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IMPACT OF NITROGEN FERTILIZATION ON BREAD WHEAT: SCREENING FOR CHANGES IN QUALITY, ANTIOXIDANT AND ESSENTIAL AMINO ACID CONTENT

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ABSTRACT

Wheat grain has a unique nutritional value and contains health-promoting and essential components in the daily human diet. Increasing consumer awareness of health and association of whole grains with several health benefits has led to a greater focus on sustainable and healthy wheat production. The aim of this study was to determine the effect of nitrogen on yield and protein characteristics as well as antioxidant capacity and essential amino acid profile of bread wheat genotypes adapted to different ecological conditions. Different nitrogen doses (0, 60, 120 and 180 kg ha^{-1}) were applied to 15 genotypes (3 lines, 1 hybrid and cultivars) with different growth habit to determine yield, quality, antioxidant and amino acid composition parameters. As a result of this study, total phenol content, antioxidant activity and gluten index of wheat decreased although grain yield potential increased in genotypes. In the results where the genetic factor is the primary focus, it was established that the responses to nitrogen fertilizer doses exhibited variability across different years particularly the case during the dry season. With regard to the YearxNitrogenxGenotype interaction, a notable increase was observed in total phenol content and antioxidant activity, while a decline was evident in yield, protein, and wet gluten parameters, particularly in the nitrogen dose applied during the dry season. The increase in protein content contributed significantly and positively to the essential amino acid composition. However, increasing the amount of some amino acids negatively affects others. The objective of this study is to identify and contribute insights into the impact of nitrogen factor on product quality, health and nutrition issues, grain yield potential of genotypes, plant breeding and agronomic studies.

Keywords: ***Triticum aestivum*** **(L.), nitrogen, protein, gluten, antioxidant, lysine, methionine**

INTRODUCTION

Wheat is a staple food and cultivated globally as supplying >20% of human calories and protein meeting the daily nutrient requirement of global population (Zhang et al., 2024). It has played an indispensable role in influencing society's culture and lifestyle and has constituted an indispensable food source for humanity throughout history. Considering the global climate change, scientists are engaged in formulating novel strategies to develop tolerated wheat varieties to sustain the crop's yield potential. The rising temperatures and irregular rainfall cause an adverse impact on crop productivity, not only leading to a decline in the potential yield of wheat but also quality (Tatar et al., 2020). The quality of the wheat grain has become a primary objective for breeders and farmers, as it is a crucial factor in achieving premium prices and meeting market demands for high-quality wheat products (Rossini et al., 2018). Aside from genetic and environmental factors, nitrogen fertilization exerts a considerable influence and serves as critical agronomic determinant in the formation of both yield and grain quality (Meng et al., 2024). Nitrogen (N) is a significant factor and essential nutrient for cereals, playing a crucial role in crop growth and yield. However, despite its importance, excessive nitrogen application does not substantially improve yields and may have detrimental effects on the environment (Kong et al., 2017; Koppensteiner et al., 2022). Therefore, the timing and application amount of nitrogen fertilization are important factors in achieving optimum yield and quality. N is not only the most important nutrient in terms of yield formation, but it also plays a crucial role in determining and involved in the formation of storage proteins in grains. The application of effective nitrogen fertilization (dosage and timing) allows for the regulation of grain N accumulation in wheat, resulting in an elevated grain protein concentration in response to an increased nitrogen fertilizer input (Massoudifar et al., 2014; Zhang et al., 2017; Zorb et al., 2018). Sohail et al. (2018) demonstrated that the application of nitrogen fertilizer during the early growth stages enhanced vegetative growth and tillering potential. Conversely, the application of nitrogen fertilizer during the stem elongation and grain filling periods led to an increase in grain protein and gluten content, underscoring the pivotal role of nitrogen in determining quality. It is also known that nitrogen (N) requirement for protein synthesis in the developing wheat grain is determined by the mobilization of pre-assimilated N in vegetative tissues (in the range of 50-70%), as well as the direct uptake and assimilation of N during grain filling. However, the mobilization and recycling ability depend on genotype and is also influenced by maturity time (Götz and Erekul, 2023). Moreover, nitrogen fertilization is vital for biochemical components that play a role in human health and nutrition as well as contributing to the agronomic and quality characteristics of wheat. Wheat-based food ingredients and food products rich in natural antioxidants, especially those produced from whole grain products, as a beneficial dietary choice. In recent years, there has been a notable increase in consumer interest in whole grains and their products, largely due to their perceived health-promoting properties (Yu, 2008).

Antioxidants and phenolic compounds are believed to have beneficial effects on human health and also attributed to have protective affect against chronic diseases suggested in epidemiological evidence. They are thought to react directly with free radicals (ROS), thereby reducing peroxides and stimulating antioxidant defense enzyme systems. Free radicals occur naturally within the body however, increasing the level of these free radicals, leading to an imbalance with the existing antioxidants, this can result from certain stress factors (Narwal et al., 2014; Sonntag et al., 2020).

The impact of nitrogen fertilization on grain antioxidant and phenol content has been the subject of several studies, with findings that vary across research. N fertilization caused inverse effects in different fractions of phenolics in wheat. This result was linked to most phenolic compounds are predominantly situated within the external layers of the grain so it is expected that increasing proportion of bran may cause higher phenolic compounds (Stumpf et al., 2015). In general, bran was confirmed as rich in phenolics and antioxidants so it is understood that a lack of nitrogen may result in an increase in the bran layer within the grain, which can be attributed to the fact that the grains are unable to form adequately due to the absence of nitrogen (Mazzoncini et al., 2015).

Wheat grain is a significant source of protein in human nutrition, in addition to its role in maintaining health. In addition to protein quality, amino acids are regarded as nutrients that provide essential amino acids for a healthy diet. Wheat proteins are characterized by a relatively low content of essential amino acids, including lysine, tryptophan and threonine, which are crucial for human nutrition. These limited amino acids in question serve to determine the quality of the protein, which in turn affects the amino acid composition of the grain (Zhang et al., 2017; Siddiqi et al., 2020). Anjum et al. (2005) provide considerable insight into newly released wheat varieties, exhibiting superior nutritional characteristics particularly in terms of essential amino acids, with notable enhancements observed in lysine levels. This may be attributed to the higher nitrogen requirement of the new varieties. Amino acid composition basically depends on the genotype and environmental factors, nitrogen application time and concentration in the soil, availability of water budget and temperature in grain filling periods (Qabaha, 2010).

Amino acids represent the primary forms in which nitrogen is remobilized from leaves to the grain during the maturity period. Therefore, to improve the nutritional value of wheat, it is essential to ensure an optimal nitrogen supply. Zhang et al. (2017) observed a notable elevation in essential amino acid concentrations in response to augmented nitrogen doses. Both essential and non-essential amino acid profiles demonstrated a linear correlation with protein content, exhibiting a concurrent increase. With in the light of this knowledge, our objective was to investigate the nitrogen and genotype-related changes in healthy compounds and nourishment properties of wheat grain, in addition to yield and quality responses. Furthermore, we focused on evaluating and gain insight into the evaluated characteristics of varieties with different growth habits and newly released lines.

MATERIALS AND METHODS

A field experiment was conducted in Aydın (37°45′22′′N 27°45′36′′E) ecological conditions during 2017 and 2018 growing seasons. The material set consist of 15 wheat genotypes with different genotypic and agronomic characteristics: 3 advanced lines, 11 cultivars and 1 hybrid wheat shown in Table 1. The genotypes were grown in five nitrogen doses (0, 60, 120 and 180 kg ha^{-1} hereafter referred to as N_0, N_{60}, N_{120} and N_{180}). The experimental soil type (sampled in the topsoil to a depth of 0.30 m) is loamy sand with 69.9% sand, 21.3% silt, and 8.65% clay with typical alkaline soil pH level (8.0). The experiment was laid out in randomized split plot design with three replications where N doses were applied as the main plot effect. Plot size and sowing distance between rows were 1,2 x 6 m and 20 cm, respectively. Experiment area consisted of 180 sub-plots, in total carried out on 3360 m^2.

Nitrogen mineral fertilization was split as follows; 30 kg ha^{-1} as 20.20.20 form applied prior to the sowing for all doses without N_0. For treatments N_{60}, N_{120}, N_{180} when first tiller visible (BBCH 21 growth period) 30 kg ha^{-1} applied for N_{60}, 45 kg ha^{-1} applied for N_{120}, and 75 kg ha^{-1} applied for N_{180}. At stem elongation stage (BBCH 31) 45 kg ha^{-1} applied for N_{120} and 75 kg ha^{-1} applied for N_{180} in the form of urea (46% N).

Weeds and diseases were controlled by using chemical control throughout the growing seasons. In tillering stage weed control was provided by herbicides: Arrat® (25% triosulfuron and 50% Dicamba) and Topcup® 240 EC (240 g/l Clodinafop-propargyl) and in the beginning of heading Sonfix® 5 EC (50 g/l Diniconazole) fungucide applied for wheat leaf rust diseases (*Puccinia* spp.) during 2017 and 2018 seasons.

Table 1. General characteristics, origin and description of genotypes

Genotype	Growth habit	Spike type	Institute/company
Golia	Facultative	White, awned	Italian origin, Tigem
Kate A	Facultative	White, awnedness	Bulgarian origin, Alfa seed
Selimiye	Winter	Red, awnedness	Trakya Agricultural Research Institute
Ceyhan 99	Facultative	White, awned	Eastern Mediterranean Agricultural Research Institute
Tosunbey	Facultative	White, awned	Field Crops Central Agricultural Research Institute
İkizce-96	Winter	White, awned	Field Crops Central Agricultural Research Institute
Müfitbey	Winter	White, awned	Transitional Zone Agricultural Research Institute
Line 1	Winter	Red	IWWIP Programme, Bahri Dağdaş International Agricultural Research Institute. CHAM//1D13.1/MLT/4/C126-6/C190-12//AU/3/TZPP/BEZ
Line 2	Winter	Red	IWWIP Programme, Bahri Dağdaş International Agricultural Research Institute. ZANDER-17/3/KAUZ*2/YACO//KAUZ
Line 3	Winter	Red	IWWIP Programme, Bahri Dağdaş International Agricultural Research Institute. PLK70/LIRA"S"/5/C126-15…/4/KRC/7/NECOMP1/5/BEZ//TOB/8156/4/ON/3/TH*6/ KF//LEE*6/K/6/TAST/SPRW..
Eraybey	Winter	White, awned	Bahri Dağdaş International Agricultural Research Institute
Bozkır	Winter	White, awned	Bahri Dağdaş International Agricultural Research Institute
Euclide	Winter	White,awned	Syngenta Seed, Quality group: A (High)
Julius	Winter	White, awnedness	KWS Seed, Quality group: A (High)
Hybery*	Winter	White, awnedness	Saaten-Union GmbH, Quality group: B (good), Hybrid

Hybery: Hybrid wheat cultivar seeds obtained for sowing in both experimental years.

Table 2. Monthly and long term (1941-2018) meteorological conditions during the growing seasons

Months	Mean Temperature (°C)			Total Precipitation (mm)		
	2017	**2018**	**1941/2018**	**2017**	**2018**	**1941/2018**
November	12.7	11.6	13.4	51.4	85.0	83.3
December	5.3	10.3	9.5	11.9	98.9	121.7
January	5.9	8.8	8.1	221.5	119.2	116.5
February	9.2	12.5	9.4	21.7	112.9	93.8
March	12.2	15.2	11.8	112.5	90.4	71.1
April	15.2	20.9	15.9	46.4	8.2	48.2
May	20.1	24.6	20.9	45.0	71.0	35.7
June	25.3	27.0	25.8	16.0	28.5	13.9
Mean T°C/mm*	13.2	16.3	14.3	526.4	501.2	584.2

*: Mean temperature and precipitation values during long term and growing seasons, Turkish State Meteorological Service (Mevbis). Mean annual temperature for long term: 17.7°C, total annual precipitation amounts for long term: 647.0 mm

Annual temperature, precipitation and long-term climate values are shown in Table 2. In 2017 the seedlings suffered from the lower (12.7, 5.3 and 5.9°C) field temperature (especially facultative growth habit cultivars) compared to long term values. In the first year of experiment, deficit rainfall amount observed in November (51.4 mm) and December (11.9 mm) but in January excess rainfall observed and caused short time flooding stress in wheat seedlings during field emergence period. In generally the weather conditions were favorable (compared to long term conditions) in 2017 during springtime and generative growth period. In terms of mean temperature, favorable values observed in vegetative development periods in 2018 compared to long term values, but it is noteworthy that March, April and May had high temperature (and also April had lowest precipitation value) values compared to long term and first year of experiment. This situation caused adverse conditions during flowering period and caused shortening of grain filling period. Furthermore, the unfavorable climatic conditions observed in 2018 resulted in a significant acceleration in the average number of days to ear emergence and the average number of days to flowering, with both occurring 12 and 11 days earlier, respectively, compared to the first year of the study.

Border lines in each plot were removed at both harvesting times (7th of June 2017 and 24th of May 2018) and the remaining area was harvested to calculate the yield. The harvested seeds were then threshed, cleaned and stored at 4°C until chemical analysis. Grain protein content (%) was determined by Velp® Dumas Nitrogen Analyzer in Aydın Adnan Menderes University Agricultural Biotechnology and Food Safety Laboratory (ADU-TARBIYOMER) according to AOAC 997.09 method. Bread making quality traits were measured following the Standard Method of the International Association of Cereal Chemistry (ICC). Wet gluten and gluten index were determined with the Bastak® 6000 (ICC Standard No. 137/1) and Bastak® Index 2002 (ICC Standard No. 155) devices with using whole grain flour (milled with Perten Laboratory Mill to 0.8 mm). For the extractions used to determine total phenolic content (and total antioxidant activity, 1 g of ground whole wheat flour sample was mixed in acidic methanol solution (HCl/methanol/water; 1:80:10, v/v) in a shaker (Gerhardt, Thermoshake) under nitrogen gas for 1 h, then centrifuged (Hettick) at 5000 rpm for 20 min. The resulting extracts were transferred to tubes and stored at +4 ºC until analysis (Beta et al., 2005; Ragaee et al., 2006; Ma et al., 2014). Total phenolic content of wheat samples was determined according to the Folin-Ciocalteu method described by Kaluza et al. (1980) and Ragaee et al. (2006) by using gallic acid as standard. The total antioxidant activity of wheat samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to the method proposed by Brand Williams et al. (1995). The absorbance measurements of total phenolic content (725 nm wavelength) and antioxidant activity (517 nm wavelength) extracts were determined by Thermo Scientific spectrophotometer. The amino acid analysis was performed by using high-performance liquid chromatography (Shimadzu Nexara, HPLC System) at the Research and Application Center of Drug development and Pharmacokinetics, Ege University. For this purpose, seven essential amino acids were identified based on the procedure chosen depends on oxidation (for methionine) and hydrolysis (threonine, valine, isoleucine, leucine, phenylalanine and lysine) analysis of samples. The experimental data were subjected to analysis of variance for each parameter and all data were analyzed by using ANOVA and LSD test techniques of Tarist statistical analysis software (Acikgoz et al. 2004). In addition, the correlogram was performed in R studio using the "metan" package (Olivoto and Lúcio, 2020).

RESULTS AND DISCUSSION

Impact of Varied Nitrogen Applications on Grain Yield and Quality traits

Results of statistical analysis for the effects of experimental factors [Year (Y), Nitrogen Applications (N) and Genotype (G)] on yield, protein and bread-making quality parameters of bread wheat with mean square values and significant levels (*: $p \leq 0.05$, **: $p \leq 0.01$) were given in Table 3. When the results of the analysis of variance of nitrogen applications and genotypes combined for two years were examined, grain yield and quality traits were affected by year (except wet gluten), nitrogen and genotypes. In addition, YxN, YxG, NxG, and YxNxG interactions were statistically significant at the $p \leq 0.05$ and 0.01 levels (Table 3).

Table 3. ANOVA results (mean square values and significance levels) for grain yield and quality traits

Experimental factors	**Grain Yield (kg ha^{-1})**	**Crude Protein Content (%)**	**Wet Gluten Content (%)**	**Gluten Index (%)**
Year (Y)	2090756.5**	570.6**	0.4	4165.4*
Nitrogen (N)	1139854.7**	70.4**	1139.9**	989.3**
Genotype (G)	75057.9**	7.7**	237.8**	1498.5**
YxN	129171.9**	6.8**	85.3*	797.2**
YxG	132242.2**	9.5**	149.6**	541.2**
NxG	28282.8**	3.3**	64.6**	314.0**
YxNxG	19116.8**	3.0**	79.3**	377.0**

Significance levels: *: $p \leq 0.05$, **: $p \leq 0.01$

In 2017, favorable climatic conditions (lower mean temperature and sufficient precipitation in generative growth stages) were conducive to wheat development. However, in the 2018 season, the mean temperature values increased from February and were observed to be higher than the long-term average values by approximately 4°C during the grain filling period. In springtime, the precipitation levels in April were below the long-term average and the previous year's levels (8.2 mm). These conditions negatively affected wheat development (occurred at an earlier period: ear emergence and flowering) and grain filling, resulting in a decrease in grain yield (Tatar et al., 2020). The occurrence of post-flowering drought (decrease in spring rainfall) in wheat resulted in a detrimental impact on yield, leading to a reduction in grain yield (Aykut Tonk et al., 2011; Tatar, 2011). The first experimental yield values demonstrated an increase due to the lower mean temperature values and a good water supply (precipitation) condition and the application of elevated nitrogen fertilizer doses (N_{120} and N_{180}). The hybrid variety (Hybery) demonstrated a remarkably high yield value in the first year of the trial, under conditions of favorable climatic circumstances (a cooler winter season) and an N_{60} nitrogen dose. Furthermore, among the genotypes evaluated in the study, and those with a winter growth habit exhibited the highest yield values at the N_{120} nitrogen dose. In general, the cultivars exhibited a good response to increasing nitrogen doses (especially N_{120} and N_{180}) in terms of yield, reaching a high yield potential at these doses (except Julius and Hybery).

The newly released wheat lines (Line, 1,2 and 3) had also high yield potential compared to other genotypes. Additionally, they demonstrated a notable capacity for responding to nitrogen fertilization. The yield potential of the lines demonstrated positive response with the increasing nitrogen fertilizer dosages (Table 4).

The protein content demonstrated a positive response to both climatic conditions and increasing nitrogen doses. In conditions of elevated temperature and water scarcity, the supply of assimilates may be constrained, leading to a reduction in starch, protein and the nutritional value of the grain. The accumulation of starch and protein is determined during the early grain-filling period, with the final size of the cells influenced by water stress during this same period. Furthermore, exposure to elevated temperatures (>30°C) accelerates the grain-filling process, leading to a shortage during this period (Dupont and Altenbach, 2003). In 2017, the protein content of the grain increased under conditions of favorable climate (enough water supply and lower mean temperature conditions in grain filling) and with an increasing dose of nitrogen fertilizer. In contrast, in 2018, due to conditions of both unfavorable climate (higher mean temperature and lower precipitation values in grain filling) and unfavorable conditions, the protein content of the grain reached a sufficient level only at the highest nitrogen dose. It was demonstrated that the method of application of nitrogen fertilizer influences the grain nitrogen content. In particular, it was observed that the protein content increased in proportion to the amount of nitrogen fertilization. Furthermore, it was indicated that the grain nitrogen content was derived from the transport of nutrients from the stem and leaf parts. Additionally, it was observed that 50% of the nitrogen was absorbed from the soil following the flowering stage (Zorb et al., 2018). Accordingly, under dry season conditions subsequent to this period result in considerable reductions in protein content. In both experimental years, the hybrid cultivar (Hybery, B Quality Class) demonstrated a linear increase (from 10.5-11.7% to 14.1-14.5%) in protein content with increasing nitrogen doses. Similarly, other varieties (Selimiye, Ceyhan 99, Tosunbey, Line 2, Julius) exhibited a considerable increase at the same level. In previous studies, a negative relationship between grain protein ratio and grain yield was generally stated, but protein ratio increased with the increase in grain yield in the study due to nitrogen fertilizer applications (Aydogan et al., 2018; Cosentino et al., 2018). It is hypothesized that the primary cause of this phenomenon is the accumulation of protein in the milk stage, which is composed of protein previously accumulated in the stems and leaves. The reduction in protein content during the dry season (2018) can be attributed to the accumulation of less dry matter in the plant, which is a consequence of suboptimal developmental periods. (Naneli et al., 2015).

Wheat grain has a unique value for nutrition and production of bakery products. The protein content determines the ability of wheat flour to perform an important role in carbon dioxide retention, dough flexibility and development, and ultimately baking quality (Khalid et al., 2023). Basically, gluten content and its stability (index) are responsible for determining baking and bakery product quality. (Sharma et al., 2020). The significance of nitrogen regarding quality was underscored by the finding that nitrogen fertilizer applied at an early stage enhanced vegetative growth and tillering potential, whereas applied during the stem elongation and grain filling periods elevated grain protein and gluten content (Sohail et al., 2018). In both growing seasons, the wet gluten content (%) was observed to be almost generally lower in the initial three applications (N_0, N_{60} and N_{120}) of nitrogen doses, while a significant increase was noted with the highest nitrogen dose (N_{180}). This situation is more clearly understood, particularly during the dry season (2018) of the study. The application of nitrogen with increasing dosages was found to result in an increase in gluten content during the dry season. In general, a reduction in the quantity of wet gluten was observed in winter growth habit genotypes as the quantity of nitrogen applied was diminished. This phenomenon was most evident in the Line 2, Eraybey, Bozkır, Line 3 and Euclide genotypes. Moreover, these genotypes exhibited a positive response to the highest nitrogen dose (N_{180}) applied, demonstrating a notable increase in the quantity of wet gluten. In regard to the quality traits of the bread, the wet gluten ratio exhibited a range of 16.6-55.0%, with an observable increase in the gluten ratio corresponding to an increase in the nitrogen fertilizer doses. Gluten index values ranged between 41.4 and 98.9%, demonstrating an increase with the application of elevated nitrogen doses. However, the highest value was observed at a nitrogen dose of N_{120} kg ha^{-1}, rather than the anticipated N_{180} kg ha^{-1} for both bread-making quality traits (Table 5). Consequently, the application of nitrogen had a considerable positive impact on the quality traits of the bread (Erekul et al., 2012a; Basyigit Koseoglu et al., 2024). The gluten index value, which is used to determine the strength of gluten, was observed to be generally higher during the dry growing season (2018) a finding that is consistent with the results reported by Barutcular et al. (2016). Furthermore, it was observed that there was an increase in gluten strength with a reduction in the quantity of wet gluten.

Table 4. Effects of nitrogen application on yield and protein content of evaluated genotypes in the period of 2017 and 2018.

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Grain Yield (kg ha^{-1})	Golia	2300	3013	6335	5967	1544	2708	4244	4490
	Kate A	3662	4736	7657	7163	1743	2721	4484	5245
	Selimiye	3783	4410	6539	5204	2624	4262	4007	4704
	Ceyhan 99	2369	2562	5767	5828	1621	1964	2346	2703
	Tosunbey	2370	2555	2491	3916	1356	2620	3605	3576
	İkizce-96	2350	2382	5802	5424	2020	2220	4521	4472
	Müfitbey	2339	2393	4360	4778	879	3263	3291	3838
	Line 1	3009	3168	4666	7524	2357	3356	3680	4603
	Line 2	2660	2634	4465	6429	1704	3864	3138	4961
	Eraybey	2780	2554	3982	4734	2139	5697	3671	4408
	Bozkır	1801	1217	5854	5765	2194	3029	3318	3513
	Line 3	3034	3703	8111	5590	1102	3592	3858	3876
	Euclide	2449	2760	7105	3162	955	2385	2313	2644
	Julius	4303	6314	5255	6767	1213	1096	2052	2028
	Hybery	6463	8349	7012	6103	465	2417	2490	1951
	Mean	3044	3516	5693	5623	1594	3012	3401	3800
	Lsd Y: 609; Lsd N: 228; Lsd YxN: 323; Lsd G: 442; Lsd YxG: 626; Lsd NxG: 885; Lsd YxNxG: 1252								
	N (kg ha^{-1}) /G	**2017**				**2018**			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Protein Content (%)	Golia	14.3	11.8	14.7	15.5	10.9	10.5	12.6	11.4
	Kate A	10.7	13.0	14.8	14.1	11.1	8.9	9.3	10.6
	Selimiye	11.3	12.7	13.7	13.7	9.3	8.3	11.0	11.3
	Ceyhan 99	12.0	12.8	14.0	14.7	9.0	8.1	11.4	12.7
	Tosunbey	11.4	14.1	14.9	14.2	9.4	10.1	10.0	12.9
	İkizce-96	13.2	14.0	15.4	14.8	11.5	9.8	11.7	12.7
	Müfitbey	13.6	14.1	14.5	14.6	9.8	8.5	10.6	11.9
	Line 1	15.0	12.1	13.9	14.4	10.2	9.5	10.3	11.3
	Line 2	12.5	12.6	12.9	13.3	9.0	8.3	10.7	14.4
	Eraybey	12.9	11.8	12.6	12.4	10.2	9.6	9.5	10.8
	Bozkır	14.1	15.7	12.3	13.0	8.8	9.2	9.9	10.3
	Line 3	12.3	11.2	11.9	13.5	9.6	8.5	9.4	11.5
	Euclide	10.4	9.0	11.5	13.1	12.6	9.4	10.8	11.4
	Julius	12.2	13.2	12.7	13.5	9.0	12.4	11.4	13.4
	Hybery	10.5	10.8	13.1	14.1	11.7	11.3	11.5	14.5
	Mean	12.4	12.6	13.5	13.9	10.1	9.5	10.7	12.1
	Lsd Y: 0.23; Lsd N: 0.40; Lsd YxN: 0.57; Lsd G: 0.79; Lsd YxG:1.12; Lsd NxG: 1.58; Lsd YxNxG: 2.24								
	color diagram: red-white-green. This sequence is characterized by an increase in value from left to right								

Table 5. Effects of nitrogen application on gluten content and gluten index of evaluated genotypes in the period of 2017 and 2018.

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Wet Gluten Content (%)	Golia	32.4	27.6	33.2	39.6	27.8	25.5	26.2	35.7
	Kate A	29.8	34.1	35.9	41.1	41.1	30.0	32.5	41.6
	Selimiye	23.6	28.0	33.3	35.3	30.9	22.6	32.8	36.2
	Ceyhan 99	25.4	32.0	27.1	33.5	27.0	18.4	30.7	37.9
	Tosunbey	37.4	42.2	38.8	30.6	23.8	31.1	27.4	31.9
	İkizce-96	35.1	37.9	25.4	41.2	36.5	29.6	32.1	36.9
	Müfitbey	27.8	28.6	33.1	46.0	30.9	19.8	31.7	38.3
	Line 1	32.9	33.8	34.1	36.4	28.1	24.6	30.2	40.7
	Line 2	28.4	22.7	55.0	29.6	27.9	20.1	28.6	40.1
	Eraybey	29.8	17.8	29.5	28.0	24.6	29.6	29.3	35.8
	Bozkır	30.1	19.6	34.2	38.3	26.9	30.4	25.8	35.8
	Line 3	28.6	26.1	32.5	35.2	30.3	41.7	36.9	34.8
	Euclide	19.5	16.6	22.0	25.0	28.6	25.6	31.9	36.8
	Julius	33.4	32.2	41.9	45.7	41.2	37.6	34.4	41.1
	Hybery	31.6	24.7	25.3	24.8	34.5	31.7	32.1	39.0
	Mean	29.7	28.3	33.4	35.3	30.7	27.9	30.8	37.5
	Lsd N: 1.4; Lsd YxN: 1.9; Lsd G: 2.7; Lsd YxG: 3.8; Lsd NxG: 5.4; Lsd YxNxG: 7.6								
	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Gluten Index (%)	Golia	67.1	81.5	67.7	75.4	86.6	84.7	92.6	81.7
	Kate A	41.4	49.7	61.2	41.9	52.2	73.2	67.8	53.3
	Selimiye	81.5	75.4	71.6	53.3	52.9	84.6	86.3	64.1
	Ceyhan 99	81.1	67.6	98.9	66.2	88.4	78.8	89.6	70.8
	Tosunbey	76.2	60.2	88.8	85.8	87.5	83.4	78.5	92.7
	İkizce-96	56.1	51.3	70.7	60.1	59.1	73.7	87.1	75.1
	Müfitbey	68.8	80.9	62.7	59.9	74.0	93.2	89.1	74.4
	Line 1	77.8	57.5	43.6	73.3	78.8	84.5	78.9	76.7
	Line 2	66.3	57.6	63.0	78.1	70.6	86.5	75.2	59.3
	Eraybey	77.4	88.1	75.1	66.2	85.7	70.8	94.4	63.1
	Bozkır	68.8	93.6	45.2	49.0	85.5	69.1	95.7	87.8
	Line 3	76.7	59.4	52.7	51.5	66.7	57.7	69.3	58.7
	Euclide	85.8	78.9	86.2	88.9	72.6	83.5	73.4	72.1
	Julius	70.9	70.3	42.2	45.4	60.3	61.3	79.0	63.4
	Hybery	89.0	55.3	87.6	76.0	65.2	65.6	73.1	48.1
	Mean	72.3	68.5	67.8	64.7	72.4	76.7	82.0	69.4

Lsd Y: 2.9; Lsd N: 2.9; Lsd YxN: 4.2; Lsd G: 5.7; Lsd YxG: 8.1; Lsd NxG: 115; Lsd YxNxG: 16.3
color diagram: red-white-green. This sequence is characterized by an increase in value from left to right

The gluten index value was observed to be generally lower at the highest nitrogen dose, and there was a concomitant decrease in gluten stability. Overall, nitrogen is found to be important factor affecting protein and gluten content and its quality largely influences dough and rheological properties of wheat (Hao et al. 2023).

Total phenolic content (µg GAE/g) and antioxidant activity (% inhibition) results

Wheat comprises a significant proportion of the human diet and has a valuable source of carbohydrates and protein and contains phytochemicals that confer significant health benefits (Punia and Sandhu, 2015). Phenolic compounds and antioxidants are the primary chemical components that directly interact with oxygen reactive species (ROS), which serve to protect cells from DNA damage. Whole grain products are well documented to be rich in phenolic compounds, which are primarily concentrated in the outer layers of grains, including aleurone, testa, and pericarp (Kerienė et al., 2015; Martini et al., 2015). The ANOVA results of total phenolic content and antioxidant activity with mean square values and significant levels (*: $p \leq 0.05$, **: $p \leq 0.01$) were given in Table 6. Total phenolic content and antioxidant activity of wheat grain were statistically significantly affected by Nitrogen Applications (N) and Genotype (G)] factors with $p \leq 0.01$ level while Year (Y) had $p \leq 0.05$ level. In addition, YxN, YxG, NxG, and YxNxG interactions were statistically significant at the $p \leq 0.01$ level.

Table 6. Total phenolic content and antioxidant activity ANOVA results (mean square values and significance levels)

Experimental factors	Total Phenolic Content (µg GAE/g)	Total Antioxidant Activity (% Inhibition)
Year (Y)	41763509.6*	2951.0*
Nitrogen (N)	5416567.0**	248.8**
Genotype (G)	1626642.0**	309.2**
YxN	1412684.2**	359.8**
YxG	1044935.6**	346.0**
NxG	949391.8**	354.4**
YxNxG	1018981.3**	340.9**

Significance levels: *: $p \leq 0.05$, **: $p \leq 0.01$

Nitrogen is an essential nutrition that causes major changes in grain chemical composition and end-use quality (Kong et al., 2017). We observed significant changes on phenolic content and antioxidant activity caused by nitrogen application.

When the year factor was taken into account, it was evident that higher values were obtained in the dry season for both parameters. It was determined that the total phenolic content and antioxidant activity of wheat grain increased in the dry season (2018). In general, lower values were obtained under favorable climatic conditions. Phenolic acids are secondary metabolites that are synthesised as a defence system against stress conditions. As with many other antioxidants, they are mainly located in the outer layers of grain. With regard to stress conditions (water deficit and high temperature), an increase in antioxidant synthesis has been observed in cereals during the grain filling period (Zrcková et al., 2018). The primary cause of this result can be attributed to the observation of elevated values during the dry and hot season following the flowering period in the second year. This situation is hypothesized to be a consequence of the expansion of the bran layer and fiber component, which is accompanied by a reduction in grain weight (Žilić et al., 2012). The total phenol content of bread wheat genotypes at varying nitrogen doses over two years exhibited in a wide range of 771.9-5179.9 µg GAE/g, with the highest value observed in the Golia variety at N_0 kg ha^{-1} nitrogen dose during the 2018 season, and the lowest value noted in the İkizce variety at N_0 kg ha^{-1} nitrogen dose during the 2017 season (Table 7). Stumpf et al. (2015) suggested that the application of nitrogen fertilizer resulted in inverse effects on the concentration of phenolic compounds are predominantly located in the outer layers of the grain, suggesting that an increase in the proportion of bran fraction within the grain may potentially lead to an elevated concentration of phenolic compounds. The phenolic content of some genotypes (Line 2, Eraybey, Bozkır, Euclide and Hybery) exhibited a notable increase in general at the highest nitrogen dose during the dry season. However, for some of the genotypes (Golia, Line 3 and Julius), the lowest dose of nitrogen resulted in the highest values. The highest antioxidant activity values were obtained from Line 2 with N_{60} kg ha^{-1} and Bozkır variety with N_0 kg ha^{-1} nitrogen applications (Table 7).

Table 7. The mean values of total phenolic content (µg GAE/g) and total antioxidant acitivty (% inhibition)

	N (kg ha^{-1}) /G	**2017**				**2018**			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Total Phenolic Content (µg GAE/g)	Golia	2118.7	1731.0	2187.8	3564.3	5179.9	2530.6	2614.4	2842.6
	Kate A	1943.0	2189.0	1788.8	1722.1	3274.1	2319.2	2404.6	2386.2
	Selimiye	2158.6	1362.9	1948.7	1905.3	2411.8	2388.3	2406.5	2884.4
	Ceyhan 99	2472.2	2508.6	2747.7	2375.6	2244.0	2217.7	2037.2	2467.1
	Tosunbey	3892.3	1405.0	1555.3	2271.2	2591.2	2393.9	2538.2	2568.6
	İkizce-96	771.9	1980.3	1806.7	1799.8	3495.8	2615.8	1960.9	2461.9
	Müfitbey	1526.4	1606.1	2136.3	2167.6	2982.0	2406.6	2310.8	2853.0
	Line 1	1727.1	2066.4	1863.5	1963.1	2392.4	2283.4	2446.0	2534.9
	Line 2	1712.7	2746.8	2239.8	1718.7	2278.7	2768.2	2368.3	4143.1
	Eraybey	1750.2	2128.0	1578.6	2166.1	2775.0	3164.6	2538.4	3976.2
	Bozkır	4553.6	2282.2	2464.5	1842.8	3143.6	3181.0	2286.5	3406.3
	Line 3	3549.4	2310.7	2347.8	2170.6	5146.7	2137.8	2384.2	2902.4
	Euclide	2051.9	1708.5	1628.9	2298.0	2561.9	2887.9	2084.4	2813.0
	Julius	1778.4	2051.1	1845.0	1819.8	4350.6	2973.7	2747.2	3136.7
	Hybery	2165.3	2005.8	1914.0	2121.9	2832.3	2619.7	2475.3	3558.8
	Mean	2278.1	2005.4	2003.5	2127.1	3177.3	2592.5	2373.5	2995.6
	Lsd Y: 620.9; Lsd N: 121.4; Lsd YxN: 171.7; Lsd G: 235.1; Lsd YxG: 332.5; Lsd NxG: 470.2; Lsd YxNxG: 664.9								

	N (kg ha^{-1}) /G	**2017**				**2018**			
		N_0	**N_{60}**	**N_{120}**	**N_{180}**	**N_0**	**N_{60}**	**N_{120}**	**N_{180}**
Total Antioxidant Activity (%)	Golia	47.1	16.9	23.4	53.9	42.4	22.4	27.3	28.4
	Kate A	20.7	22.6	18.9	20.1	17.4	27.2	30.7	33.9
	Selimiye	22.7	6.8	28.7	21.4	28.5	32.1	26.8	30.4
	Ceyhan 99	28.3	26.5	39.9	37.9	30.7	29.5	28.5	29.3
	Tosunbey	46.1	12.5	14.7	22.6	15.3	27.5	30.6	48.6
	İkizce-96	15.9	30.2	18.6	31.3	50.4	26.4	29.7	30.0
	Müfitbey	19.6	23.0	32.4	25.4	26.4	25.2	32.2	30.5
	Line 1	22.9	32.5	40.7	22.3	14.1	33.8	36.4	39.2
	Line 2	10.0	64.2	24.3	16.7	28.2	25.7	27.6	36.7
	Eraybey	39.6	19.5	20.0	44.4	32.7	30.2	26.6	34.5
	Bozkır	61.4	12.6	30.4	9.9	52.2	35.8	35.6	35.4
	Line 3	51.3	23.6	25.0	33.9	12.5	31.8	34.7	38.1
	Euclide	15.9	13.3	13.7	15.7	14.0	29.0	41.9	27.7
	Julius	18.8	20.4	11.6	13.5	29.6	44.3	28.4	36.1
	Hybery	8.9	19.7	19.6	22.6	32.9	32.7	29.2	45.0
	Mean	28.6	23.0	24.1	26.1	28.5	30.2	31.1	34.9
	Lsd Y: 4.0; Lsd N: 1.1; Lsd YxN: 1.6; Lsd G: 2.2; Lsd YxG: 3.2; Lsd NxG: 4.5; Lsd YxNxG: 6.4								

color diagram: red-white-green. This sequence is characterized by an increase in value from left to right

Similar to the results obtained for the total phenolic content, an increase in antioxidant activity values was observed in some genotypes at the highest nitrogen dose in the dry season. The similar values and responses to N applications in terms of total phenolic content and antioxidant activity results are thought to be due to the high correlation between phenolic content and antioxidant activity as noted in previous studies (Mpofu et al., 2006; Verma et al., 2008; Žilić et al., 2012; Arshad vd., 2017; Boukid et al., 2019).

Essential amino acids profile (g 100 g^{-1})

Protein quality depends on the profile of essential amino acid present in foods. The amino acid composition of cereal grains is largely determined by the endosperm, which constitutes approximately 80% of the grain's weight. The aleurone and embryo tissues of grains exhibit a higher essential amino acid content compared to other grain components (Shewry, 2007). Lysine is the most limiting amino acid in wheat. The nutritional quality is determined by the higher protein and limiting amino acid content, especially lysine (Chaudhary et al., 2022). In addition to its significance in nutritional physiology, the amino acid lysine plays a crucial role in plant stress resistance. Lysine is employed to enhance plant resilience against abiotic and biotic stresses, for the synthesis of glutamate amino acid, and most notably, for the synthesis of glutamate, which plays a pivotal role in the establishment of plant defense systems (Sumer and Erten, 2023). Statistically significant different results ($p \leq 0.01$) were observed according to YxNxG interaction in all evaluated essential amino acid parameters (Table 8). In contrast, the essential amino acid content exhibited variability in response to nitrogen and genotype factors. However, no significant influence of the year factor was observed (Lysine, Phenylalanine, Valine and Methionine) for a portion of amino acids. No interaction between year and nitrogen was observed with respect to the most valuable essential amino acids, namely lysine and methionine.

Table 8. Statistical values (ANOVA) of essential amino acid composition (mean square values and significance levels) (g 100 g^{-1})

Experimental factors	LYS	THR	PHE	ISO	LEU	VAL	MET
Year (Y)	0.016	0.037**	0.025	0.073**	0.102**	0.010	0.001
Nitrogen (N)	0.031**	0.012**	0.017**	0.008**	0.003	0.006**	0.001**
Genotype (G)	0.010**	0.004**	0.011**	0.004**	0.011**	0.003**	0.002**
YxN	0.002	0.021**	0.010**	0.002*	0.007**	0.021**	0.000
YxG	0.012**	0.002**	0.009**	0.007**	0.007**	0.007**	0.002**
NxG	0.015**	0.004**	0.010**	0.004**	0.005**	0.007**	0.002**
YxNxG	0.019**	0.004**	0.008**	0.007**	0.007**	0.005**	0.002**

Significance levels: *: $p \leq 0.05$, **: $p \leq 0.01$

Lysine: LYS; Threonine: THR; Phenylalanine: PHE; Isoleucine: ISO; Leucine: LEU; Valine: VAL; Methionine: MET

Interestingly, for higher values of the lysine amino acid was generally found in many genotypes (Golia, Kate A, Selimiye, Ceyhan 99, Tosunbey, İkizce-96, Müfitbey, Line 1, Line 2 and (Eraybey) at the highest nitrogen dose during the dry season (2018). Kate A (2017, N60 kg ha^{-1}) variety had highest lysine content (1.610 g 100 g^{-1}) than other genotypes. With regard to the specific point of lysine, the results indicated that Tosunbey exhibited higher values in all evaluated nitrogen doses and in both growing seasons. Furthermore, the stability of the lysine amino acid revealed the significance of genetic potential of Tosunbey.

The impact of the year factor on the outcomes for the amino acids threonine, isoleucine and valine is notable. The concentration of the threonine amino acid was observed to be higher during the dry season (2018), whereas the levels of the isoleucine and leucine amino acids were found to be elevated during the first year of the study (Table 10 and 11). The concentration of the threonine amino acid reached elevated levels at both the lowest (N_0) and highest (N_{180}) nitrogen doses during the dry growing season, contingent on the genotypes. Furthermore, a notable elevation in threonine amino acid levels was observed across all genotypes (with the exception of Hybery) when the nitrogen dose applied during the dry season was augmented from 120 to 180 kg ha^{-1}(Table 9). The mean values of isoleucine amino acid were analyzed, and it was determined that some genotypes (Ceyhan 99, Tosunbey, İkizce-96, Müfitbey, Line 1 and 2, Bozkır, Euclide, Julius, Hybery) with high values in the first year exhibited significant losses in isoleucine amino acid content during the dry season. It is assumed that these genotypes, which are typically winter-growing genotypes, lack an adequate level of isoleucine amino acid content in grain during the dry season. This may be attributed to a deficiency in assimilate accumulation in the plants. The values of valine, another amino acid significantly affected by the year factor, exhibited a general tendency to reach high values at a nitrogen doses of 60 (N_{60}) and 120 (N_{120}) kg ha^{-1} during the dry season (Table 10).

Noberbekova et al. (2018) reached the conclusion that, despite the differing change in amino acid amounts between the varieties in response to nitrogen fertilizer doses, the application of two times nitrogen fertilizer applications and an increasing nitrogen amount resulted in a significant increase in essential amino acids, namely valine, leucine, isoleucine, and threonine. Additionally, this resulted in an increase in the biological value of total protein. The application of nitrogen fertilizer resulted in a positive response from the phenylalanine amino acid, with an observed increase in phenylalanine content in correlation with the increase in nitrogen content. The lowest phenylalanine content was observed in genotypes that did not receive nitrogen application and exhibited low doses, while higher values were attained with increasing nitrogen doses. In the first experimental period, considerable elevations in phenylalanine concentration were discerned in Eraybey, Bozkır, Line 3, and Euclide genotypes, spanning the range of nitrogen doses from the lowest to the highest (Table 10). The leucine amino acid, which is the amino acid where the effect of the year factor is most evident, exhibited low values across all nitrogen doses (particularly for N_{120}) and genotypes, particularly during the dry season. Furthermore, a similar trend was observed in 2017, with genotypes demonstrating higher leucine amino acid values under generally suitable climatic conditions and a 60 kg ha^{-1} nitrogen dose (Table 11). Another the most limiting essential amino acid methionine takes specific and significant parts in many biochemical processes in plants. It plays a crucial role in protein synthesis and carbon metabolism, and its sulfur-bound methyl group. Methionine contributes plant physiology as cope with drought by boosting antioxidants (Maqbool et al., 2022). The methionine amino acid values exhibited a narrow spectrum distribution, with a range of 0.151-0.244 g 100 g^{-1}. The methionine content of the examined genotypes remained unaltered when subjected to different nitrogen doses, and no definitive conclusion could be drawn. In the first experimental year climatic conditions, the samples from Tosunbey and İkizce-96 exhibited elevated methionine levels across all nitrogen doses (Table 12).

Table 9. Observed lysine and threonine mean results of genotypes grown in different nitrogen applications in the period 2017 and 2018.

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Lysine Content (g 100 g^{-1})	Golia	1.139	1.208	1.286	1.283	1.219	1.289	1.229	1.265
	Kate A	1.128	1.610	1.180	1.148	1.141	1.124	1.168	1.276
	Selimiye	1.094	1.274	1.082	1.202	1.273	1.237	1.208	1.266
	Ceyhan 99	1.207	1.210	1.173	1.237	1.141	1.296	1.197	1.236
	Tosunbey	1.251	1.254	1.222	1.260	1.261	1.244	1.251	1.296
	İkizce-96	1.169	1.144	1.284	1.214	1.164	1.226	1.256	1.253
	Müfitbey	1.287	1.182	1.195	1.164	1.155	1.148	1.271	1.246
	Line 1	1.196	1.083	1.287	1.175	1.188	1.191	1.160	1.287
	Line 2	1.096	1.119	1.148	1.211	1.169	1.161	1.273	1.231
	Eraybey	1.098	1.116	1.257	1.324	1.157	1.233	1.176	1.294
	Bozkır	1.169	1.267	1.213	1.236	1.274	1.174	1.299	1.178
	Line 3	1.203	1.179	1.250	1.163	1.298	1.170	1.177	1.209
	Euclide	1.157	1.161	1.152	1.165	1.181	1.263	1.133	1.251
	Julius	1.302	1.268	1.309	1.093	1.133	1.226	1.219	1.119
	Hybery	1.090	1.182	1.107	1.308	1.132	1.270	1.297	1.122
	Mean	1.172	1.217	1.209	1.212	1.192	1.216	1.220	1.235
	Lsd N: 0.017; Lsd G: 0.033; Lsd YxG: 0.047; Lsd NxG: 0.066; Lsd YxNxG: 0.093								

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Threonine Content (g 100 g^{-1})	Golia	0.532	0.567	0.567	0.571	0.634	0.549	0.557	0.641
	Kate A	0.547	0.543	0.553	0.568	0.590	0.627	0.509	0.611
	Selimiye	0.581	0.575	0.487	0.505	0.518	0.581	0.516	0.626
	Ceyhan 99	0.567	0.519	0.546	0.539	0.59	0.550	0.512	0.585
	Tosunbey	0.582	0.562	0.517	0.566	0.582	0.511	0.544	0.500
	İkizce-96	0.534	0.531	0.583	0.611	0.524	0.534	0.578	0.612
	Müfitbey	0.573	0.529	0.564	0.543	0.623	0.620	0.504	0.606
	Line 1	0.509	0.547	0.567	0.581	0.624	0.498	0.525	0.625
	Line 2	0.492	0.597	0.553	0.596	0.641	0.514	0.528	0.571
	Eraybey	0.564	0.575	0.541	0.518	0.552	0.640	0.599	0.606
	Bozkır	0.538	0.558	0.582	0.540	0.609	0.635	0.504	0.569
	Line 3	0.577	0.542	0.594	0.527	0.638	0.584	0.492	0.541
	Euclide	0.571	0.536	0.621	0.567	0.614	0.607	0.592	0.631
	Julius	0.491	0.528	0.541	0.526	0.592	0.498	0.557	0.626
	Hybery	0.562	0.593	0.564	0.543	0.604	0.586	0.549	0.538
	Mean	0.548	0.553	0.558	0.553	0.595	0.568	0.537	0.592
	Lsd Y: 0.006; Lsd N: 0.006; Lsd G: 0.012; Lsd YxN: 0.009; Lsd YxG: 0.017; Lsd NxG: 0.024; Lsd YxNxG: 0.035								

color diagram: red-white-green. This sequence is characterized by an increase in value from left to right

Table 10. Observed phenylalanine and isoleucine mean results of genotypes grown in different nitrogen applications in the period 2017 and 2018.

	N (kg ha$^{-1)}$ /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Phenylalanine Content (g 100 g^{-1})	Golia	0.795	0.635	0.646	0.716	0.677	0.669	0.761	0.743
	Kate A	0.801	0.677	0.671	0.790	0.624	0.661	0.718	0.691
	Selimiye	0.698	0.671	0.638	0.652	0.698	0.805	0.699	0.633
	Ceyhan 99	0.642	0.646	0.717	0.724	0.624	0.686	0.771	0.801
	Tosunbey	0.728	0.697	0.738	0.721	0.730	0.718	0.733	0.820
	İkizce-96	0.673	0.646	0.686	0.677	0.711	0.767	0.807	0.734
	Müfitbey	0.733	0.724	0.798	0.786	0.657	0.815	0.753	0.744
	Line 1	0.726	0.781	0.732	0.760	0.640	0.787	0.644	0.822
	Line 2	0.734	0.813	0.676	0.664	0.759	0.816	0.74	0.703
	Eraybey	0.631	0.801	0.778	0.785	0.749	0.806	0.732	0.770
	Bozkır	0.668	0.702	0.644	0.809	0.640	0.645	0.771	0.654
	Line 3	0.666	0.639	0.718	0.781	0.633	0.675	0.724	0.815
	Euclide	0.640	0.719	0.693	0.768	0.797	0.743	0.756	0.681
	Julius	0.683	0.702	0.679	0.634	0.791	0.718	0.745	0.719
	Hybery	0.758	0.648	0.801	0.715	0.771	0.762	0.698	0.748
	Mean	0.705	0.700	0.707	0.732	0.700	0.738	0.736	0.738
	Lsd N: 0,007; Lsd G: 0,013; Lsd YxN: 0,010; Lsd YxG: 0,018; Lsd NxG: 0,026; Lsd YxNxG: 0,037								

	N (kg ha$^{-1)}$ /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Isoleucine Content (g 100 g^{-1})	Golia	0.429	0.422	0.457	0.456	0.373	0.355	0.464	0.411
	Kate A	0.459	0.358	0.357	0.484	0.420	0.364	0.455	0.323
	Selimiye	0.390	0.358	0.385	0.375	0.409	0.456	0.420	0.466
	Ceyhan 99	0.406	0.419	0.379	0.397	0.420	0.329	0.351	0.385
	Tosunbey	0.389	0.442	0.466	0.440	0.409	0.324	0.443	0.373
	İkizce-96	0.481	0.481	0.462	0.415	0.358	0.386	0.322	0.462
	Müfitbey	0.454	0.476	0.427	0.441	0.367	0.399	0.377	0.417
	Line 1	0.433	0.407	0.363	0.390	0.449	0.396	0.334	0.448
	Line 2	0.353	0.358	0.444	0.362	0.461	0.375	0.332	0.338
	Eraybey	0.465	0.379	0.453	0.473	0.458	0.366	0.418	0.338
	Bozkır	0.362	0.428	0.396	0.455	0.398	0.454	0.327	0.434
	Line 3	0.471	0.459	0.375	0.453	0.404	0.382	0.433	0.440
	Euclide	0.432	0.404	0.399	0.405	0.329	0.421	0.375	0.458
	Julius	0.466	0.461	0.443	0.459	0.378	0.417	0.340	0.396
	Hybery	0.447	0.487	0.410	0.367	0.414	0.326	0.331	0.444
	Mean	0.429	0.422	0.414	0.424	0.403	0.383	0.381	0.408
	Lsd N: 0.007; Lsd G: 0.014; Lsd YxN: 0.010; Lsd YxG: 0.020; Lsd NxG: 0.029; Lsd YxNxG: 0.040								
	color diagram: red-white-green. This sequence is characterized by an increase in value from left to right								

Table 11. Observed leucine and valine mean results of genotypes grown in different nitrogen applications in the period 2017 and 2018.

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Leucine Content (g 100 g^{-1})	Golia	0.691	0.639	0.662	0.596	0.583	0.573	0.571	0.561
	Kate A	0.568	0.716	0.635	0.562	0.638	0.619	0.611	0.597
	Selimiye	0.638	0.565	0.642	0.670	0.549	0.613	0.573	0.610
	Ceyhan 99	0.690	0.632	0.667	0.663	0.638	0.616	0.648	0.659
	Tosunbey	0.618	0.675	0.582	0.684	0.659	0.560	0.666	0.689
	İkizce-96	0.714	0.577	0.736	0.690	0.669	0.619	0.555	0.678
	Müfitbey	0.719	0.684	0.736	0.733	0.593	0.574	0.663	0.591
	Line 1	0.681	0.687	0.624	0.710	0.682	0.633	0.669	0.658
	Line 2	0.621	0.715	0.723	0.564	0.654	0.65	0.555	0.667
	Eraybey	0.572	0.591	0.584	0.672	0.605	0.608	0.583	0.653
	Bozkır	0.567	0.737	0.700	0.654	0.671	0.608	0.577	0.576
	Line 3	0.727	0.692	0.623	0.677	0.542	0.676	0.567	0.627
	Euclide	0.597	0.699	0.650	0.634	0.591	0.595	0.653	0.647
	Julius	0.655	0.712	0.674	0.605	0.661	0.603	0.661	0.694
	Hybery	0.637	0.596	0.585	0.637	0.593	0.647	0.545	0.636
	Mean	0.646	0.661	0.654	0.650	0.621	0.612	0.606	0.636
	Lsd Y: 0,013; Lsd G: 0,023; Lsd YxN: 0,017; Lsd YxG: 0,033; Lsd NxG: 0,046; Lsd YxNxG: 0,066								

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Valine Content (g 100 g^{-1})	Golia	0.686	0.639	0.625	0.689	0.631	0.629	0.655	0.593
	Kate A	0.557	0.721	0.598	0.701	0.652	0.738	0.58	0.701
	Selimiye	0.710	0.667	0.639	0.708	0.662	0.689	0.592	0.707
	Ceyhan 99	0.662	0.624	0.682	0.644	0.652	0.676	0.699	0.605
	Tosunbey	0.699	0.654	0.634	0.614	0.617	0.681	0.685	0.670
	İkizce-96	0.641	0.656	0.614	0.628	0.601	0.633	0.656	0.599
	Müfitbey	0.674	0.641	0.584	0.669	0.705	0.715	0.727	0.592
	Line 1	0.624	0.710	0.562	0.623	0.702	0.725	0.623	0.703
	Line 2	0.642	0.628	0.668	0.607	0.595	0.682	0.713	0.713
	Eraybey	0.734	0.624	0.629	0.607	0.631	0.709	0.631	0.649
	Bozkır	0.707	0.636	0.731	0.666	0.698	0.675	0.608	0.594
	Line 3	0.711	0.657	0.663	0.651	0.587	0.722	0.677	0.58
	Euclide	0.580	0.568	0.626	0.676	0.650	0.744	0.706	0.631
	Julius	0.643	0.590	0.692	0.722	0.633	0.635	0.679	0.681
	Hybery	0.674	0.645	0.635	0.642	0.668	0.708	0.708	0.660
	Mean	0.662	0.644	0.638	0.656	0.645	0.690	0.662	0.645
	Lsd N: 0.009; Lsd G: 0.017; Lsd YxN: 0.012; Lsd YxG: 0.024; Lsd NxG: 0.034; Lsd YxNxG: 0.048 color diagram: red-white-green. This sequence is characterized by an increase in value from left to right								

Table 12. Observed methionine mean results of genotypes grown in different nitrogen applications in the period 2017 and 2018.

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Methionine Content (g 100 g^{-1})	Golia	0.224	0.178	0.173	0.170	0.176	0.162	0.231	0.224
	Kate A	0.206	0.212	0.243	0.238	0.192	0.242	0.184	0.181
	Selimiye	0.203	0.220	0.229	0.190	0.209	0.186	0.206	0.192
	Ceyhan 99	0.167	0.222	0.196	0.189	0.192	0.170	0.193	0.172
	Tosunbey	0.238	0.204	0.211	0.221	0.178	0.168	0.208	0.225
	İkizce-96	0.216	0.190	0.216	0.198	0.177	0.168	0.220	0.185
	Müfitbey	0.188	0.217	0.181	0.163	0.238	0.194	0.215	0.232
	Line 1	0.244	0.198	0.220	0.172	0.225	0.176	0.164	0.163
	Line 2	0.184	0.159	0.160	0.203	0.163	0.222	0.190	0.206
	Eraybey	0.174	0.244	0.163	0.173	0.177	0.202	0.163	0.174
	Bozkır	0.193	0.166	0.240	0.226	0.196	0.213	0.185	0.178
	Line 3	0.198	0.184	0.192	0.243	0.230	0.170	0.204	0.196
	Euclide	0.167	0.176	0.156	0.206	0.188	0.200	0.214	0.214
	Julius	0.223	0.151	0.155	0.185	0.166	0.170	0.196	0.180
	Hybery	0.196	0.163	0.228	0.179	0.244	0.216	0.200	0.168
	Mean	0.201	0.192	0.197	0.197	0.196	0.190	0.198	0.192

Lsd N: 0.004; Lsd G: 0.008; Lsd YxG: 0.012; Lsd NxG: 0.016; Lsd YxNxG: 0.023

color diagram: red-white-green. This sequence is characterized by an increase in value from left to right

Relationship Between Yield, Bread-Making Quality, Health and Nourishment Properties

Figure 1. shows the relationships between the evaluated parameters grown genotypes under different nitrogen applications for the period 2017 and 2018. According to the correlation coefficient results, grain yield showed a significant positive correlation with protein content (%) and wet gluten content (%) of r=0.290** and r=0.153**, respectively. This is the most attractive result that increasing yield values caused positive effect on bread-making quality and dilution effect between yield and protein was not observed contrary to previous studies (Erekul et al., 2012; Bagulho et al., 2015). A highly positive and significant relationship was found between protein content and wet gluten content (Mut et al., 2017; Siddiqi et al., 2020). However, increasing protein and wet gluten content resulted in an adverse effect on gluten quality (gluten and gluten index, r=-0.54***). Despite an observed increase in gluten amount and protein content, the resulting dough quality and gluten strength were found to be unsatisfactory.

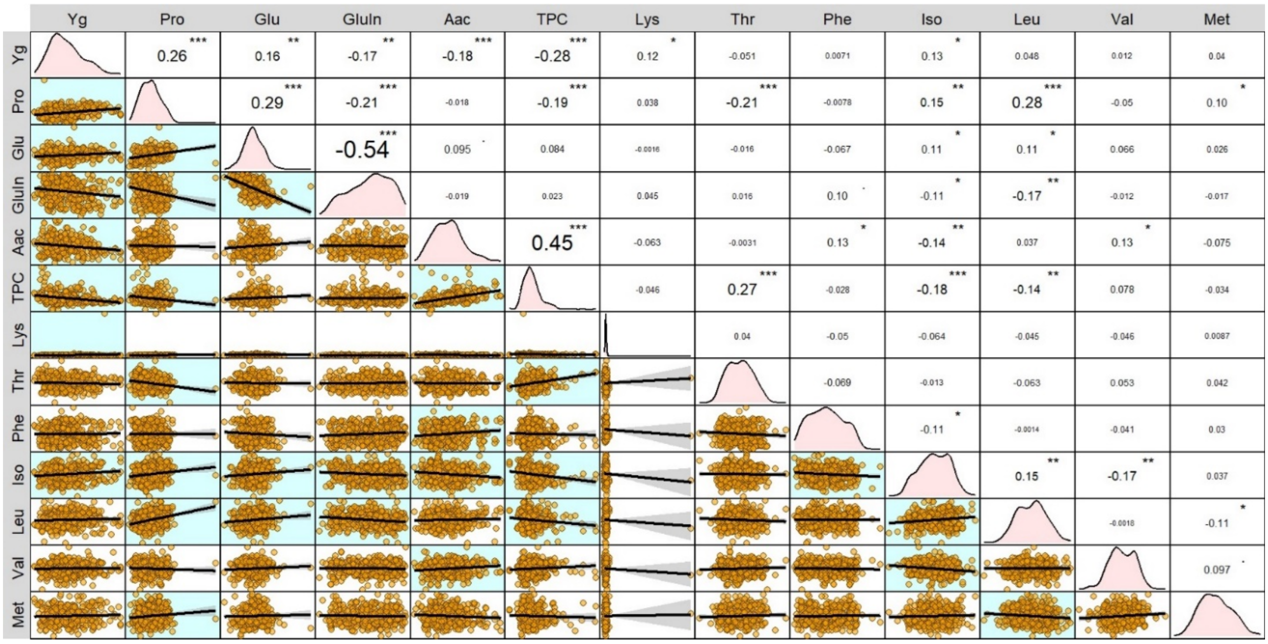

Figure 1. Correlation matrix showing the relationships between yield, bread-making quality, antioxidant and essential amino acids (Significance levels: ns: not significant, *: $p \leq 0.05$, **: $p \leq 0.01$, *** $p \leq 0.001$; Yg: Grain Yield; Pro: Protein Content; Glu: Wet Gluten Content; GluIn: Gluten Index; TPC: Total phenolic content; Aac; Total Antioxidant Activity; Lysine: Lys; Threonine: Thr; Phenylalanine: Phe; Isoleucine: Iso; Leucine: Leu; Valine: Val; Methionine: Met)

In contrast, an inverse and statistically significant correlation was observed between yield and health-related traits. The total phenol content and antioxidant activity properties demonstrated a decline with an increase in yield values.

Moreover, a notable and significant positive correlation (r=0.450**) was identified between total phenolic content and antioxidant activity, a finding that is consistent with the results reported by other researchers in the literature (Mpofu et al., 2006; Žilić et al., 2012; Zeibig et al., 2024). The increase in protein content contributed significantly and positively to the essential amino acid composition as also obtained same correlation results in our previous study (Yigit and Erekul, 2023). The amount of isoleucine (r=0.150**), leucine (0.284**) and methionine (r=0.104*) amino acids increased with the increase in protein content. Nevertheless, this is not the case for all essential amino acids (threonine; r=-0.210**), which results in a reduction in the quantity of certain amino acids, thereby indicating a complex relationship between protein and essential amino acid composition (Figure 1). Furthermore, when the interrelationships of essential amino acids are examined in general terms, it becomes evident that increases in the amount of some amino acids have negative effect on others. This illustrates that amino acid composition and physiology have complex structure.

CONCLUSION

Our study has clearly demonstrated the effects of nitrogen application on yield, bread-making, antioxidant and essential amino acid composition of bread wheat. In the research we conducted to determine the effect of nitrogen doses on genotypes with different genetic and growth habit properties, YxNxG interaction was significant in all traits due to the different climatic conditions observed in both growing seasons. The impact of climatic conditions on evaluated traits was notably identified, particularly during the second year of the study due to the prevalence of a dry growing season (2018). While a reduction was observed in yield and protein values, particularly during the dry season, an increase was noted in the gluten index, which is a key determinant of gluten strength and is therefore an important factor in bread quality, as well as in total phenolic content and antioxidant activity, which contribute to health. The total phenol content and antioxidant activity values exhibited a decline in certain genotypes because of the increased application of nitrogen fertilizers. However, an increase in health-relevant compounds in the grain was observed in some genotypes grown in the dry season (mostly winter-growth habit) with increasing nitrogen fertilizer doses. Due to the increasing sensitivities on climate change, environmental and human health and quality nutrition came to the forefront in recent years, important findings were determined by analyzing the yield, bread quality characteristics, antioxidant and amino acid composition characteristics of the results obtained within the scope of the research. Therefore, it was concluded that newly released and old genotypes have significant potential with respect to the nutritional and health traits. For this reason, these traits are important for the development of healthy products and varieties in breeding programs in the future.

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