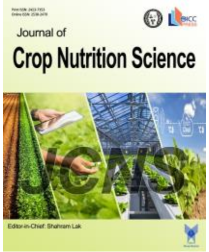

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Research Article

Assess Different Rate and Source of Nitrogen Fertilizer on Wheat (*Triticum aestivum* L.) Crop Production under Drought Stress Condition

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Abstract

Background: Climate change is expected to accelerate, leading to further drought stress in large areas of the world. Considering wheat's status as a dietary staple for a significant portion of the global populace, it becomes apparent that attaining a thorough comprehension of the fertilizers effectiveness, widely utilized in current agricultural practices, holds paramount significance for the enhancement of global food security.

Objectives: This study was done to evaluate the effect of different rate and source of Nitrogen fertilizer on quantitative and qualitative parameters of Wheat affected drought stress condition in two moderate climate regions.

Methods: Current research conducted according factorial split plot experiment based on complete randomized block design with three replications. The experimental treatments included three levels of drought stress [control or no stress, irrigation until the booting stage (booting-stage drought or BSD), and irrigation to the soft dough stage (Maturation-stage drought or MSD)] in the main plots. Different source of Nitrogen fertilizer [including Urea (U) and Ammonium Nitrate (AN)] belonged to first subplot and three level of different rate of Nitrogen [Normal (N) amount according soil test, 50% higher than normal amount (N+50), and 50% lower than normal amount (N-50)] belonged to second subplot.

Result: In addition to drought stress leading to a decrease in 1000-grain weight, the application of nitrogen fertilizer at a rate of 50 percent above normal caused an increase in 1000-grain weight of wheat compared to normal treatments and 50 percent below normal. Grain yield in the control treatment without drought stress was higher (5792 $kg.ha^{-1}$) than the two drought stress treatments, and grain yield with ammonium nitrate fertilizer application (4853 $kg.ha^{-1}$) was higher than that with urea fertilizer application (4275 $kg.ha^{-1}$). The highest biological yield was for ammonium nitrate fertilizer and in the control treatment without drought stress, at 14908 $kg.ha^{-1}$. The highest grain protein percentage of 12.81% was obtained in the irrigation treatment up to the inflorescence emergence stage and with the application of ammonium nitrate, and increasing the amount of Nitrogen fertilizer application led to a further increase in grain protein percentage.

Conclusion: Use of Baharan Wheat variety in studied region at non drought stress conditions and application of Ammonium Nitrate fertilizer at a rate 50% higher than the recommended level by soil test can be advised to producers.

Keywords: Chlorophyll, Correlation, Irrigation, Proline, Protein, Soluble sugar.

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1. Background

Wheat, a cereal crop of global significance and a primary staple food source, confronts a formidable challenge in the due to consequences of climate change (Abdolmaleki et al., 2022), particularly drought stress. This environmental issue exerts detrimental effects on wheat's growth and development (Ahmed et al., 2022). Drought stress can be characterized as a deficiency in water availability that triggers profound alterations in plants at morphologic, biochemical, physiological, and molecular levels (Ghadirnezhad Shiade et al., 2023a). Water deficit exerts adverse effects on the physio-morphologic characteristics of Wheat, encompassing parameters such as shoot length, osmoprotectant compounds, chlorophyll content, and leaf area. Additionally, drought stress disrupts water balance and interferes with cellular-level metabolic reactions (Ahmed et al., 2022). Moreover, the wheat crop exhibits sensitivity to both heat and drought stresses, with pronounced effects during flowering and grain development, ultimately resulting in reduced yield and grain quality. Alarmingly, annual production variability, estimated at approximately 40%, can be attributed primarily to heatwaves and drought occurrences in key Wheat-producing regions worldwide (Kulkarni et al., 2017). Fertilizers play a pivotal role in modern agriculture, enhancing crop productivity and ensuring food security (Fathi and Zeidali, 2020). Among the numerous fertilizers available, Ammonium Nitrate (AN) and Urea (U) are two widely utilized options (Cowan et al., 2020). Nitrogen is an essential macronutrient for plant functioning and is a key component of amino acids, which are the building blocks of plant proteins and enzymes. This element is also a component of the chlorophyll molecule, enabling the plant to capture the energy of sunlight through photosynthesis, which promotes plant growth and grain yields (Yousaf et al., 2021). In terms of chemical composition, AN (NH4NO3) consists of two major components: Ammonium ions ($NH4^+$) and nitrate ions ($NO3^-$) which are available for plants to absorb. This dual-ion composition allows for the simultaneous supply of both ammonium and nitrate forms of Nitrogen to plants (Fathi, 2022). On the other hand, U ($CO(NH_2)_2$), is a diamide of carbonic acid, containing only one form of Nitrogen. Urea must undergo a biochemical conversion process in the soil, hydrolysis, to transform into plant-available forms of Nitrogen, primarily Ammonium and Nitrate. This conversion occurs through the enzymatic activity of urease-producing microorganisms. Therefore, its nutrient release process is slower than AN (Cowan et al., 2020). Water shortage may impede Nitrogen absorption by plants, leading to this nutrient deficiency which exacerbates the drought stress consequences (Lv et al., 2021). Wierzbowska et al., (2022) studied maize (Zea mays L.) yield, which was fertilized with U and AN combined solution enriched with Phosphorus (P), Magnesium (Mg), or sulfur (S). They found that the highest grain yield was recorded by the treatments exposed with AN and U. Besides, Szmigiel et al., (2016) documented that Nitrogen fertilization enhanced biomass and grain yield in spring Wheat. Moreover, Cheng et al. (2021) found that Nitrate application under drought stress reduced Rice yield by approximately 30% less than U fertilization. Furthermore, their results revealed that the aboveground organs of Rice plants were smaller but more compact under nitrate application. Although several studies have investigated the role of Nitrogen fertilizers in enhancing crop performance and addressing drought stress consequences, there is a noticeable gap in the literature regarding a direct comparison of the effects of two widely used Nitrogen sources, AN and U, under drought stress conditions. While both these fertilizers supply Nitrogen to plants, the different forms and release mechanisms of Nitrogen might lead to varying responses of wheat to drought stress. Hence, addressing this comparative aspect is essential to provide a more comprehensive understanding of how these two Nitrogen sources impact wheat growth, physiological responses; and ultimately, yield under water-limited conditions. In this study, two hypotheses were addressed; first, the utilization of two common Nitrogen fertilizers, namely AN and U, under drought stress will yield distinct effects on the morphologic and physiological traits of Wheat. Second, whether the differences in Nitrogen source and availability influence Wheat's ability to cope with drought stress. This information is essential for understanding how fertilizer choice can impact crop resilience and yield under water scarcity conditions, contributing to more informed fertilizer recommendations for drought-prone regions.

2. Objectives

The main goals of this study was to investigate the effects of drought stress and the choice of Nitrogen fertilizers, on the morphologic and physiological characteristics of Wheat. Besides, this research should provide valuable insights for optimizing nutrient management strategies and increasing Wheat productivity under challenging environmental conditions.

3. Materials and methods

3.1. Field and Treatments Information

This study was conducted at the Research and Education Agricultural and Research Station of two moderate regions of Lorestan province (West of Iran), specifically in the cities of Borujerd (48° 76′ 0″ E, 33° 89′ 0″ N, and

1550 m above sea level) and Dorud (49° 05′ 0″ E, 33° 49′ 0″ N, and 1455 m above sea level) in the agronomic year 2017-2018. Borujerd and Dorud cities are both located in the northern part of Lorestan province and have a cold and moderate climate with deep clay soil. The average temperature and rainfall conditions of study areas are illustrated in Fig. 1. Current research was done according factorial split plot experiment based on complete randomized block design with three replications. The experimental treatments included three levels of drought stress [control or no stress, irrigation until the booting stage (booting-stage drought or BSD), and irrigation to the soft dough stage (Maturation-stage drought or MSD)] in the main plots. Different source of Nitrogen fertilizer [including Urea (U) and Ammonium Nitrate (AN)] belonged to first subplot and three level of different rate of Nitrogen [Normal (N) amount according soil test, 50% higher than normal amount (N+50), and 50% lower than normal amount (N-50)] belonged to second subplot

3.2. Farm Management

Moreover, the main irrigation plots were 2 m apart, while a distance of 0.5 m was maintained between the subplots. According to the physicochemical characteristics of the soil test at the study area (Table 1), Nitrogen (50 kg.ha^{-1} with urea), phosphorus (100 kg.ha^{-1} with triple superphosphate), and potassium (100 kg ha^{-1} with potassium sulphate) were applied as starters. The plants were irrigated as flood irrigation, and cultivated by 450 plant.m^{-2}. To apply drought stress, irrigation was stopped at the booting stage (MDS) and soft-milk doughty stage (LDS) stages. Whereas, control treatment was irrigated until the plant maturity stage. Wheat (*Triticum aestivum*), Baharan CV. seeds and Nitrogen fertilizers were provided by the Research and Education Agricultural Campus of Lorestan Province. The normal rate of Nitrogen fertilizers was determined based on the results of the soil analysis (Table 1), which was 150 kg.ha^{-1} and 165 kg.ha^{-1} in Borujerd and Dorud, respectively. Then, the ± 50% of the normal rate in both areas was calculated and applied at 225, 150, and 75 kg.ha^{-1} in Borujerd, and 247.5, 165, and 79.5 kg.ha^{-1} in Dorud, corresponding to the treatments N+50, N, and N-50.

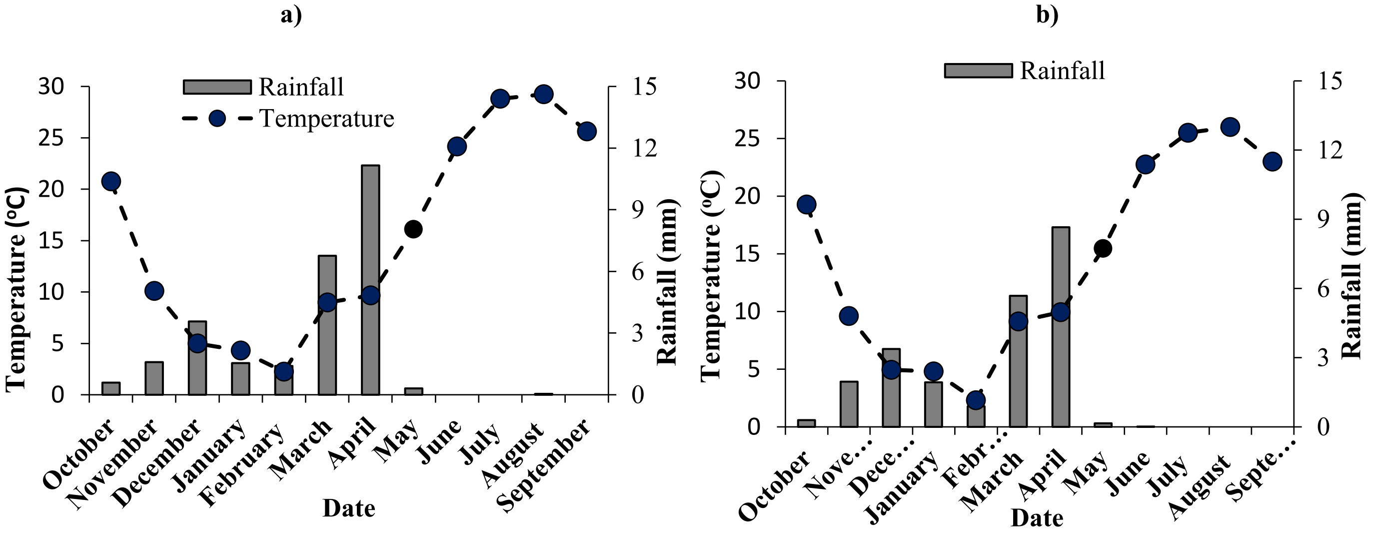

Figure 1. Average rainfall and temperature in tow study areas of a) Borujerd, and b) Dorud.

Table 1. Physicochemical properties of the soil in studied areas (Borujerd and Dorud, 0–30 cm depth)

Place	Sand (%)	Silt (%)	Clay (%)	Soil Texture	NO_3 (mg.kg^{-1})	NH_4 (mg.kg^{-1})	K (mg.kg^{-1})	P (mg.kg^{-1})	OC (%)	EC (ds.m^{-1})	Na (meq.L^{-1})	pH
Borujerd	16.11	34.75	49.14	clay	10.65	5.14	278	10.4	1.17	0.51	9.12	7.91
Dorud	18.25	46.74	35.01	Loamy clay	12.31	4.11	334	13.7	0.72	1.49	6.11	8.11

3.3. Measured Traits

Ten seedlings of each treatment were harvested at the physiological maturity phase when the grain filling was completed to determine the number of tillers per plant, plant height (cm), fertile spike number per m^2, spike length (cm), grain number per spike, 1000-grain weight (g), grain yield ($kg.ha^{-1}$), crop biomass ($kg.ha^{-1}$), harvest index (%), internode diameter (mm), and internode length (mm). Equation (1) was used to calculate harvest index (HI) (Gardner et al., 1985). Eq.1. HI = (Grain yield / biological yield) × 100

Chlorophyll content of five leaves in each plot was measured at anthesis stage by SPAD 502 device, accurately three points of leaf measured and the average of three numbers was considered. (SPAD 502, Minolta Company, Japan). This measurement was conducted on both mature and newly grown leaves of individual plants. Moreover, the determination of grain protein percentage was executed employing the Kjeldahl method. Initially, the Nitrogen content of the grains was ascertained utilizing the Kjeldahl apparatus, following which the grain protein percentage was computed by multiplying this value by a factor of 6.25 (Sosulski and Imafidon, 1990). The leaf area index (LAI) was quantified using a Leaf Area Meter device (BSLM101 Model). For the quantification of proline content, 0.5 g of freshly harvested plant leaves, initially frozen, were subjected to homogenization with 10 mL of a 3% sulfosalicylic acid solution. The resultant mixture was centrifuged, and a 2 mL aliquot of the resulting extract was subsequently subjected to a reaction involving acetic acid and ninhydrin (2 mL each) for a duration of 1 hour at 100°C. The reaction was then promptly finished in an ice bath. Subsequently, 4 mL of toluene was employed to facilitate the extraction process, and this mixture was agitated for 20 seconds. Next, the toluene phase, containing the chromophore, was allowed to reach room temperature, after which its optical density was measured at 520 nm. The quantification of proline content was achieved through reference to a standard curve spanning the range of 1.9 to 125 $\mu mol.L^{-1}$ (Bates et al., 1973). Besides, to determine total soluble sugar content, 0.1 g of the cryogenically frozen plant samples were pulverized with liquid Nitrogen and subsequently ground with 5 mL of 95% ethanol to release the sugars. Successively, 5 mL of 70% ethanol was introduced twice, followed by centrifugation at 3500 rpm for a duration of 10 minutes. The resultant supernatant was stored in a refrigerated environment for a period of one week. After this storage period, 2 mL of the stored solution was combined with 1 mL of a 5% phenol solution, after which 5 mL of sulfuric acid was added to the mixture. The resultant solution was kept at room temperature for 30 minutes, and its absorbance was then measured at 480 nm using a UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan) (Irigoyen et al., 1992).

3.4. Statistical Analysis

Data analysis was done by using the SAS software (SAS, version 9.3, SAS Institute, Cary, NC). The least significant difference (LSD) was used to compare the mean results ($P \leq 0.05$).

4. Result

Result of analysis of variance revealed effect of drought stress, different type of Nitrogen fertilizer and different rate of Nitrogen fertilizer (instead harvest index) on measured traits was significant, but interaction effect of treatments on measured traits was not significant (instead interaction effect of drought stress and different type of fertilizer on biologic yield, harvest index, leaf area index, protein, interaction effect of drought stress and different rate of fertilizer on harvest index, protein, interaction effect of drought stress and different type and rate of fertilizer on proline, soluble sugar and total chlorophyll) (Table 2). Mean comparison was observed that wheat plants cultivated in the Dorud region exhibited a modest reduction in height by 3%. In addition, several traits of the tillers' number per plant, plants height, number of fertile spikes, grain yield, and internode length decreased more significantly in the "BSD" treatment by 46%, 9%, 17%, 45%, and 22% compared to the control group. In contrast, the reduction of this characteristic in the "MSD" treatment was comparatively milder at 24%, 7%, 2%, 17%, and 7% (Table 3).

In addition, our findings indicated that the AN application yielded superior results in improving morphologic characteristics compared with U application. It is noteworthy that no statistically significant difference was found between the treatments effects with N and N+50. Whereas, our results revealed that AN decreased internode diameter, while increased its length compared with U. Furthermore, a remarkable observation was made when the fertilizer rate was reduced by 50% (N-50), which resulted in a reduction in the morphologic characteristics studied, except for the number of grains per spike, which exhibited no significant difference across the three fertilizers (Table 3).

In accordance with Fig. 2, it is evident that both biomass and LAI exhibits were influenced by fertilizer type and drought stress interaction. The most substantial values for these parameters were achieved under non-stress, or control conditions, with biomass reaching approximately 15,000 $kg.ha^{-1}$ and LAI measuring 4.5. Conversely, the introduction of water scarcity during the booting stage (BSD) had a noticeable adverse impact on these parameters, resulting in a 31% reduction in biomass and a 50% reduction at LAI. In comparison, wheat plants subjected to moderate stress during the maturity development stage (MSD) experienced a comparatively less severe reduction, with biomass decreasing by 9% and LAI by 22%

Table 2. Result of Analysis of variance effect of treatments on measured traits

S.O.V	df	No. tiller per plant	Plant height	No. fertile spike per m^2	Spike length	No. grain per spike	1000 grain weight	Grain yield	Biologic yield
Place (p)	1	3.7^{ns}	270**	70278**	42.81**	202**	59.25*	1969110**	3442908**
Block (r)	2	23.59**	226**	4460**	164.14**	330**	406**	5075138**	14683753**
Drought stress (a)	2	177.81**	730**	78165**	65.34**	1347**	291**	64999246**	210521011**
p×a	2	0.25 ^{ns}	5.36 ^{ns}	1531 ^{ns}	0.23 ^{ns}	0.73 ^{ns}	0.62 ^{ns}	13881 ^{ns}	241985 ^{ns}
Error I	2	0.92	4.00	284	14.81	21.8	22.37	39132	89879
Type of fertilizer (b)	1	21.33**	73.34*	8303**	29.03**	213**	128**	9012755**	15762074**
p×b	1	1.33 ^{ns}	1.12 ^{ns}	26 ^{ns}	0.03 ^{ns}	0.33 ^{ns}	0.14 ^{ns}	773 ^{ns}	41418 ^{ns}
a×b	2	26.77^{ns}	12.39 ^{ns}	1272 ^{ns}	1.12 ^{ns}	17.12 ^{ns}	10.84 ^{ns}	37607 ^{ns}	5659091*
p×a×b	2	1.44 ^{ns}	0.34 ^{ns}	192 ^{ns}	0.01 ^{ns}	1.02 ^{ns}	0.06 ^{ns}	4901 ^{ns}	41418 ^{ns}
Rate of Fertilizer (C)	2	918.92*	1159*	17322*	11.78*	137.43*	137.12*	1822940**	11706403**
p×c	2	0.25 ^{ns}	0.69 ^{ns}	29 ^{ns}	0.01 ^{ns}	1.67 ^{ns}	0.064 ^{ns}	2900 ^{ns}	6815 ^{ns}
a×c	4	1.48 ^{ns}	14.76 ^{ns}	356 ^{ns}	2.12 ^{ns}	10.85 ^{ns}	2.25 ^{ns}	101937 ^{ns}	593812 ^{ns}
p×a×c	4	1.73 ^{ns}	1.01 ^{ns}	191 ^{ns}	0.092 ^{ns}	0.21 ^{ns}	0.092 ^{ns}	3748 ^{ns}	2334 ^{ns}
b×c	2	4.33 ^{ns}	20.73 ^{ns}	668 ^{ns}	1.17 ^{ns}	5.45 ^{ns}	2.28 ^{ns}	15443 ^{ns}	176197 ^{ns}
p×b×c	2	0.77 ^{ns}	0.84 ^{ns}	32 ^{ns}	0.064 ^{ns}	1.86 ^{ns}	0.12 ^{ns}	2019 ^{ns}	2114 ^{ns}
a×b×c	4	1.37 ^{ns}	4.99 ^{ns}	227 ^{ns}	0.67 ^{ns}	7.85 ^{ns}	2.62 ^{ns}	29916 ^{ns}	20400 ^{ns}
p×a×b×c	4	0.73 ^{ns}	1.68 ^{ns}	49 ^{ns}	0.037 ^{ns}	2.34 ^{ns}	0.45 ^{ns}	1606 ^{ns}	1531 ^{ns}
Error II	68	2.36	14.83	87	1.42	8.64	8.95	11274	45615
CV(%)	-	2.19	4.51	28.05	3.61	7.53	7.06	7.35	5.32

$^{ns,\ *\ and\ **}$: no significant, significant at 5% and 1% of probability level, respectively.

Continue table 2.

S.O.V	df	Harvest Index	Internode diameter	Internode length	Leaf area index	protein	Proline	Soluble sugar	Total chlorophyll
Place (p)	1	36.59**	2.61**	225**	0.75**	6.81**	0.001^{ns}	136**	39 ^{ns}
Block (r)	2	50.35**	0.6 ^{ns}	47 ^{ns}	0.53**	1.08*	0.0003 ^{ns}	6.22 ^{ns}	0.08 ^{ns}
Drought stress (a)	2	607.41**	8.48**	425**	47.12**	180**	0.96 ^{ns}	1112**	26.9 ^{ns}
p×a	2	3.5 ^{ns}	0.007 ^{ns}	0.44 ^{ns}	0.02 ^{ns}	0.014 ^{ns}	0.0008 ^{ns}	0.001 ^{ns}	1.9^{ns}
Error I	2	4.62	0.003	1.44	0.1	0.0001	0.0025	7.17	1.33
Type of fertilizer (b)	1	162.04**	0.99**	142**	4.32**	27.16**	0.38 ^{ns}	137**	24.7 ^{ns}
p×b	1	0.75 ^{ns}	0.005 ^{ns}	0.59 ^{ns}	0.0000	0.08 ^{ns}	0.022 ^{ns}	5.98 ^{ns}	20.5 ^{ns}
a×b	2	34.48*	0.01 ^{ns}	8.25 ^{ns}	0.39*	7.9*	0.001 ^{ns}	0.4 ^{ns}	0.61 ^{ns}
p×a×b	2	0.79 ^{ns}	0.001 ^{ns}	0.59 ^{ns}	0	0.007 ^{ns}	0.002^{ns}	0.05 ^{ns}	0.26 ^{ns}
Rate of Fertilizer (C)	2	5.7 ^{ns}	0.83 ^{ns}	45.7 ^{ns}	1.82 ^{ns}	9.1**	0.33 ^{ns}	1.8 ^{ns}	7.19 ^{ns}
p×c	2	0.3 ^{ns}	0.002 ^{ns}	0.19 ^{ns}	0	0.16 ^{ns}	0.027 ^{ns}	0.88 ^{ns}	0.99 ^{ns}
a×c	4	11.22*	0.03 ^{ns}	1.27 ^{ns}	0.11 ^{ns}	0.86*	0.011 ^{ns}	1.22 ^{ns}	4.5 ^{ns}
p×a×c	4	0.45 ^{ns}	0.001 ^{ns}	0.22 ^{ns}	0	0.012 ^{ns}	0.00008 ^{ns}	0.05 ^{ns}	0.45 ^{ns}

Continue table 2.

S.O.V	df	Harvest Index	Internode diameter	Internode length	Leaf area index	protein	Proline	Soluble sugar	Total chlorophyll
b×c	2	0.3 ^{ns}	0.002 ^{ns}	2.56 ^{ns}	0.25 ^{ns}	0.45 ^{ns}	0.0012 ^{ns}	0.12 ^{ns}	0.38 ^{ns}
p×b×c	2	0.45 ^{ns}	0.005 ^{ns}	0.12 ^{ns}	0	0.001 ^{ns}	0.0002 ^{ns}	0.11 ^{ns}	0.1 ^{ns}
a×b×c	4	1.66 ^{ns}	0.002 ^{ns}	0.62 ^{ns}	0.02 ^{ns}	0.59 ^{ns}	0.919*	8.77*	42.04*
p×a×b×c	4	0.28 ^{ns}	0.004 ^{ns}	0.45 ^{ns}	0	0.02 ^{ns}	0.0003 ^{ns}	8.7 ^{ns}	0.08 ^{ns}
Error II	68	3.61	0.015	2.99	0.06	0.3	0.0004	1.19	1.25
CV(%)	-	5.37	5.85	6.43	7.08	5.82	4.31	8.11	6.11

$^{ns,\ *\ and\ **}$: no significant, significant at 5% and 1% of probability level, respectively.

Table 3. Mean comparison effects of treatment on morphological and physiological traits

Treatments	Tiller per plant	Height (cm)	Fertile spike per m^2	Spike length (cm)	Grain per spike
Place					
Borujerd	7.44^{*a}± 0.88	86.83^{a}±16.1	489.25^{a}±52.2	9.38^{a}±2.05	40.38^{a}±4.02
Dorud	7.07^{a}±0.75	83.66^{b}±15.1	438.24^{b}±60.1	8.12^{a}±2.90	37.64^{a}±4.03
Drought stress					
Control	9.50^{a}±0.89	90.30^{a}±13.2	495^{a}±60.2	10.11^{a}±3.51	45.77^{a}±4.01
BSD	5.05^{c}±0.75	81.66^{c}±14.8	410^{b}±99.3	7.40^{a}±3.02	33.86^{b}±3.86
MSD	7.22^{b}±0.58	83.77^{b}±13.7	485^{a}±159.2	8.70^{a}±4.02	37.41^{b}±3.85
Type of Nitrogen fertilizer					
AN	7.70^{a}±0.68	86.07^{a}±12.4	472^{a}±43.5	9.27^{a}±3.06	40.42^{a}±4.21
U	6.80^{b}±0.67	84.40^{b}±14.7	454^{b}±51.2	8.24^{b}±1.08	37.61^{b}±3.56
Rate of Nitrogen fertilizer					
N+50	7.94^{a}±0.68	87.44^{a}±11.7	483^{a}±98.2	9.36^{a}±2.95	39.77^{a}±4.25
N	7.33^{a}±0.78	85.05^{b}±12.8	467^{b}±65.4	8.69^{b}±3.20	39.41^{a}±3.98
N-50	6.50^{b}±0.85	83.25^{b}±10.9	440^{c}±96.2	8.22^{b}±3.10	37.86^{a}±3.54

BSD: Irrigation until booting stage, MSD: irrigation to soft dough stage (maturation stage), AN: ammonium nitrate, U: urea, N: normal rate of fertilizer, N+ 50: 50% higher than normal rate, N-50: 50% lower than normal rate. Data are mean ± SE. *Similar letters in each column show non-significant difference at 5% probability level via LSD test.

Continue table 3.

Treatments	1000-grain weight (g)	Grain yield (kg.ha^{-1})	Internode diameter (mm)	Internode length (mm)
Place				
Borujerd	43.09^{*a}±7.55	4699^{a}±910.65	2.98^{a}±0.12	25.44^{b}± 2.51
Dorud	41.61^{a}±7.35	4429^{a}±930.36	1.98^{b}±0.15	28.33^{a}±1.51
Drought stress				
Control	45.52^{a}±6.98	5792^{a}±911.56	2.53^{a}±0.11	29.83^{a}±2.62
BSD	40.02^{b}±6.85	3129^{c}±891.32	1.99^{b}±0.13	23.11^{c}±2.15
MSD	41.50^{ab}±7.65	4770^{b}±721.31	1.86^{c}±0.09	27.72^{b}±2.89
Type of Nitrogen fertilizer				
AN	43.44^{a}±6.58	4853^{a}±911.40	2.03^{b}±0.11	28.03^{a}±2.35
U	41.25^{b}±6.45	4275^{b}±891.36	2.22^{a}±0.12	25.74^{b}±2.42
Rate of Nitrogen fertilizer				
N+50	43.33^{a}±7.25	4799^{a}±911.36	1.98^{c}±0.14	28.05^{a}±2.65
N	42.41^{ab}±7.36	4541^{b}±891.56	2.12^{b}±0.12	26.80^{b}±2.38
N-50	41.35^{b}±6.58	4351^{c}±901.85	2.28^{a}±0.13	25.80^{c}±2.45

BSD: Irrigation until booting stage, MSD: irrigation to soft dough stage (maturation stage), AN: ammonium nitrate, U: urea, N: normal rate of fertilizer, N+ 50: 50% higher than normal rate, N-50: 50% lower than normal rate. Data are mean ± SE. *Similar letters in each column show non-significant difference at 5% probability level via LSD test.

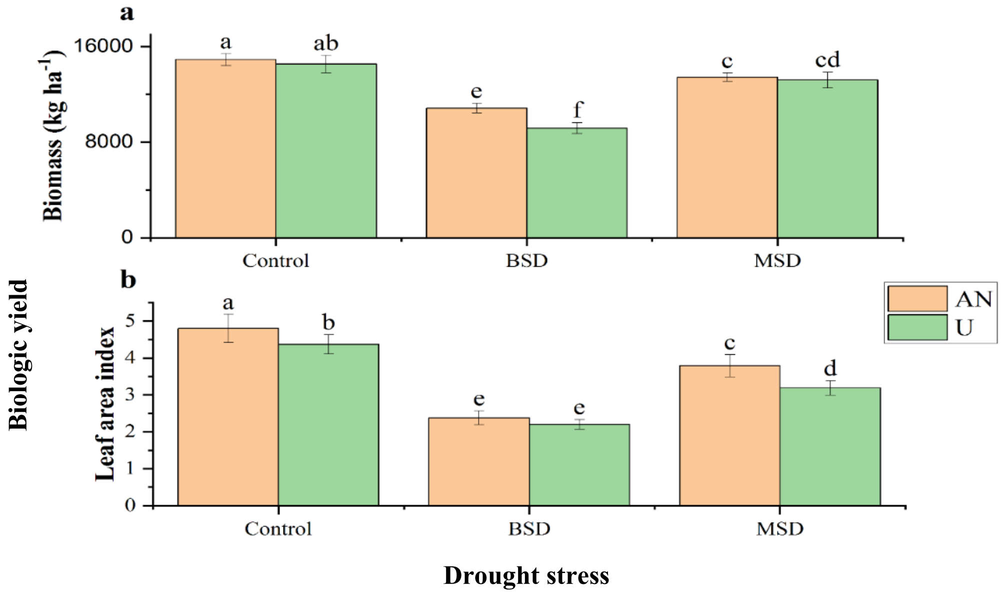

Figure 2. Mean comparison interaction effect of fertilizer type and drought stress on **a)** Biologic yield, and **b)** Leaf area index via LSD test at 5% probability level. BSD: booting stage drought, MSD: maturation stage drought. Data are mean ± SE. Similar letters in each column show non-significant difference at 5% probability level via LSD test.

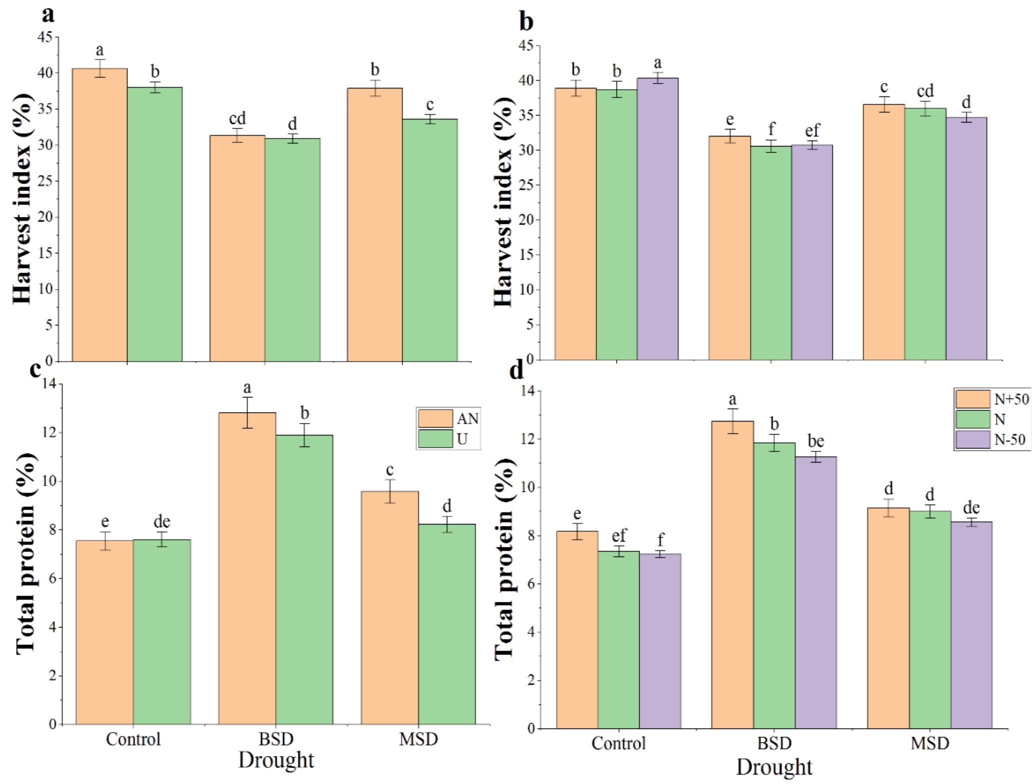

Figure 3(a). Mean comparison interaction effect of fertilizer type and drought stress on harvest index (HI) via LSD test at 5% probability level, 2(b). Mean comparison interaction effect of fertilizer rate and drought stress on HI via LSD test at 5% probability level, 3(c). Mean comparison interaction effect of fertilizer type and drought stress on protein via LSD test at 5% probability level, and 2(d). Mean comparison interaction effect of fertilizer rate and drought stress on protein via LSD test at 5% probability level. BSD: booting stage drought, MSD: maturation stage drought. Data are mean ± SE. Similar letters in each column show non-significant difference at 5% probability level via LSD test.

Fig. 3a revealed that HI was notably affected by the interaction between drought stress and nitrogen fertilizer type and rate. Across all three drought stress treatments, the highest HI was observed when the AN treatment was applied. Specifically, the application of AN in the control treatment yielded the highest HI at 40.62%, and this difference was statistically significant when compared to the utilization of urea in the same treatment (by 38%). Conversely, both the "BSD" and "MSD" treatments exhibited lower HI values, particularly when U was applied. The lowest HI was recorded at 30.91% when urea was used in combination with the "BSD" treatment. In addition, the treatment without drought stress (Fig. 3b) had the highest HI values for all fertilizer rates. It was obvious that the maximum HI was obtained in the treatment N+50 under non-drought stress conditions, reaching 40.34%. Moreover, the current results showed that the "BSD" treatment had the lowest HI value of 30.85% when the nitrogen fertilizer was applied at the normal rate (N). In particular, there was no statistically significant difference compared to the application of N-50, which resulted in a HI of 30.73%. Concerning total protein levels, as illustrated in Fig. 3c, it is evident that AN proved to be more effective in increasing the percentage of total protein in comparison to urea. Both the "BSD" and "MSD" treatments exhibited higher protein percentages when contrasted with the control treatment. Nevertheless, the highest protein percentage was recorded when AN was applied under the "BSD" treatment, yielding a percentage of 12.81%. Conversely, under the "MSD" treatment, the application of urea resulted in a higher protein percentage at 11.09%. Besides, AN resulted in the lowest protein percentage at 7.55% when compared to the other treatments. Fig. 3d revealed that within the three drought stress treatments, the highest protein percentage was observed when N+50 was applied (12.74%) in conjunction with the "BSD" treatment. This value increased by approximately 33%, compared with the control. In contrast, the lowest protein percentage was documented when N-50 was applied under control conditions, resulting in a percentage of 7.24%. As shown in Fig. 4, total chlorophyll content was affected by the triple interaction of fertilizer rate, type, and drought stress. The results revealed that wheat plants had the highest chlorophyll content under conditions without stress factors (13.8 mg.g^{-1}).

However, under "BSD" treatment, this value decreased by 21%, and under "MSD" by 27%. At two drought levels, AN application performed superior to U, except for nitrogen application in normal rate (N) under the "MSD" condition (10.7 mg.g^{-1}) compared with other treatments under the same condition. Moreover, our results revealed that higher fertilization (N+50) can improve total chlorophyll content compared with others. As for proline content, "BSD" increased this compound's level by 37% compared with the control treatment (Fig. 5).U application caused higher proline content rather than AU in all treatments. Besides, lower fertilizer application (N-50) decreased the proline content compared with higher application or normal rate. The highest proline value was recorded by N fertilization rate (0.9 mg g^{-1}) under "BSD", while the lowest values were recorded under both control and "MSD" treatments with (N-50) treatments. Regarding soluble sugars, when plants faced water stress, these compounds' production increased, which under "BSD" was higher than "MSD". AN application enhanced soluble sugars production by 50% compared with "MSD" by 15%. Interestingly, lower AN application (N-50) enhanced soluble sugar production under both drought stress levels (Fig. 6). Besides, a positive and significant correlation was found between plants' yield and morphologic traits such as height, spike number, grain per spike, tiller number, biomass, HI, and LAI (Fig. 7). While a negative correlation was recorded between yield and physiological traits including protein, proline, soluble sugar, and total chlorophyll (Fig. 7).

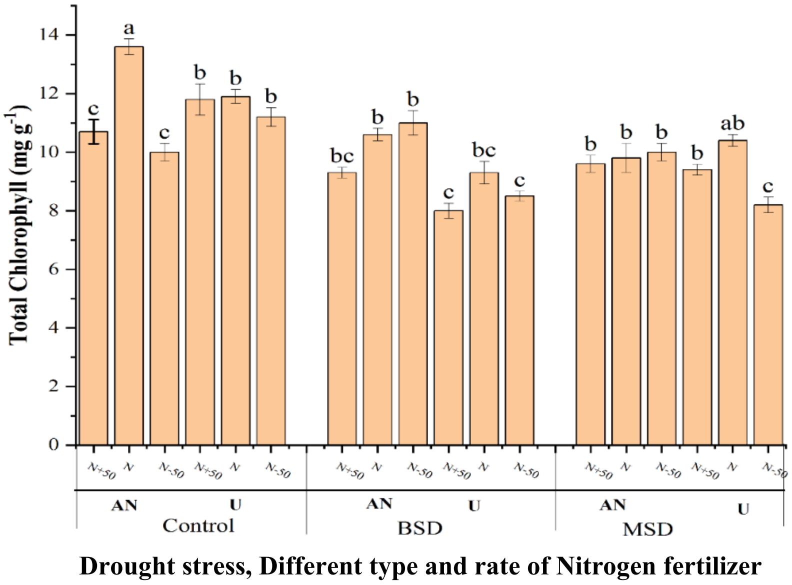

Figure 4. Mean comparison interaction effect of fertilizer rate × fertilizer type × drought stress on total chlorophyll via LSD test at 5% probability level. AU: Ammonium nitrate fertilizer, U: Urea, BSD: booting stage drought, MSD: maturation stage drought. Data are mean ± SE. Similar letters in each column show non-significant difference at 5% probability level via LSD test.

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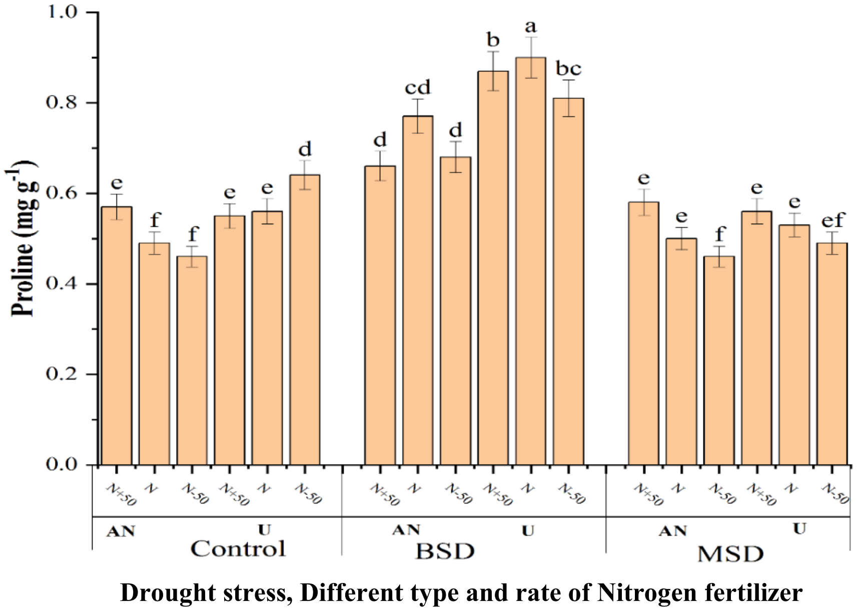

Figure 5. Mean comparison interaction effect of fertilizer rate × fertilizer type × drought stress on proline via LSD test at 5% probability level. AU: Ammonium nitrate fertilizer, U: Urea, BSD: booting stage drought, MSD: maturation stage drought. Data are mean ± SE. Similar letters in each column show non-significant difference at 5% probability level via LSD test.

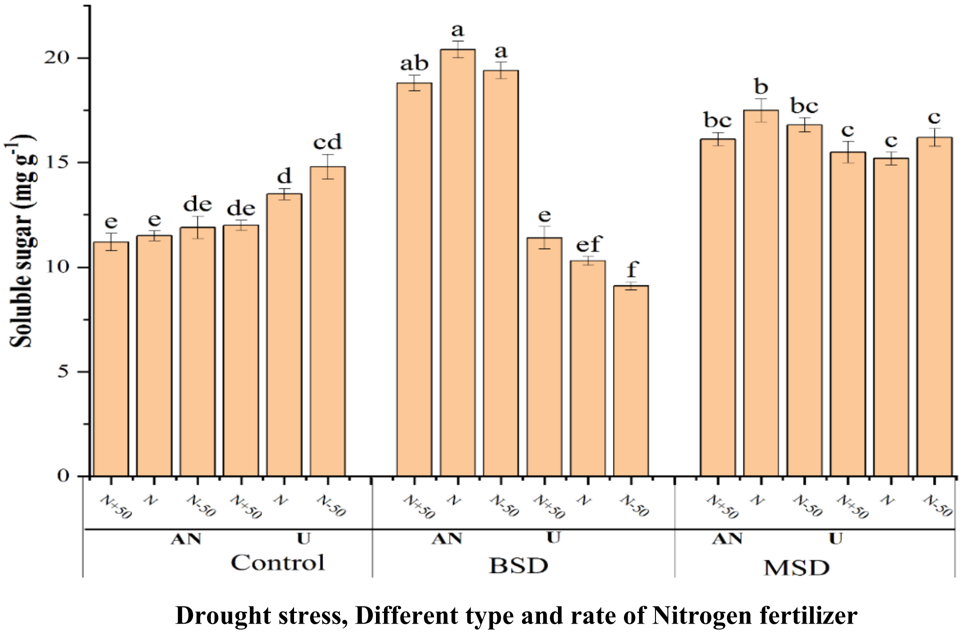

Figure 6. Mean comparison interaction effect of fertilizer rate × fertilizer type × drought stress on soluble sugar via LSD test at 5% probability level. AU: Ammonium nitrate fertilizer, U: Urea, BSD: booting stage drought, MSD: maturation stage drought. Data are mean ± SE. Similar letters in each column show non-significant difference at 5% probability level via LSD test.

Furthermore, principle component analysis (PCA) results (Fig. 8) exhibited that principal component 1 predominantly explained the variance in the variables (58.54%), indicating that the overall response of plants to fertilizers application was strongly influenced by this component. Conversely, principal component 2 accounted for 11.06% of the variance. Moreover, according to Fig. 8, under BSD stress, AN fertilizer at all levels affected on morphological parameters such as height, spike number, internode diameter, spike length, grain number per spike, 1000-grain weight, biomass, harvest index, and leaf area index. Whereas, physiological traits such as protein content, proline, soluble sugar, and total chlorophyll content affected under control and MSD level by urea application.

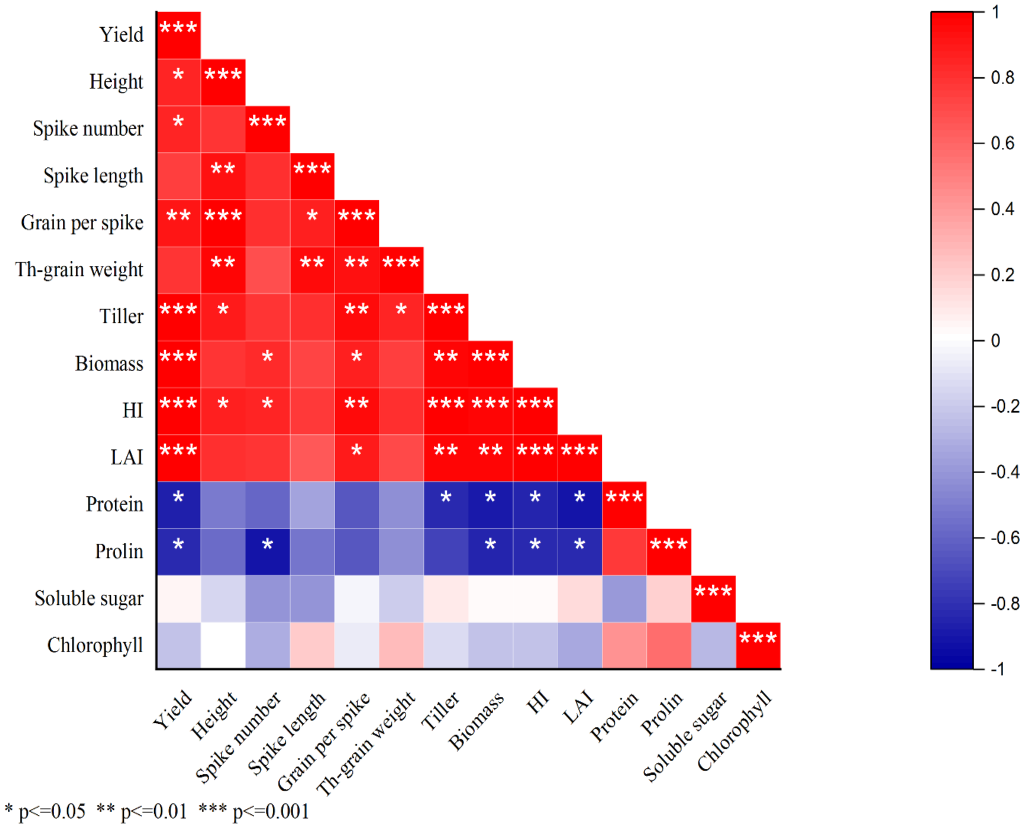

Figure 7. Pearson correlation between grin yield and measured traits.

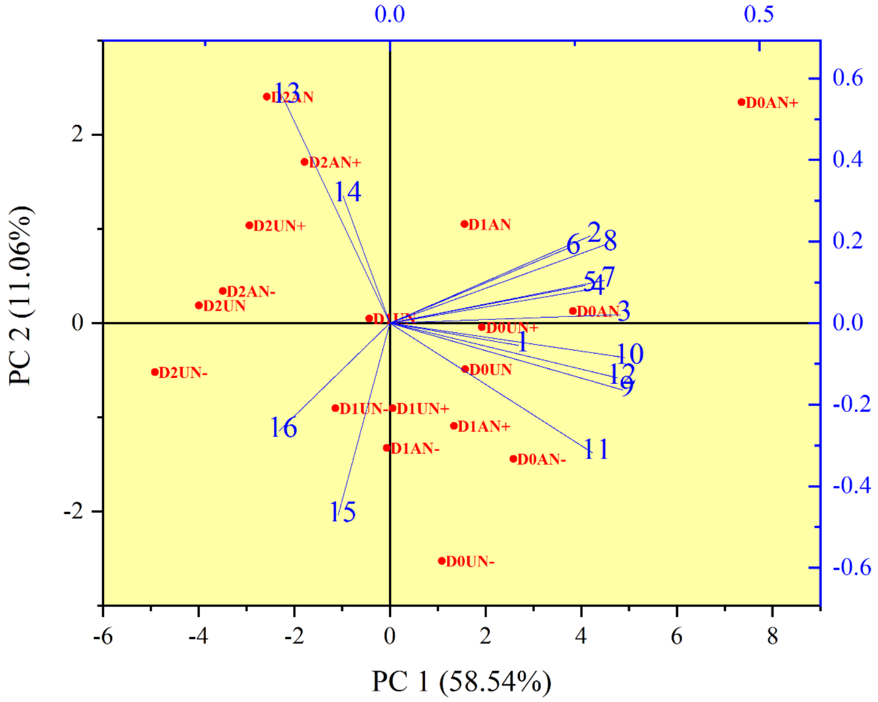

Figure 8. PCA analysis of treatments on all studied parameters. D: drought stress (D0, control, D1: BSD, D2: MSD), A: Ammonium Nitrate, U: Urea, N: fertilization rate (N+, N+50%, N: Normal rate, N-: N-50%), 1: Tiller number, 2: Height, 3: Spike number, 4: Internode diameter, 5: Internode length, 6: Spike length, 7: Grain number per spike, 8: 1000-grain weight, 9: Yield, 10: Biologic yield, 11: Harvest index, 12: Leaf area index, 13: Protein content, 14: Proline, 15: Soluble sugar, 16: Total chlorophyll content.

5. Discussion

In this study, several morphologic and physiological characteristics of wheat plants subjected to irrigation interruption at the booting stage (BSD) and at the plant maturity stage (MSD) were evaluated while nitrogenous fertilizers (AN and U) were simultaneously applied at different rates, designated as N+50, N, and N-50. Overall, the results of this study showed that "BSD" had a stronger effect on the studied characteristics compared to the two other treatments. In addition, AN was found to perform better than U fertilizer when applied under drought-stress conditions. Concerning morphologic characteristics, the findings demonstrated that the imposition of BSD-induced stress resulted in a more substantial reduction in both yield and yield component traits. Similar results were observed in Yu et al. (2023) studies, which evaluated individual and combined effects of drought stress and four nitrogen fertilizers (ammonium, nitrate, urea, and mixed nitrogen fertilizers). They found that drought inhibited plant growth. Drought stress represents a primary abiotic stressor with a substantial impact on crop yield, yield components, and various physiological traits. As outlined by Alam et al. (2021), this ecological challenge poses a significant threat to wheat production on a global scale. Drought stress manifests in a range of adverse consequences, particularly impaired stem elongation, anthesis, grain filling, and milk and dough development (Lv et al., 2021), particularly in semi-arid regions (Barati et al., 2020). The reduction extent in grain yield and biomass due to drought stress depends on factors such as the duration of the drought, the timing, and the specific growth stage of the crop (Asif et al., 2017). The deleterious effects of drought stress on wheat growth can be explained by the exacerbation of water scarcity and osmotic pressure under drought stress. In other words, a deficit in water availability may diminish the plant's ability to take up nutrients from the soil through its below-ground components and subsequently transport them to the above-ground parts of the plant, a process that is primarily hindered by restricted transpiration rates (Yu et al., 2023). It is essential to recognize the critical stages of water requirement winter wheat cultivation, which are the tillering and anthesis phases (Thapa et al., 2020). Consequently, water deficit during the booting stage may serve as a major cause of reduction in morphologic traits, subsequently leading to a decrease in the yield and yield component. It is noteworthy, however, the results of the present study revealed that irrigation stop resulted in a reduction in the tiller number per plant, plant height, number and length of spikes, 1000-grain weight, and grain yield during both booting and milk development, the application of AU seems to partially mitigate the negative effects of drought-induced stress. Ultimately, this mitigation improved the mentioned traits compared to the urea application. In addition, the application of nitrogen at a rate of N+50 demonstrated greater effectiveness in improving yield and yield component traits when contrasted with the use of a N and N-50 rate. These observations are in agreement with the findings of Soomro et al., (2016) who emphasized that excessive application of nitrogen can lead to increased production of fertile tillers per plant. In addition, our results revealed that drought stress leads to a decline in LAI. This phenomenon can be attributed to the decreased water loss that results from a reduced transpiring surface. Consequently, the detrimental effects of drought stress on wheat growth can be linked to a reduced ability to capture sunlight and a reduced area available for sunlight absorption, ultimately leading to a diminished photosynthetic capacity (Yu et al., 2023). Our results revealed that AU application resulted in a remarkable increase in biomass, LAI, and HI. This phenomenon can be explained by the facilitated assimilation of AU ions under drought conditions compared to urea. These findings are consistent with those of (Huangfu and Li, 2019), who conducted a study examining the effects of various nitrogen sources (AN and U) both individually and in combination under conditions of drought stress in wheat plants. When considering the performance of AN versus urea in improving wheat resilience under drought stress, it is essential to recognize that the form of Nitrogen supplied can influence the plant's response to water limitation. Plants absorb nitrogen primarily through their roots in the form of ions. Two common forms of nitrogen in the soil are the nitrate ($NO3^-$) and ammonium ($NH4^+$) ions. These ions are taken up by plant roots through various mechanisms and ultimately contribute to plant growth and development (Muratore et al., 2021). Nitrate ions are typically absorbed through active transport, which requires energy. These compound transporters in the root cell membrane facilitate its uptake from the soil solution into the root cells. Subsequently, nitrate ions can be transported further throughout the plant to be used for various metabolic processes, such as protein and enzyme synthesis. Ammonium ions, on the other hand, are absorbed by plants through a combination of passive and active transport processes. Root cells have specific transporters that facilitate the uptake of ammonium ions. Unlike nitrate, which is generally mobile in the plant, ammonium ions tend to accumulate in the root cells, where they are readily available for metabolic processes. Therefore, AN contains both ammonium and nitrate ions, providing advantages in terms of nitrogen uptake and utilization. Besides, the presence of nitrate ions in AN plays a crucial role in osmotic regulation, maintaining water balance, and turgor pressure during drought stress (Raddatz et al., 2020). This aids in preventing water loss through transpiration, which is particularly critical for plant survival under water-limited conditions. Furthermore, ammonium ions in AN can enhance nutrient uptake efficiency. In other words, ammonium uptake is energy-efficient and less dependent on water availability compared to nitrate uptake. Under drought stress, where water uptake may be limited, the ammonium component in ammonium nitrate provides a reliable source of nitrogen for plant growth (Huang et al., 2018). In contrast, U fertilizer primarily supplies nitrogen in the form of urea, which must undergo conversion by soil microorganisms into ammonium and subsequently into nitrate, a process that takes time and may be less efficient under drought conditions (Klimczyk et al., 2021). This delayed availability of nitrogen in the form that plants can readily use can hinder plant growth during periods of water scarcity. Therefore, it seems that the better performance of AU can be attributed to its elemental structures. The excessive use of fertilizer (N+50) performed better than the two other fertilizer rates. This effect can be explained by the crucial contribution of nitrogen to improved nutrient uptake by plants and enhanced osmotic adjustment of plants. In other words, the crucial role of nitrogen in osmotic adjustment can aid plants in maintaining turgor pressure and cell integrity. Consequently, under limited water conditions, nitrogen contributes to the accumulation of osmolytes (organic solutes) in plant cells, which contribute to osmotic adjustment. This adaptation allows the plant to maintain a suitable water potential for nutrient uptake. Nevertheless, over- application of nitrogen fertilizers, as observed in the N+50 treatment, raises concerns about nutrient runoff, groundwater contamination, and greenhouse gas emissions. Finding a balance between increased nitrogen application for drought resilience and potential environmental consequences is vital for sustainable agriculture (Bashir et al., 2013). Hence, it is important to employ strategies that limit nitrogen losses, such as controlled-release fertilizers (Fertahi et al., 2021) and split applications to reduce the risk of environmental pollution (Xiao et al., 2019). Regarding physiological traits, it was observed that drought stress had a significant impact on the studied traits, with a particularly pronounced influence evident under "BSD" conditions. Drought stress during the booting phase can significantly impair the ability of sinks to assimilate photosynthetic products, resulting in a reduction in flower fertility and an increase in flower abortion rate. Consequently, this environmental stress can trigger heightened activation of defensive mechanisms and ultimately lead to a decrease in grain yield (Abdolmaleki et al., 2022; Saffari et al., 2023). Based on our results, total protein content increased in response to drought stress. Moreover, this increase was particularly pronounced when the AU application was used in conjunction with a higher nitrogen fertilizer rate of N+50. Similar results were reported by Ghadirnezhad Shiade et al. (2020) and Ghadirnezhad Shiade et al. (2023b), who studied the rice plants exposed to abiotic stress. Plants have developed various defensive mechanisms to cope with drought stress, which is a common environmental challenge. Proline, soluble sugars, and total proteins are key components of these mechanisms. Total protein content in plant cells plays an important role in plant defense strategies against drought-induced stress. Firstly, proteins, which often serve as enzymes, are indispensable components of various cellular processes. Hence, maintaining optimal protein levels is critical for the constant functioning of vital metabolic pathways, including photosynthesis and respiration, even when faced with water shortages. Secondly, under drought conditions, plants frequently synthesize stress-responsive proteins that are responsible for various protective functions. These proteins play a crucial role in the scavenging of reactive oxygen species (ROS), repair of damaged cellular structures, and maintenance of cellular water balance, which overall contributes to plant adaptation to water scarcity (Basu et al., 2016). Besides, our findings exhibited that total chlorophyll content decreased under drought stress. Similar results were documented by Ghadirnezhad Shiade et al. (2023b). Changes in chlorophyll content can serve as a signal for the plant to activate stress responses. When chlorophyll content decreases under stress conditions, the plant may trigger specific adaptive mechanisms to cope with the stress, such as the production of stress-related proteins and the closure of stomata to reduce water loss (Ghadirnezhad Shiade et al., 2023a). The application of N-source fertilizers, especially AN, which showed a moderate improvement at the less (N-50) rate, had a positive effect on the total chlorophyll content in plants. These fertilizers are known to enhance chlorophyll synthesis, as the N nutrient is a critical component of the chlorophyll molecule. By providing an adequate supply of this nutrient element, these fertilizers facilitate the synthesis of chlorophyll, thereby improving the plant's photosynthetic efficiency. Consequently, the increased chlorophyll content can mitigate the adverse effects of drought stress by maintaining higher photosynthetic activity and promoting better growth and productivity under challenging environmental conditions. Our results were consistent with Basal and Szabó (2020) who studied N-fertilizers positive effects on chlorophyll content of soybean under drought stress conditions. Moreover, in this study, proline and soluble sugars content increased under drought stress, particularly under "BSD". The amino acid proline plays a central role in plant defense strategies against drought-induced stress. Its multiple functions include osmoprotection, in which it accumulates in plant cells during drought episodes, acting as an osmoprotectant essential for maintaining cell turgor pressure, thereby preventing cell desiccation and wilting (Li et al., 2020). In addition, proline contributes to the stabilization of cellular structures and protects vital macromolecules such as proteins and enzymes from denaturation and damage caused by water stress, so that the integrity and functionality of cells is maintained even in dry conditions (Kijowska-Oberc et al., 2023). Our results were consistent with Li et al. (2020), who studied several nitrogen supplies effects on the physiological parameters of Chinese fir seedlings under drought stress induced by polyethylene glycol (PEG). They found that proline content further increased under nitrate and ammonium treatment, to conserve plant osmotic pressure and keep water potential under water deficit conditions. Furthermore, soluble sugars such as sucrose, glucose, and fructose are integral components of plant responses to drought stress. Similar to proline, their multiple functions include osmotic adjustment. Accordingly, these compounds accumulate in plant cells in response to drought stress, to enhance the cells' osmotic potential (Yang et al., 2021). Likewise, Du et al. (2020) found similar results and reported that both soluble sugar and proline content increased under heavy nitrogen treatment in rice to improve plant tolerance against drought stress. While, contrary to our results, Li et al. (2020) reported that sugar content decreased by nitrogen fertilization. This difference can be attributed to the specific genetic makeup and adaptive responses of the studied plant species. Moreover, the correlation results revealed that wheat yield correlated positively with plant height, spike number per plant, grain number per spike, tiller number, and biomass. In other words, by selecting or cultivating wheat plants with attributes such as greater plant height, more spikes per plant, higher grain density per spike, increased tillering, and larger biomass, it is possible to enhance wheat yields. On the other hand, a significant negative correlation was found between plant yield and physiological traits such as protein and proline content, indicating that there is a trade-off between wheat quality (indicated by protein content) and quantity (yield). Moreover, PCA results revealed different effects. Accordingly, AN showed a detectable influence on morphological traits under conditions characterized by abiotic stress, especially in the case of BSD stress, while U showed a remarkable influence on physiological traits. Based on the positive and statistically significant correlation between yields and morphological attributes, it can be claimed that the application of AN, especially under the severe drought stress, can increase the resistance of plants, thus lead to an increase in overall yield. Conversely, urea application appears to be associated with a decrease in plant productivity, primarily due to its correlation with physiological traits. For wheat crop practitioners, a comprehensive understanding of this complicated relationship is essential. Informed decisions about the optimal balance between these traits are paramount and depend on the agricultural objectives. Whether the goal is to maximize yield or tailor wheat quality to specific end uses such as bread or pasta production, this understanding will guide fertilizer selection and cropping strategies accordingly.

6. Conclusion

The study showed that drought stress until the booting stage significantly reduced Wheat growth parameters like tiller number, plant height, fertile spikes, and grain yield. However, plants exposed to moderate drought stress during the maturity stage experienced less severe reductions. While drought also decreased biomass, leaf area index, and harvest index, these effects were lessened by Ammonium Nitrate, which performed better than urea, especially at higher rates. Ammonium Nitrate increased total protein, proline, and soluble sugar levels under drought, which are important for improving drought tolerance. Lower Nitrogen application rates led to poorer outcomes, emphasizing the importance of optimal Nitrogen fertilization strategies. These findings provide important insights for farmers, who should adopt optimized Nitrogen management practices, particularly the use of Ammonium Nitrate in areas prone to drought. Additionally, best practices, such as precision agriculture and controlled-release fertilizers, should be encouraged to optimize resource use and minimize Nitrogen losses. Furthermore, these results offer valuable guidance for policymakers in shaping policies and regulations that encourage the adoption of innovative technologies. Actions should include promoting the use of smart irrigation systems, incentivizing the development and use of controlled-release fertilizers, and supporting precision farming tools to enhance sustainability. These efforts should be aimed at aligning both practical agricultural practices and legislative frameworks to tackle the challenges posed by drought stress and Nitrogen management, improving sustainability, and optimizing resource use. Policymakers should also focus on developing frameworks that ensure long-term resource efficiency, providing farmers with the tools and incentives necessary to adopt these innovative and sustainable practices across the agricultural sector.

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AUTHORS' CONTRIBUTION:

Abbas Ghorbani conducted the experiment and written the first draft. Mojtaba Jafarzadeh Kenarsari supervised the project and analyzed the obtained date. Amin Farnia and Shahram Nakhjavan edited and reviewed the draft.

CONFLICT OF INTEREST:

Authors declared no conflict of interest.

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