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Evaluation of the effects of nutrient management on grain yield, quality, and rheological properties of common wheat varieties (*Triticum aestivum*, L.)

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### General abstract

The rapid rate of population growth, extreme drought, heat waves, conflicts, and pandemics are pushing food production under strong pressure. As a result of this situation, in the next years, it is expected to have the food demand around 60 to 70% higher from the current level. At the same time, the input resource is going to be limited, contributing to the slowdown of agricultural productivity and production, mainly in developing countries.

Global food demand depends on achieving sustainable cereal production, particularly rice, wheat, and maize. Widely adapted climate-resilient germplasm, and appropriate agronomic and resource management play a prominent role in sustainable crop production. Globally, the viability of limited genotypes and nutrient management, among other major challenges, must be addressed if the farmers have to improve their crop production leading to an increase in their profits, and livelihoods and fulfill the food needs of the consumers. In this research, the effects of nutrient management as a function of climate and soil variability were evaluated on common wheat varieties, through a number of experimental trials, in order to determine the cost-effectiveness of fertilization strategies along with high-yielding wheat varieties, that could contribute to crop productivity, increase quantity and quality and increase the farmer economic profitability.

The objective of Chapter 3.1 of this research is to evaluate the effects of two seeding density (SD), three nitrogen levels (NL), and two sulfur levels (SL) fertilization towards improving the grain yield (GY), rheological characteristics, and asparagine (ASN) content of 14 'old' common wheat varieties. The results showed that SL and SD treatments significantly increased grain yield (GY) without decreasing the protein content (PC), while NL significantly increased the PC without affecting GY. The dough strength (W) increased significantly with increasing SL and NL but was significantly reduced with increasing SD. Asparagine (ASN) significantly increased by 111% as the NL fertilization increased from 35 to 135 kg ha<sup>-1</sup>, while ASN significantly decreased (85.1%) with the SL treatment. The findings show that 135 kg N ha<sup>-1</sup> combined with 6.4 kg S ha<sup>-1</sup> can improve the performance of 'old' wheat wholegrain flours while maintaining the ASN as low as possible.

- The objectives of Chapter 3.2 of this thesis were aiming to (1) evaluate the impact of soil and climate on the response of winter wheat to nitrogen (N), and phosphorus (P) fertilizations; (2) quantify the specific N and P response of winter wheat for different ACZs; and (3) determine the economical application rates of N and P for the economic benefit of farmers for each considered ACZs. This trail examines the effects of nitrogen levels (NL) at 35.28, 65, 95, and 120 kg N ha<sup>-1</sup> and phosphorus levels (PL) at 0, 50, 70, and 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, respectively, in four locations (L) for two growing seasons (GS), on both yield and quality characteristics of winter wheat. The result showed that soil pH was the main environmental parameter affecting straw yield (SY), grain yield (GY), protein content (PC), and protein yield (PY). Winter wheat SY, GY, PC, and PY increased significantly (p < 0.05) with PL rates up to 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and with NL rates up to 120 kg N ha<sup>-1</sup>. NL was the most important parameter in determining PC, thus showing potential for further improvement in N management. The highest marginal rate of return was used as an index for the farmers to accept site-specific N and P fertilizer recommendations.
- The objective of Chapter 3.3 of this study aimed to evaluate the yield stability and agronomical traits of 33 common improved wheat (*Triticum aestivum* L.) varieties (V) under six environments (E), (three-locations x two-growing seasons) in Afghanistan. The combined ANOVA analysis showed that the variation due to the interaction of E x V for GY, SY, and HI was significantly larger compared to the main factor effect, environment, and variety; but the contribution of E x V for TKW and PH was smaller than the main factors. However, as average G09 showed the highest GY, followed by G31, G28, G15 and G04, whilst G31 showed wide stability, followed by G04, G15, G09, G25, in decreasing order, respectively. Interestingly, the 5-top superior stable varieties were also associated with higher GY, while G03, G01, G21, G18, and G02 varieties were identified as the most unstable with the poorest GY. Concerning locations, varieties G24, G29, G25, G15, G04 in BLK, G15, G09, G04, G23, G22 in HLM, and G31, G09, G32, G28, G27 in NGH location were identified as stable with higher GY performance. Moreover, results indicated that the highest grain yield was obtained by varieties that were grown in NGH while the lowest yield was obtained in HLM.
- The objectives of this study were to (1) quantify the effect of soil and climatic parameters on yield and quality of wheat (2) investigate the response of different wheat varieties to different N and P fertilization rates under specific climate conditions, toward to improve the yield and quality of wheat in Afghanistan. Three wheat varieties (DLN7, ZRDN, and

KBL13), three phosphorus levels (PL) at 60, 90, and 120 kg P<sub>2</sub>O<sub>5</sub> ha-<sup>1</sup>, and three nitrogen ratios (NP) at 1:1, 1.25:1, and 1.5:1, respectively, in four locations (L), were evaluated. Soil pH was the main environmental parameter affecting grain yield (GY), straw yield (SY), protein yield (PY), and starch yield (STY) similar to PL and NP management. The higher average GY, straw yield (SY), and STY were obtained by DLN7, followed, by KBL13 and ZRDN for all Ls, but statistically, no significant differences occurred between DRL7 and KBL13. Moreover, as PL increased, GY, SY, PY, and STY increased significantly in four Ls. In addition, PL significantly affected protein content (PC), gluten content (GC), and dough strength (W). NP was the most important factor to improve PC, GC, and PY. Starch (ST), STY, and amylopectin (AP) increased significantly reduced as PL increased. On the contrary, AM:AP increased significantly with increasing NP ratios, but ST and AP were significantly reduced as the NP ratio increased. The findings show that at NP1/PL120, GY, SY, ST, and AP were improved significantly; while at NP1.5:1/PL12, PC, and GC were significantly improved.

In conclusion, the results of this thesis proved that the wheat yield, quality characteristics, and rheological properties of wheat were sustainably affected by climate, soil variability, and fertilization factors. However, this result showed that to sustainably boost wheat production, the farmers need to follow proper nutrient management based on various climate zones and soil fertility. Also, the selection approach of high-yielding and stable wheat varieties based on specific agro-climatic zones can assure wheat production and farmer economic benefits, without affecting environmental pollution.

**Keywords:** wheat variety, nitrogen, phosphorus, fertilization, locations, grain yield, straw yield, starch content, amylopectin, protein, gluten, rheological properties.

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### The feature of experimental plots in the field and laboratory activities:



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### 1. General Introduction

Global agricultural production systems are being disrupted by climate change, extreme weather incidents, Covid19, and social conflicts driving up the prices of food and agricultural inputs (GAP, 2022). Since 2020, poverty rates, malnutrition, and food insecurity have jumped up rapidly, particularly in low-income countries. In 2022, 40 million people confronted emergency levels of food insecurity, twice larger compared in 2020 and six times larger than in 2016 (Food Security Information Network, 2022). In the past decade, world agricultural productivity growth showed a significant drop, from 1.99% per year (2001-2011) to 1.12% per year (2012-2020) (USDA Economic Research Service, 2022). On a global scale, in 2050 the requirement for food for 9.2 billion people in the world, food production would require an increase between 60 to 70% over current levels (Dijk et al., 2021 and Silva, 2018); while the arable land and water resources are under extreme pressure, the uncertainty of climate tent to increase (FAO, 2018). The challenge of producing food for humans in 2050 is huge, however, special investments in research and innovations possibly could contribute to overcoming this challenge and achieving sustainable food production systems (World Resource Institute, 2019; Reynolds and Braun, 2022; GAP, 2022). FAO (2017) projected that among all commodities, cereals crops will play a central role in food security by 2050, providing a major part of the total daily calorie requirements and protein of world populations (Erenstein et al., 2022). In 2022, total cereal production was forecasted to be about 2274.7 million tons, which is 6% (12.7 million tons) less than in 2021, while consumption demand for cereal was estimated at 2301.6 million tons; and the area under cereal crops was estimated to about 728.3 million hectares (World Bank, 2018).

Among cereals, wheat underlines a predominant crop for global food security. Wheat alone provides approximately 20% of all calories and protein of humans worldwide, through consumption per capita of 60.6 kg per year, as compared to rice 81 kg. Moreover, wheat is the most widely produced and stored crop in the world, cultivated on 217 million ha of land (Erenstein et al., 2022). Wheat is grown in almost all regions of the world and is an important source of food and income for millions of small farmers. According to several authors, wheat-based foods are therefore critical to global food and nutrition security (Pena-Bautista et al., 2017); it encompasses mainly two species: the hexaploid known as common wheat (*Triticum aestivum* L.) counting for approximately 95% of global wheat production, and the tetraploid known as durum wheat (*T. durum*), count for about 5% of the total production (Peng et al., 2011). In 2022, wheat production was estimated at about 780 million tons in the world, down by 1% (1.0 million tons) from 2021, while consumption was estimated at about 785 million tons (IGC, 2022). Globally, about 68% of the wheat produced is used for food (between 2016

and 2018) and 20%, 10%, and 1% are used for feed, biofuels, and other purposes, respectively (Grote et al., 2021). Wheat is the most widely traded crop, globally 25% of wheat production being exported; Russia, the United States, Canada, Ukraine, France, and Australia are known as the top producers and exporters of wheat. Wheat prices have increased by 37% in the last decade. The average wheat price was about US\$143 per ton in 1994 and US\$197 per ton in 2019 (Erenstein et al., 2022).

In Asia wheat plays a vital role in food security, as 53% of world wheat was consumed by Asians, followed by Europeans (26%), and about 10% each the Americans and Africans (Erenstein et al., 2022; Morgounov et al., 2019). Among Asian countries, common wheat is the first stable food crop in Afghanistan, counting for about one-quarter of the total agriculture GDP and 6.3% of the national GDP (Halimi, 2016). The national food security and economy are mainly dependent on common wheat production and its trade, which contributes up to 60% of the Afghan population's calorie intake with consumption per capita of about 200 kg per year (Sharma, 2019).

On one hand, the current world's growing population scenario firmly emphasizes the necessity of improving wheat production and quality to alleviate world hunger and feed future populations, on other hand, wheat productivity has stagnated in various growing areas globally (Lin and Huybers, 2012) to fulfill the anticipated increase for wheat grain in food demand for future populations. Researchers must focus on the yield gap, and determine the gap between actual farm productivity and what is hypothetically attainable under ideal management (Van Ittersum et al., 2013; Godfray et al., 2010). However, the progress of the wheat productivity rate is estimated at 1.1% per year (Dixon et al., 2009), while the forecasted demand requirement is 1.7% per year by 2050 (Rosegrant and Agcaoili, 2010). Therefore, this target would be attainable through the application of comprehensive technologies including crop management, appropriate use of input, and high-yielding, disease, and drought-resistance varieties (Bentley et al., 2022; Silva et al., 2021). As a lesson learned, wheat grain yield increased up to 5-8 fold between 1960 and 2017 through the exploitation of improved varieties along with agronomic practices in Egypt (Abdelmageed et al., 2019). Furthermore, the two dedicated international centers (International Maize and Wheat Improvement Center (CIMMYT) and the International Center of Agricultural Research for the Dry Areas (ICARDA)), have highlighted the importance of variety development, along with advanced production systems technologies, such as agronomic practices (right time and the right amount of fertilization and irrigation, optimize planting time, appropriate weed and pest control and conservation agriculture), for sustainable wheat production with high quality, efficient use of resource and possibly limiting the biotic and abiotic stresses (Shiferaw et al., 2013). Both, breeding and agronomic techniques have played a crucial role in fighting against hunger and poverty, through the improvement of food production, particularly in low-income countries (Abdelmageed et al., 2019). Foulkes et al., (2022) indicated that the only way to improve wheat productivity with no harmful effect on the environment is to develop better breeding lines and improved farm and agronomic management practices.

#### 1.1 Role of improved wheat varieties in increased production and quality

Farmers want to assure higher yields, better economic returns, and lower labor and input applications. Therefore, they could achieve this using improved and adapted varieties. Modern varieties also open up a market for farmers and support the export possibility of their production (Blecha, 2019). In the last century, researchers and farmers have been continuously working to produce, evaluate and select high-yielding and pest and disease-resistant varieties that fulfill the demand of modern life (Peng et al., 2011). Over many decades, the choice of technology, particularly improved plant varieties and their adoption was to increase productivity, production, and farmer incomes (Viatte, 2001). The dwarf-improved wheat varieties played a central role in the "Green Revolution" during 1950<sup>-1</sup>960, to successfully respond to the global food demand. These varieties were produced by the crossing of the Japanese genotype "Norin 10", with a shorter stem and higher weight of spike (Hedden, 2003). Historically, about half of common wheat enhanced production has been because of these improved genotypes, while the remaining half was because of improved agronomical practices (K-STATE, 2021). Kugbei, (2011) and Chandio and Jiang, (2018) have reported that only the use of suitable improved wheat varieties increased the grain yield by 33 and 25%, respectively. Moreover, breeding efforts in the past were significantly attributed to the improvement of both traits, grain yield, and grain quality (Laidig et al., 2017). It should be noted that improved wheat varieties contain superior quality for bakery industries (Guzmán et al., 2022).

The stress of climate change would become a serious challenge in the future; therefore, climateresilient varieties are crucial to be developed according to specific zones, which will take into account the farmer's profit as well as consumers' preferences (Poole et al., 2022). Considering optimal production, beyond a variety's yield potential, farmers must consider several factors such as climate adaptation, nutrient use efficiency, tolerance to drought, and resistance to pests and disease as well (K-STATE, 2021). Nevertheless, for successful mitigation of biotic and abiotic threats, comprehensive studies of genotype-environment-management and their interaction are crucial. Therefore, finding adaptable varieties with a higher yield that could grow in a vulnerable environment, like drought and higher temperature stresses is required (Beres et al., 2020; Bilgin et al., 2016). In developing countries, farmers who use no or limited inputs or grow crops in uncertain environments, need regional-specific adapted wheat varieties to maintain production at an optimal level (Kahram et al., 2013; Mohammadi et al., 2010; Mohammadi and Amri, 2009, 2008). Increased grain yield along with improved nutrient use efficiency, has been achieved through the release of new varieties with respect to the specific agroclimatic region in Egypt (Abdelmageed et al., 2019). In developing countries, the adoption rate of new improved varieties among wheat growers is a large challenge yet; poor education, the higher price of seed, lack of training, insufficient seed availability, and lack of credit are found to be the main drivers behind the adoption of improved varieties (Sharma and Nang, 2018; Zeleke et al., 2022; Mussei et al., 2001; Chandio and Jiang, 2018).

Generally, the improved wheat varieties require more fertilizer and more efficient use of it, compared to local varieties, which means that improved varieties absorb and exploit better the nutrients from the soil. Thus, the choice of nutrient-efficient use varieties is imperative to increase grain yield and quality and reduce fertilizer application (Karaman and Sahin, 2007). In the past decades, wheat varieties were often developed without taking into account their potential to grow and yield under low soil nutrient conditions and have been only produced for superior/high yield under high nutrient status (Wissuwa et al., 2009). On the contrary, other authors reported that the use of improving varieties can significantly contribute to improving nutrient use efficiency (NUE), as a result, it can reduce nutrient application and environmental pollution (Fageria et al., 2008). Nevertheless, local tolerant varieties against aluminium in lower pH soil existed in several crops but were characterized by lower yield potential and lower kernel quality (Ceccarelli and Grando, 2007). In the past, insufficient work has been carried out for the release of tolerant improved wheat varieties for acidic soil. Breeders only focussed on high-yielding varieties under favourable environmental conditions (Wissuwa et al., 2009). In addition, heterogeneous environments remain a big problem. Therefore, decentralized on-farm trials under various environments with a program of farmers' participatory research for the selection of superior adapted varieties, are proposed as a solid solution under field conditions (Ceccarelli and Grando, 2007).

In a specific environment, studies found that the introduction of adaptable varieties with the better characteristic of nutrient use efficiency could contribute to decreasing the level of nutrients to be applied, without reducing the grain yield (Barraclough et al., 2014). Consequently, the selection of improved wheat cultivars and the development of agronomical practices, can improve NUE, and increase economic income, while decreasing environmental pollution (Belete et al., 2018). For several decades, improved wheat varieties are used to boost production, therefore the availability of local wheat varieties is in decline. Although, the power of genetic biodiversity proved that these local landraces would play a central role in the future of wheat production (FAO, 2015). Despite that the improved varieties have superior yield potential, also they may show some disadvantageous characteristics than the local cultivars. For example, the local varieties could show better traits, particularly tolerance to biotic and abiotic stresses (Arzani and Ashraf, 2017). While other authors indicated that the improved varieties mainly were selected based on specific characteristics, such as

yield potential, higher breadmaking quality, efficient use of nutrients, and tolerance to abiotic and biotic stresses (Shewry and Hey, 2015; Atinafu et al., 2022).

### 1.2 Role of nitrogen to increase production and quality of wheat

Macro-nutrients are the most essential elements for crop growth. Mainly yield quantity and quality are affected by macro-nutrient fertilizer, particularly nitrogen (N), followed by phosphorus (P) and potassium (K) (Galloway et al., 2013). However, the intensification of agricultural production has had a consequence on an excessive overuse of N and P worldwide (Kopittke et al., 2019). Globally, about 198.2 million tons of fertilizers (N, P, K) were used in 2020/21, which was 10% more than in 2019/2020, which shows a big jump since 2010/2011 (IFA, 2021). Among all, nitrogen fertilizer plays a fundamental role in plant nutrition, as it is the main component of amino acids, proteins, enzymes, and chloroplast. The efficient application of N is crucial for crop metabolism, such as photosynthesis to produce carbohydrates for food (Brink et al., 2011). In addition, N is an essential element, needed for crop development. On average considerably more N fertilizer is used compared to any other nutrient applied to crop production, particularly wheat (Haynes et al., 1986).

Thus, the synthetic nitrogen fertilizer quantity produced and applied is quite large than the other fertilizers (Houlton et al., 2019). In 2022, the demand for nitrogen fertilizer was estimated at about 111.59 million tons, 5.77% higher than in 2016 (FAO, 2022). However, demand for nitrogen fertilizers has increased sharply in the past recent decades, with Asia leading the way, followed by European countries and North America (Sharma and Bali, 2017).

In view of the increasing demand for food, the food price increased, and the cultivated land tended to reduce. At the same time, the nitrogen fertilizer price was relatively low, this suggested that the application of nitrogen is beneficial for the optimization of farm economic return, therefore the use of high rates of N was recommended (Jensen et al., 2011). However, the overuse of N fertilizer is reported to be reducing its efficiency (Cui et al., 2010). Typically, farmers thought, that by applying more N, the yield will increase which has a higher economic return (Haynes et al., 1986). Therefore, the recovery of nitrogen in wheat is estimated to be relatively poor, which is approximately 33% of the applied nitrogen is taken up by wheat, and the remaining part is lost (Ichir et al., 2003). Overall, synthetic nitrogen fertilizers have proved major advances in food production growth, but substantial discrepancies in the globe's nitrogen balance remain a major challenge. Therefore, since the past century, developed countries have benefited more from synthetic nitrogen, compared to developing nations (Erisman et al., 2008). Many authors reported that nitrogen deficiency is the main barrier to crop productivity, specifically for wheat, and ultimately had a negative influence on global food security (Holman et al., 2016; Lima et al., 2021; Sehgal et al., 2018). Therefore, the quantity and

quality of cereal grain particularly wheat production is mainly affected by nitrogen fertilization (Wang et al., 2008). In winter wheat, on average the net unit benefit of nitrogen was estimated at up to 2.7 Euro per kg nitrogen applied in the soil, in European countries (Brink et al., 2011). A sufficient amount of nitrogen should be applied at different crop stages in order to be available for good plant growth so that it can contribute to NUE and minimize environmental risk (Stewart, 2018).

Nitrogen is known to be the key driver of plant growth, which is increasing crop yield and quality, but it is also a limiting factor for environmental quality, linked to inappropriate use of nitrogen that could be lost through leaching in the groundwater, and emission in the atmosphere (Sharma and Bali, 2017). Moreover, many studies have found that the effect of overuse of nitrogen on human health has been highlighted as a dominant challenge and contributes to respiratory disease and cancer (Townsend et al., 2003), similarly enhanced nitrous oxide emissions in the environment, driving up the global climate uncertainty and would subsequently impact the human life (Galloway et al., 2013). On one hand, synthetic nitrogen fertilizer is an essential needed resource for the optimization of production, on the other hand, it creates a serious environmental problem. Globally, researchers seek to develop responsive systems to resolve both sides of these problems: determining adequate fertilization of crops but reducing the negative effects of nitrogen in the environment (Galloway et al., 2013). In addition, access to sufficient fertilizer is imperative for crop growth, research shows that the proper use of nitrogen fertilizer, associated with proper application, and the right amount at right time significantly increases grain yield and nitrogen use efficiency (Li et al., 2021). Proper nitrogen fertilization has been strongly linked to the concentration of available nitrogen and water in the field (Ali and Akmal, 2022).

However, the accessibility of farmers to commercial nitrogen fertilizers in part of Asia, Africa, and Latin America underlines major problems. Often the farmers grow crops in depleted soil nitrogen conditions to produce their food requirements (Vitousek et al., 2009). Moreover, inadequate universal access to nitrogen fertilizer threatens food production, particularly in low-income countries, which substantially suppresses economic growth, and social resilience (Sanchez, 2010). On the contrary, overuse of nitrogen application to crops, particularly in higher-income nations, disrupted the world economy, food production, environmental quality, and biodiversity. The study reported that several hundred billion US dollars are lost annually due to excessive use of nitrogen in developed countries (Brink et al., 2011; Vitousek et al., 2009). Furthermore, researchers found that the overuse of N fertilization would result in water pollution, environmental pollution, and the destruction of soil biodiversity. On the contrary, if N application is limited to small quantities, the soils would result in lower production and soil degradation. But there are ample options to minimize these risks without reducing the profit. This can be achieved through appropriate nutrient management such as

optimizing nitrogen fertilization, better estimation of crop nitrogen requirements, increasing soil organic matter content, precision agriculture use, improving the timing of fertilization, method of nitrogen application, and reducing soil tillage (Jensen et al., 2011; Ma et al., 2019; Anas et al., 2020; Sharma and Bali, 2018). Literature reported that sustainable production and productivity of wheat, depending on the use of integrated technologies, mainly agronomical practices optimize the efficient function of nitrogen fertilization (Atinafu et al., 2022).

#### 1.3 Role of phosphorus in increasing production and quality of wheat

After nitrogen, phosphorus (P) is one of the most essential elements that play a central role in the development and growth of cereal crops including wheat. This element is not only important for the formation of proteins and enzymes but also plays a principal role in photosynthesis and the physiological process of the plant (Weeks and Hettiarachchi, 2019). As P is known to be the essential factor for crop production, therefore, to produce enough food, requires a higher amount of fertilizer (Lott et al., 2011). Additionally, phosphorus is one of the main limiting factors for wheat production in the world, and the role of phosphorus nutrients cannot be replaced by another element (Wolfe-Simon et al., 2011). However, optimal phosphorus application is associated with improved tillering and spike number per unit of area, decreasing winterkilling, and increasing water use efficiency. Therefore, common wheat requires about 10.5 kg of  $P_2O_5$  per ton of grain (Stewart, 2018). It appears that an effort should be made on the efficient use of phosphorus fertilizer at every stage by policymakers, researchers, and farmers, in order to maintain sustainable wheat production (Syers et al., 2008). Presently, phosphorus fertilizer has been widely applied for intensive cropping systems, but its recovery efficiency for crops estimated is very low, ranging between 10-30% (Syers et al., 2008). However, the global phosphorus shortage is likely to threaten the world's food security. Concerted efforts should be made soon by all nations, policymakers, industry, and society to improve the phosphorus application as efficiently as possible through agronomical practices and breeding efforts (Cordell and White, 2014).

In recent years, the demand for P was considerably high, but also the price of fertilizers was much higher. For example, the phosphorus fertilizer quantity required showed an increase of 9.39% from 2016 to 2020 (44.94 to 49.6 million tons respectively) (FAO, 2022). Although P is the major plant nutrient, the world's sources of P fertilizers are the smallest. Globally phosphorus practices should be improved to assure reducing the overuse of P, without reducing the yield (Fageria et al., 2017). Phosphorus is recognized as an essential nutrient to be applied largely for crop production. Meanwhile, P has undesirable effects on freshwater and causes negative effects on the ecosystem, including the balance of species of plants, aquatic organisms, and fishes (Syers et al., 2008). There is

a must, particularly in developing countries to improve the PUE, in order to achieve food security for their population growth (Fageria et al., 2017). The possible option to improve the P use efficiency and soil fertility depends on fertilizer management practices by modifying the topsoil surface through the application of crop residue and animal manure (Mubeen et al., 2021).

The worldwide top P user countries in 2019 are China 11.18, India 7.66, Brazil 5.42 and the United States 4.07 million tons (Statista, 2022). Since past decades, phosphorus availability has been a major limiting factor in many countries in the world (FAO, 2007).

Nowadays, the effects of fertilization on eutrophication and environmental pollution are contradictory. Typically, they are dependent on the method and overdose of applied fertilizers (Tully and Ryals, 2017), which have contributed to social and economic costs from reduced ecosystem services. Despite many studies, there is some lacking information to support a clear strategy to alleviate the overuse of phosphorus and to optimize production (IFPRI and FAO, 1995). The EU - Common Agricultural Policy (CAP) objective for 2022, has stated to identify ways to support efficient and sustainable fertilizer use whilst producing acceptable production and quality with preventing nutrient losses (EU, 2022).

Application of P to soil where P is not deficient has the consequence of no increase in yield or improve crop quality. Additionally, it will change the wheat behavior which will be more susceptible to water shortages and heat stresses (FAO, 2007). On a global scale, phosphorus must be used as efficiently as possible to save or conserve phosphorus resources, as phosphorus is a non-renewable resource (Freiling et al., 2022). Scientists found that phosphorus use efficiency can be effectively improved by applying the right amount of fertilizer, at the right time, in the right place with the associated right ratio of the other fertilizer applications (Hussain et al., 2019). More efficient use of P fertilizer in the wheat field can be achieved using the soil test, subsequently applying the right amount of P which matches the plant requirement (Gadaleta et al., 2022).

Phosphorus is involved in seed formation, root development, uniform heading, and increased tolerance of the plant to cold winter (Weeks and Hettiarachchi, 2019). The high-yielding wheat varieties require optimal phosphorus fertilization to achieve higher grain yields with better quality (Adnan et al., 2020), whereas the application of an accurate dose of P fertilizer combined with the right practices can increase fertilization efficiency and decrease the production cost (Gadaleta et al., 2022). Phosphors are required for optimal wheat yield and quality in all environments worldwide. Thus, the necessary management application is paramount, to ensure the P use efficiency at an optimal level, and to keep below the eutrophication risk, as much as possible (Edwards, 2017 and Gong et al., 2022). In view of agronomic perception, the application of an insufficient dose of phosphorus

fertilizer in the soil will hinder crop growth, while an overdose will be wasteful and could pose an environmental threat, such as eutrophication of surface water (Dobermann and White, 1999).

### 1.4 Role of sulfur to increase production and quality of wheat

Sulfur (S) is the fourth essential element in wheat production after N, P, and K, needed for higher yields and quality. The main source of S requires by plants are chemical fertilizer, animal manure, plant residue, and S gases deposits from the atmosphere (Fageria, 2009). Sulfur is the key component of cysteine and methionine amino acids which are essential for protein formation in the plant. In addition, S plays a vital role in chlorophyll formation (Zhao et al., 1999). Application of an adequate dose of S is an essential way to increase crop yield and maximize S use efficiency. Additionally, the need for S application should be specifically related to the rate of N being used, since both elements are essential for protein creation (Penn State Extension, 2021). Harward et al., (1962) reported that an adequate S ratio to N were ranging between 11:1 and 17:1 in field crops to increase the yield and quality of the grain. The S requirement of wheat is estimated between 15 to 20 kg ha<sup>-1</sup> for optimal production (Zhao et al., 1999). Global S use efficiency (SUE) for cereal crops averaged 18% between 2005 and 2014. During this period, the highest SUE (22%) was observed in 2014, while the lowest (14%) was recorded in 2005 (Aula et al., 2019). Currently, S deficiency is a major challenge to crop production in various parts of the world. Since, the functions of sulfur, in field crop nutrition are similar to nitrogen, therefore sulfur scarcity in the soil decreases crop yields but also reduces the quality of the food grain (Fageria, (2009). According to literature reviews, the management practices, which were discussed for increasing N and P use efficiencies are also adaptable for S fertilization. Furthermore, Fageria, (2009) proposed that the application of the right amount and right time of S, following proper crop rotation, application of organic manures, selection of S efficient varieties, and improvement of soil moisture may improve the SUE in the field. However, the author pointed out that S in combination with N fertilization significantly increases the grain yield of wheat (Soofizada et al., 2022; Wilson et al., 2020); similarly, another investigation demonstrated that S fertilization substantially increased the wheat grain and protein yields (Tao et al., 2018); while Guerrini et al., (2020) found that S fertilization only affected the protein content and dough alveograph but did not increase the yield.

### 1.5 Role of crop rotation and soil tillage management on wheat production

Crop rotations and soil tillage management are useful practices that impact crop productivity, soil health, fertility, disease, and pest cycle interruption and finally improve plant biodiversity. However, crop yields could be increased through breeding techniques, the application of synthetic fertilizers, and pesticides; but subsequently, it requires an increase in the crop's water demand (Lobell et al.,

2014). Gaudin et al., (2015) found that diversifying cropping systems with the application of reduced soil tillage practices can sustainably increase farmer agronomic benefits and improved soil moisture capacity in the field. Similarly, Darguza, and Gaile, (2020) indicated that winter wheat yield was significantly increased by diversifying the cropping system, but soil tillage practices did not significantly increase the yield. Therefore, the highest agronomical benefits were obtained in the following four-crop rotations: faba bean – winter wheat – oil-seed rape – barley.

Different authors (Montemurro, 2009, Carcer, et al., 2019, Hammel, 1995) indicated that the combination of both crop rotation and reduce soil tillage strategies sustainably improves wheat productivity potential (quantitively and qualitatively), as well as its response to ameliorate the soil physical-chemical and biological properties. Likewise, Jalli et. al., (2021) confirmed that among field management practices, crop rotation, and soil tillage are more influential in balancing sound wheat production and soil fertility. Therefore, they found that wheat yield increased by 30% with a diversified cropping system under no-tillage, and by 13% under the field plowing system, compared to the monoculture system. Additionally, other authors found that no-tillage practice in the legume-based rotation system can assure wheat productivity and mitigate the adverse effect of climate (higher temperature and rainfall deficiency) in the rainfed condition (Mohammad, et. al., 2012). Subsequently, Bonciarelli et al., (2016) reported that wheat yields potential declines, when wheat is grown in a continuous cropping system. Moreover, the findings of the other studies confirmed that the wheat yield increases, if wheat is followed with the oilseeds crop (Schillinger & Paulitz, 2018), legume crops (Babulicová, 2016), or root crops (Smagacz, et al., 2016) rotation systems.

Further studies are required to better understand the effect of crop rotation and soil tillage practices on wheat productivity, and the pest behavior incidence (pathogens, weeds, and insect pests) under wheat field conditions.

#### 1.5 Afghanistan agriculture status.

Afghanistan is a landlocked, and agricultural country. More than 80% of the Afghan people and approximately 90% of the poor people live in the countryside. Agriculture plays an important role in their occupation and livelihoods (Soofizada, 2017; World Bank, 2014). Therefore, the economy of the country is mainly dominated by the agriculture sector, and agriculture contributed to about 60% of the national GDP for several decades. (Waliyar, 2009).

Afghanistan has a continental climate, ranging from arid in the south and southwest areas to semiarid in the remaining part of the country. Hot summers and cold winters are common (Savage et al., 2009). The country can be sub-divided into seven main agro-climatic zones: North-Eastern, Northern, Eastern, Central, Western, Southern, and South-Western (Rahimi, 2017) In Afghanistan, the total land area is approximately 652,090 square kilometers, but only 12% is arable land and less than 6% of the arable land currently is available for crop cultivation (LandLinks, 2018). However, larger desert areas and extreme mountainous terrain limit the amount of arable land for agricultural purposes in Afghanistan (Waliyar, 2009).

Overall, agriculture production mostly depended on irrigation practices, except for a few areas, where rainfed agriculture can be practiced, due to fair precipitation, but the rainfall may not be stable across the year and locations. Over 80% of the country's water resources have been there from snowmelt, the remaining amount would come from rainfall. The average rainfall was ranging between 100 to 400 mm depending on the region (Waliyar, 2009).

The current agriculture systems in the country depend on traditional practices, as the country suffered from several decades of conflicts, severe drought periods, lack of infrastructure, poor management practices, and inadequate agriculture inputs such as chemical fertilizer, pesticides, machinery, and quality seeds (Government of Afghanistan, 2018). The field crop sub-sector in Afghanistan is quite an undiversified system and is overly focused on wheat crops, because of this farmer household remains vulnerable (Bolton, 2019).

The major agriculture crops in Afghanistan are cereals crops (wheat, rice, maize, barley, and sorghum), horticulture crops (grapes, pomegranate, almond, pistachio, apple, apricot, watermelon, melon, potatoes, onion, tomato), legume crops (bean, check peas, soybean, lentil, and mung bean), industrial crops (cotton, saffron and sugar can) (ARIA and FAO, 2018). Among all, wheat is the major stable crop and plays a central role in food security and job creation (World Bank, 2014). In Afghanistan, households, on average, spend approximately 60% of their budget on food, of which, 35% is spent on wheat (Malakhail, 2019). The total area cultivation under wheat in Afghanistan is around 2.7 million hectares (about 25% total agricultural and 80% cereals cultivated area), 55% cultivated irrigated and 45% rainfed area (Tiwari et al., 2020), with a total production of 5.2 million tones and yield per hectare about 2.5 t and 1.1 ha<sup>-1</sup> in the irrigated and rainfed field, respectively, compared to India with 3.5 t ha<sup>-1</sup> (Poole et al., 2022). While the total national wheat demand is estimated to be 6.5 million tons (NSIA, 2021).

Numerous challenges limit the rate at which wheat economic productivity can be improved. Availability of sufficient quality seed adapted high yield variety, drought and disease stress, poor agronomical practices, lack of incentive program for farmers, poor accessibility of farmers to machinery, and poor-quality fertilizers and pesticides are known as major constraints in Afghanistan's wheat sector (MAIL and FAO, 2013).

Potential opportunities to improve yield in the wheat sector of Afghanistan may include proper crop management, the wide use of improved varieties, and the use of fertilizers with the right amount and right application time (Dreisigacker et al., 2019). Many authors suggested that improving wheat productivity and quality in Afghanistan depends upon an integrated strategy. Thus, for immediate and intermediate responses: the present farmers' farm practices and management should be transformed into modern agriculture systems such as 1. improve the application of inputs and efficiency (right variety, fertilizers, labour, water, and machinery), 2. improve farmers' profits (increase productivity with a sufficient supply of inputs), 3. reduced pre-and post-harvest -losses (with smart field management and use of machinery) 4. improve soil fertility and reduce soil erosion and degradation (following proper crop rotation, cover cropping system and application of zero and/or reduce tillage). For the long-term responses: 1. rehabilitation of irrigation systems, 2. restoration of degraded lands, 3. expansion of the wheat irrigated area, 4. Developing/introducing high-yield climate resilience varieties with advance agronomic practices. Overall, the proposed strategies for the improvement of the wheat sector in Afghanistan, are only possible through research and development (Waliyar, 2009; Soofizada, 2017; ARIA and FAO, 2018; Poole et al., 2022).

### 2. Objectives

In this thesis research, the agronomic management strategies using different common wheat varieties, under irrigation and rainfed conditions were investigated in Afghanistan and Italy. The main objective of this thesis was to evaluate the effects of nutrient management as a function of climate and soil variability on common wheat varieties, in order to improve the yield and quality of wheat production. But specifically, this thesis follows three sub-objectives to assure that high yield and quality of wheat that can be obtained through the selection of high-yielding varieties and the application of the right amount of fertilizers, based on climate and soil variabilities.

- To evaluate the response of different nitrogen and sulfur fertilization strategies, associated with different seeding densities on 14 "old" wheat varieties, under the rainfed condition, in Italy.
- To evaluate the influence of the phosphorus fertilization regime with a combination of nitrogen application on common wheat varieties at four different locations under irrigated conditions in Afghanistan.
- 3) To study the yield response and stability performance of 33 common improved wheat varieties under three different climatic conditions in Afghanistan.

In fact, there has been a huge research effort on agronomical practices and nutrient management, to increase wheat production, but there are research deficits on the effect of phosphorus and nitrogen on wheat quality, particularly on starch and protein content as well as rheological characteristics. Additionally, climate-resilient varieties are not available, therefore such varieties are urgently needed in Afghanistan. However, the information about the existing studies also is not sufficient for sustainable production. In this context, a strong study effort still is required to provide more information about the sustainable wheat quality and quantity production

### 1. List of papers

One out of four papers was from the trial which was carried out at Cesa research station, Tuscany, Italy by the Department of Agriculture, Food, Environment and Forestry (DAGRI), with the collaboration of Cesa research station's researchers during 2017/2018 and 2018/2019. The field data were collected before the start of my PhD activities. According to the scope of this research, the DAGRI department delivered the data to the author of this thesis. But the analyses of the kernel quality were carried out in 2020, which was part of the PhD activities.

The remaining three papers were from the trials conducted at the Agricultural Research Institute of Afghanistan (ARIA) from 2016 to 2022. All trials were managed, and the data was collected by the author of this thesis in collaboration with local researchers. The trial of the second paper (chapter 3.2) was conducted between 2016/2017 and 2017/2018 at Baghlan, Balkh, Helmand, and Herat research stations, before starting my PhD activities. The trial of the third paper was conducted during 2017/2018 and 2019/2020 at Balkh, Helmand, and Nangarhar. Therefore, the first year of data collection was carried out before starting my PhD program, but the collection data of the second year was part of PhD activities. Finally, the trial of the fourth paper was conducted during 2020/2021 and 2021/2022, at Baghlan, Balkh, Helmand, and Herat, in Afghanistan.

First paper: Effects of Nitrogen plus Sulfur Fertilization and Seeding Density on Yield, Rheological Parameters, and Asparagine Content in Old Varieties of Common Wheat (*Triticum aestivum* L.). *Qudratullah Soofizada, Antonio Pescatore, Lorenzo Guerrini*, *Carolina Fabbri, Marco Mancini, Simone Orlandini and Marco Napoli. Published in Agronomy Journal. Agronomy* 2022, *12*(2), 351; https://doi.org/10.3390/agronomy12020351

Second paper: Evaluation of nitrogen and phosphorus responses on yield, quality, and economic advantage of winter wheat (*Triticum aestivum*, L) under four different agroclimatic zones in Afghanistan. *Qudratullah Soofizada, Antonio Pescatore, Rahmatullah Atefi, Chiara Grassi, Simone Orlandini and Marco Napoli. Published* in Agronomy Journal. *Agronomy 2023, 13(2), 345; https://doi.org/10.3390/agronomy13020345* 

Third Paper: Effects of Pedoclimate and Agronomical Management on Yield and Quality of Common Wheat Varieties (*Triticum aestivum* L.) in Afghanistan. *Qudratullah Soofizada*, *Antonio Pescatore, Simone Orlandini and Marco Napoli*. Ready to be submitted for publication. Fourth paper: Evaluation of grain yield and stability performance of 33 bread wheat varieties (*Triticum aestivum* L.) under different agroecological zones of Afghanistan. *Qudratullah Soofizada, Antonio Pescatore, Simone Orlandini and Marco Napoli.* Ready to be submitted for publication.

### 3.1 First Paper

Effects of nitrogen plus sulfur fertilization and seeding density on yield, rheological parameters and asparagine content in old varieties of Common Wheat (*Triticum aestivum*, L.)

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**Abstract:** Numerous epidemiological studies have highlighted the positive effects on the health of wholegrain bakery products made from 'old' common wheat (*Triticum aestivum* L.) varieties. However, 'old' common wheat varieties display poor rheological properties, and there is limited information on its free asparagine (ASN) content, the main precursor to acrylamide during the baking process. This paper evaluates the effects of two seeding density levels (SD: 200 and 350 seed m<sup>-2</sup>), three nitrogen levels (NL: 35, 80, and 135 kg N ha<sup>-1</sup>), and two sulfur levels (SL: 0 and 6.4 kg S ha<sup>-1</sup>) towards improving the grain yield (GY), rheological characteristics, and ASN content of 14 'old' common wheat varieties. SL and SD treatments significantly increased GY without decreasing the protein content (PC), while NL significantly increased the PC without affecting GY. The dough strength (W) increased significantly increased by 111% as NL increased from 35 to 135 kg ha<sup>-1</sup>, while ASN significantly decreased by 85.1% with the SL treatment. The findings show that 135 kg N ha<sup>-1</sup> combined with 6.4 kg S ha<sup>-1</sup> can improve the technical performance of 'old' wheat wholegrain flours while maintaining the ASN as low as possible.

**Keywords:** old winter wheat varieties; agronomic treatments; sulfur fertilization; free asparagine; rheological properties.

#### **3.1.1 Introduction**

Common wheat (*Triticum aestivum*, L.) is one of the most important cereals worldwide for both human and livestock consumption, contributing towards enhancing the global economy (Poole et al., 2021; Shewry and Hey, 2015b). Common wheat production amounted to 761 Mt in 2020 (FAO, 2021) and provides protein for the nutrition of both humans and livestock, estimated at around 60 Mt y<sup>-1</sup> as reported in (Shewry, 2009). After the Green Revolution, common wheat production has increased, attributable to intensive fertilizer use and the breeding of cultivars, respectively, characterized by higher production yields, increased tolerance to diseases and pests, higher nutrient use efficiency as well as a higher protein production per hectare, and with a gluten composition suitable for industrial processing (Arshad, 2021; L. Guerrini et al., 2020; Migliorini et al., 2016; Sanchez-Garcia et al., 2015; Sylvester-Bradley and Kindred, 2009). Conventionally, common wheat cultivars registered before the late 1960s are referred to as 'old', while those registered coinciding with the period of the Green Revolution are referred to as 'modern' (L. Guerrini et al., 2020).

In the past decades, 'old' common wheat varieties were reintroduced, and many local microeconomies have been developed around 'old' cultivars (Cappelli et al., 2018; L. Guerrini et al., 2020). In fact, the increase in pollution and food security problems led to reconsidering winter wheat production in terms not only of productivity but also of environmental and human health impact (Godfray and Garnett, 2014). Interest in low-impact and sustainable agricultural practices, combined with functional (health-promoting) products, permitted the rediscovery of 'old' common wheat varieties, considered to be more suited to unfavorable environmental factors and with improved functional value in comparison to the 'modern' varieties (Suchowilska et al., 2009). Numerous epidemiological studies have highlighted the positive effects on health and disease prevention of bread and other bakery products made from 'old' varieties (Dinelli et al., 2011; Dinu et al., 2018). In particular, the production of wholegrain bakery products is recommended as most of the bioactive compounds, associated with health benefits, are concentrated in the bran and aleurone layers, respectively (European Commission, 2021; Zilic et al., 2020). However, although the aleurone layer also contains good quality free amino acids and proteins, it also stores free ASN that is the predominant precursor of acrylamide formation in wholegrain bakery products (Seal et al., 2008; Zilic et al., 2020). As acrylamide is classified as a neurotoxin and "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC, 1994), free ASN concentration in grain should be monitored and maintained as low as possible. (Corol et al., 2016) found the free ASN contents in 150 genotypes of common wheat ranging from 0.32 to 1.56 mg g<sup>-1</sup> of dry matter (corresponding to 2.4 - 11.8

micromoles g<sup>-1</sup> of dry matter) in whole meal wheat flours. The 'old' cultivars are characterized by poor efficiency in converting assimilated nitrogen (N) to grain protein, this may contribute to an increased accumulation of ASN (Wilson et al., 2020b). Furthermore, the grain ASN content may increase in relation to stress conditions such as water logging, drought, and plant diseases, as well as either nutrient excesses or deficiencies (Lea et al., 2007). Of all the essential nutrients applied in the field, N is the most important for vegetative crop growth, productivity, and grain quality, thereby affecting plant development (Sinclair and Horie, 1989). Sulfur (S) is an essential element for wheat nutrition and S deficiency significantly affects the production and quality of wheat (Gyori, 2005). Interestingly, it was observed that ASN formation was correlated positively with N availability (Martinek et al., 2009) but was increased in presence of S deficiencies (Wilson et al., 2020b). In this context, (Wilson et al., 2020) detected free ASN concentrations ranging from 21.0 to 41.4 micromoles  $g^{-1}$  in S-deficient conditions. Aside from the effects on ASN, S affects not only N utilization and grain quality (Salvagiotti et al., 2009), but also plays an important role in baking quality (L. Guerrini et al., 2020). Thus, optimized S and N fertilization practices can be implemented to reduce the ASN concentration in wholegrain common wheat and consequently, act towards reducing the health concern of acrylamide in baked products (Curtis et al., 2018).

Despite the increased interest in old varieties for functional benefits and low input agricultural practices, these varieties are also usually characterized by a low dough strength (W) and an unbalanced ratio between dough tenacity and dough extensibility (P/L) compared to modern varieties. These rheological parameters render old varieties more difficult for baking (L. Guerrini et al., 2020). In order to improve the rheological properties of both old common and durum varieties, research on fertilizer supplements is currently being investigated (De Santis et al., 2018; Guerrini et al. 2020).

While ASN content in common wheat grain has been studied extensively on a global scale (Lea et al., 2007; Navrotskyi et al., 2018; Rapp et al., 2018; Wilson et al., 2020b), only limited information on ASN concentration in 'old' cultivars are available (Poudel et al., 2021). Given the increasing importance of 'old' cultivars and the success of crop management strategies in reducing ASN content in 'modern' cultivars, to the best of our knowledge, there is no work specifically focused on reducing the ASN concentration in the grain of 'old' cultivars. To address this aspect, the present study was aimed at investigating grain yield, dough rheology, and ASN concentration of 14 "old" Italian *Triticum aestivum*, L. varieties, in response to varying seed density (SD), as well as N and S fertilization rates. The objective was to simultaneously evaluate

the capacity of these agronomical practices in improving the technological performance of the dough whilst maintaining the lowest levels of ASN.

### 3.1.2 Material and methods

### 3.1.2.1 Field experiment

The experimental field trial was conducted at the demo-farm "Tenuta di Cesa" in Marciano della Chiana, Tuscany (Lat. 43.3095; Lon. 11.8264; 246 m asl) from September 2017 to July 2019 under rainfed conditions on an alkaline clay-loam soil (Table 1).

soil parameters	value
Sand (%)	37
Clay (%)	34
Silt (%)	27
pH	8.13
Organic matter (%)	0.88
Total N (%)	0.03
Olsen available P (mg kg <sup>-1</sup> )	0.42
Available S (mg kg <sup>-1</sup> )	3.3

Table 1. Soil properties.

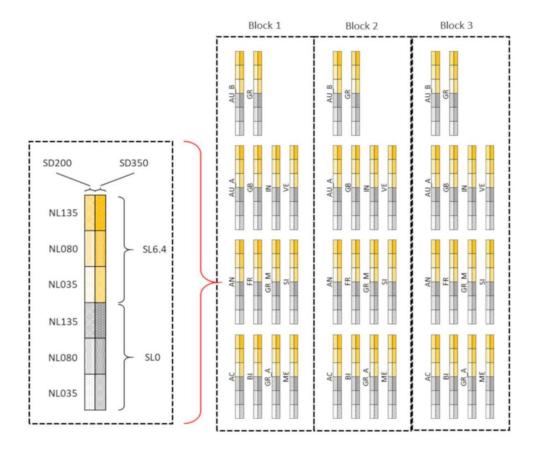
The soil was characterized by a low organic matter content and low nutrient availability. In particular, the soil was both phosphorous and sulfur-deficient, with less than 10 mg kg<sup>-1</sup> available P (Olsen et al., 1954) and S (Kilmer and Nearpass, 1960), respectively. Fourteen Italian old varieties of winter wheat (*Triticum aestivum* L.) were investigated. The old varieties were: Acciaio (AC), Andriolo (AN), Autonomia A (AU\_A), Autonomia B (AU\_B), Bianco Nostrale (BI), Frassineto 405 (FR), Gentil Bianco (GB), Gentil Rosso (GR), Gentil Rosso Aristato (GR\_A), Gentil Rosso Mutico GR\_M), Inallettabile (IN), Mentana (ME), Sieve (SI), and Verna (VE) (Table 2)

**Table 2.** Release year and origin for the wheat cultivars used in this study. Data were obtainedfrom the website of the seed bank in the Tuscany Region (Tuscany Region, 2021).

Variety	Year of Release	Origin
AC	1950	Selection of "Mara", in turn, selection of "Frassineto 405"
AN	1933	Selection of the local landrace "Andriolo"
AU_A	1938	"Frassineto 405" x "Mentana"
AU_B	1930	"Frassineto 405" x "Mentana"
BI	1927	Selection of the local Indrace "Bianco Nostrale"
FR	1932	Pureline selection of the "Gentil Rosso"
GB	1900	Local landrace dating back to the late 19th century
GR	1900	Local landrace dating back to the late 19th century
GR_A	1900	Selection of the local landrace "gentil Rosso"
GR_M	1900	Selection of the local landrace "gentil Rosso"
IN	1920	Selection of the "Hatif Inversable"
ME	1913	("Wilhelmina" x "Rieti 21") x "Akakomugi"
SI	1960	"Est Mottin 72" x "Bellevue II"
VE	1953	"Est Mottin 72" x "Mont Calme"

Five of the old varieties in the trial were derived from the older varieties that were used as parental material. These included: AU\_A and AU\_B which were derived from crossing ME x FR, and FR, GR\_A, and GR\_M, derived from the selection of the GR landrace. The characteristics of the varieties were obtained from the website of the seed bank in the Tuscany Region (Tuscany Region, 2021).

The 14 wheat genotypes (Gen), were evaluated during two growing seasons (Y) with 12 agronomic treatments comprising two seeding densities (SD) (200 and 350 seed m<sup>-2</sup>, namely SD200 and SD350, respectively), three nitrogen fertilization rates (NL) (35, 80, and 135 kg N ha<sup>-1</sup>, namely NL35, NL80, NL135, respectively), and two sulfur fertilization rates (SL) (0 and 6.4 kg S ha<sup>-1</sup>, namely SL0 and SL6.4, respectively) (Figure 1). The experiment was established as a strip-plot design with three replicate blocks per year. Gen was arranged in vertical strips as the main plot, SD was assigned to the vertical sub-plots, SL was applied horizontally in sub-sub-plots and lastly, NL was assigned to horizontal sub-sub-plots, respectively. Each sub-sub-subplot was 14.4 m<sup>2</sup> (width of 1.44 m and length of 10 m).

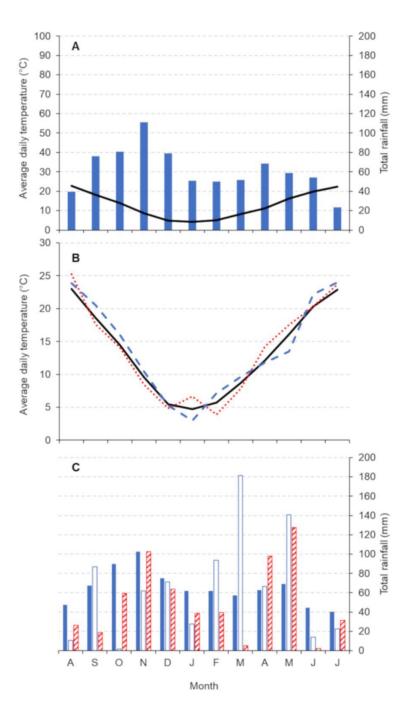


**Figure 1.** Experimental design and plot layout of trials (not in scale); on the left, the plot layout for each variety: SD200 and SD350 represent seeding density of 200 and 350 seed m<sup>-2</sup>, respectively; NL35, NL80, and NL135 represent the three nitrogen fertilization rates of 35, 80, and 135 kg N ha<sup>-1</sup>, respectively; SL0, and SL6.4 represent the two sulfur fertilization rates of 0 and 6.4 kg S ha<sup>-1</sup>, respectively. On the right is the disposition of the plots within the blocks for different varieties.

Soil tillage was carried out to a depth of 0.40 m with a mouldboard plow in both September 2017 and 2018, followed by a tandem disk harrow (0.1 m depth) to break the clods. Before seeding,  $174 \text{ kg ha}^{-1}$  of triple superphosphate (P<sub>2</sub>O<sub>5</sub>:46%) was broadcasted and immediately incorporated into the soil by means of a tandem disk harrow (0.05 m depth). The seeding was performed on 20 November, and 15 November, in the first and second years, respectively. Nitrogen application was implemented over three distinct periods. Initially, 20% nitrogen was broadcasted at seeding as ammonium nitrate (N:26%). Thereafter, 40% was spread at tillering as ammonium nitrate (N:26%) with a final 40% at the beginning of the stem elongation as urea (N:46%). As suggested in (Guerrini et al., 2020), in S6.4 a total of 6.4 kg S ha<sup>-1</sup> was distributed at booting by spraying a solution containing 20 g L<sup>-1</sup> of wettable sulfur powder (80% a.i.; Thiovit Jet 80WG®, Syngenta, Basel, Switzerland). At tillering, a broadleaf herbicide treatment was performed by distributing Manta Gold (Syngenta, Basel, Switzerland) at a dose of 2.5 L ha<sup>-1</sup> (60 g L<sup>-1</sup> fluroxipir acid, 23.3 Clopyralid, and 266.7 g L<sup>-1</sup> MCPA acid). The monocot weeds were removed from each plot by performing manual weeding at tillering and stem elongation. In both growing seasons, no crop damage by weeds, insects, or diseases, was observed. Winter wheat was harvested at commercial maturity (grain moisture < 13%) on 12 July 2018 and 5 July 2019. For each sub-subplot, the grain biomass was calculated to determine the grain yield per hectare (GY, t ha<sup>-1</sup>).

### 3.1.2.2 Meteorological conditions

The climatic conditions were typically Mediterranean, with average daily temperatures around 13 °C and approximately 750 mm of rain per year, mostly concentrated in Autumn and Spring, as well as the dry summer period (Figure 2).



**Figure 2.** (**A**) Walter-Lieth climate diagram of the study site (data 2001-2020), with monthly daily average temperature (°C, black continuous line) and monthly total rainfall amount (mm, histograms). (**B**) Comparison of the monthly daily average temperature (mm) measured during 2001–2020 (black continuous line), 1<sup>st</sup> growing season (blue dashed line), and 2<sup>nd</sup> growing season (red dotted line); (**C**) comparison of the monthly rainfall amount (mm) measured during 2001–2020 (blue color-filled histograms), 1<sup>st</sup> growing season (hollow histograms) and 2<sup>nd</sup> growing season (red diagonal-filled histograms).

The average temperature pattern during both growing seasons was consistent with the long-term temperature pattern (Figure 1). However, the average temperature values across the first and second growing seasons (13.7 and 13.9 °C, respectively) were higher than the long-term average

(13.0 °C). In both years, rainfall distribution data fluctuated significantly with respect to the long-term rainfall pattern.

During the first growing season, excess rainfall was recorded from February to May, corresponding to the tillering to flowering phenological stage of winter wheat. Then a shortage of rainfall was experienced in June, the month coinciding with grain filling. The average temperature values at flowering and grain filling in Spring 2018 were slightly warmer than the long-term averages by about 1.2 and 0.5 °C, respectively. During the second growing season, excess rainfall was recorded in April and May (from booting to flowering phenological stage of winter wheat), while a rainfall shortage was experienced in March, coinciding with stem elongation, as well as June. During the summer months of 2019, the daily average temperature at flowering was lower than the long-term average by 2.4 °C. Therefore, between May and June 2019, there was a temperature increase of 8.7 °C, which could have resulted in stress for the plant during both the initiation and grain-filling phases.

### 3.1.2.3 Analysis of kernels and dough

The 1000 kernel weight (TKW, g 1000<sup>-1</sup> seeds) and hectolitre weight (HW, kg hL<sup>-1</sup>) were determined according to ISO (2009, 2010). For each treatment, wholemeal flour samples were obtained by milling kernel samples in a grinder with a 0.5 mm screen (Cytolec 1093 lab mill, FOSS Tecator, Hoganas, Sweden) as reported in (Guerrini et al., 2020) and (Žilić et al., 2011). The wholemeal flour samples (5 mg) were analysed with a CHNS analyser (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA) to determine total nitrogen percentage and then converted to total protein percentage (PC, %) by multiplying by 5.7 according to ICC (2000). The protein yield per hectare (PY, kg ha<sup>-1</sup>) was calculated as the product of GY by PC. The ASN concentration in wholegrain flour (ASN, micromoles g<sup>-1</sup>) was determined using an enzymatic method (K-ASNAM L-Asparagine/L-Glutamine/Ammonia kit; Megazyme, Illinois, USA) followed by spectrophotometric quantification (340 nm) using a Lambda 20 UV/Vis Spectrophotometer (PerkinElmer Waltham, MA, USA) as reported by (Lecart et al., 2018).

Dough rheology was performed according to (ISO 27971, 2015). Briefly, wholegrain flour (250 g) was mixed in the Chopin alveograph chamber with a NaCl solution (2.5% w/w) for 8 min without adding yeast. The resulting dough was extruded and allowed to rest for 20 min before performing the alveographic parameters: the ratio between dough tenacity and dough extensibility (P/L) and the dough strength (W; 10<sup>-4</sup>J). TKW, HW, and PC were determined for

each sub-subplot, while ASN, W, and P/L were determined for each treatment on a bulk from the three replicates.

### 3.1.2.4 Statistical Analysis

Data were analyzed using a mixed model analysis of variance. Both year trial data were analyzed together. Data analysis was carried out in R studio (software version 1.1.456). A 4-way ANOVA was applied to determine the main effect of the four agronomical factors with their interactions. Significance was determined as: \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, n.s. = not significant. Differences between averages were compared for significance by means of the Tukey honest significant difference (Tuckey HSD) test (p < 0.05).

### 3.1.3 Result

### 3.1.3.1 Agronomic Traits and Kernel Analyses

The Y was the dominant factor for GY, followed by SL, Gen, and SD, while the NL did not significantly affect GY (Table 3).

Additionally, GY was significantly affected by the interaction  $Y \times SD$ , whilst no interactions between  $Y \times SL$  and  $Y \times NL$ , respectively, were found to be statistically significant. Statistically significant differences were detected in the interaction genotype–environment. The highest average GY was measured in AU\_A, followed by AU\_B and SI, while the lowest average GY values were measured in AC, followed by FR and GB, respectively (Table 4). SD significantly affected average GY, which increased by 5.4% from SD200 to SD350 (Table 4). Results of the present study indicated that the SL6.4 treatment increased GY by 8.2% compared to SL0.

Variability	DF	GY (t ha		H (kg ł		TK (g)		PC (%)		PY (kg h	
sources		F	sig	F	sig	F	sig	F	sig	F	sig
Year	1	80.00	***	2.49	ns	40.60	***	170.00	***	32.40	***
NL	2	0.40	ns	0.53	ns	7.91	***	83.2	***	4.00	***
SL	1	34.20	***	0.30	ns	3.00	ns	2.39	ns	39.40	***
SD	1	15.50	***	0.17	ns	0.00	ns	15.00	***	23.10	***
Gen	13	32.40	***	6.54	***	23.9	***	10.7	***	32.80	***
SL×SD	1	0.23	ns	0.01	ns	0.02	ns	0.00	ns	0.30	ns
NL×SD	2	0.52	ns	0.02	ns	0.25	ns	0.37	ns	0.61	ns
NL×SL	2	2.76	ns	0.16	ns	0.80	ns	0.17	ns	3.02	*
Y×SD	1	9.48	*	0.47	ns	0.11	ns	5.3	*	13.40	**
Y×SL	1	0.24	ns	0.23	ns	5.79	*	1.07	ns	0.39	ns
Y×NL	2	1.97	ns	0.34	ns	0.48	ns	2.36	ns	2.29	ns
Residuals	980										

**Table 3.** Results of the ANOVA for grain yield (GY), hectolitre weight (HW), thousand kernel weight (TKW), protein concentration (PC), and protein yield (PY). The table columns report the Fisher F (F) and the significance levels: \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, ns = not significant.

In the present study, Gen was the sole factor affecting HW (Table 4). Furthermore, Gen was the dominant factor for TKW, followed by Y, NL, the second-order interaction  $Y \times SD$ , and SL, respectively (Table 3). Among the 14 varieties, the highest HW was measured in AU\_A, followed by AU\_B, while the lowest HW was measured in GR\_M, followed by VE and IN, respectively (Table 4).

The highest TKW was measured in GR, followed by GR\_A and GR\_M, while the lowest TKW was measured in AN and VE (Table 4). The TKW values were found to be significantly decreased by 9.6%, with the increase from NL35 to NL135.

According to the ANOVA, the PC was significantly dominated by Y, followed by NL, SD, and Gen, while SL did not have a significant effect (Table 3). On the contrary, SL was the dominant factor for PY, followed by Gen, Y, SD, and NL (Table 3). As regards the second-order interaction, only  $Y \times SD$  affected both PC and PY, while NL  $\times$  SL significantly affected only PY (Table 3). Results indicated that sulfur application (SL6.4) increased PY by 8.7% with respect to SL0 (Table 4). The highest SD treatment significantly increased the PC and PY values with respect to the control by 1.4% and 6.6%, respectively. Furthermore, the PC and PY significantly increased by 3.8% and 4.5%, respectively, from NL35 to NL135.

**Table 4.** Grain quality parameter mean values (standard error in brackets) of 14 old common wheat varieties as a function of genotype (Gen), nitrogen (NL) and sulfur fertilization (SL), and seeding density (SD). First-order interactions are provided for SD, NL, SL, and Y. Lowercase letters represent the Tukey HSD post hoc test results. The table columns report the significance levels: \*\*\* = 0.001, ns =not significant.

X7	GY		HW		TKW	TKW			PY	
Variability	(t ha <sup>-1</sup> )		(kg hL <sup>-1</sup> )		(g)		(%)		(kg ha <sup>-1</sup> )	
sources	Average	sig	Average	sig	Average	sig	Average	sig	Average	sig
Gen	-	***	-	***	-	***		***	-	***
AC	3.36 (0.15)	e	80.10 (0.86)	ab	43.57 (0.42)	e	15.17 (0.11)	а	502.67 (21.67)	gh
AN	4.14 (0.09)	bc	79.33 (0.84)	ab	38.72 (0.33)	f	14.74 (0.1)	abcd	612.39 (15.88)	def
AU_A	5.41 (0.14)	а	81.89 (0.76)	а	46.7 (0.52)	abcd	14.64 (0.12)	bcd	788.35 (20.06)	а
AU_B	5.03 (0.12)	а	81.75 (0.9)	а	44.52 (0.54)	de	14.7 (0.11)	bcd	736.59 (16.73)	ab
BI	4.2 (0.14)	bc	79.07 (0.81)	ab	47.06 (0.49)	abcd	14.97 (0.07)	ab	629.77 (21.60)	de
FR	3.44 (0.13)	de	72.75 (1.11)	с	47.73 (0.78)	ab	14.18 (0.15)	e	483.01 (17.65)	h
GB	3.78 (0.05)	cde	79.05 (0.71)	ab	47.26 (0.72)	abc	14.92 (0.08)	abc	563.99 (8.35)	efg
GR	4.39 (0.06)	b	78.13 (0.69)	ab	48.76 (0.52)	а	14.53 (0.07)	bcde	638.10 (10.51)	cd
GR_A	3.87 (0.07)	cd	78.7 (0.59)	ab	48.56 (0.51)	а	14.31 (0.11)	de	555.01 (11.70)	fg
GR_M	3.85 (0.07)	cde	77.05 (0.89)	b	48.11 (0.43)	а	14.63 (0.09)	bcd	564.74 (11.53)	efg
IN	3.93 (0.08)	bcd	77.57 (0.99)	b	47.03 (0.81)	abcd	14.13 (0.11)	e	554.14 (11.78)	fgh
ME	4.24 (0.15)	bc	79.87 (0.78)	ab	45.15 (0.5)	bcde	14.48 (0.13)	cde	607.21 (19.84)	def
SI	4.92 (0.11)	а	78.58 (0.93)	ab	44.72 (0.54)	cde	14.31 (0.09)	de	702.15 (15.30)	bc
VE	3.84 (0.09)	cde	77.33 (1.09)	b	43.69 (0.54)	e	14.84 (0.11)	abc	567.13 (12.70)	defg
SD	-	***	-	ns	-	ns		***		***
SD200	4.06 (0.05)	b	78.75 (0.35)		45.83 (0.23)		14.51 (0.05)	b	588.12 (6.65)	b
SD300	4.28 (0.05)	а	78.56 (0.33)		45.82 (0.25)		14.71 (0.04)	а	626.92 (7.32)	а
NL		ns		ns		***		***		***
NL35	4.14 (0.06)		78.79 (0.39)		46.56 (0.30)	а	14.34 (0.05)	с	591.88 (8.04)	b
NL80	4.20 (0.06)		78.61 (0.45)		45.80 (0.31)	ab	14.61 (0.05)	b	611.89 (8.80)	ab
NL135	4.17 (0.07)		78.38 (0.41)		45.12 (0.27)	b	14.88 (0.04)	а	618.78 (8.94)	а
SL		**		ns		ns		ns		***
SL0	4.01 (0.05)	b	78.53 (0.31)		45.57 (0.25)		14.57 (0.04)		582.18 (6.70)	b
SL6.4	4.34 (0.05)	a	78.78 (0.37)		46.08 (0.24)		14.65 (0.04)		632.85 (7.19)	а
Y		***		ns		***		***		***
2018	3.92 (0.04)	b	79.02 (0.34)		46.77 (0.26)	а	14.94 (0.04)	а	584.54 (6.48)	b
2019	4.42 (0.05)	a	78.29 (0.34)		44.89 (0.22)	b	14.28 (0.04)	b	630.50 (7.53)	а

#### 3.1.3.2 Alveograph Parameters and Free Asparagine Content in Whole Flour

As regards the main factor, NL was the dominant factor for W, followed by SL, SD, Gen, and finally Y in decreasing order, respectively (Table 5). Additionally, W was strongly affected by the second-order interaction  $NL \times SL$ , while no interactions between Y and the agronomic treatments were detected.

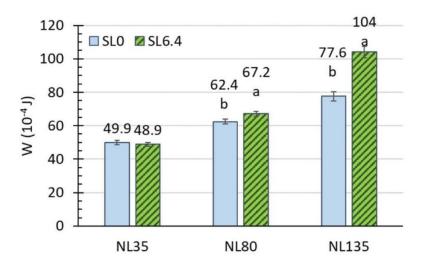
**Table 5.** Results of the ANOVA for dough strength (W), the ratio between dough tenacity and dough extensibility (P/L), and ASN concentration in whole flour. The table columns report the Fisher F (F) and the significance levels: \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, ns = not significant.

Variability	DF	W (10 <sup>-4</sup> J)		<b>P</b> /I	L	Asparagine (micromols g <sup>-1</sup> )		
sources		F	sig	F	sig	F	sig	
Year	1	11.90	**	0.29	ns	215.00	***	
NL	2	446	***	48.9	***	300.00	***	
SL	1	77.40	***	5.66	*	3966.00	***	
SD	1	67.00	***	35.50	***	0.00	ns	
Gen	13	28.20	***	62.30	***	15.90	***	
SL×SD	1	0.30	ns	11.60	***	0.00	ns	
NL×SD	2	3.40	*	0.69	ns	0.00	ns	
NL×SL	2	59.90	***	38.30	***	177.00	***	
Y×SD	1	0.20	ns	0.11	ns	0.00	ns	
Y×SL	1	0.30	ns	0.71	ns	53.70	***	
Y×NL	2	0.50	ns	0.16	ns	6.10	***	
Residuals	308							

The highest W was measured in SI, followed by GB and FR, while the lowest values were measured in AN, followed in increasing order by BI, VE, GR\_M, and ME, respectively (Table 6). The W decreased by about 19.3% as SD increased from SD200 to SD350 (Table 6). In contrast, the W value increased by 84.4% and 15.9% with the NL treatment (from N35 to N135) and the SL treatment, respectively. The S fertilization did not affect the W at N35, while W increased when S was applied at the NL80 and NL135 treatments, respectively (Figure 3). Thus, at S0, W increased from 25% at NL80 to 55.5% at NL135, while at S6.4, the W increased from 37.4% at NL80 to 112.7% at NL135 compared to the lowest N fertilization level.

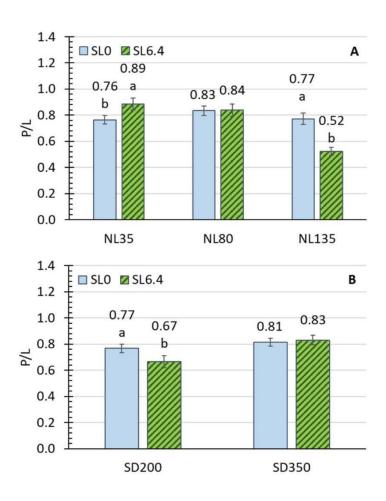
**Table 6.** Averages (standard error in brackets) of dough strength (W), the ratio between dough tenacity and dough extensibility (P/L), and asparagine concentration in whole flour as a function of genotype (Gen), nitrogen (NL), and sulfur fertilization (SL), seeding density (SD), and first-order interaction. Lowercase letters represent the Tukey HSD post hoc test results. The table columns report the significance levels: \*\* = 0.01, \*\*\* = 0.001, ns = not significant.

Variability	W (10 <sup>-4</sup> J)		P/L		Asparagine (micromols g <sup>-1</sup> )		
sources	Average	sig	Average	sig	Average	sig	
Gen		***		***		***	
AC	62.74 (3.72)	cd	0.61 (0.03)	efg	19.74 (3.5)	bcd	
AN	52.41 (3.42)	e	0.84 (0.06)	cd	19.71 (3.45)	bcd	
AU_A	78.94 (5.08)	b	0.60 (0.03)	efg	16.99 (3.06)	cde	
AU_B	78.42 (5.77)	b	0.71 (0.02)	de	17.92 (2.97)	cd	
BI	55.22 (3.41)	de	0.55 (0.02)	fg	16.52 (3.03)	de	
FR	80.70 (6.83)	ab	0.88 (0.06)	bc	17.23 (3.22)	cde	
GB	81.35 (6.25)	ab	0.71 (0.04)	de	23.69 (4.39)	ab	
GR	67.96 (2.56)	с	0.68 (0.03)	ef	22.94 (3.83)	ab	
GR_A	63.5 (2.72)	cd	0.69 (0.04)	def	25.12 (4.72)	а	
GR_M	62.16 (3.29)	cde	0.70 (0.03)	def	24.52 (4.52)	а	
IN	63.33 (3.68)	cd	0.52 (0.03)	g	21.01 (3.76)	abc	
ME	61.97 (2.83)	cde	0.71 (0.05)	de	17.92 (3.11)	cd	
SI	89.67 (7.16)	a	1.54 (0.07)	a	13.64 (2.43)	e	
VE	58.18 (3.69)	cde	1.03 (0.05)	b	17.1 (3.06)	cde	
SD		***		***		ns	
SD200	73.17 (1.13)	a	0.72 (0.02)	b	19.59 (0.77)		
SD350	63.67 (1.01)	b	0.82 (0.01)	a	19.58 (0.78)		
NL		***		***		***	
NL35	49.27 (0.48)	с	0.82 (0.02)	a	11.66 (0.56)	с	
NL80	65.16 (0.66)	b	0.84 (0.02)	a	22.48 (1.01)	b	
NL135	90.83 (1.44)	a	0.65 (0.02)	b	24.60 (1.04)	a	
SL		***		**		***	
SL0	63.37 (0.82)	b	0.79 (0.01)	а	34.08 (0.59)	а	
SL6.4	73.46 (1.27)	а	0.75 (0.02)	b	5.08 (0.12)	b	
Y		***		ns		***	
2018	70.56 (0.79)	а	0.77 (0.01)		16.23 (0.67)	b	
2019	66.28 (0.77)	b	0.77 (0.01)		22.93 (0.79)	а	



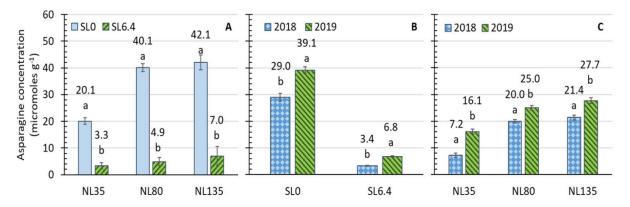
**Figure 3.** Effect of nitrogen fertilization level (NL) and sulfur fertilization level (SL) on dough strength (W). Lowercase letters represent the Tukey HSD post hoc test results.

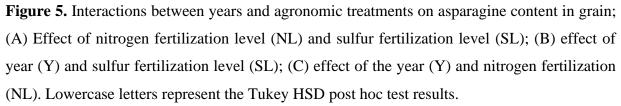
In the present trial, P/L was significantly affected by genotype. Of the 14 varieties, 10 had optimal P/L ranges, while BI and IN showed lower values, with SI and VE showing higher values, respectively (Table 6). Regardless of the variety, P/L was not affected by Y, highlighting the strong genotype effect on this characteristic. Conversely, agronomical practices affected P/L. The increase in SD significantly increased the P/L (Table 6). As the main effect, SL significantly decreased P/L. However, the SL interactions with SD and NL need to be considered. SL decreased the P/L only at the lower SD, while no significant effect was found at the higher SD (Figure 4). P/L was also decreased at the higher NL, while no significant difference was found between NL35 and NL80. Instead, there was a significant decrease in P/L at NL135 in combination with the SL treatment (Figure 4). Moreover, the P/L value was shown to be below the 0.6 thresholds with SL and NL135 treatments.



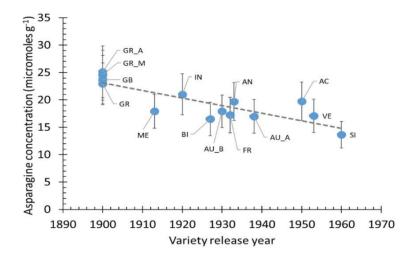
**Figure 4.** (**A**) Effect of nitrogen fertilization level (NL) and sulfur fertilization level (SL) on the ratio between dough tenacity and dough extensibility (P/L). (**B**) Effect of seeding density level (SD) and sulfur fertilization level (SL) on the ratio between dough tenacity and dough extensibility (P/L). Lowercase letters represent the Tukey HSD post hoc test results.

The SL treatment was by far the most important factor influencing the concentration of free ASN in grains, followed by NL, Y, and Gen in decreasing order, respectively (Table 5). SD was the only agronomic treatment not exerting a significant effect on free ASN concentration. The free ASN concentration in grain was affected by the second-order interaction NL  $\times$  SL, followed by  $Y \times SL$  and  $Y \times NL$  (Table 5). Free ASN concentration in grain was significantly higher in 2019 than in 2018. When combining both years, the ASN content significantly increased from 92.8% at NL80 to 111% at NL135 compared to N35. Instead, the ASN content was shown to decrease by 85.1% with the SL treatment. In the present study, S fertilization was more effective in reducing the ASN concentration in 2018 than in 2019 (Figure 5). S treatment decreased the ASN concentration by 7.5 and 4.8 times in 2018 and 2019, respectively. At the same time, during the two growing seasons, N fertilization had a contrasting effect to that of S. In particular, N fertilization increased the ASN concentration by 197% and 72% in 2018 and 2019, respectively. A more effective reduction in grain ASN concentration was observed at NL80 than at the remaining N fertilization levels (Figure 5). Particularly, the decrease in ASN content measured at SL0 and SL6.4, respectively, was not significantly different between NL35 and NL135 (6.09 and 6.01 times, respectively), while the decrease in ASN was significantly different at NL80 (8.2 times). The highest free asparagine concentration was measured in GR\_A, followed by GR\_M, GB, and GR, while the lowest values were measured in SI, followed in increasing order by BI, AU\_A, VE, and FR, respectfully (Table 6).





Interestingly, when the average ASN levels determined in the present study were plotted against the date when the varieties were released, there was a significant decline (R2 = 0.69, p < 0.01) in ASN content across the release year of the considered varieties (Figure 6).



**Figure 6.** Scatter plots of free asparagine concentration in grain as a function of the variety year of release.

# **3.1.4 Discussion**

In general, the results suggest that the N concentration in the soil of the study site was not a limiting factor for the growth and production of these 'old' common wheat genotypes. Gooding et al. (2002) and Zhang et al. (2016) found a significant interaction between N fertilization and seeding density in determining the kernel yield, whilst no interactions between  $SD \times NL$ , respectively, were found to be statistically significant in this study. Our study corroborated previous results by Valerio et al. (2013), indicating that genotypes having high tillering potential may benefit from SD up to 400 seeds  $m^{-2}$ . In contrast, Zhang et al. (2016) found that SD increased from 120 to 180 plants m-2, significantly increasing GY, with no further increases observed as SD increased from 180 to 240 plants  $m^{-2}$ . The present study suggests that the sulfur treatment can significantly increase GY. However, variable effects in response to sulfur treatment have been reported in previous literature. For example, Wilson et al. (2020) found that foliar application of 20 kg S ha<sup>-1</sup> increased GY by up to 55% compared to the control. Instead, Guerrini et al. (2020) reported that sulfur treatment did not significantly affect the GY of 'old' Italian common wheat landraces. In general, the present results corroborated those of Kilmer and Nearpass (1960) indicating that crops respond to sulfur fertilization in sulfur-deficient soils. Salvagiotti and Miralles (2008) showed that S fertilization increased grain yield in wheat by increasing nitrogen use efficiency (NUE). Further, Salvagiotti et al. (2009) suggested that sulfur fertilization can increase the NUE in sulfur-deficient soils. In the 'old' varieties used in this study, the genotypic factor predominated on the HW and TKW, corroborating previous results for Italian landraces (Guerrini et al. 2020). In contrast, in modern varieties, HW values were

shown to increase, with increasing N up to 150 kg ha<sup>-1</sup> (Litke et al. 2018). Our study indicated a strong effect of N and S fertilization on PC and PY production. These results were consistent with previous findings in 'modern' common wheat varieties (Khalil et al. 1987; Johansson et al. 2004). Likewise, Guerrini et al. (2020) reported that S and N fertilization substantially affected the PC in 'old' varieties. Yu et al. (2021) observed a reduced efficiency of sole N fertilization in increasing both protein and grain yield in sulfur-deficient soils. Further, Yu et al. (2021) suggested that sulfur application can result in protein and grain yield increases by regulating glutamine synthetase 1 and improving nitrogen-use efficiency.

Our results suggest that nitrogen fertilization may be used as a tool to modify the dough deformation energy (i.e., alveograph W) in these 'old' varieties and highlight a positive synergy between N and S. The W values were consistent with those measured in previous studies (Guerrini et al. 2020; Migliorini et al. 2016). As 'old' common wheat flours are usually characterized by a low W, any increase in this value can be regarded with interest as it improves the flour's bread-making characteristics (Guerrini et al. 2020; Parenti et al. 2020). Therefore, the observed increases in W with the NL and SL treatments, respectively, are of particular interest for 'old' common wheat varieties. The effect of S and N fertilization on W was consistent with those measured previously (Guerrini et al. 2020; Tea et al.2005). Considering all the varieties, the agronomic treatments were unsuccessful in increasing the W values above  $90 \times 10^{-4}$  J, which, according to the common classification, distinguishes biscuit flours from flours suitable for bread-making. However, the  $90 \times 10^{-4}$  J threshold was exceeded by five varieties at NL135 (132.3, 118.4, 117.8, 110.7, and  $108.6 \times 10^{-4}$  J in SI, GB, FR, AU\_B, and AU\_A, respectively), thus attaining the status of weak flours, attributable to this level of nitrogen fertilization. A P/L range of 0.6–0.8 is usually considered the optimal ratio between dough tenacity and extensibility (i.e., P/L) in bread-making flours Cappelli et al. 2020). P/L ratios exceeding 0.8 are known to be lacking in old varieties for bread-making as unrefined flours (Parenti et al. 2020). SI and VE have been extensively studied in the literature and are popular among bakers using flour from 'old' varieties, already known for high tenacity and low extensibility doughs (Parenti et al. 2020). In the literature, there has been speculation on the advantages of a blending strategy between the "poor" P/L wheat, such as BI and IN, and the most commonly used higher P/L wheat (SI and VE) in order to improve the bread-making performances, thereby promoting the valorization of local germplasm characteristics (Guerrini et al. 2020; Parenti et al. 2020). The dough parameters highlight the importance of agronomical practices in modulating the technological performance of dough in old, weak varieties. Old varieties are widely reported as having weaker dough, with unbalanced tenacity-extensibility ratios, rendering baking difficult.

Hence, the effect of agronomical practices on dough strength necessitates investigation, with careful selection of SD, NL, and SL to optimize rheological parameters for the baking industry. The ASN concentration determined in 2019 was higher than in 2018. This was attributable to the stress incurred by the higher temperatures combined with lower precipitation over the entire growing season and, in particular, during the grain-filling stage. Similar interactions between ASN content and environmental stress conditions were also reported previously (Wilson et al. 2020). Results indicated that N fertilization increased the ASN content, while sulfur fertilization was able to reduce the ASN content by up to 85.1%. This result was consistent with that observed by Wilson et al. (2020), showing an increase in ASN content in response to increasing N. Moreover, present results were similarly consistent with various studies reporting higher ASN contents in wheat grains cultivated in sulfur-deficient soils (Shewry et al. 2009; Muttucumaru et al. 2006). In contrast, in soil with satisfactory S availability, S fertilization does not impact the ASN content in grain (Rapp et al. 2018; Claus et al. 2006). Previously, it was noted that in three 'old' common wheat varieties (namely, AN, SI, and VE), the albumin, globulin, and gliadin fractions were decreased significantly, whilst the glutenin fraction was significantly increased in response to S fertilization Guerrini et al. 2020. Thus, it could be possible that these 'old' common wheat varieties were highly responsive to S deficiency and that changes in the protein composition resulted in a significant increase in ASN content. The ASN content was consistent with that measured previously for wheat (Wilson et al. 2020; Poudel et al 2021). Poudel et al. (2021) suggested that despite the absence of a legal limit for ASN concentrations in grain, this should be as low as possible. This is the first time that a negative correlation between the ASN content and the release year has been shown for old Italian common wheat varieties. Furthermore, significant correlations between free ASN and grain protein content were reported previously and shown to be higher in the old varieties (Ohm et al. 2017). Corol et al. (2016) reported a weak correlation between ASN concentration and the release year. However, those authors also found that free ASN content was positively correlated to plant height (Corol et al. 2016), which, interestingly, is generally higher in the old varieties. In contrast, more recent work, analyzing the free ASN content in grain of 19 cultivars released between 1870 and 2013 across two growing seasons in the USA, showed that the free ASN concentration in grain was significantly increased in the second growing season across the release years, whilst no trend across release year was detected during the first growing season (Poudel et al. 2021). Given the scarcity of information, the requisite for further investigating this aspect in future research programs is evidenced. Consequently, further studies involving a larger number of genotypes over a longer breeding period should be conducted to provide additional insights into the effect

of previous breeding programs on the compositional properties of 'old' common wheat varieties. Nonetheless, the preliminary results suggest that breeding programs may have inadvertently selected against free ASN content. Overall, selection by breeding programs has improved nutrient-use efficiency, increased resistance to lodging by reducing the plant height, as well as resistance to stress conditions such as water stagnation, drought, and plant diseases, which are notorious for affecting the ability of wheat to convert assimilated nitrogen (N) into free amino acids and then proteins (Wilson et al. 2020).

# **3.1.5** Conclusions

This paper was aimed at evaluating whether the grain yield and protein, rheological characteristics, as well as the ASN content in kernels of 'old' common wheat varieties grown on S-deficient soils could be improved with agronomical treatments, more specifically S fertilization, N fertilization, and SD. The experiment was conducted on 14 'old' common wheat varieties released between 1900 to 1960 in Italy. A higher seeding density was shown to increase grain yield and protein concentration. S fertilization was found to increase the grain yield without decreasing grain protein concentration, while N fertilization was found to effectively increase the grain protein concentration and the protein yield by hectare. Regarding the dough rheological parameters, SD was shown to negatively affect the dough strength in all the varieties. Instead, dough strength was significantly increased in relation to increasing S and N fertilization. Free ASN concentration in 'old' common wheat varieties was found to be comparable to other studies investigating 'old' and 'modern' genotypes with low nitrogen-use efficiency under S-deficient conditions. Interestingly, free ASN concentration was negatively correlated with the year of release in the considered varieties. This may suggest that past breeding programs may have contributed to reducing the ASN content; however, more studies on old varieties need to be conducted to further investigate this aspect. N fertilization was found to significantly increase the ASN content, whereas S application decreased the ASN content by 85.1%. In the present study, S fertilization successfully improved the grain yield and the technical parameters of the 'old' common wheat varieties while reducing the ASN concentration, thereby promoting food safety. Hence, these present results can be considered of particular interest for 'old' common wheat varieties characterized by poor technical performance when these varieties are grown on S-deficient soils. However, additional trials, including additional years within differing pedoclimatic conditions, are required in order to further evaluate the interaction between cultivars and the agronomical treatments.

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#### 3.2 Second paper

Evaluation of nitrogen and phosphorus responses on yield, quality, and economic advantage of winter wheat (*Triticum aestivum*, L) under four different agro-climatic zones in Afghanistan.

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## Abstract:

The response of winter wheat (*Triticum aestivum* L.) to the application of different rates of nitrogen (N) and phosphorus (P) on different agro-climatic zones (ACZs) has not been well studied in Afghanistan. The objectives of this study were to (1) determine the impact of soil and climate on the responses of wheat to N and P fertilization, (2) quantify the specific N and P response of winter wheat for different ACZs, and (3) determine the economical application rates of N and P for farmers for each considered ACZs. This paper evaluates the effects of nitrogen levels (NL) at 35.28, 65, 95, and 120 kg N ha<sup>-1</sup> and phosphorus levels (PL) at 0, 50, 70, and 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, respectively, in four locations (L) for two growing seasons (GS), on both yield and quality characteristics of winter wheat. Soil pH was the main environmental parameter affecting straw yield (SY), grain yield (GY), protein content (PC), and protein yield (PY). Winter wheat SY, GY, PC, and PY increased significantly (p < 0.05) with PL rates up to 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and with NL rates up to 120 kg N ha<sup>-1</sup>. NL was the most important parameter in determining PC, thus showing potential for further improvement in N management. The highest marginal rate of return was used as an index for the farmers to accept site-specific N and P fertilizer recommendations.

Keywords: N and P fertilization, kernel quality, protein yield, Kabul-13 winter wheat variety.

#### 3.2.1 Introduction

Winter wheat (Triticum aestivum L.) serves as a staple food crop in Afghanistan, followed by rice, barley, and maize, respectively. This crop contributes to about 60% of the total calories of the population's diet with an annual consumption per capita of about 181 kg (MAIL and FAO, 2013). Winter wheat is considered a pivotal factor in improving farmers' income, ensuring national food security, as well as creating jobs in Afghanistan (Halimi, 2016). Winter wheat has occupied about 70% of the total crop cultivation area in Afghanistan, accounting for 76% of national-cereal production, 49% of agriculture share in GDP, and 13.6% of national GDP (MAIL and FAO, 2013; Chabot and Policy, 2007). Winter wheat production in Afghanistan was estimated at around 3.902 million tons for 2021, which represents a reduction of 25% and 18% compared to 2020 and the last five-year average, respectively (FEWS NET, 2021). The average wheat productivity in Afghanistan is 2.5 t ha<sup>-1</sup> in irrigated fields, but only 1.0 t ha<sup>-1</sup> under rainfed conditions. Some major constraints were identified in the wheat sector of Afghanistan as follows: unfavorable climate conditions (severe drought), inadequate seed quality, poor field and nutrient management practices, and invasion of pests and diseases (MAIL and FAO, 2002). Moreover, the low quality of available fertilizers in the local market as well as poor knowledge about the supply of fertilizers were also found to be major determining factors for the low production (World Bank, 2014). Due to the above-mentioned factors, winter wheat production

Potential opportunities to improve yield in the wheat sector of Afghanistan may include proper crop management, the wide use of good quality seeds, and the use of fertilizers (Dreisigacker et al., 2019). In particular, nitrogen (N) and phosphorus (P) supply can play a vital role in plant development and optimal grain yield (Mussarat et al., 2021). In plants, N is a key component of proteins, enzymes, and chlorophyll, thus affecting photosynthesis, substance synthesis and distribution, organ construction, and physiological processes (Maathuis et al., 2009). In soil, N is mainly related to the soil organic matter (OM), as it is a component of OM and is subjected to transformation via microorganism activity (Cotrufo et al., 2013; Kallenbach et al., 2013). P is involved in cellular respiration and energy transfer via adenosine triphosphate (ATP) and participates in the formation of cellular membranes and physiologic processes such as cell division and development in the roots and the growing tip (Maathuis et al., 2009; Plaxton et al., 2011). Soils contain usually high pools of total P, but a small amount of readily available P. The P availability is mainly influenced by soil pH. In basic soils, such as those commonly occurring in Afghanistan, available monocalcium phosphate is shortly immobilized into tricalcium

does not meet the national requirement (Sharma 2019) of about 7 million metric tons.

phosphate (Lindsay et al., 1989; Sato et al., 2005). In these conditions, crop production losses related to the unavailability of phosphorus often occur (Penn et al., 2019; Devau et al., 2010).

The N and P balance represents a big challenge in optimizing local crop production (Güsewell et al., 2004). On the one hand, fertilization increases production costs, but on the other hand, the correct amount of fertilizer distributed at the right moment can enhance both wheat quality and quantity (Mandic et al., 2015; Ferrari et al., 2016). Although topdressing N application can maximize both GY and N use efficiency, attention should be paid to the optimal doses for reducing N volatilization and leaching (Barraclough et al., 2010). Furthermore, despite P being found to enhance winter wheat quality as well as quantity, it is necessary to expand the current knowledge regarding optimal dosage, given the low availability in soil (Majeed et al., 2014; Islam et al., 2017).

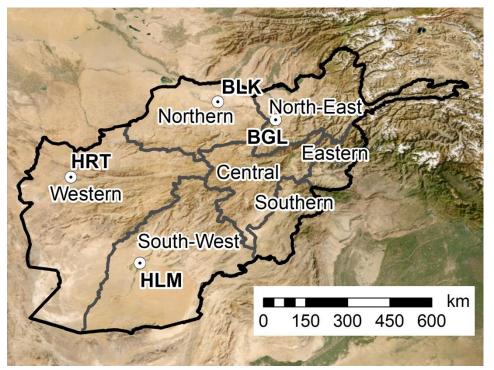
The Ministry of Agriculture Irrigation and Livestock of the Islamic Republic of Afghanistan recommended for winter wheat improved varieties that farmers apply 115 kg N ha<sup>-1</sup> and 92 kg  $P_2O_5$  ha<sup>-1</sup> that can be reduced to 46 kg  $P_2O_5$  ha<sup>-1</sup> when P fertilizers are not economically affordable. However, this recommendation does not take into consideration differences in climate and soil parameters despite them significantly affecting nutrient use efficiency and winter wheat productivity (Dargie et al., 2022; Sileshi et Al., 2022). In fact, contradictory effects in response to N and P fertilization on winter wheat have been reported by previous studies in different locations (L) of Afghanistan Agha et al., 2016; Gyeltshen et al., 2019; Obaid et al., 2019). Conversely, the best N and P levels should be selected on the basis of growth limits imposed by the different soil and climatic conditions, which can vary greatly between the 7 agroclimatic zones (ACZ) into which Afghanistan is divided.

Better matching of N and P fertilizers at rates suitable to the local climate and soil type can increase the productivity of wheat. Thus, the development of recommendations on N and P fertilization of winter wheat differentiated according to soil type, and economic returns of farmers could be a potential opportunity to improve winter wheat production in Afghanistan. Therefore, the objectives of this study were to (1) determine the impact of soil and climate on the responses of wheat to N and P fertilization; (2) quantify the specific N and P response of winter wheat for different ACZs; and (3) determine the economical application rates of N and P for farmers for each considered ACZs.

# 3.2.2 Methods and Materials

# **3.2.2.1 Field experiment**

The experimental fields were set up in Afghanistan, from September 2016 to July 2018 under irrigation, at four locations in different ACZs: Baghlan (BGL), Balkh (BLK), Helmand (HLM), and Herat (HRT), (Figure 1).



**Figure 1**: Map of the agro-climatic zones of Afghanistan with the four study sites: Baghlan (BGL), Balkh (BLK), Helmand (HLM), and Herat (HRT).

The soil at all locations was alkaline and characterized by low organic matter and poor nutrient availability, specifically N and P (Table 1). Of the four locations, the highest available P and N values were detected in BGL, while the lowest was in BLK.

Changeteristic	Locations									
Characteristic	Baghlan	Balkh	Helmand	Herat						
Location acronym	BGL	BLK	HLM	HRT						
Research station name	Poza Eshan	Dehdadi	Bolan	Urdokhan						
Latitude (N)	36°09′ N	36°65′ N	31°65′ N	34°31′ N						
Longitude (m)	68°64′ E	66°95′ E	66°96′ E	62°27′ E						
Altitude (m)	510	378	787	927						
ACZ	ACZ-NE	ACZ-N	ACZ-SW	ACZ-W						
pH	7.50	8.12	7.91	7.60						
Organic matter (%)	1.50	1.01	0.63	0.82						
Available P (mg P kg <sup>-1</sup> )	8.50	5.41	6.12	7.54						
Available K (mg K kg <sup>-1</sup> )	120	117	107	115						
Total N (g N kg <sup>-1</sup> )	1.00	0.61	0.70	0.85						
Sand (%)	36.8	18.9	24.7	63.8						
Silt (%)	37.6	52.3	53.2	26.0						
Clay (%)	25.6	28.8	22.1	10.2						
Soil texture	Loam	Silty loam	Silty loam	Sandy loan						

Table 1. Site and soil parameters	of the experimental fields in fo	our locations in Afghanistan.
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In this case, 16 treatments were obtained from the factorial combination of 4 nitrogen fertilization levels (NL) (35.28, 65, 95, and 120 kg N ha<sup>-1</sup>, namely NL35.28, NL65, NL95, and NL120, respectively) and 4 phosphorus fertilization levels (PL) (0, 50, 70 and 90 kg  $P_2O_5$  ha<sup>-1</sup>, namely, PL0, PL50, PL70, and PL90, respectively). The NL35.28 and PL0 treatments represented the control for nitrogen and phosphorous fertilization rates, respectively. One improved winter wheat variety (Kabul-13) was used for this study, originating from the international maize and wheat improvement center (CIMMYT), with the pedigree of WAXWING\*2/TUKURU. This wheat variety is resistant to Ug99 and is resistant to most rust varieties in Afghanistan. Furthermore, this variety was capable of producing an average grain yield of over six tons per hectare. For these reasons, the winter wheat variety Kabul-13 is considered the most promising for improving Afghan wheat production in the coming time. The sowing was performed at a rate of 120 kg seed ha<sup>-1</sup>. The trail was arranged in a split-plot design (SPD) with three replicates, where NL was arranged in the main plots and PL in the sub-plots, respectively. The plot size was  $1.5 \times 5$  m (7.5 m<sup>2</sup>), and each plot contained six rows with a spacing of 0.25 m. Four central rows with a net experimental unit size of 4 m<sup>2</sup> were considered for the investigation and data collection. The soil was plowed at a depth of 0.40 m and then harrowed at a depth of 0.10 m in October 2016 and September 2017, for both the first and the second growing seasons (1st GS, 2nd GS), respectively. The different farm operations, including sowing, fertilizer distribution, weeding, irrigation, pest and disease control, common wheat harvesting, and threshing, were carried out manually. In both growing seasons, although no chemicals were used to control pests and diseases, the plants were healthy, and no damage was observed. P fertilizer in form of triple superphosphate (P<sub>2</sub>O<sub>5</sub>: 46%) was applied at sowing, while the required N fertilizer was applied in the form of Urea (N:46%) in three applications as follows: 18% at sowing, 41% tillering, and 41% at stem elongation. In all locations, plots were irrigated by furrow with riverine water. A total of 500 mm of water was divided into five applications. Two irrigations were performed in the fall: about 150 mm of water were distributed before sowing to increase the soil water reserves in the topsoil layer and to obtain uniform germination. In this case, 50 mm of water was scheduled 21 days after sowing to favor the crown root initiation. Next, three irrigations (100 mm of water each) were performed in the spring by distributing water at the booting, flowering, and milking stage.

At physiological maturity, 20 plants were randomly chosen from each experiment unit to count the tiller number (TN; n) and to measure the plant height from ground level to the spike peak (PH; m). At harvesting, the above-ground biomass was collected and the weight of the kernels (GY; kg ha<sup>-1</sup> [adjusted to 12% moisture]), the straw yield (SY; kg ha<sup>-1</sup>), and the thousand kernel weight (TKW; g 1000 seeds<sup>-1</sup>) were measured for each plot. The whole meal flour samples (5 mg) were analyzed with a CHNS analyzer (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA) to determine total nitrogen percentage and then converted to total protein percentage (PC, %) by multiplying by 5.7, according to ICC Standard 167 (2000), as reported in Soofizada et al. (2022). The protein yield per hectare (PY, kg ha<sup>-1</sup>) was calculated as the product of GY by PC.

According to the recommendations of CIMMYT (1988), a partial budget was calculated as a function of total variable cost (TVC), gross benefit (GB), the net benefit (NB), and lastly the calculation of the marginal rate of return (MRR). The average market price for input (fertilizer) at sowing time and for output (GY and SY) at harvest time were considered. Accordingly, 0.48, and 0.90 US dollars were calculated per kg of N and P, and 269.8 and 126 US dollars were per metric ton of GY and SY, respectively. Meanwhile, on average one US dollar was equivalent to 72.23 Afghani (AFS). However, for the calculation of the net revenue (NR) and the percentage of the marginal rate of return (MRR), we applied the following formula:

$$NR = TR - TF$$
$$MRR = \frac{(n2 - n1)}{(f2 - f1)}x100$$

This equation is represented by the following variables as follows: TR = total revenue (total price gained from GY and SY); TF = the total cost paid for fertilizer; n2 = the higher level of net revenue; n1 = the lower level of net revenue; f2 = the higher level of fertilizer and f1 = the lower level of fertilizer, respectively.

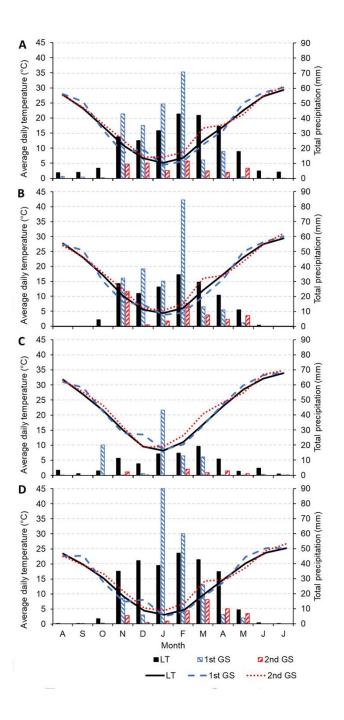
#### 3.2.2.2 Meteorology data

According to FAO (2019), all the locations were considered semi-arid, except HLM which was considered arid. The climatic conditions were further analyzed by collecting data from the NASA database (https://power.larc.nasa.gov/data-access-viewer/, accessed on 17 December 2022). Long-term (2001–2020), as well as the 1<sup>st</sup> and 2<sup>nd</sup> growing season (GS), (2017 and 2018) data of the four locations, were elaborated (Figure 2). According to the long-term data, among the experimental sites, HLM was the location with the highest average temperature (21.5 °C) and lowest precipitation (93.1 mm). Likewise, the BLK and BGL regions reported almost similar temperature patterns, but BLK (178.85 mm) had a lower average annual precipitation than BGL (245.83 mm). Moreover, HRT was the coldest and wettest of the experimental fields considered, with an average annual temperature of 14.5 °C, and an average precipitation of 253.95 mm. During the 1<sup>st</sup> GS, there was a similar temperature pattern compared to that of the long-term,

except for December. The average annual precipitation for the considered locations was not significantly different from the long-term values, but differences were detected in the pattern of monthly precipitation distribution. The 2<sup>nd</sup> GS was completely dry and hot for all the considered locations. In fact, during August, September, and October of the 2<sup>nd</sup> GS, no precipitation events occurred, and in HLM only 15 mm of precipitation occurred during the growing season. For each L and GS, the cumulated precipitation values from the tillering to grain filling period (P\_TGf) were calculated as the accumulation of daily precipitation in the same period. The growing degree day (GDD) (Salazar-Gutierrez et al., 2013) is commonly used to describe the timing of biological processes (Grassi et al.,2020, Li et al., 2012). The GDD value was daily calculated as the difference between the daily average temperature (Tavg; °C) and a crop base temperature (Tb; °C). According to Fabbri et al., (2000) and Saiyed et al., (2009), the Tb was set to 4 °C. The cumulated GDD values from the tillering to grain filling period (GDD\_TGf) were calculated as the accumulation of daily GDD over the same period.

#### **3.2.2.3 Statistical analysis**

Analyses of data were performed utilizing the RStudio version (R 4.1.1.). A multifactorial analysis of variance (ANOVA) was performed to determine the main effect of NL and PL fertilization and their interactions. In the case of significant differences, differences between means were compared using the Tukey HSD post-hoc test. Significance was determined as follows: \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, n.s. = not significant. A gradient-boosted regression tree (GBRT) statistical analysis was performed to assess the relative influence of explanatory variables on the variation of winter wheat agronomic traits (SY; GY; PC; PY). A correlation matrix was used to check for redundancy among covariates and eliminate collinear variables, using a threshold of 0.8. The BRT fit was analyzed using tenfold cross-validation. BRT model was performed using a tree complexity of 5 and a learning rate of 0.01.



**Figure 2.** Walter-Lieth diagram of the study sites. (**A**) BGL (Baghlan), (**B**) BLK (Balkh), (**C**) HLM (Helmand) and (**D**) HRT (Herat). The long-term (LT) (2001–2020), average monthly temperature (°C, black continuous line), and monthly average precipitation (mm, black histograms). The 1<sup>st</sup> growing season (1<sup>st</sup> GS), (2016/2017), average monthly temperature (°C, blue dashed line), and monthly average precipitation (mm, blue histograms). The 2<sup>nd</sup> growing season (2<sup>nd</sup> GS), (2017/2018), average monthly temperature (°C, red dotted line), and monthly average precipitation (mm, red histograms).

## 3.2.3 Results

# **3.2.3.1** Agronomic Traits and Kernel Analyses

In terms of TN per plant, L was the dominant factor followed by PL and GS, while NL did not significantly affect GY (Table 2). Additionally, TN was significantly affected by the interaction GSxL, while no other significant interactions were detected. The highest average TN was detected in HRT, followed by BLK and HLM, while the lowest average TN was recorded in BGL (Table 3). Furthermore, the mean TN at PL70 and PL90 was significantly higher than the control by 10.07% and 9.74%, respectively.

**Table 2**. Mean of tiller number (TN), plant height (PH), grain yield (GY), straw yield (SY), harvest index (HI), thousand kernel weight (TKW), protein concentration (PC), and protein yield (PY) parameter results, considering the factors, growing season (GS), location, nitrogen (NL), and phosphorus (PL) fertilization treatment. The table columns report the ANOVA result as \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, n.s. = not significant, whereas the lowercase letter shows the Tukey HSD post-hoc test results and the value inside parentheses represents standard error.

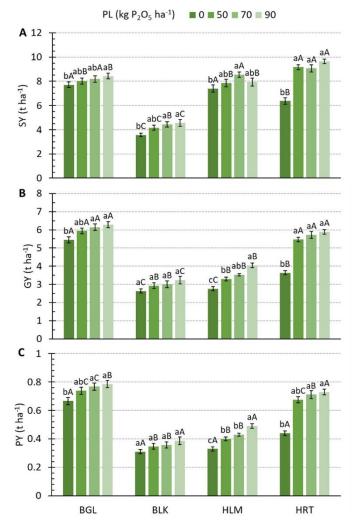
Variation source	DF	TN (N		PH (cm		SY (t ha		GY (t ha		TKV (g)	N	PC (%)		PY (t ha	
	Dr	F	, sig	F	sig	F	sig	F	sig	F (g)	sig	(70) F	sig	F	sig
GS	1	3.94	*	41.25	***	0.25	ns	138.85	***	45.01	***	364.18	***	103.60	***
L	3	338.07	***	331.19	***	268.57	***	803.29	***	332.71	***	546.52	***	819.50	***
NL	3	0.98	ns	6.79	***	0.81	ns	55.76	***	11.61	***	3901.40	***	124.67	***
PL	3	6.78	***	65.37	***	26.70	***	113.47	***	11.25	***	135.53	***	118.40	***
GSxL	3	130.04	***	47.15	***	12.49	***	12.53	***	55.14	***	42.07	***	52.86	***
GSxNL	3	0.99	ns	1.25	ns	0.16	ns	0.51	ns	7.56	***	0.25	ns	2.53	ns
LxNL	9	0.62	ns	3.33	***	1.78	ns	0.95	ns	8.19	***	5.15	***	2.71	**
GSxPL	3	1.07	ns	1.8	ns	0.40	ns	1.77	ns	2.46	ns	1.45	ns	1.72	ns
LxPL	9	1.21	ns	9.94	***	5.94	***	13.86	***	2.77	**	3.47	***	13.66	***
NLxPL	9	0.42	ns	1.06	ns	0.54	ns	0.56	ns	3.08	**	0.78	ns	0.28	ns
GSxLxNL	9	0.87	ns	1.34	ns	0.94	ns	0.91	ns	1.55	ns	1.97	*	1.27	ns
GSxLxPL	9	0.86	ns	3.35	***	3.09	**	3.74	***	2.03	*	1.69	ns	3.81	***
GSxNLxPL	9	1.00	ns	0.46	ns	1.06	ns	0.42	ns	2.38	*	0.23	ns	0.47	ns
LxNLxPL	27	1.08	ns	1.13	ns	0.96	ns	0.50	ns	2.26	**	0.91	ns	0.55	ns
GSxLxNLxPL	27	0.62	ns	0.61	ns	0.53	ns	0.51	ns	1.88	**	0.92	ns	0.51	ns
Residues	256														

The L was the principal factor affecting average PH, followed in decreasing order by PL, GS, and NL, respectively. Additionally, average PH was significantly affected by two order interactions, in particular GSxL, LxPL, and LxNL. The average PH significantly increased as PL and NL rates increased, with the highest average PH values being measured at NL120 and PL90. The highest average PH was measured in HRT, followed in decreasing order by BGL, HLM, and BLK, respectively. Lastly, the average PH measured in the second GS was significantly higher than that measured in the first GS.

**Table 3.** Mean of tiller number (TN), plant height (PH), grain yield (GY), straw yield (SY), harvest index (HI), thousand kernel weight (TKW), protein concentration (PC), and protein yield (PY) parameter results, considering the factors, growing season (GS), location, nitrogen (NL), and phosphorus (PL) fertilization treatment. The lowercase letter shows the Tukey HSD posthoc test results, and the value inside parentheses represents standard error.

Variation S	ource	Obs. (N)	TN (N)	PH (cm)	<b>SY</b> (t ha <sup>-1</sup> )	GY (t ha <sup>-1</sup> )	TKW (g)	PC (%)	PY (t ha <sup>-1</sup> )
	35.28	96	5.14 (0.21)				36.94 (0.46) bc		. /
NL	65	96	5.30 (0.22)	93.70 (0.76) ab	· · ·	· · ·	· · ·	· · ·	· · ·
(kg N ha <sup>-1</sup> )	95	96	5.36 (0.22)	93.54 (0.78) ab	7.21 (0.23)	4.56 (0.15) b	37.83 (0.61) ab	12.43 (0.03) b	0.57 (0.02) ab
	120	96	5.19 (0.21)	94.16 (0.80) a	7.33 (0.22)	4.76 (0.16) a	38.60 (0.64) a	12.74 (0.03) a	0.61 (0.02) a
	0	96	4.91 (0.21) b	89.46 (0.86) c	6.26 (0.21) b	3.62 (0.13) d	36.35 (0.50) b	11.96 (0.06) b	0.44 (0.02) b
PL	50	96	5.16 (0.21) ab	92.81 (0.81) b	7.30 (0.23) a	4.41 (0.15) c	37.84 (0.53) a	12.16 (0.06) a	0.54 (0.02) a
$(kg P_2O_5 ha^{-1})$	70	96	5.46 (0.23) a	94.94 (0.67) a	7.56 (0.23) a	4.60 (0.16) b	37.64 (0.56) a	12.20 (0.06) a	0.57 (0.02) a
	90	96	5.44 (0.22) a	96.17 (0.71) a	7.64 (0.24) a	4.86 (0.15) a	38.31 (0.59) a	12.20 (0.06) a	0.60 (0.02) a
	BGL	96	3.63 (0.09) c	95.38 (0.55) b	8.09 (0.13) b	5.95 (0.09) a	32.72 (0.24) c	12.36 (0.06) a	0.74 (0.01) a
L	BLK	96	4.94 (0.18) b	86.92 (0.48) d	4.18 (0.12) c	2.95 (0.09) d	41.68 (0.45) a	11.85 (0.06) b	0.35 (0.01) d
L	HLM	96	4.54 (0.13) b	89.38 (0.76) c	7.94 (0.16) b	3.41 (0.07) c	41.16 (0.49) a	12.05 (0.05) b	0.41 (0.01) c
	HRT	96	7.87 (0.15) a	101.71 (0.36) a	8.56 (0.18) a	5.18 (0.12) b	34.59 (0.26) b	12.26 (0.06) a	0.64 (0.02) b
GS	1st GS	192	5.34 (0.18) a	92.18 (0.53) b	7.16 (0.15)	4.67 (0.11) a	38.37 (0.47) a	12.04 (0.04) b	0.57 (0.01) a
US US	2nd GS	192	5.15 (0.12) b	94.51 (0.60) a	7.22 (0.18)	4.07 (0.11) b	36.70 (0.28) b	12.22 (0.04) a	0.50 (0.01) b

SY was firstly affected by L and secondly by PL, while the remaining principal factors did not significantly influence SY. The same two interactions, GSxL and LxPL, showed a significant influence on SY. The results indicated that the lowest SY was measured at PL0. Instead, SY at PL90, PL70, and PL50 was higher than the control by about 18.06%, 17.19%, and 14.25%, respectively. Furthermore, results indicated that L strongly influenced SY. The highest SY was recorded in HRT, followed in decreasing order by BGL, HLM, and then BLK, which showed the lowest SY. It was found that SY increased significantly as the PL increased in BLK, whereas in BGL, no significant increment was detected (Figure 3). The phosphorous fertilization significantly increased the straw production in HRT with respect to the control, while no significant difference in SY was detected between the PL fertilization rate from 50 to 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. In HLM, the highest SY was measured in PL at 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.



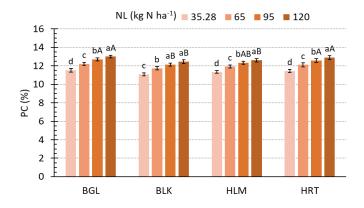
**Figure 3.** From top to bottom, the effect of phosphorous fertilization level (PL) at the four locations (BGL: Baghlan; BLK: Balkh; HLM: Helmand; HRT: Herat) on (A) the straw yield (SY), (B) grain yield (GY) and (C) protein yield (PY). Lowercase letters represent significant differences between PL levels within the same locations, and uppercase letters represent significant differences between locations within the same PL level according to the Tukey HSD post hoc test results.

ANOVA results indicated that GY was mainly affected by L, followed by GS, PL, and NL. As regards the second-order interaction, only GSxL and LxPL significantly affected GY. The results indicated that the nitrogen rate at 65, 95, and 120 kg N ha<sup>-1</sup> significantly increased GY by 9.11%, 14.69%, and 18.28%, respectively, compared to the lowest N rate (NL35.28). Furthermore, the mean GY values significantly increased by 17.91%, 21.30%, and 25.51%, for PL50, PL70, and PL90, respectively, compared to the lowest P rate (PL0). The highest average GY was measured in BGL, followed in decreasing order by HRT, HLM, and BLK, respectively. The average GY measured at the harvest of the 2<sup>nd</sup> GS was 12.8% lower than that measured in the first GS. It was

found that GY increased significantly as the PL increased in BLK and HRT, whereas no significant increment was detected in BLK (Figure 3). In HRT, the phosphorous fertilization significantly increased the grain production with respect to the control, while no significant difference in GY was detected between the PL fertilization rate from 50 to 90 kg  $P_2O_5$  ha<sup>-1</sup>.

In the present study, TKW was mainly affected by L, followed by GS, NL, and PL, the sole factor affecting HW (Table 2). Furthermore, TKW was affected by all the second-order interactions except GSxPL. BLK and HLM locations featured the highest TKW values, followed by HRT, and lastly BGL, which showed the lowest TKW value. Nitrogen fertilization increased the TKW. The TKW was increased by 2.35 and 4.30% in NL95 and NL120, respectively, compared to the control. No significant difference in TKW values was detected between NL65, NL95, and NL120, although all of the latter were significantly higher than the control. The average TKN value measured in the 1<sup>st</sup> GS was significantly higher than that measured in the 2<sup>nd</sup> GS.

According to the ANOVA, the PC was significantly dominated by NL, followed by L, GS, and then PL, respectively. On the contrary, L was the dominant factor for PY, followed in decreasing order by Gen, NL, PL, and GS. As regards the second-order interaction, PC and PY were significantly affected by GSxL, LxNL, and LxPL. The results indicated that PC significantly increased as the N rate increased. In particular, the NL65, NL95, and NL120 treatments increased the average PC by 5.8%, 9.6%, and 12.3%, respectively, compared to the control. Similarly, the NL65, NL95, and NL120 treatments increased the average PY by 18.1%, 29.5%, and 38.6%, respectively, in comparison to the control. PC and PY significantly increased between PL0 and PL50, while no further significant increase between PL50 and the other two PL levels was detected. The highest average PC was determined in BGL, with the lowest recorded in BLK. The average PC determined in BGL and HRT was significantly higher than that determined in HLM and BLK. The average PC determined in the 1<sup>st</sup> GS was higher than that determined in the 2<sup>nd</sup> GS, while the opposite was detected for the average PY. In HLM and HRT, the PC increased as NL significantly increased from NL35.28 to NL120 (Figure 4). In contrast, in BGL and BLK, the PC increased as NL increased from NL35.28 to NL95, but no further significant increment was detected between NL95 and NL120. In BLK and HRT, the PY increased significantly as the PL increased, while in BLK no significant increment was detected (Figure 3). In HRT, the phosphorous fertilization significantly increased protein production with respect to the control, while no significant difference in PY was detected between the PL fertilization rate from 50 to 90 kg  $P_2O_5$  ha<sup>-1</sup>.



**Figure 4.** The effect of nitrogen fertilization level (NL) at the four locations (BGL: Baghlan; BLK: Balkh; HLM: Helmand; HRT: Herat) on the protein concentration in grain (PC). Lowercase letters represent significant differences between PL levels within the same locations, and uppercase letters represent significant differences between locations within the same PL level according to the Tukey HSD post hoc test results.

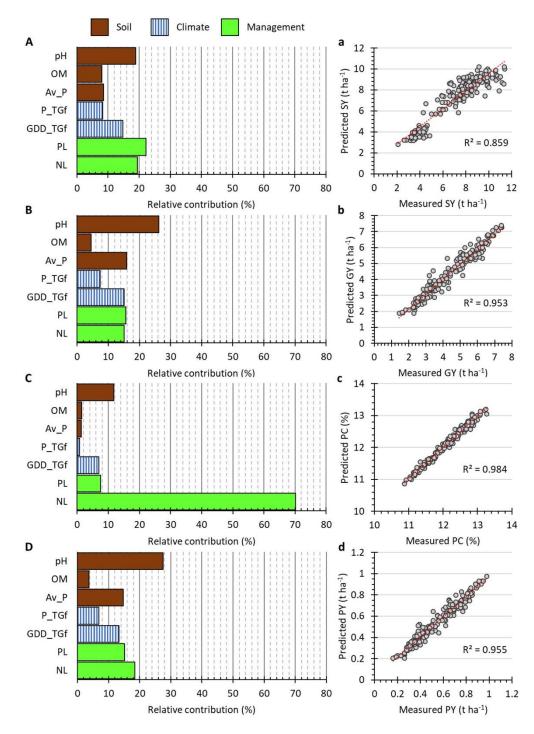
Soil pH was negatively correlated to OM, Av\_P, Av\_K, and Tot\_N (Table 4). Whereas, OM, Av\_P, Av\_K, and Tot\_N were positively correlated to each other. GDD\_TGf correlation with P\_TGf was significant and negative. GDD\_TGf was negatively correlated with OM, Av\_P, Av\_K, and Tot\_N, while positively correlated with pH. On the opposite, P\_TGf was positively correlated with OM, Av\_P, Av\_K, and Tot\_N, Av\_P, Av\_K, and Tot\_N, while negatively correlated with pH. The correlation of GY, PC, and PY with pH was significant and negative, while the correlation with OM was significant and positive. SY was negatively correlated with pH, while no significant correlation with OM was detected. The correlation of both Av\_P and Tot\_N with SY, GY, PC, and PY was positive and significant. Conversely, Av\_K was positively correlated with GY and PY, negatively correlated to SY, and not significantly correlated to PC. Both GDD\_TGf and P\_TGf were not significantly correlated to SY and PC. Further, GY and PY were negatively correlated to GDD\_TGf, while positively correlated to P\_TGf. The correlation of GY, PC, and PY with NL and PL were significant and positive. SY was positively correlated by PL, while no significant correlation with NL was detected. NL and PL were not significantly correlated to any soil and climatic parameters.

The GBRT model provided the relative contribution of each parameter on SY, GY, PC, and PY (Figure 5). Among the parameters, the management parameters were the most influential (having altogether 41.6% relative contribution to SY). NL was the most influential parameter in SY production, followed by PL, pH, and GDD\_TGf. The relative contribution of NL, PL, pH, and GDD\_TGf was 22.1%, 19.4%, 18.9%, and 14.8%, respectively, while the relative contribution

of OM, Av\_P, and P\_TGf were lower than 10%. The relative contribution of soil, climate, and management parameters to GY was 46.7%, 22.5%, and 30.8%, respectively. In particular, pH was the most important parameter affecting GY, followed by Av\_P, PL, GDD\_TGf, and NL. The relative contribution of Av\_P, PL, GDD\_TGf, and NL on GY was 26.25%, 15.9%, 15.6%, 15.1%, and 15.1%, respectively, while the relative contribution of OM and P\_TGf was lower than 10%. The range between the two leading parameters was high (58.5%), with NL being the most dominant parameter in explaining PC variability (relative contribution = 70.2%), followed by pH (relative contribution = 11.7%). PL and GDD\_TGf explained the 7.5% and 6.9% of the PC variability, while the percentage of the relative contribution of OM, Av\_P, and P\_TGf was lower than 2%. Soil, climate, and management parameters contribute to explaining the 46.1%, 20.3%, and 33.6%, respectively, of PY variability. pH was found to have the highest contribution in explaining the PY variability (27.5%), followed in decreasing order by NL, PL, Av\_P, and GDD\_TGf, with 18.5%, 15.2%, 14.8%, and 13.4%, respectively. The relative contribution of OM and P\_TGf was 3.8% and 6.9%.

**Table 4.** Correlation between soil parameters (including: pH, organic matter (OM), available phosphorous (Av\_P), available potassium (Av\_K), total nitrogen (Tot\_N), climatic parameters (including growing degree days (GDD\_TGf) and precipitation amount (P\_TGf) cumulated between tillering and grain filling), and agronomic traits (including grain yield (GY), straw yield (SY), protein concentration (PC), and protein yield (PY)). The values reporting the correlation significance are as follows: \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, ns = not significant.

	pН	ОМ	Av_P	Av_K	Tot_N	GDD_TGf	P_TGf	NL	PL	GY	SY	PC	PY
		(%)	(mg p kg <sup>-1</sup> )	(mg K ha <sup>-1</sup> )	(g N kg <sup>-1</sup> )	(° <b>C</b> )	( <b>mm</b> )	(kg N ha <sup>-1</sup> )	$(kg \ P_2O_5 \ ha^{\text{-}1})$	(t ha <sup>-1</sup> )	(t ha <sup>-1</sup> )	(%)	(t ha <sup>-1</sup> )
рН	1***	- 0.48***	-0.99***	-0.37***	-0.97***	0.35***	-0.38***	0 <sup>ns</sup>	0 <sup>ns</sup>	- 0.79***	- 0.64***	- 0.33***	- 0.78***
OM (%)	÷	1***	0.61***	0.88***	0.65***	-0.33***	0.25***	0 <sup>ns</sup>	0 <sup>ns</sup>	0.5***	-0.04 <sup>ns</sup>	0.16**	0.49***
Av_P (mg P kg <sup>-1</sup> )			1***	0.49***	1***	-0.35***	0.37***	0 <sup>ns</sup>	0 <sup>ns</sup>	0.8***	0.57***	0.33***	0.79***
Av_K (mg K kg <sup>-1</sup> )				1***	0.49***	-0.7***	0.58***	0 <sup>ns</sup>	0 <sup>ns</sup>	0.43***	-0.17**	0.11 <sup>ns</sup>	0.42***
Tot_N (g N kg <sup>-1</sup> )					1***	-0.3***	0.32***	0 <sup>ns</sup>	O <sup>ns</sup>	0.8***	0.55***	0.33***	0.78***
GDD_TGf (°C)						1***	-0.95***	0 <sup>ns</sup>	Ons	- 0.41***	0.04 <sup>ns</sup>	-0.06 <sup>ns</sup>	- 0.39***
P_TGf (mm)							1***	0 <sup>ns</sup>	Ons	0.48***	0.02 <sup>ns</sup>	0.06 <sup>ns</sup>	0.46***
NL (kg N ha <sup>-1</sup> )								1***	Ons	0.21***	0.04 <sup>ns</sup>	0.88***	0.31***
PL (kg P2O5 ha-1)									1***	0.3***	0.24***	0.16**	0.3***
GY (t ha <sup>-1</sup> )										1***	0.59***	0.51***	0.99***
SY (t ha <sup>-1</sup> )											1***	0.3***	0.58***
PC (%)												1***	0.6***
PY (t ha <sup>-1</sup> )													1***

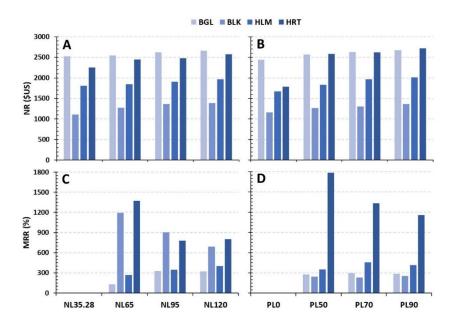


**Figure 5.** The relative contribution (%) of predictor parameters for the boosted regression tree model (BRTM) of straw yield (SY), grain yield (GY), protein concentration (PC), and protein yield (PY) are shown in (**A**–**D**), respectively. Measured and predicted annual SY, GY, PC, and PY by the BRTM model using predictors shown in (**a**–**d**), respectively.

#### **3.2.3.2** Partial economic analyses

The results indicated that a farmer distributing 90 kg  $P_2O_5$  ha<sup>-1</sup> can gain an increase of about 1089, 398, 254, and 208 US\$ in net profit as compared to PL0, at HRT, HLM, BGL, and BLK, respectively (Supplementary Table S1). Further, by distributing 120 kg N ha<sup>-1</sup>, farmers can gain an increase of about 408, 335, 202, and 195 US\$ in net profit, as compared to the NL35.28, at HRT, BLK, HLM, and BGL, respectively. Additionally, this study found that by invest of 1.4 US\$ (1 kg P), a farmer can obtain a net profit of 15, 4.1, 2.8, and 2.1 US\$, at HRT, HLM, BGL, and BLK, respectively. In addition, a farmer can obtain a net profit of 5.7, 5.4, 2.1, and 2 US\$, by invest of 0.7 US\$ (1 kg N), at HRT, BLK, HLM, and BGL, respectively.

The marginal rate of return due to N and P fertilization at each level was greater than 100% at all Ls (Figure 6), but the response to fertilization was found to differ according to L. Subsequently, in BGL the highest MRR was obtained from NL95 and PL70, indicating that in BGL the highest rates of N and P were not the best choices from an economic point of view. Nevertheless, in BLK, NL65, as well as PL90, showed the highest MRR. A similar result was evident in HLM, where NL65 showed the highest MRR for the N rate, and PL90 for the P rate, respectively. In HRT, NL120 and PL50 showed the highest MRRs for N and P rates, respectively.



**Figure 6.** Partial budget analysis of bread wheat produced in relation to the application of nitrogen (NL) and phosphorus (PL). The histogram of (**A**,**B**) represents the net revenue (NR, \$US) of NL and PL application, respectively. The

histogram of (**C**,**D**) represents the marginal rate of return (MRR, %) of NL and PL applications, respectively.

#### 3.2.4 Discussions

As reported by Hashimi et al., (2020) (Hashimi et al., 2020), Afghanistan's soils are formed under arid and semi-arid climatic conditions and are characterized by sub-alkaline to alkaline pH, high in calcium carbonate, but low in OM content. Further, deficiencies of Tot\_N and Av\_P are widespread in Afghanistan soils (Ayubi et al., 2016; Sameen et al., 2008). Our results indicate that the soil and climate conditions in Afghanistan can affect winter wheat SY, GY, and PY, as much as N and P fertilization.

Since the plots were irrigated in the 4 L, the P\_TGf effect on winter wheat growth was marginal as compared to the effect of GDD\_TGf and other soil and management parameters. This result suggested that irrigation softens the effects of high GDD\_TGf and P\_TGf shortage during the growing season. However, as suggested by Zaveri and Lobell (2019) any increase in irrigation access should also be accompanied by sustainability considerations to avoid groundwater depletion and surface water scarcity during drought periods. In general, the results can be useful for Afghan agriculture given that irrigated agriculture is the mainstay of food security and income for the majority of the rural population in Afghanistan (Rout et al., 2008). In fact, as reported by Kawasaki et al. (2012), the agricultural output in irrigated agriculture is twice or three times larger than that in rainfed one. However, our results can support the farmer's decision in irrigated land under arid climates, such as those of Afghanistan, while they should be evaluated with caution in areas where farmers can only rely on rainfall for water.

Considering the 4 Ls in this study, soil pH appeared to play a more important role in affecting GY and PY than other soil (Av\_P and OM), climate (GDD\_TGf and P\_TGf), and management (NL and PL) parameters. Further, pH was also the main environmental parameter affecting SY and PC. It is well known that pH influences the activity of microorganisms, enzymes, and the availability of nutrients, and so it plays an important role in regulating plant growth and yield. In particular, alkaline soils, such as those in BLK and HLM, generally have reduced availability of Av\_P and micronutrients such as boron, iron, manganese, and zinc. The results suggested that lowering the pH in Afghan alkaline soils could greatly boost production. However, soils containing carbonate (pH > 7.3) could require a large number of ameliorants, such as elemental sulfur, to neutralize carbonate before they can reduce soil pH. Therefore, due to the cost, the application of ameliorants to acidify soils could be more practical for horticultural crops than for field crops. While in field crops, such as winter wheat, farmers could overcome lower

availability of Av\_P and micronutrients with banded phosphorus fertilizer and chelated micronutrient applications.

P is often the most limiting nutrient for crop yield in Afghanistan. In addition, it was shown in the present study that P availability was a limiting factor for winter wheat growth. Our results indicated that winter wheat GY and PY increased significantly with PL rates (p < 0.05) in the four Ls. This is in accordance with the previous results observed by other authors (Ayubi et al., 2016; Sameen 2008; Rout et al., 2008). P was also found positively affect the winter wheat SY, with SY harvested in PL50, PL70, and PL90 being significantly higher than that in PL0. Despite the highest SY measured by distributing 90 kg  $P_2O_5$  ha<sup>-1</sup>, no significant differences were detected between PL50, PL70, and PL90. Regarding PC and PY, a significant difference was detected between PL0 and PL50, while a further increase in P application did not significantly affect both quality parameters. Our results indicate that the distribution of 50 kg  $P_2O_5$  ha<sup>-1</sup> can be considered an adequate rate so that the availability of  $P_2O_5$  is not limiting for winter wheat growth. These results were consistent with previous studies (Agha et al., 2016; Hashimi et al., 2020; Rout et al., 2008).

The present study showed a significant increase in GY with increasing rates of N application on different Ls. These results are consistent with previous research documenting significant increases in winter wheat GY to increases in N fertilization rate on different soil types (Soofizada et al., 2022; Kostíc et al., 2021, Haile et al., 2012; Guarda et al., 2004; Mansoor et al., 200). Further, the effect of the N fertilization was shown to be more effective for quality parameters as compared to the P distribution. The present results for PC and PY were consistent with those measured in previous studies showing that N fertilization substantially increased the PC and PY (Soofizada et al., 2022; Tao et al., 2022). As expected, NL was the most important factor in determining PC, compared to other considered variables including climate and soil parameters, showing potential for further improvement in N management. Further, it should be also noted that the application of urea or ammonium N fertilizer can locally induce soil acidification by the oxidation of organic compounds, loss of basic cations through ion exchange, plant uptake, and nitrification of ammonium (Cai et al., 2019). In general, this could have positive effects on alkaline soils such as those in BLK and HLM. Conversely, attention should be paid to applying animal manure as it may raise the soil pH.

The cost-benefit analysis is crucial to winter wheat growers because they are interested in observing the increased net benefit from the investment in fertilization. According to the economic training manual for farmers by CIMMYT (1988), an increase in output generally rises profit as much as the marginal rate of return is higher than the minimum rate of return, i.e., 50

to 100%. Since the marginal rate of return in all Ls due to N and P application is higher than 100%, the application of N and P fertilizers can be considered economical. When considering the net revenue, the highest values were observed at NL120 and PL90 for all Ls. These results were consistent with previous studies, showing for invest of 1 US\$ for N and P in soil, the farmer's net profit increased up to 6.25 US\$ in Afghanistan (FAO, 1971). However, in each Ls, farmers must choose the N and P fertilization levels according to the highest marginal rate of return. In fact, the highest marginal rate of return can be considered a guarantee for the farmers to accept site-specific fertilizer recommendations.

## 3.2.5 Conclusion

From the results of this study, it can be concluded that the high soil pH is the main environmental factor limiting the efficiency of N and P fertilization in irrigated winter wheat in Afghanistan. Across all four Ls, the application of 50 kg  $P_2O_5$  ha<sup>-1</sup> was sufficient for winter wheat grain production both in terms of quantity and quality. Given soil pH above 7.3, to increase the P efficiency it should be applied at planting, banded with or near the seeds. A significant increase in SY and GY with increasing rates of N application was found in all Ls. As expected, N fertilization was the most important factor in determining PC, showing potential for further improvement in N management. However, the optimal N rates in each Ls should not be calculated on the basis of the highest expected production, but on the basis of the highest marginal rate of return. Further field trials in different pedo-climatic conditions should be carried out to improve the understanding of the factors limiting the N and P fertilization efficiency.

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## 3.3 Third paper

# Effects of Pedoclimate and Agronomical Management on Yield and Quality of Common Wheat Varieties (*Triticum aestivum* L.) in Afghanistan.

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#### Abstract

The lower wheat productivity and quality are major constrain in Afghanistan. The objectives of this study were to (1) quantify the effect of soil and climatic parameters on yield and quality of wheat (2) investigate the response of different wheat varieties to different N and P fertilization rates under specific climate conditions, toward to improve the yield and quality of wheat in Afghanistan. Three wheat varieties (DLN7, ZRDN, and KBL13), three phosphorus levels (PL) at 60, 90, and 120 kg  $P_2O_5$  ha<sup>-1</sup>, and three nitrogen ratios (NP) at 1:1, 1.25:1, and 1.5:1, respectively, in four locations (L), were evaluated. Soil pH was the main environmental parameter affecting grain yield (GY), straw yield (SY), protein yield (PY), and starch yield (STY) similar to PL and NP management. The higher average GY, straw yield (SY), and STY were obtained by DLN7, followed, by KBL13 and ZRDN for all Ls, but statistically, no significant differences occurred between DRL7 and KBL13. Moreover, as PL increased, GY, SY, PY, and STY increased significantly in four Ls. In addition, PL significantly affected protein content (PC), gluten content (GC), and dough strength (W). NP was the most important factor to improve PC, GC, and PY. Starch (ST), STY, and amylopectin (AP) increased significantly with increasing PL, but the amylose to amylopectin (AM:AP) ratio was significantly reduced as PL increased. On the contrary, AM:AP increased significantly with increasing NP ratios, but ST and AP were significantly reduced as the NP ratio increased. The findings show that at NP1/PL120, GY, SY, ST, and AP were improved significantly; while at NP1.5:1/PL12, PC, and GC were significantly improved.

**Keywords:** P, NP, Variety, Soil pH, Protein, Gluten, Starch, Amylopectin, AM:AP, Grain yield, Straw yield.

#### **3.3.1 Introduction**

Agriculture is the backbone of the Afghan economy, accounting for approximately one-quarter of the national GDP, and is among the major sectors (World Bank, 2014), about 80% of the Afghan population is directly or indirectly involved in the agriculture sector (Muradi and Boz, 2015). However, agriculture growth was highlighted as an essential fact for driving the country's economy, and people's livelihood and for improving national food security (Islamic Republic of Afghanistan, 2008). Common wheat is the most dominant agricultural crop in Afghanistan, followed by horticultural crops (fruits, nuts, and vegetables), and intensive livestock production (milk, eggs, and poultry meat), therefore wheat alone accounts for one-quarter of agriculture GDP and 6.3% of the national GDP (World Bank, 2014). Common wheat grows all over the country, and it accounts for 82% of total cereal consumption (Samim et al., 2021). Despite common wheat being the principal agricultural crop in Afghanistan, domestic production fails to meet the national demand. About 2 million tons of wheat is imported annually to fulfill the national demand (7 million), as a result, Afghanistan is ranked as one of the world's top importing countries (Halimi 2016). Additionally, the quality of local wheat production is quite poor, compared to the imported grain (MAIL, 2016). The average wheat productivity per unit area is estimated to be very low (2.6 t ha<sup>-1</sup> in irrigated and 1 t ha<sup>-1</sup> under rainfed conditions) compared to India's 3.5 t ha<sup>-1</sup> (Poole et al., 2022). However variable limiting factors in response to wheat productivity and quality in Afghanistan are reported. For example, MAIL & FAO, (2013) reported that the lack of farmer awareness regarding the use of appropriate agronomic practices, and the shortage of water were among the major challenges. Moustafa et al. (2012) indicated that higher pH value and CaCO3 concentration in soil, along with lower available phosphorus, and organic matter, maintain wheat production lower in Afghanistan. Additionally, Sharma & Nang, (2018) highlighted that drought, pests, disease, and insufficient quality of seed were known to hamper wheat productivity and quality in Afghanistan. Likewise, Kazimi et al., (2018) reported, inadequate use of organic residues, poor attention to crop rotation, and heavy tillage have been mentioned to be limiting factors for yields and soil degradation in Afghanistan. Researchers must focus on the yield gap and determine the gap between actual farm productivity (quantitively and qualifiedly) and what is hypothetically achievable under ideal management (Poole et al., 2022). Additionally, ARIA et al., (2022), reported that there is a tremendous potential possibility to increase wheat productivity in Afghanistan. The research results showed through the application of improved agronomic practices (appropriate fertilization schemes, the optimal time of sowing, effective irrigation, and effective weed and pest control), would increase wheat productivity and grain quality in Afghanistan (Soofizada, 2018).

Producing optimal quality wheat, play a more substantial role in human nutrition. The grain quality depends on two main components, namely starch and protein contents. In particular, the wheat grain is constituted of 60 to 70% starch, 8 to 18% protein, 1 to 2% lipids, and 3 to 4% minerals (Zörb et al., 2018). However, it is well known that the components of stored wheat starch and proteins can greatly be improved through genetic and fertilization strategies, which together determine the final quality of wheat grain (Igrejas and Ikeda, 2020). As a result, research proposes that proper N fertilization could significantly improve wheat grain protein and gluten concentration (Stępień and Katarzyna, 2019). Since the ratio between glutenin and gliadin can determine the dough's rheological characteristics; a study result reported that under the low N and P treatments the grain gliadin content significantly improved, while a significant reduction was observed in the glutenin fractions (Tóth et al., 2020). Phosphorus fertilization is an important factor that could therefore significantly influence grain development as well as improve the starch content and its molecule components (Ni et al., 2012). Apart from the environment being an important factor, P fertilization also plays a key role in higher grain yield and improves starch and protein concentration in wheat (Kizilgeci, 2019). An appropriate N fertilization can greatly influence seed storage protein accumulation, and determine the gain processing, desirable baking value, and healthy final end-use products (Peng et al., 2022).

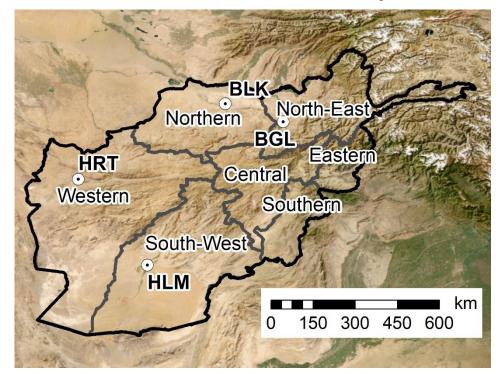
Despite huge research have been carried out in the past to assess the effect of N on wheat quality, therefore, little information is available on grain protein sub-fraction and starch content (Asthir et al., 2017); likewise, insufficient studies on starch granule distribution and its content in grain wheat were reported (Ni et al., 2012). Both N and P fertilizers are essential nutrients for normal growth, wheat quality, and productivity, which can be determined by balanced fertilization (Tóth et al., 2020).

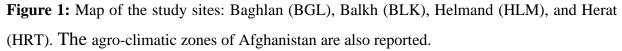
Therefore, the objectives of this study were to (1) quantify the effect of soil and climatic parameters on the yield and quality of wheat (2) investigate the response of different wheat varieties to different N and P fertilization rates under specific climate conditions, toward to improve the yield and quality of wheat in Afghanistan.

# 3.3.2 Material and Methods

## 3.3.2.1 experimental fields set-up

The research was performed over two growing seasons (GS), from September 2020 to July 2022, in four locations (L) each in different agro-climatic zones (ACZ) of Afghanistan: Baghlan (BGL) in the North-East (ACZ-NE), Balkh (BLK) in the North (ACZ-N), Helmand (HLM) in the South-West (ACZ-SW), and Herat (HRT) in the West (ACZ-W) (Figure 1).





Consistent with the description of Afghanistan's climate reported FAO and WFP (2004), BGL, BLK, and HRT experienced a semi-arid climate, while HLM was arid. Daily precipitation and NASA daily average temperature (Tavg; °C) data from the database (https://power.larc.nasa.gov/data-access-viewer/) were used to calculate the average precipitation and temperature on a monthly base for the long-term period (2001-2020), as well as for the 1<sup>st</sup> and 2<sup>nd</sup> GS. For each L and GS, the cumulated precipitation values from tillering to grain filling period (P\_TGf) were calculated as reported in Soofizada et al. (2023). The growing degree day (GDD) (Salazar-Gutierrez et al., 2013) was used to describe the timing of biological processes (Grassi et al., 2020; Fabbri et al., 2020). According to Fabbri et al. (2020), the daily GDD value was set to 0°C for Tavg at or below 4°C, whereas for Tavg higher than 4°C, it was calculated as the difference between Tavg and 4°C. Then, the daily GDD values were cumulated for the period from tillering to grain filling (GDD\_TGf).

In all Ls, soils were alkaline with a low concentration of organic matter (OM; %), available P (Av\_P; mg P kg<sup>-1</sup>), and total N (Tot\_N; %), while with a sufficient amount of available potassium (Av\_K; mg K kg<sup>-1</sup>). Among the four Ls, the BGL soil was the richest in nutrients, especially available P and total N, while the HRT soil was the poorest.

Location	pН	ОМ	Av_P	Av_K	Tot_N	Soil texture
Location	рп	(%)	(mg P kg <sup>-1</sup> )	(mg K kg <sup>-1</sup> )	(%)	
BGL	7.9	1.58	7.2	135	0.16	Silty loam
BLK	8.3	1.15	6.2	120	0.12	Silty loam
HLM	8.2	1.31	5.6	132	0.11	Silty loam
HRT	8.4	0.86	4.5	118	0.05	Sandy loam

**Table 1.** The site and soil properties of different locations

A plot experiment (plot dimensions 2.5 x 2 m; surface 5 m<sup>2</sup>) was arranged in a factorial randomized complete block design (FRBD). A total of twenty-seven treatments, with three replicates, were obtained from the factorial combination of 3 phosphorus levels (PL) (60, 90, and 120 kg P ha<sup>-1</sup>, namely PL60, PL90, and PL120, respectively), 3 nitrogen to phosphorous (NP) rates (1:1, 1.25:1 and 1.5:1; namely NP1, NP1.25, and NP1.5) (Table 2) and 3 common wheat (*Triticum aestivum* L.) varieties: Darulaman07, (DLN7) and Kabul-13 (KBL13) and Zardana-89 (ZDNA). DLN7 and KBL13 were improved varieties created by breeding by the International Maize and Wheat Improvement Center (CIMMY), while the ZDNA was an improved variety from Pakistan. The pedigree of DLN7, KBL13, and ZDNA were WEAVER/4/NAC/TH.AC//3\*PVN/3/MIRLO/BUCCID/SID:133428/10,

## WAXWING\*2/TUKURU,

and

CNO67/8156//TOB66/CNO67/4/NO/3/12300//LR64A/8156/5/PVN, respectively. DLN7 is an improved wheat variety characterized by good resistance to yellow rust and a grain yield (GY) potential of 4 t ha<sup>-1</sup> (Niane et al., 2011). KBL13 is considered the most promising variety for improving Afghan wheat production in the coming time due to its resistance to Ug99 and other rust varieties, as well as its capability of producing an average GY of over 4.4 t ha<sup>-1</sup> (Soofizada et al., 2023). Since the mid-1990s, ZDNA has been one of the most popular variety of common wheat in the northern regions of Afghanistan given the higher average yields than other local varieties (Favre, 2004; Kugbei, 2011). Because of its diffusion among farmers, ZDNA is generally used as a benchmark variety in Afghanistan (Jilani et al., 2013).

The soil was plowed at a depth of 0.40 m and then harrowed at a depth of 0.10 m in October for two consecutive growing seasons (2020/2021 and 2021/2022; namely 1<sup>st</sup> GS and 2<sup>nd</sup> GS). The sowing was performed by distributing 120 kg seed ha<sup>-1</sup> with a row spacing of 0.2 m (10 rows for

a plot). Within each plot, the six central rows (cut off 0.5 m at both ends) were used for sampling and data collection (sampling area of 1.8 m<sup>2</sup>). Although no phytosanitary products for plant protection have been used, the plants were observed to be healthy with no damage. The P was all distributed in the form of di-ammonium phosphate (DAP, P<sub>2</sub>O<sub>5</sub>: 46%) just before sowing. The N amount to be distributed in each plot was calculated taking into account the PL and the established NP ratio. The total N to be distributed in each plot was reduced by the amount of N in DAP, while the remaining N in the form of urea (N:46%) was applied at 50% at tillering and 50% at stem elongation. In all the experimental fields, furrow irrigation with riverine water was carried out to satisfy the crop's water requirements during the common wheat growing season according to the protocol reported in Soofizada et al. (2023). All the field activities were manually performed (i.e. sowing, fertilizer distribution, weeding, irrigation, harvesting, and threshing). For each plot, the GY (adjusted to 12%), the straw yield (SY; kg ha<sup>-1</sup>), and the thousand kernel weight (TKW; g 1000 seeds<sup>-1</sup>) were measured at harvesting.

**Table 2.** Phosphorus ( $P_2O_5$ ) and nitrogen (N) rates for each combination of PL and NP treatments.

Treat	tments	P rate	<b>N rate</b> (kg N ha <sup>-1</sup> ) 60 75 90 90 113				
PL	NP	(kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	(kg N ha <sup>-1</sup> )				
	NP1	-	60				
PL60	NP1.25	60	75				
	NP1.5		90				
	NP1		90				
PL90	NP1.25	90	113				
	NP1.5		(kg N ha <sup>-1</sup> ) 60 75 90 90				
	NP1		120				
PL120	NP1.25	120	150				
	NP1.5		180				

# 3.3.2.2 Analysis of kernel and dough

Wholemeal flour samples were obtained for each treatment through the use of a grinder with a 0.5 mm screen (Cytolec 1093 lab mill, FOSS Tecator, Hoganas, Sweden) (Guerrini et al., 2020; Soofizada et al., 2023). A CHNS analyzer (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA) was used to determine the total N percentage in wholemeal flour samples (5 mg per sample). The N percentage was then converted to total protein content in wholemeal (PC, %) by multiplying by 5.7, as per ICC Standard 167 (2000). The starch content in wholegrain flour (ST; %) was determined using the Total Starch Assay Kit (K-TSTA, Megazyme, Irishtown, Ireland) as reported in Cammerata et al. (2021). A Megazyme Amylose/Amylopectin Assay Kit

(Megazyme Ltd., Bray, Ireland) was used to determine the amylose (AM; %) and amylopectin (AP; %) percentage on ST strictly following the procedures indicated by the manufacturer (Wang et al., 2021). Accordingly, the AM to AP ratio (AM:AP) was calculated. Dough strength (W; 10<sup>-4</sup> J) was performed according to ISO 27971 (2015) as reported in Soofizada et al. (2022). The protein and starch yield per hectare (PY and STY; kg ha<sup>-1</sup>) were calculated as the product of GY by PC and ST, respectively.

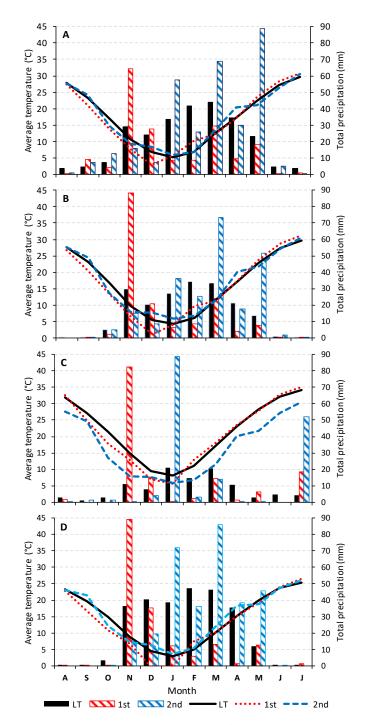
### **3.3.2.3 Statistical analysis**

A multifactorial analysis of variance (ANOVA) was performed to determine the main effect of PL and NP treatments and their interactions, utilizing the RStudio version (R 4.1.1.). In the case of significant differences, the Tukey HSD post-hoc test was used to compare the differences between means. Significance was indicated as follows: \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, and n.s. for not significant. To assess the relative influence of explanatory variables on the variation of winter wheat agronomic traits (GY; PY; GC; STY; AP), a gradient-boosted regression tree (GBRT) statistical analysis was performed. A correlation matrix was employed to eliminate collinear variables and to check for redundancy among covariates, using a threshold of 0.8. The fit of the GBRT model was analyzed using a tenfold cross validation and was performed with a tree complexity of 5 and a learning rate of 0.01.

## 3.3.3 Results

#### 3.3.3.1 Meteorology data

During the 1<sup>st</sup> GS, the average temperature pattern from Sept to Dec was slightly lower compared to the long-term for all Ls. Regarding precipitation, in the 1<sup>st</sup> GS, the average was lower for all locations (L) with respect to the long-term data. Exceptionally only in Nov, the precipitation was higher for all Ls compared to the long-term. For the 2<sup>nd</sup> GS, the temperature was similar to long-term data at BGL, BLK, and HRT, but in HLM temperature was slightly lower than in the long-term. The precipitation amount was higher in the 2<sup>nd</sup> GS for all Ls compared to the long-term data, and the 1<sup>st</sup> GS. Therefore, the months of Jan, March, and May received higher precipitation at BGL, HRT, and BLK, respectively compared to the long-term, but in HLM higher precipitation was recorded in Jan and July compared to the long-term and other locations. On average, BGL was the wettest followed by HRT in the 2<sup>nd</sup> GS, compared to BLK and HLM.



**Figure 2.** Walter-Lieth diagram of the study sites. (**A**) BGL (Baghlan), (**B**) BLK (Balkh), (**C**) HLM (Helmand) and (**D**) HRT (Herat). The long-term (LT) (2003–2022), average monthly temperature (°C, black continuous line), and monthly average precipitation (mm, black histograms). The 1<sup>st</sup> growing season (1<sup>st</sup> GS), (2020/2021), average monthly temperature (°C, red dotted line), and monthly average precipitation (mm, red histograms). The second growing season (2<sup>nd</sup> GS), (2021/2022), average monthly temperature (blue dashed line), and monthly average precipitation (mm, blue histograms).

#### 3.3.3.2 Agronomic parameters

L was the dominant factor for GY and SY, followed by PL, GS, and Gen (Table 3). NP significantly affects GY, but not SY. Additionally, GY was significantly affected in decreasing order by the following second-order interactions LxPL, GSxL, PLxNP, GSxGen, GSxNP, and LxGen. Whereas SY was significantly affected in decreasing order by the following second-order interactions: GSxL, PLxNP, LxPL, LxGen, and LxNP. The highest average GY and SY values were detected in DLN7, followed in decreasing order by KBL13 and ZRDN. The average GY and SY measured in DLN7 were significantly different from that measured in ZDNA, while no significant difference was detected between KBL13 and the other two varieties. As the PL increased, both GY and SY significantly increased, with an increment of 29.43% and 26.44%, respectively, from PL60 to PL120. The average GY and SY values measured at BGL were significantly higher than those measured in the other Ls, while the lowest average GY and SY values were measured at HRT.

In the present study, L was a dominant factor for TKW, followed by GS, and Gen, while PL and NP did not significantly affect TKW. Additionally, TKW was significantly affected by second-order interactions GSxL, LxGen, GSxPL, and PLxNP, whilst the other second-order interactions were not found to be statistically significant for TKW.

The average TKW value measured in DLN7 was significantly higher than the values measured in KBL13 and ZDNA, while no significant differences were detected between KBL13 and ZDNA.

The average TKW values measured in HLM and BLK, which showed no significant differences, were significantly higher than the values measured in BGL and HRT. Meanwhile, the lowest average TKW value was measured in HRT.

		GY	Y	SY	ζ	TKW			
Variation source	DF	(t ha	<b>1</b> <sup>-1</sup> )	(t ha	<b>1</b> <sup>-1</sup> )	( <b>g</b> )			
source		F	sig	F	Sig	F	sig		
GS	1	243.1	***	94.5	***	136.8	***		
L	3	995.1	***	613.2	***	149.6	***		
Gen	2	77.3	***	44.1	***	8.3	***		
PL	2	293.3	***	156.9	***	0.2	ns		
NP	2	8.2	***	5.9	**	0	ns		
GSxGen	2	8.1	***	1.1	ns	0.8	ns		
GSxL	3	21.3	***	37.6	***	125.5	***		
GSxNP	2	6	**	0.2	ns	0.2	ns		
GSxPL	2	2.2	ns	2.5	ns	3.4	*		
LxGen	6	5.8	***	10.2	***	7.6	***		
LxNP	6	1.6	ns	5.7	***	0.3	ns		
LxPL	6	25.1	***	10.8	***	1.4	ns		
GenxNP	4	0.4	ns	1	ns	1.3	ns		
GenxPL	4	2.6	*	0.2	ns	0.8	ns		
PLxNP	4	15.8	***	13.4	***	2.5	*		
Residuals	432								

**Table 3** Results of the ANOVA on grain yield (GY), straw yield (SY), and thousand kernel weights (TKW). The table columns report the Fisher F (F) and the significance levels: \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, ns = not significant.

The result of PL interaction with L showed that as PL increased, GY and SY increased significantly in four Ls (Figure 3, B and B), only in HLM there was no significant difference between the PL60 and PL90 for GY, PL90, and PL120 for SY. Likewise, in BLK no significant difference occurred between the PL60 and PL90 for SY, but they were significantly lower than PL120.

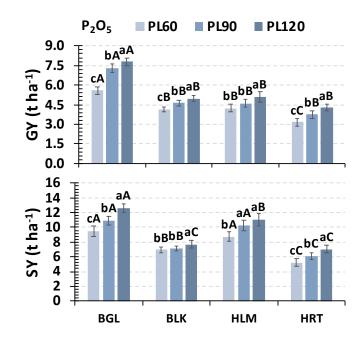
The NP ratio interaction with PL needs to be more explored. At PL60, GY, and SY significantly increased by 15% and 18.3%, respectively, after the supply of NP1.5 than that of PN1 (Figures 4, A, and B). At PL90, GY increased by 4.2%, in contrast, SY decreased by 2.96% from PN1 to PN1.5 level but statistically was not significant. In addition, at PL120, GY and SY decreased by 5.43% and 3.10% by PN1.5 compared to PN1, but the difference for SY statistically was not significant. Interestingly, the NP1/PL120 optimized substantially the GY and SY, compared to the rest of the treatments.

The interaction results of Gen with L showed that DLN7 was a superior variety for GY and SY for four Ls, but statistically, the difference was not significant between DLN7 and KBL13 for GY, at BGL, BLK, HRT, and for SY only at BGL (Figure 5, A, and B). Moreover, KBL13 also

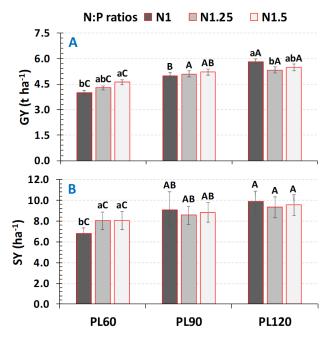
showed similar results with ZDNA for GY at BGL and HLM, and for SY only in HLM. For both, BLK and HRT, SY was not significantly affected by Gen

**Table 4.** The mean grain yield (GY), straw yield (SY), and thousand kernel weights (TKW) due to the main effect of the growing season (GS), location (L), nitrogen ratios (NP), phosphorus (PL) fertilization and varieties (Gen). The table columns report the ANOVA result as \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, n.s. = not significant, whereas, the lowercase letter shows the Tukey HSD post-hoc test results and the value inside parentheses represents standard error.

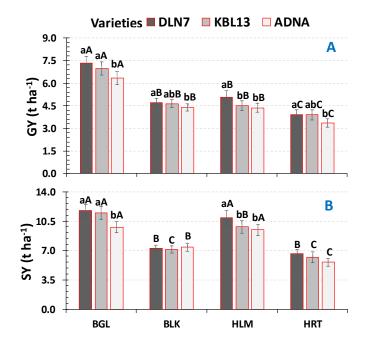
	GY		SY		TKW	
Treatments	(t ha <sup>-1</sup> )		(t ha <sup>-1</sup> )		<b>(g</b> )	
	Average	Sig	Average	sig	Average	sig
Gen	-	***	-	***	-	***
DLN7	5.26 (0.42)	А	9.12 (0.74)	а	39.81 (0.61)	а
KBL13	5.01 (0.39)	Ab	8.64 (0.73)	ab	38.66 (0.57)	b
ZDNA	4.62 (0.37)	В	8.05 (0.61)	b	38.75 (0.71)	b
PL (kg ha-1)		***		***		ns
PL60	4.28 (0.3)	С	7.6 (0.59)	с	38.97 (1.48)	
PL90	5.07 (0.41)	В	8.61 (0.65)	b	39.15 (1.27)	
PL120	5.54 (0.41)	А	9.61 (0.76)	а	39.1 (1.34)	
NP		ns		ns		ns
N1:1	4.91 (0.42)		8.4 (0.70)		39.12 (1.49)	
N1.25:1	4.89 (0.37)		8.63 (0.67)		39.03 (1.33)	
N1.5:1	5.08 (0.39)		8.78 (0.74)		39.07 (1.28)	
L		***		***		***
BGL	6.89 (0.36)	А	11 (0.73)	а	37.3 (1.11)	b
BLK	4.59 (0.19)	В	7.25 (0.39)	с	41.67 (2.03)	а
HLM	4.65 (0.28)	В	10.04 (0.68)	b	41.75 (0.95)	а
HRT	3.73 (0.25)	С	6.13 (0.51)	d	35.58 (2.03)	с
GS		***		***		***
2021	4.63 (0.35)	В	8.15 (0.62)	b	37.58 (1.51)	b
2022	5.30 (0.35)	А	9.06 (0.63)	а	40.57 (0.72)	а



**Figure 3**. From top to bottom, the effect of phosphorus fertilization rates (PL) in four locations (BGL= Baghlan, BLK= Balkh, HLM= Helmand, and HRT= Herat) on (A) the grain yield (GY), and (B) straw yield (SY). Lowercase letters represent significant differences between PL levels within the same location, and uppercase letters represent significant differences between locations within the same PL rate according to the Tukey HSD post hoc test results.



**Figure 4**. From top to bottom, the effect of nitrogen fertilization ratios (NP) on three levels of phosphorus (PL) on (A) the grain yield (GY), and (B) straw yield (SY). Lowercase letters represent significant differences between NP levels within the same level of PL, and uppercase letters represent significant differences between PL within the same NP ratio according to the Tukey HSD post hoc test results.



**Figure 5**. From top to bottom, the performance of varieties (Gen) at the four locations (BGL: Baghlan; BLK: Balkh; HLM: Helmand; HRT: Herat) on (A) the grain yield (GY) and (B) straw yield (SY). Lowercase letters represent significant differences between varieties within the same locations, and uppercase letters represent significant differences between locations within the same variety according to the Tukey HSD post hoc test results.

## 3.3.3.3 Protein, Starch, and Alveograph Parameters

Gen was the dominant factor for PC and GC, followed by L, NP, PL, and finally GS; except GC was not significantly affected by PL (table 5). Additionally, PC was significantly influenced in decreasing order by the following second-order interaction, such as Gen x NP, PL x NP, L x Gen, Gen x PL, and GS x L. While GC was influenced by PL x NP, Gen x NP, L x Gen, and lastly GS x L, in decreasing order, respectively. The highest average PC and GC were measured at ZRDA, followed by DLN7, while the lowest PC and GC were found at KBL13 (table 6). The average PC increased by 1.7% from PL60 to PL90, while further application of PL did not significantly affect the PC (Table 6). Likewise, PC increased by 2.72% from NP1 to NP1.25, but further addition of NP did not increase the PC (table 6). As the NP increased, GC significantly increased, by an increment of 4.5% from NP1 to NP1.5. The highest average PC was determined in HRT, followed by HLM, while the lowest PC was significantly higher at BGL, while no significant differences were observed between BLK, HLM, and HRT (Table 6).

PY was dominantly affected by L, followed by PL, GS, Gen, and NP (table 5). Additionally, PY is affected almost by all second-order interactions, in decreasing order, such as L x PL, GS x

PL, GS x Gen, PL x NP, L x Gen, GS x NP, Gen x NP, Gen x PL, and L x NP. The highest average PY value was measured in the DLN7, which was significantly higher than KBL13 and ZRDNA (table 6). Moreover, this study indicated that PL (from PL60 to PL120) and NP (from NP1 to NP1.5) significantly increased the average PY by 31% and 8.1%, respectively. The highest PY was observed in BGL, followed by HLM and BLK, whilst HRT showed the lowest PY.

According to the ANOVA, Gen was the main factor for W, followed by PL, NP, and L; exception W did not affect by GS (Table 5). Additionally, W was significantly affected by second-order interactions, in particular, Gen x PL, L x Gen, and Gen x NP, in decreasing order, respectively. The maximum W value was measured at ZDNA, followed by DLN7, while the lowest W was measured in BLK13 (table 6). As PL and NP increased, W significantly increased, by an increment of 5% and 4%, from PL60 to PL120 and from NP1 to NP1.5, respectively (table 6). The highest average W value was measured in HRT, which was significantly higher than BGL, but it was similar to HLM and BLK.

ST was strongly influenced by Gen, followed by NP, L, PL, and lastly GS (table 5). Additionally, ST was strongly affected by the second-order interaction, in particular PL x NP, Gen x PL, GS x Gen, GS x L, and GS x PL, respectively in decreasing order. The highest average ST was measured in the KBL13, followed by DLN7, while the lowest ST value was measured in ZDNA (table 6). As the PL increased the average ST value increased by an increment of 1.3% from PL120 to PL60 (Table 6). On contrary, as the NP ratio increased, the average ST value significantly decreased by 2.03% from NP1 to NP1.5. Furthermore, the average highest and lowest ST value was measured at BGL and HRT, respectively; while BLK and HLM statistically showed similar ST values, but lower than BGL and higher than HRT, except no significant differences occurred between HLM and HRT (table 6).

Furthermore, L was the dominant factor for STY, followed by PL, GS, and Gen. Exception STY did not influence by NP (table 5). Additionally, STY was strongly affected by the second-order interactions, such as L x PL, GS x L, PL x NP, GS x Gen, Gen x PL, GS x NP, and L x Gen, respectively in decreasing order. DLN7 showed the highest average STY, followed by KBL13, while the lowest STY was measured at ZDNA (table 6). The higher average STY was determined at BGL, followed by HLM and BLK, in decreasing order, while the lower STY was observed in HRT. Moreover, STY significantly increased by 31%, as PL application increased from PL60 to PL120. But no significant difference was occurred between PL90 and PL120.

Variable		PC		PY		GC		W		ST		STY	7	AP		AM:A	<b>NP</b>
Variable source	DF	(%)		(kg ha- <sup>1</sup> )		(%)		(10 <sup>-4</sup> J	<b>I</b> )	(%)	)	(t ha <sup>-</sup>	<sup>1</sup> )	(%)		(%)	1
source		F	sig	F	sig	F	sig	F	sig	F	sig	F	sig	F	sig	F	sig
GS	1	17.50	***	237.32	***	23.60	***	2.27	ns	99.58	***	242.36	***	144.89	***	149.16	***
L	3	202.12	***	812.08	***	238.97	***	23.85	***	613.25	***	1030.35	***	760.77	***	786.41	***
Gen	2	1196.43	***	86.91	***	6482.88	***	1082.43	***	2921.15	***	93.57	***	3096.04	***	3180.02	***
PL	2	21.91	***	355.58	***	3.35	ns	64.19	***	342.20	***	308.44	***	313.74	***	324.86	***
NP	2	126.01	***	31.43	***	130.71	***	39.91	***	909.69	***	3.88	ns	1063.88	***	1093.22	***
GSxGen	2	0.06	ns	10.15	***	0.38	ns	0.01	ns	11.89	***	7.85	***	17.06	***	17.33	***
GSxL	3	2.99	*	21.10	***	3.88	**	0.50	ns	13.10	***	21.27	***	24.35	***	24.94	***
GSxNP	2	0.15	ns	5.22	**	0.24	ns	0.89	ns	1.67	ns	6.40	**	4.23	*	3.22	*
GSxPL	2	2.59	ns	2.73	ns	2.87	ns	0.08	ns	3.72	*	2.06	ns	5.13	**	4.85	**
LxGen	6	13.30	***	6.81	***	11.12	***	5.24	***	15.55	***	5.87	***	27.87	***	24.81	***
LxNP	6	1.94	ns	2.14	*	2.01	ns	0.36	ns	1.87	ns	1.48	ns	0.83	ns	1.24	ns
LxPL	6	0.47	ns	25.21	***	0.61	ns	0.97	ns	1.82	ns	25.67	***	4.00	***	4.34	***
GenxNP	4	16.99	***	2.63	*	16.53	***	2.40	*	7.68	***	0.49	ns	72.96	***	72.46	***
GenxPL	4	3.11	*	2.49	*	2.96	ns	11.43	***	30.45	***	6.11	**	183.59	***	198.38	***
PLxNP	4	14.19	***	9.03	***	16.90	***	1.90	ns	67.37	***	18.75	***	22.10	***	22.30	***
Residuals	432																

**Table 5.** Results of the ANOVA on protein concentration (PC), protein yield (PY), gluten content (GC), dough strength (W), starch content (ST), starch yield (STY), amylopectin (AP), and amylose to amylopectin (AM:AP).

The table columns report the Fisher F (F) and the significance levels: \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, ns = not significant.

Gen was a dominant factor for AP and AM:AP, followed by NP, L, PL, and GS (table 5). Additionally, AP did significantly affect by the second-order interaction, in particular, Gen x PL, Gen x NP, L x Gen, GS x L, PL x NP, GS x Gen, GS x PL, GS x NP, and lastly L x PL, respectively in decreasing order. Similarly, AM:AP was significantly influenced by the secondorder interaction of GS x PL, Gen x NP, GS x L, L x Gen, GS x Gen, GS x PL, L x PL, and GS x NP, respectively in decreasing order. The maximum average AP value was determined in DLN7, followed by ZDNA, while the lowest AP value was determined at KBL13 (table 6). On the contrary, the maximum average AM:AP value was determined in KBL13, followed by ZDNA, and the lowest AM: AP value was measured at DLN7. As the PL increased, the average AP value was significantly increased by 2 %, but AM:AP value decreased by 3.63%, from PL60 to PL90. Contrastingly, as the NP ratio increased, the average AP value significantly decreased by 1.4%, but AM:AP value significantly increased by 6.55%, from NP1 to NP1.5 (table 6). Furthermore, the maximum average AP value was measured in HRT, while the smallest AP value was observed at BGL; but HLM and BLK showed similar AP values, significantly lower than HRT and higher than BGL. Likewise, the maximum average AM:AP value was observed at BGL, a significant difference from the other Ls. No significant differences have occurred between BLK, HLM, and HRT for AM:AP.

The PL interaction with NP needs to be further analyzed. At PL60, PC was not significantly affected by NP, while at PL90 and P120, PC increased by 3.35% and 6.57% from NP1 to NP1.5, respectively (Figure 6, A). Likewise, at PL60 and PL90, the GC trend showed a relative increase, as NP ratios increased but statistically were non-significant. Instead, GC significantly increased by 6.77% at PL120, as NP ratios increased from NP1 to NP1.5 (Data not shown).

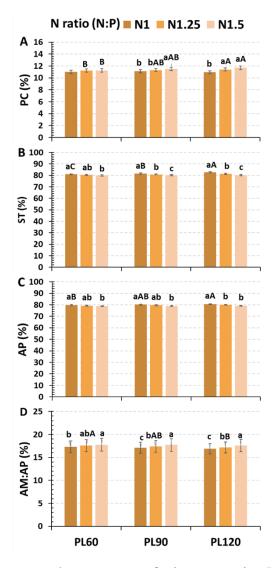
Regarding PY and W, the only significant differences were found at the PL60 level with a combination of NP ratios. Whereas the PY and W were increased by 15.3 and 4.76%, respectively, from NP1 to NP1.5 (Data not shown).

As the NP ratio increased from NP1 to NP1.5 rate at the PL60, PL90, and PL120 levels, decreased the ST and AP value significantly, by 1.2, 1.88, 3.11%, and 1.05, 1.44, and 1.72%, respectively (Figure 6, B and C). In contrast, the AM:AP value increased significantly, by 2.55, 3.41, and 4.16% at PL60, PL90, and PL120 levels, respectively, as the NP ratio increased from NP1 to NP1.5 (Figure 6, D). Regarding STY, the NP treatments were only significant at the PL60 level, in which STY increased by 12.19 from NP1 to N1.5 level (data not shown).

Variable source	(%)		PY (kg ha <sup>-1</sup> )		GC (%)		W (10 <sup>-4</sup> J)		ST (%)		STY (ha <sup>-1</sup> )		AP (%)		AM:AP (%)	
	Average	sig	Average	sig	Average	sig	Average sig		Average	sig	Average sig		Average	sig	Average	sig
Gen	-	***	-	***	-	***	_	***	-	***	-	***	_	***	-	***
DLN7	11.38 (0.13)	b	594.42 (43.47)	а	7.98 (0.09)	b	84.17 (1.26)	b	80.5 (0.29)	b	4.25 (0.35)	а	80.59 (0.22)	а	24.10 (0.35)	с
KBL13	10.5 (0.15)	с	520.47 (35.36)	b	6.95 (0.10)	с	73.35 (1.14)	с	82.52 (0.29)	а	4.14 (0.33)	а	78.7 (0.22)	с	27.07 (0.32)	а
ZDNA	11.96 (0.12)	а	550.21 (42.20)	b	9.35 (0.10)	а	90.69 (1.32)	а	79.62 (0.36)	с	3.69 (0.31)	b	79.51 (0.22)	b	25.78 (0.34)	b
PL (kg ha <sup>-1</sup> )		***		*		ns		***		***		***		***		***
PL60	11.17 (0.21)	b	474.09 (30.45)	с	8.04 (0.26)		80.31 (1.99)	b	80.38 (0.37)	с	3.45 (0.25)	с	78.31 (0.33)	b	26.11 (0.52)	а
PL90	11.30 (0.2)	ab	568.12 (41.84)	ab	8.11 (0.27)		83.59 (2.19)	а	80.87 (0.42)	b	4.11 (0.34)	b	79.58 (0.29)	ab	25.67 (0.44)	b
PL120	11.36 (0.21)	а	622.89 (40.76)	а	8.13 (0.29)		84.31 (2.39)	а	81.39 (0.5)	а	4.52 (0.35)	а	79.91 (0.25)	а	25.16 (0.39)	с
NP		***		***		**		***		***		ns		***		***
1	11.02 (0.2)	b	536.38 (42.46)	b	7.91 (1.8)	b	80.86 (2.1)	b	81.74 (0.42)	а	4.03 (0.36)		80.15 (0.29)	а	24.79 (0.45)	с
1.25	11.32 (0.19)	а	548.88 (38.62)	ab	8.12 (1.87)	ab	83.23 (2.21)	а	80.82 (0.4)	b	3.96 (0.31)		79.61 (0.27)	ab	25.63 (0.42)	b
1.5	11.49 (0.21)	а	579.84 (41.80)	а	8.25 (1.88)	а	84.12 (2.33)	а	80.08 (0.41)	с	4.08 (0.33)		79.04 (0.25)	b	26.53 (0.40)	а
L		***		***		***		***		***		***		***		***
BGL	10.78 (0.24)	с	740.05 (38.72)	а	7.71 (0.31)	b	80.62 (2.67)	b	82.01 (0.41)	а	5.65 (0.31)	а	78.83 (0.25)	с	26.88 (0.41)	а
BLK	11.34 (0.18)	b	519.60 (22.8)	b	8.14 (0.27)	а	82.87 (2.33)	ab	80.74 (0.42)	b	3.71 (0.16)	b	79.75 (0.31)	b	25.42 (0.48)	b
HLM	11.43 (0.2)	ab	530.59 (31.47)	b	8.21 (0.29)	а	83.37 (2.27)	а	80.56 (0.46)	bc	3.75 (0.23)	b	79.74 (0.29)	b	25.42 (0.44)	b
HRT	11.57 (0.19)	а	429.9 (28.15)	с	8.32 (0.28)	а	84.1 (2.23)	а	80.21 (0.47)	с	2.99 (0.21)	с	80.09 (0.31)	а	25.89 (0.48)	b
GS		***		***		***		ns		***		***		***		***
2021	11.53 (0.19)	а	519.54 (36.03)	b	8.14 (0.25)	а	82.97 (2.04)		80.72 (0.41)	b	3.75 (0.3)	b	79.72 (0.28)	а	25.46 (0.43)	b
2022	11.30 (0.18)	b	590.53 (36.65)	а	8.05 (0.25)	b	82.51 (2.01)		81.04 (0.39)	а	4.30 (0.3)	а	79.48 (0.26)	b	25.84 (0.40)	а

**Table 6.** Grain quality parameter mean values (standard error in brackets) of 3 common wheat varieties (Gen), as a function of phosphorus (PL), nitrogen ratio (NP), location (L), and growing season (GS)

The table columns report the significance levels: \*\*\* = 0.001, ns = not significant. Letters represent the Tukey HSD post hoc test results.

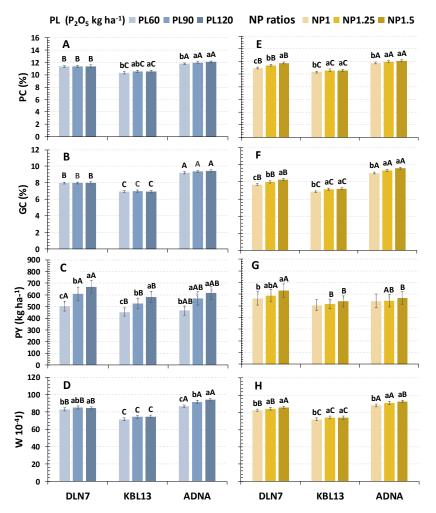


**Figure 6.** From top to bottom, the response of nitrogen ratio (NP) at the three levels of phosphorus (PL60, PL90, and PL120) on (A) protein content (PC), (B) starch content (ST), (C) amylopectin (AP), (D) amylose to amylopectin ratio (AM:AP). Lowercase letters represent significant differences between NP ratios within the same level of phosphorus, and uppercase letters represent significant differences between phosphorus levels within the same NP ratio according to the Tukey HSD post hoc test results.

The PL and NP interaction with Gen needs to be further analyzed (Figure 7). The PC increased significantly up to the application of PL90 at KBL13 and ZDNA, but no significant difference was found between PL90 and PL120 (Figure 7, A). Instead, the PL treatment did influence the PC in DLN7. The GC value tends to be increased by 2.57 and 0.4% at ZDNA and KBL13, respectively, after the supply of PL120 than the PL60, but statistically was not significant (Figure 7, B). Interestingly, the PY was principle increased by 32.9, 28.8%, and 30.8% at DLN7, KBL13, and ZDNA, respectively, as PL increased from PL60 to PL120 (Figure 7, C). Moreover, W

significantly increased by 8.65 and 1.77% at ZDNA and DLN7, respectively, as PL increased from PL60 to PL120; but W was not affected at KBL13 under PL treatment (Figure 7, D).

The PC and GC increased as NP increased up to NP1.25, in KBL13 and ZDNA, while no significant increment was observed with further application of NP in both Gen. Instead, at DLN7, PC and GC increased significantly, as increased NP, by an increment of 7.2 and 7.33% from PN1 to PN1.5 (Figure 7, E, and F). The PY was increased by 12.2% at DLN7, after the application of NP1.5 compared to NP1. Also, the trend of PY linearly increased at KBL13 and ZDNA, as the NP rate increased, but statistically was not significant (Figure 7, G). Furthermore, the W value increased significantly, as the NP ratio increased up to N1.25 for all the Gens; but no significant differences occurred between N1.25 and N1.5 (Figure 7, H).



**Figure 7.** Left diagram (blue-Gray gradient color), from top to bottom, the performance of phosphorus levels (PL), on three varieties (Gen, DLN7, KBL13, and ZDNA), on (A) protein content (PC), (B) gluten content (GC), (C) protein yield (PY), (D) dough strength (W). Right diagram, (Gold gradient color) from top to bottom, the performance of nitrogen ratios (NP), on three varieties (Gen, DLN7, KBL13, and ZDNA:), on (E) protein content (PC), (F) gluten

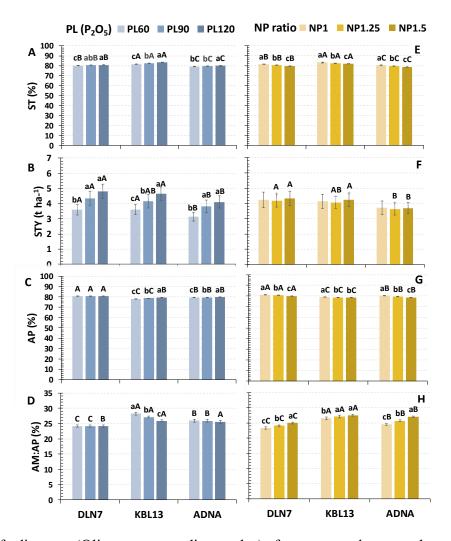
content (GC), (G) protein yield (PY), (H) dough strength (W). In both diagrams, lowercase letters represent significant differences between phosphorus level (PL) and nitrogen ratio to PL (NP) within the same variety, and uppercase letters represent significant differences between varieties within the same PL and NP level according to the Tukey HSD post hoc test results.

The PL and NP interaction with Gen significantly affected ST, AP, and AM:AP (Figure 8). ST values increased by 0.72, 1.92, and 2.04% at DLN7, KBL13, and ZDNA, respectively, as PL increased from PL60 to PL120 (Figure 8, A). Likewise, STY strongly increased by 33.70% (4.8 t ha<sup>-1</sup>), 28.42% (4.6 t ha<sup>-1</sup>), and 30.88% (4.11 t ha<sup>-1</sup>) at DLN7, KBL13, and ZDNA, respectively, after application of PL120, compared to PL60 (Figure 8, B). Further, AP increased by 1.94 and 0.3%, at KBL13 and ZDNA, respectively, as PL increased from PL60 to PL120, but AP showed no response at DLN7 under PL treatment (Figure 8, C). Additionally, AM:AP at DLN7 and ZDNA showed no response to PL treatment, but AM:AP decreased significantly by 8.5 at KBL13 after the supply of PL120 compared to PL60 (Figure 8, D).

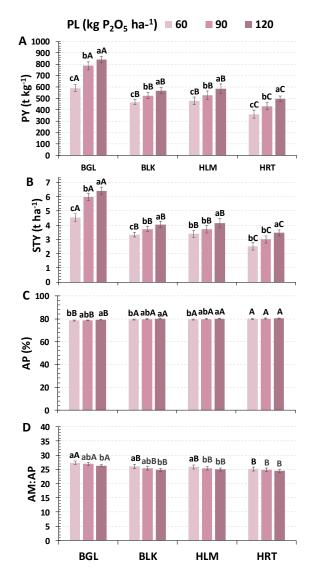
On the contrary, ST decreased significantly under NP treatment, by 2.29, 1.65, and 2.26%, at DLN7, KBL13, and ZDNA, respectively, as NP increased from NP1 to NP1.5. Additionally, NP treatment did not influence the STY at all Gens (Figure 8, E, and F). Furthermore, the AP significantly decreased by 1.37, 0.78, and 2.1% at DLN7, KBL13, and ZDNA, respectively, at the highest NP ratio than the lowest NP ratio (Figure 8, G). Interestingly, AM:AP significantly increased by 7.25, 3.77, and 10.36%, at DLN7, KBL13, and ZDNA, respectively, as NP increased from NP1 to N1.5 (Figure 8, H).

The PL interaction with L needs to be further analyzed. Although PC, GC, and ST were not significantly affected by PL application for all Ls (figure 9). But W, PY, STY, AP, and AM:AP was increased significantly with PL application at all considered Ls. W significantly increased as PL increased from PL60 to PL90, at BGL, BLK, and HLM, but no further significance was detected between the PL90 and PL120. While W was not influenced in HLM under the PL treatment (data not shown). Moreover, data showed that as PL increased, PY and STY intensively increased. Whereas PY increments were 44.43, 22.08, 22.24, and 37.40% and STY increments were 42.21, 22.1, 22.23, and 37.6 % in BGL, BLK, HLM, and HRT, respectively, from PL60 to PL120 (Figure 9, A, B). Furthermore, AP increased by 0.76, 0.97, and 0.71 % at BGL, BLK, and HLM, respectively, in the highest PL than the lowest PL; but AP showed no response at HRT under the PL treatment (Figure, C). Lastly, the results showed that as the PL rate increased, the AM:AP value was significantly reduced, by 3.66, 4.7, 3.42 and 2.87% in

BGL, BLK, HLM, and HRT, respectively; except the reduction value at HRT was statistically not significant (Figure 9, D).



**Figure 8.** Left diagram (Olive green gradient color), from top to bottom, the performance phosphorus levels (PL) on three varieties (Gen, DLN7, KBL13, and ZDNA), on (A) starch content (ST), (B) starch yield (STY), (C) amylopectin (AP), (D) amylose to amylopectin ratio (AM:AP). Right diagram (Dark gray color), from top to bottom, the performance of nitrogen ratio (NP) on three varieties (Gen, DLN7, KBL13, and ZDNA) on (E) starch content (ST), (F) starch yield (STY), (G) amylopectin (AP), (H) amylose to amylopectin ratio (AM:AP). In both diagrams, lowercase letters represent significant differences between PL and NP within the same variety, and uppercase letters represent significant differences between varieties within the same PL and NP according to the Tukey HSD post hoc test results.

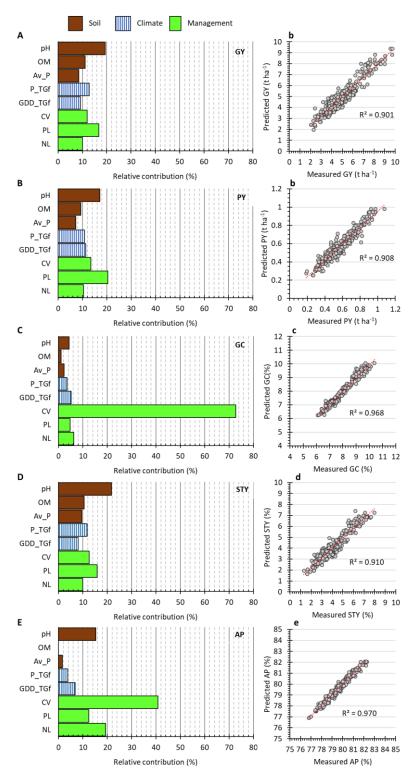


**Figure 9.** From top to bottom, the performance of three phosphorus levels (PL) in four locations (BGL: Baghlan, BLK: Balkh, HLM: Helmand, and HRT: Herat) on (A) protein yield (PY), (B) starch content (ST), ( $\mathbb{C}$ ) amylopectin (AP), ( $\mathbb{D}$ ) amylose to amylopectin ratio (AM:AP). Lowercase letters represent significant differences between PL within the same location, and uppercase letters represent significant differences between locations within the same PL according to the Tukey HSD post hoc test results.

The GBRT model was applied to determine the active relative contribution of soil, climate, and management factor on GY, PY, GC, STY, and AP (Figure 10). The relative contribution of soil and management were almost equal on GY production (39.15, and 38.85%, respectively) but the climate factor explained 22% of GY variability. The relative contribution of pH, PL, P\_TGf, and Gen on GY was 18.4, 16.84, 12.75, and 12%, respectively; while the contribution of OM, NP, GDD\_TGf, Av\_P on GY was lower than 12%. Moreover, the relative contribution of management was principally higher for PY and GC (44.2 and 83.8%, respectively) than the soil

and climate parameters on PY 33.69, 12.05%, and GC 8.6%, and 7.52%, respectively. Gen was the most important factor influencing GC accumulation (relative contribution 72.8%), but PL was the most important factor for PY explaining 20.40% of the variability. While the relative contribution of pH, Gen, NP, GDD\_TGf, P\_TGf, and Av\_P on PY was 17.17, 13.41, 10.42%, 11.31%, 11%, and 10.28%, 7.2 respectively. Instead, NP and GDD\_TGf explained the 6.3 and 5.14% of GC, while PL, P\_TGf, Av\_P, OM and pH parameters showed lower than 5% contribution on GC. Furthermore, the soil factor was more important in explaining the STY variability by 41.74 %, compared to the management at 38.44% and climate parameters at 19.82%. Particularly, pH as a soil parameter was the most influential factor that affected the STY (contributed= 21.8). The relative contribution of PL, P\_TGf, Gen, and OM on ST was found 15.88, 11.7 and 10.4% 16.75, 14.68, and 11.55%, respectively, but the relative contribution of the remaining parameters were lower than 10%. In addition, the relative contribution of management was the most influential on AP accumulation (72.58%) with respect to soil factor 21.86% and climate factor 10.54%. Specifically, Gen was the most important factor to explain 40.8% of the AP variability, while the relative contribution of NP, pH, and PL on AP was 19.28, 15.23, 12.42, respectively, while the contribution of the remaining parameters on AP was lower than 10%.

The soil pH was significant and negatively correlated with the OM, Tot\_N, AV\_P, AV\_K (table 8). OM, Tot\_N, Av\_P, and Av\_K were positively correlated among themself. GDD\_TGf was positively correlated with Tot\_N, Av\_K, Av\_P, and OM; while negatively correlated with pH. On contrary, P\_TGf negatively correlated with Tot\_N, Av\_K, Av\_P, OM, and GDD\_TGf; but positively correlated with pH. The correlation of GY, SY, PY, ST, STY, and AM:AP were strongly negative with pH, but they were positively correlated with Tot\_N, Av\_K, Av\_P, and OM. On the opposite, PC, GC, and W were positively correlated with pH, while negatively correlated with Tot\_N, Av\_K, Av\_P, and OM. Furthermore, GY, SY, PC, PY, STY, and W were positively correlated with both PL and NP; while ST, AP, AM:AP were negatively correlated with NP but positively correlated with PL. The correlation of TKW with both PL and NP was not significant, but the correlation of GC was significant, positive with NP and negative with PL.



**Figure 10.** The relative contribution (%) of predictor parameters for the boosted regression tree model (BRTM) of grain yield (GY), protein yield (PY), Gluten content (GC), starch yield (STY), and amylopectin (AP) are shown in (A–E), respectively. Measured and predicted annual GY, PY, GC, STY, and AP by the BRTM model using predictors shown in (a–e), respectively.

**Table 7.** Correlation between soil parameters (including pH, organic matter (OM), available phosphorus (AV\_P), available potassium (AV\_K) total nitrogen (Tot\_N)), climate parameters (including growing degree days (GDD\_TGF), and precipitation amount (P\_TGF) accumulated between tillering and grain filling), and agronomic traits (including straw yield (SY), grain yield (GY), thousand-grain yield (TKW), protein concentration (PC), and protein yield (PY), gluten concentration (GC), starch concentration (ST), starch yield (STY), amylopectin (AP), amylose to amylopectin ratio (AM:AP) and dough strength (W). The values reporting the correlation significance are as follows: \*\*\* = 0.0001, \*\* = 0.001, \* = 0.05, ns = not significant.

	pН	ОМ	AV_P	AV_K	Tot_N	GDD_TGf	P_TGf	PL	NP	SY	GY	TKW	PC	PY	GC	ST	STY	AP	AM:AP	W
	pii	(%)	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(%)	(°C)	(mm)		(kg ha <sup>-1</sup> )	(t ha <sup>-1</sup> )	(t ha <sup>-1</sup> )	(g)	(%)	(t ha <sup>-1</sup> )	(%)	(%)	(t ha <sup>-1</sup> )	(%)	(%)	(10 <sup>-4</sup> J)
pH	1	-0.95 ***	-0.77 ***			-0.22 ***			0 ns		-0.75 ***		0.36 ***						-0.4 ***	
OM (%)		1	0.93 ***			0.46 ***			0 ns	0.7 ***	0.71 ***	0.12 **	-0.33 ***	0.68 ***	-0.19 ***	0.35 ***	0.71 ***	-0.38 ***	0.38 ***	-0.13 **
$AV_P (mg kg^{-1})$			1	0.87 ***	0.9 ***	0.71 ***	-0.68 ***	0 ns	0 ns	0.67 ***	0.57 ***	0.29 ***	-0.25 ***	0.55 ***	-0.15 ***	0.27 ***	0.56 ***	-0.3 ***	0.3 ***	-0.1 **
AV_K (mg kg <sup>-1</sup> )				1	0.75 ***	0.6 ***	-0.4 ***	0 ns	0 ns	0.73 ***	0.61 ***	0.08 *	-0.27 ***	0.59 ***	-0.16 ***	0.29 ***	0.61 ***	-0.32 ***	0.32 ***	-0.11 **
Tot_N (%)					1	0.34 ***	-0.55 ***	0 ns	0 ns	0.6 ***	0.69 ***	0.18 ***	-0.34 ***	0.66 ***	-0.19 ***	0.35 ***	0.69 ***	-0.37 ***	0.37 ***	-0.14 ***
GDD_TGf(°C)						1	-0.67 ***	0 ns	0 ns	0.46 ***	0.09 *	0.4 ***	0.01 ns	0.11 **	0 ns	0 ns	0.08 *	-0.04 ns	0.04 ns	0 ns
P_TGf (mm)							1	0 ns	0 ns	-0.34 ***	-0.17 ***	-0.52 ***	0.07 ns	-0.18 ***	0.04 ns	-0.08 *	-0.16 ***	0.09 *	-0.09 *	0.04 ns
PL (kg ha <sup>-1</sup> )								1	0.85 ***	0.3 ***	0.34 ***	0.01 ns	0.1 *	0.38 ***	0.03 ns	0.24 ***	0.34 ***	0.22 ***	-0.22 ***	0.19 ***
NP (kg ha <sup>-1</sup> )									1	0.27 ***	0.29 ***	0 ns	0.22 ***	0.37 ***	0.1 **	-0.02 ns	0.28 ***	-0.03 ns	0.03 ns	0.24 ***
SY (t ha <sup>-1</sup> )										1	0.77 ***	0.12 **	-0.28 ***	0.76 ***	-0.23 ***	0.38 ***	0.77 ***	-0.21 ***	0.21 ***	-0.1 **
GY (t ha <sup>-1</sup> )											1	0.07 ns	-0.39 ***	0.97 ***	-0.3 ***	0.51 ***	1 ***	-0.29 ***	0.3 ***	-0.13 ***
TKW (g)												1	0 ns	0.08 *	-0.01 ns	-0.01 ns	0.06 ns	0.04 ns	-0.05 ns	0 ns
PC (%)													1	-0.16 ***	0.92 ***	-0.87 ***	-0.43 ***	0.34 ***	-0.35 ***	0.8 ***
PY (t ha <sup>-1</sup> )														1	-0.09 *	0.32 ***	0.95 ***	-0.23 ***	0.23 ***	0.07 ns
GC (%)															1	-0.83 ***	-0.35 ***	0.26 ***	-0.26 ***	0.85 ***
ST (%)																1	0.55 ***	-0.24 ***	0.24 ***	-0.68 ***
STY (t ha <sup>-1</sup> )																	1	-0.3 ***	0.3 ***	-0.17 ***
AP (%)																		1	-1 ***	0.34 ***
AM:AP (%)																			1	-0.35 ***
W (10 <sup>-4</sup> J)																				1

-1>-0.6 -0.6>-0.3 -0.3>-0.15 -0.15>0 0>0.15 0.15>0.30 0.30>0.60 0.60>1

#### **3.3.4 Discussion**

Afghanistan is an arid and semi-arid country. The annual average precipitation ranged between 200 to 400 mm (Qutbudin et al., 2019). Insufficient precipitation maintains a high amount of calcium carbonate and high pH value, and low soil organic matter content ranges from 0.2 to 2.5% in Afghanistan soil (ICARDA, 2002; Hashimi, 2020). Furthermore, the low yield for crops grown on calcareous soils in Afghanistan could be attributed to soil fertility problems related to the poor available Av\_P, Tot\_N, and OM (Moustafa, et al., 2012). These results demonstrated that the soil and climate factors can affect SY and GY, similar to the management factor, but the effect of management was prominent for PC, GC, PY, ST, STY, AP AM:AP, and W that the soil and climate factor effects.

Since the trails were irrigated at the four Ls, the influence of P\_TGf was explained by smaller variability on common wheat quality characteristics, as compared to the GDD\_TGf, pH, and management factors. Our results suggest that on the one hand, irrigation can mitigate the deficit stress of P\_TGf during the growing season, on the other hand, irrigation prolongs the GDD\_TGf possibility to accumulate more biomass. Thus, other authors Man et al. (2016) and Al-Ghzawi et al. (2018) suggested that irrigation incredibly affects the yield and quality of wheat, but a suitable amount of water applied than excessive or too little, to maintain the yield and quality of wheat, and avoid surface water scarcity and groundwater depletion during the growing season. Overall, this information can be valuable for the improvement of Afghan agriculture production (quantities and qualities), where irrigated crop production plays a central role in food security, job creation, and household income. Poole et al., (2022) reported that irrigated wheat productivity in Afghanistan is more than double the rainfed wheat productivity (2.5 compared with 1.09 t/ha). However, this result can be a solid road map to help Afghan wheat growers to make the right decision that how to manage their wheat fields under the Afghan arid and semi-arid climate, with soil of a high value of pH and CaCO3 concentration.

In this study, the result revealed that soil pH at four Ls was more importantly affected SY, GY, PC, PY, GC, ST, STY, AP, AM:AP, and W than the other soil parameters (OM and Av\_P) and climate parameters (GDD\_TGf and P\_TGf), but the effect of soil pH was comparable with PL and NP and lower than the effects of Gen. However, soil pH plays a vital role in the amount of nutrients available to plants, the activity of fauna and biota in soil, the growth of the plant as well as yield and quality of the crop. Potentially, element nutrients are easily available to plants up take when soil pH is close to neutral. Specifically, in alkaline soil like HRT and BLK, the Tot\_N, Av\_P, OM as well as micronutrients tend to be reduced as higher pH as lower their

concentration. Results suggested that lowering soil pH concentration can attribute wheat productivity in Afghanistan. Since alleviating soil pH by using a chemical element such as sulfuric acid, sulfur, and gypsum, can be more expensive in field crops, specifically in the wheat field; but reducing the adverse effect of higher soil pH, using salt-tolerant wheat varieties, banded application of P and organic amendments should be recommended to improve soil fertility and secure good yield and quality of irrigated wheat in Afghanistan. Similar to our reports, highlighted by authors (Moustafa et al., 2012, Sun et al. 2022, Soofizada et al. 2023)

The present study found that DLN7 was a superior Gen for GY, SY, WKW, PY, STY, and AP for four Ls; while ZDNA was superior for PC, GC, W, and KBL13 was superior for ST, and AM: AP. But statistically, the difference between DLN7 and KBL13 was not significant for GY, SY, and STY. This result suggests that for a country, like Afghanistan where starvation and malnutrition are a major challenge, DLN7 with higher GY and AP is suggested; while KBL13 with a higher AM: AP ratio is preferable for healthy consumptions purpose to mitigate the disease incidences. Previously, it was shown that rapid digestion of starch is required, as in case of undernutrition and hunger people, while slow and resistant digestion starches are more nutritious in addressing diabetes, gut disease, blood pressure, and cardiovascular disorder (Igrejas et al., 2020). Since ZDNA is a higher PC, GC, and W genotype, it is more preferential for baker production. In addition, BLK13 was tested in our previous paper (Soofizada et al., 2023) on 2016/2017-2017/2018, but it yielded (an average of both GS 4.37 t ha<sup>-1</sup>) relatively lower than compared with the result of this paper. This could be because of higher precipitation in 2021 and 2022 than that of the 2017 and 2018 growing seasons. Also, IndexMundi (2022) reported that the Afghan national wheat productivity and production were relatively higher in 2021 and 2022, compared to the 2017 and 2018 growing seasons.

The present study indicated that P availability is a limiting factor for wheat production in Afghanistan. As Afghan soil is mostly dominated by high pH value and CaCO3 concentration, typically, their chemical reactions control P availability in soil. In an arid climate like Afghanistan, P sorption by surfaces of clay minerals dominatingly increase in the presence of high calcium ion content, pH values, and lower organic matter in soils. The result of our previous study (Soofizada et al., 2023) was contrary to the present study results. However, the previous study found that GY, PY, and SY significantly increased up to PL50 in BGL and HRT, but no significant increments were observed with further application of PL; while this trail revealed that the GY, SY, PY, and STY increased significantly as PL increased up to PL120 in four Ls. This result could be because of the climate effect. Therefore, higher temperature with lower

precipitation was recorded for the two growing seasons of the pervious study (2016/2017-2017/2018), compared with this experiment trail's growing season (2020/2021-2021/2022). As reported by Mohammad et al. (2998), optimal precipitation and temperature during vegetative growth can mitigate the P availability stress in winter wheat fields and hence maintain wheat productivity. Also, other authors indicated that occur of deficit moisture during wheat grain filling will adversely strike late-season P accumulation as well grain yield (Pan and Hopkins 1991).

Moreover, P fertilization significantly improves the ST and AP of wheat; on the opposite, P application tends to reduce the AM:AP significantly. However, variable effects on ST, AP, and AM:AP in response to P fertilization have been reported by previous researchers. For example, Ni et al., (2012) found that P application of 160 kg ha-<sup>1</sup> increase the average ST by 13.95% and AP by 13.22% than the control PL; while the AM:AP significantly reduced under PL treatment. Li et al., (2013) and Zhang et al., (2018) indicated that PL application can increase the ST and AP in wheat kernel, but not all genotypes had the same response to PL fertilization. In addition, these results were consistent with Ali et al., (2020), who reported that optimum PC measured at 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, while further application of P did not affect the PC. But these data were in contrast with Zhang et al., (2017) and Zhu et al., (2012), showing that P fertilization tended to reduce the wheat PC. Moreover, PL treatment significantly affects W, the optimal W value observed at PL90, which was significantly higher from PL60 but statistically was similar to PL120. Our results were consistent with the previous study, which reported that dough strength significantly improved by P fertilization (Ma et al., (2018)

Our results suggest that N fertilization may be used as a tool to improve PC and GC in common wheat production. These results were consistent with those measured in previously published studies (Brill et al., 2011; Yang et al., 2022; Soofizada, 2011). Furthermore, NP significantly improved the average production of PY. These results were consistent with previous findings in common wheat varieties (Soofizada et al., 2022 and 2023; Guerrini et al. 2020). On the opposite, NP fertilization significantly reduces the ST and AP of wheat. However, variable effects in response to N treatment have been reported in previous literature. For example, Litke & Gaile, (2018) found that 1.39 and 6.85% decreases in ST were observed at 120 and 240 kg N ha<sup>-1</sup> compared to the control, respectively. Mariem et al., (2020) reported that ST decrease under higher N fertilization, but soluble sugar concentration highly increased in grain. It could be assumed that under the high N plot, grain carbohydrates tend to be stored as mono and disaccharides (glucose, sucrose, and maltose), not as starch. Xiong et al., (2014) found that an

increase in N application leads to an increase in the starch A granule, but reversely reduces the starch B granule.

In general, the interaction results of NP with PL indicated that NP1/PL120 rates were known as a breakpoint for GY and SY, while the NP1.5/PL12 for PC and GC.

# **3.3.5** Conclusions

Environmental factors, in particular, higher pH value in Afghanistan's soils were a limiting factor for common wheat production. For such soils, the nutrient availability and organic matter content are typically very low. Under this condition, the use of the salt-tolerance variety, application of organic amendments along with a banded application of P are highly recommended. The results showed that increased PL application, GY, SY, PY, and STY production increased for all Ls. In addition, PL increased the PC, and W without decreasing TKW and GC. The NP was the most important factor in determining PC, PY, GC, and W, without decreasing GY, SY, and TKW, confirming the potential for further improvement in N management. Regarding the ST properties, NP was shown to negatively affect the total ST and AP concentration in all the varieties; but AM:AP significantly improved by NP treatment, while the STY was not influenced under NP treatment. Instead, ST, AP, and STY were significantly increased with increased PL application, but AM:AP was not affected by PL treatment. The present results found that the NP1/PL120 intensively optimized the GY, SY, ST, and AP concentrations. Instead, NP1.5/PL120 strongly increased the PC and GC concentration. However, further studies, including additional year within various pedoclimatic conditions, is needed to further evaluate the interaction between soil, climate, and agronomical management.

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#### **3.4 Fourth paper**

Evaluation of grain yield and stability performance of 33 bread wheat varieties (*Triticum aestivum* L.) under different agroecological zones of Afghanistan.

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#### Abstract

Given the huge variation of common wheat productivity in Afghanistan, the lack of a breeding program and insufficient research in a varietal selection based on agro-climatic conditions were highlighted as major challenges. However, the present study aimed to evaluate the grain yield stability and agronomical traits of 33 common improved wheat (Triticum aestivum L.) varieties (V) under six environments (E) (three-locations x two-growing season; namely, BLK18, BLK20, HLM18, HLM20, NGH18, NGH20, respectively) in Afghanistan. The combined ANOVA analysis showed that the variation due to the interaction of E x V for grain yield (GY), straw yield (SY), and harvest index (HI) was significantly larger compared to the main factor effect, environment, and variety; but the contribution of E x V for thousand kernel weight (TKW) and plant height (PH) was smaller than the main factors. However, in average G09 obtained the highest GY, followed by G31, G28, G15 and G04, whilst G31 showed wide stability, followed by G04, G15, G09, G25, in decreasing order, respectively. Interestingly, the 5-top superior stable varieties were also associated with higher GY, while G03, G01, G21, G18, and G02 varieties were identified as the most unstable and the poorest GY varieties. Considering locations, varieties G24, G29, G25, G15, G04 in BLK, G15, G09, G04, G23, and G22 in HLM, and G31, G09, G32, G28, G27 in NGH location were identified as stable with higher GY performance. Moreover, results indicated that the highest grain yield was obtained by varieties that were grown in NGH while the lowest yield was obtained in HLM. In conclusion, the identified superior stable and high-yielding varieties could significantly contribute to wheat production in Afghanistan's climatic conditions. Also, this information is imperative for a future breeding program in Afghanistan.

**Keywords:** Wheat varieties, locations, grain yield, straw yield, thousand kernel weight, plant height.

#### **3.4.1** Introduction

Common wheat (*Triticum aestivum* L.) is one of the most produced, stored, and consumed food crops, contributing significantly toward the world economy (Helguera et al., 2020), playing a central role in food security after rice (Giraldo et al., 2019). Common wheat serves as the primary source of the human diet, which provides about 18% of the calories and 20% of the proteins consumed by humans globally (FAO, 2021). By 2018, common wheat was cultivated on 217 million hectares of land, which produced about 752 million tons of grain globally (Erenstein et al., 2022).

Common wheat is the first stable food crop in Afghanistan, counting for about one-quarter of the total agriculture GDP and 6.3% of the national GDP (Halimi, 2016). The national food security and economy are mainly dependent on common wheat production and its trade, which contributes up to 60% of the population's caloric intake with consumption per capita of about 200 kg per year (Sharma, 2019). Achieving sufficient wheat production in Afghanistan could significantly improve farmer livelihood and national food security (Soofizada et al., 2018). The total wheat growing area in Afghanistan is around 2.7 million hectares (25% total agricultural area) (Tiwari et al., 2020), with a total production of 5.2 million tonnes and a yield per hectare is about 2.5 t and 1.1 t in irrigated and rainfed field respectively (Poole et al., 2022), while total national wheat demand is estimated to 6.5 million tons (NSIA, 2021). Since the year 2000, the wheat production and productivity trend has shown a modest increase, but the increase in consumption has been greater, while the country's needs are fulfilled by imports (Halimi, 2016). In 2018, the total imported wheat/flour was estimated to be about 2.27 million tons, which corresponded to around 764.47 million US dollars (WITS, 2018). As a coverage, irrigated and rainfed areas are estimated at 45 and 55% of the total area, respectively. Under rainfed conditions, productivities were more vulnerable due to the poor amount of rainfall with its uncertain distribution, while irrigated field productivities were consistent (MAIL, 2013). Drought and disease stresses, insufficient improved varieties, poor quality and insufficient quantity of improved seeds, poor agronomical practices, lack of incentive program for farmers, poor accessibility of farmers to machinery, and poor-quality fertilizers and pesticides are known as major constraints in Afghanistan's wheat sector (MAIL and FAO, 2013).

The Afghanistan climate varies from semi-arid to arid, with hot summers and cold winters. The country is divided into 8 agro-climatic zones: North-Eastern, North-Western, Eastern, Central, West-Central, Western, South-Eastern, and South-Western (Rahimi, 2017). Among all, the

availability of high-yield varieties in response to variable climatic zones and the accessibility of quality seeds are highlighted as a big challenge in Afghanistan (Sharma and Nang, 2018). Similarly, reported by Sharma, (2019), that, using high-yielding varieties with adaptable characteristics appropriate to different agroclimatic conditions could significantly contribute to wheat production in Afghanistan. Kugbei, (2011), indicated that the use of improved wheat varieties in Afghanistan alone could improve the wheat potential productivity by up to 33% in the irrigated field, while the use of the quality seed, increase yield by 28%. Similarly, Akbarzai et al., (2021) have found that improved wheat varieties with good agronomical practices, increase the grain yield between 53 to 86% compared to local varieties in Afghanistan. Other researchers underscored that the adoption of new improved wheat varieties among the farmer community could contribute to boosting wheat production. It should be noted that the increase in farmer awareness of the beneficial use of improved varieties must be considered strongly (Dreisigacker et al., 2019). Moreover, other authors emphasized that variety selection as a function of climate variability is the most important technique to optimize the sustainability of wheat yield potential (Sharma et al., 2021).

The current study aimed to evaluate the grain yield and agronomical traits of 33 common wheat varieties, under varied environmental conditions in Afghanistan. This study could contribute to identifying the best high-yielding and stable varieties under variable climates in the country.

#### **3.4.2 Material and method**

#### 3.4.2.1 Variety evaluation

Thirty-three common wheat (*Triticum aestivum* L.) varieties were registered and produced from 1994 to 2017 by the Agricultural Research Institute of Afghanistan (ARIA) with the collaboration of the International Maize and Wheat Improvement Centre (CIMMYT), International Centre for Agriculture Research in the Dry Area (ICARDA), India, and French Cooperation (FC), were evaluated in this study. Indeed, these varieties have been tested with an appropriate check and have been released based on their performance. Table 1 shows the varieties' code, name, year of release, origin, and pedigree.

Table 1. Description of varieties

Code	Variety	Year	Origin	Pedigree
G01	Pamir-94	1994	CIMMYT	YMH/TOB/3/LIRA SWM16#
G02	Ghori-96	1996	ICARDA	PRL''S''/PEW CM59377- 3AP-1AP-3AP-2AP-1AP-OAP
G03	Gul-96	1996	CIMMYT	ID8009994.W./VEE 2WM- OWM-OSE-1YC-OYC
G04	Dyema-96	1996	ICARDA	HD2206/HORK//BUC/BUL
G05	Mazar-99	1999	CIMMYT	PASTURE CM85295-0101TO PY-2M-OY-OM-3Y- OM
G06	Solh-02	2002	CIMMYT	OK82282//BOW//NKT/F4/
G07	Parva-02	2002	CIMMYT	CHTO/ARDEA//SRN_2 CD74825-C-5M-1Y-040M- 2YRC-2M-0YRC
G08	PBW154	1997	India	HD2177/HD2160
G09	Darulaman-07	2007	CIMMYT	Weaver/4/Nac/ Th.ac//3*PVN/3/mirlo/buc CID/SID: 133428/104
G10	Koshan-09	2009	CIMMYT	BABAZ/LR42//BABADX*2/3VIVITSI
G11	Muqawim-09	2009	CIMMYT	OASSIS/SKAUZ//4*BCN/3/2*PASTOR
G12	Baghlan-09	2009	CIMMYT	PICAFLOR#1KIRITAI/ SERI/RAYON
G13	Auton-09	2009	FC	
G14	Exotic-09	2009	FC	
G15	MH0304-09	2009	FC	
G16	Chon#1-10	2010	CIMMYT	SERI.CERI.1B*2/3 KAUZ*2/BOW// KAUZ/4/PBW43*2/KUKUNA
G17	Kabul-13	2013	CIMMYT	WAZWIND*2/TUKURU
G18	Kohistan-13	2013	ICARDA	BB/RON//CNO67/TOTA/3/JAR
G19	Dehdadi-13	2013	CIMMYT	PYN/BAU//MILAN
G20	Amir-10	2010	ICARDA	SHAM-6/WW1402
G21	Zarin-13	2013	ICARDA/CIMMYT	TAN"S"/VEE"S"//OPATA
G22	Bakhtawar-13	2013	ICARDA	ISENGRAIN X ORNICAR
G23	Milad-13	2013	FC	(ORPICXISENGRAIN)
G24	Lalmi4	2013	CIMMYT	SLVS*2/PASTOR
G25	Afghan-15	2015	CIMMYT	WHEAR//2*PRL/2*PASTOR
G26	Wafir-15	2015	CIMMYT	BABAX/LR42//BAAX*2//3/TUKURU
G27	Bahar-15	2015	CIMMYT	CAL/NH//H567071/3/SERI/4/CAL/NH//H567071/
G28	Lalmi-15	2015	CIMMYT/ICARDA	MTRWA920161/PRINIA/5/SIRI*3//RL6010/
G29	Wahdad-15	2015	CIMMYT	KIRATATI/4/2*SERI.IB*2/3/KAUZ*2/BOW/KAUZ
G30	Elham-15	2015	CIMMYT	STARSHINA- IWWRRSN
G31	Guhar-17	2017	ICARDA	
G32	Lalmi-17	2017	CIMMYT	
G33	Shamal-17	2017	CIMMYT	

#### 3.4.2.2 Experimental design and environmental characteristic

A set of 33 common wheat varieties were evaluated in six environments (three locations "Balkh, Helmand, Herat" x two-growing seasons "2017/2018-2019/2020") namely, BLK18, BLK20, HLM18, HLM20, NGH18, and NGH20, respectively) under irrigated conditions in Afghanistan (table 1). Each trial was sown in a randomized complete block design (RCBD) with three replications. Sowing was done by hand, the size of the plots was 2 m<sup>2</sup> (4 rows, 2 m length, and 1 m width, spaced 0.25 m apart), and the net harvested area was the two central rows (1 m<sup>2</sup>). Varieties were sown using a seeding rate of 120 kg ha<sup>-1</sup>. Sowing time was in the last week of October in each environment. A total of 250 kg ha<sup>-1</sup> urea (46% N) was applied at tillering and stem-elongation stages. Diammonium phosphate, 200 kg ha<sup>-1</sup> (DAP, 18% N, 46% P<sub>2</sub>O<sub>5</sub>) was applied at sowing time for each environment. Soil tillage was carried out to a depth of 0.40 m with a tractor in September in all environments. Plot setting-up, weeds control, harvesting, and threshing were operated manually. At harvesting, the weight of the kernels (GY; kg ha<sup>-1</sup>[adjusted to 12% moisture]), the straw yield (SY; kg ha<sup>-1</sup>), harvest index (HI), and the thousand kernel weight (TKW; g 1000 seeds<sup>-1</sup>) were measured for each plot. The plant height (PH) was measured at majority time from above ground to the spike peak.

The soils were characterized in all locations as alkaline and showed poor fertility (table 2). All sites demonstrated low organic matter (OM), and poor nutrient concentration, particularly nitrogen and phosphorus contents were low in the soil of all sites. Further information about locations is shown in table 2.

**Table 2.** Soil and sites description of Balkh (BLK), Helmand (HLM), and Nangarhar (NGH). Phosphorus (P), potassium (K), and nitrogen (N)

Location	pН	Organic matter (%)	AV. P (PPM)	AV. K (PPM)	Total N (g kg <sup>-1</sup> )	Soil texture	Latitude (N)	Longitude (E)	Altitude (m)
BLK	8.01	1.51	7.24	124	0.75	Silty Ioam	36°65′ N	66°95′ E	378
HLM	8.2	1.021	7.1	132	0.65	Silty Ioam	31°65′ N	66°96′ E	787
NGH	7.56	1.56	8.5	124	1.02	Silty Ioam	34°42′ N	70°47′ E	552

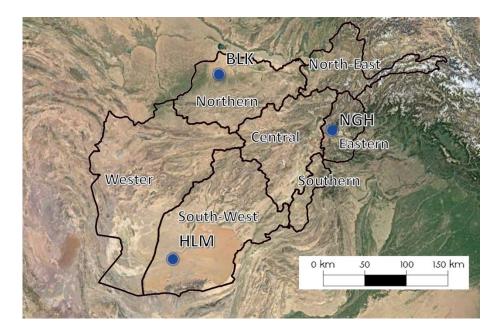
#### 3.4.2.3 Metrological data

The BLK and NGH locations are based in a semi-arid zone, but HLM was located arid zone (FAO and WFP, 2004). The climatic conditions were further analyzed by collecting data from the NASA database (https://power.larc.nasa.gov/data-access-viewer/). Long-term (2001-2020), and the six environments BLK18 (Balkh 2017/2018), BLK20 (Balkh 2019/2020), HLM18 (Helmand 2017/2018), HLM20 (Helmand 2019/2020), NGH18 (Nangarhar 2017/2018), NGH20 (Nangarhar 2019/2020) data were analyzed (two GS x three locations). According to long-term data, among the sites, HLM was the location with the lowest total annual precipitation (93.12 mm) with the highest average temperature (19.14 °C). This location was wetter compared to others. BLK had the lowest average temperature (16.94 °C) and precipitation of 178.84 mm and was a slightly colder location.

The average temperature in all environments was similar to the long-term data. But the highest temperature was measured at HLM18 and HLM20, while the lowest average temperature was measured at BLK18 and BLK20. The NGH18 and NGH20 showed a medium temperature, lower than the HLM and higher than the BLK environments. However, the 1<sup>st</sup> GS was drier in all environments (HLM18,11.28 mm; BLK18, 63.4 mm; and NGH18, 327.32 mm) compared to the 2<sup>nd</sup> GS (HLM20, 369.1 mm; BLK20, 293.26 mm; and NGH20, 745.04 mm). But the total rainfall in the NGH20 environment was higher than in the long-term, and all other environments showed lower precipitation than in the long-term.

**Table 3** Average monthly temperature (°C) and precipitation for long-term (LT) conditions (2001-2020) and environments (two growing seasons [2017/2018 to 2019/2020] x three locations) BLK18, BLK20, HLM18, HLM20, NGH18, and NGH20. For the long-term, the standard deviation is added in brackets.

		BLK		]	HLM			NGH	
Month	LT	BLK18	BLK20	LT	HLM18	HLM20	LT	NGH18	NGH20
				Тетре	rature (°C	C)			
Α	27.68 (0.79)	26.91	27.57	31.81 (0.88)	31.56	32.49	28.53 (0.88)	29.02	29.37
S	23.08 (1.22)	23.31	23.31	26.96 (1.26)	27.73	28.81	25.17 (1.26)	26.04	27.59
0	17.04 (1.97)	17.84	17.43	21.56 (1.77)	23.25	22.47	19.84 (1.77)	21.98	19.69
Ν	10.12 (2.21)	12.21	6.50	15.02 (1.43)	16.01	11.85	13.86 (1.43)	14.43	12.33
D	5.48 (1.94)	6.01	8.24	9.54 (1.51)	9.34	9.89	9.32 (1.51)	9.91	9.40
J	4.36 (2.66)	5.28	2.50	8.27 (1.84)	9.61	6.24	6.27 (1.84)	9.28	3.98
F	6.15 (2.78)	7.50	7.65	11.05 (2.21)	13.23	11.68	8.05 (2.21)	9.47	9.81
Μ	12.09 (2.15)	16.03	10.94	16.93 (1.77)	20.41	14.39	13.32 (1.77)	15.83	11.36
Α	17.38 (1.76)	16.92	15.49	23.07 (1.42)	24.27	19.92	18.55 (1.42)	20.17	16.97
Μ	23.05 (1.77)	21.61	21.79	28.51 (1.69)	27.42	26.04	23.59 (1.69)	22.94	21.28
J	27.49 (1.21)	27.34	26.48	32.12 (1.11)	33.44	31.03	28.06 (1.11)	29.20	25.91
J	29.45 (0.91)	30.97	29.07	33.93 (0.91)	34.84	33.61	29.85 (0.91)	30.66	28.94
				Precipi	tation (mr	n)			
Α	0.01 (0.03)	0	0	3.23 (5.92)	0	0	57.19 (36.92)	37.75	38.01
S	0.08 (0.19)	0	0	1.12 (3.37)	0	0	33.80 (3.37)	12.13	6.42
0	4.51 (11.83)	0	1.07	2.86 (5.13)	0	4.96	26.14 (5.13)	2.35	20.95
Ν	28.57 (28.39)	23.39	41.11	11.23 (22.34)	2.11	57.67	28.15 (22.34)	8.75	59.69
D	22.1 (14.09)	1.05	36.05	7.67 (10.24)	0.21	4.84	19.97 (10.24)	13.33	4.31
J	26.36 (23.92)	3.59	9.21	14.17 (13.71)	0	15.55	37.92 (13.71)	5.15	51.49
F	34.59 (25.01)	15.47	26.09	14.6 (11.75)	3.97	19.2	77.77 (11.75)	52.46	42.61
Μ	29.54 (17.79)	7.7	52.76	19.27 (30.8)	1.74	142.62	84.71 (30.8)	48.51	190.74
Α	20.93 (18.03)	4.89	61.43	10.83 (24.69)	2.77	116.66	84.90 (24.69)	51.76	180.95
Μ	11.22 (15.49)	7.3	65.03	2.69 (2.79)	0.96	7.22	44.22 (2.79)	49.39	91.64
J	0.85 (1.72)	0.01	0	4.58 (11.05)	0	0.1	31.58 (11.05)	17.99	36.19
J	0.08 (0.22)	0	0.51	0.87 (4.48)	0.06	0.28	49.30 (4.48)	27.75	22.04



**Figure 1**. Map of the agro-climatic zones of Afghanistan with the three study sites: Balkh (BLK), Helmand (HLM), and Nangarhar (NGH).

#### 3.4.2.4 Statistical analysis

A 2-way ANOVA was used to test the main effect of grain yield traits as a function of environments and variety and their interactions. The Tukey HSD post-hoc test was used when the result showed significance was set up: \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, n.s. = not significant. The varieties' stability traits were calculated according to their average performance. High stability was recognized according to the higher average performance of the varieties within the locations and across the locations. The higher and lower stability of the varieties is indicated with a ranking number (the variety with the highest value stability received a rank of 1).

#### 3.4.3 Results

#### 3.4.3.1 Agronomical traits

The combined ANOVA analyses revealed that the main effects due to environment (E), variety (V), and their interactions (E x V) were strongly significant for all studied parameters (table 4). However, the greatest variation for GY, SY, and HI (37, 34, and 33%, respectively) was observed by interaction E x V, followed by environment, while the largest variation (50%) in PH was demonstrated by the environment, followed by varieties. Variation due to variety for GY, SY, and HI (24, 10, and 14%, respectively) was smaller with respect to other factors; but TKW showed the greatest variation (39%) by environment than variety and E x V interactions.

According to ANOVA, the mean range between the varieties (averaging varieties over environments) was larger than the mean ranges between environments for all studied parameters (table 5). Varieties grown in the NGH18 environment yielded the highest GY, associated with lower TKW, and medium SY, HI, and PH mean values. Subsequently, a comparable mean GY showed in BLK20, HLM18, and NGH20 environments, but it was associated with the largest TKW, SY in HLM18, HI in BLK, and PH in NGH20. Likewise, HLM20 yielded the lowest mean for GY, followed by BLK18. In addition, BLK18 and NGH20 indicated similar SY with the lowest mean values compared to other environments. Also, statistically, HI demonstrated similar values in BLK18, HLM20, and NGH20, but significantly lower than in the other environments. In terms of TKW result was statistically similar in BLK18 and HLM20, but lower than the other locations, while PH with the lowest mean values was detected in HLM18 and HLM20 (table 5).

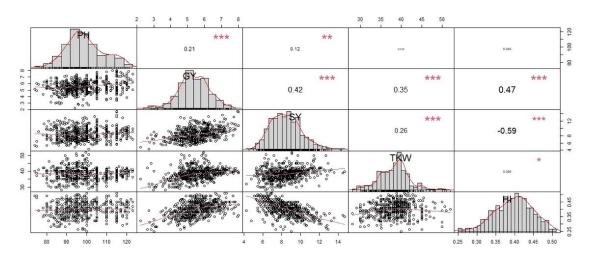
Concerning wide adaptation of varieties over the country, results of analysis of variance and Tukey-test (p<0.05) stratified the varieties within 11 classes (A to K), from higher to lower yielding potential (table 5). However, the average GY (averaging environments) between the varieties ranged from 3.69 to 6.15 t ha<sup>-1</sup>. Varieties G09 and G31 (A), G31 and G28 (AB), G15 (ABC), and G04 (ABCD) were characterized as the highest yielding and widely adaptable varieties in the country, in which G09 surpassed all the varieties. But G03 (K), G01 and G21 (J), G18 (IJ), and G02 (HIJ) showed poor grain yield (table 5). Regarding SY, varieties are classified into six classes (A to F); whereas the range between varieties for SY varied from 7.16 to 9.84 t ha<sup>-1</sup>. The maximum SY noted by variety G32 (A), followed by G28 (AB) and G10 (ABC), and G27 (ABCD) over the country; while G03 (F), G18, G33 (EF), and G13 (DF) produced the poorest SY (table 5). In respect to TKW, varieties distinguished for 11 classes (A to K), ranging from 32.00 to 42.24 g. Varieties G02 (A), G09 (AB), G33 (ABC), and G06 (ABCD) were characterized with the highest TKW; though varieties G01 (K), G21 (JK), and G03 (IJK) showed the lowest TKW. Concerning HI, varieties were categorized into 7 classes (A to G), which ranged from 0.34 to 0.43%. The highest HI were identified in G33 (A), G04 (AB), G31 (ABC), and G23 (ABCD); while varieties G03 (H), G01 (GH), and G32 (FGH) showed the poorest HI (table 5). Equally, varieties were classified into 14 categories for PH (A to N), which ranged from 83.60 to 107.56 cm. Therefore, varieties G31 (A), G28 (AB), G32 (ABC), and G30 (ABCD) were taller; while G12 and G13 (N), G19 (M), and G22 (LM) were shorter varieties.

				MS		
Variability source	DF	GY	SY	TKW	HI	PH
	-	(t ha <sup>-1</sup> )	(t ha <sup>-1</sup> )	(g)	(%)	(cm)
Environment (E)	5	29.192	106.7	649.4	0.06753	6298
Variety (V)	32	4.116	4.96	51.8	0.00636	561
ExV	160	1.257	3.46	10.5	0.00294	51
Residuals	396	0.181	1.02	4.2	0.001	15

**Table 4.** Effect of the environment (E), variety (V), and their interaction (E x V) on grain yield (GY), straw yield (SY), 1000 kernel weight (TKW), harvest index (HI), and plant height (PH).

\*\*\* All the effects shown are highly significant (p<0.001). The table columns report the mean of squares (MS).

The pair-wise correlation coefficient study stated that the investigated traits showed a significant correlation (p<0.05) (figure 2); PH exhibited no significant relations between TKW and HI. On the other hand, HI displayed a negatively significant relation with SY, while the relationship between HI and TKW measured negligible. Furthermore, GY showed significant relation with SY, HI, and TKW; also, SY and TKW were well correlated; but PH showed a poor relationship with GY and SY.



**Figure 2.** Correlation coefficients among the mean values of grain yield (GY), straw yield (SY), thousand kernel weight (TKW), harvest index (HI), and plant height (PH). Significance was set up to: \* = 0.05, \*\* = 0.01, \*\*\* = 0.001, n.s. = not significant.

Variability	GY		SY		тк₩		HI		PH	
source	(t ha-1)		(t ha-1)		(g)		(%)		(cm)	
Variety		***		***		***		***		***
G01	4.81 (0.77)	j	8.37 (1.98)	bcde	34.01 (3.02)	k	0.37 (0.04)	gh	96.59 (9.26)	hij
G02	5.21 (0.81)	hij	8.23 (1.96)	cdef	42.25 (4.75)	а	0.39 (0.05)	abcd	101.74 (9.08)	bcde
G03	3.70 (1.04)	k	7.17 (1.40)	f	36.08 (4.54)	ijk	0.34 (0.06)	h	98.98 (9.49)	fghi
G04	5.98 (0.78)	abcd	8.32 (1.89)	bcde	37.28 (3.62)	efgh	0.42 (0.05)	ab	101.14 (8.66)	cde
G05	5.48 (0.96)	cdef	8.15 (1.76)	cdef	36.55 (3.85)	hijk	0.41 (0.04)	abcd	102.23 (8.90)	bcd
G06	5.45 (0.86)	efgh	8.21 (1.29)	cdef	39.96 (3.52)	abcd	0.40 (0.06)	abcd	95.25 (6.38)	ijkl
G07	5.36 (0.74)	ghij	8.74 (1.53)	abcd	39.30 (2.76)	cdef	0.38 (0.05)	cdef	100.98 (8.6)	def
G08	5.52 (0.83)	cdef	8.39 (1.07)	bcde	38.08 (2.32)	defg	0.40 (0.03)	abcd	96.44 (9.59)	hijk
G09	6.15 (0.92)	а	8.59 (1.53)	abcd	42.09 (5.56)	ab	0.42 (0.04)	abcd	104.54 (9.7)	abc
G10	5.48 (0.97)	cdef	9.18 (1.79)	abc	38.64 (4.34)	cdef	0.38 (0.05)	efgh	97.35 (8.6)	ghi
G11	5.70 (1.05)	abcd	8.26 (1.64)	cdef	39.47 (3.56)	cde	0.41 (0.04)	abcd	101.57 (10.09)	cde
G12	5.41 (0.67)	fghi	8.13 (1.48)	cdef	37.69 (2.68)	defg	0.40 (0.04)	abcd	83.61 (5.04)	n
G13	5.38 (0.79)	ghi	7.87 (1.66)	def	36.69 (3.36)	ghij	0.41 (0.04)	abcd	84.35 (7.49)	n
G14	5.22 (1.02)	hij	8.67 (1.89)	abcd	36.51 (3.78)	hijk	0.38 (0.05)	efgh	97.88 (8.60)	fgh
G15	6.02 (0.59)	abc	8.8 (1.08)	abcd	38.42 (2.39)	cdef	0.41 (0.03)	abcd	101.54 (7.94)	cde
G16	5.73 (0.75)	abcd	8.45 (1.89)	bcde	39.49 (3.46)	bcde	0.41 (0.06)	abcd	101.27 (12.12)	cde
G17	5.70 (0.83)	abcd	8.29 (1.47)	bcde	38.12 (2.67)	defg	0.41 (0.04)	abcd	97.45 (9.29)	ghi
G18	5.15 (0.55)	ij	7.67 (1.08)	ef	38.34 (4.95)	cdef	0.40 (0.03)	abcd	100.09 (9.87)	efg
G19	5.47 (0.88)	defg	8.74 (1.13)	abcd	36.74 (3.16)	fghi	0.38 (0.04)	bcde	89.56 (5.97)	m
G20	5.61 (0.68)	bcde	7.88 (1.14)	def	39.63 (2.38)	abc	0.42 (0.04)	abcd	100.88 (9.5)	def
G21	4.82 (1.08)	i	7.91 (1.97)	cdef	35.58 (3.83)	jk	0.38 (0.07)	defg	94.01 (9.69)	klm
G22	5.93 (0.65)	abcd	8.52 (1.31)	bcde	38.65 (2.98)	cdef	0.41 (0.03)	abcd	92.93 (6.01)	lm
G23	5.98 (0.66)	abcd	8.42 (1.65)	bcde	38.38 (2.55)	cdef	0.42 (0.04)	abcd	96.47 (7.80)	hijl
G24	5.81 (1.11)	abcd	8.55 (1.76)	bcde	38.63 (4.07)	cdef	0.41 (0.04)	abcd	95.25 (9.77)	ijk
G25	5.95 (1.01)	abcd	8.8 (1.44)	abcd	37.55 (3.2)	defg	0.41 (0.04)	abcd	94.62 (8.25)	, jkl
G26	5.43 (0.92)	fghi	8.62 (1.76)	abcd	37.88 (3.06)	defg	0.39 (0.05)	abcd	97.49 (7.98)	fgh
G27	5.78 (0.83)	abcd	9.03 (1.86)	abcd	38.05 (2.69)	defg	0.39 (0.04)	abcd	102.23 (10.1)	bcd
G28	6.10 (1.13)	ab	9.57 (2.20)	ab	39.20 (2.98)	cdef	0.39 (0.04)	abcd	106.46 (9.71)	ab
G29	5.84 (0.53)	abcd	8.40 (1.60)	bcde	39.35 (3.30)	cdef	0.41 (0.05)	abcd	101.57 (10.54)	cde
G30	5.38 (0.77)	ghi	8.11 (1.61)	cdef	37.96 (2.59)	defg	0.40 (0.06)	abcd	105.26 (9.63)	abc
G31	6.12 (0.79)	ab	8.47 (1.73)	bcde	39.11 (3.16)	cdef	0.42 (0.05)	abc	107.57 (9.42)	a
G32	5.81 (1.15)	abcd	9.85 (1.84)	а	38.95 (2.91)	cdef	0.37 (0.05)	fgh	105.95 (7.52)	abo
G33	5.70 (0.89)	abcd	7.73 (1.52)	ef	40.75 (2.02)	abc	0.43 (0.04)	a	102.36 (12.26)	bcd
nvironment		***		***		***		***		***
BLK18	5.34 (0.90)	с	8.15 (1.57)	с	35.27 (2.86)	е	0.40 (0.04)	с	98.47 (7.45)	с
BLK20	5.57 (0.64)	b	7.25 (1.22)	d	40.02 (3.49)	b	0.44 (0.03)	a	93.63 (7.63)	d
HLM18	5.55 (0.77)	b	9.91 (1.31)	a	42.02 (3.29)	a	0.36 (0.04)	d	92.04 (6.01)	e
HLM10	4.90 (0.87)	d	7.42 (1.01)	d	35.89 (3.19)	e	0.40 (0.04)	c	92.24 (5.42)	de
NGH18	4.50 (0.87) 6.54 (0.78)	a	9.27 (1.78)	b	39.14 (2.08)	c	0.42 (0.03)	b	103.19 (9.07)	b
NGH20	5.41 (0.98)	bc	8.56 (1.24)	c	37.73 (2.31)	d	0.39 (0.04)	c	112.34 (8.07)	a

**Table 5** grand mean of grain yield (GY), straw yield (SY), 1000 kernel weight (TKW), harvest index (HI), and plant height (PH) of 33 improved bread wheat varieties.

Lowercase letters represent the Tukey HSD post hoc results, \*\*\*= represents highly significant (p=<0.001).

#### 3.4.3.2 Variety of stability and recommendation

#### 3.4.3.2.1 Grain yield (GY)

The variation described for grain yield (GY) by E x V interaction was 27.43 and 34.51% greater than the variation taken by main factors, environment, and variety, respectively (table 4). These results confirmed, where the mean GY values varied from 2.58 (G03, BLK18) to 7.85 t ha<sup>-1</sup> (G09, GNH20), (supplement table 1), in comparison to ANOVA grand mean GY values (averaging environments) 3.69 (G03) to 6.99 t ha<sup>-1</sup> (G04), (table 5). According to the stability table (table 6), any variety showing a lower cross-over interaction has higher stability. G31 (6.11 t ha<sup>-1</sup>), G04 (6 t ha<sup>-1</sup>), G15 (6.02 t ha<sup>-1</sup>), G09 (6.15 t ha<sup>-1</sup>), and G25 (5.95 t ha<sup>-1</sup>) with the greatest

mean rank value identified as superior stable and high yielding varieties over the locations, while G03 ( $3.7 \text{ t} \text{ ha}^{-1}$ ), G01 ( $4.8 \text{ t} \text{ ha}^{-1}$ ), G21 ( $4.82 \text{ t} \text{ ha}^{-1}$ ) with the lowest mean rank value and lower yield identified as unstable varieties (table 6, and supplement table 1). Meantime G24 ( $6.16 \text{ t} \text{ ha}^{-1}$ ), G29 ( $6.15 \text{ t} \text{ ha}^{-1}$ ), G25 ( $6.06 \text{ t} \text{ ha}^{-1}$ ), G15 ( $5.9 \text{ t} \text{ ha}^{-1}$ ), and G04 ( $5.89 \text{ t} \text{ ha}^{-1}$ ) varieties in BLK, G15 ( $6.18 \text{ t} \text{ ha}^{-1}$ ), G04 ( $5.95 \text{ t} \text{ ha}^{-1}$ ), G09 ( $6.03 \text{ t} \text{ ha}^{-1}$ ), G23 ( $5.74 \text{ t} \text{ ha}^{-1}$ ) and G22 ( $5.51 \text{ t} \text{ ha}^{-1}$ ) varieties in HLM, and G28 ( $7.53 \text{ t} \text{ ha}^{-1}$ ), G32 ( $7.04 \text{ t} \text{ ha}^{-1}$ ), G09 ( $7 \text{ t} \text{ ha}^{-1}$ ), G31 ( $6.91 \text{ t} \text{ ha}^{-1}$ ) and G11 ( $6.79 \text{ t} \text{ ha}^{-1}$ ) varieties in NGH were determined superior stable and high yielding varieties. While, G03 ( $3.74 \text{ t} \text{ ha}^{-1}$ ), G01 ( $4.58 \text{ t} \text{ ha}^{-1}$ ), G18 ( $4.76 \text{ t} \text{ ha}^{-1}$ ), G19 ( $4.94 \text{ t} \text{ ha}^{-1}$ ), and G06 ( $4.88 \text{ t} \text{ ha}^{-1}$ ) in BLK, G03 ( $2.91 \text{ t} \text{ ha}^{-1}$ ), G03 ( $4.43 \text{ t} \text{ ha}^{-1}$ ), G02 ( $4.83 \text{ t} \text{ ha}^{-1}$ ) and G10 ( $4.91 \text{ t} \text{ ha}^{-1}$ ) in NGH showed the poorest stability with lower yield. Among the 5-top varieties, G04 and G15 together appeared in BLK and HLM, G09 in HLM and NGH, G25 in BLK, and G31 in NGH locations, they all appeared in the grand mean rank, showing more stability compared to other varieties (table 6).

#### 3.4.3.2.2 Straw Yield (SY)

The E x V interaction counted for 5.88 and 70.59% higher variation than the main factors effect (environment and variety) for straw yield (SY), respectively (Table 4). The varieties mean for SY values over six environments ranged between 5.09 (G03, BLK18) and 13.7 t ha<sup>-1</sup> (G28, NGH18), (supplement table 2), in contrast to ANOVA mean table (table 5) SY mean values (averaging environments) varied from 7.16 (G03) to 9.84 t ha<sup>-1</sup> (G32). Considering stability traits, G32 (9.84 t ha<sup>-1</sup>), G28 (9.27 t ha<sup>-1</sup>), G10 (9.14 t ha<sup>-1</sup>), G19 (8.47 t ha<sup>-1</sup>), and G27 (8.7 t ha<sup>-1</sup>) <sup>1</sup>) demonstrated wide stability over the locations with higher SY, while G03 (7.16 t ha<sup>-1</sup>), G33 (7.7 t ha<sup>-1</sup>), G18 (8.03 t ha<sup>-1</sup>), G13 (8.21 t ha<sup>-1</sup>) and G21 (8.24 t ha<sup>-1</sup>) were identified as unstable varieties with lower SY compared to other varieties (table 6 and supplement table 2). Considering the location, G26 (9.23 t ha<sup>-1</sup>), G14 (9.7 t ha<sup>-1</sup>), G32 (8.67 t ha<sup>-1</sup>), G29 (8.51 t ha<sup>-1</sup>), and G19 (8.38 t ha<sup>-1</sup>) in BLK, G32 (9.17 t ha<sup>-1</sup>), G21 (8.1 t ha<sup>-1</sup>), G30 (7.58 t ha<sup>-1</sup>), G01 (8.20 t ha<sup>-1</sup>) and G27 (8.08 t ha<sup>-1</sup>) in HLM and G28 (7.54 t ha<sup>-1</sup>), G07 (7.86 t ha<sup>-1</sup>), G17 (7.24 t ha<sup>-1</sup>), G32 (9.22 t ha<sup>-1</sup>) and G09 (9.77 t ha<sup>-1</sup>) in NGH were identified as higher stability with higher SY; while G21 (6.50 t ha<sup>-1</sup>), G33 (6.61 t ha<sup>-1</sup>), G18 (6.49 t ha<sup>-1</sup>), G05 (6.58 t ha<sup>-1</sup>) and G11 (6.96 t ha<sup>-1</sup>) varieties in BLK, G33 (7.72 t ha<sup>-1</sup>), G13 (10.17 t ha<sup>-1</sup>), G03 (8.92 t ha<sup>-1</sup>), G11 (9.33 t ha<sup>-1</sup>) <sup>1</sup>) and G04 (8.75 t ha<sup>-1</sup>) varieties in HLM, and G21 (10. 7 t ha<sup>-1</sup>), G26 (8.74 t ha<sup>-1</sup>), G14 (7.88 t ha<sup>-1</sup>) and G30 (7.91 t ha<sup>-1</sup>) varieties in NGH were observed as unstable with lower SY than the others varieties. Among the 5-top, G32 appeared in all locations along the grand mean rank, this

suggested a more stable variety, while varieties which included G28 in the NGH, G19 in BLK, G27 in NGH, and all occurred in the grand mean rank, showed good stability for SY (table 6).

#### 3.4.3.2.3 Thousand kernel weights (TKW)

The E x V interaction established 48.26% lower variation than the main effect of the environment but showed equal variation with the variety of main effects for TKW (table 4). The varieties TKW over six environments varied from 29.67 (G18, BLK18) to 50.67 g (G02, HLM18), (supplement table 3), compared to ANOVA mean table (table 5), TKW values (averaging environments) ranged between 34.00 (G01) to 42.24 g (G02). Regarding stability traits, G33 (40.75 g), G20 (39.60 g), G16 (39.49), G09 (42.09 g), and G07 (39.3 g) were characterized superior stable varieties with higher TKW over the locations, contrastingly G01 (34.00 g), G03 (36.08 g), G05 (36.55 g), G14 (36.51 g) and G21 (35.58 g) showed poor stability with lower TKW value in respect to other varieties (table 6, supplement table 3). Concerning locations, G02 (42.83 g), G20 (40.36 g), G33 (40.16 g), G06 (39.6 g), and G26 (39 g) in BLK, G02 (45.5 g), G09 (44.5 g), G18 (40.83 g), G16 (40.83 g) and G33 (41 g) in HLM and G33 (41.1 g), G09 (40.23 g), G11 (40.68 g), G07 (39.94 g), G29 (40.02 g) in NGH showed remarkable stability with TKW; though G01 (32.73 g), G03 (34.1 g), G19 (35 g), G04 (35 g) and G05 (35.46 g) in BLK, G01 (34.16 g), G21 (35 g), G05 (36.5 g), G26 (37 g) and G14 (36.16 g) in HLM and G01 (35.10 g), G21 (34.29 g), G10 (36.85 g), G14 (36.78 g) and G19 (37.22 g) in NGH showed poor stability along lower TKW, compared to other varieties (table 6 and supplement table 3). Out of 5-top varieties, G33 emerged in all locations along with grand mean rank, was the best stable variety; while G02 and G09 appeared in two locations (BLK, HLM) and (HLM, NGH), respectively, along the grand mean rank, they were more stable varieties than others (table 6).

#### 3.4.3.2.4 Harvest index (HI)

The E x V interaction contributed 27.2 and 57.58% further variation than the main factor effect, environment, and variety for HI, respectively (table 4). However, HI varied from 0.26 (G03, HLM18) to 0.49% (G13, BLK20), (supplement table 4). However, the ANOVA mean table (table 5) and the stability mean table (table 6) showed the same results for the 5-top and the lowest varieties for HI (G33, G04, G23, G20, G09). Regarding locations, varieties G20, G12, G33, G25, and G13 (0.44%) in BLK, G04, G09, and G17 (0.42%) and G02, G15 (0.41%) in HLM and G33, G29, G23 (0.43%), G31, G26 (0.44%) in NGH showed higher stability with higher HI. But G03 (0.36%), G14, G26, G06, and G19 (0.37%) in BLK, G03 (0.27%), G32 and

G21 (0.32%), G01 and G10 (0.34%) in HLM and G03 (0.34%), G01 (0.37%), G10, G05 (0.38%), G12 (0.39%) in NGH showed poor stability with lower HI value (table 6 and supplement table 4). Among 5-top varieties, G33 emerged in two locations (BLK and NGH), G04, G20 in BLK, G09 in HLM, and all in grand mean rank, which means they were more stable varieties (table 6).

#### 3.4.3.2.5. Plant height (PH)

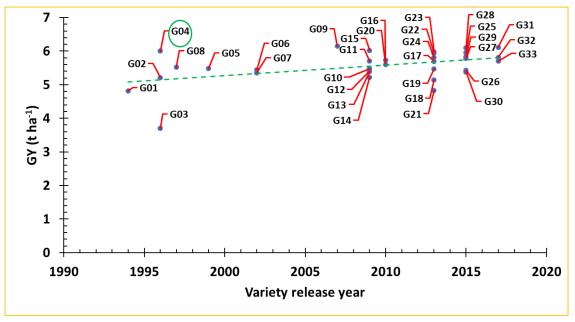
The main effect of the environment on PH was 74 and 44% greater than the effect of variety and E x V interaction, respectively (table 4). Thus, the varieties' mean PH values in the six environments varied from 74.67 (G13, BLK20) to 120.67 cm (G33, NGH20) (supplement table 5). However, varieties G28 (106.5 cm), G32 (106 cm), G31 (107.6 cm), G30 (105.3 cm), and G09 (104.5 cm) were found to be stable with taller PH over all locations, whereas G12 (83.6 cm), G13 (84.4 cm), G19 (89.6 cm), G21 (94 cm) and G22 (93 cm) varieties were unstable with shorter PH value than the other varieties (table 6). Furthermore, G32 (104 cm), G27 (103 cm), G28 (103.6 cm), G31 (109 cm), and G29 (101.3 cm) in BLK, G32 (99.5 cm), G28 (98.5), G14 (98.3 cm), G30 (98.1 cm), and G02 (97.4 cm) in HLM and G33, G09 and G28 (117.1 cm) and G31 (116.6 cm) in NGH showed high stability and higher PH, while G13 (80 cm), G12 (81.6 cm), G24 (89.3 cm), G22 (91 cm) and G19 (89 cm) in BLK, G12 (81.6 cm), G19 (84.6 cm), G13 (82.3 cm), G21 (85.8 cm) and G25 (87.5 cm) in HLM and G12 (87.5 cm), G13 (90.8 cm, G19 (95 cm), G22 (98.6 cm) and G06 (101.6 cm) in NGH exhibited poor stability with shorter PH (table 6, and supplement table 5). Among 5-top taller varieties, G20 appeared in all locations along grand mean rank, which was characterized as the most stable; while G32 and G31 appeared in two locations (BLK and HLM) and (BLK and NGH), respectively, G30 in HLM, G01 in NGH, along all in the grand mean rank, were more stable varieties for PH than the others (table 6).

Thousa		Inci w	cigin	$(\mathbf{I}\mathbf{K}\mathbf{v}),$	nai ves		<i></i> /	, and p	nam ner	gint (1	11).									
Variety			GY			:	SY			Т	KW				ні			I	РН	
variety	BLK	HLM	NGH	Mean	BLK	HLM	NGH	Mean	BLK	HLM	NGH	Mean	BLK	HLM	NGH	Mean	BLK	HLM	NGH	Mean
G01	20.0	27.0	32.5	22.7	13.0	9.5	24.5	13.4	32.5	31.5	30.0	26.9	21.0	27.5	26.0	21.3	22.0	19.5	11.0	15.0
G02	20.0	14.5	28.0	17.9	13.0	22.5	25.5	17.4	1.5	1.5	19.0	6.3	23.0	9.0	22.5	15.6	16.5	6.5	8.0	8.9
G03	31.0	33.0	32.0	27.4	19.0	26.5	32.0	22.1	31.0	22.0	22.5	21.6	32.0	32.5	24.5	25.4	15.5	17.5	9.0	12.0
G04	10.0	5.0	12.5	7.9	19.5	24.0	10.0	15.3	28.5	16.0	20.5	18.6	9.5	4.5	16.5	8.7	13.0	10.0	9.0	9.1
G05	18.0	15.5	18.0	14.7	28.0	23.0	11.5	17.9	26.5	28.5	22.5	22.1	11.5	11.0	25.0	13.6	7.5	12.0	5.0	7.0
G06	26.5	12.5	17.5	16.1	12.0	23.0	20.0	15.7	9.0	9.5	9.0	7.9	30.0	10.0	15.5	15.9	19.5	20.0	14.0	15.3
G07	16.5	15.0	27.5	16.9	17.5	18.5	5.5	11.9	10.5	14.5	7.5	9.3	19.0	14.0	33.0	18.9	14.2	9.5	8.0	9.1
G08	16.5	22.5	13.5	15.0	14.0	20.0	17.0	14.6	21.5	21.5	8.5	14.7	25.0	16.0	16.5	16.4	16.0	26.0	12.5	15.6
G09	19.0	5.5	5.0	8.4	20.5	17.5	7.5	13.0	13.0	1.5	6.5	6.0	14.5	7.5	11.0	9.4	9.0	9.0	2.5	5.8
G10	13.5	23.0	19.0	15.9	10.0	10.0	13.0	9.4	14.5	11.5	27.5	15.3	24.5	25.5	25.0	21.4	19.0	20.0	11.0	14.3
G11	23.0	21.5	11.5	16.0	25.0	25.5	8.0	16.7	14.0	13.0	7.0	9.7	11.5	17.5	15.0	12.6	9.0	14.0	7.0	8.6
G12	15.0	16.5	26.5	16.6	21.5	13.5	21.0	16.0	23.0	16.0	24.0	18.0	7.0	16.5	24.5	13.7	29.0	32.5	18.5	22.9
G13	17.0	21.0	22.5	17.3	24.0	29.0	15.5	19.6	25.5	24.0	25.0	21.3	8.0	12.0	23.5	12.4	29.5	30.5	17.5	22.1
G14	13.5	21.5	28.0	18.0	3.5	19.5	27.0	14.3	21.5	27.0	27.0	21.6	31.5	22.5	14.5	19.6	22.0	5.0	11.5	11.0
G15	10.0	3.0	16.0	8.3	16.0	12.5	13.0	11.9	17.5	18.0	12.5	13.7	10.5	9.0	22.5	12.0	15.5	7.5	8.5	9.0
G16	17.0	17.0	12.5	13.3	20.5	12.5	17.5	14.4	16.0	7.5	9.0	9.3	10.5	20.0	13.0	12.4	9.4	20.5	4.5	9.8
G17	25.0	15.0	15.0	15.7	24.5	19.0	7.0	14.4	16.0	15.5	20.0	14.7	12.0	8.0	23.5	12.4	18.0	19.5	10.5	13.7
G18	28.0	21.5	21.0	20.1	28.0	21.0	19.5	19.6	20.5	6.5	17.5	12.7	14.5	13.5	20.5	13.9	8.6	17.0	11.0	10.5
G19	26.5	15.0	14.5	16.0	9.5	10.5	19.0	11.1	29.0	20.5	25.5	21.4	29.5	19.5	11.0	17.1	24.0	31.0	16.5	20.4
G20	12.0	19.5	16.5	13.7	22.5	22.0	19.0	18.1	4.0	11.5	17.0	9.3	4.0	13.5	15.0	9.3	13.0	12.0	8.0	9.4
G21	29.5	19.5	28.0	22.0	28.5	6.5	29.5	18.4	19.0	29.5	29.0	22.1	16.0	28.5	11.5	16.0	23.5	29.5	13.0	18.9
G22	10.5	9.5	15.5	10.1	12.5	15.5	14.0	12.0	10.0	14.0	22.0	13.1	14.5	11.5	14.0	11.4	25.0	24.5	15.0	18.4
G23	10.5	8.0	16.5	10.0	12.0	18.0	21.0	14.6	11.0	23.0	16.0	14.3	12.0	12.5	7.0	9.0	17.5	25.0	11.5	15.4
G24	4.5	24.5	10.0	11.1	17.5	18.5	15.5	14.7	14.0	15.0	22.0	14.6	8.0	23.0	16.0	13.4	25.0	22.0	11.5	16.7
G25	8.5	14.0	10.5	9.4	16.0	14.5	11.5	12.0	23.5	24.5	16.5	18.4	7.5	14.5	22.0	12.6	22.5	27.0	13.5	18.0
G26	16.5	20.0	19.0	15.9	2.5	16.5	28.5	13.6	9.0	27.0	23.5	17.0	30.0	24.0	9.0	18.0	19.0	20.0	12.5	14.7
G27	13.0	19.0	9.0	11.7	14.5	9.5	15.5	11.3	25.0	18.0	14.0	16.3	19.0	22.5	17.0	16.7	4.5	19.0	6.5	8.6
G28	16.5	18.5	1.5	10.4	15.5	10.0	4.0	8.4	13.0	11.5	12.0	10.4	21.5	22.0	17.0	17.3	4.5	4.0	2.5	3.1
G29	6.0	16.5	16.0	11.0	8.5	15.5	25.5	14.1	13.0	12.5	7.5	9.4	19.5	17.0	6.5	12.3	5.5	17.5	7.5	8.7
G30	19.0	11.0	26.0	16.0	21.0	9.0	25.5	15.9	17.5	26.0	17.0	17.3	18.0	18.0	14.0	14.3	6.6	5.0	7.5	5.4
G31	12.0	10.0	5.0	7.7	15.0	15.5	15.5	13.1	15.5	10.5	11.5	10.7	13.5	15.5	6.0	10.0	4.5	8.0	3.0	4.4
G32	13.0	27.0	4.0	12.6	8.0	2.0	7.0	4.9	10.0	23.5	9.5	12.3	25.5	31.5	17.0	21.1	3.5	3.0	4.5	3.1
G33	23.5	18.5	10.5	15.0	28.0	30.5	15.0	21.0	5.0	8.5	2.5	4.6	7.0	11.0	5.5	6.7	17.7	17.0	1.5	10.4

**Table 6.** Ranking\* of the performance stability of 33 improved wheat varieties within and across locations. The mean grain yield (GY), straw yield (SY), Thousand kernel weight (TKW), harvest index (HI), and plant height (PH).

*Ranking\*: the variety with the highest value, received a rank of 1* 

*BLK= Mean of BLK18 and BLK20, HLM= mean of HLM18 and HLM20, NGH= mean of NGH18 and NGH20 environments Grand mean= averaging of all environments (BLK18, BLK20, HLM18, HLM20, NGH18, NGH20) The 5-top high stable varieties in every location and the grand mean for across the locations are bolded.*  This study found that the introduction of new improved wheat varieties under Afghanistan agroclimatic conditions significantly increased the GY potential ( $R^2$ = 0.25, P< 0.01) as compared to the old, improved varieties, except for G04 (1996) which has comparable GY with recently released varieties (Figure 2).



**Figure 3** scatter plots of the varieties' grain yield (GY) potential as a function of the variety year of release.

#### 3.4.4 Discussion

Despite wheat being an important food crop in Afghanistan, the country is dependent on imported wheat to fulfill domestic demand (Tiwari et al., 2020). Over the past two-decade efforts have resulted in a slight increase in wheat production but with this increase, the country has not reached self-sufficiency (Sharma, 2019). Among all, insufficient high-yielding varieties adapted to variable agro-climate in Afghanistan were underlined as a big challenge. However, identifying superior yield and stable variety for particular climate conditions is known as the best approach to rise wheat production (Chowdhury et al., 2021).

In this study, we aimed to identify superior stable high yielding common wheat varieties for different agro-climatic zones, in Afghanistan. The grain yield and yield characteristics of 33 common wheat varieties were studied in six different environments (three locations x twogrowing seasons). This study found that the variation due to interaction E x V for GY, SY, and HI was significantly larger as compared to the environment and variety effect; but the contribution of E x V for TKW and PH was smaller than the main factors effect. These results were consistent with the results of previous studies (Omrani et al., 2022; Ahmadi et al., 2012; Wardofa et al., 2019; Sakin et al., 2011). Overall, varieties grown in NGH, produced higher grain yields compared to other locations, while the lowest grain yield was obtained in the HLM location (table 6, and supplement table 1). These results were in line with previous assessment series reports (Jilani A et al., 2013), which stratified NGH in higher and HLM in lower wheat potential productivity in Afghanistan compared to the other locations. Moreover, HLM is in arid climatic conditions with higher temperatures, probably the heat stress could be a limiting factor for wheat production. Higher temperature contributes to modifying wheat phenology, causing shortening the number of days to reach anthesis and physiological maturity, subsequently reducing the number of days in which plants can intercept light for photosynthesis, which derives reducing biomass and grain yield. This was confirmed by many other authors Athar Khan et al., (2020) and Asseng et al., (2016). Furthermore, results detected huge variations among the performance of varieties, G09, G31, G28, G15, and G04 varieties showed the highest GY in decreasing order, respectively over the locations (table 5). According to the stability table (table 6), G31, G04, G15, G09, and G25 varieties exhibited wide stability with superior GY over the locations in decreasing order, compared to other varieties (table 6). The following varieties, G03, G01, G21, G18, and G02 showed the lowest stability as well as GY. Interestingly, G31, G09, and G04 appeared in the 5-top highest stable and GY varieties (table 5 and table 6). Among all, G09 also was a pioneer for TKW, HI, and PH; G04 was only for HI and G31 for PH over the locations (table 6). These results were in line with the findings of Sayed et al., (2022), who reported that among 31 genotypes, 5 genotypes had wide stability and higher grain yield in multiple environments in Egypt.

Furthermore, varieties G24, G29, G25, G15, and G04 in the BLK, G15, G09, G04, G23, and G22 in the HLM, and G31, G09, G32, G28, G27 in the NGH location were superior for stability and GY than the other varieties. Among 5-top varieties, varieties G15 and G04 together appeared in two locations (BLK and HLM), also G09 emerged in two locations (HLM and NGH). Additionally, G29 was associated with higher SY and taller PH and G25 showed a higher percentage of HI in the BLK location. G09 was characterized by higher TKW and HI, and G04, and G15 were characterized by higher HI in the HLM location. G09 showed higher SY, TKW, and taller PH, G28 showed higher SY and taller PH, and G31 demonstrated taller PH in the NGH location. In relevance to the farmers' benefits, SY after GY is the second most important product for livestock in Afghanistan, which have its economic importance. Despite, G29 in BLK and G09, G28 in the NGH location was identified as a superior stable variety along with higher GY and SY. According to climate variability, many authors detected that the adaptability and yield

performance of wheat genotypes varied among the locations (Munaro et al., (2014); Mohammadi et al., (2010), Singh et al., (2020); Kahram et al., (2013) and Mądry et al., (2017)). Likewise, Mikó et al., (2014) stated that TKW and PH substantially are affected by environment rather than genotype.

Interestingly, this result has revealed that there has been continuous progress in wheat genetic gain on yield during the past 25 years in Afghanistan. However, the "recent" improved wheat varieties showed higher grain yield with respect to the "old" improved wheat varieties. The explanation behind this could probably be that the "old" improved varieties relatively have lost their yield potential within a certain period of time; on the other hand, the breeders may have attempted to further improve the genetic gain of the new varieties. These results were in line with the result of other similar studies: Sharma et al., (2021) discovered that in the past 14 years (2002 to 2015) the wheat genetic gain for yield was improved by about 0.12 t ha<sup>-1</sup> in Afghanistan while Mohammadi et al., (2010) has detected that the genetic gain for yield was only about 0.31 t ha<sup>-1</sup> per decade in Turkey.

#### 3.4.5 Conclusion

This study determined the actual yield and stability performance of 33 improved wheat varieties (V) within and over six environments (E), (three locations x two growing seasons) in Afghanistan. According to the results, yield and yield characteristics were significantly affected by interaction E x V, followed by the main factor, environment, and variety. The high-yielding and stable varieties were identified and were underlined in particular locations as well as across the locations. The continuous progress of genetic gain for yield was found paramount for the last 24 years in Afghanistan. The obtained results could be useful for the farmers to find their ideal wheat variety. Additionally, this information is vital for breeders to continue developing new high-yielding genotypes for diverse climate conditions in Afghanistan. Furthermore, there is sufficient room for breeders to improve genetic gain and therefore improve the yield potential.

#### **Supplemental information**

Supplemental information is included with this paper, which describes the detailed status performance of the varieties within the environments (locations and years).

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## 4. General Conclusions

The objectives of this thesis were to evaluate the productivity, quality, rheological properties, and economic benefits (sustainability of crop production) of bread wheat that can be achieved through the development of agronomic management strategies, particularly efficient management of nitrogen, phosphorus, and sulfur and using of high-yielding varieties, as a function of various climate and soil variability. Among four experiments, three were conducted in Afghanistan at five different pedoclimatic sites (BGL: Baghlan, BLK: Balkh, HLM: Helmand, HRT: Herat, and NGH: Nangarhar) and one experiment was conducted at Cesa research farm, Tuscany, Italy.

The trial of the first paper was conducted with three and two levels of nitrogen (NL) and sulfur (SL), and two seeding densities (SD) on 14 "old" bread wheat varieties released in Italy between 1900 and 1960. The objective was to evaluate whether grain yield and protein, rheological properties, and asparagine (ASN) content in grains of "old" bread wheat varieties could be improved by agronomical management, particularly sulfur, and nitrogen fertilizations and seed densities, in central Italy. The results showed that high SD application improved grain yield (GY) and protein concentrations (PC). Sulfur fertilization increased only grain yield (GY), while NL significantly increased PC and protein yield (PY) per hectare but did not affect the grain yield. However, for all varieties, the rheological parameters of the dough were reduced, while the seed density increased. Dough strength (W) was significantly increased with increasing SL and NL fertilizations. Interestingly, the concentration of free ASN was negatively correlated with the year of release in the cultivars studied. The ASN content of new varieties was lower as compared to the old varieties. This may indicate that previous breeding programs have contributed to a reduction in ASN content; however, further studies on old varieties are needed to investigate this aspect. Similarly, the results showed that ASN concentration was significantly reduced by SL fertilization, while NL fertilization significantly increased ASN content.

Moreover, since ASN is the predominant precursor of acrylamide formation in wholegrain bakery products, and acrylamide is classified as a neurotoxin, therefore free ASN concentration in grain should be monitored and maintained as low as possible. In this case, these results will provide a technical basis for the potential use of N and S to manage the quality of wheat yield with a lower concentration of ASN in central Italy, also these results suggest avoiding traditional fertilization practices considered inefficient. In conclusion, the present study suggested that 135 kg N ha<sup>-1</sup> combined with 6.4 kg S ha<sup>-1</sup> can improve the quality and technical performance of "Old" wheat verities 'wholegrain flours while maintaining the ASN concentration as low as possible, which contributes to promoting food safety. Therefore, it is important to know for all varieties that food safety can be assured by reducing ASN concertation. This information can be, as well used by policymakers so that in the future high ASN content will be not allowed in human food consumption, because of its health hazards.

Furthermore, *the finding of this study may also help policymakers to encourage the production of healthy wheat in the future, to improve social life as healthy as possible.* Additional research, including more years in different pedoclimate, and agronomical management is recommended to discover the more secret effect of nutrient management, particularly, N and S fertilization on yield, quality as well as free ASN content of wheat.

The experiment of the second paper was conducted with four nitrogen levels (NL) and four phosphorus levels (PL) at four climatic conditions on an improved wheat variety in Afghanistan. The objectives of this study were to (1) determine the impact of soil and climate on the responses of wheat to N and P fertilization; (2) quantify the specific N and P response on winter wheat for different agroclimatic zones (ACZ); and (3) determine the economical application rates of N and P for farmers for each considered ACZ. From the results of this study, we concluded that the high soil pH was the main environmental factor that limited the efficiency of N and P fertilization in irrigated winter wheat and caused a reduction in the grain and quality of wheat. Across all four locations, the application of 50 kg  $P_2O_5$  ha<sup>-1</sup> was sufficient for winter wheat grain production both in terms of quantity and quality. Given that soil pH is above 7.3, in order to increase the P efficiency, we have to apply the P fertilizer at planting time, banded with or near the seeds. We noticed that a significant increase in SY and GY appeared, while the N fertilization rates increased. As expected, N fertilization was the most important factor in determining PC, showing potential for further improvement in N management. However, the optimal N rate in each location should not be calculated on the basis of the highest expected yield production, but on the highest marginal rate of return. Therefore, the results showed the highest rate of return determined at N 60 and  $P_2O_5$  50 kg ha<sup>-1</sup> in all locations.

In conclusion, this result suggested that  $50 \text{ kg } P_2O_5 \text{ ha}^{-1}$  can be sufficient for optimal grain yield, straw yield, and protein yield production of bread wheat in all locations, but 120 kg N ha<sup>-1</sup> was the optimum rate for protein concentration in the kernel for all locations. Further field trials in different pedoclimatic conditions should be carried out to improve the understanding of the factors limiting the N and P fertilization efficiency in Afghanistan.

The trial of the third paper was conducted with three bread wheat varieties (DLN7: Darulaman-07, KBL13: Kabul-13, and ZRDN: Zardana-89), three phosphorus levels (PL) at 60, 90, and 120 kg P<sub>2</sub>O<sub>5</sub> ha-<sup>1</sup>, and three nitrogen ratios (NP) at 1:1, 1.25:1, and 1.5:1, respectively, in four locations (L). The objectives of this study were to (1) quantify the effect of soil and climatic parameters on yield and quality of wheat (2) investigate the response of different wheat varieties to different N and P fertilization rates under specific climate conditions, to improve the yield and quality of wheat in Afghanistan. However, higher pH value in Afghanistan's soils was the main environmental limiting factor for common wheat production. For such soils, the nutrient availability and organic matter content are typically very low. However, under this condition, the use of salt-tolerance wheat varieties, application of organic amendments along with a banded application of P are crucial. The result showed that increased PL application, grain yield (GY), straw yield (SY), protein yield (PY), and starch yield (STY) production increased in all locations. In addition, PL increased the protein content (PC), and dough strength (W), without decreasing thousand kernel weight (TKW) and gluten content (GC). The NP was the most important factor in determining PC, PY, GC, and W, without decreasing GY, SY, and TKW, confirming the potential for further improvement in N management. Regarding the starch content (ST) properties, NP was shown to negatively affect the total ST and amylopectin (AP) concentration in all the varieties; but amylose to amylopectin (AM:AP) ratio was significantly improved by NP treatment, while the STY was not influenced under NP treatment. Instead, ST, AP, and STY were significantly increased with increased PL application, but AM:AP was not affected by PL treatment.

In conclusion, the present results suggested that the NP1/PL120 intensively optimized the GY, SY, ST, and AP concentrations. Instead, NP1.5/PL120 strongly increased the PC and GC concentration. This result was in contrast, with the result of the second paper (the optimum GY was obtained at  $P_2O_5$  50 ka ha<sup>-1</sup> for all locations). However, we found this was because of the adverse effect of climate. The trial of the second paper, during both growing seasons, faced lower precipitation and higher temperature, compared to the trial of the third paper's growing seasons. This result concluded that the optimal soil moisture and temperature during the vegetative growth of wheat can improve significantly N and P fertilization in winter wheat fields.

But further studies, including additional year within various pedoclimatic conditions, is needed to quantify more the effect of interactions between soil, climate, and agronomical management on wheat yield and quality in Afghanistan.

Both the second and the third papers of this thesis, found that high pH values were the main environmental factors limiting wheat productivity and quality in Afghanistan. Since, it is well known that pH influences the activity of microorganisms, enzymes, and the availability of nutrients, and therefore it acts as an important role in regulating plant growth and yield. Alleviating soil pH by using chemical elements such as sulfuric acid, sulfur, and gypsum, can be more expensive in field crops, specifically in the wheat field; but reducing the adverse effect of higher soil pH, using salt-tolerant wheat varieties, banded application of P and organic amendments are recommended to improve soil fertility and to sustain wheat yield and quality in Afghanistan. Additionally, before sowing, having knowledge about soil fertility is an important matter for adjusting the fertilization schedule, specifically for nitrogen and phosphorus application rates.

Furthermore, the results of both mentioned papers, revealed that efficient management of N and P based on climate and soil-specific conditions can substantially increase the wheat yield and quality in Afghanistan. Therefore this information can be useful for policymakers to make solid decisions in order to reduce the yield gaps as well as improve wheat production, ultimately it can possibly attribute the wheat self-sufficiency, toward aiming to reduce food insecurity in Afghanistan.

Since wheat contributes to about 60% of the total calories of the Afghan's population diet with an annual consumption per capita of about 181 kg. Thus, lower productivity, with poor quality and insufficient total production, are major challenges, and it directly contributes to food insecurity and malnutrition, in the current situation of Afghanistan. To overcome these challenges, the practical agronomical information of this thesis can help the policy maker to mainstream wheat production in the country.

As regards, the dissemination and the adoption of these technologies depend on the public farmer services; however, we suggested that the policy maker should establish a responsive extension service system, in order to make sure that the technologies can reach the wheat farmers.

The trial of the fourth paper evaluated 33 improved wheat varieties in six environments (three locations x two growing seasons) in Afghanistan. The results indicated that the yield traits were significantly affected by the interaction between  $E \times V$ , followed by the main factors, environment, and varieties, respectively. The high-yielding and stable varieties were identified, particularly within the locations as well as across the locations. In this study, we found that continuous progress in genetic gain was paramount in Afghanistan. Therefore, a positive correlation was found between yield and the year of release of the varieties. It means the new varieties showed superior yield than the old varieties. But there was also an exception, for some varieties, for instant, the yield of the G04 old variety was comparable with the yield of new varieties.

On one hand, the obtained results of this paper could be useful for farmers to find their ideal wheat varieties in a specific climate, on the other hand, this information is vital for breeders to continue developing new high-yielding genotypes for the diverse climate in Afghanistan.

In conclusion, this study found that the varieties G31, G04, G15, G09, and G25 had higher adaptability and stability, associated with superior grain yields in all locations in Afghanistan. But most of the varieties had regional-specific adaptabilities, such as varieties G24, G29, G25, G15, and G04 in Balkh; G15, G09, G04, G23, G22 in Helmand, and G31, G09, G32, G28, G27 in Nangarhar had high yield with superior stability performance. Therefore, the identified superior stable, and high-yielding varieties could significantly contribute to wheat production in Afghanistan, which will assure food security as well as improve the farmer's livelihood.

Afghanistan is constituted of seven macro and many microclimates. In the current situation, the insufficient stable high-yielding varieties based on agro-climatic conditions is a big constraint. The selection of high-yielding climate-resilient varieties can meet the farmers' requirements and consumer preferences. Furthermore, this study found that the productivity and stability performance of wheat varieties varied as a function of climate variabilities. However, through this study, the high-yielding and stable varieties were identified in particular locations as well as across the locations.

This information could be a useful result for policymakers, seed enterprises, farmers' groups, and end users to support the use of those varieties in suitable environments.

But further studies with more locations and years are needed to understand more about the yield and stability behaviour of these varieties in relation to the climate change context in the future.

# 5. General References

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# 7. Supplementary tables

## 7.1 Paper two: Supplementary table

**Table 1.** Partial budget analysis of bread wheat produced in relation to the application of nitrogen(NL) and phosphorus (PL).

	NL and PL	Fert cost	Grain yield price	Straw yield	Total	Net revenu
Locations	(kg ha- <sup>1</sup> )	(US Dollar ha-1)	(US Dollar ha-1)	price (US Dollar)	revenue (US Dollar)	(US Dollar
	-	-	NL	-	-	
BGL	35.28	24.58	1944.52	1090.67	3035.19	3010.62
	65	45.28	2074.46	1025.59	3100.05	3054.76
	95	66.18	2195.51	1024.30	3219.82	3153.63
	120	83.60	2283.96	1006.42	3290.38	3206.78
BLK	35.28	24.58	872.77	470.67	1343.44	1318.86
	65	45.28	1047.92	525.28	1573.20	1527.91
	95	66.18	1137.41	561.94	1699.34	1633.16
	120	83.60	1151.31	586.43	1737.74	1654.14
HLM	35.28	24.58	1087.20	1017.48	2104.68	2080.10
	65	45.28	1188.65	993.50	2182.15	2136.87
	95	66.18	1268.67	1008.83	2277.50	2211.32
	120	83.60	1316.66	1049.46	2366.12	2282.52
HRT	35.28	24.58	1645.79	1039.41	2685.20	2660.62
	65	45.28	1803.09	1135.14	2938.24	2892.95
	95	66.18	1901.84	1101.01	3002.85	2936.67
	120	83.60	2038.47	1114.70	3153.17	3069.57
			PL			
BGL	0	0.00	1944.85	988.41	2933.26	2933.26
	50	74.42	2119.37	1026.80	3146.17	3071.76
	70	104.18	2191.24	1050.81	3242.05	3137.87
	90	133.95	2242.99	1080.97	3323.96	3190.01
BLK	0	0.00	939.32	456.76	1396.08	1396.08
	50	74.42	1044.68	533.92	1578.60	1504.19
	70	104.18	1070.75	569.16	1639.91	1535.73
	90	133.95	1154.66	584.48	1739.14	1605.19
HLM	0	0.00	983.33	948.40	1931.72	1931.72
	50	74.42	1174.21	1008.03	2182.25	2107.83
	70	104.18	1257.45	1095.21	2352.66	2248.48
	90	133.95	1446.20	1017.62	2463.82	2329.87
HRT	0	0.00	1296.53	816.31	2112.84	2112.84
	50	74.42	1951.92	1175.17	3127.09	3052.67
	70	104.18	2040.89	1161.91	3202.79	3098.61
	90	133.95	2099.85	1236.88	3336.73	3202.78

## 7.2 Paper four: Supplement tables

Variatu						Envir	onment						Mean
Variety	BLK1	8	BLK2	0	HLM1	8	HLM	120	NGH	18	NGH20	)	wean
G01	5.27 (0.33)	abcdef	5.44 (0.09)	bcde	5.32 (0.64)	bcde	4.19 (0.13)	fghij	5.14 (0.15)	gh	3.53 (0.12)	h	4.815
G02	5.58 (0.59)	abcdef	5.12 (0.37)	de	5.63 (0.45)	abcde	5.16 (0.16)	abcdefg	6.05 (0.11)	cdefgh	3.73 (0.15)	gh	5.210
G03	2.58 (0.31)	g	4.92 (0.26)	de	2.83 (0.19)	f	3.02 (0.13)	j	5.02 (0.48)	h	3.85 (0.35)	fgh	3.701
G04	6.17 (0.21)	а	5.63 (0.74)	abcde	6.53 (0.61)	ab	5.39 (0.37)	abcdef	7.09 (0.16)	abcde	5.19 (0.21)	cde	5.999
G05	4.31 (0.15)	def	6.03 (0.26)	abcde	6.04 (0.24)	abcd	4.64 (0.39)	cdefgh	6.82 (0.31)	abcdef	5.06 (0.69)	defg	5.482
G06	4.66 (0.66)	bcdef	5.12 (0.13)	de	5.55 (0.63)	abcde	5.39 (0.33)	abcdef	7.01 (0.39)	abcde	4.96 (0.31)	defg	5.447
G07	4.57 (0.23)	cdef	6.23 (0.29)	abcd	5.86 (0.43)	abcde	4.87 (0.16)	bcdefgh	5.87 (0.61)	defgh	4.74 (0.34)	efgh	5.355
G08	4.63 (0.21)	cdef	6.09 (0.23)	abcde	5.29 (0.44)	bcde	4.89 (0.48)	bcdefgh	6.91 (0.26)	abcde	5.31 (0.17)	cde	5.522
G09	5.51 (0.36)	abcdef	5.33 (0.65)	bcde	5.89 (0.21)	abcde	6.19 (0.61)	ab	7.85 (0.15)	а	6.16 (0.25)	abcd	6.155
G10	5.88 (0.87)	abc	5.45 (0.46)	abcde	5.43 (0.59)	abcde	4.38 (0.42)	efghij	7.02 (0.11)	abcde	4.71 (0.27)	efgh	5.478
G11	5.78 (0.65)	abcd	4.79 (0.27)	е	4.83 (0.71)	de	5.23 (0.62)	abcdefg	6.22 (0.72)	cdefgh	7.38 (0.18)	а	5.703
G12	6.01 (0.51)	abc	5.21 (0.13)	cde	5.53 (0.62)	abcde	5.12 (0.65)	abcdefgh	6.08 (0.26)	cdefgh	4.51 (0.22)	efgh	5.410
G13	5.71 (0.49)	abcde	5.36 (0.61)	bcde	5.72 (0.21)	abcde	4.19 (0.27)	fghij	6.38 (0.43)	bcdefg	4.96 (0.36)	defg	5.385
G14	4.68 (0.14)	bcdef	6.58 (0.24)	ab	5.83 (0.33)	abcde	3.71 (0.13)	hij	5.78 (0.62)	efgh	4.78 (0.53)	efgh	5.224
G15	6.13 (0.28)	ab	5.68 (0.48)	abcde	6.32 (0.62)	ab	6.05 (0.37)	abc	6.71 (0.35)	abcdef	5.23 (0.27)	cde	6.020
G16	4.97 (0.43)	abcdef	5.94 (0.35)	abcde	5.35 (0.13)	bcde	5.33 (1.11)	abcdefg	6.58 (0.48)	abcdef	6.24 (0.33)	abcd	5.734
G17	5.49 (0.34)	abcdef	4.84 (0.32)	е	5.17 (0.16)	bcde	6.48 (0.09)	а	7.01 (0.16)	abcde	5.18 (0.21)	cdef	5.696
G18	4.66 (0.44)	bcdef	4.86 (0.47)	е	5.36 (0.17)	bcde	4.79 (0.52)	bcdefgh	5.86 (0.17)	defgh	5.35 (0.52)	cde	5.146
G19	5.05 (0.45)	abcdef	4.85 (0.26)	е	6.17 (0.23)	abcd	4.44 (0.39)	defghi	6.63 (0.58)	abcdef	5.71 (0.71)	bcde	5.473
G20	5.91 (0.21)	abc	5.47 (0.43)	abcde	5.46 (0.52)	abcde	4.85 (0.03)	bcdefgh	6.78 (0.21)	abcdef	5.19 (0.28)	cdef	5.608
G21	4.11 (0.13)	f	5.06 (0.47)	de	6.07 (0.18)	abcd	3.12 (0.09)	ij	5.85 (0.33)	defgh	4.76 (0.53)	efgh	4.828
G22	5.88 (0.89)	abc	5.88 (0.53)	abcde	6.27 (0.08)	abc	5.16 (0.23)	abcdefg	5.93 (0.79)	defgh	6.49 (0.55)	abc	5.932
G23	5.43 (0.51)	abcdef	6.79 (0.28)	а	6.26 (0.64)	abc	5.23 (0.54)	abcdefg	5.98 (0.21)	defgh	6.22 (0.42)	abcd	5.985
G24	6.34 (0.34)	а	5.99 (0.67)	abcde	5.35 (0.79)	bcde	4.33 (0.14)	efghij	7.57 (0.19)	ab	5.27 (0.23)	cde	5.807
G25	6.31 (0.45)	а	5.83 (0.24)	abcde	6.79 (0.36)	а	4.24 (0.22)	efghij	7.04 (0.16)	abcde	5.48 (0.51)	bcde	5.951
G26	5.54 (0.46)	abcdef	5.47 (0.46)	abcde	5.86 (0.33)	abcde	3.94 (0.18)	ghij	6.68 (0.13)	abcdef	5.07 (0.55)	def	5.426
G27	5.02 (0.23)	abcdef	6.51 (0.12)	abc	5.32 (0.25)	bcde	5.18 (0.37)	abcdefg	7.11 (0.17)	abcd	5.52 (0.56)	bcde	5.777
G28	5.85 (0.31)	abc	5.29 (0.49)	bcde	5.45 (0.25)	abcde	4.96 (0.29)	bcdefgh	7.61 (0.49)	ab	7.47 (0.39)	а	6.102
G29	6.33 (0.08)	а	5.97 (0.63)	abcde	5.18 (0.19)	bcde	5.64 (0.44)	abcde	6.08 (0.64)	cdefgh	5.82 (0.36)	bcde	5.837
G30	4.24 (0.47)	ef	6.02 (0.13)	abcde	5.47 (0.29)	abcde	5.87 (0.87)	abcd	5.57 (0.62)	fgh	5.13 (0.66)	def	5.382
G31	5.84 (0.52)	abc	5.87 (0.55)	abcde	5.67 (0.22)	abcde	5.48 (0.45)	abcdef	7.59 (0.25)	ab	6.24 (0.25)	abcd	6.116
G32	6.34 (0.44)	а	5.15 (0.38)	de	4.58 (0.17)	е	4.73 (0.39)	cdefgh	7.37 (0.59)	abc	6.72 (0.57)	ab	5.814
G33	5.47 (0.72)	abcdef	4.97 (0.47)	de	4.94 (0.41)	cde	5.44 (0.71)	abcdef	6.67 (0.53)	abcdef	6.72 (0.48)	ab	5.700

**7.2.1 Table 1.** The variety performance mean grain yield at each environment, and grand mean over the environment. Lowercase letters represent the Tukey HSD post hoc test results.

						Environr	nent						
Variety	BLK18		BLK20		HLM18	3	HLM2	0	NGH18		NGH20		Mean
G01	9.91 (2.19)	abc	6.89 (0.77)	ab	10.51 (0.83)	abc	7.85 (0.12)	а	9.23 (1.54)	bcde	5.87 (0.51)	b	8.38
G02	10.93 (2.08)	а	6.55 (0.39)	b	9.21 (2.12)	abc	7.01 (0.17)	а	8.32 (0.45)	cde	7.41 (1.88)	ab	8.24
G03	5.09 (1.01)	d	8.25 (1.37)	ab	8.18 (0.81)	С	6.98 (0.95)	а	7.18 (1.51)	e	7.32 (0.27)	ab	7.17
G04	7.83 (0.86)	abcd	7.21 (1.66)	ab	9.81 (0.64)	abc	6.61 (0.98)	а	8.61 (0.49)	cde	9.84 (3.41)	а	8.32
G05	6.69 (0.86)	bcd	6.48 (0.61)	b	9.29 (0.55)	abc	6.86 (0.52)	а	10.82 (1.22)	abcd	8.74 (0.41)	ab	8.15
G06	9.18 (1.43)	abc	7.25 (0.43)	ab	9.25 (1.13)	abc	6.94 (0.87)	а	7.39 (0.51)	е	9.24 (0.42)	а	8.21
G07	7.11 (1.38)	abcd	8.11 (1.45)	ab	8.67 (0.81)	bc	8.13 (0.89)	а	10.86 (1.11)	abcd	9.57 (0.54)	а	8.74
G08	7.37 (1.56)	abcd	8.41 (0.64)	ab	8.98 (1.06)	abc	7.44 (0.35)	а	9.56 (0.07)	bcde	8.59 (0.26)	ab	8.39
G09	7.67 (1.21)	abcd	7.17 (1.25)	ab	9.71 (0.62)	abc	7.31 (0.78)	а	10.35 (0.83)	bcde	9.34 (1.12)	а	8.59
G10	9.63 (0.43)	abc	7.38 (0.56)	ab	12.07 (0.6)	а	7.11 (0.11)	а	10.11 (0.48)	bcde	8.79 (0.77)	ab	9.18
G11	7.56 (1.25)	abcd	6.38 (0.99)	b	9.67 (0.63)	abc	6.61 (0.32)	а	9.72 (0.71)	bcde	9.62 (0.49)	а	8.26
G12	8.49 (0.58)	abcd	5.95 (0.67)	b	10.3 (0.85)	abc	7.39 (0.65)	а	8.69 (0.93)	cde	7.96 (0.26)	ab	8.13
G13	8.13 (1.15)	abcd	5.64 (0.71)	b	8.28 (0.79)	bc	6.81 (0.74)	а	10.32 (0.59)	bcde	8.05 (1.21)	ab	7.87
G14	9.49 (2.92)	abc	9.93 (0.52)	а	10.5 (1.57)	abc	6.63 (0.46)	а	7.93 (0.64)	cde	7.55 (1.03)	ab	8.67
G15	8.71 (0.52)	abcd	7.15 (0.84)	ab	9.28 (1.61)	abc	8.96 (0.37)	а	9.59 (0.15)	bcde	9.14 (0.83)	ab	8.80
G16	7.21 (1.61)	abcd	7.39 (1.73)	ab	10.92 (1.23)	abc	7.17 (1.11)	а	10.25 (0.75)	bcde	7.76 (0.51)	ab	8.45
G17	7.84 (1.08)	abcd	5.99 (0.73)	b	9.03 (0.11)	abc	7.52 (0.91)	а	9.72 (0.26)	bcde	9.65 (0.32)	а	8.29
G18	6.34 (0.59)	cd	6.64 (1.07)	b	8.15 (0.35)	с	7.88 (0.78)	а	8.21 (0.78)	cde	8.79 (0.29)	ab	7.67
G19	9.12 (1.09)	abc	7.65 (0.35)	ab	9.98 (1.11)	abc	8.56 (1.88)	а	8.47 (0.31)	cde	8.66 (0.67)	ab	8.74
G20	8.11 (0.96)	abcd	6.03 (0.28)	b	8.74 (0.26)	abc	7.32 (0.73)	а	8.79 (1.02)	cde	8.31 (0.28)	ab	7.88
G21	6.89 (0.88)	bcd	6.11 (0.74)	b	11.59 (0.11)	ab	7.74 (0.67)	а	7.88 (1.16)	cde	7.24 (1.53)	ab	7.91
G22	7.96 (0.22)	abcd	7.96 (0.76)	ab	10.23 (1.28)	abc	7.18 (1.25)	а	8.17 (0.61)	cde	9.61 (0.53)	а	8.52
G23	7.91 (1.06)	abcd	8.21 (1.08)	ab	11.14 (0.55)	abc	6.62 (0.68)	а	7.69 (1.34)	de	8.95 (0.74)	ab	8.42
G24	7.66 (1.18)	abcd	7.51 (0.66)	ab	10.48 (0.88)	abc	6.84 (0.28)	а	11.07 (0.46)	abc	7.73 (0.25)	ab	8.55
G25	8.71 (0.64)	abcd	7.17 (1.57)	ab	9.64 (0.85)	abc	8.09 (1.86)	а	10.12 (1.38)	bcde	9.08 (0.25)	ab	8.80
G26	10.28 (1.28)	ab	8.37 (0.46)	ab	11.07 (0.99)	abc	6.73 (0.44)	а	7.31 (0.79)	е	7.93 (0.82)	ab	8.62
G27	7.48 (0.64)	abcd	8.32 (1.15)	ab	10.01 (1.28)	abc	8.65 (0.61)	а	12.25 (0.51)	ab	7.48 (0.46)	ab	9.03
G28	8.42 (0.47)	abcd	7.21 (0.51)	ab	10.22 (0.94)	abc	8.38 (0.83)	а	13.69 (0.73)	а	9.48 (0.16)	а	9.57
G29	9.17 (1.05)	abc	7.87 (1.43)	ab	10.65 (1.33)	abc	6.86 (0.44)	а	7.38 (1.25)	е	8.45 (1.17)	ab	8.40
G30	6.58 (0.55)	bcd	7.65 (1.24)	ab	10.36 (0.17)	abc	8.32 (1.51)	а	7.57 (1.82)	е	8.21 (1.03)	ab	8.11
G31	8.19 (0.53)	abcd	7.28 (1.06)	ab	10.98 (1.32)	abc	6.85 (0.21)	а	8.24 (1.37)	cde	9.26 (1.95)	а	8.47
G32	9.83 (0.91)	abc	7.52 (0.23)	ab	11.25 (1.46)	abc	9.12 (0.49)	а	12.23 (2.11)	ab	9.15 (0.09)	ab	9.85
G33	7.53 (0.46)	abcd	5.69 (0.68)	b	8.89 (1.57)	abc	6.56 (0.27)	а	8.11 (0.69)	cde	9.59 (0.28)	а	7.73

**7.2.2 Table 2.** the variety performance means straw yield (SY) at each environment and grand mean over the environment. Lowercase letters represent the Tukey HSD post hoc test results.

Veriety						Inviror	nment						Maan
Variety	BLK18	}	BLK20		HLM	18	HLM2	20	NGH18		NGH2	0	Mean
G01	30.61 (0.01)	ef	45.5 (2.48)	ab	36.67 (2.08)	С	31.67 (1.53)	efg	37.93 (0.63)	abcd	32.28 (1.66)	f	34.00
G02	40.17 (0.78)	а	35.77 (3.09)	cd	50.67 (1.15)	а	40.33 (0.58)	ab	39.21 (0.44)	abcd	37.62 (1.59)	abcde	42.25
G03	32.4 (2.42)	cdef	36.33 (3.78)	cd	42.33 (2.52)	bc	30.67 (1.15)	fg	39.82 (1.47)	abcd	35.48 (2.35)	cdef	36.08
G04	33.67 (2.48)	bcdef	38.9 (4.06)	bcd	42.67 (2.52)	abc	35.01 (1.02)	bcdefg	39.51 (0.99)	abcd	36.49 (1.31)	bcdef	37.28
G05	32.03 (2.63)	def	43.87 (1.63)	abc	39.67 (0.58)	bc	33.33 (4.16)	cdefg	39.37 (1.68)	abcd	36.01 (0.81)	bcdef	36.55
G06	35.33 (3.72)	abcdef	40.37 (5.2)	abcd	42.01 (2.65)	bc	39.01 (1.01)	abcd	41.74 (1.01)	а	37.82 (2.37)	abcde	39.96
G07	37.23 (1.21)	abcd	37.07 (2.54)	bcd	40.67 (1.15)	bc	37.67 (2.52)	abcde	41.65 (0.61)	а	38.24 (1.84)	abcde	39.30
G08	35.6 (2.78)	abcdef	48.83 (1.55)	а	39.67 (0.58)	bc	36.67 (1.53)	abcdef	40.34 (1.05)	abcd	39.16 (0.99)	abcde	38.08
G09	34.23 (1.86)	abcdef	39.27 (1.78)	bcd	47.01 (6.08)	ab	42.01 (2.65)	а	40.01 (1.69)	abcd	40.46 (1.54)	ab	42.09
G10	37.23 (1.72)	abcd	41.47 (1.75)	abcd	46.01 (5.29)	ab	35.67 (2.08)	abcdefg	38.85 (1.27)	abcd	34.85 (0.41)	ef	38.65
G11	34.67 (2.31)	abcdef	38.6 (4.61)	bcd	43.01 (2.65)	abc	36.33 (3.21)	abcdef	39.96 (1.28)	abcd	41.41 (0.53)	а	39.48
G12	34.97 (0.15)	abcdef	38.73 (1.52)	bcd	40.67 (1.15)	bc	37.33 (2.52)	abcde	37.02 (1.88)	bcd	37.54 (1.41)	abcde	37.69
G13	33.07 (2.14)	bcdef	40.23 (0.32)	abcd	41.01 (1.01)	bc	33.01 (2.65)	defg	36.88 (1.34)	cd	37.44 (1.98)	abcde	36.69
G14	32.9 (1.95)	bcdef	40.07 (2.57)	bcd	40.67 (1.15)	bc	31.67 (3.51)	efg	36.72 (1.41)	cd	36.86 (0.41)	abcdef	36.51
G15	35.13 (1.51)	abcdef	40.73 (3.48)	abcd	39.01 (1.02)	bc	38.01 (1.73)	abcde	41.02 (0.58)	а	37.31 (1.47)	abcde	38.43
G16	34.67 (3.06)	abcdef	39.07 (2.31)	bcd	44.01 (2.01)	abc	37.67 (1.15)	abcde	39.85 (0.79)	abcd	40.01 (1.02)	abc	39.49
G17	36.27 (2.39)	abcde	41.27 (4.82)	abcd	41.01 (1.01)	bc	37.33 (2.08)	abcde	40.01 (0.89)	abcd	35.07 (2.01)	def	38.13
G18	29.67 (0.58)	f	37.13 (2.35)	bcd	43.67 (3.21)	abc	38.01 (0.02)	abcde	38.69 (1.59)	abcd	38.73 (1.71)	abcde	38.34
G19	32.87 (2.21)	bcdef	42.73 (0.41)	abcd	41.33 (1.15)	bc	34.67 (1.15)	bcdefg	38.87 (1.27)	abcd	35.57 (0.73)	cdef	36.74
G20	38.01 (2.01)	abcd	37.9 (1.01)	bcd	41.67 (2.89)	bc	38.01 (1.02)	abcde	38.01 (0.87)	abcd	39.41 (1.13)	abcde	39.64
G21	37.01 (1.73)	abcde	40.33 (1.53)	abcd	40.33 (0.58)	bc	29.67 (1.53)	g	32.36 (0.81)	е	36.23 (1.86)	bcdef	35.58
G22	37.67 (2.52)	abcd	41.67 (1.53)	abcd	43.01 (2.65)	abc	36.01 (1.73)	abcdefg	36.51 (2.16)	d	38.37 (0.87)	abcde	38.65
G23	35.27 (2.61)	abcdef	38.73 (1.27)	bcd	39.02 (1.73)	bc	36.67 (0.58)	abcdef	38.17 (1.79)	abcd	39.51 (1.32)	abcde	38.38
G24	38.57 (1.96)	abc	38.87 (1.96)	bcd	45.01 (2.01)	ab	34.01 (3.61)	bcdefg	39.63 (1.72)	abcd	35.87 (3.04)	bcdef	38.64
G25	34.33 (2.32)	abcdef	40.67 (3.79)	abcd	40.67 (1.15)	bc	34.33 (4.04)	bcdefg	39.97 (0.85)	abcd	37.15 (1.38)	abcde	37.55
G26	37.33 (2.08)	abcd	38.1 (2.13)	bcd	40.33 (1.53)	bc	33.67 (1.53)	cdefg	39.11 (0.89)	abcd	36.17 (1.04)	bcdef	37.88
G27	34.6 (0.53)	abcdef	40.47 (1.64)	abcd	41.67 (1.53)	bc	36.01 (1.02)	abcdefg	39.97 (1.02)	abcd	37.97 (1.78)	abcde	38.05
G28	35.4 (1.11)	abcdef	41.17 (5.29)	abcd	43.33 (2.89)	abc	36.67 (1.53)	abcdef	39.37 (0.55)	abcd	39.98 (0.62)	abc	39.20
G29	35.23 (1.33)	abcdef	40.13 (1.8)	abcd	42.33 (2.52)	bc	37.33 (1.15)	abcde	40.68 (0.96)	abc	39.37 (1.24)	abcde	39.35
G30	35.01 (1.01)	abcdef	39.57 (0.95)	bcd	40.33 (0.58)	bc	34.67 (0.58)	bcdefg	37.87 (1.25)	abcd	39.77 (0.68)	abcd	37.96
G31	35.93 (1.98)	abcdef	40.01 (1.01)	bcd	44.33 (2.08)	abc	36.67 (2.08)	abcdef	40.11 (0.56)	abcd	38.03 (1.52)	abcde	39.11
G32	39.01 (2.01)	ab	42.33 (1.72)	abcd	40.67 (5.13)	bc	35.01 (0.01)	bcdefg	40.87 (1.11)	ab	38.14 (1.81)	abcde	38.95
G33	38.01 (1.01)	abcd	0.00	Letters	42.33 (2.52)	bc	39.67 (0.58)	abc	41.51 (1.47)	а	40.68 (0.73)	ab	40.76

**7.2.3 Table 3.** The variety performance means thousand kernel weight (TKW) at each environment and grand mean over the environment. Lowercase letters represent the Tukey HSD post hoc test results.

						Environ	ment						
Variety	BLK18		BLK20		HLM18	3	HLM20	)	NGH18	3	NGH20		Mean
G01	0.35 (0.04)	bc	0.44 (0.03)	abcd	0.34 (0.02)	abcd	0.35 (0.01)	abcd	0.36 (0.04)	cd	0.38 (0.02)	а	0.37
G02	0.34 (0.02)	с	0.44 (0.02)	abcd	0.39 (0.07)	abc	0.43 (0.01)	ab	0.42 (0.01)	abcd	0.34 (0.06)	а	0.39
G03	0.34 (0.04)	с	0.38 (0.02)	d	0.26 (0.01)	d	0.31 (0.03)	cd	0.42 (0.08)	abcd	0.34 (0.03)	а	0.34
G04	0.44 (0.02)	ab	0.44 (0.04)	abcd	0.39 (0.04)	ab	0.45 (0.05)	ab	0.45 (0.01)	abcd	0.36 (0.08)	а	0.42
G05	0.39 (0.03)	abc	0.48 (0.01)	а	0.39 (0.03)	ab	0.41 (0.01)	abcd	0.39 (0.03)	abcd	0.37 (0.04)	а	0.41
G06	0.34 (0.01)	с	0.41 (0.01)	abcd	0.38 (0.05)	abc	0.44 (0.05)	ab	0.48 (0.03)	а	0.35 (0.01)	а	0.40
G07	0.39 (0.03)	abc	0.44 (0.04)	abcd	0.41 (0.04)	ab	0.37 (0.02)	abcd	0.35 (0.05)	d	0.33 (0.03)	а	0.38
G08	0.39 (0.04)	abc	0.42 (0.01)	abcd	0.37 (0.05)	abc	0.39 (0.02)	abcd	0.42 (0.01)	abcd	0.38 (0.02)	а	0.40
G09	0.42 (0.03)	abc	0.43 (0.02)	abcd	0.38 (0.02)	abc	0.46 (0.04)	ab	0.43 (0.02)	abcd	0.41 (0.03)	а	0.42
G10	0.38 (0.04)	abc	0.42 (0.04)	abcd	0.31 (0.03)	bcd	0.38 (0.02)	abcd	0.41 (0.01)	abcd	0.35 (0.03)	а	0.38
G11	0.44 (0.02)	ab	0.43 (0.03)	abcd	0.33 (0.02)	abcd	0.44 (0.04)	ab	0.39 (0.01)	abcd	0.43 (0.02)	а	0.41
G12	0.41 (0.02)	abc	0.47 (0.02)	abc	0.35 (0.01)	abcd	0.41 (0.05)	abc	0.41 (0.03)	abcd	0.36 (0.01)	а	0.40
G13	0.41 (0.03)	abc	0.49 (0.01)	а	0.41 (0.02)	ab	0.38 (0.01)	abcd	0.38 (0.03)	bcd	0.38 (0.05)	а	0.41
G14	0.34 (0.06)	с	0.39 (0.02)	bcd	0.36 (0.04)	abc	0.36 (0.01)	abcd	0.42 (0.01)	abcd	0.39 (0.06)	а	0.38
G15	0.41 (0.01)	abc	0.45 (0.02)	abcd	0.41 (0.05)	а	0.41 (0.03)	abcd	0.41 (0.02)	abcd	0.37 (0.01)	а	0.41
G16	0.41 (0.06)	abc	0.45 (0.05)	abcd	0.33 (0.03)	abcd	0.43 (0.09)	ab	0.39 (0.03)	abcd	0.44 (0.03)	а	0.41
G17	0.41 (0.03)	abc	0.45 (0.02)	abcd	0.37 (0.01)	abc	0.46 (0.03)	а	0.42 (0.01)	abcd	0.35 (0.02)	а	0.41
G18	0.42 (0.01)	abc	0.42 (0.04)	abcd	0.41 (0.01)	ab	0.38 (0.04)	abcd	0.42 (0.02)	abcd	0.38 (0.02)	а	0.41
G19	0.36 (0.01)	bc	0.39 (0.01)	cd	0.38 (0.03)	abc	0.34 (0.05)	bcd	0.44 (0.02)	abcd	0.39 (0.03)	а	0.38
G20	0.42 (0.03)	abc	0.47 (0.03)	ab	0.38 (0.02)	abc	0.41 (0.02)	abcd	0.43 (0.02)	abcd	0.38 (0.03)	а	0.42
G21	0.38 (0.03)	abc	0.46 (0.03)	abcd	0.34 (0.01)	abcd	0.29 (0.01)	d	0.43 (0.04)	abcd	0.41 (0.08)	а	0.38
G22	0.42 (0.05)	abc	0.43 (0.01)	abcd	0.38 (0.03)	abc	0.42 (0.03)	ab	0.42 (0.04)	abcd	0.41 (0.04)	а	0.41
G23	0.41 (0.02)	abc	0.45 (0.02)	abcd	0.36 (0.03)	abc	0.44 (0.05)	ab	0.44 (0.04)	abcd	0.41 (0.03)	а	0.42
G24	0.46 (0.03)	а	0.44 (0.01)	abcd	0.34 (0.03)	abcd	0.39 (0.01)	abcd	0.41 (0.01)	abcd	0.41 (0.01)	а	0.41
G25	0.42 (0.02)	abc	0.46 (0.04)	abcd	0.41 (0.03)	а	0.35 (0.04)	abcd	0.41 (0.03)	abcd	0.38 (0.03)	а	0.41
G26	0.35 (0.03)	bc	0.39 (0.02)	bcd	0.35 (0.03)	abcd	0.37 (0.01)	abcd	0.48 (0.03)	ab	0.39 (0.05)	а	0.39
G27	0.41 (0.01)	abc	0.44 (0.04)	abcd	0.35 (0.02)	abcd	0.37 (0.03)	abcd	0.37 (0.01)	cd	0.43 (0.01)	а	0.39
G28	0.41 (0.02)	abc	0.42 (0.02)	abcd	0.35 (0.01)	abcd	0.38 (0.04)	abcd	0.36 (0.02)	cd	0.44 (0.01)	а	0.39
G29	0.41 (0.03)	abc	0.43 (0.03)	abcd	0.33 (0.03)	abcd	0.45 (0.04)	ab	0.45 (0.03)	abc	0.41 (0.04)	а	0.41
G30	0.39 (0.01)	abc	0.45 (0.04)	abcd	0.35 (0.01)	abcd	0.42 (0.08)	ab	0.43 (0.07)	abcd	0.39 (0.07)	а	0.40
G31	0.41 (0.02)	abc	0.45 (0.02)	abcd	0.34 (0.02)	abcd	0.45 (0.03)	ab	0.48 (0.03)	а	0.41 (0.05)	а	0.42
G32	0.39 (0.02)	abc	0.41 (0.02)	abcd	0.29 (0.03)	cd	0.34 (0.03)	bcd	0.38 (0.03)	cd	0.42 (0.02)	а	0.37
G33	0.42 (0.03)	abc	0.47 (0.01)	abc	0.36 (0.03)	abc	0.45 (0.04)	ab	0.45 (0.04)	abc	0.41 (0.02)	а	0.43

**7.2.4 Table 4.** the variety performance means harvest index (HI) at each environment and grand mean over the environment. Lowercase letters represent the Tukey HSD post hoc test results.

						Inviro	nment						
Variety	BLK18	3	BLK20		HLM1	8	HLM2	20	NGH18		NGH20	)	Mean
G01	88.67 (6.81)	fghi	91.06 (2.01)	efgh	92.73 (2.33)	abcdef	91.17 (2.25)	abcdefgh	103.33 (7.64)	abcd	111.67 (2.89)	abcd	96.44
G02	104.67 (5.03)	abcd	90.33 (0.58)	ghi	97.55 (2.32)	abcd	97.23 (2.72)	abcd	104.08 (8.66)	abc	115.67 (4.04)	abc	101.59
G03	97.01 (3.61)	bcdefgh	94.67 (0.58)	efg	97.85 (2.15)	abc	85.31 (0.98)	fgh	106.67 (5.77)	ab	112.33 (6.43)	abcd	98.97
G04	103.01 (2.65)	abc	91.08 (2.01)	efgh	95.39 (1.23)	abcdef	94.42 (3.87)	abcdef	104.08 (5.01)	abc	114.05 (3.03)	abc	100.34
G05	104.01 (0.02)	abc	95.33 (0.58)	defg	94.73 (0.64)	abcdef	93.97 (1.05)	abcdef	106.67 (2.89)	ab	117.67 (2.52)	ab	102.06
G06	93.67 (7.09)	cdefghi	92.33 (1.15)	efgh	92.94 (6.92)	abcdef	89.21 (5.19)	bcdefgh	100.01 (0.01)	abcd	103.33 (2.89)	defg	95.25
G07	98.33 (2.89)	bcdefg	94.67 (1.53)	efg	98.52 (1.77)	abc	92.71 (2.52)	abcdefgh	105.01 (5.01)	abc	116.67 (2.89)	ab	100.98
G08	97.33 (1.53)	bcdefg	94.67 (0.58)	efg	89.27 (1.77)	cdefg	85.71 (4.49)	fgh	98.33 (7.64)	abcd	113.33 (2.89)	abc	96.44
G09	100.07 (3.61)	abcde	100.01 (1.01)	bcd	95.26 (2.37)	abcdef	96.63 (2.99)	abcde	114.05 (2.01)	а	119.33 (1.15)	а	104.23
G10	93.33 (2.89)	cdefghi	94.01 (1.02)	efgh	88.77 (2.08)	cdefg	93.01 (2.69)	abcdefg	103.33 (7.64)	abcd	111.67 (2.89)	abcd	97.35
G11	97.08 (1.01)	bcdefg	102.33 (1.15)	b	93.17 (2.02)	abcdef	93.91 (1.21)	abcdefg	101.67 (10.41)	abcd	120.33 (2.52)	а	101.42
G12	81.67 (2.89)	i	81.67 (1.53)	k	80.84 (2.79)	gh	82.47 (5.81)	h	83.33 (5.77)	d	91.67 (2.89)	h	83.61
G13	85.01 (4.05)	hi	74.67 (0.58)	I	78.11 (2.11)	h	86.65 (1.73)	efgh	85.01 (0.02)	cd	96.67 (2.89)	gh	84.35
G14	101.01 (3.61)	abcde	81.33 (1.15)	k	97.47 (1.76)	abcd	99.13 (5.46)	abc	106.67 (2.89)	ab	101.67 (2.89)	efg	97.88
G15	100.33 (1.15)	abcdef	94.01 (1.73)	efgh	93.63 (1.32)	abcdef	99.61 (3.82)	а	106.67 (2.89)	ab	115.01 (3.01)	abc	101.54
G16	103.05 (1.01)	abcd	95.33 (2.52)	defg	85.18 (3.94)	fgh	94.47 (4.88)	abcdef	108.33 (7.64)	ab	120.33 (1.53)	а	101.12
G17	104.33 (5.03)	abcd	85.67 (0.58)	ijk	90.75 (4.55)	abcdefg	92.27 (2.05)	abcdefgh	109.01 (5.01)	ab	101.67 (2.89)	efg	97.28
G18	101.08 (1.02)	abcde	97.01 (2.01)	cde	95.22 (2.75)	abcdef	90.33 (2.52)	abcdefgh	100.01 (14.01)	abcd	116.01 (2.65)	ab	99.94
G19	87.33 (4.04)	ghi	90.67 (1.15)	fghi	85.71 (3.24)	fgh	83.63 (1.35)	gh	90.02 (5.02)	bcd	100.02 (0.02)	fgh	89.56
G20	98.33 (2.08)	bcdefg	95.33 (0.58)	defg	91.83 (3.17)	abcdef	97.77 (2.54)	abcd	103.33 (7.64)	abcd	118.67 (3.21)	ab	100.88
G21	91.67 (6.66)	efghi	90.67 (1.15)	fghi	86.43 (2.53)	efgh	85.31 (2.84)	fgh	98.33 (2.89)	abcd	111.67 (2.89)	abcd	94.01
G22	92.67 (2.08)	defghi	89.33 (1.15)	hij	90.48 (7.59)	bcdefg	87.77 (2.04)	defgh	95.02 (5.01)	abcd	102.33 (2.52)	efg	92.93
G23	95.33 (5.51)	bcdefgh	94.67 (0.58)	efg	88.95 (3.17)	cdefg	88.23 (3.15)	defgh	105.01 (0.02)	abc	106.67 (2.89)	cdef	96.48
G24	93.67 (3.21)	cdefghi	85.01 (0.02)	jk	86.97 (3.33)	defgh	92.53 (2.51)	abcdefgh	101.67 (5.77)	abcd	111.67 (2.89)	abcd	95.25
G25	93.67 (2.31)	cdefghi	90.67 (0.58)	fghi	86.17 (2.47)	fgh	88.87 (1.21)	cdefgh	98.33 (2.89)	abcd	109.07 (0.01)	bcde	94.46
G26	97.67 (4.93)	bcdefg	92.01 (2.01)	efgh	91.7 (3.25)	abcdef	91.93 (4.01)	abcdefgh	101.67 (7.64)	abcd	110.01 (5.01)	bcde	97.50
G27	105.33 (0.58)	abc	100.33 (1.53)	bcd	91.83 (3.82)	abcdef	92.23 (3.09)	abcdefgh	103.33 (2.89)	abcd	120.33 (2.52)	а	102.23
G28	111.33 (1.53)	а	96.01 (5.29)	de	98.41 (2.85)	abc	98.71 (3.63)	abc	114.01 (0.04)	а	119.33 (2.08)	а	106.30
G29	107.01 (2.65)	ab	95.67 (1.15)	def	88.67 (2.31)	cdefgh	96.42 (1.33)	abcde	101.67 (5.77)	abcd	119.02 (1.23)	а	101.41
G30	101.67 (4.93)	abcde	111.33 (0.58)	а	97.01 (2.47)	abcde	99.21 (0.72)	ab	105.01 (17.32)	abc	117.33 (2.52)	ab	105.26
G31	104.01 (2.65)	abcd	113.05 (1.73)	а	100.37 (1.8)	ab	93.69 (1.63)	abcdefg	113.33 (2.89)	а	119.04 (1.07)	а	107.25
G32	106.67 (2.52)	ab	101.33 (1.15)	bc	101.14 (2.79)	а	97.91 (1.93)	abcd	110.03 (5.01)	ab	118.67 (2.08)	ab	105.96
G33	103.01 (5.29)	abcde	90.67 (0.58)	fghi	94.39 (6.25)	abcdef	91.77 (5.56)	abcdefgh	113.67 (3.21)	а	120.67 (2.08)	а	102.36

**7.2.5 Table 5.** the variety performance means plant height (PH) at each environment and grand mean over the environment. Lowercase letters represent the Tukey HSD post hoc test results.