Physiological Traits and Quantitative Trait Loci Associated with Nitrogen Use Efficiency in Soft Red Winter Wheat

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Crop and Soil Environmental Sciences

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March 15, 2019

Blacksburg, Virginia

Keywords: *Triticum aestivum*, wheat, nitrogen, nitrogen use efficiency, quantitative trait loci, linkage mapping, marker-assisted selection

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ACADEMIC ABSTRACT

Development of winter wheat (*Triticum aestivum* L.) cultivars capable of more efficient uptake and utilization of applied nitrogen (N) has the potential to increase grower profitability and reduce negative environmental consequences associated with N lost from the plant-soil system. The first study sought to evaluate genotypic variation for N use efficiency (NUE) and identify lines consistently expressing high or low NUE under two or more N rates in a total of 51 N-environments. The results indicated that significant genotype by N rate interactions were frequently observed when trials utilized at least three N rates and identified wheat lines with high and stable yield potential that varied in performance under low N conditions. In addition, NUE was associated with above-ground biomass at physiological maturity were found to be both highly heritable across multiple N supplies. In the second study, two bi-parental mapping populations having a common low ('Yorktown') and two high (VA05W-151 and VA09W-52) NUE parents were characterized to dissect the genetics underlying N response. The populations were evaluated in eight N-environments and genotyped using single-nucleotide polymorphism data derived from a genotyping-by-sequencing protocol to identify quantitative trait loci (QTL) associated with high NUE. Six QTL for NUE were identified on chromosomes 1D, 2D, 4A, 6A, 7A, and 7D that were associated with N use efficiency. The QTL on 2D and 4A co-localized with known loci governing photoperiod sensitivity and resistance to Fusarium head blight (caused by the fungal pathogen *Fusarium graminearum* Schwabe), respectively. Three of the identified QTL (6A, 7A, and 7D) were associated with NUE in previous investigations, while the QTL on 1D was novel. The final experiment employed a small panel of soft red winter wheat lines to study the effects of photoperiod alleles on chromosome 1D (*Ppd-D1*) on yield-related traits under three or five N rates that were variably split over two growth stages in eight environments. The results validated the effect of a photoperiod sensitive allele (*Ppd-D1b*) that was associated with increased grain yield across N rates in half of the Virginia testing environments and under low N rates in all Ohio testing sites at the expense of grain N content. Yield advantages conferred by the *Ppd-D1b* allele were attributable to increased floret fertility and kernel number per spike. The findings from these studies have direct application for winter wheat breeding programs targeting NUE improvements.

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GENERAL AUDIENCE ABSTRACT

Wheat (*Triticum aestivum* L.) products account for a significant percentage of the total dietary calories and protein consumed globally. To meet production demands, wheat requires efficient nitrogen (N) management to ensure continued grower profitability and to reduce negative environmental impacts of N lost from agricultural systems. This dissertation sought to evaluate variation among wheat lines for N use efficiency (NUE), assess the performance of wheat lines under multiple N supplies, validate traits that are associated with NUE, investigate the role of photoperiod sensitivity genes on N response, and identify regions of the wheat genome associated with high N use efficiency. These studies were conducted using panels of winter wheat lines grown under two or more N conditions over a combined 32 location-years.

Results of Chapter I identified variation in cultivar response to N rates was more frequently observed when a greater number of N rates were used in trials of wheat N response. The first chapter also identified variation among wheat lines for NUE and identified lines that consistently produce high grain yields over N-location-years. In addition, above-ground biomass at physiological maturity was found to be strongly associated with grain yield under all N rates and was highly heritable in both studies. Chapter II utilized a combination of genetic and observable trait data to perform genetic analysis in two bi-parental populations grown in eight N-location-years. The study identified reproducible and significant genetic markers associated with NUE for application in wheat breeding programs. Upon analysis of photoperiod sensitive versus insensitive wheat lines in Chapter III, photoperiod sensitive wheat lines had a significant yield advantage under N-limited conditions in Ohio and across N treatments in half of the Virginia testing location-years. This resulted from an increased number of kernels per spike and fertile florets in photoperiod sensitive wheat lines. Results from this dissertation suggest that active breeding and selection for N response may be achieved through the employment of high NUE genes and the continued identification of adapted high NUE wheat parental lines.

DEDICATION

This dissertation is dedicated to those that have supported me through my graduate education, especially Brian, Rhena, Alyson, Sam, and Duke. None of this would be possible without your kindness and support.

ACKNOWLEDGEMENTS

The results presented in this dissertation are the product of many amazing people. I am grateful for the contributions of many individuals and it has been my most sincere privilege to work with all of you on the nitrogen use efficiency projects.

First, I am indebted to my advisor, Dr. Carl Griffey, who provided an amazing opportunity to tackle the challenge of nitrogen use efficiency. Dr. Griffey taught me what it means to be an impactful breeder and mentor through his leadership and actions. Finally, Dr. Griffey allowed me to develop studies that were too big for a single graduate student to complete but provided the incredible amount of assistance required to complete the project.

I would also like to thank the members of the Virginia Tech Small Grains Breeding Group, including Wynse Brooks, Limei Liu, Subas Malla, Josh Fitzgerald, Luciana Rosso, Anthony Christopher, John Seago, Jon Light, Brian Ward, Neal Carpenter, Jordan Ulrich, Nick Meier, Camron Clark, William Myers, Sam Lawton, Inam Jameel, and Bradley Cole. These individuals were instrumental to the completion of this dissertation. I will never forget our unique experiences traveling across the state each summer.

Furthermore, this dissertation would be incomplete without the assistance of the incredible staff at the Agricultural Research and Extension Centers including Bob Pitman, Joseph Oakes, Mark Vaughn, Scooter Dunaway, Lin Barrack, Mary Beahm, Robin Markham, Michelle Lee, Maria Balota, and the countless others that made the spring and summer months an adventure to remember. I would also like to thank the technical staff in Blacksburg that kept this dissertation moving along, including Liz Rucker, Steve Nagle, Harry Behl, and Mike Brosius.

I would also like to thank the friends that helped me maintain some degree of sanity throughout my time in Blacksburg, including Sam Winter, Alexis Eliot, Brian Ward, Neal Carpenter, Jordan Ulrich, Nick Meier, Bee Chim, Luke Boyd, Bishal Tamang, Jasper Alpuerto, Suman Lamichhane, and the many others that made Blacksburg an amazing place.

Finally, I would like to thank my graduate committee; Dr. Wade Thomason, Dr. Takeshi Fukao, and Dr. Jason Holliday. Their contributions to my dissertation and experience at Virginia Tech have helped me grow as a crop scientist and a plant breeder.

ATTRIBUTIONS

The guidance and assistance of many colleagues and collaborators aided in the design of experiments, data collection, analysis, and interpretation of findings included in this dissertation. Their contributions are described by chapter.

Chapter I: Photoperiod Response Gene *Ppd-D1* Affects Nitrogen Use Efficiency in Soft Red Winter Wheat

Bishal Tamang, PhD is a postdoctoral research scholar at the University of Minnesota in Minneapolis, MN. Dr. Tamang measured chlorophyll content and gas exchange on a subset of the plots in the Warsaw, VA environments.

Neal Carpenter, PhD is a postdoctoral research scholar at Purdue University in West Lafayette, IN. Dr. Carpenter assisted in plant biomass sampling in many of the testing environments in the USDA-NIFA *Triticeae* Coordinated Agricultural Project (TCAP) Nitrogen Use Efficiency Validation study.

Takeshi Fukao, PhD is an assistant professor at Virginia Tech. Dr. Fukao measured chlorophyll content and gas exchange on a subset of the plots in the Warsaw, VA, provided guidance on data analysis, and offered editorial comments.

Mark Reiter, PhD is an associate professor at Virginia Tech and is stationed at the Eastern Shore Agricultural Research and Extension Center (AREC) in Painter, VA. Dr. Reiter managed all trials conducted in the Painter testing environments and provided technical assistance.

Robert Pitman, MS is a retired superintendent of the Eastern Virginia AREC in Warsaw, VA. Mr. Pitman was an excellent source of agricultural management knowledge and provided amazing management of all trials conducted in the Warsaw testing environment.

Clay Sneller, PhD is a professor at the Ohio State University in Wooster, OH. Dr. Sneller was a co-PI on the TCAP studies and assisted in the development of the experimental design for the TCAP Nitrogen Use Efficiency Validation study and managed the trials conducted in Ohio.

Wade Thomason, PhD is a professor at Virginia Tech. Dr. Thomason was a co-PI on the TCAP studies, assisted in the development of the experimental design for the TCAP Nitrogen Use Efficiency Validation study, provided invaluable assistance on the data analysis in this study, and offered editorial comments.

Carl Griffey, PhD is the W.G. Wysor professor of crop breeding and genetics at Virginia Tech. Dr. Griffey was a co-PI on the TCAP studies and a PI on the checkoff board grants that supported this project. He provided assistance on the execution, analysis, and interpretation of findings for the TCAP Nitrogen Use Efficiency Validation study in addition to providing editorial comments on the manuscript.

Chapter II: Multi-Environment Assessment of Genotypic Variation and Stability for Nitrogen Use Efficiency in Winter Wheat

Joseph Oakes, PhD is the superintendent of the Eastern Virginia AREC in Warsaw, VA. Dr. Oakes managed trials conducted in Suffolk, VA, assisted with data collection in Warsaw and Suffolk, and offered editorial comments.

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Mark Reiter, PhD is an associate professor at Virginia Tech and is stationed at the Eastern Shore AREC in Painter, VA. Dr. Reiter managed all trials conducted in the Painter testing environments and provided technical assistance.

Ned Jones is the farm manager at the Southern Piedmont AREC in Blackstone, VA. Mr. Jones managed the trials conducted in Blackstone.

Robert Pitman, MS is a retired superintendent of the Eastern Virginia AREC in Warsaw, VA. Mr. Pitman was an excellent source of agricultural management knowledge and provided amazing management of all trials conducted in the Warsaw testing environment.

Clay Sneller, PhD is a professor at the Ohio State University in Wooster, OH. Dr. Sneller was a co-PI on the TCAP studies and assisted in the development of the experimental design for the nitrogen use efficiency studies and managed the trials conducted in Ohio.

Wade Thomason, PhD is a professor at Virginia Tech. Dr. Thomason was a co-PI on the TCAP studies, assisted in the development of the experimental design for the nitrogen use efficiency studies, provided invaluable assistance on the data analysis in this study, and offered editorial comments.

Carl Griffey, PhD is the W.G. Wysor professor of crop breeding and genetics at Virginia Tech. Dr. Griffey was a co-PI on the TCAP studies and a PI on the checkoff board grants that supported this project. He provided assistance on the design, execution, analysis, and interpretation of findings for the nitrogen use efficiency studies in addition to providing editorial comments on the manuscript.

Chapter III: Identification of Quantitative Trait Loci for Nitrogen Use Efficiency in Soft Red Winter Wheat

Brian Ward, PhD is a postdoctoral research scholar at North Carolina State University in Raleigh, NC. Dr. Ward provided technical expertise on genomic data generated via genotyping-by-sequencing in addition to providing editorial comments on the study.

Joseph Oakes, PhD is the superintendent of the Eastern Virginia AREC in Warsaw, VA. Dr. Oakes managed trials conducted in Warsaw and Suffolk, VA, assisted with data collection in both locations, and offered editorial comments.

Maria Balota, PhD is an associate professor at Virginia Tech and is stationed at the Tidewater AREC in Suffolk, VA. Dr. Balota assisted with the management of the trial in Suffolk.

Paul Davis, MS is an incredible grower of agronomic agricultural and horticultural crops in New Kent, VA. Mr. Davis managed the trial in New Kent and provided valuable insights into practical research applications.

Myron Fountain, PhD is a breeder and geneticist at the United States Department of Agriculture (USDA) Agricultural Research Service in Raleigh, NC. Dr. Fountain managed the trial conducted in Kinston, NC.

Gina Brown-Guedira, PhD is a professor at North Carolina State University in Raleigh, NC, director of the USDA Eastern Regional Small Grains Genotyping Lab. Dr. Brown-Guedira's lab generated the genotyping-by-sequencing data used in this study.

Clay Sneller, PhD is a professor at the Ohio State University in Wooster, OH. Dr. Sneller managed the trials conducted in Ohio and assisted with data collection.

Wade Thomason, PhD is a professor at Virginia Tech. Dr. Thomason assisted in the development of the experimental design for the genetic mapping study, provided invaluable assistance on the data analysis in this study, and offered editorial comments.

Carl Griffey, PhD is the W.G. Wysor professor of crop breeding and genetics at Virginia Tech. Dr. Griffey was a PI on the checkoff board grants that supported this project. He provided assistance on the design, execution, analysis, and interpretation of findings for the mapping study in addition to providing editorial comments on the manuscript.

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CHAPTER I:

GENOTYPIC VARIATION AND STABILITY FOR NITROGEN USE EFFICIENCY IN WINTER WHEAT

Kyle Brasier, Joseph Oakes, Maria Balota, Mark Reiter, Ned Jones, Robert Pitman, Clay Sneller, Wade Thomason, and Carl Griffey

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Abbreviation: BB, Blacksburg, VA, location; BS, Blackstone, VA, location; CS, Custar, OH, location; FR, Fremont, OH, location; G, genotype; GDD, growing degree days; GS92, physiological maturity; N, nitrogen; NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NUtE, nitrogen utilization efficiency; PT, Painter, VA, location; WR, Warsaw, VA, location; WS, Wooster, OH, location.

Abstract

Wheat (Triticum aestivum L.) products account for roughly 20 % of the total dietary calories and protein consumed globally, thus requiring efficient nitrogen (N) management systems for optimal grower profitability and to reduce negative environmental impacts of non-target fertilization. This investigation sought to evaluate genotypic variation for N use efficiency (NUE), assess yield stability, and validate proxy traits for indirect improvement of crop performance over N rates using three separate studies that varied in the number of N rates a nd testing environments. Studies I, II, and III were conducted using small panels of winter wheat lines grown under five N rates over three site-seasons, three N rates over six site-seasons, and two N rates over nine site-seasons, respectively. Grain yield was assessed in all studies while above-ground biomass, harvest index, grain N content, NUE, N uptake efficiency, and N utilization efficiency were evaluated in studies II and III. Genotype by N rate interactions were more frequently identified for grain yield when three or more N rates were used in the experimental design. The three studies identified significant genotypic variation for NUE and identified wheat lines that consistently expressed high grain yields over N-environments. Above-ground biomass at physiological maturity was strongly associated with grain yield in Study II when 67 kg N ha^{-1} ($r = 0.66$, $P < 0.05$) was applied and in Study III when 67 kg N ha^{-1} ($r = 0.71$, $P < 0.05$) and 134 kg N ha^{-1} ($r = 0.89$, $P < 0.001$) was applied and expressed high heritability in both studies ($H^2 = 0.83$ and 0.80). Findings from this investigation inform soft red winter wheat breeders on diverse sources of breeding materials and target traits to improve N use efficiency.

Introduction

Wheat (Triticum aestivum L.) is among the most important food crops with production exceeding 700 million t yr^{-1}, accounting for roughly 20 % of the total dietary calories and protein consumed globally (Shiferaw et al., 2013). Demand for wheat products is expected to increase collinearly with world population, requiring a combination of increased genetic gains, land availability, and agricultural efficiency to sustainably meet production needs. Global cereal yields have increased by 230 % since the 2nd half of the 20th century, while nitrogen (N) use efficiency (NUE), defined as the ratio of grain produced per unit N applied (Moll et al., 1982), has consequently decreased by a similar percentage during the same period of time (Tilman et al., 2002). The reduction in NUE has resulted in greater total N losses from the plant-soil system, which may reduce grower profit potential and have negative environmental consequences. This unutilized N can return to the atmosphere as unreactive N or nitrous oxides through denitrification (Galloway et al., 2004) or become subject to runoff and leaching, contributing to the degradation of aquatic and terrestrial ecosystems (Hamilton et al., 2016; Sinha et al., 2017). Thus, the efficient use of N is critical to promote grower profitability and ensure environmentally sustainable increases in grain production.

The effects of locations, growing seasons, and agronomic management practices are frequently reported to have greater effects on quantitative traits such as N use efficiency than the cultivar being grown (Bhatta et al., 2017; Krupnik et al., 2015; Rozbicki et al., 2015). These results imply that grower level improvements in NUE are most readily achieved through the adoption of more efficient nutrient management practices (Zhang et al., 2015), management of abiotic and biotic stresses (Carretero et al., 2011; Zhou et al., 2011), and improvements in soil health (Brennan et al., 2014; Tilak et al., 2005). However, significant genotypic effects on yield

and N-traits were also reported in the aforementioned studies, indicating the potential to select cultivars with improved N use efficiency under a range of N conditions.

Breeding progress for grain yield in wheat between the onset of the Green Revolution (1950) and 1985 was estimated at 1.1 % yr^{-1} under low and standard N conditions and 1.9 % yr^{-1} under high N rates (Ortiz-Monasterio R. et al., 1997). The authors attributed the higher rates of yield gains under high N supplies to improved lodging resistance conferred through the introduction of alleles conferring reduced plant height into modern germplasm. However, these gains have since slowed to approximately 0.37 % yr^{-1} and 0.30 % yr^{-1} under low and standard N rates, respectively (Cormier et al., 2013). In an effort to further increase rates of genetic gains, Cormier et al. (2016) hypothesized that selecting wheat lines under multiple N rates may increase yearly improvements, particularly in trials conducted under conditions of moderate N stress. However, recent investigations of N response in wheat have not led to a consensus regarding the occurrence of significant genotype by N rate interactions for NUE and N-traits in elite wheat germplasm (Bhatta et al., 2017; Büchi et al., 2016; Kubota et al., 2018; Russell et al., 2017). The contrasting results have prompted some researchers to explore exotic germplasm for sources of genetic variation and led others to doubt the merits of conducting trials under multiple N rates all together (Hawkesford, 2017; Van Deynze et al., 2018). It is therefore crucial to establish optimized testing conditions to identify consistent variation in yield response under multiple N supplies.

To better understand variation underlying NUE, Moll et al. (1982) decomposed the trait into two components: N-uptake efficiency (the ratio of aboveground N at harvest per unit of N applied, NUpE) and N-utilization efficiency (the amount of grain produced per total aboveground N in the plant at harvest, NUtE). Previous investigations of winter wheat generally

attribute a greater proportion of the variation in NUE to greater NUpE under low N supplies and NUtE under high N rates (Latshaw et al., 2016; Le Gouis et al., 2000). In contrast, other studies have attributed gains in NUE to the maintenance of high NUpE (Dhugga and Waines, 1989; Hitz et al., 2017) or high NUtE (Barraclough et al., 2010; Brasier et al., 2018; Cormier et al., 2013) across N rates. The conflicting findings reported in these studies are likely attributable to differences in testing environments, genetic material, wheat class, and management practices beyond that of total N applied.

Continuing the theme of trait dissection, breeders can also apply elements of physiology theory to increase genetic gains by selecting on physiological traits associated with NUE under multiple N rates as opposed to exclusively selecting on yield per se (Brancourt-Hulmel et al., 2005; Donald, 1968). For instance, traits such as increased biomass production and N accumulation tend to be frequently discussed as targets for NUE improvements under multiple N conditions (Gaju et al., 2014; Hitz et al., 2017; Tamang et al., 2017), while other traits including senescence dynamics often vary geographically (Alhabbar et al., 2018; Gaju et al., 2011; Górny et al., 2006). Differing wheat classes, end uses, and growing regions targeted by each breeding program will likely require independent validation of target traits to be used for indirect improvements of N use efficiency.

Despite the many previous investigations of NUE in winter wheat, availability of genotypic variation and breeding schemes required for selection of NUE are relatively unestablished in the Eastern United States. Therefore, the objectives of the present study are to: (i) explore genotypic variation for NUE and N-related traits under three selection schemes, (ii) assess yield stability over multiple N-environments, and (iii) to validate target traits for indirect improvements of NUE under varying N conditions.

Materials and Methods

Three similar studies (I, II, and III) were conducted under rainfed conditions in the Eastern United States (Figure 1.1) to address the stated objectives. Each study differed in the number of testing environments (defined as a site-season) and N rates but maintained a similar number of winter wheat lines, N-environments, and experimental plots (Table 1.1). Seeds were treated with Raxil MD (triazole, Bayer Crop Science) and Gaucho XT (imidacloprid, Bayer Crop Science) in all studies to control diseases and insects, respectively. Foliar pesticides and herbicides were applied as necessary throughout the growing seasons in all environments to further mitigate plant pests and diseases (Supplemental Table 1.1). All studies utilized a randomized complete block design with sowing dates occurring under optimal environmental conditions at a seeding density of 520 seeds m^{-2} (Alley et al., 1996). Specific experimental methods for studies I, II, and III are detailed in sections *2.1.* and *2.2.* below.

Total precipitation and cumulative growing degree days (GDD) were determined for each testing environment from planting to harvest. Growing degree days were calculated as:

$$GDD = \left(\frac{T_{max} + T_{min}}{2}\right) - T_{min}$$

Where T_{max} and T_{min} are the daily maximum and minimum temperatures, respectively. A floor of 0 °C for T_{min} and a ceiling of 25 °C for T_{max} was used in each environment (McMaster and Wilhelm, 1997).

Testing Environments and Experimental Design

Study I was conducted at the Northwest Agricultural Research Center near Custar, OH in 2013-2014 and 2014-2015 (CS; 41°21' N, 83°76' W) and the Ohio Agriculture Research and

Development Center near Wooster, OH in 2014-2015 (WS; 40°77' N, 81°90' W). Experimental units, seven-row yield plots that measured 2.74 × 1.78 m, were replicated four times in each environment. Five spring N rates, ranging from 45 to 134 kg N ha^{-1}, were applied to 12 winter wheat lines resulting in 60 plots per replication (Table 1.1). Total precipitation ranged from 655 to 1,757 mm in 15CS (where "15" refers to the season 2015-2016) and 15WS, respectively, while cumulative GDD was similar in all three environments (Figure 1.1).

Study II was conducted at the Eastern Shore Agricultural Research and Extension Center (AREC) near Painter, VA in 2013-2014 and 2014-2015 (PT; 37°58' N, 75°82' W), the Eastern Virginia AREC near Warsaw, VA in 2013-2014, 2014-2015, and 2015-2016 (WR; 37°99' N, 76°78' W), and Kentland Farm near Blacksburg, VA in 2015-2016 (BB; 37°11' N, 80°34' W). Experimental units consisted of seven-row yield plots that measured 2.74 × 1.78 m in PT and 2.74 × 1.52 m in BB and WR. Each experimental unit was replicated three times in all environments except 16WR, which consisted of two replicates. Three spring N rates, ranging from 67 to 134 kg N ha^{-1}, were applied to 12 wheat lines resulting in 36 plots per replication (Table 1.1). Total precipitation and GDDs were similar over growing seasons at each site (Figure 1.1).

Study III was conducted at the Eastern Virginia AREC near WR in 2015-2016 and 2016-17, Kentland Farm near BB in 2015-2016 and 2016-2017, the North Central Agricultural Research Center near Fremont, OH in 2015-2016 (FR; 41°31' N, 83°17' W), the Northwest Agricultural Research Center near CS in 2015-2016, the Ohio Agriculture Research and Development Center near WS in 2015-2016, and the Southern Piedmont AREC near Blackstone, VA in 2015-2016 and 2016-2017 (BS; 37°09' N, 77°97' W). Experimental units were seven-row yield plots that measured 2.74 × 1.78 m in the CS, FR, PT, and WS locations and 2.74 × 1.52 m

in the BB and WR locations. Four replications were used in 16CS, 16FR, and 16WS, while three replications were used in all remaining environments except for 16WR, which consisted of two replicates. Two spring N rates, 67 and 134 kg N ha^{-1}, were applied to 11 wheat lines in Study III, resulting in 22 plots per replication (Table 1.1). Total precipitation ranged from 665 to 1,284 mm in 16WR and 16BS, respectively (Figure 1.1). Cumulative GDD was highest at 17BS (3,301 °C) while GDD in the remaining environments ranged from 2,439 to 2,905 °C in 17BB and 16WR, respectively.

Plant Materials

Plant materials used in Study I and II (Table 1.2) consisted of 12 regionally diverse soft red winter wheat lines derived from the Triticeae Coordinated Agricultural Project's (http://www.triticeaecap.org/) Eastern Elite Mapping Panel of 280 genotypes from six public breeding programs (The Ohio State University, University of Illinois, University of Kentucky, University of Maryland, University of Missouri, and Virginia Tech). Study III shares six common wheat lines with Study I and II, while line numbers 1-6 were replaced with five Virginia adapted cultivars (lines 13-17).

Physiological and N Traits

In Study II and III, a 1.0 m above-ground biomass sample was cut from the center row of each plot at physiological maturity (GS92) in all Virginia testing environments (Supplemental Table 1.2). Biomass samples were oven dried at 60 °C for 72 hours, and weighed to determine above-ground biomass yield for each experimental unit. Samples were then threshed to separate grain from straw tissue, and their respective weights were recorded to estimate harvest index.

Grain and straw tissues were then ground and homogenized to estimate tissue-specific N contents via combustion analysis using a Vario Max CNS macro elemental analyzer (Elementar Analysensysteme). Results of the combustion analysis were used to estimate NUE, NUpE, and NUtE, and grain N content. Plots were combine harvested in all studies at harvest maturity (grain moisture content ≤ 160 g kg^{-1}) and adjusted to 0 g moisture kg^{-1} to determine grain yield.

Statistical Analysis

Analysis of variance (ANOVA) was first performed over environments using the *lme4* package (Bates et al., 2015) in the R statistical computing environment (Team, 2015):

$$Y_{ijkl} = \mu + G_i + N_j + E_k + R_l(E_k) + GN_{ij} + GE_{ik} + NE_{jk} + GNE_{ijk} + \varepsilon_{ijkl}$$

Where the trait response (Y_{ijkl}) is a function of the overall mean (μ), the fixed effect of the *i*th wheat line (G_i), the fixed effect of the *j*th N rate (N_j), the random effect of the *l*th replication (R_l) nested within the *k*th environment (E_k), the interactions of the *i*th wheat line with the *j*th N rate and the *k*th environment (GN_{ij} and GE_{ik}), the interaction of the *j*th N rate and the *k*th environment NE_{jk}), their 3-way interaction (GNE_{ijk}), and the residual error (ε_{ijkl}). As interactions with E_k were significant for most traits, a second ANOVA model was then used within testing environments to determine significant effects ($P < 0.05$) of wheat lines (**G**), N rates (**N**), and G × N interactions within each environment. Means comparisons were conducted using least significant differences for wheat lines, N rates, and their interactions.

Yield stability was assessed in each study using a principal component analysis method (Laffont et al., 2013) and the regression method described by Eberhart and Russell (1966) to estimate two stability parameters: the regression slope (β_i) which measures the response of the *i*th line to varying growing conditions and the sum of absolute deviations regression (δ_d^2) for the

*d*th wheat line in each N-environments. Wheat lines with wide adaptability have a β_i = 1.0 while those above and below this threshold express specific adaptability to favorable and unfavorable environments, respectively. A stable wheat line is defined as having a near zero δ_d^2 value. A bivariate correlation analysis was conducted using wheat line means under each N rate in Study II and III to calculate Pearson's Correlations using the *cor* function in the R statistical computing environment (Team, 2015). Broad-sense heritability (H^2) was calculated with two approaches using the variance component method where all components were treated as random effects (Falconer, 1960):

$$H^2 = \frac{\sigma_G^2}{\left[\sigma_G^2 + \left(\frac{\sigma_{G\times E}^2}{n}\right) + \left(\frac{\sigma_{G\times N}^2}{e}\right) + \left(\frac{\sigma_\varepsilon^2}{ner}\right)\right]}$$

and

$$H^2 = \frac{\sigma_G^2}{\left[\sigma_G^2 + \left(\frac{\sigma_{G\times NE}^2}{s}\right) + \left(\frac{\sigma_\varepsilon^2}{sr}\right)\right]}$$

Where σ_G^2 is the genotypic variance, $\sigma_{G\times E}^2$ is the genotype by environment variance, $\sigma_{G\times N}^2$ is the genotype by N rate variance, $\sigma_{G\times NE}^2$ is the genotype by N-environment (environments and N rates) variance, σ_ε^2 is the error variance, *n* is the number of N rates, *e* is the number of environments, *s* is the number of N-environments, and *r* is the number of replications.

Contributions of variation in NUE component traits NUpE and NUtE to variation in product trait NUE were calculated according to the method described by Moll et al. (1982), where the component and product traits are log transformed and the fraction of the hybrid sum of squared is calculated for each component trait:

$$1.0 = \left(\frac{\sum_k y_k x_k}{\sum_k y_k^2}\right) + \left(\frac{\sum_k y_k x'_k}{\sum_k y_k^2}\right)$$

Where the relative contribution of each component trait (x_k) is calculated by dividing the sums of the cross products of each term by the sum of squares for the product trait (y_k) for *k*th experimental unit.

Results

The combined ANOVA identified significant interactions between genotype and N rate in Study I and significant environmental interactions for grain yield in all studies except the three-way interaction in Study III (Table 1.3). Thus, the ANOVA for grain yield was broken down by testing environment within each study to assess the effects of genotype, N rate, and their interactions.

Genotypic Variation and Stability for Grain Yield

Mean grain yield, range, and standard deviation was calculated for wheat lines under each of the five N rates for each Study I environment in Table 1.4. Significant G × N rate interactions ($P < 0.05$) were observed in 14CS and 15CS. In the 14CS environment, MD03W485-10-10 yielded roughly 5,000 g ha^{-1} across N rates, while MO080864 had significantly lower yields than MD03W485-10-10 at lower N rates but similar yields at the two highest N rates (Supplemental Table 1.3). OH08-161-78 and KY06C-1003-139-8-3 were among the highest yielding lines in the 15CS environment under 45 kg N ha^{-1}, producing 3,983 and 4,097 kg ha^{-1}, respectively. However, KY06C-1003-139-8-3 was able to maintain similarly high yields (roughly 4,000 kg ha^{-1}) with incremental additions of N applications, while OH08-161-78 yielded roughly 3,550 kg ha^{-1} at N rates greater than 45 kg N ha^{-1}. Significant N rate effects

($P < 0.05$) occurred in 14CS and 15WS where high N rates increased and decreased grain yields, respectively. The grain yield decrease in 15WS is attributable to lodging (data not shown), and the mean grain yield reduction between the standard (134 kg N ha^{-1}) and half (67 kg N ha^{-1}) N rates were 0.1 % in Study I. Significant effects of genotype ($P < 0.05$) on grain yield were observed in all three testing environments.

In Study II, mean grain yields for each N rate are presented with range and standard deviation for wheat lines within N rate in Table 1.5. Significant G × N rate interactions ($P < 0.05$) were observed in two of the six testing environments (14PT and 14WR), while two additional environments (15PT and 16BB) were significant at $P \leq 0.1$ level. In the two testing environments exhibiting significant G × N interactions ($P < 0.05$), VA08MAS-369 had the highest grain yield under 134 kg N ha^{-1} and among the lowest when 67 kg N ha^{-1} was applied (Supplemental Table 1.4). Within the 14PT and 14WR environments, OH08-172-42 was among the highest yielding lines under the lowest N rate (67 kg N ha^{-1}) and had yields similar to VA08MAS-369 under the highest N rate (134 kg N ha^{-1}). Grain yields were directly related to N rate in all six testing environments, while N application rates of 101 and 134 kg N ha^{-1} were not significantly different ($P < 0.05$) in the 15PT and 16BB environments. Over the six Study II testing environments, grain yield reductions averaged 17.4 % between the standard and 50 % N rates. Similar to the findings of Study I, genotypic variation for grain yield was observed in all Study II testing environments.

Mean, range, and standard deviation for grain yields are presented for wheat lines within each N rate at each Study III environment in Table 1.6. Unlike the results of Study I and II, no significant G × N rate interactions ($P < 0.05$) were observed in Study III. However, G × N interactions were significant in 16FR and 17WR at the $P \leq 0.1$ level. The limited number of

significant interactions may be due to fewer N rates tested or the differing panels of wheat lines tested. Significant effects due to N rate ($P < 0.05$) were observed in seven of the nine environments where 134 kg N ha^{-1} produced higher grain yields than the half N rate (67 kg N ha^{-1}), while mean yield reduction between the standard and half N rates was 10.0 % in Study III. Consistent with Study I and II, significant genotypic variation for grain yield ($P < 0.05$) was observed in every testing environment.

Despite the availability of genotypic variation for grain yield, and thus NUE, it is crucial for breeders to identify lines with consistently high yields across N conditions and environments to produce improvements at the grower level. Yield stability was assessed using two stability indices (β_i and δ_d^2) over 15 (Study I) or 18 (Study II and III) N-environments (Figure 1.2). Study I and II had the highest and lowest mean δ_d^2, respectively. OH08-172-42 (line 10), MD03W485-10-10 (line 8), and KY06C-1003-139-8-3 (line 3) were among the highest yielding and most stable lines over N-environments in Study I. However, line performance and stability changed for two of the top three wheat lines between Study I and II where OH08-172-42 (line 10), VA08MAS-369 (line 12), and OH08-161-78 (line 9) maintained the highest yields and stability across N-environments. Genotypic variation for stability parameter δ_d^2 in Study III was similar to that of Study I. The stability analysis identified two wheat lines: VA08MAS-369 (line 12) and VA09W-73 (line 16) in Study III as consistently maintaining high grain yields.

Variation in N Traits and Identification of Proxy Traits for Indirect Selection

Significant G × N × E effects ($P < 0.05$) were observed in Study II for harvest index and NUtE, and in Study III for NUE in the over-environment ANOVA for physiological and N traits (Table 1.7). Genotype and N rate interactions with environment were significant for several of

the traits in Study II and III, while G × N interactions were not significant for any trait in the combined analysis. However, significant G × N interactions ($P < 0.05$) were identified when the ANOVA was conducted with each environment (Supplemental Tables 1.7 – 1.21) in Study II (harvest index in 15PT, NUtE in 15PT, above-ground biomass in 16BB, harvest index in 16BB, NUE in 16BB, and NUtE in 16BB) and in Study III (grain N content in 16BB, NUpE in 16BB, NUtE in 16BB, harvest index in 17WR, and NUE in 17WR). Significant single effects (genotype, N rate, and environment) were observed in the combined analysis for all traits except genotype effects on NUpE in both studies.

A correlation analysis was conducted over environments for each N rate in Study II and III (Figure 1.3). Significant positive associations between above-ground biomass and grain yield were observed under 67 kg N ha^{-1} in Study II ($r = 0.66$, $P < 0.05$) and under both 67 kg N ha^{-1} ($r = 0.71$, $P < 0.05$) and 134 kg N ha^{-1} ($r = 0.89$, $P < 0.001$) in Study III. Harvest index was significantly and negatively associated with grain N content when 67 kg N ha^{-1} was applied in Study II ($r = -0.58$, $P < 0.05$) and Study III ($r = -0.81$, $P < 0.05$). Similarly, significant negative correlations (r = -0.70 to -0.98) were observed between NUtE and grain N content under all N rates except 101 kg N ha^{-1} in Study II. Significant negative correlations (r = -0.51 to -0.69) were also observed between NUpE and NUtE in both studies at all N rates except for 134 kg N ha^{-1} in Study III.

Trait Heritability and Variance Components

Genotype was a major contributor to variability in all traits evaluated in the three studies except for grain yield in Study I and NUpE in Study II (Table 1.8). Genotype by N rate effects were observed in Study II for harvest index, NUpE, and NUtE in addition to grain yield in both

studies. The percent contribution of genotype by environment interaction ranged from 9 to 61 % over all traits in studies II and III with the exception of NUpE (0%) in both studies and GNC in Study II (0 %). The lowest broad sense heritability was observed for NUpE in Study II (0.00) and III (0.05), while grain N content was the most heritable trait in Study II (0.90) and III (0.86). However, single effects of N rate, environment, and error variances were generally greater than that of the wheat lines themselves (Supplemental Table 1.5).

Upon analysis by N-environment, genotypic variance was observed for all traits in Studies I, II, and III (Supplemental Table 1.6). Variance due to G × N-environment was observed for all traits except grain N content in both studies and NUtE in Study III. Similar to the results in Table 1.8, heritability was lowest for NUpE in Study II (0.28) and III (0.22) and highest for grain N content (0.91 in both studies) when analyzed for variance due to N-environments.

Contributions of NUpE and NUtE to Variation in NUE

The component trait analysis described by Moll et al. (1982) revealed genotypic variation in the contribution of NUpE and NUtE to NUE in Study II and III (Figure 1.4). Across N rates, VA08MAS-369 (line 12) and Bess (line 8) were consistently among the highest and lowest NUE lines, respectively. There were no clear trends emphasizing a greater importance of NUpE or NUtE to the variation in NUE among the highest and lowest performing lines. However, the mean contribution of NUpE to NUE was higher under 67 kg N ha^{-1} (66.4 and 83.8 % in studies II and III, respectively) than 134 kg N ha^{-1} (34.3 and 73.0 % in studies II and III, respectively).

Discussion

Results are based on performance data of winter wheat lines evaluated in a combined 51 N-environments over three studies for N use efficiency. Grain yield and NUE were then decomposed into components traits to assess physiological contributors underlying the two traits. The results therefore provide winter wheat breeders with information on genotypic variation in elite germplasm, yield stability over N-environments, heritability of N traits, and target traits for indirect selection of N use efficiency.

Breeding Schemes for NUE in Elite Germplasm

Plant breeders targeting improvements in NUE must first identify sources of genotypic variation for N response in available germplasm through the detection of genotype by N rate interactions. In the present studies, G × N rate interactions for grain yield were most commonly observed when lines were tested under three or five N rates. While exceptions exist (Kubota et al., 2018; Russell et al., 2017), recent investigations of NUE in wheat generally follow a similar trend where significant interactions between wheat line and N rate for grain yield were more frequently reported when more than two N rates were utilized (Barraclough et al., 2010; Büchi et al., 2016; Gaju et al., 2011; Guttieri et al., 2017; Latshaw et al., 2016; Mahjourimajd et al., 2016; Ul-Allah et al., 2018). The G × N interactions observed in these investigations may be attributable to a combination of increased lodging resistance for some wheat lines under high N supplies (Ortiz-Monasterio R. et al., 1997) and a moderate mean grain yield reduction (roughly 20 %) between N rates (Presterl et al., 2003). These findings have led many breeders to advocate for direct selection of NUE under reduced N systems in locations that are exclusively subjected to N stress each growing season (Brancourt-Hulmel et al., 2005; Cormier et al., 2013; Muellner

et al., 2014; Ranjitha et al., 2018). However, Büchi et al. (2016) and Hasegawa (2003) argued that crop varieties selected under reduced N rates are not necessarily better adapted to low input conditions and that breeders should instead devote a majority of their resources to multi-environment testing. This observation may eventually prove that direct selection under low N supplies is too hindered by the stability of crop performance. Overall, it is agreed that trials conducted over multiple sites may incidentally incorporate multiple N stress conditions and thus continue to result in genetic gains over diverse environments and sources of plant stress. However, the identification of significant G × N interactions for grain yield in the present investigation may still justify testing of advanced wheat lines under multiple N rates in locations that are routinely limited by N availability.

Stability of N Response

A stability analysis was conducted over N-environments in the present investigation to identify wheat lines that consistently produced high grain yields regardless of environment and N availability. Interestingly, wheat lines that expressed the highest mean grain yields were also among the most stable within each of the three studies. Similar findings were reported in a panel of 25 spring barley (*Hordeum vulgare* L.) varieties by Anbessa et al. (2009) and eight wheat lines by Mahjourimajd et al. (2016) who reported that the most stable lines generally experienced lower grain yield reduction between high and low N conditions than did the least stable lines. The present investigation also reported significant variation in the adaptability of wheat lines over the tested N-environments, indicating the existence of genotypic variation in broad vs. specific adaptability to favorable and unfavorable environments.

Previous assessments of NUE stability in spring barley (Anbessa et al., 2009), dry bean (*Phaseolus vulgaris* L.)(Farid et al., 2016), and winter wheat (Büchi et al., 2016) advocated for a greater emphasis on testing environments than N management schemes as the authors note that yield gains across N rates occur regardless of testing conditions. This recommendation has some merit following the biplot analysis for grain yield in the current investigation often placed greater emphasis on testing environment than N rate (Supplemental Figures 2.1 – 2.3). However, the biplots also illustrate variation in genotypic performance due to N rates within environments. Stagnari et al. (2013) reported similar results in a study of durum (*Triticum durum* L.) cultivars grown under two N rates where the variation in stability under each N rate and the detection of N rate by environment interactions justified the establishment of breeding efforts in N-deficient conditions. Building on these interactions, Elía et al. (2018) reported significant interactions between temperature and N rate on grain yields in adapted wheat cultivars. The authors further explained that grain yield penalties under warmer post-anthesis temperatures were increased under higher N supplies, warranting the need to develop wheat varieties with reduced N requirements as climates continue to become warmer or more variable.

Similar to the results of the present investigation, Hitz et al. (2017) identified the wheat line KY06C-1003-139-8-3 as consistently expressing high grain yields across N rates and locations in the Eastern United States. Another recent assessment of genotypic variation in soft red winter wheat lines for NUE was conducted under greenhouse conditions in Virginia using three equivalent N rates and four common genotypes (line numbers 2, 4, 6, and 11) included in Study II of the current investigation (Tamang et al., 2017). The authors identified ‘Sisson’ (line 11) as expressing the highest grain yields and protein content under the three N rates in this panel and consequently suggested its use as a parent in breeding for N use efficiency. However, Sisson

did not perform as well relative to the testing panels in the present studies. Within the Eastern United States, two previously mentioned lines (KY06C-1003-139-8-3 and Sisson) in addition to those identified in the present study (VA08MAS-369, VA09W-73, OH08-161-78, and OH08-172-42) may serve as good parents or check varieties for NUE breeding efforts.

Traits Underlying Variation in NUE

Selection based on physiological traits associated with N response may increase genetic gains through more targeted breeding (Gaju et al., 2011). Building on this application of physiology theory, recent investigations of NUE in winter wheat have identified significant trait associations with NUE across N rates including NUtE (Cormier et al., 2013) and total biomass at physiological maturity (Hitz et al., 2017). Similar findings were observed in the present study where above-ground biomass at maturity was significantly associated with grain yield when 67 kg N ha^{-1} was applied in Study II and both N rates in Study III. Unlike the findings in Hitz et al. (2017), this trait appeared to be highly heritable in Study II (0.83) and Study III (0.80) indicating that above-ground biomass with maintained harvest index may serve as a useful trait for indirect selection of grain yield, and thus N use efficiency. Active selection on increased NUtE to realize gains in NUE, as suggested by Cormier et al. (2013) and Gaju et al. (2011), will likely have pleiotropic effects. For instance, higher NUtE came at the expense of reduced grain N content under most N rates in the present investigation. Interestingly, many breeding programs have reported yield gains at the cost of grain protein content (a trait that is strongly associated with grain N content) over the past three decades (Laidig et al., 2017). The authors are also quick to point out that despite these reductions in grain protein content, the baking quality was actually improved over this period. Going forward, breeders and physiologists will need to identify

additional traits for continued NUE gains without sacrificing quality or drastically changing plant morpho-physiology traits.

Acknowledgments

Funding for this project was provided by the USDA National Institute of Food and Agriculture Triticeae Coordinated Agricultural Project no. 2011-68002-30029 and the Virginia Small Grains Board. The authors would also like to thank Neal Carpenter, John Seago, Anthony Christopher, Jon Light, Sam Lawton, Brian Ward, Michelle Lee, and William Myers for assistance collecting field data and Steve Nagle for providing technical assistance in the analysis of tissue N content.

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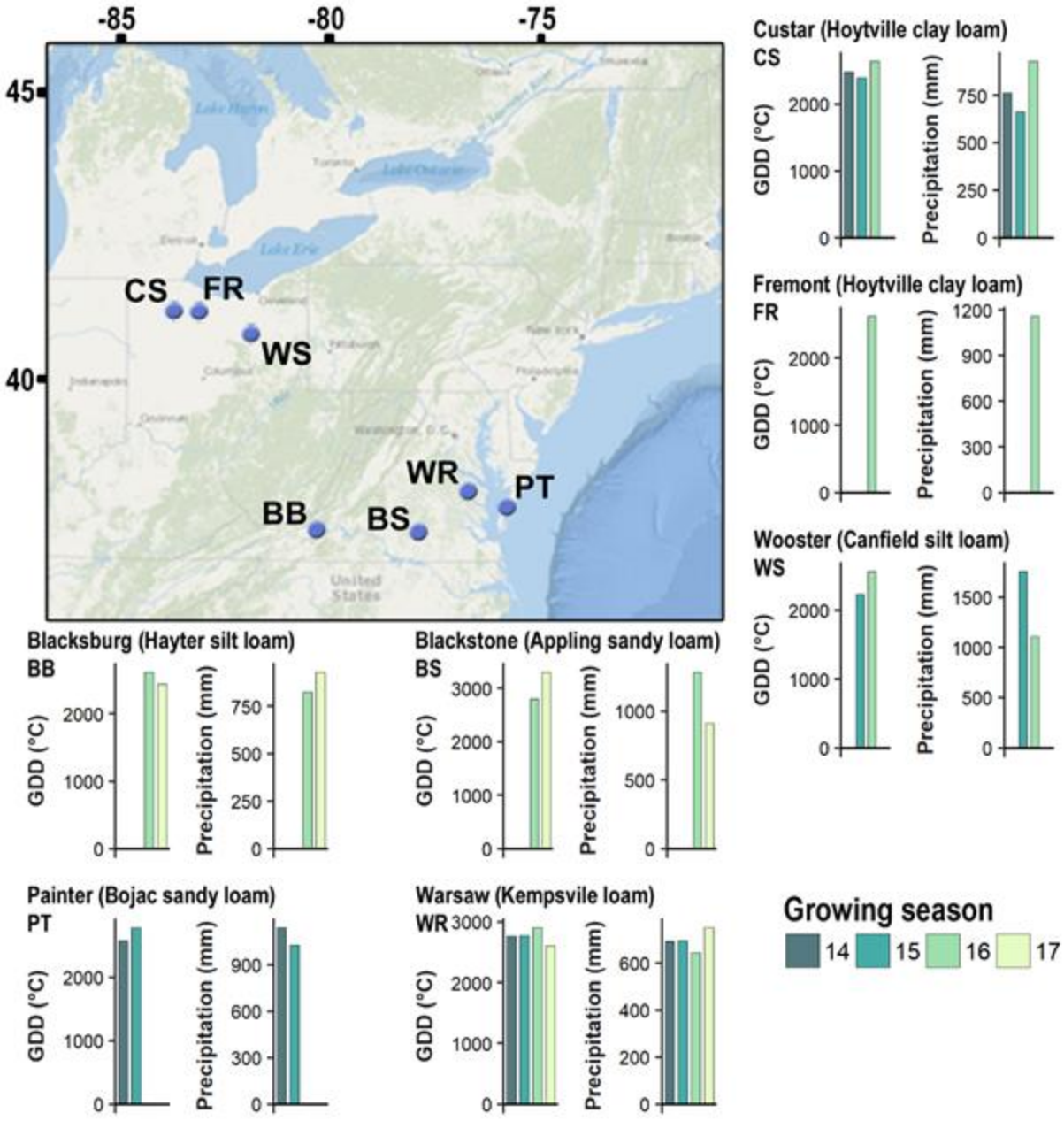


Figure 1.1. Site locations, soil series and type, total precipitation (mm), and growing degree days (GDDs) accumulated from planting to harvest for each trial environment in studies I, II, and III. Values on the top and left of the image indicate latitude and longitude, respectively.

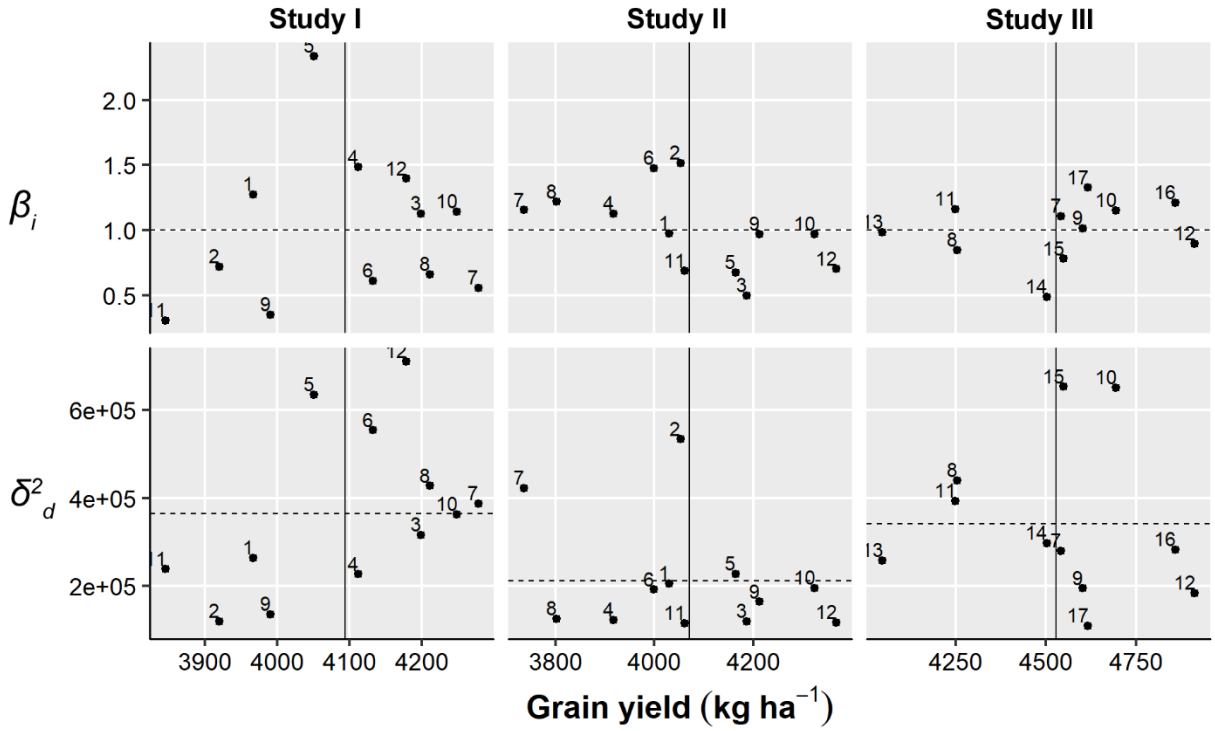


Figure 1.2. Yield stability parameters slope (βi) and deviation from regression (δ^2_d) against grain yield over N-environments for wheat lines within each study. Points with corresponding line numbers represent each genotype tested while dashed and solid lines represent means of the two stability parameters and yields in each study, respectively.

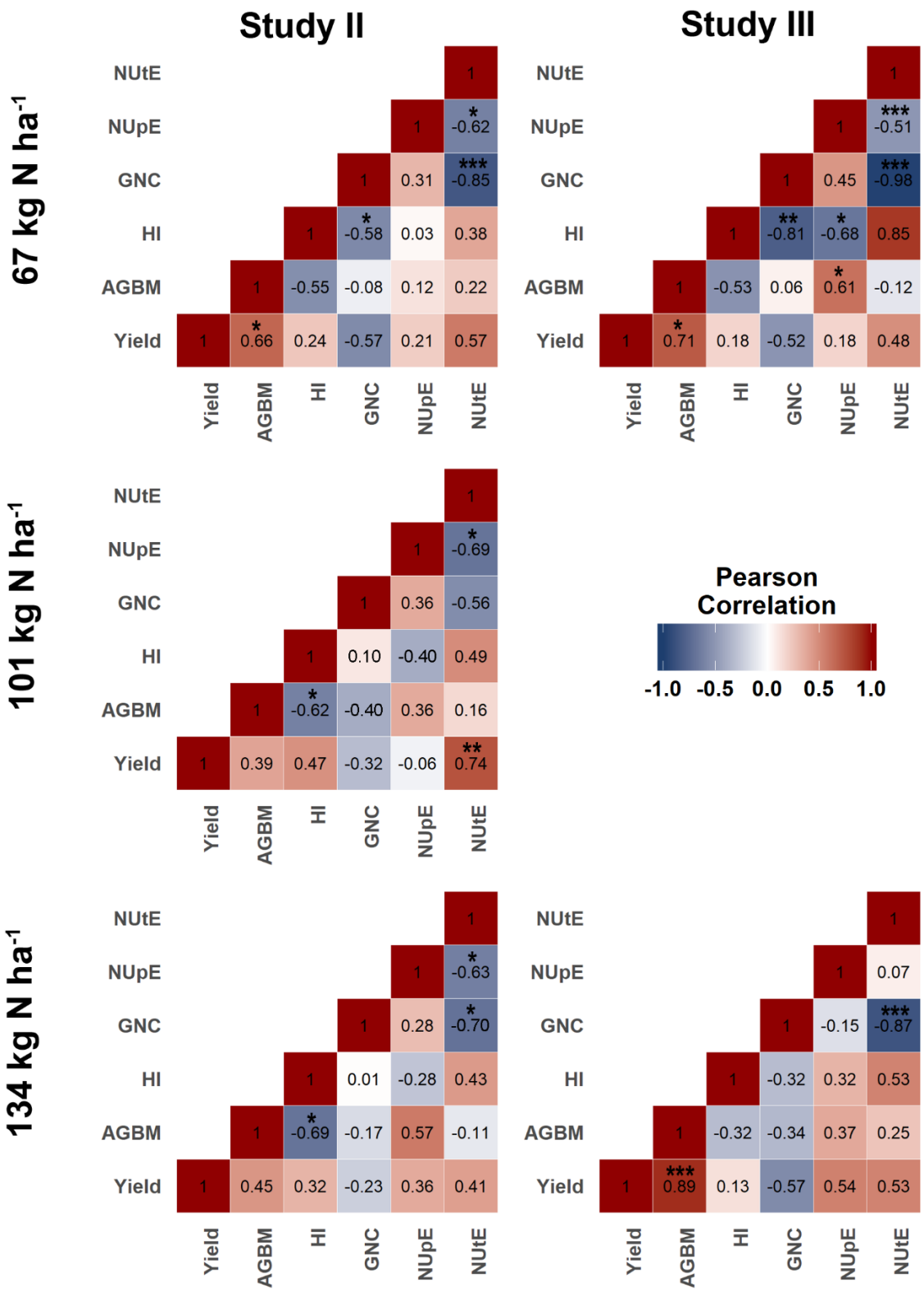


Figure 1.4. Correlation matrix for traits in Study II at three N rates over four environments and Study III at two N rates over six environments. Above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), NUpE and NUtE were assessed. Results significant on the 0.05 (*), 0.01 (**), and 0.001 (***) levels.

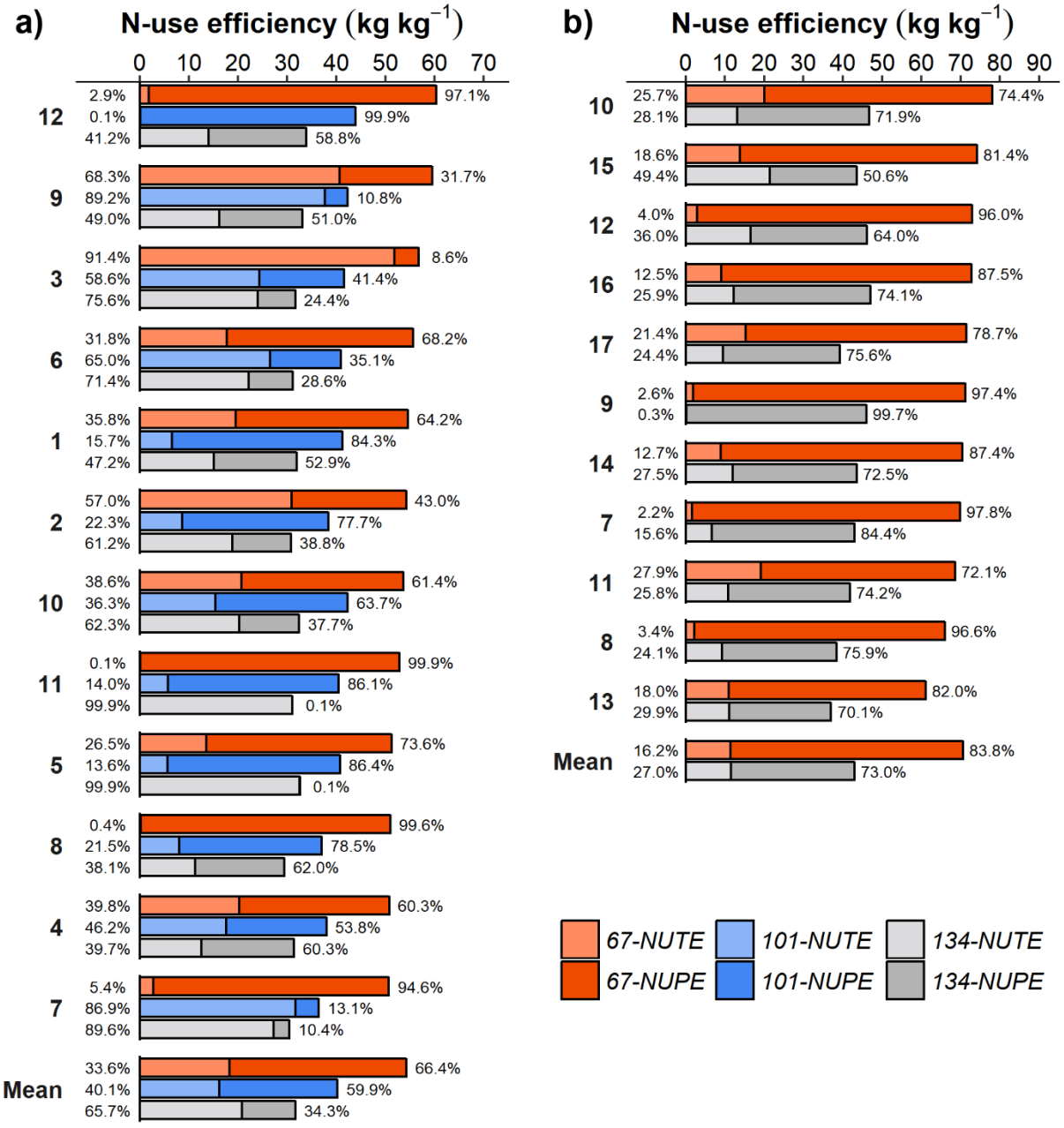


Figure 1.4. Contribution of NUpE and NUtE to the variation in NUE by wheat line and N rate in a) Study II and b) Study III. Line numbers listed on the left of each set of bars correspond with those provided in Table 1.2.

Table 1.1. Description of number of lines evaluated, N-environments (N-Env.), experimental units (Exp. units), replications, and N rates used in each study.

Study	Wheat lines	N-Env.	Exp. units	Replications	GS25 N[a]	GS30 N	Total spring N
						kg N ha^{-1}	
I[b]	12	15	720	4	22	22	45
					33	33	67
					45	45	90
					56	56	112
					67	67	134
II	12	18	612	2 - 3	33	33	67
					33	67	101
					33	101	134
III	11	18	704	2 - 4	33	33	67
					67	67	134

[a] Nitrogen split applied at Zadoks growth stage 25 (GS25) and 30 (GS30).
[b] Study I, II, and III represent three separate studies of N response grown under the conditions described.

Table 1.2. Description of plant materials and their respective pedigrees used in each study.

Line No.	Line[a]	Study			Pedigree
		I	II	III	
1	IL02-19483B	✓	✓		Patton/Cardinal//IL96-2550
2	IL07-4415	✓	✓		P96169RE2-3-6-4/IL01-34159
3	KY06C-1003-139-8-3	✓	✓		Truman/McCormick//25R37
4	MD05W10208-11-8	✓	✓		Tribute/25R42//Chesapeake
5	MO080864	✓	✓		981020//P92201D5-2/98072
6	OH06-150-57	✓	✓		P.92201D5-2-29/OH708
7	Bess	✓	✓	✓	MO11769/Madison
8	MD03W485-10-10	✓	✓	✓	USG3209/Tribute//USG3342"S"
9	OH08-161-78	✓	✓	✓	OH751/OH738
10	OH08-172-42	✓	✓	✓	Douglas/Jekyl
11	Sisson	✓	✓	✓	Coker9803/Freedom
12	VA08MAS-369	✓	✓	✓	McCormick/GA881130LE5
13	SS520			✓	FFR555W/GA-Gore
14	VA05W-151			✓	Pioneer26R24/McCormick
15	VA07W-415			✓	VA98W-895/GA881130LE5//VA98W-627RS
16	VA09W-73			✓	SS520/VA99W-188//Tribute
17	Yorktown			✓	SS520/VA99W-188//Tribute

[a] Plant introduction number for Bess is PI 642794 (McKendry et al., 2007), Sisson is PI 617053 (Griffey et al., 2003), SS520 (‘38158’) is PI 619052, VA05W-151 (‘5187J’) is PI 665039, VA07W-415 (‘72014415’) is PI 669571, VA09W-73 (‘Featherstone 73’) is PI 669572, and Yorktown is PI 667643 (Griffey et al., 2012).

Table 1.3. Analysis of variance with F-values for grain yield in each study.

Effect	Study I		Study II		Study III	
	df	*F*-value	df	*F*-value	df	*F*-value
G[a]	11	12.87 ***	11	15.60 ***	10	26.25 ***
N	4	6.81 ***	2	243.08 ***	1	217.95 ***
E	2	1,111.77 ***	5	655.13 ***	9	1,056.75 ***
G × N	44	2.38 ***	22	1.33 ns[b]	10	1.47 ns
G × E	22	24.72 ***	55	4.11 ***	90	5.37 ***
N × E	8	15.76 ***	10	10.50 ***	9	15.32 ***
G × N × E	88	2.68 ***	110	1.32 *	90	0.84 ns

[a] Effects of genotype (G), N rate (N), testing environment (E), and their interactions.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[b] ns, not significant.

Table 1.4. Analysis of variance and *F*-values for grain yield in Study I. Summary statistics for grain yield presented in each testing environment. Mean yield, range, and standard deviation (**SD**) presented for the panel of wheat lines under each N rate.

N rate	2013-14 Custar			2014-15 Custar			2014-15 Wooster		
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
	----------Grain yield (kg ha^{-1})----------								
45	4,118 d[a]	3,460 - 4,914	454	3,308 a	2,665 - 4,097	516	4,518 a	4,253 - 5,102	392
67	4,417 c	3,976 - 4,978	372	3,438 a	2,823 - 4,378	470	4,487 a	3,982 - 5,143	428
90	4,551 bc	4,027 - 5,294	436	3,277 a	2,443 - 4,064	506	4,474 a	3,940 - 5,314	450
112	4,717 a	4,140 - 5,207	365	3,325 a	2,851 - 3,780	491	4,394 ab	3,869 - 5,193	429
134	4,685 ab	4,178 - 5,222	429	3,405 a	2,666 - 4,088	519	4,298 b	3,816 - 4,958	412
G[b]	32.95 ***			9.73 ***			31.50 ***		
N	45.88 ***			1.73 ns[c]			5.22 ***		
G × N	2.23 ***			3.74 ***			0.99 ns		

[a] The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.
[b] Effects of genotype (G), N rate (N), and their interactions.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
[c] ns, not significant.

Table 1.5. Analysis of variance and *F*-values for grain yield in Study II. Summary statistics for grain yield presented in each testing environment. Mean yield, range, and standard deviation (SD) presented for the panel of wheat lines under each N rate.

N rate	2013-14 Painter			2013-14 Warsaw			2014-15 Painter		
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
	----------Grain yield (kg ha^{-1})----------								
67	2,609 c[a]	2,423 - 2,791	288	4,573 c	4,055 - 5,333	473	3,224 b	2,729 - 3,652	522
101	3,211 b	1,800 - 3,601	579	5,247 b	4,604 - 5,828	417	3,457 a	2,864 - 4,053	477
134	3,656 a	3,006 - 4,137	452	5,789 a	5,024 - 6,423	525	3,595 a	3,204 - 3,997	445
G[b]	3.80 ***			14.32 ***			3.12 **		
N	74.69 ***			170.56 ***			8.54 ***		
G × N	2.19 ***			2.02 *			1.53 [c]		

N rate	2014-15 Warsaw			2015-16 Blacksburg			2015-16 Warsaw		
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
	----------Grain yield (kg ha^{-1})----------								
67	4,246 c	3,362 - 4,789	532	4,367 b	3,715 - 4,955	392	2,994 c	2,523 - 3,305	265
101	4,878 b	4,355 - 5,466	427	4,575 a	3,897 - 4,941	375	3,339 b	2,864 - 3,765	349
134	5,163 a	4,467 - 5,596	490	4,681 a	4,074 - 5,117	392	3,661 a	3,301 - 4,092	306
G	7.70 ***			20.00 ***			4.43 ***		
N	58.10 ***			19.00 ***			45.79 ***		
G × N	0.90 ns[d]			1.54 [c]			1.14 ns		

[a] The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.
[b] Effects of genotype (G), N rate (N), and their interactions.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
[c] Significant at the 0.1 probability level.
[d] ns, not significant.

Table 1.6. Analysis of variance and *F*-values for grain yield in Study III. Summary statistics for grain yield presented in each testing environment. Mean yield, range, and standard deviation (SD) presented for the panel of wheat lines under each N rate.

	2015-16 Custar			2015-16 Fremont			2015-16 Wooster		
N rate	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
	----------Grain yield (kg ha^{-1})----------								
67	4,828 b[a]	4,365 - 5,831	536	5,648 b	5,078 - 6,468	475	5,887 b	5,015 - 7,203	743
134	5,262 a	4,463 - 5,715	476	5,926 a	5,528 - 6,370	438	6,502 a	5,354 - 7,455	806
G[b]	6.12 ***			4.50 ***			22.28 ***		
N	26.67 ***			12.97 ***			53.61 ***		
G × N	1.34 ns[c]			1.97 ns[d]			1.24 ns		
	2015-16 Blacksburg			2015-16 Blackstone			2015-16 Warsaw		
N rate	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
	----------Grain yield (kg ha^{-1})----------								
67	4,530 a	3,972 - 5,180	552	1,879 b	1,437 - 2,268	532	3,273 b	3,049 - 3,561	259
134	4,536 a	3,858 - 5,295	557	2,391 a	1,826 - 3,017	484	4,311 a	3,895 - 4,738	308
G	7.08 ***			4.06 ***			4.52 **		
N	0.00 ns			38.83 ***			252.15 ***		
G × N	0.67 ns			1.06 ns			0.55 ns		
	2016-17 Blacksburg			2016-17 Blackstone			2016-17 Warsaw		
N rate	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
	----------Grain yield (kg ha^{-1})----------								
67	5,838 b	4,837 - 6,697	670	2,394 a	1,976 - 2,855	404	4,433 b	3,918 - 4,807	442
134	6,036 a	4,527 - 7,112	814	2,397 a	1,653 - 3,026	491	5,436 a	4,813 - 6,086	420
G	22.2 ***			5.88 ***			6.74 ***		
N	5.53 *			0.00 ns			178.14 ***		
G × N	0.97 ns			0.38 ns			1.92 ns[d]		

[a] The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.
[b] Effects of genotype (G), N rate (N), and their interactions.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
[c] ns, not significant.
[d] Significant at the 0.1 probability level.

Table 1.7. Analysis of variance and F-values for physiological and N traits in Study II and III.

Effect	df	Above-ground biomass	Harvest index	Grain N content	NUE[a]	NUpE	NUtE
Study II							
G[b]	11	7.25 ***	7.30 ***	11.16 ***	3.73 ***	1.38 ns	4.48 ***
N	2	55.52 ***	10.33 ***	60.94 ***	428.37 ***	112.85 ***	52.78 ***
E	5	139.44 ***	101.51 ***	96.09 ***	139.01 ***	132.12 ***	154.58 ***
G × N	22	0.81 ns[c]	1.28 ns	0.83 ns	0.60 ns	1.55 ns	1.28 ns
G × E	55	1.29 ns	2.16 ***	1.02 ns	1.75 **	1.01 ns	1.91 **
N × E	10	4.62 ***	2.86 **	4.01 ***	7.33 ***	7.52 ***	2.75 *
G × N × E	110	1.14 ns	1.41 **	0.99 ns	1.03 ns	1.09 ns	1.46 *
Study III							
G	10	6.86 ***	6.56 ***	8.99 ***	5.16 ***	1.10 ns	7.10 ***
N	1	24.17 ***	13.92 ***	138.54 ***	811.43 ***	282.67 ***	102.57 ***
E	5	196.27 ***	323.37 ***	50.52 ***	233.13 ***	117.88 ***	50.62 ***
G × N	10	0.56 ns	0.68 ns	0.51 ns	0.63 ns	0.89 ns	1.02 ns
G × E	50	1.38 ns	1.96 ***	1.40 ns	2.60 ***	1.10 ns	1.56 *
N × E	5	8.83 ***	3.81 **	0.94 ns	31.20 ***	12.78 ***	0.90 ns
G × N × E	50	0.69 ns	0.67 ns	0.77 ns	1.44 *	1.01 ns	0.84 ns

[a] NUE, NUpE, and NUtE were collected in four of the six Study II environments.

[b] Effects of genotype (G), N rate (N), testing environment (E), and their interactions.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

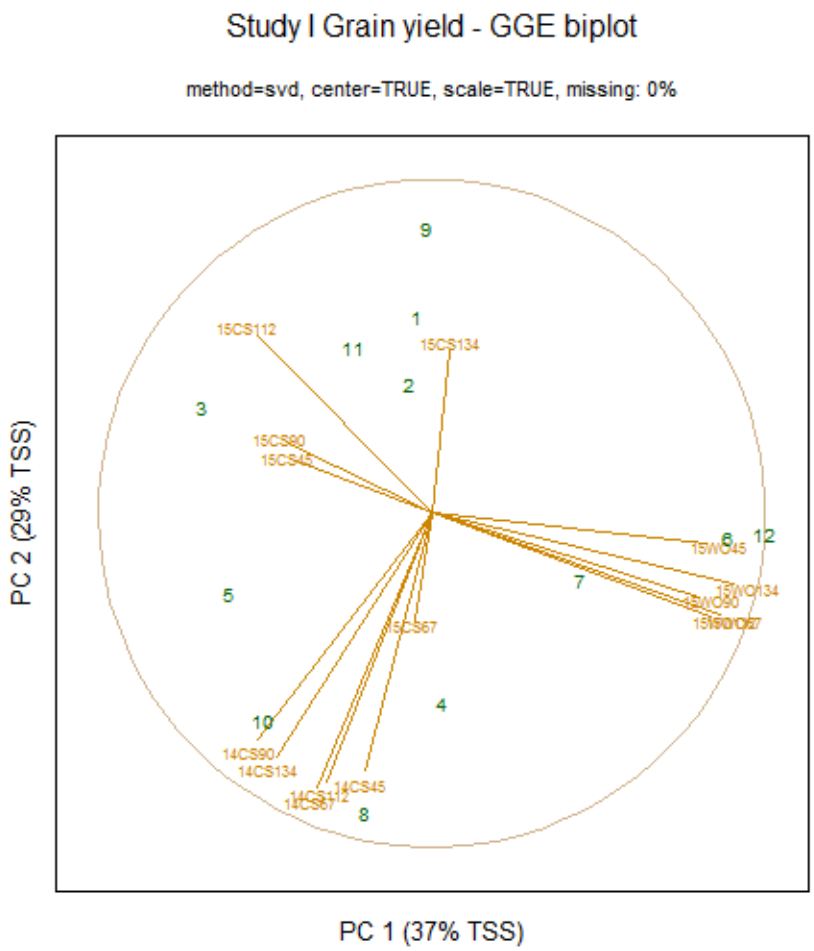
*** Significant at the 0.001 probability level.

[c] ns, not significant.

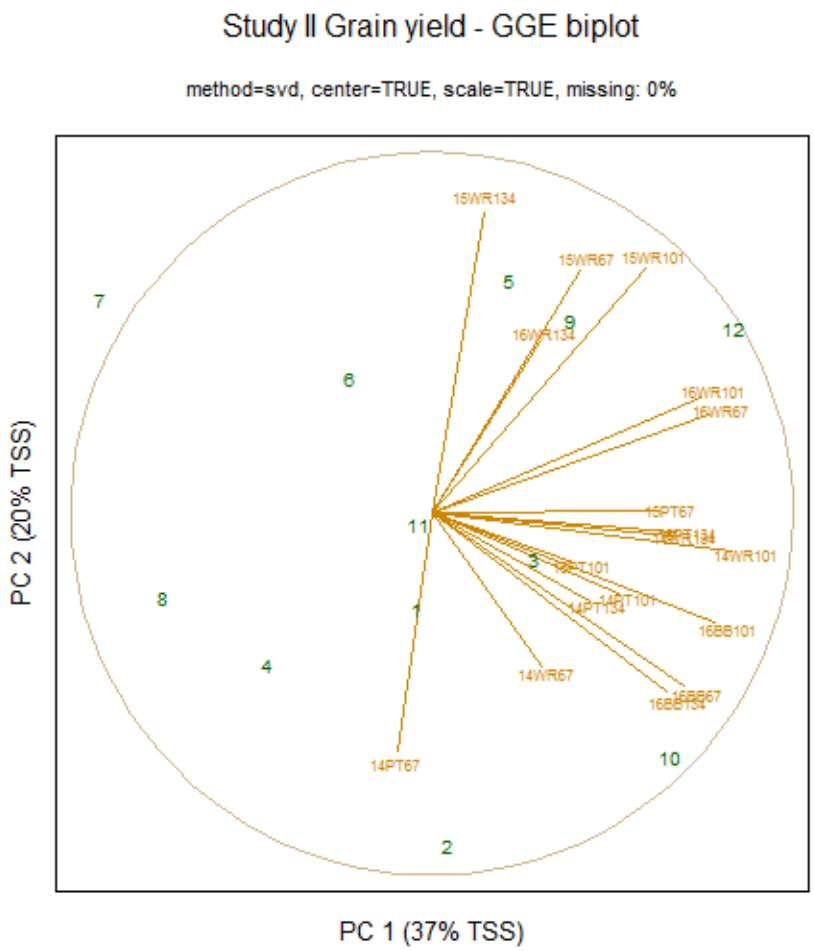
Table 1.8. Percent contribution of variance components to grain yield, above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE) with heritabilities for wheat lines grown in Study II and III.

Trait	Variance components			H^{2b}
	G (%)[a]	G × N (%)	G × E (%)	
Study I				
Grain yield	0	7	93	0.00
Study II				
Grain yield	41	1	58	0.74
AGBM	86	0	14	0.83
HI	43	5	52	0.68
GNC	100	0	0	0.90
NUpE	0	100	0	0.00
NUtE	40	14	46	0.54
Study III				
Grain yield	37	2	61	0.79
AGBM	73	0	27	0.80
HI	44	0	56	0.69
GNC	91	0	9	0.86
NUpE	100	0	0	0.05
NUtE	67	0	33	0.80

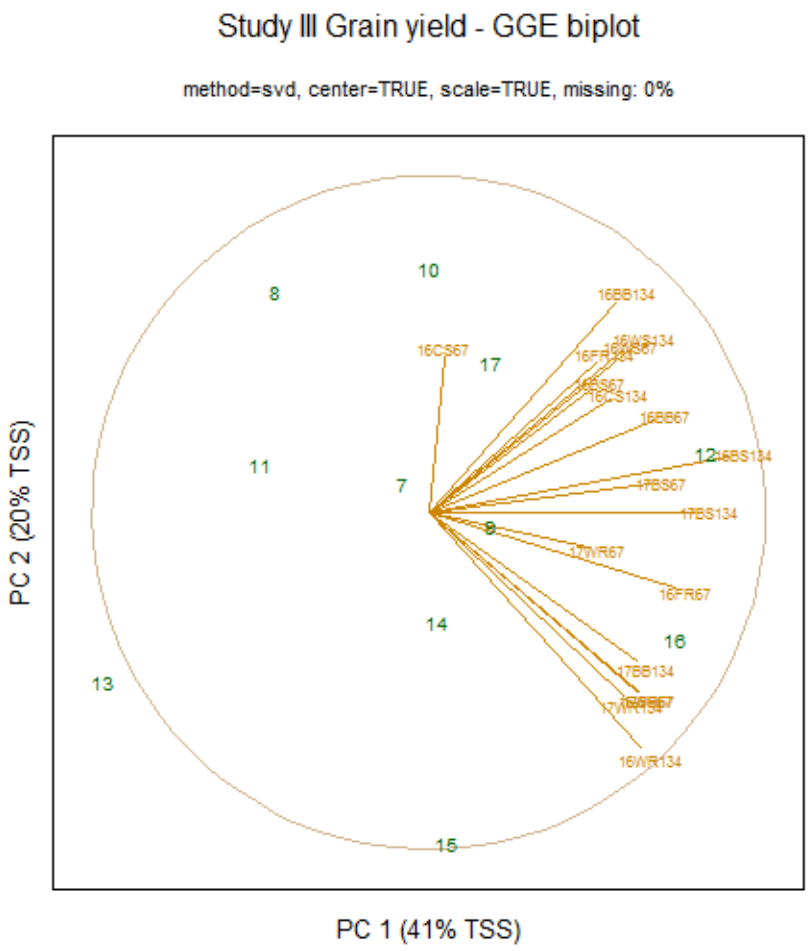
[a] G (%) is the percent genotypic variance; G × N (%) is the percent genotype × nitrogen variance; G × E (%) is the percent genotype × environment variance; H^2 is the broad sense heritability.
[b] Number of testing environments used for each trait in studies II and III shown in Supplemental Table 1.2.



Supplemental Figure 1.1. GGE biplot for grain yield in Study I with line numbers (green color) and N-environments (brown color). The line designations are given in Table 1.2 and N-environment descriptions are in Appendix Table 1.4.



Supplemental Figure 1.2. GGE biplot for grain yield in Study II with line numbers (green color) and N-environments (brown color). The line designations are given in Table 1.2 and N-environment descriptions are in Appendix Table 1.4.



Supplemental Figure 1.3. GGE biplot for grain yield in Study III with line numbers (green color) and N-environments (brown color). The line designations are given in Table 1.2 and N-environment descriptions are in Appendix Table 1.4.

Supplemental Table 1.1. Management practices in each Virginia testing environment including product, application rate of fertilizer, active ingredient (A.I.), and application date.

Season	Location	Product	Application rate	Application date
2013-14	Painter	Pre-plant fertilizer	34-0-0-0[a]	10/23/2013
		Planting	520 seeds m^{-2}	10/24/2013
		GS25 UAN	Variable[‡]	03/10/2014
		Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/12/2014
		G30 UAN	Variable	04/03/2014
		Harvest	NA	06/16/2014
	Warsaw	Pre-plant fertilizer	34-67-67-2	10/18/2013
		Planting	520 seeds m^{-2}	10/22/2013
		GS25 UAN	Variable	03/01/2014
		Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/01/2014
		Starane™	0.21 kg A.I. ha^{-1}	03/11/2014
		GS30 UAN	Variable	04/04/2014
		Palisade EC	0.08 kg A.I. ha^{-1}	04/04/2014
		Tilt	0.07 kg A.I. ha^{-1}	04/10/2014
		Prosaro	0.14 kg A.I. ha^{-1}	05/08/2014
		Tilt	007 kg A.I. ha^{-1}	05/08/2014
		Harvest	NA	06/24/2014
2014-15	Painter	Pre-plant fertilizer	34-0-0-0	10/20/2014
		Planting	520 seeds m^{-2}	10/23/2014
		GS25 UAN	Variable	03/17/2015
		Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/25/2015
		GS30 UAN	Variable	04/30/2015
		Harvest	NA	06/22/2015
	Warsaw	Pre-plant fertilizer	34-67-67-5	10/06/2014
		Planting	520 seeds m^{-2}	10/21/2014
		GS25 UAN	Variable	03/19/2015
		Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/24/2015
		Starane™	0.21 kg A.I. ha^{-1}	03/24/2015
		GS30 UAN	Variable	04/01/2015
		Palisade EC	0.08 kg A.I. ha^{-1}	04/06/2015
		Tilt	0.07 kg A.I. ha^{-1}	04/10/2015
		Prosaro	0.14 kg A.I. ha^{-1}	05/14/2015
		Tilt	007 kg A.I. ha^{-1}	05/14/2015
		Harvest	NA	06/22/2015
2015-16	Blacksburg	Pre-plant fertilizer	34-67-67-5	10/16/2015
		Planting	520 seeds m^{-2}	10/16/2015
		Harmony Extra SG®	0.02 kg A.I. ha^{-1}	11/16/2015
		GS25 UAN	Variable	03/08/2016
		Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/23/2016
		GS30 UAN	Variable	03/23/2016
		Palisade EC	0.08 kg A.I. ha^{-1}	03/31/2016
		Tilt	0.04 kg A.I. ha^{-1}	03/31/2016
		Prosaro	0.14 kg A.I. ha^{-1}	05/12/2016
		Tilt	0.10 kg A.I. ha^{-1}	05/26/2016
		Harvest	NA	06/27/2016
	Blackstone	Planting	520 seeds m^{-2}	10/20/2015
		GS25 UAN	Variable	02/10/2016

Season	Location	Product	Application rate	Application date
		Harmony Extra SG®	0.02 kg A.I. ha^{-1}	02/10/2016
		GS30 UAN	Variable	03/09/2016
		Prosaro	0.07 kg A.I. ha^{-1}	04/11/2016
		Mustang® Maxx	0.03 kg A.I. ha^{-1}	04/25/2016
		Prosaro	0.07 kg A.I. ha^{-1}	04/29/2016
		Harvest	NA	06/15/2016
	Warsaw	Lime	2242 kg ha^{-1}	09/24/2015
		Pre-plant fertilizer	34-67-67-5	10/14/2015
		Planting	520 seeds m^{-2}	10/21/2015
		Starane™	0.21 kg A.I. ha^{-1}	12/06/2015
		GS25 UAN	Variable	02/19/2016
		Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/08/2016
		Starane™	0.15 kg A.I. ha^{-1}	03/08/2016
		GS30 UAN	Variable	03/12/2016
		Palisade EC	0.06 kg A.I. ha^{-1}	03/30/2016
		Fitness	0.12 kg A.I. ha^{-1}	03/30/2016
		Fitness	0.12 kg A.I. ha^{-1}	04/19/2016
		Prosaro	0.14 kg A.I. ha^{-1}	05/20/2016
		Harvest	NA	06/22/2016
2016-17	Blacksburg	Lime	3363 kg ha^{-1}	09/28/2016
		Pre-plant fertilizer	15-40-60-8-3B	10/10/2016
		Planting	520 seeds m^{-2}	10/12/2016
		Harmony Extra SG®	0.02 kg A.I. ha^{-1}	11/15/2016
		GS25 UAN	Variable	02/17/2017
		Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/24/2017
		GS30UAN	Variable	03/24/2017
		Palisade EC	0.08 kg A.I. ha^{-1}	03/27/2017
		Prosaro	0.14 kg A.I. ha^{-1}	05/08/2017
		Tilt	0.10 kg A.I. ha^{-1}	05/30/2017
		Harvest	NA	06/22/2017
	Blackstone	Pre-plant fertilizer	34-34-34-0	10/16/2016
		Planting	520 seeds m^{-2}	10/08/2016
		GS25 UAN	Variable	02/02/2017
		Harmony Extra SG®	0.02 kg A.I. ha^{-1}	02/02/2017
		GS30 UAN	Variable	03/01/2017
		Mustang® Maxx	0.03 kg A.I. ha^{-1}	03/01/2017
		Harvest	NA	06/15/2017
	Warsaw	Pre-plant fertilizer	30-70-60-5	10/17/2016
		Planting	520 seeds m^{-2}	10/18/2016
		Quelex®	0.01 kg A.I. ha^{-1}	12/04/2016
		GS25 UAN	Variable	02/06/2017
		Tilt	0.07 kg A.I. ha^{-1}	03/09/2017
		Tombstone	0.03 kg A.I. ha^{-1}	03/09/2017
		GS30 UAN	Variable	03/13/2017
		Palisade EC	0.08 kg A.I. ha^{-1}	03/17/2017
		Tilt	0.07 kg A.I. ha^{-1}	04/05/2017
		Prosaro	0.14 kg A.I. ha^{-1}	04/20/2017
		Tombstone	0.03 kg A.I. ha^{-1}	04/28/2017
		Harvest	NA	06/12/2017

[a]Nitrogen, phosphorous, potassium, and sulfur pre-plant fertilizer applied, respectively.

Supplemental Table 1.2. Testing environments, N rates, traits evaluated, and number of genotypes in each study.

Study number	Sites	Seasons observed	Environment designation	N rates	Traits evaluated[a]	Balanced genotypes
I	Custar, OH	2013-14	14CS	5	Grain yield	12
		2014-15	15CS			
	Wooster, OH	2014-15	15WS			
II	Painter, VA	2013-14	14PT	3	Grain yield, AGBM, HI, GNC	12
	Warsaw, VA	2013-14	14WR			
II	Blacksburg, VA	2015-16	16BB	3	Grain yield, AGBM, HI, GNC, NUE, NUpE, NUtE	12
	Painter, VA	2014-15	15PT			
	Warsaw, VA	2014-15	15WR			
		2015-16	16WR			
III	Custar, OH	2015-16	16CS	2	Grain yield	11
	Fremont, OH	2015-16	16FR			
	Wooster, OH	2015-16	16WS			
III	Blacksburg, VA	2015-16	16BB	2	Grain yield, AGBM, HI, GNC, NUE, NUpE, NUtE	11
		2016-17	17BB			
	Blackstone, VA	2015-16	16BS			
		2016-17	17BS			
	Warsaw, VA	2015-16	16WR			
		2016-17	17WR			

[a] Traits evaluated include grain yield, above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

Supplemental Table 1.3. Significant genotype × N rate interactions ($P \leq 0.05$) for grain yield in Study I at two testing environments under five N rates.

Wheat line	45 kg N ha^{-1}	67 kg N ha^{-1}	90 kg N ha^{-1}	112 kg N ha^{-1}	134 kg N ha^{-1}
			kg ha^{-1}		
Custar 2013-2014					
Bess	4,168 p-u[a]	4,333 m-t	4,175 p-u	4,790 d-j	4,869 c-i
IL02-19483B	3,749 w-y	4,263 n-u	4,495 j-q	4,422 k-s	4,394 k-s
IL07-4415	4,085 s-w	4,151 q-v	4,462 j-r	4,528 i-o	4,178 p-u
KY06C-1003-139-8-3	4,082 s-w	4,573 h-n	4,654 f-m	4,690 e-l	4,880 b-h
MD03W485-10-10	4,914 b-h	4,978 a-f	5,145 a-c	4,923 b-f	5,018 a-e
MD05W10208-11-8	4,208 o-u	4,606 g-n	5,044 a-d	4,999 a-f	4,987 a-f
MO080864	4,306 n-u	4,462 j-r	4,593 g-n	4,996 a-f	5,222 ab
OH06-150-57	4,317 m-u	4,299 n-u	4,184 o-u	4,514 j-p	4,480 j-r
OH08-161-78	3,819 v-x	3,976 u-x	4,027 t-x	4,140 r-v	4,186 o-u
OH08-172-42	4,591 g-n	4,867 c-i	5,294 a	5,207 a-c	5,161 a-c
Sisson	3,460 y	4,181 p-u	4,335 m-t	4,712 d-k	4,495 j-q
VA08MAS-369	3,724 yx	4,312 m-u	4,205 o-u	4,683 e-l	4,347 l-t
Custar 2014-2015					
Bess	3,077 l-t	4,378 a	3,472 e-m	3,438 f-n	3,472 e-m
IL02-19483B	3,039 l-t	2,823 q-u	3,409 g-o	3,475 e-m	3,961 a-e
IL07-4415	3,249 h-q	3,349 f-p	2,733 r-u	3,431 f-n	3,413 f-o
KY06C-1003-139-8-3	4,097 ab	3,601 b-k	4,064 a-c	3,780 b-f	4,088 a-c
MD03W485-10-10	3,164 j-t	3,765 b-g	3,277 h-q	2,851 p-u	3,066 l-t
MD05W10208-11-8	3,502 d-l	3,297 f-q	2,443 u	2,926 o-u	3,168 j-s
MO080864	3,657 b-j	3,433 f-n	3,762 b-g	3,231 i-r	2,666 s-u
OH06-150-57	2,858 p-u	3,209 i-r	2,745 r-u	2,946 n-t	3,750 b-h
OH08-161-78	3,983 a-d	3,515 d-l	3,536 d-l	3,537 d-l	3,590 c-k
OH08-172-42	3,179 j-r	3,453 f-m	3,365 f-o	3,682 b-i	3,365 f-o
Sisson	2,665 tu	3,234 i-r	3,201 i-r	3,599 b-k	3,195 i-r
VA08MAS-369	3,222 j-r	3,204 i-r	3,315 e-q	2,998 m-t	3,130 k-s

[a] The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

Supplemental Table 1.4. Significant genotype × N rate interactions ($P \leq 0.05$) for grain yield in Study II at two testing environments under three N rates.

Line	2013-2014 Painter			2013-2014 Warsaw		
	67 kg N ha^{-1}	101 kg N ha^{-1}	134 kg N ha^{-1}	67 kg N ha^{-1}	101 kg N ha^{-1}	134 kg N ha^{-1}
	kg ha^{-1}			kg ha^{-1}		
Bess	2,528 k[a]	1,800 l	3,006 e-k	4,562 n-q	4,925 i-n	5,669 c-e
IL02-19483B	2,677 i-k	3,231 d-i	3,629 a-d	4,055 r	5,089 g-l	5,276 e-j
IL07-4415	2,608 jk	3,197 d-j	3,697 a-d	5,333 e-i	5,287 e-j	6,151 ab
KY06C-1003-139-8-3	2,677 i-k	3,601 a-d	3,312 c-h	4,755 k-p	5,552 d-f	6,157 ab
MD03W485-10-10	2,542 k	3,494 b-f	3,863 a-c	4,342 p-r	4,604 m-p	5,024 h-m
MD05W10208-11-8	2,791 g-k	3,173 d-j	3,756 a-d	4,396 p-r	5,011 h-n	5,423 d-h
MO080864	2,507 k	3,530 b-e	3,827 a-c	4,699 l-p	5,198 f-j	6,091 a-c
OH06-150-57	2,617 jk	3,376 c-g	3,313 c-h	4,130 qr	4,867 j-n	5,415 d-h
OH08-161-78	2,423 k	3,559 a-e	3,464 b-f	4,572 m-p	5,382 d-h	5,442 d-h
OH08-172-42	2,768 h-k	3,489 b-f	3,870 a-c	5,008 h-n	5,828 b-d	6,358 a
Sisson	2,721 h-j	2,902 f-k	4,001 ab	4,603 m-p	5,512 d-g	6,036 a-c
VA08MAS-369	2,449 k	3,185 d-j	4,137 a	4,427 o-r	5,711 b-e	6,423 a

[a] The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

Supplemental Table 1.5. Variance components and heritability for grain yield, above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE) for wheat lines grown in Study II and III.

Trait	Variance components						H^{2b}
	σ^2 G[a]	σ^2 N	σ^2 E	σ^2 (G × N)	σ^2 (G × E)	σ^2 ε	
Study I							
Grain yield	3.127e-10	2.778e+03	4.080e+05	6.917e+03	8.650e+04	1.280e+05	0.00
Study II							
Grain yield	27,588	145,582	750,645	674	39,034	150,203	0.74
AGBM	2,585	5,624	26,048	0	435	22,458	0.83
HI	1.833e-04	8.291e-05	1.855e-03	2.151e-05	2.240e-04	2.061e-03	0.68
GNC	7.582e-03	1.208e-02	3.478e-02	0.000	0.000	4.232e-02	0.90
NUpE	0.000	5.457e-02	7.108e-02	2.691e-03	0.000	6.744e-02	0.00
NUtE	2.725	14.045	53.049	0.966	3.064	39.359	0.54
Study III							
Grain yield	54,835	92,887	2,184,287	2,651	91,935	172,552	0.79
AGBM	5,872	3,733	102,483	0	2,153	36,412	0.80
HI	1.437e-04	7.219e-05	5.465e-03	0.000	1.850e-04	1.102e-03	0.69
GNC	8.327e-03	2.871e-02	2.565e-02	0.000	2.622e-03	3.019e-02	0.86
NUpE	2.038e-04	1.937e-01	2.647e-01	0.000	0.000	1.229e-01	0.05
NUtE	3.683	12.264	17.986	0.000	1.815	20.307	0.80

[a] σ^2 G genotypic variance; σ^2 N nitrogen variance; σ^2 E environmental variance; σ^2 (G × N) genotype × nitrogen variance; σ^2 (G × E) genotype × environment variance; σ^2 ε residual variance; H^2 broad sense heritability.

[b] Number of testing environments used for each trait in studies II and III shown in Supplemental Table 1.2.

Supplemental Table 1.6. Variance components and heritability for grain yield, above-ground biomass, harvest index, grain N content, NUpE, and NUtE under each N-environment for wheat lines grown in studies I, II, and III.

Trait	Variance components				H^2[b]
	σ^2 G[a]	σ^2 N-env	σ^2 (G × N-env)	σ^2 ε	
Study I					
Grain yield‡	10,658	306,677	106,148	90,559	0.55
Study II					
Grain yield	32,429	781,285	47,198	120,344	0.87
Above-ground biomass	2,610	28,022	1,064	20,639	0.86
Harvest index	2.096e-04	1.742e-03	4.112e-04	1.828e-03	0.80
Grain N content	7.649e-03	4.114e-02	0.000	4.036e-02	0.91
NUpE	7.443e-04	1.023e-01	3.209e-03	6.037e-02	0.28
NUtE	3.37	54.42	6.86	35.30	0.76
Study III					
Grain yield	62,441	2,142,783	89,737	143,935	0.89
Above-ground biomass	6,300	99,638	700	33,733	0.86
Harvest index	1.617e-04	5.063e-03	8.970e-05	1.128e-03	0.81
Grain N content	8.745e-03	3.832e-02	0.000	3.229e-02	0.91
NUpE	8.319e-04	3.603e-01	1.337e-15	1.041e-01	0.22
NUtE	3.94	23.07	0.00	21.80	0.87

[a] σ^2 G genotypic variance; σ^2 E environmental variance; σ^2 (G × N-env) genotype × N-environment variance; σ^2 ε residual variance; H^2 broad sense heritability.

[b] Number of testing environments used for each trait in studies I, II, and III shown in Supplemental Table 1.2.

Supplemental Table 1.7. Trait means for wheat lines and N rates in Study II at Painter during the 2013-14 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	770 d[b]	0.34 de	17.3 b	-	-	-
IL02-19483B	827 b-d	0.38 a-c	18.0 ab	-	-	-
IL07-4415	818 b-d	0.39 ab	16.1 c	-	-	-
KY06C-1003-139-8-3	900 a-d	0.36 b-d	17.1 b	-	-	-
MD03W485-10-10	943 ab	0.35 c-e	18.3 a	-	-	-
MD05W10208-11-8	849 b-d	0.38 a-d	18.1 ab	-	-	-
MO080864	908 a-c	0.36 b-d	16.0 c	-	-	-
OH06-150-57	983 a	0.32 e	17.5 ab	-	-	-
OH08-161-78	898 a-d	0.35 c-e	17.3 ab	-	-	-
OH08-172-42	900 a-c	0.38 a-d	16.1 c	-	-	-
Sisson	791 cd	0.40 a	17.2 b	-	-	-
VA08MAS-369	831 b-d	0.39 ab	17.3 ab	-	-	-
N rate						
67	765 b	0.34 c	16.7 b	-	-	-
101	897 a	0.37 b	17.5 a	-	-	-
134	943 a	0.40 a	17.4 a	-	-	-
Effect						
G (df = 11)	1.91 ns[c]	3.25 **	4.81 ***	-	-	-
N (df = 2)	16.06 ***	11.03 ***	5.48 **	-	-	-
G × N (df = 22)	0.73 ns	0.55 ns	1.23 ns	-	-	-

[a] Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.8. Trait means for wheat lines and N rates in Study II at Warsaw during the 2013-14 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	1288 de[b]	0.39 b	21.2 bc	-	-	-
IL02-19483B	1298 de	0.37 cd	21.3 bc	-	-	-
IL07-4415	1338 b-e	0.42 a	20.8 bc	-	-	-
KY06C-1003-139-8-3	1430 ab	0.38 bc	22.0 b	-	-	-
MD03W485-10-10	1345 b-d	0.35 e	22.1 b	-	-	-
MD05W10208-11-8	1378 b-d	0.36 de	24.7 a	-	-	-
MO080864	1518 a	0.35 de	19.8 c	-	-	-
OH06-150-57	1387 b-d	0.35 e	20.7 bc	-	-	-
OH08-161-78	1316 c-e	0.39 b	21.2 bc	-	-	-
OH08-172-42	1334 b-e	0.43 a	21.0 bc	-	-	-
Sisson	1240 e	0.43 a	20.4 bc	-	-	-
VA08MAS-369	1402 bc	0.39 b	21.4 bc	-	-	-
N rate						
67	1235 c	0.37 c	2.03 b	-	-	-
101	1369 b	0.38 b	2.10 b	-	-	-
134	1465 a	0.40 a	2.28 a	-	-	-
Effect						
G (df = 11)	4.08 ***	27.20 ***	3.11 **	-	-	-
N (df = 2)	40.51 ***	19.69 ***	13.12 ***	-	-	-
G × N (df = 22)	1.30 ns[c]	1.67 ns	0.68 ns	-	-	-

[a] Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.9. Trait means for wheat lines and N rates in Study II at Painter during the 2014-15 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	998 d[b]	0.32 a-d	16.5 bc	32.2 c	0.89 b	37.3 ab
IL02-19483B	1044 cd	0.33 a	18.3 a-c	35.0 bc	0.97 ab	36.2 ab
IL07-4415	1109 b-d	0.32 a-c	16.9 a-c	35.1 bc	0.95 ab	37.2 ab
KY06C-1003-139-8-3	1142 a-d	0.32 a-d	15.7 c	38.8 ab	1.01 ab	38.1 a
MD03W485-10-10	1238 ab	0.27 cd	19.4 a	35.1 bc	1.07 ab	32.6 b
MD05W10208-11-8	1146 a-d	0.31 a-d	18.9 ab	36.1 a-c	1.05 ab	35.2 ab
MO080864	1306 a	0.26 d	16.7 a-c	35.2 bc	1.02 ab	35.1 ab
OH06-150-57	1198 a-d	0.27 b-d	17.8 a-c	33.9 c	0.94 ab	36.3 ab
OH08-161-78	1242 ab	0.31 a-d	16.1 bc	39.2 ab	1.02 ab	38.2 a
OH08-172-42	1269 ab	0.31 a-d	16.6 bc	39.2 ab	1.06 ab	39.1 a
Sisson	1206 a-c	0.28 a-d	18.2 a-c	35.2 bc	1.09 a	32.0 b
VA08MAS-369	1185 a-d	0.33 ab	17.2 a-c	39.8 a	1.04 ab	39.2 a
N rate						
67	1168 a	0.29 b	16.2 b	47.8 a	1.24 a	39.9 a
101	1128 a	0.32 a	16.3 b	34.2 b	0.92 b	38.1 a
134	1224 a	0.30 ab	19.6 a	26.7 c	0.88 b	31.1 b
Effect						
G (df = 11)	1.68 ns[c]	1.44 ns	1.30 ns	2.39 *	0.85 ns	1.57 ns
N (df = 2)	1.95 ns	2.22 ns	14.49 ***	192.80 ***	37.60 ***	24.88 ***
G × N (df = 22)	1.14 ns	1.99 *	1.30 ns	1.52 ns	1.12 ns	1.82 *

[a] Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.10. Trait means for wheat lines and N rates in Study II at Warsaw during the 2014-15 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	1147 abc[b]	0.43 a	15.2 b-d	50.4 ab	0.98 bc	51.7 a
IL02-19483B	1055 c	0.44 a	18.1 a	48.8 ab	1.08 ab	44.7 c-e
IL07-4415	1180 abc	0.35 c	15.4 b-d	42.0 b	0.96 c	44.0 de
KY06C-1003-139-8-3	1254 a	0.39 abc	15.4 b-d	50.6 ab	1.02 a-c	49.4 a-c
MD03W485-10-10	1181 abc	0.37 bc	18.2 a	45.4 ab	1.12 a	40.8 e
MD05W10208-11-8	1108 bc	0.41 ab	17.5 a	45.9 ab	1.05 a-c	43.6 de
MO080864	1261 a	0.42 ab	14.3 d	46.6 ab	1.02 a-c	53.2 a
OH06-150-57	1197 ab	0.42 a	15.4 b-d	53.1 a	1.02 a-c	51.9 a
OH08-161-78	1188 ab	0.43 a	15.9 b	53.5 a	1.10 a	48.8 a-c
OH08-172-42	1208 ab	0.40 abc	14.5 cd	42.7 b	0.98 bc	50.5 ac
Sisson	1202 ab	0.40 abc	15.8 bc	49.7 ab	1.07 a-c	46.2 b-d
VA08MAS-369	1222 ab	0.43 a	15.4 b-d	54.8 a	1.11 a	48.9 a-c
N rate						
67	1035 c	0.42 a	14.2 c	59.3 a	1.19 a	53.6 a
101	1208 b	0.41 a	16.2 b	48.4 b	1.05 b	46.9 b
134	1308 a	0.40 a	17.4 a	38.2 c	0.89 c	43.0 c
Effect						
G (df = 11)	1.61 ns[c]	2.16 *	8.34 ***	1.54 ns	1.86 ns	5.69 ***
N (df = 2)	35.63 ***	1.16 ns	51.77 ***	38.35 ***	55.18 ***	41.44 ***
G × N (df = 22)	1.57 ns	1.09 ns	1.09 ns	0.91 ns	1.24 ns	1.10 ns

[a] Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.11. Trait means for wheat lines and N rates in Study II at Blacksburg during the 2015-16 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	972 g[b]	0.40 b-e	16.5 c	41.5 g	1.57 bc	27.5 cd
IL02-19483B	1162 b-e	0.42 a-c	17.0 bc	51.6 ab	1.70 a-c	32.2 a-c
IL07-4415	1144 c-e	0.43 a	15.6 d	52.6 a	1.43 c	37.5 a
KY06C-1003-139-8-3	1105 d-f	0.42 ab	16.4 cd	49.2 bc	1.51 bc	34.1 ab
MD03W485-10-10	1107 d-f	0.38 e-g	18.0 a	44.8 f	1.65 a-c	27.6 cd
MD05W10208-11-8	1092 ef	0.40 d-f	18.2 a	46.3 ef	1.44 c	32.0 a-c
MO080864	1195 a-c	0.37 g	16.4 cd	47.1 c-f	1.88 a	25.9 d
OH06-150-57	1185 a-d	0.38 e-g	16.1 cd	48.1 c-e	1.53 bc	31.9 bc
OH08-161-78	1239 ab	0.38 fg	17.4 ab	48.8 cd	1.53 bc	32.0 a-c
OH08-172-42	1261 a	0.40 c-f	16.7 bc	53.7 a	1.53 bc	36.5 ab
Sisson	1046 fg	0.42 a-d	17.6 ab	46.5 d-f	1.73 ab	27.0 cd
VA08MAS-369	1205 a-c	0.40 c-f	16.9 bc	51.4 ab	1.50 bc	34.4 ab
N rate						
67	1121 b	0.39 b	1.62 c	65.2 a	2.00 a	34.5 a
101	1131 b	0.41 a	1.67 b	45.3 b	1.51 b	30.6 b
134	1176 a	0.40 ab	1.78 a	34.9 c	1.24 c	29.5 b
Effect						
G (df = 11)	8.08 ***	6.41 ***	6.09 ***	16.91 ***	1.65 ns	3.75 ***
N (df = 2)	4.07 *	3.35 *	27.63 ***	1290.02 ***	56.44 ***	7.11 **
G × N (df = 22)	2.10 *	2.30 **	1.42 ns[c]	1.89 *	1.62 ns	2.07 *

[a] Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.12. Trait means for wheat lines and N rates in Study II at Warsaw during the 2015-16 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	973 de[b]	0.32 c	18.7 a-c	32.8 c-e	1.29 a	28.5 b
IL02-19483B	1076 bc	0.31 cd	20.3 a	35.1 bc	1.15 a	31.7 ab
IL07-4415	986 de	0.34 a-c	16.2 de	34.9 b-d	1.12 a	35.5 ab
KY06C-1003-139-8-3	986 de	0.33 a-c	17.8 b-e	34.8 b-d	1.05 a	28.3 b
MD03W485-10-10	1129 ab	0.27 e	20.0 ab	31.5 e	1.08 a	29.1 b
MD05W10208-11-8	974 de	0.32 c	19.2 a-c	32.0 de	1.03 a	30.8 ab
MO080864	1133 ab	0.32 c	17.0 c-e	37.3 ab	1.14 a	34.1 ab
OH06-150-57	1161 a	0.29 de	17.4 c-e	35.2 bc	1.03 a	34.4 ab
OH08-161-78	1045 cd	0.35 a	17.6 c-e	38.5 a	1.28 a	32.9 ab
OH08-172-42	962 e	0.35 ab	15.5 e	35.6 a-c	1.04 a	34.7 ab
Sisson	1027 c-e	0.32 bc	17.8 b-e	34.3 c-e	1.00 a	34.8 ab
VA08MAS-369	1144 ab	0.32 bc	18.1 a-d	38.3 a	1.05 a	37.7 a
N rate						
67	997 c	0.30 b	17.3 b	44.7 a	1.33 a	35.7 a
101	1047 b	0.32 a	17.7 ab	33.1 b	1.03 b	32.8 ab
134	1105 a	0.33 a	18.9 a	27.3 c	0.95 b	29.6 b
Effect						
G (df = 11)	6.96 ***	6.21 ***	3.04 **	5.12 ***	0.80 ns	1.30 ns
N (df = 2)	14.05 ***	11.19 ***	4.02 *	318.40 ***	13.25 ***	5.32 **
G × N (df = 22)	1.66 ns[c]	1.52 ns	0.89 ns	1.47 ns	1.07 ns	1.10 ns

[a] Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.13. Significant genotype by N rate interaction effects in Study II under 67, 101, and 134 kg N ha^{-1} in Painter (15PT) and Blacksburg (16BB) during the 2014-15 and 2015-16 growing seasons, respectively.

Wheat line	Harvest index in 15PT			N-utilization efficiency in 15PT		
	67	101	134	67	101	134
	%			kg kg^{-1}		
Bess	0.33 b-f[a]	0.34 a-f	0.30 c-f	32.6 b-f	29.1 c-f	23.9 f
IL02-19483B	0.25 f	0.42 ab	0.34 b-f	31.0 b-f	34.7 a-f	29.3 c-f
IL07-4415	0.44 a	0.27 d-f	0.26 d-f	36.2 a-f	29.3 c-f	41.0 a-d
KY06C-1003-139-8-3	0.33 b-f	0.30 c-f	0.34 b-f	28.6 c-f	30.1 b-f	26.1 ef
MD03W485-10-10	0.26 d-f	0.28 d-f	0.26 d-f	31.4 b-f	27.8 d-f	28.0 d-f
MD05W10208-11-8	0.24 f	0.34 b-f	0.36 a-e	34.6 b-f	28.3 d-f	29.3 c-f
MO080864	0.25 ef	0.27 d-f	0.26 d-f	28.5 c-f	38.8 a-e	35.0 a-f
OH06-150-57	0.28 d-f	0.27 d-f	0.27 d-f	39.5 a-d	35.2 a-f	28.5 c-f
OH08-161-78	0.30 c-f	0.36 a-e	0.27 d-f	33.4 b-f	36.0 a-f	29.2 c-f
OH08-172-42	0.23 f	0.40 a-c	0.30 c-f	43.3 ab	30.5 b-f	30.2 b-f
Sisson	0.25 f	0.28 d-f	0.32 b-f	41.5 a-c	34.5 b-f	28.2 d-f
VA08MAS-369	0.30 c-f	0.33 b-f	0.36 a-d	47.9 a	39.0 a-e	26.3 ef
Wheat line	Above-ground biomass in 16BB			Harvest index in 16BB		
	67	101	134	67	101	134
	g m^{-2}			%		
Bess	919 m	988 k-m	1010 j-m	0.40 c-j	0.40 f-l	0.40 c-j
IL02-19483B	1232 b-e	1098 e-l	1156 c-i	0.38 h-m	0.45 ab	0.44 a-e
IL07-4415	1211 b-e	1122 c-l	1099 e-k	0.40 f-l	0.43 a-f	0.46 a
KY06C-1003-139-8-3	1020 i-m	1072 g-l	1222 b-e	0.42 b-h	0.44 a-c	0.41 b-i
MD03W485-10-10	1144 c-j	1067 g-l	1111 d-l	0.36 lm	0.39 g-l	0.40 e-k
MD05W10208-11-8	1127 c-k	1032 h-m	1116 c-l	0.38 h-m	0.41 b-i	0.40 e-j
MO080864	1119 c-l	1240 b-e	1225 b-e	0.38 g-m	0.38 i-m	0.35 m
OH06-150-57	1222 b-e	1174 c-h	1159 c-i	0.36 lm	0.39 g-l	0.39 g-l
OH08-161-78	1156 c-i	1141 c-j	1420 a	0.38 h-m	0.39 g-l	0.36 k-m
OH08-172-42	1184 c-g	1345 ab	1256 bc	0.42 b-g	0.37 j-m	0.41 b-i
Sisson	980 lm	1052 g-m	1105 d-l	0.41 c-i	0.44 a-d	0.40 d-j
VA08MAS-369	1134 c-j	1247 b-d	1235 b-e	0.41 c-i	0.39 g-l	0.39 g-l
Wheat line	N-use efficiency in 16BB			N-utilization efficiency in 16BB		
	67	101	134	67	101	134
	kg kg^{-1}			kg kg^{-1}		
Bess	55.5 g	38.6 l-n	30.4 r	26.7 g-j	28.2 e-j	27.5 f-j
IL02-19483B	68.5 b-d	48.7 h	37.6 m-o	29.5 c-j	35.0 b-g	32.2 b-i
IL07-4415	71.8 ab	48.0 hi	37.9 l-n	53.0 a	30.0 b-j	29.5 c-j
KY06C-1003-139-8-3	63.6 ef	47.0 hi	37.2 m-p	31.8 b-i	33.2 b-h	37.3 b-e
MD03W485-10-10	60.7 f	40.7 k-m	32.9 qr	29.6 c-j	23.3 ij	30.0 b-j
MD05W10208-11-8	63.6 ef	41.9 j-l	33.4 p-r	37.9 b-d	31.5 b-i	26.7 f-j
MO080864	63.9 ef	45.8 h-j	31.6 r	24.8 h-j	31.2 b-j	21.7 j
OH06-150-57	65.1 de	45.6 h-k	33.4 o-r	38.3 bc	28.8 c-j	28.5 d-j
OH08-161-78	65.4 c-e	43.9 i-k	37.2 m-p	36.4 b-f	29.3 c-j	30.3 b-j
OH08-172-42	74.0 a	48.9 h	38.2 l-n	38.3 bc	39.4 b	31.9 b-i
Sisson	60.6 f	45.9 h-j	33.2 p-r	29.7 c-j	26.5 g-j	25.0 h-j
VA08MAS-369	69.5 bc	48.5 h	36.3 n-q	38.3 bc	30.9 b-j	34.0 b-h

[a] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

Supplemental Table 1.14. Trait means for wheat lines and N rates in Study III at Blacksburg during the 2015-16 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	1309 d[b]	0.31 cd	19.9 a-c	70.5 de	2.04 a-c	34.2 d
MD03W485-10-10	1448 bc	0.32 a-d	20.5 ab	76.2 c-e	2.12 a-c	36.2 cd
OH08-161-78	1532 ab	0.31 cd	21.0 a	83.6 a-d	2.32 ab	35.6 cd
OH08-172-42	1463 b	0.32 a-c	17.0 d	82.7 a-d	1.78 c	45.5 a
Sisson	1355 cd	0.33 ab	20.3 a-c	93.1 a	2.52 a	36.7 cd
SS520	1297 d	0.30 d	19.7 a-c	63.6 e	1.88 bc	34.9 cd
VA05W-151	1440 bc	0.33 ab	18.6 b-d	87.8 a-c	2.09 a-c	41.5 ab
VA07W-415	1260 d	0.32 b-d	19.1 a-d	75.8 c-e	1.92 bc	38.8 bc
VA08MAS-369	1572 a	0.33 a	17.3 d	91.0 ab	2.19 a-c	42.6 ab
VA09W-73	1516 ab	0.31 cd	18.6 b-d	75.4 c-e	2.06 a-c	36.4 cd
Yorktown	1496 ab	0.32 b-d	18.4 cd	90.9 ab	2.31 a-c	39.3 bc
N rate						
67	1431 a	0.32 a	18.2 b	103.9 a	2.59 a	40.8 a
134	1421 a	0.32 a	20.1 a	58.0 b	1.64 b	35.9 b
Effect						
G (df = 10)	8.05 ***	2.68 *	3.43 **	3.51 **	1.41 ns	5.48 ***
N (df = 1)	0.19 ns[c]	0.72 ns	21.35 ***	227.08 ***	75.17 ***	29.12 ***
G × N (df = 10)	1.12 ns	0.59 ns	2.97 *	2.27 ns	2.49 *	4.03 **

[a] Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.15. Trait means for wheat lines and N rates in Study III at Blackstone during the 2015-16 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	814 b-d[b]	0.27 ab	19.5 d	27.1 a-c	0.76 b-d	35.3 a
MD03W485-10-10	958 a-c	0.23 c-e	22.3 b	24.3 b-d	0.85 a-d	27.9 b
OH08-161-78	904 a-d	0.26 ab	19.1 d	27.7 ab	0.80 a-d	35.1 a
OH08-172-42	744 d	0.26 ab	19.2 d	31.7 a	0.97 ab	31.9 ab
Sisson	946 a-d	0.21 e	25.1 a	17.1 e	0.77 a-d	22.3 c
SS520	801 cd	0.21 de	21.5 bc	18.9 de	0.67 d	28.3 b
VA05W-151	950 a-d	0.23 c-e	20.5 cd	29.0 ab	0.91 a-c	31.2 ab
VA07W-415	793 cd	0.25 a-c	20.8 b-d	27.0 a-c	0.89 a-d	30.7 ab
VA08MAS-369	1097 a	0.24 b-e	19.9 cd	20.6 c-e	0.70 cd	30.2 ab
VA09W-73	908 a-d	0.27 a	19.5 d	32.4 a	0.96 ab	33.6 a
Yorktown	1018 ab	0.24 a-d	20.6 b-d	30.2 ab	0.99 a	30.6 ab
N rate						
67	759 b	0.25 a	19.5 b	31.0 a	0.93 a	33.8 a
134	1047 a	0.24 b	21.9 a	21.0 b	0.76 b	27.5 b
Effect						
G (df = 10)	2.15 *	3.99 ***	8.92 ***	4.66 **	2.19 ns	4.30 **
N (df = 1)	43.16 ***	4.45 *	46.51 ***	47.56 ***	13.65 **	34.69 ***
G × N (df = 10)	1.36 ns[c]	0.42 ns	2.11 ns	1.37 ns	2.07 ns	1.95 ns

a Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.16. Trait means for wheat lines and N rates in Study III at Warsaw during the 2015-16 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	939 a-c[b]	0.41 a-d	17.7 c	48.2 ab	1.18 a-d	40.6 bc
MD03W485-10-10	1028 ab	0.34 f	19.0 ab	41.6 b-d	1.20 a-c	34.6 f
OH08-161-78	928 bc	0.42 ab	16.3 d	51.7 a	1.16 a-d	44.3 a
OH08-172-42	807 d	0.44 a	16.3 d	48.2 ab	1.10 b-d	43.5 ab
Sisson	880 cd	0.41 a-d	18.6 a-c	45.7 a-c	1.23 a-c	37.3 d-f
SS520	910 b-d	0.39 b-d	18.6 a-c	39.4 cd	1.08 cd	36.3 ef
VA05W-151	1001 ab	0.38 cd	19.2 a	41.6 b-d	1.17 a-d	35.6 f
VA07W-415	999 ab	0.41 a-c	18.0 a-c	51.0 a	1.29 ab	39.4 c-e
VA08MAS-369	1048 a	0.38 de	18.5 a-c	42.3 b-d	1.14 b-d	37.2 d-f
VA09W-73	1003 ab	0.41 a-d	17.9 bc	53.7 a	1.33 a	40.0 dc
Yorktown	1058 a	0.35 ef	18.0 a-c	37.0 d	0.99 d	36.9 d-f
N rate						
67	859 b	0.38 b	17.2 b	53.5 a	1.32 a	40.5 a
134	1068 a	0.41 a	18.8 a	37.5 b	1.02 b	36.9 b
Effect						
G (df = 10)	3.71 **	7.59 ***	4.76 **	3.68 **	2.14 ns	7.68 ***
N (df = 1)	74.15 ***	10.13 **	36.21 ***	85.44 ***	59.79 ***	26.59 ***
G × N (df = 10)	0.63 ns[c]	1.91 ns	0.32 ns	0.98 ns	0.53 ns	0.98 ns

[a] Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.17. Trait means for wheat lines and N rates in Study III at Blacksburg during the 2016-17 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	1326 bc[b]	0.47 a	20.3 cd	77.0 ab	2.02 a-c	38.0 ab
MD03W485-10-10	1214 c	0.40 bc	24.3 a	58.4 d	2.05 a-c	28.3 d
OH08-161-78	1535 a-c	0.41 bc	20.6 cd	76.4 ab	2.11 a-c	35.6 a-c
OH08-172-42	1434 bc	0.43 ab	19.5 d	86.0 a	2.25 ab	38.5 ab
Sisson	1205 c	0.41 a-c	22.3 b	63.8 cd	1.98 bc	31.6 cd
SS520	1307 bc	0.43 ab	20.7 b-d	75.0 a-c	2.11 a-c	34.8 a-c
VA05W-151	1397 bc	0.42 ab	21.4 bc	76.0 a-c	2.30 a	32.7 cd
VA07W-415	1609 ab	0.44 ab	19.3 d	76.6 ab	1.91 c	39.7 a
VA08MAS-369	1563 ab	0.40 bc	21.5 bc	76.2 ab	2.26 ab	33.8 bc
VA09W-73	1784 a	0.38 bc	20.5 cd	72.9 bc	2.08 a-c	34.9 a-c
Yorktown	1786 a	0.35 c	21.2 bc	58.1 d	1.82 c	32.0 cd
N rate						
67	1490 a	0.40 a	19.8 b	93.9 a	2.53 a	37.7 a
134	1449 a	0.42 a	22.4 a	50.9 b	1.63 b	31.4 b
Effect						
G (df = 10)	3.11 **	2.09 *	5.70 ***	4.05 ***	2.06 ns	3.60 **
N (df = 1)	0.34 ns[c]	2.49 ns	55.97 ***	275.31 ***	204.83 ***	34.58 ***
G × N (df = 10)	0.82 ns	0.70 ns	0.47 ns	0.87 ns	0.69 ns	0.49 ns

[a] Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.18. Trait means for wheat lines and N rates in Study III at Blackstone during the 2016-17 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	525 d[b]	0.41 a	18.4 d-f	53.4 a-c	1.66 b	31.8 ab
MD03W485-10-10	677 a-c	0.37 b-d	21.5 ab	53.4 a-c	1.98 ab	27.3 cd
OH08-161-78	747 ab	0.37 a-d	18.0 ef	60.0 ab	1.78 ab	32.9 a
OH08-172-42	531 d	0.40 ab	17.7 f	51.1 bc	1.73 b	30.1 a-c
Sisson	547 cd	0.40 ab	19.9 b-d	52.7 a-c	1.81 ab	29.0 a-c
SS520	544 cd	0.34 d	22.3 a	43.4 c	1.87 ab	22.7 e
VA05W-151	658 a-d	0.36 cd	21.2 ab	52.5 a-c	2.18 a	24.4 de
VA07W-415	642 a-d	0.40 ab	18.9 c-f	57.4 ab	1.83 ab	30.5 a-c
VA08MAS-369	768 a	0.38 a-d	19.4 c-f	61.6 a	2.04 ab	30.1 a-c
VA09W-73	630 b-d	0.40 a-c	19.5 c-e	53.4 a-c	1.95 ab	28.0 b-d
Yorktown	698 ab	0.38 a-d	20.2 bc	54.6 ab	1.95 ab	28.7 a-d
N rate						
67	645 a	0.37 a	18.5 b	68.6 a	2.28 a	30.8 a
134	622 a	0.39 a	21.0 a	39.3 b	1.50 b	26.6 b
Effect						
G (df = 10)	3.34 **	2.08 *	5.94 ***	1.76 ns	1.05 ns	3.87 ***
N (df = 1)	0.65 ns[c]	3.03 ns	46.79 ***	179.24 ***	80.51 ***	20.55 ***
G × N (df = 10)	0.40 ns	0.46 ns	0.84 ns	0.72 ns	0.31 ns	0.39 ns

[a] Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.19. Trait means for wheat lines and N rates in Study III at Warsaw during the 2016-17 growing season. Analysis of variance and F-values provided for each effect and their interaction.

Wheat line	AGBM[a]	HI	GNC	NUE	NUpE	NUtE
	g m^{-2}	%	g kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}
Bess	1028 d[b]	0.45 bc	17.2 b	64.9 c-e	1.69 a-d	38.3 bc
MD03W485-10-10	1020 d	0.43 de	18.4 a	61.8 c-f	1.78 a-c	34.8 d
OH08-161-78	1056 d	0.44 c-e	16.4 b	54.6 f	1.47 d	37.7 b-d
OH08-172-42	1064 cd	0.46 bc	15.1 c	77.2 a	1.89 ab	40.4 ab
Sisson	1056 d	0.46 ab	17.1 b	61.3 c-f	1.62 b-d	37.6 b-d
SS520	1120 b-d	0.43 e	16.7 b	56.7 ef	1.52 cd	37.1 cd
VA05W-151	1167 a-c	0.45 bc	16.6 b	57.8 d-f	1.52 cd	38.6 a-c
VA07W-415	1058 d	0.48 a	16.4 b	67.7 a-d	1.63 a-d	41.8 a
VA08MAS-369	1231 a	0.44 c-e	16.9 b	68.3 a-c	1.85 ab	36.9 cd
VA09W-73	1185 ab	0.45 b-d	17.0 b	74.1 ab	1.93 a	38.2 bc
Yorktown	1163 a-c	0.42 e	17.1 b	64.0 c-f	1.75 a-d	36.8 cd
N rate						
67	1030 b	0.43 b	15.4 b	77.7 a	1.94 a	40.3 a
134	1179 a	0.46 a	18.3 a	51.1 b	1.45 b	35.8 b
Effect						
G (df = 10)	3.81 ***	6.47 ***	4.62 ***	3.98 ***	2.23 *	2.57 *
N (df = 1)	44.73 ***	62.08 ***	171.64 ***	154.29 ***	61.97 ***	41.5 ***
G × N (df = 10)	1.53 ns[c]	2.10 *	1.62 ns	2.21 *	1.74 ns	1.62 ns

[a] Trait abbreviations include above-ground biomass (AGBM), harvest index (HI), grain N content (GNC), N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE).

[b] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[c] ns, not significant.

Supplemental Table 1.20. Significant genotype by N rate interaction effects in Study III under 67 and 134 kg N ha^{-1} in Blacksburg during the 2015-16 growing season.

Wheat line	Grain N content		N-uptake efficiency		N-utilization efficiency	
	67	134	67	134	67	134
	g kg^{-1}		kg kg-1		kg kg-1	
Bess	18.3 d-g[a]	21.5 a-c	2.49 b-d	1.59 e-g	35.94 e-j	32.42 h-j
MD03W485-10-10	20.1 a-e	21.0 a-d	2.64 a-d	1.59 e-g	37.39 d-i	35.03 f-j
OH08-161-78	19.6 a-f	22.4 a	2.58 b-d	2.07 d-f	39.03 c-g	32.12 ij
OH08-172-42	14.6 h	19.5 b-f	2.07 d-f	1.49 fg	51.61 a	39.43 c-g
Sisson	19.0 b-g	21.7 ab	3.14 ab	1.90 d-g	39.16 c-g	34.32 g-j
SS520	21.1 a-d	18.4 d-g	2.56 b-d	1.20 g	32.30 ij	37.59 d-i
VA05W-151	16.9 f-h	20.4 a-e	2.26 c-e	1.93 d-g	48.09 ab	34.96 f-j
VA07W-415	17.7 e-g	20.5 a-e	2.39 b-d	1.45 fg	41.94 b-e	35.65 e-j
VA08MAS-369	17.7 e-g	16.9 f-h	2.87 a-c	1.50 fg	41.03 c-f	44.08 bc
VA09W-73	16.3 gh	20.9 a-d	2.09 d-f	2.03 d-f	42.75 b-d	30.12 j
Yorktown	18.7 c-g	18.2 d-g	3.36 a	1.25 g	39.87 c-g	38.73 c-h

[a] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

Supplemental Table 1.21. Significant genotype by N rate interaction effects in Study III under 67 and 134 kg N ha^{-1} in Warsaw during the 2016-17 growing season.

Wheat line	Harvest index		N-use efficiency	
	67	134	67	134
	%		kg kg-1	
Bess	0.44 e-g[a]	0.46 b-e	79.42 b-d	50.47 f
MD03W485-10-10	0.40 hi	0.46 c-f	75.51 cd	48.14 f
OH08-161-78	0.43 gh	0.45 d-g	55.85 ef	53.36 ef
OH08-172-42	0.45 d-g	0.47 b-d	99.77 a	54.73 ef
Sisson	0.45 d-g	0.48 ab	74.60 cd	47.96 f
SS520	0.43 gh	0.43 fg	66.73 de	46.59 f
VA05W-151	0.43 gh	0.48 a-c	67.05 de	48.57 f
VA07W-415	0.46 b-e	0.50 a	82.74 bc	52.75 ef
VA08MAS-369	0.43 gh	0.45 d-g	83.59 bc	52.91 ef
VA09W-73	0.44 d-g	0.45 c-g	90.77 ab	57.51 ef
Yorktown	0.39 i	0.46 c-f	78.88 b-d	49.08 f

[a] The LSD at $P \leq 0.05$ is used to compare genotype and treatment means; trait means followed by the same letter are not significantly different.

CHAPTER II:

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR NITROGEN USE EFFICIENCY IN SOFT RED WINTER WHEAT

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Abbreviation: AGBM, above-ground biomass; DH, doubled haploid; FHB, Fusarium head blight; G, genotype; GBS, genotyping-by-sequencing; GWAS, genome-wide association study; HN, high nitrogen rate; LN, low nitrogen rate; LOD, logarithm of odds; N, nitrogen; NK, New Kent, VA location; NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NUte, nitrogen utilization efficiency; QTL, quantitative trait loci; RIL, recombinant inbred line; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; WR, Warsaw, VA location.

Abstract

Maintaining winter wheat (*Triticum aestivum* L.) productivity with fewer or more efficient nitrogen (**N**) inputs will enable growers to increase profitability and reduce the negative environmental impacts associated with intensive agriculture. However, yield trials conducted under multiple N conditions are expensive and not feasible for wheat breeders, who would benefit greatly from the identification and application of genetic markers associated with nitrogen use efficiency (**NUE**). To investigate the genetic regulation of N response, two bi-parental mapping populations were developed and grown in four site-seasons under low and high N rates. Both populations utilized a high NUE parent (VA05W-151 and VA09W-52) and shared a common low NUE parent, 'Yorktown.' The two populations comprised of 136 RILs (Yorktown × VA05W-151) and 138 DHs (Yorktown × VA09W-52). Phenotypic data was collected on parental lines and their progeny for 11 N-related traits and genotypes were characterized using a genotyping-by-sequencing platform to detect more than 2,400 high quality single nucleotide polymorphisms in each population. A total of 130 quantitative trait loci (**QTL**) were detected on 20 chromosomes six of which were associated with NUE in multiple testing environments. Three of the six QTL for NUE were associated with known photoperiod and disease resistance genes, two did not co-localize with known disease or morphological genes and were reported in previous investigations, and one QTL, *QNue.151-1D*, appeared novel. The reproducible NUE QTL on 1D, 6A, 7A, and 7D had LOD scores ranging from 2.63 to 8.33 and explained 0.97 to 18.1 % of the phenotypic variation. The QTL identified in this study have potential for marker-assisted breeding for NUE in soft red winter wheat.

Introduction

Wheat (*Triticum aestivum* L.) is among the most widely grown crops in the world and accounts for roughly 20 % of the total dietary calories and protein consumed per annum (Shiferaw et al., 2013). It is therefore crucial to continue improvement of wheat productivity and quality to meet grain demands in an era of increasing human population. Since the onset of the Green Revolution in the mid-20th century, wheat yield gains in the Eastern United States' soft red winter wheat growing region have been largely attributed to active selection for performance under intensified nitrogen (**N**) management conditions (Tilman et al., 2002). However, a majority of the N applied to agricultural crops in this region is not harvested and thus subject to loss from the plant-soil system (Swaney et al., 2018). The non-harvested N can be associated with emissions of nitrous oxides (Galloway et al., 2004), runoff and leaching of nitrate (Liu et al., 2019), and the degradation of aquatic and terrestrial ecosystems (Hamilton et al., 2016; Sinha et al., 2017). Therefore, the development of wheat cultivars that more efficiently take up and utilize applied N provides a means of promoting grower profitability and ensuring environmentally sustainable increases in wheat production.

Genetic improvement of N use efficiency (**NUE**), defined as the ratio of harvested grain per unit N applied (Moll et al., 1982), in wheat has been previously reported under a range of N conditions in European (Cormier et al., 2013), Central American (Ortiz-Monasterio R. et al., 1997), and the United States (Guttieri et al., 2017). While the extent of genetic improvement under each N condition varied by study, the authors tend to agree that direct selection under multiple N rates will accelerate gains in NUE. This hypothesis was further validated by the detection of significant genotype (**G**) × N rate interactions for grain yield and N traits in recent studies of genotypic variation in wheat (Cormier et al., 2013; Büchi et al., 2016; Kubota et al.,

2018). However, the authors also note that direct selection under multiple N rates may be cost-prohibitive for wheat breeders. In response to this dilemma, Cormier et al. (2016) proposed identifying genomic regions associated with N response, known as quantitative trait loci (**QTL**), to enable more efficient cultivar selection. Through this approach, breeders can quickly screen germplasm for genetic markers associated with N response to assist in the development of high NUE cultivars.

Previous studies of cereal crops have searched for novel NUE traits and alleles in adapted breeding materials (Fontaine et al., 2009), closely related species (Hu et al., 2015), and landraces (Pozzo et al., 2018; Van Deynze et al., 2018). While these authors have successfully identified QTL, genes, and genotypes conferring high NUE, it stands to reason that additional sources of genetic variation exist within currently unexplored germplasm. This hypothesis has withstood testing in European (Bogard et al., 2013; Laperche et al., 2006; Monostori et al., 2017; Zheng et al., 2010) and Chinese (An et al., 2006; Cui et al., 2014; Li et al., 2011; Sun et al., 2013) wheat leading to the successful identification of several QTL associated with N response. However, identification of novel alleles in North American winter wheat remains relatively unexplored despite known post-domestication selection amongst geographic regions, classes of wheat, and growth habit (Cavanagh et al., 2013; Chao et al., 2010; Chao et al., 2007). Guttieri et al. (2017) and Hitz et al. (2017) began the process of dissecting the genetic variation underlying NUE in United States wheat germplasm by conducting genome-wide association studies (**GWAS**) for N traits in the central and eastern Unites States, respectively. It is therefore crucial to follow up these investigations using bi-parental mapping populations to enrich potentially rare NUE alleles for subsequent QTL analysis and to validate the findings from the aforementioned GWAS investigations.

Successful QTL mapping for complex traits including NUE is dependent on the selection of suitable parents, appropriate population size, multi-environment testing, and the development of high-density genetic maps (Han et al., 2015). Utilization of genotyping-by-sequencing (Elshire et al., 2011)(**GBS**) derived single nucleotide polymorphisms (**SNP**) markers aligned to the recently published wheat reference genome (International Wheat Genome Sequencing Consortium, 2018) has the potential to increase marker density and may thus improve the quantity and accuracy of QTL identified for N-related traits. Recently, GBS derived SNPs have been used to detect QTL underlying qualitative and quantitative traits such as flag leaf architecture (Hussain et al., 2017), disease resistance (Kariyawasam et al., 2018), and yield components (Würschum et al., 2018) in winter wheat – resulting in the identification of novel QTL and improved marker quality.

The present study sought to: i) construct a high density genetic map using GBS derived markers; and ii) identify and validate QTL associated with N-related traits under normal and reduced N conditions. Phenotypic data was collected on recombinant inbred lines (**RILs**) and doubled haploids (**DHs**), and genotypes were sequenced via GBS to produce high-resolution genetic maps for two soft red winter wheat populations. This study aims to detect stable and impactful marker-trait-associations for NUE and N-related traits with direct application for marker-assisted breeding programs.

Materials and Methods

Plant Materials

The present study employed a population of 136 RILs, derived from a cross between 'Yorktown' and VA05W-151 (herein known as "YT×151"), and a population of 138 DHs, derived from a cross between parental lines Yorktown and VA09W-52 (herein known as "YT×52"). VA05W-151 (PI 665039), a high NUE parent, was derived from the cross Pioneer brand '26R24' (PI 614110) / 'McCormick' (PI 632691). The other high NUE parent, VA09W-52, is a breeding line derived from a three-way cross, GF921221E16 / McCormick // VA99W-200. Parentage of GF921221E16 is GA83519 / GA85240 // GA861278. Both mapping populations shared a common low NUE parent, Yorktown (PI 667643), which is a product of the three-way cross '38158' (PI 619052) / VA99W-188 // 'Tribute' (PI 632689). Parentage of sib lines VA99W-188 and VA99W-200 is VA91-54-343 (IN71761A4-31-5-48 // VA71-54-147 (CItr 17449) / 'McNair 1813' (CItr 15289) / VA91-54-222 (sib of 'Roane', PI612958). VA91-54-343 is a sib of VA 92-51-39, which is one of the parents of sibs McCormick and Tribute. The parents used in this study were developed at Virginia Tech for high yield potential under intensive management systems and were assessed for N response in previous investigations of soft red winter wheat (Huang et al., 2016; Pavuluri et al., 2015).

Experimental Design

Both populations were grown under rainfed conditions in four Eastern Virginia testing environments (defined as a "site-season") that are described in **Table 2.1**. Testing sites included the Eastern Virginia Agricultural Research and Extension Center near Warsaw, VA in 2015-2016, 2016-2017, and 2017-2018 (**WR**; 37°99' N, 76°78' W) and a commercial field near New

Kent, VA in 2017-2018 (**NK**; 37°54' N, 76°89' W). Seeds were treated with Raxil MD (triazole, Bayer Crop Science) and Gaucho XT (imidacloprid, Bayer Crop Science) in all testing environments to control diseases and insects, respectively. Foliar pesticides and herbicides were applied throughout the growing season in all environments to further mitigate pest pressure (**Supplemental Table 2.1**). Experimental units, seven-row yield plots that measured 2.74 × 1.52 m in WR and 4.88 × 1.78 m in NK, were sown under optimal environmental conditions at a seeding rate of 480 seeds m^{-2} to ensure normal stand establishment.

The two populations utilized a non-replicated type-2 modified augmented design (Lin and Poushinsky, 1985; You et al., 2016) in all environments due to limited seed and land availability. The design was comprised of 12 statistical blocks with each block consisting of 30 plots (three rows and 10 columns per block) to facilitate spatial adjustments. Each block consisted of a centered primary check line (OH08-161-78), two randomized secondary check lines (OH08-172-42 and 'Sisson', PI 617053 (Griffey et al., 2003)), and 12 randomized experimental lines with each check and experimental line being grown under two N rates in adjacent plots. Parental lines were replicated three times per population under each N rate per environment. Wheat lines in each block were grown under high (134 kg N ha^{-1}; **HN**) and reduced (67 kg N ha^{-1}; **LN**) spring N supplies that were foliar applied as liquid urea ammonium nitrate split over Zadoks (Zadoks et al., 1974) growth stages 25 and 30. Phosphorous, potassium, and sulfur were applied according to soil test results at planting (**Supplemental Table 2.1**).

Phenotypic Measurements

In the WR location, anthesis date was recorded when half of the anthers had emerged from the florets. Maturity date was recorded in 17WR (where "17" refers to the growing season)

and 18WR when 75 % of the peduncles turned yellow. Before harvest in all testing environments, plant height was measured from two random locations within the center rows of each yield plot.

A 1.0 m above-ground biomass (**AGBM**) sample was cut from the center row of each plot at harvest maturity (grain moisture content ≤ 160 g kg^{-1}) in all Virginia testing environments. Biomass samples were oven dried at 60 °C for 72 hours, and weighed to estimate AGBM yield for all experimental line and check plots. Samples were then threshed to estimate harvest index (calculated as the ratio of grain biomass per unit of total AGBM) by separating grain from straw tissue and recording their respective weights. Grain and straw tissues were ground and homogenized to estimate tissue N content via combustion analysis using a Vario Max Cube elemental analyzer (Elementar Analysensysteme). Results of the combustion analysis enabled calculations of grain N content, NUE, N uptake efficiency (**NUpE**), and N utilization efficiency (**NUtE**). Moll et al. (1982) defined the terms NUpE and NUtE as quotients of NUE where NUpE is calculated as the ratio of aboveground N at harvest per unit of N applied and NUtE as the amount of grain produced per total aboveground N in the plant at harvest. Plots were combine harvested in all testing environments at harvest maturity and adjusted to 0 g moisture kg^{-1} to determine grain yield.

Statistical Analysis

An analysis of variance (ANOVA) was performed for the replicated alleles and parents in each testing environment using the *lme4* package (Bates et al., 2015) in the R statistical computing environment (R Core Team, 2015):

$$Y_{ijkl} = \mu + G_i + N_j + E_k + R_l(E_k) + GN_{ij} + GE_{ik} + NE_{jk} + GNE_{ijk} + \varepsilon_{ijkl}$$

Where the trait response (Y_{ijkl}) is a function of the overall mean (μ), the fixed effect of the *i*th wheat line (G_i), the fixed effect of the *j*th N rate (N_j), the random effect of the *l*th replication (R_l) nested within the *k*th environment (E_k), the interactions of the *i*th wheat line with the *j*th N rate and the *k*th environment (GN_{ij} and GE_{ik}), the interaction of the *j*th N rate and the *k*th environment NE_{jk}), their 3-way interaction (GNE_{ijk}), and the residual error (ε_{ijkl}). This was followed with an ANOVA for parental lines within environment as effects of test environment and its interactions were significant for most traits. Means comparisons were conducted using least significant differences for single effects and their interactions. Following the ANOVA, a bivariate correlation analysis calculated Pearson's correlation coefficients using trait means for progeny in each population under LN and HN conditions.

QTL Mapping

Genotyping

Genomic DNA was isolated from seedlings of RILs, DHs, and parents at the three-leaf stage using an LGC 218 Genomics Oktopure™ robotic extraction platform with sbeadex™ magnetic microparticle 219 reagent kits at the USDA-ARS Eastern Regional Small Grains Genotyping Center. Genotyping-by-sequencing was performed using an Illumina HiSeq 2500 following the protocol of DNA digestion with restriction enzymes, PstI and MspI (Poland et al., 2012). Sequence reads were aligned to the wheat reference genome (International Wheat Genome Sequencing Consortium, 2018) using the Burrows-Wheeler Aligner v0.7.17-r1188 (Li and Durbin, 2009). The GBS derived data was used to generate consensus calls from replicated parents and filtered to retain SNPs with less than 20 % missing data frequencies and less than 5 % heterozygous calls using Beagle v. 5.0 (Browning, 2018). Missing data was not imputed and

only SNPs with differing parental homozygous calls were retained. The remaining genomic DNA was used to identify polymorphic markers from a set of 116 SNP and simple sequence repeat (**SSR**) markers used in routine screening of the uniform and regional breeding nurseries (**Supplemental Table 2.2**).

Construction of Genetic Maps and Detection of QTL

Polymorphic markers were used to construct high-density linkage maps for the YT×151 and YT×52 populations. The SNP calls were converted to an ABH parent-based format for the construction of linkage maps in JoinMap v. 4.0 (Van Ooijen, 2006). Within JoinMap, map distance was determined using the Kosambi mapping function (Kosambi, 2016) and linkage groups were constructed based on a minimum logarithm of odds (**LOD**) threshold value of 3.0. Linkage maps retained all markers to better facilitate future validation studies and meta-analysis. IciMapping v. 4.1.0 (Meng et al., 2015) was used to identify QTL in both populations using composite interval mapping for traits under LN and HN conditions at each environment. The critical threshold to declare a QTL significant ($P < 0.05$) was based on 1000 permutations (Doerge and Churchill, 1996) with a LOD value of 2.5 for traits within each N-environment. Linkage maps were drawn using MapChart v. 2.2.3 (Voorrips, 2002).

Results

Phenotypic Variation and Trait Correlations

ANOVAs for the replicated parental lines, N rates, and their interactions within each environment are described in **Supplemental Tables 2.3 and 2.4**. Variance of parents and

progeny for all traits at each N-environment for the YT×151 and YT×52 populations are reported in **Supplemental Tables 2.5 and 2.6**, respectively. Phenotypic variation was observed in the two populations under each N rate for a majority of the yield and N-related. The parents in both populations expressed similar plant heights, anthesis dates, and maturity dates within each testing environment despite variation at the *Ppd-D1* loci in the YT×52 population (**Supplemental Table 2.2**). Parents of the YT×151 population differed significantly in NUE under LN supplies in all testing environments, while their NUE was not statistically different under the HN rate (**Figure 2.1**). The YT×52 population expressed a similar trend but the parents only differed significantly under the LN supply in the 18NK environment. All progeny reached anthesis over a seven and 10 day period in the YT×151 and YT×52 populations, respectively (**Supplemental Tables 2.5 and 2.6**).

In both populations, NUE was strongly and positively associated with AGBM, harvest index, and N uptake efficiency under LN and HN rates (**Tables 2.2 and 2.3**). Within the YT×52 population, NUE was significantly correlated with grain N content (LN: r = -0.32, P < 0.001; HN: r = -0.32, P < 0.001) and NUtE (LN: r = -0.31, P < 0.001; HN: r = -0.38, P < 0.001). Under HN rates, lodging was associated with grain yield (r = -0.40, P < 0.001), grain N content (r = 0.24, P < 0.01), harvest index (r = -0.37, P < 0.001), NUtE (r = -0.21, P < 0.05), and plant height (r = 0.23, P < 0.01) in the YT×151 population, while the trait was only associated with plant height (r = 0.44, P < 0.001) in the YT×52 population. Nitrogen uptake efficiency was negatively correlated with NUtE under LN and HN rates in both populations. However, this association was greater for RILs in the YT×151 population under LN (r = -0.78, P < 0.001) and HN (r = -0.82, P < 0.001) rates compared to DHs in the YT×52 population under LN (r = -0.52, P < 0.001) and HN (r = -0.47, P < 0.001) conditions.

QTL Analysis

Linkage Map Construction

After filtering for low-quality markers, linkage maps comprised of 3,918 markers spanning 2,962.8 cM and 3,147 markers spanning 2,491.7 cM in the YT×151 and YT×52 populations, respectively (**Table 2.4**). Within the YT×151 population, chromosome 6B had the lowest coverage (7.8 cM) despite being comprised of 92 genetic markers, while 7A had the greatest coverage (238.6 cM) and consisted of 366 markers. The YT×52 population's shortest linkage map on chromosome 4D consisted of 12 markers spanning 17.0 cM and its longest was on chromosome 5A having 249 markers that spanned 227.4 cM. Marker densities ranged from 4.18 (3D) to 0.08 (6B) cM per marker for the YT×151 and from 2.69 (3D) to 0.32 (1A) cM per marker for the YT×52 population. The YT×151 linkage map had gaps greater than 30.0 cM on 3B, 3D, and 4A, while the longest gap in the YT×52 population was on 5D (28.0 cM). In both populations, the A, B, and D genomes had good marker density and high coverage.

QTL in the YT×151 Population

The YT×151 linkage map was employed to detect QTL with a LOD score greater than 2.5 for 11 traits. A total of 54 QTL were identified for traits in one N-environment (**Supplemental Table 2.7**) and 12 QTL that were reproducible in two or more N-environments (**Table 2.5**). The combined 66 QTL mapped to the A (24), B (17), and D (25) genomes. Reproducible QTL mapped to chromosomes 1D, 2B, 3B, 4A, 5A, 6A, and 7D individually explained 4.7 to 27.5 % of the phenotypic variation. The LOD values among reproducible QTL ranged from 2.61 to 21.85 for *QAgbm.151-2B* in 17WR-LN and 18WR-HN, respectively. The

QTL on 3B (*QHi.151-3B*) was located within 11 cM of the SNP, IWA4755, conferring resistance to Fusarium head blight (**FHB**, caused by the fungal pathogen *Fusarium graminearum*) that was identified in the wheat cultivar 'Bess,' PI 642794 (McKendry et al., 2007). The reproducible QTL including *QAgbm.151-2B, QNue.151-1D, QNue.151-7D,* and *QMd.151-5A* were not located near any of the 116 haplotype markers with known trait associations.

Figure 2.2 illustrates partial linkage maps of the QTL on 4A and 6A. The reproducible QTL including *QNupe.151-4A* and *QNute.151-4A* clustered around the FHB 4A locus identified in 'NC-Neuse,' PI 633037 (Murphy et al., 2004; Petersen et al., 2016). *QNue.151-4A* and *QHi.151*-4A mapped to a position more than 15 cM away from the FHB 4A locus. The other QTL cluster centralized around the marker IWA4036 which is known to associate with FHB 6A locus, identified in NC-Neuse (Petersen et al., 2016). *QAgbm.151-6A, QNue.151-6A,* and *QMd.151-6A* were within 18 cM of the QTL for FHB resistance on 6A. Additionally, the QTL on 4A and 6A were associated with non-reproducible trait QTL (**Supplemental Table 2.7**). The remaining two reproducible NUE QTL (*QNue.151-1D* and *QNue.151-7D*) are illustrated in **Figure 2.3**. The reproducible NUE QTL on 1D colocalized with the non-reproducible QTL, *QAgbm.151-1D,* in the 17WR-LN environment and the reproducible NUE QTL on 7D colocalized with non-reproducible QTL, *QHgt.151-7D* and *QHi.151-7D*, in the 17WR-LN and 18WR-LN environments, respectively (**Supplemental Table 2.7**).

QTL in the YT×52 Population

The YT×52 population linkage maps were employed to detect QTL in the same eight N-environments as the YT×151 population. Within this population, 64 QTL were identified with a

LOD score greater than 2.5 (**Table 2.6** and **Supplemental Table 2.8**) and mapped to the A (25), B (19), and D (20) genomes. However, only eight of these QTL were reproducible in two or more N-environments and were located on chromosomes 1A, 2D, 4A, and 7A (**Table 2.6**). The reproducible QTL individually explained 4.9 to 52.1 % of the phenotypic variation, while LOD values ranged from 2.57 for *QHgt.52-2D* in 18NK-LN to 26.50 for *QAd.52-2D* in 17WR-HN. *QNue.52-7A* and *QHgt.51-1A* were not located near any of the 116 tested SNP and SSR markers with known trait associations and explained 4.9 to 7.7 % and 10.7 to 13.2 % of the phenotypic variation, respectively.

Figure 2.4 illustrates the reproducible QTL clusters on chromosomes 2D and 4A. The first cluster was found within 14 cM of the photoperiod response locus, *Ppd-D1* (Beales et al., 2007), and was comprised of *QAgbm.vt-2D*, *QNue.vt-2D*, *QAd.vt-2D*, *QHgt.vt-2D*. Similar to the results of the YT×151 population, a second QTL cluster was within 10 cM of the FHB 4A locus. The 4A cluster consisted of *QAgbm.vt-4A*, *QNue.vt-4A*, and *QNute.vt-4A*. Additionally, both QTL clusters in the YT×52 population were linked to trait QTL significant in only one testing environment. A third reproducible NUE QTL, *QNue.52-7A*, was identified in the YT×52 population (**Figure 2.5**). However, this QTL did not colocalize with any non-reproducible QTL observed in this population (**Supplemental Table 2.8**).

Effects of QTL Combinations on NUE

Reproducible QTL and their combinations were assessed for NUE under LN and HN conditions. Within the YT×151 population, the presence of *QNue.151-1D* and *QNue.151-6A* significantly increased NUE over testing environments under LN by 1.8 and 1.7 kg kg^{-1}, respectively (**Table 2.7**). However, the QTL on 7D, *QNue.151-7D*, produced a significant

increase in NUE under both LN (1.8 kg kg^{-1}) and HN (1.2 kg kg^{-1}) supplies. Nitrogen use efficiency further increased with two or three combined QTL from the YT×151 population. The YT×52 QTL, *QNue.52-2D*, mapped near *Ppd-D1* did not improve NUE under either N rate - indicating a significant influence due to testing environment (**Table 2.8**). The second positive QTL, *QNue.52-7A*, significantly increased NUE under the reduced N rate and resulted in higher NUE than its combination with *QNue.52-2D*. Finally, the FHB resistance QTL on 4A was associated with decreased NUE in both populations and, therefore, this repulsion linkage will have a negative effect on improving NUE in the presence of the FHB-4A QTL. (**Table 2.7** and **Table 2.8**).

Discussion

Trait Variation and Associations in the Mapping Populations

Transgressive segregates were identified for all traits evaluated in both mapping populations. Furthermore, trait means within N-environment were frequently intermediate of the parents, thus suggesting polygenic inheritance of a majority of the studied yield and N traits. Habash et al. (2007) and Fontaine et al. (2009) also noted non-Mendelian inheritance for NUE traits in bi-parental wheat populations grown under similar N conditions. It therefore stands to reason that the parents of the YT×151 and the YT×52 populations possess both favorable and unfavorable alleles for yield and N traits. Additional sources of beneficial NUE alleles likely exist within the eastern United States' soft red winter wheat germplasm as genotypic variation from unrelated lineages has been reported in recent field (Hitz et al., 2017) and greenhouse

(Tamang et al., 2017) studies of N response. Wheat lines from these panels may thus serve as a basis for future NUE mapping studies.

The present investigation also decomposed grain yield into harvest index and AGBM and thereby identified a strong association between AGBM and NUE under LN and HN conditions in both populations. A similar finding was reported by Reynolds et al. (2012) who suggested that improving source capacity will be required to continue yield improvement as breeders approach the theoretical maximum harvest index. In a previous investigation of 225 European wheat lines, AGBM was further shown to be highly heritable (h^2 = 0.79) over environments (Cormier et al., 2013), but traditional phenotyping proved to be exceptionally laborious. Frels et al. (2018) sought to overcome this constraint by identifying high-throughput vegetative indices that were predictive of AGBM, enabling more efficient phenotyping. While future work is required to improve the predictive ability of current AGBM models, the trait appears to be a suitable target for NUE breeding in winter wheat.

The present study also identified a strong relationship between NUE and its component trait, NUpE, under LN and HN rates in both populations, while the other component trait, NUtE, was only associated with NUE in the YT×52 population. Several investigations of wheat (Barraclough et al., 2010; Dhugga and Waines, 1989; Latshaw et al., 2016; Le Gouis et al., 2000) previously weighed the contribution of each component trait to the variation in NUE and generally concluded that: 1) improvements in both traits are necessary to improve NUE; and 2) the influence of each trait is dependent on the environment, management practices, and genetic material being tested. It is therefore up to wheat breeders within specific regions to identify traits that most limit NUE and those for which phenotypic assessment is feasible.

Identification of QTL for NUE Traits

As expected, the GBS genotyping platform increased the number of polymorphic SNPs available in the present study compared to previous investigations that utilized the 90K-SNP array for soft red winter wheat (Carpenter et al., 2017; Kolmer et al., 2018). The use of GBS increases marker density through a whole genome scan approach as opposed to screening a select number of potential polymorphisms. The present study detected similar numbers of SNPs, chromosome coverage, and marker densities as a previous bi-parental population of winter wheat that utilized GBS (Hussain et al., 2017). However, map length was exceptionally low on chromosome 6B in the YT×151 population, thus indicating reduced allelic diversity on 6B between the two parents.

Previous wheat NUE mapping studies have identified QTL on every chromosome for N traits (Cormier et al., 2014; Laperche et al., 2007; Mahjourimajd et al., 2016; Sun et al., 2013; Zheng et al., 2010). It therefore becomes challenging to identify which of these QTL have potential implication for marker-assisted breeding. In response to this challenge, Quraishi et al. (2011) conducted a meta-analysis of QTL for NUE to identify 11 major chromosomal regions linked to N response. *QNue.151-6A* identified in the present study was located near the QTL on 6A described in the aforementioned study. Additional QTL on 6A were reported to be associated with NUtE and kernel weight per spike (Xu et al., 2014) and kernel weight (Cui et al., 2016) in previous studies of N response. The authors further linked this QTL on 6A to *TaGW2*, which influences grain size and weight (Simmonds et al., 2016; Su et al., 2011). The IWA4036 marker located near the reproducible QTL for NUE on chromosome 6A flanks a known FHB resistance locus (Petersen et al., 2016). However, the other flanking marker, IWA3483, was not segregating in the population and may therefore indicate that the genetic regions on 6A

governing FHB resistance and improved NUE and N traits are different in the YT×151 population (**Supplemental Table 2.2**).

The QTL, *QNue.151-1D,* inherited from Yorktown was shown to have a strong effect on increasing NUE in multiple N-environments and AGBM in one N-environment within the VA05W-151 background. Bordes et al. (2013) identified a QTL with close proximity to *QNue.151-1D* in bread wheat. The high-molecular-weight glutenin subunit gene, *Glu-D1*, was located more than 25 cM from this QTL and, therefore, is not likely the candidate gene (D'ovidio et al., 1995). Sun et al. (2013) also reported a QTL on 1D associated with root and shoot weight in wheat seedlings grown in hydroponic culture at a similar mapping interval as the *QNue.151-1D* and, therefore, may indicate a role in seedling vigor. *QNue.52-7A* was found within the same mapping interval as QTL previously linked to grain yield and spikes per m^{-2} (Monostori et al., 2017) and a QTL associated with NUtE and spikes per m^{-2} (Xu et al., 2014). A majority of the QTL identified in the present study validated findings from previous studies, yet *QNue.151-7D* was not commonly observed in recent investigations of N response and may represent a novel QTL. While previous mapping studies have identified QTL within proximity to *QNue.151-7D*, they are primarily associated with a vernalization allele located more than 50 million nucleotides from the presently identified QTL (Bogard et al., 2011).

QTL Co-Segregating with Known Genes

In addition to evaluation for parental variation in N response, the parents shared similar alleles for vernalization (*Vrn-A1*, *Vrn-B1*, and *Vrn-D1*), photoperiod (*Ppd-A1*, *Ppd-B1*, and *Ppd-D1*), and plant height (*Rht-B1* and *Rht-D1*). However, the *Vrn-A1* and *Ppd-D1* loci differed between Yorktown and VA09W-52 and the resulting allelic variation on 2D resulted in a strong

effect QTL that explained relatively large percentages of the variation for NUE, grain yield, plant height, and anthesis date in multiple environments and N supplies. Indeed, this result is consistent with previous findings of wheat grown in the Eastern (Brasier et al., 2018) and Central (Guttieri et al., 2017) United States, Northwestern Mexico (García-Suárez et al., 2010), and Western Europe (Bogard et al., 2011). It is postulated that the presence of the *Ppd-D1* sensitivity allele confers reduced frost damage (Foulkes et al., 2004) and maintains radiation use efficiency during grain filling (Shearman et al., 2005). Worland et al. (1998) further observed strong interactions between testing environment and the *Ppd-D1* locus where near-isogenic lines (*D1a* and *D1b*) had increased or reduced grain yields (-16.0 to 28.4 %) over a 10-year period. Similarly, the over environment effects of the *Ppd-D1* allele in the present study were not significant under LN or HN conditions despite the identification of strong effect QTL in 16WR and 17WR.

The negative effects on NUE conferred by the QTL identified near FHB 4A was much higher than expected upon development of the mapping populations. Indeed, associations between FHB resistance and yield *per se* is not uncommon as previous investigations found a small negative impact on grain yield (Salameh et al., 2011) and grain quality (McCartney et al., 2007) conferred through a FHB resistance locus on chromosome 5A. The known yield penalties associated with FHB resistance have discouraged some winter wheat breeders from utilizing exotic sources of resistance including that of spring wheat variety ‘Sumai-3’ (Balut et al., 2013; Steiner et al., 2017; Wilde et al., 2007). However, the mechanisms governing a reduction in NUE by the QTL linked to FHB 4A in the present study requires further investigation to determine if this linkage can be broken.

Implications for Breeding

The QTL identified in this study that were not linked to the FHB 4A or *Ppd-D1* may merit use in marker-assisted-breeding programs as they were not associated with major physiological traits or known sources of disease resistance. Genetic markers for high NUE were previously reported near *QNue.151-1D*, *QNue.151-6A*, and *QNue.52-7A* and, therefore, offer the most potential for immediate marker deployment. The other QTL, *QNue.151-7D*, requires further investigation to determine its value in breeding for N response, which will require screening in unrelated populations. All QTL identified in this study will also benefit from introgression into diverse backgrounds to gauge their overall breeding value.

Acknowledgments

Funding for this project was provided by the Virginia Small Grains Board. The authors would like to thank John Seago, Anthony Christopher, Jon Light, Michelle Lee, and William Myers for assistance collecting field data and Steve Nagle for providing technical assistance in the analysis of tissue N content.

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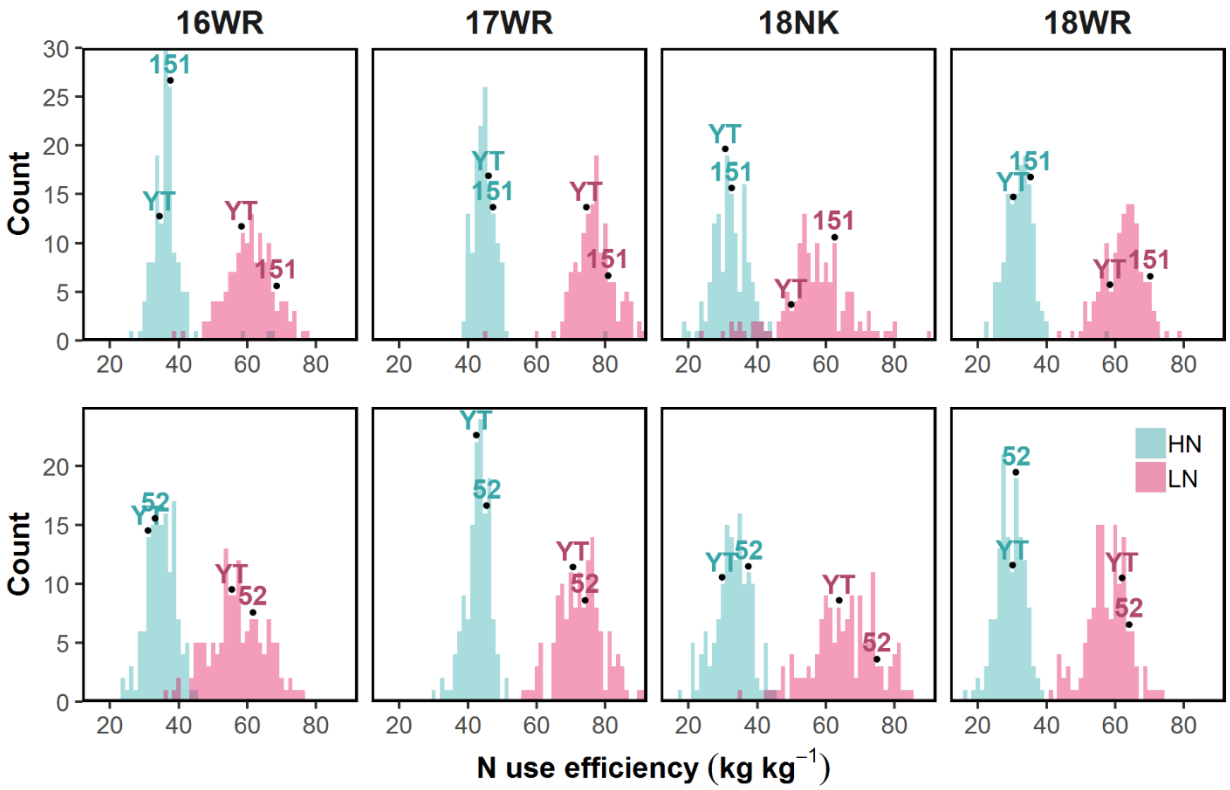

Figure 2.1. Nitrogen use efficiency histograms of wheat parents and progeny from the Yorktown × VA05W-151 (top) and Yorktown × VA09W-52 (bottom) populations grown under low (LN) and high (HN) N rates in each testing environment. Parent means at each N rate are shown for Yorktown (YT), VA05W-151 (151), and VA09W-52 (52). Detailed statistical analysis of parents and progeny in Supplemental Tables 2.5 and 2.6.

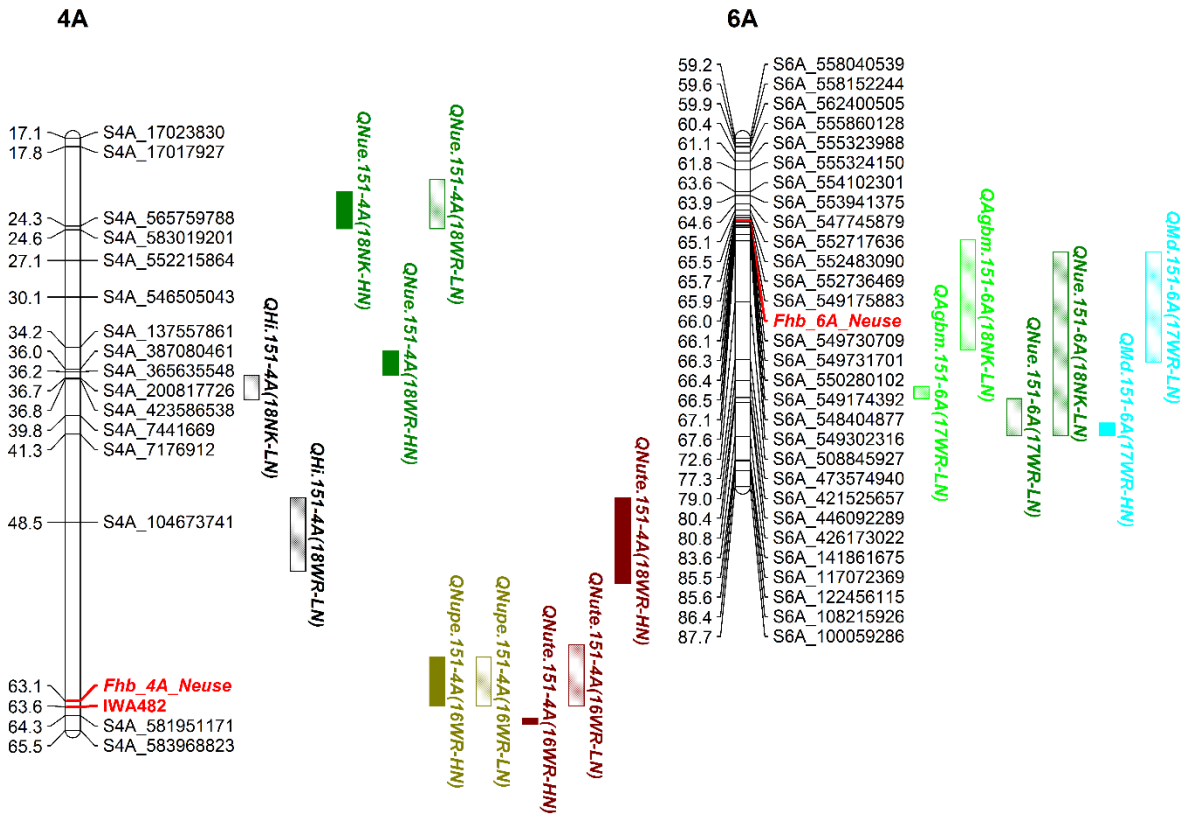

Figure 2.2. Partial linkage maps of QTL clusters on chromosomes 4A and 6A in the Yorktown × VA05W-151 wheat population. Blocks represent trait confidence intervals.

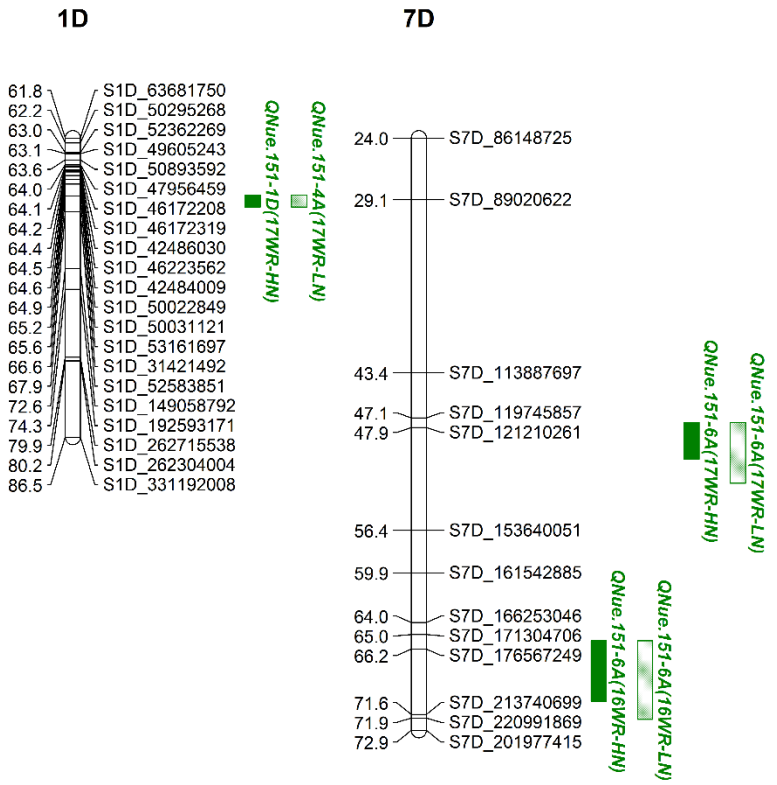

Figure 2.3. Partial linkage maps of reproducible QTL clusters on chromosomes 1D and 7D in the Yorktown × VA05W-151 wheat population. Blocks represent trait confidence intervals.

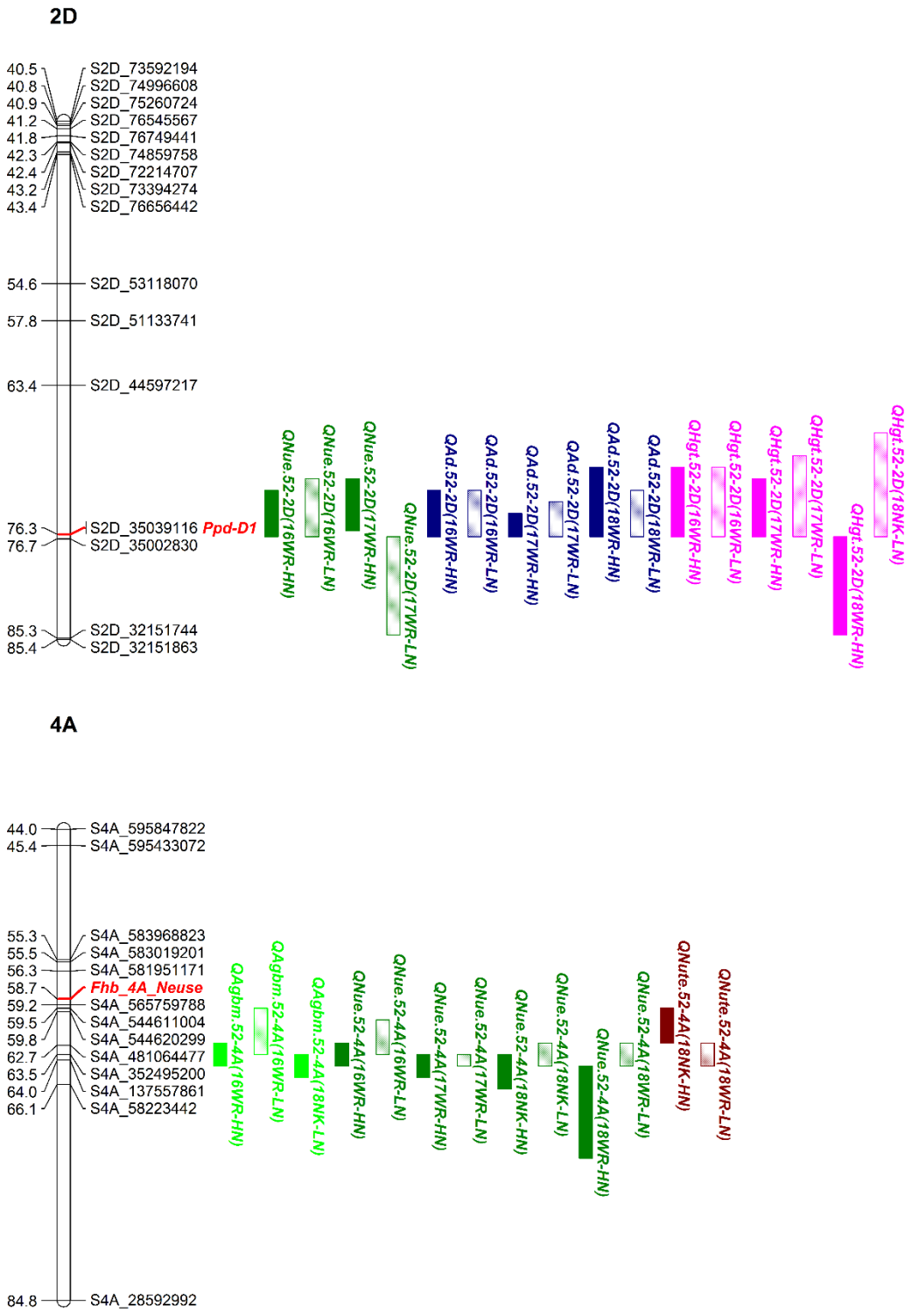

Figure 2.4. Partial linkage maps of QTL clusters on chromosomes 2D and 4A in the Yorktown × VA09W-52 wheat population. Blocks represent trait confidence intervals.

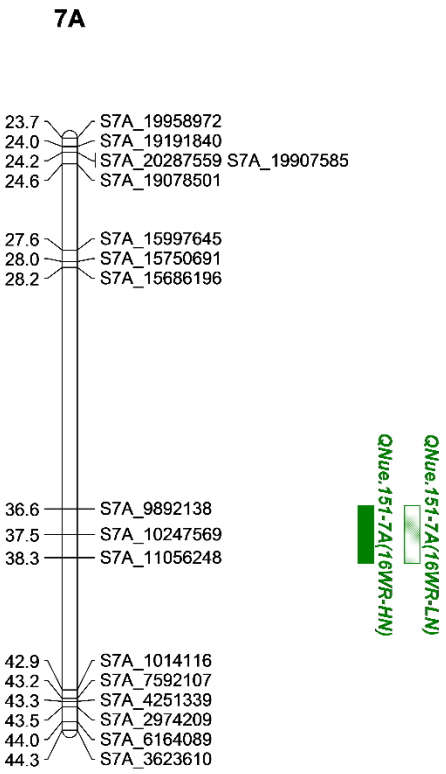

Figure 2.5. Partial linkage maps of reproducible QTL clusters on chromosome 7A in the Yorktown × VA09W-52 wheat population. Blocks represent trait confidence intervals.

Table 2.1. Description of environments (Env.) used to test mapping populations.

Location	Season	Env.	Soil series	Soil type	Cumm. Precip.†	Cumm. GDD†	NO_3^- N‡	NH_4^+ N‡	Total N§	Total C§
					mm	°C	mg kg^{-1}		mg g^{-1}	
Warsaw	15-16	16WR	Kempsville	Loam	645	2,905	5.9	1.1	0.5	4.1
	16-17	17WR	Kempsville	Loam	752	2,603	3.9	1.6	0.6	4.4
	17-18	18WR	Kempsville	Loam	1,002	3,379	7.1	1.6	0.5	5.7
New Kent	17-18	18NK	Altavista	Sandy loam	938	3,199	22.0	6.6	1.0	11.2

† Cumulative precipitation and growing degree days (GDD) from planting to harvest.
‡ Soil nitrate (NO_3^-) and ammonium (NH_4^+) were determined by analysis of KCl filtrates on a Lachat 8500 Flow Injection Analyzer (Knepel, 2001; Hofer, 2001).
§ Total N and organic C determined by combustion analysis.

Table 2.2. Trait correlations under low (bottom left) and high (top right) N rates in the Yorktown × VA05W-151 wheat population. Pearson's correlation coefficients calculated from means of the 136 RILs over testing environments.

	GY†	GNC	AGBM	HI	NUE	NUpE	NUtE	AD	HGT	LDG
GY		-0.23**	0.60***	0.48***	0.99***	0.36***	0.16	0.02	0.02	-0.40***
GNC	-0.12		-0.00	-0.28**	-0.23**	0.31***	-0.51***	0.10	0.07	0.24**
AGBM	0.74***	0.01		-0.39***	0.60***	0.66***	-0.32***	-0.09	0.20*	-0.10
HI	0.46***	-0.25**	-0.15		0.48***	-0.29***	0.53***	0.10	-0.19*	-0.37***
NUE	0.98***	-0.13	0.80***	0.45***		0.35***	0.15	0.02	0.02	-0.40***
NUpE	0.45***	0.30***	0.58***	-0.10	0.46***		-0.82***	0.04	0.26**	-0.01
NUtE	0.06	-0.47***	-0.16	0.38***	0.08	-0.78***		-0.04	-0.26**	-0.21*
AD	-0.03	-0.05	-0.02	-0.01	0.01	0.06	-0.05		0.08	-0.11
HGT	0.18*	0.07	0.29***	-0.20*	0.14	0.28***	-0.17	0.11		0.23**
LDG	0.00	0.15	0.09	-0.09	0.01	0.11	-0.11	-0.05	0.31***	

† Trait abbreviations for grain yield (YLD), grain N content (GNC), above-ground biomass (AGBM), harvest index (HI), N use efficiency (NUE), N uptake efficiency (NUpE), N utilization efficiency (NUtE), anthesis date (AD), plant height (HGT), and lodging (LDG).

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table 2.3. Trait correlations under low (bottom left) and high (top right) N rates in the Yorktown × VA09W-52 wheat population. Pearson's correlation coefficients calculated from means of the 138 DHs over testing environments.

	GY†	GNC	AGBM	HI	NUE	NUpE	NUtE	AD	HGT	LDG
GY		-0.33***	0.49***	0.34***	0.99***	0.46***	0.36***	0.11	0.09	-0.01
GNC	-0.32***		-0.10	-0.16	-0.32***	0.21*	-0.47***	-0.19*	-0.28***	0.04
AGBM	0.70***	-0.12		-0.24**	0.49***	0.71***	-0.01	0.05	0.06	0.01
HI	0.50***	-0.31***	-0.24**		0.34***	-0.16	0.42***	-0.18*	-0.12	-0.07
NUE	0.99***	-0.32***	0.70***	0.50***		0.46***	0.38***	0.09	0.08	-0.02
NUpE	0.48***	0.36***	0.50***	0.03	0.48***		-0.47***	-0.03	-0.10	-0.01
NUtE	0.30***	-0.45***	0.12	0.29***	0.31***	-0.52***		0.08	0.18*	-0.04
AD	0.03	-0.11	0.13	-0.14	0.02	-0.07	0.07		0.42***	0.04
HGT	0.21*	-0.10	0.29***	-0.06	0.21*	0.05	0.10	0.39***		0.44***
LDG	0.13	0.05	0.09	0.04	0.12	0.12	-0.05	0.05	0.30***	

† Trait abbreviations for grain yield (YLD), grain N content (GNC), above-ground biomass (AGBM), harvest index (HI), N use efficiency (NUE), N uptake efficiency (NUpE), N utilization efficiency (NUtE), anthesis date (AD), plant height (HGT), and lodging (LDG).

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table 2.4. Description of the novel genetic linkage maps for the Yorktown × VA05W-151 and Yorktown × VA09W-52 wheat populations.

Chromosome	Yorktown × VA05W-151				Yorktown × VA09W-52			
	No. of markers	Coverage	Average Spacing	No. of linkage groups	No. of markers	Coverage	Average Spacing	No. of linkage groups
		cM				cM		
1A	67	68.0	1.01	3	202	65.3	0.32	1
1B	264	132.6	0.50	2	152	107.6	0.71	3
1D	111	126.1	1.14	2	74	91.2	1.23	2
2A	367	218.3	0.59	3	255	117.3	0.46	3
2B	134	38.0	0.28	2	281	145.8	0.52	2
2D	138	160.6	1.16	1	61	97.3	1.60	2
3A	216	175.2	0.81	3	240	184.3	0.77	1
3B	300	218.0	0.73	2	299	161.3	0.54	2
3D	48	200.4	4.18	4	30	80.8	2.69	2
4A	268	188.9	0.70	3	65	128.6	1.98	2
4B	159	110.4	0.69	2	108	92.5	0.86	1
4D	30	100.5	3.35	2	12	17.0	1.42	1
5A	180	128.1	0.71	3	249	227.4	0.91	1
5B	273	166.1	0.61	1	245	154.8	0.63	2
5D	70	146.3	2.09	3	56	150.7	2.69	2
6A	325	175.3	0.54	1	183	93.7	0.51	2
6B	92	7.8	0.08	2	65	59.4	0.91	2
6D	28	60.6	2.16	2	70	78.8	1.13	2
7A	366	238.6	0.65	3	241	173.6	0.72	3
7B	372	204.6	0.55	3	187	129.3	0.69	2
7D	110	98.4	0.89	3	72	135.0	1.88	2
A genome	1,789	1,192.4	0.72	19	1,435	990.2	0.81	13
B genome	1,594	877.5	0.49	14	1,337	850.7	0.69	14
D genome	535	892.9	2.14	17	375	650.8	1.81	13
Total	3,918	2,962.8	1.12	50	3,147	2,491.7	1.10	40

Table 2.5. Quantitative trait loci (QTL) associated with N and agronomic traits in 2 or more N-environments in the Yorktown × VA05W-151 wheat population.

Trait	QTL	Chr.†	N-Env.‡	Pos.	Left marker	Right marker	LOD§	PVE¶	Add#
								%	
AGBM††	*QAgbm.151-2B*	2B	17WR-HN	2	S698090811	S699106811	2.86	4.7	-21.19
			17WR-LN	1	S699106811	S701094516	2.61	6.2	-22.01
			18WR-HN	3	S687334185	S683005457	21.85	27.5	-134.54
	QAgbm.151-6A	6A	17WR-LN	80	S421525657	S446092289	4.91	11.7	31.12
			18NK-LN	72	S549302316	S508845927	4.84	13.5	85.26
HI	*QHi.151-3B*	3B	16WR-LN	71	S418440403	S452107771	3.56	13.1	0.01
			18WR-LN	80	S58771568	S520444036	2.76	7.3	-0.01
	QHi.151-4A	4A	18NK-HN	37	S423586538	S7441669	3.15	10.0	-0.01
			18WR-HN	49	S104673741	Fhb_4A_Neuse	4.75	14.6	-0.02
NUE	*QNue.151-1D*	1D	17WR-HN	67	S31421492	S52583851	7.40	17.1	1.25
			17WR-LN	67	S31421492	S52583851	8.33	18.1	2.42
	QNue.151-4A	4A	18NK-HN	24	S17017927	S565759788	4.67	13.7	-1.87
			18WR-HN	35	S137557861	S387080461	4.33	13.2	-1.46
			18WR-LN	24	S17017927	S565759788	3.37	10.3	-1.71
	QNue.151-6A	6A	17WR-LN	81	S426173022	S141861675	4.85	9.3	1.77
			18NK-LN	74	S508845927	S473574940	3.18	8.7	3.86
	QNue.151-7D	7D	16WR-HN	66	S171304706	S176567249	3.52	12.6	1.07
			16WR-LN	67	S176567249	S213740699	2.80	7.5	1.78
			17WR-HN	48	S121210261	S153640051	5.25	10.9	0.99
			17WR-LN	48	S121210261	S153640051	2.86	5.3	1.29
NUpE	*QNupe.151-4A*	4A	16WR-HN	63	S104673741	Fhb_4A_Neuse	3.91	12.3	-0.18
			16WR-LN	63	S104673741	Fhb_4A_Neuse	10.84	12.7	-0.59
NUtE	*QNute.151-4A*	4A	16WR-HN	65	S581951171	S583968823	3.61	11.2	-2.74
			16WR-LN	62	S104673741	Fhb_4A_Neuse	10.95	20.0	-6.51
			18WR-HN	49	S104673741	Fhb_4A_Neuse	3.02	9.5	-1.55
MD	*QMd.151-5A*	5A	17WR-HN	7	S511963634	S544461754	4.55	11.9	-0.52
			17WR-LN	9	S511963634	S544461754	5.63	11.2	-0.58
	QMd.151-6A	6A	17WR-HN	84	S141861675	S117072369	6.91	15.8	0.58
			17WR-LN	84	S141861675	S117072369	7.85	14.8	0.65

† Chromosome (Chr.).

‡ Numbers indicate years 2015–2016 (16), 2016-2017 (17), and 2017-2018 (18); letters indicate locations Warsaw (WR) and New Kent (NK); low (LN) and high (HN) N rates within environment.

§ Logarithm of odds.

¶Percentage of phenotypic variation explained by the QTL.

#Level of additivity. A positive sign indicates that alleles from Yorktown increased the trait value and a negative sign indicates that alleles from VA05W-151 increased the trait value.

††Trait abbreviations for above-ground biomass (AGBM), harvest index (HI), N use efficiency (NUE), N uptake efficiency (NUpE), N utilization efficiency (NUtE), and maturity date (MD). Grain yield was excluded from the results as its QTL were identical to those found for NUE.

Table 2.6. Quantitative trait loci (QTL) associated with N and agronomic traits in two or more N-environments in the Yorktown × VA09W-52 wheat population.

Trait	QTL	Chr.†	N-Env.‡	Pos.	Left marker	Right marker	LOD§	PVE¶	Add#
								%	
AGBM††	*QAgbm.52-4A*	4A	16WR-HN	64	S352495200	S137557861	4.70	14.7	-73.15
			16WR-LN	62	S544620299	S481064477	4.40	10.0	-45.95
			18NK-LN	64	S352495200	S137557861	3.67	11.6	-57.96
NUE	*QNue.52-2D*	2D	16WR-HN	76	S44597217	S35039116	6.75	13.6	1.64
			16WR-LN	76	S44597217	S35039116	3.86	8.6	2.49
			17WR-HN	76	S44597217	S35039116	3.60	9.5	1.17
			17WR-LN	82	S35002830	S32151744	3.22	8.1	2.03
	QNue.52-4A	4A	16WR-HN	63	S481064477	S352495200	12.09	26.2	-2.29
			16WR-LN	63	S481064477	S352495200	8.35	20.0	-3.82
			17WR-HN	64	S352495200	S137557861	6.79	18.6	-1.64
			17WR-LN	64	S352495200	S137557861	6.55	15.7	-2.83
			18NK-HN	65	S137557861	S58223442	4.33	11.6	-1.86
			18NK-LN	64	S352495200	S137557861	8.01	23.9	-4.79
			18WR-HN	67	S58223442	S28592992	9.44	19.0	-1.74
			18WR-LN	63	S481064477	S352495200	10.75	24.0	-2.83
	QNue.52-7A	7A	16WR-HN	38	S10247569	S11056248	2.63	4.9	0.97
			16WR-LN	38	S10247569	S11056248	3.43	7.7	2.31
NUtE	*QNute.52-4A*	4A	18NK-HN	60	S544620299	S481064477	2.83	9.1	-0.94
			18WR-LN	63	S481064477	S352495200	2.68	6.6	-1.01
AD	*QAd.52-2D*	2D	16WR-HN	76	S44597217	S35039116	25.15	52.1	1.61
			16WR-LN	76	S44597217	S35039116	20.09	49.2	1.42
			17WR-HN	76	S44597217	S35039116	26.50	50.8	1.40
			17WR-LN	76	S44597217	S35039116	19.55	46.2	1.23
			18WR-HN	76	S44597217	S35039116	7.33	21.2	0.64
			18WR-LN	76	S44597217	S5039116	16.90	37.8	0.98
HGT	*QHgt.52-1A*	1A	18NK-HN	63	S367438005	S261248932	4.96	13.2	-2.62
			18NK-LN	63	S367438005	S261248932	3.91	10.7	-2.16
	QHgt.52-2D	2D	16WR-HN	75	S44597217	S35039116	8.83	18.4	3.24
			16WR-LN	76	S44597217	S35039116	7.89	19.8	3.28
			17WR-HN	76	S44597217	S35039116	13.85	25.1	2.68
			17WR-LN	76	S44597217	S35039116	5.18	12.8	2.31
			18NK-LN	83	S35002830	S32151744	2.57	7.6	1.83
			18WR-HN	76	S44597217	S35039116	5.27	14.9	1.87

† Chromosome (Chr.).

‡ Numbers indicate years 2015–2016 (16), 2016-2017 (17), and 2017-2018 (18); letters indicate locations Warsaw (WR) and New Kent (NK); low (LN) and high (HN) N rates within environment.

§ Logarithm of odds.

¶Percentage of phenotypic variation explained by the QTL.

#Level of additivity. A positive sign indicates that alleles from Yorktown increased the trait value and a negative sign indicates that alleles from VA09W-52 increased the trait value.

††Trait abbreviations for above-ground biomass (AGBM), N use efficiency (NUE), N utilization efficiency (NUtE), anthesis date (AD), and plant height (HGT). Grain yield was excluded from the results as its QTL were identical to those found for NUE.

Table 2.7. Single and combination QTL effects on NUE (kg grain kg N^{-1}) for RILs in the Yorktown ×VA05W-151 wheat population over four testing environments.

Single Loci	Low N	P.I.†	High N	P.I.	Combinations	Low N	P.I.	High N	P.I.
	kg kg N^{-1}	%	kg kg N^{-1}	%		kg kg N^{-1}	%	kg kg N^{-1}	%
QNue.151-1D					*1D + 6A*				
a (75)‡	64.8 a§	2.9	36.5 a	2.2	*aa* (46)	65.5 a	4.1	36.5 a	2.5
b (52)	63.0 b		35.7 a		*bb* (23)	62.9 b		35.6 a	
QNue.151-4A					*1D + 7D*				
a (91)	63.5 b	-3.3	35.7 b	-4.2	*aa* (34)	65.7 a	5.9	37.1 a	5.7
b (36)	65.6 a		37.2 a		*bb* (22)	62.0 b		35.1 b	
QNue.151-6A					*6A + 7D*				
a (78)	64.7 a	2.7	36.2 a	0.8	*aa* (32)	66.2 a	5.6	36.7 a	4.6
b (48)	63.0 b		35.9 a		*bb* (19)	62.7 b		35.1 b	
QNue.151-7D					*1D + 6A + 7D*				
a (64)	65.1 a	2.8	36.8 a	3.3	*aaa* (19)	66.6 a	5.0	36.9 a	4.2
b (59)	63.3 b		35.6 b		*bbb* (9)	63.4 b		35.4 b	

† Percent increase (P.I.) conferred through the ‘a’ allele.

‡ The ‘a’ and ‘b’ alleles are inherited from Yorktown and VA05W-151, respectively. Number of individuals per allele provided in parentheses.

§ The LSD at $P \leq 0.05$ is used to compare allele groupings within N rates over four testing environments; means within a single or combination of QTL followed by the same letter are not significantly different.

Table 2.8. Single and combination QTL effects on NUE (kg grain kg N^{-1}) for DHs in the Yorktown × VA09W-52 population over four testing environments.

Single Loci	Low N	P.I.†	High N	P.I.
QNue.52-2D	kg kg N^{-1}	%	kg kg N^{-1}	%
a (83) ‡	63.5 a§	1.0	35.2 a	2.0
b (53)	62.9 a		34.5 a	
QNue.52-4A				
a (56)	59.6 b	-10.2	33.0 b	-9.7
b (82)	65.7 a		36.2 a	
QNue.52-7A				
a (61)	64.3 a	3.0	35.5 a	3.2
b (63)	62.4 b		34.4 b	

Combination	Low N	P.I.	High N	P.I.
2D + 7A	kg kg N^{-1}	%	kg kg N^{-1}	%
aa (38)	64.1 a	4.7	35.5 a	5.3
bb (22)	61.2 b		33.7 b	

† Percent increase (P.I.) conferred through the 'a' allele.

‡ The 'a' and 'b' alleles are inherited from Yorktown and VA09W-52, respectively. Number of individuals per allele provided in parentheses.

§ The LSD at $P \leq 0.05$ is used to compare allele groupings within N rates over four testing environments; means within a single or combination of QTL followed by the same letter are not significantly different.

Supplemental Table 2.1. Management practices in each testing environment including product name, application of fertilizer or active ingredient (A.I.), and date of application.

Environment	Product	Application rate	Application date
16WR	Lime	2242 kg ha^{-1}	09/24/2015
	Pre-plant fertilizer	34-67-67-5S[†]	10/14/2015
	Planting	480 seeds m^{-2}	10/21/2015
	Starane™	0.21 kg A.I. ha^{-1}	12/06/2015
	GS25 UAN	Variable[‡]	02/19/2016
	Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/08/2016
	Starane™	0.15 kg A.I. ha^{-1}	03/08/2016
	GS30 UAN	Variable[‡]	03/12/2016
	Palisade EC	0.06 kg A.I. ha^{-1}	03/30/2016
	Fitness	0.12 kg A.I. ha^{-1}	03/30/2016
	Fitness	0.12 kg A.I. ha^{-1}	04/19/2016
	Prosaro	0.14 kg A.I. ha^{-1}	05/20/2016
	Harvest	-	06/22/2016
17WR	Pre-plant fertilizer	30-70-60-5S[†]	10/17/2016
	Planting	480 seeds m^{-2}	10/18/2016
	Quelex®	0.01 kg A.I. ha^{-1}	12/04/2016
	GS25 UAN	Variable[‡]	02/06/2017
	Tilt	0.07 kg A.I. ha^{-1}	03/09/2017
	Tombstone	0.03 kg A.I. ha^{-1}	03/09/2017
	GS30 UAN	Variable[‡]	03/13/2017
	Palisade EC	0.08 kg A.I. ha^{-1}	03/17/2017
	Tilt	0.07 kg A.I. ha^{-1}	04/05/2017
	Prosaro	0.14 kg A.I. ha^{-1}	04/20/2017
	Tombstone	0.03 kg A.I. ha^{-1}	04/28/2017
	Harvest	-	06/12/2017
18WR	Lime	2242 kg ha^{-1}	10/04/2017
	Pre-plant fertilizer	30-80-80-5S[†]	10/19/2017
	Planting	480 seeds m^{-2}	10/19/2017
	Harmony Extra SG®	0.02 kg A.I. ha-1	11/30/2017
	Winter fertilizer	33 kg N ha^{-1}	12/07/2017
	GS25 UAN	Variable[‡]	02/09/2018
	GS30 UAN	Variable[‡]	03/10/2018
	Palisade EC	0.08 kg A.I. ha^{-1}	03/17/2018
	Tilt	0.07 kg A.I. ha^{-1}	03/17/2018
	Boron fertilizer	0.01% (w/v) B as $Na_2B_4O_7$ - H_2O	03/27/2018
	Fitness	0.12 kg A.I. ha^{-1}	04/11/2018
	Prosaro	0.14 kg A.I. ha^{-1}	05/01/2018
	Harvest	-	06/17/2018
18NK	Pre-plant fertilizer	30-50-60[†]	09/26/2017
	Paraquat	0.4 kg A.I. ha^{-1}	10/15/2017
	Planting	480 seeds m^{-2}	10/22/2017
	Quelex®	0.01 kg A.I. ha^{-1}	12/04/2017
	Winter fertilizer	22 kg N ha^{-1}	12/08/2017
	Axial XL	0.06 kg A.I. ha^{-1}	12/18/2017
	GS25 UAN	Variable[‡]	01/21/2018
	GS30 UAN	Variable[‡]	02/14/2018
	Palisade EC	0.08 kg A.I. ha^{-1}	03/28/2018

Environment	Product	Application rate	Application date
	Prosaro	0.14 kg A.I. ha^{-1}	05/05/2018
	Harvest	-	06/21/2018

† Nitrogen (UAN), phosphorous, potassium, and sulfur re-plant fertilizer applied, respectively.
‡ Spring N application rates split applied as 67 or 134 kg N ha^{-1}.

Supplemental Table 2.2. Marker report from the Eastern Regional Small Grains Genotyping Center's suite of 116 haplotyping markers that were screened in both wheat populations.

Marker name	Parent genotype			Marker name	Parent genotype		
	YT[†]	52	151		YT	52	151
KASP_cim_RhtB1_SNP	X:X	X:X	X:X	KASP_IWA886	Y:Y	Y:Y	Y:Y
KASP_RhtD1	Y:Y	Y:Y	Y:Y	KASP_IWA3805	X:X	Y:Y	X:X
Rht-B1	B1a	B1a	B1a	Fhb_1A_Neuse	no	1A	no
Rht-D1	D1b	D1b	D1b	KASP_IWA2793	Y:Y	Y:Y	Y:Y
KASP_Ppd-A1prodel	Y:Y	Y:Y	Y:Y	KASP_IWA2900	Y:Y	X:X	Y:Y
Ppd-A1	insens	insens	insens	KASP_IWA402	Y:Y	Y:Y	Y:Y
KASP_TaPpdBJ001	X:X	X:X	X:X	KASP_IWA482	Y:Y	X:X	X:X
KASP_TaPpdBJ003	no call	no call	no call	Fhb_4A_Neuse	4A	no	no
Ppd-B1	**	**	**	KASP_IWA3483	X:X	X:X	X:X
KASP_TaPpdDD001	X:X	Y:Y	X:X	KASP_IWA4036	X:X	X:X	Y:Y
Ppd-D1	**	insens	**	Fhb_6A_Neuse	no	no	no
KASP_vrn-A1_exon4	Y:Y	X:X	Y:Y	KASP_IWA4606	X:X	Y:Y	X:X
KASP_vrn-A1_exon7	Y:Y	X:X	Y:Y	KASP_IWA5830	X:X	X:X	X:X
vrn-A1	A1	short	A1	Fhb_2B_Bess	no	no	no
vrn-A1_copy_number	A1	copy	A1	KASP_IWA4755	X:X	Y:Y	Y:Y
KASP_5A-585397830	Y:Y	Y:Y	Y:Y	KASP_IWA6381	X:X	X:X	X:X
vrn-A1_MTA	short	short	short	KASP_IWB65344	X:X	X:X	X:X
KASP_vrn-B1_AGS2K	X:X	X:X	X:X	Fhb_3B_Bess	no	no	no
vrn-B1	B1	B1	B1	KASP_Lr34	X:X	X:X	X:X
KASP_Vrn-A1_9K001	X:X	X:X	X:X	KASP_Lr34jagger	X:X	X:X	X:X
KASP_Vrn-A1b-Marq	X:X	X:X	X:X	Lr34/Yr18	no	no	no
Vrn-A1	A1	A1	A1	KASP_Lr37_A	X:X	X:X	X:X
KASP_Vrn-B1_D-I	X:X	X:X	X:X	Yr17/Lr37/Sr38	no	no	no
KASP_Vrn-B1_B	X:X	X:X	X:X	KASP_Lr9_A	Y:Y	no call	no call
KASP_Vrn-B1_C	no call	no call	no call	Lr9	Lr9	no	no
Vrn-B1	B1	B1	B1	GbFd	**	**	**
KASP_Vrn-D1-D1a	no call	no call	no call	Lr19/Sr25	no	no	no
Vrn-D1	D1	D1	D1	KASP_Sr24	no call	no call	Y:Y
KASP_Fhb1	Y:Y	Y:Y	Y:Y	Sr24/Lr24	no	no	24
KASP_snp3BS-8	X:X	X:X	X:X	KASP_Sr2_ger9_3p	X:X	X:X	X:X
Fhb1	no	no	no	Sr2	no	no	no
KASP_Fhb3Bc_6105	X:X	X:X	X:X	KASP_Sr36_8085	X:X	X:X	X:X
KASP_Fhb3Bc_8137	X:X	X:X	X:X	wmc477Fd	159	163	159
Fhb_3B_Massey	no	no	no	Sr36/Pm6	no	no	no
gwm304Fd	198	198	198	KASP_1RS_6110	X:X	X:X	X:X
wmc705Pd	160	160	162	KASP_1RS_8035	Y:Y	X:X	Y:Y
Fhb_5A_Ernie	no	no	no	KASP_IWA5194	Y:Y	Y:Y	Y:Y
Fhb_5A_Ning7840	no	no	no	1RS	1RS	no	1RS
cfd233Fd	274	282	274	KASP_UN_95397731	X:X	X:X	X:X
gwm539Hd	137	135	133	H13	no	no	no
Fhb_2DL_Wuhan1/W14	no	no	no	cfa2153Fd	**	203	**
KASP_IWB43992	Y:Y	Y:Y	X:X	H9	no	no	no
KASP_IWA6259	Y:Y	Y:Y	X:X	Bdv3Fd	184	184	184
KASP_IWA7594	X:X	X:X	X:X	Bdv2/3	no	no	no
Fhb_1B_Jamestown	no	no	no	KASP_wsnp198467	Y:Y	X:X	X:X
KASP_IWA1587	Y:Y	Y:Y	Y:Y	Sbm1	no	Sbm1	Sbm1
KASP_Tsn1_A	no call	no call	no call	Pinb-D1	soft	soft	soft
Tsn1	no	no	no	grain texture	soft	soft	soft
bx7oeFd	400	400	400	KASP_TaSus2-2B	X:X	X:X	X:X
Glu-B1	no	no	no	sucrose synthase	no	no	no
umn19Fd	341	359	341	KASP_Tamyb10-A1_H	X:X	Y:Y	X:X

Marker name	Parent genotype			Marker name	Parent genotype		
	YT	52	151		YT	52	151
Glu-A1	Ax2*	Ax1	Ax2*	KASP_Tamyb10_Nor17	X:X	X:X	X:X
KASP_Glu-D1d_SNP	X:X	X:X	X:X	Tamyb10-A1	R-A1	white	R-A1
umn25Nd	296	296	296	KASP_Tamyb10-B1	Y:Y	Y:Y	Y:Y
Glu-D1	2+12	2+12	2+12	Tamyb10-B1	white	white	white
KASP_pina-D1a	X:X	X:X	X:X	KASP_Tamyb10-D1	Y:Y	Y:Y	Y:Y
Pina-D1	soft	soft	soft	Tamyb10-D1	R-D1	R-D1	R-D1
KASP_pinb-wild	X:X	X:X	X:X	combined kernel color	RrR	rrR	RrR

†Genotypic data for Yorktown (YT), VA09W-52 (52), and VA05W-151 (151).

Supplemental Table 2.3. ANOVA for agronomic and N traits of wheat parent lines Yorktown and VA05W-151 within each testing environment.

Env.	Effect	YLD†	GNC	AGBM	HI	AD	MD	HGT	LDG	NUE	NUpE	NUtE
16WR‡	G§	*	ns	ns	ns	***	-	ns	ns	*	ns	ns
	N	**	ns	ns	ns	ns	-	ns	ns	***	*	ns
	G × N	ns¶	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
17WR	G	*	ns	ns	**	ns	ns	ns	ns	*	ns	ns
	N	***	*	*	*	ns	ns	ns	ns	***	**	*
	G × N	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
18WR	G	**	ns	**	ns	ns	ns	ns	ns	***	ns	ns
	N	ns	ns	**	*	ns	*	ns	***	***	**	**
	G × N	*	ns	ns	*	ns	ns	ns	ns	**	ns	ns
18NK	G	*	ns	ns	ns	-	-	ns	ns	ns	ns	*
	N	*	**	ns	ns	-	-	ns	ns	***	**	*
	G × N	ns	ns	ns	ns	-	-	ns	ns	ns	ns	ns

† Trait abbreviations for grain yield (YLD), grain N content (GNC), above-ground biomass (AGBM), harvest index (HI), anthesis date (AD), maturity date (MD), plant height (HGT), lodging (LDG), N use efficiency (NUE), N uptake efficiency (NUpE), and N utilization efficiency (NUtE).

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

‡ Numbers indicate years 2015–2016 (16), 2016-2017 (17), and 2017-2018 (18); letters indicate locations Warsaw (WR) and New Kent (NK).

§ G, parent genotype; N, N rate.

¶ ns, not significant.

Supplemental Table 2.4. ANOVA for agronomic and N traits of wheat parent lines Yorktown and VA09W-52 within each testing environment.

Env.	Effect	YLD†	GNC	AGBM	HI	AD	MD	HGT	LDG	NUE	NUpE	NUtE
16WR‡	G§	ns¶	ns	ns	*	*	-	ns	ns	ns	*	*
	N	*	***	ns	ns	ns	-	ns	ns	**	ns	ns
	G × N	ns	**	ns	ns	ns	-	ns	ns	ns	ns	ns
17WR	G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	N	***	**	*	ns	ns	ns	ns	ns	***	***	***
	G × N	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
18WR	G	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns
	N	ns	ns	ns	ns	ns	ns	**	ns	***	***	ns
	G × N	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
18NK	G	**	*	ns	ns	-	-	ns	ns	*	ns	ns
	N	ns	ns	ns	ns	-	-	ns	ns	***	***	ns
	G × N	ns	ns	ns	ns	-	-	ns	ns	ns	ns	ns

† Trait abbreviations for grain yield (YLD), grain N content (GNC), above-ground biomass (AGBM), harvest index (HI), anthesis date (AD), maturity date (MD), plant height (HGT), lodging (LDG), N use efficiency (NUE), N uptake efficiency (NUpE), and N utilization efficiency (NUtE).

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

‡ Numbers indicate years 2015–2016 (16), 2016-2017 (17), and 2017-2018 (18); letters indicate locations Warsaw (WR) and New Kent (NK).

§ G, parent genotype; N, N rate.

¶ ns, not significant.

Supplemental Table 2.5. Summary statistics of wheat parents and RILs for each trait in the Yorktown × VA05W-151 population.

Trait	Env.	Parents				RILs					
		Yorktown		VA05W-151		Mean		Range		SD	
		LN	HN	LN	HN	LN	HN	LN	HN	LN	HN
Grain yield (kg ha^{-1})	16WR	3,907 c	4,901 ab	4,468 b	5,187 a	4,078	4,824	2,731–5,214	3,442–6,074	438	424
	17WR	5,059 b	5,947 a	5,392 b	6,159 a	5,162	5,980	4,050–6,123	5,255–6,928	365	379
	18WR	3,899 c	4,307 b	4,685 b	4,381 ab	4,166	4,264	2,928–5,837	2,999–5,410	383	483
	18NK	3,205 b	4,194 a	4,146 a	4,475 a	3,786	4,311	833–6,023	2,523–5,837	757	657
Grain N content (g kg^{-1})	16WR	1.64 a	1.76 a	1.70 a	1.80 a	1.95	2.13	1.39-2.63	1.53-2.84	0.35	0.35
	17WR	1.58 ab	1.72 a	1.44 b	1.71 a	1.45	1.72	1.10-1.85	1.36-2.12	0.15	0.16
	18WR	2.20 b	2.77 a	2.12 b	3.14 a	2.46	2.62	1.93-2.98	1.95-3.23	0.25	0.24
	18NK	2.54 a	2.65 a	2.29 a	2.40 a	2.14	2.80	1.70-3.22	2.22-3.87	0.26	0.35
Above-ground biomass (g m^{-2})	16WR	1,152 a	1,408 a	1,489 a	1,321 a	1,115	1,296	881–1,482	744–2,424	116	154
	17WR	1,220 ab	1,328 a	1,089 b	1,297 ab	1,168	1,313	957–1,408	1,143–1,548	85	86
	18WR	1,039 c	1,160 b	1,190 b	1,271 a	1,083	1,159	868–1,346	688–1,748	74	148
	18NK	909 b	1,142 ab	1,135 ab	1,245 a	1,045	1,157	148–1,718	681–1,644	224	165
Harvest index (g g^{-1})	16WR	0.34 a	0.35 a	0.31 a	0.39 a	0.35	0.38	0.27-0.44	0.20-0.61	0.03	0.04
	17WR	0.42 c	0.45 b	0.45 ab	0.47 a	0.44	0.46	0.37-0.49	0.36-0.50	0.02	0.02
	18WR	0.38 b	0.37 b	0.41 a	0.35 b	0.36	0.37	0.28-0.42	0.28-0.46	0.03	0.03
	18NK	0.35 a	0.37 a	0.36 a	0.38 a	0.39	0.37	0.28-0.45	0.23-0.65	0.03	0.06
Anthesis date (Julian)	16WR	111 b	111 b	112 a	112 a	111	111	108-114	108-115	1.3	1.2
	17WR	111 a	111 a	111 a	111 a	110	110	106-112	107-112	1.2	1.3
	18WR	120 a	121 a	120 a	120 a	120	120	117-123	117-123	0.9	1.0
	18NK	-	-	-	-	-	-	-	-	-	-
Maturity date (Julian)	16WR	-	-	-	-	-	-	-	-	-	-
	17WR	149 a	150 a	149 a	149 a	149	149	144-151	145-152	1.4	1.3
	18WR	153 a	153 a	150 a	150 a	150	151	149-155	149-157	1.0	2.0
	18NK	-	-	-	-	-	-	-	-	-	-
Height (cm)	16WR	81.3 a	85.2 a	79.2 a	83.1 a	81.6	85.1	68.6-95.7	71.6-101.3	5.0	5.2
	17WR	69.5 a	69.5 a	66.7 a	67.6 a	65.3	65.4	55.1-72.9	54.3-99.9	4.3	3.8
	18WR	87.6 a	87.8 a	86.7 a	88.1 a	85.7	87.5	75.4-95.7	76.2-99.9	4.1	4.1
	18NK	75.1 a	76.6 a	77.6 a	79.7 a	75.8	80.3	56.7-90.6	61.9-95.8	6.5	6.1
Lodging (0-9)	16WR	0 a	0 a	0 a	0 a	0.0	0.1	0.0-0.0	0.0-2.0	0.0	0.2
	17WR	0 a	0 a	0 a	0 a	0.0	0.0	0.0-0.0	0.0-0.0	0.0	0.0
	18WR	1 b	4 a	1 b	5 a	1.3	3.8	0.0-7.7	0.0-9.0	1.6	2.2
	18NK	0 a	0 a	0 a	0 a	0.2	1.4	0.0-6.0	0.0-7.5	0.8	1.7
N-use efficiency (kg kg^{-1})	16WR	58.3 b	36.6 c	66.7 a	38.7 c	60.9	36.1	40.8-77.8	25.7-45.3	6.6	3.2
	17WR	75.5 b	44.4 c	80.5 a	46.0 c	77.0	44.6	60.5-91.4	39.2-51.7	5.5	2.8
	18WR	58.1 b	32.1 c	69.9 a	32.7 c	62.2	31.8	43.7-78.3	18.8-43.6	5.7	3.6

Trait	Env.	Parents				RILs					
		Yorktown		VA05W-151		Mean		Range		SD	
		LN	HN	LN	HN	LN	HN	LN	HN	LN	HN
	18NK	47.8 b	31.3 c	61.9 a	33.4 c	56.0	32.2	7.70-89.9	22.4-40.4	11.9	4.9
N-uptake efficiency	16WR	2.84 ab	1.58 b	4.10 a	1.98 b	2.27	1.51	1.03-5.09	0.77-3.37	0.95	0.54
(kg kg^{-1})	17WR	1.74 a	1.07 b	1.58 a	1.12 b	1.66	1.16	1.08-3.21	0.84-1.79	0.37	0.21
	18WR	1.92 ab	1.38 c	2.12 a	1.59 bc	1.95	1.35	1.45-3.03	0.91-2.41	0.29	0.27
	18NK	1.89 ab	1.30 b	2.09 a	1.31 b	2.12	1.32	0.29-3.54	0.68-1.83	0.47	0.21
N-utilization	16WR	22.4 a	27.9 a	17.2 a	19.5 a	30.7	26.6	12.1-55.6	11.0-45.5	10.7	8.4
efficiency	17WR	44.9 ab	42.0 b	51.2 a	41.2 b	48.2	39.5	27.0-71.7	23.4-54.9	8.4	6.2
(kg kg^{-1})	18WR	30.4 a	23.3 b	33.4 a	21.1 b	32.3	24.4	17.8-39.9	17.4-30.7	4.1	4.7
	18NK	25.9 b	24.0 b	29.9 a	25.6 b	26.5	24.6	20.7-34.0	12.8-34.2	2.5	2.5

† The LSD at $P \leq 0.05$ is used to compare parental lines across N rates within an environment; means within an environment followed by the same letter are not significantly different.

Supplemental Table 2.6. Summary statistics of wheat parents and DHs for each trait in the Yorktown × VA09W-52 population.

Trait	Env.	Parents				DHs					
		Yorktown		VA09W-52		Mean		Range		SD	
		LN	HN	LN	HN	LN	HN	LN	HN	LN	HN
Grain yield (kg ha^{-1})	16WR	3,659 c	4,867 a	4,068 bc	4,607 ab	3,837	4,670	2,407–5,067	3,216–6,098	542	562
	17WR	4,924 b	5,832 a	5,092 b	6,106 a	4,876	5,730	3,750–6,151	3,937–6,834	450	500
	18WR	4,120 ab	3,883 b	4,247 a	4,027 ab	3,864	3,936	2,756–4,912	2,111–5,139	408	548
	18NK	4,250 b	4,038 b	5,081 a	4,950 a	4,372	4,372	2,355–5,717	2,428–6,014	650	741
Grain N content (g kg^{-1})	16WR	1.69 c	1.89 b	1.51 d	2.03 a	1.67	1.90	1.27-2.02	1.57-2.43	0.16	0.15
	17WR	1.68 b	2.11 a	1.57 b	1.94 a	1.72	2.04	1.28-2.49	1.52-2.66	0.16	0.19
	18WR	2.56 a	2.59 a	2.71 a	2.98 a	2.39	2.79	1.81-3.20	2.11-3.49	0.29	0.26
	18NK	2.54 ab	2.61 a	2.45 b	2.48 ab	2.46	2.55	1.97-2.96	1.98-3.06	0.20	0.19
Above-ground biomass (g m^{-2})	16WR	1,101 ab	1,306 a	910 bc	1,076 ab	1,004	1,165	555–1,487	726–2,343	140	191
	17WR	1,108 b	1,239 ab	1,119 bc	1,325 a	1,129	1,257	860–1,531	342–1,777	138	150
	18WR	1,299 a	1,107 a	1,124 a	1,186 a	1,085	1,112	737–1,682	619–1,673	156	148
	18NK	1,156 a	968 a	1,204 a	1,092 a	1,089	1,108	628–1,475	541–1,507	168	193
Harvest index (g g^{-1})	16WR	0.33 c	0.37 bc	0.45 a	0.43 ab	0.38	0.40	0.30-0.48	0.24-0.58	0.04	0.04
	17WR	0.46 a	0.47 a	0.46 a	0.47 a	0.44	0.46	0.30-0.52	0.28-0.64	0.04	0.04
	18WR	0.33 a	0.35 a	0.38 a	0.34 a	0.36	0.35	0.21-0.52	0.21-0.49	0.04	0.04
	18NK	0.37 a	0.43 a	0.42 a	0.46 a	0.40	0.40	0.30-0.49	0.30-0.55	0.03	0.05
Anthesis date (Julian)	16WR	111 a	112 a	108 a	108 a	112	112	108-117	107-117	2.1	2.2
	17WR	109 a	109 a	109 a	109 a	110	110	106-113	106-115	1.8	1.9
	18WR	121 a	120 a	120 a	120 a	121	121	118-125	116-124	1.6	1.5
	18NK	-	-	-	-	-	-	-	-	-	-
Maturity date (Julian)	16WR	-	-	-	-	-	-	-	-	-	-
	17WR	149 a	149 a	150 a	150 a	149	149	145-152	146-153	1.6	1.3
	18WR	153 a	152 a	150 a	152 a	151	152	149-156	148-158	1.7	2.5
	18NK	-	-	-	-	-	-	-	-	-	-
Height (cm)	16WR	78.8 a	87.5 a	81.0 a	84.1 a	82.0	86.4	60.6-98.7	64.7-101.1	7.2	6.5
	17WR	69.1 a	65.3 a	65.7 a	68.7 a	68.8	69.3	49.9-89.7	57.1-85.0	5.7	5.1
	18WR	82.0 b	88.8 a	88.8 a	91.3 a	87.1	88.1	70.7-98.6	71.8-104.0	5.5	5.0
	18NK	82.4 a	83.8 a	81.6 a	83.5 a	84.2	87.5	71.4-102.7	73.9-107.0	6.5	6.7
Lodging (0-9)	16WR	0 a	0 a	0 a	0 a	0	0.0	0.0-1.0	0.0-6.0	0.1	0.6
	17WR	0 a	0 a	0 a	0 a	0	0.0	0.0-0.0	0.0-0.0	0.0	0.0
	18WR	2 b	3 ab	4 ab	6 a	1.4	4.2	0.0-6.3	0.0-9.0	1.5	2.7
	18NK	0 a	0 a	0 a	1 a	0.4	1.3	0.0-5.8	0.0-7.1	0.8	1.6
N-use efficiency (kg kg^{-1})	16WR	54.6 a	36.3 b	60.7 a	34.4 b	57.2	34.9	35.9-75.6	24.0-45.5	8.1	4.2
	17WR	73.5 a	43.5 b	76.0 a	45.6 b	72.8	65.3	56.0-91.8	35.1-85.3	6.7	9.7
	18WR	61.5 a	29.0 b	63.4 a	30.4 b	57.7	29.4	41.1-73.3	15.8-38.4	6.1	4.1

Trait	Env.	Parents				DHs					
		Yorktown		VA09W-52		Mean		Range		SD	
		LN	HN	LN	HN	LN	HN	LN	HN	LN	HN
	18NK	63.4 b	30.1 c	74.5 a	36.9 c	65.3	32.6	35.1-85.3	18.1-44.9	9.7	5.3
N-uptake efficiency	16WR	2.66 a	1.44 b	1.27 b	1.01 b	1.66	1.12	0.98-3.80	0.67-2.23	0.62	0.31
(kg kg^{-1})	17WR	1.63 a	1.21 b	1.56 a	1.23 b	1.68	2.42	1.26-2.63	1.50-3.73	0.20	0.41
	18WR	2.77 a	1.25 b	2.60 a	1.53 b	2.11	1.38	1.40-3.54	0.85-4.64	0.33	0.36
	18NK	2.62 a	1.22 b	2.69 a	1.30 b	2.42	1.31	1.50-3.73	0.71-1.73	0.41	0.21
N-utilization	16WR	21.7 b	27.8 b	48.0 a	34.2 ab	38.5	32.9	16.7-59.0	17.3-47.6	10.1	7.2
efficiency	17WR	45.1 a	36.0 b	48.7 a	37.3 b	42.8	27.2	29.4-51.0	20.6-35.7	3.7	2.9
(kg kg^{-1})	18WR	22.3 a	23.7 a	25.6 a	20.0 a	27.8	22.0	17.5-39.3	7.1-33.4	4.1	3.7
	18NK	24.3 a	25.2 a	27.7 a	28.5 a	27.2	25.1	20.6-35.7	18.7-33.3	2.9	3.1

† The LSD at $P \leq 0.05$ is used to compare parental lines across N rates within an environment; means within an environment followed by the same letter are not significantly different.

Supplemental Table 2.7. Quantitative trait loci (QTL) associated with N and agronomic traits in one N-environments in the Yorktown × VA05W-151 wheat population.

Trait	Chr.†	N-Env.‡	Pos.	Left marker	Right marker	LOD§	PVE¶	Add#
							%	
AD††	1D	16WR-HN	99	S331192008	S96704215	2.51	9.35	0.39
	4A	16WR-HN	37	S423586538	S7441669	3.33	9.65	-0.40
	5B	16WR-LN	165	S706353953	S685218875	4.19	10.08	0.46
	7A	16WR-LN	39	S101469749	S89976042	5.43	13.72	-0.54
	7A	17WR-HN	85	S700115395	S710197438	2.58	8.49	-0.38
	7B	16WR-HN	53	S739405563	S730299386	2.52	6.53	-0.34
AGBM	1D	17WR-HN	105	S331192008	S96704215	8.90	17.70	42.85
	1D	17WR-LN	67	S31421492	S52583851	5.14	12.82	31.78
	2B	18WR-HN	1	S44752993	S44754542	2.60	2.27	-38.31
	2D	17WR-HN	16	S19051559	S18166508	2.57	4.31	-20.32
	7B	17WR-HN	131	S10160986	S5980505	4.19	7.33	-26.37
GNC	1A	18WR-LN	44	S536172066	S536294689	2.66	7.73	-0.07
	2D	16WR-LN	117	S406151907	S459517523	3.40	9.58	-0.12
	5D	16WR-LN	33	S396986910	S405321115	3.12	7.69	-0.11
	5D	17WR-HN	0	S540196427	S546020057	2.85	9.37	0.05
	5D	18WR-LN	0	S254497728	S143735165	2.98	8.70	0.08
HGT	1B	16WR-HN	7	S25475412	S26433743	4.19	13.09	2.00
	3B	18WR-LN	91	S598995503	S236353481	3.66	12.04	1.84
	7A	16WR-HN	31	S660537877	S661534491	2.52	7.28	1.38
	7D	17WR-LN	72	S220991869	S201977415	2.99	10.2	-1.31
HI	3D	18NK-HN	28	S430360367	S442814795	2.54	7.31	-0.01
	4A	18WR-LN	0	S40243263	S40252565	6.46	17.00	0.01
	5B	17WR-LN	165	S706353953	S685218875	3.07	11.22	0.01
	5D	17WR-HN	42	S556723663	S562030409	3.73	8.16	0.01
	6A	18NK-LN	54	S617207033	S556967794	5.40	16.26	-0.01
	7A	17WR-HN	14	S621213341	S639296223	5.18	11.74	-0.01
	7A	17WR-HN	13	S30064864	S23108024	2.55	5.42	-0.01
	7D	18WR-LN	71	S176567249	S213740699	3.60	9.11	0.01
LDG	4D	18NK-HN	53	S480514255	S482954488	2.68	9.00	-0.51
MD	1A	18WR-LN	7	S532668674	S536067277	3.09	7.82	-0.49
	1B	17WR-LN	4	S22056647	S17836570	4.25	7.46	-0.48
	2B	18WR-HN	11	S41442050	S37359247	4.38	14.26	0.74
NUE	2B	17WR-HN	20	S27008007	S28419889	2.91	6.11	0.74
	2B	17WR-LN	1	S699106811	S701094516	2.51	4.71	1.23
	2D	17WR-HN	15	S19847217	S19051559	2.56	5.10	-0.68
	3B	18WR-LN	95	S269253850	S621935545	2.67	9.66	-1.71
	3D	18WR-LN	44	S595066861	S594754857	4.09	12.01	1.86
	4D	16WR-LN	0	S509798252	S499573720	3.45	8.98	1.97
	4D	18WR-HN	17	S389572522	S28548806	3.00	8.22	-1.14
	5D	18NK-HN	31	S556844077	S556723663	2.64	7.06	-1.34
	7A	17WR-LN	23	S115834359	S112241400	3.69	6.91	-1.48
	7B	16WR-LN	5	S682597816	S693793240	4.25	12.35	2.32
NUpE	2A	18NK-LN	73	S678190504	S637991282	3.86	11.16	0.15
	2D	16WR-HN	4	S21934481	S21785248	2.76	8.56	0.14
	3B	16WR-HN	82	S779966541	S788042552	4.40	13.81	0.18
	6A	18NK-LN	73	S508845927	S473574940	4.40	13.11	0.17
NUtE	1B	18NK-HN	21	S648133593	S653887678	2.90	7.40	0.72
	2D	16WR-HN	4	S21934481	S21785248	2.76	8.22	-2.17
	2D	16WR-LN	15	S19847217	S19051559	3.91	5.82	-3.25
	4A	18NK-HN	25	S583019201	S552215864	5.93	15.63	-1.05
	4A	18WR-LN	0	S40243263	S40252565	3.46	11.48	-1.39

Trait	Chr.†	N-Env.‡	Pos.	Left marker	Right marker	LOD§	PVE¶	Add#
							%	
	5A	16WR-LN	57	S436192529	S436264964	3.51	5.04	-3.20
	6D	16WR-HN	0	S323045603	S317855626	4.25	12.76	2.70
	7D	16WR-HN	38	S89020622	S113887697	2.75	10.30	2.40

† Chromosome (Chr.).

‡ Numbers indicate years 2015–2016 (16), 2016-2017 (17), and 2017-2018 (18); letters indicate locations Warsaw (WR) and New Kent (NK); low (LN) and high (HN) N rates within environment.

§ Logarithm of odds.

¶Percentage of phenotypic variation explained by the QTL.

#Level of additivity. A positive sign indicates that alleles from Yorktown increased the trait value and a negative sign indicates that alleles from VA05W-151 increased the trait value.

††Trait abbreviations for grain N content (GNC), above-ground biomass (AGBM), harvest index (HI), N use efficiency (NUE), N uptake efficiency (NUpE), N utilization efficiency (NUtE), anthesis date (AD), maturity date (MD), plant height (HGT), and lodging (LDG). Grain yield was excluded from the results as its QTL were identical to those found for NUE.

Supplemental Table 2.8. Quantitative trait loci (QTL) associated with N and agronomic traits in one N-environments in the Yorktown × VA09W-52 wheat population.

Trait	Chr.[†]	N-Env.[‡]	Pos.	Left marker	Right marker	LOD[§]	PVE[¶]	Add[#]
							%	
AD[††]	1B	17WR-HN	89	S563121178	S562485837	2.81	3.47	0.36
	4A	17WR-HN	125	S7176912	S2786681	4.42	6.06	-0.48
	4A	18WR-LN	61	S544620299	S481064477	4.73	8.60	0.47
	5A	16WR-HN	73	S596570258	S595425852	3.28	4.74	0.48
	5A	17WR-LN	99	S567114202	S564579859	3.71	6.88	-0.46
	5D	17WR-HN	68	S601232900	S598810451	3.53	4.42	-0.40
	7B	16WR-LN	31	S19536241	S34848225	2.51	4.39	-0.42
AGBM	2D	17WR-LN	76	S44597217	S35039116	2.84	7.81	40.77
	3B	16WR-LN	84	S39455478	S29498148	3.06	8.48	-41.34
	5B	16WR-LN	153	S670064794	S669690464	4.15	9.41	43.55
	6A	16WR-LN	3	S52019595	S51375303	4.15	9.32	43.4
GNC	5A	16WR-HN	208	S9395730	S7493203	2.99	11.74	0.05
	5A	18WR-LN	157	S458562272	S455344314	3.88	10.18	0.10
	7B	18NK-LN	15	S707714032	S706277402	2.85	8.33	-0.06
	7D	17WR-HN	31	S527727944	S524324303	3.31	10.46	0.06
HGT	1A	17WR-HN	52	S497201595	S478424706	4.34	6.84	-1.38
	2B	18WR-HN	21	S775486182	S775329395	3.19	8.72	1.41
	3B	16WR-HN	82	S39455478	S29498148	2.90	6.28	1.86
	4A	16WR-HN	49	S595433072	S583968823	3.71	7.60	-2.06
	5A	18NK-HN	95	S567502385	S567534925	4.28	11.09	-2.35
	5A	18WR-HN	82	S585018041	S584664469	2.68	7.11	-1.27
	5D	17WR-LN	112	S396986910	S63267701	2.75	10.34	2.04
	7B	16WR-HN	71	S210333999	S483533955	5.30	9.80	-2.32
	7D	17WR-HN	75	S86148725	S73368696	4.52	6.91	1.38
	7D	17WR-HN	101	S55405801	S53710322	8.43	13.76	-1.95
	7D	17WR-HN	0	S5068391	S15199996	3.01	4.44	-1.11
HI	2A	18NK-LN	3	S25669457	S22128148	4.19	11.36	0.01
	2B	17WR-LN	0	S67433042	S67431792	2.61	8.65	0.01
	4A	18NK-HN	66	S137557861	S58223442	5.13	15.92	-0.02
	4A	18WR-LN	87	S28592992	S24753888	3.24	8.12	-0.01
	4B	18WR-LN	75	S88225322	S31876652	6.28	16.48	-0.02
LDG	2B	18WR-LN	53	S707177604	S705673880	2.75	8.90	0.43
	2D	18NK-HN	41	S75260724	S75756585	2.93	9.66	0.50
	3B	18WR-HN	23	S647967657	S602479682	4.34	11.96	0.97
	5D	18WR-HN	95	S409542642	S396986910	2.66	7.89	0.78
MD	3D	17WR-LN	42	S460249827	S431289828	5.26	14.13	-0.69
	4D	17WR-LN	0	S456054449	S455763126	3.50	7.30	0.50
	5D	17WR-HN	85	S421533203	S409542642	2.75	7.39	-0.39
	5D	18WR-HN	132	S254497728	S251953300	3.09	7.96	0.73
	7B	18WR-HN	71	S210333999	S483533955	3.39	9.05	0.77
	7D	17WR-HN	31	S527727944	S524324303	3.65	9.66	-0.44
NUE	1A	18NK-HN	56	S475625631	S394817972	2.54	6.80	1.41
	1B	18NK-HN	23	S656423128	S655908290	2.59	6.61	1.40
	3A	18WR-HN	75	S571169985	S568645681	6.58	12.21	1.38
	3B	18WR-HN	92	S27484145	S27493643	2.62	4.50	0.83
	5A	18WR-LN	157	S458562272	S455344314	2.53	5.13	-1.27
	7A	18WR-HN	35	S112439521	S111912514	4.19	7.5	1.08
NUpE	1A	18NK-HN	55	S464836988	S475625631	3.07	8.88	0.07
	2D	18NK-HN	5	S556333501	S442052650	2.88	8.94	0.07
	7B	18NK-LN	15	S707714032	S706277402	3.66	11.48	-0.14
NUtE	2A	18NK-LN	3	S25669457	S22128148	3.00	9.65	0.91

Trait	Chr.†	N-Env.‡	Pos.	Left marker	Right marker	LOD§	PVE¶	Add#
							%	
	3B	16WR-LN	65	S45271380	S44274197	2.53	8.08	-2.84
	3B	18WR-LN	30	S586509678	S523569541	2.78	6.90	-1.01
	3B	18WR-LN	42	S762639074	S760963490	2.73	6.81	-1.00
	5A	18WR-LN	157	S458562272	S455344314	3.96	10.37	-1.23
	7D	17WR-HN	32	S529393082	S529349312	4.37	13.80	-1.70
	7D	18WR-LN	85	S86148725	S73368696	3.26	8.17	-1.10

† Chromosome (Chr.).

‡ Numbers indicate years 2015–2016 (16), 2016-2017 (17), and 2017-2018 (18); letters indicate locations Warsaw (WR) and New Kent (NK); low (LN) and high (HN) N rates within environment.

§ Logarithm of odds.

¶Percentage of phenotypic variation explained by the QTL.

#Level of additivity. A positive sign indicates that alleles from Yorktown increased the trait value and a negative sign indicates that alleles from VA09W-52 increased the trait value.

††Trait abbreviations for grain N content (GNC), above-ground biomass (AGBM), harvest index (HI), N use efficiency (NUE), N uptake efficiency (NUpE), N utilization efficiency (NUtE), anthesis date (AD), maturity date (MD), plant height (HGT), and lodging (LDG). Grain yield was excluded from the results as its QTL were identical to those found for NUE.

CHAPTER III:

PHOTOPERIOD RESPONSE GENE PPD-D1 AFFECTS NITROGEN USE EFFICIENCY IN SOFT RED WINTER WHEAT

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Abbreviation: BVA, Blacksburg, VA, location; COH, Custar, OH, location; GS, growth stage; NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NUtE, nitrogen utilization efficiency; PVA, Painter, VA, location; WVA, Warsaw, VA, location.

Abstract

Optimal N application is critical to ensure profitable soft red winter wheat (Triticum aestivum L.) production and limit negative environmental effects. This study was conducted using 12 wheat lines grown in two Ohio and six Virginia environments to determine the effects of differentially split N rates on grain yield, yield components, and N-related traits. Upon analysis of wheat lines in two contrasting photoperiod groups, it was discovered that wheat lines containing the photoperiod sensitivity allele (*Ppd-D1b*) conferred a significant ($P < 0.05$) yield advantage under N-limited conditions in Ohio and across N treatments in half of the Virginia testing environments. This resulted from increased harvest index, grains per square meter, number of kernels per spike, and floret fertility in *Ppd-D1b* lines relative to lines with the insensitive allele (*Ppd-D1a*). However, wheat genotypes in the photoperiod-insensitive allelic group had significantly higher ($P < 0.05$) grain N concentration in all Virginia testing sites. A spring split N application of 33 kg N ha^{-1} at Zadoks growth stage (GS) 25 and 101 kg N ha^{-1} at Zadoks GS 30 produced the highest grain yields and grain N contents in five of the six Virginia environments. Nitrogen utilization efficiency was the primary contributor to variation in N use efficiency under higher N rates, whereas N uptake efficiency was the greatest contributor under low N rates. The contribution of N utilization and uptake efficiencies varied among wheat lines in the *Ppd-D1a* group when 67 kg N ha^{-1} was applied at Zadoks GS 25 under the moderate and standard N rates.

Introduction

Wheat (*Triticum aestivum* L.) yield improvement during the second half of the 20th century is largely attributed to a combination of intensified N management and the introduction of semidwarf cultivars expressing a positive response to added N (Evenson and Gollin, 2003). However, yield gains derived exclusively from greater rates of N fertilization may reduce grower profits and contribute to eutrophication, the release of greenhouse gasses, and degradation of aquatic and terrestrial ecosystems (Heisler et al., 2008; Howarth, 2008). It has been estimated that 50 to 70% of applied N is not taken up by plants and is lost from agricultural production systems (Good et al., 2004). In addition, over one-third of the annual N load going into estuaries within the Northeast and Mid-Atlantic regions of the United States is derived from agricultural sources (Moore et al., 2011). Plant breeders, agronomists, and growers are thus faced with the challenge of developing highly N-efficient cultivars and production systems to feed growing populations while also fostering the principles of environmental stewardship.

Improvements in nitrogen use efficiency (NUE) are most readily achieved at the on-farm level through the adoption of more efficient nutrient management practices (e.g., N rate, source, timing, and placement) (Zhang et al., 2015). In Virginia, spring-applied N is typically split over two development stages to increase NUE by meeting plant demands during periods of high N uptake. The first application of spring N in Virginia generally occurs when winter wheat breaks dormancy in late February, demanding N fertilizer for tiller production (Alley et al., 1996). The stem elongation phase begins ~30 d after breaking dormancy, requiring a second application of N for vegetative growth and grain fill. Yield advantages from splitting N applications over multiple developmental stages were also reported in winter wheat grown in the South Central region of the United States (Raun et al., 2002) and Iran (Abedi et al., 2011). However,

management guidelines are dependent on the class of wheat, end uses, and growing environment. For instance, in an investigation of hard red spring wheat in the Northern Great Plains of the United States, N applied before the five-leaf stage resulted in greater tiller production than an equally split N application of the same rate at preplant, the five-leaf stage, and postanthesis, but the increased tiller number did not translate into a significant increase in grain yield (Otteson et al., 2007). The authors further reported that the wheat lines themselves were the most significant factor in grain yield determination; however, the genetics and physiology underlying the observed genotypic variation was not investigated further.

Genes governing photoperiod response have been shown to provide adaptability to challenging environments by regulating flowering date and offering avoidance to abiotic stresses. A photoperiod gene, *Ppd-D1*, on wheat chromosome 2D has been shown to act pleiotropically to affect a number of traits including flowering date, spikelet number, and floret fertility (González et al., 2005). In a study of near-isogenic wheat lines in the United Kingdom, the photoperiod insensitivity allele (*Ppd-D1a*) functioned by reducing the duration of preanthesis developmental stages and consequently time to flowering (Foulkes et al., 2004). The authors also reported higher grain yields and greater preanthesis water uptake in photoperiod-sensitive (*Ppd-D1b*) lines under both irrigated and nonirrigated conditions, associating photoperiod response with abiotic stress adaptation. Furthermore, genetic markers associated with NUE and N-related traits have been identified near the *Ppd-D1* locus in several genetic mapping studies (Laperche et al., 2007; Bogard et al., 2013; Cormier et al., 2014; Mahjourimajd et al., 2016), suggesting a link between photoperiod sensitivity and NUE.

Contributions of yield building components to crop productivity have been a prevalent topic of investigation in recent years, which have resulted in a better understanding of

morphophysiological stress responses and facilitated breeding directions. In a study of wheat response to N stress, a direct relationship between grain number per spike and grain yield was reported under both optimal and reduced N conditions (Lu et al., 2015). Similar findings were also reported for durum wheat (*Triticum durum* Desf.) grown in a Mediterranean climate (Ferrante et al., 2010) and soft red winter wheat grown in the eastern United States (Tamang et al., 2017). In contrast, Albrizio et al. (2010) found that grain number per square meter, as influenced by N availability, accounted for much of the observed variation in grain yield. However, a combination of modifications in spike morphology, aboveground structure, and nutrient partitioning is required for robust genetic gains in grain yield across varying levels of N fertility (Foulkes et al., 2009; Barraclough et al., 2014; Gaju et al., 2014). Identification of N-responsive morphological traits may enable wheat breeders to more effectively develop broadly adapted N-efficient cultivars.

Nitrogen use efficiency, classically defined as the amount of grain yield produced per unit of N applied, is the product of two component traits: N uptake (NUpE) and N utilization (NUtE) efficiencies (Moll et al., 1982). Nitrogen uptake efficiency is calculated as the aboveground N at harvest per unit of N applied, whereas NUtE is the amount of grain produced per total aboveground N in the plant at harvest. To date, several studies have investigated variation in the relative contribution of NUpE and NUtE to NUE for wheat lines under a range of N regimes and growing environments (Le Gouis et al., 2000; Barraclough et al., 2010; Gaju et al., 2011; Latshaw et al., 2016; Hitz et al., 2017). Although these investigations report significant genotypic variation and differences by N rate, the effects of photoperiod sensitivity under differentially split N to the percentage contribution of each component trait to NUE has not yet been reported.

Despite extensive research and improvements in NUE, investigations into the effects of N rates and spring splitting ratios on NUE in diverse breeding stocks and photoperiod groups are extremely limited. The objectives of this study were (i) to validate traits associated with NUE, and (ii) to evaluate the effects of differentially split spring N rates and subsequently photoperiod alleles on grain yield, yield components, and N-related traits.

Materials and Methods

Testing Environments and Experimental Design

Field experiments were performed under rainfed conditions at one Ohio and three Virginia locations (Supplemental Table S1.1.). These included the Northwest Agricultural Research Center near Custar, OH, in 2013–2014 and 2014– 2015 (COH; 41°17′ N, 83°07′ W), Kentland Farm near Blacksburg, VA in 2015–2016 (BVA; 37°11′ N, 80°34′ W), the Eastern Shore Agricultural Research and Extension Center near Painter, VA, in 2013–2014 and 2014–2015 (PVA; 37°35′ N, 75°49′ W), and the Eastern Virginia Agricultural Research and Extension Center near Warsaw, VA in 2013–2014, 2014–2015, and 2015–2016 (WVA; 37°59′ N, 76°46′ W). Seeds were treated with Raxil MD (triazole, Bayer Crop Science) and Gaucho XT (imidacloprid, Bayer Crop Science) to control diseases and insects, respectively. Planting dates were dependent on optimal environmental conditions at each site, ranging from 2 to 29 October (Supplemental Table S1.1.), and plots were sown at a seeding density of 520 seeds m^{-2} on the basis of kernel weight. A randomized complete block design was used for all testing environments in which five N treatments were applied to 12 winter wheat genotypes, totaling 60 plots per replication. Each experimental unit, a seven-row yield plot measuring 2.74 × 1.52 m at

Blacksburg and Warsaw or 2.74 × 1.78 m at Custar and Painter, was replicated four times in Ohio and three times in the Virginia testing sites. Preplant fertilizer was applied at a rate of 33 kg N ha^{-1} in all testing environments, and spring split N treatments were foliar applied as liquid urea ammonium nitrate at Zadoks (Zadoks et al., 1974) growth stages 25 (GS 25) and 30 (GS 30) centered on established Ohio and Virginia winter wheat management recommendations (134 kg N ha^{-1}, Figure 3.1.). Herbicides and foliar pesticides were applied throughout the growing season as necessary to mitigate weed and disease pressure (Supplemental Table S1.2.).

Weather Conditions

Cumulative growing degree days and daily precipitation for each testing environment are compared with the 10-yr average in Supplemental Figure S1.1. Total rainfall was similar to the 10-yr average throughout the growing season in 14COH, 14PVA, and 14WVA (where "14" refers to the year 2013–2014). The spring in 15COH was drier than normal, accumulating only 34 mm between Days 110 and 145, prior to a series of major rain events that occurred around Day 150. However, heavy spring rainfall in 15PVA, totaling 200 mm, occurred between Days 99 and 116 and delayed the Zadoks GS 30 N application. Furthermore, 35 mm of rainfall unexpectedly occurred in 15PVA shortly after the Zadoks GS 30 N application. In the 16BVA environment, precipitation events remained normal throughout the season. Finally, 16WVA had a few heavy late winter and early spring precipitation events until Day 123, which accumulated 59 mm of rainfall.

Plant Materials

The plant materials used in this study (Table 3.1.) were a subset of 12 regionally diverse wheat lines from the Triticeae Coordinated Agricultural Project's (http://www.triticeaecap.org/)

Eastern Elite Mapping Panel of 280 genotypes from six regional wheat breeding programs (The Ohio State University, University of Illinois, University of Kentucky, University of Maryland, University of Missouri, and Virginia Tech). The lines were selected to encompass a broad range of breeding programs and are known to have alleles on chromosome 2D conferring photoperiod insensitivity (*Ppd-D1a*) or sensitivity (*Ppd-D1b*). Diagnostic DNA markers were used to validate the *Ppd-D1* photoperiod (Beales et al., 2007) and reduced height alleles (*Rht-B1* and *Rht-D1*) (Ellis et al., 2002).

Phenotypic Measurements and Plant Sampling

Heading date and plant height data were collected in all Warsaw and Blacksburg testing environments. Heading date was recorded as the day of year (Julian) when 50% of the heads fully emerged from the boot. Plant height was measured prior to harvest from two random locations within the center rows of each plot.

Grain yield data were collected in all Virginia and Ohio sites and adjusted to 0% moisture. In the six Virginia environments, a 1.0-m aerial biomass sample was cut at ground level from one of the center rows at physiological maturity, dried at 60°C for 72 h, and weighed. Spikes were removed from the biomass samples, counted, threshed, and weighed to determine spikes per square meter, grains per square meter, kernel weight, and harvest index (calculated as the proportion of grain yield to the total above-ground biomass). Ten representative spikes were retained from each plot before threshing to determine floret fertility and number of seeds per spike.

Cut and dried straw samples and grain harvested at physiological maturity were ground to estimate their respective N contents via combustion analysis using a Vario Max CNS macro

elemental analyzer (Elementar Analysensysteme). However, in the 14PVA and 14WVA environments, straw samples were lost due to processing errors and N content was not analyzed. Results from combustion analysis were used to determine grain N content, NUE, NUpE, NUtE, and N harvest index (grain N yield per unit of aboveground N yield).

Due to time constraints, chlorophyll content and gas exchange were observed on a randomly selected subset of six wheat lines under low (Treatment 1) and standard (Treatment 5) N rates using 20 flag leaves per plot at anthesis in the 14WVA and 15WVA testing environments. Traits were measured using a handheld chlorophyll meter (CCM-300, Opti-Sciences) and a portable photosynthesis analysis system (LI-6400XT, LI-COR). The rates of photosynthesis (based on CO_2 assimilation), stomatal conductance, and transpiration were assessed at 400 μmol CO_2 mol^{-1} under 1000 μmol m^{-2} s^{-1} irradiation at 23°C with relative humidity maintained to 50%. After photosynthetic measurements, leaf area was recorded and used for standardization.

Statistical Analysis

Analysis of variance and *F* values were produced using the GLIMMIX procedure in SAS 9.4 (SAS Institute, 2013) with replication treated as a random effect to determine significant ($P < 0.05$) effects of photoperiod group (*Ppd*), N treatment, and *Ppd* × N treatment interactions for each site-season. Results are presented by testing environment, as its interaction effects were highly significant for most traits. Comparison of means within site-season was conducted by calculating least squares means for treatments and genotypes. Phenotypic trait correlations were calculated using the CORR procedure in SAS 9.4. Contributions of variation in component traits (NUpE and NUtE) to variation in the resultant trait (NUE) were calculated according to the

method described by Moll et al. (1982), where the resultant trait and its component traits are log transformed with the fraction of the hybrid sum of squares calculated for each component trait.

Results

Grain Yield and Grain Nitrogen Content

The ANOVA did not identify significant *Ppd* × N treatment interactions for grain yield or grain N content within any of the six Virginia testing environments, although significant effects ($P < 0.05$) due to *Ppd* alleles were detected for grain yield in three environments and grain N content in all testing environments (Table 3.2.). The *Ppd-D1b* allele group produced significantly higher grain yields in 14WVA, 15WVA, and 16WVA than the *Ppd-D1a* group, providing a yield advantage of 377, 148, and 199 kg ha^{-1}, respectively (Figure 3.2.). In contrast, the *Ppd-D1a* genotypes had higher grain N contents than the *Ppd-D1b* lines in all environments, ranging from an increase of 0.080% in 16BVA ($P < 0.05$) to 0.142% grain N content in 15WVA ($P \leq 0.001$).

Grain yields were directly related to total spring N rate in the 14PVA, 15WVA, and 16BVA environments (Figure 3.3.), whereas splitting spring N produced significantly higher grain yields when 33 kg N ha^{-1} was applied at Zadoks GS 25 under the moderate N rates (Treatments 2 and 3) in 14WVA and under the standard N rates (Treatments 4 and 5) in the 16WVA environment. In contrast, significantly higher grain yields were observed in the 15PVA environment when 67 kg N ha^{-1} was applied at Zadoks GS 25 under both the moderate and standard N rates. As was the case for grain yield, N rates by application splitting effects were observed in 14WVA, 15PVA, and 16WVA, whereas increases in grain N content were directly related to total spring N rates in the remaining environments. In the 14WVA and 15PVA

environments, the highest grain N contents were reported when 33 kg N ha^{-1} was applied at Zadoks GS 25 under the standard N rates, whereas genotypes in the 16WVA environment produced higher grain N content when 67 kg N ha^{-1} was applied at Zadoks GS 25 under the moderate and standard N rates.

To evaluate yield response of the *Ppd* allele groups to a greater number of N rates, the same 12 winter wheat lines were evaluated over two growing seasons in Custar under five N rates, ranging from 45 to 134 kg N ha^{-1} (Figure 3.1.). The results indicate that the *Ppd-D1a* allele group produced significantly lower grain yields than the *Ppd-D1b* group under the lowest N rate (45 kg N ha^{-1}, Figure 3.4.), while yields became statistically similar as N rate increased.

Grain yield was positively associated with heading date in the *Ppd-D1a* lines under the low N rate in Virginia (Treatment 1, Supplemental Figure S 1.2.a) and negatively associated with heading date in *Ppd-D1b* lines when 67 kg N ha^{-1} was applied at Zadoks GS 25 under moderate and standard N rates (Treatments 2 and 4, Supplemental Figure S1.2.b). Likewise, a positive association between grain yield and plant height was observed for the *Ppd-D1a* allelic class under low N rates (Supplemental Figure S1.3.a) and negatively correlated for the *Ppd-D1b* lines under Treatments 2 and 4 (Supplemental Figure S1.3.b).

Harvest Index, Biomass, and Yield Components

Harvest index in 16BVA was the only yield-related trait to exhibit a significant *Ppd* × N interaction effect ($P < 0.05$, Table 3.2.), where N treatment means ranged from 36.9 to 40.5% and 37.8 to 38.8% for *Ppd-D1a* and *Ppd-D1b* groups, respectively (Table 3.3.). In considering main effects, the photoperiod-sensitive group of wheat lines had significantly higher harvest index and grains per square meter than the photoperiod-insensitive group and significantly lower

kernel weight and spikes per square meter in two of the six testing environments (Table 3.4.). The number of kernels per spike was significantly higher for the *Ppd-D1b* than the *Ppd-D1a* group in 15PVA, 15WVA, and 16BVA where five additional kernels per spike were produced in 15PVA and 15WVA, whereas three additional kernels per spike were produced in 16BVA in the *Ppd-D1b* group. Among yield components, floret fertility was most consistently affected by the photoperiod alleles, with *Ppd-D1b* having a significantly ($P \leq 0.001$) higher percentage of fertile florets than the *Ppd-D1a* group in all 2014–2015 and 2015–2016 testing environments.

Aerial biomass was directly related to total spring N rate in 14PVA, 14WVA, 15WVA, and 16WVA, whereas splitting effects were observed in 15PVA (Table 3.5.). Similar to the N treatment effects on grain yield in 15PVA, aerial biomass benefited from higher rates of N application at Zadoks GS 25 under both moderate and standard N rates. Generally, grains per square meter and spikes per square meter were directly related to the total spring N rate in significant environments. Under the standard N rate in 16WVA, grains per square meter increased by 2171 and an additional 53 spikes m^{-2} were produced when 33 kg N ha^{-1} was applied at Zadoks GS 25, resulting in higher grain yields due to splitting ratios. In 14WVA under the standard N rate and 16BVA under the moderate N rate, floret fertility was greater when higher N rates were applied at Zadoks GS 25 than at GS 30. In the other significant environments (15WVA and 16WVA), floret fertility increased in response to higher N rates.

Nitrogen Use Efficiency and Nitrogen-Related Traits

Significant *Ppd* × N interaction effects were observed for NUE in 16WVA ($P < 0.01$) and NUtE in 15PVA ($P < 0.05$, Table 3.6.). The *Ppd-D1b* group had significantly higher NUE than the *Ppd-D1a* group in 16WVA by 4.8 and 4.2 kg kg^{-1} under Treatments 1 and 2, respectively

(Table 3.7.). An increase in NUtE of 6.0 kg kg^{-1} for the *Ppd-D1b* wheat lines over those in the *Ppd-D1a* group occurred at the lowest N rate (Treatment 1).

During the 2015–2016 growing season, the *Ppd-D1b* group had significantly higher NUE (1.3 kg kg^{-1} in 16BVA [$P < 0.05$] and 2.3 kg kg^{-1} in 16WVA [P □ 0.001]) than the *Ppd-D1a* group (Table 3.8.). Nitrogen utilization efficiency was significantly higher in 15PVA (2.0 kg kg^{-1}, $P < 0.05$) and 15WVA (3.2 kg kg^{-1}, $P \leq 0.001$) in wheat lines with the *Ppd-D1b* than in those with the *Ppd-D1a* allele. In general, increases in total spring N rate were accompanied by significant decreases in NUE, NUpE, and NUtE in all testing environments except for 16WVA (Table 3.9.), where NUtE was 5.2 kg kg^{-1} higher when 67 kg N ha^{-1} was applied at GS 25 under moderate spring N rates.

A strong positive relationship between grain yield and NUtE was observed in the *Ppd-D1a* ($r = 0.83$, $P < 0.05$) and *Ppd-D1b* ($r = 0.80$, $P < 0.10$) groups (Table 3.10.). In the *Ppd-D1a* group of lines, a strong association was observed between grain yield and harvest index ($r = 0.79$, $P < 0.10$). Furthermore, NUtE had a significant negative correlation with grain N content ($r = -0.83$, $P < 0.05$) and NUpE ($r = -0.82$, $P < 0.05$) in photoperiod-insensitive (*Ppd-D1a*) lines while being insignificant in the *Ppd-D1b* lines. Aerial biomass was positively associated with N harvest index ($r = 0.80$, $P < 0.10$) in the *Ppd-D1b* group and negatively associated with grain N content ($r = -0.79$, $P < 0.10$) in the *Ppd-D1a* group of wheat lines.

Among the two photoperiod allelic groups, the contribution to variation in NUE and its components within the *Ppd-D1a* and *Ppd-D1b* groups was similar across testing environments when 33 kg N ha^{-1} was applied at Zadoks GS 25 (Figure 3.5.). However, variation in NUtE for the *Ppd-D1a* allele group was 19.8% higher than the *Ppd-D1b* allele group under Treatment 2, but was 23.2% lower under Treatment 4. The contribution of NUpE to NUE ranged from 69.3%

under Treatment 1 for the *Ppd-D1a* group to 25.1% under Treatment 4 for the *Ppd-D1b* group, generally decreasing with higher spring N rates. In contrast, the contribution of NUtE to the variation in NUE increased with total spring N rate and was the greatest contributor under all standard N rates (Treatments 4 and 5).

Associations between Chlorophyll Content, Gas Exchange, and Grain Yield

Chlorophyll content and three photosynthetic traits were measured on a subset of six wheat lines and two N rates (Treatments 1 and 5) in 14WVA and 15WVA to validate their associations with grain yield and N content (Table 3.11.). Unsurprisingly, a strong negative relationship was observed between grain yield and grain N content under low ($r = -0.97$, $P < 0.01$) and standard ($r = -0.90$, $P < 0.05$) N rates. Chlorophyll content had a strong positive correlation with grain yield under the low N rate ($r = 0.91$, $P < 0.05$), but the correlation was not significant under the standard N rate ($r = 0.78$, $P < 0.10$).

Discussion

A combination of high-NUE cultivars and efficient N management is crucial to minimize N losses and achieve optimal grain yields and end-use quality in winter wheat. In the present study, *Ppd* × N treatment interaction effects were only significant for three traits: harvest index in 16BVA ($P < 0.05$), NUE in 16WVA ($P < 0.01$), and NUtE in 15PVA ($P < 0.05$). The limited interaction effects allow for comparisons of individual effects of photoperiod alleles (*Ppd-D1a* and *Ppd-D1b*), N rate, and split N ratios in addition to a discussion of the implications for developing winter wheat cultivars exhibiting high NUE.

Morphophysiological Effects of the Ppd-D1 Allele on Nitrogen Use Efficiency

The effects of the *Ppd-D1b* allele on grain yield in the present study were dependent on environment, ranging from a loss of 0.1% in 14PVA to an increase of 7.5% in 14WVA. Similarly, Worland et al. (1998) reported variability in grain yield over a span of 10 growing seasons for wheat carrying *Ppd-D1* alleles in Germany (−13.5 to +28.4%) and the United Kingdom (−16.0 to +9.0%). The variable effects of the *Ppd-D1* alleles on grain yield are often dependent on geographic areas and are attributed to a number of environmentally influenced traits governed by photoperiod response, including crop adaptation and optimal timing of stem elongation, heading, and maturity. Significant grain yield advantages, conferred by the *Ppd-D1b* allele vs. the *Ppd-D1a* allele were observed in half of the testing environments in Virginia, consistent with findings by Gaju et al. (2011), who attributed these effects to increased aerial biomass at maturity. In the present study, a stronger yet nonsignificant association between grain yield and biomass was observed for lines containing *Ppd-D1b* vs. *Ppd-D1a*. In all Virginia environments, the *Ppd-D1b* allele group had significantly lower grain N content than the *Ppd-D1a* allele group, ranging from a reduction of 4.9% in 16BVA to 8.7% in 15WVA. Similarly, the *Ppd-D1a* locus was previously shown to be positively associated with grain protein and negatively associated with flour extraction n in a study of milling and end-use quality in Australian wheat (Maphosa et al., 2013). These findings suggest that differences in milling and baking quality of soft red winter wheat may be associated with specific alleles at the *Ppd-D1* locus, as well as environmental effects.

Historically, negative associations between grain yield and protein content have been reported in investigations of winter wheat genotypes grown under a range of N rates in field

(Terman et al., 1969) and greenhouse (Tamang et al., 2017) conditions. Interestingly, the negative correlation between grain yield and grain N content in the present study was much higher for wheat lines containing *Ppd-D1a* ($r = -0.71$) than *Ppd-D1b* ($r = -0.09$). This finding may be due to the earlier maturation associated with the *Ppd-D1a* allele, more strongly affecting source/sink dynamics. A greater antagonistic relationship between grain yield and grain protein in wheat lines with the *Ppd-D1a* allele was also reported by Bogard et al. (2013), which they attributed to reduced senescence durations. These findings may be explained by the observation that earlier senescing lines are associated with reduced C assimilation, and higher N remobilization thereby producing grains with higher protein contents that are less "diluted" by carbohydrates (Gregersen et al., 2008).

Averaged over environments, lines in the photoperiod-sensitive (*Ppd-D1b*) group were later maturing and taller than those with the photoperiod-insensitive (*Ppd-D1a*) allele, despite linkage between *Ppd-D1* and a minor effect dwarfing gene *Rht8c* in some genetic backgrounds (Chebotar et al., 2013). Furthermore, the *Ppd-D1b* group was primarily composed of wheat lines containing *Rht-D1a*, which may account for the small effect on plant height in the *Ppd-D1* groups (Butler et al., 2005). Under the low N rate, positive associations between grain yield with heading date and plant height were observed for lines containing the *Ppd-D1a* allele. Similarly, Foulkes et al. (2007) reported a significant association under drought stress between grain yield and delayed flowering in a doubled-haploid population differing in *Ppd-D1* alleles, which they attributed to larger root systems that were derived from longer developmental periods. However, an inverse relationship was observed in the *Ppd-D1b* allelic group where taller and later heading lines produced lower grain yields when 67 kg N ha^{-1} was applied at Zadoks GS 25. This may be a result of greater tiller production due to the *Ppd-D1b* allele itself (Li et al., 2002) and higher

GS 25 N rates (Weisz et al., 2001), making the plant weaker and more prone to lodging. Upon decomposition of grain yield components, floret fertility was significantly higher in the *Ppd-D1b* group of lines in four of the six Virginia environments ($P \leq 0.001$), validating the findings of previous studies in wheat (Worland et al., 1998; Börner et al., 1993; González et al., 2005). However, differences between photoperiod allele groups for the remaining yield components were only significant in a limited number of test environments.

Increased NUE and NUtE in the *Ppd-D1b* allele group in the present study were consistent with a previous study of winter wheat under low and high N rates (Gaju et al., 2011). Grain yield, and thus NUE, was derived from a strong relationship with NUtE in the photoperiod-sensitive ($r = 0.80$, $P < 0.10$) and -insensitive ($r = 0.83$, $P < 0.05$) groups, reflecting similar trends reported in winter wheat (Barraclough et al., 2010; Gaju et al., 2011). Furthermore, the contribution of NUpE to NUE declined with increasing N rates, consistent with results from previous investigations of spring (Ortiz-Monasterio R. et al., 1997) and winter (Le Gouis et al., 2000) wheat. These observations may reflect the suppression of N absorption under high N availability, whereas N assimilation is still maintained. Interestingly, the contributions of NUpE and NUtE to total NUE between *Ppd-D1* allelic groups differed when 67 kg N ha^{-1} was applied at Zadoks GS 25 (Treatments 2 and 4). The decreased contribution from NUpE in the *Ppd-D1a* group under Treatments 2 and 4 may have resulted from reduced absorption of N when excess N was supplied at the five-leaf stage as compared with the *Ppd-D1b* group.

Efficient Nitrogen Management for Wheat Production

Grain yields were highest when a greater proportion of the spring split N rate was applied at Zadoks GS 30 under the standard N rate (134 kg N ha^{-1}) in all environments except 15PVA.

The conflicting findings in 15PVA were likely due to a large rainfall event that occurred shortly after the GS 30 N application and likely resulted in a decrease in the amount of available N as the season progressed. Similar yield advantages from unequal N splits at Zadoks GS 25 and 30 were reported in Virginia winter barley (*Hordeum vulgare* L.), which matched N supply with crop demand to minimize fertilizer loss (Thomason et al., 2012). In the current study, 101 kg N ha^{-1} applied at Zadoks GS 30 also resulted in the highest grain N contents in all environments under the standard spring N rates. These effects are in agreement with previous studies of hard red winter wheat in Nebraska (Bhatta et al., 2017) and Virginia (Thomason et al., 2007), which reported improved end-use qualities with higher N application rates later in plant development. However, higher grain N, in the form of increased protein content, either has a negative or neutral effect on end-use quality in soft red winter wheat, which is largely dependent on protein quality and environment.

Upon dissection of yield components, harvest index, aerial biomass, grains per square meter, spikes per square meter, kernels per spike, and floret fertility were associated with total spring N rate in most test environments, whereas consistent effects were not observed due to different N splitting ratios. Kernel weight was the only yield component that was not significantly affected by N treatment in any Virginia environment, likely due to high heritability of kernel weight in wheat (Sadras and Slafer, 2012). Nitrogen use efficiency, NUpE, and NUtE were significantly affected by total spring N rate in a majority of the test environments. However, different N splitting ratios did not have a consistent effect on the two components of NUE in the present study, unlike the findings of other wheat (Haile et al., 2012) and maize (*Zea mays* L.) N rate × timing studies (Abbasi et al., 2012). The limited N splitting effects in the present study are likely a result of high N demands at these two stages.

Breeding for Increased Nitrogen Use Efficiency in Soft Red Winter Wheat

Recent breeding progress has increased winter wheat grain yields (+0.45% yr^{-1}) relatively equally under high and low N conditions (Cormier et al., 2013) but requires further improvements in traits such as sink and source relationships, senescence dynamics, and root architecture to reach the estimated +2.50% yr^{-1} yield increase needed to meet global food demands by 2050. In the present study, a strong positive correlation between flag leaf chlorophyll content at heading and grain yield under low ($r = 0.91$, $P < 0.05$) and standard ($r = 0.78$, $P < 0.10$) N rates offers a potential avenue for crop improvement. The association and diminishing advantage of greater chlorophyll content under higher rates of N are well studied in durum (Debaeke et al., 2006), winter wheat (Bavec and Bavec, 2001), and maize (Abdel-Ghani et al., 2013). Identification of additional proxy traits and alleles associated with NUE is necessary to further improve genetic gains under both N stress and normal growing conditions. However, it is important to ensure that large changes to crop physiology comply with regional agronomic production systems. For instance, preference for early-maturing soft red winter wheat cultivars to optimize the wheat–soybean [*Glycine max* (L.) Merr.] double cropping system may explain the somewhat limited introgression of the photoperiod-sensitive allele *Ppd-D1b* in the Mid-Atlantic (45.9%) relative to the Northeastern (54.3%) region (Benson et al., 2012). Similarly, the prevalence of *Ppd-D1b* within the US Great Plains is higher in northern (90%) than southern (56%) germplasm, suggesting a role in latitudinal adaptation and temperature stress avoidance (Grogan et al., 2016). Further genetic improvements in NUE must consider effects on the entire cropping system and regional adaptation for profitable grain production.

In conclusion, cultivars with the photoperiod sensitive allele (*Ppd-D1b*) used N fertilizer to produce higher grain dry matter rather than increasing grain N concentrations, across

treatments, compared with cultivars with the insensitive allele (*Ppd-D1a*). Furthermore, when 67 kg N ha^{-1} was applied at Zadoks GS 25, variation in NUtE was the greatest contributor to variation in NUE under moderate N (Treatment 2), whereas NUtE and NUpE contributed equally under standard N (Treatment 4) in the *Ppd-D1a* allele group. Under the same split N ratios and rates, the contribution of NUpE and NUtE in the *Ppd-D1b* allele group was more similar to both allelic groups when 33 kg N ha^{-1} was applied at Zadoks GS 25 (Treatments 3 and 5). The differences in contributions of component traits, NUpE and NUtE, to variation in NUE in the *Ppd-D1a* group may be a result of reduced N absorption under conditions when excess N was supplied compared with the *Ppd-D1b* group.

Acknowledgments

Funding for this project was provided by the USDA National Institute of Food and Agriculture Triticeae Coordinated Agricultural Project no. 2011-68002-30029 and the Virginia Small Grains Board. We'd also like to thank Sam Lawton, Brian Ward, Mark Vaughn, John Seago, and Anthony Christopher for assistance collecting field data.

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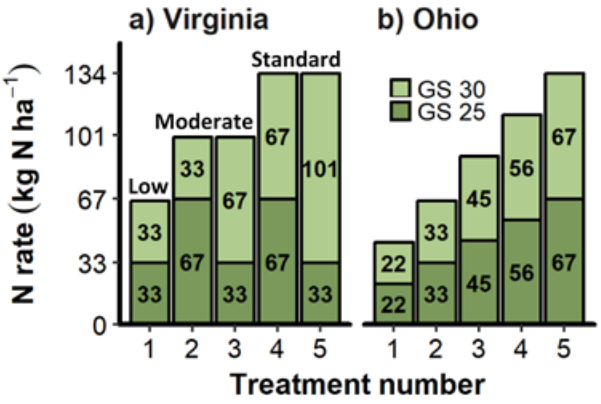


Figure 3.1. Nitrogen treatment structure for (a) Virginia used low (Treatment 1), moderate (Treatments 2 and 3), and standard (Treatments 4 and 5) spring N rates split applied at Zadoks growth stages (GS) 25 and 30 to wheat plots, whereas (b) Ohio treatments were centered on total spring N rates.

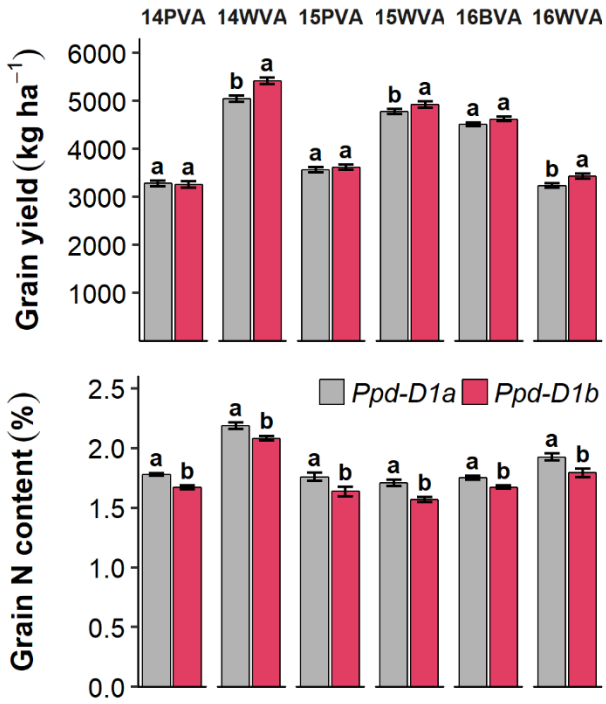


Figure 3.2. Grain yield (top) and grain N content (bottom) for groups of wheat lines with photoperiod sensitivity (*Ppd-D1b*) and insensitivity (*Ppd-D1a*) alleles in six Virginia environments (Painter [PVA], Warsaw [WVA], and Blacksburg [BVA]; 14, 15, and 16 indicate years 2014, 2015, and 2016, respectively). The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

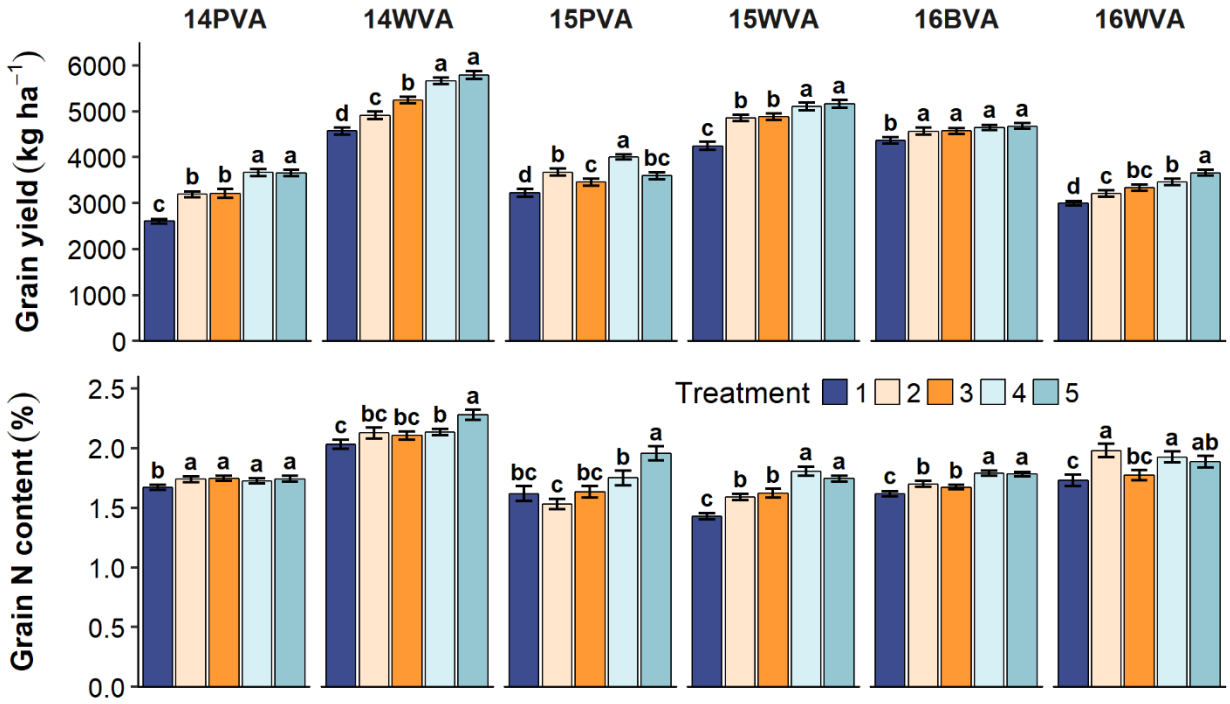


Figure 3.3. Grain yield (top) and grain N content (bottom) of wheat lines under five spring-split N (kg N ha^{-1}) Treatments 1 (33 + 33 kg N ha^{-1}), 2 (67 + 33 kg N ha^{-1}), 3 (33 + 67 kg N ha^{-1}), 4 (67 + 67 kg N ha^{-1}), and 5 (33 + 101 kg N ha^{-1}) in six Virginia environments (Painter [PVA], Warsaw [WVA], and Blacksburg [BVA]; 14, 15, and 16 indicate years 2014, 2015, and 2016, respectively). The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

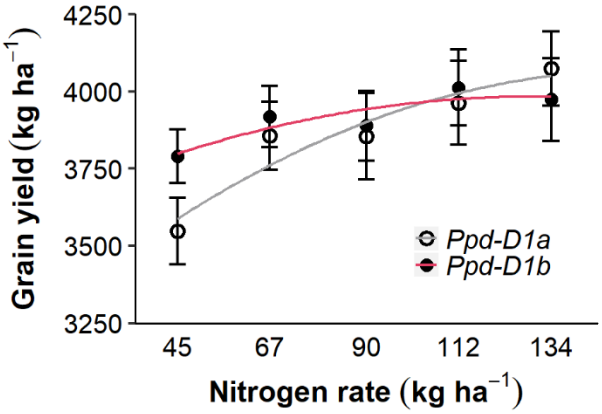


Figure 3.4. Regression of grain yield on five spring N fertilizer rates for photoperiod-sensitive ($y = -0.0259x^2 + 6.6974x + 3550.4$, $R^2 = 0.80$, *Ppd-D1b*) and -insensitive ($y = -0.0402x^2 + 12.3970x + 3111.5$, $R^2 = 0.91$, *Ppd-D1a*) groups of wheat lines over two growing seasons in Custar, OH.

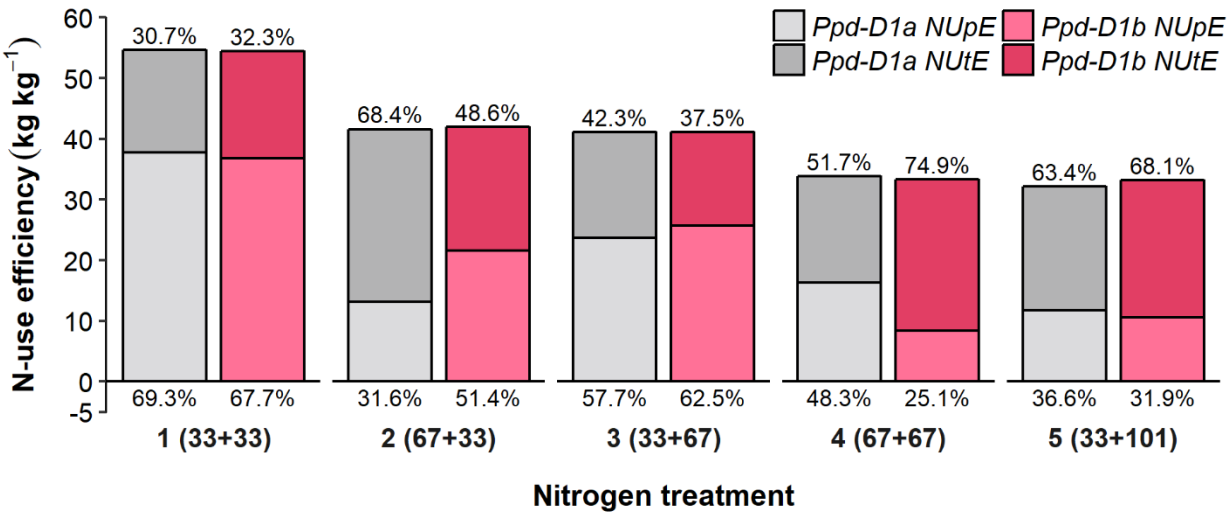


Figure 3.5. Contribution of variation in N uptake (lower percentage, NUpE) and utilization (upper percentage, NUtE) efficiency to variation in N use efficiency for each photoperiod allele group of wheat lines (*Ppd-D1a* and *Ppd-D1b*) at five N treatments across four Virginia testing environments. Percentages below and above boxes represent the contributions of NUpE and NUtE, respectively.

Table 3.1. Wheat lines grouped by photoperiod insensitivity (*Ppd-D1a*) and sensitivity (*Ppd-D1b*) and for reduced plant height alleles (*Rht-B1b* and *Rht-D1b*), field heading dates and plant heights, and pedigrees for the 12 soft red winter wheat lines. Plant heights and heading dates represent means across treatments in four Virginia environments (Warsaw in 2013–2014, 2014–2015, and 2015–2016 and Blacksburg in 2015–2016).

***Ppd* allele groups**	***Rht-B1***	***Rht-D1***	**Heading date**	**Plant height**	**Pedigree**
			Julian d	cm	
Ppd-D1a					
IL02-19483B	*Rht-B1b*	*Rht-D1a*	121	82	Patton/Cardinal//IL96–2550
KY06C-1003-139-8-3	*Rht-B1b*	*Rht-D1a*	123	80	Truman/McCormick//25R37
MD03W485-10-10	*Rht-B1a*	*Rht-D1b*	122	77	USG3209/Tribute//USG3342"S"
MD05W10208-11-8	*Rht-B1a*	*Rht-D1b*	123	77	Tribute/25R42//Chesapeake
OH06-150-57	*Rht-B1b*	*Rht-D1a*	124	88	P.92201D5–2-29/OH708
Sisson†	*Rht-B1a*	*Rht-D1b*	122	77	Coker9803/Freedom
Ppd-D1b					
Bess†	*Rht-B1b*	*Rht-D1a*	124	83	MO11769/Madison
IL07-4415	*Rht-B1b*	*Rht-D1a*	121	81	P96169RE2–3-6–4/IL01–34159
MO080864	*Rht-B1b*	*Rht-D1a*	127	90	981020//P92201D5–2/98072
OH08-161-78	*Rht-B1a*	*Rht-D1a*	124	89	OH751/OH738
OH08-172-42	*Rht-B1b*	*Rht-D1a*	125	85	Douglas/Jekyl
VA08MAS-369	*Rht-B1a*	*Rht-D1b*	122	78	McCormick/GA881130LE5

† The plant introduction number for Bess is PI 642794 (McKendry et al., 2007) and for Sisson is PI 617053 (Griffey et al., 2003).

Table 3.2. Analysis of variance and *F* values for photoperiod group, treatment, and interaction effect on grain yield, grain N content, and yield components for soft red winter wheat lines grown in six Virginia environments.

Environment†	Effect‡	df	Grain yield	Grain N content	Harvest index	Aerial biomass	Kernel weight	Grains m^{-2}	Spikes m^{-2}	Kernels $spike^{-1}$	Floret fertility
14PVA	*Ppd*‡	1	0.20ns§	34.99***	2.68ns	0.43ns	0.31ns	1.86ns	0.51ns	0.90ns	0.60ns
	N	4	33.83***	2.24ns	6.64***	60.23***	2.11ns	0.78ns	0.39ns	1.12ns	1.89ns
	Ppd × N	4	0.54ns	0.59ns	0.78ns	0.31ns	0.72ns	0.93ns	0.42ns	0.60ns	1.34ns
14WVA	*Ppd*	1	36.93***	10.31**	28.28***	0.51ns	0.85ns	1.37ns	1.70ns	0.00ns	0.21ns
	N	4	53.39***	6.00***	3.47**	15.88***	0.48ns	0.37ns	0.57ns	0.88ns	2.58*
	Ppd × N	4	0.27ns	0.63ns	0.28ns	0.97ns	0.51ns	0.56ns	1.04ns	1.34ns	1.21ns
15PVA	*Ppd*	1	0.69ns	6.52*	0.41ns	0.24ns	9.32**	1.97ns	13.65***	34.82***	30.84***
	N	4	14.48***	9.58***	1.90ns	10.67***	0.45ns	3.82**	6.78***	1.36ns	0.66ns
	Ppd × N	4	0.40ns	1.07ns	1.38ns	0.85ns	0.82ns	1.85ns	0.66ns	1.28ns	1.14ns
15WVA	*Ppd*	1	4.56*	30.90***	1.11ns	0.34ns	4.85*	5.21*	22.35***	63.33***	60.18***
	N	4	22.15***	26.45***	2.09ns	24.21***	0.51ns	10.94***	7.72***	4.27**	5.58***
	Ppd × N	4	0.13ns	0.68ns	1.00ns	1.20ns	0.82ns	0.60ns	0.39ns	0.07ns	0.96ns
16BVA	*Ppd*	1	3.75ns	20.74***	0.46ns	3.23ns	2.56ns	6.85**	0.04ns	16.49***	12.62***
	N	4	3.32*	13.97***	1.22ns	2.39ns	0.09ns	2.63*	2.82*	4.32**	3.60**
	Ppd × N	4	0.37ns	1.47ns	3.14*	1.57ns	0.72ns	0.47ns	1.69ns	0.31ns	0.12ns
16WVA	*Ppd*	1	14.14***	10.46**	11.95***	1.16ns	0.04ns	3.27ns	0.01ns	3.41ns	17.51***
	N	4	18.19***	5.18***	4.83**	6.13***	0.78ns	4.60**	2.73*	2.52*	4.50**
	Ppd × N	4	2.44ns	1.57ns	0.65ns	1.05ns	0.19ns	0.56ns	0.26ns	0.69ns	0.84ns

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Numbers indicate years 2013–2014 (14), 2014–2015 (15), and 2015–2016 (16); PVA, Painter, VA; WVA, Warsaw, VA; BVA, Blacksburg, VA.
‡ *Ppd*, photoperiod allele grouping; N, N rate.
§ ns, not significant.

Table 3.3. Least square means of wheat harvest index interaction effects in the 2015–2016 Blacksburg, VA, testing environment.

	Harvest index	
N treatment	***Ppd-D1a***	***Ppd-D1b***
kg N ha^{-1}	%	
1 (33 + 33)†	36.9c‡	38.4bc
2 (67 + 33)	37.3bc	38.8abc
3 (33 + 67)	40.5a	37.8bc
4 (67 + 67)	39.0ab	38.2bc
5 (33 + 101)	39.1ab	38.1bc

† Nitrogen treatments (kg N ha^{-1}) were split applied at Zadoks growth stages 25 and 30.

‡ The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

Table 3.4. Least squares means of yield component traits for groups of wheat lines having either photoperiod-insensitive (*Ppd-D1a*) or -sensitive (*Ppd-D1b*) alleles in significant Virginia testing environments. Values represent means for two photoperiod groups within testing environments.

	Photoperiod group	
Trait and environment†	***Ppd-D1a***	***Ppd-D1b***
Harvest index (%)		
14WVA	37.1b‡	39.6a
16WVA	30.3b	32.3a
Kernel weight (mg)		
15PVA	34.3a	32.7b
15WVA	35.6a	34.6b
Grains m^{-2}		
15WVA	18,472b	19,930a
16BVA	26,062b	28,131a
Spikes m^{-2}		
15PVA	625a	569b
15WVA	698a	618b
Kernels $spike^{-1}$		
15PVA	33b	38a
15WVA	27b	32a
16BVA	32b	35a
Floret fertility (%)		
15PVA	84.3b	87.4a
15WVA	81.6b	85.8a
16BVA	84.2b	86.4a
16WVA	77.9b	81.3a

† In environment names, numbers indicate years 2013–2014 (14), 2014–2015 (15), and 2015–2016 (16); WVA, Warsaw, VA; PVA, Painter, VA; BVA, Blacksburg, VA.

‡ The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

Table 3.5. Least squares means of wheat yield component traits for five spring split N Treatments 1 (33 + 33 kg N ha^{-1}), 2 (67 + 33 kg N ha^{-1}), 3 (33 + 67 kg N ha^{-1}), 4 (67 + 67 kg N ha^{-1}), and 5 (33 + 101 kg N ha^{-1}) in significant Virginia testing environments. Values represent means over treatment.

	Nitrogen treatment‡				
Trait and environment†	**1**	**2**	**3**	**4**	**5**
Harvest index (%)					
14PVA	34.4c§	36.3b	36.6b	37.9ab	38.9a
14WVA	37.1c	37.7bc	38.4abc	38.6ab	39.7a
16WVA	30.3bc	29.7c	32.0ab	31.3bc	33.3a
Aerial biomass (g m^{-2})					
14PVA	1,127c	1,442b	1,442b	1,658a	1,614a
14WVA	1,255c	1,353b	1,444b	1,561a	1,552a
15PVA	1,168c	1,301b	1,133c	1,426a	1,224bc
15WVA	1,117c	1,315b	1,287b	1,447a	1,388a
16WVA	907c	1,133ab	1,083b	1,115ab	1,173a
Grains m^{-2}					
15PVA	18,986c	20,944abc	20,535bc	23,060a	22,039ab
15WVA	15,642d	18,580c	19,159bc	21,627a	20,994ab
16BVA	25,453b	26,903ab	26,154b	29,091a	27,881ab
16WVA	14,026c	16,100b	16,490cb	16,248b	18,419a
Spikes m^{-2}					
15PVA	548b	572b	576b	638a	648a
15WVA	589d	630cd	650bc	719a	701ab
16BVA	741b	766ab	805a	807a	761ab
16WVA	566c	619ab	591abc	585bc	638a
Kernels $spike^{-1}$					
15WVA	27b	30a	29a	30a	30a
16BVA	33bc	34ab	31c	35ab	35a
16WVA	25c	26bc	28ab	27ab	28a
Floret fertility (%)					
14WVA	81.4ab	82.7a	82.1ab	83.6a	80.2b
15WVA	81.3b	83.6a	83.9a	84.5a	85.2a
16BVA	84.7bc	85.7ab	83.5c	85.7ab	86.9a
16WVA	77.9b	77.6b	80.5a	79.9ab	82.1a

† In environment names, numbers indicate years 2013–2014 (14), 2014–2015 (15), and 2015–2016 (16); WVA, PVA, Painter, VA; Warsaw, VA; BVA, Blacksburg, VA.

‡ Treatments were split applied at Zadoks growth stages 25 and 30.

§ The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

Table 3.6. Analysis of variance and *F* values for *Ppd* group, treatment, and interaction effect on N use efficiency (NUE), N uptake efficiency (NUpE), N utilization efficiency (NUtE), and N harvest index for soft red winter wheat lines grown in six Virginia environments.

Environment†	Effect‡	df	NUE	NUpE	NUtE	N harvest index
15PVA	*Ppd*	1	1.14ns§	0.64ns	4.96*	2.49ns
	N	4	98.68***	21.88***	15.51***	2.11ns
	Ppd × N	4	0.61ns	0.88ns	2.49*	0.87ns
15WVA	*Ppd*	1	2.50ns	6.03*	15.05***	0.42ns
	N	4	134.12***	35.91***	25.26***	0.46ns
	Ppd × N	4	0.17ns	0.52ns	0.48ns	1.63ns
16BVA	*Ppd*	1	4.46*	0.01ns	1.00ns	2.44ns
	N	4	329.24***	40.24***	5.04***	0.67ns
	Ppd × N	4	0.73ns	0.57ns	0.85ns	2.19ns
16WVA	*Ppd*	1	19.35***	0.19ns	3.16ns	0.40ns
	N	4	172.29***	10.51***	5.77***	3.45*
	Ppd × N	4	3.78**	0.13ns	0.44ns	1.14ns

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† In environment names, numbers indicate years 2014–2015 (15), and 2015–2016 (16); PVA, Painter, VA; WVA, Warsaw, VA; BVA, Blacksburg, VA.
‡ *Ppd*, photoperiod allele grouping; N, N rate.
§ ns, not significant.

Table 3.7. Least squares means of wheat N use efficiency interaction effects in Warsaw, VA, in 2015–2016 and N utilization efficiency interaction effects in Painter, VA, in 2014–2015.

	N use efficiency		N utilization efficiency	
Treatment†	***Ppd-D1a***	***Ppd-D1b***	***Ppd-D1a***	***Ppd-D1b***
kg N ha^{-1}		kg kg^{-1}		
1 (33 + 33)	43.6b‡	48.4a	39.9cd	45.9a
2 (67 + 33)	31.0d	35.2c	40.6bcd	43.7ab
3 (33 + 67)	33.8c	34.9c	39.8cd	42.5abc
4 (67 + 67)	27.3ef	27.0f	37.3de	35.9e
5 (33 + 101)	27.9ef	29.3de	34.6e	33.7e

† Treatments were split applied at Zadoks growth stages 25 and 30.

‡ The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means for a trait followed by the same letter are not significantly different.

Table 3.8. Least squares means of N-related traits for groups of wheat lines having a photoperiod allele (*Ppd-D1*) in significant Virginia testing environments. Values represent means for two photoperiod groups within testing environments.

	Photoperiod group	
Trait and environment†	***Ppd-D1a***	***Ppd-D1b***
	kg kg^{-1}	
N use efficiency		
16BVA	44.4b‡	45.7a
16WVA	32.7b	35.0a
N uptake efficiency		
15WVA	1.14a	1.09b
N utilization efficiency		
15PVA	38.4b	40.4a
15WVA	42.0b	45.2a

† In environment names, numbers indicate years 2014–2015 (15), and 2015–2016 (16); BVA, Blacksburg, VA; WVA, Warsaw, VA; PVA, Painter, VA.

‡ The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

Table 3.9. Least squares means of wheat N use efficiency traits for spring split N Treatments 1 (33 + 33 kg N ha^{-1}), 2 (67 + 33 kg N ha^{-1}), 3 (33 + 67 kg N ha^{-1}), 4 (67 + 67 kg N ha^{-1}), and 5 (33 + 101 kg N ha^{-1}) in significant Virginia testing environments. Values represent means over treatment.

	Nitrogen treatment‡				
Trait and environment†	**1**	**2**	**3**	**4**	**5**
N use efficiency (kg kg^{-1})					
15PVA	47.8a§	36.4b	34.3b	29.7c	26.7c
15WVA	64.1a	49.1b	49.4b	38.9c	39.4c
16BVA	65.2a	45.2b	45.3b	34.7c	34.9c
16WVA	46.0a	33.1b	34.4b	27.2c	28.6c
N uptake efficiency (kg kg^{-1})					
15PVA	1.19a	0.90b	0.87b	0.85b	0.83b
15WVA	1.29a	1.11b	1.14b	1.02c	0.99c
16BVA	2.13a	1.64b	1.64b	1.39c	1.36c
16WVA	1.25a	1.14a	0.96b	0.84b	0.88b
N utilization efficiency (kg kg^{-1})					
15PVA	42.9a	42.2a	41.2a	36.6b	34.1b
15WVA	50.3a	45.2b	43.9b	38.3c	40.1c
16BVA	32.8a	28.7b	28.8b	26.2b	27.8b
16WVA	39.1a	31.1c	36.2ab	32.7bc	33.0bc
N harvest index (%)					
16WVA	59.4ab	56.9b	60.8a	56.9b	59.7a

† In environment names, numbers indicate years 2014–2015 (15), and 2015–2016 (16); PVA, Painter, VA; WVA, Warsaw, VA; BVA, Blacksburg, VA.

‡ Treatments were split applied at Zadoks growth stages 25 and 30.

§ The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

Table 3.10. Pearson correlation coefficients between traits amongst photoperiod-sensitive (*Ppd-D1b*) and -insensitive (*Ppd-D1a*) wheat groups (above and below the diagonal, respectively). Means are over four testing environments (Painter in 2014–2015, Warsaw in 2014–2015 and 2015–2016, and Blacksburg in 2015–2016) and five N treatments.

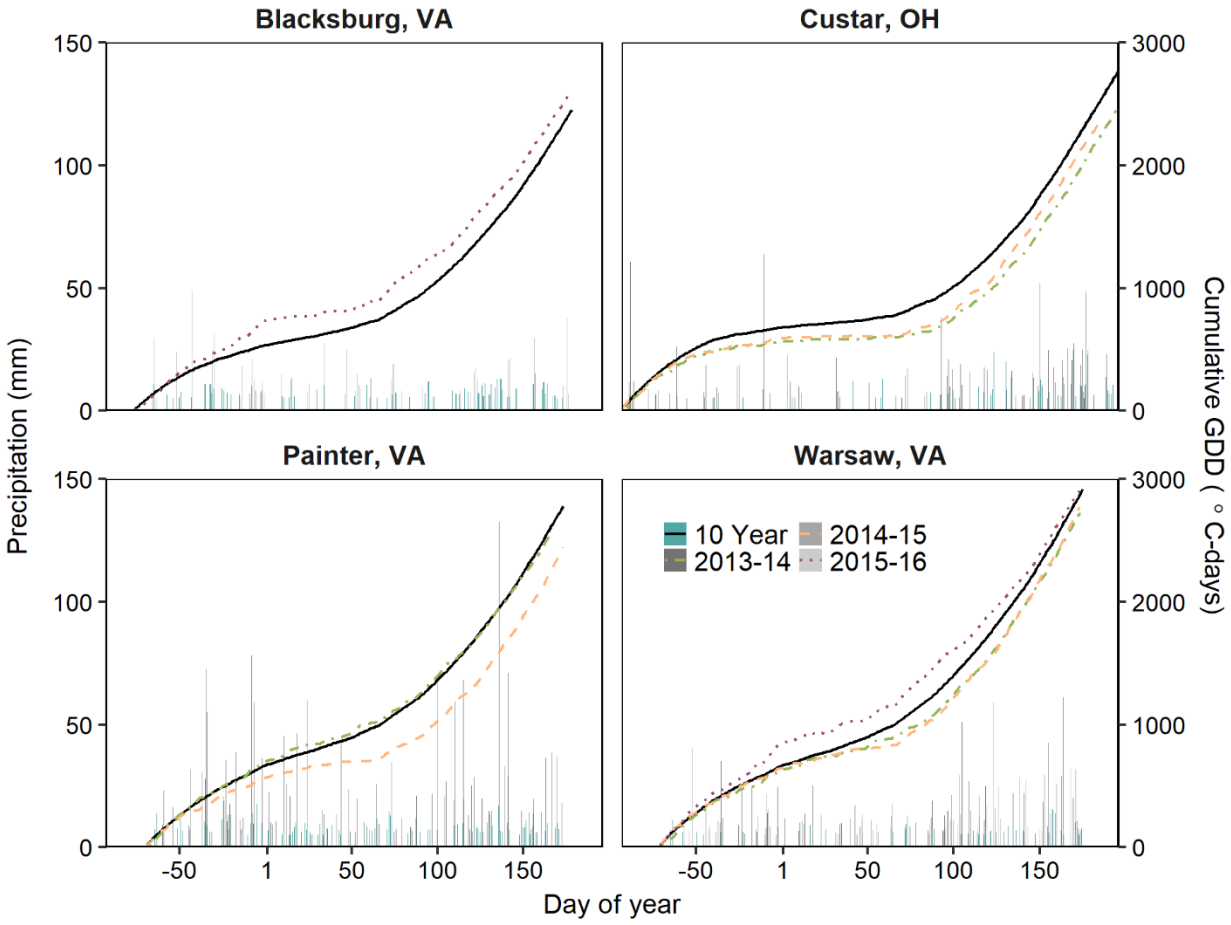
Trait†	Grain yield	Grain N content	Harvest index	Aerial biomass	N harvest index	NUpE	NUtE
Grain yield		−0.09	0.32	0.41	0.65	0.41	0.80‡
Grain N content	−0.71		0.66	−0.45	0.61	0.37	−0.44
Harvest index	0.79‡	−0.22		−0.63	−0.05	0.66	0.10
Aerial biomass	0.15	−0.79‡	−0.39		0.80‡	−0.08	0.27
N harvest index	0.42	0.22	−0.65	0.62		0.49	0.29
NUpE	−0.39	0.57	−0.46	0.13	−0.17		−0.21
NUtE	0.83*	−0.83*	0.46	0.37	0.29	−0.82*	

* Significant at the 0.05 probability level.
† NUpE, N uptake efficiency; NUtE, N utilization efficiency.
‡ Significant at the 0.10 probability level.

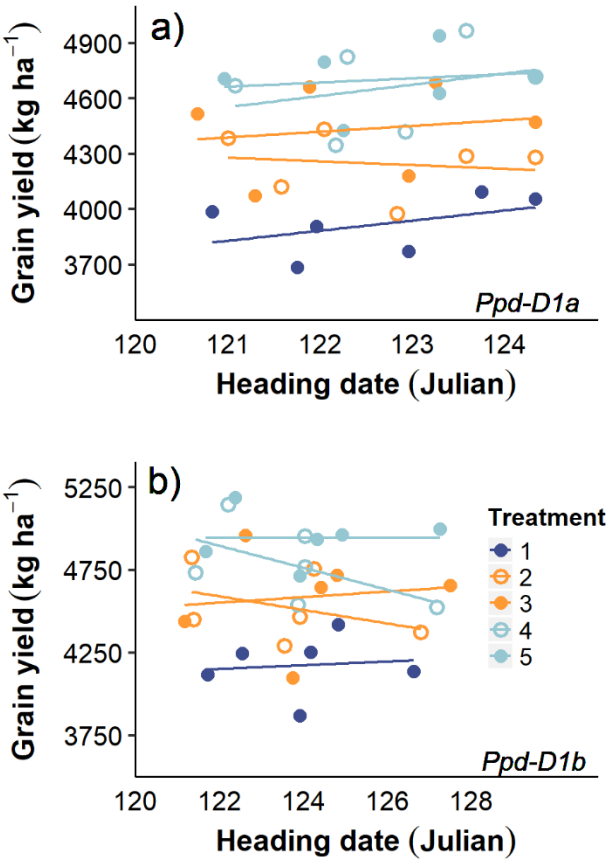
Table 3.11. Pearson correlation coefficients for photosynthetic traits under low (67 kg N ha^{-1}) and standard (134 kg N ha^{-1}) N rates (above and below the diagonal, respectively). Treatments were split applied at Zadoks growth stages 25 and 30 1:1 under the low rate (Treatment 1) and 1:3 under the standard rate (Treatment 5). Means are over two testing environments (Warsaw, VA, in 2013–2014 and 2015–2016) and six wheat lines (IL07-4415, MD03W485-10-10, MD05W10208-11-8, MO080864, OH06150-57, and Sisson).

Trait	Grain yield	Grain N content	Photosynthetic rate	Stomatal conductance	Transpiration rate	Chlorophyll content
Grain yield		−0.97**	−0.45	−0.59	−0.56	0.91*
Grain N content	−0.90*		0.47	0.53	0.40	−0.85*
Photosynthetic rate	0.20	−0.02		0.39	0.67	−0.67
Stomatal conductance	0.25	−0.13	0.89*		0.68	−0.73
Transpiration rate	0.01	0.23	0.94**	0.83*		−0.81†
Chlorophyll content	0.78†	−0.72	−0.38	−0.35	−0.52	

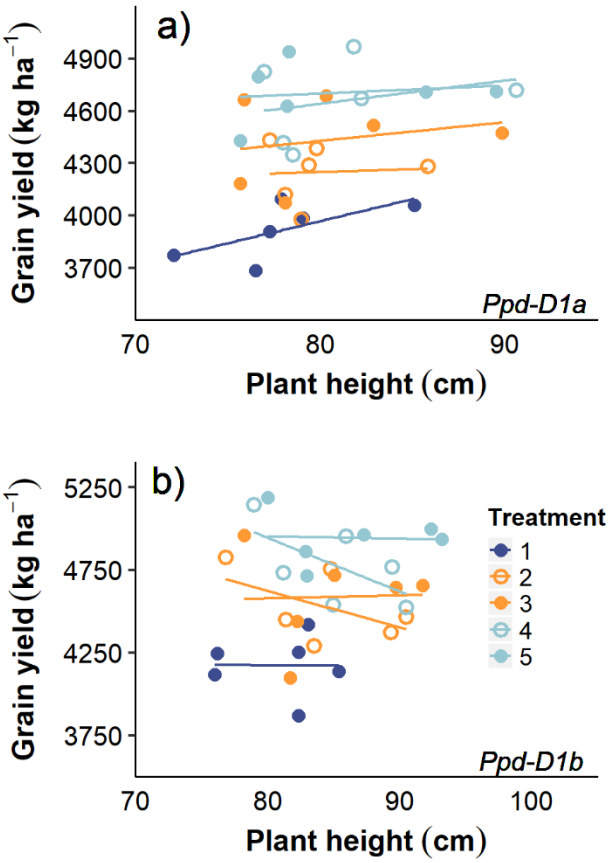
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† Significant at the 0.10 probability level.



Supplementary Figure 3.1. Daily precipitation (bars) and cumulative growing degree days (lines; GDD) at Blacksburg, Custar, Painter, and Warsaw during the 2013-14 (dark grey bars and green dot-dashed lines), 2014-15 (grey bars and orange dashed lines), and 2015-16 (light grey bars and purple dotted lines) growing seasons and 10 year averages (blue bars and black solid lines) from 2006 to 2016. January 1st represents the first day of year with weather data collected from sowing to harvest for each testing environment.



Supplementary Figure 3.2. Grain yield by heading date for wheat lines within each photoperiod group under five spring-split nitrogen treatments over four testing environments (14WVA, 15WVA, 16BVA, and 16WVA). Regressions of photoperiod (a) insensitive (*Ppd-D1a*) under treatments 1 ($y = 57.641x - 3{,}153.5$; $R^2 = 0.19$), 2 ($y = -35.248x + 8{,}572.1$; $R^2 = 0.06$), 3 ($y = 49.243x - 1{,}590.8$; $R^2 = 0.06$), 4 ($y = 67.094x - 3{,}570.6$; $R^2 = 0.12$), and 5 ($y = 31.734x + 815.51$; $R^2 = 0.04$) and (b) sensitive (*Ppd-D1b*) under treatments 1 ($y = 11.417x + 2{,}718.9$; $R^2 = 0.01$), 2 ($y = -44.037x + 9{,}966.5$; $R^2 = 0.22$), 3 ($y = 17.443x + 2{,}420.7$; $R^2 = 0.02$), 4 ($y = -66.511x + 12{,}632$; $R^2 = 0.30$), and 5 ($y = -8.5072x + 5{,}998.7$; $R^2 = 0.01$).



Supplementary Figure 3.3. Grain yield by plant height for wheat lines within each photoperiod group under five spring-split nitrogen treatments over four testing environments (14WVA, 15WVA, 16BVA, and 16WVA). Regressions of photoperiod (a) insensitive (*Ppd-D1a*) under treatments 1 ($y = 25.361x + 1{,}939.4$; $R^2 = 0.44$), 3 ($y = 10.777x + 3{,}566.2$; $R^2 = 0.05$), 5 ($y = 4.5735x + 4{,}333.9$; $R^2 = 0.02$), 2 ($y = 3.3444x + 3{,}981.2$; $R^2 = 0.00$), and 4 ($y = 13.335x + 3{,}574.8$; $R^2 = 0.08$ and (b) sensitive (*Ppd-D1b*) under treatments 1 ($y = -0.257x + 4{,}194.2$; $R^2 = 0.00$), 2 ($y = -22.272x + 6{,}407.4$; $R^2 = 0.28$), 3 ($y = 1.8001x + 4{,}433.9$; $R^2 = 0.01$), 4 ($y = -32.452x + 7{,}541.7$; $R^2 = 0.37$), and 5 ($y = -1.4302x + 5{,}067.4$; $R^2 = 0.00$).

Supplementary Table 3.1. Experimental summary of wheat NUE study grown in six Virginia (VA) and two Ohio (OH) environments.

Season	Testing site	Environment designation	Soil series	Soil texture	Planting date	Harvest date
2013-14	Custar, OH	14COH	Hoytville	Clay loam	02 - Oct.	15 - July
	Painter, VA	14PVA	Bojac	Sandy loam	24 - Oct.	16 - June
	Warsaw, VA	14WVA	Kempsville	Loam	22 - Oct.	24 - June
2014-15	Custar, OH	15COH	Hoytville	Clay loam	29 - Sept.	06 - July
	Painter, VA	15PVA	Bojac	Sandy loam	23 - Oct.	22 - June
	Warsaw, VA	15WVA	Kempsville	Loam	21 - Oct.	22 - June
2015-16	Blacksburg, VA	16BVA	Hayter	Silt loam	16 - Oct.	27 - June
	Warsaw, VA	16WVA	Kempsville	Loam	21 - Oct.	22 - June

Supplementary Table 3.2. Management practices in each Virginia testing environment including product name, application rate of fertilizer or active ingredient (A.I.), and date of application. Experiments were grown during the 2013-14 season at Painter (14PVA) and Warsaw (14WVA), the 2014-15 season at Painter (15PVA) and Warsaw (15WVA), and during the 2015-16 season at Blacksburg (16BVA) and Warsaw (16WVA).

Environment	Product	Application rate	Application date
14PVA	Pre-plant fertilizer	34-0-0-0 kg nutrient ha^{-1}[†]	10/23/2013
	GS25 UAN	Variable[‡]	03/10/2014
	Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/12/2014
	G30 UAN	Variable	04/03/2014
14WVA	Pre-plant fertilizer	34-67-67-2 kg nutrient ha^{-1}	10/18/2013
	GS25 UAN	Variable	03/01/2014
	Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/11/2014
	Starane™	0.21 kg A.I. ha^{-1}	03/11/2014
	GS30 UAN	Variable	04/04/2014
	Palisade EC	0.08 kg A.I. ha^{-1}	04/04/2014
	Tilt	0.07 kg A.I. ha^{-1}	04/10/2014
	Prosaro	0.14 kg A.I. ha^{-1}	05/08/2014
	Tilt	007 kg A.I. ha^{-1}	05/08/2014
15PVA	Pre-plant fertilizer	34-0-0-0 kg nutrient ha^{-1}	10/20/2014
	GS25 UAN	Variable	03/17/2015
	Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/25/2015
	GS30 UAN	Variable	04/30/2015
15WVA	Pre-plant fertilizer	34-67-67-5 kg nutrient ha^{-1}	10/06/2014
	GS25 UAN	Variable	03/19/2015
	Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/24/2015
	Starane™	0.21 kg A.I. ha^{-1}	03/24/2015
	GS30 UAN	Variable	04/01/2015
	Palisade EC	0.08 kg A.I. ha^{-1}	04/06/2015
	Tilt	0.07 kg A.I. ha^{-1}	04/10/2015
	Prosaro	0.14 kg A.I. ha^{-1}	05/14/2015
	Tilt	007 kg A.I. ha^{-1}	05/14/2015
16BVA	Pre-plant fertilizer	34-67-67-5 kg nutrient ha^{-1}	10/16/2015
	Harmony Extra SG®	0.02 kg A.I. ha^{-1}	11/16/2015
	GS25 UAN	Variable	03/08/2016
	Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/23/2016
	GS30 UAN	Variable	03/23/2016
	Palisade EC	0.08 kg A.I. ha^{-1}	03/31/2016
	Tilt	0.04 kg A.I. ha^{-1}	03/31/2016
	Prosaro	0.14 kg A.I. ha^{-1}	05/12/2016
	Tilt	0.10 kg A.I. ha^{-1}	05/26/2016

Environment	Product	Application rate	Application date
16WVA	Pre-plant fertilizer	34-67-67-5 kg nutrient ha^{-1}	10/14/2015
	Starane™	0.21 kg A.I. ha^{-1}	12/06/2015
	GS25 UAN	Variable	02/19/2016
	Harmony Extra SG®	0.02 kg A.I. ha^{-1}	03/08/2016
	Starane™	0.15 kg A.I. ha^{-1}	03/08/2016
	GS30 UAN	Variable	03/12/2016
	Palisade EC	0.06 kg A.I. ha^{-1}	03/30/2016
	Fitness	0.12 kg A.I. ha^{-1}	03/30/2016
	Fitness	0.12 kg A.I. ha^{-1}	04/19/2016
	Prosaro	0.14 kg A.I. ha^{-1}	05/20/2016

†Nitrogen (UAN), phosphorous, potassium, and sulfur re-plant fertilizer applied, respectively.
‡Spring N application rates applied according to treatments in Figure 3.1.

CHAPTER IV:

CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions

The first chapter reported significant genotypic variation for grain yield, grain N content, physiological traits, and N traits under a range of N supplies and testing environments (Table 4.1). Most of the traits were highly heritable and had high genotypic contributions to the total observed variation except for NUpE (heritability ranged from 0.00 to 0.05). Two wheat lines, VA08MAS-369 and VA09W-73, expressed high yield stability over N-environments and high mean grain yields – providing regional breeders with sources of high NUE germplasm to begin breeding for N response. Finally, chapter two emphasized the importance of testing wheat lines under more than three N conditions per location to identify significant genotype by N rate interactions. Future trials for NUE in soft red winter wheat will require breeders to optimize the number of testing sites and N rates per site to assess both yield stability and interaction effects.

The second chapter used two mapping populations to identify significant marker-trait-associations for 11 N-related traits (**Table 4.1**). The study employed a genotyping-by-sequence approach to produce more than 2,400 high-density polymorphic genetic markers for the parents and progeny in both populations and detected a combined 130 quantitative trait loci (QTL). The reproducible QTL for NUE mapped to chromosomes 1D, 2D, 4A, 6A, 7A, and 7D. Two of the six QTL (2D and 4A) were also associated with known photoperiod response and a QTL associated with resistance to Fusarium head blight. Three of the QTL (6A, 7A, and 7D) were identified at similar mapping intervals in previous NUE mapping studies in wheat, while the QTL on 1D was not previously reported. A majority of the QTL for NUE identified in this study

explained less than 20 % of the observed phenotypic variation. Upon further validation in unrelated germplasm, these markers may be implementable in NUE marker-assisted breeding programs. Breeders may benefit from including *QNue.151-1D* and *QNue.151-7D* into MAS programs as they were reproducible over multiple site seasons, explained a large percentage of the variation, and conferred an increase of 5.9 % and 5.7 % in NUE when combined. Continued selection on Fhb_6A_Neuse will also enable genetic improvements for FHB resistance without reducing NUE as was observed for the QTL, Fhb_4A-Neuse.

Chapter three of this dissertation emphasizes the importance of accounting for major genes and effects of different alleles governing major morpho-physiological traits including heading date and plant height when conducting nitrogen use efficiency (NUE) studies. Using the methodology described in Table 4.1, it was observed that wheat (*Triticum aestivum* L.) lines with the photoperiod sensitivity allele on chromosome 1D (*Ppd-D1b*) had higher grain yields, floret fertility, and kernels per spike at the expense of grain nitrogen (N) content. The rate of N applied at the five tiller and stem elongation stages also contributed to grain yield, several yield components, grain N content, and N-traits such as NUE, N uptake efficiency (NUpE), and N utilization efficiency (NUtE). The contribution of component traits (NUpE and NUtE) to NUE was similar between photoperiod allele groups at a given N rate when 33 kg N ha^{-1} was applied at the five tiller stage. However, a relative decrease in the contribution from NUpE in the *Ppd-D1a* group was observed when 67 kg N ha^{-1} was applied. This may have resulted from reduced absorption of N when excess N was supplied at the five tiller stage as compared with the *Ppd-D1b* group. Finally, in the first chapter it also was observed that NUtE and flag leaf chlorophyll content were significantly associated with grain yield across N supplies and may serve as target traits for future NUE improvement.

Future Directions

The findings reported in this dissertation will enable breeders to develop diallel crosses using several high and low NUE parental lines to determine the merits of actively breeding for N response. Through this approach, breeding for improved N response may be of merit if the progeny from high × high NUE crosses express higher grain yield and quality under a range of N conditions than low × low NUE crosses. The current study determined that certain phenotypes are strongly associated with NUE improvement under low N conditions and may benefit from development of the "ideal phenotype" that combines desirable plant architecture, chlorophyll content, senescence dynamics, and head morphology. The ideotype strategy would employ optimized N acquisition, transportation, remobilization, and assimilation to produce crop varieties with strong agronomic performance, disease resistance, and high NUE. Wheat breeding for NUE within the Eastern United States may also benefit from the exploration of secondary and tertiary germplasm to identify novel N response alleles. Previous investigations have identified significant genetic variation amongst geographic regions, classes of wheat, wheat growth habits, and related species that may confer significant genetic gains in N response with introgression into adapted germplasm.

A less conventional direction would require breeders to dive further into the realm of molecular biology by developing a nested-association mapping (NAM) panel of 20 to 30 populations to identify quantitative trait loci (QTL) that confer meaningful improvements in NUE across genetic materials. The Virginia Tech Small Grains Breeding Program has already begun development of such a panel that currently consists of five doubled haploid populations

that share a common low NUE parent, '38158', PI 619052 (Table 4.2). An allele report for the parents of these populations is provided in Table 4.3. However, the 38158 NAM panel will likely require the addition of more populations to identify QTL with greater application to the breeding program. Results of the mapping study mentioned in this dissertation combined with those from the NAM panel may then be followed up with fine mapping and cloning of genes conferring a positive N response.

Another approach to identify QTL was recently employed in rice (*Oryza sativa* L.) using a genome wide scan of two subspecies to identify a major contributor to NUE that arose upon divergence. Wheat breeders can similarly investigate the potential closely related species for improved N response by conducting genome wide scans in its many wild relatives for eventual gene identification. The cloned genes from such approaches may then be introduced into adapted lines using conventional backcrossing or by implementing a CRISPR based strategy.

Breeders targeting improved NUE may also benefit from implementing genomic prediction models into their programs. This will require the tactful development of a suitable panel of lines that takes into account population structure and the addition of newer plant material. The implementation of genomic selection may indeed prove to be more valuable than QTL mapping alone as we expect that NUE, much like yield *per se*, is a complex quantitative trait controlled by several to many genes. Wheat breeders have already begun to utilize genomic selection for similarly complex traits including grain yield, disease resistance, and end use quality.

Table 4.1. Summary of methodology and key findings in each study.

Chapter	Analysis	Study	Traits	Methods
I	Allelic effects	I	13	Two *Ppd-D1* allele groups were grown under five N rates (Ohio) or N rate by timing (Virginia) treatments in a total of eight environments.
		II	1	
II	Genotypic variation	I	6	Two panels of wheat lines were tested under two, three, or five N rates to detect G × N interactions in a combined 51 N-environments.
		II	6	
		III	1	
III	QTL mapping	I	11	Two bi-parental mapping populations sharing a low NUE parent ('Yorktown') were grown under two N rates in four environments.
		II	11	

Table 4.2. Summary of the '38158' (derived from FFR/GA-Gore) doubled haploid (DH) populations developed at Virginia Tech and the number of DHs (No. of DHs).

Low NUE line	High NUE line	High NUE line pedigree	No. of DHs
38158	Sisson	Coker9803/Freedom	103
38158	VA05W-151	Pioneer26R24/McCormick	174
38158	VA08MAS-369	McCormick/GA881130LE5	116
38158	MD03W665-10-5	USG3209/Tribute//Chesapeake	32
38158	OH08-161-78	OH751/OH738	63

Table 4.3. Allele report for the 38158 NAM population parents from a suite of haplotyping markers. The 12 selected markers were shown to confer major physiological effects in the present dissertation, Brian P. Ward's dissertation, and many of the previous investigations discussed in Chapters 1, 2, and 3.

Wheat line	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D3*	*Ppd-A1*	*Ppd-B1*	*Ppd-D1*	*Fhb1*	*Sr36*	*TaSus2-2B*	*1RS*¶
38158	a†	b‡	b	b	b	b	b	a	a	a	a	no
Sisson	a	b	b	b	b	a	a	b	a	b	b	1BL
VA05W-151	a	b	b	b	b	a	b	b	a	a	a	no
VA08MAS-369	a	b	a	b	a	a	b	b	a	a	a	1AL
MD03W665-10-5	a	b	b	b	b	b	NC§	a	a	b	b	1BL
OH08-161-78	a	a	b	b	b	b	a	b	a	b	b	no

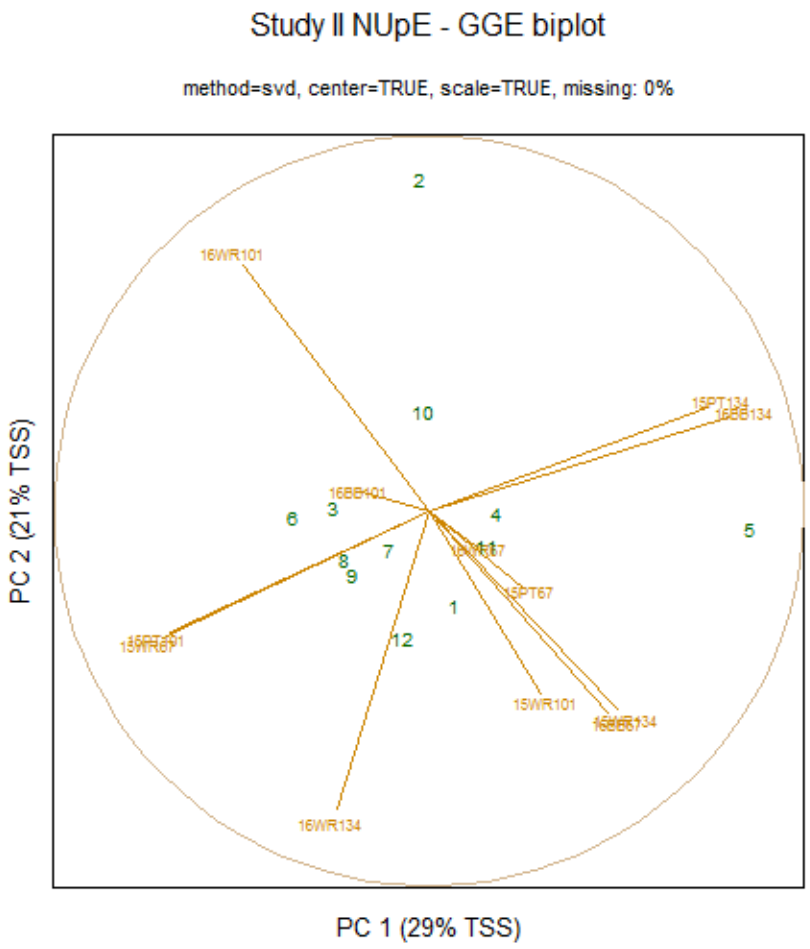
† The 'a' allele represents the absence of a dwarfing allele (*Rht-B1* and *Rht-D1*), short or early vernilization genes (*Vrn-A1*, *Vrn-B1*, and *Vrn-D3*), photoperiod insensitivity (*Ppd-A1*, *Ppd-B1*, and *Ppd-D1*), the absence of resistance alleles at *Fhb1* and *Sr36*, and the absence of the sucrose synthase allele (*TaSus2-2B*).

‡ The 'b' allele represents the presence of a dwarfing allele (*Rht-B1* and *Rht-D1*), long or late vernilization genes (*Vrn-A1*, *Vrn-B1*, and *Vrn-D3*), photoperiod sensitivity (*Ppd-A1*, *Ppd-B1*, and *Ppd-D1*), the presence of resistance alleles at *Fhb1* and *Sr36*, and the presence of the sucrose synthase allele (*TaSus2-2B*).

§ No call.

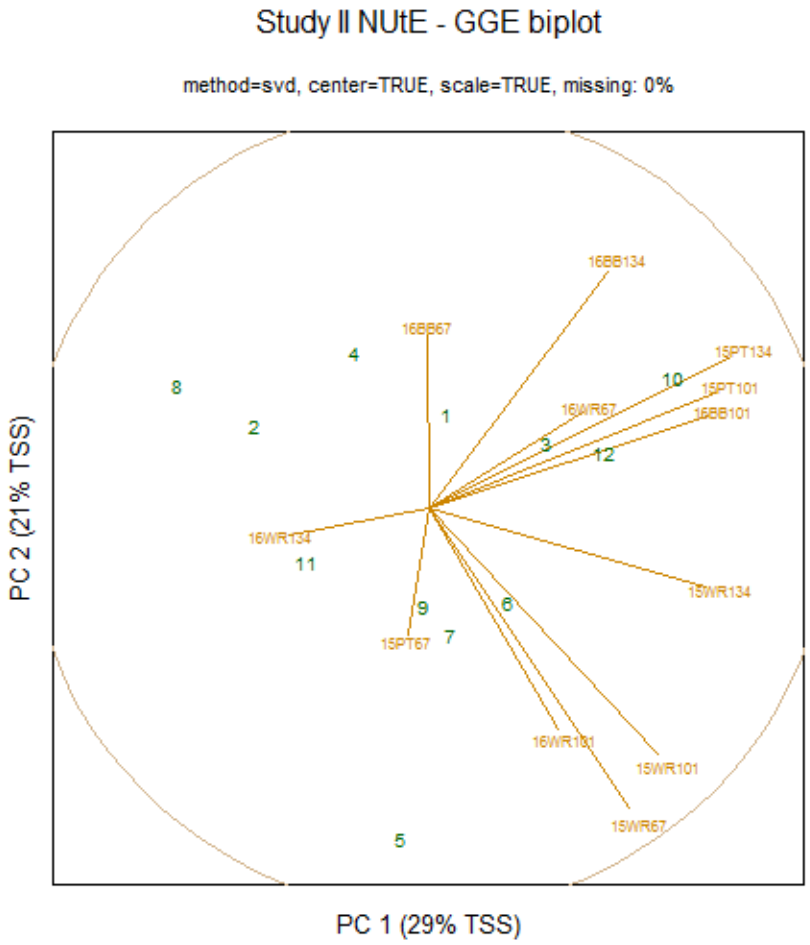
¶ Wheat-rye translocations on 1AL and 1BL.

APPENDIX A: GGE BIPLOT FOR STUDY II NUPE IN CHAPTER I



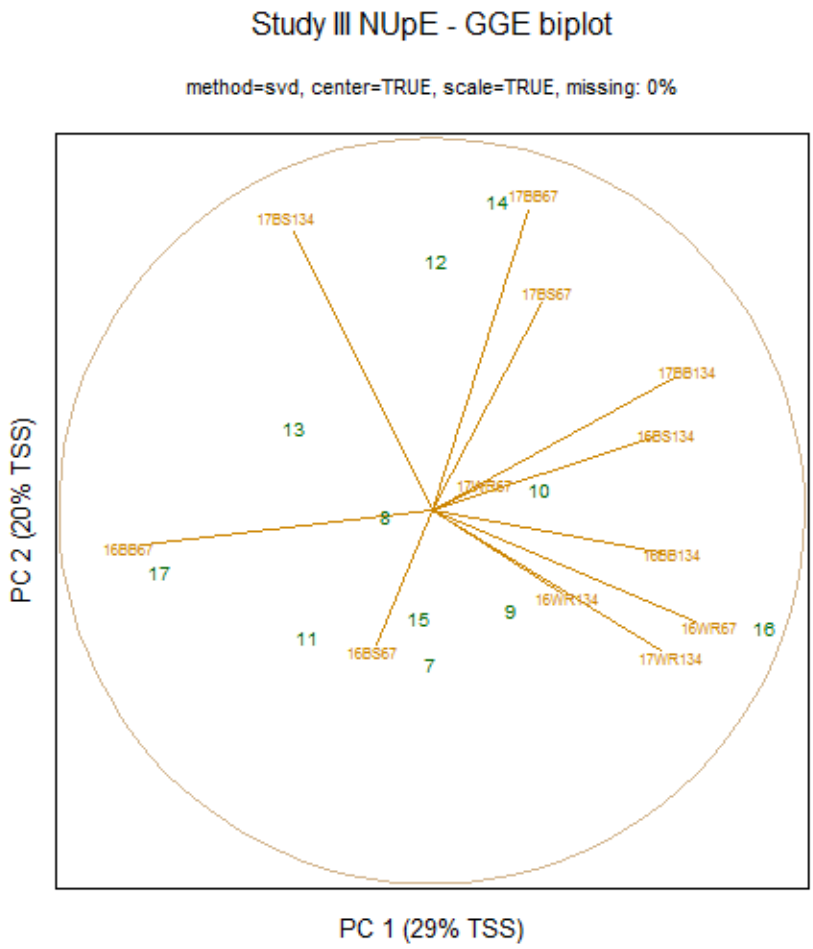
Appendix Figure 1.1. GGE biplot for N-uptake efficiency in Study II with line numbers (green color) and N-environments (brown color). The line designations are given in Table 1.2 and N-environments are described in Appendix Table 1.4.

APPENDIX B: GGE BIPLOT FOR STUDY II NUTE IN CHAPTER I



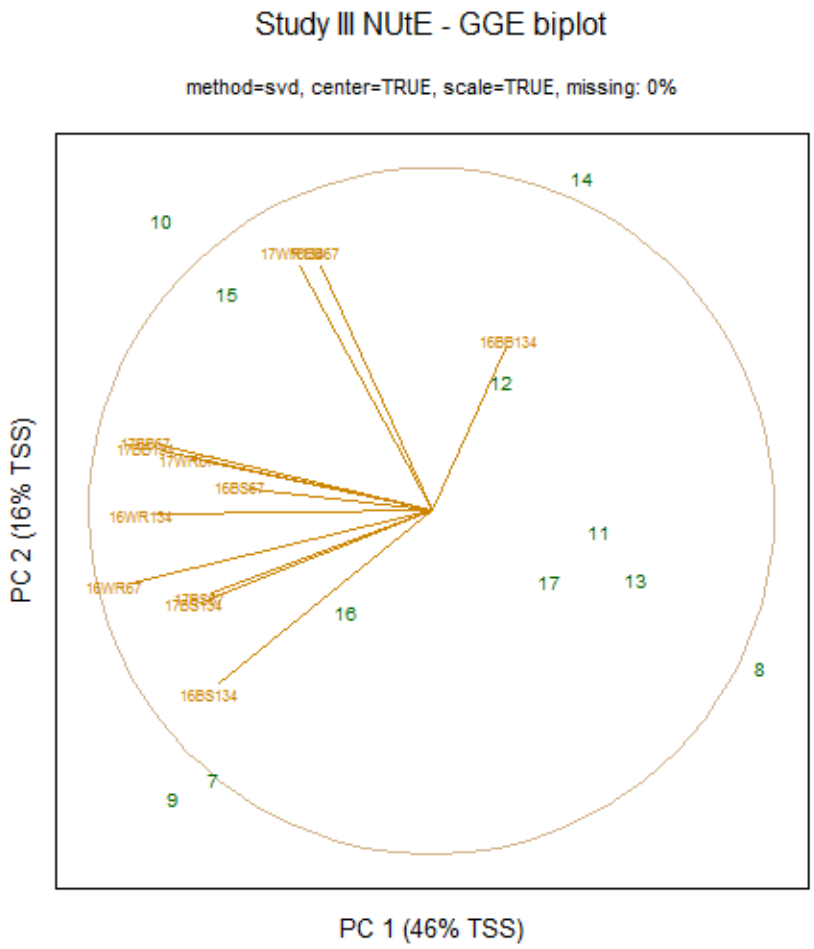
Appendix Figure 1.2. GGE biplot for N-utilization efficiency in Study II with line numbers (green color) and N-environments (brown color). The line designations are given in Table 1.2 and N-environments are described in Appendix Table 1.4.

APPENDIX C: GGE BIPLOT FOR STUDY III NUPE IN CHAPTER I



Appendix Figure 1.3. GGE biplot for N-uptake efficiency in Study III with line numbers (green color) and N-environments (brown color). The line designations are given in Table 1.2 and N-environments are described in Appendix Table 1.4.

APPENDIX D: GGE BIPLOT FOR STUDY III NUTE IN CHAPTER I



Appendix Figure 1.4. GGE biplot for N-utilization efficiency in Study III with line numbers (green color) and N-environments (brown color). The line designations are given in Table 1.2 and N-environments are described in Appendix Table 1.4.

APPENDIX E: STUDY I GRAIN YIELDS IN CHAPTER I

Appendix Table 1.1. Grain yields for wheat lines over five N rates in Study I at each testing environment.

Wheat line	14CS[a]	15CS	15WS
	----------Grain yield (kg ha^{-1})----------		
Bess	-	-	4,799 b[b]
IL02-19483B	-	-	4,294 de
IL07-4415	-	-	4,243 ef
KY06C-1003-139-8-3	-	-	4,094 f
MD03W485-10-10	-	-	4,412 cd
MD05W10208-11-8	-	-	4,499 c
MO080864	-	-	4,086 f
OH06-150-57	-	-	4,936 b
OH08-161-78	-	-	4,310 de
OH08-172-42	-	-	4,311 de
Sisson	-	-	4,122 f
VA08MAS-369	-	-	5,106 a

[a] 2013-14 Custar (14CS), 2014-15 Custar (15CS), and 2014-15 Wooster (15WS) environments.
[b] The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

APPENDIX F: STUDY II GRAIN YIELDS IN CHAPTER I

Appendix Table 1.2. Grain yields for wheat lines over three N rates in Study II at each testing environment.

Wheat line	14PT[a]	14WR	15PT	15WR	16BB	16WR
	----------Grain yield (kg ha^{-1})----------					
Bess	-	-	3,070 e[b]	4,856 b-d	3,895 f	3,103 d-f
IL02-19483B	-	-	3,379 b-e	4,609 d-f	4,849 ab	3,353 cd
IL07-4415	-	-	3,247 de	4,061 g	4,914 a	3,331 c-e
KY06C-1003-139-8-3	-	-	3,618 a-c	4,876 b-d	4,661 bc	3,274 d-f
MD03W485-10-10	-	-	3,262 c-e	4,361 fg	4,197 e	3,034 f
MD05W10208-11-8	-	-	3,486 a-d	4,451 ef	4,322 de	3,061 ef
MO080864	-	-	3,306 c-e	5,105 ab	4,380 de	3,569 a-c
OH06-150-57	-	-	3,231 de	5,013 a-c	4,483 cd	3,357 b-d
OH08-161-78	-	-	3,668 ab	5,068 a-c	4,600 c	3,651 a
OH08-172-42	-	-	3,759 a	4,753 c-e	5,004 a	3,311 c-f
Sisson	-	-	3,329 b-e	4,767 b-d	4,379 de	3,296 c-f
VA08MAS-369	-	-	3,750 a	5,229 a	4,806 ab	3,637 ab

[a] 2013-14 Painter (14PT), 2014-15 Warsaw, (14WR), 2014-15 Painter (15PT), 2014-15 Warsaw (15WR), 2015-16 Blacksburg (16BB), and 2015-16 Warsaw (16WR) environments.

[b] The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

APPENDIX G: STUDY III GRAIN YIELDS IN CHAPTER I

Appendix Table 1.3. Grain yields for wheat lines over two N rates in Study III at each testing environment.

Wheat line	16CS[a]	16FR	16WS
	----------Grain yield (kg ha^{-1})----------		
Bess	5,292 b[b]	5,953 bc	6,734 b
MD03W485-10-10	4,795 de	5,691 b-d	5,988 c
OH08-161-78	4,795 de	5,618 cd	6,410 b
OH08-172-42	5,766 a	5,766 bc	7,329 a
Sisson	5,192 bc	5,629 b-d	5,429 de
SS520	4,414 e	5,377 d	5,223 e
VA05W-151	5,060 b-d	5,731 b-d	5,572 de
VA07W-415	5,043 b-d	5,612 cd	5,714 cd
VA08MAS-369	5,223 bc	5,980 b	6,665 b
VA09W-73	4,865 cd	6,419 a	6,688 b
Yorktown	5,051 b-d	5,880 bc	6,386 b
	16BB	16BS	16WR
	----------Grain yield (kg ha^{-1})----------		
Bess	4,056 cd	2,152 a-c	3,827 a-d
MD03W485-10-10	4,605 b	2,168 a-c	3,494 e
OH08-161-78	4,719 b	2,347 ab	3,938 ab
OH08-172-42	4,698 b	1,946 cd	3,533 de
Sisson	4,455 bc	1,900 cd	3,610 c-e
SS520	3,931 d	1,632 d	3,575 c-e
VA05W-151	4,744 b	2,079 bc	3,871 a-c
VA07W-415	3,986 d	1,901 cd	4,138 a
VA08MAS-369	5,238 a	2,496 a	3,939 ab
VA09W-73	4,697 b	2,439 ab	4,122 a
Yorktown	4,735 b	2,423 ab	3,666 b-e
	17BB	17BS	17WR
	----------Grain yield (kg ha^{-1})----------		
Bess	6,084 cd	2,145 c-e	4,623 cd
MD03W485-10-10	4,724 f	2,448 b-d	4,377 d
OH08-161-78	6,226 c	2,713 ab	4,650 cd
OH08-172-42	6,199 cd	2,129 de	4,872 bc
Sisson	4,927 f	2,183 c-e	4,908 bc
SS520	5,654 e	1,814 e	4,796 bc
VA05W-151	5,813 de	2,338 b-d	5,303 a
VA07W-415	6,864 a	2,584 ab	5,094 ab
VA08MAS-369	6,304 bc	2,940 a	5,423 a
VA09W-73	6,684 ab	2,507 bc	5,300 a
Yorktown	5,826 de	2,640 ab	4,937 bc

[a] 2015-16 Custar (16CS), 2015-16 Fremont, (16FR), 2015-16 Wooster (16WS), 2015-16, 2015-16 Blacksburg (16BB), 2015-16 Blackstone (16BS), 2015-16 Warsaw (16WR), 2016-17 Blacksburg (17BB), 2016-17 Blackstone (17BS), and 2016-17 Warsaw (17WR) environments.
[b] The LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.

APPENDIX H: ENVIRONMENT CODES FOR GGE BIPLOTS IN CHAPTER I

Appendix Table 1.4. Environment codes for N-environments in each study for GGE biplots†.

Study	Environment abbreviation	Season	Location	Replications	N rate
I	14CS45	2013-14	Custar, OH	4	45
I	14CS67	2013-14	Custar, OH	4	67
I	14CS90	2013-14	Custar, OH	4	90
I	14CS112	2013-14	Custar, OH	4	112
I	14CS134	2013-14	Custar, OH	4	134
I	15CS45	2014-15	Custar, OH	4	45
I	15CS67	2014-15	Custar, OH	4	67
I	15CS90	2014-15	Custar, OH	4	90
I	15CS112	2014-15	Custar, OH	4	112
I	15CS134	2014-15	Custar, OH	4	134
I	15WS45	2014-15	Wooster, OH	4	45
I	15WS67	2014-15	Wooster, OH	4	67
I	15WS90	2014-15	Wooster, OH	4	90
I	15WS112	2014-15	Wooster, OH	4	112
I	15WS134	2014-15	Wooster, OH	4	134
II	14PT67	2013-14	Painter, VA	3	67
II	14PT101	2013-14	Painter, VA	3	101
II	14PT134	2013-14	Painter, VA	3	134
II	14WR67	2013-14	Warsaw, VA	3	67
II	14WR101	2013-14	Warsaw, VA	3	101
II	14WR134	2013-14	Warsaw, VA	3	134
II	15PT67	2014-15	Painter, VA	3	67
II	15PT101	2014-15	Painter, VA	3	101
II	15PT134	2014-15	Painter, VA	3	134
II	15WR67	2014-15	Warsaw, VA	3	67
II	15WR101	2014-15	Warsaw, VA	3	101
II	15WR134	2014-15	Warsaw, VA	3	134
II	16BB67	2015-16	Blacksburg, VA	3	67
II	16BB101	2015-16	Blacksburg, VA	3	101
II	16BB134	2015-16	Blacksburg, VA	3	134
II	16WR67	2015-16	Warsaw, VA	2	67
II	16WR101	2015-16	Warsaw, VA	2	101
II	16WR134	2015-16	Warsaw, VA	2	134
III	16CS67	2015-16	Custar, OH	4	67
III	16CS134	2015-16	Custar, OH	4	134
III	16FR67	2015-16	Fremont, OH	4	67
III	16FR134	2015-16	Fremont, OH	4	134
III	16WS67	2015-16	Wooster, OH	4	67
III	16WS134	2015-16	Wooster, OH	4	134
III	16BB67	2015-16	Blacksburg, VA	3	67
III	16BB134	2015-16	Blacksburg, VA	3	134
III	16BS67	2015-16	Blackstone, VA	3	67
III	16BS134	2015-16	Blackstone, VA	3	134
III	16WR67	2015-16	Warsaw, VA	2	67
III	16WR134	2015-16	Warsaw, VA	2	134
III	17BB67	2016-17	Blacksburg, VA	3	67
III	17BB134	2016-17	Blacksburg, VA	3	134
III	17BS67	2016-17	Blackstone, VA	3	67

Study	Environment abbreviation	Season	Location	Replications	N rate
III	17BS134	2016-17	Blackstone, VA	3	134
III	17WR67	2016-17	Warsaw, VA	3	67
III	17WR134	2016-17	Warsaw, VA	3	134

[a] The R (R Core Team, 2018) package 'gge' (Wright and Laffont, 2018) was used to produce genotype + genotype-by-N-environment interaction plots (Yan et al., 2000; Yan, 2001; Yan and Kang, 2003) for NUpE, NUtE, and grain yield. The genotypes correspond with line numbers from Table 1.1 and were generated using arithmetic means.

APPENDIX I: STUDY II STABILITY PARAMETERS IN CHAPTER I

Appendix Table 1.5. Mean N-uptake (NUpE) and N-utilization (NUtE) efficiencies with stability parameters slope (βi) and deviation from regression (δ^2_d) over N-environments for wheat lines in Study II.

Wheat line	Trait	Mean	βi	δ^2_d
Bess	NUpE	1.15	2.02	0.06
IL02-19483B		1.22	1.76	0.04
IL07-4415		1.10	0.69	0.08
KY06C-1003-139-8-3		1.21	0.97	0.06
MD03W485-10-10		1.23	1.01	0.03
MD05W10208-11-8		1.14	-0.16	0.03
MO080864		1.27	0.78	0.11
OH06-150-57		1.13	1.37	0.04
OH08-161-78		1.23	1.12	0.06
OH08-172-42		1.15	0.62	0.04
Sisson		1.22	1.15	0.04
VA08MAS-369		1.17	0.67	0.04
Bess	NUtE	36.17	1.50	19.88
IL02-19483B		36.21	1.27	25.91
IL07-4415		38.53	1.92	169.07
KY06C-1003-139-8-3		37.49	1.02	38.49
MD03W485-10-10		32.52	0.32	11.51
MD05W10208-11-8		35.39	-0.28	31.72
MO080864		37.05	0.35	87.13
OH06-150-57		38.62	1.79	19.57
OH08-161-78		37.99	0.96	24.69
OH08-172-42		40.23	1.60	44.31
Sisson		35.03	0.76	26.44
VA08MAS-369		40.07	0.80	30.92

APPENDIX J: STUDY III STABILITY PARAMETERS IN CHAPTER I

Appendix Table 1.6. Mean N-uptake (NUpE) and N-utilization (NUtE) efficiencies with stability parameters slope (βi) and deviation from regression (δ^2_d) over N-environments for wheat lines in Study III.

Wheat line	Trait	Mean	βi	δ^2_d
Bess		1.56	0.85	0.02
MD03W485-10-10		1.66	1.48	0.03
OH08-161-78		1.61	0.83	0.12
OH08-172-42		1.62	1.00	0.13
Sisson		1.66	0.68	0.10
SS520	NUpE	1.52	0.80	0.07
VA05W-151		1.70	1.39	0.13
VA07W-415		1.58	0.91	0.02
VA08MAS-369		1.70	1.12	0.04
VA09W-73		1.72	0.73	0.11
Yorktown		1.63	1.20	0.21
Bess		36.37	1.13	19.70
MD03W485-10-10		31.52	0.70	7.51
OH08-161-78		36.87	0.68	21.11
OH08-172-42		38.33	1.06	10.56
Sisson		32.42	0.77	26.42
SS520	NUtE	32.37	0.72	28.50
VA05W-151		34.01	0.67	31.15
VA07W-415		36.82	1.00	12.50
VA08MAS-369		35.15	1.10	20.50
VA09W-73		35.20	1.36	17.99
Yorktown		34.05	1.83	19.51

APPENDIX K:

ANOVA AND F-VALUES FOR HEADING DATE AND

PLANT HEIGHT IN CHAPTER III

Appendix Table 3.1. Analysis of variance and *F*-values for photoperiod group, treatment, and interaction effects on heading date and height at physiological maturity for soft red winter wheat lines grown in four Virginia environments in Chapter I.

		df	Heading date		Plant height	
14WVA	*Ppd*‡	1	9.25	**	36.06	***
	N	4	0.43	ns†	23.97	***
	Ppd × N	4	0.26	ns	0.11	ns
15WVA	*Ppd*	1	52.26	***	19.70	***
	N	4	0.19	ns	4.18	**
	Ppd × N	4	0.23	ns	0.15	ns
16BVA	*Ppd*	1	14.92	***	37.86	***
	N	4	0.22	ns	0.43	ns
	Ppd × N	4	0.34	ns	0.31	ns
16WVA	*Ppd*	1	14.46	***	7.42	***
	N	4	0.36	ns	1.00	ns
	Ppd × N	4	0.30	ns	1.20	ns

†ns, not significant.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
‡*Ppd*, photoperiod allele grouping; N, nitrogen rate.

APPENDIX L:

HEADING DATE AND PLANT HEIGHT FOR PPD-D1 ALLELES IN CHAPTER III

Appendix Table 3.2. Least square means heading date and plant height at maturity for groups of wheat lines containing either the photoperiod insensitive (*Ppd-D1a*) or sensitive (*Ppd-D1b*) allele in significant Virginia testing environments. Values represent means for two photoperiod groups within testing environments.

	Photoperiod group	
	Ppd-D1a	*Ppd-D1b*
Heading date (Julian)		
14WVA	128.3 b†	129.1 a
15WVA	126.3 b	128.0 a
16BVA	122.4 b	123.2 a
16WVA	113.4 b	115.2 a
Plant height (cm)		
14WVA	79.2 b	82.7 a
15WVA	78.1 b	81.2 a
16BVA	81.9 b	88.4 a
16WVA	81.0 b	84.8 a

†LSD at $P \leq 0.05$ is used to compare photoperiod group means within an environment; means within an environment followed by the same letter are not significantly different.

APPENDIX M: PLANT HEIGHT BY TREATMENT IN CHAPTER III

Appendix Table 3.3. Least square means of heading date and plant height at maturity for five spring-split nitrogen treatments, 1 (33+33), 2 (67+33), 3 (33+67), 4 (67+67), and 5 (33+101), in significant Virginia testing environments. Values represent means over treatment.

	Nitrogen treatment (kg N ha^{-1})				
	1 (33+33)†	2 (67+33)	3 (33+67)	4 (67+67)	5 (33+101)
Plant height (cm)					
14WVA	76.6 d‡	78.9 c	82.0 b	83.0 ab	84.8 a
15WVA	76.9 b	80.3 a	79.9 a	81.1 a	80.1 a

†Treatments were split applied at Zadoks growth stages 25 and 30.
‡LSD at $P \leq 0.05$ is used to compare treatment means within an environment; means within an environment followed by the same letter are not significantly different.