WP: 57.1-61.3%) in the E/V34 population. Mohammadi, et al., (2015) reported this region on 5H (*QAa.StMo-5H.2; QAa.HaMo-5H; QS/T.HaMo-5H*) as explaining significant amounts of PVE in six bi-parental breeding populations that represent both 2-row and 6-row breeding material from four different programs (Montana State University, University of Minnesota, USDA-Aberdeen Idaho, and Washington State University). Harrington / 'Morex' and 'Steptoe' / Morex were the initial populations where this region was reported (Mohammadi, et al., 2015). Endeavor was derived from the cross ORWM8406 / Harrington (Obert, et al., 2009), explaining the likely source of this genomic region. The 5H region identified in the E/V34 population was not significant in the V/V34 population; therefore, MAS for this region is only relevant when Endeavor or other Harrington derivatives are used as a parent (Table 3 and S. Figure 5). Two regions on chromosome 1H accounted for 10.8-12.0% and 10.5-10.8% PVE for ME in the V/V34 population, both of these regions have previously shown significant marker-trait associations for ME, reported in GrainGenes 3.0 (Table 3 and S. Figure 2). The first V/V341H QTL with the JHI-Hv50k-2016-18720 and JHI-Hv50k-2016-19015 flanking markers, was reported to have significant malt

quality marker-trait associations in three trials listed in GrainGenes 3.0 (SPY1_Malt_2011_Morris', Beta GlucanTest-VT_2009_Madison, & SPY1_Malt_2011_Crookston). The second V/V34 1H QTL with the JHI-Hv50k-2016-44645 and JHI-Hv50k-2016-46344 flanking markers was reported to have significant malt quality marker-trait associations in five trials listed in GrainGene 3.0 (SPY1_Malt_2011_Morris, TCFW6-NUEhighN_2013_Corvallis, SPY1_Malt_2011_Crookston, CAPIIYT_2007_Corvallis, & Beta GlucanTest_2008_Madison) The region on 4H, *QDp.DiMo-4H* (Mohammadi, et al., 2015) explained a major proportion of the PVE for DP, 12.1 - 42.2% (E/V34) and 30.0 - 55.7% (V/V34) (Table 3, Figures 1 and 2, and S. Figure 2). DP is a major predictor of overall malting quality, which encompasses the collective activity of four starch-degrading enzymes, namely α-amylase, β-amylase, limit dextrinase, and α-glucosidase (Fang, et la., 2019). It is important to note that the QTL region on 4H also influenced heading date in both populations and mature plant height in the E/V34 population. Additional malt quality PVE was explained by a separate region on 1H in the E/V34 population, 6.0-7.3% for BG, 4.2% for DP (W17 only), and 9.6-26.9% for S/T (Table 3 and S. Figure 1). This region was previously reported to have significant marker-trait associations in two trials (Beta Glucan Test- OR_2006_Madison & Beta GlucanTest_2007_Madison) in GrainGenes 3.0. Another region on 7H explained 6.4-7.9% PVE for ME in the E/V34 population (Table 3 and S. Figure 5), also previously reported in GrainGenes 3.0 in five separate trials (PYT1_2009_Crookston, PYT1_2009_StPaul, SPY1_Malt_2011_Morris, SPY1_Malt_2011_Crookston, & Beta GlucanTest_2008_Madison).

Additive effects of QTL were observed in both populations (Tables 4 and 5). Least-squares means and Fisher's LSD ($P<0.05$) were generated to determine the significance between the groups in the R statistical environment (R CoreTeam, 2016, R Studio, 2014). Depending on site-

year, 2-row DH lines with Endeavor alleles at the three-malt quality QTL (1H, 5H, and 7H) had 3.5-5.3 % higher average malt ME, 17.4-47.2 DU higher average AA, and 252.9-446.2 ppm lower average BG. Similarly, 6-row DH lines had 3.5-46 % higher average ME, 17.3-48.8 DU higher average AA, and 204.5-364.5 ppm lower average BG. The groups of DH lines with a combination of Endeavor and VA09B-34 alleles mostly had average least square means that were in between the groups with all three Endeavor alleles and all three VA09B-34 alleles (Table 4). The 5H region reported by Mohammadi, et al., (2015) (*QAa.StMo-5H.2; QAa.HaMo-5H; QS/T.HaMo-5H*) was by far the best predictor of ME, BG, and AA. The DH lines with the Endeavor allele at 5H were much closer to meeting AMBA recommendations for ME and BG (AA was too high for most lines with the Endeavor allele), while the lines with the VA09B-34 allele would only be suitable as feed types, due to very high BG and low ME. The 1H and 7H QTL regions also influenced malt quality, the effect of the Endeavor alleles from these two regions alone would not be enough to meet AMBA recommendation for this population for ME or BG, without favorable alleles from the 5H region.

Similar results were observed in the V/V34 population for the two QTL on 1H for ME and BG. Depending on site-year, 2-row DH lines with Violetta alleles at the two 1H malt quality QTL had 1.9-2.0 % higher average malt ME and 136.8-179.1 ppm lower average BG. Similarly, 6-row DH lines had 1.4-1.5 % higher average ME and 113.3-142.2 ppm lower average BG. There were only seven DH lines that had E plus V34 or V34 plus E allelic combinations for the two QTL on 1H. This indicates the presence of linkage between these two regions (Table 5).

Row number and Malt Extract

Row number (2 vs. 6) was not a significant (P<0.05) predictor of malt extract in the E/V34 population (Figure 3). However, a significant pleiotropic effect was observed in the V/V34

population, but not as expected. Least square means for 6-row DH lines were greater than 2-row lines for malt extract (%) (P<0.05) at both locations (Figure 4).

Conclusions

Developing acceptable malt barley cultivars (AMBA, 2019) is a significant challenge for malt barley breeders in the Eastern U.S. Understanding the genetic architecture of relevant breeding populations can offer insight into genomic regions important to malting quality. Diagnostic DNA markers associated with malt quality traits and the favorable breeding parents could benefit breeders that need to utilize feed barley breeding material as a base. The telomeric region on chromosome 5H (*QAa.StMo-5H.2; QAa.HaMo-5H; QS/T.HaMo-5H*) is an important region to target when crosses include breeding material from Western U.S. programs, such as Endeavor or Harrington. The 4H region, *QDp.DiMo-4H* (Mohammadi, et al., 2015), should be targeted when the objective is to increase enzyme activity, which is very important if the goal is to develop barley cultivars for the adjunct brewing market (AMBA, 2019). Table 6 includes QTL, marker, and favorable parent information for the six QTL described in this report. Eastern barley breeders should focus on including the best malt quality lines derived from such 'malt x feed' populations in order to maximize genetic gains for malting performance, while retaining superior agronomic characteristics of the feed type parents. Supplementary Table 3 includes a list of doubled haploid winter barley lines that had exceptional malting quality (>80% malt extract, between 9-12% barley protein, and <150 ppm β-glucan) at one or more site-years, and should be considered as elite malt breeding lines.

Supplemental material

S. Tables 1 and 2 include information about the growing locations and management practices. S. Table 3 includes a list of doubled haploid winter barley lines that show exceptional quality and

could be used as high quality malt breeding parental lines. S. Figures 1-5 include partial linkage maps of barley chromosomes 1H, 4H, 5H, and 7H displaying the QTL described in this report.

Conflict of interests

The authors declare no conflicts of interest.

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Figure 1. Boxplot representing single marker trait pairwise comparison of least-square means (Fisher's LSD, *P*<0.05) for diastatic power (DU) of QTL region associated with *QDp.DiMo-4H* (Mohammadi, et al., 2015) between progeny with the Endeavor (n=65) allele and the VA09B-34 (n=65) allele in the Endeavor / VA09B-34 doubled haploid winter barley population at all three site-years (values connected by the same letter are not significantly different at α=0.05).

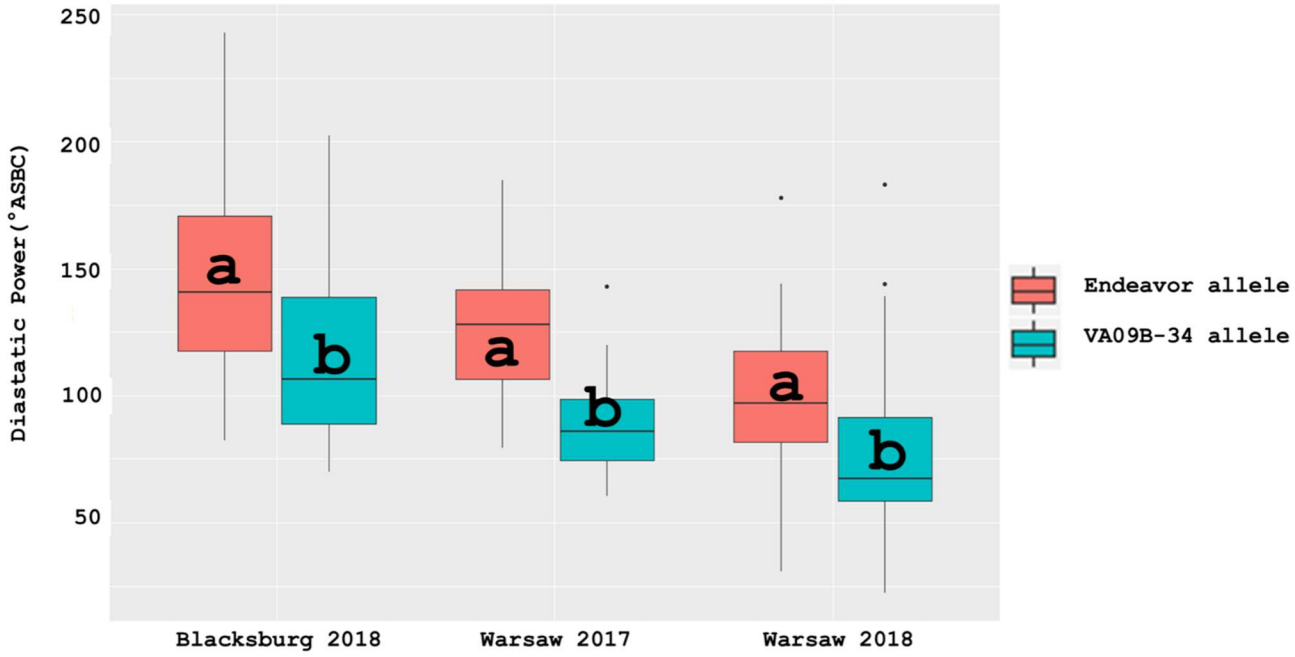


Figure 2. Boxplot representing single marker trait pairwise comparison of least-square means (Fisher's LSD, *P*<0.05) for diastatic power (DU) of QTL region associated with *QDp.DiMo-4H* (Mohammadi, et al., 2015) between progeny with the Violetta (n=58) allele and the VA09B-34 (n=83) allele in the Violetta / VA09B-34 doubled haploid winter barley population at both site-years (values with same letter are not significantly different at α=0.05).

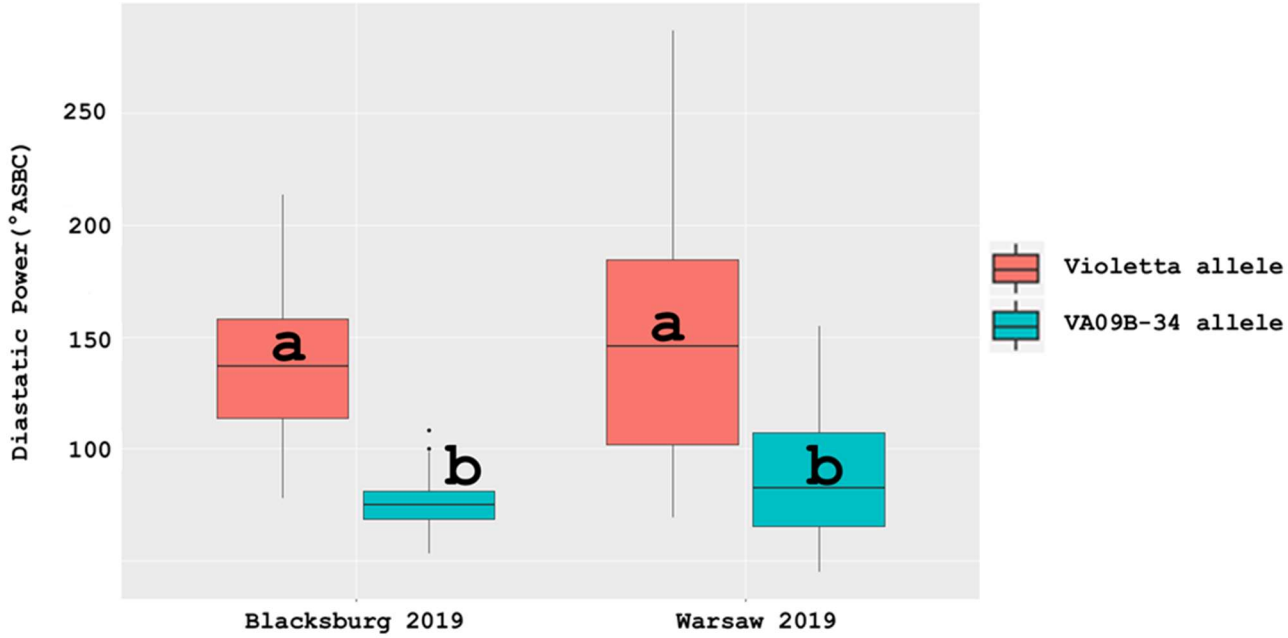


Figure 3. Boxplot representing pairwise comparison of least-square means (Fisher's LSD, *P*<0.05) in 2-row (n=45) vs. 6-row (n=85) DH lines for malt extract (%) in the Endeavor / VA09B-34 doubled haploid winter barley population at both site-years (values with the same letter are not significantly different at α=0.05).

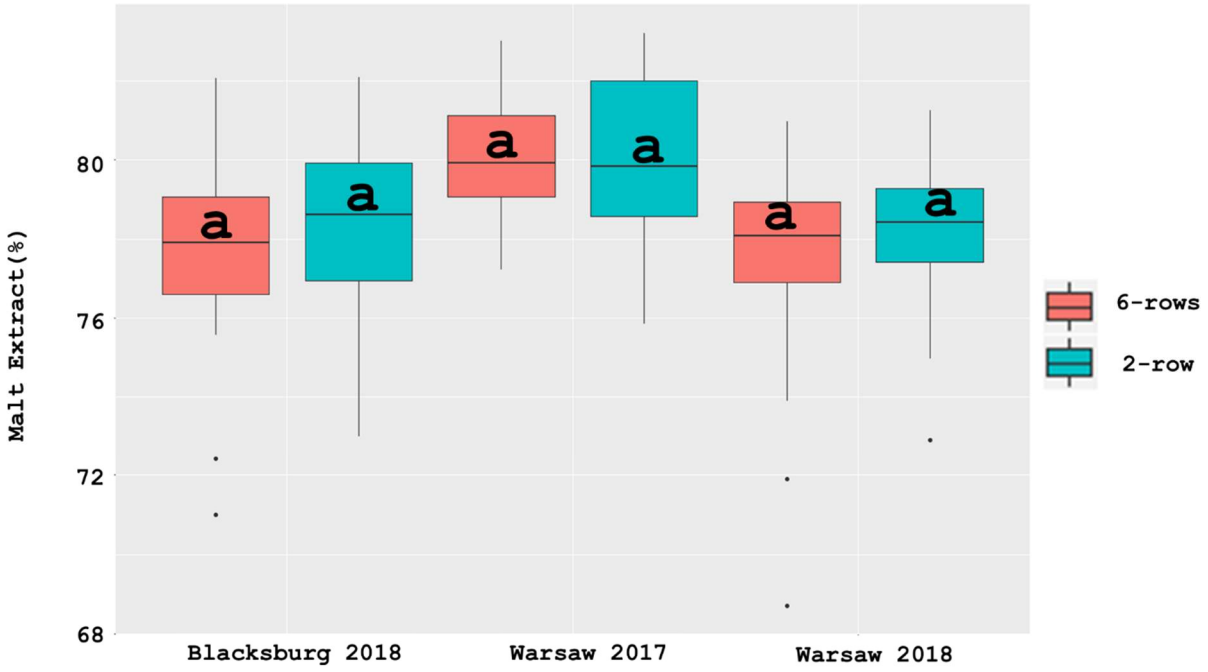


Figure 4. Boxplot representing pairwise comparison of least-square means (Fisher's LSD, *P*<0.05) in 2-row (n=72) vs. 6-row (n=69) DH lines for malt extract (%) in the Violetta / VA09B-34 doubled haploid winter barley population at both site-years (values connected by the same letter are not significantly different at α=0.05).

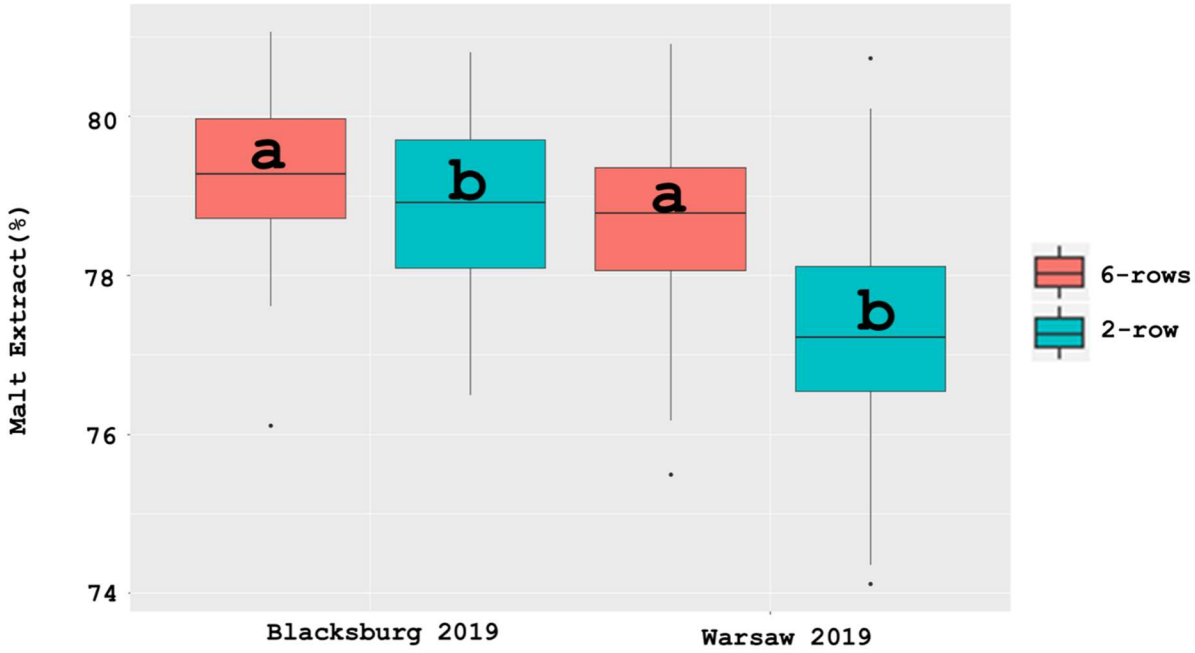


Table 1. Pearson correlations for Endeavor / VA09B-34 doubled haploid winter barley population using averages for all three site-years among 14 traits based on least square means of each doubled haploid genotype.

Trait[a]	KW	PL	ME	BP	WP	ST	DP	AA	BG	FAN	Y	TW	HT	HD
KW	1.00													
PL	0.87*	1.00												
ME	0.06	0.07	1.00											
BP	0.48*	0.42*	-0.56*	1.00										
WP	0.39*	0.30*	0.37*	0.35*	1.00									
ST	0.01	-0.04	0.76	-0.38*	0.72*	1.00								
DP	-0.01	0.00	-0.10	0.17	0.10	-0.04	1.00							
AA	-0.11	-0.18	0.43	-0.12	0.66*	0.74*	0.16	1.00						
BG	0.17	0.17	-0.54	0.24*	-0.61*	-0.79*	-0.06	-0.65*	1.00					
FAN	0.21	0.18	0.48	0.14	0.92*	0.80*	0.03	0.72*	-0.74*	1.00				
Y	0.08	0.27*	0.26	-0.24*	-0.02	0.13	0.19	-0.06	-0.06	0.03	1.00			
TW	0.65*	0.75*	0.17	0.16	-0.02	-0.18	-0.03	-0.37*	-0.32*	0.09	0.44*	1.00		
HT	0.55*	0.52*	0.13	0.26*	0.25*	0.04	0.10	-0.03	0.14	0.13	0.20	0.43*	1.00	
HD	0.45*	0.39*	-0.10	0.33*	0.22	-0.01	0.02	-0.01	0.06	0.11	-0.06	0.08	0.59*	1.00

*Indicates a significant (P<0.001) Pearson trait correlation using R with Bonferroni multiple comparison adjustment

[a]KW kernel weight (mg), PL kernel plumpness 6/64", ME malt extract (%), BP barley grain protein (%), WP wort protein (%), ST soluble/total Nitrogen, DP diastatic power (DU), alpha-amylase (DU), BG beta-glucan (ppm), FAN free amino nitrogen, Y yield (%), TW test weight (kg hL-1), HT mature plant height (cm), HD heading date (Julian))

Table 2. Pearson correlations for Violetta / VA09B-34 doubled haploid winter barley population using averages for two site-years among 14 traits based on least square means of each doubled haploid genotype.

Trait[a]	KW	PL	ME	BP	WP	ST	DP	AA	BG	FAN	Y	TW	HT	HD
KW	1.00													
PL	0.91*	1.00												
ME	-0.39*	-0.28*	1.00											
BP	0.83*	0.75*	-0.64*	1.00										
WP	0.74*	0.67*	-0.39*	0.86*	1.00									
ST	-0.66*	-0.59*	0.69*	-0.80*	-0.42*	1.00								
DP	0.60*	0.57*	-0.36*	0.64*	0.63*	-0.43*	1.00							
AA	-0.11	-0.12	0.11	0.03	0.33*	0.34*	0.08	1.00						
BG	0.16	0.18	-0.30*	0.21	-0.04	-0.47*	-0.09	-0.46*	1.00					
FAN	0.53*	0.48*	-0.18	0.63*	0.90*	-0.12	0.46*	0.56*	-0.21	1.00				
Y	-0.65*	-0.55*	0.52*	-0.69*	-0.73*	0.45*	-0.47*	-0.04	-0.10	-0.63*	1.00			
TW	0.83*	0.84*	-0.20	0.73*	0.60*	-0.61*	0.42*	-0.18	0.28	0.39*	-0.39*	1.00		
HT	0.46*	0.41*	-0.13	0.37*	0.25*	-0.38*	0.19	-0.05	0.21	0.15	-0.02	0.46	1.00	
HD	0.13	0.14	-0.15	0.12	0.19	0.01	0.23*	0.11	-0.01	0.19	-0.22	0.04	-0.04	1.00

*Indicates a significant (P<0.001) Pearson trait correlation using R with Bonferroni multiple comparison adjustment

[a]KW kernel weight (mg), PL kernel plumpness 6/64", ME malt extract (%), BP barley grain protein (%), WP wort protein (%), ST soluble/total Nitrogen, DP diastatic power (DU), alpha-amylase (DU), BG beta-glucan (ppm), FAN free amino nitrogen, Y yield (%), TW test weight (kg hL-1), HT mature plant height (cm), HD heading date (Julian))

Table 3. List of QTL identified in Endeavor / VA09B-34 and Violetta / VA09B-34 doubled haploid winter barley populations.

Trait[a]	site-year	Chromosome	Left Marker	Right Marker	Reference Position	Initial Report	LOD	PVE (%)	Add	Positive Parent
BG	W17*	1H	19505[a]	20069	69749686-80267507	GrainGenes 3.0 (Beta Glucan Test-OR_2006_Madison & Beta GlucanTest_2007_Madison)	9.8	7.3	-50.1	Endeavor
	B18		19505	23021			3.7	6.0	-49.5	Endeavor
DP	W17		20069	23021			3.1	4.2	-5.8	Endeavor
S/T	W17		19505	20069			17.0	26.9	2.9	Endeavor
	B18		19505	20069			7.1	9.6	1.7	Endeavor
ME	B19	1H	18720	19015	35728427-59789397	GrainGenes 3.0 (SPY1_Malt_2011_Morris, Beta GlucanTest-VT_2009_Madison, & SPY1_Malt_2011_Crookston,)	10.5	10.8	0.4	Violetta
ME	W19		18720	19015			6.9	10.5	0.5	Violetta
FAN	B19	1H	45646	46344	520679879-523953393	GrainGenes 3.0 (SPY1_Malt_2011_Morris, TCFW6-NUEhighN_2013_Corvallis, SPY1_Malt_2011_Crookston, CAPIIYT_2007_Corvallis, & Beta GlucanTest_2008_Madison)	3.4	5.1	-5.4	Violetta
ME	B19		45319	45675			11.3	12.0	0.4	Violetta
ME	W19		46073	46344			7.0	10.8	0.5	Violetta
Y	W19		45646	46344			23.7	65.4	13.7	Violetta
DP	W17	4H	274999	273845	641157502-643754756	*QDp.DiMo-4H (Mohammadi, et al., 2015)*	21.6	42.6	18.6	Endeavor
	B18		275586	274999			5.7	18.8	16.2	Endeavor
	W18		274999	273845			3.6	12.1	10.6	Endeavor
HD	W17		274999	273845			5.5	4.7	0.9	Endeavor
	W18		274999	273845			4.3	3.8	0.8	Endeavor
	B18		274999	273845			3.4	3.6	0.8	Endeavor
HT	W17		274999	273845			13.9	24.2	1.6	Endeavor
DP	B19	4H	275904	273856	641157956-644434992	*QDp.DiMo-4H (Mohammadi, et al., 2015)*	45.6	55.7	27.2	Violetta
HD	B19		275904	273856			12.0	22.7	0.9	Violetta
DP	W19		275904	273856			24.9	30.0	28.8	Violetta
AA	W17	5H	366116	366429	666497489-667342288	*QAa.StMo-5H.2; QAa.HaMo-5H; QS/T.HaMo-5H (Mohammadi, et al., 2015)*	36.1	59.0	16.9	Endeavor
	B18		366116	366429			17.2	45.3	15.2	Endeavor
	W18		366116	366429			8.3	18.3	6.6	Endeavor
BG	W17		366116	366429			38.7	54.2	-36.2	Endeavor
	B18		366116	366429			15.6	32.2	-15.0	Endeavor
	W18		366116	366429			14.6	40.1	-22.0	Endeavor
FAN	W17		366116	366429			20.6	53.3	45.0	Endeavor
	B18		366116	366429			43.4	77.6	48.4	Endeavor
	W18		366116	366429			23.5	56.1	51.7	Endeavor
HD	W18		366116	366429			4.9	4.2	0.8	Endeavor
ME	W17		366116	366429			22.7	42.3	1.0	Endeavor
	B18		366116	366429			15.2	28.3	1.0	Endeavor
	W18		366116	366429			7.0	19.3	0.8	Endeavor

S/T	W17		366116	366429			27.8	56.2	4.2	Endeavor
	B18		366116	366429			26.2	51.3	3.9	Endeavor
	W18		366116	366429			41.1	66.9	5.8	Endeavor
TW	W17		366116	366429			4.3	6.6	-0.7	Endeavor
	W18		366116	366429			8.4	9.7	-1.0	Endeavor
WP	W17		366116	366429			27.1	57.1	0.4	Endeavor
	B18		366116	366429			27.4	60.2	0.5	Endeavor
	W18		366116	366429			30.2	61.3	0.7	Endeavor
ME	W17	7H	491428	491539	537914633-549006824	GrainGenes 3.0 (PYT1_2009_Crookston, PYT1_2009_StPaul, SPY1_Malt_2011_Morris, SPY1_Malt_2011_Crookston, & Beta GlucanTest_2008_Madison)	5.8	7.8	0.4	Endeavor
	B18		491428	491539			5.4	7.9	0.5	Endeavor
	W18		491158	491200			2.6	6.4	0.5	Endeavor
S/T	W18		491428	491539			2.8	2.0	1.0	Endeavor

*indicates site-harvest year: W=Warsaw, VA, B=Blacksburg, VA, 17=2017, 18=2018, 19=2019

[a]iSelect InfiniumTM SNP genotyping assay with 50,000 barley SNPs (Illumina, San Diego, CA, USA) (Bayer, et al., 2017) (JHI-Hv50k-2016-"#")

Table 4. Single marker analysis of malt extract (%), α-amylase (DU), and β-glucan (ppm) for QTL on Chromosomes 1H, 5H, and 7H for Endeavor / VA09B-34 doubled haploid winter barley population.

		2-rows				6-rows		
		Warsaw 2017	Blacksburg 2018	Warsaw 2018		Warsaw 2017	Blacksburg 2018	Warsaw 2018
Parental Alleles*	n	Least Square Means			n	Least Square Means		
Malt extract (%)								
E,E,E	8	82.6a(a)	81.1a	80.3a	7	81.8a	80.2a	79.7a
E,E,V34	5	81.6ab	80.1ab	79.2ab	17	80.9b	78.8b	78.0bc
V34,E,E	5	81.2abc	78.8bc	77.9bcd	9	80.7bc	78.8ab	78.7ab
V34,E,V34	4	80.0bcd	78.3bc	78.5bc	9	79.9cd	77.9bc	78.2abc
E,V34,E	6	79.8cd	78.7bc	78.3bc	12	79.5de	77.0cd	77.1cd
V34,V34,E	10	79.0d	77.3cd	77.9bcd	9	78.7ef	76.4de	76.8cd
E,V34,V34	1	78.1de	76.4cd	76.4cd	11	79.8cd	77.8bc	78.2abc
V34,V34,V34	6	77.3e	76.0d	76.8d	9	78.3f	75.8e	76.1d
α-amylase (DU)								
E,E,E	8	94.8a	82.9ab	68.8a	7	95.4ab	80.8a	71.4ab
E,E,V34	5	88.4ab	81.9ab	69.4a	17	94.7a	92.2a	75.0a
V34,E,E	5	82.9b	89.5a	63.5ab	9	94.8ab	94.8a	78.1a
V34,E,V34	4	76.8b	82.5ab	63.9abc	9	82.8b	90.6a	67.5abc
E,V34,E	6	56.5cd	58.5c	58.0abc	12	60.8c	62.9b	64.2bc
V34,V34,E	10	59.5c	56.9c	53.4bc	9	52.6cd	58.9b	61.3bc
E,V34,V34	1	42.7cd	49.8bc	45.2bc	11	59.7c	57.3b	58.1bc
V34,V34,V34	6	47.6d	53.1c	51.4c	9	46.6d	55.9b	54.1c
β-glucan (ppm)								
E,E,E	8	184.6c	238.6d	124.3e	7	324.9c	343.4c	133.7d
E,E,V34	5	148.9c	285.8cd	154.2de	17	258.3c	323.5c	192.6cd
V34,E,E	5	290.1bc	466.2bc	289.8bcd	9	318.9c	304.8c	167.0d
V34,E,V34	4	274.3bc	371.3bcd	182.1de	9	321.4c	311.1c	156.2d
E,V34,E	6	585.4a	549.5ab	443.2ab	12	491.7b	494.6b	363.8ab
V34,V34,E	10	614.2a	702.9a	463.6a	9	611.9a	665.9a	496.0a
E,V34,V34	1	443.8ab	441.8abcd	319.3abc	11	463.0b	343.1c	312.4bc
V34,V34,V34	6	630.8a	491.5b	388.1abc	9	640.4a	547.9ab	498.2a

*indicates parental source of allele (1H, 5H, 7H) for each QTL; E=Endeavor and V34=VA09B-34

[a]values connected by the same letter are not significantly different (α=0.05)

Table 5. Single marker analysis of malt extract (%) and β-glucan (ppm) for both QTL on Chromosome 1H for Violetta / VA09B-34 doubled haploid winter barley population.

		2-rows			6-rows	
		Blacksburg 2019	Warsaw 2019		Blacksburg 2019	Warsaw 2019
Parental Alleles*	n	Least Square Mean		n	Least Square Means	
malt extract (%)						
V,V	43	77.8a	80.0a	43	79.1a	79.6a
V,V34	3	77.3a	78.7b	2	78.5a	79.3a
V34,V	1	77.1a	78.7b	1	78.7a	79.4a
V34,V34	24	75.8b	78.1b	23	77.6b	78.2b
β-glucan (ppm)						
V,V	43	292.1b	288.7b	43	258.9a	292.4b
V,V34	3	296.7b	293.6b	2	275.8ab	347.6ab
V34,V	1	314.2b	330.3b	1	297.4ab	309.7b
V34,V34	24	428.9a	467.8a	23	372.2a	435.2a

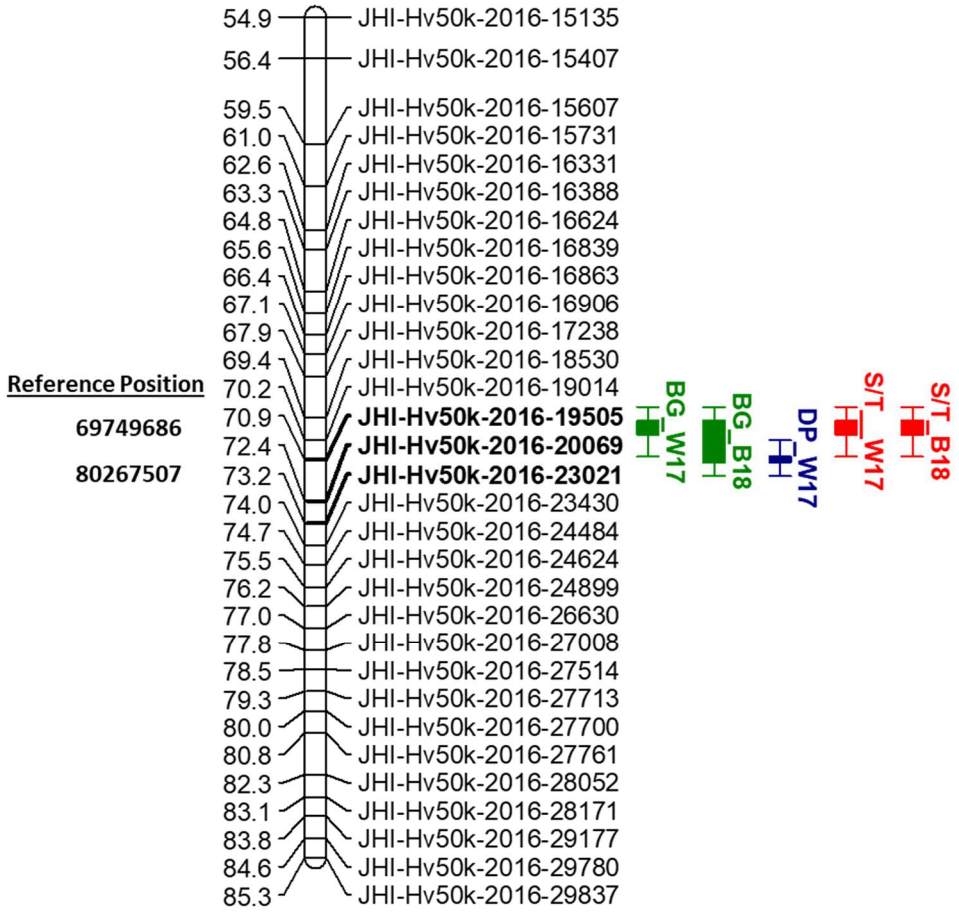
*indicates parental source of 1H alleles for each QTL; V=Violetta and V34=VA09B-34

[a]values connected by the same letter are not significantly different (α=0.05)

Table 6. Summary of markers for each QTL and favorable parents for marker assisted selection

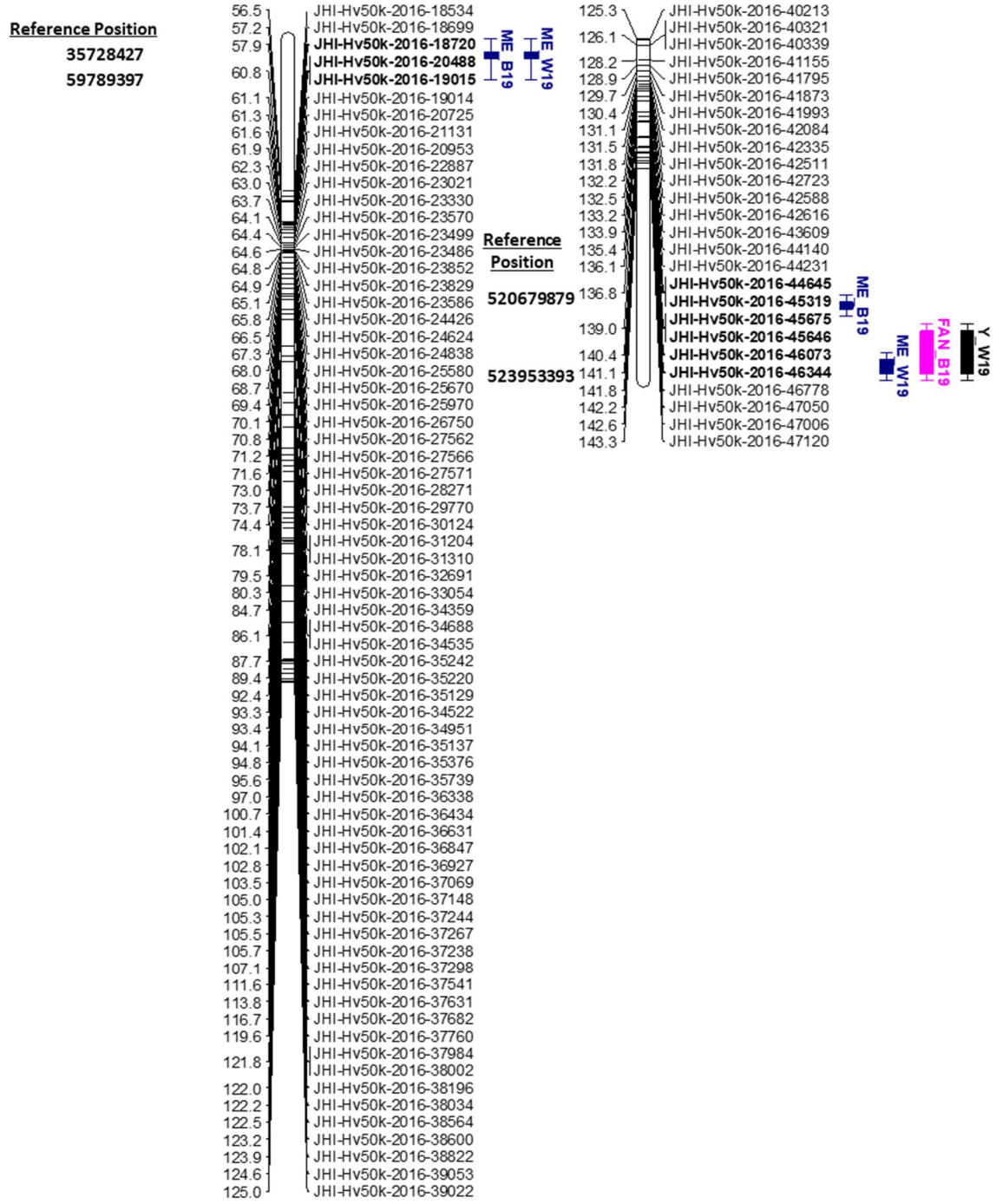
Traits	Chromosome	Right Marker	Left Marker	Favorable Parent
β-glucan, diastatic power, soluble/total nitrogen	1H	JHI-Hv50k-2016-19505	JHI-Hv50k-2016-23021	Endeavor
malt extract	1H	JHI-Hv50k-2016-18720	JHI-Hv50k-2016-19015	Violetta
malt extract	1H	JHI-Hv50k-2016-45319	JHI-Hv50k-2016-46344	Violetta
diastatic power	4H	JHI-Hv50k-2016-275586	JHI-Hv50k-2016-273845	Endeavor or Violetta
α-amylase, β-glucan, free amino nitrogen, malt extract, soluble/total nitrogen, wort protein, test weight	5H	JHI-Hv50k-2016-366116	JHI-Hv50k-2016-366429	Endeavor
malt extract, soluble/total nitrogen	7H	JHI-Hv50k-2016-491158	JHI-Hv50k-2016-491539	Endeavor

Supplemental Figure 1. Partial linkage map displaying the malt quality QTL on the 1H chromosome from Endeavor / VA09B-34 winter barley mapping population along with the corresponding reference positions (GrainGenes 3.0).



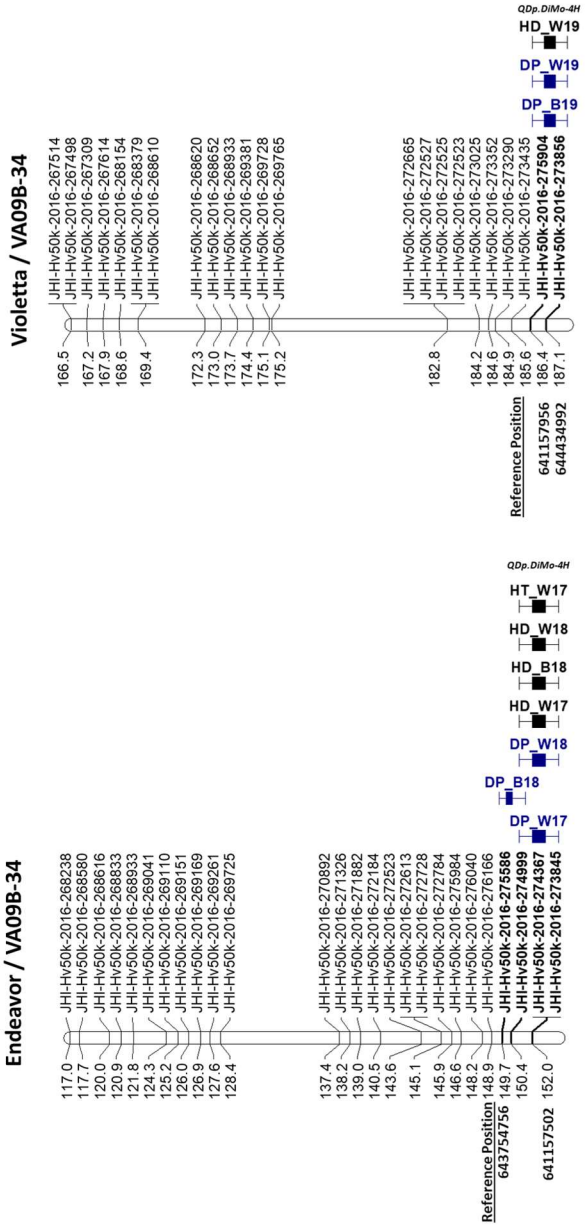
Marker-trait association is the trait abbreviation: BG=β-glucan, DP=diastatic power, S/T=soluble/total nitrogen followed by site-harvest year: W=Warsaw, VA, B=Blacksburg, VA, 17=2017, 18=2018.

Supplemental Figure 2. Partial linkage map displaying the two-malt quality QTL identified on the 1H chromosome from Violetta / VA09B-34 doubled haploid winter barley mapping population along with the corresponding reference positions (GrainGenes 3.0).



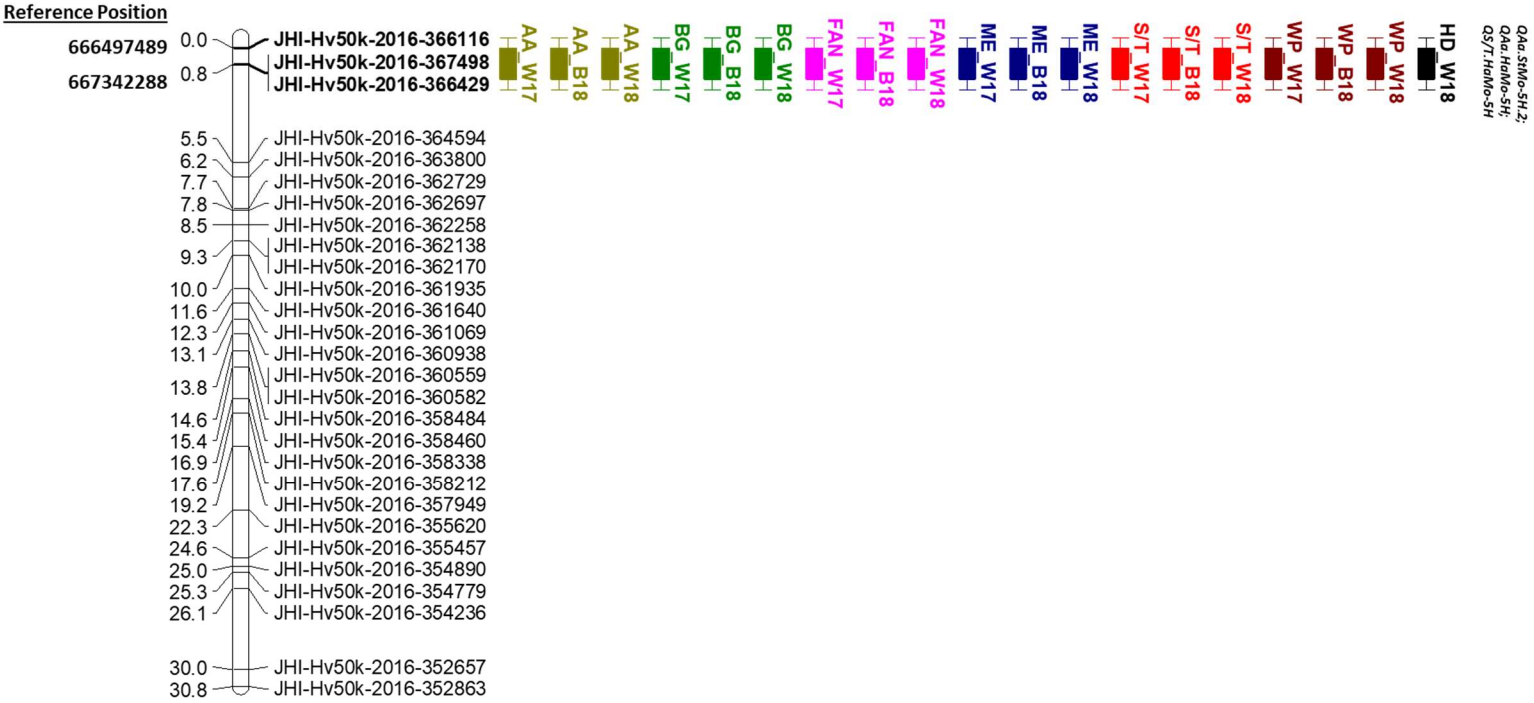
Marker-trait association is the trait abbreviation: FAN=free amino nitrogen, ME=malt extract (%), Y=grain yield followed by site-harvest year: W=Warsaw, VA, B=Blacksburg, VA, 19=2019.

Supplemental Figure 3. Partial linkage map displaying the malt quality QTL identified on the 4H chromosome from both the Endeavor / VA09B-34 and Violetta / VA09B-34 doubled haploid winter barley mapping populations along with the corresponding reference positions (GrainGenes 3.0).



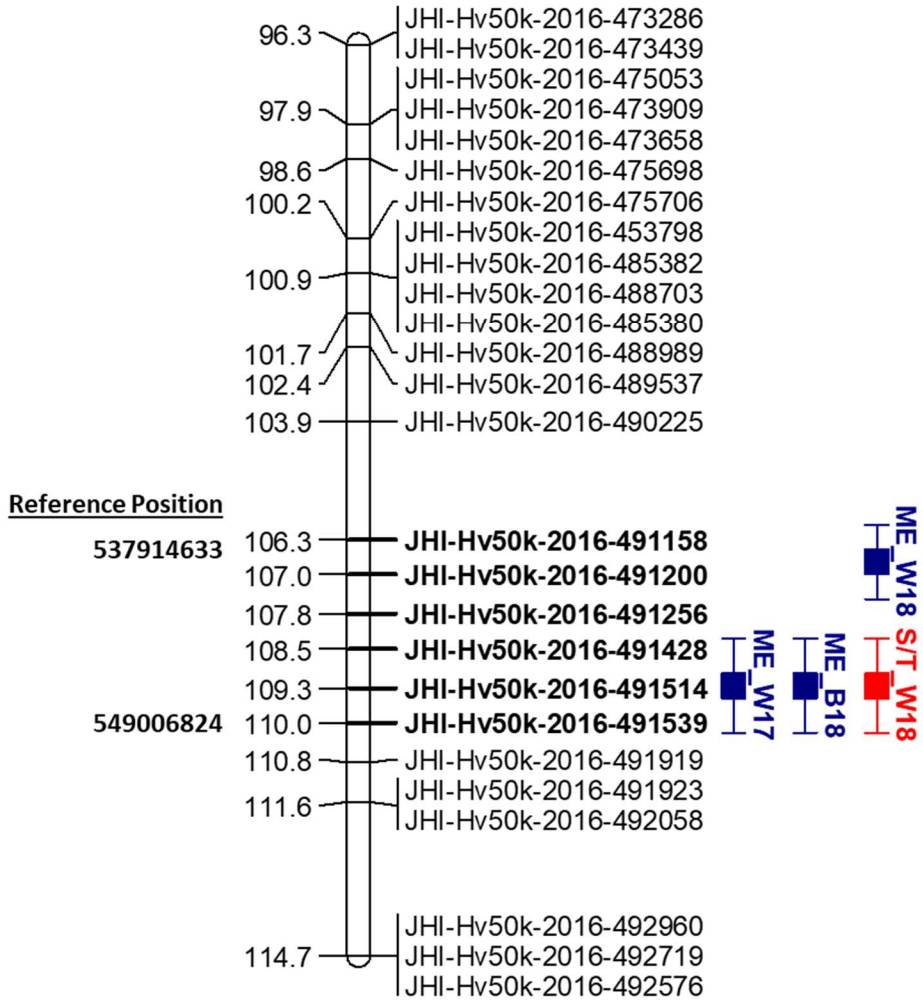
Marker-trait association is the trait abbreviation: DP=diastatic power (ASBC°), HD=heading date (Julian), HT=mature plant height (cm) followed by site-harvest year: W=Warsaw, VA, B=Blacksburg, VA, 17=2017, 18=2018, 19=2019.

Supplemental Figure 4. Partial linkage map displaying the malt quality QTL identified on the 5H chromosome from the Endeavor / VA09B-34 doubled haploid winter barley mapping population along with the corresponding reference positions (GrainGenes 3.0).



Marker-trait association is the trait abbreviation: AA=α-amylase (DU), BG=β-glucan (ppm), DP=diastatic power (ASBC°), FAN=free amino nitrogen, HD=heading date (Julian), ME=malt extract (%), S/T=soluble/total nitrogen, WP=wort protein followed by site-harvest year: W=Warsaw, VA, B=Blacksburg, VA, 17=2017, 18=2018.

Supplemental Figure 5. Partial linkage map displaying the malt quality QTL identified on the 7H chromosome from the Endeavor / VA09B-34 doubled haploid winter barley mapping population along with the corresponding reference positions (GrainGenes 3.0).



Marker-trait association is the trait abbreviation: ME=malt extract (%), S/T=soluble/total nitrogen followed by site-harvest year: W=Warsaw, VA, B=Blacksburg, VA, 17=2017, 18=2018.

Supplemental Table 1. Soil type, elevation, and tillage type for each site-year.

Location	Soil type	Elevation	Tillage
Warsaw, VA	Kempsville Sandy Loam	36 m	conventional
Blacksburg, VA	Hayter loam	518 m	conventional

Supplemental Table 2. Management practices by site-year including fertilizer, active ingredient (A.I.) growth regulator, and application dates.

Season	Location	Harvest area	Product	Application rate	Application date
2016-17	Warsaw, VA	1.5 x 2.7 m	Planting	-	10/14/2017
			Pre-plant fertilizer	30-80-80-5	10/11/2018
			UAN*	33.6 kg ha-1	12/3/2017
			Harmony Extra SG®	0.014 A.I. kg ha-1	3/9/2019
			Tilt®	0.07 A.I. kg ha-1	3/22/2018
			Palisade EC®	0.14 A.I. kg ha-1	3/22/2018
			UAN	33.6 kg ha-1	3/24/2019
			Fitness®	0.07 A.I. kg ha-1	4/19/2018
			Prosaro®	0.14 A.I. kg ha-1	4/30/2018
			Harvest	-	6/10/2019
2017-18	Warsaw, VA	1.5 x 2.7 m	Planting	-	10/21/2017
			Pre-plant fertilizer	30-80-80-5	10/19/2018
			UAN	33.6 kg ha-1	12/7/2017
			Harmony Extra SG®	0.014 A.I. kg ha-1	3/6/2019
			Tilt®	0.07 A.I. kg ha-1	3/17/2018
			Palisade EC®	0.14 A.I. kg ha-1	3/17/2018
			UAN	56.1 kg ha-1	3/19/2018
			Fitness®	0.07 A.I. kg ha-1	4/11/2018
			Prosaro®	0.14 A.I. kg ha-1	5/1/2018
			Harvest	-	6/7/2019
2018-19	Warsaw, VA	1.5 x 2.7 m	Planting	-	10/18/2018
			Pre-plant fertilizer	30-80-100	10/10/2018
			UAN	28.0 kg ha-1	12/6/2018
			UAN	28.0 kg ha-1	1/31/2019
			Harmony Extra SG®	0.018 A.I. kg ha-1	3/13/2019
			UAN	33.6 kg ha-1	3/18/2019
			Fitness®	0.07 A.I. kg ha-1	3/25/2019
			Starane®	0.17 A.I. kg ha-1	3/29/2019

			Palisade EC®	0.21 A.I. kg ha-1	3/30/2019
			Endigo ZC®	0.09 A.I. kg ha-1	4/11/2019
			Fitness®	0.07 A.I. kg ha-1	4/18/2019
			Prosaro®	0.14 A.I. kg ha-1	5/3/2019
			Harvet	-	5/31/2019
2017-18	Blacksburg, VA	1.5 x 2.7 m	Planting	-	10/2/2017
			Pre-plant fertilizer	47-120-150-S/10-B/3	9/30/2017
			Harmony Extra SG®	0.014 A.I. kg ha-1	11/17/2017
			UAN	44.7 kg ha-1	4/6/2018
			Harmony Extra SG®	0.012 A.I. kg ha-1	4/6/2018
			UAN	22.4 kg ha-1	3/15/2018
			Harvest	-	6/15/2018
2018-19	Blacksburg, VA	1.5 x 2.7 m	Planting	-	10/7/2018
			Pre-plant fertilizer	30-50-50-S/10-B/3-ZN/2	10/5/2018
			UAN	33.6 kg ha-1	3/6/2019
			UAN	56.1 kg ha-1	3/24/2019
			Harmony Extra SG®	0.014 A.I. kg ha-1	3/6/2019
			Tilt®	0.14 A.I. kg ha-1	4/10/2019
			Palisade EC®	0.21 A.I. kg ha-1	4/10/2019
			Prosaro®	0.09 A.I. kg ha-1	4/29/2019
			Harvest	-	6/4/2019

*UAN=urea and ammonium nitrate

Supplemental Table 3. List of doubled haploid winter barley lines with >80% malt extract, between 9.0-12.0% barley, and <150 ppm β-glucan

Line Designation	row number	Population
VA17M-DH141818	6	Endeavor / VA09B-34
VA17M-DH141476	6	Endeavor / VA09B-34
VA17M-DH141819	6	Endeavor / VA09B-34
VA17M-DH150265	2	Endeavor / VA09B-34
VA17M-DH141483	6	Endeavor / VA09B-34
VA16M-DH14BDH13-12	2	Endeavor / VA09B-34
VA16M-DH14BDH12-75	6	Endeavor / VA09B-34
VA18M-DH170705	2	Violetta / VA09B-34
VA18M-DH161725	6	Violetta / VA09B-34
VA18M-DH162226	6	Violetta / VA09B-34
VA18M-DH161699	6	Violetta / VA09B-34
VA18M-DH162061	2	Violetta / VA09B-34
VA18M-DH170056	6	Violetta / VA09B-34