



Article Interactive Effects of Nitrogen and Potassium Fertilizers on Quantitative-Qualitative Traits and Drought Tolerance Indices of Rainfed Wheat Cultivar

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Abstract: Increasing global food requirements and global warming are two challenges of future food security. Water availability and nutrient management are two important factors that affect high-yield and high-quality wheat production. The main and interactive effects of nitrogen and potassium fertilizers on quantitative-qualitative properties and drought tolerance of an Iranian rainfed cultivar of wheat, Azar-2, were evaluated. Four rates of nitrogen (N0, N30, N60, and N90 kg/ha), along with four concentrations of potassium (K0, K30, K60, and K90 kg/ha), were applied in rainfed (drought stress) and non-stress conditions. The interactive effect of N × K was significant on nitrogen and protein contents of grains at 5% and 1% probability levels, respectively. Different trends of SSI, STI, K1STI, and K2STI indexes were observed with the interactive levels of nitrogen and potassium. The lowest SSI index (0.67) was observed in N30K30, whereas the highest STI (1.07), K1STI (1.46), and K2STI (1.51) indexes were obtained by N90K60 and N90K90. The obtained results could be useful to increase yield and quality of winter rainfed wheat cultivars under drought stress with cool-rainfed areas. N60K30 and N90K60 can be recommended to increase the grain yield and protein content of rainfed wheat under drought stress and non-stress conditions, respectively.

Keywords: drought stress; protein content; rainfed; yield stability; wheat

1. Introduction

Increasing the grain yield and quality of wheat (*Triticum aestivum* L.), as the most widely distributed cereal crop and major staple food crop in the world, is crucial to meet the growing demands of increasing human population [1,2]. Drought stress is a very important abiotic stress that constricts wheat production, especially in arid and semiarid regions of the world, such as Iran [3]. Significant decreased nest photosynthesis, stomatal conductance, relative water contents, 100-grain weight, and grain yield have been reported for 14 bread wheat genotypes under drought stress [4]. Low amounts of mineral nutrients in the soil is the second important constraint for wheat production in arid and semi-arid regions [5]. For an increasing human population, ever increasing global food requirements, and global warming, the development of basic and applied research on drought stress—as one of the most important treats of world food security—is very important [6–8]. Finding plant genotypes with high yield under drought stress and/or enhancing tolerance of drought sensitive genotypes are the proficient approaches to deal with drought stress-induced losses [9]. Selection and breeding of plants are the most economic and effective ways to overcome abiotic stresses [10]. Direct selection of drought



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). tolerance is not effective, as it is a quantitative character with low heritability. Drought tolerance indices (DTI), such as tolerance (TOL) [11], mean productivity (MP) [11], stress susceptibility index (SSI) [12], geometric mean productivity (GMP) [13], harmonic mean (HARM) [14], relative drought index (RDI) [15], stress tolerance index (STI) [13], yield index (YI) [16], and yield stability index (YSI) [17], which provide a measure of drought based on yield loss under drought condition in comparison to normal condition, are useful tools to assess the tolerance of different genotypes to stressful conditions [18]. Yield-based DTI have been widely applied by researchers to assess the drought tolerance of various important crops, such as oilseed rape (*Brassica napus* L.) [19], teff (*Eragrostis tef* (Zucc.) *Trotter*) [20], upland cotton (*G. hirsutum* L.) [21], and wheat [3,9,22–24].

As aforementioned, enhancing tolerance of drought sensitive genotypes is the second breeding strategy to cope with detrimental outcomes of drought stress. There are some adjuvants that their exogenous supply can increase the plant's ability to protect itself under stressful conditions [25]. The exogenous supply of some nutrient elements can reduce the inhibitory effects of drought stress on different plants. Nitrogen (N), phosphorous (P), and potassium (K) are three important plant stress ameliorants [26]. Creation of favorable growing conditions buffers plant stress, and long-term grain yield stability of winter wheat can be obtained through balanced nutrient supply [27]. Nitrogen (N)—as vital structural component of proteins, Rubisco, nucleic acids, chlorophyll system, and some hormones [28]—is an important nutrient that has a significant role in stimulating plant growth, development, and increasing crop productivity under environmental stresses [26]. In addition to drought stress, nitrogen deficiency is one of the primary factors limiting productivity of wheat [29]. Agami et al. [30] investigated the effect of exogenous nitrogen supply, N-fertilizer (0.3 and 0.6 g N/kg soil), on drought tolerance of wheat plants and reported that under deficit irrigation condition (60% of ETc), nitrogen-treated plants had higher growth and yield characteristics compared to the untreated plants. They also reported that nitrogen-treated plants were significantly better than untreated plants in terms of grain yield, photosynthetic pigments, and antioxidant enzymes activities. Applied N-fertilizer alleviated the adverse effects of drought stress in wheat plants through keeping higher relative water content and water use efficiency, higher osmoprotectants (soluble carbohydrates, soluble proteins, total soluble phenols, and free proline) and antioxidant systems (peroxidase, superoxide dismutase, glutathione reductase, and catalase) [30]. Sedri et al. [3] assessed the effect of different concentrations of nitrogen fertilizer (0, 30, 60, 90, and 120 kg/ha) on DTI of mean productivity (MP), GMP, TOL, SSI, STI, and modified stress tolerance index (MSTI), in a rainfed wheat cultivar and reported that drought tolerance of nitrogen-treated plants was significantly more than control plants, and STI was the best index for drought tolerance assessment. Potassium is another important nutrient element involved in grain weight, yield, and drought resistance of cereals [31,32]. The significantly higher content of endogenous K in a drought-resistant variety than a drought-sensitive variety of wheat has been reported previously [33]. Potassium is also involved in biotic stress tolerance of wheat [34]. Therefore, it seems that exogenous supply of this nutrient element can lead to positive results in wheat under water deficiency conditions. The positive effects of K fertilizer on grain filling and drought resistance of wheat have been reported [35,36]. Potassium (K⁺) has significant effects on enzyme activation, protein synthesis, photosynthesis, stomatal movement, and water-relation (turgor regulation and osmotic adjustment) in plants [37]. The co-application of N and K can prevent the harmful effects of drought stress through different routes. Application of potassium-nitrate-containing chitosan/montmorillonite microparticles led to significant increased root and shoot length, shoot fresh and dry weight, chlorophyll a, carotenoids, total soluble proteins, soluble sugars, potassium, and phosphorous concentrations of spinach (Spinacia oleracea L.) under severe drought stress [5].

In addition to the crop yield, there is an increasing attention to grain quality, especially the grain protein content, in modern intensive agricultural production [38]. In arid and semi-arid environments, wheat grain quality could be greatly affected by drought stress [39].

One of the major challenges in producing high-quality wheat is inconsistency and instability of wheat grain quality during grain filling [40]. Drought stress has an adverse effect on wheat grain development through affecting booting and anthesis stages, disrupting meiosis, reducing pollination efficiency, aborting ovules and seeds, reducing days to anthesis and maturity, and resulting in early seed maturity [41].

Decreased grain yield and quality are the most important challenges of wheat production under drought stress in arid and semi-arid regions. In addition to alleviating the adverse effects of drought stress, the co-application of nitrogen with phosphorus, potassium, and sulfur nutrients can reduce the rates of N fertilizer required by wheat, improve grain yield and protein content, grain yield and grain protein content stability, and subsequently reduce environmental pollution [42]. The present study was conducted to investigate the main and interactive effects of nitrogen and potassium fertilizers on yield components, qualitative characteristics, and yield-based DTI of an Iranian rainfed cultivar (Azar-2) of wheat and find the best interacting levels of N and K on mentioned characteristics under non-stress and drought stress conditions. We hypothesized that yield components, qualitative characteristics, and DTI of Azar-2 would all be affected by the interactions of N and K fertilizers.

2. Materials and Methods

2.1. Experimental Design and Treatments

Two field experiments were conducted, under rainfed (drought stress) and supplemental irrigation (non-stress) conditions, to assess the main and interactive effects of different concentrations of N \times K fertilizers on quantitative-qualitative properties, and drought tolerance of Azar-2 rainfed cultivar of wheat, in two continuous growing seasons (2014–2015 and 2015–2016). The treatments consisted of four rates of N (N0, N30, N60, and N90 kg/ha) and K (K0, K30, K60, and K90 kg/ha) fertilizers. Factorial experiments, based on randomized complete block design (RCBD), were conducted in Arid Land Agricultural Research Station of Qamloo in Kurdistan province, Iran (47°29' E longitudes and 35°9' N latitude). The climate of this region is dry and cool [3].

For soil test, soil samples (6 samples, each being a combination of 10 samples) were taken from 0–30 cm depth, before planting. The physic-chemical soil analysis of experimental sites is presented in Table 1. Urea (46% N) and potassium chloride (60% K₂O) fertilizers were used as the source of N and K nutrients, respectively. Half of the mentioned concentrations of N and K fertilizers were broadcasted uniformly in experimental plots; before cultivation in fall, and remaining half was broadcasted in plots at tillering stage of wheat in spring as top-dress fertilizer. Azar-2 rainfed cultivar of wheat was sown at 150 kg seed per ha in plots of size 4 m \times 5 m.

Environment	SP (%)	${ m Ec} imes 10^{-3}$ (ds/m)	pН	T.N.V (%)	OC (%)	Total.N (%)	P.ava (mg/kg)	K.ava (mg/kg)	Texture
Drought stress	37.820 37.930 39.760	0.590 0.570 0.620	7.900 7.900 7.950	14.380 25.140 15.000	0.700 0.650 0.670	0.080 0.080 0.070	15.900 16.700 16.400	210.000 200.000 180.000	Clay Clay Clay
Non- stress	39.190 38.320 39.080	0.600 0.580 0.590	7.900 7.900 7.900	15.250 14.880 15.250	0.670 0.680 0.690	0.080 0.070 0.080	16.100 14.700 14.700	160.000 170.000 160.000	Clay Clay Clay

Table 1. Soil chemical and physical properties at arid land agricultural research station of Qamloo.

In non-stress conditions, supplemental irrigations were conducted in flowering and grain filling developmental steps. The volumetric soil water contents at depth 0–30 cm (rooting zone) and 30–60 cm were calculated using a time domain reflectometry (TDR)

probe in different phonological stages. The net depth of irrigation (mm) to bring the soil to field capacity was calculated using Equation (1) [43].

$$I_n = (\theta_{FC} - \theta_i) \times D_z \tag{1}$$

where, I_n is the net depth of irrigation (mm), is the volumetric soil water content at field capacity (cm³/cm⁻³), is the volumetric soil water content before irrigation (cm³/cm⁻³), and D_z is the rooting depth (mm).

2.2. Measurements

The main and interactive effects of N and K fertilizers were assessed on quantitative (grain yield, straw yield, and 1000-seed weight) and qualitative (nitrogen and potassium concentrations in flag leaves, nitrogen, potassium, and protein content of seeds) characteristics of Azar-2 cultivar under both drought stress and non-stress conditions. Flag leaf samples were gathered at the heading stage to measure their nitrogen and potassium concentrations. Kjeldahl method was applied to determine the nitrogen content in flag leaf and combined seed samples. Protein content (%) of seed samples was determined by multiplying N content by 6.25 [25]. Atomic absorption spectrum using a flame photometer was applied to estimate the potassium concentration of flag leaf and combined seed samples according to Yoshida et al. [44].

Grain yield, straw yield, and 1000-seed weight (TSW) were measured from randomly selected samples at harvest stage.

2.3. Grain Yield and Grain Protein Content Stability

Stability of grain yield and protein content under interactive effects of applied N and K fertilizers was evaluated using coefficient of variation (CV) index (Equation (2)) [45].

Coefficient of variance (CV) =
$$(\sigma/\bar{\Upsilon}) \times 100$$
 (2)

where, CV is the coefficient of variance, σ is the standard deviation (t/ha), and Υ is the average crop yield (t/ha).

2.4. Drought Tolerance Assessment

To assess the interactive effects of applied N and K fertilizers on drought tolerance of Azar-2 cultivar of wheat, different yield-based DTI—including *MP*, *GMP*, *TOL*, *SSI*, *STI*, and modified stress tolerance index (MSTI)—were calculated. Potential yield in normal (the obtained seed yield in non-stress condition) (Yp) and drought stress condition (Ys), the average performance in non-stress condition (\overline{Y}_p), and drought stress condition (\overline{Y}_s), were measured and then the mentioned DTI were calculated according to the following Equations (3)–(7) [11–13].

$$Mp = \frac{Yp + Ys}{2} \tag{3}$$

$$GMP = \sqrt{Yp \times Ys} \tag{4}$$

$$TOL = Yp - Ys \tag{5}$$

$$SSI = \frac{1 - (Ys/Yp)}{1 - (\overline{Ys}/\overline{Yp})}$$
(6)

$$STI = \frac{Y_S \times Y_p}{\overline{Y_p^2}} \tag{7}$$

Modified stress tolerance index were calculated for non-stress (defined as K1STI), and drought stress (defined as K2STI) conditions, according to Equations (8) and (9) [46], respectively

$$K1 = Y2p/\tilde{y}^2P \tag{8}$$

$$K2 = Y2s/\tilde{y}^2s \tag{9}$$

2.5. Statistical Analysis

Statistical analyses, including the analysis of variance (ANOVA) and means comparison analysis were carried out using the MSTATC software. Least significant difference (LSD) test at 1% ($p \le 0.01$) and 5% ($p \le 0.05$) probability levels was used for means comparison analysis. The coefficient of variance and drought tolerance indices were calculated using Excel 2010 software. Simple correlation analysis was conducted using SAS[®] software (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussions

3.1. Effect of the Mineral Content of the Soil on Morphological and Qualitative Parameters of the Azar-2 Wheat Cultivar

The correlation coefficient analysis of measured mineral contents of field soil with quantitative and qualitative characteristics of Azar-2 wheat cultivar showed that soil minerals such as N, P, and K had significant correlation with grain yield and grain protein content of Azar-2 cultivar in both stress and non-stress condition (Table 2). As the present study was conducted in an open field, the obtained results are valuable for the future field experiments to predict the grain yield and quality of the cultivated crop.

Table 2. Correlation coefficients of basic mineral content of the field soil with the quantitative and qualitative parameters of the Azar-2 wheat cultivar.

	Soil Mineral Contents								
Plant Characteristics	Total.N (%)	P.ava (mg/kg)	K.ava (mg/kg)	Total Neutralizing Value (T.N.V)					
Grain yield	0.745 **	0.591 **	0.432 *	0.081 ns					
Straw yield	0.230 ns	0.415 *	0.123 ns	0.341 ns					
1000-seed weight	0.653 **	0.471 *	0.215 ns	0.182 ns					
Grain N content	0.823 **	0.302 ns	0.243 ns	0.214 ns					
Grain K content	0.146 ns	0.214 ns	0.732 **	0.124 ns					
Grain protein content	0.516 **	0.504 **	0.361 ns	0.632 **					

**, *: Significant at 1% and 5% probability level, respectively. ns: Not significant.

3.2. Main and Interactive Effects of N and K Fertilizers on Quantitative Properties of Wheat under Non-Stress and Drought Stress Conditions

The results of combined ANOVA showed that, under drought stress condition, the main effect of year was significant on grain yield and straw yield at 1% probability level; however, it was not affected 1000-seed weight trait (Table 3). Under non-stress condition, the main effect of year was significant on grain yield, straw yield, and 1000-seed weight of Azar-2 cultivar of wheat at 1% probability level (Table 3). Assessing yield trends in wheat dryland farming and under different fertilization conditions is important to recommend the best fertilizer rates and keep yield increases [47]. Crop yield stability can be assessed through the CV, sustainable yield index (SYI), and some statistical methods such as additive main effects and multiplicative interactions (AMMI). Obtained CV percentage of grain yield under drought stress condition (15.59) was more than that of non-stress condition (10.75) (Table 3). These results indicate higher variation degree and lower stability of average grain yield of Azar-2 cultivar of wheat during 2014–2015 and 2015–2016 growing seasons under drought stress conditions. Unlike grain yield, the CV percentage of straw yield under drought stress conditions (22.6) was lower than calculated CV under non-stress condition (23.25) (Table 3). It is obvious that environmental factors, such as water availability and nutrients, can affect both crops yield and yield stability. In regions with variable waterdeficit years, additional water inputs led to more stable yield productivity in different crops, such as wheat, maize, rice, and soybean, with [48]. Balanced nutrient supply can also support long-term crop yield stability [27].

		 Mean Squares								
Source of Variation	df ^a		Drought Stress		Non-Stress					
		Grain Yield	Straw Yield	1000-Seed Weight	Grain Yield	Straw Yield	1000-Seed Weight			
Year (Y)	1	25,043,094.000 **	463,457,153.760 **	0.076ns	6,043,077.042 **	306,159,695.010 **	283.800 **			
Year \times Block	4	235,071.146 *	753,715.948 ns	11.898*	1,941,093.104 **	4,435,583.292 **	25.351 **			
Nitrogen (N)	3	1,570,010.069 **	186,390.955 ns	63.570**	4,827,021.917 **	945,981.038 ns	14.306 *			
$Y \times N$	3	171,841.917 ns	328267.622 ns	4.778 ns	642,481.125 **	503,941.872 ns	4.887 ns			
Potassium (K)	3	20,432.819 ns	547,642.955 ns	2.226 ns	5886.944 ns	1,035,804.233 ns	17.604 ns			
Y×K	3	143,145.944 ns	1,253,567.399 ns	5.846 ns	31,186.819 ns	2,319,904.622 ns	0.608 ns			
$N \times K$	9	79,243.440 ns	163,752.890 ns	5.099 ns	55,293.694 ns	1,114,630.612 ns	5.808 ns			
$Y \times N \times K$	9	111,048.639 ns	808,701.927 ns	1.765 ns	125,138.421 ns	2,078,221.446 ns	5.968 ns			
Error	60	80,213.690	1,003,141.292	4.206	66,966.582	1,063,045.258	4.356			
CV (%)		15.590	22.600	5.170	10.750	23.250	4.380			

Table 3. Combined analysis of variance of the main and interactive effects of nitrogen and potassium fertilizers on quantitative characteristics of Azar-2 cultivar of wheat under drought stress and non-stress environments.

^a degree of freedom; **, *: Significant at 1% and 5% probability level, respectively. ns: Not significant.

The trend of wheat grain yield stability, based on calculated CV (%), with interactive effects of different rates of N and K fertilizers is depicted in Figure 1a. The highest and lowest CV percentages were obtained from N0 \times K30 (30.14%) and N90 \times K60 (18.17%) (Figure 1a). These results indicate positive effect of combined use of N and K fertilizers on grain yield stability of rainfed Azar-2 wheat. Azar-2 is a well-known dryland wheat cultivar in Iran, with acceptable levels of water use efficiency, grain yield, and biomass production under water-limited conditions [49]. At present, Azar-2 has the second rank of cultivated area in dryland conditions of cold and temperate regions of Iran. This cultivar can be useful for cultivation in arid and semi-arid regions of the world. The present study conducted in two constitute years to assess the drought response of this cultivar under the effect of N and K fertilizers. Azar-2 cultivar in rotation with a plant species that enriches the soil with nitrogen, such as chickpea (*Cicer arietinum*), is ideal for rainfed agriculture in countries with water scarcity. This rotation can improve nitrogen uptake, grain protein, soil nitrogen, soil properties, and elevated availability of nutrients [50]. Liu et al. [51] investigated the effects of different rates of N fertilizer (100%, 75%, 50%, 25%, and 0%) and two tillage patterns (conventional tillage and no-tillage) on wheat and maize productivity in a long-term experiment (10-year). Authors concluded that higher yield stability, according to the calculated SYI, in both wheat and maize cropping system were obtained by application of 75% and 50% N [51]. In terms of yield stability, the combined use of different fertilizers can be more effective than application of one kind of fertilizer alone [52]. In rainfed winter wheat, using a long-term fertilization experiment (20-year), it was revealed that higher grain yield and sustaining the productivity were obtained by integrated use of N and phosphor (P) fertilizers than the sole application of N and/or P [47]. Han et al. [45] assessed the effects of inorganic fertilizers (N, NP, NPK) and organic manure (M) on the yield stability of rice and wheat during a long term (34-year) field experiment and reported that the combined use of both organic manure and inorganic fertilizer led to lower CV of wheat yield and straw biomass. The yield stability analysis of winter wheat in a long-term fertilization experiment (36-year), using AMMI method, it was revealed that 62.3%, 26.3%, and 11.4% of sums of squares were attributable to fertilization effect, environmental effect, and fertilization \times environmental interaction effect, respectively. In addition, authors concluded that the combination of organic and inorganic fertilization led to more stable yields than applied organic or inorganic fertilization alone [53].

In both drought stress and non-stress conditions, the main effect of N fertilizer on grain yield was significant at 1% probability level (Table 3). The main effect of N fertilizer on 1000seed weight trait was significant under both drought stress and non-stress conditions, at 1% and 5% probability levels, respectively (Table 3). However, this effect was not significant on straw yield under non-stress and drought stress environments (Table 3). Results of means comparison analysis using LSD test showed that grain yield of Azar-2 cultivar of wheat was increased with increasing levels of N fertilizer, under both drought stress and



non-stress environments. However, obtained grain yields in the non-stress environment were more than those obtained under drought stress conditions (Table 4).

Figure 1. Grain yield and grain protein content stability of rainfed Azar-2 cultivar of wheat. (a) Trend of grain yield stability of wheat Azar-2 cultivar under interaction effects of different rates of nitrogen and potassium fertilizers. (b) Trend of grain protein content stability of wheat Azar-2 cultivar under interaction effects of different rates of nitrogen and potassium fertilizers.

Table 4. Means comparison analysis of the main effect of applied concentrations of nitrogen fertilizer on quantitative characteristics of Azar-2 cultivar of wheat under drought stress and non-stress environments.

	Droug	ht Stress	Non-Stress			
Nitrogen (kg/ha)	Grain Yield (kg/ha)	1000-Seed Weight (g)	Grain Yield (kg/ha)	1000-Seed Weight (g)		
N0	1473.000	41.580	1893.000	48.820		
N30	1788.000	40.320	2279.000	47.500		
N60	1941.000	39.000	2657.000	27.250		
N90	2066.000	37.820	2857.000	47.160		
LSD (5%)	163.500	1.184	149.400	1.205		
LSD (5%)	217.500	1.575	198.700	1.603		

Zhang et al. [54] assessed the effect of irrigation and nitrogen application on grain yield and quality of winter wheat over three cropping seasons and reported that N fertilizer significantly increased grain yield, grain protein and the total, essential, and non-essential amino acid content. The results of a long-term (20-years) evaluation of effects of N fertilizer on durum wheat revealed that grain yield was remarkably increased by N fertilizer application [55]. It has been reported that the effect of nitrogen fertilization on grain yield variation of winter wheat is more important than annual weather conditions [27]. Nitrogen affects canopy formation, photosynthesis, and subsequently wheat grain yield. Although growth, grain yield, and quality of wheat absolutely depend upon substantial N inputs [56], finding the optimum concentration and avoid over and under-use of N fertilizer is very important to keep maximum productivity with reduced costs, resource waste, and environmental pollution [51].

The interaction effect of N \times K was not significant on grain yield, straw yield, and 1000-seed weight of wheat under both drought stress and non-stress environments (Table 3). These results may relate to the K deficiency in the soil of experimental site (Table 1). In soils with high concentrations of P, K, and S, less N fertilizer might be required for crops to reach rainfall-limited yield potentials. In addition, genetic variation and weather conditions can

also affect N \times nutrient(s) interactions [42]. In addition, water deficiency and drought stress may prevent the absorption of potassium in wheat plants. In comparison with control treatment (N0K0), grain yield of Azar-2 cultivar of wheat increased by 52% with application of N90K60 (Figure 2a). The highest positive increases were then obtained by N90K90 (46%) and N90K0 (42%) interactive effects, respectively (Figure 2a).



Figure 2. Changes of grain yield and protein content of wheat Azar-2 cultivar under applied interactive rates of N and K fertilizers versus control treatment. (a) Differences of grain yield achieved by interactive effects of N and K fertilizers versus control treatment. (b) Differences of grain protein content achieved by interactive effects of N and K fertilizers versus control treatment. Means followed by the same letters within columns are not significantly different at the 5% level.

Based on the results of combined ANOVA, the main effect of K fertilizer, its interactive effects with year (year \times potassium) and with N fertilizer (nitrogen \times potassium), were not significant on all investigated quantitative characteristics of Azar-2 cultivar of wheat neither in drought stress nor in non-stress environments (Table 3). In addition, the interactive effect of year \times nitrogen \times potassium was not significant on grain yield, straw yield, and 1000-seed weight traits, under both drought stress and non-stress conditions (Table 3). It can be related to the non-significant main effect of potassium. In the hierarchy of nutrients for wheat, carbon, N and P are more important than K [57]. Although it has been reported that K fertilizer can affect grain filling and grain yield of wheat under drought stress conditions [32]; however, the main effect of K and its interactive effect with N fertilizer were not significant on grain yield and 1000-seed yield of wheat in the present study. It can be related to the cool condition of the present study, as the previous study was conducted in a controlled dry condition [32]. Assessing the interactive effects of N fertilizer with other components involved in tolerance to cold and chilling stresses, such as brassinosteroids [58], could be more helpful to increase both drought-cold tolerance in wheat under the environments similar to the present study.

3.3. Main and Interactive Effects of N and K Nutrients on Qualitative Properties of Wheat under Non-Stress and Drought Stress Conditions

According to the results of combined ANOVA, potassium concentration of flag leaves and nitrogen and protein contents of grains were changed during to evaluated growing seasons, under drought stress condition (Table 5). Vazquez et al. [59] also reported the significant effect of the year on grain yield and grain protein concentration of wheat in two growing seasons of 2012 and 2013. The obtained CV percentage of grain protein concentration under drought stress (16.35) was lower than that of non-stress conditions (20.42) (Table 5). These results indicate the higher stability of grain protein content of Azar-2 cultivar of wheat under drought stress environment. Giunta et al. [60] evaluated grain protein stability in old and modern durum wheat cultivars, grown under different cropping systems in terms of soil fertility, sowing date, sowing rate, and nitrogen rate, and reported that the greatest genotype and year interaction (G \times Y) was obtained by the more favorable years. They also reported that the interaction of rainfall, during post-anthesis, with nitrogen availability was the main cause of G \times Y interaction for grain protein percentage [60].

Table 5. Combined analysis of variance of the main and interactive effects of nitrogen and potassium fertilizers on qualitative characteristics of Azar-2 cultivar of wheat under drought stress and non-stress environments.

		Mean Squares											
	16.2		Drought Stress					Non-Stress					
Source of Variation	df ^a	Flag L	eaves		Grain			Leaves		Grain			
		Ν	К	Ν	K	Protein	Ν	К	Ν	K	Protein		
Year (Y)	1	0.013 ns	4.263 **	4.438 **	0.001 ns	144.158 **	3.323 **	0.672 **	3.046 **	0.015 ns	105.169 **		
Year \times Block	4	0.957 **	0.090 ns	1.475 **	0.045 **	47.927 **	1.042 **	0.336 **	3.674 **	0.012 ns	120.638 **		
Nitrogen (N)	3	0.068 ns	0.058 ns	0.093 ns	0.003 ns	3.073 ns	0.436 ns	0.280 *	0.092 ns	0.006 ns	2.435 ns		
Y×N	3	0.098 ns	0.140 *	0.029 ns	0.012 ns	0.932 ns	0.303 ns	0.064 ns	0.187 ns	0.008 ns	7.067 ns		
Potassium (K)	3	0.113 ns	0.047 ns	0.084 ns	0.002 ns	2.722 ns	0.184 ns	0.044 ns	0.057 ns	0.001 ns	2.584 ns		
Y×K	3	0.070 ns	0.021 ns	0.003 ns	0.003 ns	0.083 ns	0.222 ns	0.073 ns	0.280 ns	0.003 ns	10.751 ns		
$N \times K$	9	0.131 ns	0.039 ns	0.271 **	0.006 ns	8.820 **	0.271 ns	0.070 ns	0.126 ns	0.003 ns	3.992 ns		
$Y \times N \times K$	9	0.199 ns	0.082 ns	0.206 *	0.007 ns	6.638 *	0.151 ns	0.050 ns	0.205 ns	0.004 ns	7.533 ns		
Error	60	0.196	0.044	0.099	0.005	3.199	0.208	0.091	0.197	0.006	6.405		
CV (%)		15.40	16.61	16.360	24.320	16.350	14.090	23.170	20.350	20.740	20.420		

^a degree of freedom; **, *: Significant at 1% and 5% probability level, respectively. ns: Not significant.

The main effects of N and K fertilizers were not significant on investigated qualitative characteristics, except in non-stress condition that the main effect of N fertilizer on potassium content of flag leaves was significant at 5% probability level (Table 5). Flag leaves are an important source of N metabolites, including amino acids, which then transport them into the developing kernels [61]. Under drought stress conditions, the interactive effect of N \times K fertilizers was significant on nitrogen and protein contents of wheat grain at a 1% probability level; however, this effect was not significant on all investigated qualitative characteristics under non-stress condition (Table 5). The interactive effect of year \times N \times K was only significant on nitrogen and protein contents of wheat grains at a 5% probability level under drought stress conditions (Table 5). These results indicate the importance of applied fertilizers on qualitative characteristics of wheat grain under water deficiency condition. The use of different nutrients is one of the practical strategies to maintain wheat flour quality. Tao et al. [40] investigated the effect of sulfur fertilization on wheat grain production and wheat flour proteins and reported that grain and protein yields; grain weight; total protein, albumin, gliadin, glutenin, and globulin contents; and total starch were increased using sulfur fertilization. In terms of grain protein concentration stability, the lowest calculated CV was obtained from interaction of N90 \times K60 (7.77); however, the highest CV percentage was obtained from the sole application of K fertilizer (N0 \times K30) (31.52) (Figure 1b). Therefore, the co-application of N and K fertilizers led to the higher grain protein content stability than the individually applied fertilizers (Figure 1b). Balanced nutrition—co-application of N, P, and K, nutrients—may protect proteins against protein dilution as yields increase [42]. Water availability and nutrients supply both can affect the grain N concentration and grain protein content of wheat. Yan et al. [62] reported the

different responses of the grain N concentration of winter wheat to the changing rates of fertilization and irrigation. Authors stated that the grain N concentration was firstly increased and then decreased with increasing rate of fertilization, whereas increasing irrigation rate first led to decrease and then increase of this trait.

Based on the means comparison analysis of the combined data of 2014–2015 and 2015–2016 growing seasons, using LSD test, the highest means of nitrogen and protein contents of wheat grains were obtained from interactive effects of N60 × K0 treatment (Figure 3a,b). The lowest means of these two qualitative characteristics were obtained from N0 × K0 treatment (Figure 3a,b). These results indicate the superiority of N than K in wheat grain quality. In comparison with control treatment (N0K0), protein content of wheat grains increased by 2.90% with application of N90K0 (Figure 2b). However, in contrast to N0K0, protein content changes were 0.24%, -2.28%, and -0.44% with N90K30, N90K60, and N90K90 fertilizers, respectively (Figure 2b). The reduced protein content of wheat grains with increasing levels of K fertilizer, in a constant level of N fertilizer (N90), can be related to the disturbed nutritional balance of wheat under drought stress conditions.



Figure 3. The means comparison analysis of the interactive effects of nitrogen and potassium fertilizers on qualitative characteristics of Azar-2 cultivar of wheat under drought stress condition using LSD test at 5% and 1% probability levels. (a) The interactive effects of nitrogen and potassium fertilizers on nitrogen content of grains. (b) The interactive effects of nitrogen and potassium fertilizers on protein content of grains.

The positive effect of nitrogen fertilization on nitrogen and protein content of wheat grain has been reported previously [63]. Evaluation of long-term (1966–2016) experimental data revealed that grain-nitrogen concentration of wheat increased linearly with increases in N, whereas it reduced with increases in P and K rate [64]. Nitrogen regime has important role in the concentration of storage protein in wheat grains and bread-making quality [59]. Increasing grain protein concentration with increasing levels of N fertilizer has been reported in wheat [65]. Zhang et al. [66] reported that expression of glutamine synthetase genes (*GS1* and *GS2*) in wheat cultivars were significantly increased with applied high

levels of N fertilizer. They also reported that high nitrogen resulted in increased grain yield, grain protein content, and protein fraction. Grain protein concentration is an important factor that determines baking quality of wheat flour. The protein content of wheat grains is more than other important cereals—such as maize (*Zea mays*) millet (*Pennisetum glau*-

is more than other important cereals—such as maize (*Zea mays*), millet (*Pennisetum glaucum*), rice (*Oryza sativa*), and rye (*Secale cereale*)—which makes wheat flour valuable for the production of bread, pasta, and other bakery products [56]. Obtained grain protein concentration under drought stress condition (12.24%) (Figure 3b) is suitable for bakery products, as recently it has been reported that baking volume of wheat varieties with a 1–2% lesser protein content than varieties with high raw protein content (13–16%) was similar [56].

3.4. Main and Interactive Effects of N and K Nutrients on Yield-Based Drought Tolerance Indices of Wheat

Estimated values of YP, YS, MP, GMP, TOL, SSI, STI, K1STI, and K2STI in Azar-2 cultivar of wheat under the interactive effects of N and K fertilizers are presented in Table 6. The highest YP and YS values were obtained from N90 × K90 and N90 × K60, respectively (Table 6). The highest MP, GMP, STI, K1STI, and K2STI drought tolerance indices were obtained from the interaction of N90 × K60; whereas the lowest values of the mentioned DTI were obtained from N0 × K60 interactive effect (Table 6). The highest and lowest estimated TOL and SSI values were obtained from the N90 × K30 and N0 × K90 interactive effects, respectively (Table 6). The obtained results indicate the greater importance of N fertilizer than K fertilizer in drought tolerance of wheat. These results also showed the high interaction effects of investigated concentrations of N and K fertilizers on DTI of Azar-2 cultivar of wheat. Correlation coefficient of drought tolerance indices and grain yield is a suitable criterion for screening the best indices [67].

Table 6. Drought indexes in different rates of nitrogen and potassium fertilizers in rainfed wheat over two years.

Nitrogen (kg/ha)	Potassium (kg/ha)	Ys (kg/ha)	Yp (kg/ha)	МР	GMP	TOL	SSI	STI	K2STI	K1STI
	K0	1483	1881	1682	1670	398	0.86	0.48	0.31	0.29
NIO	K30	1537	1853	1695	1688	317	0.70	0.49	0.35	0.28
INU	K60	1411	1924	1667	1648	512	1.08	0.47	0.28	0.29
	K90	1461	1700	1580	1576	238	0.57	0.43	0.27	0.21
	K0	1898	2356	2127	2114	458	0.79	0.77	0.83	0.72
N30	K30	1885	2254	2070	2061	369	0.67	0.73	0.78	0.63
	K60	1638	2230	1934	1911	592	1.08	0.63	0.50	0.53
	K90	1731	2278	2005	1986	547	0.98	0.68	0.61	0.59
	K0	1835	2731	2283	2238	896	1.34	0.86	0.87	1.08
NKO	K30	2060	2646	2353	2335	585	0.90	0.94	1.19	1.10
IN60	K60	1906	2514	2210	2189	609	0.99	0.83	0.89	0.88
	K90	1962	2737	2350	2317	775	1.15	0.93	1.06	1.17
	K0	2110	2748	2429	2408	638	0.95	1.00	1.33	1.27
N90	K30	1880	2880	2380	2327	1000	1.41	0.93	0.98	1.30
	K60	2142	2896	2519	2491	754	1.06	1.07	1.46	1.51
	K90	2129	2903	2516	2486	774	1.09	1.07	1.44	1.51
	Mean	1817	2408	2112	2090	591	0.98	0.77	0.82	0.83

Yp: grain yield under non-stress condition; Ys: grain yield under drought-stress condition; MP: mean productivity; GMP: geometric mean productivity; TOL: tolerance; SSI: stress susceptibility index; STI: stress tolerance index; K1STI: modified stress tolerance index in non-stress condition; K2STI: modified stress tolerance index in drought stress condition.

The simple correlation of calculated yield-based drought tolerance indices with grain yield under the non-stressed (Yp) and moisture-stressed (Ys) conditions are shown in Table 7. K2STI showed the highest positive correlation with grain yield under drought stress

condition (r = 0.96 **), following with GMP and STI indices (r = 0.94 **) (Table 7). K1STI, MP, GMP, and STI showed the highest positive correlation with grain yield under non-stress condition (Table 7). Nitrogen can improve drought tolerance of wheat through affecting physiological and biochemical processes such as accumulation of osmoprotectants and activity of antioxidant enzymes [26]. There is a positive correlation between water deficit stress and accumulation of reactive oxygen species (ROS) in cellular organelles [1,68]. Some cellular components, such as osmoprotectants and antioxidants, can stabilize cell membrane and proteins under stressful conditions through scavenging cellular ROS accumulation and keeping cell osmotic pressure [7,69]. In other hands, it has been reported that exogenous nitrogen supply led to higher osmoprotectants, antioxidant system, and lower relative membrane permeability in wheat plants [30]. Therefore, ROS detoxification and osmotic adjustment are the possible physiological mechanisms through which N-fertilizer can alleviate damages of drought stress in wheat and other plants.

 Table 7. Simple correlations of yield-based drought tolerance indices and grain yield of Azar-2 cultivar of wheat.

	Ys	Yp	MP	GMP	TOL	SSI	STI	K2STI	K1STI
Ys	1								
Yp	0.85 **	1							
МР	0.92 **	0.97 **	1						
GMP	0.94 **	0.95 **	0.99 **	1					
TOL	0.56 *	0.86 **	0.77 **	0.75 **	1				
SSI	0.23 ns	0.63 **	0.48 ns	0.45 ns	0.90 **	1			
STI	0.94 **	0.95 **	0.99 **	0.99 **	0.75 **	0.45 ns	1		
K2STI	0.96 **	0.94 **	0.98 **	0.99 **	0.73 **	0.43 ns	0.99 **	1	
K1STI	0.89 **	0.99 **	0.98 **	0.97 **	0.83 **	0.57 *	0.97 **	0.96 **	1

Yp: grain yield under non-stress condition; Ys: grain yield under drought-stress condition; MP: mean productivity; GMP: geometric mean productivity; TOL: tolerance; SSI: stress susceptibility index; STI: stress tolerance index; K1STI: modified stress tolerance index in non-stress condition; K2STI: modified stress tolerance index in drought stress condition. **, *: Significant at 1% and 5% probability level, respectively. ns: not significant.

Estimated SSI values from the interaction of different rates of N fertilizer across applied rates of K fertilizer showed decreasing trend in interaction of lowest concentration of N (N30) with increased concentration of K (K0 to K30), and then it was increased with increasing levels of K (Figure 4a). Estimated STI values during two investigated growing seasons showed an increasing trend with increasing rates of N fertilizer across applied rates of K fertilizer (Figure 4b). The intersection point of STI trend curve was observed in $N60 \times K30$ and $N90 \times K30$ (Figure 4b). Therefore, there is no significant difference between these two interactive treatments in terms of grain yield under drought stress condition. However, the highest STI values were obtained by N90K60 and N90K90. K1STI was also had uniform trends with changing rates of N fertilizer across different rates of K fertilizer (Figure 4c). Unlike K1STI, K2STI showed variable trends, especially with N60 and N90 across different concentrations of K fertilizer (Figure 4d). The highest values of K1STI and K2STI were also obtained by interactive rates of N90K60 and N90K90 (Figure 4c,d). At all, it can be concluded that N90K60 and N90K90 were the best interactive rates of N and K fertilizers in terms of drought tolerance indices. However, N90K60 is the best, as the intersection point of SSI was also obtained in K60 (Figure 4a).

Drought stress indices are mainly applyied by researchers to find most tolerant genotypes among huge number of studied genotypes. However, in the present study, DTI were applied to find the best interaction(s) of N and K fertilizers for enhance drought tolerance of a rainfed cultivar, Azar-2, cultivar of wheat. Sedri et al. [3] used drought stress indices of MP, GMP, TOL, SSI, STI, K1STI, and K2STI to evaluate effect of different concentrations of N fertilizer on drought tolerance of rainfed wheat and reported that application of 60 kg/ha N fertilizer improved both grain yield and drought tolerance of wheat. Khan and Mohammad [70] used stress tolerance indices of TOL, MP, harmonic mean (HM), SI, GMP, STI, YI, YSI, low nitrogen tolerance index (LNTI), nitrogen stress index (NSI), nitrogen index (NI), stress susceptibility percentage index (SSPI), K1STI, K2STI, and relative nitrogen index (RNI) to assess grain yield of wheat varieties under with (N+) and without nitrogen (N0) conditions. They reported that only NI had a positive correlation with grain yield under stress conditions [70].



Figure 4. Trend of drought tolerance indices of Azar-2 cultivar of wheat under interactive effects of nitrogen and potassium fertilizers. (**a**) Trend of estimated SSI values under interactive effects of different concentrations of nitrogen and potassium fertilizers. (**b**) Trend of estimated STI values under interactive effects of different concentrations of nitrogen and potassium fertilizers. (**c**) Trend of estimated K1STI values under interactive effects of different concentrations of nitrogen and potassium fertilizers. (**d**) Trend of estimated K2STI values under interactive effects of different concentrations of nitrogen and potassium fertilizers.

Although yield-based DTI are most applied tools to identify tolerant genotypes in different crops, however, their discrimination efficiency depends on stress severity. Mohammadi [71] applied STI, GMP, MP, TOL, SSI, YSI, and YI drought tolerance indices to identify tolerant genotypes of durum wheat under mild, moderate, and severe levels of drought stress and reported that, under severe stress, discrimination among the genotypes was better than mild stress condition.

4. Conclusions

The exogenous supply of some nutrients, especially nitrogen, can improve grain yield, grain quality, and yield stability of wheat. In addition, these nutrients can alleviate drought stress-induced yield loss of wheat. The interactive effects of N and K fertilizers were not significant on grain yield trait, under both drought stress and non-stress environments; however, the main effect of N was significant on grain yield in both investigated environments. The interactive effects of N and K fertilizers, under drought stress conditions, significantly affected the grain protein content. In comparison with control treatment (N0K0), the highest increases of grain yield and grain protein content were obtained from the interactive rates of N90K60 and N90K0, respectively. The grain yield stability, based on calculated CV percentage of grain yield, of Azar-2 rainfed cultivar of wheat in drought stress condition was lower than non-stress condition. Calculated CV percentage of grain protein content that obtained from non-stress con-

dition. In terms of grain yield and grain protein content stability, the combined use of N and K fertilizers (interaction of N90 \times K60) was better than N and K fertilizers individually. The interaction of N90 \times K60 led to the highest estimated MP, GMP, STI, K1STI, and K2STI drought tolerance indices. K2STI, GMP, and STI had the highest positive correlation with grain yield of Azar-2 rainfed cultivar of wheat under drought stress condition.

The positive effect of N fertilizer on yield and drought tolerance of wheat was significant in the present study. However, using other components involved in chilling tolerance—such as brassinosteroids and hydrogen sulfide—along with N fertilizer could enhance drought-chilling tolerance of wheat and increase its productivity under environments similar to the environment of the present study (cool and dry).

The present study could be expanded to include other types of fertilizers (individually or combined) and other wheat genotypes in future experiments. Different tolerance-enhancing components could be used along with conventional fertilizers to enhance biotic and abiotic stress tolerance of wheat genotypes. However, there are some limitations for both in-door and open filed assessments in this regard. Genotype \times environment interaction is an important influencing factor that should be considered in stress tolerance assessment experiments. Many phenological, morphological, physiological, and biochemical characteristics must be assessed to find the best interaction(s) of applied stress tolerance-enhancers. This creates a multi-factorial condition, which is difficult to interpret. Advanced machine learning algorithms could be useful to make the right decisions in these situations.

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