Published online November 7, 2017

Interaction of Genetics, Environment, and Management in Determining Soft Red Winter Wheat Yields

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ABSTRACT

The complex interaction of genetics, environment, and management in determination of crop yields can interfere with selection progress in breeding programs. Specifically, the impact on selection for nitrogen use efficiency (NUE) in soft red winter (SRW) wheat (*Triticum aestivum* L.) can be confounded by these interactions. We utilized a multi-environment trial in Lexington and Princeton, KY, from 2013 to 2015 to assess variation in traits associated with NUE based on interactions of genotype × environment × management (G×E×M). The NUE traits were measured on 10 genotypes under three management levels and four levels of N fertility. Genotype and genotype × environment interactions were significant for NUE traits ($p < 0.001$) but no genotype × N rate interactions were significant. Reduced N rates had no negative effect on grain yield for any genotype. Incremental application of N rates increased yield and postanthesis N uptake significantly. The utility of incorporating management treatments into breeding programs, specifically geared to low-input systems, could help drive progress for development of increased NUE in wheat cultivars.

Core Ideas

- Reducing N rates did not have significantly reduce wheat yields.
- Incremental application of N increased wheat yields over conventional application.
- Earlier maturing wheat genotypes had greater postanthesis N uptake than later lines.

Published in Agron. J. 109:2463–2473 (2017)
doi:10.2134/agronj2017.02.0126

Coupled with the challenge of increasing yields to meet global food demands is the need to produce crops in a sustainable manner while maintaining end use quality and value. To keep up with population demands it is estimated that cereal crop production must increase by 940 million Mg by 2050 (Hatfield and Walthall, 2015). Yields of major commodity crops have increased over time due to three major factors: improved genetics, improved management, and environmental adaptations. Current annual yield gains in wheat are estimated at roughly 1% and will not be sufficient to meet the 38% increase needed per population projections (Ray et al., 2013; Hatfield and Walthall, 2015). Fischer (2009) provided an analysis of historical Australian wheat yields over 100 yr and found average wheat yield has increased at a rate of 1.3% annually. He attributed 0.2% of the annual yield gains to the environmental components, 0.5% to genetics and the interaction of genetics with management, and 0.6% to management alone. The environmental factors are primarily associated with increases in carbon dioxide; the management component is primarily a response to fertilizer. When extrapolated to total yield increases over time genetics contribute about 30%, environment about 15%, and management about 55% to overall yields (Porter et al., 2012; Fischer, 2009).

Selection of high yielding wheat varieties is complicated by the inherent variability of the environment. Plant breeders generally measure genotypic performance across multiple environments to understand the stability of traits including yield. Hammond (1947) suggested that selection progress can be attained more quickly when the environment is favorable for expression of a particular trait. Genotypic performance in breeding programs is typically characterized under high-input management systems with yield being the primary criterion for selection. However, most geneticists recognize that genotypic performance in a favorable environment would not translate to improved performance in an unfavorable environment unless the two environments were highly correlated, and performance was heritable in both environments (Falconer and Latyszewsky, 1952). Thus, modern varieties bred for high-input production systems may not perform optimally under low-input conditions (Fess et al., 2011).

Varietal improvements and implementation of intensive management has greatly improved SRW wheat yields in the

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Abbreviations: G×E×M, genotype × environment × management; NDVI, normalized difference vegetation index; NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NutE, nitrogen utilization efficiency; PANU, postanthesis nitrogen uptake; REMN, nitrogen remobilized at maturity; SRW, soft red winter.

southeastern United States (Lee et al., 2009). With the adoption of high-input management systems there are increasing concerns for regulatory issues associated with nitrate in ground water (van Grinsven et al., 2015). Since 1960, the application of N fertilizers has increased sevenfold, with human inputs equaling the natural inputs to the global N cycle. Additionally, more than half of the N fertilizers applied to crop production are entering non-agricultural ecosystems (Tilman, 1998). Thus, it is increasingly important to understand response to low-input systems.

Nitrogen use efficiency has been defined as the ratio of grain yield to the supply of available N from both soil and fertilizer sources (Moll et al.,1982). A large amount of variation is known to exist among genotypes for NUE which is directly linked to grain yield potential. In theory, by breeding for yield, we are inadvertently breeding for NUE. The components of NUE include: nitrogen uptake efficiency (NUpE), the ability to remove N from the soil; and nitrogen utilization efficiency (NutE), the ability to remobilize N to yield (Moll et al., 1982, Li et al., 2015). Several authors have found that more variation in grain yield is attributed to NUtE than NUpE across multiple N rates. Barraclough et al. (2010) reported this across 39 varieties and five N rates. Gaju et al. (2011) found that NUtE was the most important component of NUE to explain phenotypic variation in 16 genotypes in two locations. Gaju et al. (2014) attribute 32 to 70% of the phenotypic variation in NUtE among genotypes to the onset of senescence in decreased N environments. However, Van Sanford and MacKown (1986) attributed 54% of genotypic variation in NUE to NUpE in a study of 25 wheat genotypes.

Previous researchers have indicated that indirect selection for yield remains the most appropriate approach for progress in NUE (Sadras and Richards, 2014; Cormier et al., 2016). Nitrogen use efficiency as a phenotypic trait can be difficult to ascertain. To breed for NUE there are a number of strategies proposed including breeding specifically in a low N environment (Fess et al., 2011; Raun and Johnson 1999). Performance in a low-input system would also allow flexibility for producers to reduce costs when conditions are not favorable for intense management (Pimentel et al., 1989).

The G×E×M interaction is a major concern in confounding selection progress and in development of measures for stability (Yan and Kang, 2003). Due to its complexity, the interaction G×E×M is generally difficult to quantify in multi-environment field studies. The objective of this study was to evaluate the response of SRW wheat cultivars to reduced production inputs in a multi-environment trial (MET), by assessing the variation in traits associated with NUE for grain yield across an array of genotypes, management intensities, and N rates.

MATERIALS AND METHODS

Site Description and Experimental Design

We evaluated 10 locally adapted SRW wheat breeding lines and cultivars across 5 site-years. Entries were selected based on variation in growth habit, disease resistance, maturity, lodging resistance, biomass production, and yield potential. The genotypes evaluated were: Truman, Pembroke 2008, Pembroke 2014, Shirley, KY93-1238-17-1, Pioneer Brand 25R32, Dyna-Gro Dinah, Southern States 8700, Southern States MPV57, and Branson. The experiment included three management systems and four N rates arranged in a randomized split-split plot design in which management system represented the main plot effect with the genotype and N rate representing the split and split-split plot effects, respectively. All treatments had three replications. The experimental unit at each location was a single six-row yield plot measuring 3.3 m in length and 1.2 m wide.

The study was grown over the 2013, 2014, and 2015 harvest years. Planting dates were: 18 Oct. 2012 at the University of Kentucky Spindletop Research Farm in Lexington, KY (38.1304 N, −84.4913 W), for the 2012–2013 season (LEX13) and 14 Oct. (LEX14) and 12 Nov. 2013 (2LEX14) at two locations on the same farm for the 2013–2014 season. An additional location was planted on 14 Oct. 2013 (PRN14) at the West Kentucky Research and Educational Center in Princeton, KY (37.1017 N, −87.8571 W). The soil type at the Lexington site is a Maury silt loam (fine, mixed, semi active, mesic Typic Paleudalf), whereas the soil type at Princeton is a Crider silt loam (fine-silty, mixed, active, mesic, Typic Paleudalf). A final experiment was planted 27 Oct. 2014 (LEX15) at the University of Kentucky Spindletop Research Farm in Lexington, KY. This experiment was grown under the control management only, as described below.

Management Systems

The management systems evaluated included a high, control and low input levels (Table 1). All management levels received a pre-plant seed treatment consisting of an insecticide and fungicide to control for soil borne diseases and insects. The seed treatment was a combination of Cruiser Insecticide (thiamethoxam, 3-[(2-chloro-1,3-thiazol-5-yl)methyl]-5-methyl-N-nitro-1,3,5-oxadiazinan-4-imine, 45 g a.i. ha^{-1}, Syngenta Basil, Switzerland) and Dividend Fungicide (difenoconazole, 1-((2-(2-chloro-4-(4-chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-2-yl)methyl)-1H-1,2,4-triazole, 95 g a.i. ha^{-1}, and metalaxyl, 22 g a.i. ha^{-1}, Syngenta Basil, Switzerland). All management levels received an herbicide application to control for early season weeds (Harmony, thifensulturon-methyl, methyl 3-[[[[(4-methoxy-6-methyl-I,3,5- triazin-2-yl) amino] carbonyl] amino] sulfonyl]-2-thiophenecarboxylate, 21 g a.i. ha^{-1}, Dupont Wilmington, DE) combined with a foliar insecticide to control for early season insects (Warrior, lambda-cyhalothrin, [1a(S*),3a(Z)]-cyano(3-phenoxyphenyl) methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2- dimethylcyclopropanecarboxylate, 13.6 g a.i. ha^{-1}, Syngenta Basil, Switzerland). Additional inputs according to management intensity include: early season foliar fungicide applied at Feekes 6.0 (Headline, Pyraclostrobin, 113 g a.i. ha^{-1}, BASF Research Triangle Park, NC), late season fungicide applied at Feekes 10.5 (Prosaro, Prothioconazole, 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)- 2-hydroxypropyl]-1, 2-dihydro-3H-1, 2, 4-triazole-3-thione, 86 g a.i. ha^{-1}, and Tebuconazole, α-[2-(4-chlorophenyl) ethyl]-α-(1, 1-dimethylethyl)-1H-1, 2, 4-triazole-1-ethanol, 86 g a.i. ha^{-1}, Bayer Crop Science Montvale, NJ) and a plant growth regulator applied at Feekes 6.0 (Palisade, Trinexapac-ethyl, 50 g a.i. ha^{-1}, Syngenta Basil, Switzerland), The control management level is based on University of Kentucky wheat production recommendations (Lee et al., 2009).

Table 1. Management systems and inputs for wheat genotype × environment × management studies grown at Lexington and Princeton, KY, 2013 to 2015 growing seasons.

Input	Brand name	Management system		
		High	Control	Low
Seed applied fungicide	Dividend	At planting	At planting	At planting
Seed applied insecticide	Cruiser	At planting	At planting	At planting
Foliar insecticide	Warrior	Feekes 6.0	Feekes 6.0	Feekes 6.0
Herbicide	Harmony	Feekes 6.0	Feekes 6.0	Feekes 6.0
Foliar fungicide	Prosaro	Feekes 10.5	Feekes 10.5	–
Foliar fungicide	Headline	Feekes 6.0	–	–
Plant growth regulator	Palisade 2 EC	Feekes 6.0	–	–
Nitrogen rates†		Feekes 3.0 and 5.0	Feekes 3.0 and 5.0	Feekes 3.0 and 5.0

† 68, 112 kg ha^{-1} Incremental N rates, 2015 only, eight applications Feekes 3.0 to Feekes 10.0.

Nitrogen Treatments

The split-split plot treatments consisted of four levels of N applied at rates of 0, 68, 112, and 168 kg ha^{-1} across all genotype × management combinations for the 2013 to 2015 seasons. Nitrogen rates were selected based on University of Kentucky recommendations with targeted rates below, at, and above recommended rates (Murdock and Ritchey, 2014). Nitrogen was applied as pelleted urea (46–0–0) in 2013 and as liquid urea ammonium nitrate (28–0–0) formulation for the 2014 and 2015 seasons using a backpack sprayer (R&D Sprayers, Opelousas, LA) and TeeJet flat fan nozzles (TeeJet, Glendale Heights, IL). In 2014–2015 season there were two additional N rates in which N was applied incrementally from Feekes 3 to Feekes 10.5 for a total of 68 and 112 kg ha^{-1}. Soil pH averaged 6.9 in both locations during the period of this study, soil organic matter ranges from 2 to 2.4% at these locations, and P and K levels were maintained based on University of Kentucky recommendations (Murdock and Ritchey, 2014).

The NUE calculations were performed based on partial factor productivity {NUE = Yield (kg ha^{-1})/[Soil N + N Applied (kg ha^{-1})]}, [NUpE = Total plant N/soil N (pre-N soil N and N applied (kg ha^{-1})], (NUtE = yield/total plant N), (PANU = Total Plant N (kg ha^{-1}) – N uptake at anthesis (kg ha^{-1})(NUpA)), (REMN = Grain N (kg ha^{-1}) – PANU (kg ha^{-1}) (Moll et al., 1982).

Soil Sampling

Soil nitrate analysis was conducted using mixed-bed ion exchange resin bags biweekly from 10 Mar. 2013 to 29 May 2013 across genotype × N plot combinations in one replication of control treatment in 2012–2013. Resin bags were constructed using nylon fabric cut into 10 by 10 cm squares with 15 mL of mixed bed exchange resin and bound with a plastic zip tie. Two resin bags were placed in each plot, 15-cm deep, for a total of 80 resin bags in 40 field plots. Bags were removed from the field after 2 wk and replaced with new bags in the same location. Bags were transported to the laboratory on ice in a cooler, cleaned of soil residue using nanopure water, and extracted in the laboratory using 40 mL of 2 M KCl and placed on a shaker plate for 1 h at 200 rpm. After extraction, the liquid was filtered through filter paper (Fisherbrand Filter Paper, Pittsburgh PA) with fine porosity pre-wetted with deionized water and stored in the refrigerator overnight. The following day 1 mL of solution was pipetted into cluster tubes and analyzed using KCl extraction analysis (Giblin et al., 1994) for nitrate and ammonium.

Soil samples were collected within each replication in the control management across each N rate at three stages: prior to N application, anthesis, and physiological maturity for all locations in the 2014–2015 seasons. For each sampling, six soil cores were taken at a depth of 30.48 cm with a 1.6 cm diam. soil probe. The cores were combined, air dried, and ground using a soil grinder.

Ammonium and nitrate were extracted from each soil sample using the KCl method (Giblin et al., 1994). A 2 mol KCl solution was prepared by diluting 150 g KCl in 1000 mL of deionized water. Ten grams of soil were combined with 25 mL of 2 mol KCl in 113.4 g specimen cups. The solution was mixed for 30 min by shaking on a reciprocal shaker at 200 rpm. One milliliter of solution was transferred to cluster tubes by pipette and cluster tubes are centrifuged for 27 min. Then 15 μL of each sample and calibration standards were pipetted into the wells of two microplates, one for the ammonium analysis and one for the nitrate analysis.

Agronomic Traits and Nitrogen Sampling

Heading dates were recorded for each plot when 50% of the plants in the plot had visible spikes emerged from the flag leaf sheath. Anthesis date was recorded when 50% of the spikes in the plot had extruded anthers. Disease ratings for Fusarium head blight and leaf rust based on incidence and severity were taken approximately 20 d after anthesis. Height was recorded prior to senescence and row length was recorded just before harvest. Lodging ratings were taken when needed prior to harvest.

Nitrogen status was collected using normalized difference vegetation index (NDVI) measurements with a hand-held canopy spectral reflectance (CSR) Crop Circle device (Holland Scientific, Lincoln, NE) held approximately 50 cm above the plot generating 60+ readings per plot. The NDVI is calculated based on wavelength using the following formula: $(R_{NIR} - R_{VIS})/(R_{NIR} + R_{VIS})$ with R_{NIR} representing light reflection in near infrared spectra and R_{VIS} representing light in the visible spectrum (Crippen, 1990). Readings were taken at each location prior to N application, 2 wk after N application, anthesis, and mid-grain filling. Anthesis NDVI measurements were used to estimate total biomass across treatments.

Ten flag leaves were removed randomly from each plot at anthesis and maturity to sample for N concentration. Flag leaves were oven dried and ground to a powder using a cyclone mill (UDY One, Fort Collins, CO). Vegetative material was analyzed for % N content using a FlashEA 1112 combustion analyzer. Whole grain samples were analyzed for % N and % protein

content using near infrared reflectance (NIR) on a DA7200 analyzer with a 950 to 1650 nm wavelength range (Perten, Hägersten, Sweden).

Statistical Analysis

GGE Biplot software (Yan and Kang, 2003) was used to graphically represent the response of genotypes to specific management systems and N environments using principal component analysis. The principal components (PC1 and PC2) represent genotype (G = PC1) and the interaction of genotype × environment (GE = PC2). The combination of PC1 and PC2 represent GGE (Yan and Kang, 2003).

Analysis of variance was performed using the General Linear Models procedure of SAS (SAS Institute, 2011). The model used was:

$$Y_{ijklm} = \mu + L_i + R(L)_{ij} + N_k + G_l + M_m + L_i \times N_k + L_i \times G_l + L_i \times M_m + G_l \times N_k + M_m \times N_k + L_i \times N_k \times G_l + L_i \times N_k \times M_m + N_k \times G_l \times M_m + L_i \times N_k \times G_l \times M_m + E_{ijklm}$$

where Y_{ijkm} = the observation in the lth genotype in the jth rep in the ith location at the kth N rate and mth management system, μ = the overall mean, $N_k \times G_l$ = the effect of the interaction of the kth N rate and the lth genotype, and E_{ijkl} = the residual error. Least squares means were estimated to measure treatment differences among genotypes. Treatment and interaction effects were considered significant if $P \leq 0.05$.

Combined analysis was performed except in those cases where significant genotype × treatment interaction was observed. Variance components were estimated using Procedure Varcomp of SAS (SAS Institute, 2011).

RESULTS AND DISCUSSION

Characterization of Environments

The GGE biplot was used to visualize any treatment × environment interactions and to characterize genotypes within individual environments for yield and N traits. A polygon view displays mega-environments and cultivar response to the specified environments. The 5 site-year locations for this study represent five distinct environments characterized by location within the state, seasonal variation in weather, planting date, and soil characteristics. The GGE biplot (Fig. 1) shows that LEX13 and LEX15 comprise one mega-environment and LEX14, PRN14, and 2LEX14 comprise a separate mega-environment. The criteria for characterizing mega-environments requires that the same cultivar does not display optimal performance in every test environment and the among group variation is greater than within group variation. The locations in this study appear to be separating into mega-environments based on baseline soil nutrient data determined as soil N prior to fertilizer N application. Both LEX13 and LEX15 had high baseline soil nitrate levels (HN) whereas the remaining three locations had low baseline soil N environment (LN). These soil N levels become apparent in the mid-season sampling (Table 2). Additionally, LEX13 and LEX15 had above average rainfall totals during the seed-filling

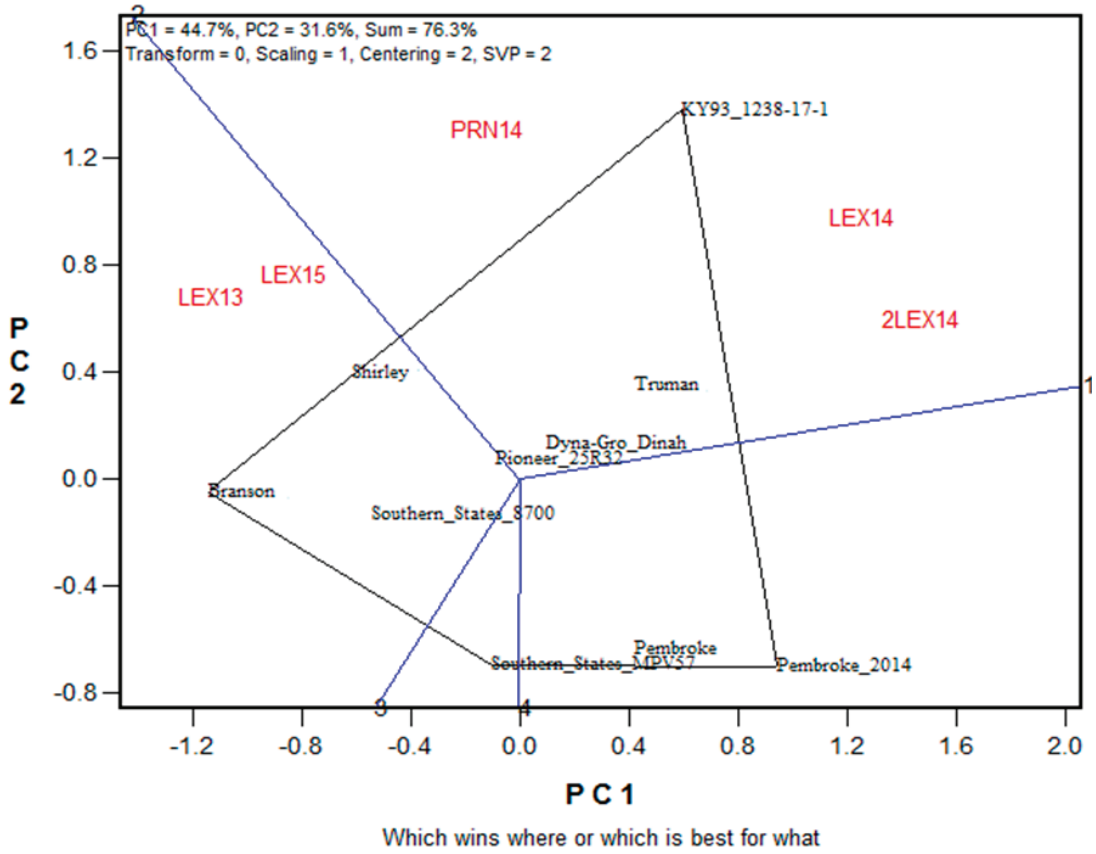

Fig. 1. Polygon view of the GGE biplot, showing which cultivar yielded best in which environment. Individual environments are separated by axis denoting sectors with two mega-environments. Perpendicular lines drawn on the polygon divide the biplot into sectors. The vertex cultivar in each sector is the highest-yielding cultivar in the environment(s) that fell into each sector.

period compared to the other environments. Adequate rain combined with the HN environment in these locations resulted in significantly higher yields compared to all locations, further confirming the separation of mega-environments (Fig. 1). Both PRN14 and LEX15 had above average precipitation during the period after N application which may have contributed to high N losses through leaching in the soil profile. However, the high precipitation in LEX15 recorded from Feekes 6 to Feekes 8 was primarily due to one large rainfall event rather than consistent rains over time (Fig. 2).

Management, Nitrogen Rate, and Genetic Effects

Analysis of variance was performed to determine the effects of N level, management system, genotype and location and all interactions on grain yield and numerous N traits related to crop performance. Averaged over all genotypes, the high and control management both resulted in an 11% increase in yield compared to the low management level, with no significant increase in yield from the control to high management treatment. This lack of response from control to high-input over 4 site-years is the rationale for removing management as a treatment in 2015 and investigating response to the control management level.

Table 2. Soil mineral N (kg N ha^{-1}) across N treatments of wheat genotype × environment × management studies, Lexington and Princeton KY, 2013 to 2015, pre N application, mid-season and Maturity sample dates are shown.

	N Treatment, kg ha^{-1}			
	0	68	112	168
Location	Soil mineral N, kg ha^{-1}			
	Pre N application			
2013				
Lexington	12.38	–	–	–
2014				
Princeton	19.08	–	–	–
Lexington	13.97	–	–	–
Lexington-Late Planting	30.83	–	–	–
2015				
Lexington	29.52	–	–	–
	Mid-season			
2013				
Lexington	15.38	299.92	330.46	426.62
2014				
Princeton	10.01	76.78	119.15	177.85
Lexington	10.74	94.66	139.44	178.87
Lexington-Late planting	6.40	75.26	121.52	185.94
2015				
Lexington	22.88	144.62	184.38	291.99
	Maturity			
2013				
Lexington	20.91	185.93	340.70	518.77
2014				
Princeton	18.51	82.75	130.97	181.89
Lexington	17.83	87.35	135.31	192.79
Lexington-Late planting	12.62	84.27	129.62	190.43
2015				
Lexington	13.83	84.08	128.18	185.81

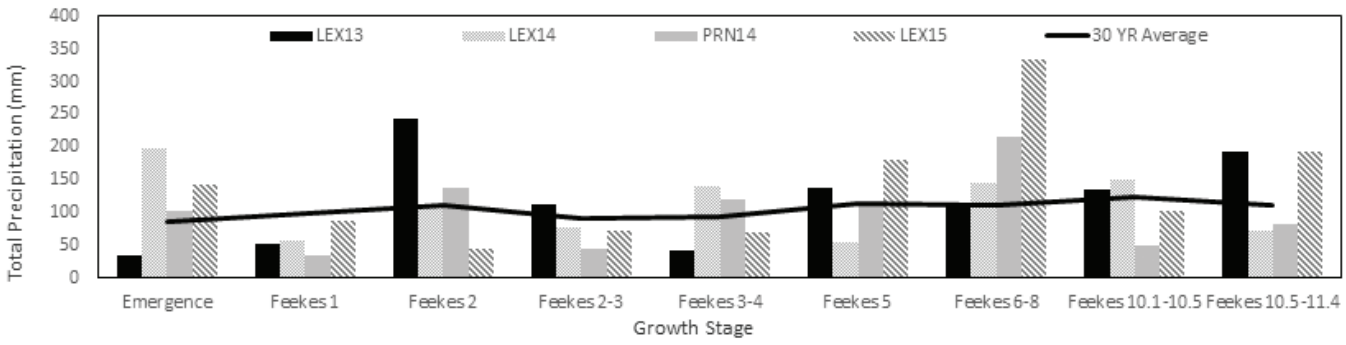

Fig. 2. Annual recorded rainfall (mm) by environment and 30-yr average (2012–2015).

The genotype × N interaction was not significant for yield, NUE, or the various N traits measured indicating all genotypes performed similarly in response to N rate (Table 3). Interestingly, there were no significant increases in grain yield when N rate was increased beyond the low N treatment (68 kg ha^{-1}) for all genotypes. However, there were significant yield gains from the 0 N kg ha^{-1} treatment to any additional N level (Table 4).

Incremental N treatments resulted in yields greater than or equal to the high N treatment ($p < 0.001$) (Table 4). The 68 kg ha^{-1} incremental rate increased yield by 7.2% compared to the same rate applied in a split application, and increased yield by 3.6% compared to the high rate (168 kg ha^{-1}) applied in a split application (Table 4). Grain protein was significantly increased in the incremental N rate treatments ($p < 0.001$). The 68 kg ha^{-1} incremental rate increased grain protein 6.6% compared to the split application; while the 112 kg ha^{-1} incremental rate increased grain protein by 9% compared to the split application, with genotypes combined across locations (Table 4). Nitrogen utilization efficiency was decreased in the incremental N treatments due to high total plant nitrogen (TPN) compared to the split application treatments (Table 4). While NUpE was not significant, postanthesis nitrogen uptake (PANU) in the 68 and 112 kg ha^{-1} incremental rates were 30 to 32% greater compared to the split application at the same rates (Table 4). Variation in PANU among genotypes ranged from 17.23 to 30.33 kg ha^{-1} which was significantly correlated to yield (Tables 5 and 6).

The ANOVA indicates no significant differences among genotypes for NUpE with highly significant differences for NUtE. This is further confirmed by the N content in the vegetative tissue sampled at both anthesis and maturity. There were no genotypic differences found in N content at anthesis but there were differences at maturity and in total plant N across genotypes (Table 3). When considered in the context of yield performance, several genotypes display a less than average NUpE but an above average NUtE. For example, Southern States 8700 had the third highest yield in the 0 kg ha^{-1} N level but ranked eighth in NUpE. This entry could overcome the limitations in N uptake by having above average NUtE, ranking second. A specific genotype may take up N efficiently but without the ability to remobilize the N, yield will suffer. This is important to understand from a plant breeding perspective as some research used root morphology and root N transporter systems as phenotypic traits that may contribute to NUE through NUpE. (Cormier et al., 2016). However, these specific physiological traits are likely not indicative of remobilization ability and additional consideration should be made to vegetative N traits.

Due to the uniform response to N rate increases across genotypes, it was appropriate to evaluate cultivar performance within each N treatment to understand the genetic response to reduced N. When evaluating cultivar performance in various environments the large variation due to the environment main effect is not relevant (Yan and Kang, 2003). The remaining components

Table 3. Type 3 tests of fixed effects for N traits†, yield and total plant N in wheat genotype × environment × management studies grown at Lexington and Princeton, KY, 2013 to 2015.

Source	Yield	NUE†	Total plant N	NHI	NUtE	NUpE	PANU	NRE
	kg ha^{-1}		kg ha^{-1}					
Nitrogen (N)	***	***	***	ns	***	***	***	***
Genotype (G)	***	***	**	***	***	ns	***	***
G×N	ns	ns	ns	ns	ns	ns	ns	ns
Environment (E)	***	***	***	***	***	***	***	***
N×E	***	***	***	***	***	***	***	ns
G×E	***	***	***	***	***	ns	ns	ns
G×E×N	ns	**	ns	ns	ns	ns	ns	ns

** $p < 0.01$.

*** $p < 0.001$.

† NUE, Nitrogen use efficiency; NHI = Nitrogen harvest index, NUtE = Nitrogen utilization efficiency, NUpE = Nitrogen uptake efficiency, PANU = Postanthesis nitrogen uptake, NRE = Nitrogen remobilization efficiency.

Table 4. Mean response to N level across genotypes in wheat genotype × environment × management studies grown at Lexington and Princeton, KY, 2013 to 2015.

	Trait†‡						
N Rate environment	Yield	Grain N	Protein	NUtE	NUpE	TPN	PANU
	kg ha^{-1}		%	kg ha^{-1}			
0	4368.00d	81.37c	9.62d	48.62a	5.67a	96.40e	15.89d
68	5772.30c	112.79b	10.46c	44.57b	1.58b	135.52d	22.95c
68-Incremental§	6220.10a	122.08a	11.20b	42.38c	1.64b	147.31bc	34.08a
112	5908.20bc	117.87ab	10.69c	42.82c	1.11c	144.94c	24.81bc
112- Incremental§	5912.50bc	121.81a	11.75a	39.38d	1.12c	150.47ab	35.90a
168	5992.60b	121.56a	11.23b	39.95d	0.82d	153.46a	29.23b
Mean	5695.62	112.91	10.83	42.95	1.99	138.01	27.14

† NUtE, nitrogen utilization efficiency; NUpE, nitrogen uptake efficiency; TPN, total plant nitrogen; PANU, postanthesis nitrogen uptake.

‡ Means followed by different letters are significantly different at $p < 0.05$ levels.

§ 68, 112 kg ha^{-1} Incremental N rates, 2015 only.

of phenotypic expression are genotype and genotype × environment interaction which are relevant for cultivar evaluation. The GGE biplot uses a ranking plot analysis to show the performance of genotypes in each environment identified for analysis. A comparison to both ideal N environment and ideal cultivar was performed. Biplots are based on two principle components resulting from data scaling by the factor-centered and within-factor standard deviation.

The ranking plot analysis for grain yield displays the test environments in comparison to an ideal environment, to show discriminating ability and representativeness (Fig. 3). The proximity and the vector angle of the 68 kg ha^{-1} environment to the axis, and the circle indicating the ideal environment on the axis, signifies this environment as the most representative of an ideal test environment for the dataset. This confirms the results from the ANOVA in which no genotypes exhibited significant yield increases to additional N beyond the 68 kg ha^{-1} treatment (Table 4). The implications for this result are profound. Environmental impacts due to excess N fertilizers and fertilizer loss are known to have lasting effects on both terrestrial and aquatic ecosystems as a result of agricultural applications (Foley et al., 2005). Anthropogenic sources of N, at least partly through fertilizer release, are believed to contribute to increases in greenhouse gas emissions (GHG), specifically nitrous oxide (N_20) (Vitousek et al., 1997). Of the GHG emissions associated with wheat production, 70% are attributed to N fertilizer (Gaju et al., 2011). Aquatic environments are particularly sensitive to N inputs and nitrate is the most universal chemical contaminant in the world's freshwater aquifers (Spalding and Exner, 1993). Excess N will cause eutrophication and anoxic conditions in aquatic ecosystems which contribute to fish kills and algal blooms (Cameron et al., 2013). This result demonstrates that many genotypes and environments could respond to decreased fertilizer N without a significant reduction in grain yield.

The cultivars were ranked within the individual N treatments with the 68 kg ha^{-1} environment being the most interesting for ascertaining genetic response to N, as this treatment was found

Table 5. Agronomic and N traits†‡ by genotype in wheat genotype × environment × management studies grown at Lexington and Princeton, KY, 2013–2015.

Genotype	Yield	Anthesis date	PANU	NUtE	NUpE	Veg. N anthesis	Biomass
	kg ha^{-1}	DOY	kg ha^{-1}	kg ha^{-1} grain/kg ha^{-1} plant N	kg ha^{-1} TPN/kg ha^{-1} soil N	—— kg ha^{-1} ——	
KY93-1238-17-1	5995.00a	135.3bc	29.70abc	45.39abc	2.18	116.81	3113.95b
Dinah	5667.00b	136.8b	27.77abc	42.50cde	2.24	119.01	3161.59ab
Truman	5648bc	138.3a	24.44bcd	43.9bcd	2.25	123.3	3289.30a
Pioneer 25R32	5554.80bcd	137.0b	23.80cd	44.51abc	2.23	122.01	3177.27ab
Shirley	5481.5bcd	137.2ab	28.86abc	43.47bcd	2.21	123.66	3159.22ab
Pembroke 14	5445.7cde	134.2c	30.33a	40.71e	2.26	118.00	3184.08ab
Branson	5420.70de	135.7bc	23.05cd	43.53bcd	2.18	126.87	3122.75b
Pembroke	5413de	135.9bc	20.15de	44.65abc	2.13	118.72	3205.78ab
Southern States 8700	5401.90de	137.6ab	20.22de	46.07abc	2.05	119.15	3226.07ab
Southern States MPV57	5253.70de	137.4ab	17.23e	41.96de	2.18	125.77	3248.83ab
Mean	5528.13	136.0	24.56	43.67	2.19	121.33	3188.88

† PANU, Postanthesis nitrogen uptake; NUtE, nitrogen utilization efficiency; NUpE, nitrogen uptake efficiency; Veg. N anthesis, vegetative nitrogen content at anthesis; DOY, day of year.

‡ Means followed by different letters are significantly different at $p < 0.05$ levels.

Table 6. Correlations of agronomic and N traits† across genotypes and managements in high (168 kg ha^{-1}) and low N rates (0 kg ha^{-1}) in wheat genotype × environment × management studies grown at Lexington and Princeton, KY, 2013 to 2015.

N Rate environment		0 kg ha^{-1} N Rate environment								
		Yield	ADOY†	Veg. N anthesis	TPN	PANU	NUE	NUtE	NUpE	REMN
168 kg ha^{-1} N Rate environment	Yield		−0.546**	0.731**	0.922**	0.575**	0.916**	0.352**	0.831**	0.684**
	ADOM	−0.668**		−0.370**	−0.382**	−0.207	−0.581**	−0.607**	−0.459**	−0.396
	Veg. Ant. N	0.427**	−0.299		0.791**	−0.057	0.756**	−0.0.028	0.788**	0.922**
	TPN	0.674**	−0.452**	0.766**		0.633**	0.811**	−0.0.02	0.853**	0.638**
	PANU	0.382**	−0.457**	−0.262**	0.269**		0.535**	0.022	0.583**	−0.131
	NUE	0.996**	−0.695**	0.458**	0.686**	0.347**		0.288**	0.979**	0.369**
	NUtE	0.478**	−0.335*	−0.359**	−0.309**	0.121	0.463**		0.115	0.165
	NUpE	0.717**	−0.495**	0.774**	0.994**	0.251**	0.735**	−0.024**		0.337**
	REMN	0.564**	−0.263	0.847**	0.628**	−0.409**	0.588**	−0.02	0.662**	

* $p < 0.05$.
** $p < 0.01$.
† ADOY, Anthesis day of year; Veg. N anthesis, vegetative nitrogen content at anthesis; TPN, total plant nitrogen; PANU, postanthesis nitrogen uptake; NUE, nitrogen use efficiency; NUtE, nitrogen uilization efficiency; NUpE, nitrogen uptake efficiency; REMN, nitrogen remobilization efficiency at maturity.

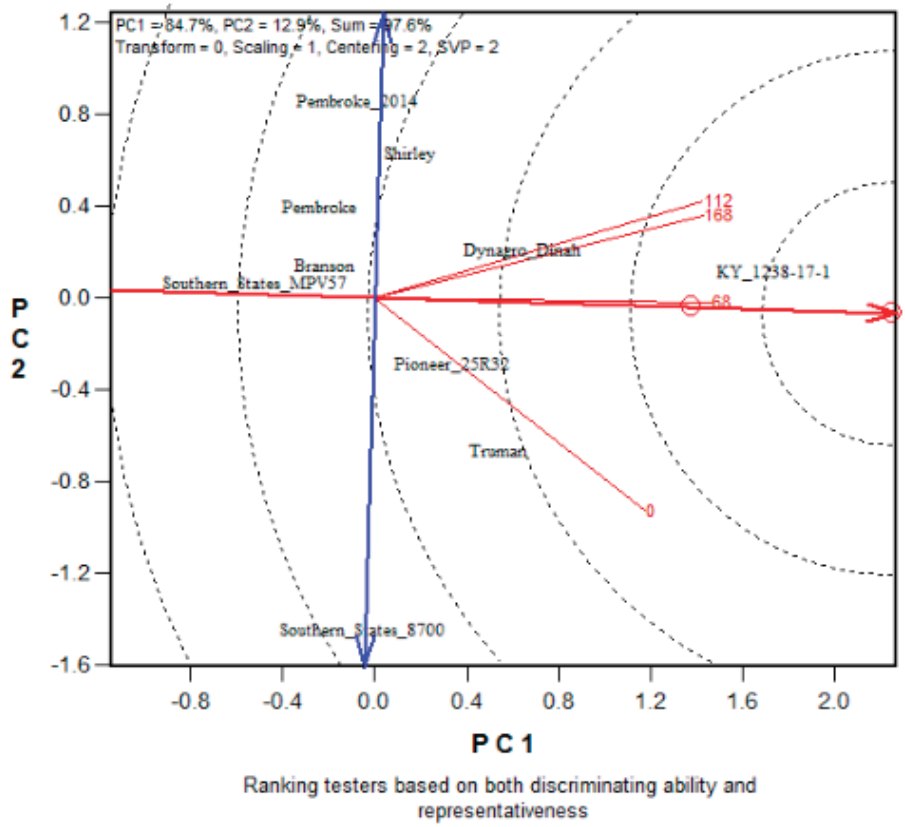

Fig. 3. Comparison of N environments with the ideal environment. The ideal environment is represented by the small circle on the red average environment coordinate (AEC) axis. The environments are ranked on their distance from the ideal environment with the 68 kg ha^{-1} environment being the most representative due to proximity to the AEC axis.

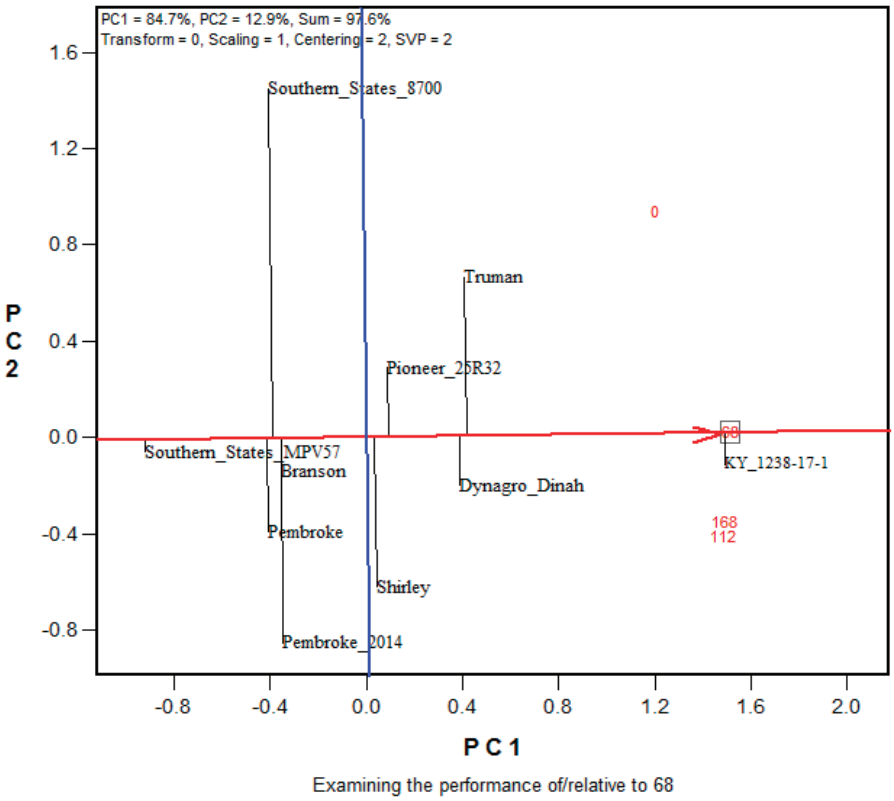

Fig. 4. Comparison of cultivar yield performance in the 68 kg ha^{-1} N environment across five locations. The red line represents the 68 kg ha^{-1} N axis. The blue line perpendicular to the red line separates cultivars with below-average yield from those with above-average yields. Cultivar rank: KY93-1238-17-1 > Truman > Dyna-Gro Dinah > Pioneer 25R32 > Shirley > Branson≈ Pembroke 2014 ≈ Southern States 8700 ≈ Pembroke > Southern States MPV57.

to be an ideal test environment (Fig. 4). The principal components for yield in the 68 kg ha^{-1} environment are G (84.7%) and GE (12.9%) with GGE (97.6%) combined for the dataset. For the 68 kg ha^{-1} environment KY93-1238-171 has the highest average yield followed by Truman, Dyna-Gro Dinah, Pioneer 25R32, and Shirley. These genotypes produced greater than average yields in the environment as displayed by their vector location compared to the blue line representing the mean yield for the environment. The length of vector for each cultivar is an indicator of stability within the environment. Longer vectors from the axis indicate greater variability and less stability, or a greater G×E interaction for the dataset. Thus, KY93-1238-17-1 ranks as a high yielding, stable cultivar in a low N environment. Pioneer 25R32 and Dyna-Gro Dinah also rank as stable with greater than average yields for the 68 kg ha^{-1} environment despite having lower average yields than a cultivar like Truman. A larger set of cultivars would make the stability analysis more useful for making recommendations on cultivar selection within management system.

The GGE biplot proved useful to assess genotypic performance and stability under various N environments. The utility of GGE biplot for this dataset allowed for further insight into a MET dataset that if evaluated by ANOVA alone would lose the complex genotypic information that was present. The economic and environmental benefits from developing genotypes with yield stability in a low N environment would be substantial. While the physiological mechanisms that drive NUE are still elusive it seems relevant to adopt N screening into breeding programs (Hitz et al., 2016).

To incorporate low-input systems into breeding programs there will be a need to utilize high-throughput phenotyping tools to remain cost effective. Instruments that gather phenotypic data associated with estimates of grain yield without the need for harvest are a practical option when utilizing multiple management approaches. As a tool for high-throughput phenotyping, NDVI can be useful to evaluate N status and genotypic performance in low vs. high input systems. The NDVI at mid-grain fill in this study was well correlated with grain yield ($r = 0.73$, $R^2 = 0.56$) (Fig. 5). Previous research has also shown strong correlations for CSR with wheat grain yield, biomass, and N traits (Hitz et al., 2016; Prasad et al., 2007; Raun et al., 2001)

Similar to the findings of Barbottin et al. (2005) the nitrogen uptake at anthesis (NUpA) was well correlated to the amount of nitrogen remobilized at maturity (REMN) (Fig. 6). While the total N measured in the vegetative tissue at anthesis did not vary significantly among this set of genotypes it should be considered as a potential measure of NUE. However, sampling tissue across many genotypes in a breeding program is not an efficient means to assess N status. Highly significant correlation coefficients between REMN and N concentration of vegetative tissues at anthesis, in both 0 and 168 kg ha^{-1} environments suggests an alternate approach (Table 6). Use of canopy spectral reflectance at anthesis as a measure of N content will likely be a good predictor of REMN and grain yield potential when calibrated to a model based on vegetative N content.

Maturity was significantly negatively correlated to PANU and most N traits among genotypes (Table 6). Early maturing genotypes, those that are photoperiod insensitive, display greater PANU than late maturing, or photoperiod sensitive, genotypes (Table 6). Selection based on allelic variation at the photoperiod sensitivity loci combined with response to incremental N applications could allow varietal improvements in overall NUE.

Assessing crop performance as an interaction of genetics, environment and management has demonstrated the potential effectiveness in screening genotypes in low N and high N environments to classify yield response. While KY93-1238-17-1 had optimal performance in three locations across all N rates it did not have the best yield in the two locations with high baseline N. KY93-1238-17-1 would be well adapted to a low N environment whereas Branson would respond to a high N environment (Fig. 1). Evaluating management across multiple genotypes can give insight into the stability of that management system. The consistent performance of a genotype across environments will be important information for growers who might consider low-input management systems. However, fluctuation in grain prices will likely drive the adoption of low-input management systems over the use of high-input management, which may control other yield limiting variables. While the alleles responsible for

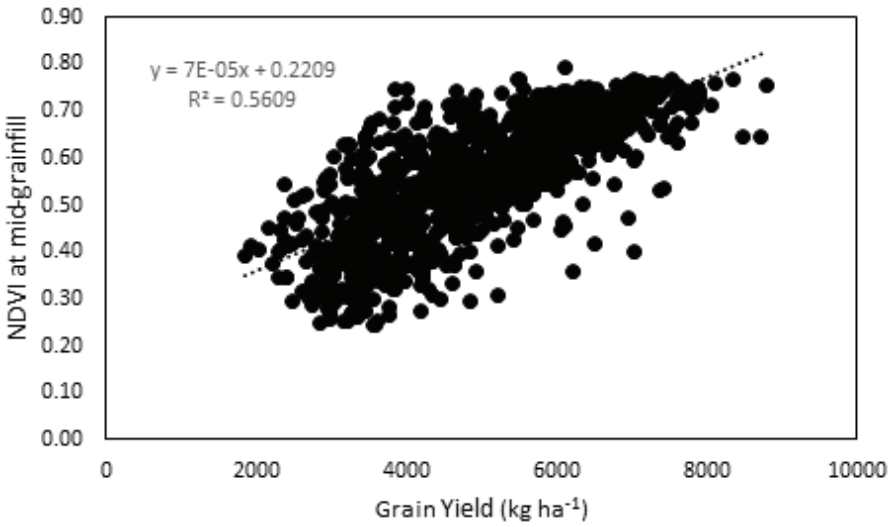

Fig. 5. Correlation of yield and normalized difference vegetation index (NDVI) at mid-grain fill in wheat genotype × environment × management studies grown at Lexington and Princeton, KY, 2013 to 2015.

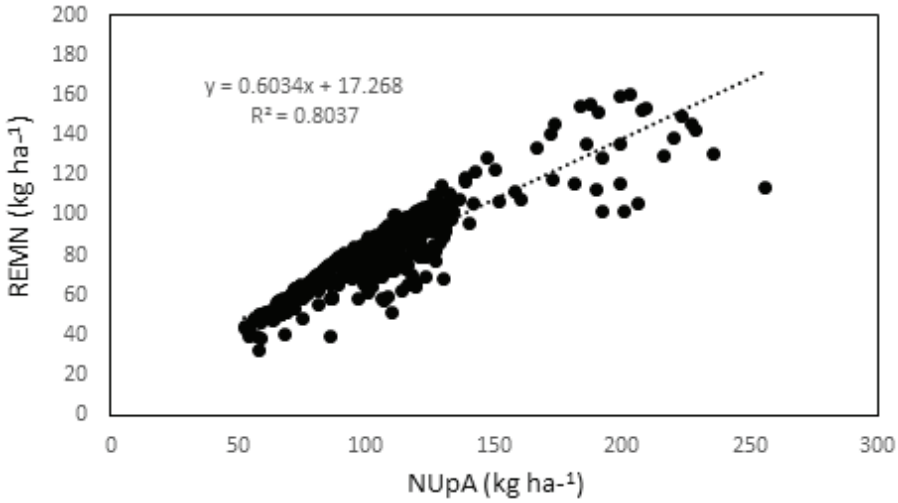

Fig. 6. Correlation of total N uptake at anthesis to nitrogen remobilized at maturity in wheat genotype × environment × management studies grown at Lexington and Princeton, KY, 2013 to 2015.

maximum yield in low input systems may be different from those in high-input systems, breeding programs tend to focus on specific target environments (Atlin and Frey, 1989). Breeders, however, will benefit from a streamlined approach to assessing genotypic performance across managements as a precautionary approach to future regulatory concerns.

Currently, it is not common practice to screen genotypes based on specific management packages but rather to test for response to single treatments. However, it will be important to understand response to low input systems to balance yield and profitability as we attempt to increase global crop yields. If we are selecting based on yield, there may not be a net return on investment by continuing to intensify our production systems when the crop use has a new optimum lower threshold. While this strategy may increase yield gaps in low-risk cropping systems there exists a necessary trade-off for economic and environmental returns (Sadras and Denison, 2016). An increase in NUE by just 1% is estimated to save more than US$2,000,000 in fertilizer costs (Raun and Johnson, 1999).

The benefit of utilizing breeding programs to screen breeding lines for response to specific management allows a more robust set of genotypes than are typically utilized in agronomic management studies. Based on the findings from this MET, focus on wheat NUE in this region should be on traits that benefit N utilization, photoperiod sensitivity, and PANU. Some potential examples of traits that can optimize NUtE may be leaf architecture and canopy interception, postanthesis remobilization, and delayed senescence (Cormier et al., 2016).

CONCLUSION

While our study utilized a relatively small set of genotypes, the genetic variation among those tested was wide when considering maturity, disease resistance, and growth habit. Despite this variation, this study demonstrates that reducing N rates may not have a negative effect on wheat yields across multiple environments and management levels. Future research into this area should provide insight into the potential for reduction of N use in wheat, particularly in areas that are prone to leaching into ground water or run-off into surface water, without sacrificing yield or quality. There appears to be utility in exploring incremental N applications, made possible through irrigation and precision agriculture for exploration of genetic variation in PANU. Incremental N applications would need to be assessed for economic viability. Reduction in N rates and variety specific management could bring solutions to regulatory concerns and produce positive environmental outcomes in the future.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Kentucky Small Grain Growers Association for their generous support of this research. We thank James Dollarhide and John Connelley for technical assistance; R.L. McCulley for laboratory use and sample N determinations; E.A. Carlisle and J.A. Nelson for assistance in generating N analysis; and G. Netto, C. Huntemann, L. Vitantonio, and E. Gonzaga for sample preparation and analysis.

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