agriculture

MDPI

Article

Variation of Grain Yield, Grain Protein Content and Nitrogen Use Efficiency Components under Different Nitrogen Rates in Mediterranean Durum Wheat Genotypes

Sawsen Ayadi [1,*], Salma Jallouli [1], Zoubeir Chamekh [2], Inès Zouari [1,2,3], Simone Landi [4,*], Zied Hammami [5], Fatma Ezzahra Ben Azaiez [1], Mokhtar Baraket [6], Sergio Esposito [4] and Youssef Trifa [1]

[1] National Institute of Agronomy of Tunisia, Carthage University, LR-14-AGR-01, Cite Mahragène, Tunis 1082, Tunisia; salmajallouli@gmail.com (S.J.); ines.zouari28@gmail.com (I.Z.); zahra.azaiez@gmail.com (F.E.B.A.); youssef.trifa@gmail.com (Y.T.)
[2] National Institute of Agronomic Research of Tunisia, Carthage University, LR20-INRAT-02, Menzah I, Tunis 1084, Tunisia; zoubeir.chamekh@iresa.agrinet.tn
[3] High Institute of Agronomy of Chott Mariam, Sousse University, Chott-Mariem 13, Sousse 4042, Tunisia
[4] Dipartimento di Biologia, Complesso Universitario Monte Sant'Angelo, Universita di Napoli Federico II, 80126 Naples, Italy; sergio.esposito@unina.it
[5] International Center for Biosaline Agriculture, Dubai P.O. Box 14660, United Arab Emirates; z.hammami@biosaline.org.ae
[6] National Research Institute of Rural Engineering, Water and Forests (INRGREF), Tunis 1004, Tunisia; mokhtar.baraket@iresa.agrinet.tn
* Correspondence: sawsen.ayadi@gmail.com (S.A.); simone.landi@unina.it (S.L.)

Citation: Ayadi, S.; Jallouli, S.; Chamekh, Z.; Zouari, I.; Landi, S.; Hammami, Z.; Ben Azaiez, F.E.; Baraket, M.; Esposito, S.; Trifa, Y. Variation of Grain Yield, Grain Protein Content and Nitrogen Use Efficiency Components under Different Nitrogen Rates in Mediterranean Durum Wheat Genotypes. *Agriculture* **2022**, *12*, 916. https://doi.org/10.3390/agriculture12070916

Academic Editors: Jose L. Gabriel and Diana Martín-Lammerding

Received: 12 May 2022
Accepted: 22 June 2022
Published: 23 June 2022

Abstract: Nitrogen (N) is a crucial nutrient for plant growth and development. To optimize agricultural environments, N fertilizers represent a critical tool to regulate crop productivity. The improvement of nitrogen use efficiency (NUE) represents a promising tool that may enable cereal production to meet future food demand. Wheat reported contrasting behaviors in N utilization showing specific abilities depending on genotype. This study selected two landraces and two improved genotypes from Northern Africa to investigate grain yield (GY), grain protein content (GPC) and NUE. Plants were grown under three levels of N supply: 0, 75, 150 kg N ha^{-1} and for two consecutive years. Results reported a better NUE (0.40 kg kg N^{-1}) obtained under 150 kg N ha^{-1}, while N utilization efficiency (NUtE) showed a 13% increase using 75 kg N ha^{-1} compared with 150 kg N ha^{-1}. Under low nitrogen rate (0 N), crop N supply (CNS) and N uptake efficiency (NUpE) were shown as determinant factors for improved genotypes GY (R^2 = 0.72), while NUtE represented the most determinant component for GPC in landraces (R^2 = 0.92). Multivariate regression models explained the dependence in GPC on NUE, NUpE, and NUtE. In conclusion, our results recognize GPC and NUtE as suitable selection traits to identify durum wheat with higher NUE.

Keywords: landraces; *Triticum durum*; improved; nitrogen; utilization efficiency; stepwise analysis

1. Introduction

The intensive and global use of mineral N fertilizers in agriculture has induced tremendous detrimental effects on both the environment and human health [1,2]. Currently, worldwide consumption of N fertilizers per year has reached 170 million tons [3]. Inappropriate use of N fertilizers can result in the leaching of unabsorbed nitrate in groundwaters, leading to eutrophication and hypoxia of soil waters. Furthermore, the excessive use of N fertilizers increases the emission of N_2O, significantly contributing to the greenhouse effect [4].

The optimization of crop yield and nitrogen use efficiency (NUE) [5,6] reduces N leaching [7,8], thus decreasing both the cost of crop production and environmental impact. NUE is a complex trait regulated by several factors and changing in different crop species, namely climatic conditions, soil texture, crop rotation, plant growth stage and N fertilizer ionic composition [2]. More specifically, NUE can be described as a sum of two main components, N uptake efficiency (NUpE) and N utilization efficiency (NUtE) [9]. In cereals, these factors represent the routes for the translocation of N from soil to roots (NUpE) and then to grains (NUtE) through N metabolic pathways [10]. Important cereal quality parameters and grain protein content (GPC) are influenced by N fertilization, which is critical in food production and quality [11,12]. Other important aspects positively influenced by N application are shoot dry matter, grain harvest index, N harvest index, wheat grain yield and chlorophyll metabolism [13–16].

Conversely, durum wheat (*Triticum durum Desf.)* has been shown to exhibit contrasting behavior under changing N fertilization, especially comparing improved genotypes and landraces [17]. An improved grain yield (GY) is often associated with both NUE components in wheat cultivars [10]; however, an excess of N fertilization could decrease the number of kernels per spike, reducing GY in specific genotypes [9,18].

Furthermore, an excess of N application delays plant senescence, reducing the grain filling rate and GY [19]. A higher nitrogen rate will extend the grain filling period and potentially increase the chances of abiotic stresses such as drought and heat, increasing its vulnerability to changing climatic conditions. Significant genetic variability for NUE has been observed in barley [20], maize [21], rice [13], winter wheat [6], and durum wheat [15]. Such genotypic variability could be used in wheat breeding programs to improve yield and NUE [22]. Yield components such as kernel number per spike are strictly regulated by genetic control [23].

In recent years, N fertilizers have acquired critical importance considering the persistent climate change scenario [14,23]. This phenomenon induced a general reduction in crop yields, especially in semi-arid regions as well as the southern Mediterranean area and northern Africa [14,23,24]. These phenomena change species' variability and reduce biodiversity in non-agricultural systems [1,19,25]. Therefore, the equilibrium research on the efficient use of nitrogen, in order to maximize grain yields, is crucial for the agricultural system to sustain cereals crop production, especially in these regions. Particularly, in the Mediterranean basin, durum wheat represents a staple food [24]. In this region, farmers actively cultivated wheat, and this practice selects different local landraces varying their behavior in term of NUE and yield [14,24,26]. These genotypes have gained increasing economic value in their respective countries during the years.

One of the main challenges for sustainable agriculture is to manage NUE in order to improve (or maintain equal) yields under reduced N inputs. This can be attempted by an improved recovery of soil and N fertilizer and by regulating the use of N in plants.

This process could be in part resolved by the study of local durum wheat genotypic variability in terms of NUE under Mediterranean conditions. To meet this challenge, two genotypic pools (landrace and improved durum wheat genotypes) were cultivated under different nitrogen supplies in northern Africa under open field conditions.

This study aims to compare the main growth and grain quality parameters of these different durum wheat genotypes. This manuscript focuses on the relationships between different agronomical parameters, namely GPC, NUE (in both components) and GY, cultivating these genotypes under different N supplies in sub-humid areas of Tunisia.

2. Materials and Methods

2.1. Plant Material, Sites and Experimental Design

Four Tunisian durum wheat genotypes (*Triticum durum* Desf.) were selected for this study: two landraces, Bidi and Azizi, and two improved genotypes (modern varieties), Om Rabiaa and Khiar. These varieties have been previously reported to exhibit significant differences in those enzymes involved in N metabolism, namely nitrate reductase (NR), glutamine synthetase (GS), glutamine oxoglutarate aminotransferase (GOGAT) and glutamate dehydrogenase (GDH), showing interesting performance upon N deprivation conditions [26]. The selected genotypes were provided by the laboratory of genetics and cereal breeding (LR-14-AGR-01, Carthage University, Tunis); further details of these four cultivars are described in Table 1. Experiments were conducted in the sub-humid area of Mateur (Northern Tunisia, 37°03′15.48″ N, 9°37′14.73″ E). Two experiments were carried out under rainfed conditions from November to June in two consecutive years (2010, 2011) on the same site Mateur. The experiments performed in the first and second years were renamed S1 (2010) and S2 (2011). Plants were exposed to three N treatments, by supplying 0, 75 and 150 kg N ha^{-1}. The maximum N supply used in this study was chosen because it has been previously demonstrated that exceeding N fertilizer supply could induce yield losses [15]. Nitrogen fertilizer was applied as ammonium nitrate granules (33.5% N) in three splits: 30% at early tillering, 40% at stem elongation and 30% at the second node stage. Both S1 and S2 were conducted in split plot design with three replications for genotypes and treatments (total plots = 36). Each plot represents one replication for each genotype and N rate. Plot size was 6 m^2 (3 m length and 2 m width), and each plot was sown with ten rows of 3 m, spaced by 0.20 m. Seeds were manually sown (300 seeds/m^2). Plants were randomly hand harvested in each plot in both S1 and S2 experiments.

Table 1. Origin and release history of landraces and improved genotypes used in this study [24].

Genotypes	Origin, Selection or Release History
Bidi (landrace)	Local landrace introduced from Morocco, pure line selection started in 1908.
Azizi (landrace)	Local landrace of various origins, present pre-1893, pure line selection started in 1908.
Om Rabiaa (improved)	From cross made at ICARDA, introduced as fixed line in 1987, registered in 1996.
Khiar (improved)	From cross made at CIMMYT, introduced in 1987, registered in 1992.

2.2. Soil and Environmental Conditions

The experimental site's soil and environmental characteristics in *Mateur* (Tunis) were investigated. The soil of the S1 and S2 at the experimental station consisted essentially of silt loam (Table 2). Clay content (20.86%) may indicate a high water and nutrient holding capacity. The soil was alkaline (pH = 8.5) in all evaluated soil layers.

Table 2. Soil properties at the experimental fields in 2010 and 2011 before sowing.

Soil Layer (cm)	Clay (%)	Silt (%)	Sand (%)	Limestone (%)	pH
0–10	22.5	57.3	17.3	20.1	8.3
10–30	21.6	57.3	18.3	20.9	8.4
≥30	18.5	52	16.1	19.9	8.5

Abbreviations: pH, pH of soil.

As shown in Figure 1, average temperatures in S2 were generally cooler than those observed in S1, particularly between March and May, at both flowering and grain filling, respectively. Total rainfall from November to June for S1 and S2 were 630.1 and 405.5 mm, respectively. In April, the maximum precipitation was observed in S1 (190.5 mm), higher than that observed in S2 (35.7 mm). In contrast with S1, the S2 field was characterized by a longer thermal period and an unevenly distributed rainfall.

2.3. Data Collection and Measurements

The soil characteristics of the experimental site are described in Table 2. Three soil cores were collected from each plot at 90 cm depth before sowing and after harvesting. Samples were analyzed for nitrate and ammonium content according to Devarda's Alloy reagent method [27]; ammonium was measured using the distillation–titration proceeding method [28]. Temperature and rainfall data were collected from a weather station installed on site (Figure 1). Three central rows, one linear meter each, were randomly hand-harvested in each plot. Grains were collected using a shredder (LD-180, Wintersteiger, Ried im Innkreis, Austria). Grain yield was measured. Grain N concentration was determined using the Kjeldahl procedure [29]. The Kjeldahl method was used to determine grain protein content (GPC) and then converted to crude grain protein as described in Ortiz-Monasterio et al. [8].

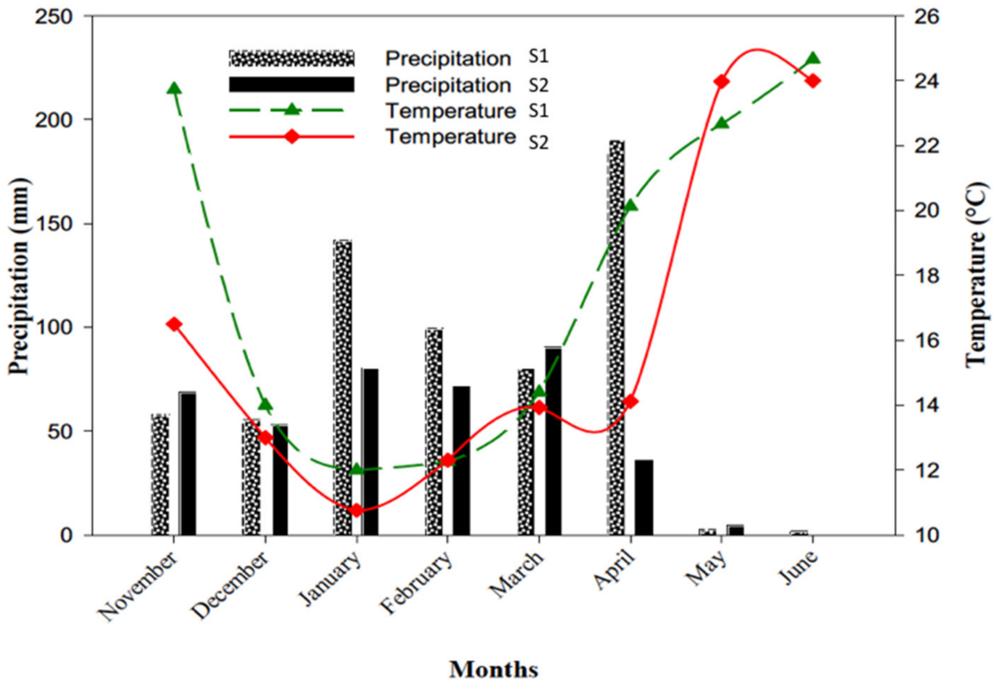

Figure 1. Meteorological data (temperature and rainfall) in S1 (2010) and S2 (2011) experimental years.

The following NUE components were calculated: (i) crop N supply (CNS; kg N ha^{-1}), represented by the sum of soil nitrogen at sowing, mineralized N and N fertilizers [5]; mineralized N was measured as the difference between pre-sowing and post-harvest plant and soil NO_3^--N in one plot; (ii) Nitrogen uptake efficiency (NUpE; kg N^{-1}), calculated as the total N uptake/crop N supply; (iii) N utilization efficiency (NUtE; kg N^{-1}), calculated as the GY/total N uptake; (iv) N use efficiency (NUE; kg kg^{-1}), which is defined as GY per unit of N supply in the soil (NUE = GY/CNS) [29]. Total N in the biomass and grain samples was utilized to analyze the NUE according to Kichey and Mona stereo [5,9].

2.4. Statistical Analysis

Statistical analyses were carried out using the SAS proc GLM procedure [30]. Comparisons between genotypes and different N treatments were made using Tukey's test. Correlation matrixes were calculated for all measured and estimated components during the two years under the different N treatments, and the resultant correlation coefficients were tested at a 95% probability level. This correlation matrix was generated to evaluate the linear relationships among all parameters. Significant correlations were visualized using Cystoscopes 3.4.0 [31]. The significance threshold for correlations between traits was set at $r > 0.6$ for positive correlations and $r < -0.6$ for negative correlations, with a p value <0.001 in both cases. Stepwise multiple linear regression analysis was developed, considering GY and NUE as the dependent variables and the other traits as independent variables. Stepwise multiple linear regression was carried out using SPSS 16 statistical package (SPSS Inc., Chicago, IL, USA) and Sigmaplot 11 (Sysat Software Inc., Point Richmond, CA, USA).

3. Results

3.1. Variation in Grain Yield (GY) and Grain Protein Content (GPC) under Different Nitrogen Rates

N rates considerably impacted GY and GPC in both landraces and improved genotypes. As shown in Table 3, GY is significantly affected by different nitrogen rates in each analyzed genotype (G). This parameter was influenced by the experimental year (S), nitrogen rate (N), and by the interactions S×G, S×N and G×N. GY was higher in S1 with respect to S2. GY gradually and significantly increased with increasing N rates to reach 5.7 t ha^{-1} at 150 kg N ha^{-1} (Table 3). No significant change was observed in GY among different genotypes by averaging N doses and years. When the interaction N×S is considered for the GY parameter, more evident effects of N levels were observed in S1, with an increase of 37% compared with S2 (Table 3).

Table 3. Mean squares of grain yield (GY; t ha^{-1}), grain protein content (GPC; %), nitrogen uptake efficiency (NUpE, kg kg N^{-1}), nitrogen utilization efficiency (NUtE; kg kg N^{-1}), nitrogen use efficiency (NUE; kg kg N^{-1}) and crop N supply (CNS; kg N ha^{-1}) of durum wheat genotype at different N rates in two experimental years.

		GY	GPC	NupE	NutE	NUE	CNS
Experimental year (S)							
2010 (S1)		4.6 ^{a}	9.75 ^{b}	0.17 ^{b}	59.34 ^{a}	9.42 ^{b}	476.40 ^{a}
2011 (S2)		3.3 ^{b}	12.76 ^{a}	0.52 ^{a}	44.94 ^{b}	20.92 ^{a}	158.05 ^{b}
Nitrogen Rate (N)							
0 N		2.4 ^{c}	10.73 ^{b}	0.32 ^{b}	53.24 ^{a}	13.27 ^{b}	240.21 ^{c}
75 N		3.4 ^{b}	11.12 ^{b}	0.31 ^{b}	54.72 ^{a}	14.92 ^{b}	320.19 ^{b}
150 N		5.7 ^{a}	11.91 ^{a}	0.40 ^{a}	48.46 ^{b}	17.34 ^{a}	391.27 ^{a}
Genotypes (G)							
Landrace genotypes							
Azizi		3.2 ^{a}	11.49 ^{a}	0.34 ^{a}	51.50 ^{b}	14.75 ^{a}	326.85 ^{a}
Bidi		4.2 ^{a}	11.78 ^{a}	0.40 ^{a}	49.59 ^{b}	16 ^{a}	311.46 ^{a}
Means		3.7	11.63	0.37	50.54	15.37	319.15
Improved genotypes							
Khiar		3.9 ^{a}	9.97 ^{b}	0.32 ^{a}	57.03 ^{a}	15.86 ^{a}	312.02 ^{a}
Om Rabiaa		3.8 ^{a}	11.78 ^{a}	0.32 ^{a}	50.43 ^{b}	14.09 ^{a}	318.55 ^{a}
Means		3.85	10.87	0.32	53.73	14.97	315.28
	DF	Mean Square					
Year (S)	1	27.28 ***	162.32 ***	2.2321 ***	3497 ***	2379.8 ***	1,823,051 ***
Nitrogen Rate (N)	3	70.95 ***	8.54 **	0.0657 *	250 ***	100.5 ***	136,864 ***
Genotype (G)	2	0.33 ^{ns}	13.46 ***	0.0220 ^{ns}	199 ***	15.0 ^{ns}	929 ^{ns}
S×G	3	1.65 **	4.20 *	0.0167 ^{ns}	48 ^{ns}	19.2 ^{ns}	929 ^{ns}
S×N	2	4.00 ***	1.05 ^{ns}	0.0372 ^{ns}	94 **	31.7 *	159 ^{ns}
G×N	6	1.86 ***	0.40 ^{ns}	0.0110 ^{ns}	84 ***	13.9 ^{ns}	703 ^{ns}
S×G×N	6	0.60 ^{ns}	0.91 ^{ns}	0.0075 ^{ns}	62 **	6.9 ^{ns}	703 ^{ns}

Asterisks (*), (**) and (***) indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. ns, no significant differences ($p > 0.05$). Letters indicated significant differences.

However, the response of genotypes to different N rates changed significantly (Figure 2A–C). Using the lower nitrogen level (0 kg N ha^{-1}), landraces and improved genotypes showed no significant differences. On the contrary, significant variations were reported comparing S1 and S2 experimental years. In landraces, GY sharply increased with the initial increase in N rate (Figure 2A–C). At a higher nitrogen level (150 kg N ha^{-1}), improved genotypes showed a greater response than landraces (Figure 2A,B). Particularly, in both S1 and S2 experimental years, landraces showed a higher GY (4.5 t ha^{-1}) compared with improved genotypes (3.45 t ha$^{-1)}$ at 75 kg N ha^{-1} (Figure 2C). Conversely, in S2 experimental year (but not in S1), improved genotypes reported a significant increasing in GY (30%) compared with landraces at 150 kg N ha^{-1} (Figure 2B).

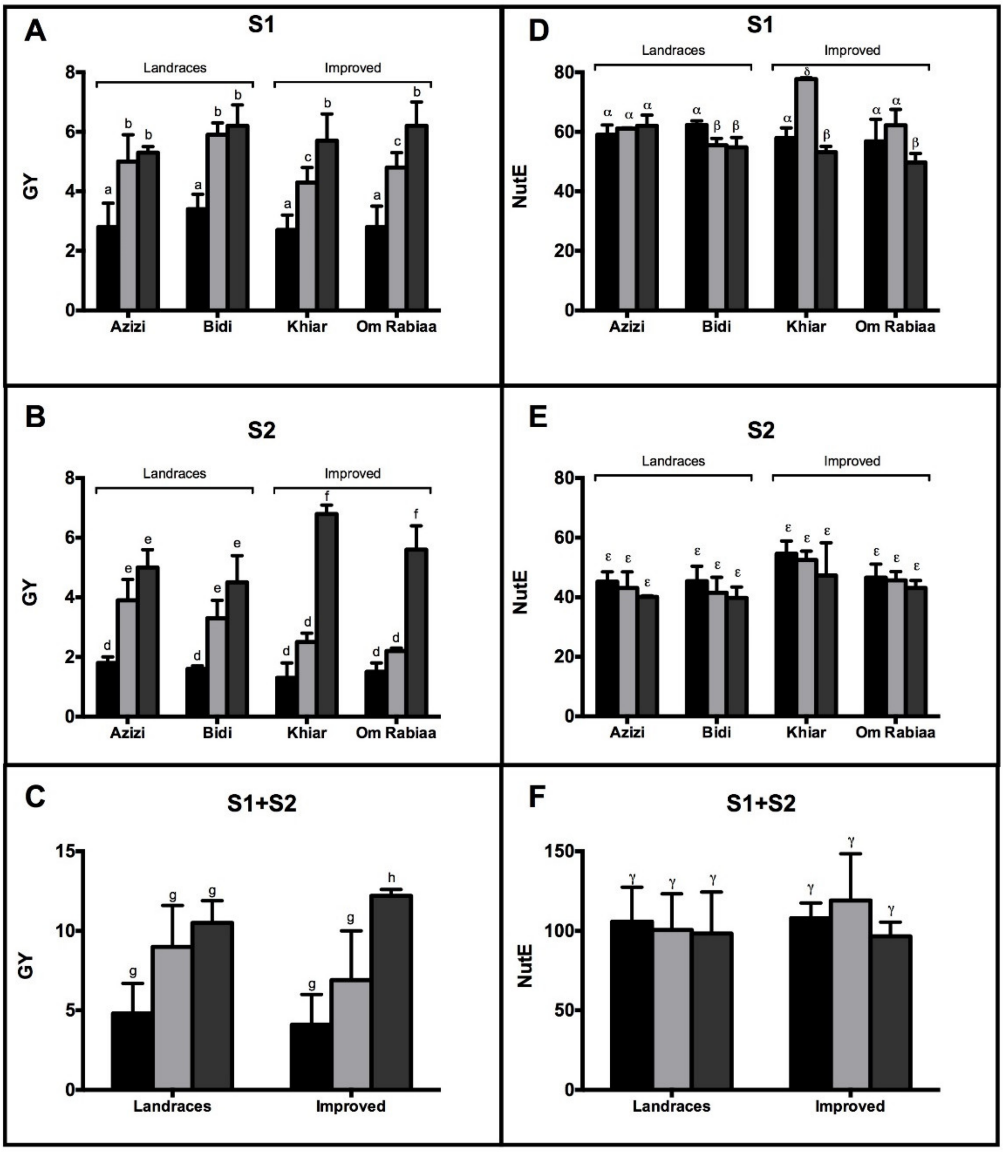

Figure 2. Variation in grain yield (GY) and of NutE for different genotypes (**A**,**B**,**D**,**E**) and for each genotypic pool (landraces and improved (**C**,**F**)) at different nitrogen rates 0 (black bars), 75 (light grey bars), 150 (dark grey bars) kg N ha^{-1}. S1 and S2 correspond to 2010 and 2011, respectively. Each value is the mean SD for each genotype and nitrogen supply (n = 3 for genotypes and n = 3 for N supplies). Letters indicate significant differences between treatments for different graphs.

GPC was significantly affected by the experimental year (S), the nitrogen rate (N), the genotypes (G), and the interaction S×G. In response to N rates, GPC increased only when 150 kg N ha^{-1} was applied. GPC was 30% higher in the experimental S2 as compared with S1 (Table 3). The highest GPC was registered in the two landraces Azizi (13.41%) and Bidi (13.64%) in S1. The minimum GPC was observed in the improved genotype Khiar (8.66%) (Table 4).

Table 4. Means of grain yield (GY; t ha^{-1}), grain protein content (GPC; %) and nitrogen utilization efficiency (NUtE; kg kg N^{-1}) of durum wheat genotype at different N rates in S1 (2010) and S2 (2011) (S×N, S×G and S×G×N, respectively).

		Experimental Year (S)					
N rates			**S1—2010**			**S2—2011**	
GY	0 N		2.89 ^{c}			1.57 ^{d}	
	75 N		4.97 ^{b}			2.98 ^{c}	
	150 N		5.85 ^{a}			5.48 ^{b}	
	LSD (0.05)		†: 370.61			‡: 343.62	
Genotypes							
GPC	Azizi		9.57 ^{de}			13.41 ^{a}	
	Bidi		9.91 ^{cde}			13.64 ^{a}	
	Khiar		8.66 ^{e}			11.29 ^{bc}	
	Om Rabiaa		10.88 ^{cd}			12.69 ^{ab}	
	LSD (0.05)		†: 0.38			†: 0.77	
		0 N	75 N	150 N	0 N	75 N	150 N
NutE	Om Rabiaa	56.8 ^{bcde}	62.1	49.7 ^{defghi}	46.6 ^{fghij}	45.7 ^{ghij}	43 ^{hij}
	Khiar	57.9 ^{bcd}	77.7 ^{a}	53.2 ^{bcdefg}	54.6 ^{bcdefg}	52.5 ^{cdefgh}	47.2 ^{bcg}
	Bidi	62.2 ^{b}	55.5 ^{bcdef}	54.7 ^{bcdefg}	45.4 ^{ghij}	41.4 ^{ij}	39.8 ^{j}
	Azizi	58.9 ^{bcd}	61.1 ^{bc}	62 ^{bc}	45.2 ^{ghij}	43 ^{hij}	40.05 ^{ij}
	SE			2.5			

Different letters indicate significant differences between year (within row) and treatments (within column) at $\alpha = 0.05$. Legend: † comparison of year means; ‡ comparison of N treatment means.

3.2. Variation in NUE Traits

Nitrogen rates (N) and experimental years (S) significantly affected NUpE and NUtE, which both globally contribute to NUE ($p \leq 0.001$, Table 3). NUtE is always affected by all effects and interactions, with the exception of S×G (Table 3). The general trend showed a decrease in NUtE with an increase in N doses (Table 4). As shown in Figure 2, significant differences were reported comparing the S1 and S2 experimental year for all selected genotypes. Particularly, in Table 3, S2 showed a lower NUtE value (44.94 kg kg N^{-1}) compared with S1 (59.34 kg kg N^{-1}). This experimental year (S2) showed differences in NUtE between genotypes (Figure 2D). Azizi landrace maintained a similar NUtE value all N rates. This genotype showed a significantly higher value (47.3 kg kg N^{-1}) of NUtE at 150 kg N ha^{-1} compared with the other genotypes. Conversely, Khiar-improved genotype showed a significantly higher value of NUtE (54.6 kg kg N^{-1}) at 75 kg N ha^{-1} (Figure 2E).

NUpE was significantly affected by the experimental year and nitrogen rate. By comparing nitrogen rates and genotypes, the highest NUpE value 0.52 kg kg N^{-1} was recorded under S2, while under S1, it was only 0.17 kg. kgN^{-1}. NUpE was the highest at the maximum nitrogen level (Table 3). Regarding CNS, an increase of 66.8% was recorded in S1 in comparison with S2 (Table 3). CNS showed a significant increase when N rates varied from 0 to 150 kg N ha^{-1} (Table 3).

NUE value was more than twice higher in S2 with respect to S1 when N rates and genotypes were compared (Table 3). When the comparison was made among the experimental year and genotypes, NUE was statistically similar under 0 and 75 N, significantly increasing by 30.6% under 150 N. In contrast, the interaction (S×N) showed a significant effect on NUE ($p \leq 0.05$).

3.3. Stepwise Analysis and Relationships among GY, GPC and NUE Components under Different Nitrogen Rates

Multiple regression analysis using a stepwise procedure was performed for each N rate applied, using grain yield as the genotype-dependent variable to assess each independent variable's contribution to GY prediction.

The first trait selected was CNS for all N doses and genotypes (Table 5). NUpE and NUE ranked second for 0 N in improved genotypes and 75 N in landraces, respectively (Table 5).

Table 5. Multivariate regression models explaining grain yield variation from NUE traits (NUE) across genotypes under different N rates.

	Genotypes	Variable	R^2	Significance
GY_{N0}	Landraces	CNS	0.696	**
	Improved	CNS	0.628	**
		CNS, NupE	0.742	**
GY_{N75}	Landraces	CNS	0.679	**
		CNS, NUE	0.877	**
	Improved	CNS	0.887	**
GY_{N150}	Landraces	CNS	0.451	**
	Improved	–	–	–

Asterisks (**) indicate significant differences at $p < 0.01$.

A correlation network was built using $r > 0.6$ or $r < -0.6$ and $p < 0.001$ in order to visualize the correlations existing between the different parameters measured (Figure 3A). GPC was shown to be surrounded by the NUE components. GPC was negatively correlated with CNS. CNS was also closely and positively correlated with GY, while NUE components were not. Based on the correlation results, the relationships of GPC with NUE, NUpE and NUtE were plotted using the averaged values across the experimental year and nitrogen rates for landrace and improved genotypes (Figure 3B). GPC showed higher correlations with NUE and NutE in landrace genotypes ($r^2 = 0.859$; 0.920) than in improved ones ($r^2 = 0.505$; 0.475).

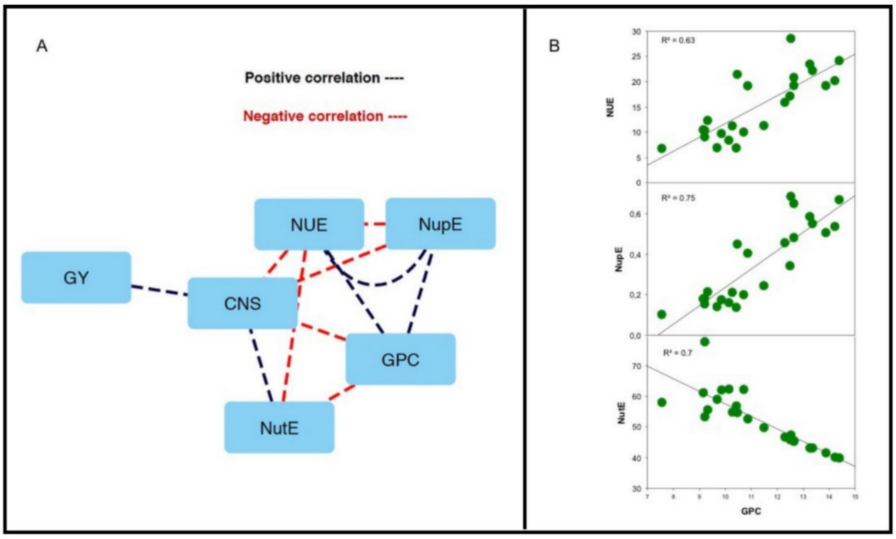

Figure 3. (**A**) Correlation network for NUE traits in durum wheat using four different genotypes and three nitrogen treatments. Line colors represent positive correlations (black) between traits in blue (Pearson's $r > 0.6$; $p < 0.001$) and negative correlations (red). Pearson's $r < -0.6$; $p < 0.001$. (**B**) Significant correlations between yield and NUE components ($n = 72$). Abbreviations: NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NutE, nitrogen utilization efficiency; CNS, crop nitrogen supply; GPC, grain protein content; GY, grain yield.

4. Discussion

4.1. Environment and Nitrogen Supply Impacts on Grain Yield (GY), Crop N Supply (CNS) and NUE

Nitrogen is a key nutrient for durum wheat growth and grain yield (GY) [32]. In order to take advantage of N effects in terms of crop growth and productivity, breeding programs have developed cultivars that efficiently respond to high N rates [33]. In this study, we investigated the differential responses of durum wheat landrace (Azizi and Bidi) and improved genotypes (Khiar and Om Rabiaa) in order to dissect the relationships between GY, CNS, and NUE in the two different experimental years.

In S1, CNS, which is the total nitrogen from the soil available for the plant, was higher by 201.4% with respect to S2, while GY was higher by 39.39%, and this resulted in significantly lower NUE in S1. The significant variability between the two experimental years and the non-significant N fertilizer effect in S1 could be attributed to N leaching, as rainfall was important during vegetative growth stages and higher average temperature (Figure 1). Our results showed an increase in GY associated with a decrease in NUE. This trend results from increasing N inputs, as already reported by Foulkes et al. [34] and corresponds to the plant response when N supply is much higher than crop N demand [9]. Moreover, our results agree with Foulkes et al. [34] who reported that seasonal variations affected yields. Our results showed that CNS, NUE, NUpE and GY increased by rising N fertilizer levels. The results suggest that plants were more able to take up and assimilate N fertilizer from surface soil, than to uptake N from the deeper stock in the soil [8]. This was previously reported in studies on modern wheat cultivars, but it was generally ascribed to the reduced root systems in selected genotypes [35,36]. In this study, NUpE and NUtE as dependent NUE components showed different trends in their response to N levels. NUpE showed a slight increase as the N rate increased from 0 to 150 kg N ha^{-1} and NUtE showed a decrease with the increase in N rates. These results are in accord with similar previous data [37,38], suggesting that NUpE is the most remarkable component of NUE at low N supply, while NUtE is more significant in determining NUE at high N supply.

4.2. How Genotypic Variability Impacts Grain Yield (GY), Grain Protein Content (GPC) and NUE and Their Relationships

Genotypic variation in GY under N fertilization has been previously observed in wheat and other cereals [8,39]. Such variability was observed in our set of cultivars for GY and NUtE under different N supplies. Improved genotypes showed a greater GY at high N, but these varieties require higher N and an enhanced uptake efficiency [40]. Conversely, landraces would be better able to take up N mineralized from the soil. This would suggest that improved genotypes show a better response to nitrogen fertilization; in landraces, this behavior is possibly due to missing dwarf genes associated with high N supply [15,40].

Our data indicate a genotypic variability in GPC and NUtE as a response to N fertilizer application. The improved genotype Khiar showed the lowest GPC and the highest NUtE. At a given level of N supply, cultivars with higher yield potential have lower protein content than cultivars with lower yield potential [8,19]. Moreover, improved genotypes showed the highest NUtE, while the landraces recorded the lowest value. This is in accord with previous studies showing the genotypic variability of NUtE in wheat [8,39]. Furthermore, high NUtE is essential for high grain yield because the improved genotypes utilize N efficiently [19]. Usually, GY and GPC yield are negatively correlated. Conversely, another important parameter is grain protein deviation (GPD), which refers to a grain protein content (GPC) greater than expected for any particular yield. This is a desirable trait that may be linked to anthesis date and post-anthesis N uptake [7]. GPC is affected by partitioning, as a large fraction of grain N comes from remobilization from vegetative tissues [7]. This could explain the relations between GPC and NUtE shown by our results. However, genetic variation in GY and GPC is mainly controlled by pre-anthesis N accumulation more than post-anthesis N remobilization [11], thus suggesting a prominent role of NUpE in the GY process. NUpE increases with N application rates up to a certain threshold level; then, NUpE declines even if the N supply increases [40]. However, the results indicated that NUpE was higher at low N application but decreased at higher N supply. Generally, NUpE was not affected in durum wheat genotypes [9]. The genetic effect on N uptake on NUE has not been detected while assessing 195 bread wheat genotypes in multi-environmental experiments [39]. Our results are in accordance with previous observations [5,9], indicating that NUpE is a more important component of NUE at a low N supply, while NUtE is more important in determining NUE at a high N supply [9,40].

The importance of GPC and its correlation with NUE components require further investigation. According to genotype origin, the correlation network and multiple linear regression analysis showed different relationships between NUE and GPC. GPC is closely related to NUtE regardless of the durum wheat genotype origin. Thus, NUpE for improved genotypes could be the most determinant component for GPC and NUE for landraces. The GY of improved cultivars seems more related to the ability of N uptake by roots and its utilization for plant growth rather than to the increasing protein content in the grains [40].

The direct and positive correlation between GPC, NUpE and NUE in durum wheat genotypes has been confirmed. The regression analysis showed a significant and positive relationship between GPC and NupE in improved genotypes. Therefore, GPC is assumed to be positively associated with NUE for landraces and negatively related to NutE. This could be explained by previous findings demonstrating that in wheat, 50% to 95% of the grain N at harvest comes from the remobilization of N stored before anthesis [6,19]. Furthermore, it has previously been shown that a low N uptake by roots during grain filling induced the rapid remobilization of N stored in vegetative tissues into seeds [40]. Therefore, our results suggest that the selection for better NUE may have value in durum wheat breeding programs for higher GY, NUE and GPC.

5. Conclusions

The present study emphasizes the contrasting response to N fertilization between Tunisian durum wheat landrace and improved genotypes. Our results highlight the ability of landrace genotypes (Bidi and Azizi) to demand less N fertilizer in comparison with improved ones (Om Rabiaa and Khiar), confirming the adaptation of landraces to a low input environment.

Genotypic variability significantly influenced GY in wheat subjected to different N levels. The maximum GY of about 5.7 t. ha^{-1} was obtained during the first experimental year in the presence of 150 kg N ha^{-1}, but landraces showed minor differences in terms of GY changing the N levels from 75 to 150 kg N ha^{-1}. GY in landraces was mostly related to NUE, while the GY of improved genotypes was related to their ability in N uptake from the soil. Furthermore, landraces showed higher GPC compared with improved genotypes, reporting 13.41 and 13.64 in Azizi and Bidi, respectively.

The direct and positive correlation between GPC, NUpE and NUE suggests that NUpE and NUE are potential candidate selection traits for the improvement of GPC in durum wheat genotypes. Deciphering NUE components showed that NUpE is the most determinant component at low N supply, while NutE is more important in determining NUE at high N supply. Our results also suggest the integration of NutE and NupE as valuable screening traits in durum wheat breeding programs for higher GY and GPC.

Author Contributions: Conceptualization, S.A., S.E., and Y.T.; Data curation, S.J. and F.E.B.A.; Formal analysis, S.A., S.J., and Z.C.; Funding acquisition, S.E. and Y.T.; Investigation, S.J., Z.C., I.Z., S.L., and M.B.; Methodology, S.J., Z.C., I.Z., Z.H., and F.E.B.A.; Supervision, S.E. and Y.T.; Validation, S.A. and S.J.; Writing—original draft, S.A.; Writing—review and editing, S.L., S.E., and Y.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Tunisian Ministry of Agriculture and the Tunisian Ministry of Higher Education and Scientific Research.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available within the article.

Acknowledgments: We would like to thank Hager Slim Amara, director of the laboratory of Genetics and Cereal breeding (LR14AGR01), for her generous gift of the landrace durum wheat genotypes.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chen, X.; Zhou, J.; Wang, X.; Blackmer, A.; Zhang, F. Optimal rates of nitrogen fertilization for a winter wheat corn cropping system in northern China. *Commun. Soil Sci. Plant Anal.* **2004**, *35*, 583–597. [CrossRef]
2. Hirel, B.; Foulkes, J.; Cromier, F.; Gouche, D.; Moênne-Loccoz, Y.; Le Gouis, J. Breeding for increased nitrogen-use efficiency: A review for wheat (*Triticum aestivum* L.). *Plant Breed.* **2016**, *135*, 255–278.
3. FAO. Available online: https://www.fao.org/documents/card/fr/c/cb1447fr (accessed on 1 April 2022).
4. Moll, R.H.; Kamprath, E.J.; Jackson, W.A. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *J. Agron.* **1982**, *74*, 562–564. [CrossRef]
5. Kichey, T.; Hirel, B.; Heumez, E.; Dubois, F.; Le Gouis, J. In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisationto the grain correlates with agronomic traits and nitrogen physiological markers. *Field Crops Res.* **2007**, *102*, 22–32. [CrossRef]
6. Hawkesford, M.J. Reducing the reliance on nitrogen fertilizer for wheat production. *J. Cereal Sci.* **2014**, *59*, 276–283. [CrossRef] [PubMed]
7. Hawkesford, M.J.; Griffiths, S. Exploiting genetic variation in nitrogen use efficiency for cereal crop improvement. *Curr. Opin. Plant Biol.* **2019**, *49*, 35–42. [CrossRef] [PubMed]
8. Ortiz-Monasterio, J.I.; Sayre, K.D.; Rajaram, S.; Mcmahon, M. Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. *Crop. Sci.* **1997**, *37*, 898–904. [CrossRef]
9. Ortiz-Monasterio, J.I.; Manske, G.G.B.; van Ginkel, M. Nitrogen and phosphorus use efficiency. In *Application of Physiology in Wheat Breeding*; Reynolds, M.P., Ortiz-Monasterio, J.I., Mcnab, A., Eds.; CIMMYT: El Batan, Mexico, 2002; pp. 200–207.
10. Gaju, O.; Allard, V.; Martre, P.; Le Gouis, J.; Moreau, D.; Bogard, M.; Hubbar, S.; Foulkes, M.J. Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. *Field Crops Res.* **2014**, *155*, 213–223. [CrossRef]
11. Fageria, N.K. Yield physiology of rice. *J. Plant. Nutr.* **2007**, *30*, 843–879. [CrossRef]
12. Ata-Ul-Karim, S.T.; Zhu, Y.M.; Cao, Q.; Rehmani, M.I.A.; Cao, W.; Tang, L. In-season assessment of grain protein and amylose content in rice using critical nitrogen dilution curve. *Eur. J. Agr.* **2017**, *90*, 139–151. [CrossRef]
13. Fageria, N.K.; Baligar, V.C. Enhancing nitrogen use efficiency in crop plants. *Adv. Agron.* **2005**, *88*, 97–185.
14. Ayadi, S.; Karmous, C.; Hammami, Z.; Trifa, Y.; Rezgui, S. Variation of durum wheat yield and nitrogen use efficiency under Mediterranean rainfed environment. *Int. J. Agr. Crop. Sci.* **2014**, *7*, 693–699.
15. Ayadi, S.; Karmous, C.; Chamekh, Z.; Hammami, Z.; Baraket, M.; Esposito, S.; Rezgui, S.; Trifa, Y. Effects of nitrogen agronomic efficiency of Durum wheat genotypes under different environments. *Ann. Appl. Biol.* **2016**, *168*, 264–273. [CrossRef]
16. Ata-Ul-Karim, S.T.; Cao, Q.; Zhou, Y.; Tang, L.; Rehmani, M.I.A.; Cao, W. Non-destructive Assessment of Plant Nitrogen Parameters Using Leaf Chlorophyll Measurements in Rice. *Front. Plant. Sci.* **2016**, *7*, 1829. [CrossRef] [PubMed]
17. Ben Mariem, S.; González-Torralba, J.; Collar, C.; Aranjuelo, I.; Morales, F. Durum Wheat Grain Yield and Quality under Low and High Nitrogen Conditions: Insights into Natural Variation in Low- and High-Yielding Genotypes. *Plants* **2020**, *9*, 1636. [CrossRef]
18. Chardon, F.; Noêl, V.; Masclaux-Daubresse, C. Exploring NUE in crops and in *Arabidopsis* ideotypes to improve yield and seed quality. *J. Exp. Bot.* **2012**, *63*, 3401–3412. [CrossRef]
19. Anbessa, Y.; Juskiw, P.; Good, A.; Nyachiro, J.; Helm, J. Genetic variability in nitrogen use efficiency of spring barley. *Crop. Sci.* **2009**, *49*, 1259–1269. [CrossRef]
20. Canãs, R.A.; Amiour, N.; Quillere, I.; Hirel, B. An integrated statistical analysis of the genetic variability of nitrogen metabolism in the ear of three maize inbred lines (*Zea mays*). *J. Exp. Bot.* **2011**, *62*, 2309–2318. [CrossRef]
21. Gorny, A.G.; Banaszak, Z.M.; Lugowska, B.; Ratajcka, D. Inheritance of the efficiency of nitrogen uptake and utilisation in winter wheat (*Triticum aestivum*) under diverse nutritional levels. *Euphytica* **2011**, *177*, 191–206. [CrossRef]
22. Guendouz, A.; Guessoum, S.; Maamari, K.; Hafsi, M. Effects of supplementary irrigation on grain yield, yield components and some morphological traits of durum wheat (*Triticum durum*) Cultivars. *Adv. Environ. Biol.* **2012**, *6*, 564–572.
23. Jallouli, S.; Ayadi, S.; Landi, S.; Capasso, G.; Santini, G.; Chamekh, Z.; Zouari, I.; Azaiez, F.B.A.; Trifa, Y.; Esposito, S. Physiological and molecular osmotic stress responses in three durum wheat (*Triticum turgidum* ssp Durum) genotypes. *Agronomy* **2019**, *9*, 550. [CrossRef]
24. Ayadi, S.; Jallouli, S.; Landi, S.; Capasso, G.; Chamekh, Z.; Cardi, M.; Paradisone, V.; Lentini, M.; Karmous, C.; Trifa, Y.; et al. Nitrogen assimilation under different nitrate nutrition in Tunisian durum wheat landraces and improved genotypes. *Plant Biosyst.* **2020**, *154*, 1–21. [CrossRef]
25. Landi, S.; Capasso, G.; Ben Azaiez, F.E.; Jallouli, S.; Ayadi, S.; Trifa, Y.; Esposito, S. Different roles of heat shock proteins (70 kda) during abiotic stresses in barley (*Hordeum vulgare*) genotypes. *Plants* **2019**, *8*, 248. [CrossRef] [PubMed]
26. Deghaïs, M.; Kouki, M.; Gharbi, M.S.; El Faleh, M. *Les Variétés des Céréales Cultivées en Tunisie*; Institut National de la Recherche Agronomique de Tunisie: Tunis, Tunisia, 2007; p. 445.
27. Rhine, E.D.; Sims, G.K.; Mulvaney, R.L.; Pratt, E.J. Improving the Berthelot reaction for determining ammonium in soil extracts and water. *Soil Sci. Soc. Am. J.* **1998**, *62*, 473–480. [CrossRef]
28. Cataldo, D.A.; Schrader, L.E.; Youngs, V.L. Analysis by digestion and colorimetric assay of total nitrogen in plant tissues high in nitrate. *Crop. Sci.* **1974**, *14*, 854–856. [CrossRef]

29. Good, A.; Shrawat, A.K.; Muench, D. Can Less Yield More? Is Reducing Nutrient Input into The Environment Compatible with Maintaining Crop Production? *Trends Plant Sci.* **2004**, *9*, 597–605. [CrossRef]
30. SAS Institute. *The SAS System for Windows Version 9.2.*; SAS Institute: Cary, NC, USA, 1999.
31. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin, N.; Schlwikowski, B.; Ideker, T. Cytoscape a software environment for integrated models of biomolecular Interaction networks. *Genome Res.* **2003**, *13*, 2498–2504. [CrossRef] [PubMed]
32. Roy, R.N.; Finck, A.; Blair, G.J.; Tandon, H.L. *Plant Nutrition for Food Security: A Guide for Integrated Nutrient Management. FAO Fertilizer and Plant Nutrition Bulletin 16*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2006; 368p.
33. Lopez-Bellido, L.; Lopez-Bellido, R.J.; Lopez-Bellido, F.J. Fertilizer nitrogen efficiency in durum wheat under rain fed Mediterranean conditions: Effect of split application. *Agric. J.* **2006**, *98*, 55–62.
34. Foulkes, M.J.; Sylvester-Bradley, R.; Scott, R.K. Evidence for differences between winter wheat cultivars in acquisition of soil mineral nitrogen and uptake and utilization of applied fertilizer nitrogen. *J. Agric. Sci.* **1998**, *130*, 29–44. [CrossRef]
35. Le Gouis, J.; Béghin, D.; Heumez, E.; Pluchard, P. Genetic differences for nitrogen uptake and nitrogen utilization efficiencies in winter wheat. *Eur. J. Agron.* **2000**, *12*, 163–173. [CrossRef]
36. Wang, R.F.; An, D.G.; Hu, C.S.; Li, L.H.; Zhang, Y.M.; Jia, Y.G.; Tong, Y.P. Relationship between nitrogen uptake and use efficiency of winter wheat grown in the North China Plain. *Crop. Pasture Sci.* **2011**, *62*, 504–514. [CrossRef]
37. Cormier, F.; Faure, S.; Dubreuil, P.; Heumez, E.; Beauchêne, K.; Lafarge, S.; Praud, S.; Le Gouis, J. A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (*Triticum aestivum*). *Theor. Appl. Genet.* **2013**, *126*, 3035–3048. [CrossRef] [PubMed]
38. Worku, M.; Bänziger, M.; Erley, G.S.; Friesen, D.; Diallo, A.O.; Horst, W.J. Nitrogen uptake and utilization in contrasting nitrogen efficient tropical maize hybrids. *Crop. Sci.* **2007**, *47*, 519–528. [CrossRef]
39. Jat, R.S.; Choudhary, M. Nitrogen utilization efficiency variability in genotypes of Indian mustard (*Brassica juncea*) under contrasting N supply. *J. Plant Nutr.* **2019**, *42*, 2435–2446. [CrossRef]
40. Martre, P.; Porter, J.R.; Jamieson, P.D.; Triboi, E. Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. *Plant Physiol.* **2003**, *133*, 1959–1967. [CrossRef]