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RESEARCH PAPERS

Effect of Foliar and Soil Application of Zinc on Grain Filling, Yield and Some Physiological Traits of Wheat (*Triticum aestivum* L.) under Salinity Stress

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Abstract—Salinity is a global challenge issue that is drastically reducing agricultural production and limiting the uptake of essential nutrients such as zinc (Zn) in plants. A better understanding of wheat (*Triticum aestivum* L.) for improved yield and physiological responses may help in main programs, increasing growth and yield under stress conditions. Thus, factorial experiment was conducted based on a randomized complete block design with three replications under greenhouse conditions during 2020–2021. Experimental factors included salinity levels (no salinity or control, salinity 30, 60, and 90 mM NaCl) and Zn application methods (no Zn as control, soil applied Zn as $ZnSO_4$, foliar application of nano Zn oxide (ZnO), and combined soil and foliar application). The results demonstrated that the maximum of shoot and stem dry matter remobilization, and the contribution of stem reserves to the grain yield and electrical conductance were observed in salinity severe stress and no Zn application. The combined soil and foliar application of Zn at no salinity increased chlorophyll index (69.83%), stomatal conductance (50.34%), quantum yield (45.78%), relative water content (RWC; 80.19%), leaf area index (46.89%), grain filling period (20.2%), effective grain filling period (29.22%), and grain yield (34.56%) compared to no Zn application at the highest salinity level. Based on the results of this study, it seems that combined soil and foliar application can increase the grain yield of wheat under salinity stress due to improving grain filling components and some physiological traits such as RWC, SPAD and stomatal conductance.

Keywords: *Tririсum*, chlorophyll index, electrical conductance, nanoparticles, quantum yield, salinity stress, stomatal conductance, zinc

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INTRODUCTION

Salinity is a global challenge that affects agricultural production worldwide. More than 800 million hectares of agricultural land suffer from soil salinity [1]. The increased salinity of agricultural land is expected to have destructive global impacts, resulting in the loss of up to half of the arable land by the middle of the twenty-first century [2]. The adverse effects of salinity have been attributed to the increase of Na^+ and Cl^-, which are considered to be the most important ions that induce several disorders in the physiological processes of different plants [3]. Salinity increases ion toxicity levels while reducing water availability to plants and absorption of essential nutrients [4].

Intense salinity stress may trigger the acceleration of senescence, enzymatic and metabolic dysfunctions, including a decline in photosynthetic carbon dioxide assimilation and electron transport activity, chlorophyll degradation, the accumulation of reactive oxygen species (ROS), and membrane damage, leading to plant death during prolonged exposure [2]. Khalilzadeh et al. [5] stated that salinity stress decreases chlorophyll content, quantum yield, relative water content (RWC) and grain filling parameters, but increases leaf electrical conductance (EC) and dry matter remobilization in wheat.

Zinc (Zn) is known as important micronutrients, and its deficiency is recognized as a critical problem in plants, especially grown in saline conditions with high pH values [6]. Zn nano-particles restrict the entry of Na^+ and Cl^- ions and reduce the accumulation of ROS and abscisic acid, in exchange for an increase in

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Abbreviations: EC—leaf electrical conductance; LAI—leaf area index; RWC—relative water content; SPAD—chlorophyll index.

chlorophyll content, antioxidant activities, leaf gas exchange parameters, osmolytes, and hormone accumulation, resulting in a substantial improvement in plant growth and grain yield [7]. Zn ions are also known to be powerful inhibitors of enzymes generating oxygen radicals and protect salt-stressed plants from damaging attacks of these compounds [8].

In recent years, a considerable improvement in salinity tolerance has been achieved in some crop species by nanotechnology [9]. On the other hand, the application of nano-fertilizers is one of the proper ways for increasing resource use efficiency and plant production while reducing environmental pollution [10]. Babaei et al. [11] reported that the application of nano Zn-iron oxide increased nearly 17.40% from the grain yield in comparison with the lack of its application at the highest salinity level. Hence, Zn deficiency is frequently a major limiting factor for crops production worldwide, especially under salinity stress and high pH values. Shemi et al. [12] demonstrated that the application of Zn increased the grain yield of maize by increasing the RWC, stomatal conductance, chlorophyll content, and leaf area index (LAI), along with improving the photosynthetic processes of the plant. Sattar et al. [13] also reported that the foliar application of Zn improved the grain yield of wheat by improving RWC and stomatal conductance. Hence, a better understanding of wheat physiological responses under salinity may help in programs whose objective is to improve the grain yield under salinity stress. Therefore, this study sought to evaluate the effects of the foliar and soil application of Zn on grain filling components, yield, and some physiological traits of wheat under salinity stress.

MATERIALS AND METHODS

Experimental design, plant material and growth conditions. A factorial experiment was performed according to randomized complete block design with three replications under greenhouse conditions from 2020 to 2021. Experimental factors encompassed salinity at four levels [no salinity (S_0) as control, salinity 30 (S_1), 60 (S_2), and 90 (S_3) mM NaCl (equivalent to 1.02, 2.76, 5.53, and 8.3 dS/m, respectively) and Zn application methods at four levels [no application as control (Z_1), soil application Zn as zinc sulfate ($ZnSO_4$) (Z_2), foliar application nano ZnO (Z_3), combined soil, and foliar application of Zn (Z_4)]. The soil application of Zn and foliar application of nano ZnO were 120 kg/ha as $ZnSO_4$ at the planting stage and 1 g/L respectively. Saline irrigation water with three salinity levels (except for control) was prepared by adding sodium chloride to irrigation water to achieve the target salinity level. The temperature of the greenhouse varied between 20 and 30°C during the day and 18 to 21°C at night, and its maximum and minimum relative humidity levels were 60 and 67%, respectively. The soil type was silty with a pH rate of about 7.8. The wheat cultivar "Zagross" was used in the experiment. Its optimum density was 350 seeds/m^2. Thus, 31 seeds were sown in every pot with a 2 cm depth and 40 cm diameter, filled almost with 18 kg soil. Foliar application with nano ZnO was performed in two stages of the growth period, namely (BBCH 13) and (BBCH 41–43). Nano ZnO was produced in China and prepared by Jahan Kimia Company (Iran). Nano ZnO had an average particle size of less than 30 nm and a special particle surface of more than 30 m^2/g. Nano ZnO powder was added to deionized water and placed on ultra-sonic equipment (100 W and 40 kHz) on a shaker for a better solution [14]. To ensure the penetration of nano ZnO into the plant organ, the scanning electron microscopy images of the flag leaf of wheat under the foliar application of nano ZnO at different levels of salinity stress were obtained (Fig. 1) using a Leo-1430 VP Scanning Electron Microscope (Carl Zeiss AG, Germany). Details of the region are presented as a yellow circle, showing the presence of nanoparticles in flag leaf samples.

Relative water content. At the mid of the booting stage (BBCH 43), the flag leaves of the plants were selected to measure the chlorophyll index, stomata conductance, RWC, quantum yield and leaf EC. The RWC of the flag leaves was calculated based on the following formula [15]:

$$\mathrm{RWC}(\%) = [(\mathrm{FW} - \mathrm{DW})/(\mathrm{TW} - \mathrm{DW})] \times 100, \quad (1)$$

where FW—fresh weight, DW—dry weight, and TW—turgid weight.

Leaf electrical conductance. The EC of the flag leaf was determined based on the standard method of previous research [16]. From each pot, two developed flag leaves were randomly selected, and after placing them in aluminum foils, they were transferred to the laboratory very quickly. Then, the flag leaf samples were kept in flasks containing 25 mL of distilled water (with specific EC) for a period of time. It was placed at room temperature for 24 h, and then electrical conductivity was measured using an Mi180 Bench meter (Milwaukee Instruments Inc., USA). To measure the electrical conductivity of the flag leaf, it was attempted to homogenize the leaf samples.

Chlorophyll index (SPAD). The chlorophyll index of the flag leaves was calculated based on the method of a previous study [17]. A device chlorophyll meter SPAD-502 (Konica Minolta Sensing Inc., Japan) was used, and in each plant, measurements were taken at three spots on each leaf, two on each side of the midrib on the flag leaf.

Stomatal conductance. Stomata conductance was measured with an AP4 porometer system (Delta-T Devices Ltd., UK) according to the manual instructions. It was computed on the flag leaves of four different plants from each treatment.

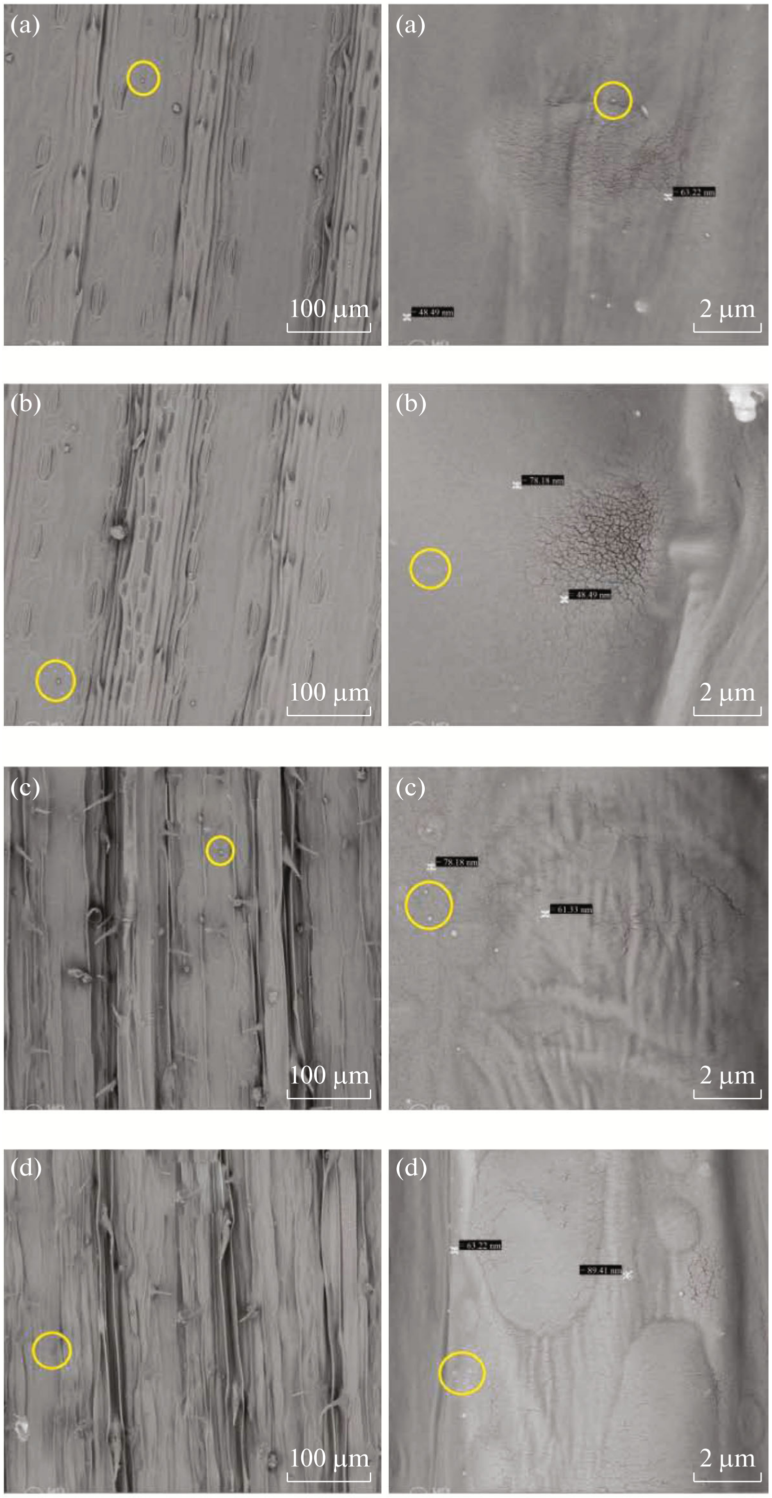

Fig. 1. SEM micrograph of ZnO nanoparticles. (a) Without nano ZnO (S_0) as a control, (b) nano ZnO under salinity 30 (S_1), (c) 60 (S_2), and (d) 90 (S_3) mM NaCl conditions. SEM—scanning electron microscopy. ZnO—Zinc oxide. Nanoparticles are marked with yellow circles.

Quantum yield of PSII electron transport (ØPSII). This trait was determined on the flag leaves by the uppermost fool expanded leaf using a chlorophyll fluorometer OS30P+ (Opti Sciences Inc., USA). For this purpose, the plants were adapted to darkness for 20 min by using one special clamp, and then the fluorescence amounts were measured in 1000 μM photon/m^2 s using the following formula:

$$\varnothing \mathrm{PSII} = (F_\mathrm{m} - F_0)/F_\mathrm{m}, \tag{2}$$

where F_m—is the maximum fluorescence after a saturated light pulse on plants adapted to darkness, and F_0—represents the minimal fluorescence in the adapted light, which was determined by illumination with FR.

Leaf area index. This trait was measured at the heading stage using a leaf area meter LI-3000C (LI-COR Biosciences, USA).

Rate and grain filling period assay. Two plants in each pot were taken for studying grain filling parameters in each sampling. The first sampling was taken on day 8 after heading, and the other samplings steps were taken in 4-day intervals to determine the accumulation of grain weight. At each sampling, the grains were manually removed from spikes and dried at 75°C for 48 h. The total duration of grain filling for each treatment combination was calculated through fitting a bilinear model [18]:

$$\mathrm{GW} = \begin{cases} \mathrm{a} + \mathrm{gfr}(\mathrm{daa}), \ldots \ldots \mathrm{if} \ldots \mathrm{daa} < P_\mathrm{m} \\ \mathrm{a} + \mathrm{gfr}(P_\mathrm{m}), \ldots \ldots \mathrm{if} \ldots \mathrm{daa} \geq P_\mathrm{m} \end{cases}, \tag{3}$$

where GW is the grain dry weight, a—the GW-intercept, gfr—the slope of grain weight indicating the grain filling rate, daa—the days after earring, and pm—physiological maturity.

Borrás et al. [19] determined grain filling using a bilinear model. The effective grain filling period (EGFD) was estimated from the following equation:

$$\mathrm{EGFD} = \mathrm{HGW}/\mathrm{RGF}, \tag{4}$$

where HGW and RGF are highest grain weight (g) and ratio of grain filling (g/day), respectively.

Conversely, an increase in kernel weight in the filling period was calculated using the above-cited equation in statistical software SAS 9.2 via Proc NLIN and the DUD method.

Remobilization of stem reserves to grain yield assay. The dry matter and remobilization of stem reserves to grain yield were evaluated as follows [20]:

$$\begin{aligned} &\text{Dry matter remobilization to grain}(\text{g/plant}) = [\text{Maximum shoot dry matter after anthesis}(\text{g/plant})] \\ &\quad - [\text{Shoot dry matter}(\text{except grains}) \text{ in maturity}(\text{g/plant})], \end{aligned} \tag{5}$$

$$\text{Dry matter contribution of assimilates to grain}(\%) = (\text{Remobilization/Grain yield}) \times 100, \tag{6}$$

$$\begin{aligned} &\text{Stem reserves remobilization to grain yield}(\text{g/plant}) \\ &= [\text{Maximum stem dry matter after anthesis}(\text{g/plant})] - [\text{Stem dry matter in maturity}(\text{g/plant})], \end{aligned} \tag{7}$$

$$\text{Stem reserve contribution to grain yield}(\%) = (\text{Stem dry matter remobilization/Grain yield}) \times 100. \tag{8}$$

Overall, five plants of each pot were randomly harvested to measure grain yield per plant.

Statistical analysis. The analysis of variance and mean comparisons were performed using the SAS computer software package. The main effects and interactions were tested using the least significant difference test at the $P < 0.05$ probability level.

RESULTS

Considering that there were no significant differences between the two studied years (2020–2021), the averaged data from the two years were used for statistical analyses. The results indicated that the effect of Zn × salinity stress was significant on all traits such as RWC, chlorophyll index, stomata conductance, EC, quantum yield, LAI, maximum grain weight, and grain filling rate. The other affected parameters were effective grain filling period, grain filling period, dry matter remobilization from the stem, the contribution of remobilization from shoots to grain, stem reserve contribution in grain yield, and grain yield (Table 1).

Relative Water Content and Leaf Electrical Conductance

RWC decreased under salinity stress. The application of salinity as S_4 decreased RWC by 18.7, 31.4, and 55.88% compared to its application as S_3, S_2, and S_1, respectively (Fig. 2). RWC was 77.67% in combined soil and foliar application of Zn and decreased to 64.65% in control (Fig. 2). On the other hand, there was a decrease of about 20% in the application of Zn_1 compared to Zn_4. The interaction effect between salinity and Zn application demonstrated that the highest RWC (92.06%) was obtained in no salinity with Zn application as Zn_4 while the lowest RWC (51.09%) was obtained at Zn_1S_4 (Table 2). Moreover, there was a decrease of about 80% RWC in the highest salinity level and no application of Zn (S_4Zn_1) in comparison with (S_1Zn_4) (Table 2).

Based on the results, the use of salinity as S_4 increased EC by 153.8, 74.4, and 37% compared to its application as S_1, S_2, and S_3, respectively (Fig. 2). Additionally, the application of Zn as Zn_1 increased

Table 1. Variance analysis of the effects of salinity and zinc on some agro-physiological traits of wheat (mean of two years, 2020 and 2021)

Source of variation	df	Mean square														
		RWC	EC	SPAD	SC	F_v/F_m	LAI	MGW	GFR	EGFP	GFP	DMRG	DMRS	CRSG	SRCG	GY
Replication	2	134*	83^{ns}	24.1^{ns}	108**	0.003^{ns}	0.49**	0.00086**	1.3×10^{-6}**	433.2**	528.2**	0.039**	0.019**	390**	215**	0.43**
Salinity (S)	3	1043**	15515**	359.6**	29**	0.053**	0.15**	0.0004**	2.3×10^{-7}**	13.2**	12.8**	0.013**	0.0033**	450**	164**	0.043**
Zinc (Zn)	3	246**	3099**	95**	51**	0.013**	0.56**	0.00009**	2.9×10^{-8}**	16.6**	20.9**	0.0069**	0.0034**	199**	43**	0.044**
S × Zn	9	130**	701**	39**	5*	0.0044**	0.04*	0.00002**	2×10^{-8}**	8.3**	8.1**	0.0001^{ns}	0.0006**	23**	22**	0.012*
Error	30	40	169	10.2	2	0.0013	0.016	0.000007	6.4×10^{-9}	2.5	2.3	0.0004	0.00019	6	5	0.0055
C.V (%)	–	8.55	12.2	7.02	6.23	5.21	5.73	7.78	5.69	6.1	5.1	9.4	8.51	10.4	12.85	8.03

NS, * and ** show no significant and significant differences at $P < 0.05$ and $P < 0.01$, respectively. RWC—relative water content; EC—electrical conductivity; SPAD—chlorophyll index; SC—stomatal conductance; LAI—leaf area index; MGW—maximum of grain weight; GFR—grain filling rate; EGFP—effective grain filling period; GFP—grain filling period; DMRG—dry matter remobilization to grain; DMRS—dry matter remobilization from stem; CRSG—contribution of remobilization from shoots to grain; SRCG—stem reserve contribution in grain yield; GY—grain yield.

Table 2. Means comparison of the effects of salinity and zinc on some physiological traits of wheat (mean of two years, 2020 and 2021)

Treatment compound	RWC, %	EC, µS/cm	SPAD	SC, mmol $H_2/(m^2 s)$	F_v/F_m	LAI
$S_1 \times Zn_1$	80.66 ± 0.6	81.5 ± 2.15	47.79 ± 0.56	24.5 ± 2.88	0.753 ± 0.0076	2.22 ± 0.15
$S_1 \times Zn_2$	86.01 ± 1.83	65.9 ± 1.01	52.16 ± 0.75	22.53 ± 4.6	0.804 ± 0.004	2.14 ± 0.28
$S_1 \times Zn_3$	89.7 ± 0.71	58.63 ± 1.54	53.7 ± 0.61	27.16 ± 2.6	0.812 ± 0.0025	2.55 ± 0.21
$S_1 \times Zn_4$	92.06 ± 1.71	52.66 ± 2.38	55.86 ± 0.47	28.46 ± 2.98	0.831 ± 0.006	2.6 ± 0.21
$S_2 \times Zn_1$	65.73 ± 1.55	122.8 ± 2.49	43.17 ± 0.43	21.5 ± 2.62	0.68 ± 0.0065	1.94 ± 0.16
$S_2 \times Zn_2$	70.54 ± 0.46	92.18 ± 1.95	46.51 ± 0.5	22.3 ± 2.38	0.718 ± 0.003	2.15 ± 0.17
$S_2 \times Zn_3$	75.7 ± 1.25	86.91 ± 1.73	50.3 ± 0.3	24.96 ± 2.84	0.732 ± 0.004	2.19 ± 0.22
$S_2 \times Zn_4$	81.9 ± 1.69	74.51 ± 2.78	50.93 ± 0.25	26.16 ± 2.77	0.785 ± 0.004	2.5 ± 0.23
$S_3 \times Zn_1$	61.11 ± 1.29	137.67 ± 2.8	36.31 ± 0.31	19.96 ± 2.21	0.62 ± 0.0037	1.85 ± 0.09
$S_3 \times Zn_2$	63.5 ± 0.7	128.76 ± 1.75	38.9 ± 0.74	22.56 ± 2.54	0.632 ± 0.002	2.3 ± 0.39
$S_3 \times Zn_3$	68.23 ± 1.22	105.25 ± 6.56	40.48 ± 0.47	23.23 ± 2.56	0.662 ± 0.005	2.26 ± 0.16
$S_3 \times Zn_4$	72.56 ± 0.4	105.3 ± 8.79	45.81 ± 0.27	25.7 ± 2.78	0.694 ± 0.003	2.36 ± 0.19
$S_4 \times Zn_1$	51.09 ± 1.98	180.1 ± 2.06	32.89 ± 0.84	18.93 ± 2.05	0.57 ± 0.004	1.77 ± 0.06
$S_4 \times Zn_2$	52.75 ± 0.66	169.27 ± 2.81	35.17 ± 0.28	22.4 ± 3.2	0.592 ± 0.0052	2.02 ± 0.17
$S_4 \times Zn_3$	55.52 ± 1.33	158.16 ± 2.25	36.44 ± 0.5	24.73 ± 3.85	0.605 ± 0.0055	2.32 ± 0.28
$S_4 \times Zn_4$	64.16 ± 2.12	149.15 ± 3.35	38.2 ± 0.22	22.03 ± 2.56	0.649 ± 0.0025	2.32 ± 0.18
LSD	2.08	5.745	0.828	2.449	0.096	0.212

LSD – least significant difference. S_1, S_2, S_3, and S_4 are no salinity, while 30, 60, and 90 mM represent salinity. Zn_1, Zn_2, Zn_3, and Zn_4 are no Zn as control, soil application Zn as $ZnSO_4$, foliar application nano ZnO and combined soil, and foliar application of Zn. RWC—relative water content; EC—electrical conductivity; SPAD—chlorophyll index; SC—stomatal conductance; LAI—leaf area index.

EC by 36.8, 19.5, and 7.1% compared to its application as Zn_4, Zn_3 and Zn_2, respectively (Fig. 2). However, EC content significantly decreased when applying Zn was. The highest (180.1 µS/cm) and lowest (52.66 µS/cm) levels of EC were obtained in S_4Zn_1 and S_1Zn_4, respectively (Table 2). There was a decrease of about 242% in the EC content in S_4Zn_1 in comparison with S_1Zn_4 (Table 2).

Chlorophyll Index and Stomata Conductance

Chlorophyll index and stomata conductance were significantly affected by salinity stress, in other words, salinity decreased these two parameters (Fig. 2). The lowest chlorophyll index (35.67) was observed at 90 mM salinity. Salinity as S_4 decreased the chlorophyll index by 13.17, 33.8, and 46.84%, respectively, in compari-

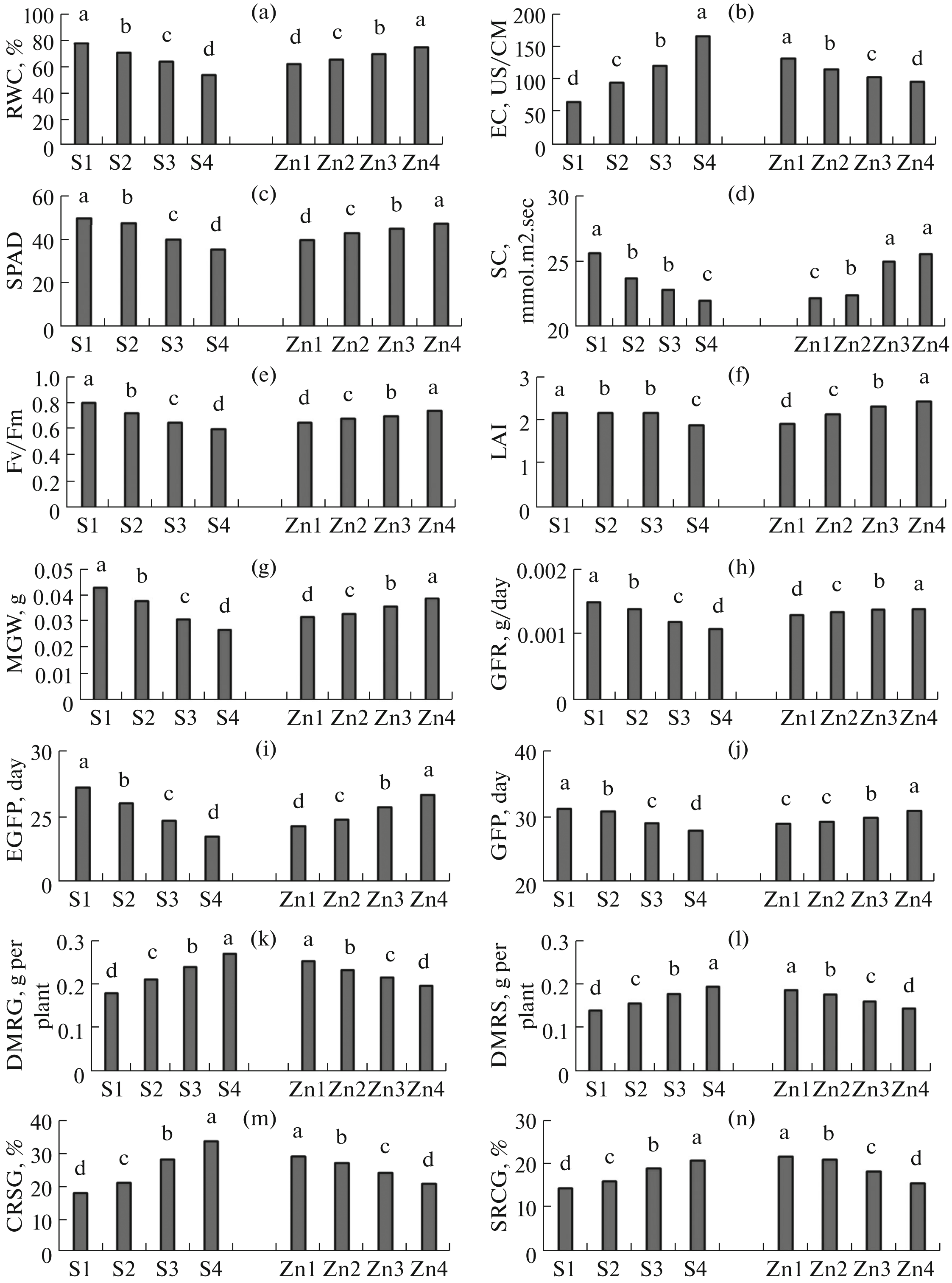

Fig. 2. Means comparison of the effects of salinity and zinc on some agro-physiological traits of wheat (mean of two years, 2020 and 2021). (a) Relative water content; (b) electrical conductivity; (c) chlorophyll index; (d) stomata conductance; (e) quantum yield; (f) leaf area index; (g) maximum of grain weight; (h) grain filling rate; (i) effective grain filling period; (g) grain filling period; (k) dry matter remobilization to grain; (l) dry matter remobilization from stem; (m) contribution of remobilization from shoots to grain; (n) stem reserve contribution in grain yield. ZnO—Zinc oxide.

son with S_3, S_2, and no salinity (S_1) (Fig. 2). The application of Zn increased the chlorophyll index under salinity stress (Fig. 2). Of course, the combined soil and foliar application of Zn (Zn_4) increased this index by 5.25, 10.46, and 19.13% in comparison with the application of Zn as Zn_3, Zn_2, and Zn_1 (Fig. 2). Conversely, stomatal conductance decreased with increasing salinity levels (Fig. 2) so that salinity 90 mM (S_4) had the lowest value of stomatal conductance (22.02 mmol H_20/m^2 s). However, the application of Zn as Zn_4 increased stomatal conductance by about 2.27, 13.98, and 15.21% in comparison with Zn_3, Zn_2, and Zn_1 (Fig. 2). The interaction effect between salinity and Zn represented that salinity 90 mM decreased chlorophyll index and stomata conductance. The highest chlorophyll content (55.86) and stomata conductance (28.46 mmol H_20/m^2 s) were observed as S_1Zn_4 while the minimum levels of these values (32.89 and 18.93 mmol H_20/m^2 s respectively) were found as S_4Zn_1 (Table 2).

Quantum Yield and Leaf Area Index

Quantum yield and LAI decreased significantly under salinity stress (Fig. 2). The highest salinity level (S_4) had the lowest value of quantum yield (0.6) so that 90 mM salinity decreased the quantum yield by 33.33% in comparison with no salinity condition (Fig. 2). Contrarily, the application of Zn increased the quantum yield (Fig. 2) so that the application of Zn as Zn_4 increased the quantum yield by 5.71, 8.82, and 13.84% in comparison with Zn_3, Zn_2, and Zn_1 (Fig. 2). LAI was progressively decreased with increasing salinity levels (Fig. 2). No salinity (S_1) increased LAI about 25.26% in comparison with S_4. Compared with the non Zn treatment (Zn_1), the Zn treatment Zn_2, Zn_3, and Zn_4 decreased LAI by 4.72, 13.47, and 25.77% respectively (Table 2).

Rate and Grain Filling Period

The results revealed that the highest grain filling rate was obtained under no salinity and application of Zn as Zn_4 (Fig. 3, Tables 3 and 4). The highest level of salinity stress (90 mM) had the lowest grain filling components such as maximum grain weight (0.027 g), grain filling rate (0.0011 g/day), grain filling period (27.84 days), and effective grain filling period (23.52 days). On the other hand, salinity as S_4 caused a decrease of 59.25, 36.36, 12.21, and 16.02%, respectively, in the maximum grain weight, grain filling rate, grain filling period, and effective grain filling period in comparison with no salinity (S_1). The grain filling component was increased in response to Zn usage (Fig. 2). The application of Zn as Zn_4 under the highest salinity level (Zn_4S_4) increased the maximum grain weight, grain filling rate, effective grain filling period, and grain filling period by approximately 25.1, 9.4, 14.3, and 10.5%, respectively, compared to no Zn application under the same salinity level (Zn_1S_4) (Table 4).

Dry Matter and Stem Reserves Mobilization to Grain Yield

The results showed that the highest salinity level (S_4) increased dry matter remobilization from stem and shoots yield (38 and 50%, respectively) and the contribution of these processes to grain yield (43 and 70%, respectively) in comparison with no salinity. However, the Zn application demonstrated senescence and retarded such mobilization. The highest dry matter remobilization from the stem (0.205 g/plant), stem reserve contribution in the grain yield (27.15%), and the contribution of remobilization from shoots to grain (38.27%) was observed in S_4Zn_1 (Table 5). Contrarily, the lowest level of the mentioned traits was found in S_1Zn_4 (Table 5). On the other hand, there was an increase of about 71, 25, and 55%, respectively, in the contribution of remobilization from shoots to grain, dry matter remobilization from the stem, and stem reserve contribution in the grain yield at the 90 mM salinity level and Zn treated plants as Zn_4 (S_4Zn_4) in comparison with S_1Zn_1 (Table 5).

Grain Yield

Grain yield was significantly affected by salinity stress levels (Fig. 2) and decreased under salinity stress (Fig. 2) so that salinity 90 mM (S4) had the lowest value of grain yield (0.796 g/plant). Salinity as S_4 decreased 6.15, 22.73, and 23.36%, respectively, of the grain yield in comparison with S_3, S_2, and no salinity (S_1) (Fig. 2). The application of Zn increased the grain yield under salinity stress (Fig. 2). Of course, the combined soil and foliar application of Zn (Zn_4) could increase the grain yield by 4.54, 6.42, and 8.88% in comparison with the application of Zn as Zn_3, Zn_2, and Zn_1 (Fig. 2). The highest grain yield (1.016 g/plant) was found as S_1Zn_4, whereas its minimum value (0.755 g/plant) was observed as S_4Zn_1 (Table 5).

DISCUSSION

Relative Water Content and Leaf Electrical Conductance

RWC decreased significantly under salinity stress (Fig. 2). These results may be attributed to the accumulation of toxic ions such as Na^+ and Cl^-, reducing leaf expansion, LAI and stomata conductance (Table 2) and apparently decreasing stomata conductance or stomata closure, leading to a reduction in intracellular CO_2 partial pressure [21]. Chattha et al. [7] reported that salinity reduces the ability of plants to take up water and decrease in leaf RWC. Further, it could be related to low water availability under stress conditions or to root systems, which are unable to compensate for

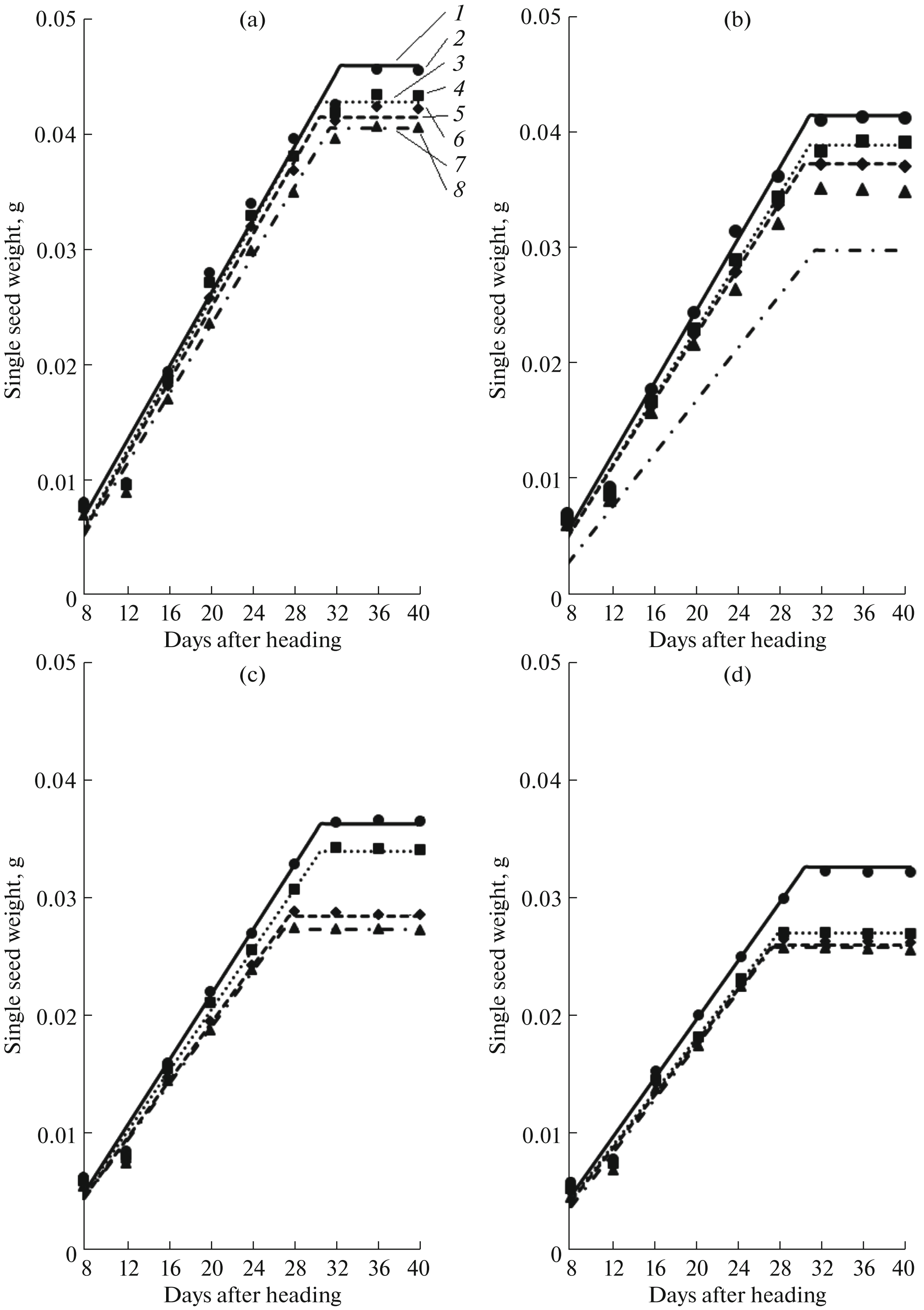

Fig. 3. Effect of Zinc and salinity stress on grain filling of wheat (mean of two years, 2020 and 2021). (a) No salinity, (b) salinity 30 mM NaCl, (c) salinity 60 mM NaCl and (d) salinity 90 mM NaCl. (*1*) Estimated (application $ZnSO_4$ and nano ZnO); (*2*) observed (application $ZnSO_4$ and nano ZnO); (*3*) estimated (application nano ZnO); (*4*) observed (application nano ZnO); (*5*) estimated (application $ZnSO_4$); (*6*) observed (application $ZnSO_4$); (*7*) estimated (no application Zn); (*8*) observed (no application Zn). ZnO—Zinc oxide.

Table 3. Means comparison of the effects of salinity and zinc on grain filling of wheat (mean of two years, 2020 and 2021)

Treatment compound	Sampling stages (days after heading)								
	8	12	16	20±	24	28	32	36	40
$S_1 \times Zn_1$	0.0069c ± 0.0014	0.00897c ± 0.0018	0.0170d ± 0.0035	0.0236cd ± 0.0048	0.0299d ± 0.0061	0.0348de ± 0.0071	0.0395bc ± 0.0081	0.0406cd ± 0.0083	0.0405cd ± 0.0083
$S_1 \times Zn_2$	0.00747b ± 0.0015	0.00946b ± 0.0019	0.0182c ± 0.0037	0.0258b ± 0.0053	0.03199bc ± 0.0065	0.0368bc ± 0.0075	0.041ab ± 0.0084	0.0423bc ± 0.0087	0.0421bc ± 0.0086
$S_1 \times Zn_3$	0.00777b ± 0.0015	0.00966ab ± 0.0019	0.0188b ± 0.0038	0.0271a ± 0.0055	0.0328ab ± 0.0067	0.038b ± 0.0078	0.0418a ± 0.0086	0.0433ab ± 0.0089	0.0432ab ± 0.0088
$S_1 \times Zn_4$	0.0817a ± 0.0016	0.00986a ± 0.002	0.0194a ± 0.0039	0.028a ± 0.0057	0.0339a ± 0.0069	0.0395a ± 0.0081	0.0425a ± 0.0087	0.0455a ± 0.0093	0.0454a ± 0.0093
$S_2 \times Zn_1$	0.00607ef ± 0.0012	0.00817ef ± 0.0016	0.0157gh ± 0.0032	0.0216fg ± 0.0044	0.0263gh ± 0.0054	0.03199gh ± 0.0065	0.0349ef ± 0.0071	0.0348fg ± 0.0071	0.0347fg ± 0.0071
$S_2 \times Zn_2$	0.00647d ± 0.0013	0.00867cd ± 0.0017	0.0163ef ± 0.0033	0.0224ef ± 0.0046	0.0278ef ± 0.0057	0.0335ef ± 0.0069	0.037d ± 0.0076	0.037ef ± 0.0076	0.0368ef ± 0.0075
$S_2 \times Zn_3$	0.00657d ± 0.0013	0.00867cd ± 0.0017	0.0167de ± 0.0034	0.0229de ± 0.0047	0.0289de ± 0.0059	0.0342ef ± 0.007	0.0382cd ± 0.0078	0.039de ± 0.008	0.0389de ± 0.008
$S_2 \times Zn_4$	0.00707c ± 0.0014	0.00936b ± 0.0019	0.0177c ± 0.0036	0.0243c ± 0.005	0.03129c ± 0.0064	0.036cd ± 0.0074	0.0408ab ± 0.0084	0.0411bcd ± 0.0084	0.041bcd ± 0.0084
$S_3 \times Zn_1$	0.00548hi ± 0.0011	0.00747hij ± 0.0015	0.0144j ± 0.0029	0.0187jk ± 0.0036	0.0239jkl ± 0.0049	0.0274kl ± 0.0056	0.0273hi ± 0.0056	0.0273ij ± 0.0056	0.0272ij ± 0.0055
$S_3 \times Zn_2$	0.00568gh ± 0.0011	0.00757hi ± 0.0015	0.01475ij ± 0.003	0.0195hij ± 0.004	0.0242jk ± 0.0049	0.0288jk ± 0.0059	0.0287h ± 0.0059	0.0286i ± 0.0058	0.0286i ± 0.0058
$S_3 \times Zn_3$	0.00598efg ± 0.0012	0.00807fg ± 0.0016	0.01544gh ± 0.0031	0.0211gh ± 0.0043	0.0256hi ± 0.0052	0.0306hi ± 0.0063	0.0342f ± 0.007	0.0341gh ± 0.007	0.034gh ± 0.007
$S_3 \times Zn_4$	0.00627de ± 0.0012	0.00847de ± 0.0017	0.0159fg ± 0.0032	0.022efg ± 0.0045	0.027fg ± 0.0055	0.0328fg ± 0.0067	0.0363de ± 0.0074	0.0365f ± 0.0075	0.0364f ± 0.0075
$S_4 \times Zn_1$	0.00448k ± 0.0009	0.00687k ± 0.0014	0.0137k ± 0.0028	0.0173l ± 0.0035	0.0224m ± 0.0046	0.0257m ± 0.0052	0.0257i ± 0.0052	0.0256j ± 0.0052	0.0255j ± 0.0052
$S_4 \times Zn_2$	0.00498j ± 0.001	0.00717jk ± 0.0014	0.0142jk ± 0.0029	0.0178kl ± 0.0036	0.0226lm ± 0.0046	0.0264lm ± 0.0054	0.0262i ± 0.0053	0.0262j ± 0.0053	0.0261j ± 0.0053
$S_4 \times Zn_3$	0.00528ij ± 0.001	0.00737ij ± 0.0015	0.0144j ± 0.0029	0.0181kl ± 0.0037	0.023klm ± 0.0047	0.027lm ± 0.0055	0.027hi ± 0.0055	0.0269ij ± 0.0055	0.0269ij ± 0.0055
$S_4 \times Zn_4$	0.00578fgh ± 0.0011	0.00777gh ± 0.0015	0.0152hi ± 0.0031	0.02003hi ± 0.0041	0.0249ij ± 0.0051	0.0299ij ± 0.0061	0.0321g ± 0.0066	0.032h ± 0.0066	0.032h ± 0.0066
LSD	0.0004	0.0003	0.0006	0.0011	0.0013	0.0015	0.0021	0.0023	0.0023

LSD – least significant difference. S_1, S_2, S_3, and S_4 are no salinity, while 30, 60, and 90 mM represent salinity. Zn_1, Zn_2, Zn_3, and Zn_4 are no Zn as control, soil application Zn as $ZnSO_4$, foliar application nano ZnO and combined soil, and foliar application of Zn. Means with similar letters in each column are not significantly different based on LSD test.

Table 4. Means comparison of the effects of salinity and zinc on some agro-physiological traits of wheat (mean of two years, 2020 and 2021)

Treatment compound	MGW, g	GFR, g/day	EGFP, day	GFP, day
$S_1 \times Zn_1$	0.0408 ± 0.0081	0.0015 ± 0.0003	27.2 ± 5.44	31.42 ± 6.28
$S_1 \times Zn_2$	0.042 ± 0.0085	0.00159 ± 0.00031	26.72 ± 5.34	30.73 ± 6.14
$S_1 \times Zn_3$	0.0435 ± 0.0087	0.00164 ± 0.00032	26.52 ± 5.3	30.46 ± 6.09
$S_1 \times Zn_4$	0.0457 ± 0.0091	0.00159 ± 0.00031	28.74 ± 5.74	32.36 ± 6.47
$S_2 \times Zn_1$	0.0351 ± 0.007	0.0014 ± 0.00028	25.07 ± 5.01	31.51 ± 6.3
$S_2 \times Zn_2$	0.0372 ± 0.0074	0.00143 ± 0.00028	26.01 ± 5.2	30.21 ± 6.04
$S_2 \times Zn_3$	0.0392 ± 0.0078	0.00147 ± 0.00029	26.66 ± 5.33	31 ± 6.2
$S_2 \times Zn_4$	0.0413 ± 0.0082	0.00156 ± 0.00031	26.47 ± 5.29	30.91 ± 6.18
$S_3 \times Zn_1$	0.0275 ± 0.0055	0.00121 ± 0.00024	22.72 ± 4.54	27.04 ± 5.4
$S_3 \times Zn_2$	0.0289 ± 0.0057	0.00123 ± 0.00024	23.49 ± 4.69	27.68 ± 5.53
$S_3 \times Zn_3$	0.0344 ± 0.0068	0.0013 ± 0.00026	26.46 ± 5.29	30.59 ± 6.11
$S_3 \times Zn_4$	0.0367 ± 0.0073	0.0014 ± 0.00028	26.21 ± 5.24	30.72 ± 6.14
$S_4 \times Zn_1$	0.0258 ± 0.0051	0.00116 ± 0.00023	22.24 ± 4.44	26.92 ± 5.38
$S_4 \times Zn_2$	0.0265 ± 0.0053	0.00115 ± 0.00023	23.04 ± 4.6	27.19 ± 5.43
$S_4 \times Zn_3$	0.0271 ± 0.0054	0.00116 ± 0.00023	23.36 ± 4.67	27.5 ± 5.5
$S_4 \times Zn_4$	0.0323± 0.0064	0.00127 ± 0.00025	25.43 ± 5.08	29.75 ± 5.95
LSD	0.0022	0.000056	0.62	0.61

LSD – least significant difference. S_1, S_2, S_3, and S_4 are no salinity, while 30, 60, and 90 mM represent salinity. Zn_1, Zn_2, Zn_3, and Zn_4 are no Zn as control, soil application Zn as $ZnSO_4$, foliar application nano ZnO and combined soil, and foliar application of Zn. MGW—maximum of grain weight; GFR—grain filling rate; EGFP—effective grain filling period; GFP—grain filling period.

water lost by transpiration through a reduction of the absorbing surface [22]. They also concluded that the amelioration role of Zn in RWC maintenance might be attributed to the improvement of vascular tissue. Similarly, other researchers indicated that nano ZnO application decreased the leaf EC while increasing the leaf RWC of triticale [23]. Part of the increase in the RWC due to the application of Zn can be due to a decrease in EC (Table 2). Al-Zahrani et al. [24] also attributed the increase in RWC due to the application of Zn in salt stress conditions, to the reduction of electrolyte leakage.

Khalilzadeh et al. [5] found that under salinity stress, plant membranes are subject to changes often associated with increases in permeability and loss of integrity, but Zn application under such conditions decreased EC [23]. They demonstrated that the Zn application improved cell membrane stability in the triticale plant as a consequence of enhancing nutrient uptake, extension of the root system, and water status of the plants [23]. Al-Zahrani et al. [24] reported that salinity induced secondary oxidative stress by producing a raised level of ROS, damaged the membrane, and increased electrolyte leakage. The application of Zn increased the maintenance of plasma membrane integrity and thus controlled the toxic ions uptake under salinity stress [8].

Chlorophyll Index and Stomata Conductance

Altuntaş et al. [25] found that the reduction in the chlorophyll content under salinity stress was due to nutrient deficiency, diminished chlorophyll biosynthesis, and enhanced activity of chlorophyll destroying an enzyme named chlorophyllase enzyme. In saline conditions, reducing the RWC by disrupting the transpiration rate and photosynthetic processes causes a decrease in stomatal conductance [7]. It seems that the soil and foliar application of Zn by improving RWC and F_v/F_m (Table 2) has increased the chlorophyll index and stomatal conductance (Table 2). These results are in line with the findings of previous research [13], representing that the application of Zn by improving the RWC increases the chlorophyll

Table 5. Means comparison of the effects of salinity and zinc on dry matter remobilization and grain yield of wheat (mean of two years, 2020 and 2021)

Treatment compound	CRSG, %	DMRS, g/plant	SRCG, %	GY, g/plant
$S_1 \times Zn_1$	22.38 ± 4.47	0.164 ± 0.032	17.48 ± 3.49	0.938 ± 0.187
$S_1 \times Zn_2$	19.1 ± 3.82	0.148 ± 0.029	15.11 ± 3.02	0.979 ± 0.195
$S_1 \times Zn_3$	16.13 ± 3.22	0.13 ± 0.026	13.02 ± 2.6	0.998 ± 0.199
$S_1 \times Zn_4$	15.25 ± 3.05	0.127 ± 0.025	12.5 ± 2.5	1.016 ± 0.203
$S_2 \times Zn_1$	22.59 ± 4.51	0.184 ± 0.036	17.32 ± 3.46	1.062 ± 0.212
$S_2 \times Zn_2$	23.24 ± 4.64	0.165 ± 0.033	18.09 ± 3.61	0.912 ± 0.182
$S_2 \times Zn_3$	20.96 ± 4.19	0.147 ± 0.029	15.4 ± 3.08	0.954 ± 0.19
$S_2 \times Zn_4$	18.75 ± 3.75	0.133 ± 0.026	13.55 ± 2.71	0.981 ± 0.196
$S_3 \times Zn_1$	33.75 ± 6.75	0.199 ± 0.039	25.06 ± 5.01	0.794 ± 0.158
$S_3 \times Zn_2$	29.86 ± 5.97	0.198 ± 0.039	24.23 ± 4.84	0.817 ± 0.163
$S_3 \times Zn_3$	26.69 ± 5.33	0.178 ± 0.035	20.13 ± 4.02	0.884 ± 0.176
$S_3 \times Zn_4$	23.19 ± 4.63	0.141 ± 0.028	15.87 ± 3.17	0.888 ± 0.177
$S_4 \times Zn_1$	38.27 ± 7.65	0.205 ± 0.041	27.15 ± 5.43	0.755 ± 0.151
$S_4 \times Zn_2$	37.36 ± 7.47	0.202 ± 0.04	26.57 ± 5.31	0.76 ± 0.152
$S_4 \times Zn_3$	33.71 ± 6.74	0.193 ± 0.038	24.83 ± 4.96	0.777 ± 0.155
$S_4 \times Zn_4$	26.65 ± 5.33	0.184 ± 0.036	20.6 ± 4.12	0.893 ± 0.178
LSD	2.42	0.0093	1.671	0.029

LSD—least significant difference. S_1, S_2, S_3, and S_4 are no salinity, while 30, 60, and 90 mM represent salinity. Zn_1, Zn_2, Zn_3, and Zn_4 are no Zn as control, soil application Zn as $ZnSO_4$, foliar application nano ZnO and combined soil, and foliar application of Zn. DMRG—dry matter remobilization to grain; DMRS—dry matter remobilization from stem; CRSG—contribution of remobilization from shoots to grain; SRCG—stem reserve contribution in grain yield; GY—grain yield.

content and stomatal conductance of wheat. Chattha et al. [7] concluded that the application of Zn increased the leaf chlorophyll content and stomatal conductance of wheat by improving the RWC and reducing electrolyte leakage. Rasouli et al. [26] attributed that the increase in the chlorophyll content due to the application of Zn under salinity stress conditions, to the improvement of antioxidant enzyme activity, an increase in F_v/F_m, and a reduction of electrolyte leakage.

Chattha et al. [7] indicated that the main reason for the decrease in the chlorophyll content may be the degradation by ROS. Low chlorophyll content under salinity stress was reported as a result of lower chlorophyll synthesis, destroying the PSII reaction center, inhibiting carbonic anhydrase and nitrate reductase activities, creating an imbalance in the ion flux inside plants, affecting the membrane stability index, and decreasing RWC [27]. The application of nano ZnO increased the chlorophyll, highlighting the Zn impact on reducing the stress effect; furthermore, it seems that the major reason for the reduction in chlorophyll may be degradation by ROS [11]. The completion and formation of chlorophyll are finally facilitated in the presence of Zn. Of course, the Zn is not directly effective in the formation of chlorophyll but may have an impact on the concentration of food elements involved in the formation of chlorophyll or substances that are part of the chlorophyll molecule, including Fe and Mg. In fact, Zn is required for the activity of enzymes that are involved in chlorophyll biosynthesis [28]. Shemi et al. [12] demonstrated that the application of Zn increased the stomatal conductance of maize by increasing the RWC and improving the photosynthetic processes of the plant. Sattar et al. [13] also reported that the foliar application of Zn improved the photosynthetic conditions and stomatal conductance of wheat leaves by improving the antioxidant defense system and increasing the chlorophyll content and RWC.

Quantum Yield and Leaf Area Index

Probably, the higher levels of salinity (S_4) could decrease the chlorophyll index, RWC and stomatal conductance of the flag leaf which might have decreased the quantum yield (Fig. 2). In this regard, Khalilzadeh et al. [5] attributed the decrease in the quantum yield to the decline in the chlorophyll content, RWC, and stomatal conductance. Damage to PSII can lead to a change in chlorophyll fluorescence. Thus, chlorophyll fluorescence has been used as a powerful and reliable non-invasive method for assessing the changes in the function of PSII and reflecting the primary photosynthetic processes under environmental stress conditions [26]. Another common damage anticipated under stress conditions is the accumulation of excessive ROS [26], which can cause serious damage to organelles such as chloroplasts, mitochondria, and plasma membranes [29]. Photosynthetic pigments present in photosystems are believed to be damaged by stress factors, resulting in the reduced light-absorbing efficiency of both photosystems (PSI and PSII) and hence a reduced photosynthetic capacity. Babaei et al. [11] reported that the application of Zn on wheat increased the quantum yield. Rasouli et al. [26] also attributed the increase in the leaf quantum yield, due to the application of Zn under salt stress conditions, to the increase in the chlorophyll content and a decrease in electrolyte leakage.

Kherizadeh Arough et al. [30] stated that Zn treatment significantly increased LAI against the control. Moreover, it seems that the presence of high amounts of salt in the environment reduces the amount of water available for the plant and limits cell division, and causes a decrease in cell mass, thus reducing the leaf area [31]. Kheirizadeh Arough et al. [30] found that the foliar application of nano ZnO increased the LAI of triticale by modulating the effect caused by stress and increasing the supply of nutrients and photosynthetic materials. It seems that the soil and foliar applications of Zn have increased LAI by improving the chlorophyll content (Table 2). Lalarukh et al. [32] also attributed the increase in wheat LAI, due to the application of Zn under salt stress conditions, to the increase in the chlorophyll content.

A Zn-enhancement CA activity is highly beneficial for plants in order to facilitate the supply of CO_2 from the stomatal cavity to the site of CO_2 fixation [33]. Babaei et al. [11] concluded that Zn increased the quantum yield under salinity stress. Plants that grow under Zn deficiency conditions have low quantum yield performance, and damage to the components of chloroplast is irreparable [34]. Therefore, it seems that the application of Zn under salinity conditions can increase the quantum yield.

Rate and Grain Filling Period

The decrease in RWC, stomatal conductance, chlorophyll index, and quantum yield (Fig. 2) can have major effects on grain filling components (Fig. 2), which was consistent with the findings of other researchers, reporting that under salinity stress, low grain filling components of wheat are as a result of a decrease in RWC, stomatal conductance, chlorophyll content, and quantum yield [5]. Francois et al. [35] suggested that salinity reduces grain weight due to the shortage of the seed-filling period and accelerates the maturation of seeds. Munns and James [36] demonstrated that salinity stress decreases the duration of vegetative and reproductive growth of wheat genotypes, which reduces grain-filling components compared to normal conditions.

Although Zn is a micronutrient, Zn application can affect the susceptibility of plants to stress [37]. Seyed Sharifi et al. [38] indicated that the application of Zn increased grain filling components such as the effective grain filling period and grain filling rate of barley (*Hordeum vulgare* L.). Baniabbass et al. [39] represented that the reasons for the essential role of Zn in the structure of phospho-ethanol pyruvate carboxylase and its importance in the direct synthesis of growth material such as auxin cause photovoltaic power and consequently carbohydrate levels increase in the presence of Zn, leading to further production of dry matter and its storage in grain as a reservoir.

Dry Matter and Stem Reserves Mobilization to Grain Yield

The stress tolerance efficiency of cereals relies not only on the assimilation of dry matter and stem reserves but also on the effective partitioning of these reserves to grains. Khalilzadeh et al. [5] found that contributions become greater when plants are cultivated under salinity stress rather than no salinity treatment. The amount of dry matter remobilization and its contribution to grain yield are more affected by the source and sink relations and environmental conditions [40]. It seems that one rationale behind this may be improving the chlorophyll index (Fig. 2) under favorable conditions and accessibility to sufficient resources, which can increase current photosynthesis. However, accessibility to nutrient sources (e.g., Zn) is limited when the plants are under salinity conditions. In such conditions, an increase in the grain weight under the application of Zn is mainly attributed to the higher number of endosperm cells that cause reduced sink strength. Similar results have been reported by previous researchers, indicating that the application of nano ZnO decreased dry matter remobilization to grain, contributing stem reserves to grain [38].

Grain Yield

Babaei et al. [11] concluded that the application of Zn under salinity stress conditions increased the quantum yield, RWC, chlorophyll index, and grain yield. Kheirizadeh Arough et al. [23] also indicated that the application of Zn could increase chlorophyll content, quantum yield, RWC, and grain yield under stress conditions. Based on the findings of Seyed Sharifi et al. [38], the application of nano ZnO increased the grain filling components (e.g., effective grain filling period, grain filling period, and grain filling rate) of triticale. It seems that the soil and foliar application of Zn, by improving RWC, chlorophyll content, and stomatal conductance (Table 2) while reducing EC (Table 2), could increase the grain yield (Table 5). Chattha et al. [7] reported that the application of Zn under salinity stress conditions improved the rate of photosynthesis and increased grain yield of wheat by reducing electrolyte leakage while increasing quantum yield, chlorophyll content, and stomatal conductance.

The results revealed that the maximum of the shoot and stem dry matter remobilization, the contribution of stem reserves to the grain yield, and EC were observed in S_4Zn_1. Salinity severe stress as S_4 decreased RWC, stomata conductance, chlorophyll index, LAI, and quantum yield (F_v/F_m), whereas the application of Zn improved these traits under salinity stress and normal conditions. Thus, the highest levels of RWC, stomata conductance, chlorophyll index, LAI, and quantum yield were observed in S_1Zn_4, which caused a 34.56% increase in the grain yield in comparison with S_4Zn_1. Hence, it seems that the application of Zn, especially combined soil and foliar application of Zn, can be recommended for profitable wheat production under soil salt stress conditions.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving people as objects of research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Hamed Narimani and Raouf Seyed Sharifi equally contributed to this work.

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