Winter Barley as a Commodity Cover Crop in the Mid-Atlantic Coastal Plain and Evaluation of Soft Red Winter Wheat Nitrogen Use Efficiency by Genotype, and its Prediction of Nitrogen Use Efficiency through Canopy Spectral Reflectance in the Eastern USA

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ABSTRACT

To understand the impact of N management on harvestable cover crop systems, seven research trials compared: 1) standard intensive management (SIM) (both fall and spring N application), 2) No fall N, a single spring N application, and 3) Cover N (no N application) effects on winter barley (*Hordeum vulgare* L.) plant biomass (PB), plant N uptake (PNU), grain yield, residual soil nitrate (RSN), and ammonium (RSA). In general, at winter dormancy, SIM resulted in increased PB and PNU but not RSN or RSA. At cover crop termination; SIM and the No fall N practices increased PNU, and at harvesting stage; they produced higher grain yields than the Cover N practice with little significant effect on RSN or RSA values, under normal climatic conditions. While overall yields for the No fall N treatment were lower (8%) than SIM yields, partial net return was similar due to decreased fertilizer input.

Nitrogen use efficiency (NUE) of soft red winter wheat (SRWW) can be improved by characterizing genotypes for NUE using canopy spectral reflectance [(CSR), a cheap, rapid and non-destructive remote sensing tool]. The other objectives of this study were to evaluate the predictive potential of vegetative reflection indices for wheat nitrogen use efficiency (NUE) by genotype and the appropriate stages of CSR sensing. An elite panel of 281 regionally developed SRWW genotypes was screened under low and normal N regimes in two crop seasons for grain yield, N uptake, nitrogen use efficiency for yield (NUEY) and nitrogen use efficiency for protein (NUEP). The best models incorporating CSR data at wheat heading explained a significant proportion of total variation in grain yield, N uptake, NUEY and NUEP. Based on the best linear

unbiased predictor values, genotypes were ranked and grouped into quartiles and the most efficient and responsive genotypes were identified. A significant proportion of the genotypes with high NUEY under high N conditions also had high NUEY under N stress; however, this was not the case for NUEP. Similarly, a significant proportion of genotypes with high NUEY also had high NUEP under both normal and low N conditions.

Dedication

This dissertation is dedicated to my parents, Dr. Pavuluri Krishna Prasad and Rathna and to all parents who dedicated their life for their children.

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CHAPTER I

INTRODUCTION

Crop growth and yield is the product of the interaction of genotype with soil, weather and biotic stress factors. Assuming appropriate protection from biotic stress factors, crop yield is limited by water, nutrients and environmental factors (Spiertz and De Vos, 1983). Among plant nutrients, N is considered as the most common limiting factor in cereal crop production across the world (Andrews et al., 2004; Nielsen and Halvorson, 1993). Nitrogen also plays an important role in the synthesis of amino acids, proteins and nucleic acids. Consequently, demand for N fertilizer has increased enormously in the past century (Frink et al., 1999) and the current (2013) estimation is141.5 million tons (Mt). This is projected to rise to 154.2 Mt by 2017 (Heffer and Prud'homme, 2013) and to 250 Mt by 2050 (Tillman et al.,2011). Sixty percent of N fertilizer is allocated to cereal production at a global scale (Raun and Johnson, 1999). The Projected 38% increase in cereal demand by 2025 may require 30% more N usage in cereal cropping systems (Dobermann and Cassman, 2005).

The increasing N fertilizer use in cereals has fed more people but also prompted fears of polluted water resources and air quality (Malakoff, 1998; National Academy of Sciences, 1995). Howarth, (2008) reviewed sources of coastal ecosystem N pollution globally, and reported agriculture as the largest source of marine N pollution. Accordingly, the Chesapeake Bay Foundation estimated sources of N pollution from different sectors and attributed agriculture as the cause of 38% N loading into watershed.

The current study attempted to address this problem in Mid-Atlantic coastal plains by two different approaches. One approach is to increase the area under cover crop cultivation during winter fallow periods. Cover crop cultivation has numerous advantages. Some of them are increased soil organic carbon content (Kuo et al., 1997), enhanced cation exchange capacity (Dabney et al., 2001), suppressed weed growth (Evers, 1983), increased aggregate stability and consequently reduced soil erosion, sedimentation, and residual soil nitrate (Dabney, 1998). Residual soil nitrate is more effectively scavenged by cereal cover crops than legumes (Reeves and Wood, 1994). Despite these advantages, farmer's reluctance to increase cover crops plantings is due to the additional costs associated with planting and killing of cover crops, and labor constraints (Dabney et al., 2001). To overcome these limitations, several cost share programs were initiated by state and federal agencies in Mid-Atlantic USA. In the current study, with a broad objective of enhancing cereal cover crop usage, an attempt was made to understand the impact of a barley (*Hordeum vulgare L.*) cover crops in relation to N management practices on residual soil nitrate content.

Improving the nitrogen use efficiency, (NUE) of soft red winter wheat (*Triticum aestivum* L.) genotypes is another potential avenue to reduce N inputs and negative environmental effects. Current world wheat production is estimated to be 708 Mt (USDA, 2013). Approximately, 1.5 billion people depend on wheat for their calories (Reynolds, et al., 1999). Available soil N and N fertilizer application affects yield levels, protein quality, quantity, and therefore net returns. Nitrogen influences these parameters via different biochemical (nitrogen assimilation costs at various plant parts), physiological and morphological processes (tiller, leaf panicle development) (Novoa and Loomis, 1981). Nitrogen also influences yield levels through its interaction with soil moisture and vegetative growth, especially in early growth stages (Ritchie and Johnson, 1990). Numerous transformation pathways and the multitude of factors affecting the dynamics of N in soils, renders it a complex plant nutrient to study. Nitrogen is also one of the most expensive inputs in wheat production and is considered as the most limiting growth factor after moisture stress in wheat (Ehdaie et al., 2001). Thus, it is essential to increase NUE of wheat genotypes by either increasing N uptake efficiency or by increasing N utilization efficiency. This can be done by either matching plant N requirements with N supply (N management strategies) or by improving NUE of new wheat genotypes. The use of improved NUE genotypes can reduce N loading by either using less N per unit quantity of yield or by more yield per unit quantity of N input or by both. Wheat evolved under various climatic and soil conditions. This resulted in wide variation in germplasm for NUE (Ortiz-Monasterio et al., 1997). Genotype characterization is necessary to understand and thereby selecting the efficient genotypes for high NUE. Such genotype characterization especially in early breeding generation trials, requires easy, portable, and fast sampling tools. This study attempted to characterize germplasm for NUE and evaluate remote sensing of canopy Reflectance for predicting NUE of wheat genotypes.

Thus with a broad objective of reducing the cereal N loads into Chesapeake bay, following specific objectives were framed for the current study.

1) Evaluate three distinct N management strategies on winter barley plant biomass, plant nitrogen uptake, grain yield, residual soil nitrate, and residual soil ammonium in the Coastal Plain of Virginia.
2) Evaluate vegetative reflectance indices for predicting soft red winter wheat grain yield, N uptake, nitrogen use efficiency for yield (NUEY), and nitrogen use efficiency for protein (NUEP), and determine the optimum growth stage for collection of spectral reflectance measurements in soft red winter wheat.
3) Identify the soft red winter wheat genotypes with high NUEY, NUEP, or both; and correlate the genotypes performance for NUEY and NUEP under low and normal N regimes.

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CHAPTER II

Winter Barley as a Commodity Cover Crop in the Mid-Atlantic Coastal Plain

ABSTRACT

Interest in harvestable cover crop systems, where producers apply no fertilizer until after March 1, has increased in the mid-Atlantic region in response to greater demand for winter cover crops. However little is known about how this practice will affect barley yield and residual soil nitrogen (N). Seven research trials over two years compared: 1) standard intensive management (SIM) (both fall and spring N application); 2) No fall N, a single spring N application; and 3) Cover N (no N application) effects on winter barley (*Hordeum vulgare* L.) plant biomass (PB), plant N uptake (PNU), grain yield, residual soil nitrate (RSN), and ammonium (RSA). At winter dormancy (December), SIM resulted in increased PB and PNU in 2010 but not in 2011 and in higher RSN or RSA in only three of 18 observations. At cover crop termination, SIM and the No fall N practice (five and four sites respectively, out of seven) significantly increased PNU but not RSN compared to the Cover N treatment. Similarly, SIM and No fall N produced significantly higher grain yields than the Cover N practice under normal climatic conditions (five of seven sites) with little significant effect on RSN or RSA values (seven out of 120 samples). While overall yields for the No fall N treatment were lower (8%) than SIM yields, partial net return was similar due to decreased fertilizer input. However at sites where SIM yields were significantly higher than No fall N yields (two of seven), net income for the SIM practice over N fall N was $\$88\ ha^{-1}$.

Abbreviations

PB	Above Ground Plant Biomass
PNU	Above Ground Plant Nitrogen Uptake
RSA	Residual Soil Ammonium
RSN	Residual Soil Nitrate
SIM	Standard Intensive Management

INTRODUCTION

Improved water quality, and the role of agriculture in maintaining water quality in the Chesapeake Bay has been a long-term concern in Virginia and other mid-Atlantic states (Staver, 2002). In 2010, President Obama issued Executive Order 13508 that outlined strategies for reaching water quality goals in the Chesapeake Bay (Obama, 2009). Based on the guidelines in section 202(b) of the Executive Order, agriculture in Virginia is credited with reaching 50% of its targeted pollution (N, phosphorus, and sediment) reduction goal. Consequently, Virginia has identified selected agricultural best management practices, including expanded cover crop plantings, that can enable the state to reach the mandated pollution reductions (2012). Virginia estimated that cover crops were planted on 48,000 ha (Chesapeake Bay Program, 2009) but an increase to over 125,000 ha is needed to meet the agreed-upon water quality improvement goals (2012).

Previous research in the mid-Atlantic region has documented the ability of cereal cover crops, especially rye, to capture residual soil N and reduce N losses from agricultural fields (Staver and Brinsfield, 1998) (Meisinger et al., 1991). Cover crop effects on N cycling and availability have also been assessed and in general have documented net N immobilization with cereals and mixtures of cereals and legumes but N mineralization and increased cash crop production with legume cover crops (Vaughan and Evanylo, 1998).

Fall N application is generally recommended for profitable small grain production in Virginia (Alley et al., 2009) however, recent work conducted by Kratochvil et al. (2005) reported little or no benefit of fall N application on grain yield of two cultivars of hard red winter wheat. Recent work on soft red winter wheat, also in Maryland, reported no yield benefit to fall N application when preplant soil NO_3-N levels were greater than 15 mg kg^{-1} but below this, the authors observed significant yield increases at 18 of 21 sites when 34 kg N ha^{-1} was applied in fall (Forrestal, 2011).

Cost share programs for cereal grain cover crops exist in Virginia and other states in the mid-Atlantic region. In Virginia, while numerous species and mixtures of cover crops are planted, only winter barley, wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), triticale (*Triticale hexaploide* Lart.) and oats (Avena sativa L.) supported with cost share. These programs are typically based on a tiered structure where the highest cost share (up to $123 ha^{-1} for early planted barley in Virginia in 2013) is provided for cereal grain cover crops that are seeded early, unfertilized, and killed in spring prior to planting a summer crop. At the other end of the spectrum are programs where the grower is allowed to harvest the cover crop for grain and to apply N fertilizer after March 1, but receives a much lower cost share rate. This is commonly called the commodity cover crop practice. Commodity cover crops differ from traditional cover crops in that they may be harvested for grain, hay, or silage and may receive nutrient applications after March 1 (Simpson and Weammert, 2008). No fall N is applied, so that the crop scavenges only available soil N, achieving water quality benefits similar to a tradition cover crop for the fall and winter portion of the production cycle. Both approaches have merit in a cover crop system where N retention and cycling are emphasized. However to develop effective cover crop adoption strategies, a clearer understanding of the impact of these N management schemes is needed.

Among cereal grain cover crops, barley has been found to produce biomass at comparatively faster rates than other cereals when planted early (Hively et al., 2009) and is often favored by growers due to lower seed cost, greater seed availability and existing grain markets.

The objective of this study was to evaluate three N management strategies on winter barley PB, PNU, grain yield, RSN, and RSA in the Coastal Plain of Virginia.

MATERIALS AND METHODS

Winter barley (*Hordeum vulgare* L.) research trials were established at four and three diverse sites in Virginia in 2010-11 and 2011-12, respectively. Site details, soil series, and sampling dates are included in Table 1. While experiments were conducted in similar geographic areas (Dinwiddie, Essex, and Petersburg) in both years, the same field was not used. In all instances barley followed corn (*Zea mays* L.) in a corn:barley:doublecrop soybean (*Glycine max* L.) rotation that is common in the region. Experimental locations and planting dates were chosen to represent the common range of barley growing conditions of Virginia; though planting at Dinwiddie in 2011-12 was delayed due to excess rainfall. Trials employed a randomized complete block design with four replications and three treatments: 1) SIM practices; 2) No fall N; and 3) Cover N management. In the SIM practice, a total of 145 kg N ha^{-1} was applied in three split applications that included 33 kg N ha^{-1} at planting, 56 kg N ha^{-1} at Zadoks growth stage (GS) 25 (typically mid-February) and 56 kg N ha^{-1} at GS 30 (typically mid-March) (Zadoks et al., 1974) based on Virginia Tech recommendations (Alley et al., 2009). The No fall N treatment received no nutrient application in the fall and 89 kg N ha^{-1} on, or shortly after, March 1 which follows the protocol set out for a commodity cover crop (Simpson and Weammert, 2009) . The Cover N management treatment received no N application at any time during the season. Urea ammonium nitrate solution (UAN; 300 g N kg^{-1}) was applied using a CO_2 sprayer to supply N as dictated by treatment protocols. Preplant N in the SIM treatment was broadcast over the plot area two to three days prior to seeding barley. Other than delayed planting at Dinwiddie 2011-12, seeding rates, dates and agronomic practices followed were those recommended for Virginia winter barley with the exception of N fertilization rates (Brann et al., 2000). ‘Thoroughbred’ winter barley was planted in 19 cm rows at 323 seeds m^{-2} into corn stover with a no-till grain drill at all sites and individual plot size for N rate treatments was 12 m by 6 m.

Soil samples were collected to a depth of 60 cm in increments of 0-15, 15-30, and 30-60 cm two to three days prior to seeding, at winter dormancy in December, after cover crop PB harvest in March, and after grain harvest in June. Preplant samples consisted of 15, 1.9 cm soil cores taken randomly from the experimental area and were composited for analysis. In December, in March or April (at the time spring PB collection) and after grain harvest, 10, 1.9 cm soil cores were collected from each plot and composited. Soil samples were air dried and ground to pass through a 2 mm screen. Samples were extracted using 2M KCl (Bremner, 1965) and analyzed for RSA and RSN using automated flow injection analysis (Lachat Inst., Milwaukee, WI). All PB was hand clipped from a 0.3 m^2 area, to the side of the center area maintained for grain harvest, from each plot in December and from an adjacent area in March. These sampling times represent fall growth and total growth cover crop at the time of termination, respectively. Aboveground biomass samples were dried in a forced air oven at 60^{o}C for 48 hours and then ground to pass through a 2 mm screen using a Wiley (Thomas Scientific, Swedesboro, NJ) sample mill. Total C and N were determined by dry combustion (VarioMax CNS macro elemental analyzer, Elementar, Hanau, Germany). Fertilizer N recovery in SIM and No fall N treatments was calculated by subtracting the PNU from the Cover N treatment. Grain was harvested from the center 1.5 m and the entire length of each plot using a Massey Ferguson 8XP plot combine and plot weights were measured using a GrainGage™ system (Juniper Systems, Logan, UT). A grain subsample of approximately 250 g was collected from each plot upon which test weight and moisture were determined using a Dickey-John GAC2100 grain sampler (DICKEY-john, Auburn, IL). Grain yields are reported on a 135 g kg^{-1} moisture basis.

Statistical analysis

Statistical analyses were performed using the GLIMMIX procedure (proc) in SAS 9.2 (SAS.Institute.Inc, 2010). Due to significant interaction of site and treatments, grain yield, PB and PNU were analyzed by site and year. Residual soil nitrate and RSA were analyzed by site, year and depth. Mean comparisons using Tukey's Honestly Significant Difference (HSD) (*alpha*=0.05) were made to separate treatment effects when F-tests indicated that significant differences existed ($P<0.05$).

RESULTS AND DISCUSSION

December PB and N uptake were influenced by N management at two of the four sites where there was adequate fall/winter barley growth to collect a sample (Table 2). March crop biomass production was affected by treatment in three of seven sites while N uptake was found to differ in six of seven sites. Similarly, grain yield was significantly affected by N management in five of seven sites (Table 2).

Above Ground Biomass

December

Plant biomass was higher in the SIM treatment compared with No fall N and Cover N at the Dinwiddie 2010-11 (674 and 682 kg ha^{-1} greater, respectively) and Petersburg 2010-11 (379 and 441 kg ha^{-1} greater, respectively) sites (Table 3). Samples were not collected at Prince George and Essex in 2010 and at Dinwiddie in 2011 due to insufficient barley growth which is attributable to late planting at the Dinwiddie site and limited rainfall at Essex. No differences in PB were measured at the Essex 2011-12 or Petersburg 2011-12 sites (Table 3). When the low yielding Essex 2011-12 site was excluded, barley winter PB over sites and treatments where no N was applied in fall was 788 kg ha^{-1}. Similar to our results, Hively et al. (2009) reported an average of 810 kg ha^{-1} PB when no fall N was applied to sandy loam soils from research locations on Maryland's Eastern Shore (25 field samples).

Cover Crop Termination - March

Across sites, March PB averaged 4005, 3347, and 1920 kg ha^{-1} for the SIM, No fall N, and Cover N treatments, respectively in 2010-11 (four sites) and 1798, 1141, and 1052 kg ha^{-1} in 2011-12 (three sites). Measured PB was relatively lower in 2011-12 than in 2010-11, likely due to lower rainfall and higher temperatures during March coupled with earlier sampling in 2011-

12. During March 2012, average temperature was 6°C higher than the 30-year average (10.7°C) at Dinwiddie and Petersburg, and 5.5°C higher that the historical average (9.1°C) at Essex. Average temperatures during March 2011, were 0.9°C (Dinwiddie and Petersburg) and 0.1 °C (Essex) higher that the 30-year mean for the month. Similarly, during March 2012, rainfall was 48 mm lower than the historical average (100 mm) at Dinwiddie and Petersburg, and 51 mm lower at Essex. During March 2011, rainfall was 14 (Dinwiddie and Petersburg) and 7 mm (Essex) higher than the historical monthly average. Average sampling dates were April 2 in 2011, and March 24 in 2012. No differences were found among treatments for PB at four of the seven sites (Table 3). At Essex 2011-12 and Petersburg 2011-12, PB in the SIM treatment was greater than the treatments receiving No fall N or Cover N treatments, which both had similar PB. At the Dinwiddie 2011-12 site PB was highest for SIM, followed by No fall N and Cover N (Table 3). Significant differences in March PB production among treatments were not observed at any site in 2010-11, but at all in 2011-12. This is likely a result of lower rainfall, warmer winter and early spring of 2011-12 compared to the first year of the study.

Nitrogen Uptake

December

At the Dinwiddie 2010-11and Petersburg 2010-11 sites, PNU in December was greater for the SIM treatment than No fall N or Cover N treatments (15.5 kg N ha^{-1} higher in both cases) (Table 4); similar to what was observed with PB (Table 3). Hively et al. (2009) reported an average of 14 kg ha^{-1} PNU for winter barley cover crop studies conducted on Maryland's Eastern Shore (25 field samples) with no N application, under varied soil N conditions in fall 2005. When the average N uptake in the No fall N and Cover N treatments was subtracted from the N uptake in the SIM treatment (to remove the effect of soil-supplied N) 37 and 20% of fall applied total N was observed in December PB in 2010-11 and 2011-12, respectively. The variation among years reflects the higher PB measured in 2010-11 as biomass N concentration was similar between years (data not shown).

Cover Crop Termination - March

In 2010-11, PNU ranged from 10 (Cover N) to 130 kg ha^{-1} (No fall N) and in 2011-12, ranged from 6 (Cover N) to 75 kg ha^{-1} in the SIM treatment (Table 4). Similar to PB, N uptake in all treatments was generally higher in 2010-11 than 2011-12. Across sites, 29 kg ha^{-1}of PNU was measured from the Cover crop treatment at termination. Again, similar values of winter barley PNU were measured (average of 22 kg ha^{-1} from 25 different fields under varied soil N conditions) by Hively et al. (2009) in Maryland. Coale et al. (2001) also investigated the effect of RSN and different cover crops on PNU in the mid-Atlantic Coastal Plain. Values reported for barley PNU (20 kg ha^{-1}) for the control in that experiment were also comparable to the 29 kg ha^{-1}of PNU obtained in this study.

At the time of typical cover crop termination, significant differences in PNU were observed among treatments at all sites, except Essex 2010-11 (Table 4). Relatively high initial RSN values (6, 10 and 16 mg kg^{-1} at 0-15, 15-30 and 30-60 cm soil depths, respectively) could have contributed to the lack of differences among treatments at Essex 2010-11. A similar lack of crop N response with high RSN was also reported by Gravelle et al. (1988) for winter wheat in the mid-Atlantic. Nitrogen uptake in the Cover N treatment ranged from 69 kg N ha^{-1} at Essex 2010-11 to 6 kg N ha^{-1}at Dinwiddie 2011-12 (Table 4). At five and four sites, respectively, SIM and No fall N resulted in significantly higher N uptake than the Cover N practice (Table 4).

Grain Yield

Similar to PB and PNU, average grain yield over sites was nearly double in 2010-11 (4395 kg ha^{-1}) compared to 2011-12 (2318 kg ha^{-1}) (Table 5). Standard intensive management practices produced significantly higher grain yields than the Cover N practice at five of seven sites (Table 5). Grain yields at Dinwiddie 2011-12 and Essex 2011-12 were abnormally low (1816 and 1514 kg ha^{-1}) due to environmental factors coupled with delayed planting at Dinwiddie. At three of the five responsive sites, the No fall N practice produced grain yields similar to the SIM treatment (Table 5). At the remaining two sites, Dinwiddie and Essex in 2010-11 the SIM treatment produced significantly higher grain yields than No fall N practice.

Averaged over all sites, grain yields were 4133, 3801, and 2585 kg ha^{-1} for the SIM, No fall N, and Cover N practices. Each kg of N applied under SIM and No fall N treatments produced 29 and 42 kg of grain yield, respectively. Over sites and years, average yield of the No fall N treatments was 92% of the grain yield of the SIM practices. Similarly, Kratochvil et al. (2005) reported little or no benefit of fall N application on grain yield of two cultivars of hard red winter wheat in Maryland. Assuming five year average prices of \$213 Mg^{-1} for barley and \$1.27kg N^{-1} for liquid urea ammonium nitrate (30% N) (USDA Economic Research Service, 2012), the SIM treatment grossed \$71 ha^{-1} more than the No fall N practice, but the additional N cost \$69 ha^{-1} would have resulted in a similar net return for a grower. At the two sites where yield of the SIM treatment was significantly higher than the No fall N treatment, Dinwiddie and Essex 2010-11, net income increase due to N fertilizer was \$134 and \$43 ha^{-1}, respectively. Economically, the SIM practice generated higher income than the No fall N practice in two of seven sites and no difference in the other five. This indicates that most growers would likely opt for the SIM practice over No fall N if no subsidy for the latter is provided.

Residual Soil Nitrate and Ammonium

Planting

In general, RSN and RSA at planting were low (less than 10 mg kg^{-1}) except at Essex and Prince George in 2010-11 (Table 1). Coale et al. (2001) reported similar RSN values (0.42 to 10 mg kg^{-1}) at cover crop planting time in the mid-Atlantic Coastal Plain. The ranges observed among sites are likely due to variations in climate across the region, previous crop N management practices, and previous crop yields. Varied effects of N management and cropping systems on RSN at the time of cereal rye cover crop planting was also reported by Brandi-Dohrn et al. (1997).

December

No significant differences among N management practices for RSN and RSA were observed at 16 and 17 of 18 possible depth-by-site combinations, respectively (Table 6). Soil nitrate was significantly affected by N management at Essex and Petersburg in 2011-12 at the 30-60 cm depth (Table 7). No significant differences for RSA in response to fall N management were observed except at the Essex 2011-12 site at the 15-30 cm depth (Tables 6 and 7). At those sites where differences in soil nitrate and ammonium were detected in 2011-12, neither PB nor PNU were significantly different among treatments (Tables 2 and 3). The differences observed among treatments were generally less than 2 mg kg^{-1} (Table 7). These differences, then, likely reflect a slight oversupply of N in these environments. December PB at the Essex 2011-12 site averaged approximated 300 kg ha^{-1} and the higher RSN observed at the 15-30 cm depth agrees with previous work by Hively et al. (2009) who reported increased early winter RSN values when cover PB was less than 1000 kg ha^{-1}. However, this explanation does not hold for Petersburg 2011-12 where PB was nearly 1000 kg ha^{-1}.

March

Mean RSN values over all seven sites at 0-15, 15-30 and 30-60 cm were 3, 1 and 1 mg kg^{-1} respectively. Relatively low RSN values (5 to 1 mg kg^{-1}) were also reported by Thomason et al. (2009) for barley cover crop in March in Virginia. Total RSN concentration from the sampled profile was doubled from December to March (10 vs 5 mg kg^{-1}). In the current study, no significant differences for RSA were observed among treatments at any site (Table 6). Differences among treatments for RSN were seen at Dinwiddie 2011-12 at 0-15 and 15-30 cm and at Essex 2011-12 at 0-15 cm (Table 7). In these cases, the SIM treatment resulted in significantly greater RSN than the Cover N treatment. Soil nitrate did not differ between Cover N and No fall N management in any case (Table 7). Similar results at the time of cover crop termination among different initial RSN treatments were also observed by Coale et al. (2001) in barley, wheat, rye and triticale (*Triticale hexaploide* Lart.) under mid-Atlantic conditions (Coale et al., 2001) (Coale et al., 2001). The lack of differences among N management practices likely indicate the rapid uptake of spring applied N by barley during March. Similarly, Thomason et al. (2009) also reported increased RSN values at the time of cover crop termination with decreased early barley PB in Virginia during 2005. In general, additions of recommended rates fall and spring N did not significantly increase RSN.

June

Mean RSN over the seven sites at 0-15, 15-30 and 30-60 cm were 4, 3 and 3 mg kg^{-1} respectively. Similar increased RSN values from March to June for several types of small grains were made by Coale et al. (2001). We support their hypothesis that this increase in RSN could be due soil N mineralization and reduced N uptake rate as the crop nears senescence.

Significant differences in RSN in response to N treatments were observed at only one site-sampling depth (Dinwiddie 2010-11; 15-30 cm), where SIM resulted in greater RSN than the Cover N treatment (Table 7). Though statistically significant, the differences observed among treatments were less than 2 mg kg^{-1} (Table 7) which likely means this is not biologically significant in this environment. No other significant differences were observed for RSN or RSA among N management practices (Table 6). Similar to our findings, very small increases (less than 1.1 mg kg^{-1}) in RSN values were observed with increasing N applications up to the economic optimum rate (142 kg ha^{-1}) in winter barley under varied soil conditions (sandy to clay loam) in Kentucky (Richards et al., 1996). However, N application should not exceed agronomic optimum rate because increased RSN values were observed in the Richards et al. study when the applied N exceeded agronomic optimum rate.

Overall RSN and RSA

In total, at seven out of 120 site-by-depth combinations, we found significantly higher RSN or RSA values for SIM than the Cover N practice (Table 6). Six of the observed instances of differences were from the 2011-12 cropping season and were mainly at Dinwiddie and Essex (Table 6). At these two sites, no significant differences in grain yields (Table 5) among treatments were observed. The observed grain yields (Table 5) and PNU (at the time of normal cover crop kill time) (Table 4) were low at these two sites. Similarly, at Petersburg 2011-12 in December there was no significant difference in PNU among treatments (Table 4). In the seven instances where soil residual N was influenced by N management, differences were between the SIM practice and one of the other treatments. There were no significant differences in RSN and RSA values between the No fall N and Cover N practices (Table 7)

These results indicated that statistically higher RSN and RSA contents for the SIM practice compared to the Cover N practice mainly appear when environmental conditions limit yield potential for PB, PNU and grain as occurred in 2011-12.

Averaged across sites at the time of cover crop termination 51 and 47 % of total N applied was observed in the PB for the SIM N and No fall N treatments respectively in 2010-11. In 2011-12, only 27, and 18 % of the N applied was observed in PB for the SIM N and No fall N treatments respectively. Nitrogen recovery was high in 2010-11 likely due to more favorable growing conditions. Barley research in Canada found that, 49% of the applied N was recovered in the barley PB and grain at the time of harvest under good growing conditions with normal rainfall (Kucey, 1986) however at the same location, fertilizer N recovery was reduced to 22% during a dry year. Similarly, N recovery by barley was 57% on sandy soils in Belgium (Khanif et al., 1984). Meisinger et al. (1991) reported 60% reduction in mean RSN and RSA values by cereal cover crops with high PNU. Approximately one half (2010-11) and one quarter (2011-12) of the applied N was recovered by barley in March. The applied N not accounted for can be attributed to N in roots and various N loss processes such as leaching beyond the sampling depth, de-nitrification, immobilization and ammonia volatilization losses.

Other researchers reported that when N fertilizer was applied at a rate of 140 kg N ha^{-1}in spring wheat in sandy soils in north west China (Wang et al., 2010) and 100 kg N ha^{-1}in barley on clay soils in the southeast part of Norway, residual soil nitrate N concentration did not increase (Lyngstad, 1975). In the current study, fertilizer N rates of 145 kg N ha^{-1} did not exceed plant N requirements and did not result in excess RSN in the soil profile. Correspondingly, in simulation studies conducted in South central Colorado (Delgado et al., 2001) and Spain, (Gabriel et al., 2012) barley utilized nitrogen efficiently with no increase in RSN.

CONCLUSIONS

Cover N management resulted in grain yields that were lower than the SIM and No fall N treatment at five of seven sites. Averaged over all seven sites, the yield of the No fall N treatments produced 92% of the grain yield of the SIM treatment. Considering the additional cost of fertilizer in the SIM treatment, net returns to the two systems were similar. However, at the two sites where yields were higher for SIM than No fall N, returns were higher for SIM. In general, nitrogen applied in the fall resulted in more fall and early winter barley biomass and N uptake during a normal rainfall year, though this does not always result in higher grain yields. Similar results were obtained for spring PB and PNU indicating the importance of fall N to adequately support fall and early winter barley plant growth.

At two out of 18 samples collected in December 2011-12, RSN (30- 60 cm soil depth) and RSA (15-30 cm soil depth) were higher for the SIM treatment; however these differences were never greater than 2 mg kg^{-1} suggesting that the biological significance of this difference is questionable. Spring N applications significantly increased PNU in late March without significantly effecting RSN and RSA contents (0-60 cm soil depth) except at two out of 21 samples (Dinwiddie and Essex in 2011-12) indicating efficient crop uptake of spring-applied N. Overall soil nitrate and ammonium values measured in December were low across all treatments. By March, there were higher amounts of nitrate and ammonium associated with the SIM practice in some cases (three of 42 instances) compared to the Cover N practice, but not when compared to the No fall N practice. At only one sample depth at one site did RSN at harvest vary among the three treatments. No effect of N management treatment on RSA was measured at any site or depth in June. In general, we found very few differences in soil N changes between SIM and the No fall N practice. Soil nitrate and ammonium were generally lower in spring for the Cover N treatment compared to the other two treatments, but grain yields were significantly lower in most cases as well. In order to avoid the risk of lost grain yield and income, growers should carefully consider field-specific conditions, such as residual soil N and yield potential before adopting a reduced N management such as the No fall N practice evaluated in the current study.

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Table 1. Experimental sites, locations, descriptions, sampling dates and initial soil ammonium and nitrate, 2010-11 and 2011-12.

						Biomass sampling		Grain	Preplant Soil Nitrate, mg kg^{-1}			Preplant Soil Ammonium, mg kg^{-1}		
Year	Site	Latitude	Longitude	Soil series, texture, and slope	Planting date	Winter	Spring	Harvest	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm
2010-11	Dinwiddie	37°7'N	77°27' W	Mattaponi sandy loam, 0-2%	15-Oct	20 Dec.	29-Mar	9-Jun	3.2	2.2	4.3	0.9	1.6	0.9
	Essex	38°1' N	76°56' W	State fine sandy loam, 0-2%	11-Oct	not collected	10-Apr	1-Jun	6.3	10.3	16.1	1.2	0.8	1.4
	Petersburg	37°13' N	77°26’ W	Norfolk fine sandy loam, 0-2%	8-Oct	20 Dec.	29 Mar.	3-Jun	3.3	2.9	3.1	1.0	0.9	0.5
	Prince George	37°14' N	77°0' W	Pamunkey loam, 0-2%	22-Oct	not collected	30-Mar	1-Jun	11.4	16.6	6.1	2.5	1.6	1.5
2011-12	Dinwiddie	37°07' N	77°27' W	Mattaponi sandy loam, 0-2%	2-Dec	20-Dec	23-Mar	31-May	2.4	1.2	1.3	0.8	1.1	1.1
	Essex	37°56' N	76°59' W	Emporia sandy loam, 2-6%	17. Oct	15 Dec.	15-Mar	29-May	5.2	3.2	3.0	1.2	1.4	0.9
	Petersburg	37°13' N	77°26' W	Norfolk fine sandy loam, 2-6%	15-Oct	16-Dec	24-Mar	6-Jun	4.1	1.9	1.1	1.3	1.1	1.2

Table 2. Analysis of variance for N management effects on grain yield, above ground biomass, and N uptake in barley at the time of winter dormancy (December) and at time of normal cover crop termination (March) in each of seven sites, 2010-11 and 2011-12.

	Above ground biomass		Barley N uptake		Grain yield
Experimental Site	Dec	Mar	Dec	Mar	
Dinwiddie 2010-11	***	ns	*	*	***
Essex 2010-11	-	ns	-	ns	***
Petersburg 2010-11	**	ns	*	**	***
Prince George 2010-11	-	ns	-	*	**
Dinwiddie 2011-12	-†	***	-	***	ns
Essex 2011-12	ns	*	ns	**	ns
Petersburg 2011-12	ns	*	ns	**	***

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

ns, Nonsignificant at the 0.05 probability level.

†samples were not collected.

Table 3. Least squares means and standard errors of above ground barley biomass as influenced by N management practice at the time of winter dormancy (December) and time of normal cover crop termination (March).

Time of sampling	Site	SIM ‡	No fall N	Cover N
		-------------- kg ha^{-1} --------------		
Dec	Dinwiddie 2010-11	1397 ± 22 [A†]	723 ± 70 [B]	715 ± 46 [B]
	Petersburg 2010-11	1193 ± 109 [A]	814 ± 16 [B]	752 ± 86 [B]
	Essex 2011-12	382 ± 49 [A]	256 ± 43 [A]	268 ± 23 [A]
	Petersburg 2011-12	1250 ± 244 [A]	883 ± 126 [A]	842 ± 171 [A]
Mar	Dinwiddie 2010-11	3204 ± 859 [A]	1294 ± 157 [A]	869 ± 197 [A]
	Essex 2010-11	4290 ± 443 [A]	3785 ± 272 [A]	3818 ± 363 [A]
	Petersburg 2010-11	4478 ± 686 [A]	3568 ± 499 [A]	2368 ± 798 [A]
	Prince George 2010-11	4047 ± 603 [A]	4741 ± 521 [A]	3624 ± 269 [A]
	Dinwiddie 2011-12	958 ± 89 [A]	656 ± 67 [B]	315 ± 24 [C]
	Essex 2011-12	708 ± 91 [A]	493 ± 92 [B]	502 ± 98 [B]
	Petersburg 2011-12	3728 ± 192 [A]	2275 ± 249 [B]	2340 ± 323 [B]

†Means within each row followed by the same letter are not significantly different ($P > 0.05$).
‡ Standard intensive management

Table 4. Least squares means and standard errors of barley N uptake as influenced by N management practice at the time of winter dormancy (December) and time of normal cover crop termination (March), 2010-2011 and 2011-12.

Time of sampling	Site	SIM ‡	No fall N	Cover N
		---------------- kg ha^{-1}		--------------
Dec	Dinwiddie 2010-11	32 ± 4 ^{A†}	17 ± 3 ^{B}	15 ± 1 ^{B}
	Petersburg 2010-11	43 ± 8 ^{A}	27 ± 4 ^{B}	29 ± 5 ^{B}
	Essex 2011-12	12 ± 1 ^{A}	7 ± 2 ^{A}	7 ± 0 ^{A}
	Petersburg 2011-12	28 ± 7 ^{A}	19 ± 4 ^{A}	19 ± 5 ^{A}
Mar	Dinwiddie 2010-11	67 ± 17 ^{A}	37 ± 2 ^{AB}	10 ± 3 ^{B}
	Essex 2010-11	117 ± 15 ^{A}	98 ± 13 ^{A}	69 ± 5 ^{A}
	Petersburg 2010-11	103 ± 20 ^{A}	80 ± 13 ^{A}	33 ± 13 ^{B}
	Prince George 2010-11	121 ± 15 ^{AB}	130 ± 19 ^{A}	67 ± 5 ^{B}
	Dinwiddie 2011-12	32 ± 3 ^{A}	25 ± 3 ^{B}	6 ± 1 ^{C}
	Essex 2011-12	24 ± 2 ^{A}	15 ± 2 ^{B}	11 ± 1 ^{B}
	Petersburg 2011-12	75 ± 6 ^{A}	50 ± 6 ^{B}	24 ± 4 ^{C}

†Means within each row followed by the same letter are not significantly different ($P > 0.05$).
‡ Standard intensive management

Table 5. Least squares means and standard errors of barley grain yield as influenced by N management practice at each site, 2010-11 and 2011-12.

Site	SIM ‡	No fall N	Cover N
	-------------------	kg ha^{-1}	-------------------
Dinwiddie 2010-11	$4889 \pm 221^{A†}$	3931 ± 204^{B}	1872 ± 392^{C}
Essex 2010-11	4009 ± 191^{A}	3480 ± 176^{B}	3203 ± 152^{C}
Petersburg 2010-11	5940 ± 115^{A}	5844 ± 156^{A}	4409 ± 325^{B}
Prince George 2010-11	5670 ± 52^{A}	5371 ± 152^{A}	4118 ± 526^{B}
Dinwiddie 2011-12	2443 ± 690^{A}	2106 ± 276^{A}	902 ± 179^{A}
Essex 2011-12	1629 ± 166^{A}	1656 ± 50^{A}	1257 ± 38^{A}
Petersburg 2011-12	4353 ± 95^{A}	4217 ± 207^{A}	2335 ± 191^{B}

†Means within each row followed by the same uppercase letter are not significantly different ($P > 0.05$).
‡ Standard intensive management

Table 6. Analysis of variance for N management effects on residual soil nitrate and ammonium at three soil depths at the time of winter dormancy (December), normal cover crop termination time (March), and at the time of barley grain harvest (June) at seven sites, 2010-11 and 2011-12.

Time of sampling	Dec.			Mar.			June		
Depth, cm	0-15	15-30	30-60	0-15	15-30	30-60	0-15	15-30	30-60
	Residual soil nitrate, mg kg^{-1}								
Dinwiddie 2010-11	ns	ns	ns	ns	ns	ns	ns	*	ns
Dinwiddie 2011-12	ns	ns	ns	*	*	ns	ns	ns	ns
Prince George 2010-11	-†	-	-	ns	ns	ns	ns	ns	ns
Essex 2010-11	ns	ns	ns	ns	ns	ns	ns	ns	ns
Essex 2011-12	ns	ns	*	**	ns	ns	ns	ns	ns
Petersburg 2010-11	ns	ns	ns	ns	ns	ns	ns	ns	ns
Petersburg 2011-12	ns	ns	*	ns	ns	ns	ns	ns	ns
	Residual soil ammonium, mg kg^{-1}								
Dinwiddie 2010-11	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dinwiddie 2011-12	ns	ns	ns	ns	ns	ns	ns	ns	ns
Prince George 2010-11	-	-	-	ns	ns	ns	ns	ns	ns
Essex 2010-11	ns	ns	ns	ns	ns	ns	ns	ns	ns
Essex 2011-12	ns	*	ns	ns	ns	ns	ns	ns	ns
Petersburg 2010-11	ns	ns	ns	ns	ns	ns	ns	ns	ns
Petersburg 2011-12	ns	ns	ns	ns	ns	ns	ns	ns	ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level; ns Nonsignificant at the 0.05 probability level.

†samples were not collected.

Table 7. Least squares means and standard error of residual soil nitrate and ammonium as influenced by N management practice and sites at three soil depths at the time of winter dormancy (December), spring (March), and at the time of barley harvest (June), 2010-2011 and 2011-12.

N management practices			SIM ‡	No fall N	Cover
Time of sampling	Depth, cm	Site			
			------------Residual soil nitrate, mg kg^{-1}------------		
Dec.	30-60	Essex 2011-12	3.1 ± 0.4^{A†}	1.8 ± 0.3^{B}	2.4 ± 0.2^{B}
		Petersburg 2011-12	3.1 ± 0.4^{A}	1.4 ± 0.1^{B}	2.2 ± 0.2^{B}
Mar.	0-15	Dinwiddie 2011-12	5.5 ± 1.1^{A}	3.8 ± 0.4^{AB}	1.3 ± 0.2^{B}
		Essex 2011-12	10.1 ± 1.1^{A}	6.3 ± 0.9^{AB}	2.2 ± 0.5^{B}
	15-30	Dinwiddie 2011-12	2.1 ± 0.5^{A}	1.1 ± 0.1^{AB}	0.7 ± 0.2^{B}
June	15-30	Dinwiddie 2010-11	1.5 ± 0.3^{A}	0.8 ± 0^{AB}	0.6 ± 0.1^{B}
			------Residual soil ammonium, mg kg^{-1}-------		
Dec.	15-30	Essex 2011-12	2 ± 0.2^{A}	1.3 ± 0.3^{B}	1.7 ± 0.1^{AB}

†Means within each row followed by the same uppercase letter are not significantly different (P > 0.05).

‡ Standard intensive management

CHAPTER III

Canopy Spectral Reflectance Can Predict Grain Nitrogen Use Efficiency in Soft Red Winter Wheat

ABSTRACT

Canopy spectral reflectance (CSR) is a cheap, rapid, and non-destructive remote sensing and selection tool that can be employed in high throughput plant phenotypic studies. The objectives of the current study were to evaluate the predictive potential of vegetative indices for nitrogen use efficiency (NUE) in soft red winter wheat (SRWW) (*Triticum aestivum* L.) and determine the optimum growth stage for employing CSR. A panel of 281 regionally developed SRWW genotypes was screened under low and normal N regimes in two crop seasons for grain yield, N uptake, nitrogen use efficiency for yield (NUEY) and nitrogen use efficiency for protein (NUEP). Vegetative indices were calculated from CSR and the data were analyzed by year and over years. Multiple regression technique and Pearson's correlation were used to obtain the best predictive models and vegetative indices. The chosen models explained 84% and 83% of total variation in grain yield and N uptake respectively, over two crop seasons. Models further accounted for 85% and 77% of total variation in NUEY, and 85%, and 81% of total variation in NUEP under low and normal N conditions, respectively. In general, yield, NUEY and NUEP were predicted with greater than 0.6 R^2 values in in 2011-12 but not in 2012-13. Differences between years are likely a result of saturation of CSR indices due to high yields in 2012. Heading was found to be the most appropriate crop growth stage to sense SRWW CSR data for predicting grain yield, N uptake, NUEY, and NUEP.

Abbreviations

CSR	Canopy Spectral Reflectance
CV	Coefficient of Variation
NIR	Near Infrared
NUE	Nitrogen Use Efficiency
NUEP	Nitrogen Use Efficiency for Protein
NUEY	Nitrogen Use Efficiency for Yield
PRESS	Predicted Residual Sum of Squares
RMSE	Root Mean Square Error
SRWW	Soft Red Winter Wheat
VIF	Variance Inflation Factor

INTRODUCTION

Wheat is one of the most widely grown cereal crops in the world due to its importance for various food and feed products, and its wide genetic adaptability to varying environmental conditions such as temperature, moisture and light (Slafer and Rawson, 1994). Genetic yield potential improvement is responsible for more than 50% of wheat yield gains in the USA (Feyerherm et al., 1984) and across the world, and will continue to be the most effective solution to meet increasing wheat grain demands (Reynolds et al., 1999). Current wheat yield improvements are at one percent per year; lagging behind by 30 to 40% (Rosengrant et al., 1995), the improvement that will be required to meet the projected wheat demand for increasing world population (Kruse, 2010). In winter wheat, genetic yield improvement rate per year ranges from 0.56% to 1.4% in eastern Virginia (Green et al., 2012) and 0.45% in Europe (Cormier et al., 2013). In cereals, yield improvement has been mainly achieved through increased biomass and improved harvest index (Van den Boogaard et al., 1996). However, in wheat, yield improvement has been mainly through the latter due to the focus on yield *per se* (Reynolds et al., 1999; Siddique et al., 1989). Some scientists speculate that wheat harvest index has reached its theoretical maximum limit of 0.6 (Austin, 1980). This has resulted in increasing focus on secondary traits such as water, radiation and nitrogen use efficiencies to meet anticipated wheat production goals (Araus et al., 2009; Reynolds and Pfeiffer, 2000). Parallel to the relatively low yield improvement rate observed after the green revolution, little increase in NUE was documented by Reynolds et al. (1999). Consequently, efforts to improve NUE of cereals through breeding (Foulkes et al., 2009; Ortiz-Monasterio., 2001) and agronomic strategies (Raun et al., 2005) are being actively pursued and can result in either increased yields or reduced N fertilizer usage (Mortimer et al., 2004).

Genetic association between grain yield and NUE [grain dry matter yield per unit available N, Moll et al., (1982)] and variation in NUE among genotypes has been widely recognized in various studies (Foulkes et al., 2009; Van Sanford and Mackown, 1986). Traits contributing to variation in NUE among genotypes were reviewed by Foulkes et al. (2009). Some listed traits are root size and morphology (root dry weight, length, density, depth, longevity and root membrane N transporter systems), physiological factors (glutamine synthetase and rubiscose activity) and morphological factors (vertical N distribution with leaf layer, leaf posture, post anthesis leaf photosynthetic rate, stem N storage, stay-green trait, post anthesis N uptake and N remobilization). Association between genotypic and corresponding phenotypic trait data is essential for understanding the genetic basis of NUE. Recent progress in DNA marker assay technology has helped in reducing the cost of high throughput genotyping of many lines (Peleman and van der Voort, 2003). However, phenotyping of genotypes for NUE especially in early stages of the breeding and selection process is a costly (Gaju et al., 2011) and a time-consuming task that also typically involves destructive sampling.

Canopy spectral reflectance is a cheap, rapid and non- destructive remote sensing technique (Montes et al., 2007) employed widely in plant nutrition management (Raun et al., 2005; Thomason et al., 2011), ecological (Chappelle et al., 1992) and physiological studies (Gitelson et al., 2005). Other studies have established the potential of CSR indices in predicting grain N uptake (Eitel et al., 2007), biomass (Chen et al., 2009) and N concentration (Barnes et al., 2000). However, these studies were conducted under varied N rates as treatments. Babar et al. (2006b) and Prasad et al. (2007) employed CSR for screening bread wheat genotypes for grain yield and biomass in plant breeding trials. However, the number of genotypes employed in those studies was small (25). Recently, Kipp et al. (2014) employed CSR in discriminating winter wheat genotypes but for early plant vigor. There is a need to validate CSR as a tool for estimating grain yield and NUE in SRWW and to assess the robustness of this approach over diverse genotypes.

The current study, comprising 281 genotypes evaluated the prediction potential of CSR data for NUE in SRWW under mid-Atlantic conditions. The objectives of this work were to: 1) evaluate vegetative reflectance indices for predicting SRWW grain yield, N uptake, nitrogen use efficiency for yield (NUEY), and nitrogen use efficiency for protein (NUEP), and 2) determine the optimum growth stage for collection of spectral reflectance measurements in SRWW.

MATERIALS AND METHODS

Experiment layout

A panel of regionally developed SRWW genotypes [281 lines, (Illinois-38, Indiana-37, Kentucky-36, Maryland-36, Missouri-37, Ohio-39, Virginia-38, Michigan-8, New York-8, Arkansas-2, Pioneer-1) and one check 'Branson'] was screened for NUE via CSR at Eastern Virginia Agricultural Research & Extension Center, Warsaw, VA (37°59′ N, 76°46′ W, and 40.5 m elevation) during 2011-12 and 2012-13 on a Kempsville sandy loam soil. The above genotype panel was replicated twice under low N (67 kg ha^{-1}) and once under normal N (134 kg ha^{-1}) regimes. Each replicaton contained five main blocks of 64 plots. The main blocks were arranged in an 8 row x 8 column (square lattice) format. The entry composition of each main block was identical in each replication of the trial. Each block was set up with the cultivar 'Branson' as a check orthogonally, appearing once in each row and column. Thus, each block contained 64 plots (56 lines grouped by similar maturity and 8 plots of Branson). Entries were assigned to main blocks based on predicted heading date.

Agronomic data

Seeding rates, planting dates and other agronomic practices were conducted according to the intensively managed, high-yielding small grain production recommendations of Virginia Cooperative Extension (Brann et al., 2000). Each experimental plot was seeded at 520 seeds m^{-2} in seven rows, 2.7 m in length with 15.2 cm spacing between rows. The entire trial received a uniform application of 33 kg N ha^{-1} preplant in a surface broadcast application. Under the normal N regime, 134 kg N ha^{-1} was applied in three additional split applications that included 33.5 kg N ha^{-1} at Zadoks (Zadoks et al., 1974) growth stage (GS) 20, 33.5 kg N ha^{-1} at GS 25 (typically mid-February) and 67 kg N ha^{-1} at GS 30 (typically mid-March) based on Virginia

Tech recommendations (Alley et al., 1996). Under the low N regime, a total of 67 kg N ha^{-1} was applied in two additional split applications that included 33.5 kg N ha^{-1} at GS 20 and 33.5 kg N ha^{-1} at GS 30.

Grain was harvested at maturity from the entire plot with a plot combine. A subsample of approximately 250 g was collected from each plot and used for test weight and moisture using a Dickey-John GAC2500 grain sampler (DICKEY-john, Auburn, IL). Grain yields were reported on a 135 g kg^{-1} moisture basis. Whole grain concentrations of protein and starch were estimated on cleaned subsamples from each plot using an XDS Rapid Content Analyzer (Foss NIR Systems, Inc. Laurel, MD). These estimates were calibrated by analyzing select duplicate samples for total N content via dry combustion (Leco FPS 528, Leco Corporation, St. Joseph, MI, AOAC 4.2.08). Grain protein estimates were converted to grain N by dividing by a conversion factor of 5.83 (Kent and Evers, 1994). Grain N uptake was determined as the product of dry matter grain yield and grain N concentration. The panel was evaluated for grain yield, grain N uptake, NUEY (Moll et al., 1982) [(grain dry weight (kg ha^{-1}) divided by fertilizer N supplied to the crop (kg ha^{-1})] and NUEP (Van Sanford and MacKown, 1986) [total grain N (kg ha^{-1}) divided by fertilizer N supplied to the $crop^{-1}$ (kg ha^{-1})]. The assumption was made that N supplied by soil was the same across the experimental area and for all varieties as done in similar research by Le Gouis and Pluchard (1996).

Canopy spectral reflectance measurements

Canopy spectral reflectance data were collected using JAZ portable field spectrometer (Ocean Optics, Dunedin, FL) from 400-900 nm (at 0.3 nm band widths) with a 25^{o} field of view from the nadir position at a height of 40 to 50 cm above the plant canopy as done in similar research by Prasad et al. (2007). Incident spectrum was determined from the light reflected from a white reference ($BaSO_4$) plate and the reflectance and vegetative indices were calculated from the ratio of reflected light from the crop canopy and the total radiance reflectance from the white reference plate. The white reference plate was prepared according to the protocol given by Knighton and Bugbee (2005). Reflectance data were collected through a continuous scanning method, which took the average reflectance values over 100 points in each plot. Reflectance was sensed during cloudless periods once from all plots occurring closely on days corresponding to stem elongation [Zadoks growth stage (GS) 31-35], booting (GS 39-47), heading (GS 55-69) and grain filling (GS 75-83). A difference of two to 10 days with respect to growth stages was observed among genotypes while collecting CSR data. The spectro radiometer was recalibrated by collected white plate reflectance approximately every 15 minutes during the process. Data collection was restricted to the period from 0900 to 1600 hours. Reflectance at a specific wavelength was calculated as the average of reflectance of two nm above and below that specific wavelength.

Vegetative indices

Sixty-three published vegetative indices {20 vegetative indices [listed in table 2 of Hatfield et al. (2008)], 26 vegetative indices [listed in table 1 of Royo and Villegas (2011) (except water indices)] and 17 vegetative indices [listed in table 2 of Ray et al. (2010)]} were calculated. These vegetative indices were selected to collect the information pertaining to chlorophyll a, b, c, canopy biomass, and N content. The vegetative indices, chosen by the final models in the current study, to predict parameters of interest are reported in Table 1.

Statistical analysis

The dependent variables grain yield, N uptake, NUEY, and NUEP were analyzed both by year and combined over both years as done in similar research by Kipp et al. (2014). Data were analyzed year wise to evaluate the predictive ability of CSR data when the variation in parameters of interest was low. Two crop year's data were combined to add robustness to the confidence intervals of the predictive values generated by the regression models as done in similar research by Mourtzinis et al. (2013).

All calculated vegetative indices were treated as independent variables. Due to 63 number of independent variables, the stepwise model building approach available in JMP®10 (SAS Institute, 2012) was used in the analysis. In stepwise model building, the option of "all possible models" was selected and all given models were analyzed comprehensively.

Models were evaluated through R^2, adjusted R^2 (Cameron, 1993), root mean square error (RMSE) , coefficient of variation (CV), residual sum of squares (RSS), predicted residual sum of squares (PRESS), Mallow's criterion [C(p)], and variance inflation factor (VIF). An independent variable significant at $\alpha \leq 0.01$ was incorporated into the model and was retained at $\alpha \leq 0.001$. Models with relatively high R^2 and adjusted R^2 values, low RMSE [predictions match closely with actual values (Sheiner and Beal, 1982)], low CV (small variation with respect to population mean), low RSS (better fit), low PRESS (better model fit to a proportion of total dataset) and low Mallow's criterion [C(p), most appropriate predictors] were preferred.

Multi-collinearity was addressed by discarding models having independent variables with VIF higher than 10. Non-intercept models were avoided to not inflate the R^2 values (UCLA, 2012; Mourtzinis et al., 2013). Potential outliers in y space (dependent variable) were identified by plotting studentized residuals against predicted values. Potential outliers in x space (independent variable) were identified by calculating Hat values (Hoaglin and Welsch, 1978). Highly influential points were identified by calculating Cook's D values (Cook, 1997). Final chosen models were evaluated for assumptions of linear regression, such as non-constant variance and non-normal errors through scatter plots of the studentized residuals versus predicted values, normal quantile plot (Goodness-of- Fit test, showing the distribution of the residuals), and leverage plots. Association between dependent variables and raw reflectance as well as all 63 vegetative indices at the afore-mentioned four different growth stages was estimated through Pearson's correlation.

RESULTS AND DISCUSSION

Crop seasons

Favorable rainfall and temperatures for wheat growth occurred in 2012-13 resulting in higher grain yields than 2011-12. During March 2012, average temperature was 5.6°C higher than the 30-year average (9.1°C) while during March 2013, temperature were 3.3°C lower than the historical average. Similarly, during March 2012, rainfall was 51 mm lower than the historical average (101 mm) while during March 2013, rainfall was 16 mm higher than the historical monthly average. These temperature differences in March could have resulted in accelerated growth rate and consequently reduced duration of spike, spikelet, and floret (reproductive stage) initiation and/ or development stages in 2011-12. Consequently, these differences in weather parameters resulted in wide variation among genotypes for yield, N uptake, NUEY and NUEP (Table 2). Such increased growth rate response of winter wheat to increased temperatures was also reported by Slafer and Rawson (1994).

Raw reflectance

The relationship between grain yield, N uptake, and unprocessed canopy spectral reflectance was estimated through Pearson's correlation under both low and normal N conditions at different wavelengths (Fig.1). As expected, reflectance in the visible range (430 to 690 nm) decreased with increasing grain filling and N uptake likely due to decreased canopy chlorophyll content. With increasing grain N uptake, under both low and normal N conditions, raw reflectance slightly increased in near infrared (NIR) range (750- 900 nm), possibly due to increased scattering caused by increased biomass. These results are not uncommon and have been reported in various studies (Hansen and Schjoerring, 2003; Hatfield et al., 2008; Liew et al., 2008).

Under low N conditions, there was a narrow depression at 710-720 nm and broad depression at 550 nm (Fig 1A). This resulted in the best-correlated vegetative indices occurring in red-edge region (710-730 nm). This sensitivity is likely due to absorption associated with chlorophyll a, slightly beyond the red region. Similar patterns and relationships between raw reflectance and chlorophyll or canopy N content were obtained by Hansen and Schjoerring, (2003) in wheat; Gitelson et al. (2005) in maize and soybean canopies; Sims and Gamon, (2002) across a wide range of species; and Datt (1999) in several *Eucalyptus* species. Under normal N conditions, best correlation coefficients were observed from red edge region but continued into NIR region (Fig.1B). Under the normal N regime, the trough observed near 550 nm in the low N regime was not obvious. This could be due to a saturation effect caused by pigment content due to their high absorption coefficients as previously reported by Hatfield et al. (2008).

Most of the variation in grain yield, N uptake, NUEY and NUEP was explained by three selected vegetative indices. This is likely because most of the genotypes' CSR differences were at visible, red edge and NIR regions. High degree of correlation among vegetative indices (multi collinearity) was observed whenever their number exceeded four in the multiple regression models.

Correlation coefficients

In general, the highest correlations of vegetative indices for parameters of interest were obtained at heading, followed by booting, stem elongation and grain filling (Table 3). The difference between r values for these vegetative indices at different growth stages was more obvious when the data were analyzed by year (Data not shown). Differences between growth stages were not obvious when the data were combined over two crop seasons (Table 3). High variation among genotypes due to varied environmental conditions could have contributed to this pattern. Results support the conclusion that the optimum growth stage to collect reflectance readings is heading. However, r values obtained at other growth stages was also more than 0.5 when the data were combined over crop seasons. In general, the worst relationship was obtained at grain filling (Table 3). Lower correlation values at grain filling could be due to the senescence of green tissue that occurs during this time. Similar higher associations of infrared-based vegetative indices with biomass at heading were observed by Babar et al. (2006a) in spring bread wheat in Mexico.

The top three vegetative indices having highest correlation coefficient values for parameters of interest at heading are shown in Table 4 by year and over years. Better relationships were obtained for vegetative indices when the data were analyzed over years than when evaluated by year. Kipp et al. (2014) reported r value of 0.98 between canopy spectral reflectance index and early plant vigor obtained through the analysis of green pixels in winter wheat in Germany. Similar to our findings, r values were reduced in their experiment, when the data was analyzed by year wise. Lower r values for selected vegetative indices were obtained for the normal N regime than for the low N regime for all parameters of interest during each year (Table 4). Overall, weaker correlations were observed in 2012-13 compared to 2011-12 (Table 4). These differences are likely due to the saturation effect of vegetative indices due to high yields and N uptake in 2012-13.

The vegetative indices R_{780}/ R_{740}, normalized difference index 1 and 2 were more frequently correlated with parameters of interest than other calculated vegetative indices (Table 3). The R_{780}/ R_{740} index was selected in most models under both low and high N in both crop seasons. This index was developed by Mistele and Schmidhalter in Germany in 2010 for wheat who reported a R^2 value of 0.9 with canopy N content at Zadoks 37. The strong relationship between this index and canopy N could be due to the potential information pertaining to both the amount of chlorophyll and biomass especially in dense crop stands. Guyot (1990) reported that the red edge inflection point is mainly depended on the reflectance values at 780 and 740 nm. Increased nitrogen uptake should have enhanced leaf elongation rate through increased epidermal and mesophyll cell number and elongation (MacAdam et al., 1989). Increased cell number likely resulted in increased NIR scattering and reflectance at R_{780} nm. The red edge position was also reported to be a robust index for variation caused by solar elevations (Broge et al., 2003); leaf surface heterogeneities like trichome densities, and structural variations. Red edge region was also reported to be a good predictor of nitrogen stress levels (Liew et al., 2008).

Association of normalized difference index 1 and 2 to grain N uptake are likely due to the chlorophyll insensitivity with reflectance at 780 and 850 nm and sensitivity at 710 and 680 nm (Datt, 1999). These indices showed high sensitivity (R^2=0.86) to a wide range of chlorophyll content in *Eucalyptus* species. Normalized difference indices 1 and 2 were also insensitive to the effects of light scattering due to external leaf structure causing optical heterogeneous surfaces (Datt, 1999). Insensitivity to the noise caused by heterogeneous surfaces is particularly important when screening large number of genotypes.

Other vegetative indices that exhibited strong relationships with NUE and yield were red edge model index, modified simple ratio and integrated index, MCARI/OSAVI (Table 4). In the current study, the red edge model index was one of the three most predictive indices at three instances for estimating grain N uptake and NUEP (Table 4). Similar to these findings, Gitelson et al., (2005) observed correlation coefficient of 0.92 between red edge model index and canopy chlorophyll content under contrasting canopy architectures such as in soybean and corn and under varied environmental conditions. In the current study, when data were analyzed over years, modified simple ratio was associated with NUEP under normal N conditions with an r value of 0.77. Similar to our results, modified simple ratio was selected to estimate chlorophyll content by accounting for the noise caused by leaf surface specular reflectance by Sims and Gamon (2002). Noise reduction was done by subtracting reflectance at 445 nm from both the reference (800 nm) and index terms (680 nm) of simple ratio. They obtained an R^2 value of 0.74 with total chlorophyll content for modified simple ratio across a wide range of species and therefore wide leaf structures. In the current study, the integrated index MCARI/OSAVI was found to be the 2nd best index for estimating NUEP (r= 0.39) under normal N conditions during 2012-13. This index was also been used to estimate wheat chlorophyll content under noisy background conditions such as shadow, soil reflectance and non-photosynthetic materials (Wu et al., 2008).

Regression models

The use of combined vegetation indices resulted in better prediction values between spectral parameters and N measurements. Similar to correlation coefficients, developed regression models were significant at α= 0.001 at all growth stages and models with highest R^2 and adjusted R^2 values were obtained at heading (Tables 5 through 7). Similarly, models developed at heading had the lowest RMSE, CV, RSS, PRESS and Mallow's C(p) (Tables 5 through 7). Considering the noise caused by solar azimuth angles and minor differences in growth stage among cultivars, model having an adjusted R^2 greater than 0.6 was assumed a good fit.

Grain yield

Several multiple linear regression models were evaluated and the best ones were picked (selected models, Table 8) based on the statistical parameters mentioned in materials and methods. When data were combined over two crop years, selected models for grain yield were characterized by adjusted R^2 values of 0.83, 0.81, 0.79, and 0.69 at heading, booting, stem elongation and grain filling stages, respectively (Table 5). Selected model at heading stage is shown in Table 8. In 2011-12, selected models were characterized by adjusted R^2 values of 0.62, 0.51, 0.51, and 0.51 at heading, booting, stem elongation and grain filling stages, respectively. In 2012-13, selected models were characterized by adjusted R^2 values of 0.43, 0.39, 0.36, and 0.32 at heading, booting, stem elongation and grain filling stages, respectively. In all the cases, highest adjusted R^2 values were observed at the heading growth stage.

Results indicated that grain yield can be predicted at heading with selected model (Table 8) as the coefficient of determination value obtained for data over two years and for data in 2011-12 is greater than 0.6. Several studies confirmed the prediction potential of vegetative indices for grain yield, but the source of variation in those studies is from more than two different N treatments (Raun, 2005; Royo, 2011; Thomason, 2011). The current study results imply that CSR has the potential to predict grain yield when the source of variability is just from genotypes and two N treatments. Similar conclusions were drawn by Babar et al. (2006b) and Prasad et al (2007).

However, R^2 values obtained in 2012-13 were less than 0.5. This is not encouraging enough to employ the developed models immediately without further refinement. One of the limitations in the current study may be low signal to noise ratio. This could be due to varied azimuth and zenith angles, wind velocities and imperceptible cloud coverage. There is an opportunity for further research to develop or evaluate CSR tool at higher grain yields by using active remote sensing, tools that do not rely on incident sunlight. Based on the results from the current study, such active remote sensing sensors should be able to capture information pertaining to red edge region.

Grain N uptake

When data were combined over two crop years; selected models for grain N uptake were characterized by adjusted R^2 values of 0.84, 0.80, 0.76, and 0.76 at heading, booting, stem elongation and grain filling, respectively (Table 5). Selected model at heading stage are shown in Table 8. In 2011-12, selected models were characterized by adjusted R^2 values of 0.75, 0.65, 0.61, and 0.66 at heading, booting, stem elongation and grain filling, respectively. In 2012-13, selected models were characterized by adjusted R^2 values of 0.64, 0.61, 0.60, and 0.60 at heading, booting, stem elongation and grain filling stages, respectively.

Unlike grain yields, results confirm CSR predictive potential for both years, when the data were analyzed by year and over both years. In general, R^2 values greater than 0.6 were obtained for grain N uptake compared to yield. Higher correlation with grain N uptake is not uncommon, as CSR indices capture information pertaining to both canopy biomass and chlorophyll content. Several studies confirmed the prediction potential of vegetative indices for grain N uptake, but the source of variation in those studies is from more than two different N treatments (Fitzgerald et al., 2010; Lukina et al., 2001). The current study results imply that CSR has the potential to predict grain N uptake when the source of variability is just from genotypes. Similar conclusions were reported in wheat by Naser, (2012) under Colorado conditions. However, before employing CSR in regular breeding programs, one should evaluate how sensitive this tool is especially when discriminating genotypes with little variation in grain N uptake.

NUEY under low N conditions

When data were combined over two crop years, selected models were characterized by adjusted R^2 values of 0.85, 0.81, 0.79, 0.70 at heading, booting, stem elongation and grain filling stages respectively (Table 6). Selected regression model at heading stage are shown in Table 8. In 2011-12, selected models were characterized by adjusted R^2 values of 0.56, 0.48, 0.39, and 0.43 respectively at heading, booting, stem elongation and grain filling stages respectively. In 2012-13, selected models were characterized by adjusted R^2 values of 0.44, 0.42, 0.41, and 0.37 at heading, booting, stem elongation and grain filling stages, respectively.

NUEY under normal N conditions

When data were combined over two crop years, selected models were characterized by adjusted R^2 values of 0.77, 0.77, 0.74, and 0.66 at heading, booting, stem elongation and grain filling stages respectively (Table 6). Selected model at heading stage is shown in Table 8. Normalized difference red edge index was chosen by the models for predicting NUEY under both low and normal N conditions (Table 8). The red portion of the spectrum is one of the areas where chlorophyll strongly absorbs light and the NIR is where the leaf cellular structure produces a strong reflection. Therefore, variations in both the chlorophyll content and the leaf structure are often reflected in the red edge band (Liew et al., 2008). Fitzgerald et al. (2010) observed an R^2 value of 0.66 between canopy N uptake and normalized difference red edge under rainfed conditions at Victoria in Australia at Zadoks GS 30. Eitel et al. (2010) also observed that this index has improved the estimates of abiotic stress induced variation in chlorophyll concentration ($R^2 > 0.73$, RMSE < 1.69) when compared to those without this index ($R^2 = 0.57$, RMSE = 2.11). The importance of the red edge region in estimating wheat biomass N concentration ($R^2 = 0.68$) was also recently confirmed by Siegmann et al. (2013). In 2011-12, selected models were characterized by adjusted R^2 values of 0.46, 0.45, 0.34, and 0.38 at heading, booting, stem elongation and grain filling stages, respectively. In 2012-13, selected models were characterized by adjusted R^2 values of 0.39, 0.33, 0.31, and 0.31 at heading, booting, stem elongation and grain filling stages, respectively.

Similar to grain yield results, greater than 0.7 R^2 values were obtained for data over two crop years; indicating the potential of CSR tool in discriminating wide varying genotypes for NUEY. However, this also implies that data from a single year or environment may not be highly predictive, thus, data from multiple years/environments may be needed. Similarly higher R^2 values were obtained for NUEY under low N conditions than normal N conditions in both crop years. The R^2 values that were obtained under normal N conditions, while analyzing the data by crop season were less than 0.5. These values are not sufficient to employ developed CSR models (Table 8) routinely in screening genotypes for NUEY.

NUEP under low N conditions

When data were combined over two crop years, selected models were characterized by adjusted R^2 values of 0.85, 0.82, 0.77, and 0.76 respectively at heading, booting, stem elongation and grain filling stages respectively (Table 7). Selected model at heading stage is shown in Table 8. In 2011-12, selected models were characterized by adjusted R^2 values of 0.71, 0.54, 0.59, and 0.54 at heading, booting, stem elongation and grain filling stages, respectively. In 2012-13, selected models, were characterized by adjusted R^2 values of 0.69, 0.68, 0.66 and 0.62 respectively at heading, booting, stem elongation and grain filling stages respectively.

NUEP under normal N conditions

When data were combined over two crop years, selected models were characterized by adjusted R^2 values of 0.81, 0.77, 0.67, and 0.74 at heading, booting, stem elongation and grain filling stages, respectively. Selected model at heading stage is shown in Table 8. In 2011-12, selected models were characterized by adjusted R^2 values of 0.68, 0.48, 0.45, and 0.56 at heading, booting, stem elongation and grain filling stages respectively. In 2012-13, selected models were characterized by adjusted R^2 values of 0.54, 0.49, 0.43 and 0.42 at heading, booting, stem elongation and grain filling stages respectively.

Greater than 0.8 adjusted R^2 values were obtained for NUEP when the data was analyzed over both years under both low and normal N conditions. Even, when the data was analyzed year wise, adjusted R^2 values were greater than 0.6 under low N conditions. Greater than 0.6 adjusted R^2 values indicated that CSR could be employed for predicting NUEP especially under low N conditions. However, adjusted R^2 values obtained under normal N conditions, when analyzing the data by crop season were less than 0.6 in 2012-13. As discussed for yield and NUEY, this value is not sufficient to employ developed CSR models (Table 8) routinely in screening genotypes for NUEY under normal N conditions. Similar to the discussion mentioned for grain yield; by employing active remote sensing technology, there is an opportunity to develop CSR indices before employing them to screen SRWW wheat genotypes regularly for NUEP.

In general, for all the selected models (Table 8), adjusted R^2 was almost equal to R^2 (Tables. 5 through 7), suggesting that none of the parameters in the model should be removed (model was not over parameterized). In all the selected models, VIF of each predictor was less than eight (Table 8), indicating that multi-collinearity was not a problem. Figures 2 through 7 depicts the scatter plots of two years actual grain yield, N uptake, and NUEY under low and normal N conditions, and NUEP under low and normal N conditions respectively against chosen model predicted values. Virtually, all the observations fell inside the 95% prediction limits. No patterns were observed in the graphs overlaid by crop year with respect to the studentized residuals chosen by the selected models against model predicted values (homogeneity of variance). Mallow's C(p) developed for different models ideally should be equal to the number of parameters in that model. Due to higher independent variables (63 vegetative indices), all the developed models in this work have relatively high Mallow's C(p) (Tables 5 through 7).

When data were combined over years, the best-fit models explained 83% and 84% of total variation in grain yield and N uptake respectively (Table 5). They further contributed to 85% and 77% of total variation in NUEY (Table 6); 85%, and 81% of total variation in NUEP under low and normal N conditions (Table 7), respectively. The selected models were robust due to wide (Table 2) parameter of interests' boundaries as presented in research by Mourtzinis et al., 2013.

When data were analyzed by year, similar to the patterns observed for correlation coefficients, parameters of interest were better (higher R^2 values) predicted in 2011-12 than in 2012-13. In general, parameters of interest were better predicted under low N conditions than under normal N conditions. Similar conclusions were reported by Royo and Villegas in their 2011 review on field measurement of canopy spectra for biomass assessment of small grain cereals.

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CONCLUSIONS

The current study evaluated CSR prediction potential for SRWW grain yield, N uptake, NUEY, and NUEP under a wide range of genotypic variability. The best selected multiple linear regression models explained 84% and 83% of total variation in SRWW grain yield and N uptake respectively, over the data from two crop seasons. Models further contributed to 85% and 77% of total variation in NUEY and 85%, and 81% of total variation in NUEP under low and normal N regimes, respectively. These results confirm the ability of CSR to broadly discriminate among genotypes for grain yield, grain N uptake, NUEY, and NUEP, provided wide variation exists in the germplasm. However, when the data were analyzed by N regime and crop season, during 2012-13, the obtained R^2 values were less than 0.6 for grain yield and NUEY under both N regimes, and for NUEP under the normal N regime. This implies that CSR could be used to discriminate genotypes for N uptake under both N regimes and NUEP under low N regimes but not for yield and NUEY. Parameters were better (higher R^2 values) predicted under low N than under normal N regime. These differences are likely a result of saturation of vegetative indices at higher yield levels. Measures of CSR from a single year or environment may not be sufficient to discriminate among genotypes because of these factors. Further research is essential to confirm the latter results and to assess the ability of active sensors to improve these estimates. Heading was found to be the optimum growth stage to collect SRWW CSR data for predicting grain N uptake, NUEY, and NUEP, however a strong relationship was also observed at the flowering stage, which indicates a reasonable window for implementation of these measurements.

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Table 1. Formulae and references of the vegetative indices selected in this study to predict grain N uptake, nitrogen use efficiency for yield (NUEY), and nitrogen use efficiency for protein (NUEP).

Vegetative index	**Formula†**	**Reference**
R_{780}/R_{740}	R_{780}/R_{740}	Mistele and Schmidhalter (2010)
Double difference index	$(R_{750}-R_{720})-(R_{700}+R_{670})$	Le Maire et al. (2004)
Modified Chlorophyll Absorption Ratio Index (MCARI) / Optimal Soil Adjusted Vegetation Index (OSAVI) [705,750]	$\frac{1.5[2.5(R_{800}-R_{670})-1.3(R_{800}-R_{500})]}{\sqrt{[(2R_{800}+1)^2-(6R_{800}-5\sqrt{R_{670}})-0.5]}}$	Wu et al. (2008)
Photo chemical Reflectance index	$(R_{550}-R_{531})/(R_{550}+R_{531})$	Gamon et al., (1992)
Normalized difference red edge	$(R_{790}-R_{720})/(R_{790}+R_{720})$	Barnes et al., (2000)
Ratio analysis of reflectance spectra b	$R_{675}/(R_{650}*R_{700})$	Chappelle at al., (1992)
Ratio analysis of reflectance spectra c	R_{760}/R_{500}	Chappelle at al., (1992)
Red Edge Triangular Vegetation index (RETVI)	$100(R_{750}-R_{730})-10(R_{750}-R_{550})\sqrt{\frac{R_{700}}{R_{670}}}$	Chen et al., (2009)
Chlorophyll index green	$(R_{845}/R_{550})-1$	Wu et al., (2010)
Normalized difference index 1	$(R_{780}-R_{710})-(R_{780}+R_{680})$	Datt (1999)
Normalized difference index 2	$(R_{850}-R_{710})-(R_{850}+R_{680})$	Datt (1999)
Red edge model index	$(R_{750}-R_{720})-1$	Gitelson et al., 2005
Modified simple ratio	$(R_{750}-R_{445})-(R_{705}+R_{445})$	Sims and Gamon (2002)
Modified normalized index	$(R_{750}-R_{705})-(R_{750}+R_{705}-2*R_{445})$	Sims and Gamon (2002)
Pigment specific normalized difference	$(R_{800}-R_{470})-(R_{800}+R_{470})$	Blackburn (1998)
R^{-1}_{700}	R^{-1}_{700}	Gitelson et al., 1999

†R and the subindex indicate the reflectance of light at that specific wavelength in nm.

Table 2. Simple means for grain N uptake, nitrogen use efficiency for yield (NUEY), nitrogen use efficiency for protein (NUEP) by year and N regime†.

N regime	Year	Minimum	25% quartile	Mean	Median	75% quartile	Maximum
			Grain yield, kg ha^{-1}				
Low	2011-12	1083	3979	4530	4476	5043	7262
	2012-13	4177	6521	6994	7013	7466	9122
Normal	2011-12	3577	4884	5344	5348	5781	7292
	2012-13	5054	6829	7348	7330	7891	9087
			N uptake, kg ha^{-1}				
Low	2011-12	25	83	96	94	108	158
	2012-13	89	131	148	147	164	224
Normal	2011-12	70	93	104	104	114	157
	2012-13	96	131	145	145	159	208
			NUEY, grain yield (kg ha^{-1}) applied N $^{-1}$(kg ha^{-1})				
Low	2011-12	11	40	45	45	50	73
	2012-13	42	65	70	70	75	91
Normal	2011-12	21	29	32	32	35	44
	2012-13	30	41	44	44	47	54
			NUEP, grain N uptake (kg ha^{-1}) applied N^{-1}(kg ha^{-1})				
Low	2011-12	0.25	0.83	0.96	0.94	1.08	1.58
	2012-13	0.89	1.31	1.48	1.47	1.64	2.24
Normal	2011-12	0.42	0.56	0.62	0.62	0.68	0.94
	2012-13	0.57	0.78	0.87	0.87	0.95	1.25

† N regime; low = 67 kg ha^{-1} spring N; normal = 134 kg ha^{-1} spring N.

Table 3. Pearson's correlation coefficients between the best vegetative index and parameters of interest [N uptake, nitrogen use efficiency yield (NUEY), nitrogen use efficiency for protein (NUEP)] at different growth stages of soft red winter wheat, 2011-13.

	Growth stage			
Parameter	**Stem elongation**	**Booting**	**Heading**	**Grain filling**
Yield, kg ha^{-1}	0.7021	0.7497	0.7902	0.6129
N uptake, kg ha^{-1}	0.6991	0.7704	0.8182	0.6838
NUEY (Low N conditions†), kg grain yield kg^{-1} N applied	0.7222	0.738	0.8061	0.6251
NUEY (Normal N conditions†), kg grain yield kg^{-1} N applied	0.6688	0.712	0.7278	0.5265
NUEP (Low N conditions), kg grain N kg^{-1} N applied	0.7330	0.7865	0.8304	0.7239
NUEP (Normal N conditions), kg grain N kg^{-1} N applied	0.6230	0.7419	0.7756	0.5727

† N regime; low = 67 kg ha^{-1} spring N; normal = 134 kg ha^{-1} spring N.

Table 4. Best estimate vegetative indices with highest r (Correlation coefficient) value in relation to parameters of interest by year and over years.

Year	Vegetative index - 1	r	Vegetative index - 2	r	Vegetative index - 3	r
			Yield, kg ha^{-1}			
2011-12	Normalized difference index 1	0.56	R_{780}/R_{740}	0.55	Normalized difference index 2	0.54
2012-13	R_{780}/r_{740}	0.33	Normalized difference index 1	0.33	Normalized difference index 2	0.32
2011-13	Normalized difference index 1	0.79	Normalized difference index 2	0.78	R_{780}/R_{740}	0.78
			N uptake, kg ha^{-1}			
2011-12	R_{780}/r_{740}	0.69	Normalized difference index 1	0.69	Normalized difference index 2	0.66
2012-13	R_{780}/r_{740}	0.51	Normalized difference index 1	0.44	Red edge model index	0.43
2011-13	R_{780}/r_{740}	0.82	Normalized difference index 1	0.77	Normalized difference index 2	0.77
			NUEY (Low N conditions†), kg grain yield kg^{-1} N applied			
2011-12	Normalized difference index 2	0.49	Normalized difference index 1	0.48	R_{780}/R_{740}	0.47
2012-13	Normalized difference index 1	0.34	Normalized difference index 2	0.34	R_{780}/R_{740}	0.33
2011-13	Normalized difference index 1	0.81	Normalized difference index 2	0.8	R_{780}/R_{740}	0.78
			NUEY (Normal N conditions†), kg grain yield kg^{-1} N applied			
2011-12	R_{780}/r_{740}	0.43	Normalized difference index 1	0.43	Normalized difference index 2	0.42
2012-13	R_{780}/r_{740}	0.31	Normalized difference index 1	0.31	Normalized difference index 2	0.3
2011-13	R_{780}/r_{740}	0.73	Normalized difference index 1	0.72	Normalized difference index 2	0.72
			NUEP (Low N conditions), kg grain N kg^{-1} N applied			
2011-12	Normalized difference red edge 2	0.65	R_{780}/R_{740}	0.62	Normalized difference index 1	0.62
2012-13	Red edge model index	0.59	R_{780}/R_{740}	0.53	Normalized difference index 2	0.3
2011-13	R_{780}/r_{740}	0.83	Normalized difference index 1	0.79	Red edge model index	0.78
			NUEP (Normal N conditions), kg grain N kg^{-1} N applied			
2011-12	Normalized difference red edge 2	0.63	Red edge model index	0.63	Modified normalized index	0.62
2012-13	R_{780}/r_{740}	0.39	MCARI/OSAVI	0.38	Modified simple ratio	0.38
2011-13	R_{780}/r_{740}	0.78	Modified simple ratio	0.77	Normalized difference red edge 2	0.74

Table 5. Comparison of selected models for soft red winter wheat grain yields and N uptake at different growth stages (2011-12 and 2012-13). Note that lower values of root mean square error (RMSE), coefficient of variation (CV), residual sum of squares (RSS), predicted residual sums of square (PRESS), and Mallow's C(p) denote a better fitting model.

Growth stage	**Stem elongation**	**Booting**	**Heading**	**Grain filling**
Fit statistic		**Yield, kg ha^{-1}**		
Model P > F	≤0.0001	≤0.0001	≤0.0001	≤0.0001
R^2	0.7859	0.8076	0.8344	0.6855
Adjusted R^2	0.7855	0.8073	0.8341	0.6851
RMSE	644.75	608.29	565.29	780.57
CV	11.20	10.20	9.66	13.08
RSS	659,296,629	703,396,885	546,130,571	1,161,931,233
PRESS	662,718,734	706,500,260	548,844,141	1,167,714,075
Mallow's C(p)	248	376	269	745
Fit statistic		**N uptake, kg ha^{-1}**		
Model P > F	≤0.0001	≤0.0001	≤0.0001	≤0.0001
R^2	0.7601	0.8004	0.8403	0.7587
Adjusted R^2	0.7596	0.8000	0.8401	0.7583
RMSE	14.81	13.34	12.02	14.67
CV	13.39	11.75	10.68	12.93
RSS	346,760	337,466	256,396	408,886
PRESS	348,489	339,091	258,112	410,992
Mallow's C(p)	258	370	335	849

Table 6. Comparison of selected models for soft red winter wheat nitrogen use efficiency for yield (NUEY) under low and normal N conditions at different growth stages (combined 2011-12 and 2012-13). Note that lower values of root mean square error (RMSE), coefficient of variation (CV), residual sum of squares (RSS), predicted residual sums of square (PRESS), and Mallow's C(p) denote a better fitting model.

Growth stage	**Stem elongation**	**Booting**	**Heading**	**Grain filling**
Fit statistic	**NUEY (Low N conditions), kg grain yield kg $^{-1}$ N applied**			
Model P > F	≤0.0001	≤0.0001	≤0.0001	≤0.0001
R^2	0.7936	0.8076	0.8470	0.7053
Adjusted R^2	0.7929	0.8072	0.8466	0.7046
RMSE	9.21	9.43	8.16	11.71
CV	11.52	10.95	9.84	13.61
RSS	141,023	112,623	71,304	174,284
PRESS	141,541	113,307	71,867	175,377
Mallow's C(p)	415	319	276	764
	NUEY (Normal N conditions), kg grain yield kg $^{-1}$ N applied			
Model P > F	≤0.0001	≤0.0001	≤0.0001	≤0.0001
R^2	0.74	0.7706	0.7747	0.6779
Adjusted R^2	0.7388	0.7695	0.7737	0.6591
RMSE	4.676	4.39	4.34	5.2077
CV	9.87	9.26	9.16	11.27
RSS	13,884	12,210	11,952	18,119
PRESS	14,077	12,381	4,207	18,500
Mallow's C(p)	82	63	56	267

Table 7. Comparison of selected soft red winter wheat nitrogen use efficiency for protein (NUEP) models under low and normal N conditions at different growth stages, (combined 2011-12 and 2012-13). Note that lower values of root mean square error (RMSE), coefficient of variation (CV), residual sum of squares (RSS), predicted residual sums of square (PRESS), and Mallow's C(p) denote a better fitting model.

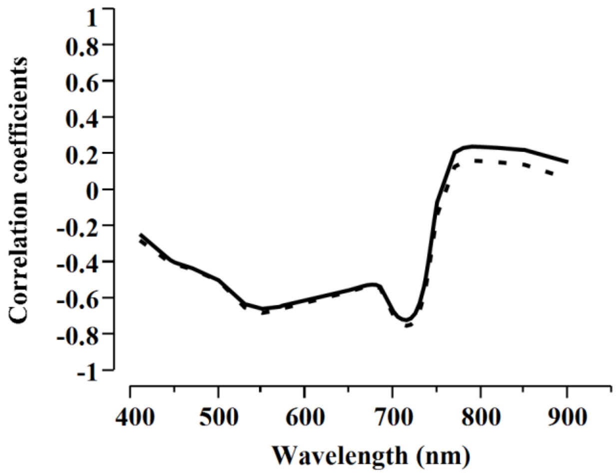
Growth stage	**Stem elongation**	**Booting**	**Heading**	**Grain filling**
Fit statistic	**NUEP (Low N conditions), kg grain N kg $^{-1}$ N applied**			
Model P > F	≤0.0001	≤0.0001	≤0.0001	≤0.0001
R2	0.7725	0.8177	0.8521	0.7582
Adjusted R2	0.7718	0.8172	0.8517	0.7576
RMSE	0.23	0.19	0.17	0.22
CV	13.23	11.77	10.81	13.57
RSS	48.1	45.2	33.0	60.2
PRESS	48.5	46.0	33.0	60.6
Mallow's C(p)	158	259	278	741
	NUEP (Normal N conditions), kg grain N kg $^{-1}$ N applied			
Model P > F	≤0.0001	≤0.0001	≤0.0001	≤0.0001
R2	0.6719	0.7751	0.8102	0.7425
Adjusted R2	0.6704	0.7741	0.8093	0.7413
RMSE	0.11	0.09	0.09	0.10
CV	10.23	10.19	9.36	10.91
RSS	7.74	5.70	4.81	6.53
PRESS	7.84	5.78	4.88	6.63
Mallow's C(p)	122	72	64	163

Table 8. Final selected models for parameters of interest using selected vegetative indices collected at heading (combined 2011-12 and 2012-13).

Variable	**Coefficient**	**VIF**
Yield, kg ha^{-1}		
Intercept	9035.614	
Normalized difference red edge	18243.72	5.3
Pigment specific normalized difference	-11971.6	2.1
R^{-1}700	-4833.02	5.5
N uptake, kg ha^{-1}		
Intercept	-154.538	
R780/ R740	172.4593	4.1
Double difference index	2.557	6.3
Modified Chlorophyll Absorption Ratio Index (MCARI) / Optimal Soil Adjusted Vegetation Index (OSAVI) [705,750]	-0.2418	7.9
NUEY (Low N regime), kg grain yield kg^{-1} N applied		
Intercept	34.76861	
Normalized difference red edge	132.9922	6.7
Photo chemical reflectance index	-369.345	1.1
Ratio analysis of reflectance spectra b	-53.6935	6.9
NUEY (Normal N regime), kg grain yield kg^{-1} N applied		
Intercept	3.44671	
Normalized difference red edge	72.49507	3.3
Ratio analysis of reflectance spectra c	-0.47029	1.5
Red edge triangular vegetation index	0.00367	4
NUEP (Low N regime), kg grain N kg^{-1} N applied		
Intercept	-2.527	
R_{780}/ R_{740}	1.99312	7.4
Chlorophyll index green	-0.02969	7.9
Double difference index	0.01541	3.4
NUEP (Normal N regime), kg grain N kg^{-1} N applied		
Intercept	-0.634	
R780/ R740	0.79556	2.7
Ratio analysis of reflectance spectra c	-0.00454	1.7
Red edge triangular vegetation index	0.00011	3.7

Fig. 1. Raw reflectance, averaged over all genotypes and years, at different wavelengths and correlation coefficient obtained through linear regression analysis of canopy reflectance against grain yield, kg ha^{-1} (broken lines) and N uptake, kg ha^{-1} (solid line), under A) low N conditions and B) normal N conditions.

A)



B)

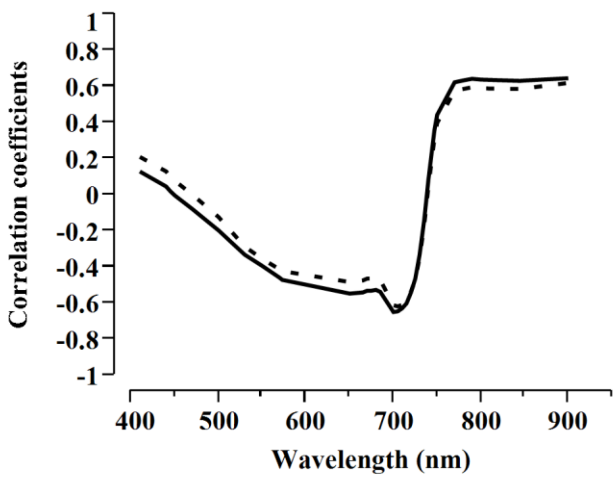


Fig. 2. Predicted vs. actual soft red winter wheat grain yields at heading stage. Dashed and solid lines denote mean and predicted linear fits respectively. Shaded region represent the 95% prediction intervals (4741, 6961 kg ha^{-1}). Black dots and gray dots represent 2011-12 and 2012-13 data points respectively**.**

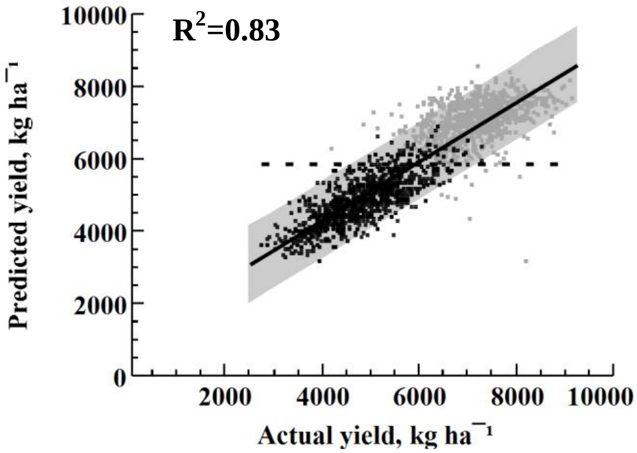


Fig. 3. Predicted vs. actual soft red winter wheat N uptake at heading stage. Dashed and solid lines denote mean and predicted linear fits respectively. Shaded region represent the 95% prediction intervals (89, 136 kg ha^{-1}). Black dots and gray dots represent 2011-12 and 2012-13 data points respectively**.**

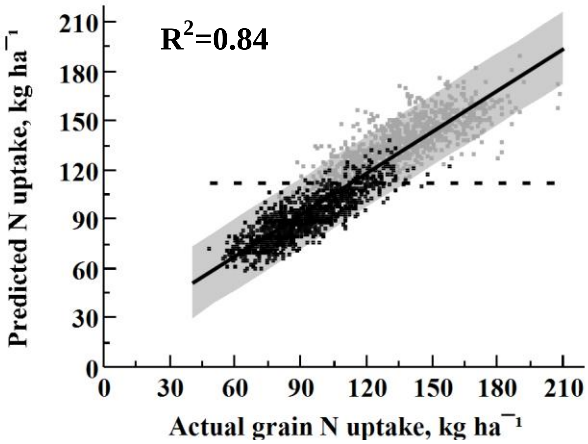


Fig. 4. Predicted vs. actual soft red winter wheat Nitrogen use efficiency for yield (NUEY), under low N conditions, at heading stage. Dashed and solid lines denote mean and predicted linear fits respectively. Shaded region represent the 95% prediction intervals (44, 66 kg ha^{-1}). Black dots and gray dots represent 2011-12 and 2012-13 data points respectively.

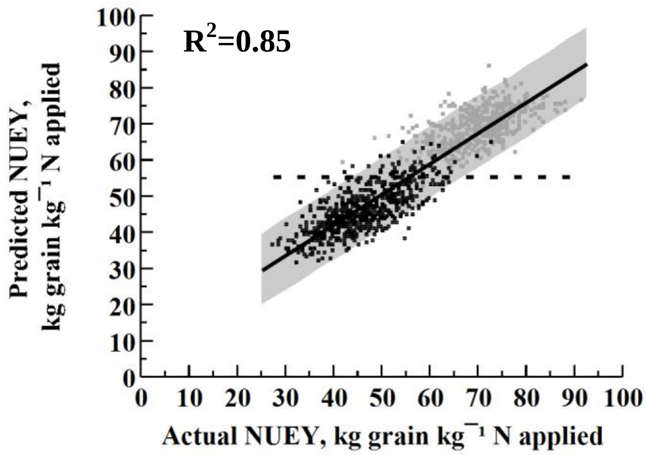


Fig. 5. Predicted vs. actual soft red winter wheat Nitrogen use efficiency for yield (NUEY), under normal N conditions, at heading stage. Dashed and solid lines denote mean and predicted linear fits respectively. Shaded region represent the 95% prediction intervals (31, 45 kg ha^{-1}). Black dots and gray dots represent 2011-12 and 2012-13 data points respectively.

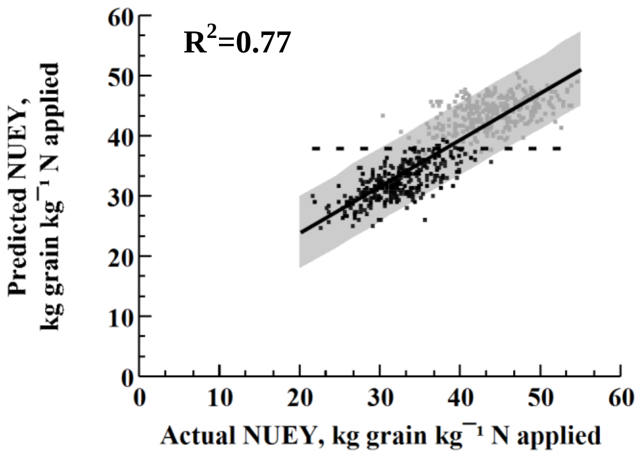


Fig. 6. Predicted vs. actual soft red winter wheat Nitrogen use efficiency for protein (NUEP), under low N conditions, at heading stage. Dashed and solid lines denote mean and predicted linear fits respectively. Shaded region represent the 95% prediction intervals (0.83, 1.28 kg ha^{-1}). Black dots and gray dots represent 2011-12 and 2012-13 data points respectively.

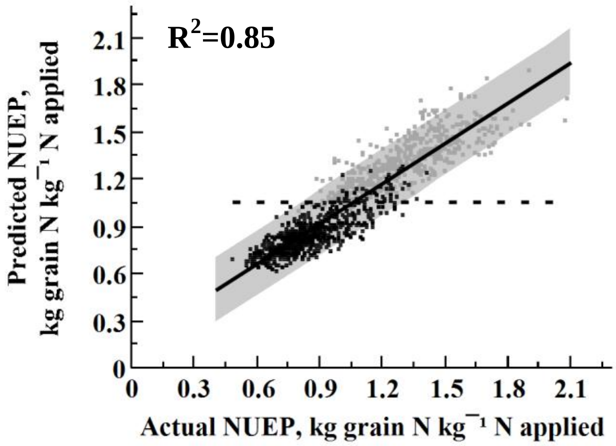
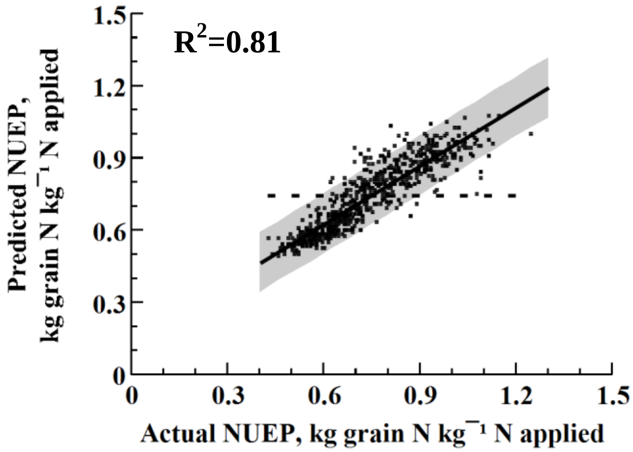


Fig. 7. Predicted vs. actual soft red winter wheat Nitrogen use efficiency for protein (NUEP), under normal N conditions, at heading stage. Dashed and solid lines denote mean and predicted linear fits respectively. Shaded region represent the 95% prediction intervals (0.60, 0.88 kg ha^{-1}). Black dots and gray dots represent 2011-12 and 2012-13 data points respectively.



CHAPTER IV

Nitrogen Use Efficiency of Regionally Developed Soft Red Winter Wheat Genotypes in the Eastern US

ABSTRACT

To reduce the impact of N pollution and production costs in a sustainable agricultural system, it is essential to increase nitrogen use efficiency (NUE) of cereal crops. Characterization of genotypes for NUE will facilitate selection in breeding programs. One of the objectives of the current study is to characterize soft red winter wheat (SRWW) (*Triticum aestivum* L.) genotypes for NUE under contrasting N conditions. An elite panel of 281 regionally developed (Illinois, Indiana, Kentucky, Maryland, Missouri, Ohio, and Virginia) SRWW genotypes was screened using a lattice square design under low (67 kg ha^{-1}) and normal (134 kg ha^{-1}) spring nitrogen (N) conditions at the Eastern Virginia Agricultural Research Extension Center near Warsaw, VA for two crop years. Other questions of interest were the consistency of NUE, i.e. do genotypes with high NUE under normal N conditions behave the same under N stress conditions, and the association between NUEY and NUEP. Genotypes were analyzed for NUE by N regime and by year using a combination of experimental design information (blocks, rows, columns) and spatial analysis. Significant variation among genotypes was observed for nitrogen use efficiency for yield (NUEY) during both crop seasons; and for nitrogen use efficiency for protein (NUEP) during 2012-13 (normal rainfall year). Based on the genotype's best linear unbiased predictor (BLUP) values, genotypes were ranked and grouped into quartiles. The genotype "VA05W-151" (cultivar 5187J) was identified as the most efficient and responsive genotype for both NUEY and NUEP over both crop seasons. Ten genotypes were identified for their responsiveness and efficiency for NUEY over both crop seasons and four of them were

developed in Virginia. Similarly, six genotypes were noted for their responsiveness and efficiency for NUEP over both seasons and two of them were developed in Maryland. Greater consistency of genotype performance between low and high N conditions was observed for NUEY (r= 0.64) than for NUEP (r=0.43). A significant proportion of the genotypes with high NUEY under high N conditions also had high NUEY under N stress; however, this was not the case for NUEP. Similarly, a significant proportion of genotypes with high NUEY also had high NUEP under both normal and low N conditions.

INTRODUCTION

Increasing world population demands 70% more food by 2050 (FAO, 2009). Approximately, 1.5 billion people depend on wheat for their nutritional needs (Reynolds, et al., 1999). Crop yield improvement through plant breeding is a cost effective strategy for sustainable food security (Tester and Langridge, 2010). Several studies reported that grain yield improvement in wheat ranges from 0.5 to 1.5% per year (Schmidt, 1984; Cox et al.,1988; Graybosch and Peterson, 2009; Green et al., 2012; Cormier et al., 2013). Grain yield improvement can be achieved through selection either directly for a primary trait like grain yield (empirical breeding) or indirectly for a secondary trait associated with grain yield (analytical or physiological breeding) (Araus et al., 2009). Yield improvement in wheat has been mainly due to empirical breeding i.e., through improved harvest index by increasing grain number (Reynolds et al., 1999). However, harvest index in most modern cultivars has nearly reached its theoretical maximum of 60% (Austin, 1980). Hence, many breeding efforts are on physiological approaches like nitrogen and radiation use efficiencies. These parameters have the potential to be evaluated via high throughput selection tools (Reynolds and Pfeiffer, 2000). Tester and Langridge (2010) review on breeding technologies suggested that plant breeders should focus on traits like NUE with greater potential to improve grain yields. Identification of such NUE efficient genotypes to develop progeny/germplasms and breed nutrient-efficient wheat varieties is essential for sustainable agriculture (Fageria et al., 2008; Kant et al., 2010; Baresel et al., 2008).

Cereal grain production is limited by the availability of N (Lopes and Araus, 2006) and the current cereal NUE is estimated to be 33% worldwide (Raun and Johnson, 1999). Nitrogen use efficiency can be improved by either maintenance of higher yields at lower N supply and/or enhanced yields at a reduced N supply. Due to high costs and extensive use of N in cereals (Raun and Johnson, 1999), and environmental contamination concerns (Dubey and Lal, 2009), efforts to improve NUE of cereals through both breeding (Foulkes et al., 2009; Ortiz-Monasterio et al., 2001) and agronomic strategies (Raun et al., 2005) are being actively pursued. These concerns, coupled with interest in mitigating the effects of climate change, have motivated policy makers and plant breeders to pursue development of N efficient genotypes (Le Gouis and Pluchard, 1996)

Parallel to low yield improvements after the green revolution, modest increase in NUE was observed by Reynolds et al. (1999). Similarly, Cormier et al. (2013) evaluated 195 wheat varieties in eight trials for NUE in Europe and estimated 0.45%, 0.39%, and 0.12% improvement rate in grain yield, grain N uptake and N harvest index, respectively per year over a period of the past 25 years. Limited genetic variability in winter wheat was reported by FAO in 1997. However, variation in wheat genotypes for NUE and NUE components like N uptake and N utilization efficiencies was reported by Van Sanford and MacKown (1986), Van Ginkel et al. (2001); Baresel et al., (2008); and Cormier et al. (2013). High heritability of complex traits such as N utilization efficiency, N harvest index, NUEY, NUEP in various wheat genotypes was also reported by Cormier et al. (2013). In view of such high heritability, one of the initial steps in developing N efficient genotypes is to identify and characterize the best performing genotypes among existing germplasm for NUE. Genotype characterization is expected to be useful for analyzing genotype and environmental interaction and stress tolerance patterns and assessing genetic diversity for nitrogen stress tolerance.

Nitrogen use efficiency is an integrative trait (Hirel et al., 2007), composed of two parameters such as N uptake efficiency, proportion of total N uptake to N availability in the soil, and N utilization efficiency, grain mass formed per unit of N absorbed (Oritz-Monasterio et al., 2001). While, N uptake efficiency plays an important role under low N conditions (Foulkes et al., 2009; Gaju et al. 2011), N utilization efficiency plays significant role under high N conditions (Ortiz-Monasterio et al., 1997) for genotypic variance for NUE. Several studies reported interaction of genotype and N rate for NUE (Le Gouis et al., 2000; Barraclough et al., 2010; Górny et al., 2011). Selection of genotypes at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico under moderate to normal N regimes simultaneously improved their performance under limited input environments (Reynolds et al., 2000). Current SRWW wheat breeding programs in the eastern USA region also conducted evaluations under nutrient rich conditions to obtain maximum net returns. In general, limited efforts have been made by breeders to directly develop varieties adapted to low N conditions. The reasons for this, according to Górny et al. (2011), are physio-morphological complexity for NUE trait and limited data available for variation among the existing germplasm. Low N conditions may also demand plant characteristics, which are quite different to the plant characteristics adapted to high N conditions (Murphy et al. 2007). Plant breeders doubt whether variation exists in the current germplasm for plant characteristics suitable to low N conditions to guarantee progress in developing more efficient cultivars (Górny et al., 2011). They further concluded that N fertilization levels have critical influence on the expression of gene actions governing NUE.

Therefore, evaluating regionally developed SRWW genotypes for NUEY and NUEP under both low and normal N conditions may facilitate a better understanding of the genetic diversity of current germplasm for NUE and in discerning whether genotypes performing well under normal N conditions simultaneously perform well under low N conditions. Thus, the objectives of this work are to: 1) identify SRWW genotypes with high NUEY, NUEP, or both; and 2) Correlate genotype performance for NUEY and NUEP under low and normal N regimes.

MATERIALS AND METHODS

Experiment layout

Trials consisting of a panel of regionally developed SRWW genotypes [281 lines, (Illinois-38, Indiana-37, Kentucky-36, Maryland-36, Missouri-37, Ohio-39, Virginia-38, Michigan-8, Newyork-8, Arkansas-2, Pioneer-1) and one check 'Branson'] were conducted at the Eastern Virginia Agricultural Research & Extension Center, Warsaw, VA during 2011-12 and 2012-13 (Kempsville sandy loam soil, 37° 59'N, 76° 46'W, 40.5 m elevation). Each year, the panel was replicated twice under low N (67 kg ha^{-1}) and once under normal N (134 kg ha^{-1}) regimes. Each replication contained five main blocks of 64 plots. The main blocks were arranged in an 8 row x 8 column square lattice design. The entry composition of each main block was identical in each replication of the trial. Each block was set up with the cultivar Branson orthogonally as a check, appearing once in each row and column. Thus, each block contained 64 plots (56 lines grouped by similar maturity and 8 plots of Branson). Entries were assigned to main blocks based on predicted heading date.

Agronomic data

Seeding rates, planting dates and other agronomic practices were according to the intensively managed, high-yielding small grain production recommendations of Virginia Cooperative Extension (Brann et al., 2000). Experiment plots were planted on 17 October in both 2011 and 2012. Each experimental plot was seeded at 520 seeds m^{-2} in seven rows, 2.7 m in length with 15.2 cm spacing between rows. Seed was treated with Raxil® -Thiram (tebuconazole 0.6% and thiram 20%) at 2.3 ml and with imidacloprid at a rate of 0.37 ml a.i. kg^{-1} of seed.

The entire trial received a uniform application of 33 kg N ha^{-1} shortly before planting. Under the normal N regime, 134 kg N ha^{-1} was applied in three split applications that included 33.5 kg N ha^{-1} at Zadoks (Zadoks et al., 1974) growth stage (GS) 20, 33.5 kg N ha^{-1} at GS 25, and 67 kg N ha^{-1} at GS 30 based on Virginia Tech recommendations (Alley et al., 1996). Under the low N regime, a total of 67 kg N ha^{-1} was applied in two split applications that included 33.5 kg N ha^{-1} at GS 20 and 33.5 kg N ha^{-1} at GS 30. Pre-plant applications of P, K, S, and lime were based upon Virginia Tech soil testing recommendations.

Weed control was achieved through herbicide application of Harmony-Extra SG® (thifensulfuron-methyl and tribenuron-methyl) at rates recommended by Virginia Cooperative extension (Hagood and Herbert, 2010). Pest control was based on current Virginia Cooperative Extension recommendations (Hagood and Herbert, 2010) and thresholds. Tilt® (propiconazole) fungicide was applied between GS 30 to GS 45 (in 2011, once, on 15th March @ 140 g a.i. ha^{-1} and in 2012 twice, on March 20 and April 25 @ 280 g a.i. ha^{-1}) to control powdery mildew [*Blumeria graminis* (DC.) E.O. Speerf. sp. *tritici* Em. Marchal; syn *Erysiphe graminis* f. sp. *tritici*]. Prosaro (prothioconazole and tebuconazole) was applied at a rate of 212.8 g a.i. ha^{-1} at spike emergence (GS 50) to control leaf rust (*Puccina triticina* f. sp. *tritici*) and fusarium head blight (*Fusarium graminearum*). As much as possible, grain yield and cultivar performance differences due to variation in disease resistance was minimized by these measures. Grain was harvested at maturity from the entire plot with a plot combine. A subsample of approximately 250 g was taken from each plot for test weight and moisture determination using a Dickey-John GAC2500 grain sampler (DICKEY-john, Auburn, IL). Grain yields were reported on a 135 g kg^{-1} moisture basis. Whole grain concentrations of protein and starch were estimated on cleaned subsamples from each plot using an XDS Rapid Content Analyzer (Foss NIR Systems, Inc.

Laurel, MD). These estimates were calibrated by analyzing select duplicate samples for total N content via dry combustion (Leco FPS 528, Leco Corporation, St. Joseph, MI, AOAC 4.2.08). Grain protein estimates were converted to grain N by dividing by a conversion factor of 5.83 (Kent and Evers, 1994). Grain N uptake was determined as the product of dry matter grain yield and grain N concentration. The elite panel was evaluated for grain yield, grain N uptake, NUEY (Moll et al., 1982) [(grain dry weight (kg ha^{-1}) divided by fertilizer N supplied to the crop (kg ha^{-1})] and NUEP (Van Sanford and MacKown, 1986) [total grain N (kg ha^{-1}) divided by fertilizer N supplied to the crop (kg ha^{-1})]. The assumption was made that N supplied by soil was the same across the experimental area and for all varieties as done in similar research by Le Gouis and Pluchard (1996).

Statistical analysis

Due to the significant interaction effect of genotypes, N regime and growing seasons, data were analyzed by year and N regime for NUEY and NUEP using a combination of design information (blocks, sub blocks) and spatial analysis (an iterative and sequential process) using ASREML (Gilmour et al.1999) software. While characterizing genotypes, Qiao et al. (2000) observed that chosen spatial models (combination of design information and spatial trends) enhanced average relative efficiency by 138% over randomized complete block design. Spatial analyses generally increase the precision of genotype estimates (Gilmour et al., 1997). Therefore, all genotypes were spatially analyzed for NUEY and NUEP in the current study. Spatial analysis was conducted according to the protocol published by Burgueño et al. (2000). Blocks, rows, columns and genotypes were treated as random effects similar to the approach of Mackay (2011). Best linear unbiased predictor (BLUP) values, a best estimate for genotype performance in future trials (Burgueño et al. 2000) were obtained for each genotype. Models were developed according to the patterns observed in variogram and trellis plots (Gilmour et al., 1997). Final models were chosen based on their likelihood ratio test and error mean square values. In general, most of the final selected linear mixed spatial models consisted of overall mean, genotype, block and row effects. The residual component contained local trend [modeled by the two dimensional auto-regressive integrated moving average (ARIMA) procedures in the direction of row and columns] and error term (after adjusting all other terms in the model). Based on BLUP values, genotypes were ranked for NUEY and NUEP. Genotypes were then grouped into upper-quartile (Q) (top 25%), 1st inter-Q (26-50%), 2nd inter Q (51-75%), and lower Q (76 to 100%) as done in similar research by Barraclough et al. (2010). Year wise association of genotype performance between the two N regimes was measured through Pearson's correlation coefficients.

RESULTS AND DISCUSSION

Crop seasons

More favorable rainfall and temperatures for wheat growth occurred in 2012-13 resulting in higher grain yields than in 2011-12 (Table 1). During March 2012, average temperature was 5.6°C higher than the 30-year average (9.1°C) while during March 2013; it was 3.3°C lower than the historical average. Similarly, during March 2012, rainfall was 51 mm lower than the historical average (101 mm) while during March 2013; it was 16 mm higher than the historical monthly average. These temperature differences in March could have resulted in accelerated growth rate and consequently reduced growth duration at spike, spikelet, and floret (reproductive stage) initiation and/ or development stages in 2011-12. Consequently, these differences in weather parameters resulted in wide variation among genotypes for yield, N uptake, NUEY and NUEP (Table 2). Such increased growth rate response of winter wheat to increased temperatures was reported by Slafer and Rawson (1994).

Genotype performance

An interaction among genotypes, N levels and crop seasons resulted in genotype ranking differences between N levels and crop seasons. Similar interactions between N levels and winter wheat genotypes for NUE were observed by Le Gouis and Pluchard (1996), Otto (2007), Gaju et al. (2011) and Khalilzadeh et al. (2013).

Genotype variance was significant in all cases except NUEP under the normal N regime during 2011-12. Genotypic differences for NUE were also reported in SRWW by Van Sanford and MacKown (1982) and May et al. (1991) and in winter bread wheat by Le Gouis and Pluchard (1996), Ortiz-Monasterio et al. (1997), Otto (2007), and Gaju et al. (2011). Factors such as reduced soil moisture and unfavorably warm weather conditions during 2011-12 likely resulted in the genotypes' non-responsiveness to N rich conditions.

A summary of each genotype ranking for NUEY and NUEP at low and normal N regimes during 2011-12 and 2012-13 is shown in Tables 3 through 10 and Appendices A through H. Tables 3 through 9 depict each genotype panel (Illinois, Indiana, Kentucky, Maryland, Missouri, Ohio, and Virginia respectively) for NUEY and NUEP, and Table 10 depicts selected genotypes from Michigan, New York and Arkansas breeding programs.

The effects of genotype ranks and N rate on NUEY and NUEP are shown in Fig. 1A and Fig. 1B, respectively. Nitrogen rate had considerable effect on NUEY and NUEP (Fig. 1). Higher NUEY and NUEP values were recorded under low N treatment; which was also reported by Cormier et al. (2013). The significance and magnitude of N rate effect on grain yield comparison among genotypes and crop seasons was also reported by Barraclough et al. (2010) and Cormier et al. (2013). Mean genotype performance frequency distribution for NUEY under low and normal N conditions is shown in Fig. 2A and Fig. 2B, respectively. They are normally distributed for both NUEY and NUEP.

Grain yield, grain N uptake, and grain N concentration

Relationships among grain yield, grain N uptake and grain N concentration are depicted in Fig.3. Under the low N regime, yield was positively associated with N uptake (Fig. 3A) with correlation coefficients of 0.86 and 0.72, during 2011-12 and 2012-13, respectively. Under normal N regime, they were 0.72 and 0.78. Correlation coefficients of 0.74 and 0.95 during two different crop seasons between grain yield and grain N uptake in winter wheat were reported by Heitholt et al. (1990).

Grain N uptake defined as the amount of total N in the grain yield was positively associated with grain N concentration (% of N per unit weight of grain weight) (Fig. 3B) with correlation coefficients of 0.54 and 0.81 under the low N regime during 2011-12 and 2012-13, respectively. Under the normal N regime, they were 0.47 and 0.66, respectively. Similar to these results, a correlation coefficient of 0.31 between grain N uptake and grain N concentration in winter wheat was observed by Heitholt et al. (1990) in Oklahoma.

There was no significant relationship between grain yield and grain N concentration (Fig. 3C) in 2011-12 under low N and in 2012-13 under normal N regimes. Cormier et al. (2013) also reported that grain N concentration was stable in the varieties released over the last 25 years in Europe meaning no association between grain yield and grain N concentration. For the remaining cases in the current study, even though there was a significant relationship between grain yield and grain N concentration, the negative correlation coefficients were less than 0.25. Barraclough et al. (2010) and Heitholt et al. (1990) also reported negative association between grain yield and grain N concentration at a fixed N rate application among winter wheat genotypes in southern England and Oklahoma, respectively.

NUEY and NUEP

Gerloff (1977) proposed a genotype classifying system for nutrient use efficiency based on genotype performance under low and nutrient rich conditions. He grouped germplasm into 1) efficient, responders; 2) efficient, non-responders; 3) inefficient, responders; and 4) inefficient, non-responders. Current work employed this classification to categorize and distinguish soft red winter wheat genotypes to N stress.

Genotypes that were in the upper quartile for NUEY and NUEP in both crop seasons under normal N condition were identified as stable responding genotypes. In total, there were 28 for NUEY and 29 for NUEP (Tables 2 through 9). The majority (36%) of these responding NUEY genotypes were from the Virginia panel (Fig. 4A). This can be expected, because these genotypes are adapted to Virginia conditions and in the current study, genotypes were evaluated under Virginia conditions. However, the majority (28%) of responding NUEP genotypes were from the Maryland panel (Fig. 4B). This could be due to the differences in parental lines used in Maryland versus Virginia breeding programs.

Similar to stable responding genotypes; stable efficient genotypes were identified but under low N conditions. In total, there were 27 (Table 2 through 9) for NUEY and 23 (Table 2 through 7) for NUEP. Again as expected, a majority (41%) of these responding NUEY genotypes were from the Virginia panel (Fig. 5A). This could be due to the importance given to yield *per se* in the Virginia breeding program and the location of the current study. However, a majority (41%) of efficient NUEP genotypes were from the Maryland panel (Fig. 5B).

Few genotypes were common in both responsive and efficient category for NUEY, meaning responding and efficient genotypes for NUEY. They were IL07-19334 from Illinois (Table 3); 0566A1-3-1-67 from Indiana (Table 4); KY03C-2314-08 from Kentucky (Table 5); MD03W665-10-5 and MD03W485-10-2 from Maryland (Table 6); OH08-269-58 from Ohio (Table 8); and VA09W-110, VA05W-151, VA10W-123, and MERL from Virginia (Table 9).

Similarly, six genotypes were common in responsive and efficient category for NUEP, meaning responding and efficient genotypes for NUEP. They were KY02C-1076-07 from Kentucky (Table 5); MD04W249-11-7 and MD05W479-B-11-3 from Maryland (Table 6); RED RUBY from Michigan (Table 10); OH08-161-4 from Ohio (Table 8), and VA05W-151 from Virginia (Table 9).

The genotypes that were in the upper quartile category for both NUEY and NUEP in both crop seasons and under both N conditions, were identified as responding and efficient genotypes for both NUEY and NUEP. Only one genotype (VA05W-151 from Virginia) out of 281 genotypes was identified in this category (Table 9). The low number of genotypes in this category could be due to the interaction of genotype and environment (Araus et al., 2002; Barraclough et al., 2010) especially due to the presence of an environment characterized by moisture stress during the 2011-12 crop season. The genotype VA05W-151 can be considered as the most stable genotype for both NUEY and NUEP. For this genotype, genetic factors could be over-riding the external factors such as N availability and crop season. This genotype was developed from a cross between Pioneer brand '26R24' (PI 614110 PVPO) / 'McCormick' (PI 632691) at Virginia Tech and was released as cultivar 5187J in 2012. Genotype VA05W-151 was categorized as a high yielding, early maturing, short height semi-dwarf (gene *Rht2*) variety adapted to varied climatic conditions (Griffey et al., 2012a). Similar to these results, Griffey et al., (2012b) reported the highest three-year (2008-2010) average grain yield of 5778 kg ha^{-1} for this genotype in Virginia state variety trials (seven environments over three years). This genotype was also ranked first in grain yield in 2009-10 (27 locations) and in 2010-11 (26 locations) in United States Department of Agriculture (USDA) uniform eastern SRWW nursery trials (Griffey et al., 2012a). Such phenotypic data can be used in future association analysis studies to identify quantitative trait loci for yield, stability, and nitrogen use efficiency.

Low and normal N regimes correlation in genotypic performance

Correlation coefficients of genotype performance between low and normal N conditions for NUEY were 0.66 during 2011-12 and 0.62 during 2012-13 crop seasons (Fig. 6 and 7 , respectively). For NUEP, they were 0.45 for 2011-12 and 0.42 for 2012-13 crop seasons. All these correlation coefficients were significant at $\alpha=0.05$. A correlation coefficient of 0.84 for genotype grain yield between low and high N conditions was observed in wheat in Europe (Gaju et al., 2011). These correlation coefficients were high when compared with the values (almost no relationship) obtained by Barralough et al. (2010). In the latter study, strong interaction between genotype and N treatment was observed. Unlike in the current study (all genotypes are soft red winter wheat), their experiment consisted of different wheat classes (bread, pastry, animal feed, historical tall, spring, and continental types). This could be the reason for these contrasting correlation observations.

High correlations for NUEY between low and normal N regimes indicate that the genotypes developed under normal N conditions were also performing well for NUEY under low N conditions. This has also been observed in other studies under varied moisture stress conditions in wheat (Calderini and Slafer, 1999; Slafer and Araus, 2007; Cattivelli et al., 2008) and in barley (Tambussi et al., 2005). Low correlations for NUEP compared to NUEY could be due to the preference given by the plant breeders for yield *per se* over N uptake or NUEP in SRWW.

CONCLUSIONS

Grain yield, N uptake, NUEY, and NUEP were affected by genotype, N levels and crop seasons. Genotype variance was found to be significant in all cases except NUEP under normal N regime during 2012-13. Based on BLUP values, all genotypes were characterized for their response to N and assigned to four different quarter categories. Among the regional genotypes, only one genotype, VA05W-151, was ranked consistently in the upper quarter category, during both crop seasons, for both NUEY and NUEP. Ten genotypes performed better under both low and normal N conditions and during both crop seasons for NUEY. Similarly, six genotypes were identified in common over both crop seasons, for their efficiency and responsiveness for NUEP. Further research is needed on agronomic or physiological attributes leading to efficiency or responsiveness of the above selected genotypes. This genotype characterization data will be used in association analysis for NUE in SRWW. A significant correlation of 0.64 was observed between low and normal N conditions for genotype performance for NUEY. For NUEP, the correlation was lower (0.43) but significant. This means that genotypes bred under nutrient rich conditions also performed well under nutrient stress conditions for NUEY.

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Table 1. Mean monthly air temperature, total precipitation and total snowfall for the two growing seasons and historical (30 years) averages.

Month	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**March**	**April**	**May**	**June**
				Precipitation (cm)					
2012-13	17.4	7.6	4.2	9.0	4.6	11.7	20.1	5.5	19.4
2011-12	7.5	10.4	5.3	2.3	7.4	5.0	7.8	7.1	3.6
Historical average	9.0	8.5	7.9	7.6	6.7	10.1	8.0	10.4	9.3
				Temperature (°C)					
2012-13	16.8	10.6	10.3	5.2	3.3	5.8	14.3	19.9	23.7
2011-12	16.1	11.9	8.1	5.7	7.1	14.7	14.2	21.3	23.0
Historical average	15.4	10.4	5.1	3.3	5.0	9.1	14.5	19.1	23.8
				Total monthly snowfall (cm)					
2012-13	0	0	0	9	1	6	0	0	0
2011-12	0	0	0	0	2	8	0	0	0
Historical average	0	0.8	7	13	10	2	0.4	0	0

†Climate data obtained from http://www.sercc.com/climateinfo/historical/historical_va.html

Table 2. Simple statistics for grain yield, N uptake, nitrogen use efficiency for yield (NUEY), and nitrogen use efficiency for protein (NUEP) by year and level of N application.

N regime	Year	Minimum	25% quartile	Mean	Median	75% quartile	Maximum
			Grain yield, kg ha^{-1}				
Low	2011-12	1083	3979	4530	4476	5043	7262
	2012-13	4177	6521	6994	7013	7466	9122
Normal	2011-12	3577	4884	5344	5348	5781	7292
	2012-13	5054	6829	7348	7330	7891	9087
			N uptake, kg ha^{-1}				
Low	2011-12	25	83	96	94	108	158
	2012-13	89	131	148	147	164	224
Normal	2011-12	70	93	104	104	114	157
	2012-13	96	131	145	145	159	208
			NUEY, kg grain yield kg^{-1} N applied				
Low	2011-12	11	40	*45*	45	50	73
	2012-13	42	65	70	70	75	91
Normal	2011-12	21	29	32	32	35	44
	2012-13	30	41	44	44	47	54
			NUEP, kg grain N kg^{-1} N applied				
Low	2011-12	0.25	0.83	0.96	0.94	1.08	1.58
	2012-13	0.89	1.31	1.48	1.47	1.64	2.24
Normal	2011-12	0.42	0.56	0.62	0.62	0.68	0.94
	2012-13	0.57	0.78	0.87	0.87	0.95	1.25

Table 3. Performance of Illinois wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP). Genotypes were ranked and accordingly grouped into Upper-Q (top 25% -), 1st inter-Q (26-50%), 2nd inter Q (51-75% -), and lower Q (76 to 100% -). Genotypes that were in top quartile for both responsiveness and efficiency for NUEY were highlighted with bold font.

	NUEY				**NUEP**			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON								
IL00-8109								
IL00-8530								
IL00-8633								
IL01-11934								
IL02-18228								
IL02-19483B								
IL04-24668								
IL04-9942								
IL05-4236								
IL06-13072								
IL06-13708								
IL06-13721								
IL06-14262								
IL06-14303								
IL06-14325								
IL06-23571								
IL06-31053								
IL06-7550								
IL06-7653								
IL07-12948								
IL07-16075								
IL07-19334								
IL07-20728								
IL07-20743								
IL07-21847								
IL07-23420								
IL07-24841								

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
IL07-4415								
IL07-6861								
IL08-12174								
IL08-12206								
IL08-22075								
IL08-31639								
IL08-33373								
IL08-33951								
IL08-34020								
IL08-9266								
IL99-26442								

Table 4. Performance of Indiana wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP). Genotypes were ranked and accordingly grouped into Upper-Q (top 25% -), 1st inter-Q (26-50%), 2nd inter Q (51-75% -), and lower Q (76 to 100% -). Genotypes that were in top quartile for both responsiveness and efficiency for NUEY were highlighted with bold font.

	NUEY				**NUEP**			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON								
91193								
92201								
011007A1-14-16-50								
0175A1-37-4-1								
02444A1-23-1-3								
03207A1-7-3-1								
03549A1-18-25								
03633A1-69-2-5								
04606RA1-1-7-1								
04606RA1-1-7-1-6								
04620A1-1-7-4								
04702A1-18								
04719A1-16-1-1-7								
0513A1-1-3								
05219A1-8-21-2-4								
05222A1-1-2-1								
05247A1-7-3-120								
05247A1-7-3-27								
05247A1-7-7-3-1								
05251A1-1-136-9-5								
05264A1-1-3-2								
05287A1-1-13								
0537A1-12								
0537A1-3-12								
0566A1-3-1-65								
0566A1-3-1-67								
0570A1-2-39-5								

	NUEY				NUEP			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
06403A1-4								
0722A1-1-7								
07287RA1-14								
07290A1-12								
0762A1-2-8								
9346A1-2-5-5-2-1								
Clark								
INW0411								
INW0412								
INW1021								

Table 5. Performance of Kentucky wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP). Genotypes were ranked and accordingly grouped into Upper-Q (top 25% -), 1st inter-Q (26-50%), 2nd inter Q (51-75% -), and lower Q (76 to 100% -). Genotypes that were in top quartile for both responsiveness and efficiency for NUEY were highlighted with bold font.

	NUEY				**NUEP**			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON								
ALLEGIANCE								
FOSTER	NA†		NA				NA	
KY02C-1058-03								
KY02C-1076-07								
KY02C-1121-11								
KY02C-1121-75								
KY02C-1122-06								
KY02C-2215-02								
KY02C-3004-07								
KY02C-3005-25								
KY03C-1002-02								
KY03C-1192-37								
KY03C-1195-10-1-5								
KY03C-1221-01								
KY03C-1221-06								
KY03C-1221-22								
KY03C-1237-01								
KY03C-1237-15								
KY03C-1237-32								
KY03C-2047-02								
KY03C-2047-06								
KY03C-2049-02								
KY03C-2314-08								
KY03C-2399-02								
KY04C-1128-38-1-5								
KY04C-2006-41-1-1								
KY04C-2151-40								

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
KY04C-2151-41								
KY04C-3006-33-14-3								
KY05C-1007-2-12-5								
KY05C-1105-42-20-1								
KY05C-1381-77-7-5								
KY05C-1617-17-17-3								
KY06C-1003-139-8-3								
KY93C-1238-17-1								
PEMBROKE								

†NA Data unavailable due to poor seed germination.

Table 6. Performance of Maryland wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP). Genotypes were ranked and accordingly grouped into Upper-Q (top 25% -), 1st inter-Q (26-50%), 2nd inter Q (51-75% -), and lower Q (76 to 100% -). Genotypes that were in top quartile for both responsiveness and efficiency for NUEY were highlighted with bold font.

	NUEY				**NUEP**			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON								
CATOCIN								
CHESAPEAKE								
MD01W270-10-3								
MD03W104-10-2								
MD03W151-10-12								
MD03W485-10-10								
MD03W485-10-12								
MD03W485-10-2								
MD03W485-10-8								
MD03W61-11-2								
MD03W61-11-3								
MD03W64-10-3								
MD03W665-10-3								
MD03W665-10-5								
MD04W1197-11-13								
MD04W249-11-12								
MD04W249-11-13								
MD04W249-11-5								
MD04W249-11-7								
MD04W359-11-10								
MD04W8-11-4								
MD05W10208-11-13								
MD05W10208-11-14								
MD05W10208-11-3								
MD05W10208-11-6								
MD05W10208-11-7								
MD05W10208-11-8								

	NUEY				NUEP			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
MD05W1292-11-1								
MD05W1292-11-4								
MD05W1317-11-4								
MD05W479-B-11-3								
MD07W272-11-5								
MD07W419UM5-11-11								
MD07W419UM5-11-12								
MD665-09-6								
MD665-09-6								

Table 7. Performance of Missouri wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP). Genotypes were ranked and accordingly grouped into Upper-Q (top 25% -), 1st inter-Q (26-50%), 2nd inter Q (51-75% -), and lower Q (76 to 100% -).

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON								
BESS								
ERNIE								
Milton								
MO050921								
MO080103								
MO080104								
MO080584								
MO080589								
MO080864								
MO081163								
MO081280								
MO081537								
MO081559								
MO081652								
MO081699								
MO090574								
MO090581								
MO090821								
MO091011								
MO091159								
MO100172								
MO100231								
MO100265								
MO100519								
MO100535								
MO100539								
MO100647								
MO100745								

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
MO101142								
MO101202								
MO101207								
MO101278								
MO101329								
MO101358								
MO101361								
MO101571								
TRUMAN								

Table 8. Performance of Ohio wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP). Genotypes were ranked and accordingly grouped into Upper-Q (top 25% - ■), 1st inter-Q (26-50% ■), 2nd inter Q (51-75% - □), and lower Q (76 to 100% - □). Genotypes that were in top quartile for both responsiveness and efficiency for NUEY were highlighted with bold font in green color.

	NUEY				**NUEP**			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON	1st inter-Q	Upper-Q	2nd inter-Q	Upper-Q	Lower-Q	Upper-Q	Lower-Q	2nd inter-Q
Becker	2nd inter-Q	2nd inter-Q	1st inter-Q	1st inter-Q	1st inter-Q	1st inter-Q	1st inter-Q	Upper-Q
BROMFIELD	1st inter-Q	2nd inter-Q	2nd inter-Q	2nd inter-Q	Upper-Q	2nd inter-Q	1st inter-Q	1st inter-Q
MALABAR	1st inter-Q	Lower-Q	Upper-Q	1st inter-Q	Upper-Q	Lower-Q	1st inter-Q	1st inter-Q
OH05-200-74	2nd inter-Q	2nd inter-Q	Upper-Q	Upper-Q	1st inter-Q	2nd inter-Q	Upper-Q	Upper-Q
OH06-150-57	2nd inter-Q	2nd inter-Q	Lower-Q	Lower-Q	2nd inter-Q	Lower-Q	Lower-Q	Lower-Q
OH06-180-57	2nd inter-Q	Upper-Q	2nd inter-Q	Upper-Q	2nd inter-Q	Upper-Q	2nd inter-Q	1st inter-Q
OH07-166-41	1st inter-Q	Lower-Q	2nd inter-Q	2nd inter-Q	2nd inter-Q	Lower-Q	2nd inter-Q	1st inter-Q
OH07-166-49	Lower-Q	2nd inter-Q	Lower-Q	2nd inter-Q	1st inter-Q	2nd inter-Q	Lower-Q	1st inter-Q
OH07-174-11	1st inter-Q	Upper-Q	1st inter-Q	1st inter-Q	Upper-Q	Upper-Q	1st inter-Q	2nd inter-Q
OH07-238-15	Upper-Q	Lower-Q	Upper-Q	Lower-Q	1st inter-Q	Lower-Q	1st inter-Q	Lower-Q
OH07-254-11	Upper-Q	1st inter-Q	1st inter-Q	2nd inter-Q	Upper-Q	1st inter-Q	1st inter-Q	Upper-Q
OH07-263-3	Upper-Q	Upper-Q	Upper-Q	1st inter-Q	Upper-Q	2nd inter-Q	Upper-Q	2nd inter-Q
OH07-94-70	Lower-Q	Lower-Q	Lower-Q	Lower-Q	1st inter-Q	1st inter-Q	Lower-Q	1st inter-Q
OH07-95-7	Lower-Q	Lower-Q	Lower-Q	Lower-Q	1st inter-Q	Lower-Q	2nd inter-Q	Upper-Q
OH07-98-21	Lower-Q	1st inter-Q	2nd inter-Q	1st inter-Q	Lower-Q	1st inter-Q	Lower-Q	Upper-Q
OH08-101-57	2nd inter-Q	Lower-Q	1st inter-Q	2nd inter-Q	Upper-Q	Lower-Q	1st inter-Q	Upper-Q
OH08-101-72	1st inter-Q	Lower-Q	2nd inter-Q	Lower-Q	Lower-Q	Lower-Q	Lower-Q	Lower-Q
OH08-107-16	Lower-Q	Lower-Q	Lower-Q	Lower-Q	Lower-Q	1st inter-Q	Lower-Q	Lower-Q
OH08-133-25	1st inter-Q	Upper-Q	1st inter-Q	Upper-Q	1st inter-Q	Upper-Q	2nd inter-Q	Upper-Q
OH08-141-6	1st inter-Q	Lower-Q	2nd inter-Q	Lower-Q	Upper-Q	Lower-Q	2nd inter-Q	2nd inter-Q
OH08-149-11	Upper-Q	Upper-Q	2nd inter-Q	Upper-Q	1st inter-Q	1st inter-Q	Upper-Q	1st inter-Q
OH08-161-4	Upper-Q	Upper-Q	1st inter-Q	Upper-Q	Upper-Q	Upper-Q	Upper-Q	Upper-Q
OH08-161-78	Upper-Q	1st inter-Q	Upper-Q	1st inter-Q	2nd inter-Q	Upper-Q	Upper-Q	1st inter-Q
OH08-170-66	2nd inter-Q	Lower-Q	Upper-Q	2nd inter-Q	Lower-Q	Lower-Q	1st inter-Q	2nd inter-Q
OH08-172-42	2nd inter-Q	1st inter-Q	Upper-Q	Upper-Q	Lower-Q	Lower-Q	2nd inter-Q	Lower-Q
OH08-178-52	Upper-Q	1st inter-Q	Upper-Q	2nd inter-Q	Lower-Q	2nd inter-Q	1st inter-Q	Lower-Q
OH08-180-48	Upper-Q	1st inter-Q	Upper-Q	Upper-Q	Upper-Q	2nd inter-Q	Upper-Q	Upper-Q

	NUEY				**NUEP**			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
OH08-182-4								
OH08-199-1								
OH08-206-19								
OH08-207-33								
OH08-234-4								
OH08-235-33								
OH08-246-15								
OH08-254-22								
OH08-256-47								
OH08-265-37								
OH08-269-58								
OH08-98-13								

Table 9. Performance of Virginia wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP). Genotypes were ranked and accordingly grouped into Upper-Q (top 25% -), 1st inter-Q (26-50%), 2nd inter Q (51-75% -), and lower Q (76 to 100% -). Genotypes that were in top quartile for both responsiveness and efficiency for NUEY were highlighted with bold font.

	NUEY				**NUEP**			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON	1st inter-Q	Upper-Q	2nd inter-Q	Upper-Q	Lower Q	Upper-Q	Lower Q	2nd inter-Q
JAMESTOWN	Upper-Q	1st inter-Q	Upper-Q	2nd inter-Q	Upper-Q	Upper-Q	1st inter-Q	Upper-Q
MASSEY	Lower Q	Lower Q	Lower Q	Lower Q	2nd inter-Q	Upper-Q	Lower Q	Upper-Q
MERL	Upper-Q	Upper-Q	Upper-Q	Upper-Q	Upper-Q	1st inter-Q	Upper-Q	1st inter-Q
ROANE	2nd inter-Q	Lower Q	Lower Q	Lower Q	1st inter-Q	1st inter-Q	1st inter-Q	Lower Q
SHIRLEY	1st inter-Q	Upper-Q	2nd inter-Q	Upper-Q	2nd inter-Q	Upper-Q	Lower Q	Upper-Q
SISSON	1st inter-Q	1st inter-Q	Upper-Q	Upper-Q	1st inter-Q	1st inter-Q	Upper-Q	Upper-Q
SS520	1st inter-Q	Upper-Q	Lower Q	1st inter-Q	Lower Q	1st inter-Q	2nd inter-Q	1st inter-Q
SS5205	1st inter-Q	Upper-Q	Upper-Q	Upper-Q	2nd inter-Q	1st inter-Q	Upper-Q	2nd inter-Q
SSMPV57	Upper-Q	Upper-Q	Lower Q	Upper-Q	1st inter-Q	Upper-Q	Upper-Q	Upper-Q
TRIBUTE	Lower Q	Upper-Q	2nd inter-Q	2nd inter-Q	Upper-Q	1st inter-Q	2nd inter-Q	2nd inter-Q
USG3209	1st inter-Q	Upper-Q	2nd inter-Q	1st inter-Q	2nd inter-Q	Upper-Q	2nd inter-Q	1st inter-Q
USG3315	1st inter-Q	Lower Q	1st inter-Q	Upper-Q	2nd inter-Q	Lower Q	Lower Q	1st inter-Q
USG3555	Upper-Q	Upper-Q	2nd inter-Q	Upper-Q	Upper-Q	Upper-Q	2nd inter-Q	Upper-Q
VA05W-151	Upper-Q	Upper-Q	Upper-Q	Upper-Q	Upper-Q	Upper-Q	Upper-Q	Upper-Q
VA05W-251	1st inter-Q	1st inter-Q	Upper-Q	Upper-Q	Lower Q	Upper-Q	Lower Q	2nd inter-Q
VA06W-412	2nd inter-Q	Upper-Q	Upper-Q	1st inter-Q	1st inter-Q	Upper-Q	Upper-Q	2nd inter-Q
VA07W-415	Upper-Q	Upper-Q	Upper-Q	1st inter-Q	Upper-Q	1st inter-Q	Upper-Q	Lower Q
VA08MAS-369	2nd inter-Q	1st inter-Q	Upper-Q	Upper-Q	Upper-Q	1st inter-Q	1st inter-Q	Upper-Q
VA08W-176	Upper-Q	Upper-Q	1st inter-Q	Upper-Q	Upper-Q	1st inter-Q	Upper-Q	2nd inter-Q
VA08W-294	2nd inter-Q	1st inter-Q	2nd inter-Q	Upper-Q	1st inter-Q	Upper-Q	1st inter-Q	1st inter-Q
VA08W-613	2nd inter-Q	Lower Q	Lower Q	Lower Q	1st inter-Q	Lower Q	2nd inter-Q	Lower Q
VA09W-110	Upper-Q	Upper-Q	Upper-Q	Upper-Q	1st inter-Q	2nd inter-Q	2nd inter-Q	2nd inter-Q
VA09W-112	1st inter-Q	2nd inter-Q	1st inter-Q	1st inter-Q	1st inter-Q	2nd inter-Q	2nd inter-Q	1st inter-Q
VA09W-114	2nd inter-Q	Upper-Q	Upper-Q	1st inter-Q	1st inter-Q	Upper-Q	Upper-Q	2nd inter-Q
VA09W-188WS	Upper-Q	1st inter-Q	Upper-Q	Upper-Q	1st inter-Q	Lower Q	1st inter-Q	2nd inter-Q
VA09W-46	1st inter-Q	1st inter-Q	Upper-Q	1st inter-Q	Lower Q	2nd inter-Q	Upper-Q	2nd inter-Q
VA09W-52	Upper-Q	Upper-Q	Upper-Q	2nd inter-Q	Upper-Q	Upper-Q	Upper-Q	1st inter-Q

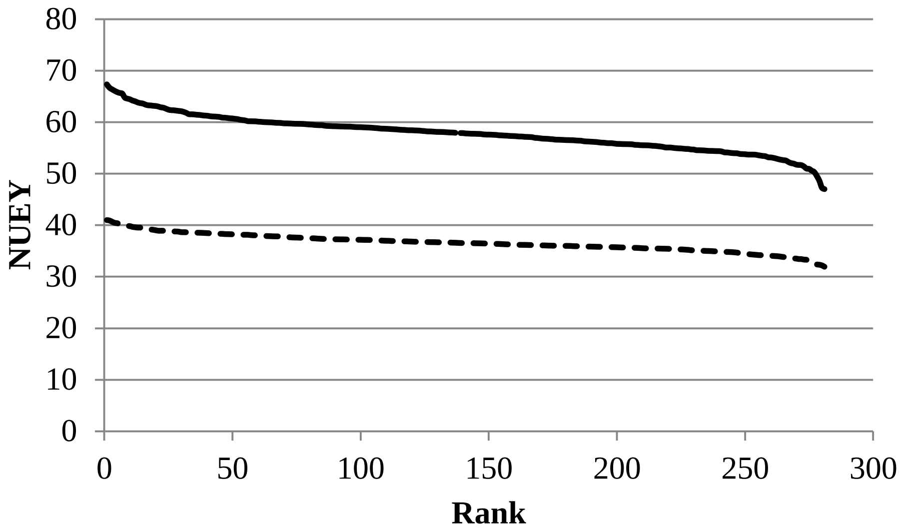
	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
VA09W-69								
VA09W-73								
VA09W-75								
VA10W-119								
VA10W-123								
VA10W-125								
VA10W-140								
VA10W-21								
VA10W-28								
VA10W-663								
VA96W-247								

Table 10. Performance of wheat genotypes at other breeding programs, under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP). Genotypes were ranked and accordingly grouped into Upper-Q (top 25% -), 1st inter-Q (26-50%), 2nd inter Q (51-75% -), and lower Q (76 to 100% -).

		NUEY				**NUEP**			
		Normal N		**Low N**		**Normal N**		**Low N**	
Breeding Program	**Name**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
AR-UAR	Jaypee								
MI-MSU	CRYSTAL								
MI-MSU	D6234								
MI-MSU	D8006								
MI-MSU	E2041								
MI-MSU	E5011								
MI-MSU	E5024								
MI-MSU	E6012								
MI-MSU	REDRUBY								
NY-Cornell	CALEDONIA								
NY-Cornell	CAYUGA								
NY-Cornell	Hopkins								
NY-Cornell	Medina								
NY-Cornell	NY103-208-7263								
NY-Cornell	NY91017-8080								
NY-Cornell	NY96009-3037								
NY-Cornell	NY99066-3444								
Pioneer	PIO 25R26								
Syngenta	BRANSON								

Fig.1. Genotype rankings for 1A) NUEY and 1B) NUEP under low and normal N regimes. Ranking is by average performance over years under each N regime. Solid and broken lines indicate low and normal N conditions, respectively.

1 A)



1 B)

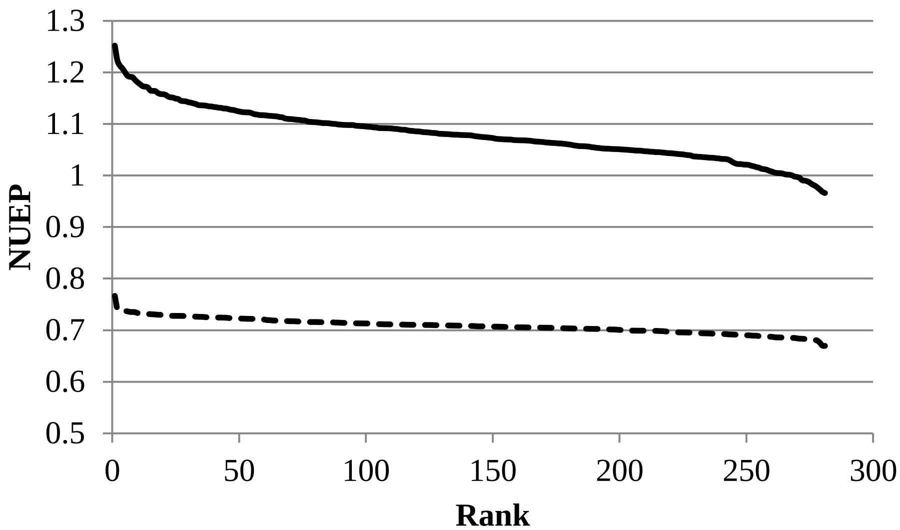
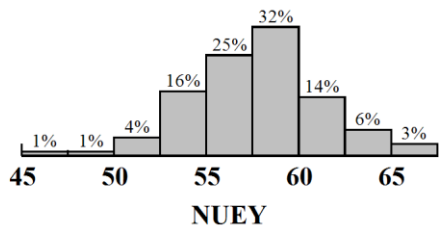


Fig.2. Mean genotype frequency distribution for NUEY under A) low and B) normal N conditions. NUEY bars are labelled with frequency percentages.

2 A)



2 B)

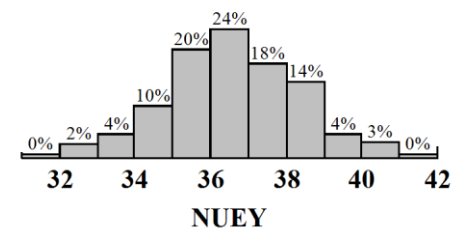
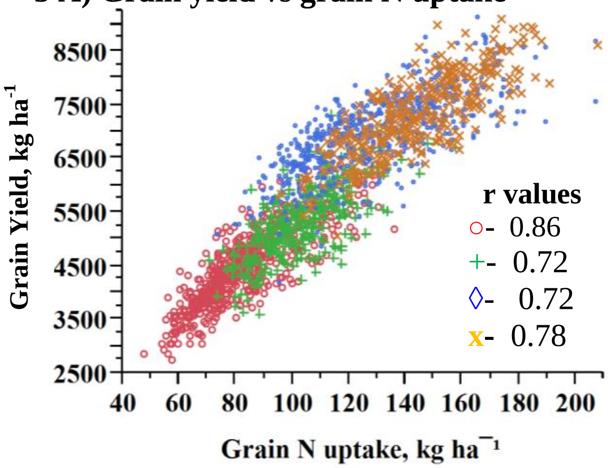
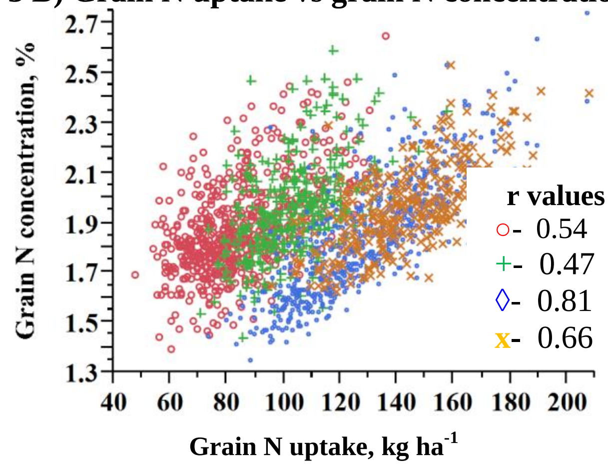


Fig. 3. Relationships between A) grain yield vs N uptake B) grain yield vs grain N concentration and C) grain N uptake vs grain N concentration during 2011-12 under low N (○), and under normal N (+); and during 2012-13 under low N(◊), and under normal N (x) regimes.

3 A) Grain yield vs grain N uptake



3 B) Grain N uptake vs grain N concentration



3 C) Grain yield vs grain N concentration

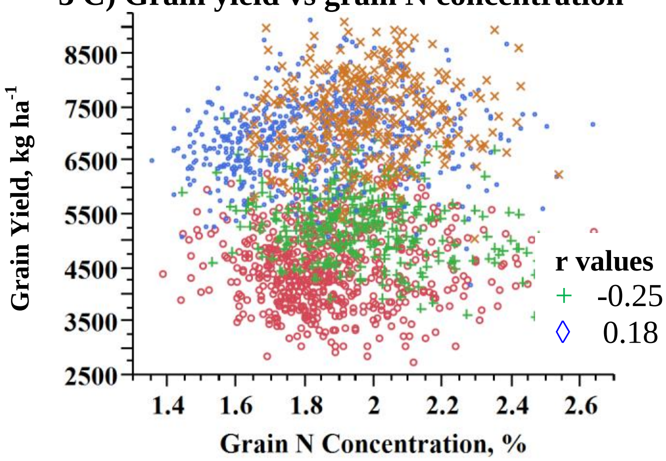


Fig.4. Region wise proportion of the selected responding (in top quartile - normal N conditions) genotypes for A) nitrogen use efficiency for yield (NUEY) and B) nitrogen use efficiency for protein (NUEP).

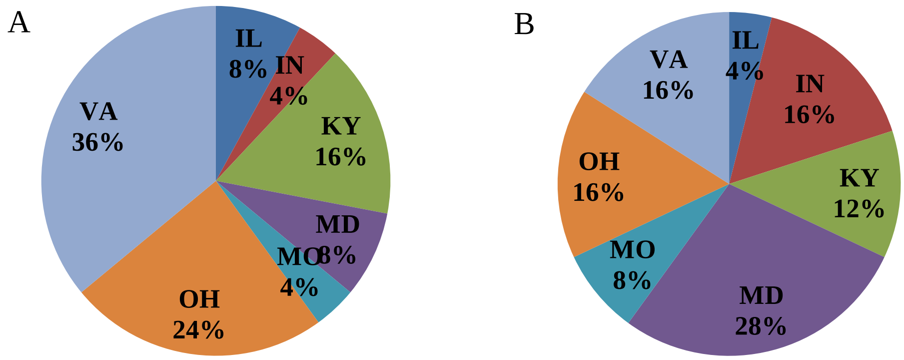


Fig.5. Region wise proportion of the selected efficient (in top quartile- low N conditions) genotypes for A) nitrogen use efficiency for yield (NUEY) and B) nitrogen use efficiency for protein (NUEP).

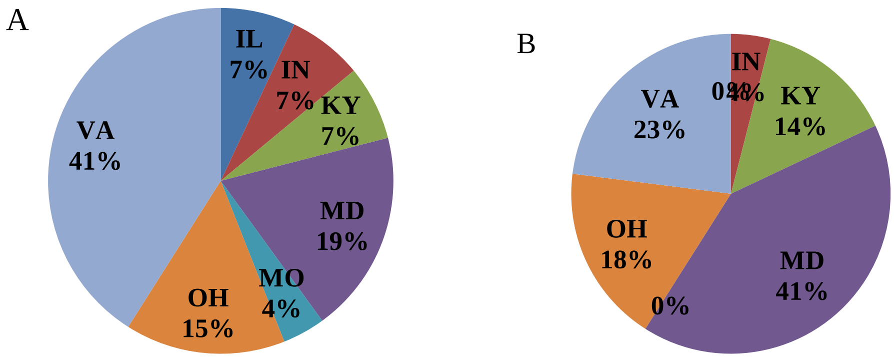


Fig.6. Genotype's nitrogen use efficiency for yield (NUEY) under normal and low N conditions during 2011-12 crop season.

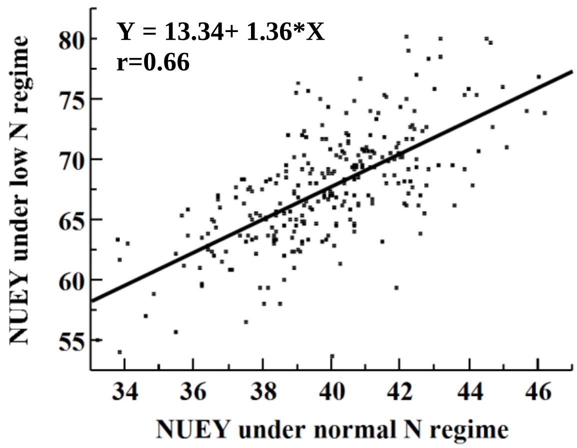
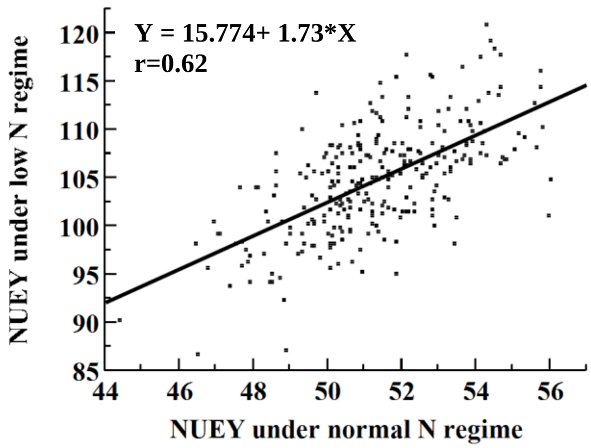


Fig.7. Genotype's nitrogen use efficiency for yield (NUEY) under normal and low N conditions during 2012-13 crop season**.**



Appendix A. Performance of Illnois wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP).

	NUEY				**NUEP**			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON	32.15	44.77	44.02	73.86	0.6223	0.8342	0.822	1.2898
IL00-8109	28.74	41.06	44.06	68.5	0.6232	0.7624	0.8436	1.2304
IL00-8530	30.69	41.19	43.09	73.03	0.6238	0.7798	0.8657	1.3584
IL00-8633	34.46	41.06	50.82	71.77	0.6238	0.7434	0.8731	1.2644
IL01-11934	33.66	41.71	47.81	70.59	0.6238	0.7977	0.8674	1.2857
IL02-18228	31.27	38.98	43.19	69.8	0.6239	0.7685	0.8677	1.3835
IL02-19483B	27.95	44.95	39.38	70.26	0.6235	0.8525	0.8271	1.2989
IL04-24668	31.39	42.92	42.17	67.51	0.6239	0.7965	0.8428	1.2213
IL04-9942	29.63	42.64	41.82	68.92	0.6231	0.8082	0.8153	1.2731
IL05-4236	31.37	43.23	41.74	73.94	0.6235	0.8286	0.8279	1.3153
IL06-13072	34.32	39.7	46.35	67.15	0.6243	0.779	0.8851	1.2517
IL06-13708	31.22	40.21	41.74	70.01	0.6236	0.7566	0.813	1.3297
IL06-13721	32.53	41.78	43.51	71.13	0.6239	0.7988	0.8545	1.2514
IL06-14262	34.05	41.78	46.5	71.2	0.6237	0.7906	0.8354	1.2371
IL06-14303	31.85	42.26	42.35	70.62	0.6238	0.7968	0.8367	1.2563
IL06-14325	34.26	41.88	43.94	67.98	0.6239	0.8086	0.8327	1.2843
IL06-23571	32.28	40.06	41.07	67.27	0.6239	0.7681	0.8212	1.265
IL06-31053	31.13	40.59	43.55	72.38	0.6235	0.7802	0.8394	1.2791
IL06-7550	37.06	40.66	49.45	70.13	0.6238	0.7552	0.8425	1.2531
IL06-7653	31.3	41.07	51.1	70.31	0.6239	0.7873	0.8942	1.2965
IL07-12948	30.99	42.64	43.26	70.6	0.6239	0.803	0.8371	1.3299
IL07-16075	30.77	38.2	45.53	69.59	0.6235	0.7157	0.8486	1.1716
IL07-19334	36.07	42.37	50.87	72.77	0.624	0.82	0.8746	1.3557
IL07-20728	33.73	40.78	47.25	72.6	0.6237	0.7744	0.8674	1.3163
IL07-20743	32.93	40.33	44.94	64.39	0.6239	0.8072	0.8593	1.2213
IL07-21847	32.6	42.02	45.79	70.94	0.6237	0.7737	0.8553	1.2756
IL07-23420	31.93	39.82	45.85	70.83	0.6235	0.7466	0.856	1.3142
IL07-24841	34.03	42.39	49.55	68.87	0.6236	0.7985	0.8731	1.2431
IL07-4415	32.14	40.34	42.13	70.14	0.6236	0.751	0.7909	1.2735
IL07-6861	33.92	41.84	45.62	71.17	0.6242	0.8073	0.8811	1.3191

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
IL08-12174	29.95	40.54	42.69	69.27	0.6235	0.755	0.823	1.3042
IL08-12206	30.11	40.83	42.36	68.14	0.6231	0.759	0.795	1.1937
IL08-22075	31.28	39.97	41.85	64.75	0.6239	0.7847	0.8392	1.2433
IL08-31639	32.08	43.41	45.01	74.13	0.6238	0.7989	0.8339	1.2855
IL08-33373	32.04	40.26	44.84	69.78	0.6241	0.7834	0.8394	1.2976
IL08-33951	30.12	40.63	42.71	64.55	0.6237	0.7912	0.7838	1.2106
IL08-34020	32.94	41.35	47.39	71.96	0.6239	0.7729	0.8951	1.3241
IL08-9266	28.67	40.72	47.39	71.96	0.6232	0.8116	0.8951	1.3241
IL99-26442	33.76	37.54	45.72	64.06	0.6238	0.7484	0.8324	1.2666

Appendix B. Performance of Indiana wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP).

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON	32.15	44.77	44.02	73.86	0.6223	0.8342	0.822	1.2898
91193	29.44	39.79	44.67	65.66	0.6235	0.7644	0.8623	1.2573
92201	27.75	39.18	38.19	61.83	0.6233	0.7557	0.7986	1.162
011007A1-14-16-50	30.04	40.97	45.79	71.82	0.6236	0.7826	0.8637	1.321
0175A1-37-4-1	30.74	40.89	42.71	63.77	0.6235	0.7875	0.857	1.1952
02444A1-23-1-3	32.14	42.4	46.2	68.05	0.6238	0.7936	0.864	1.2528
03207A1-7-3-1	32.2	41.98	47.08	67.95	0.6237	0.8345	0.8495	1.2542
03549A1-18-25	30.98	39.02	41.49	72.04	0.6239	0.7815	0.8551	1.417
03633A1-69-2-5	29.31	38.87	43.53	63.03	0.6235	0.7867	0.8553	1.2605
04606RA1-1-7-1	33.13	41.37	49.1	66.01	0.6235	0.7969	0.8711	1.2201
04606RA1-1-7-1-6	32.79	40.49	47.46	68.07	0.6237	0.7817	0.8733	1.2557
04620A1-1-7-4	31.19	39.77	45.11	67.44	0.6236	0.7478	0.8733	1.1775
04702A1-18	32.97	40.61	46.5	66.66	0.6238	0.7793	0.8488	1.2014
04719A1-16-1-1-7	31.64	41.22	44.13	66.57	0.6234	0.7973	0.8007	1.2743
0513A1-1-3	32.9	41.01	47.62	66.42	0.624	0.807	0.8869	1.2573
05219A1-8-21-2-4	31.86	41.07	46.08	67.12	0.6239	0.8109	0.8624	1.3049
05222A1-1-2-1	31.14	40.44	42.49	66.64	0.6235	0.7848	0.828	1.2581
05247A1-7-3-120	30.18	42.38	45.28	67.74	0.6236	0.8015	0.835	1.1991
05247A1-7-3-27	33.43	41.9	47.26	69.64	0.6239	0.8261	0.8841	1.2613
05247A1-7-7-3-1	34.04	40.6	46.92	69.39	0.6238	0.7971	0.8568	1.2787
05251A1-1-136-9-5	30.81	40.79	46.03	73.25	0.6231	0.7735	0.8672	1.3713
05264A1-1-3-2	31.47	41.29	48.11	67.81	0.6232	0.7868	0.8697	1.2759
05287A1-1-13	32.99	41.45	47.05	70.38	0.6237	0.8071	0.8911	1.3673
0537A1-12	33.78	40.89	44.44	78.81	0.6239	0.7884	0.8547	1.4552
0537A1-3-12	32.61	41.75	44.88	72.68	0.6238	0.7873	0.8681	1.281
0566A1-3-1-65	32.96	40.19	48.27	72.52	0.6239	0.7578	0.8992	1.2889
0566A1-3-1-67	33.37	43.12	50.39	72.71	0.6239	0.8115	0.9519	1.282
0570A1-2-39-5	30.96	39.63	43.94	70.38	0.6235	0.7821	0.8528	1.2936
06403A1-4	33.34	41.44	46.01	70.78	0.624	0.8019	0.8679	1.3861
0722A1-1-7	30.61	41.61	39.77	63.66	0.6235	0.7754	0.797	1.1987

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
07287RA1-14	28.45	40.94	41.64	69.17	0.6235	0.7945	0.8063	1.3241
07290A1-12	32.17	39.81	44.26	67.34	0.6236	0.7615	0.8236	1.2603
0762A1-2-8	33.1	42.09	47.24	72.16	0.6239	0.7961	0.8965	1.2594
9346A1-2-5-5-2-1	28.44	38.93	37.33	63.12	0.6239	0.7648	0.8116	1.274
Clark	30.4	41.68	43.83	67.59	0.6236	0.7819	0.8471	1.237
INW0411	30.32	40.29	42.5	68.52	0.6239	0.7996	0.8524	1.3039
INW0412	30.3	41.61	41.67	65.83	0.6241	0.8576	0.8544	1.2987
INW1021	31.5	39.55	44.6	73.63	0.6237	0.7512	0.8404	1.3018

Appendix C. Performance of Kentucky wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP).

	NUEY				**NUEP**			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON	32.15	44.77	44.02	73.86	0.6223	0.8342	0.822	1.2898
ALLEGIANCE	33.86	43.39	46.34	75.59	0.6238	0.8261	0.8521	1.3748
FOSTER	†NA	50.15	NA	98.06	NA	0.9731	NA	1.816
KY02C-1058-03	32.01	40.37	46.1	70.7	0.624	0.7677	0.8798	1.3083
KY02C-1076-07	32.82	43.11	46.48	75.14	0.6239	0.8374	0.892	1.3795
KY02C-1121-11	26.64	38.89	36.82	63.62	0.6235	0.79	0.81	1.2554
KY02C-1121-75	33.17	41.32	49.42	71.06	0.6237	0.7747	0.9082	1.2392
KY02C-1122-06	29.2	38.6	42.8	69.7	0.6235	0.7599	0.8616	1.3188
KY02C-2215-02	33.74	42.14	47.04	74.3	0.6242	0.8253	0.8737	1.3377
KY02C-3004-07	30.09	38.01	37.8	62.86	0.6237	0.7621	0.7918	1.2111
KY02C-3005-25	32.61	42.45	43.37	66.94	0.6237	0.7949	0.8261	1.242
KY03C-1002-02	32.12	44.76	44.88	77.7	0.6237	0.8357	0.8578	1.4173
KY03C-1192-37	32.69	41.88	45.85	72.27	0.6236	0.8038	0.8471	1.3472
KY03C-1195-10-1-5	30.17	39.49	43.76	65.11	0.6234	0.7861	0.8705	1.1906
KY03C-1221-01	29.34	43.22	41.59	71.66	0.6237	0.8214	0.8597	1.2586
KY03C-1221-06	28.63	40.62	41.03	71.11	0.6236	0.7978	0.8511	1.3189
KY03C-1221-22	31.02	40.67	46.28	71.97	0.6239	0.7939	0.9054	1.2978
KY03C-1237-01	33.35	40.16	45.52	64.11	0.6239	0.7895	0.8415	1.2224
KY03C-1237-15	30.8	42.11	42.92	73.87	0.6236	0.8201	0.8364	1.4274
KY03C-1237-32	32.46	44.74	48.19	76.6	0.6237	0.8249	0.8856	1.3695
KY03C-2047-02	32.27	43.89	47.64	71.71	0.6239	0.8322	0.8745	1.2579
KY03C-2047-06	30.23	41.79	42.39	71.84	0.6237	0.7811	0.8423	1.3448
KY03C-2049-02	30.81	38.24	42.42	64.28	0.6239	0.7899	0.8833	1.2975
KY03C-2314-08	33.97	42.39	47.98	77.32	0.6237	0.7857	0.8521	1.3492
KY03C-2399-02	32.01	40.34	42.7	71.11	0.6236	0.7827	0.8332	1.2986
KY04C-1128-38-1-5	36.61	43.22	49.61	71.37	0.6243	0.8046	0.8951	1.3324
KY04C-2006-41-1-1	33.98	40.71	45.33	71.07	0.6236	0.7927	0.8222	1.3325
KY04C-2151-40	29.33	39.62	43.39	65.77	0.6235	0.7985	0.854	1.2509
KY04C-2151-41	27.11	37.78	41.28	66.43	0.6235	0.7279	0.8419	1.2498
KY04C-3006-33-14-3	30.46	41.37	45.24	72.35	0.6238	0.8245	0.9293	1.4296

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
KY05C-1007-2-12-5	32.42	40.15	46.86	70.72	0.6234	0.7612	0.8583	1.3072
KY05C-1105-42-20-1	29.07	41.52	42.6	72.23	0.6235	0.7942	0.8305	1.3534
KY05C-1381-77-7-5	30.53	41.34	43.66	68.73	0.6234	0.7957	0.8273	1.2944
KY05C-1617-17-17-3	31.42	40.16	42.37	68.99	0.6236	0.8129	0.8303	1.3627
KY06C-1003-139-8-3	33.55	42.67	46.91	72.34	0.6242	0.7863	0.8979	1.2977
KY93C-1238-17-1	31.88	39.99	48.79	70.67	0.6234	0.74	0.8811	1.2668
PEMBROKE	32.63	41.02	43.24	75.54	0.624	0.7618	0.8439	1.3657

†NA Data unavailable due to poor seed germination.

Appendix D. Performance of Maryland wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP).

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON	32.15	44.77	44.02	73.86	0.6223	0.8342	0.822	1.2898
CATOCIN	29.75	38.39	40.77	64.49	0.6237	0.7911	0.875	1.3003
CHESAPEAKE	30.55	40.12	43.5	70.77	0.6237	0.7888	0.852	1.3128
MD01W270-10-3	31.88	41.86	48.01	75.08	0.6235	0.8257	0.8894	1.3159
MD03W104-10-2	29.84	41.55	45.3	72.76	0.6238	0.8462	0.9029	1.443
MD03W151-10-12	34.64	40.82	52.57	72.58	0.6237	0.7756	0.9051	1.3389
MD03W485-10-10	31.57	41.99	46.09	68.49	0.624	0.8422	0.9352	1.3353
MD03W485-10-12	29.42	40.8	44.93	69.93	0.6235	0.8231	0.8958	1.3655
MD03W485-10-2	34.28	43.54	48.74	73.5	0.6241	0.8486	0.8923	1.2828
MD03W485-10-8	32.31	43.06	47.36	77.98	0.6236	0.8502	0.8651	1.4489
MD03W61-11-2	34.32	40.45	52.46	68.58	0.624	0.7671	0.9187	1.25
MD03W61-11-3	32.04	42.16	47.49	70.5	0.6239	0.7953	0.8794	1.3466
MD03W64-10-3	28.61	41.07	43.82	67.98	0.6239	0.8109	0.902	1.3332
MD03W665-10-3	32.26	40.99	47	68.77	0.6235	0.7876	0.8851	1.2724
MD03W665-10-5	35.86	43.75	48.69	79.25	0.6237	0.8413	0.8526	1.4362
MD04W1197-11-13	31.39	41.42	43.47	68.31	0.6236	0.8216	0.8553	1.3469
MD04W249-11-12	31.65	40.35	46.37	67.82	0.6239	0.8026	0.9047	1.4081
MD04W249-11-13	31.55	39.52	42.47	70.58	0.6239	0.7995	0.8382	1.3025
MD04W249-11-5	31.02	43.46	45.88	72.77	0.6234	0.8076	0.8566	1.2745
MD04W249-11-7	32.37	43.63	44.54	79.87	0.6239	0.8278	0.8901	1.4828
MD04W359-11-10	31.19	41.93	42.92	72.35	0.6237	0.8287	0.8037	1.3286
MD04W8-11-4	31.73	41.17	45.6	72.72	0.6234	0.7535	0.8306	1.1831
MD05W10208-11-13	34.6	40.74	46.55	69.81	0.6245	0.7919	0.9364	1.325
MD05W10208-11-14	32.21	40.82	43.25	72.32	0.6241	0.8063	0.8902	1.4288
MD05W10208-11-3	31	40.03	46.58	71.73	0.6237	0.7654	0.9076	1.2851
MD05W10208-11-6	31.42	39.29	47.12	66.47	0.6243	0.7673	0.9278	1.2388
MD05W10208-11-7	31.43	40.57	44.33	68.79	0.6243	0.7893	0.9058	1.3076
MD05W10208-11-8	31.46	40.21	43.3	72.52	0.6239	0.7925	0.8971	1.4543
MD05W1292-11-1	32.39	38.43	47.18	64.9	0.6235	0.7465	0.8547	1.2621
MD05W1292-11-4	29.18	43.88	42.15	78.81	0.6238	0.8197	0.8719	1.4948

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
MD05W1317-11-4	29.17	40.7	41.9	74.08	0.6238	0.8099	0.8758	1.4789
MD05W479-B-11-3	30.24	41.16	45.16	67.03	0.624	0.8243	0.9271	1.3556
MD07W272-11-5	34.18	41.82	44.9	68.9	0.624	0.8313	0.851	1.3297
MD07W419UM5-11-11	29.38	40.19	43.14	68.17	0.6239	0.793	0.8971	1.2351
MD07W419UM5-11-12	29.48	37.76	44.25	66.48	0.6238	0.7398	0.9101	1.3564
MD665-09-6	33.29	41.32	42.3	68.38	0.6243	0.8314	0.8516	1.3185
MD665-09-6	31.1	40.6	44.01	68.7	0.6235	0.797	0.8785	1.3134

Appendix E. Performance of Missouri wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP).

	NUEY				**NUEP**			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON	32.15	44.77	44.02	73.86	0.6223	0.8342	0.822	1.2898
BESS	30.51	41.69	38.86	70.57	0.6234	0.8077	0.7957	1.1819
ERNIE	30.43	39.28	39.75	66.8	0.6237	0.7523	0.8078	1.2774
Milton	33.89	41.52	45	70.49	0.6243	0.8055	0.8886	1.3218
MO050921	32.47	40.55	47.86	67.57	0.6235	0.7681	0.8708	1.2543
MO080103	30.66	42.11	45.77	72.04	0.6238	0.8276	0.8892	1.2818
MO080104	32.73	43.94	46.81	71.64	0.6237	0.8465	0.9058	1.3116
MO080584	31.59	44.93	45.61	67.71	0.6234	0.8456	0.8223	1.1689
MO080589	31.2	42.86	44.99	65.78	0.6236	0.8298	0.8447	1.2118
MO080864	32.65	40.34	46.4	72.48	0.6235	0.7639	0.8522	1.2491
MO081163	34.16	44.4	42.79	73.11	0.6239	0.8106	0.8073	1.3647
MO081280	30.99	40.38	40.24	66.8	0.6238	0.7727	0.8007	1.1981
MO081537	31.26	42.19	43.53	70.3	0.6235	0.7818	0.836	1.3163
MO081559	32.41	42.74	48.41	68.76	0.6237	0.8315	0.8543	1.22
MO081652	31.31	41.9	45.42	71.4	0.6235	0.8252	0.8704	1.3512
MO081699	31.41	42.6	43.73	72.38	0.6239	0.822	0.8721	1.3443
MO090574	32.38	41.78	45.81	71.45	0.6239	0.8293	0.8943	1.2648
MO090581	30.61	40.83	42.62	74.2	0.6236	0.7883	0.8392	1.3243
MO090821	28.74	38.43	42.7	63.02	0.6234	0.7485	0.8615	1.2422
MO091011	31.74	41.84	43.31	68	0.6238	0.8025	0.8295	1.2822
MO091159	32.5	37.25	44.15	65.69	0.6235	0.7438	0.8253	1.2144
MO100172	32.33	44.18	44.32	72.36	0.6237	0.8137	0.8473	1.254
MO100231	34.18	40.09	48.47	72.05	0.6239	0.7567	0.8701	1.3157
MO100265	32.68	40.11	46.52	66.43	0.6238	0.7831	0.8611	1.2634
MO100519	29.08	39.22	40.01	65.68	0.6237	0.7839	0.8082	1.3159
MO100535	32.01	40.88	46.32	70.17	0.6236	0.7482	0.8668	1.3553
MO100539	34.91	40.45	46.52	72.04	0.6235	0.7758	0.8353	1.2016
MO100647	35.48	40.24	50.46	71.31	0.6238	0.7745	0.8588	1.3932
MO100745	30.05	41.31	44.41	70.41	0.6235	0.7741	0.8644	1.208
MO101142	30.74	38.37	42.49	65.32	0.6233	0.7409	0.7916	1.2818

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
MO101202	31.76	39.86	44.56	68.83	0.6233	0.7449	0.8004	1.2041
MO101207	31.57	40.45	44.18	66.01	0.6233	0.8047	0.8154	1.2584
MO101278	33.05	41.82	47.35	74.59	0.6239	0.7743	0.8972	1.3067
MO101329	31.23	39.13	47.05	67.28	0.6235	0.7482	0.8335	1.2489
MO101358	33.94	39.83	48.66	67.16	0.6236	0.7364	0.8302	1.1545
MO101361	33.3	41.41	48.17	70.21	0.6235	0.7932	0.8563	1.2996
MO101571	27.14	40.06	36.22	67.75	0.6238	0.7717	0.7877	1.263
TRUMAN	33.29	40.8	50.42	70	0.6237	0.7513	0.8583	1.1832

Appendix F. Performance of Ohio wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP).

	NUEY				**NUEP**			
	Normal N		**Low N**		**Normal N**		**Low N**	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON	32.15	44.77	44.02	73.86	0.6223	0.8342	0.822	1.2898
Becker	31.74	40.28	45.54	71.72	0.6238	0.8077	0.8744	1.3692
BROMFIELD	32.68	40.4	44.41	68.79	0.6239	0.7782	0.868	1.3374
MALABAR	32.62	39.71	50.13	71.25	0.624	0.7662	0.8717	1.304
OH05-200-74	31.46	40.77	48.49	72.78	0.6237	0.7842	0.8827	1.34
OH06-150-57	31.18	40.27	40.88	66.92	0.6236	0.764	0.823	1.2367
OH06-180-57	30.9	43.26	44.42	72.92	0.6236	0.8117	0.8382	1.3304
OH07-166-41	32.17	38.57	44.76	69.59	0.6236	0.7476	0.8471	1.3222
OH07-166-49	29.69	40.41	40.72	68.44	0.6237	0.7903	0.8293	1.3362
OH07-174-11	33.1	42.78	46.48	71	0.6239	0.8152	0.8737	1.2682
OH07-238-15	33.81	37.63	48.03	67.26	0.6237	0.7168	0.8658	1.2401
OH07-254-11	35.2	41.19	46.38	70.37	0.6248	0.8077	0.8732	1.3874
OH07-263-3	35.78	42.63	53.39	71.8	0.624	0.7743	0.9143	1.2608
OH07-94-70	29.07	39.57	39.87	65.12	0.6237	0.8023	0.8254	1.3196
OH07-95-7	29.28	38.72	41.75	65.1	0.6237	0.7679	0.8453	1.3859
OH07-98-21	30.3	42.18	43.88	71.27	0.6235	0.7952	0.8325	1.3738
OH08-101-57	31.38	38.96	46.34	69.15	0.6239	0.7594	0.8594	1.3679
OH08-101-72	33.04	38.14	44.67	65.76	0.6234	0.7493	0.7936	1.2011
OH08-107-16	27.32	39.1	42.26	63.41	0.6232	0.793	0.8293	1.2053
OH08-133-25	32.09	42.54	46.1	73.98	0.6237	0.838	0.8432	1.3849
OH08-141-6	32.01	38.83	44.68	67.26	0.6242	0.7705	0.8555	1.2733
OH08-149-11	33.85	42.67	44.32	73.63	0.6238	0.8093	0.8855	1.3271
OH08-161-4	33.69	42.99	46.96	74.31	0.6241	0.8248	0.882	1.3382
OH08-161-78	35.68	41.72	53.56	71.85	0.6236	0.8134	0.9111	1.3243
OH08-170-66	31.26	39.76	50.61	69.05	0.6233	0.7669	0.8629	1.2869
OH08-172-42	31.94	41.19	48.02	74.84	0.6235	0.7589	0.8563	1.2408
OH08-178-52	35.2	41.42	50.44	69.7	0.6233	0.7811	0.8679	1.2189
OH08-180-48	33.96	41.6	48.82	77.27	0.6241	0.7789	0.9176	1.36
OH08-182-4	31.91	40.83	44.83	65.3	0.6235	0.7898	0.8707	1.2613
OH08-199-1	31.29	41.04	45.49	69.9	0.6239	0.7965	0.8809	1.2411

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
OH08-206-19	32.62	42.72	46.6	75.07	0.6235	0.8152	0.8796	1.3512
OH08-207-33	29.85	41.76	44.75	67.97	0.6237	0.7965	0.8785	1.3243
OH08-234-4	35.51	43.44	47.4	71.97	0.6243	0.8206	0.8663	1.3716
OH08-235-33	33.66	43.55	45.62	71.93	0.6241	0.8231	0.8432	1.3273
OH08-246-15	31.9	39.59	45.16	68.25	0.6235	0.7741	0.8613	1.3366
OH08-254-22	31.42	42.34	43.42	77.5	0.6238	0.8272	0.8587	1.5438
OH08-256-47	30.77	39.01	43.73	70.82	0.6236	0.7794	0.8717	1.3245
OH08-265-37	32.6	40.21	42.91	66.28	0.6239	0.788	0.8208	1.2397
OH08-269-58	33.81	43.84	50.22	76.09	0.6239	0.7902	0.9216	1.3488
OH08-98-13	34.32	40.77	45.47	69.2	0.6239	0.7884	0.8409	1.2696

Appendix G. Performance of Virginia wheat genotypes under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP).

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
BRANSON	32.15	44.77	44.02	73.86	0.6223	0.8342	0.822	1.2898
JAMESTOWN	33.66	41.09	47.18	67.75	0.624	0.8313	0.866	1.3492
MASSEY	29.52	40.18	41.22	65.81	0.6235	0.8351	0.8348	1.376
MERL	34.03	44.24	48.59	73.46	0.6239	0.8004	0.885	1.2987
ROANE	31.97	40.19	42.48	65.42	0.6238	0.7982	0.8644	1.1969
SHIRLEY	32.69	43.85	44.31	76.61	0.6236	0.8384	0.8368	1.3753
SISSON	32.76	41.26	51.4	76.85	0.6238	0.7967	0.9436	1.3978
SS520	32.09	42.46	35.99	71.27	0.6235	0.7989	0.8476	1.3066
SS5205	32.49	43.42	47.76	74.11	0.6235	0.8055	0.8819	1.2845
SSMPV57	33.59	42.78	39.72	75.97	0.6238	0.8401	0.8844	1.3971
TRIBUTE	30.22	42.47	43.55	69.84	0.6239	0.8079	0.8422	1.2908
USG3209	32.43	43.08	44.98	71.92	0.6235	0.8286	0.8552	1.3002
USG3315	32.36	39.85	45.53	76.26	0.6235	0.769	0.8193	1.3208
USG3555	33.77	42.51	44.4	74.98	0.6241	0.8198	0.8498	1.4099
VA05W-151	35.28	43.45	50.85	78.67	0.6243	0.841	0.9606	1.3628
VA05W-251	32.87	41.27	47.3	74.54	0.6235	0.8186	0.8311	1.2823
VA06W-412	31.53	44	50.69	71.56	0.6237	0.831	0.9168	1.291
VA07W-415	33.82	43.03	53.75	71.61	0.624	0.7962	0.9052	1.2466
VA08MAS-369	31.06	41.8	48.26	78.83	0.6239	0.8068	0.8764	1.4173
VA08W-176	33.42	43.26	46.82	73.55	0.624	0.8034	0.8993	1.2711
VA08W-294	31.3	41.21	44.76	73.31	0.6238	0.818	0.873	1.335
VA08W-613	30.86	39.58	38.86	66.47	0.6238	0.76	0.8422	1.2245
VA09W-110	36.19	43.17	47.59	74.29	0.6237	0.7863	0.8496	1.256
VA09W-112	32.71	40.98	47.15	71.9	0.6238	0.7867	0.8525	1.3026
VA09W-114	31.79	43.87	50.22	71.33	0.6237	0.8354	0.9158	1.2554
VA09W-188WS	33.58	41.29	47.59	75.93	0.6238	0.7634	0.8672	1.26
VA09W-46	32.44	41.27	49.46	71.46	0.6235	0.7858	0.8971	1.2613
VA09W-52	36.92	42.43	51.48	69.35	0.6242	0.8172	0.8898	1.3315
VA09W-69	33.05	40.18	44.49	69.65	0.624	0.7773	0.8212	1.278
VA09W-73	31.9	40.43	44.86	73.97	0.6236	0.7628	0.8581	1.3466

	NUEY				NUEP			
	Normal N		Low N		Normal N		Low N	
Name	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
VA09W-75	34.96	41.86	44.28	75.9	0.6238	0.8041	0.8499	1.4074
VA10W-119	34.04	40.41	51.6	72.65	0.6239	0.7813	0.8995	1.3476
VA10W-123	34.62	44.61	53.57	75.47	0.6236	0.7832	0.9106	1.2801
VA10W-125	32	42.36	49.76	71.03	0.6237	0.7941	0.8906	1.2806
VA10W-140	29.02	43.14	40.91	72.63	0.6232	0.7843	0.8145	1.2849
VA10W-21	29.99	43.54	45.73	80.97	0.6236	0.8119	0.8646	1.3922
VA10W-28	33.01	41.32	49.59	74.22	0.6236	0.8015	0.897	1.4575
VA10W-663	30.14	40.59	45.22	66.6	0.6235	0.8029	0.8354	1.2666
VA96W-247	34.12	41.1	44.29	74.94	0.624	0.798	0.8603	1.4656

Appendix H. Performance of genotypes from other breeding programs, under both normal and low N conditions during 2012 and 2013 crop seasons for nitrogen use efficiency for yield (NUEY) and protein (NUEP).

		NUEY				**NUEP**			
		Normal N		**Low N**		**Normal N**		**Low N**	
Breeding Program	**Name**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
AR-UAR	Jaypee	32.15	44.77	44.02	73.86	0.6223	0.8342	0.822	1.2898
MI-MSU	CRYSTAL	32.21	40.91	43.28	68.69	0.6237	0.7948	0.83	1.3324
MI-MSU	D6234	32.6	40.54	46.37	67.75	0.6237	0.7575	0.863	1.2577
MI-MSU	D8006	30.76	41.23	43.95	70.2	0.6237	0.8022	0.853	1.2997
MI-MSU	E2041	33.28	42.9	47.04	71.49	0.6239	0.7746	0.8581	1.2659
MI-MSU	E5011	30.73	44.65	42.16	72.4	0.624	0.9084	0.8662	1.4008
MI-MSU	E5024	33.87	42.54	52.91	71.82	0.6235	0.7954	0.8677	1.3105
MI-MSU	E6012	33.7	42.76	49.82	70.71	0.6242	0.8499	0.9025	1.3115
MI-MSU	REDRUBY	32.06	43.61	45.16	76.15	0.6234	0.8367	0.8597	1.3806
NY-Cornell	CALEDONIA	32.57	42.85	46.44	72.18	0.6241	0.8173	0.8967	1.4079
NY-Cornell	CAYUGA	35.37	41.58	45.42	68.14	0.6238	0.7857	0.8048	1.2475
NY-Cornell	Hopkins	32.78	39.85	46.54	65.56	0.6243	0.8209	0.9126	1.2978
NY-Cornell	Medina	33.55	38.24	45.96	65.87	0.6235	0.7512	0.8418	1.2671
NY-Cornell	NY103-208-7263	31.18	35.59	41.76	60.46	0.6234	0.7169	0.8049	1.2394
NY-Cornell	NY91017-8080	31.53	40.43	46.68	65.97	0.6232	0.7764	0.8366	1.2094
NY-Cornell	NY96009-3037	27.08	39.21	42.41	58.33	0.6231	0.7676	0.8612	1.1482
NY-Cornell	NY99066-3444	31.47	38.77	48.14	68.03	0.6237	0.7385	0.8716	1.2456
Pioneer	PIO 25R26	32.44	37.32	45	58.05	0.6237	0.7426	0.8276	1.1488
Syngenta	BRANSON	30.55	40.82	45.64	71.42	0.6235	0.7739	0.8419	1.2912