

CONTRIBUTION OF EAR AND FLAG LEAF TO GRAIN FILLING OF WHEAT IN RESPONSE TO NITROGEN UNDER LATE-SEASON WATER DEFICIT

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Abstract. In a two-year field experiment, the assessment of leaves and ears photosynthetic contribution to grain filling in winter wheat genotypes was made under nitrogen fertilizer application and water deficit condition. A split plot factorial experiment was conducted based on randomized complete block design with three replications during 2014 and 2015 growing seasons. Treatments were irrigation levels including full irrigation (I₁) as control and irrigation withholding at heading stages (I₂), nitrogen (120 kg.ha⁻¹) splitting method into different phonological stages including 20 kg.ha⁻¹ at sowing and 100 kg.ha⁻¹ at mid-tillering stage (N₁), 20 kg.ha⁻¹ at sowing, 50 kg.ha⁻¹ at mid-tillering and 50 kg.ha⁻¹ at stem elongation stage (N₂) and 20 kg.ha⁻¹ at sowing, 50 kg.ha⁻¹ at mid-tillering and 50 kg.ha⁻¹ at 50% of heading stage (N₃) and four winter bread wheat genotypes including zarrin, Pishgam, Orum, and Mihan. Results showed that water deficit stress decreased the photosynthetic contribution of the flag leaf, ear and leaves below the flag leaf to grain filling, 1000-grain weight, number of grains per ear and seed yield of wheat. Furthermore, ear photosynthetic contribution (EPC) was higher compared to the flag leaf (FLPC) and leaves below the flag leaf (OLPC) to grain filling. Higher grain yield of Mihan and Pishgam genotypes can be associated with more grains per ear, 1000-grain weight and higher photosynthetic contribution of flag leaf and ear to grain filling. Such genotypic and environmentally driven differences were associated with changes in most of the traits. According to the results, partitioning of N fertilizer application to different phonological times can be a proper tool for increasing wheat yield under water deficit condition on different genotypes.

Keywords: grain yield, leaves below flag leaf, nitrogen splitting, phonological stages, water limitation

Introduction

Wheat is considered as the most important crop grown in the semi-arid regions where water deficit stress seems to be one of the most limiting factors for agricultural products (Maccaferri et al., 2011). That is to say, such a strategic crop usually suffers from water deficit stress during grain-filling period affecting the yield of the plant. According to Maccaferri et al. (2011), the reaction and response of plants to water deficit are evaluated based on genetic, biochemical, and morpho-physiological features of a plant.

The variations in the crop on the water uptake capacity during or shortly before the post-anthesis phase can have major effects on grain yield because growing grain number and grain weight are set during this stage.

Final grain weight is in relation to the duration of grain filling period and rate, and the respective interaction in-between (Sadras and Egli, 2008). In C3 cereals, such as wheat and barley, grain filling process is determined by current photosynthesis of the upper parts of the plant, i.e. both the flag leaf and the ear (Tambussi et al., 2007) and by redistribution of assimilates stored in the stem (Ehdaie et al., 2008). Further, the leaf photosynthesis value is affected by the position of the leaf on the plant. The flag leaf and the second leaf are the most active leaves in the photosynthesis rate in the wheat (Olszewski et al., 2008). According to Maydup et al. (2010) ear, photosynthesis plays a key role in the grain yield of wheat under both stress and non-stress conditions.

N in the plant is in the structure of the protein and nucleic acids, which are the most important building and information substances of every cell. In addition, N is also found in chlorophyll enabling the plant to transfer energy from sunlight through the process of photosynthesis. So, the value of N supply to the plant will influence the amount of protein, amino acids, protoplasm, and chlorophyll formed. Moreover, it influences cell size, leaf area, and photosynthetic activity (Uribe Larrea et al., 2009; Diacono et al., 2013). Dordas et al. (2008) reported that higher rates of N increase photosynthetic processes, leaf area production, leaf area duration as well as grain filling period. Lawrence et al. (2008) also reported that the reduction in photosynthesis caused by drought occurs more in the lower leaves than in the upper ones, and this reduction also was reported to be lower in the ear compared to the leaves. In the same line, under low nitrogen supply, plants use photosynthesis to maintain life functions which are not related to the yield-forming process. Under high nitrogen supply, the photosynthetic activity of ears causes the grain yield to increase (Olszewski et al., 2008). Soleymani fard and Sidat (2011) suggested that yield and yield components of safflower are increased by increasing the rate of applied nitrogen.

A better understanding of growth parameters may of help in programs the objective of which is to improve the grain yield under water limitation. Determination of response of wheat to N application is very important to maximize yield and economic profitability of wheat production in a particular environment. Considering the abovementioned facts, the present study was undertaken to know the effects of nitrogen rates on growth parameters and yield quantity of wheat under drought stress.

Material and methods

The field experiment was conducted as split factorial plot based on randomized complete block design with three replications during 2013-2014 and 2014-2015 growing seasons in the agriculture research station of Miandoab in West Azarbayjan province, Iran. The area is located at (46°6' N latitude and 36° 58' E longitude; alt. 1143 m, Miandoab, West Azarbaijan Province, Iran). Climatically, the area is placed in a semi-arid temperate zone with a pretty cold winter and a hot summer. The mean temperature and precipitation wheat growing season in 2014-2015 are presented in *Figure 1*.

In the field of the present study, each plot was included 6 rows with 4-m long. Besides, plots and blocks were separated by 3 m unplanted distances. The wheat cultivars were sown with a density of 400 seeds per m². Regarding the treatments, irrigation levels were set as the variables of the investigation where Full Irrigation (I1) was considered as

control while Irrigation Withholding during heading stages (I2), in main plots and the time of the application of nitrogen fertilizer and varieties were allocated to subplots as the treatments. Based on the results of the meteorological station located at the site of the experiment and collecting the meteorological data, amount of water were determined according to evaporation from class A pan and, using relevant calculations, the water volume required for irrigation was determined. To irrigate the volume of irrigation water in the plots, a pressure meter and pressurized water pipes were used to maintain the water volume consumed per plot in accordance with the evaporation rate of 100 mm evaporation from class A pan. Thus, the volume of water consumed for each round was about 650 m³/ha. Furthermore, Nitrogen fertilizer, 120 kg in total, was applied in three different arrangements as follow: 20 kg.ha⁻¹ nitrogen fertilizer in sowing and 100 kg.ha⁻¹ at mid-tillering stage (N1), 20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid-tillering and 50 kg.ha⁻¹ at stem elongation stage (N2), and 20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid-tillering and 50 kg.ha⁻¹ at 50% of heading stage (N3). In regard to the type of the varieties, four genotypes of winter bread wheat, Zarrin, Pishgam, Orum, and Mihan were utilized in the study.

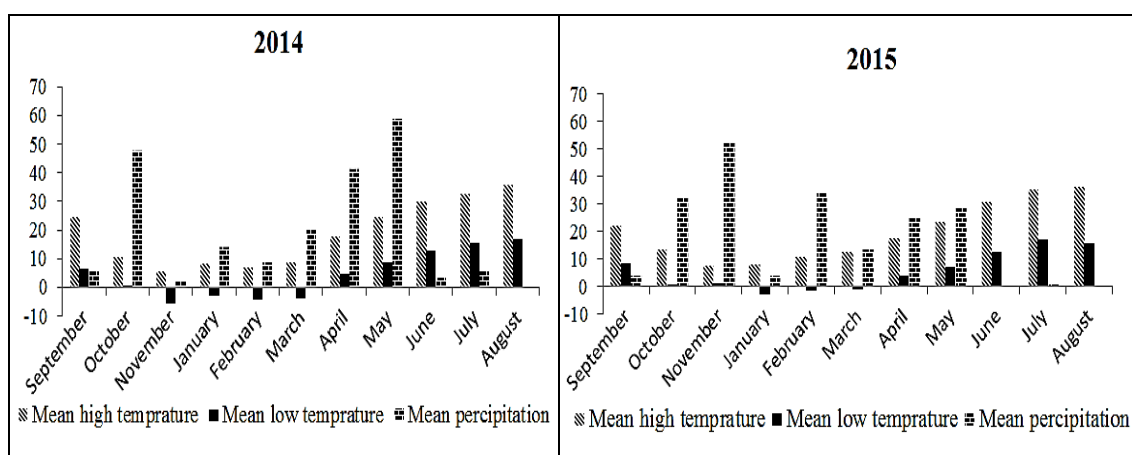


Figure 1. Mean temperature and precipitation wheat growing season at 2014-2015

Irrigation, weeding, and all other agronomic practices except those under study were kept consistent and uniform for all treatments. The soil samples were taken from the 30 cm depth of surface soil with soil auger 20 days before the beginning of the experiment in both formerly mentioned growing seasons. Moreover, the physicochemical properties of the soil samples have analyzed the result of which are presented in *Table 1*.

Table 1. Soil physico-chemical properties at depth of 0-30 cm

K available (p.p.m)	P available (p.p.m)	N total (%)	O.C. (%)	Texture	pH	Depth of sampling (cm)	Year
255	8.05	0.13	0.78	Silty-loam	8	0-30	2014
180	8.4	0.9	0.61	Silty-loam	9	0-30	2015

Some physiological traits were determined for the measurement of growth parameters. In the first sampling procedures, ten samples were taken at flowering stages in both time periods. In order to determine the weight of spike, peduncle, and internode, the samples were dried in an oven for 48 h at 80 °C. Then, the samples were treated for determining the phenotypic manipulations components. This was followed by the removal of all the leaves below the flag leaf, the flag leaf, and half of the spike. At the maturity stage, both labeled plants and 10 other randomly selected plants were harvested to determine the yield components and traits such as total dry matter, ear weight, grain weight per ear, and grain number per ear, 1000-grain weight and seed yield. Then the photosynthetic contributions of different plant parts (ear, flag leaf and leaves below the flag leaf) to grain filling in bread wheat genotypes were calculated as follows:

$$\text{EPC} = (\text{ear weight in flowering} - (\text{ear weight in physiological maturity} - \text{ear grain weight})) / \text{grain yield}$$

$$\text{FLPC} = (\text{ear grain weight} - \text{ear grain weight in flag leaf removal plant}) / \text{grain yield}$$

$$\text{OLPC} = (\text{ear grain weight} - \text{ear grain weight in other leaf removal plant}) / \text{grain yield}$$

where EPC, FLPC, and BFLPC refer to photosynthetic contributions of the ear, flag leaf and leaves below the flag leaf to grain filling, respectively.

The analysis of variance for the collected data was calculated applying SAS software a the main effects and interactions were tested using the Duncan's multiple range tests ($p \leq 0.05$).

Results and discussion

The results obtained from the combined (two-year data) analysis of variance, showed a significant effect of the interaction $I \times N \times G$ on photosynthetic contribution of the flag-leaf to grain filling, photosynthetic contribution of the ear to grain filling and number of grains per-ear in wheat plants in the field condition (Table 2). It was also revealed that the interaction of $Y \times I \times N \times G$ significantly affects the photosynthetic contribution of the leaves below the flag-leaf to grain filling and 1000-grain weight (Table 2). The photosynthetic contribution of the ear to grain filling was affected by the interaction of $Y \times I \times N$ (Table 2). Furthermore, the number of grains per ear and grain yield also were affected by the interaction of $Y \times I \times G$ (Table 2). The findings also showed that the number of grains per ear can be affected by the interaction of $Y \times N \times G$ (Table 2).

Photosynthetic contribution of the flag leaf to grain filling

As Amiri et al. (2013) stated in their article, due to the proximity of Flag-leaf to the spike, and the longer green life of it, the photosynthetic contribution of the flag-leaf is one of the most important sources for higher grain yield production during grain filling period. In this study, the highest photosynthetic contribution of the flag-leaf to grain filling (18.48%) was observed in Pishgaam genotype G2 (I1N2G2) under normal irrigation condition as I1 and the application of N2 (Table 3). The lowest value (10.22%) was obtained at I2N3G1 (Table 3).

Our results also showed that among the studied genotypes, Pishgaam genotype had the highest contribution of the flag-leaf to grain filling. Such results suggested that the flag-leaf contribution depends not only on sink strength but also on genotypic variations. In this line, the increase in the assimilate production was linked to the long green life of flag-leaf. N2 fertilizer treatments increased the photosynthetic contribution of the flag-leaf compared to the other nitrogen treatments. Low photosynthesis under water deficit stress was reported to be the result of lower chlorophyll synthesis and higher chlorophyll breakdown (Osman, 2014). Chlorophyll loss can also be due to damage to mesophyll chloroplasts leading to a lower photosynthetic rate (Wang and Xiao, 2009). The importance of the flag leaf in determining the mean grain weight, at mid-tillering and stem elongation of the plant is well-known. Sanjeri et al. (2008) showed that in water deficit condition, chlorophyll content of wheat genotypes decrease and this reduction is significant among genotypes.

Table 2. Summary of the analysis of variance for all the analyzed parameters

Source of variation	Df	Mean square					
		Photosynthetic contribution of the flag leaf to grain filling	Photosynthetic contribution of the leaves below the flag leaf to grain filling	Photosynthetic contribution of the ear to grain filling	1000-grain weight	Number of grains per ear	Grain yield
Year (Y)	1	71.30*	752.03**	1428.21*	25.84ns	1936.000*	21834592.56**
Replication/year	4	22.38	3.18	220.20	60.31	204.97	1693022.33
Irrigation (I)	1	0.001 ns	64.34**	173.27ns	770.06**	26.69 ns	151850167.56**
I × Y	1	56.163*	148.88**	461.10ns	189.06 ns	38.02 ns	72061706.174**
Error a	4	8.45	2.65	153.20	13.292	116.52	43517.13
Nitrogen (N)	2	58.31**	4.33 ns	1.83 ns	69.71**	521.69**	5594760.43**
Y × N	2	18.20ns	61.10**	6.96 ns	60.007*	40.58 ns	164618.77 ns
I × N	2	36.96*	0.60 ns	24.45 ns	5.81 ns	57.69 ns	960658.56ns
Y × I × N	2	3.31 ns	42.97*	115.12*	30.271ns	44.19 ns	60796.75ns
Genotype (G)	3	97.49**	33.07*	113.34*	215.80**	150.74ns	44327283.563**
Y × G	3	67.30**	37.92*	13.71 ns	34.35*	436.88**	2116660.50**
I × G	3	8.46 ns	19.51ns	6.62 ns	19.87ns	51.65 ns	1299497.581*
Y × I × G	3	20.98ns	11.67ns	34.45 ns	8.17 ns	232.17*	7220776.007**
N × G	6	10.13 ns	14.20ns	80.92*	38.87**	22.04 ns	908637.41*
Y × N × G	6	4.81 ns	27.08*	28.70 ns	3.55 ns	144.47*	255124.24 ns
I × N × G	6	28.92*	0.20 ns	71.51*	8.65 ns	189.60*	162713.22 ns
Y × I × N × G	6	16.38ns	49.71**	21.10 ns	43.24**	28.00 ns	138935.28 ns
Error b	88	10.82	11.44	46.29	12.54	64.87	426042.78
C.V.		14.55	13.22	14.28	9.95	13.55	9.73

ns: not significant at $P \leq 0.05$; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$

Photosynthetic contribution of the ear to grain filling

In the present study, in terms of grain filling, in all wheat genotypes, treatments, the time of nitrogen fertilizer application, and water deficit conditions, the photosynthetic contribution of the ear was better than the flag-leaf. The highest photosynthetic

contribution of the ear to grain filling (35.08%) was observed in normal irrigation as I1, application of N3, and Mihan genotype as G4 (I1N3G4) (Table 3). The lowest value (21.52%) of the photosynthetic contribution of the ear to grain filling was obtained at I2N2G2 (Table 3).

Table 3. Comparison of means for the experimental factors including genotypes and nitrogen application on photosynthetic contribution of the ear and flag leaf to grain filling

Treatment		Photosynthetic contribution of the flag leaf to grain filling(%)				Photosynthetic contribution of the ear to grain filling (%)			
Water limitation	Nitrogen application	G1	G2	G3	G4	G1	G2	G3	G4
I1	N1	11.23cde	12.28b-e	11.56cde	13.12b-e	27.73a-d	25.21b-d	33.71ab	27.48a-d
	N2	13.22b-e	18.48a	12.40b-e	18.44a	28.18a-d	27.04a-d	30.73a-d	28.64a-d
	N3	10.44e	12.21b-e	13.57b-e	14.08b-e	23.99cd	32.32abc	29.25a-d	35.08a
I2	N1	12.74b-e	16.68ab	11.28cde	13.51b-e	26.33a-d	29.19a-d	26.43a-d	26.98a-d
	N2	11.13cde	15.34a-d	15.48a-d	12.87b-e	24.88bcd	21.52d	30.70a-d	31.72abc
	N3	10.22b-e	14.49a-e	11.06de	15.71abc	23.23cd	29.83a-d	26.50a-d	25.72a-d

Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using Duncan's Multiple Range Test

I1 and I2 indicate normal irrigation and irrigation withholding in heading stages. N1, N2 and N3 indicate (20 kg.ha⁻¹ nitrogen fertilizer in sowing and 100 kg.ha⁻¹ at mid tillering stage), (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at stem elongation stage) and (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at 50% of heading stage) respectively
G1, G2, G3 and G4 indicate Zarrin, Peshgam, Orum and Mihaan genotypes respectively

According to the findings of the present investigation, the photosynthetic contribution of the ear to grain filling varied from 21% to 35%. Nonetheless, under water deficit conditions, the photosynthetic contribution of the ear in all genotypes was not significant (Table 5). According to a comparable study run by Mydup et al. (2010), the ear photosynthetic may act as a buffer in preventing serious damages under water deficit condition. Probably this can mainly be attributed to high durability of ear photosynthesis and the close relationship between grain and the ear. Considering the fact that ear benefits from a long-term spike photosynthetic activity with awn, the photosynthetic contribution of the ear is greater (Amiri et al., 2013). The decreased photosynthesis of the plant under water-stressed could be explained by the stomatal closure, which reduces CO₂ diffusion. Abebe et al. (2003) indicated that an early decrease in the photosynthesis during drought season is due to an increase in stomatal resistance. The Study conducted in the years 2014-2015, showed that maximum photosynthetic contribution of the ear (32.50 g) was obtained in Y2I2N2 (Table 4), whereas the minimum value (20.33 g) was observed in Y1I2N3 (Table 6). In 2015, due largely to a good agronomical condition, the photosynthetic contribution of the ear to grain filling increased up to 58%, which was higher considering the application of N3I2 in the same year (Table 4). These results indicated that the ear, as a photosynthetic organ, is better adapted than the flag-leaf to environmental factors. Even under good agronomical conditions, the ear may be more important than the flag-leaf during grain filling (Tambussi et al., 2007).

Table 4. Comparison of means for the experimental factors including genotypes and water limitation on photosynthetic contribution of the ear to grain filling of wheat under water limitation in two years, 2014-2015

Treatment		Photosynthetic contribution of the ear to grain filling (%)		
Year	Water limitation	N ₁	N ₂	N ₃
Y ₁ = 2014	I ₁	25.02bcd	28.42abc	29.82abc
	I ₂	23.70cd	21.91d	20.33d
Y ₂ = 2015	I ₁	32.05a	28.87abc	30.50ab
	I ₂	30.77ab	32.50a	32.31a

Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using Duncan's Multiple Range Test

I₁ and I₂ indicate normal irrigation and irrigation withholding in heading stages. N₁, N₂ and N₃ indicate (20 kg.ha⁻¹ nitrogen fertilizer in sowing and 100 kg.ha⁻¹ at mid tillering stage), (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at stem elongation stage) and (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at 50% of heading stage) respectively

The photosynthetic contribution of the leaves below the flag leaf to grain filling

It was well-established that the flag leaf and the ear are not the only sources of assimilates to grain filling (Tambussi et al., 2007). This study reported that the photosynthetic contribution of the ear was greater than that of the flag leaf and the leaves below the flag leaf. Owing to a good environmental condition in 2014 compared to the same period in 2015, among all genotypes, the photosynthetic contribution of the leaves below the flag leaf to grain filling was higher, and the photosynthetic contribution of the other leaves to grain filling was lower in Mihan genotype largely due to the lower height of the plants (Table 5). Indeed, the effect of the photosynthetic contribution to grain filling of flag leaf is an important parameter to assess rather than the leaves below flag leaf. The results showed that the maximum photosynthetic contribution of the leaves below the flag leaf to grain filling (19.45%) was obtained in Y1I2N2G1 (Table 5). But the lowest value (3.25%) was observed in Y2I2N3G4 (Table 5).

1000-kernel weight

Grain yield in wheat is the result of the number of grains per ear and grain weight (Ahmad et al., 1988). Some studies in 2013-2014 showed that a maximum of 1000-grain weight (53.33 g) was obtained in Y1I1N3G4 and Y1I1N2G4 (Table 5). But the minimum value (33.33 g) was observed in Y1I2N3G1 (Table 5). Mihan and Pishgaam genotypes were higher 1000-kernel weight under water deficit condition. In 2014 under water deficit condition, 1000-kernel weight decreased about 22% in I1N3G4 compared to I2N3G4 (Table 5). It seems that the environmental factors have a high influence on the 1000-kernel weight, and this trait, especially in 2015, is significantly affected by water deficit and nitrogen fertilizer division. These results are in line with the results of the study practiced by Ahmadi et al. (2006). Akinirinde (2006) and Alam et al. (2007) reported that the increase in 1000-kernel weight resulted from the increase in the photosynthesis. In the present study, the photosynthetic contribution of the leaves, flag leaf, and ear during grain filling period could correlate with grain yield. Azarpanah et al.

(2013) stated that the reduction of crop yield is related to the reduction of rate and amount of photosynthesis and thousand seeds weight.

Table 5. Comparison of means for the experimental factors including genotypes and nitrogen application on thousand grain weight and photosynthetic contribution of the leaves below the flag leaf to grain filling of wheat under water limitation in two years, 2014-2015

Year	Treatment		1000-grain weight (g)				Photosynthetic contribution of the leaves below the flag leaf to grain filling (%)			
	Water limitation	Nitrogen application	G ₁	G ₂	G ₃	G ₄	G ₁	G ₂	G ₃	G ₄
Y ₁ =2014	I ₁	N ₁	41.67f-n	45.33c-k	48.33a-g	48.67a-f	14.56a-g	17.59abc	13.14a-j	10.59c-l
		N ₂	44d-l	51.67abc	49.33a-d	53.33a	14.90a-e	14.67a-f	14.65a-f	14.51a-g
		N ₃	45.33c-k	53.00ab	47.00a-i	53.33a	18.19ab	16.80a-d	10.24d-m	13.47a-j
	I ₂	N ₁	35.67no	41.67f-n	41.33g-n	39.00k-o	8.007f-n	11.43c-l	6.81j-n	8.99e-n
		N ₂	41.67f-n	43.67d-l	42.67d-m	45.67c-k	19.45a	10.87c-l	13.33a-j	7.50h-n
		N ₃	33.33o	46.67a-j	45.33c-k	41.33g-n	14.70a-h	12.23b-k	11.89b-l	8.61e-n
Y ₂ =2015	I ₁	N ₁	42.00e-n	44.67c-l	49.00a-e	47.00a-i	7.13i-n	7.12i-n	5.90k-n	11.60b-l
		N ₂	46.67a-j	43.33d-m	43.33d-m	46.00b-k	12.46b-k	7.41h-n	5.76k-n	7.72g-n
		N ₃	36.33mno	47.67a-h	48.67a-f	48.67a-f	3.96mn	7.81f-n	10.15d-m	7.34h-n
	I ₂	N ₁	43.33d-m	39.67j-o	43.67d-l	40.33i-n	10.82c-l	11.42c-l	13.93a-i	7.83f-n
		N ₂	37.67l-o	40.67h-n	44.67c-l	43.00d-m	5.26lmn	9.03e-n	5.70k-n	9.90e-n
		N ₃	43.00d-m	46.67a-j	45.67c-k	47.00a-i	7.02j-n	8.39e-n	10.19d-m	3.25n

Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using Duncan's Multiple Range Test

I₁ and I₂ indicate normal irrigation and irrigation withholding in heading stages. N₁, N₂ and N₃ indicate (20 kg.ha⁻¹ nitrogen fertilizer in sowing and 100 kg.ha⁻¹ at mid tillering stage), (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at stem elongation stage) and (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at 50% of heading stage) respectively. G₁, G₂, G₃ and G₄ indicate Zarrin, Peshgam, Orum and Mihaan genotypes respectively

The number of grains ear

The number of grain ears is an important factor that affects the grain yield in wheat (Wei-Wei et al., 2012). The comparison of the effects of the interaction means of rates and time of N application and genotypes on the number of grains per ear in 2014 and 2015 indicated that the highest value (73.67) belonged to Y2N2G4, and the minimum number of grains per ear (45.50) was recorded at Y1N3G3 (Table 6). The highest number of grains per ear (66.83) was recorded at limited irrigation, application of N2 and Orum genotype (I2N2G3), and the lowest values of this trait (50.83) were recorded at I2N3G1 treatments (Table 7). The maximum number of grains per ear (70.11) was recorded at Orum genotype in normal irrigation condition in 2015 as Y2I1G3, and the minimum of this trait was recorded at Orum genotype and water limitation in 2014 as Y1I2G3 treatment (Table 8).

This reduction was probably due to the reduction of the numbers of the endosperm cells leading to the reduction of sink strength. This, in turn, could confer a critical survival advantage for few versus many seeds in terminal drought environments by reducing sink numbers at a key point in development and thus secure a sufficient sucrose supply for maturation of a few remaining seeds as observed under irrigation conditions. This is supported by a previous report showing that 180 kg.ha⁻¹ urea increased significantly the number of grains per head in safflower (Seyed Sharifi, 2011).

Dawadi and Sah (2012) suggested that a decrease in the number of grains per ear under lower N fertilizer application at stem elongation might be attributed to the poor development of sinks and the reduction of photosynthates translocation. In this study, high N rates increased the photosynthesis and delayed the appearance of phenological stages. It seems that high N rate can be one of the reasons of increasing the number of grains per ear row. Similar results have been reported by Alam and Haidar (2006). Increase in the number of grains per ear at higher N rates might be due to the lower competition for the nutrients allow the plants to accumulate more biomass with higher capacity to convert more photosynthesis into sinks (Zeidan et al., 2006). Uribelarrea et al. (2009) suggested that the effect of N stress on stem elongation on grain number occurs indirectly through its effect on photosynthesis and also on phenology stages. Seyed Sharifi (2011) suggested that increasing the number of grains may be due to the delay of the duration of the vegetative and reproductive period and lengthening of grain filling duration.

Table 6. Comparison of means for the experimental factors including genotypes and nitrogen application on number of grains per ear of wheat in two years, 2014-2015

Treatment		Number of grains per ear			
Year	Nitrogen application	G ₁	G ₂	G ₃	G ₄
Y ₁ = 2014	N ₁	51.67efg	55.00c-g	51.50fg	53.50d-g
	N ₂	66.17abc	62.83a-e	55.00c-g	58.17c-f
	N ₃	54.00d-g	55.00c-g	45.50g	61.00b-f
Y ₂ = 2015	N ₁	58.67c-f	60.83b-f	64.17a-d	64.33a-d
	N ₂	55.50c-g	63.00bcd	71.67ab	73.67a
	N ₃	58.67c-f	59.33c-f	64.50a-d	63.00a-d

Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using Duncan's Multiple Range Test

N₁, N₂ and N₃ indicate (20 kg.ha⁻¹ nitrogen fertilizer in sowing and 100 kg.ha⁻¹ at mid tillering stage), (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at stem elongation stage) and (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at 50% of heading stage) respectively

G₁, G₂, G₃ and G₄ indicate Zarrin, Peshgam, Orum and Mihaan genotypes respectively

Grain yield

Grain yield is a function of interaction amongst various yield components affected differently by the growing conditions and crop management practices (Cheema et al., 2010). The data presented in *Table 8* shows that the interactions of the studied experimental factors (year, irrigation, and genotype) had significant effects on grain yield of wheat. The highest figure for grain yield (9020 kg/ha) was observed in 2014, normal irrigation (I₁) and Mihaan genotype (G₄) (*Table 8*). Whereas the lowest figure regarding of the abovementioned trait (3277 kg.ha⁻¹) was obtained in Y₂I₂G₃ (*Table 8*). The seed yield decreased in water deficit condition except for the Y₁I₂G₃ treatment (*Table 8*). It is assumed that the obtained reasons are as the result of lower current photosynthesis, stomata conduction, and assimilation in water deficit condition.

Table 7. Comparison of means for the experimental factors including genotypes and nitrogen application on number of grains per ear of wheat under water limitation

Treatment		Number of grains per ear			
Water limitation	Nitrogen application	G ₁	G ₂	G ₃	G ₄
I ₁	N ₁	51.50c	58.17abc	59.67abc	57.50abc
	N ₂	64.33ab	65.00ab	59.83abc	66.33a
	N ₃	61.83abc	52.33c	55.83abc	66.17a
I ₂	N ₁	58.83abc	57.67abc	56.00abc	60.33abc
	N ₂	57.33abc	60.83abc	66.83a	65.50ab
	N ₃	50.83c	62.00abc	54.17bc	57.83abc

I₁ and I₂ indicate normal irrigation and irrigation withholding in heading stages

Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using Duncan's Multiple Range Test

N₁, N₂ and N₃ indicate (20 kg.ha⁻¹ nitrogen fertilizer in sowing and 100 kg.ha⁻¹ at mid tillering stage), (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at stem elongation stage) and (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at 50% of heading stage) respectively

G₁, G₂, G₃ and G₄ indicate Zarrin, Peshgam, Orum and Mihaan genotypes respectively

Table 8. Comparison of means for the interaction of factors including genotype, water limitation and year on number of grains per ear and grain yield of wheat

Treatment		Number of grains per ear				Grain yield (kg/ha)			
Year	Water limitation	G ₁	G ₂	G ₃	G ₄	G ₁	G ₂	G ₃	G ₄
Y ₁ =2014	I ₁	57.56cdf	58.78b-e	54.56def	59.67b-e	6143fg	8537ab	5969g	9020a
	I ₂	57.00c-e	56.44cde	46.78f	55.44cde	5180h	7471e	6634f	7828cde
Y ₂ =2015	I ₁	60.89b-e	58.22cde	70.11a	67.00ab	7680de	8294bcd	7775de	8464abc
	I ₂	54.33ef	63.89abc	63.44a-d	67.00ab	3409i	5794g	3277i	5857g

Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using Duncan's Multiple Range Test

I₁ and I₂ indicate normal irrigation and irrigation withholding at heading stages. G₁, G₂, G₃ and G₄ indicate refer to Zarrin, Pishgam, Orum and Mihaan genotypes, respectively

Mihaan and Pishgam genotypes had the highest seed yield in contrast to Zarrin genotype showing the lowest seed yield. The high seed yield can be associated with grains per ear, 1000-grain weight, and high photosynthetic contribution of flag leaf and ear to grain filling. These factors indicate that these genotypes are more tolerant of drought stress than the others. Yield reduction was significant in 2015 under water deficit condition resulting in a decrease of 57% in seed yield in Y2I1G3 compared to Y2I2G3 (Table 8). Results also showed that in normal irrigation (I1), wheat genotypes as Y1G4 had 7% more grain yield than Y2G4 (Table 8). However, the available literature has classically considered the flag leaf, leaf below the flag leaf and the ear

(Tambussi et al., 2007) as the main photosynthetic contributors during grain filling. Water deficit stress affects plant metabolism leading to a decrease in plant growth and grain yield. In the grains of the crops, both current assimilations were transferred directly to grains and remobilization of assimilates were stored in vegetative plant parts contributing to grain yield (Arduini et al., 2006).

Conclusion

In this research, Mihan and Pysgham cultivars with the highest seed weight and the transfer of more photosynthetic materials from flag leaf and spike had the highest seed yield in in water deficit conditions. According to the results, the application of N fertilizer in different doses and times, on different genotypes can be a proper tool for increasing wheat yield under water deficit condition.

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APPENDIX

Appendix 1. Study site. The pollination stage in wheats are shown. In this stage samples were treated for determining the phenotypic manipulations components, e.g. all the leaves below the flag leaf, flag leaf and half of the spike were removed in samples



Appendix 2. *Experimental design: a split plot based randomized complete block design with three replications*

I2	Mihan	Orum	Orum	Orum	Pesh	Pesh	Pesh	Pesh	Pesh	Zrrin	Pesh	Zrrin
	Orum	Pesh	Zrrin	Zrrin	Mihan	Mihan	Mihan	Mihan	Mihan	Orum	Mihan	Orum
	Pesh	Zrrin	Pesh	Pesh	Zrrin	Zrrin	Zrrin	Zrrin	Zrrin	Mihan	Zrrin	Mihan
	Zrrin	Mihan	Mihan	Mihan	Orum	Orum	Orum	Orum	Orum	Pesh	Orum	Pesh
I1	Mihan	Orum	Orum	Orum	Pesh	Pesh	Pesh	Pesh	Pesh	Zrrin	Pesh	Zrrin
	Orum	Pesh	Zrrin	Zrrin	Mihan	Mihan	Mihan	Mihan	Mihan	Orum	Mihan	Orum
	Pesh	Zrrin	Pesh	Pesh	Zrrin	Zrrin	Zrrin	Zrrin	Zrrin	Mihan	Zrrin	Mihan
	Zrrin	Mihan	Mihan	Mihan	Orum	Orum	Orum	Orum	Orum	Pesh	Orum	Pesh
	F1	F2	F3	F4	F2	F4	F1	F3	F3	F1	F4	F2
	R1				R2				R3			

I1 and I2 indicate normal irrigation and irrigation withholding in heading stages. N1, N2 and N3 indicate (20 kg.ha⁻¹ nitrogen fertilizer in sowing and 100 kg.ha⁻¹ at mid tillering stage), (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at stem elongation stage) and (20 kg.ha⁻¹ nitrogen fertilizer in sowing, 50 kg.ha⁻¹ at mid tillering and 50 kg.ha⁻¹ at 50% of heading stage) respectively

Zarrin, Peshgam, Orum and Mihan genotypes respectively