

Do nitrogen and zinc application alleviate the adverse effect of heat stress on wheat (*Triticum aestivum* L.)?

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Abstract

Late-season heat stress (LSH) is a limiting factor for wheat production. Besides, low zinc and poor protein diet usually is a problem in low-income countries. The primary calorie source in such countries is prepared from bread. This study aims to mitigate heat stress by zinc and nitrogen application and improve zinc and protein content in wheat grain. We did the field experiments as a split-split-plot based on a randomized complete block design with four replications to assess zinc and nitrogen's possible mitigation effect on LSH and protein and zinc enrichment of wheat grain during two years. Factors included LSH by delay in planting date (optimum, late, and very late) as the main plot, nitrogen (0, 75, 150, and 225 kg ha⁻¹) in subplots, and zinc (0, 10, and 20 kg ha⁻¹) as sub-subplots. We measured yield, yield components, physiological traits, zinc, and protein contents in the grain. Results showed that the highest relative water content and cell-membrane thermal stability were attained at the optimum planting date, 150 kg N ha⁻¹ and 20 kg Zn ha⁻¹. The maximum chlorophyll a and carotenoids contents in wheat cells were recorded in the optimum planting date, 225 kg N ha⁻¹, and 20 kg Zn ha⁻¹. Heat stress reduced the grain yield. In the second year of the experiment, the grain number per unit area was more than that of the first year; however, the highest grain yield was achieved in the first year owing to the higher mean grain weight. Nitrogen application decreased the adverse effects of heat stress on grain yield by increasing the grain number. Zinc application diminished the adverse effects of heat stress by increasing the mean grain weight. The adverse impact of the LSH on grain yield was more than that of biological yield. Heat stress reduced the hectolitre weight and zinc content of the grain. Meanwhile, it increased grain protein. In general, under LSH, the application of 225 kg N ha⁻¹ and 20 kg Zn ha⁻¹ can reduce the adverse effects of heat on the grain quality and quantity.

Keywords: biofortification; cell-membrane thermal stability; fertilizers; planting date

Abbreviations: Car., Carotenoids; Chl a, chlorophyll a; Chl b, Chlorophyll b; CMTS, Cell membrane thermal stability; DW, Dry weight; FW, Fresh weight; GN, Grain number per unit area; GP, grain protein content; GW, 1000-grain weight; GY, grain yield; GZ, grain zinc content; HL, grain hectolitre; HSP, Heat shock protein; LPD, Late planting date; LSH, Late-season heat stress; N, Nitrogen; OPD, Optimum planting date; RCBD, Randomized complete block design; RWC Relative water content; TW, Turgid weight; VLPD, Very late planting date; Zn, Zinc

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Introduction

As the primary source of humans' daily calorie intake, wheat plays a significant role in food security worldwide. This crop is also a critical source of protein, mineral nutrients, and vitamins, particularly B-group vitamins (Halim *et al.*, 2018). Considering a close relationship among soils, crops, and human health nutrition, plant growth in zinc-deficient soils results in low zinc seeds, causing zinc deficiency in humans and animals fed such seeds, which will result in many health-related side effects (Niyigaba *et al.*, 2019).

In the southwest of Iran, wheat is cultivated during late autumn. Under such conditions, the vegetative wheat growth coincides with cool-season precipitations, and no environmental limitations related to weather affect the vegetative growth. However, in the late-season of wheat growth, there exist limitations related to increased air temperature and water deficit, negatively impacting crop growth and production. Moshatati and Mousavi (2017) observed that late-season heat stress reduced wheat grain yields up to 48%. They reported that the highest and lowest grain yield belonged to the early November and early January planting dates. Delay in planting can cause a 65% reduction in grain yield because of late-season heat stress (Asakereh-Nezhad and Lak, 2017). It is concluded that heat stress reduces the biological yield by imposing premature aging on photosynthetic organs and lowering the current assimilates (Pireivatlou *et al.*, 2010). Cell membrane damage, electrical leakage from thylakoid, and reduced chlorophyll content are the adverse effects of heat stress, ultimately decreasing the photosynthesis performance (Rehman *et al.*, 2016). It is essential to use nitrogen and zinc efficiently to mitigate the undesirable influences of heat on crop yield and growth (Hafez and Badawy, 2018).

Nutrient deficiency is an important environmental limitation experienced by wheat during its growth and development. Zinc deficiency in the soil reduces wheat yield and grain Zn content (Niazkhani *et al.*, 2018). Almost 60% of the cultivated soils globally, especially soils in arid and semi-arid regions, are zinc deficient, leading to a more than 50% reduction in crop yield (Yang *et al.*, 2014). Zinc deficiency leads to the inefficiency of metabolic and enzymatic defence systems associated with stress resistance. Zinc improves the growth of the root and stem of cereals, hence produce vigour plants that will be more tolerant against stress and have more grain yield (Bechoff and Dhuique-Mayer, 2016). Besides, zinc deficiency in soils and plants is a global micronutrient deficiency problem reported in many countries, and it is registered as a risk factor for human health universally. Confirming acceptable levels of zinc in diets and its intake should be a key constituent in reducing child illness, improving physical growth, and decreasing death in developing countries (Chiranjib and Kumar, 2010). Zinc increases the grain protein content by activating protein synthesis enzymes (Saleem *et al.*, 2015).

High temperatures reduce photosynthesis because of damage to chlorophyll pigments, a decline in leaf nitrogen, obstruction of PSII reaction centre and electron flow, decreased quantum efficiency, and down-regulation of PSII photochemistry (Fahad *et al.*, 2017). In addition to the role of nitrogen in plant growth, it has been shown that high nitrogen regimes induce more heat shock proteins (mitochondrial Hsp60 and chloroplastic Hsp24) that confer heat tolerance to plant (Heckathorn *et al.*, 1996). In irrigated wheat fields, where no nitrogen deficiencies occur, the most influential factor on the grain protein content is the air temperature during the grain-filling period. It has been reported that high temperature enhanced the wheat protein content (Velu *et al.*, 2016).

As the essential food source, wheat experiences high temperatures and low precipitation during the grain filling period in Iran. Also, large areas of wheat cultivated lands have low available zinc for plants. Hence, the current research was designed to assess the probable heat stress mitigation effect of zinc and nitrogen fertilizers on wheat yield and zinc bio-fortification for improving wheat grain quality (protein and zinc content) under control and the heat stress conditions.

Materials and Methods

This research was carried out during two successive growing seasons of 2018 and 2019 in the research field (31°36'N, 48°53'E, and 34m above sea level) of Khuzestan University of Agriculture and Natural Resources, Molassani, Iran. The experimental site was placed in a semi-arid area with an average annual temperature and precipitation of 26.6 °C and 213 mm, respectively. Some chemical and physical features of the soil, meteorological data of the experimental site, as well as the trend of Tmax during the experiments in relation to wheat growth stages during the study period are given in Tables 1 and 2, and Figure 1 respectively.

Table 1. Soil physicochemical properties of the experimental farm

Year	Texture	pH	EC (dS cm ⁻¹)	Organic matter (%)	N (%)	P ₂ O ₅ (%)	Zn (mg kg ⁻¹)	Fe (%)	K (%)
The First year	Silty clay loam	6.99	2.59	1.33	0.0336	0.05	0.014	0.016	0.378
The second year	Silty clay loam	6.80	2.80	0.53	0.0328	0.04	0.012	0.013	0.365

Table 2. Some meteorological data of the experimental site during 2017-2018 and 2018-2019 growing seasons

	Temperature (°C)				Relative humidity (%)				Sunshine sum		Rainfall (mm)	
	Min		Max		Min		Max		Mean	Mean	Mean	Mean
Months	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
December	9.3	12.5	22.5	21.8	15.9	17.1	56.7	77.5	206.9	157.8	18.7	103.6
January	8.8	9.7	23.2	18.2	16.0	14.0	53.1	74.5	218	126	1.5	27.1
February	8.8	9.7	23.3	20.6	16.6	15.2	53.5	67.3	190.5	198.8	8.0	33.3
March	14.2	10.7	27.4	23.5	20.8	17.1	50.4	54.4	275.2	239.8	36.6	11.8
April	18.1	16.6	33.1	29.7	25.6	23.1	43.3	52	199.6	220.7	32.7	45.4
May	22.6	21.3	36.3	36.7	29.5	29.0	30.4	34	263.3	334.5	12.2	2.2

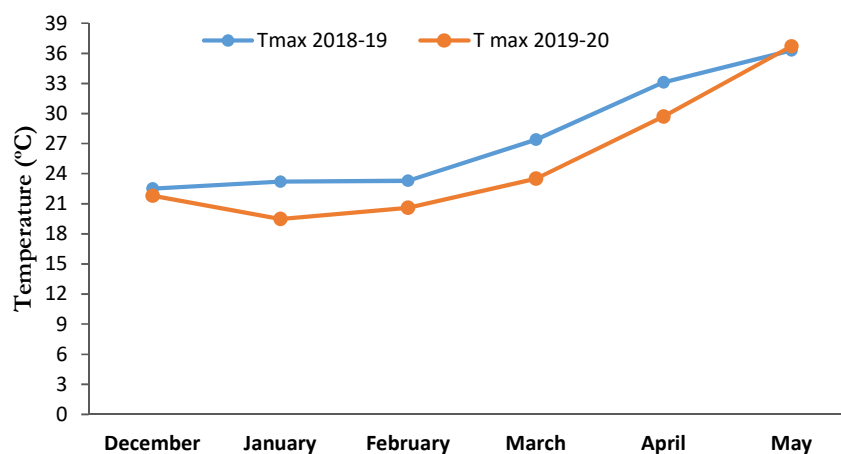


Figure 1. The trend of Tmax during the experiments in relation to wheat growth stages

Experimental design

The research was conducted using a split-split plot based on a randomized complete block design with four replications. Experimental factors were three planting dates, including November 22 (as an appropriate planting date), December 11 (late planting date), and December 31 (very late planting date) as main plots, four nitrogen levels, including 0, 75, 150, and 225 kg ha⁻¹ as subplots, and three zinc levels, namely 0, 10.0, and 20.0

kg ha⁻¹ as sub-sub plots. Urea (containing 46% N) and zinc sulfate (containing 14.7% zinc) were used as fertilizer sources. The field experiment had 144 plots. Each plot area was 4 m² (2 m × 2 m with 10 planting rows of 2 m lengths), with 60 cm, 90 cm, and 300 cm wide ridge between two neighbouring sub-sub plots, subplots, and main plots, respectively. The entire experimental plots were 144 each year.

Crop management

Wheat (Chamran2 cultivar) was cultivated on three planting dates. The planting density was 400 plants m⁻². Chamran2 is a cultivar resistant to rust and lodging. Field preparation (plowing and land levelling), weed and pest control, and irrigation were carried out according to typical region practices. All plots were given identical applications of P at 100 kg ha⁻¹ as superphosphate triple and K at 100 kg ha⁻¹ as potassium sulfate, as recommended by soil analysis. Nitrogen treatments were applied three times: one-third before planting at land preparation time, one-third at tillering stage, and one-third at the onset of the stem extension period. Zinc treatment was applied at the mid-stage of tillering. Zinc sulfate was dissolved in water, and the obtained solution was added to the soil close to the plant crown.

Measuring indices and methods

Growth stage recording

For all treatments, time to reach a specific growth stage was recorded (Table 3).

Table 3. The time of occurring growing stages of wheat at different planting dates

Growth stage	First planting date (22Nov)		Second planting date (11 Dec)		Third planting date (31 Dec)	
	2018	2019	2018	2019	2018	2019
Seedling emergence	Nov 28 (7*)	Dec 31 (10)	Dec 18 (7)	Dec 23 (11)	Jan 8 (8)	Jan 12 (11)
Tillering	Jan 6 (46)	Jan 16 (56)	Jan 27 (47)	Feb 6 (55)	Feb 26 (27)	Mar 8 (35)
Stem elongation	Feb 10 (81)	Feb 20 (91)	Feb 26 (77)	Mar 8 (85)	Mar 14 (73)	Mar 24 (81)
Flowering	Feb 23 (94)	Mar 7 (106)	Mar 15 (94)	Mar 27 (105)	Mar 25 (85)	Apr 10 (98)
Grain maturation	Apr 13 (143)	Apr 10 (156)	Apr 24 (134)	May 7 (146)	Apr 27 (117)	May 17 (135)

* indicates the number of days after planting

Yield

At maturity (when the moisture content of grains reached 14%), all plants in each plot were harvested; above-ground biomass, the number of seed per unit area, weight of 1000-grain, and final grain yields were further determined. Each plot's harvest index was measured by dividing the grain yield by the above-ground biomass (Dai *et al.*, 2016).

Physiological traits

At the flowering stage, cell membrane thermal stability (CMTS), carotenoid content, chlorophyll a, chlorophyll b, relative water content, and wheat canopy temperature were measured as the physiological responses of wheat to the experimental treatments. CMTS was determined based on a method presented by Agarie *et al.* (1995). For each plot, the flag leaves of 10 plants were selected to measure carotenoids content, chlorophyll a, and chlorophyll b with a spectrophotometer (SPEKOL2000) using the Arnon method (1967). Relative water content was measured using a 5 cm² surface of the flag. The samples' fresh weight was measured; the samples were floated on distilled water for seven hours in darkness. The turgid weight of samples was determined. Afterward, to specify the dry weight of samples, they were dried at 75 °C for 48 h. Relative water was measured using the following equation:

$$RWC = [(FW-DW) / (TW-DW)] \times 100$$

Where DW is dry weight, FW is fresh weight, TW and is the turgid weight.

Grain quality

Protein (%) and zinc (mg kg^{-1}) contents were determined as grain quality indices. Protein content was measured by the NIR grain analyzer (7250, Perten Company, Sweden). Atomic absorption (240FS AA, Agilent Company, USA) was used to measure grains' zinc content. Hectolitre was determined, according to Hill's (1975) method.

Data analysis

A combined analysis of the data about yield and yield components, physiological traits, and grain quality was carried out using SAS ver.9.4 (SAS Institute Inc., Cary, NC, USA). Because of page limitations, ANOVA tables are not included in the manuscript. However, means comparison is shown. The mean comparisons of various treatments were carried out utilizing Duncan's Multiple Range Test.

Results

Relative water content (RWC) of wheat

The compound analysis showed that year and interaction of planting date \times nitrogen \times zinc on RWC were significant ($P \leq 0.01$, Table 10). Delay in planting caused a significant reduction in leaf RWC, where late and very late planting dates, respectively, had 3.28% and 5.55% lower RWC than the optimal planting date (Table 4). The highest RWC (83.40%) was obtained at optimal planting date using 150 kg ha^{-1} N and 20 kg ha^{-1} Zn. The lowest RWC (61.64%) belonged to the very late planting date with no usage of N and zinc fertilizers (control), which was 26% lower than that of the highest value (Table 4). RWC of the second year of the experiment was higher than that of the first year (Table 5). When wheat was sowed late (late planting date), the highest RWC was attained using 225 kg ha^{-1} N and 10 kg ha^{-1} Zinc. Under the conditions of very late planting, the highest RWC was achieved with the application of 150 kg ha^{-1} N and 20 kg ha^{-1} zinc (Table 4).

Cell membrane thermal stability (CMTS)

According to the results, year and interaction of planting date \times nitrogen \times zinc significantly affected CMTS ($P \leq 0.01$, Table 10). Delay in planting reduced CMTS, where late and very late planting dates caused 6.45% and 13.1% reductions in CMTS, respectively. The mean comparison of the data showed that the highest CMTS ($52.25 \mu\text{mhos.cm}^{-1}$) belonged to the treatment with optimal planting date, 150 kg ha^{-1} N and 20 kg ha^{-1} zinc. However, the lowest CMTS ($21.63 \mu\text{mhos.cm}^{-1}$) was observed in a very late planting date with no N and Zinc application (control treatment). There was a 65.79% reduction in CMTS in the second year compared to the first year of the experiment (Table 5). Regarding the interaction of planting date \times fertilizer, 225 kg ha^{-1} N; 20 kg ha^{-1} Zinc, 75 kg ha^{-1} N; 10 kg ha^{-1} Zinc and 225 kg ha^{-1} N and 20 kg ha^{-1} Zinc were required to achieve the highest CMTS under the conditions of optimal, late, and a very late planting dates, respectively (Table 4).

Carotenoids, chlorophyll a, and chlorophyll b

The effect of year and interaction of planting date \times nitrogen \times zinc on carotenoids, chlorophyll a, and chlorophyll b was significant ($P \leq 0.01$, Table 10). Late planting date had a negligible effect on chlorophyll a content, where the difference between optimal and late planting date was only 5% regarding chlorophyll a content. However, a very late planting date (with a difference of 26.27%) significantly reduced chlorophyll a (Table 4). The same trend was observed in chlorophyll b content, where the difference of optimal planting date with late and very late planting dates was 6.5% and 32%, respectively. Delay in planting resulted in increased carotenoid content, with the flag leaf of wheat having 0.83% and 5.8% higher carotenoids at optimal planting date than the late and very late planting dates, respectively (Table 4). The mean comparison of the interaction of planting date \times nitrogen \times zinc showed that the highest content of chlorophyll a (27.22 mg g^{-1}) was obtained

in optimal planting date treatment with 225 kg ha⁻¹ N and 20 kg ha⁻¹ Zinc. No fertilizer application led to the lowest (12.73 mg g⁻¹) chlorophyll content of wheat leaf at a very late planting date (Table 4). The highest carotenoid content of flag leaf (27.29 mg g⁻¹) was observed at the first planting date with the application of 225 kg ha⁻¹ N and 20 kg ha⁻¹ zinc. With no fertilizers applied and a very late wheat planting, the leaf had the lowest (8.70 mg g⁻¹) carotenoids.

Regarding the effect of year, there was a considerable difference ($P \leq 0.01$) between the two years of experiment in terms of chlorophyll a and carotenoids content; specifically, the chlorophyll and carotenoids contents of flag leaf were higher in the first year (3% and twice more than that of the second year, respectively) (Table 5). When wheat was planted with delay, 225 kg ha⁻¹ N and 10 kg ha⁻¹ Zinc had to be applied for high chlorophyll content. Furthermore, to maximize wheat resistance to high-temperature stress through maximizing carotenoid content, 225 kg ha⁻¹ N and 20 kg ha⁻¹ Zinc had to be utilized under late planting conditions (Table 4). However, this amount was reduced to 150 kg ha⁻¹ N and 10 kg ha⁻¹ Zinc at the very late planting date (Table 4). The interaction of year \times planting date significantly influenced the chlorophyll b content ($P \leq 0.01$). The highest content of chlorophyll b in the two years of the experiment belonged to the LPD. However, the very late planting date had the lowest chlorophyll b content (Figure 2).

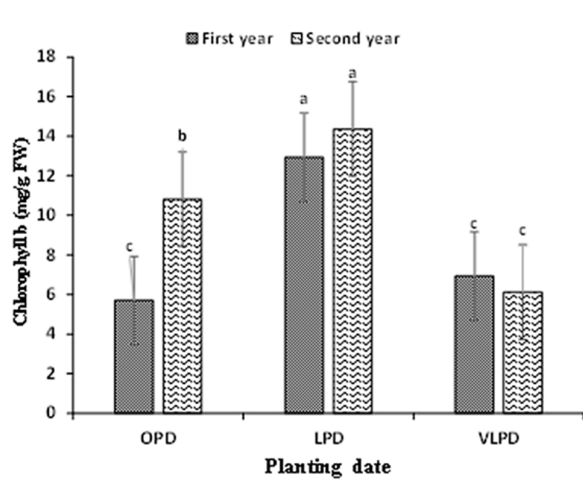


Figure 2. Chlorophyll b content of wheat leaf in different planting dates of two years of experiment OPD, LPD, and VLPD indicate optimum, late, and very late planting dates, respectively. Different letters represent a significant difference ($P \leq 0.01$) based on Duncan's multiple range test. Standard errors are also showed in the figure

Grain yield

The compound analysis revealed that the year's effect and interaction of planting date \times nitrogen \times zinc on the wheat grain yield were significant ($P \leq 0.01$, Table 10). In the second year, the wheat grain yield was significantly ($P \leq 0.01$) greater than that of the first year (Table 5). The mean comparison showed that the grain's highest yield was acquired when wheat was planted at optimal planting date using 225 kg N ha⁻¹ and 20 kg Zn ha⁻¹. A very late planting date with no fertilizer application resulted in the lowest grain yield. Delay in planting was associated with reduced grain yield. At late and very late planting dates, the achieved grain yield was 14% and 35% lower than that of the optimal planting date, respectively. However, under late and very late planting conditions, usage of 225 kg N ha⁻¹ and 150 kg ha⁻¹ and 20 kg Zn ha⁻¹ resulted in the highest grain yield (Table 6).

Grain yield components were reduced by delayed planting; specifically, grain weight and number per unit area experienced 7.86% and 13.37% reduction at the late planting and 12.7% and 25.58% reduction at the very late planting dates, respectively (Table 6). The highest grain weight (47.13 g) belonged to the optimal planting date applying 150 kg N ha⁻¹ and 20 kg Zn ha⁻¹. The smallest grains were observed (31.29 g) in the very late planting date with no fertilizers applied, showing a reduction of 33% (Table 6). Using 225 kg N ha⁻¹ and

20 kg Zn ha⁻¹ at the optimal planting date resulted in the highest grain number per unit area; in other words, a very late planting date without the use of nitrogen and zinc fertilizers yielded the lowest value of this trait (Table 6). To maximize the grain weight at late and very late planting dates, 150 kg N ha⁻¹ and 10 kg Zn ha⁻¹ was needed. Furthermore, to maximize the grain number per unit area at late and very late planting dates, 150 kg N ha⁻¹, 10 kg Zn ha⁻¹, and 225 kg N ha⁻¹, 10 kg Zn ha⁻¹ had to be applied, respectively (Table 6).

Table 4. Means comparison for interaction between planting date and N-Zn fertilizers on some physiological properties of wheat

Planting date	Nitrogen (kg ha-1)	Zinc (kg ha-1)	RWC (%)		CMTS (µmhos.cm-1)		Chlorophyll a (mg g-1FW)		Chlorophyll b (mg g-1FW)		Carotenoids (mg g-1FW)	
OPD	0	0	79.10	a-c	43.08	a-c	19.81	b-k	5.02	pq	8.95	e
OPD	0	10	78.69	a-c	51.17	ab	21.99	aj	4.82	q	16.86	a-e
OPD	0	20	75.78	a-d	50.53	ab	19.12	c-l	7.01	k-p	18.39	a-d
OPD	75	0	80.16	a-c	51.61	ab	18.33	f-l	9.84	c-h	20.81	a-d
OPD	75	10	82.04	ab	53.86	ab	22.48	b-i	8.91	d-l	11.98	de
OPD	75	20	80.33	a-c	44.38	ab	18.75	d-l	7.82	h-o	13.87	b-e
OPD	150	0	80.16	a-c	52.01	ab	25.82	a-c	10.33	b-f	15.45	b-e
OPD	150	10	78.10	a-c	48.86	ab	23.79	a-h	9.32	c-j	15.35	b-e
OPD	150	20	83.40	a	53.58	ab	16.58	i-l	7.42	j-o	20.10	a-d
OPD	225	0	80.28	a-c	52.54	ab	21.91	aj	11.28	a-c	14.13	b-e
OPD	225	10	77.58	a-c	47.92	ab	18.54	e-l	12.51	a	18.56	a-e
OPD	225	20	76.49	a-c	57.25	a	27.22	A	12.30	ab	27.29	a
LPD	0	0	75.14	a-d	46.43	ab	13.11	k-l	6.89	l-q	13.33	c-e
LPD	0	10	73.16	b-d	48.92	ab	14.08	k-l	8.74	e-m	17.63	a-e
LPD	0	20	78.57	a-c	49.18	ab	21.99	aj	9.10	dk	16.34	b-e
LPD	75	0	71.35	cd	51.30	ab	23.63	a-h	9.68	c-i	12.16	de
LPD	75	10	77.34	a-c	51.83	ab	24.45	a-g	10.08	c-e	18.35	a-e
LPD	75	20	78.45	a-c	50.58	ab	24.66	a-f	10.98	a-d	15.83	b-e
LPD	150	0	76.10	a-d	48.63	ab	21.32	aj	8.41	fo	13.69	b-e
LPD	150	10	78.76	a-c	50.37	ab	24.51	a-g	10.91	a-o	21.52	a-d
LPD	150	20	77.13	a-c	51.19	ab	25.46	a-e	10.71	a-e	14.15	b-e
LPD	225	0	77.76	a-c	49.65	ab	26.36	ab	8.71	e-m	18.58	a-e
LPD	225	10	78.88	a-c	49.31	ab	25.56	a-d	9.99	c-g	17.62	a-e
LPD	225	20	76.25	a-d	50.19	ab	22.14	aj	9.87	d-h	24.26	ab
VLPD	0	0	61.64	e	21.63	d	12.73	L	2.17	r	8.70	e
VLPD	0	10	76.16	a-d	44.80	ab	13.60	kl	5.20	pq	16.20	b-e
VLPD	0	20	76.50	a-c	48.25	ab	15.54	j-l	6.58	n-q	19.14	a-e
VLPD	75	0	66.85	de	28.59	cd	17.45	h-l	5.22	pq	14.52	b-e
VLPD	75	10	72.25	cd	43.08	a-c	17.89	f-l	7.63	i-o	18.70	a-e
VLPD	75	20	78.43	a-c	46.08	ab	18.37	f-l	7.51	j-o	21.80	a-d
VLPD	150	0	76.15	a-d	39.01	bc	16.29	i-l	6.34	o-q	15.47	b-e
VLPD	150	10	76.55	a-c	47.12	ab	18.23	fl	7.12	k-p	23.74	a-c
VLPD	150	20	81.56	ab	48.83	ab	17.66	g-l	8.48	f-n	18.39	a-e
VLPD	225	0	78.91	a-c	46.80	ab	13.35	kl	6.76	m-q	18.36	a-e
VLPD	225	10	76.76	a-c	48.45	ab	18.38	f-l	7.40	j-o	20.27	a-d
VLPD	225	20	77.56	a-c	49.05	ab	17.64	g-l	7.90	g-o	18.03	a-e

*OPD, LPD, and VLPD indicate optimum, late, and very late planting dates, respectively. Means followed with at least one common letter in each column are not significantly different according to Duncan's multiple range tests (P<0.01).

Table 5. Grain yield, weight, and number per unit area of wheat over the two years of the experiment

Year	RWC (%)	Chl a (mg g ⁻¹ FW)	Carotenoids (mg g ⁻¹ FW)	CMTS (μ mos.cm ⁻¹)	Grain yield (kg ha ⁻¹)	1000-Grain weight (g)	Grain number per unit area (m ²)
2018	72.49 ^b	20.52 ^a	23.60 ^a	71.04 ^a	4902 ^a	39.9 ^a	15927 ^b
2019	81.41 ^a	19.97 ^b	10.76 ^b	24.30 ^b	4030 ^b	37.2 ^b	17808 ^a

*Different letters indicate a significant difference ($P \leq 0.01$) according to Duncan's multiple range test.

Table 6. Means comparison for interaction between planting date and N-Zn fertilizers on grain yield and yield component of wheat

Planting date	Nitrogen (kg ha ⁻¹)	Zinc (kg ha ⁻¹)	1000-grain weight (g)		Grain yield (kg ha ⁻¹)		Grain number per unit area (m ²)	
OPD*	0	0	37.66	e-l	3634.3	k-n	1138	op
OPD	0	10	40.63	b-e	4567.9	d-k	15032	j-o
OPD	0	20	40.71	b-e	4233.3	f-m	15762	h-n
OPD	75	0	39.79	c-g	4539.4	e-k	17709	d-k
OPD	75	10	43.07	b	5995.9	a-c	19585	b-i
OPD	75	20	42.23	bc	5873.0	a-c	21345	a-c
OPD	150	0	40.44	b-f	5005.1	c-j	19851	b-g
OPD	150	10	46.77	a	5988.4	a-c	22628	ab
OPD	150	20	47.13	a	6506.9	ab	21693	a-d
OPD	225	0	38.45	d-j	5563.7	b-c	21775	a-c
OPD	225	10	40.61	b-c	5779.9	a-d	21836	a-c
OPD	225	20	41.96	bc	6823.6	a	24122	a
LPD	0	0	36.35	i-n	3097.3	mn	10519	p
LPD	0	10	38.59	c-j	3246.3	l-n	12563	n-p
LPD	0	20	39.52	e-h	3453.3	k-n	13460	l-p
LPD	75	0	36.19	j-n	3936.0	i-m	16289	f-n
LPD	75	10	38.24	d-k	5095.6	c-i	17359	e-l
LPD	75	20	38.68	d-j	4545.0	e-k	17818	c-k
LPD	150	0	37.41	fl	4552.7	e-k	16799	fl
LPD	150	10	41.84	bc	5352.4	b-g	20084	b-f
LPD	150	20	40.95	b-d	5436.7	b-f	19756	b-h
LPD	225	0	35.74	j-n	4931.7	c-j	18325	c-j
LPD	225	10	36.99	g-l	5196.8	c-h	18639	b-j
LPD	225	20	39.68	d-h	5499.4	b-c	19781	b-h
VLPD	0	0	31.29	o	1839.0	o	5917	q
VLPD	0	10	36.49	h-m	2656.3	no	10270	p
VLPD	0	20	37.30	g-l	3139.0	mn	12627	m-p
VLPD	75	0	33.45	no	3850.3	j-n	12519	n-p
VLPD	75	10	36.24	j-n	3037.1	mn	13904	l-p
VLPD	75	20	36.80	g-l	3567.3	k-n	16533	f-n
VLPD	150	0	35.35	k-n	3652.3	k-n	15682	i-n
VLPD	150	10	38.27	d-k	4086.0	h-m	16634	f-m
VLPD	150	20	37.15	g-l	4422.0	e-l	17160	f-l
VLPD	225	0	33.50	m-o	3620.1	k-n	16030	g-n
VLPD	225	10	35.11	l-n	3894.5	i-m	19515	b-i
VLPD	225	20	37.52	fl	4154.2	g-m	16230	g-n

*OPD, LPD, and VLPD indicate optimum, late, and very late planting dates, respectively. Means followed with at least one common letter in each column are not significantly different according to Duncan's multiple range tests ($P \leq 0.01$).

Hectolitre weight

According to the compound analysis, the main effect of year and interaction of planting date × nitrogen × zinc on hectolitre weight was significant ($P \leq 0.01$, Table 10). The wheat grain's hectolitre weight was lower in the first year compared with the second year (Table 7).

Table 7. Some quality traits of wheat grain over the two years of experiment

Year	Grain hectolitre weight (g)	Grain protein (%)	Grain zinc content (mg kg ⁻¹)
2018	79.79 ^b	10.22 ^b	1.53 ^a
2019	81.52 ^a	12.22 ^a	1.42 ^b

*Different letters indicate a significant difference ($P \leq 0.01$) according to Duncan's multiple range test.

Table 8. Means comparison for interaction between planting date and N-Zn fertilizers on some grain quality traits

Planting date	Nitrogen (kg ha ⁻¹)	Zinc (kg ha ⁻¹)	Protein (%)		Zinc (mg kg ⁻¹)		Hectoliter weight (g)	
OPD	0	0	7.58	l	1.11	t	79.14	j-n
OPD	0	10	8.23	j-l	1.42	j-p	80.56	d-l
OPD	0	20	10.12	f-j	1.32	n-q	80.84	c-j
OPD	75	0	9.01	i-l	1.29	p-r	81.63	a-g
OPD	75	10	9.20	i-l	1.43	i-o	82.41	a-c
OPD	75	20	10.5	e-i	1.52	g-l	82.92	ab
OPD	150	0	10.80	e-i	1.39	k-p	82.39	a-c
OPD	150	10	11.32	c-h	1.66	c-f	82.69	ab
OPD	150	20	11.27	d-h	1.68	b-e	83.25	a
OPD	225	0	11.74	a-g	1.52	g-k	82.24	a-d
OPD	225	10	12.00	a-f	1.80	ab	83.00	ab
OPD	225	20	11.74	a-g	1.90	a	82.39	a-d
LPD	0	0	7.87	kl	1.16	st	79.69	h-m
LPD	0	10	9.05	i-l	1.39	m-q	78.95	k-n
LPD	0	20	10.78	e-i	1.38	m-q	80.80	c-j
LPD	75	0	10.40	e-i	1.26	q-s	79.86	g-m
LPD	75	10	10.22	e-i	1.53	f-j	81.28	b-i
LPD	75	20	10.53	e-i	1.44	i-n	81.80	a-f
LPD	150	0	12.17	a-e	1.39	l-q	80.34	e-l
LPD	150	10	11.80	a-g	1.71	b-d	81.36	b-h
LPD	150	20	11.97	a-f	1.55	e-i	81.95	a-e
LPD	225	0	13.22	a-d	1.41	j-p	80.64	c-k
LPD	225	10	13.24	a-d	1.71	b-d	80.82	c-j
LPD	225	20	12.92	a-d	1.78	bc	81.97	a-e
VLPD	0	0	10.40	e-i	1.17	r-t	77.40	n
VLPD	0	10	9.18	g-k	1.29	p-s	79.63	h-m
VLPD	0	20	9.62	h-k	1.30	o-q	79.85	g-m
VLPD	75	0	11.48	b-h	1.26	q-s	79.20	j-n
VLPD	75	10	11.37	c-h	1.55	f-j	79.28	j-m
VLPD	75	20	12.13	a-e	1.47	h-m	78.75	l-n
VLPD	150	0	13.47	ab	1.36	m-q	80.16	e-m
VLPD	150	10	13.71	a	1.68	b-e	79.50	i-m
VLPD	150	20	13.29	a-c	1.59	d-h	79.61	h-m
VLPD	225	0	13.67	a	1.47	h-m	78.93	k-n
VLPD	225	10	13.68	a	1.64	c-g	78.40	mn
VLPD	225	20	13.68	a	1.61	c-g	79.98	f-m

*OPD, LPD, and VLPD indicate optimum, late, and very late planting dates, respectively. Means followed with at least one common letter in each column are not significantly different according to Duncan's multiple range tests ($P \leq 0.01$).

Delayed planting caused a reduction in hectolitre weight. The highest hectolitre weight (83.00 g) belonged to optimal planting date and the application of 225 kg N ha⁻¹ and 10 kg Zn ha⁻¹. Very late planting date and no fertilizer treatment (control) yielded the lowest (77.40 g) hectolitre weight of grain, showing a 7% reduction (Table 7). At the late planting date, the fertilizer treatment of 150 kg ha⁻¹ N and 20 kg ha⁻¹ zinc, and at the very late planting date, the fertilizer treatment of 150 kg ha⁻¹ N without zinc enhanced the hectolitre weight of wheat grain (Table 8).

Grain protein

The compound analysis showed that the grain protein was meaningfully ($P \leq 0.05$) influenced by the year and interaction of planting date \times nitrogen \times zinc (Table 10). The grain protein was lower in the first year compared with the second year (Table 7). Delayed sowing increased the protein content; in other words, late and very late planting dates resulted in 14.67% and 17.98% more accumulation of protein in wheat grain than the optimal planting date. Mean comparison revealed that the highest protein content of grain (13.71%) belonged to the very late planting date with the usage of 150 kg ha⁻¹ N and 10 kg ha⁻¹ zinc. Furthermore, the lowest values (7.58%) were recorded under optimal planting date and no fertilizer use, showing a 44% reduction. However, at optimal planting date, 225 kg ha⁻¹ N and 10 kg ha⁻¹ zinc improved the grain protein (Table 8).

Grain zinc content

The main effect of year and interaction of planting date \times nitrogen \times zinc had a meaningful influence ($P \leq 0.01$) on the grain zinc content (Table 10). Heat stress, induced by delayed planting, did not affect the zinc content. In the first year, the amount of grain zinc was higher (Table 7). The highest zinc content (1.9 mg kg⁻¹) was achieved under optimal planting date and use of 225 kg ha⁻¹ N and 20 kg ha⁻¹ zinc. The lowest value (1.17 mg kg⁻¹) was recorded when wheat was planted very late, and no fertilizers (nitrogen and zinc) were applied.

Wheat grains had higher protein content (20%) and hectolitre weight (3%) in the second year than the first year (Table 7). However, wheat's zinc content in the first year was more significant than in the second year (Table 7).

Discussion

Physiological traits

Damage to pigments such as chlorophyll is one of the effects of heat stress on plants. Reduction in chlorophyll content under heat stress is due to the increased activity of chlorophyllase (Kamanga *et al.*, 2018). In the present research, delayed planting reduced the chlorophyll content by inducing heat stress. However, the application of nitrogen fertilizer resulted in increased chlorophyll content. Since nitrogen is one of the main elements of chlorophyll structure, its presence in plants increases chlorophyll synthesis. By affecting the chlorophyll concentration, nitrogen directly influences the photosynthesis rate (Evans, 1983) hence plant growth and yield.

Heat stress reduced chlorophyll biosynthesis and increased its degradation. The chlorophyll biosynthesis inhibition under high temperature is attributed to various enzymes' deactivation (Dutta *et al.*, 2009), such as 5-aminolevulinatase, an essential enzyme in the pyrrole biosynthesis pathway, that its activity decreases in wheat under heat stress (Mohanty *et al.*, 2006).

Cell membrane usually is the first part to be damaged by environmental stresses, so CMTS is a good indicator of heat stress damage to leaf cells. Delayed planting reduced CMTS (Table 4) as the wheat was exposed to more heat stress. This finding is consistent with the results of Damaris *et al.* (2016). The decrease in RWC was correlated with a reduction in CMTS (Table 10). Delayed planting and the fact that some growth

stages of wheat coincided with heat stress resulted in reduced RWC; this may be attributed to the increasing effect of heat on evaporation and transpiration. When RWC was reduced and stomata closed, the wheat's cooling power decreased, leading to an increase in the temperature of the whole plant. With the rise in the canopy temperature, the internal temperature of cells was further increased, negatively affecting the whole cell, especially the membrane system. The changes in carotenoids content (Table 4) confirms these findings. Carotenoids play a role in the chloroplast as a pigment. However, their most important function is an antioxidant activity because they are responsible for preventing lipid peroxidation and alleviating environmental stress's adverse effects (Altuntas *et al.*, 2018). Accordingly, the increased carotenoid content at delayed planting dates indicates that wheat experienced heat stress at late and very late planting dates, generating more carotenoids to reduce its heat stress effects on cell membranes.

Adverse effects of heat stress on RWC, chlorophyll content, and CMTS were reduced with nitrogen and zinc application (Table 4). Nitrogen can overshadow water losses by affecting stomatal behaviour and leaf area duration and postponing plant maturation (Holík *et al.*, 2018). Zinc application has increased RWC in maize (Tohidi Moghadam *et al.*, 2013). Increasing the amount of nitrogen and zinc resulted in the synthesis of more protein molecules in the cell membrane structure, reducing cellular leakage and increasing membrane stability under stress conditions (Gomez-Coronado *et al.*, 2017). Zinc is involved in the activation of enzymes related to carbohydrate metabolism, maintenance of the integrity of cellular membranes, protein synthesis, regulation of auxin synthesis, and pollen formation (Marschner, 1995). Also, regulation and conservation of the gene expression required for environmental stress tolerance are dependent on zinc (Cakmak, 2000).

Grain yield

The results showed that grain yield was negatively affected by a delay in planting. The induced heat stress by delayed sowing reduced the plant's photosynthetic potential, leading to reduction of chlorophyll content, RWC (Table 4), and grain filling-period (Mondal *et al.*, 2015; Moshatati and Mosavi, 2017). These processes ultimately reduced grain number per unit area and the mean grain weight as two main wheat grain yield components. In the second year, the grain number per unit area was more than that of the first year. However, grain yield was lower in the first year, which can be attributed to the lower grain weight in the first year. It seems that climatic parameters are useful in determining wheat grain yield. In the second year, precipitation and relative humidity were significantly higher than the first year (Table 2), resulting in yellow rust disease in the post-pollination stage (data not shown). This adverse condition and reduced sunny hours reduced assimilate allocation to grains, hence grain weight reduction (Table 6).

Grain weight depends on the rate and duration of the grain-filling period and is strongly affected by the environment. Therefore, any factor that reduces the duration or rate of the grain filling period will reduce grain weight (Joshi *et al.*, 2016). Another reason for the loss of grain weight under heat conditions is the plant's inability to transport and convert photosynthetic materials to starch under heat stress conditions (Ahmed *et al.*, 2017).

A decrease in the grain-filling period reduces mean grain weight under heat stress conditions. Although the grain filling rate increases under such an adverse condition, this increase cannot reimburse reducing grain weight due to the reduced grain filling period (Gupta *et al.*, 2015). By inducing kernel abortion, heat stress leads to grain number and grain yield reduction (Zaki and Radwan, 2011). Similarly, in the present study, delayed planting caused heat stress on wheat and reduced the number of grains per unit area.

Nitrogen application alleviated the adverse effects of heat stress on grain number per unit area; specifically, at late and very late planting dates, with the usage of 225 kg ha⁻¹N, the number of grains per unit area enhanced by 55% and 21%, respectively. Given the positive correlation between grain number per unit area and grain yield (Table 9), nitrogen increased grain yield by 60% and 53% under late and very late planting dates, respectively (Table 6). There is a reciprocal relationship between the number of grains per unit area and mean grain weight (Kutlu and Olgun, 2015); therefore, nitrogen application led to grain weight reduction in the present study. Another reason for the decrease in grain weight under increased nitrogen application is the

prolonged vegetative growth period. This exposes the grain filling period to higher temperatures and exacerbated heat stress effects (Lotfi, 2013).

Table 9. Pearson correlation coefficients for measured traits

	GY	GN	GW	RWC	CMTS	Chl a	Chl b	Car.	GP	GZC	HL
GY	1										
GN	0.63**	1									
GW	0.62**	0.45**	1								
RWC	0.10 ^{ns}	0.36**	0.09 ^{ns}	1							
CMTS	0.41**	-0.02 ^{ns}	0.41**	0.41**	1						
Chl a	0.27**	0.20**	0.07 ^{ns}	0.000 ^{ns}	0.15**	1					
Chl b	0.29**	0.26*	0.08 ^{ns}	0.10 ^{ns}	0.17**	0.85**	1				
Car.	0.20**	-0.10 ^{ns}	0.14*	-0.26**	0.66**	0.16**	0.21**	1			
GP	-0.04 ^{ns}	0.29**	-0.25**	0.24**	-0.42**	0.01 ^{ns}	0.04 ^{ns}	-0.12*	1		
GZC	0.49**	0.42**	0.31**	0.01 ^{ns}	0.30**	0.35**	0.41**	0.44**	0.246**	1	
HL	0.34**	0.47**	0.33**	0.46**	-0.24**	0.18**	0.19**	-0.16*	0.15*	0.15*	1

GY: grain yield; GN: Grain number per unit area; GW: 1000-grain weight; RWC: Relative water content; CMTS: Cell membrane thermal stability; Chl a: chlorophyll a; Chl b: Chlorophyll b; Car.: Carotenoids content; GP: grain protein; GZ: grain zinc content; HL: grain hectolitre. ns: not a significant difference; **, * Significant at 0.01 and 0.05 of probability level, respectively.

Zinc has positive effects on the enzymatic systems of the plant, having catalytic or structural parts in protein synthesis and increased production capacity of photo-assimilates; therefore, zinc plays a significant role in spike formation and increasing yield of the grain through enhancing the yield components (Ahmadi *et al.*, 2017; Singh, 2014). Application of zinc under heat stress conditions increases the grain yield (Kamaei *et al.*, 2018). Zinc can also mitigate the effects of environmental stresses on plants by improving cellular stability (Torrión and Stougaard, 2017) and enhancing antioxidant activity (Yadavi *et al.*, 2014). In the recent study, zinc alleviated the adverse effects of heat stress on grain yield components; in other words, at late and very late planting dates, the weight of the grain and grain number per unit area was improved by 7%, 11% and 26% 33%, respectively. This ultimately led to a 26% (late planting date) and 33% (very late planting date) increase in grain yield. Because zinc had been applied when the plant had not yet experienced the heat stress; so maybe additionally stored zinc in the plant could play a beneficial role in increasing the grain weight (probably through increasing assimilates transfer to grain (Ma *et al.*, 2017) and participate in the biosynthesis of growth regulators (Zand *et al.*, 2009).

Grain quality

In the second year of the experiment, the grain's protein content was greater than that in the first year, which can be attributed to the smaller grain (Table 6) in the second year. About 70% of the grain weight of wheat comprises starch; thus, decreasing the accumulation of starch in the grain results in smaller and lighter grains (Hu *et al.*, 2018). The enzymes involved in starch production are susceptible to high temperatures during the period of grain-filling. Reducing these enzymes' activity at temperatures above 22 °C results in the reduction of starch accumulation (Faroq *et al.*, 2011). There is an inverse relationship between the amount of starch and grain protein. Also, protein production has a lower sensitivity to higher temperatures than starch (Kutlu and Olgun, 2015); therefore, grains had lower starch and higher protein in the second year of the test (Table 7). Nitrogen fertilizers increase protein storage compared to carbohydrates in the grain (Joshi *et al.*, 2016). El-Habbal *et al.* (2010) concluded that the increase in the grain's protein content using nitrogen fertilizer was related to the increase in essential amino acids, leading to higher nutritional values.

Table 10. Compound analysis of variance (Mean squares) of the effects of nitrogen and zinc application under heat stress (induced via different planting dates) on some traits of wheat during two growing seasons (2017-18 and 2018-19)

SOV	DF	RWC	CMTS	Chlorophyll a	Chlorophyll b	Carotenoid	1000-grain weight	Grain yield
YE	1	5722.27**	157328.71**	13.59**	88.00**	1186.55**	554.50**	54768534.4**
B×YE	6	21.80	18.39	7.03	63.43	46.58	9.70	421876.7
PD	2	474.38 ^{ns}	1833.90**	312.16 ^{ns}	1356.83 ^{ns}	134.84 ^{ns}	843.33 ^{ns}	85360334.8 ^{ns}
PD×YE	2	33.27 ^{ns}	8.89 ^{ns}	690.11**	2154.20**	1725.32**	222.96**	6817349.6**
E1	12	23.27	41.52	8.90	17.32	65.56	4.10	727501.5
N	3	190.90	381.60 ^{ns}	159.78	307.47 ^{ns}	273.47 ^{ns}	140.21 ^{ns}	46698751.9
N×YE	3	20.11 ^{ns}	59.13 ^{ns}	17.36**	53.90	62.31 ^{ns}	60.10**	2043038.8
PD×N	6	91.54 ^{ns}	135.52 ^{ns}	6.52 ^{ns}	36.27 ^{ns}	29.85 ^{ns}	17.57 ^{ns}	517398.3 ^{ns}
PD×N×YE	6	53.80	154.51**	6.89 ^{ns}	14.35 ^{ns}	24.32 ^{ns}	18.26	532010.4 ^{ns}
E2	54	19.80	24.50	3.19	14.17	26.78	6.16	526148.9
Z	2	230.34	860.08 ^{ns}	26.17 ^{ns}	45.38 ^{ns}	532.73**	370.44 ^{ns}	15680389.3 ^{ns}
Z×YE	2	8.90 ^{ns}	252.93**	44.70**	186.51**	3.05 ^{ns}	151.15**	5894717.1**
Z×PD	4	152.27 ^{ns}	502.36 ^{ns}	24.49 ^{ns}	23.07 ^{ns}	129.74 ^{ns}	3.12 ^{ns}	1509869.9 ^{ns}
Z×D×YE	4	100.79**	142.36**	5.60 ^{ns}	61.62	81.04	2.62 ^{ns}	671092.2 ^{ns}
Z×N	6	97.79 ^{ns}	221.41 ^{ns}	11.52 ^{ns}	36.83 ^{ns}	100.28 ^{ns}	12.32 ^{ns}	599584.4 ^{ns}
Z×N×YE	6	94.36**	96.10**	14.05**	63.43**	104.95**	9.52**	2194838.7**
Z×N×PD	12	70.90**	115.71**	5.45**	47.94**	79.91**	6.54	11457780**
Z×N×PD×YE	12	59.62**	164.23**	31.41	2.93 ^{ns}	78.12**	6.11	984292.1
E3	144	19.57	18.76	3.19	14.80	24.07	3.08	459217.7
CV (%)		5.74	9.08	19.26	21.50	28.55	4.55	15.17
SOV	DF	Grain number per unit area		Protein content		Zinc content	Hectoliter weight	
YE	1	254761267**		289.52**		0.99**	217.06**	
B×YE	6	6112269		2.10		0.02	2.59	
PD	2	59441727 ^{ns}		86.80		0.7 ^{ns}	180.39 ^{ns}	
PD×YE	2	140999011**		4.28 ^{ns}		0.48**	56.04**	
E1	12	9694431		1.75		0.02	0.66	
N	3	860674711		190.95 ^{ns}		1.86	28.72 ^{ns}	
N×YE	3	40923992		33.06**		0.08**	16.68**	
PD×N	6	4014021 ^{ns}		2.00 ^{ns}		0.04 ^{ns}	10.10 ^{ns}	
PD×N×YE	6	6056780 ^{ns}		2.51 ^{ns}		0.02 ^{ns}	4.05 ^{ns}	
E2	54	12550608		1.22		0.01	3.36	
Z	2	203777704 ^{ns}		8.11 ^{ns}		1.84 ^{ns}	22.44 ^{ns}	
Z×YE	2	176734713**		13.43**		0.67**	2.99 ^{ns}	
Z×PD	4	2666030 ^{ns}		2.94 ^{ns}		0.02 ^{ns}	4.10 ^{ns}	
Z×PD×YE	4	4447540 ^{ns}		3.08**		0.02 ^{ns}	1.81 ^{ns}	
Z×N	6	15152889 ^{ns}		4.19 ^{ns}		0.02 ^{ns}	1.77 ^{ns}	
Z×N×YE	6	20623652**		2.78**		0.02	3.50 ^{ns}	
Z×N×PD	12	10386830		2.39**		0.02	3.53	
Z×N×PD×YE	12	10630140		2.64**		0.01 ^{ns}	2.19 ^{ns}	
E3	144	5464479		0.40		0.008	1.76	
CV (%)		13.85		5.69		6.35	1.64	

ns, *, **: non-significant and significant at 5% and 1% probability levels.

YE: year; B: Block; PD: Planting date; E1: Error1; N: Nitrogen; E2: Error 2; Z: Zinc; E3: Total Error.

Zinc can effectively determine the protein content of grain. In other words, it can increase the grain protein content associated with increased enzymatic activity (Aziz *et al.*, 2019). Zandipour *et al.* (2018) showed the positive effect of zinc on the increased protein content of wheat grain. The present study's findings follow this because the correlation between protein and zinc content was positive and significant (Table 9). Giunta *et al.* (2019) concluded that zinc sulfate could increase the grain protein content, which agrees with the present research work results. However, the influence of zinc on the protein content of grain was affected by heat stress. At optimal and late planting dates, zinc application resulted in a 13% and 6% increase in the grain protein content, respectively; however, zinc had no positive influence on the wheat grain protein content at a very late

planting date. In other words, the highest effect of zinc on the wheat grain protein content occurred under optimal planting dates and no heat stress imposed on the plant.

Hectolitre weight of grains is an indicator of grain yield and depends on uniformity in shape, density, and moisture content. Heat stress may reduce hectolitre weight, and grains damaged by stress also have a lower weight (Motaghi *et al.*, 2012). Similar results were observed in the present study, where delayed planting (which induced heat stress on wheat) caused a reduction in hectolitre weight. This can be due to the negative effect of heat on starch accumulation and the increased bran to endosperm ratio (Ghodyeh Zarinabadi and Ehsanzadeh, 2014). On the other hand, nitrogen had no meaningful influence on hectolitre weight. In other words, nitrogen could not alleviate the negative influences of heat stress on hectolitre weight. These findings are in line with the results of Kazemzadeh *et al.* (2013), who stated that nitrogen had no effects on the hectolitre weight of wheat ('Alvand' cultivar). The present research work results showed a positive relationship between hectolitre weight and grain protein content (Table 9), which is consistent with Kazemzadeh *et al.* (2013).

Conclusions

Quantitative (grain kg/ha) and qualitative yields (protein and zinc content) of wheat were improved by integrating nitrogen and zinc fertilizers. The extent to which these fertilizers are used to maximize quantity and quality depends on the planting date. To achieve the highest grain yield, optimal planting date (November 22) and 225 kg ha⁻¹ N and 20 kg ha⁻¹ zinc is the best treatment. The highest protein content was obtained at a very late planting date with 150 kg ha⁻¹ N and 10 kg ha⁻¹ zinc. When zinc is considered a qualitative trait of wheat grain, the most optimal treatment is the first planting date (22 November), 225 kg ha⁻¹ N and 20 kg ha⁻¹ zinc. The treatment recommended for the maximum hectolitre weight is optimal planting date, 225 kg ha⁻¹ N, and 10 kg ha⁻¹ zinc. The role of nitrogen in protein synthesis (HSPs to increase heat tolerance and gluten to increase seed quality) and development of area and photosynthesis efficiency is essential here. Zinc, by its various roles in cell metabolism, especially enzymatic reactions and carbohydrate metabolism, improved the quantity and quality of yield under normal and heat stress conditions.

According to the results of this two-year experiment, wheat's grain yield is affected by climatic conditions. Thus, the wheat grain yield was higher in the first year because, in the second year, the climatic parameters did not fully correspond to the plant's needs. In the second year of the experiment, climatic conditions such as higher rainfall, less sunshine, and higher relative humidity during the grain filling period resulted in reduced plant photosynthetic potential and increased pest damage (yellow rust, especially flag leaf data not shown), reducing the grain yield.

Authors' Contributions

Conceptualization: HRE and SNM; Investigation: NA and AM; Methodology: HRE, AM, and NA; Formal analysis: AI; Writing-original draft: SNM; and Writing-review and editing: HRE and SNM.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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