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Research Article/Araştırma Makalesi

Effects of post-flowering drought on nitrogen mobilization and growth of bread wheat (*Triticum aestivum* L.) using stable ^{15}N isotope

Ekmeklik buğdayda (Triticum aestivum L.) ^{15}N izotopu kullanılarak çiçeklenme sonrası kuraklığın azot mobilizasyonu ve gelişimine etkileri

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ABSTRACT

Water and nitrogen shortage are one of the main limiting factors of crop productivity such as wheat and cereals. Increased variation and changes in climate conditions are expected to dominate yield potential of wheat. Nitrogen isotope technique widely used in recent years provides useful information about mobilization and nitrogen use efficiency under environmental constraints. This study aimed to determine the effects of drought conditions applied during different growing periods on nitrogen uptake by using stable ^{15}N isotope, yield and quality properties and stomatal conductivity of bread wheat. Environmental variation was obtained by designing 4 artificial practices (irrigated condition, rainfed condition, early drought (flowering-harvest) and late drought (grain filling-harvest) by rainout shelter with covering progress about drought in different growing periods. Number of grains per spike, 1000 grain weight, single spike yield, spike numbers per square meter, plant height, grain yield, biomass yield, stomatal conductance, protein, ash content and stem $\delta^{15}N$ (‰), flag leaf $\delta^{15}N$ (‰), grain $\delta^{15}N$ (‰) values were determined. The drought period from the beginning of flowering till harvest of plants had adverse impact on grain yield and yield components. The results clearly indicated the practices that may cause drought stress in the generative period should be avoided. In addition, nitrogen use efficiency of bread wheat was disrupted with the decrease in the amount and efficiency of plant water use during drought periods. The results also revealed that contribution of nitrogen to crop yield decreased due to less consumption of nitrogen in plant metabolic activities during drought periods. Based on the results additional water supply decreased $\delta^{15}N$ content in mature grains from 13420 ‰ to 9278 ‰. Nitrogen applied in stem elongation period had greater contribution to grain $\delta^{15}N$ content (15269 ‰) compared to tillering growth stage (8975 ‰). Nitrogenous fertilizer application time suggested not to be delayed to improve nitrogen contribution in metabolic activities and to prevent postponing the tillering and stem elongation periods. The application of nitrogen improved mobilization and efficiency of nitrogen contribution to different plant parts during generative development stages of bread wheat.

Key Words: *Triticum aestivum* L., drought, nitrogen isotope ^{15}N, yield, quality

ÖZ

Su ve azot eksikliği buğday ve diğer tahıllar gibi bitkilerin ürün verimliliğini etkileyen ana faktörlerden birisidir. İklim koşullarındaki artan değişiklikler nedeniyle buğday bitkisinin verim potansiyelinin açığa çıkması engellenmektedir. Son yıllarda kullanımı artan azot izotop tekniği sayesinde olumsuz çevre koşulları altında azot taşınımı ve azot kullanım verimliliği hakkında önemli bilgiler sağlanmaktadır. Bu çalışmada ekmeklik buğdayda ^{15}N izotopu kullanılarak farklı gelişme dönemlerinde uygulanan kuraklık koşullarının azot taşınımı, verim ve kalite özellikleri

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ile stoma iletkenliği üzerindeki etkilerinin belirlenmesi amaçlanmıştır. Farklı gelişme dönemlerinde (sulu ve kuru koşul, erken kuraklık (çiçeklenme-hasat) ve geç kuraklık (tane dolum dönemi-hasat)) yağmur korunağı kullanılarak uygulanan yapay kuraklık koşulları ile çevresel varyasyon elde edilmiştir. Çalışmada; başakta tane sayısı, bin tane ağırlığı, tek başak verimi, metrekarede başak sayısı, bitki boyu, tane verimi, biyomas verimi, stoma iletkenliği, tane protein ve kül oranı, sap $\delta^{15}N$ (‰), bayrak yaprak $\delta^{15}N$ (‰) ve tane $\delta^{15}N$ (‰) içerikleri değerlendirilmiştir. Elde edilen sonuçlara göre; verim ve verim öğeleri generatif dönem boyunca uygulanan kuraklık uygulamalarından olumsuz yönde etkilenmiştir ve bu nedenle ekmeklik buğday üretiminde generatif dönem kuraklık stresine neden olabilecek uygulamalardan kaçınılması gerektiği sonucuna ulaşılmıştır. Ayrıca azot kullanım etkinliğinin bitki su kullanımının kısıtlanması ile azaldığı ve kuraklık dönemi boyunca su kullanım etkinliğinin azaldığı anlaşılmıştır. Tüm bunlara ek olarak kuraklık dönemi boyunca bitki metabolik aktivitelerinin azalması sonucu azot tüketiminin kısıtlanmasına bağlı olarak azotun verim potansiyeline katkısının azaldığı sonucuna ulaşılmıştır. Sulu koşulda tane $\delta^{15}N$ içeriğinin sulama ile kuru koşula göre 13420 ‰ değerinden 9278 ‰ değerine azaldığı sonucuna ulaşılmıştır. Ayrıca sapa kalkma döneminde uygulanan azotun tane $\delta^{15}N$ değerinin (15269 ‰) kardeşlenme döneminde uygulanan $\delta^{15}N$ içeriğine (8975 ‰) oranla daha yüksek azot içeriğine ulaşılmasını sağlamıştır. Azotun metabolik faaliyetlere etkin bir şekilde katılması için kardeşlenme ve sapa kalkma dönemlerinde azotlu gübrelemenin uygun zamanda uygulanması ve özellikle sapa kalkma döneminde uygulanan azotun generatif dönem boyunca buğday bitkisinin farklı kısımlarında daha etkin azot taşınımına ve azot kullanımına neden olduğu sonucuna ulaşılmıştır.

Anahtar Kelimeler: *Triticum aestivum* L., kuraklık, azot izotopu ^{15}N, verim, kalite

Introduction

Drought in wheat cultivation is one of the major abiotic stress factors and limits wheat yield potential. Inadequate precipitation and irregular distribution of rainfall during the year cause limited water conditions and drought. Although drought periods are observed in different development stages due to inadequate and irregular distribution of precipitation, drought stress generally starts in the period of flowering and increases its effect during the grain filling period (Öztürk, 1999). Depending on the duration and severity of drought stress, wheat plants may react differently during different development stages. Drought stress in the stem elongation period caused a decrease in the number of spikelets and the number of grain per spike, the drought in the anthesis and grain filling period also decreased the number of fertile spikelets and dry matter transport to the grain resulting in reduced yield values (Tonk et al., 2011). Besides grain yield and components affected by drought grain quality is also influenced by environmental modifications including temperature fluctuations (heat stress and heat shock) and distribution of precipitation. Heat stress and drought in grain filling period cause reductions in protein functionality (starch deposition, bread quality and end-use quality) whilst grain protein content increases in heat stress (Erekul and Köhn 2006; Farooq et. al 2011).

Nitrogen is an important and basically necessary plant nutrient to obtain optimum growth and maximize grain yield. In addition to plant growth nitrogen fertilizer is also one of the most important key factor to obtain high quality (related to protein) products (Borghi, 1999). The wheat plant takes up nitrogen from the soil in ammonium (NH_4^+) and nitrate (NO_3^-) forms. The absorbed nitrate (NO_3) can be reduced to ammonium (NH_4) directly in the roots, or preferably in the shoots catalyzed by the nitrate and nitrate reductase enzymes. These converted forms of nitrogen are reduced to the amine (NH_2) form in the plant tissues. The reduced nitrogen form combines with fatty acids to constitute amino acids. Proteins are formed by the incorporation of amino acids. With drought stress, NO_3 nitrogen is prevented from transforming into useful form for plants. Reduction of nitrate reduction activity is achieved by decreasing nitrate reduction enzyme activity (Kutlu, 2010). On the other hand, Rubisco (ribulose-1,5-bisphosphate carboxy/oxygenase), which plays a role in carbon assimilation, is significantly affected by nitrogen deficiency (Seemann et al., 1987).

Nitrogen isotope analysis as a nuclear technique to determine genotypic differences in drought resistance has been applied by several researchers (Kiss et al., 1990; Bort et al., 1998; Lopes and Araus, 2006; Fraser et al., 2011; del Pozo et al., 2014; Liu et al., 2015; Luo et al., 2015).

^{15}N and ^{14}N, which are stable isotopes of nitrogen, are found in nature at levels 0.366% and 99.634% respectively (Halitligil, 1996). Nitrogen isotopes have been considered as a tool to study nitrogen plant dynamics and understand the pathway of nitrogen sources in plant tissues. Water availability and nitrogen source affect the natural abundance of N isotopes (Sanchez-Bragado et al., 2017). Nitrogen isotope analysis determines differences between genotypes in terms of nitrogen uptake and nitrogen utilization efficiency. Nitrogen use efficiency in bread wheat has been reported to have a positive and significant correlation with plant water use, water use efficiency and grain carbon isotope discrimination (Dalal et al., 2013; Quemada and Gabriel, 2016). In addition, nitrogen isotope composition values of wheat can be used to separate the total nitrogen obtained from nitrogenous fertilization and the nitrogen taken from the soil (Dalal et al., 2013). This study aimed to determine the effects of different drought periods on nitrogen uptake and assess the performance and relative contribution of nitrogen N content associated with ^{15}N isotope to yield and quality parameters.

Material and Method

Ceyhan 99 spring bread wheat cultivar was chosen as genetic material in the study. A field experiment was carried out at Aydın Adnan Menderes University located about 33 m (37°45´22´´N 27°45´36´´E) above sea level with typical Mediterranean climate conditions during 2015/16 growing season (sowed in 24th of November 2015 and harvested in 03rd of June 2016). The experiment was set up according to the Randomized Block Design with three replications. The plots were 3x5 m in dimensions and 20 cm in rows. Four artificial drought periods were applied in the plots:

1. Irrigated conditions: Surface irrigation method was applied to plots in the flowering period (about 40% of the available water in the soil has been consumed from the sowing to flowering period). The gravimetric method was used to measure soil moisture (Black, 1965).
2. Rainfed conditions: The plants were grown under natural conditions (without additional water supply), irrigation and covering were not applied.
3. Early drought: The plots were covered from the beginning of the flowering stage (ZGS 61) until the maturity stage (ZGS 99). The covers were placed 1.5 m high from the soil surface and 2 m out of the plot edges.
4. Late drought: The plots were covered from the beginning of the grain filling stage (ZGS 71) to the maturity stage (ZGS 99). The covering process was carried out as mentioned in the early drought. 95% light-permeable polyethylene rainout shelter was used for covering plots for artificial drought (Öztürk, 1999).

Deficit rainfall (4.0 mm) and low temperature (6.2°C) were observed in December compared to the long-term climate values in the early growth stages of plants. In general, the weather conditions were favorable during vegetative and generative growth periods while the flowering period in April obtained low rainfall value (21.8 mm) compared to long-term precipitation value (48.2 mm). In general terms, 2015/16 growing season sufficient rainfall and favorable temperature conditions have been observed in flowering and grain filling periods (Table 1).

Table 1. Climate data for 2015/16 growing season in Aydın

Months	Prec (mm)	LT Pre (mm)	MT (°C)	LTMT (°C)
November	85.2	83,3	13.3	13.4
December	4.0	121.7	6.2	9.5
January	139.0	116.5	7.3	8.1
February	37.4	93.8	12.0	9.4
March	100.8	71.1	12.0	11.8
April	21.8	48.2	17.6	15.9
May	35.2	35.7	19.7	20.9
June	13.4	13.9	26.8	25.8

Prec: Precipitation; LT Pre: Long Term Precipitation; MT: Mean Temperature; LTMT: Long Term Mean Temperature

The experiment field had sandy-loam texture with alkaline reaction (pH: 8.05) and the organic matter content was low (%1.02). In the trial, 180 kg ha^{-1} nitrogen and 80 kg ha^{-1} phosphorus and potassium were applied to all plots. In addition, 0.25 m^2 micropilots were formed in each plot and 3 g of ^{15}N isotopes ($^{15}NH_4NO_3$ 96.4 atom%, Chemotrade, Leipzig, Germany) mixed with 300 ml of water were applied to the micro plots during tillering and stem elongation periods (Kiss et al., 1990). Stem, leaf and grain samples collected from micro plots at the time of harvest and milled in 0.5 mm thickness (UDY Corporation Cyclone Sample mill). The nitrogen isotope ratios of the samples were analyzed at the TÜBİTAK UME (The Scientific and Technological Research Council of Turkey National Metrology Institute).

Test method and procedure

Nitrogen isotope analysis was carried out according to TLM-05-G3OK-04-64 "Experiment Instruction of Carbon and Nitrogen Isotope ratios of Solid Samples at Isoprime IRMS EA Device". For the determination of $\delta^{15}N$ air isotope ratios in wheat samples, 0.1 µg, 1000-5000 µg of sample and 1000 µg of reference material (USGS32) were weighed separately in 3.5x4 mm tin capsules. The capsules were folded and placed in the oven auto-sampler neck connected to the Elemental Analyzer. The experiment was started in the Elemental Analyzer device connected to the Isoprime IRMS EA device. The formed gases were transported with IRMS gas with 99.999% purity He gas and δ^{15}Nair values were determined according to Nair scale.

δ^{15}N (‰): According to the formula used by Bort et al., (1998), Fraser et al., (2011) and Liu et al. (2015);

$$\delta^{15}N\ (‰) = [(Rsample / Rstandard) - 1] \times 1000.$$

Here;

$\delta^{15}N$ (‰): N isotope composition of plant sample,

R: represents the ^{15}N / ^{14}N ratio.

According to IAEA (International Atomic Energy Agency) standards, N_1 (δ^{15}Nair = 0.4‰) and ($\delta^{14}N_{air}$ = 8.44 ‰), N-2 ($\delta^{15}N_{air}$ = 20.3‰).

Grain yield (GY) was determined in 4.8 m^2 of each plot. Plant height (PH), number of spike per square meter (S/m^2), 1000 grain weight (TGW), number of grains per spike (G/S), single spike yield (SSY), and biomass yield (BY) were also measured. Whole milled wheat samples were scanned for crude protein and ash (%) ratio by using Near Infrared Reflected Spectroscopy (NIRS) method with Bruker MPA device (Germany) (Oliveira and Franca, 2011).

Stomatal conductance, SC (mmol $m^{-2}s^{-1}$)

Stomatal conductivity (SC) was determined by the portable photosynthesis system Decagon SC-1 porometer device (Decagon, Inc., Pullman, WA). This device measures the time the air passes through the leaf. If the number of stomata in the leaf and the number of open stomata are low, the air transfer rate is slow. If the number of stomata and the number of open stomata are high, air passes faster. The higher the permeability of the leaf, the faster the air passage (Rebetzke et al., 2001). The measurements were replicated in ZGS 55 (SC1) and ZGS 65 (SC2) periods between 11:00 am and 4:00 pm during the day. (Reynolds et al., 1998). Statistical analyses were performed using SPSS 19 statistical software (SPSS Inc. Chicago, USA). The effects of drought treatments on parameters determined were assessed by variance analysis (ANOVA). When the ANOVA indicated a significant difference between treatments, the mean values for each trait were grouped using least significant difference (LSD) test.

Results and Discussion

Agronomic and physiological parameters

Agronomic and physiological characteristics examined under drought and rainfed conditions are presented in Table 2. The effects of drought treatments on number of spikes per square meter, thousand grain weight, spike yield, grain yield, biological yield, stomatal conductance in flowering period were statistically significant (SC 2), protein and ash content. Drought applications had no significant effect on plant height, number of grains per spike, harvest index and stomatal conductance in ear emergence period (SC 1) parameters.

Table 2. Variance analysis of agronomic and physiological characters

	Mean Square					
	Plant Height (cm)	Number of spike (spike/m²)	Number of grains per spike	Thousand kernel weight (g)	Spike yield (g)	Grain Yield (kg/ha)
Replication	16.368	7687.600	35.668	5.368	0.101	5982.658
Drought	163.841	18820.222*	23.328	442.619**	0.967**	45995.586**
Error	50.734	6122.089	25.882	16.390	0.083	3255.890
Total	58.016	8118.696	27.676	69.589	0.202	9423.408
	Biological Yield (kg/ha)	Stomatal conductance (ZGS 55)	Stomatal conductance (ZGS 65)	Crude Protein (%)	Crude Ash (%)	
Replication	59428.400	795.245	2113.126	0.063	0.113	
Drought	360306.444**	603.280	3211.479*	3.820*	0.441**	
Error	11492.044	1279.906	898.540	1.198	0.080	
Total	67410.522	1086.289	1464.268	1.294	0.134	

*: *P*<0.05; **: *P*<0.01

The drought conditions in generative growth periods did not affect plant height due to drought treatments. The average plant height was higher (94.23 cm) in rainfed condition, and early drought period had lower value (84.22 cm) compared to other treatments (Table 3). Some researchers had similar results; Ayrancı (2012) and Öztürk (1999) indicated that plant height had higher value in irrigated conditions compared to early drought and late drought periods.

Table 3. Mean values of Ceyhan 99 bread wheat cultivar for investigated characters

	Plant Height (cm)	Number of spike (spike/m²)	Number of grains per spike	Thousand kernel weight (g)	Spike yield (g)	Grain Yield (kg/ha)
Irrigated	92.58±3.13	693±56 A	32.10±2.17	45.55±0.63 A	1.73±0.11 A	4452.5±43.8 A
Rainfed	94.23±3.84	643±27 AB	28.08±3.14	35.08±1.48 B	1.05±0.14 B	3504.2±515.3 B
Early drought	84.22±0.95	599±36 AB	30.98±0.67	25.95±1.10 C	0.88±0.04 B	2572.9±141.1 C
Late drought	84.66±1.72	563±38 B	28.37±1.44	29.32±1.74 C	0.89±0.04 B	2666.5±170.6 C
LSD	ns	96.34	ns	4.985	0.355	702.57
	Biological Yield (kg/ha)	Stomatal conductance (ZGS 55)	Stomatal conductance (ZGS 65)	Crude Protein (%)	Crude Ash (%)	
Irrigated	14350±467 A	119.49±10.01	156.88±18.73 A	15.77±0.75 AB	1.26±0.09 AB	
Rainfed	12540±608 B	110.22±3.21	150.02±8.05 A	14.59±0.09 B	1.40±0.11 A	
Early drought	9120±238 C	131.59±22.03	108.31±3.25 B	16.52±0.29 A	0.78±0.14 C	
Late drought	9730±193 C	110.65±20.80	121.13±17.52 AB	15.77±0.18 AB	1.05±0.05 BC	
LSD	1319.93	ns	36.908	1.348	0.348	

*: *P*<0.05; **: *P*<0.01

The number of spikes per square meter was significantly (*p*<0.01) different among the drought treatments (Table 3). Drought conditions during the grain filling period had the lowest value (563) compared to flowering period drought condition (599), rainfed condition (643) and irrigated condition (693) (Table 3). Ayrancı (2012) stated that the number of spike per square meter was 1002 in irrigated conditions, 846 in early drought and 924 in late drought periods. Öztürk (1999) reported that the number of spike per square meter as 528.3 in irrigated conditions, 448.3 in dry conditions, 415.8 in early drought period and 511.7 in late drought period.

Number of grains per spike is one of the main contributor component to grain yield and it is determined during earlier stages of wheat growth. Therefore, drought treatments during the grain filling stage didn't significantly affect the number of grains per spike (Tatar et al., 2020). In our study, related to number of grains per spike already formed in early developments it is found that there was no statistically significant changes belong to number of grains per spike under different drought treatments. Irrigated conditions had higher number of grains per spike (32.10) value while dry conditions had lower number of grains per spike (28.08) value (Table 3). Although there was no statistically significant difference, the number of grains per spike showed a tendency to increase by surface irrigation in the flowering period. Our data in arrangement with several findings as given above higher number of grains per spike could be attributed to irrigation and sufficient soil moisture that may cause higher fertility during early generative stages of growth. Mirbahar et al. (2009) reported that water stress throughout in vegetative and reproductive growth stages caused a significant reduction in number of grains per spike in wheat.

Thousand grain weight significantly decreased (43.0%) under grain filling drought condition compared to irrigation condition. In similar, in the previous study the effect of drought and well-watered regimes on 1000 grain weight was displayed as in drought stressed environment reduced 21.7% decrease of 1000 grain weight (Kılıç ve Yağbasanlar, 2010). Surface irrigation in flowering period mainly contributed to grain development with higher grain size. The average of highest 1000 grain weight was 45.55 g in irrigated condition, whereas the lowest 1000 grain weight was 25.95 g in early drought period (Table 3). Irrigation at generative growth periods contributes grain development with effective grain filling and so irrigation at anthesis and grain filling periods causes higher seed weight than non-irrigated and rainfed treatments. Xue et al. (2006) reported that 1000 grain weight had the highest value (33.7 g) in irrigated condition (supplemental irrigation in booting and grain filling stages), and rainfed condition had the lowest 1000 grain weight value (29.2 g).

The highest single spike yield was significantly affected and recorded (1.73 g) in irrigated condition and followed by rainfed condition. The lowest single spike yield (0.88 g, 0.89 g and 1.05 g) was obtained from the early and late drought periods but rainfed condition had also the same statistical group (Table 3). Irrigation in flowering period caused a statistically significant increase in single spike yield as well as 1000 grain weight contributed to higher yield values. Spike productivity becomes most important for yield followed by the size and number of grains per spike under drought conditions that occur in crucial growth stages of wheat (Petrova and Penchev, 2014). As it obtained in the study, single spike yield and 1000 grain weight in rainfed condition had greater impact on grain yield than drought conditions.

Analysis of variance showed that higher grain yield (p<0.01) value was obtained from irrigated condition. Post flowering drought conditions had the lowest yield values, whereas 2572.9 kg ha^{-1} in early drought and 2666.5 kg ha^{-1} in late drought condition (Table 3). Grain yield values almost decreased approximately two-fold (42.2% early and 59.88% late drought condition) in drought conditions compared to irrigated conditions. Grain yield was greater in well-watered conditions than in the drought conditions as a consequence of obtained more number of spike per square meter values, heavier grains, and longer grain filling period (Kılıç and Yağbasanlar, 2010). Results in the study related to the decrease in grain yield during early drought period are consistent with the findings of Ayrancı (2012) and Öztürk (1999). Achieving higher yields in irrigated conditions can be explained by the role of additional water other than precipitation with the combination of transport nitrogen and plant nutrients from roots to the grain.

Achieving high 1000 grain weight values in irrigated conditions led to get higher grain yield values. Yield loss was observed in both drought periods due to the generative period stress caused by less precipitation due to the covering processes applied from the beginning of flowering period to harvest in early drought and from the beginning of grain filling period to harvest in late drought.

The relative decline in the mean biological yield under drought stress was 36.44% (early drought) and 32.19% (late drought), and irrigation in flowering period had greater contribution to biomass development of wheat than rainfed conditions. Statistically significant differences in biological yield were found among tested environmental conditions (Table 3). While the highest biological yield (14350 kg ha^{-1}) was obtained from irrigated condition, the lowest biological yield value (9120 kg ha^{-1}) was achieved in early drought condition (Table 3). Ayrancı (2012) observed the biological yield values as 21110 kg ha^{-1} in irrigated conditions, 15760 kg ha^{-1} in early drought period and 17780 kg ha^{-1} in late drought period. On the other hand, Öztürk (1999) reported 12468 kg ha^{-1} in irrigated conditions, 9713 kg ha^{-1} in dry conditions 9155 kg ha^{-1} in the early drought period and 11004 kg ha^{-1} in the late drought period. The inclination of biomass related to photosynthetic organs above the ground and biomass production is directly proportional to photosynthesis but leaf senescence is hastened in water stress conditions (Khatiwada et al. 2020). The decrease in the biological yield in the early drought period is supported by the findings of previous studies.

Stomatal conductance

Stomatal conductance mean values decreased with elevated drought conditions were 156.88 mmol $m^{-2}s^{-1}$ in irrigated treatments and 108.31 mmol $m^{-2}s^{-1}$ in early drought conditions. Stomatal conductance decreased more in early drought condition than late drought treatment and more increased with irrigated treatment and rainfed condition (Table 3). Stomatal conductance in early drought condition (SC1) was not affected significantly compared to irrigated condition and drought treatments in heading period (ZGS 55).

Drought conditions may cause to reduction in leaf water potential that results with lowering turgor, closing stomata, and thereby reducing stomatal conductance, photosynthesis and finally lessening plant growth and yield potential of wheat. Root-shoot relationship is important to develop drought-tolerant wheat varieties. In water scarcity conditions lessened stomatal conductance may appear where shoot moisture content remains the same. Root-sourced signals assist plants to detect water deficit in roots and are expressed as a change of stomatal conductance in leaves (Ahmad et al., 2018). We found decreased stomatal conductance values between two generative phenological stages and stomatal conductance reduced in flowering period compared to heading.

Stomatal closure limits water loss through transpiration and helps plants to conserve water during stress but as a result of low transpiration stomatal closure may also reduce uptake of water from the soil reducing grain yield (Wasaya et al., 2021). Ayrancı (2012) reported stomatal conductivity values obtained during the heading period as 96.83 mmol $m^{-2}s^{-1}$ in irrigated conditions, 37.51 mmol $m^{-2}s^{-1}$ in the early drought period, and 46.86 mmol $m^{-2}s^{-1}$ in the late drought period.

Protein content

The lowest grain protein content (14.59%) was obtained in rainfed condition while the highest value (16.52%) was recorded in the early drought treatment. With irrigated and rainfed conditions, reductions in the protein content were expected and this may be explained by dilution effect as a result of higher grain yield values (Erekul et al., 2009). Quality characters of wheat are affected by genotypes, environmental conditions and their interactions.

Adverse climatic conditions during the anthesis and grain filling period have been a major restriction to wheat quality. Shortening grain filling period caused by drought conditions may result in changes for protein composition of the grains and distribution of starch, ash and fiber granules (Barutçular et al., 2016). Protein content is significantly influenced by applied drought treatments.

Ash content

Ash content which refers to mineral content of grain depends on agronomic practices, variety, fertilization and irrigation conditions. The results indicated after post-anthesis growth period drought caused an increase in grain ash content and wheat grain ash content was greatly affected by soil water conditions (Zhao et al. 2009). However, drought treatments had an adverse effect on grain ash content in our study. The highest ash content was observed in rainfed condition with 1.40% value and lowest value was obtained from early drought condition with the value of 0.78% (Table 3).

Nitrogen mobilization stem to grain with δ15 isotope

In wheat advances in management and cultivation practices, such as sowing time adjustment and flowering phenology as drought escape mechanisms may probably have contributed more to improving cereal growth in water scarcity conditions than genetic gain (Onyemaobi et al., 2021). Nitrogen management also contributes to controlling phenology and lack of nitrogen limits the productivity of wheat and other cereals. Available nitrogen in soil defines as a major contributor to grain yield per unit area for maximum yield. However, the cost of nitrogen fertilizers and increasing environmental constraints are expected to limit N fertilization and utilization in the future (Serret et al., 2008). Few studies consider natural abundance and mobilization of $δ^{15}N$ from stem to grain under drought treatments after post-anthesis. Our study reported here assessed nitrogen mobilization soil to grain under drought and irrigated conditions by applying in different phenological stages. Variance analysis of $δ^{15}N$ (‰) stem, $δ^{15}N$ (‰) leaves and $δ^{15}N$ (‰) grain characters are given in Table 3. According to the results stem ($p<0.01$), leaf ($p<0.01$) and grain ($p<0.05$) $δ^{15}N$ (‰) content changed statistically significant by drought applications. Nitrogen application time affected the amount of $δ^{15}N$ (‰) in stem and grain except in flag leaf. Drought and $δ^{15}N$ application time interaction had statistically significant affect on stem $δ^{15}N$ (‰) content (Table 4).

Table 4. Variance analysis of $δ^{15}N$ (‰) stem, $δ^{15}N$ (‰) leaf and $δ^{15}N$ (‰) grain characters

Mean square				
	df	$δ^{15}N$ (‰) Stem	$δ^{15}N$ (‰) Leaf	$δ^{15}N$ (‰) Grain
Replication	2	2694168	617152	5652641
Drought	3	39070569**	25001836**	21977348*
Error-1	6	514458	2198310	2744390
^{15}N app. time	1	127946308**	1702935	237648853**
Drought x ^{15}N app. time	3	13769529**	2309568	6872223
Error	8	458481	896320	7374260
Total	23	12983023	4575298	17867968
*: $P<0.05$; **: $P<0.01$				

The drought treatment significantly affected the stem $δ^{15}N$, $δ^{15}N$ application time and drought x $δ^{15}N$ application time interaction (Table 4). The stem $δ^{15}N$ values of the Ceyhan 99 bread wheat cultivar are given in Table 5. The highest stem $δ^{15}N$ value (15165 ‰) was obtained under rainfed condition and the lowest stem $δ^{15}N$ value (9619 ‰) was determined in irrigated and post-anthesis drought conditions. On the other hand, applied $δ^{15}N$ in the stem elongation period had the highest stem nitrogen content (13684 ‰) while tillering period had the lowest mean stem $δ^{15}N$ value (9066 ‰) (Table 5). Nitrogen mobilization from soil to stem increased by application of nitrogen isotope in the stem elongation period. Besides, rainfed condition had the highest $δ^{15}N$ value in both tillering and stem elongation $δ^{15}N$ application times. Post-anthesis drought treatments caused a markedly decline in stem $δ^{15}N$ value so it can be assumed that without additional water and rainfall resulted in less nitrogen mobilization to stem of wheat. Stem is an important vegetative part of plant that is one of the main sources of nitrogen grain. The current nitrogen uptake, excess of stem nitrogen and nitrogen released by leaf senescence are the main sources that supply wheat grain nitrogen (Götz et al., 2008). As our findings, application of nitrogen in stem elongation period was the period of rapid N accumulation under rainfed condition. Before flag leaf just visible third application of nitrogen promotes protein buildup in the ears. In modern wheat cultivars, protein content is required to be 12% dry matter and more which needs to synthesize high amounts of amino acids in vegetative tissues and transported to developing grain (Zörb et al., 2018). Otherwise, obtaining higher nitrogen levels applied in the stem elongation period contributes to get higher protein values in grain.

Table 5. Stem $\delta^{15}N$ value (‰) under drought and application time treatments

	$\delta^{15}N$ application time		
Drought Treatments	Tillering	Stem Elongation	Mean
Irrigated	7138 f	12099 b	9619 B
Rainfed	10803 cd	19527 a	15165 A
Early drought	8954 ef	11924 bc	10439 B
Late drought	9368 e	11184 bcd	10276 B
Mean	9066 B	13684 A	
Lsd Drought: 1013.85; Lsd $\delta^{15}N$ application time: 637.72; Lsd Drought x $\delta^{15}N$ application time: 1275.45			

Flag leaf $\delta^{15}N$ value

Flag leaves contribute the majority of assimilates for grains during post-anthesis stages and increased N supply results in increased leaf area, leaf nitrogen and chlorophyll content in wheat flag leaves (Tamang et al. 2017). Flag leaf senescence and photosynthesis performance are highly regulated by N supply which plays a vital role in the grain-filling process and alters dry matter accumulation (Ma et al. 2022). Although flag leaf $\delta^{15}N$ results showed that flag leaf nitrogen increased to comparably extents under drought conditions. Stomatal conductance decreased markedly in the flowering period in drought conditions compared to irrigation condition (Table 4). However, there is a possible explanation for this result as decreased stomatal conductance and photosynthetic parameters result in limited nitrogen mobilization from flag leaf to grain and so increased flag leaf nitrogen content results were obtained in irrigated condition but further studies are needed to explain this situation. According to statistical analysis results of the flag leaf $\delta^{15}N$ value of Ceyhan 99 cultivar were examined, drought factor was statistically significant but ^{15}N application time and drought x ^{15}N application time was not affected flag leaf $\delta^{15}N$ content (Table 3). Leaf $\delta^{15}N$ values of Ceyhan 99 bread wheat varieties are given in Table 6. The highest flag leaf $\delta^{15}N$ value (15378 ‰) was obtained in the early drought period and the lowest flag leaf $\delta^{15}N$ value (10867 ‰) was obtained in irrigated conditions. It can be seen from the same table that the mean flag leaf $\delta^{15}N$ value (14113 ‰) was obtained higher than the ^{15}N application in the tillering period but the difference was not significant when compared with the stem elongation period (Table 6).

Table 6. Flag leaf $\delta^{15}N$ value (‰) under drought and application time treatments

	$\delta^{15}N$ application time		
Drought Treatments	Tillering	Stem Elongation	Mean
Irrigated	11147	10587	10867 B
Rainfed	15547	14273	14910 A
Early drought	16139	14617	15378 A
Late drought	13618	14843	14231 A
Mean	14113	13580	
Lsd Drought: 2095.75, Lsd $\delta^{15}N$ application time: ns			

Grain $\delta^{15}N$ value

According to the statistical analysis results obtained for the grain $\delta^{15}N$ value of Ceyhan 99 cultivar, drought factor and ^{15}N application time were significant, drought x ^{15}N application time was insignificant (Table 4). The grain $\delta^{15}N$ values of the Ceyhan 99 bread wheat varieties are given in Table 7. The highest grain $\delta^{15}N$ value (13420 ‰) was obtained in dry conditions, while the lowest grain $\delta^{15}N$ value (9278 ‰) was determined in irrigated conditions. Götz et al. (2017) also reported the same result that ^{15}N content decreased slightly in mature grains with additional water supply. On the other hand, the mean grain $\delta^{15}N$ value obtained in the tillering period was determined as 8975 ‰, while the mean grain $\delta^{15}N$ value measured in the stem elongation period was 15269 ‰ (Table 7). Nitrogen applied in the stem elongation growth stage had greater contribution to grain nitrogen content.

Table 7. Grain $\delta^{15}N$ values (‰)

	$\delta^{15}N$ application time		
Drought Treatments	Tillering	Stem Elongation	Mean
Irrigated	7077	11479	9278 B
Rainfed	8833	18008	13420 A
Early drought	10510	15481	12996 A
Late drought	9481	16108	12794 A
Mean	8975 B	15269 A	
Lsd Drought: 2341.65, Lsd $\delta^{15}N$ application time: 2557.60			

In the study, stem, leaf and grain $\delta^{15}N$ values obtained in irrigated conditions were found to be significantly lower than the artificial drought periods. This can be explained by the fact that the nitrogen taken by the plant from the soil is used more in the metabolic activities of the plant in irrigated conditions, whereas absorbed nitrogen is consumed less in drought periods. Dalal et al. (2013) reported that nitrogen use efficiency in bread wheat has a positive and significant correlation with plant water use, water use efficiency and grain carbon isotope discrimination. Therefore, obtaining high nitrogen efficiency in irrigated conditions can be related to the high use of nitrogen. On the other hand, stem and grain $\delta^{15}N$ values were significantly lower applied in the tillering period compared to the stem elongation period. The reason for this can be explained as since the time from tillering to harvest is longer, the nitrogen taken into the plant is spent more by participating in metabolic activities.

Conclusions

Yield and its components were negatively affected as a result of exposure to drought during the generative period from the beginning of flowering to harvest and this period was found to be more severe results than other drought periods. It might be explained that additional water except for rainfall which is effective in transporting nitrogen and other nutrients from the soil to plants allows for obtaining higher yield values under irrigated conditions. Therefore, it can be emphasized that it is necessary to avoid practices that may cause drought stress in the plant in the generative period, especially in bread wheat production. Furthermore, it can be said that nitrogen use efficiency is disrupted due to the decrease in plant water use and water usage efficiency during drought periods. In addition, it can be stated that nitrogen's contribution to yield potential decreases due to less consumption of nitrogen in plant metabolic activities during drought periods. In order to unlimit the time to participate of nitrogen in metabolic activities, it can be concluded that nitrogenous fertilizer application should not be delayed in the period of tillering and it is suggested that stem elongation nitrogen application appears to be effective for nitrogen mobilization and contribution of different plant parts to grain.

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