

Trait-based drivers of nitrogen responsiveness in winter wheat: The role of phenotypic plasticity
of yield, grain protein deviation, and maturity

by

Mariana de Oliveira Mota

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Approved by:

Major Professor
Dr. Romulo P. Lollato

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Abstract

Wheat (*Triticum aestivum* L.) is a primary source of energy and protein in human nutrition, and improving both grain yield and quality remains a major challenge closely linked to nitrogen (N) supply. However, excessive N fertilization can lead to environmental concerns, including nitrate leaching and greenhouse gas emissions. Since cultivars differ in their response to N availability, a better understanding of how key agronomic traits influence these responses can help improve both yield and environmental outcomes. Among these traits, phenotypic plasticity of grain yield, grain protein deviation (GPD), and maturity have been suggested to influence wheat agronomic response to nitrogen. Phenotypic plasticity reflects the ability of a genotype to modify its performance across environments. We hypothesize that cultivars with greater yield plasticity will exhibit greater yield response to increasing N availability. Grain protein concentration is typically negatively related to yield, but some cultivars deviate from this trend, exhibiting positive or negative GPD. We hypothesize that cultivars with higher GPD will require greater N inputs to maximize grain yield due to increased protein accumulation per unit of grain yield. Finally, cultivar maturity influences the environmental conditions experienced during critical developmental stages. We hypothesize that early-maturing cultivars avoid high-temperature stress during grain filling and therefore show greater responsiveness to N. Our objectives were to validate whether the selected cultivars exhibit the expected levels of yield plasticity, GPD, and maturity, to test whether these trait differences are associated with variation in cultivar responsiveness to N availability, and to explain the physiological basis of cultivar-specific N response. Rainfed field experiments were conducted across 14 environments in Kansas during the 2023–2024 and 2024–2025 growing seasons. A split-plot design evaluated seven N rates (0, 33.6, 67, 101, 135, 168, and 202 kg N ha⁻¹) applied at green-up (Zadoks 30) as

whole plots and eight winter wheat cultivars as subplots (SY Wolverine, LCS Julep, WB4401, WB4699, Bob Dole, LCS Chrome, WB4269, and SY Monument) contrasting in yield plasticity, GPD, and maturity. Phenotypic plasticity cultivar selection was validated using reaction norm slopes, GPD was validated as the residual from the relationship between grain protein concentration and grain yield, and maturity was validated through heading date scores. Linear mixed effects models were used to evaluate the effects of plasticity, GPD, maturity, and total N availability on grain yield across environments. A Bayesian quadratic-plateau model was applied to estimate agronomic optimum nitrogen availability (AONA) as a function of grain yield and total N availability, accounting for genotype-specific plasticity. Additionally, linear regressions were used to relate N-based yield response to mean grain filling temperature to assess differences among maturity groups. Cultivars classified as high-plasticity yielded more than their counterparts in five environments, without a yield trade-off ($\approx 0.06 \text{ Mg ha}^{-1}$ more yield), and exhibited slightly higher AONA in ten environments compared to low-plasticity cultivars. High GPD cultivars had greater grain protein concentration in nine environments and did not present greater AONA across environments. Early and late-maturity cultivars did not differ in yield response to N, except for one environment. Overall, our results suggest that phenotypic plasticity of grain yield is the most promising cultivar trait for enhancing nitrogen-responsive yield across environments, when compared with grain protein deviation and maturity.

Keywords: winter wheat, phenotypic plasticity of yield, grain protein deviation, maturity, nitrogen availability, nitrogen response.

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Dedication

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Chapter 1 - Literature Review

1.1 Wheat Production and Its Global Importance

Wheat (*Triticum aestivum* L.) is one of the most broadly cultivated cereal crops worldwide and, together with rice and maize, provides a substantial proportion of the calories and protein consumed by the global population (Erenstein et al., 2022). Due to its critical role in food security, global wheat demand is expected to increase in the coming decades (Shewry and Hey, 2015; Kruseman et al., 2025). In parallel with rising demand, global wheat production has shown a constant increase, reaching approximately 800 million metric tons in the 2024/25 season (FAO, 2025). China leads global production with 140 million metric tons, followed by the European Union (122 million metric tons), India (113 million metric tons), Russia (81 million metric tons), and the United States (54 million metric tons), which accounts for about 7% of total global production (FAO, 2025).

The global population is estimated to reach approximately 9 billion by 2050, requiring a 70% increase in overall food production (FAO, 2009). As a key cereal crop, wheat production must increase accordingly. For instance, Kettlewell et al. (2023) suggested that global wheat production may need to increase by approximately 45% to meet projected demand by 2050 if wheat maintains its current contribution to global diets. However, current projections suggest production will increase by only 18.3%, which is well below the level required to meet future demand (Neupane et al., 2022). Historical trends also indicate that production growth may be insufficient, with global wheat production increasing by approximately 30% between 1991 and 2018, representing gains but remaining relatively slow and variable, which raises concerns about the ability to meet projected demand by 2050 (Fischer et al., 2014; Erenstein et al., 2022;

Neupane et al., 2022). Given continued population growth, meeting future food demand will require crucial and sustainable intensification of wheat production, alongside the adoption of improved management practices to enhance productivity, ensuring global food security (Erenstein et al., 2022; Shiferaw et al., 2013).

Within this global context, the United States plays a crucial role as one of the world's leading wheat producers and exporters. In 2025, wheat was grown on approximately 18 million hectares in the U.S., with a total production of about 54 million metric tons. Among producing states, Kansas and North Dakota led national production with approximately 9 million metric tons each, followed by Montana (5 million metric tons), Washington (4 million metric tons), and Idaho and Oklahoma (3 million metric tons each) (USDA, 2025). U.S. wheat production is geographically diverse and includes six classes of wheat: hard red winter, hard white, soft red winter, soft white, hard red spring, and durum (USDA, 2025). Despite this diversity, approximately 70% of total production is concentrated in the Great Plains region, where wheat is predominantly grown under rainfed systems (Fischer et al., 2014). In this region, the major wheat classes include hard red winter, hard red spring, hard white, and durum (Paulsen and Shroyer, 2008).

Among producing states, Kansas is particularly known as the “Wheat State” for historically leading wheat production in the country (NASS, 2022). Wheat production in Kansas occurs primarily under dry climates and is characterized by yield variability driven by weather conditions such as drought, late spring freeze damage, and heat stress during grain filling (Fischer et al., 2014). Although wheat yields have increased compared with two decades ago, yield gaps remain between attainable and actual yields across the state. For example, Lollato et al. (2017) reported differences of up to 3 Mg ha⁻¹ between western and eastern Kansas. Other

studies have also shown that improvements in management practices can increase actual yields, particularly in regions where yield progress has stagnated (Lollato et al., 2019a). Among the main constraints to achieving higher productivity, nitrogen availability and nitrogen management have been associated positively to increase wheat yield (Walsh et al., 2018; Lollato et al., 2021; Jaenisch et al., 2022). Due to its significant contribution to national and global wheat supplies, improving wheat productivity and nutrient management practices in Kansas represents an important opportunity to help mitigate the gap between projected supply and future wheat demand.

1.2 Nitrogen Management in Winter Wheat

Nitrogen is the primary nutrient limiting yield in cereal crops (Fageria and Baligar, 2005) and plays a central role in crop production by influencing leaf area development, radiation interception, photosynthetic capacity, and dry matter accumulation (Duan et al., 2019). To be more specific, nitrogen is strongly connected to wheat grain yield since nitrogen affects tiller density, head number, and kernel number and weight (Otteson et al., 2007; Slafer et al., 2014). Also, nitrogen is the main component for plants to build grain protein content (Giordano et al., 2024; Lollato et al., 2021; McDonalds, 1992; Walsh and Walsh, 2020). Consequently, management practices that enhance N uptake are critical for increasing both grain yield and grain protein concentration (Lollato et al., 2021). Significant factors modulating effective nitrogen management include the use of appropriate application rates, sources, methods, and timing that align with periods of maximum crop nutrient demand (Fageria and Baligar, 2005).

1.3 Physiological Role of Nitrogen in Winter Wheat

Nitrogen is an important component of chlorophyll, amino acids, nucleic acids, and metabolism (O'Brien et al., 2016; Wang et al., 2012). The plants can obtain nitrogen from soil in available forms of nitrate (NO_3^-) and ammonium (NH_4^+) (Fageria and Baligar, 2005). Several studies have demonstrated that nitrogen availability is closely linked to leaf area expansion, canopy development, and, consequently, the photosynthetic capacity of wheat plants. Increases in the cumulative rate of photosynthesis are strongly associated with greater wheat yield potential (Parry et al., 2011). For example, higher nitrogen application rates promote greater nitrogen uptake, which in turn enhances leaf photosynthesis (Lemaire et al., 2008; Zhang et al., 2022). Improved photosynthetic rate in response to nitrogen occurs because nitrogen accumulates in both the green leaf area and biomass components of the plant, reflecting its role in photosynthetic and metabolic processes as well as in structural tissue formation (Olesen et al., 2002). Conversely, nitrogen deficiency reduces leaf expansion rates, leading to lower leaf area index and diminished photosynthetic capacity (Lemaire et al., 2008).

As nitrogen has a strong effect on the accumulation of biomass, allowing a greater capture of light by the plant and improved photosynthesis rates, the potential yield is consequently affected. Many studies have shown that nitrogen fertilization influences crop performance and, when combined with the appropriate fertilizer timing and wheat variety, significantly alters yield components (heads per unit area, kernels per head, and kernel weight), grain yield, and grain protein content (Alley et al., 2009; Lollato et al., 2019a; Woodard and Bly, 1998). To illustrate these changes, a global meta-analysis by Wang et al. (2023a) reported that nitrogen addition increased grain yield by 14.9% and GPC by 6.6%. However, excessive nitrogen applications can significantly reduce grain yield (Zorb et al., 2018).

1.4 Wheat Response to Nitrogen

While nitrogen management involves rate, timing, source, and placement, this section focuses exclusively on nitrogen rate, as it is the primary focus of this study. Nitrogen rate is a key determinant of wheat yield, and several methods can be used to estimate crop nutrient requirements, including soil tests, field trials, nutrient removal estimates, and plant tissue analysis (Whitney, 1997; Zhang et al., 2018). Among these, analyzing soil samples collected in the field is the most commonly used and reliable approach, as it accounts for expected yield, cropping system, soil texture, and nitrogen available in the soil profile (Whitney, 1997).

To achieve maximum yields, the optimal nitrogen rate can be estimated based on the yield response curve as a function of nitrogen application rate. Cerrato and Blackmer (1990) were among the first to compare different response models, such as linear plateau, quadratic plateau, quadratic, exponential, and square root, to determine which best fit yield response data and identify the nitrogen rate that maximizes yield. Their results suggested that the quadratic-plateau model was the most appropriate. However, no single model is suitable for all cases because site-specific conditions strongly influence the optimal nitrogen rate (Bullock and Bullock, 1994). Therefore, selecting the model that best fits the observed data is generally recommended rather than adopting a single model considered optimal in other studies (Miguez and Poffenbarger, 2022). This modeling approach has been widely documented for several crops, including maize, wheat, and sugar beet across different production regions (Oglesby et al., 2022; Ghimire and Maharjan, 2024; Baum et al., 2025; Leitão et al., 2025).

The agronomic optimum nitrogen rate is the minimum amount of nitrogen required to achieve maximum crop yield (Camberato et al., 2022). The relationship between grain yield and

nitrogen application rate is commonly described by a quadratic or plateau response, indicating the existence of an optimal nitrogen rate beyond which yield declines due to excessive nitrogen inputs (Duan et al., 2019). In quadratic models, the agronomic optimum nitrogen rate corresponds to the vertex of the regression curve, whereas in plateau models it corresponds to the breakpoint of the curve (Leitão et al., 2025). These points represent the stage at which additional nitrogen no longer increases yield because the marginal yield response to nitrogen has declined or stabilized.

Previous studies show that grain yield significantly increases with increasing nitrogen rates up to an optimal nitrogen rate, after which grain yield decreases (Hawkesford et al., 2014; Howard et al. 1994; Lollato et al., 2019a). For instance, Wang et al. (2017) identified an application rate of 185 kg ha⁻¹ as a threshold for achieving high yield, whereas Zhang et al. (2009) recommended an optimal nitrogen range of 240–270 kg ha⁻¹ for wheat production in Shandong Province, China. Expectedly, the optimal nitrogen rate will depend on the yield potential of each region and year.

The optimal nitrogen rate has been studied for decades in the Great Plains of the United States, a region where yield is limited by water stress (Lollato et al., 2019b). For example, Halvorson et al. (2004) showed that wheat yield was maximized with N rates of 84 to 112 kg ha⁻¹, and in subsequent years, when unfavorable weather conditions occurred, the optimal N rate decreased to between 56 and 84 kg ha⁻¹, which also resulted in lower grain yields. Sadras et al. (2022) confirmed the negative effects of temperature during the critical period for yield determination on wheat response to nitrogen. Likewise, water availability and residual nitrogen can impact winter wheat response to nitrogen. For example, winter wheat had lower yields and higher nitrogen rate (94 to 112 kg ha⁻¹) after sorghum as compared to after soybeans (70 to 94 kg

ha⁻¹) owing to the sorghum's residue and nitrogen immobilization as well as more water usage than soybeans. However, in subsequent years, the optimal N doses were reversed due to low water availability (Staggenborg et al., 2003). Additionally, Lollato et al. (2019) showed that in rainfed environments in Oklahoma, the optimal nitrogen rates were stable between 86 and 92 kg ha⁻¹.

Although many studies have identified an optimal nitrogen rate for maximizing wheat yield, increasing nitrogen application rates generally leads to a considerable decline in nitrogen use efficiency (NUE), defined as the amount of grain yield produced per unit of nitrogen available (Moll et al., 1982). This indicates that, despite the progressive increase in nitrogen fertilizer inputs in wheat production systems, gains in crop growth rate and yield have not kept pace with the rate of nitrogen application (Duan et al., 2019). Consequently, achieving both high grain yield and high NUE simultaneously remains challenging.

1.5 Environmental and Economic Implications of Nitrogen Management

Exceeding the optimal nitrogen rate, in addition to increasing the possibility of reduced wheat yield, has significant negative environmental and economic consequences. From an environmental perspective, excessive nitrogen inputs can lead to nitrate leaching and increased emissions of nitrous oxide (N₂O), contributing to environmental nitrogen pollution and greenhouse gas emissions (Zorb et al., 2018). In addition, economic considerations should guide nitrogen management decisions, as identifying the most cost-effective nitrogen strategies can enhance farm profitability without compromising crop performance (Vaughan et al., 1990). Together, these environmental and economic concerns underscore the need for a more comprehensive investigation into N management strategies for wheat production.

1.6 Key Agronomic Traits Relevant to Nitrogen Response

1.6.1 Phenotypic Plasticity

Phenotypic plasticity (PP) is defined as the “environment-dependent expression of phenotypes” (DeWitt and Scheiner, 2004). This concept explains traits about the conventional approach of genotype $\times$ environment $\times$ management ($G \times E \times M$) interactions (DeWitt & Scheiner, 2004; Scheiner & Goodnight, 1984). In this approach, genotype–environment interactions lead individuals to express phenotypic traits that may differ from expectations based on population means (e.g., race or species). Phenotypic plasticity can therefore be interpreted as how the environment affects gene expression (West-Eberhard, 1989), or the ability of an organism to produce different phenotypes, such as morphological, physiological, hormonal, or behavioral, in response to varying environmental conditions during growth and development (Schlichting & Pigliucci, 1998; West-Eberhard, 1989).

PP is part of the evolutionary theory and relates to an organism’s adaptive ability. In the past, PP was associated with noise, error, outliers, or “unfortunate defect” (West-Eberhard, 1989; Schlichting & Pigliucci, 1998). However, modern perspectives in crop ecology and plant breeding recognize PP as an important concept of crop adaptation (DeWitt & Scheiner, 2004; Giordano et al., 2024; Sadras et al., 2013; Sadras and Rebetzke, 2013). While earlier studies associated PP mostly with the emergence of new races or species at the population level (DeWitt & Scheiner, 2004; Schlichting & Pigliucci, 1998; Schmid & Guillaume, 2017; Willmer et al., 2006), more recent work has applied the concept to understand the evolution of artificially selected crop varieties (Giordano et al., 2025a), as well as to support crop management strategies

that seek to improve profitability (Giordano et al., 2025a; Lollato et al., 2020; Sadras et al., 2016; Veenstra et al., 2021).

Although PP is often considered an adaptive or competitive advantage, higher plasticity is not necessarily associated with greater ability of traits to express superior performance in different environmental conditions and under strong competition (West-Eberhard, 1989). In some cases, increased plasticity allows individuals to express phenotypes closer to an environmental optimum (Schmid and Guillaume, 2017), but negative relationships between phenotypic plasticity and trait expression have also been reported (DeWitt & Scheiner, 2004; Sadras et al., 2016). For example, studying PP in chickpea (*Cicer arietinum* L.), Sadras et al. (2016) observed a linear negative relationship between harvest index phenology and harvest index phenotypic plasticity. Nevertheless, multiple works report other types of regressions beyond the linear relation between trait responses and PP, such as non-linear (e.g., quadratic regressions) and non-plastic (flat slope) relationships (Schlichting and Pigliucci, 1998). These findings highlight that the adaptive value of plasticity is dependent on the specific trait, genotype, and environmental context.

Across many studies evaluating phenotypic plasticity, it is common to find statements about the limitations of the dataset, particularly the number of environments and genotypes evaluated. These limitations can be associated with the methodology and induce misinterpretation if not well understood. Also, it is important to state that the values obtained in the PP analyses are always limited to the dataset present in the study, the set of environments, and the selected genotype. However, PP evaluation remains a powerful methodology to explain the interaction complexities in $G \times E \times M$ datasets from an agronomic and ecologic perspective (Giordano et al., 2024; Sadras et al., 2013; Veenstra et al., 2021). Thus, PP has the potential to

support decision-makers regarding genotype selection and management strategies appropriate to specific environments (Bastos et al., 2020; Sadras et al., 2013), such as planting density (Veenstra et al., 2021) or fertilizer management (Giordano et al., 2024). Phenotypic plasticity is also a useful tool for breeders (Giordano et al., 2025a; Nicotra et al., 2010; Sommer, 2020), since it can support the explanation of the significant $G \times E \times M$ interactions. In practical approaches, the PP framework can allow for an understanding of the environmental effect on the genetic pool (crop), individual (genotype), and the “environmental optimum” rate of this specific locale (DeWitt & Scheiner, 2004; Schmid & Guillaume, 2017).

Given its complexity, different methods have been proposed to quantify PP of a given trait (Valladares et al., 2006). In crop ecology, plasticity is interpreted as the opposite of stability and has been quantified using the slope of the reaction norm methodology, which describes how a genotype responds across environmental gradients (Dingemanse et al., 2010; Pemac and Tucic, 1998; Schlichting and Pigliucci, 1998). Additionally, PP has been estimated through the variance ratio methodology, which quantifies how much a genotype varies across environments relative to other genotypes grown under the same conditions (Giordano et al. 2024; Lollato et al., 2020; Sadras et al., 2016). Slopes derived using the variance ratio approach are highly correlated with slopes derived from the reaction norm (Sadras and Richards, 2014).

1.6.2 Phenotypic Plasticity of Yield

Phenotypic plasticity of yield refers to the variation in grain yield of a given genotype across environments or growing conditions. Recent publications reported a positive correlation between grain yield and phenotypic plasticity of grain yield (Giordano et al., 2024; Lollato et al., 2020), suggesting that genotypes with greater yield plasticity are more effective in utilizing

environmental resources for grain production in high-yielding environments while showing no tradeoff under stress. A similar relationship was observed for grain yield components (Sadras and Rebetzke, 2013), indicating that plasticity can contribute to yield performance. However, new research showed a different approach when correlated phenotypic plasticity with a modern statistical method to capture different perspectives about yield plasticity in interaction with environments. Sadras et al (2026) defined phenotypic plasticity as the agronomically adaptive plasticity where cultivars consistently achieve superior yield across environments without a yield penalty under stress. They showed that phenotypic plasticity in oat yield was adaptive in Australian environments, while wheat yield was maladaptive in Mexican environments, providing a new method for integrating biological concepts with statistical analysis.

Caldereni and Slafer (1999) showed that wheat breeding efforts worldwide have increased yield potential, often accompanied by a reduction in yield stability among genotypes. More recently, Giordano et al. (2025) reinforced this concept by showing that more recent wheat genotypes exhibit a stronger association between yield and plasticity in high-yielding environments in the U.S. Great Plains.

Stone and Savin (1999) proposed different response phases for grain yield and grain protein concentration as a function of the total nitrogen available to the crop. Utilizing this concept, Giordano et al (2024) explored a framework linking this relationship to phenotypic plasticity to evaluate genotype $\times$ nitrogen interactions. Their results suggested that yield plasticity under non-nitrogen-limiting environments was correlated with higher grain yield but was not related to grain protein concentration. So, evaluating yield plasticity provides a useful background for interpreting nitrogen-driven yield responses across variable environments.

1.6.3 Grain Protein Deviation

Grain quality is an important component of wheat production and is determined by the composition of the grain - including proteins, carbohydrates, and lipids (Stone and Savin, 1999). Among these components, carbohydrates and proteins are the most abundant and play key roles in determining both grain yield and end-use quality. Carbohydrates account for approximately 65 to 75% of the wheat grain and largely influence yield, whereas protein typically accounts for approximately 7 to 18% of the grain mass (Stone and Savin, 1999; Dupont and Altenbach, 2003; Guzman et al., 2022). Wheat protein quality influences both the nutritional value of the grain and the functional properties required for final wheat products such as pasta, bread, and cakes (Godfrey et al., 2010). Grain protein contains approximately 16% nitrogen, and its concentration is strongly influenced by nitrogen availability and by complex interactions among genetic, environmental, and management factors (Dupont et al., 2006; Duncan et al., 2018).

The accumulation of carbohydrates and proteins in wheat grain occurs during the grain development process. Wheat grain development can be divided into three stages: cell division, cell expansion, and desiccation (Altenbach, 2012). Following fertilization, the grain goes through rapid cell division and differentiation into the endosperm, embryo, and testa. During the grain filling phase, endosperm cells expand and accumulate starch and protein. When the grain reaches physiological maturity, the deposition of starch and protein ends, moisture content declines rapidly, and the endosperm tissue suffers apoptosis (Altenbach, 2012).

Carbohydrates accumulated in the grain are derived from carbon dioxide (CO₂) fixed through photosynthesis during the grain filling period, and most of this carbohydrate is deposited as starch (Jenner et al., 1991). Photosynthates produced by the flag leaf and the spike are major contributors to grain filling. The rate of grain filling, therefore, depends on the balance between

the capacity of plants to produce assimilates (source) and the ability of grains to utilize and store them (sink). In some situations, the supply of assimilates is greater than grain growth, meaning that the sink becomes the limiting factor for starch accumulation (Triboi et al., 2006; Jenner et al., 1991).

Protein accumulation in the grain follows a different physiological pathway and is closely associated with nitrogen uptake and remobilization within the plant. Nitrogen absorbed during vegetative growth is transported to leaves, where it is reduced and incorporated into amino acids such as glutamate, which serves as a key precursor for protein synthesis (Jenner et al., 1991). During grain filling, the nitrogen stored in vegetative tissues is remobilized and transported to the developing grain. Leaves and stems can contribute approximately 30% of the protein deposited in the grain, while additional nitrogen may be supplied for greater protein accumulation into the grain (Jenner et al., 1991). In contrast to starch accumulation, protein synthesis tends to be more limited by nitrogen supply from the source tissues, whereas starch synthesis is more often limited by grain storage capacity (Triboi et al., 2006).

These physiological processes contribute to the widely observed negative relationship between grain yield and grain protein concentration. In general, higher grain yields are associated with lower grain protein concentrations (Fowler et al., 2003; Simmonds, 1995; Woodard and Bly, 1998; Giordano et al., 2024). This relationship is largely explained by source–sink relationships and protein dilution. When yield increases through greater kernel number or grain mass, the available nitrogen must be distributed among a larger number of kernels, reducing the concentration of protein in each grain (Holford, 1992; Duncan et al., 2018; Kibite and Evans, 1984; Lollato and Edwards, 2015; Lollato et al., 2021). Consequently, environments

or management practices that promote high yield often lead to lower grain protein concentrations due to dilution of the available nitrogen within the grain.

Since it is important to achieve both high yields and grain quality simultaneously, the negative association between yield and protein has been widely examined in the literature. It is well established that nitrogen management can influence this relationship, helping to boost protein levels. For instance, Woodard and Bly (1998) showed that higher protein concentrations can be achieved without yield penalties through increased nitrogen rates and split applications. Shortly after flowering, the kernels start to develop, and nitrogen stored in the leaves is redirected to the kernel during grain filling for protein synthesis (Paulsen, 1997). Lollato et al. (2021) further demonstrated that this trade-off between yield and protein can be mitigated by applying higher nitrogen rates and split applications when crop nitrogen demand peaks during grain filling. However, Ghimire et al. (2021) reported no effect of nitrogen split application on grain protein content, although higher nitrogen doses increased protein levels. Additionally, concentrated spring nitrogen application has been shown to promote higher grain yield and protein levels compared to fall or split applications (Vaughan et al., 1990). A global meta-analysis by Giordano et al. (2023) suggests that late-season nitrogen application can raise grain protein by about 4% without affecting overall crop yield.

To better describe genotypic differences in this yield–protein relationship, Monaghan et al. (2001) proposed the concept of grain protein deviation (GPD). Grain protein deviation is typically quantified as the residual from the regression between grain yield and grain protein concentration. Genotypes with positive GPD maintain higher protein concentrations than expected for a given yield level, whereas genotypes with negative GPD exhibit lower protein concentrations than predicted by the yield–protein relationship (Monaghan et al., 2001; Latshaw

et al., 2016). This trait captures genotypic differences in the ability to sustain grain protein concentration as yield increases and has been associated with physiological mechanisms such as enhanced post-anthesis nitrogen uptake and more efficient nitrogen remobilization to the grain (Monaghan et al., 2001). Consequently, GPD provides a useful framework for identifying wheat lines that combine high grain yield with improved grain protein concentration under varying nitrogen availability (Monaghan et al., 2001; Latshaw et al., 2016).

In earlier years, studies have shown that breeding can help to mitigate the diminution of grain protein with new cultivars. Ortiz-Monasterio R. et al (1997) indicated that semidwarf germplasm from CIMMYT's wheat breeding program increases yield, keeping grain protein content constant. While Giordano et al. (2024) suggested that genetic improvement can select varieties with high or low potential for protein content, and in environments without nitrogen limitation, these varieties can reach high protein levels, however, this may result in low yield rates (Giordano et al., 2024). According to Huang et al. (2025) meta-analysis of data since 1980, the negative correlation between yield and protein is still a challenge, but breeding efforts could improve yield and grain quality under high nitrogen supply for new cultivars represented by the ones released after 1998.

1.6.4 Phenology Influenced by Environmental Factors

Yield components in wheat are produced during the crop cycle, from seedling emergence to maturity, but this production is not constant during the whole growth cycle. There is a period when the yield is sensitive to growth or development changes, named the critical period, a developmental window for yield determination (Slafer et al., 2021; Carrera et al., 2024), and considerably impacted by abiotic and biotic stress (Cann et al., 2023).

In an experiment in earlier years in Australia and Mexico, Fischer (1985) demonstrated that shading and heat stress applied at different stages of the crop cycle uncovered the critical period right before flowering (20 days before anthesis) to one week after anthesis. During this developmental window, changes in grain number and grain weight were observed, and yield components associated with yield formation (Fischer, 1985; Slafer et al., 2021).

Grain filling begins during the development of the endosperm, where cell divisions happen repeatedly and the tissue becomes organized into a cellular structure. This is followed by a phase of cell expansion or grain filling, characterized by increased water uptake and the accumulation of starch and proteins in the grain. The final storage capacity of each grain depends on the number of endosperm cells established early during grain filling (Dupont and Altenbach, 2003; Altenbach, 2012). As development proceeds, cell expansion and water accumulation decline while starch and protein deposition continue, gradually replacing cellular water (Altenbach, 2012). The grain reaches physiological maturity when maximum dry weight is achieved, and storage deposition ceases (Dupont and Altenbach, 2003).

Physiological maturity is defined as the stage at which wheat grains reach their maximum weight (Hanft and Wych, 1982). In this stage, kernels rapidly lose moisture until reaching approximately 10 to 15% water content, which corresponds to harvest readiness (Dupont and Altenbach, 2003). Quickly identifying or predicting this moment is essential to avoid yield losses due to early or delayed harvesting (Zhuo et al., 2020). A common physiological maturity visual indicator is the loss of green color in the flag leaf and spikes (Acevedo et al., 2002; Hanft and Wych, 1982).

An extended literature review conducted by Dupont and Altenbach (2003) gathered studies that covered weather and environment impacts during critical and grain filling periods.

Temperature, water availability, fertilizer, and their interactive effects were explored, reinforcing the influences during these periods.

Among these factors, temperature plays a key role by affecting both the rate and duration of grain filling. Optimal wheat yields are generally achieved under temperatures between 15 and 20 °C, which favor a longer grain-filling period and greater starch accumulation per kernel (Dupont and Altenbach, 2003). In contrast, when a U.S. wheat variety was grown under a moderate and very high temperature regimen, high temperature had a limited effect on the grain fill rate, but it significantly reduced the duration and mature kernel weights. This study also revealed that the timing of all stages of grain development was affected by the higher temperature regimens. The higher temperature resulted in a grain filling duration of 26 days, whereas the moderate temperature lasted 44 days, emphasizing that at higher temperatures, starch and protein accumulation begin earlier, and the time needed to reach maximum grain water content and dry weight is shorter (Altenbach et al., 2003; Altenbach, 2012). Therefore, wheat crops adapt to temperature changes and drought during grain filling by accelerating and reducing the developmental stages and preserving the ability to produce a viable seed (Altenbach et al., 2012).

Several studies demonstrated that drought decreased kernel size by shortening the duration, but not the rate of grain fill. The combination of high temperature and drought reduced the duration of grain filling even more (Dupont and Altenbach, 2003). Also, Gooding et al. (2003) tested water stress during grain filling period and observed that the plants exposed to drought in the first 14 days after anthesis (represented by the cell division) had the shortest period. Other studies also found similar results, confirming that abiotic stresses such as high

temperatures and water deficit can accelerate the crop development process, resulting in yield losses (Acevedo et al., 2002; Mondal et al., 2015).

Regarding fertilizer effects on critical and grain filling periods, Altenbach et al. (2003) demonstrated that post-anthesis fertilizer had only minor effects on the grain filling period, although it influenced the duration of protein deposition and timing of leaf senescence (Altenbach et al., 2003). Other studies documented that wheat loses nitrogen during the grain filling period (Daigger et al., 1976). This loss can be correlated with grain formation during periods of high temperatures, negatively affecting both yield and grain protein accumulation (Farhad et al., 2023; Lollato et al., 2017; Lollato et al., 2021).

Focusing on the critical period, Cossani and Sadras (2021) found that elevated temperature reduced the duration of the critical period by approximately 5 degree days, and was the largest driver of reduced grain number and yield. When temperature was associated with nitrogen fertilizer, unfertilized plots had a superior yield at temperatures above 13 °C than fertilized plots. Sadras et al. (2022) found a negative association between yield response to N and temperature during the critical period in wheat growth in the US and Australia. He observed that high temperatures shortened the critical period affecting yield in soils with low nitrogen, while in soils with high nitrogen, the relation was positive, benefiting the grain number, but not grain fill.

Additionally, weather and environmental changes also impact starch and protein accumulation in the grains. High temperatures are responsible for substantial losses in grain yield due to a reduction in starch accumulation. For example, Australian varieties exposed to temperatures above 35 °C presented a decline in starch content, which can be attributed to the heat inactivation of a key enzyme involved in starch synthesis (Dupont and Altenbach, 2003). However, other studies show that these relationships could be more complex, where the observed

decrease in the starch amount per kernel resulted from a reduction in the interval of starch accumulation (Altenbach et al., 2003).

Other studies indicate that fertilizer and temperature may affect the amount, composition, and polymerization of gluten proteins. Increases in grain protein concentration have been observed with higher applications of nitrogen fertilizer (Dupont and Altenbach, 2003; Dupont et al., 2006). This finding is supported by Barneix (2007), who noted that the most common method for enhancing protein levels in wheat grain is to add nitrogen fertilizer. However, he also pointed out that there is a limit to how much protein concentration can increase, once the plant reaches its capacity to uptake nitrogen, any additional fertilizer will result in increased nitrogen levels in the straw rather than in the grain.

Furthermore, the duration of crop development is dependent on genotype, day length, and sowing date (Acevedo et al., 2002). Genetic variation in wheat maturity leads to the existence of early, optimum, and late maturing varieties, which strongly influence crop performance during the grain filling period, particularly under stressful environmental conditions. For example, studies conducted in South Asia and Mexico showed that early genotypes performed better than late genotypes, because they avoided exposure to extreme heat stress at the end of grain filling (Mondal et al., 2015). Similarly, late sowing delayed the heading date and exposed crops to higher temperatures compared with an early sowing date in Texas, with late genotypes showing a grain yield reduction of 35.3 to 91 kg ha⁻¹ for each day of delay in reaching the heading stage (Tewolde et al., 2006). Early varieties reach the grain formation and filling stage earlier, being exposed to moderate temperatures, while late genotypes require a longer time to reach grain maturity and are consequently exposed to higher temperatures, which justifies the reduction in yield (Modal et al., 2015; Singh et al., 2024; Tewolde et al., 2006).

Chapter 2 – Trait-based drivers of nitrogen responsiveness in winter wheat: The role of phenotypic plasticity of yield, grain protein deviation, and maturity

2.1 Introduction

Wheat (*Triticum aestivum* L.) is a primary source of energy and protein in human nutrition (Shewry and Hey, 2015). Enhancing both wheat yield and quality is crucial and intrinsically linked to nitrogen supply (Wieser and Seilmeier, 1998; Hawkesford, 2014; Zorb et al., 2018). Nonetheless, the excessive use of nitrogen fertilizers can lead to environmental challenges, including nitrate leaching and increased greenhouse gas emissions (Gastal et al., 2015; Angus and Grace, 2017). Optimizing nitrogen use efficiency (i.e., grain yield per unit nitrogen available, NUE; Moll et al., 1982) has therefore become a critical consideration in wheat production. Since wheat cultivars seem to respond differently to nitrogen availability (Nehe et al., 2018), improved understanding of the impact of agronomic traits on wheat nitrogen responses can help optimize both agricultural practices and environmental outcomes (Ortiz-Monasterio et al., 1997; Hawkesford, 2014).

The response of wheat to nitrogen availability is strongly influenced by genetic and physiological variation (McAllister et al., 2012). Genotypes differ in their ability to uptake, assimilate, and allocate nitrogen to various plant organs, which contributes to variation in yield and grain protein under different nitrogen conditions (Monaghan et al., 2001; Masclaux-Daubresse et al., 2010; Hawkesford, 2014; Gastal et al., 2015; Giordano et al., 2023).

Understanding the interaction between genotype, environment, and management ($G \times E \times M$)

can help identify traits associated with improved nitrogen responsiveness and inform breeding and management decisions aimed at enhancing NUE (Sanford and MacKown, 1987; Gaju et al., 2011; Nehe et al., 2020; Lollato et al., 2021).

Among cultivar traits of interest, previous data suggest that phenotypic plasticity of grain yield, grain protein deviation (GPD), and cultivar maturity may influence wheat agronomic response to nitrogen. Phenotypic plasticity refers to the changes in how a genotype expresses its traits in response to environmental conditions (Dewitt and Scheiner, 2004) and offers valuable insights into the $G \times E \times M$ interactions (Sadras et al., 2009; Sadras et al., 2016; Lollato et al., 2020; Diouf et al., 2020; Giordano et al., 2024; Giordano et al., 2025a). The degree of plasticity varies with genotype, trait, and the environmental factors causing variation (Bradshaw, 1965; Dewitt and Scheiner, 2004). Phenotypic plasticity of yield reflects how responsive a cultivar is, in terms of grain yield, to environmental variation. Previous evidence suggests that cultivars with high yield plasticity associate with greater yield in high-yielding environments and non-nitrogen-limiting conditions without tradeoffs under stress (Grogan et al., 2016; Jaenisch et al., 2019; Lollato et al., 2020; Giordano et al., 2024, 2025a, 2025b). Thus, we hypothesize that cultivars with greater phenotypic plasticity of grain yield will have greater grain yield at a similar level of nitrogen supply, increasing system's NUE.

Grain protein concentration is another critical trait that reflects both the rheological quality of wheat (Raj et al., 2023) and the nitrogen status of the crop (Goos et al., 1982; Stone and Savin, 1999). Protein synthesis depends directly on nitrogen uptake and remobilization (Borghi, 1999). While nitrogen is crucial for grain yield and protein formation, the association between yield and protein is usually negative (Simmonds, 1995; Giordano et al., 2023; Giordano et al., 2024) due to the nitrogen dilution by compounds of carbon (Stone and Savin, 1999;

Acreche and Slafer, 2009). Selected cultivars seem to deviate from this negative trend, with positive (greater than expected) or negative (lower than expected) grain protein deviation (Latshaw et al., 2016). This deviation has a genetic origin and can be masked by the $G \times E$ interaction (Monaghan et al., 2001). Accordingly, we hypothesize that cultivars with higher GPD will require more nitrogen to maximize grain yield as compared to low GPD cultivars since high GPD cultivars produce more protein per unit yield.

Finally, the third trait we hypothesize can impact wheat response to nitrogen is maturity. Cultivar maturity affects the environment during critical stages of crop development. In winter wheat in the U.S. Great Plains, early-maturing cultivars are usually more vulnerable to frost damage during anthesis (Paulsen and Heyne, 1983), while late-maturing cultivars are more exposed to heat stress during grain filling (Bergkamp et al., 2018). While frost damage substantially decreases wheat yield, it is beyond the scope of this work since most varieties in the region have optimized phenology to avoid freezing events (Maeoka et al., 2020). Thus, we focus on heat stress. Heat stress can significantly reduce wheat yield (Acevedo et al., 2002; Mondal et al., 2015) by shortening critical developmental periods and can even interrupt reproduction and grain growth during stressful temperatures (Sadras and Dreccer, 2015; Cossani and Sadras, 2021; Sadras et al., 2022). As a result, cultivar maturity can modulate crop productivity. For instance, Cossani and Sadras (2021) found that crops exposed to heat stress (whether via delayed sowing dates or cultivar maturity) had a reduced response to nitrogen fertilization and a shorter critical period, negatively impacting crop yield. Therefore, we hypothesize that early-maturing cultivars generally avoid high-temperature stress during the critical period and grain filling, enabling greater nitrogen responsiveness.

We performed nitrogen response field experiments in multiple environments to test the three hypotheses above using eight winter wheat cultivars that represented all combinations of variation in phenotypic plasticity of grain yield (high/low), grain protein concentration (high/low), and maturity (early/late). A comprehensive data mining exercise was used to select the cultivars based on pre-existing long-term variety performance data that identified their contrasting performances across multiple environments (Munaro et al., 2020; Giordano et al., 2025a). Our objectives were to: (i) validate whether the selected cultivars exhibit the expected levels of phenotypic plasticity of grain yield, grain protein deviation, and maturity; (ii) test whether these trait differences are associated with variation in cultivar responsiveness to nitrogen availability, and (iii) explain potential physiological basis of cultivar-specific nitrogen response.

2.2 Materials and Methods

2.2.1 Sites and Crop Management

Field experiments were conducted in fourteen environments across the state of Kansas, U.S., resulting from a combination of two growing seasons (2023–24 and 2024–25) and seven locations (**Table 2.1**). All experiments were established under rainfed conditions. Different crop rotations and tillage practices used in each experiment represented the predominant commercial practice in each region (**Table 2.1**).

Wheat was sown from late September through November using a Great Plains 606 no-till drill with a row spacing of 0.19 m. Individual plots consisted of seven rows and were approximately 9 meters long. Di-ammonium phosphate (DAP 18-46-0) was applied in-furrow at sowing at a rate of 56 kg ha⁻¹. Nitrogen treatments were broadcast as granulated urea (46-0-0) at spring green up (at growth stage GS25-30; Zadoks et al., 1974). At the N application time, all the

experiments also received 17 kg ha⁻¹ of sulfur (S) as calcium sulfate to avoid sulfur deficiency and its interaction with N availability (Jaenisch et al., 2019, 2021). Weeds were controlled throughout the growing season with commercially available pesticides to avoid a confounding factor. Foliar fungal diseases were controlled using 118.8 g ha⁻¹ propiconazole, 28 g ha⁻¹ fluxapyroxad, and 190.5 g ha⁻¹ pyraclostrobin and a non-ionic surfactant (292 g a.i. ha⁻¹ of Phosphatidylcholine, methylacetic acid, and alkyl polyoxyethylene ether) applied after the flag leaf was fully emerged and no later than anthesis (GS39-59; Zadoks et al., 1974). Fungicides were applied to minimize confounding effects associated with variety-specific disease resistance (Cruppe et al., 2021; de Oliveira Silva et al., 2020).

2.2.2 Treatments and Experiment Design

Field experiments were arranged in a split-plot design with three or four replications. Treatments were organized in a complete factorial structure in which seven nitrogen rates (0, 34, 67, 101, 135, 168, and 202 kg N ha⁻¹) were assigned to the whole plots, and eight winter wheat cultivars were assigned to the subplots.

The wheat cultivars were selected based on the three agronomic traits of interest: (i) yield phenotypic plasticity, defined as the variance ratio and calculated with long-term cultivar trial data (Giordano et al., 2025a) with cultivars classified as high or low plasticity; (ii) grain protein deviation (GPD), defined as the residuals from the protein-yield regression (deviation in grain protein concentration), with cultivars classified as high or low GPD; and (iii) maturity, defined as heading date, with cultivars classified as early or late.

To quantify phenotypic plasticity of grain yield of winter wheat varieties, we collected data from around 800 replicated variety trials in Kansas, Oklahoma, and Colorado as previously

explored by Munaro et al. (2020). This database had approximately 100,000 observations of winter wheat grain yield. For accurate estimates of phenotypic plasticity of yield, one variety must be tested across multiple environments. Therefore, the first step was to filter the database for varieties observed in at least 25 environments across years. The resulting database contained data from around 240 cultivars meeting this minimum criterion. Then, we calculated the phenotypic plasticity of grain yield of each cultivar using the reaction norm method. This method regresses each cultivar's grain yield over the mean grain yield of all cultivars tested in the same environment. The resulting estimates of phenotypic plasticity ranged from 0.66 in the most stable cultivar to 1.32 in the least stable cultivar, as reported by Giordano et al. (2025a). To select cultivars for this study, we created a list of varieties meeting the criteria for high plasticity (consisting of those placed approximately within the top decile across the 240 tested cultivars, resulting in cultivars with phenotypic plasticity ranging roughly from 1.15 to 1.32) and low plasticity (consisting of the lowest decile across the 240 tested cultivars, resulting in cultivars with phenotypic plasticity of yield ranging roughly from 0.66 to 0.8). The varieties within these two contrasting groups were then further compared for the other traits of interest.

Grain protein concentration usually decreases linearly with increasing grain yield at a constant level of resource availability (environment). Grain protein deviation is a trait that represents how far a given variety is from the expected protein reduction line. In other words, varieties with high protein deviation fall above the regression line (positive residuals) while varieties with negative grain protein deviation fall below the regression line (negative residuals). All cultivars selected for high and low phenotypic plasticity in step 1 received a rating for grain protein deviation using the scores available on <http://www.ramwheatdb.com/database.php>. This website is the most robust database for winter wheat grain yield available in the US Great Plains.

Regarding grain protein deviation, winter wheat varieties were rated using 3,876 total datapoints across 15 years (2003-2017) and across the Colorado State University (CSU) Elite Trial and the CSU dryland and irrigated variety trials, with a dataset comprising 280 different varieties and experimental lines and 91 total year-location-trial combinations.

The selected group of varieties, as described above, received a relative maturity score based on the same source as grain protein deviation and extensive observations collected by the co-authors over the years, summarized on yearly extension publications (i.e., using previous versions of Andersen Onofre et al., 2025).

After filtering varieties for their plasticity group and assigning them classifications regarding grain protein deviation and relative maturity, the final group of varieties was selected to ensure that all combinations of the three different traits occurred in a balanced way (e.g., high plasticity, high grain protein deviation, late maturity; high plasticity, high grain protein deviation, early maturity, etc.). Once a few varieties meeting all of the criteria above were identified, the final step was to ensure that the selected varieties included in the experiment were modern, commercially available, and relevant to producers in the region. This final step was done by consulting the annual surveys of winter wheat varieties planted in Kansas reported by the USDA NASS (2025). The selected cultivars and their respective traits are shown in **Table 2.2**.

2.2.3 Measurements

Soil samples were collected at each location from 0 to 15 and 15 to 60 cm depth at sowing, except for three environments (BEL.S24, MHN24, MCP24) where the maximum sampled depths were shallower (45 and 30 cm) due to dry subsoil conditions preventing deeper sampling. Samples were submitted to the Soil Testing Laboratory at Kansas State University for

chemical and physical property analyses (**Table 2.3**). For each experimental unit, the total nitrogen available was calculated by the sum of nitrate and ammonia in the soil measured at sowing and the applied nitrogen rate. Nitrogen use efficiency (NUE) was calculated as the ratio of grain yield over total N available (N soil + N rate) (Moll et al., 1982).

Heading progression was monitored between the flag leaf and heading stages (GS39-59; Zadoks et al., 1974) during the 2024-25 growing season. Heading was visually assessed in each plot by estimating the percentage of emerged heads, using a scale from 0 to 100%, where 0% indicated no visible heads and 100% indicated complete heading. Field assessments were conducted at approximately three-day intervals. Linear regressions between field assessments at the plot level were conducted to identify the first day that each plot achieved 50% of emerged heads (**see section 2.2.4.1.3 Maturity**). These measurements allowed for validating the classification of cultivars into early- and late-maturing groups.

At anthesis (GS59; Zadoks et al., 1974), one linear meter of aboveground biomass was collected using an electric grass shear. Samples were dried at 60 °C in an airflow dryer until constant weight, weighed, ground using a 2 mm mesh, and analyzed for carbon and nitrogen concentration using a LECO TruSpec CN combustion analyzer and reported on a weight percent basis. Similarly, one linear meter of aboveground biomass was collected and dried at physiological maturity. Each sample was dried and weighed, and these samples were used for yield component measurements, including shoot mass, harvest index (HI), spike m⁻², kernels m⁻², kernels spike⁻¹, and thousand kernel weight, following the procedure described by Jaenisch et al. (2022). At the maturity stage (GS92), the entire experimental unit was harvested using a Massey Ferguson 8XP small plot combine and adjusted to dry matter (moisture at 0%) from which we retrieved grain weight, moisture concentration, and test weight. Grain yield was calculated using

the area from the center of the plot to the center of the plot (1.8 m width) to account for border effects and avoid yield overestimation. Grain yield was also estimated at physiological maturity from the biomass sample, which was compared with the combine-harvested yield (**Appendix Figure A.2**). Grain protein concentration was measured using a near-infrared spectrometer and adjusted to a dry basis.

2.2.4 Data Analysis

All statistical analyses were performed using R software version 4.1.1 (R Core Team, 2022).

2.2.4.1 Crop Modeling

A mechanistic crop simulation model (Simple Simulation Model Wheat; Soltani & Sinclair, 2012) was used to characterize each environment in terms of major water and temperature stressors in relation to crop phenology. This crop model has been calibrated and widely validated for winter wheat potential growth conditions in this region (Lollato et al., 2017; Sciarresi et al., 2019; Couedel et al., 2021, 2025; Andrade et al., 2026). Simulations used measured daily weather data and soil parameters. Daily weather data for precipitation, maximum and minimum daily temperatures, incident solar radiation, and reference evapotranspiration (ET_o) were obtained from nearby Kansas Mesonet meteorological stations (Patrignani et al., 2020). Crop transpiration was estimated based on effective daily vapor pressure deficit and crop daily dry matter production using a crop-specific transpiration efficiency coefficient, and evaporation from the soil was calculated based on a two-stage model based on the potential evaporation rate calculated using a modified Priestly and Taylor model (Amir and Sinclair, 1991; Priestly and Taylor, 1972; Ritchie, 1998; Tanner & Sinclair, 1983). Crop evapotranspiration

(ET_c) was calculated as the sum of transpiration and evaporation. Plant available water at sowing (PAWS) was derived from non-growing season precipitation (Lollato et al., 2016).

2.2.4.2 Validation of Agronomic Traits

Since our cultivar selection was performed before the start of the experiment, using existing cultivar performance data, comprehensive data repositories, and extension bulletins, the first step was to validate whether our trait-based selection was appropriate to test our hypotheses.

2.2.4.1.1 Phenotypic Plasticity

The validation of the estimates of phenotypic plasticity of yield was done using the slopes of the reaction norm as an indicative of trait plasticity (Woltereck, 1909). Following Eberhart and Russel (1966), the slope was assessed through the linear model fitted between the yield of the cultivars and the yield environmental index, according to Equation 1. In this analysis, environmental index was calculated for the interaction of environment and nitrogen.

$$Y_{ij} = \mu_i + \beta_i E_j + \delta_{ij} \quad (1)$$

where Y_{ij} is the yield of i th cultivar ($i = 1, 2, \dots, 8$) at j th environment ($j = 1, 2, \dots, 98$), μ_i is the mean yield for cultivar i across environments, β_i is the regression coefficient that measures the response of cultivar i to varying environments, E_j is the yield environmental index calculated as the mean of all cultivars at environment j minus the grand mean, and δ_{ij} is the deviation from regression of cultivar i at environment j .

2.2.4.1.2 Grain Protein Deviation

Grain protein deviation (GPD) was used to quantify differences in grain protein concentration response to yield increases among cultivars. GPD was calculated as the residual from a linear regression of grain protein concentration with grain yield (Monaghan et al., 2001). Linear regressions were fitted separately for each environment and nitrogen rate to account for environment- and nitrogen-specific effects on the relationship between grain protein concentration and grain yield (Lollato et al., 2021). The residuals from these regressions were defined as GPD, with positive values indicating higher-than-expected grain protein concentration for a given yield and negative values indicating lower protein concentration. Calculating GPD values for each environment-nitrogen combination resulted in $n = 98$ GPD estimates per cultivar. These estimates were subsequently analyzed using a linear mixed-effects model (ANOVA) considering cultivar, nitrogen rate, environment, and their interactions as fixed effects and replication nested within environment and N rate nested within replication and environment as random effects to account for the experimental design. Significant cultivar differences were determined by Tukey pairwise comparison at a significance level of $\alpha = 0.05$. These least square GPD means were used to validate the GPD groups previously established during the selection of cultivars for this experiment (**Table 2.2**).

2.2.4.1.3 Maturity

Heading date was estimated by interpolating heading progression over time for each experimental unit (**Appendix Figure A.1**), with heading date defined as the date when 50% heading occurred (GS55, Zadoks et al., 1974). Heading dates were expressed in days of the year (DOY) and used as a quantitative measure of maturity. To assess varietal differences in maturity,

heading dates were analyzed using a linear mixed effects model. Cultivar, nitrogen rate, environment, and their interactions were included as fixed effects. Random effects were considered as replication nested within environment and N rate nested within replication and environment. ANOVA was performed, and significant differences among cultivars were determined using pairwise comparisons with Tukey's test at a significance level of $\alpha = 0.05$. These predicted heading dates were used to validate the maturity groups previously established during the selection of cultivars for this experiment (**Table 2.2**).

2.2.4.3 Effect of Agronomic Traits on Wheat Response to Nitrogen

Variable responses, such as grain yield, biomass, spikes m^{-2} , HI, TKW, kernels spike $^{-1}$, kernels m^{-2} , protein concentration, anthesis N uptake, and NUE were analyzed using linear mixed-effects models to assess the effects of cultivar trait groups classified by phenotypic plasticity (high and low yield plasticity; PP-groups), grain protein deviation (high and low GPD; GPD-groups), and maturity (early and late maturity; MAT-groups) across environments. Because the total nitrogen availability varied across experimental units and environments, responses for the evaluated traits were analyzed using a two-way analysis of covariance (ANCOVA), similar to previous propositions for other continuous variables (Mumford et al., 2023). For each response variable, trait group, environment (E), and their interaction were included as fixed effects, while total nitrogen availability was included as a continuous covariate. Cultivar nested within trait group and replication nested within environment were included as random effects. Separate ANCOVA models were fitted for each trait group (PP, GPC, and MAT).

Models were fitted using R packages “*lme4*” (Bates et al., 2015) and “*car*” (Fox & Weisberg, 2018), with Type-III error rate to evaluate the significance of fixed effects (Fox,

2015). When significant effects were detected ($p \leq 0.05$), estimated marginal means were calculated for trait groups within each environment. Pairwise comparisons among groups were performed using Tukey's test at a significance level of $\alpha = 0.05$.

2.2.4.4 Hierarchical Bayesian Approach and Agronomic Optimum Nitrogen Available

Hierarchical Bayesian approach is used to capture complex relationships between predictors and response variables while incorporating prior knowledge and uncertainty (Cho et al., 2023; Hooten & Hefley, 2019). Bayesian models offer the possibility of accounting for multiple uncertainties in the model (e.g., limiting known predictors' effects by probabilistic assumption), which is recommended for stochastic systems, as is the nature of grain yield (Gelman et al., 1995; Hooten & Hefley, 2019). Here, grain yield was modeled as a function of total nitrogen available (soil + fertilizer) for each cultivar in each environment.

We implemented a Bayesian hierarchical framework to model the yield-total nitrogen available relationship as affected by environment and cultivar grain yield plasticity, focusing on continuous and measurable variables instead of grouping by categorical variables (e.g., “cultivar”, “variety”, “maturity group”). Studies on wheat response to total nitrogen available suggest the presence of an asymptotic relationship (Giordano et al., 2024; Ouedraogo & Brorsen, 2018). Thus, the model was written as follows:

$$y_{ij} \sim N(\mu_{ij}, \sigma_{ij}^2), \quad (2)$$

$$\mu_{ij} = \begin{cases} \beta_{1ij}s_{ij} + \beta_{2ij}s_{ij} & \text{if } s_{ij} \leq AONA_{ij} \\ \beta_{1ij}AONA_{ij} + \beta_{2ij}AONA_{ij} & \text{otherwise,} \end{cases} \quad (3)$$

$$\beta_{1ij} = \alpha_{0j} + \alpha_{2j}p_i, \text{ and} \quad (4)$$

$$AONA_{ij} = \alpha_{3j} + \alpha_{4j}p_i. \quad (5)$$

In Equation 1, y_{ij} represents an observation of the response variable grain yield at the i th plot ($i=1, 2, \dots, n$) in the j th environment ($j:1, 2, \dots, 14$) and follows a normal distribution with an expected value μ_{ij} and variance σ_{ij}^2 . Here, μ_{ij} follows a quadratic-plateau function with parameters terms β_{1ij} , β_{2ij} and agronomic optimum nitrogen available ($AONA_{ij}$). Parameter β_{2ij} can be expressed as a function of β_{1ij} and $AONA_{ij}$ using algebra:

$$\beta_{2ij} = \frac{-\beta_{1ij}}{2 AONR_{ij}}. \quad (6)$$

The proposed model novelty relies on the assumption that β_{1ij} and $AONA_{ij}$ depend on the grain yield phenotypic plasticity (p_i) of the k th cultivar ($k:1, 2, \dots, 8$). Thus, α_{0j} represents the intercept for linear term β_{1ij} and α_{1j} the linear effect of p_i on β_{1ij} . Similarly, as proposed in our objective we modeled how $AONA_{ij}$ changed with p_i in Eq. 4 where α_{0j} represents the intercept for linear term $AONA_{ij}$ and α_{3j} the linear effect of p_i on $AONA_{ij}$.

Also, we defined the uninformative priors as follows:

$$\sigma_{ij} \sim (\Gamma(1, 10^{-9}))^{-\frac{1}{2}} \quad (7)$$

$$\alpha_{0j}, \sim N(1.0, 1.7^2), \quad (8)$$

$$\alpha_{1j} \sim N(0.02, 0.06^2), \quad (9)$$

$$\alpha_{2j} \sim N(130, 50^2), \text{ and} \quad (10)$$

$$\alpha_{3j} \sim N(0, 50^2). \quad (11)$$

The Bayesian model was fit using JAGS (Plummer, 2003) through the “*rjags*” package in R. For each cultivar, the model was fitted independently by subsetting the data by cultivar and

estimating environment-specific residual variance. The model ran in three Monte Carlo-Markov Chains (Hooten & Hefley, 2019; Johansen et al., 2010) for 2,000 iterations, followed by 5,000 sampling iterations per chain. Posterior samples were retained for inference after the adaptation phase. Model parameters included nitrogen-level coefficients describing the intercept, linear nitrogen response, and agronomic optimum nitrogen availability (AONA), as well as environment-specific residual standard deviations. Simulations to obtain the posterior distributions were summarized using posterior means and 95% credible intervals (2.5th and 97.5th percentiles) for each combination of phenotype, environment, and total nitrogen available, and created a data frame for the next analysis. Finally, AONA was calculated by the ratio of the maximum grain yield attainable at the minimum total nitrogen available (breaking point) for each cultivar. Regressions were plotted by extreme cultivars, representing the highest and lowest grain yield plasticity or GDP, using the “*geom_raster*” function in the R package “*ggplot2*” (Wickham et al., 2016).

2.2.4.5 Relationship Between AONA and Grain Yield Across Environments

To address whether differences in AONA between high and low plasticity (or GDP) cultivars explained differences in grain yield achieved at AONA across environments, mean AONA and mean grain yield at AONA (GY_{AONA}) were first calculated separately in each environment for each cultivar classified as having high or low phenotypic plasticity (or GDP) using the hierarchical Bayesian approach described above. We then computed the $\Delta AONA$ and ΔGY_{AONA} , where Δ represents the difference between the highest and lowest plasticity (or GDP) cultivars within the same environment (e.g., $\Delta AONA$ was calculated as the AONA of the highest plasticity cultivars minus the AONA of the lowest plasticity cultivar). A simple linear regression

model was fitted with ΔGY_{AONA} as the response variable and $\Delta AONA$ as the explanatory variable using the “*lm*” function.

2.2.4.6 Nitrogen Responsiveness and Temperature Stress

N responsiveness was calculated by comparing each N fertilization rate with the unfertilized control according to Equation 12:

$$V_{\text{resp}}(\%) = 100 \times \frac{(V_N - V_0)}{V_0} \quad (12)$$

where V represents the analyzed variable (grain yield, yield components, anthesis nitrogen uptake), and subscripts N and 0 indicate fertilized and unfertilized treatments, respectively (Sadras et al., 2022).

We defined the grain filling period for each cultivar in each environment as starting at 100 °Cd after anthesis and lasting through 600 °Cd after anthesis, using a base temperature of 0 °C for winter wheat (Dupont et al., 2006).

We performed a linear mixed-effects model to compare grain-filling temperature between early- and late-maturing groups (ANOVA), with maturing group, environment, and their interaction as fixed effects. Cultivar nested within maturity group, repetition nested within environment, and N rate nested within repetition and environment were used as random effects. The relationship between grain yield responsiveness to N and temperature during grain filling was evaluated using a linear mixed-effects model. Because the temperature during grain filling varied across environments, a two-way analysis of covariance (ANCOVA) was performed. Nitrogen rate, environments, and maturing group cultivars were included as fixed effects, while grain filling temperature was included as a continuous covariate. Random effects included cultivar nested within maturity group, repetition nested within environment, and N rate nested

within repetition and environment. Slopes of grain yield responsiveness to N were estimated to assess the strength and direction of these relationships within environments and N rates.

Models were fitted using R packages “*lme4*” (Bates et al., 2015) and “*car*” (Fox & Weisberg, 2018), with Type-III error rate to evaluate the significance of fixed effects (Fox, 2015). When significant effects were detected ($p \leq 0.05$), estimated marginal means were calculated using pairwise comparisons performed using Tukey’s test at a significance level of $\alpha = 0.05$.

2.2.4.7 Relationships Between Grain Yield and Yield Components

Relationships between wheat grain yield (kg ha^{-1}) and biomass, HI, spikes m^{-2} , kernels spike $^{-1}$, thousand kernel weight (TKW), kernels m^{-2} , grain protein concentration, grain test weight, and nitrogen uptake at anthesis, were evaluated across all environments and nitrogen rates. The impact of the agronomic traits of interest (PP-, GPD-, and maturity groups) on the relationship between yield and the different yield components was evaluated using simple linear regression models. When linear regressions for the two groups were statistically different, two regressions were maintained; otherwise, a single regression was used.

2.3 Results

2.3.1 Weather Conditions and Yield Levels

Across the fourteen environments, growing-season precipitation ranged from 221 to 641 mm and plant-available water at sowing ranged from 106 to 159 mm, resulting in a seasonal water supply ranging from 352 to 769 mm (**Figure 2.1**). Meanwhile, seasonal ET_c ranged from 263 to 475 mm with large mismatches between water supply and crop water demand in 24-

BEL.S, 24-HAYS, 24-HOI, 24-PHG, and 25-HAYS. Overall, the fall, winter, and early spring were colder and drier in 2023-24, delaying early spring phenology. By the time spring temperatures increased; daylength was sufficient for all cultivars to progress with reproductive development, which was hastened. The contrasting environments resulted in growing season length ranging from 233 to 267 days. Mean grain yield across sources of variation averaged 3.0 Mg ha⁻¹ and ranged from 0.05 to 5.87 Mg ha⁻¹ (**Figure 2.2A**). Mean grain yield varied considerably as a function of site-year, ranging from 0.49 to 4.27 Mg ha⁻¹, with overall greater yields in 2025 than in 2024 (3.29 *versus* 2.72 Mg ha⁻¹) and large location-to-location variability within year (3.78 Mg ha⁻¹ in 2024 and 1.72 Mg ha⁻¹ in 2025) (**Figure 2.2B**). Grain yield also ranged as a function of N rate, with the zero N control averaging 2.5 Mg ha⁻¹ and the 202 kg N ha⁻¹ averaging 3.5 Mg ha⁻¹ (**Figure 2.2C**), with less variation as function of cultivar (3.0 to 3.4 Mg ha⁻¹, **Figure 2.2D**).

2.3.2 Validation of the Hypothesized Agronomic Traits

Overall, the data measured in this study aligned well with our hypothesized cultivar traits that were based on independent preliminary data (i.e., cultivars classified as high plasticity, high GPD, and late maturity using preliminary data had greater slopes of reaction norms, higher GPD, and later heading dates than those classified as low plasticity, low GPD, and early maturity).

2.3.1.1 Phenotypic Plasticity of Grain Yield

The mean grain yield for different cultivars across environments (i.e., the intercept of the reaction norm on **Figure 2.3**) was highest in WB4401 (3.25 Mg ha⁻¹) and SY Wolverine (3.19 Mg ha⁻¹) and lowest in Bob Dole (2.81 Mg ha⁻¹) and LCS Chrome (2.79 Mg ha⁻¹) (**Table 2.3**).

The slope of the reaction norm of grain yield against the environmental index confirmed our hypothesized cultivar classification, with the cultivars originally classified as high plasticity resulting in slopes ranging from 1.02 to 1.04, and the cultivars hypothesized as stable resulting in slopes of 0.91 through 1.02 (**Table 2.4; Figure 2.3**).

2.3.1.2 Grain Protein Deviation

Across environments and nitrogen rate combinations, grain protein deviation ranged from -0.58% to 0.56% depending on cultivar. Cultivars previously classified as high grain protein deviation had positive mean GPD values (range: 0.21% to 0.56%), whereas cultivars classified as low grain protein deviation had negative mean GPD values (range: -0.15% to -0.58%) (**Table 2.5**). Analysis of variance indicated a significant environment $\times$ cultivar interaction on GPD (**Appendix Table A.1**), primarily driven by a few environments in which the cultivar LCS Julep (a high GPD cultivar) had negative GPD; and likewise, a few environments in which the cultivars WB4269 and SY Monument (low GPD cultivars) had positive GPD (**Table 2.5**). Despite these small environment-specific cultivar GPD performances, there was a consistent pattern in which the hypothesized high-GPD cultivars had greater GPD than low-GPD cultivars.

2.3.1.3 Maturity

Interpolation of heading progression against DOY provided estimated heading dates for each experimental unit (**Appendix Figure A.1**). Analysis of variance indicated that heading date differed significantly among cultivars and environments, as well as for the cultivar $\times$ nitrogen rate and cultivar $\times$ environment interactions (**Appendix Table A.1**). Predicted heading dates ranged from day of year 122 to 127 among cultivars (**Table 2.6**). Cultivars previously classified

as early maturity resulted in earlier heading dates (range: 122 to 123 DOY) while cultivars classified as late maturity had heading dates ranging from 125 to 127 DOY, with clear separation between early- and late-maturity groups (**Table 2.6**). Cultivar interactions with environment and with nitrogen rates were subtle and overall did not change the initial classification of the cultivars, with early cultivars heading approximately 5 days earlier than late-maturing ones in 2024-25.

2.3.3 Effects of Wheat Cultivars Traits on Nitrogen Response

Total N available modulated all crop response variables measured (grain yield, yield components, protein concentration, anthesis N uptake, and NUE) in all analyses irrespective of cultivar trait being evaluated (**Tables 2.7, 2.9, and 2.11**).

2.3.3.1 Phenotypic Plasticity

The agronomic trait yield PP, which considered cultivars as random effects nested within PP group, interacted with the environment to modulate all traits except biomass, head number, and anthesis N uptake, which were only affected by environment and not PP (**Table 2.7**). High-PP varieties had greater grain yield than their low-PP counterparts in five environments (24-BE.S, 25-HAYS, 25-HOI, 25-MNH, and 25-RUS). The effect of yield PP on yield components was more variable, with higher biomass in one environment, greater HI in six environments, more kernels per spike in two environments, and higher NUE in five environments (24-BE.S, 25-AB, 25-HAYS, 25-MNH, 25-RUS). Of interest, there was no tradeoff (i.e., the low-PP did not show greater trait expression in any environment) (**Table 2.8**).

The response of grain yield to total nitrogen availability was markedly different between the highest and the lowest PP cultivars (**Figure 2.4; A-C**). The quadratic plateau models in the three environments where the trait was statistically significant suggested that the cultivar with the highest PP had considerably greater yield plateaus at a slightly greater AONA. This is portrayed across the 14 selected environments, where the highest PP cultivar produced approximately 0.06 Mg ha⁻¹ more yield with no more than ~11 kg N ha⁻¹ greater AONA (**Figure 2.4; D**). The Δ grain yield was always positive for plasticity, and delta AONA was positive in 10 out of 14 sites. The slope of this relationship suggested that more plastic cultivars produced 63 kg more grain per kg N ha⁻¹ at the AONA.

2.3.3.2 Grain Protein Concentration

The agronomic trait GPD, with cultivars treated as random effects nested within GPD group, interacted with the environment to modulate all variables except anthesis N uptake and NUE, which were influenced by environment but not by GPD (**Table 2.9**). High-GPD cultivars exhibited greater grain protein concentration in nine environments and higher anthesis N uptake in one environment. Low-GPD cultivars achieved higher grain yield in one environment and showed variable responses for yield components across environments: biomass and NUE were greater in three environments, head number and kernels per spike were higher in one environment, and kernels per m² were greater in two environments. Notably, cultivars with higher-GPD achieved similar grain yields compared to low-GPD cultivars in all environments except 25-HAYS, indicating no consistent yield penalty associated with increased GPD (**Table 2.10**).

The response of grain yield to total N availability differed between GPD cultivar groups only in the 25-HAYS environment (**Figure 2.5; A**). The quadratic–plateau model indicated that the cultivar with highest GPD reached a yield plateau of 2.7 Mg ha⁻¹ and an AONA of 216 kg N ha⁻¹, while the cultivar with the lowest GPD needed a slightly higher AONA of 221 kg N ha⁻¹ to achieve a 3.8 Mg ha⁻¹ yield plateau. Across the 14 environments (**Figure 2.5; B**), Δ grain yield was negative in all except one, and Δ AONA was negative in 10 out of 14 sites, suggesting that the highest GPD cultivar underperformed the lowest GPD cultivar with respective lower AONA.

2.3.3.3 Maturity

The interaction between maturity group and environment was statistically significant for all response variables except biomass, anthesis N uptake, and NUE, which were more strongly influenced by environment (**Table 2.11**). Least-square means between maturity groups did not differ in grain yield, showing only slight differences in yield components. Early-maturing cultivars showed greater biomass and anthesis N uptake in one environment and higher HI and NUE in two environments. Late maturing cultivars exhibited higher anthesis nitrogen uptake in one environment (**Table 2.12**).

Maturity effects on yield response to nitrogen were evaluated in relation to mean temperature during the grain-filling period. Late-maturing cultivars experienced slightly higher grain-fill temperatures than early-maturing cultivars (18.4 °C vs. 18.1 °C; **Figure 2.6A**). However, the relationship between yield response to N and grain-fill temperature was environment-dependent. In 25-MNH, the only environment showing a maturity effect, early-maturing cultivars exhibited greater responsiveness to N than late-maturing ones (82.5% vs. 75.0%; **Figure 2.6B**) and yield response to N was greater under cooler grain-fill temperatures,

declining as temperature increased (a 62% reduction in yield responsiveness per 1 °C increase) (**Figure 2.6C**). Across all environments and N rates, however, most regressions between yield response to N and grain-fill temperature were not significant (**Figure 2.6D**).

2.3.4 Variety Trait Influence on the Relationships Between Wheat Grain Yield and Yield Components

Across all sources of variation, grain yield was tightly related to shoot biomass at maturity, kernels m^{-2} , and spikes m^{-2} , it was less but still significantly related to anthesis N uptake, and showed weak to no relationship with kernels spike $^{-1}$, thousand kernel weight, and grain test weight (**Figure 2.7**). Cultivar yield plasticity affected the relationships among yield and biomass, spikes m^{-2} , and kernels m^{-2} . High plasticity cultivars had steeper slopes for the relations of yield and biomass (3.9 vs. 3.6 $\text{kg} \cdot \text{ha}^{-1} \text{ g} \cdot \text{m}^{-2}$) and spikes m^{-2} (4.5 vs. 3.9 $\text{kg} \cdot \text{ha}^{-1} \text{ spikes} \cdot \text{m}^{-2}$), and greater intercepts for the relation of yield and kernels m^{-2} (**Figure 2.7; A, C, F**). For all other yield components, a single regression portrayed both cultivar groups well. Likewise, cultivars with higher GPD had greater grain yield and slopes for spikes m^{-2} (4.7 vs. 3.6 $\text{kg} \cdot \text{ha}^{-1} \text{ m}^{-2}$), kernel spike $^{-1}$ (114.3 vs. 89.9 $\text{kg} \cdot \text{ha}^{-1} \text{ m}^{-2}$), and kernels m^{-2} (0.3 vs. 0.2 $\text{kg} \cdot \text{ha}^{-1} \text{ m}^{-2}$), while relationships with the other variables were similar between groups (**Figure 2.8; C, D, F**). Early-maturity cultivars showed greater grain yield for biomass, kernel spike $^{-1}$, kernels m^{-2} , and anthesis N uptake, but all other traits were not affected. Early group cultivars had steeper slopes for biomass (3.9 vs. 3.6 $\text{kg} \cdot \text{ha}^{-1} \text{ g} \cdot \text{m}^{-2}$), kernel spike $^{-1}$ (128.6 vs. 85.2 $\text{kg} \cdot \text{ha}^{-1} \text{ m}^{-2}$), kernels (0.3 vs. 0.2 $\text{kg} \cdot \text{ha}^{-1} \text{ m}^{-2}$), and anthesis N uptake (13.2 vs. 10.7 $\text{kg} \cdot \text{ha}^{-1} \text{ kg} \cdot \text{ha}^{-1}$) (**Figure 2.9; A, D, F, H**).

2.3.5 Nitrogen Responsiveness of Grain Yield and Its Components

Yield responsiveness to N associated positively with responsiveness to N of shoot biomass, spikes m^{-2} , kernels m^{-2} , kernels spike $^{-1}$, and anthesis N uptake (**Figures 2.10 and 2.11**). For these yield components, increasing N rates resulted in greater slopes of yield responsiveness to N versus yield component responsiveness to N (**Figures 2.10 and 2.11**). Among these yield components, the only that was cultivar-dependent was kernels m^{-2} , for which more plastic cultivars had greater responsiveness than less plastic ones (**Figure 2.11; C**). Yield responsiveness to N was not associated with the responsiveness to N of TKW or HI. The relationships between yield responsiveness to N and yield component responsiveness to N did not differ within other trait groups (GPD- or maturity, **Appendix Figures A.3 and A.4**).

2.4 Discussion

2.4.1 Validation of Agronomic Trait Hypotheses

The validation of hypothesized agronomic traits across multiple environments confirmed the accuracy of cultivar classifications based on preliminary data. The alignment between measured and expected trait behavior (PP, GPD, and maturity) supported the initial selection approach and highlighted the stability of these traits under the rainfed field conditions in Kansas. This suggests that the evaluated traits may be highly heritable and therefore cultivar selection based on these traits may yield consistent (Monaghan et al. 2001; Oury and Godin 2007; Rapp et al., 2018). Cultivars classified as high plasticity had steeper slopes in their reaction norms, meaning their yields were more responsive to changes in environmental conditions (Eberhart & Russell, 1966; Sadras and Richards, 2014; Giordano et al., 2024, 2025a). The exception was that

the most plastic stable cultivar (WB4269), and the most stable plastic cultivar (LCS Julep), had similar slopes (1.02). This contrasts with the preliminary data used for cultivar selection, where their slopes were 0.11 units apart (Giordano et al., 2025a). We believe that, to some extent, this is a function of using only eight cultivars to quantify plasticity in the current analysis. Plasticity estimates of a group of cultivars tested in the same environments mathematically average one, whether estimated as slopes of the reaction norm or as variance ratio. Therefore, while the most extreme cultivars still segregate well, the cultivars towards the middle are more likely to overlap. Still, we maintained these cultivars in their original plasticity group because the cultivar selection exercise used a more robust database.

Cultivars previously identified as high grain protein concentration maintained consistently positive GPD values while those classified as low GPC showed negative values across environments. Applying the GPD approach to measure cultivars that achieve higher GPC than expected for a given yield level across environments and nitrogen rates allowed us to identify cultivars that deviated most favorably for this trait. Variation in heading date is driven by genetic controls regulating vernalization and photoperiod sensitivity, which influence the duration of vegetative development and the timing of reproductive transition (Hyles et al., 2020). Environmental conditions also influence heading date, as temperature accumulation and photoperiod are closely associated with wheat development (Gonzalez et al., 2002; Sadras & Monzon, 2006). Consistent with previous studies, environmental variation influenced the timing of heading among cultivars. Despite our results being limited by the small sample size, as expected in experimental trials (Wang et al., 2023b), the combination of eight cultivars, seven N rates, and fourteen environments, suggested that the cultivar selection was appropriate to test our hypothesis.

2.4.2 Effects of Wheat Cultivar Traits on Nitrogen Response

2.4.2.1 Phenotypic Plasticity

In this study, we hypothesized that cultivars with greater phenotypic plasticity of grain yield would exhibit a greater yield response to increasing nitrogen availability, and our results support this hypothesis. Overall, our results showed that cultivars classified as high plasticity yielded more than their counterparts and exhibited a greater response to nitrogen compared to low plasticity cultivars. The high yield plasticity group achieved greater grain yields than the low plasticity group in high-yielding environments, aligning with recent literature suggesting that phenotypic plasticity of yield and yield are associated in high-yielding environments in the US Great Plains (Grogan et al., 2016; Lollato et al., 2020; Giordano et al., 2024; 2025a). More plastic cultivars also requested slightly higher total available nitrogen to achieve their respective grain yield plateaus, suggesting that the $G \times E$ interaction appears to be a leading factor on wheat response to nitrogen, as observed in the literature (Lemaire and Ciampitti, 2020). The slope of delta yield versus delta AONA indicated that more plastic cultivars had greater yields at the plateau at $63 \text{ kg.yield kg.N}^{-1}$. This parameter represents a system's nitrogen use efficiency that can be achieved via cultivar selection. Although high yield plasticity cultivars required more nitrogen to achieve their yield plateau, as observed before by Lenoir et al. (2023), this NUE estimate falls near the upper limit of the range of long-term estimates of wheat NUE in the region (range: -10.3 to 74.4 kg.grain kg.N⁻¹, mean: 12.1 kg.grain kg.N⁻¹; Lollato et al., 2019a), suggesting an avenue for improved NUE via cultivar selection. These results suggest that phenotypic plasticity of yield may not only be an effective tool for maximizing the use of the

total nitrogen available in the system, but also that this trait may be sought after in breeding programs due to its predictability and genetic control (Giordano et al., 2025a).

2.4.2.2 Grain Protein Deviation

Cultivars with higher GPD were hypothesized to require greater nitrogen inputs to maximize grain yield than low GPD cultivars because they produce more protein per unit yield, but our results do not support this hypothesis. Instead, the agronomic optimum nitrogen availability was similar or slightly lower for high GPD cultivars relative to low GPD cultivars, indicating that higher GPD did not translate into a higher N requirement to reach maximum yield primarily driven by slightly lower yields. There was one environment where grain yield was statistically lower for high GPD-cultivars, and high GPD cultivars had numerically lower grain yield than low GPD cultivars at most sites, indicating that while GPD may be positively correlated with GPC it may not be with yield, as previously suggested (Rapp et al., 2018; Giordano et al., 2024). The delta AONA also indicated that high-GPD cultivars had slightly lower AONA. For instance, the highest GPD cultivar (LCS Chrome) had an AONA of 216.2 as compared to 221.3 kg ha⁻¹ for the lowest GPD cultivar (WB4699). This contrasts with previous literature suggesting that higher GPD cultivars may require higher nitrogen rates to achieve their maximum grain yield (Woodard and Bly, 1998; Ghimire et al., 2021; Lollato et al., 2021). But again, within the context of our data, these differences are likely due to a slightly lower grain yield in higher GPD cultivars in the current research. The significant interaction of GPD group × environment on all response variables except for anthesis N uptake and NUE, which were led by the environmental conditions and the total N available, suggests that differences in cultivar GPD may result from post-anthesis N uptake, as observed elsewhere (Nehe et al, 2018, 2020). At a

first glance, the high GPD group had greater grain protein concentration in nine out of 14 environments and comparable grain yield across all environments except for one, indicating that this trait can be effective in mitigating the negative relation between protein and yield (Barneix, 2007; Lollato et al., 2021; Ghimire et al., 2021). These results suggest that GPD can be used as a cultivar selection trait for preserving adequate protein percentage to manage the usual reduction in grain protein concentration at a given grain yield level (Giordano et al., 2024).

2.4.2.3 Maturity

We hypothesized that early-maturing cultivars would avoid high-temperature stress during grain filling, thereby enhancing yield responsiveness to nitrogen. Overall, our results supported that early-maturing cultivars were exposed to slightly cooler temperatures during grain filling; however, this did not consistently translate into greater responsiveness to N across environments. In fact, a greater nitrogen responsiveness to nitrogen of earlier cultivars only occurred in one environment, in which grain fill temperature was negatively related to nitrogen responsiveness, indicating that while our hypothesis was correct, it was not widespread.

Our hypothesis was established based on previous studies reporting that heat stress during the critical period can reduce crop responsiveness to nitrogen. For instance, Cossani and Sadras (2021) and Sadras et al. (2022) reported a reduction in grain yield responsiveness to N with increasing mean temperatures during the critical period for yield determination in wheat. We used grain fill temperature instead of critical period temperature due to the importance of heat stress during grain filling in determining wheat yield in this region (Lollato et al., 2017; Bergkamp et al., 2018), but results using the critical period temperature were extremely similar to those using grain fill temperature (**Appendix Figure A.5**). Other studies highlight wheat

maturity as a crucial factor driving yield, with earlier maturing cultivars reducing the exposure to later-season stress factors, such as water deficit stress and high temperatures during grain filling (Acevedo et al., 2002; Tewolde et al., 2006; Mondal et al., 2015).

In our study, however, most environments showed no significant relationship between grain-fill temperature and N responsiveness, suggesting that temperature alone was not a dominant driver of N response. One potential explanation for the differences between our results and the previous literature is that some of the growing seasons studied had relatively long and moist grain fill period, which potentially benefited later-maturing varieties due to a slightly longer reproductive period. This may explain the lack of significant difference between early (exposed to cooler temperatures) and late-maturing cultivars (exposed to a more positive water balance). A second potential explanation relates to the experimental design. While Cossani and Sadras (2021) and Sadras et al. (2022) had experiments that imposed heat treatments, water stress levels, and sowing dates within environments, avoiding these confounding factors, in our case the only driver for different temperatures was cultivar maturity. Since the difference in maturity was not extremely large (i.e., 3-4 days to heading), the difference in magnitudes of within-year temperatures among treatments may not have been enough to capture this effect. This suggests that, for current cultivars adapted to this region, which have been optimized for maturity (Maeoka et al., 2020), grain fill temperature differences are likely not large enough to modulate nitrogen responses.

2.5. Conclusion

This study evaluated the effect of cultivar traits – yield plasticity, grain protein deviation, and maturity – on winter wheat yield response to nitrogen across multiple environments in

Kansas. Our results suggested that phenotypic plasticity of grain yield is a promising cultivar trait for enhancing systems-level nitrogen use efficiency, since highly plastic cultivars produced substantially more grain, requiring a slightly increased minimum amount of nitrogen to maximize grain yield. Yield plasticity consistently conferred yield or efficiency advantages without yield tradeoffs, indicating agronomic benefit under varying N availability. In contrast, grain protein deviation and cultivar maturity appeared to have more limited potential to modulate wheat nitrogen response. Cultivars with higher GPD had greater grain protein concentration but were slightly lower yielding, resulting in similar to slightly lower AONA than cultivars with lower GPD. Likewise, although early-maturing cultivars were exposed to cooler temperatures during the grain filling period, they showed a greater yield response to N in only one environment, likely owing to optimized maturity across cultivars resulting in a small range of temperatures within environment.

Table 2.1 Description of the fourteen environments where field experiments were conducted, including year, location, environment, longitude, latitude, soil type, previous crop, tillage (NT, no-till; CT, conventional till), sowing date, nitrogen (N) application date, and harvest date.

Year	Location	Environment	Lat. (degrees)	Long. (degrees)	Soil type	Previous Crop	Tillage	Initial Soil N Content (kg ha ⁻¹)	Sowing Date	N Application Date	Harvest Date
2023-24	Ashland Bottoms	24-AB	39.15	-96.63	Silt Loam	Soybeans	NT	126.5	9/21/2023	3/19/2024	6/29/2024
2023-24	Belleville	24-BEL.S	39.81	-97.67	Silty Clay Loam	Soybeans	NT	71.0	10/20/2023	3/19/2024	6/26/2024
2023-24	Hays	24-HAYS	38.85	-99.34	Silt Loam	Forage	CT	352.2	9/27/2023	4/11/2024	7/5/2024
2023-24	Hoisington	24-HOI	38.62	-98.96	Loam	Fallow	CT	454.7	9/26/2023	4/2/2024	6/14/2024
2023-24	Manhattan	24-MHN	39.22	-96.59	Silty Clay Loam	Soybeans	NT	83.9	10/11/2023	3/18/2024	7/8/2024
2023-24	McPherson	24-MCP	38.24	-97.57	Silty Clay Loam	Soybeans	NT	144.0	11/2/2023	3/20/2024	6/15/2024
2023-24	Phillipsburg	24-PHG	39.73	-99.27	Silt Loam	Fallow	CT	303.4	9/29/2023	3/22/2024	6/25/2024

2024-25	Ashland Bottoms	25-AB	39.14	-96.63	Silt Loam	Soybeans	NT	60.5	10/24/2024	3/26/2025	6/30/2025
2024-25	Belleville	25-BEL.S	39.81	-97.63	Silt Loam	Soybeans	NT	116.8	10/15/2024	3/28/2025	7/4/2025
2024-25	Hays	25-HAYS	38.85	-99.34	Silty Clay Loam	Sorghum – Fallow	NT	209.0	10/8/2024	3/28/2025	6/27/2025
2024-25	Hoisington	25-HOI	38.63	-98.86	Silty Clay Loam	Sorghum – Fallow	NT	305.2	9/27/2024	4/10/2025	6/21/2025
2024-25	Hutchinson	25-HUT.O	37.92	-98.02	Loam	Oats (no N)	CT	162.0	11/16/2024	4/2/2025	6/29/2025
2024-25	Manhattan	25-MHN	39.21	-96.58	Silty Clay Loam	Soybeans	NT	100.5	10/21/2024	3/26/2025	7/4/2025
2024-25	Russel	25-RUS	39.14	-96.63	Silty Clay Loam	Sorghum	NT	105.3	10/14/2024	4/3/2025	6/22/2025

Table 2.2 Varieties selected based on agronomic traits (phenotypic plasticity of yield -PP, grain protein concentration, and maturity), and validation of traits based on their performance across environments.

Variety	Phenotypic Plasticity of yield	GPC	Maturity
SY Wolverine	High	High	Early
WB4401	High	Low	Early
WB4699	High	Low	Late
LCS Julep	High	High	Late
WB4269	Low	Low	Early
SY Monument	Low	Low	Late
LCS Chrome	Low	High	Late
Bob Dole	Low	High	Early

Table 2.3 Soil fertility measured at sowing in the 0-15 or 0-30 and 15-45 or 15-60 cm depths in all environments.

Sample Name	Depth	OM	pH	NO ₃ N	NH ₄ N	P-M	K	S	Ca	Cu	Mg	Mn	Na	Zn	Fe	Cl	CECS	Sand	Silt	Clay
	cm	%	----- ppm -----														Meq 100g ⁻¹	----- % -----		
24-AB	0 to 15	1.7	6.1	24.4	5.1	65.0	198.0	4.0	1283	0.6	119.0	10.1	16.0	1.0	43.2	5.6	8.00	35	53	12
24-AB	15 to 60	1.4	7.7	5.4	4.0	34.0	154.0	2.4	2118	0.6	87.0	4.5	13.0	0.9	29.6	2.8	11.80	35	53	12
24-BEL.S	0 to 15	3.2	5.0	14.7	5.5	54.0	478.0	7.1	1561	1.8	197.0	43.5	18.0	1.4	157.3	4.6	21.50	16	61	23
24-BEL.S	15 to 30	2.9	5.7	5.3	4.7	35.0	435.0	4.3	2756	1.7	376.0	23.4	37.0	0.7	92.4	5.1	26.20	17	48	35
24-HAYS	0 to 15	2.4	5.2	53.2	6.9	39.0	441.0	6.4	1482	1.2	177.0	48.8	27.0	0.7	62.5	12.3	18.20	9	70	21
24-HAYS	15 to 60	2.1	6.6	33.5	3.0	17.0	298.0	3.1	2533	0.9	369.0	19.4	24.0	0.4	24.2	6.1	19.40	16	67	17
24-HOI	0 to 15	2.1	7.2	43.0	10.6	28.0	177.0	11.3	4339	0.9	186.0	12.1	21.0	1.1	33.2	5.6	23.80	31	46	23
24-HOI	15 to 60	1.9	8.0	31.0	24.6	17.0	147.0	6.2	5624	0.6	167.0	5.7	20.0	0.3	12.3	4.7	30.00	32	40	28
24-MHN	0 to 15	4.6	6.8	9.4	11.5	27.0	234.0	6.6	4505	1.5	275.0	23.4	21.0	2.4	67.5	6.1	29.30	16	56	28
24-MHN	15 to 30	3.7	7.2	6.2	9.5	13.0	218.0	4.9	5499	1.6	277.0	13.8	22.0	0.9	54.5	6.4	30.50	14	53	33
24-MCP	0 to 15	2.9	6.5	9.4	14.4	36.0	330.0	6.8	3123	1.3	576.0	16.0	49.0	4.6	59.6	21.6	25.50	16	49	35
24-MCP	15 to 45	2.4	6.8	10.7	10.6	19.0	319.0	5.7	3585	1.3	788.0	11.2	115.0	2.7	47.4	9.6	29.30	15	45	40
24-PHG	0 to 15	2.5	6.1	40.6	2.5	97.0	651.0	6.1	1920	1.1	234.0	19.3	14.0	2.5	45.3	4.6	17.70	20	56	24

24-PHG	15 to 60	2.2	6.9	31.4	2.7	110.0	702.0	5.8	2676	0.9	367.0	9.7	16.0	1.9	20.5	5.2	18.30	18	53	29
25-AB	0 to 15	1.8	6.4	5.6	3.2	25.1	236.4	2.4	1409	0.7	153.3	10.2	20.3	0.6	3.4	54.0	9.02	26	62	12
25-AB	15 to 60	1.1	7.3	2.5	2.9	17.7	144.8	1.7	1366	0.5	121.3	3.6	21.0	0.3	2.9	20.4	8.30	28	62	10
25-BEL.S	0 to 15	2.6	5.3	12.5	4.1	37.8	340.6	4.7	1418	1.3	189.2	35.8	31.0	1.0	6.6	135.4	19.92	14	62	24
25-BEL.S	15 to 60	2.6	6.3	6.1	6.0	20.9	266.8	3.1	2159	1.3	273.0	16.9	45.1	0.5	5.7	87.2	19.77	14	58	28
25-HAYS	0 to 15	2.5	6.7	17.2	5.2	65.5	578.8	5.4	2555	1.4	418.6	16.1	21.6	0.9	6.8	41.6	17.84	16	52	32
25-HAYS	15 to 60	1.9	7.5	20	5.4	54.0	521.3	6.0	3802	1.4	505.2	11.4	27.9	0.5	6.8	29.7	24.68	12	53	35
25-HOI	0 to 15	2.6	6.0	32.1	4.1	32.0	367.8	5.2	2129	1.3	363.1	26.4	33.3	0.7	8.1	80.9	20.89	16	57	27
25-HOI	15 to 60	2.1	7.1	29.4	7.3	33.6	415.9	5.6	3251	1.3	570.2	13.1	70.8	0.6	10.1	46.7	22.38	10	52	38
25-HUT.O	0 to 15	1.7	5.9	7.6	3.5	60.8	195.8	5.4	1347	1.0	168.0	20.4	21.3	0.4	3.5	70.4	14.65	39	43	18
25-HUT.O	15 to 60	1.8	8.0	16.7	4.7	8.0	176.0	4.3	5403	0.5	214.4	3.1	25.6	0.1	4.2	16.8	29.36	36	36	28
25-MHN	0 to 15	3.1	6.8	8.2	6.6	21.8	197.9	3.6	3579	1.6	337.0	18.8	26.3	1.6	3.7	63.1	21.33	14	60	26
25-MHN	15 to 60	2.6	7.8	3.9	6.1	11.0	233.2	3.0	5816	1.3	356.7	7.0	30.7	0.6	5.7	30.8	32.79	12	58	30
25-RUS	0 to 15	2.8	6.4	8.2	5.1	38.3	544.7	5.1	2243. 9	1.3	281.6	24.3	22.4	1.3	9.0	70.7	15.06	14	62	24
25-RUS	15 to 60	1.9	7.2	4.8	6.5	15.7	439.6	4.0	3174. 8	1.0	480.9	12.0	37.0	1.6	9.8	30.8	21.17	12	54	34

Table 2.4 Parameters of the linear relationship between wheat grain yield and environmental index (EI) for eight winter wheat cultivars evaluated under seven nitrogen rates and 14 Kansas environments during the 2024 and 2025 harvest seasons. The hypothesized cultivar yield phenotypic plasticity (PP) classification used preliminary data is shown. Values represent the intercepts and slopes followed by standard error of the estimate, and R^2 . All relationships were statistically significant at $p < 0.001$.

Cultivar	PP	Intercept	Slope	R²
Bob Dole	low	2.81 ± 0.03	0.91 ± 0.02	0.83
LCS Chrome	low	2.79 ± 0.02	0.96 ± 0.02	0.89
SY Monument	low	2.87 ± 0.02	0.97 ± 0.02	0.92
WB4269	low	3.06 ± 0.02	1.02 ± 0.02	0.88
LCS Julep	high	3.00 ± 0.02	1.02 ± 0.02	0.90
WB4699	high	3.02 ± 0.03	1.04 ± 0.02	0.87
WB4401	high	3.25 ± 0.03	1.04 ± 0.02	0.86
SY Wolverine	high	3.19 ± 0.03	1.04 ± 0.02	0.87

Table 2.5 Least square mean of grain protein deviation (GPD) as affected by cultivar (LCS Chrome, Bob Dole, LCS Julep, SY Wolverine – classified as high grain protein content; WB4269, SY Monument, WB4401, WB4699 – classified as low grain protein content) and environment. Means within the same environment, followed by different lowercase letters differ significantly according to Tukey's test at $p \leq 0.05$.

Environment	High GPC				Low GPC			
	LCS Chrome	Bob Dole	LCS Julep	SY Wolverine	WB4269	SY Monument	WB4401	WB4699
24-AB	0.38 ab	0.56 a	-0.02 b	0.14 ab	0.28 ab	0.05 b	-0.71 c	-0.68 c
24-BE.S	0.33 ab	0.53 a	-0.14 bc	0.32 ab	0.26 ab	-0.16 bc	-0.46 c	-0.62 c
24-HAYS	0.98 a	0.47 bc	0.68 ab	0.33 bc	0.13 c	-0.66 d	-0.91 d	-0.83 d
24-HOI	0.27 cd	0.86 ab	0.48 bc	1.08 a	-1.33 f	-0.6 e	0.01 d	-0.81 e
24-MCP	1.36 a	0.66 b	0.65 b	0.31 b	-0.6 cd	-0.2 c	-1.37 e	-0.82 d
24-MNH	0.37 a	0.52 a	0.46 a	0.11 ab	-0.22 bc	0.26 ab	-0.82 d	-0.58 cd
24-PHG	0.49 a	0.33 ab	-0.43 de	0.29 ab	0.08 abc	-0.61 e	-0.04 bcd	-0.2 cde
25-AB	0.48 ab	0.58 a	0.24 abc	-0.02 bcd	-0.15 cde	-0.14 cde	-0.42 de	-0.57 e
25-BE.S	0.74 a	0.38 ab	0.16 b	-0.1 bc	-0.12 bc	-0.07 bc	-0.58 c	-0.42 c
25-HAYS	0.25 a	0.1 abc	-0.05 abc	0.14 ab	0.3 a	-0.2 bc	-0.21 bc	-0.33 c
25-HOI	0.35 a	0.41 a	0.2 ab	0.1 abc	0.06 abc	-0.16 bcd	-0.4 cd	-0.57 d
25-HUT.O	1.35 a	0.69 b	0.76 b	0.02 c	-0.92 d	-0.12 c	-0.68 d	-1.11 d
25-MNH	0.1 a	0.19 a	0.11 a	-0.07 a	0.1 a	-0.08 a	-0.21 a	-0.15 a
25-RUS	0.35 a	0.33 a	0.09 a	0.25 a	0.02 ab	-0.04 ab	-0.64 c	-0.36 bc
Mean	0.56 a	0.47 a	0.23 b	0.21 b	-0.15 c	-0.20 c	-0.53 d	-0.58 d

Table 2.6 Least square means of heading date as affected by cultivar and nitrogen rate, and cultivar and environment interactions. Cultivars were classified according to maturity group as late maturity (LCS Julep, SY Monument, LCS Chrome, WB4699) and early maturity (Bob Dole, WB4401, WB4269, SY Wolverine). Means followed by different lowercase letters within nitrogen rate or within environment differ significantly according to Tukey's test at $p \leq 0.05$.

Nitrogen rate (kg ha ⁻¹)	Late				Early			
	LCS Julep	LCS Chrome	SY Monument	WB4699	Bob Dole	WB4269	WB4401	SY Wolverine
0	127 a	126 b	126 b	125 b	123 c	122 d	122 d	122 d
34	127 a	126 ab	126 bc	125 c	124 d	123 e	123 e	122 e
67	127 a	126 b	126 b	125 b	124 c	123 cd	122 d	122 d
101	127 a	126 a	125 b	125 b	123 c	123 cd	122 e	122 de
135	127 a	126 a	126 a	126 a	123 b	122 c	122 c	122 c
168	127 a	126 ab	125 c	126 bc	123 d	123 de	122 e	122 e
202	127 a	126 ab	125 b	126 b	124 c	123 cd	122 d	122 d
Environment								
25-AB	127 a	126 a	125 bc	125 b	124 c	124 c	120 d	121 d
25-BE.S	131 a	130 b	131 a	131 a	128 c	127 c	128 c	126 d
25-HAYS	126 a	123 c	126 a	124 b	122 d	120 e	120 e	121 d
25-HOI	123 a	122 a	123 a	123 a	120 b	119 cd	119 d	120 bc
25-HUT.O	128 b	130 a	123 d	126 c	123 d	124 d	123 d	123 d
25-MNH	130 ab	130 a	129 b	126 c	127 c	125 d	126 cd	124 e
25-RUS	124 a	123 b	123 b	123 ab	121 c	120 d	119 d	119 d
Mean	127 a	126 b	126 c	125 c	123 d	123 e	122 f	122 f

Table 2.7 Analysis of covariance (ANCOVA) for yield, yield components, protein, and nitrogen-related traits of wheat as affected by phenotypic plasticity (PP), environment (E), and their interaction, with total nitrogen availability (N total) included as a covariate. Values represent *p*-values for grain yield (Mg ha⁻¹), shoot biomass (g m⁻²), head number (m⁻²), harvest index (HI), thousand kernel weight (TKW), kernels per spike, kernels per unit area (m⁻²), grain protein (%), nitrogen uptake (g m⁻²), and nitrogen use efficiency (NUE).

Source	Grain Yield (Mg ha ⁻¹)	Shoot Biomass (g m ⁻²)	Head number (m ⁻²)	HI	TKW	Kernels spike ⁻¹	Kernels (m ⁻²)	Protein (%)	N uptake (kg ha ⁻¹)	NUE (kg kg ⁻¹)
Intercept	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PP	0.24	0.94	0.90	0.07	0.73	0.48	0.50	0.08	0.75	0.43
E	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N total	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PP x E	<0.001	0.23	0.15	<0.001	<0.001	<0.001	0.01	<0.001	0.66	<0.001

Table 2.8 Means for grain yield, yield components, grain protein, and nitrogen-related traits of wheat as affected by phenotypic plasticity (PP) and environments. Values represent the mean of yield (Mg ha⁻¹), shoot biomass (g m⁻²), head number (m⁻²), harvest index (HI), thousand kernel weight (TKW), kernels per spike, kernels per unit area (m⁻²), grain protein (%), nitrogen uptake (g m⁻²), and nitrogen use efficiency (NUE). Means followed by different lowercase letters indicate significant differences according to Tukey's test at $p \leq 0.05$.

Environment	PP	Grain Yield (Mg ha ⁻¹)	Shoot Biomass (g m ⁻²)	Head number (m ⁻²)	HI	TKW	Kernels spike ⁻¹	Kernels (m ⁻²)	Protein (%)	N uptake (kg ha ⁻¹)	NUE (kg kg ⁻¹)
24-AB	high	3.99a	1135a	700a	0.44a	31.7a	22.6a	15798a	12.2a	118.6a	18.7a
24-AB	low	3.86a	1132a	707a	0.41a	30.9a	21.6a	15071a	12.8a	120.1a	18.3a
24-BE.S	high	3.47a	1363a	826a	0.41a	31.1a	22.2a	18437a	12.6a	122.1a	23.3a
24-BE.S	low	3.15b	1320a	828a	0.38b	29.9a	20.3a	16727a	13.3a	117.4a	21.3b
24-HAYS	high	0.51a	294a	233a	0.47a	26.9a	22.7a	5268a	12.8a	31.0a	1.2a
24-HAYS	low	0.46a	261a	233a	0.46a	26.2a	19.9a	4577a	13.2a	29.8a	1.0a
24-HOI	high	0.86a	535a	550a	0.21a	24.8a	8.20a	4828a	18.7a	83.2a	1.6a
24-HOI	low	0.87a	519a	571a	0.2a	22.4a	8.30a	5052a	18.3a	80.9a	1.6a
24-MCP	high	3.53a	1048a	677a	0.45a	31.3a	22.7a	15265a	14.3a	122.7a	15.7a
24-MCP	low	3.55a	1049a	700a	0.43a	30.6a	21.4a	14810a	14.9a	124.2a	15.8a
24-MNH	high	3.35a	1142a	771a	0.43a	30.7a	21.2a	16462a	11.7a	114.6a	20.1a
24-MNH	low	3.21a	1089a	753a	0.40a	29.7a	19.9a	14984a	12.3a	105.1a	19.3a
24-PHG	high	4.37a	1432a	976a	0.38a	30.1a	18.6a	18366a	12.2a	151.3a	11.0a
24-PHG	low	4.17a	1398a	1037a	0.36a	28.0a	17.2a	17993a	12.8a	146.6a	10.5a
25-AB	high	3.48a	1103a	709a	0.44a	32.0a	21.8a	15469a	10.5a	121.0a	25.2a
25-AB	low	3.31a	1102a	734a	0.41b	30.7a	20.1a	14666a	11.0a	117.5a	23.8b
25-BE.S	high	2.87a	816a	528a	0.43a	32.5a	20.7a	11026a	10.6a	87.1a	14.1a
25-BE.S	low	2.77a	809a	575a	0.40a	30.1a	18.9a	10864a	11.2a	82.4a	13.6a
25-HAYS	high	3.88a	1406a	852a	0.40a	30.9a	22.0a	18806a	10.8a	142.9a	13.1a
25-HAYS	low	2.80b	1299b	905a	0.35b	27.9a	18.3b	16297a	11.6a	143.4a	9.5b
25-HOI	high	4.42a	1476a	874a	0.39a	29.2a	23.2a	20035a	11.2a	170.4a	11.1a
25-HOI	low	4.05b	1432a	920a	0.37a	27.6a	21.1a	19208a	11.7a	175.5a	10.2a
25-HUT.O	high	3.36a	878a	464a	0.51a	32.4a	30.5a	14027a	13.0a	134.2a	13.7a
25-HUT.O	low	3.42a	918a	521a	0.48b	31.0a	27.9a	14333a	13.5a	140.4a	14.0a
25-MNH	high	3.73a	1210a	758a	0.44a	33.2a	21.5a	16519a	9.7a	116.0a	19.6a
25-MNH	low	3.39b	1213a	798a	0.41b	30.9a	20.2a	15997a	10.3a	114.0a	17.8b
25-RUS	high	2.73a	917a	614a	0.45a	29.2a	23.2a	14171a	11.9a	95.8a	14.5a
25-RUS	low	2.31b	850a	669a	0.41b	26.7a	19.7b	12984a	12.6a	91.3a	12.3b

Table 2.9 Analysis of covariance (ANCOVA) for yield, yield components, protein, and nitrogen-related traits of wheat as affected by grain protein concentration (GPC), environment (E), and their interaction, with total nitrogen availability (N total) included as a covariate. Values represent *p*-values for grain yield (Mg ha⁻¹), shoot biomass (g m⁻²), head number (m⁻²), harvest index (HI), thousand kernel weight (TKW), kernels per spike, kernels per unit area (m⁻²), grain protein (%), nitrogen uptake (g m⁻²), and nitrogen use efficiency (NUE).

Source	Grain Yield (Mg ha ⁻¹)	Shoot Biomass (g m ⁻²)	Head number (m ⁻²)	HI	TKW	Kernels spike ⁻¹	Kernels (m ⁻²)	Protein (%)	N uptake (kg ha ⁻¹)	NUE (kg kg ⁻¹)
Intercept	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GPC	0.39	0.12	0.92	0.29	0.92	0.94	0.96	0.02	0.55	0.45
E	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N total	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GPC x E	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.17	0.01

Table 2.10 Means for grain yield, yield components, grain protein, and nitrogen-related traits of wheat as affected by grain protein concentration (GPC) and environments. Values represent the mean of yield (Mg ha⁻¹), shoot biomass (g m⁻²), head number (m⁻²), harvest index (HI), thousand kernel weight (TKW), kernels per spike, kernels per unit area (m⁻²), grain protein (%), nitrogen uptake (g m⁻²), and nitrogen use efficiency (NUE). Means followed by different lowercase letters indicate significant differences according to Tukey's test at $p \leq 0.05$.

Environment	GPD	Grain Yield (Mg ha ⁻¹)	Shoot Biomass (g m ⁻²)	Head number (m ⁻²)	HI	TKW	Kernels spike ⁻¹	Kernels (m ⁻²)	Protein (%)	N uptake (kg ha ⁻¹)	NUE (kg kg ⁻¹)
24-AB	high	3.98a	1103a	707a	0.43a	31.2a	22.1a	15476a	12.2b	117.8a	18.8a
24-AB	low	3.86a	1165a	701a	0.42a	31.4a	22.2a	15402a	12.8a	120.9a	18.3a
24-BE.S	high	3.33a	1385a	865a	0.39a	29.9a	21.7a	18682a	12.7a	123.0a	22.6a
24-BE.S	low	3.29a	1298b	788a	0.39a	31.1a	20.8a	16480a	13.2a	116.5a	22.1a
24-HAYS	high	0.50a	305a	240a	0.48a	26.1a	23.6a	5611a	12.4b	31.7a	1.1a
24-HAYS	low	0.47a	251a	225a	0.46a	27.0a	18.9b	4225a	13.6a	29.0a	1.1a
24-HOI	high	0.84a	539a	598a	0.20a	23.3a	8.0a	5122a	17.8b	86.2a	1.5a
24-HOI	low	0.88a	516a	522a	0.21a	23.8a	8.5a	4761a	19.1a	77.9a	1.6a
24-MCP	high	3.56a	1053a	709a	0.45a	30.3a	22.5a	15680a	13.8b	123.2a	15.9a
24-MCP	low	3.51a	1043a	668a	0.43a	31.6a	21.7a	14403a	15.3a	123.7a	15.6a
24-MNH	high	3.33a	1164a	806a	0.43a	28.9a	21.8a	17649a	11.6b	111.0a	20.0a
24-MNH	low	3.23a	1068b	719a	0.41a	31.5a	19.3a	13797b	12.4a	108.7a	19.4a
24-PHG	high	4.29a	1440a	1039a	0.37a	29.1a	18.2a	18907a	12.2a	146.8a	10.8a
24-PHG	low	4.24a	1391a	974a	0.36a	29.1a	17.7a	17453a	12.7a	151.0a	10.7a
25-AB	high	3.49a	1145a	784a	0.43a	31.0a	20.9a	16293a	10.3b	117.7a	25.3a
25-AB	low	3.30a	1061b	659b	0.41a	31.8a	20.9a	13841b	11.2a	120.7a	23.7b
25-BE.S	high	2.91a	835a	578a	0.42a	30.4a	20.3a	11759a	10.5b	82.9a	14.3a
25-BE.S	low	2.73a	790a	525a	0.41a	32.2a	19.3a	10132a	11.3a	86.6a	13.4a
25-HAYS	high	3.61a	1365a	893a	0.37a	29.6a	20.3a	17829a	10.9a	145.6a	12.2a
25-HAYS	low	3.08b	1340a	864a	0.38a	29.2a	20.0a	17274a	11.4a	140.7a	10.4b
25-HOI	high	4.24a	1422a	901a	0.38a	28.2a	22.0a	19573a	11.2a	169.9a	10.7a
25-HOI	low	4.23a	1486a	892a	0.38a	28.6a	22.2a	19671a	11.7a	175.9a	10.7a
25-HUT.O	high	3.39a	864a	486a	0.51a	31.4a	29.8a	14215a	12.5b	131.7b	13.8a
25-HUT.O	low	3.39a	933a	499a	0.49a	32.1a	28.5a	14143a	13.9a	142.8a	13.9a
25-MNH	high	3.70a	1233a	830a	0.43a	31.4a	20.6a	17108a	9.7b	115.3a	19.5a
25-MNH	low	3.43a	1190a	726a	0.42a	32.7a	21.2a	15408a	10.3a	114.7a	17.9b
25-RUS	high	2.51a	886a	658a	0.43a	27.9a	21.2a	13656a	12.0a	94.1a	13.4a
25-RUS	low	2.53a	881a	625a	0.43a	27.9a	21.7a	13500a	12.5a	93.0a	13.4a

Table 2.11 Analysis of covariance (ANCOVA) for yield, yield components, protein, and nitrogen-related traits of wheat as affected by maturity (MAT), environment (E), and their interaction, with total nitrogen availability (N total) included as a covariate. Values represent *p*-values for grain yield (Mg ha⁻¹), shoot biomass (g m⁻²), head number (m⁻²), harvest index (HI), thousand kernel weight (TKW), kernels per spike, kernels per unit area (m⁻²), grain protein (%), nitrogen uptake (g m⁻²), and nitrogen use efficiency (NUE).

Source	Grain Yield (Mg ha ⁻¹)	Shoot Biomass (g m ⁻²)	Head number (m ⁻²)	HI	TKW	Kernels spike ⁻¹	Kernels (m ⁻²)	Protein (%)	N uptake (kg ha ⁻¹)	NUE (kg kg ⁻¹)
Intercept	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MAT	0.41	0.37	0.39	0.29	0.22	0.25	0.68	0.75	0.67	0.46
E	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N total	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MAT x E	0.03	0.37	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.27	0.33

Table 2.12 Means for grain yield, yield components, grain protein, and nitrogen-related traits of wheat as affected by maturity (MAT) and environments. Values represent the mean of yield (Mg ha⁻¹), shoot biomass (g m⁻²), head number (m⁻²), harvest index (HI), thousand kernel weight (TKW), kernels per spike, kernels per unit area (m⁻²), grain protein (%), nitrogen uptake (g m⁻²), and nitrogen use efficiency (NUE). Means followed by different lowercase letters indicate significant differences according to Tukey's test at $p \leq 0.05$.

Environment	MAT	Grain Yield (Mg ha ⁻¹)	Shoot Biomass (g m ⁻²)	Head number (m ⁻²)	HI	TKW	Kernels spike ⁻¹	Kernels (m ⁻²)	Protein (%)	N uptake (kg ha ⁻¹)	NUE (kg kg ⁻¹)
24-AB	early	3.98a	1152a	728a	0.43a	32.7a	21.2a	15189a	12.6a	120.5a	18.8a
24-AB	late	3.87a	1116a	680a	0.42a	29.9a	23.1a	15689a	12.4a	118.3a	18.3a
24-BE.S	early	3.42a	1349a	845a	0.4a	31.7a	20.7a	17330a	13.0a	120.0a	23.1a
24-BE.S	late	3.19a	1334a	809a	0.38a	29.4a	21.8a	17847a	12.9a	119.5a	21.6b
24-HAYS	early	0.56a	283a	233a	0.48a	27.6a	21.4a	4890a	12.9a	30.5a	1.3a
24-HAYS	late	0.41a	273a	233a	0.46a	25.5a	21.1a	4952a	13.0a	30.3a	0.9a
24-HOI	early	1.00a	567a	608a	0.23a	25.0a	8.9a	5637a	18.4a	82.6a	1.8a
24-HOI	late	0.73a	487b	512a	0.17b	22.2a	7.7a	4242a	18.6a	81.5a	1.3a
24-MCP	early	3.64a	1053a	702a	0.46a	31.6a	22.1a	15249a	14.2a	124.7a	16.2a
24-MCP	late	3.44a	1044a	676a	0.43a	30.3a	22.0a	14834a	14.9a	122.1a	15.3a
24-MNH	early	3.26a	1088a	773a	0.42a	30.4a	19.9a	15384a	11.9a	110.7a	19.5a
24-MNH	late	3.30a	1144a	751a	0.41a	30.0a	21.2a	16063a	12.1a	109.0a	19.8a
24-PHG	early	4.38a	1439a	1020a	0.39a	30.7a	18.1a	18581a	12.5a	145.6a	11.0a
24-PHG	late	4.15a	1391a	993a	0.34b	27.5a	17.7a	17779a	12.5a	152.3a	10.5a
25-AB	early	3.51a	1128a	741a	0.43a	32.5a	20.5a	15161a	10.7a	119.3a	25.3a
25-AB	late	3.29a	1077a	702a	0.41a	30.2a	21.3a	14974a	10.8a	119.1a	23.7b
25-BE.S	early	2.82a	801a	542a	0.42a	32.6a	19.3a	10453a	10.8a	84.4a	13.8a
25-BE.S	late	2.82a	824a	562a	0.41a	30.0a	20.3a	11437a	11.0a	85.1a	13.9a
25-HAYS	early	3.44a	1358a	860a	0.38a	31.1a	19.7a	16559a	11.2a	147.8a	11.7a
25-HAYS	late	3.24a	1348a	897a	0.37a	27.7a	20.6a	18545a	11.1a	138.5b	11.0a
25-HOI	early	4.36a	1463a	867a	0.39a	30.0a	22.3a	19015a	11.5a	174.0a	11.0a
25-HOI	late	4.11a	1445a	927a	0.37a	26.8a	21.9a	20228a	11.5a	171.9a	10.4a
25-HUT.O	early	3.43a	900a	511a	0.51a	32.8a	28.2a	14165a	13.0a	132.2b	14.0a
25-HUT.O	late	3.35a	896a	474a	0.48a	30.7a	30.2a	14193a	13.4a	142.3a	13.7a
25-MNH	early	3.62a	1209a	780a	0.44a	33.8a	20.3a	15744a	9.9a	115.0a	18.9a
25-MNH	late	3.51a	1213a	776a	0.41a	30.3a	21.5a	16772a	10.1a	115.1a	18.4a
25-RUS	early	2.56a	916a	669a	0.43a	29.3a	20.4a	13440a	12.2a	97.0a	13.7a
25-RUS	late	2.48a	851a	615a	0.42a	26.6a	22.5a	13716a	12.3a	90.1a	13.1a

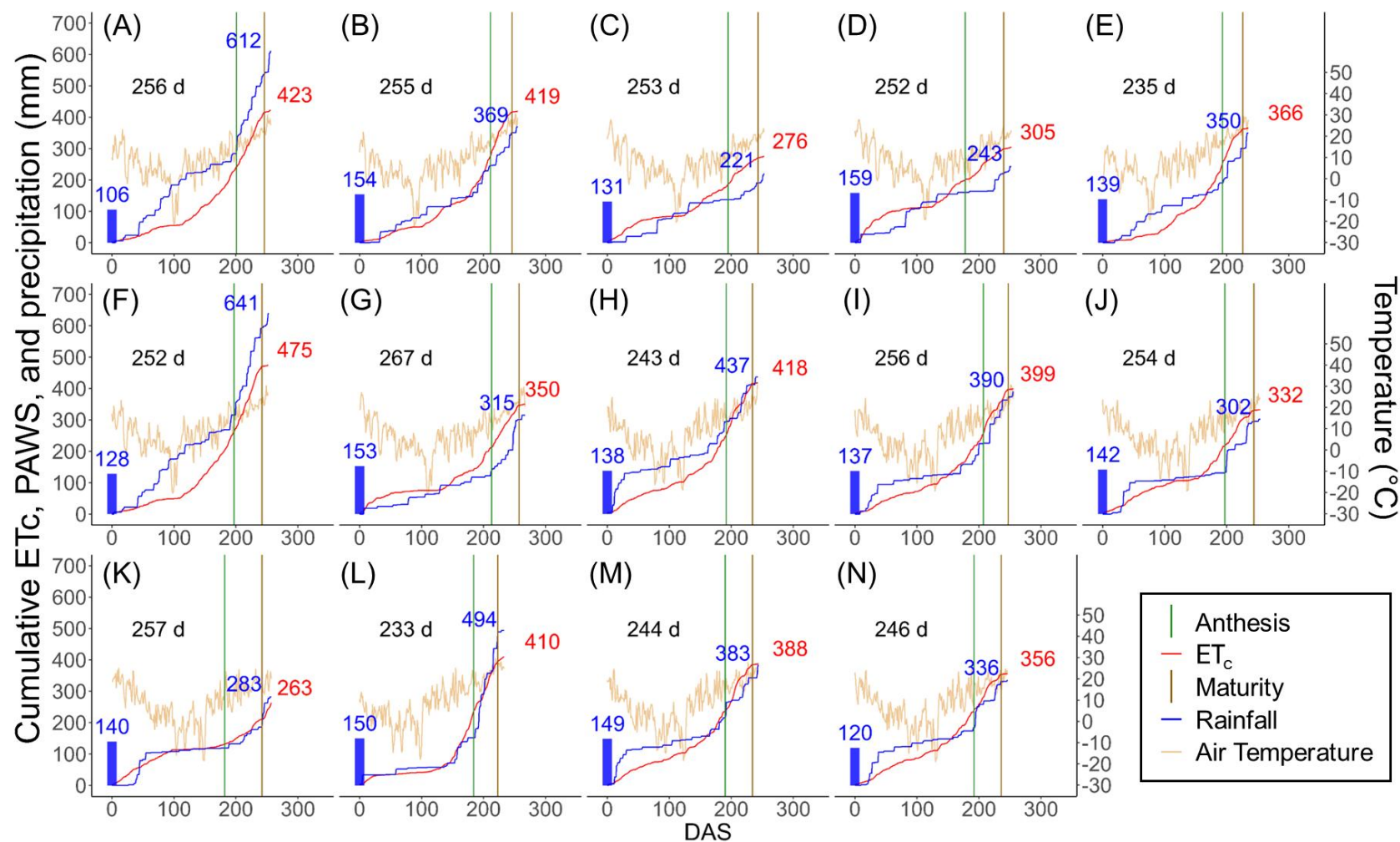


Figure 2.1 Weather conditions experienced during the winter wheat-growing season at the fourteen Kansas environments including 24-AB (A), 24-BEL.S (B), 24-HAYS (C), 24-HOI (D), 24-MCP (E), 24-MHN (F), 24-PHG (G), 25-AB (H), 25-BEL.S (I), 25-HAYS (J), 25-HOI (K), 25-HUT.O (L), 25-MHN (M), and 25-RUS (N). Plant available water at sowing (blue bars and associated numbers), precipitation (blue lines and numbers), cumulative simulated crop evapotranspiration (ET_c, red lines and numbers), crop season length (black numbers), and mean temperature (yellow lines) are shown. The green vertical lines denote anthesis date (Zadoks 60), and the brown vertical lines denote the end of grain filling (Zadoks 90).

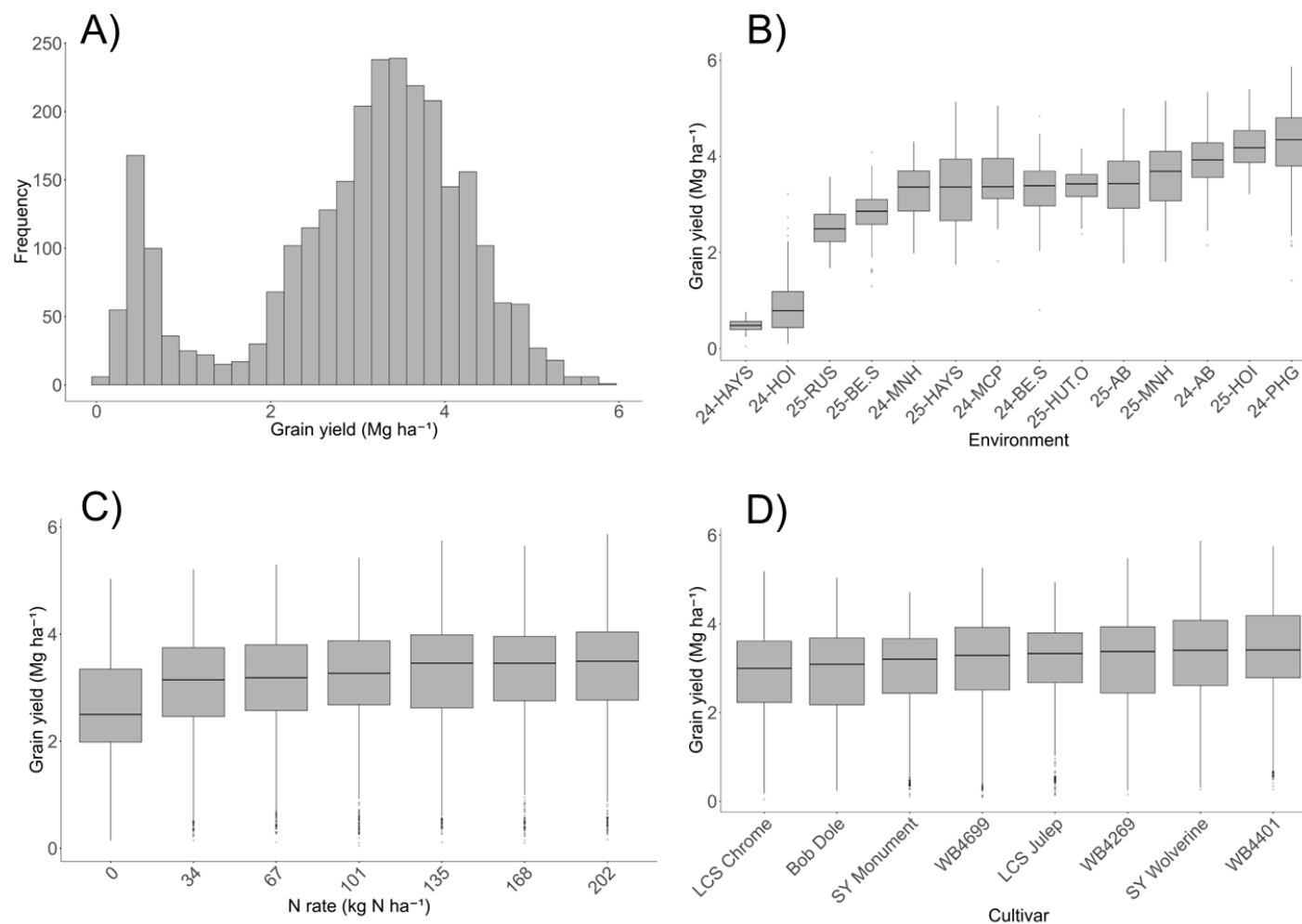


Figure 2.2 Yield variability as influenced by $G \times E \times M$ across all observations from the 2023–2024 and 2024–2025 seasons. (A) Distribution of grain yield (Mg ha⁻¹) considering all sources of variation. (B) Variation in grain yield (Mg ha⁻¹) across the fourteen environments. (C) Variation in grain yield (Mg ha⁻¹) across the seven nitrogen rates. (D) Variation in grain yield (Mg ha⁻¹) across the eight cultivars.

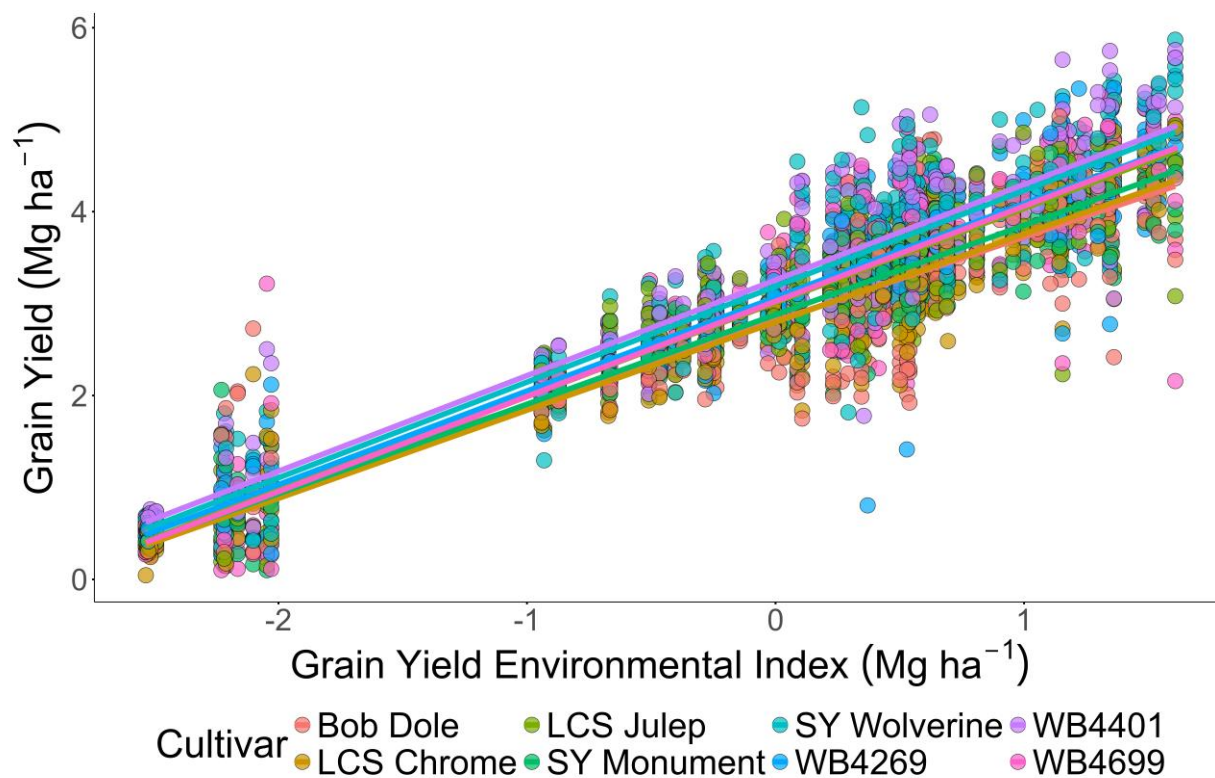


Figure 2.3 Linear relationship between grain yield and environmental index (EI) for the eight winter wheat cultivars evaluated under seven nitrogen rates in 14 Kansas environments during the 2023-24 and 2024-25 harvest seasons.

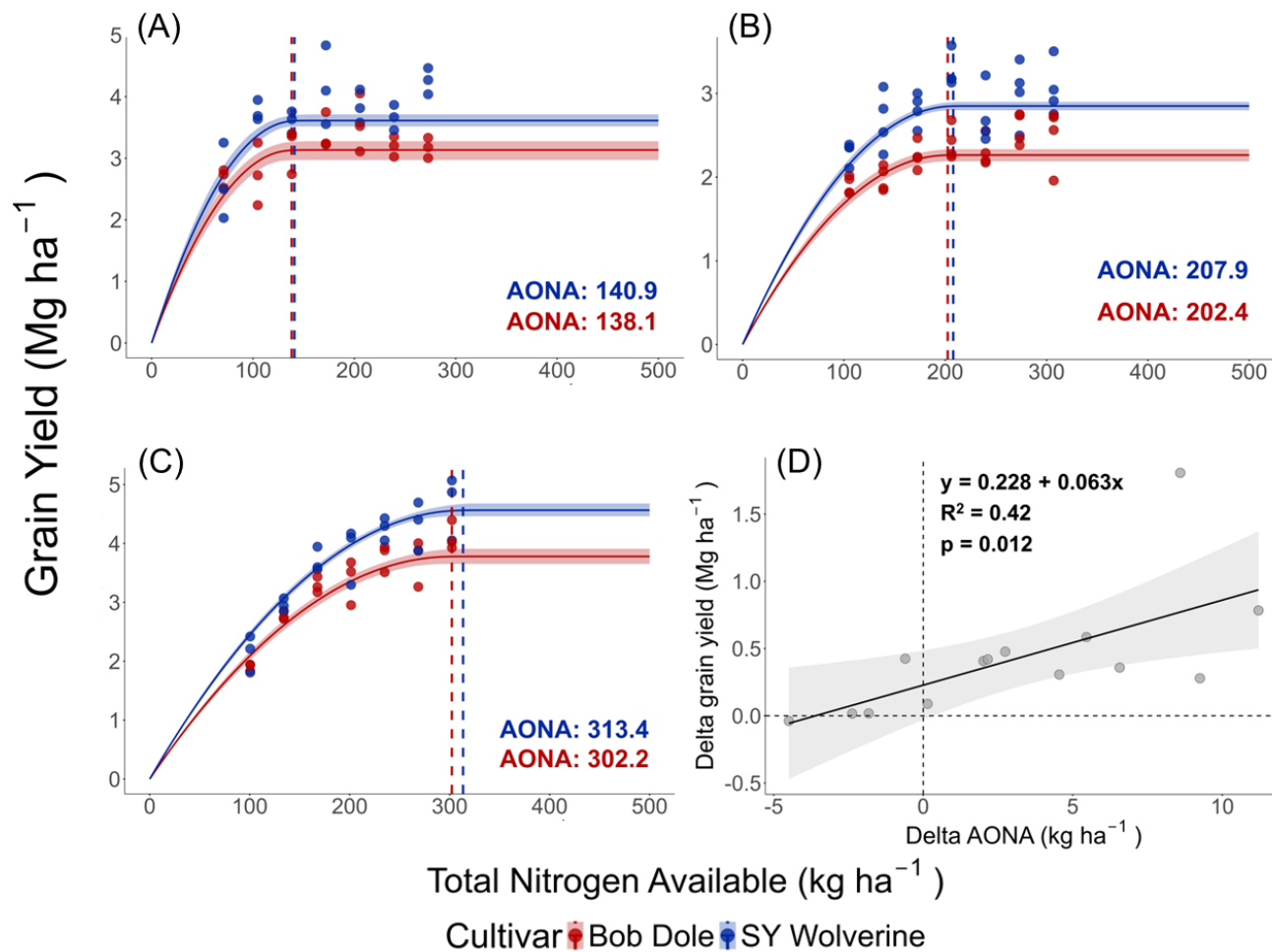


Figure 2.4 Quadratic-plateau relationships between grain yield and total nitrogen availability for cultivars with highest and lowest phenotypic plasticity (PP) of yield in three selected Kansas environments. (A) BE.S-24; (B) RUS-25; (C) MNH-25. Blue lines represent high-PP cultivars, whereas red lines represent low-PP cultivars. Dashed lines indicate the agronomic optimum nitrogen availability (AONA). Linear regression of the difference in grain yield at agronomic optimum nitrogen availability (AONA) and the difference in AONA between highest and lowest PP cultivars across all evaluated environments (D).

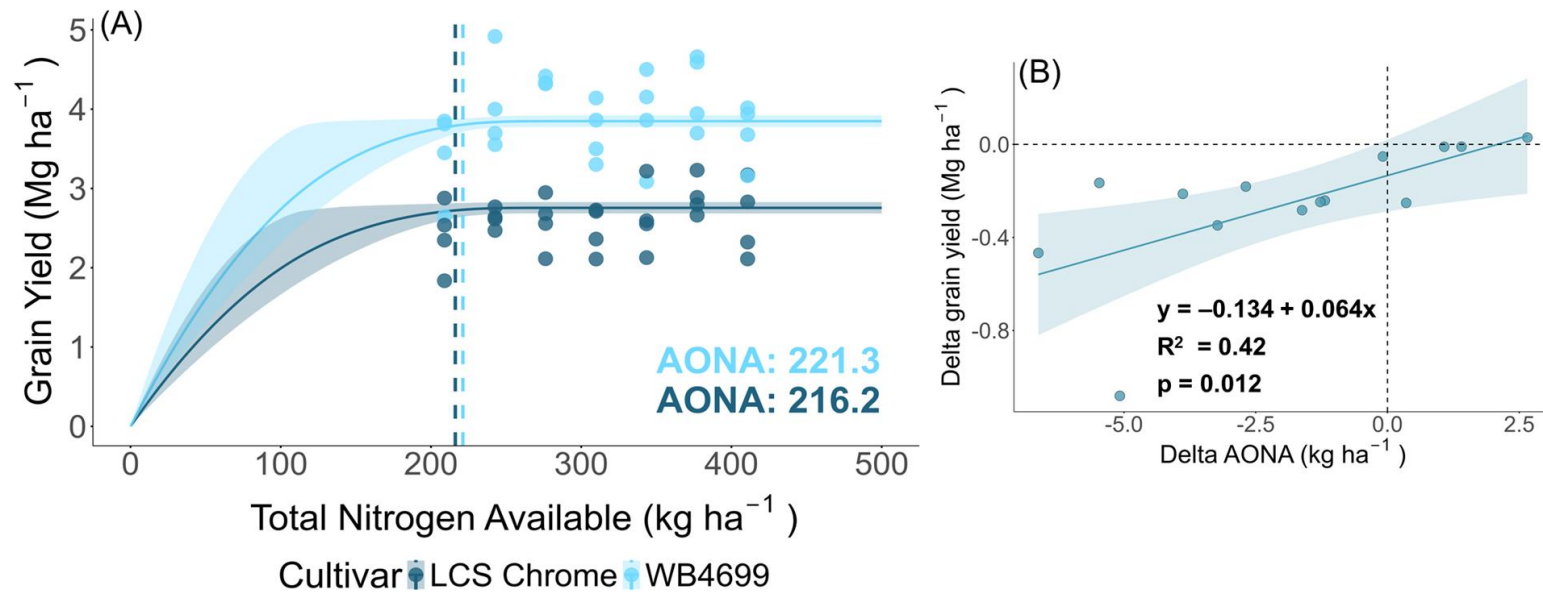


Figure 2.5 Quadratic-plateau relationships between grain yield and total nitrogen availability for cultivars with the highest and lowest grain protein deviation (GPD) in one Kansas environment, HAYS-25 (A). Dark blue color represents high-GPD cultivar, and light blue color represents low-GPD cultivar. Dashed lines indicate the agronomic optimum nitrogen availability (AONA). Linear regression of the difference in grain yield at AONA and the difference in AONA between highest and lowest GPD cultivars across all evaluated environments (B).

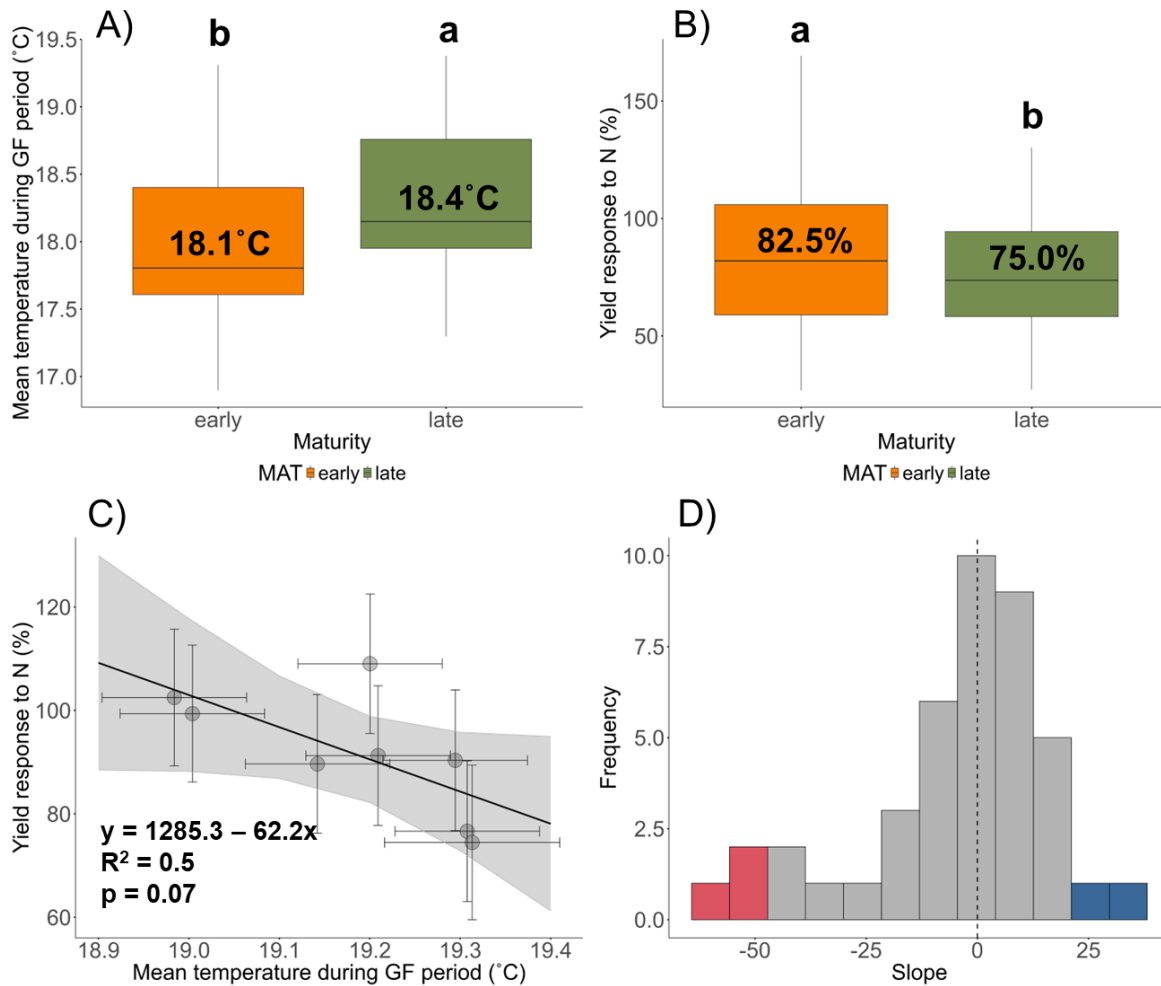


Figure 2.6 Temperature during grain filling and its relationship with yield responsiveness to nitrogen as affected by cultivar maturity. (A) distribution of mean temperature during grain filling by maturity group; (B) distribution of yield responsiveness to nitrogen by maturity group in Manhattan during 2025 (means followed by different lowercase letters indicate significant differences according to Tukey's test at $p \leq 0.05$); (C) relationship between mean temperature during grain filling and yield responsiveness to nitrogen at Manhattan 2025 at 168 kg N ha⁻¹; (D) distribution of slopes from regressions between yield responsiveness to nitrogen and mean temperature during grain filling for each environment at each nitrogen rates. Colored bars indicate significant slopes (red = negative, blue = positive).

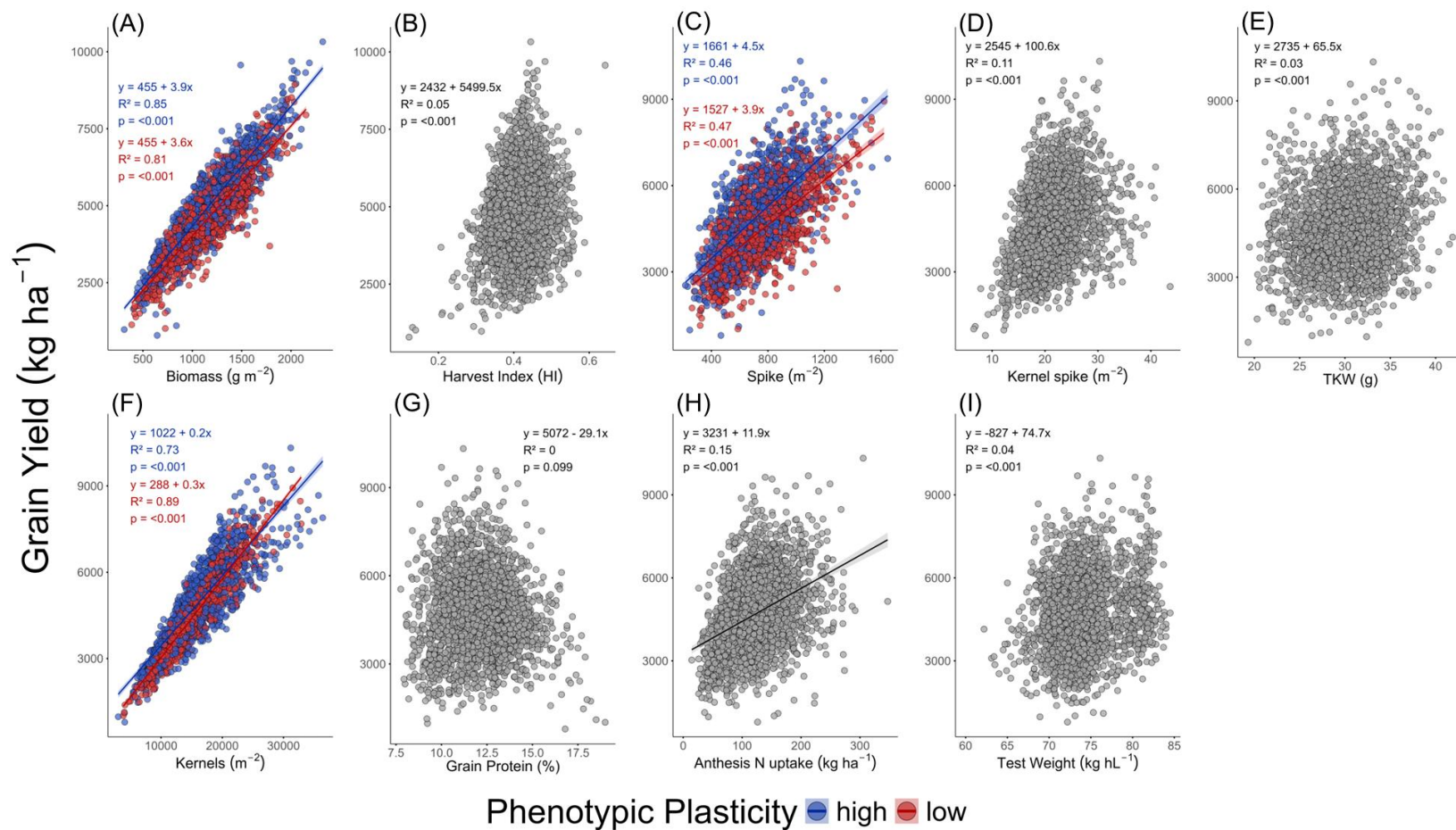


Figure 2.7 Relationships between wheat grain yield (kg ha⁻¹) and yield components; (A) biomass, (B) Harvest Index, (C) spikes m⁻², (D) Kernel spike⁻¹ m⁻², (E) thousand kernels weight, (F) kernels m⁻², (G) grain protein (%), (H) nitrogen uptake at anthesis (kg ha⁻¹), (I) test weight (kg hL⁻¹); across all environments for cultivar phenotypic plasticity groups. Blue color represents high-PP cultivars, and red color represents low-PP cultivars. Regression lines are not presented when R² < 0.15, even if p < 0.01, as statistical significance may be driven by large sample size.

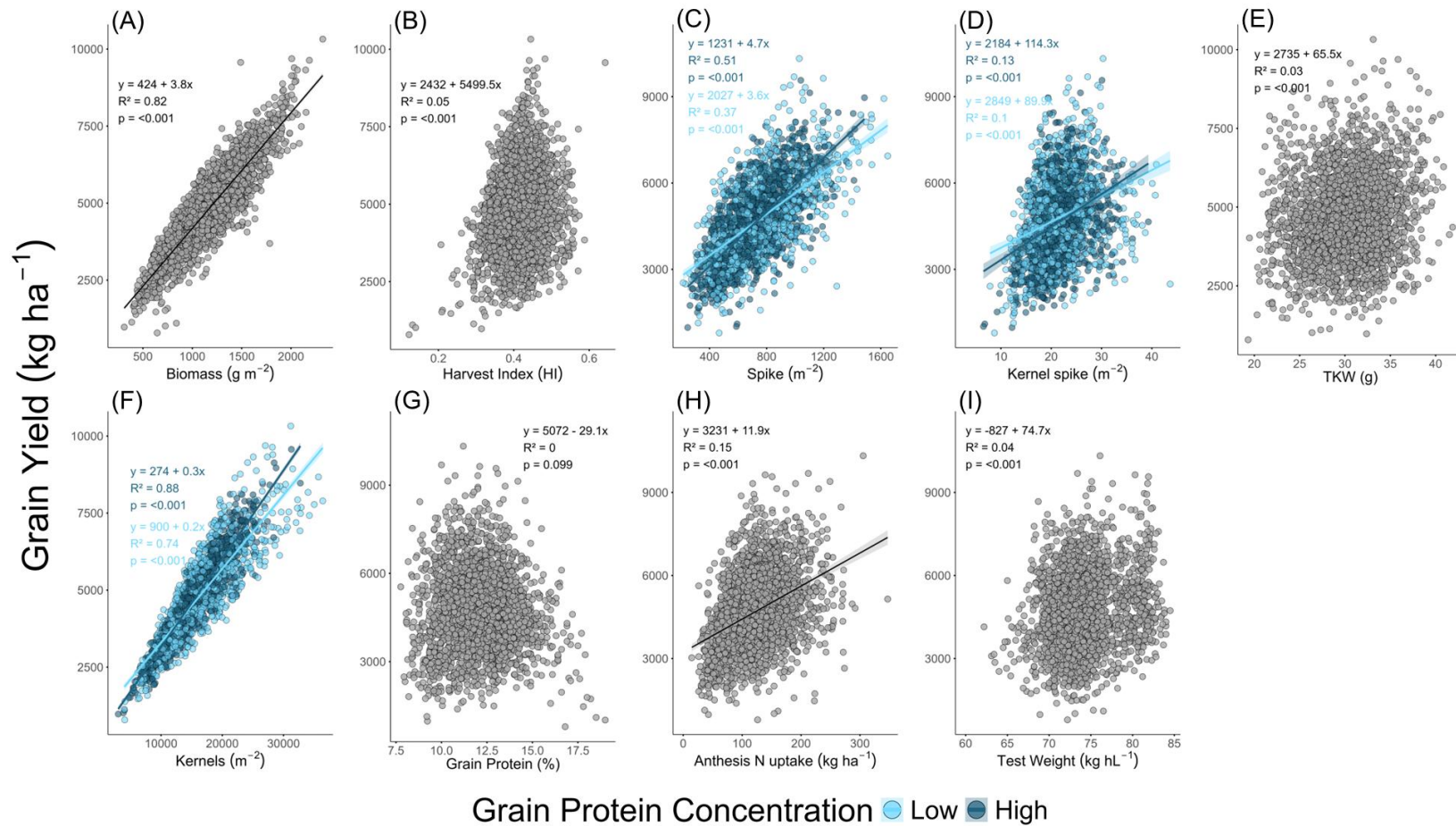


Figure 2.8 Relationships between wheat grain yield (kg ha⁻¹) and yield components; (A) biomass, (B) Harvest Index, (C) spikes m⁻², (D) Kernel spike⁻¹ m⁻², (E) thousand kernels weight, (F) kernels m⁻², (G) grain protein (%), (H) nitrogen uptake at anthesis (kg ha⁻¹), (I) test weight (kg hL⁻¹); across all environments for cultivar grain protein concentration groups. Dark blue color represents high-GPC cultivars, and light blue color represents low-GPC cultivars. Regression lines are not presented when $R^2 < 0.15$, even if $p < 0.01$, as statistical significance may be driven by large sample size.

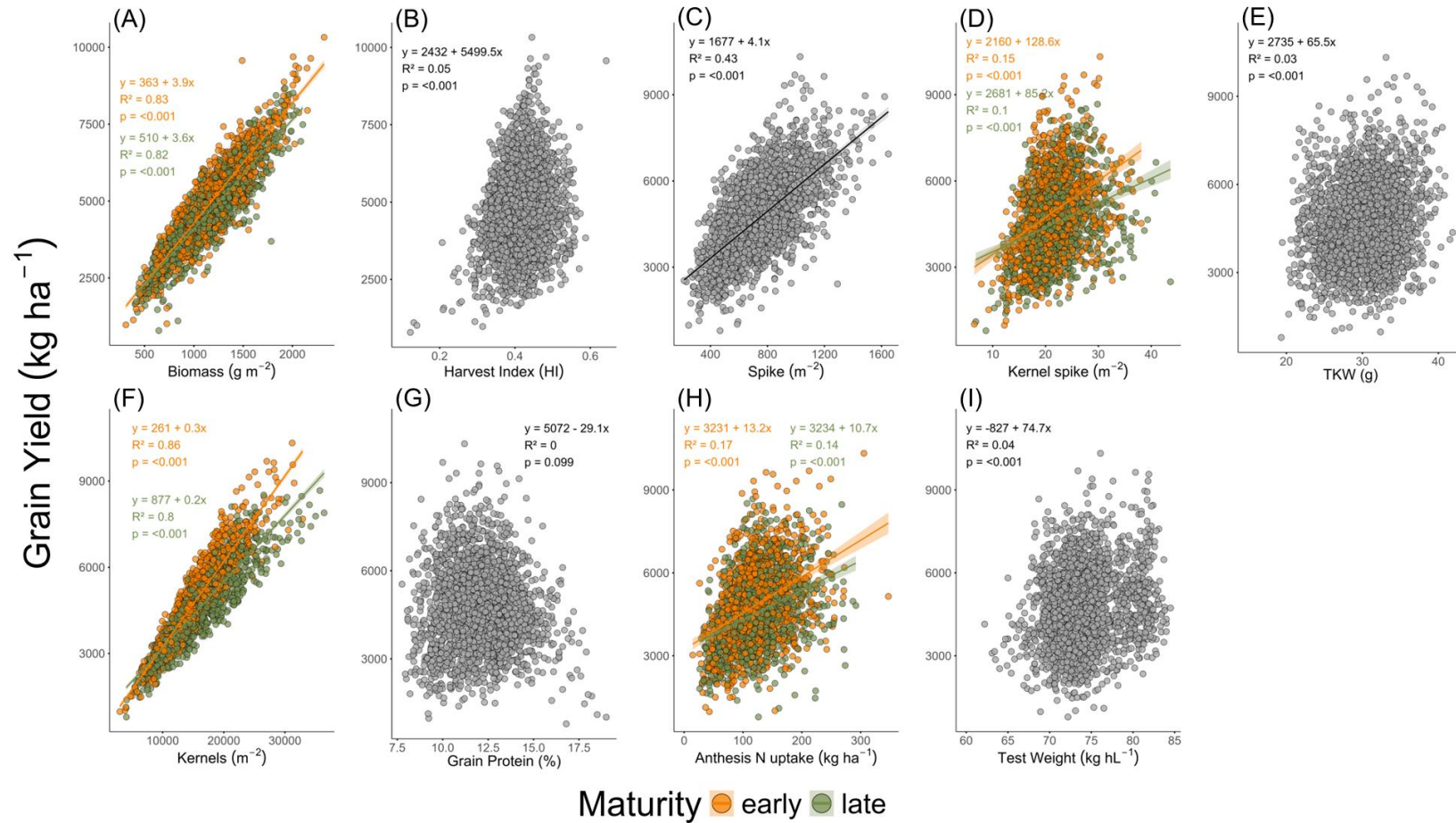


Figure 2.9 Relationships between wheat grain yield (kg ha⁻¹) and yield components; (A) biomass, (B) Harvest Index, (C) spikes m⁻², (D) Kernel spike⁻¹ m⁻², (E) thousand kernels weight, (F) kernels m⁻², (G) grain protein (%), (H) nitrogen uptake at anthesis (kg ha⁻¹), (I) test weight (kg hL⁻¹); across all environments for cultivar maturity groups. Orange color represents early-MAT cultivars, and olive color represents late-MAT cultivars. Regression lines are not presented when $R^2 < 0.15$, even if $p < 0.01$, as statistical significance may be driven by large sample size.

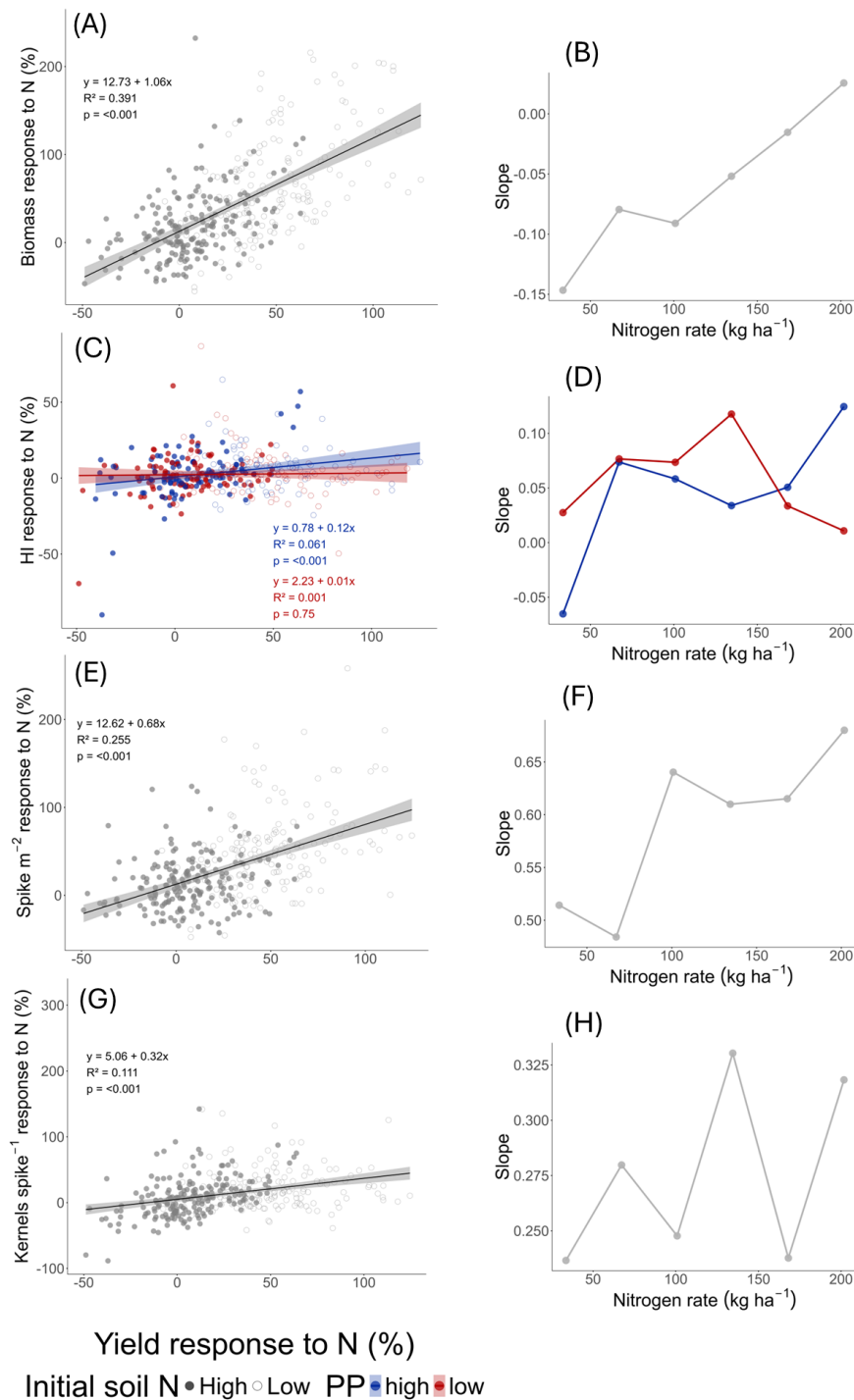


Figure 2.10 Linear regressions between N responsiveness of yield components and grain yield response to N (%) at the highest N rate (202 $kg\ ha^{-1}$) across all environments; (A) biomass, (C) harvest index, (E) spike m^{-2} , (G) kernels spike $^{-1}$; Open symbols indicate low initial soil N and closed symbols indicate high initial soil N; Blue color represents high-PP cultivars, and red color represents low-PP cultivars; (B), (D), (F) and (H) represent the slopes of linear regressions for N fertilizer rates of 34, 67, 101, 135, 168 and 202 $kg\ N\ ha^{-1}$.

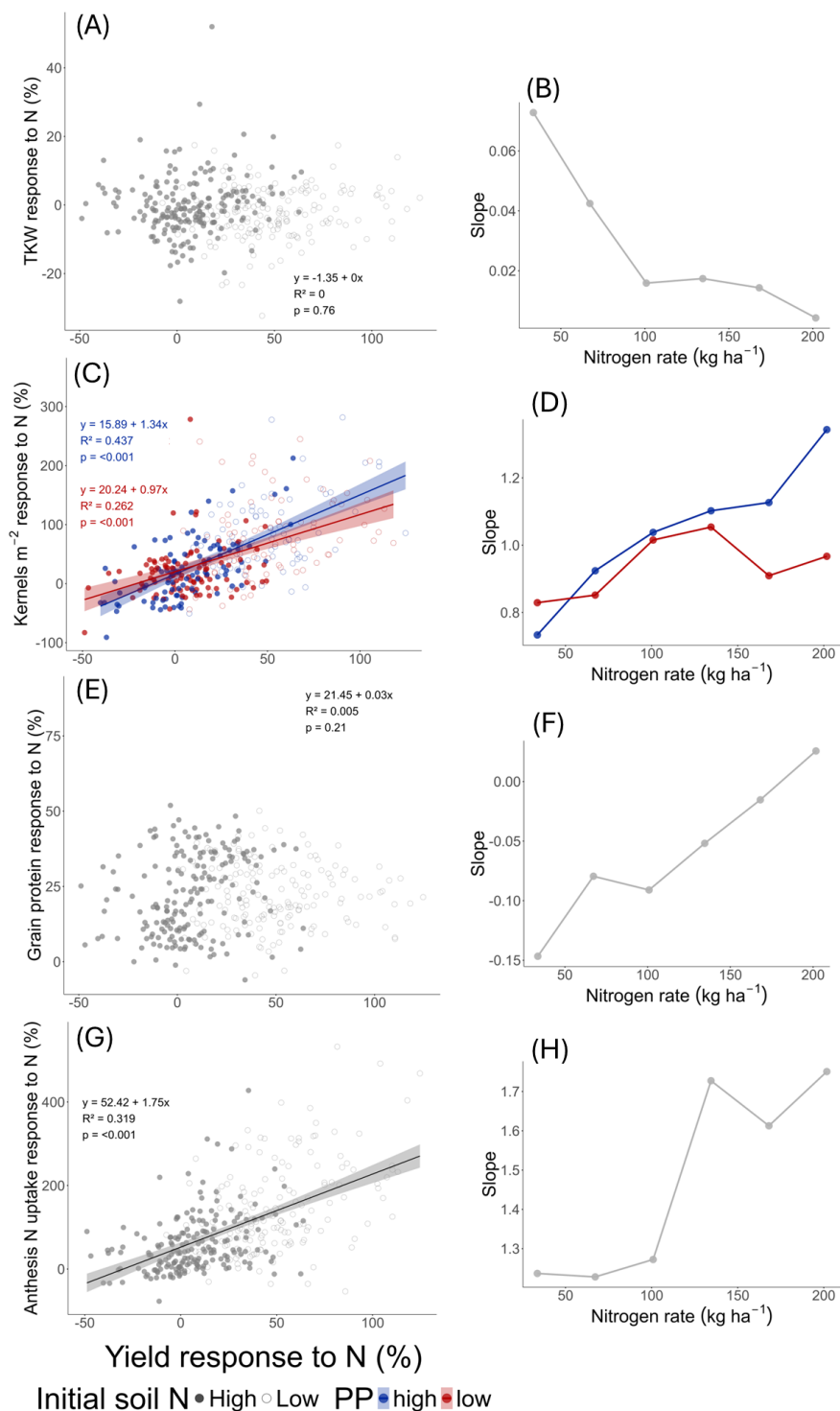


Figure 2.11 Linear regressions between N responsiveness of yield components and grain yield response to N (%) at the highest N rate (202 kg ha⁻¹) across all environments; (A) thousand kernel weight, (C) kernel m⁻², (E) grain protein, (G) anthesis N uptake; Open symbols indicate low initial soil N and closed symbols indicate high initial soil N; Blue color represents high-PP cultivars, and red color represents low-PP cultivars; (B), (D), (F) and (H) represent the slopes of linear regressions for N fertilizer rates of 34, 67, 101, 135, 168 and 202 kg N ha⁻¹.

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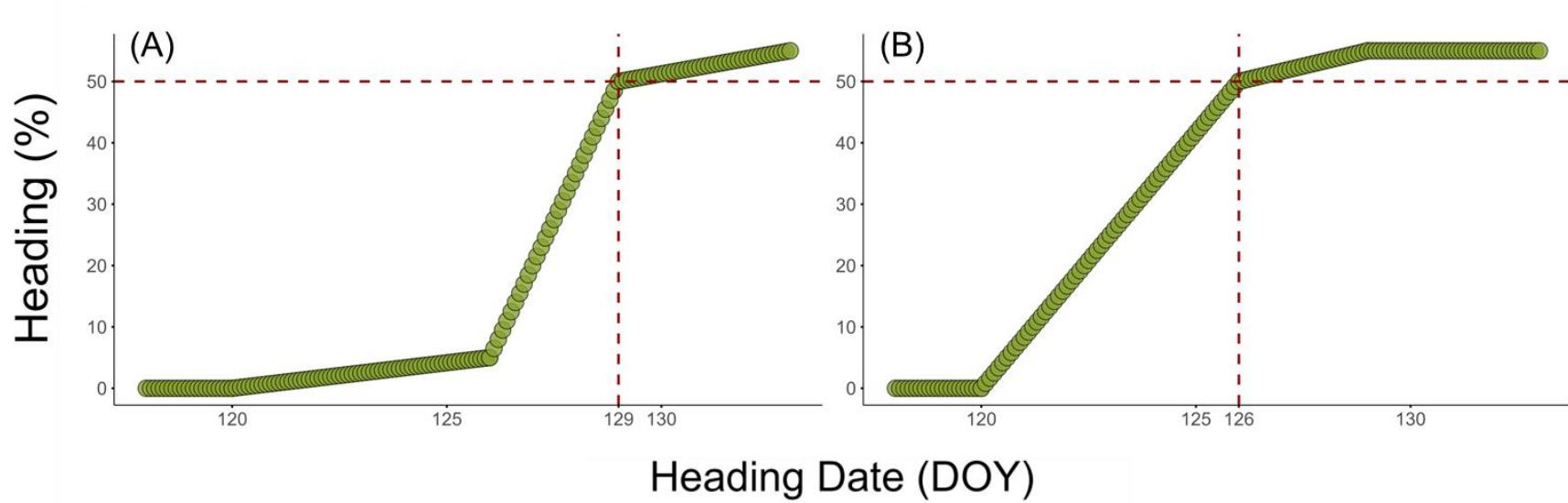
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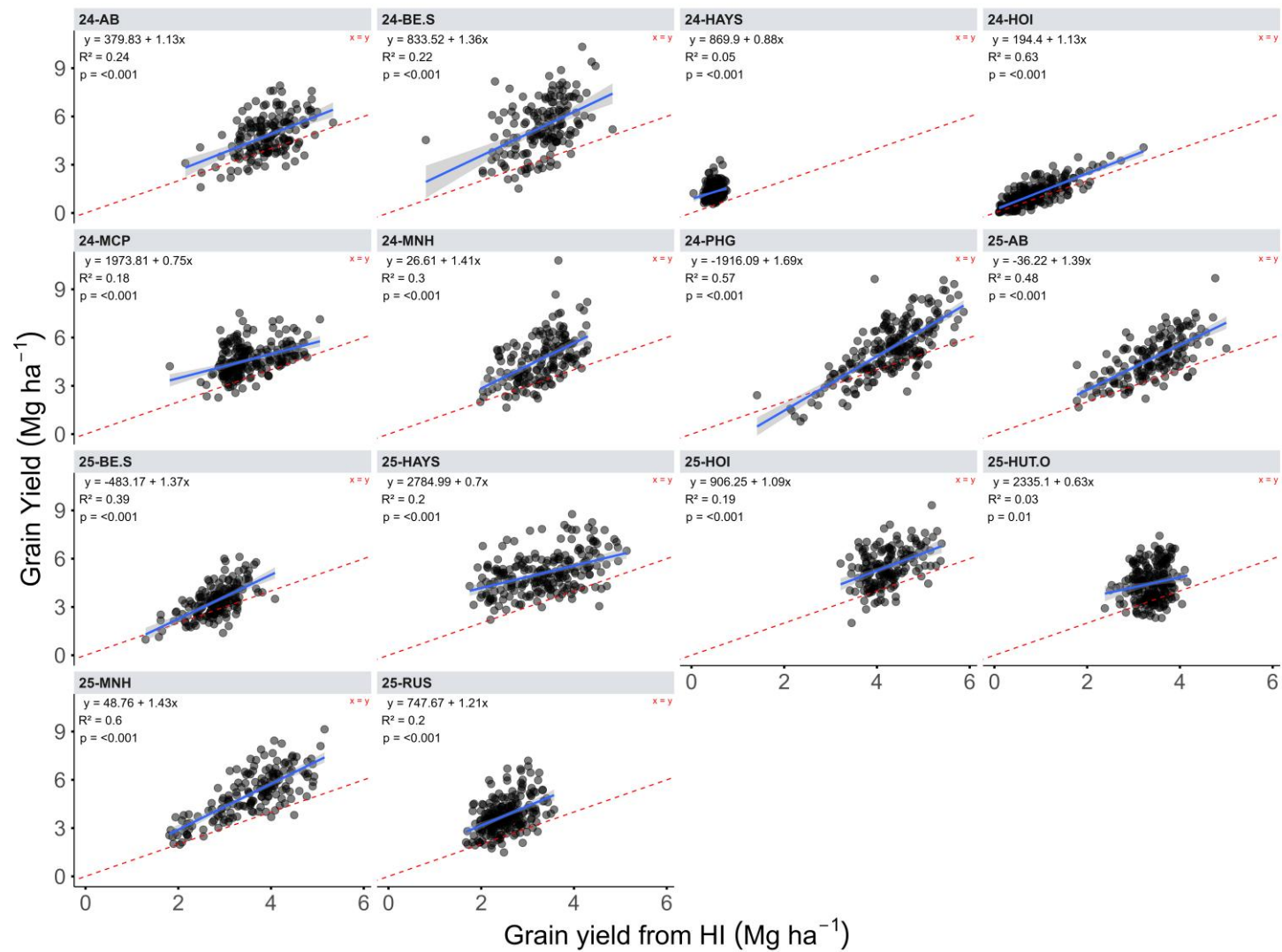
Appendix A - Chapter 2 Supplementary Material

Appendix Table Error! No text of specified style in document..1 Analysis of variance (ANOVA) for grain protein deviation (GPD) and heading date, expressed as day of the year (DOY), as affected by cultivar, nitrogen rate, environment, and their interactions used to validate cultivar grain protein concentration (GPC) and maturity (MAT) classification. Values represent p-values for grain protein deviation and heading dates.

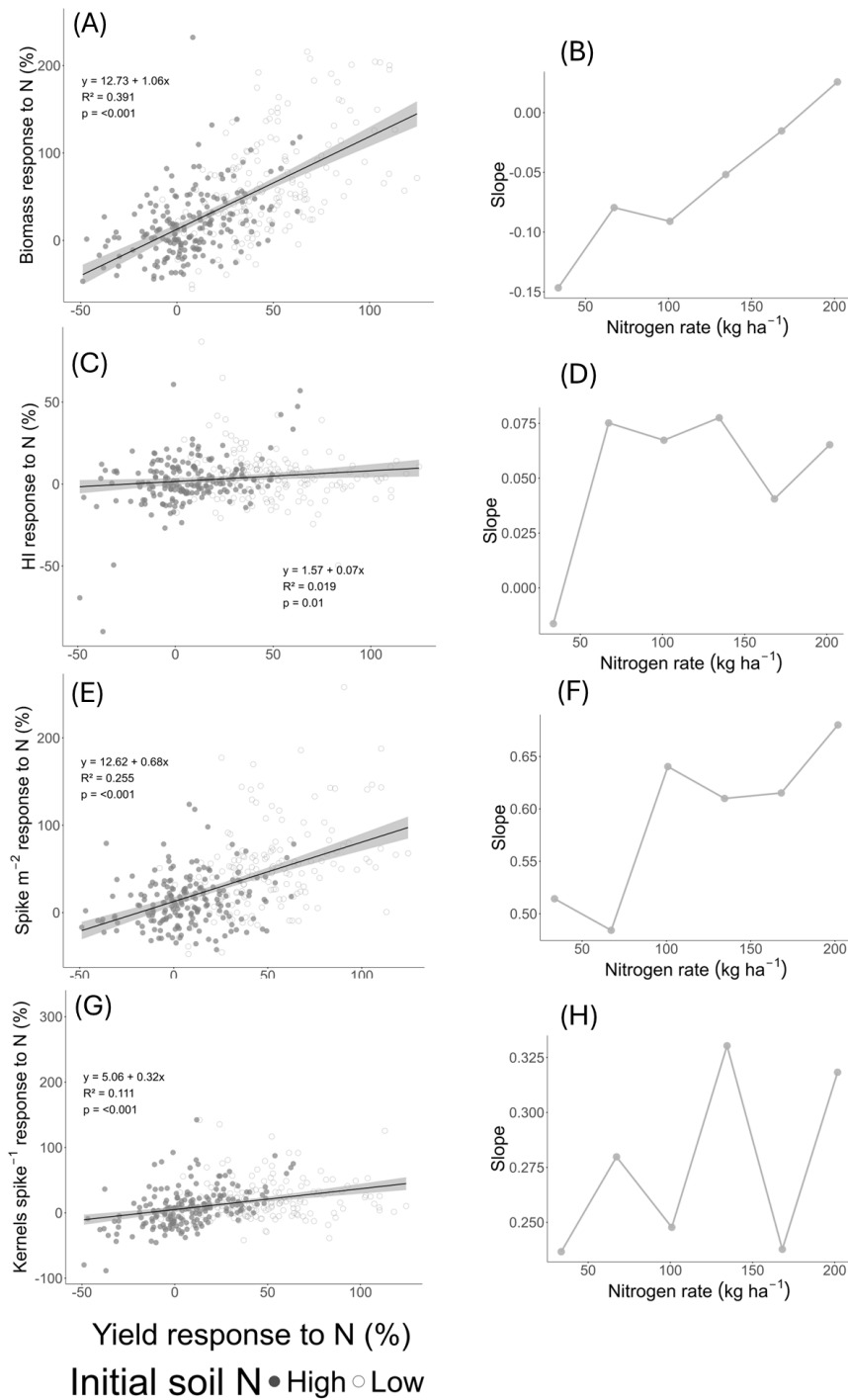
Source of Variation	GPD	Heading Date
(Intercept)	0.024	<0.001
Cultivar (C)	<0.001	<0.001
Nitrogen rate (Nrate)	0.807	0.565
Environment (E)	0.716	<0.001
C : Nrate	0.934	<0.001
C : E	<0.001	<0.001
Nrate : E	0.988	0.742
C : Nrate : E	0.432	0.824



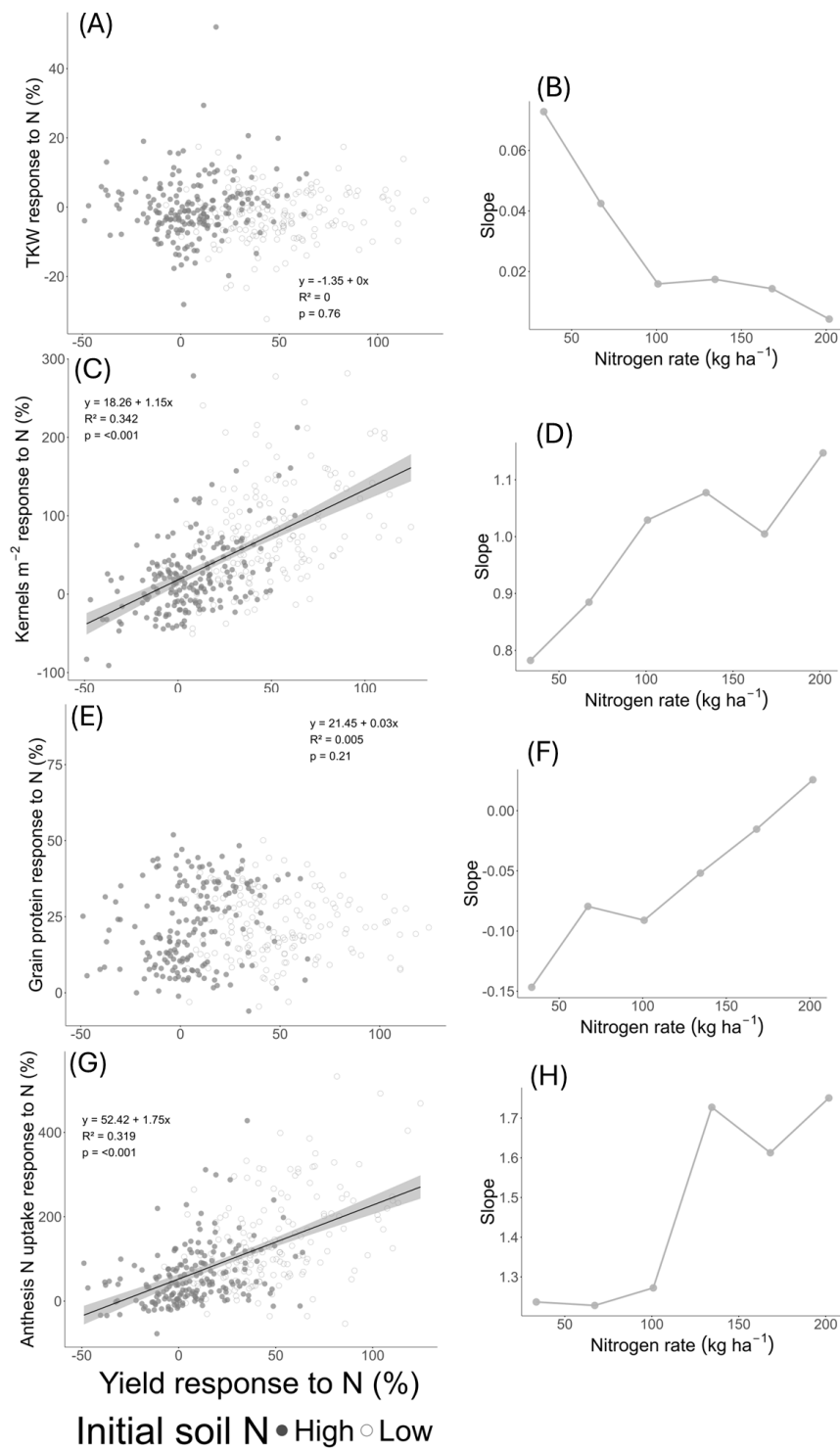
Appendix Figure A.1 Interpolated heading progression (% heads) plotted against day of the year (DOY) for two experiment units during the 2024-25 season in Manhattan, KS. (A) LCS Chrome – late maturity cultivar; (B) WB4401 – early maturity cultivar. Dashed lines denote the estimated 50% heading date, which occurred at DOY 129 (A) and DOY 126 (B).



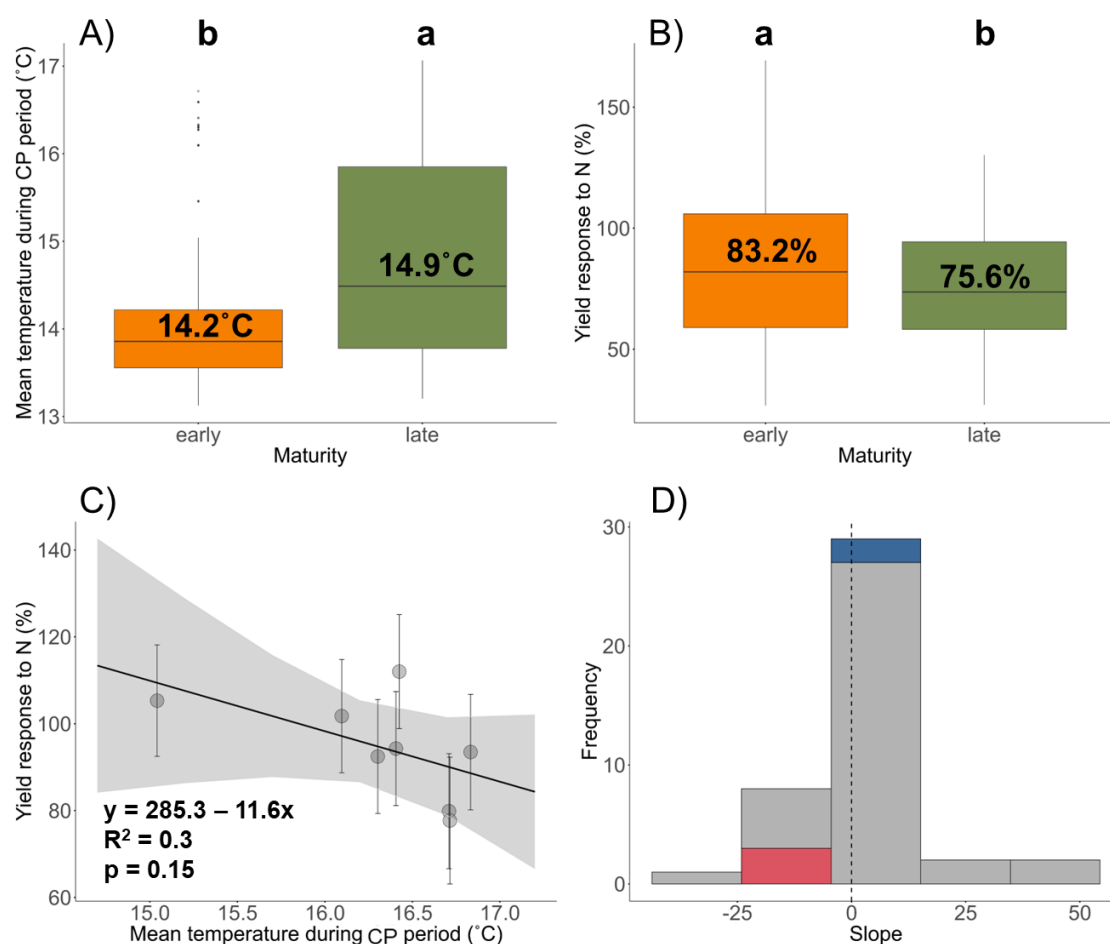
Appendix Figure A.2 Grain yield obtained from maturity samples from each experiment unit (x-axis) compared with combine-harvested yield (y-axis)



Appendix Figure A.3 Linear regressions between N responsiveness of yield components and grain yield response to N (%) at the highest N rate (202 kg ha⁻¹) across all environments; (A) biomass, (C) harvest index, (E) spike m⁻², (G) kernels spike⁻¹; Open symbols indicate low initial soil N and closed symbols indicate high initial soil N; (B), (D), (F) and (H) represent the slopes of linear regressions for N fertilizer rates of 34, 67, 101, 135, 168 and 202 kg N ha⁻¹.



Appendix Figure A.4 Linear regressions between N responsiveness of yield components and grain yield response to N (%) at the highest N rate (202 kg ha⁻¹) across all environments; (A) thousand kernel weight, (C) kernel m⁻², (E) grain protein, (G) anthesis N uptake; Open symbols indicate low initial soil N and closed symbols indicate high initial soil N; (B), (D), (F) and (H) represent the slopes of linear regressions for N fertilizer rates of 34, 67, 101, 135, 168 and 202 kg N ha⁻¹



Appendix Figure A.5 Temperature during the critical period and its relationship with yield responsiveness to nitrogen as affected by cultivar maturity. (A) distribution of mean temperature during grain filling by maturity group; (B) distribution of yield responsiveness to nitrogen by maturity group in Manhattan during 2025 (means followed by different lowercase letters indicate significant differences according to Tukey's test at $p \leq 0.05$); (C) relationship between mean temperature during grain filling and yield responsiveness to nitrogen at Manhattan 2025 at 168 kg N ha⁻¹; (D) distribution of slopes from regressions between yield responsiveness to nitrogen and mean temperature during grain filling for each environment at each nitrogen rates. Colored bars indicate significant slopes (red = negative, blue = positive).

Appendix B - Chapter 2 Supplementary Material

Materials and Methods

Relationships between Grain Protein and Nitrogen Use Efficiency

The relationship between grain protein concentration and NUE was evaluated using regression analyses across environments and agronomic plasticity groups (PP). Linear and quadratic regression models were fitted with NUE as the response variable and grain protein concentration as the explanatory variable, and analyses were conducted separately for each environment and PP group.

All regression models were fitted using the “*lm*” function in the R “*stats*” package. Quadratic models included a second-order polynomial term for grain protein concentration. Model performance was assessed using the coefficient of determination (R^2), and the significance of regression terms was evaluated at a significance level of $\alpha = 0.05$.

Results

Nitrogen Use Efficiency

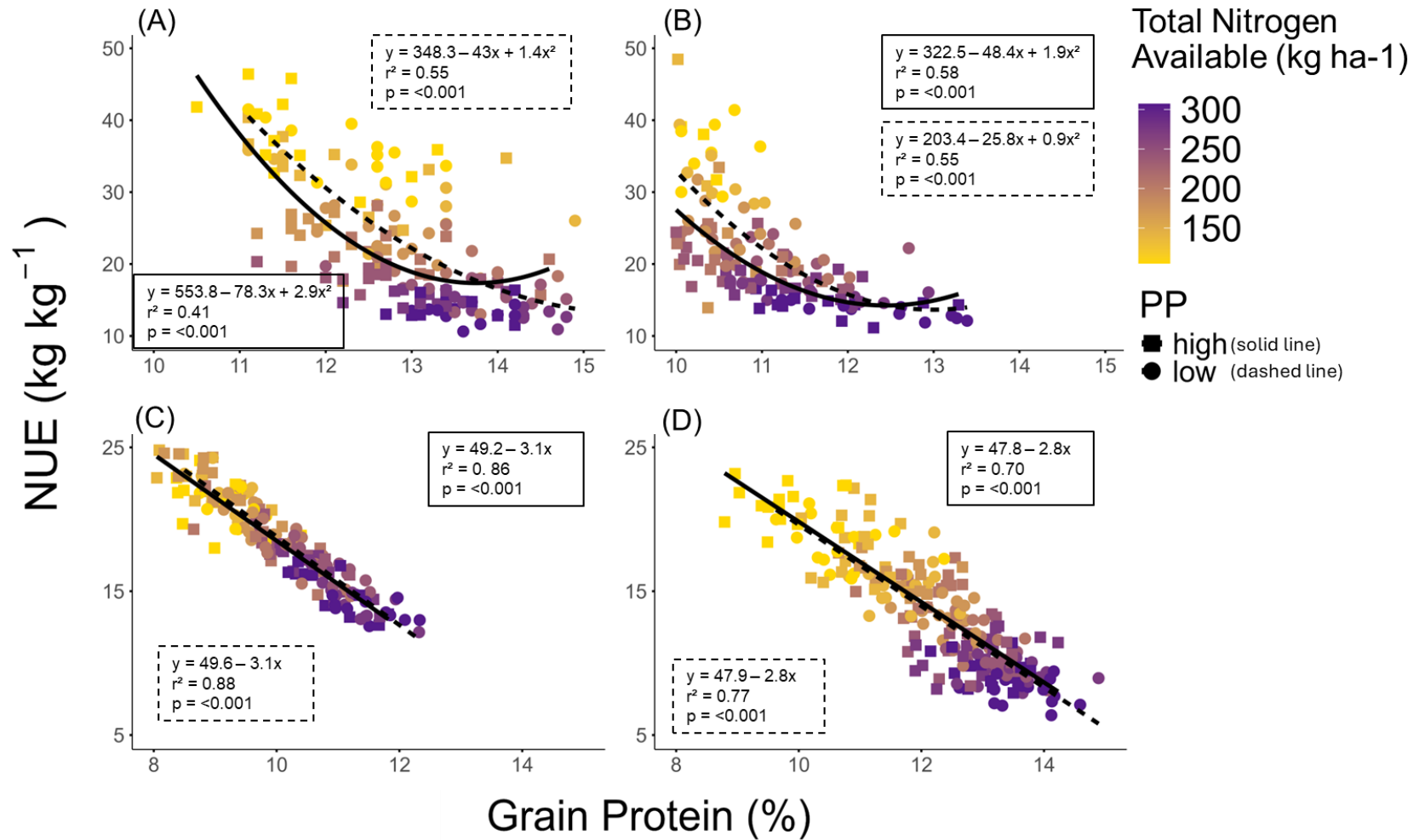
The quadratic and linear relationships between nitrogen use efficiency (NUE) and grain protein was influenced by PP-group and total nitrogen availability (**Appendix Figure B.1**). NUE ranged from 0.1 to 48.4 kg N ha⁻¹ and grain protein concentration from 7.7 to 22.5%. Higher N availability increased grain protein concentration and reduced NUE across environments. High-PP cultivars displayed lower protein concentration, and at comparable protein levels, exhibited lower or no difference NUE than low-PP cultivars (**C and D, Appendix Figure B.1**). This pattern suggests that the previously observed higher NUE in high-PP cultivars (**Table 2.8**)

primarily results from their lower grain protein concentration rather than inherent differences in nitrogen use efficiency

Discussion

Nitrogen Use Efficiency

Our results indicated that high phenotypic plasticity associated with high yield potential under increasing nitrogen availability but was accompanied by a trade-off in grain protein concentration and NUE at equivalent protein levels. The combination of yield response and nitrogen management highlights the agronomic advantage of selecting high-PP cultivars under environments where N supply can be optimized.



Appendix Figure B.1 Nitrogen use efficiency (NUE) as a function of grain protein concentration, PP-group, and total N available in four selected environments. (A) Belleville-24; (B) Ashland Bottom-25; (C) Manhattan-25; (D) Russel-25. Higher N availability and grain protein concentration reduced NUE. High-PP cultivars had lower protein concentration and, at similar protein levels, lower NUE than stable cultivar

