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REGULAR ARTICLE

Effect of predicted climate change on growth and yield performance of wheat under varied nitrogen and zinc supply

Muhammad Asif · Cevza Esin Tunc · Mustafa Atilla Yazici · Yusuf Tutus · Raheela Rehman · Abdul Rehman · Levent Ozturk

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Abstract

Background and aims Sustainable crop production is crucial to address global food security and requires a solid input of chemical fertilizers containing macro (e.g. nitrogen: N) and micro (e.g. zinc: Zn) nutrients. However, climatic factors beyond farmers' management capabilities determine the final crop yields, and world's climate has been changing more rapidly than ever due to human activities such as industrialization and deforestation. This study evaluates the interactive effects of predicted climate change and N and Zn supply on performance of bread wheat as a model staple food crop.

Methods Bread wheat (*T. aestivum* cv. Ceyhan-99) was cultivated in soil fertilized with adequate or low N and Zn in pots under ambient climate (ambient CO_2 and temperature) or predicted climate (700 μmol mol^{-1} CO_2 and 3 °C temperature rise) conditions in dedicated plant growth chambers. Plants were harvested at full maturity and grain yield, and yield attributes along with Zn and N status of grains were determined.

Results Predicted climate (PC) treatment significantly accelerated plant growth rate resulting in early onset of successive growth stages and maturity. In both PC and ambient climate (AC) conditions, adequate supply of N and Zn significantly increased straw and grain yield by increasing number of spikes per plant and number of grains per spike, whereas PC significantly reduced straw and grain yield through reducing number of spikes per plant, particularly in plants supplied with adequate N. Effect of adequate Zn or PC treatments were significant only under adequate N supply. Adequate N not only increased grain protein concentration but also grain Zn, particularly under adequate Zn application. In general, PC tended to increase grain Zn concentration, but the effect was non-significant. PC had no effect on grain protein concentration, whereas it significantly reduced grain protein yield (i.e. total mass of protein in whole grains of a single plant).

Conclusion The future climate with elevated CO_2 and raised temperature can dramatically reduce duration of time to complete successive growth stages in wheat. Plants cultured under PC conditions had significantly lower straw and grain yield, although supplied with ample fertilization. Whereas the PC and adequate Zn treatments enhanced main spike gain yield and number of grains per spike, PC declined overall grain yield, particularly due to severe reduction in number of spikes per plant. We conclude that sustaining a higher number of spikes per plant along with adequate nutrition with N and Zn are key factors to benefit from an elevated CO_2 atmosphere and to minimize adverse effects of rising temperatures on wheat yield and quality.

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M. Asif · C. E. Tunc · M. A. Yazici · Y. Tutus · R. Rehman · L. Ozturk (✉)
Faculty of Engineering and Natural Sciences, Sabanci University, 34956 Istanbul, Turkey
e-mail: Lozturk@sabanciuniv.edu

A. Rehman
Department of Agronomy, University of Agriculture, Faisalabad, Pakistan

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Introduction

Feeding the ever-increasing human population with sufficient yet nutritious food is a challenging problem of the twenty-first century. Currently, one in nine people does not have access to sufficient food to provide enough calories, and even more are suffering from malnutrition (FAO, Rome 2014). As the main source of food and animal feed, staple crops play a pivotal role in meeting global food security. Among the major staples, wheat is fundamental to humans. On a global basis, the share of wheat protein in human diet (21%) is even more than the sum of rice (13%) and corn (4%) (FAOSTAT 2011). It is the most widely cultivated crop plant throughout the world (Shiferaw et al. 2013) and constitutes the main source of calories in large populations, especially in the developing world (Cakmak 2008). However, wheat production is influenced by a variety of biotic (diseases, insects and weeds) and abiotic (diverse climate, water and nutrient availability) stress factors (Shiferaw et al. 2013). Sustaining the productivity of wheat undoubtedly requires addressing the short- or long-term effects of these multiple stressors, which often occur at the same time (e.g. heat and drought stress) under field conditions.

Climate change (rising atmospheric CO_2 and temperatures, changing precipitation patterns etc.) appears to be the new "norm" for scientists and breeders to develop new climate-resilient cultivars. Of the climate change components, changes in atmospheric CO_2 concentration is exceptionally distinct. Atmospheric CO_2 has been increasing at an unprecedented rate since the onset of industrialization in the 1800s and is thought to be responsible for increasing global surface temperatures (IPCC 2013). Currently, atmospheric CO_2 has reached 400 μmole mole^{-1} and is predicted to increase up to 700 μmole mole^{-1} with an average increase of 3 °C in atmospheric temperature by the end of this century (IPCC 2013). Individual components of climate change will have variable effects on productivity of wheat. For example, in C_3 species the principal photosynthetic enzyme RuBisCO is currently not functioning at high efficiency because it is not saturated with one of its major substrates, i.e. CO_2. Therefore, elevated CO_2 levels improve photosynthetic efficiency in wheat resulting in higher biomass production and grain yield (Leakey et al. 2009; Wang et al. 2013). On the other hand, increased temperatures accelerate growth and development rate with concomitant reduction in leaf area, carbon fixation and growth (Shiferaw et al. 2013), reducing the grain yield by 3–4% with each °C increase above the optimum (Wardlaw et al. 1989). However, combined effects of climate change components are not well known and complicates the efforts for breeding climate-resilient cultivars.

Fertilization with mineral nutrients is a key agricultural practice to maximize the yield potential of modern cultivars. Nitrogen (N) is the most extensively used fertilizer to improve wheat yield throughout the agriculture world (Bell et al. 1995; Makino 2011). Nitrogen fertilization has a profound effect on biomass buildup at anthesis by enhancing leaf area index and intercepted radiation, which ultimately translates into increased number of spikes, grain number and finally grain yield (Caviglia and Sadras 2001; Salvagiotti and Miralles 2008). Although required at much lower quantities by crop plants as compared to N, zinc (Zn) is among the most extensively used micronutrient fertilizers. Ample nutrition with Zn is a prerequisite to maximize photosynthesis, enzyme activation, protein synthesis and carbohydrate metabolism, and grain yield in wheat (Cakmak 2000; Bagci et al. 2007; Rehman et al. 2017). Combined application of N and Zn increases wheat productivity as well as nutritional quality of the grains (Erenoglu et al. 2011; Jan et al. 2013). It is therefore, highly important to study the influence of predicted climate (PC) on N and Zn nutrition of wheat to reveal the interactions on grain yield and quality.

An extensive number of studies are available regarding the interaction of N application and elevated CO_2 (Li et al. 2003; Pal et al. 2005; Reich et al. 2006; Duval et al. 2012), and elevated CO_2, elevated temperature and N (Mitchell et al. 1993; Delgado et al. 1994; Martínez-Carrasco et al. 2005) on wheat. For example, Dias de Oliveira et al. (2013) found that only climate treatment (elevated CO_2 and 2 °C temperature rise) increased grain yield in wheat while this effect was negligible when temperature was risen by 4 and 6 °C. Similarly, Zhang et al. (2014) found that the positive effect of elevated CO_2 on wheat was more prominent at ambient temperature as compared to elevated temperature. However, no information is available on the impact of elevated CO_2 and increased temperature and Zn application, as well as interactive effect of predicted climate (elevated CO_2 and elevated temperature) with N and Zn application on wheat yield. Recently, Asif et al. (2017) studied the interactive effect of elevated CO_2, Zn application and terminal drought and found that elevated CO_2 ameliorates the effect of low Zn as well as terminal drought on wheat yield. Considering the importance of climate change on wheat productivity and role of N and Zn fertilization in improving wheat yield to ensure food security, there is a dire need to investigate the interactive effect of predicted climate, N and Zn fertilization on growth and grain yield of wheat.

The aim of this study was to investigate the interactions of PC and N and Zn supply on grain yield, yield determinants (number of spikes, grains per spike, thousand grain weight, harvest index etc.) and nutritional quality (grain Zn and protein concentration) of wheat. Bread wheat (*Triticum aestivum* cv. Ceyhan-99) was grown in pots filled with soil fertilized with all combinations of low and adequate N and Zn in identical growth chambers set to ambient climate (AC) or predicted climate (PC) conditions. Duration of time (number of days after sowing) required for the onset of different stages including tillering, flag leaf emergence, heading, anthesis and maturity were recorded and at full maturity, plants were harvested as main tiller spikes, secondary tiller spikes and shoots to determine the aforementioned attributes.

Materials and methods

Plant material and growth conditions

Plants were grown in two identical climate chambers at Sabanci University, Turkey. The climatic conditions in growth chambers were set to either ambient climate (AC: 400 ± 40 µmol CO_2 mol^{-1}, day/night temperature: 12 ± 0.5 °C/7 ± 0.5 °C) or predicted climate (PC: 700 ± 40 µmol CO_2 mol^{-1}, day/night temperature: 15 ± 0.5 °C/10 ± 0.5 °C) scenario with a 3 °C of increased temperature and an elevated CO_2 at 700 µmol mol^{-1} (IPCC 2013). The day and night temperatures were gradually (1 °C per day) raised by 8 °C in both AC and PC treatments at the onset of spike emergence and thereafter kept constant until full maturity. Other growth conditions in both chambers were set as follows: photon flux density: 450 ± 15 µmol m^{-2} s^{-1}; light/dark period: 16 h/8 h; humidity: $65 \pm 5\%$ / $75 \pm 5\%$. The soil was calcareous (18% $CaCO_3$) with an alkaline pH (pH: 8.0 by 1:1 water extraction), clayey-loam texture, low organic matter content (1.5%) and very low DTPA-extractable Zn (0.1 mg kg^{-1}). Before sowing, the soil was fertilized with the following basal treatments: 150 mg N kg^{-1} as $Ca(NO_3)_2$ and 100 mg P kg^{-1} as KH_2PO_4. For the adequate Zn treatment, the soil was amended with 3 mg Zn kg^{-1} whereas low Zn treatment received 0.3 mg Zn kg^{-1} as $ZnSO_4$. Adequate N plants were top-dressed with 150 mg N kg^{-1} thrice at tillering, booting and anthesis stages, while low N plants were top-dressed only once at the booting stage in the form of $Ca(NO_3)_2$. Initially ten seeds of bread wheat (*Triticum aestivum* cv. Ceyhan-99) were planted in each plastic pot containing 2.0 kg of soil and germinated seedlings were thinned to four per pot at the two-leaf stage. Pots were irrigated twice a day during whole growth period. Plant age (i.e. days after sowing: DAS) at the onset of specific growth stages was recorded. Plant height was determined at 60 DAS and maturity using a meter rod.

Harvesting and yield parameters determination

At full maturity, number of spikes per plant was determined and plants were harvested in three parts as spikes of main tillers, spikes of secondary tillers and the remaining above-ground biomass (i.e. straw). Harvested plant parts were dried in a drying oven at 60 °C until a constant weight. After weighing, spikes were threshed using a laboratory thresher (LD 180, Wintersteiger AG, Austria). Spike length and grain yield per spike were determined from the main spikes whereas over all grain yield and number of grains per spike were determined from whole spikes per plant. All grains obtained from each plant were counted manually and thousand grain weight was determined by unitary method (grain yield / number of grains * 1000).

Determination of grain Zn and protein concentration

Whole grains were ground to fine powder using a metal-free agate vibrating cup mill (Pulverisette 9, Fritsch GmbH, Germany) and 200 ± 1 mg of ground sample was acid-digested in 2 mL of 30% H_2O_2 (*w*/*v*) and 5 mL of 65% HNO_3 (w/v) using a closed-vessel microwave reaction system (MarsExpress, CEM Corp., Matthews, NC, USA). After diluting the digested samples to 20 mL using ultrapure (18.2 MΩ) water, Zn concentrations were determined using inductively coupled plasma optical emission spectrometer (Vista-Pro Axial, Varian Pty

Ltd., Mulgrave, Australia). Total nitrogen concentration (% N) in flour samples was analyzed using an automated N analyzer (TruSpec CN, LECO Corp., Michigan, USA) and the results were converted to protein concentration using the conversion factor of 5.36 (Mosse 1990).

Experimental design and statistical analysis

The experimental designed was a completely randomized under full factorial with four independent pot replicates. Analysis of variance (ANOVA) was applied to evaluate the significance of the effects of different treatments and their interactions using JMP® (5.0.1) SAS Institute Inc., Cary, NC, USA. The significant difference between the treatment means were determined using Tukey's honestly significant difference HSD test at 5% confidence interval ($p \leq 0.05$).

Results

Predicted climate (PC) treatment accelerated growth and development of plants causing them to reach full maturity in 111 days after sowing (DAS) as compared to ambient climate (AC) treated plants, which reached to full maturity in 138 DAS (Table 1). Within 60 DAS, average plant height at PC reached to 55 cm compared to 40 cm at AC (Fig. 1 a) resulting in a significant effect of climate on plant phenology as illustrated in Fig. 2 at 95 DAS. Accordingly, plants grown under PC reached to each successive growth stage earlier compared to those under AC (Table 1, Fig. 2). Plants grown under PC reached to tillering stage as early as in 20 DAS, flag leaf emergence at 47 DAS, heading at 68 DAS, anthesis at 74 DAS and maturity at 111 DAS while plants grown under AC reached these stages at 32, 71, 87, 91 and 138 DAS respectively (Table 1). The PC-induced reduction in time to complete a growth stage was more distinct in the earlier stages of the plant life cycle (i.e. tillering and flag leaf) (Table 1).

At 60 DAS, plant height was significantly affected ($p < 0.001$) by climate, N, and Zn treatments (Fig. 1 a). Plant height was particularly enhanced by PC, although adequate N and Zn treatments also increased plant height significantly (Fig. 1 a). Adequate Zn supply resulted in a significant increase in height of plants grown with adequate N and AC, while the effect of Zn was non-significant within all other treatments (Fig. 1 a). At AC, adequate N supply significantly increased plant height in both low and adequate Zn plants whereas under PC conditions, the effect of N application was significant only under adequate Zn supply (Fig. 1 a). Moreover, N treatment significantly interacted with Zn ($p < 0.01$) as well as climate ($p < 0.05$) (Fig. 1 a).

Plant height at maturity was also significantly affected by climate ($p < 0.05$), N ($p < 0.01$) and Zn treatments ($p < 0.001$), although none of the interactions were significant (Figs. 1 b). In mature plants, plant height was shorter in adequate N (i.e. 73.9 cm) compared to low N treatment (i.e. 77.5 cm) (Fig. 1 b and Fig. 3). However, plant height was significantly increased by adequate Zn (i.e. from 71.8 cm to 79.6 cm) and PC (i.e. from 74.1 cm to 77.4 cm) compared to low Zn and AC treatments respectively (Fig. 1 b and Fig. 3). Overall, adequate Zn, low N and AC treated plants were tallest (82 cm) while the low Zn, adequate N and AC treated plants were the shortest of all (66 cm) (Fig. 1 b and Fig. 3).

Grain yield was significantly affected ($p < 0.001$) by Zn, N and climate treatments (Fig. 4 a). The PC treatment decreased overall grain yield by 12%, whereas adequate Zn and N treatments increased grain yield by 38 and 90% respectively (Fig. 4 a). The N treatment significantly interacted with climate ($p < 0.001$) and Zn ($p < 0.001$) (Fig. 4 a), resulting in highest grain yield (7.92 g plant^{-1}) in plants grown under AC conditions supplied with adequate N and Zn followed by those grown under PC (6.49 g pant^{-1}) with the same fertilization treatments (Fig. 4 a). The interaction between climate and Zn was non-significant, and adequate Zn increased grain yield of AC and PC treated plants at a similar rate when supplied with adequate N (Fig. 4 a).

Table 1 Duration of time (DAS: days after sowing) required to complete successive growth stages in wheat (*Triticum aestivum* cv. Ceyhan-99) at ambient (AC: ambient CO_2 and temperature) and predicted climate (PC: 700 µmol mol^{-1} CO_2 and 3 °C temperature rise)

Growth stage	AC	PC	Reduction in time by PC
	(DAS)		(%)
Tillering	32	20	38
Flag leaf	71	47	34
Heading	87	68	22
Anthesis	91	74	19
Maturation	138	111	20

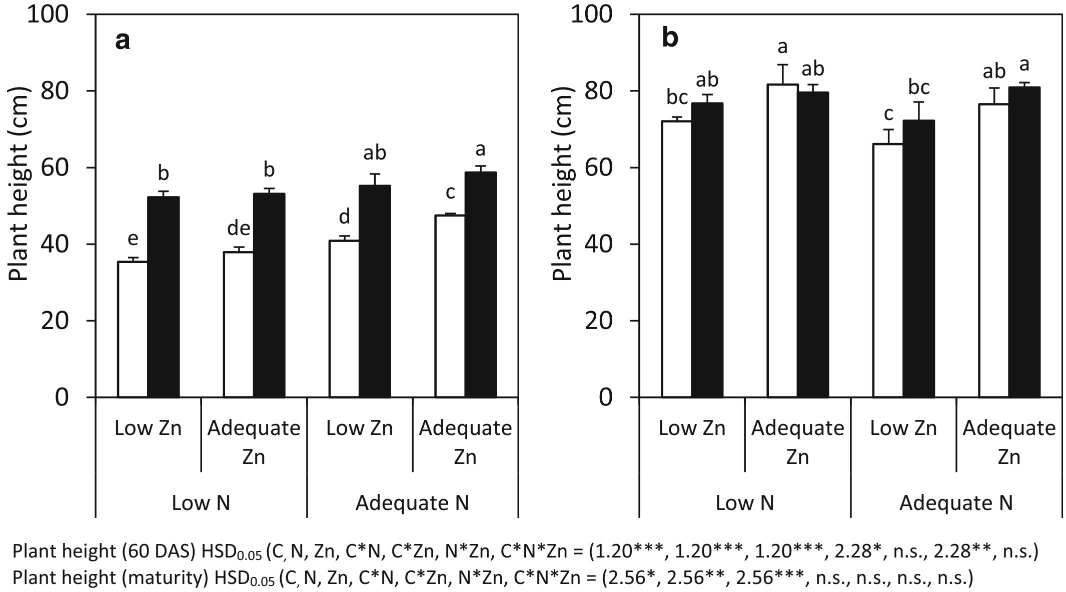


Fig. 1 Plant height of bread wheat plants (*Triticum aestivum* cv. Ceyhan-99) at 60 days after sowing (**a**) and maturity (**b**) as affected by varied N, Zn and climate treatments (open bars: ambient climate, solid bars: predicted climate). In each panel, bars not showing the same letter are significantly different from each other (Tukey's HSD, $p \leq 0.05$). All values are means ± SD ($n = 4$)

Low N plants neither responded to Zn nor climate treatments (Fig. 4 a), suggesting that N was the main determinant of grain yield in this study.

Number of spikes per plant was significantly affected by climate as well as N ($p < 0.001$), but not with varied Zn supply (Fig. 4 b). Overall, average number of spikes per plant was reduced from four at AC to three at PC (Fig. 4 b). However, none of the interactions were found significant except that of climate and N ($p < 0.001$). Highest number of spikes per plant resulted in adequate N plants grown under AC followed by those grown under PC, whereas N starvation severely reduced number of spikes per plant irrespective of Zn and climate treatments (Fig. 4 b).

In line with grain yield, straw yield was also significantly affected by climate, N and Zn treatments ($p < 0.001$) (Fig. 4 c). Overall, PC reduced straw yield by 13% as compared to AC, whereas adequate N and Zn increased straw yield by 68 and 24% as compared to low N and low Zn treatments respectively (Fig. 4 c). Nitrogen significantly interacted with climate ($p < 0.001$), resulting in highest straw yield in plants grown under adequate N and AC followed by those under PC, whereas low N plants had the lowest straw yield irrespective of climate treatments. Nitrogen also interacted with Zn ($p < 0.001$) with higher response to Zn supply in adequate N compared to low N plants. The three-way interaction was non-significant for straw yield (Fig. 4 c).

Harvest index was not affected by climate or Zn treatments, whereas N had a significant effect ($p < 0.01$) resulting in higher overall harvest index in adequate N (44.4%) as compared to low N plants (42.1%) (Fig. 4 d). Zinc treatment interacted with N ($p < 0.05$) by increasing the harvest index in adequate N but not in low N plants (Fig. 4 d). Overall, plants grown with adequate N and Zn under PC had the highest harvest index (47%) while those grown with low N and under AC had the least (41%) (Fig. 4 d).

Figure 4 shows yield parameters related to spike (main spike length, main spike grain yield) and grains (number of grains per spike and thousand grain weight). Increasing Zn supply significantly increased the length of main spikes from 97 to 103 mm ($p < 0.001$), whereas N and climate treatments had no clear effect (Fig. 5 a). Moreover, Zn treatment significantly interacted with climate, increasing the spike length from 94 mm in low Zn and AC treated plants to 101 and 103 mm in adequate Zn plants grown under PC and AC respectively (Fig. 5 a). Overall, plants grown under AC with adequate N and Zn had the longest main spike (107 mm) and those grown under adequate N and low Zn had shortest main spike (93 mm) (Fig. 5 a).

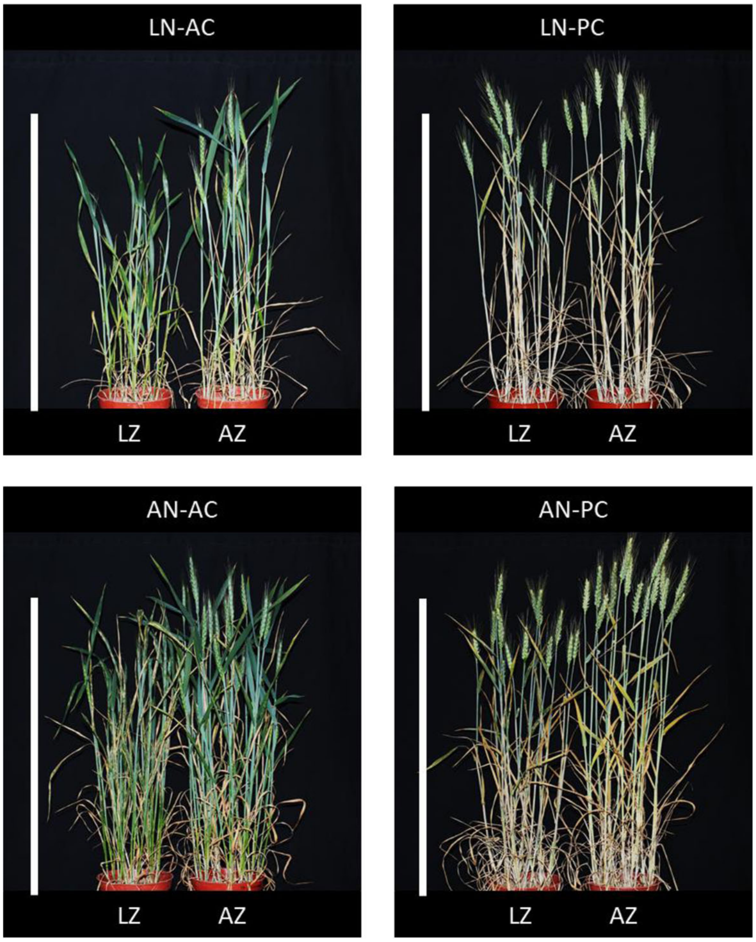


Fig. 2 Bread wheat plants (*Triticum aestivum* cv. Ceyhan-99) at 95 days after sowing as affected by varied N (AN: adequate N, LN: low N), Zn (LZ: low Zn, AZ: adequate Zn) and climate (AC: ambient climate, PC: predicted climate) treatments. Plants were photographed from same distance and exposure settings. Scale bar on left indicates a height of 70 cm

Unlike grain yield per plant, main spike grain yield was not influenced by varied N supply, however both climate and Zn treatments had a profound effect ($p < 0.001$) (Fig. 5 b). Overall, main spike grain yield increased from 1.44 g spike^{-1} in AC to 1.75 g spike^{-1} in PC and from 1.30 g spike^{-1} in low Zn to 1.80 g spike^{-1} in adequate Zn treated plants (Fig. 5 b). Moreover, Zn treatment significantly interacted with N ($p < 0.05$) causing a higher increase in main spike grain yield at adequate N compared to low N plants (Fig. 5 b). Overall, plants grown under PC with adequate N and Zn had the highest main spike grain yield (2.0 g spike^{-1}), whereas plants grown under AC with adequate N and low Zn had the lowest (1.2 g spike^{-1}) main spike grain yield (Fig. 5 b).

The changes in number of grains per spike presented a very similar pattern as grain yield of main spike (Figs 4 b and c). Both PC as well as adequate Zn treatments significantly increased ($p < 0.001$) the number of grains per spike, while increasing the N supply had no apparent effect (Fig. 5 c). Number of grains per spike increased from 33 in AC to 41 in PC and from 32 in low Zn to 42 in adequate Zn treatments (Fig. 5 c). There was a significant interaction ($p < 0.05$) between Zn and N resulting from a higher increase in number of grains per spike due to Zn at adequate N as compared to low N treated plants (Fig. 5 c). Overall, plants grown under PC with adequate N and Zn had the highest number of grains per spike (46) while those grown under AC with adequate N and low Zn had the lowest (26) (Fig. 5 c).

There was no change in thousand grain weight by climate or N treatments, however adequate Zn treatment increased thousand grain weight ($p < 0.05$) to 34.9 g from 33.4 g in low Zn treatment (Fig. 5 d). Zinc treatment significantly ($p < 0.01$) interacted with climate resulting in a significant increase (i.e. from 32 g to 36 g) in thousand grain weight under PC but not AC conditions (Fig. 5 d). Moreover, interaction between climate and N application was highly significant ($p < 0.001$) due to the

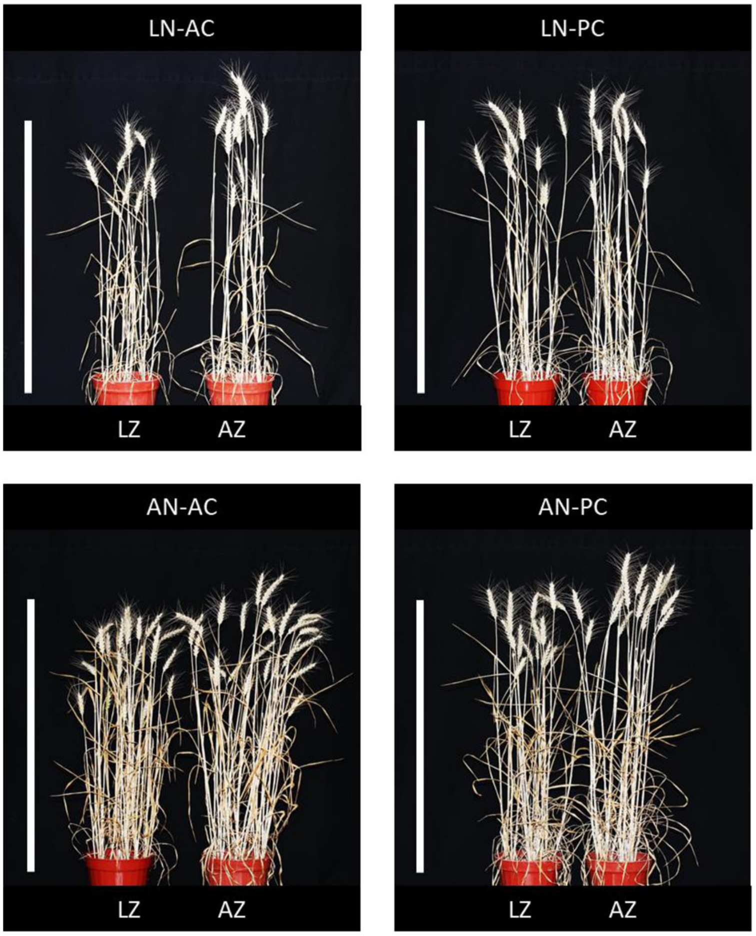


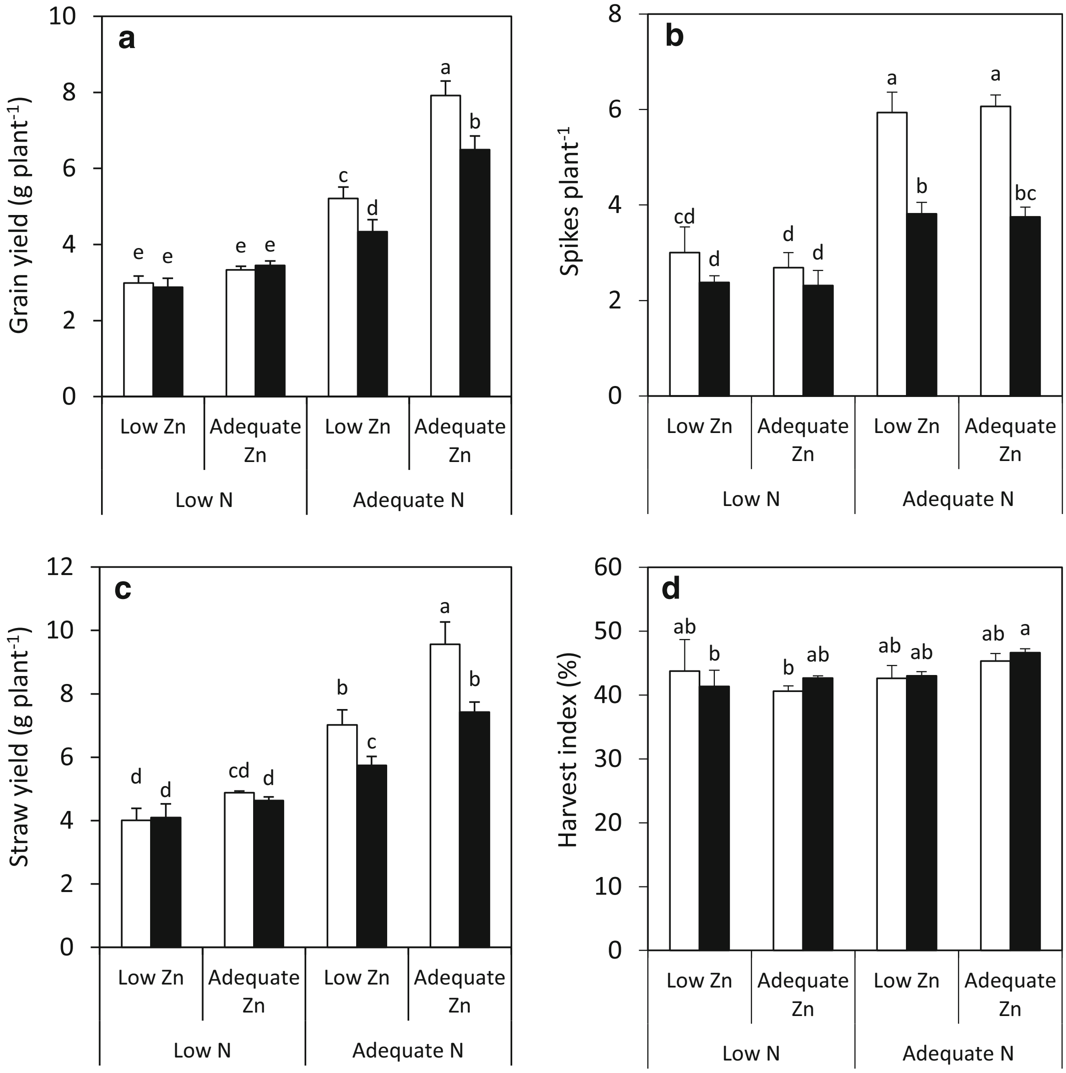
Fig. 3 Bread wheat plants (*Triticum aestivum* cv. Ceyhan-99) at maturity as affected by varied N (AN: adequate N, LN: low N), Zn (LZ: low Zn, AZ: adequate Zn) and climate (AC: ambient climate, PC: predicted climate) treatments. Plants were photographed from same distance and exposure settings. Scale bar on left indicates a height of 70 cm

fact that in low N treatment thousand grain weight was higher in AC whereas in adequate N it was higher in PC treatment (Fig. 5 d). Overall, adequately fertilized plants grown in PC conditions had the highest thousand grain weight (37 g) and inadequately fertilized (low N and Zn) plants grown under PC conditions had the least thousand grain weight (32 g) (Fig. 5 d).

Grain Zn concentration was significantly affected ($p < 0.001$) by climate, N and Zn treatments (Fig. 6 a). As expected, adequate Zn supply to plants increased grain Zn concentration in all treatments of climate and N fertilization (Fig. 6 a). Over all, PC increased grain Zn concentration from 9.9 mg kg^{-1} in AC plants to 11.4 mg kg^{-1} in PC plants; however within the same treatment of Zn application, PC did not cause a significant effect, resulting in a non-significant interaction ($p = 0.073$) between Zn and climate (Fig. 6 a). Similarly, N application significantly increased grain Zn concentration from 9.3 mg kg^{-1} in low N plants to 12.0 mg kg^{-1} in adequate N plants (Fig. 6 a). In contrast to climate, N application interacted with Zn ($p < 0.05$) with a higher response to Zn amendment in adequate N compared to low N plants (Fig. 6 a). The interaction between climate and N as well as three-way interaction (climate x N x Zn) was non-significant. Considering all treatments, plants supplied with adequate N and Zn and grown under PC had the highest grain Zn concentration while inadequately fertilized (low N and low Zn) plants had the lowest (Fig. 6 a).

Grain Zn yield (i.e. total mass of Zn in whole grains of a single plant) was significantly ($p < 0.001$) affected by Zn and N, but not climate treatments (Fig. 6 b). Among different treatments, the only significant interaction was between N and Zn ($p < 0.001$) in which grain Zn yield was much more enhanced by Zn amendment in adequate N compared to low N treatment (Fig. 6 b).

Grain protein concentration was markedly increased by adequate N treatment ($p < 0.001$) as expected. However, adequate Zn had a reducing effect



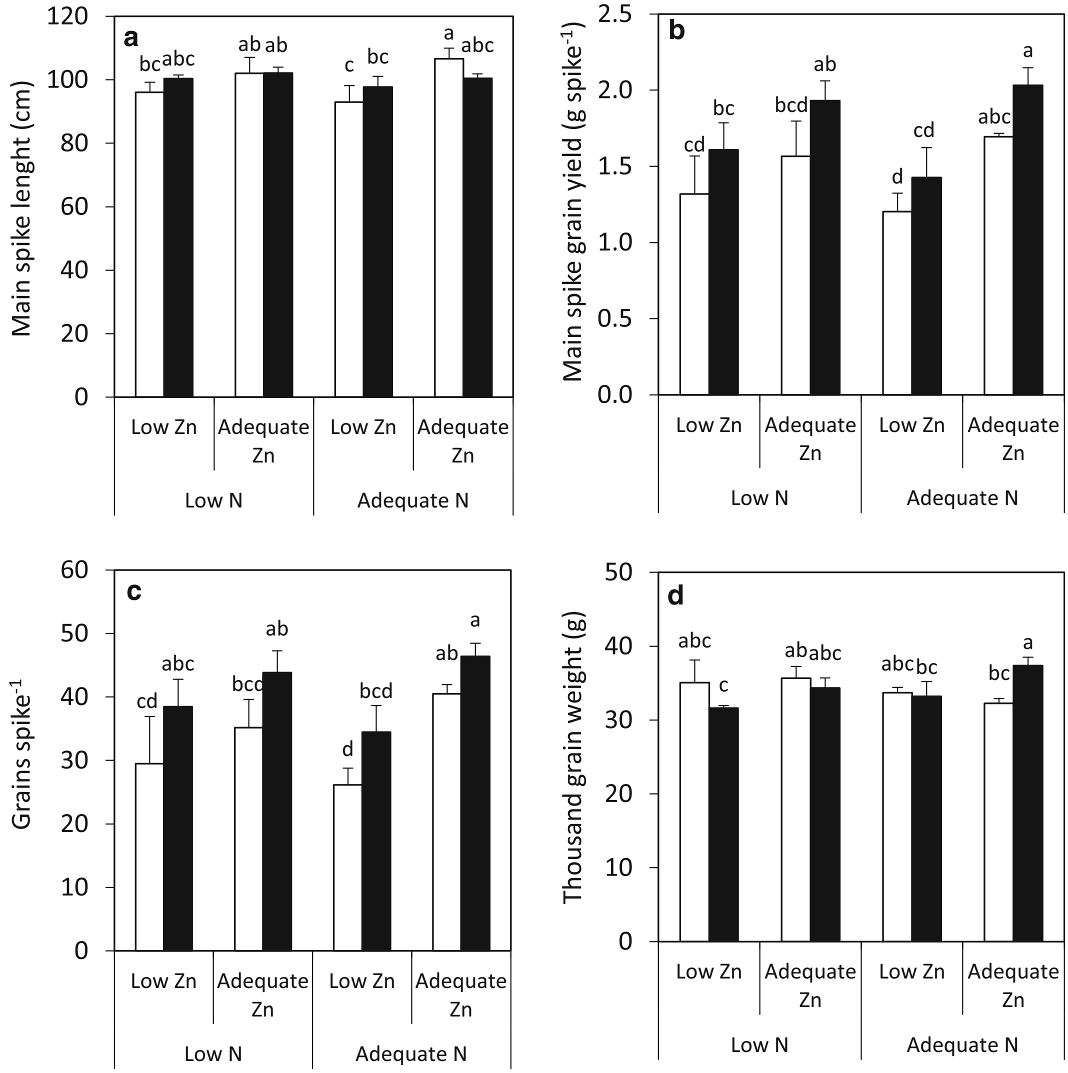
Grain yield $HSD_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (0.21***, 0.21***, 0.21***, 0.39***, n.s., 0.39***, 0.66*)
Spikes $plant^{-1}$ $HSD_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (0.23***, 0.23***, n.s., 0.45***, n.s., n.s., n.s.)
Straw yield $HSD_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (0.28***, 0.28***, 0.28***, 0.54***, 0.54*, 0.54***, n.s.)
Harvest index $HSD_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (n.s., 1.59**, n.s., n.s., n.s., 3.01*, n.s.)

Fig. 4 Grain yield (**a**), spikes per plant (**b**), straw yield (**c**) and harvest index (**d**) of bread wheat (*Triticum aestivum* cv. Ceyhan-99) plants as affected by varied N, Zn and climate treatments (open bars: ambient climate, solid bars: predicted climate). In each panel, bars not showing the same letter are significantly different from each other (Tukey's HSD, $p \leq 0.05$). All values are means ± SD ($n = 4$)

($p < 0.001$) whereas PC had no significant impact on grain protein concentration (Fig. 7 a).

Although PC treatment did not affect grain protein concentration, it significantly interacted with Zn ($p < 0.05$) causing a reduction of grain protein concentration of low Zn plants when grown under PC as compared to AC conditions, while the effect was not visible in adequate Zn plants (Fig. 7 a). There was also a significant interaction between N and Zn treatments ($p < 0.05$) on grain protein concentration, in which the negative effect of adequate Zn on grain protein was more evident in adequate N, but not in low N treated plants (Fig. 7 a). The three-way interaction of treatments on grain protein concentration was non-significant.

Grain protein yield (i.e. total mass of protein in whole grains of a single plant) was significantly ($p < 0.001$)



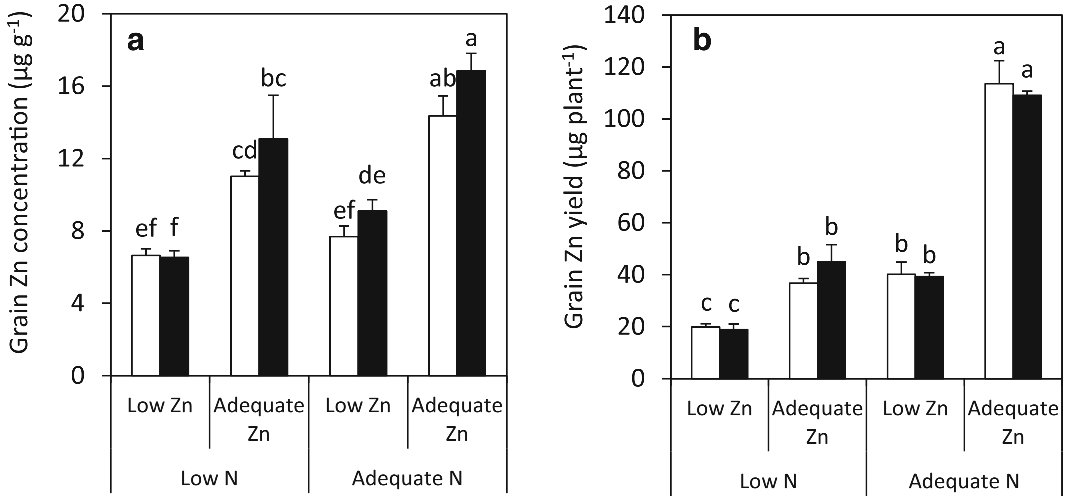
Spike length HSD$_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (n.s., n.s., 1.44***, n.s., 2.74**, n.s., n.s.)
Main spike grain yield HSD$_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (0.12***, n.s., 0.12***, n.s., n.s. 0.23*, n.s.)
Grains per spike HSD$_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (3.0***, n.s., 3.0***, n.s., n.s. 5.7*, n.s.)
Thousand grain weight HSD$_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (n.s., n.s., 1.15*, 2.18***, 2.18**, n.s., n.s.)

Fig. 5 Spike length (**a**) and grain yield (**b**) of main spike, grains per spike (**c**) and thousand grain weight (**d**) of bread wheat (*Triticum aestivum* cv. Ceyhan-99) plants as affected by varied N, Zn and climate treatments (open bars: ambient climate, solid bars: predicted climate). In each panel, bars not showing the same letter are significantly different from each other (Tukey's HSD, p ≤ 0.05). All values are means ± SD (n = 4)

affected by all treatments, viz., varied climate, N and Zn (Fig. 7 b). Adequate N treatment greatly enhanced grain protein yield up to four-fold compared to low N plants. Whereas adequate Zn had an additive effect ($p < 0.05$), PC resulted in a significant reduction ($p < 0.05$) in grain protein yield of adequate N plants (Fig. 7 b). Such effects of Zn or climate treatments were not evident in low N plants as the grain protein yield was already severely reduced by low N treatment (Fig. 7 b).

Discussion

In this study, plants under PC treatment grew taller during the early growth stages (Fig. 1a) whereas the

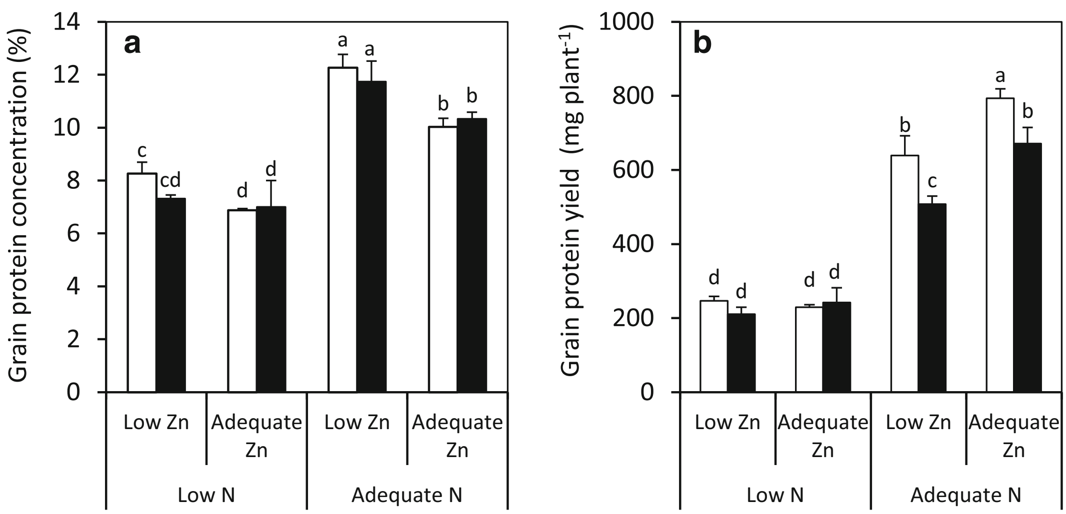


Grain Zn concentration $HSD_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (0.75***, 0.75***, 0.75***, n.s., n.s., 1.43*, n.s.)
Grain Zn yield $HSD_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (n.s., 3.25***, 3.25***, n.s., n.s. 6.16***, 10*)

Fig. 6 Grain Zn concentration (**a**) and grain Zn yield (**b**) of bread wheat (*Triticum aestivum* cv. Ceyhan-99) plants as affected by varied N, Zn and climate treatments (open bars: ambient climate, solid bars: predicted climate). In each panel, bars not showing the same letter are significantly different from each other (Tukey's HSD, p ≤ 0.05). All values are means ± SD (n = 4)

final plant height at maturity did not differ significantly (Fig. 1b). This response is ascribed to accelerated plant growth and development under the PC conditions (Fig. 1 a) with a distinct effect on plant phenology. Consequently, the PC-treated plants reached and completed each successive growth stage earlier as compared to AC plants (Table 1, Fig. 2). It was noted that the reduction in time to complete a growth stage due to PC was more evident in the earlier stages of the plant life cycle (i.e. tillering and flag leaf) (Table 1).

Grain yield is dependent on different yield parameters. In this study, number of spikes per plant, number of



Grain protein conc. $HSD_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (n.s., 0.38***, 0.38***, n.s., 0.72*, n.s., 0.72*, n.s.)
Grain protein yield $HSD_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (23***, 23***, 23***, 43***, n.s. 43***, n.s.)

Fig. 7 Grain Protein concentration (**a**) and protein yield (**b**) of bread wheat (*Triticum aestivum* cv. Ceyhan-99) plants as affected by varied N, Zn and climate treatments (open bars: ambient climate, solid bars: predicted climate). In each panel, bars not showing the same letter are significantly different from each other (Tukey's HSD, p ≤ 0.05). All values are means ± SD (n = 4) grains per spike and grain yield per spike played the most important roles in determining the final grain yield per plant. Different treatments viz. climate, N and Zn affected the final grain yield through influencing different yield parameters. For example, level of N supply played the most crucial role in determination of grain yield (Fig. 4 a) through enhancing the number of spikes per plant (Fig. 4 b). Overall, adequate N increased grain yield by 90% and number of spikes per plant by 89% as compared to low N (Fig. 4 a, b). The importance of N fertilization in increasing grain yield through increasing leaf area index, canopy photosynthesis and ultimately number of tillers/spikes per plant is a well-known fact (López-Bellido and López-Bellido 2001; Kim et al. 2003; Otteson et al. 2007). However, when N level was increased from low to adequate, response of grain yield was highly dependent on climate as well as Zn treatments (Fig. 4 a). For example, under AC environment, adequate N treatment increased grain yield by 74% as compared to low N plants under low Zn conditions, whereas under adequate Zn conditions this increase was 138% (Fig. 4 a). Similarly, under PC conditions this increase was 51 and 88% in low Zn and adequate Zn plants respectively, although the N-induced increase in number of spikes was similar in low Zn and adequate Zn plants (Fig. 4 a, b). This differential response in grain yield is due to combined effect of increased spike length, main spike grain yield and number of grains per spike (Fig. 5). A significant interaction between N and Zn application has been reported for yield and yield parameters previously (Jan et al. 2013) suggesting the combined application of N and Zn for improving wheat productivity.

An important aspect of the results presented here is that under low N conditions, influence of Zn and climate become irrelevant for grain yield. Under adequate N, PC significantly reduced grain yield (Fig. 4 a) through decreasing the number of spikes per plant (Fig. 4 b). Although PC treatment increased main spike length, main spike grain yield and number of grains per spike within different treatments, the effect was non-significant and could not compensate the grain yield loss brought by the severe reduction in number of spikes per plant (Fig. 5 and Fig. 4). Number of tillers and spikes depend on different factors, one of which is total radiation acquired by plants during vegetative growth (Evans 1978; Rawson 1986; Dias de Oliveira et al. 2013). Since PC conditions hastened plant development and reduced duration of time to complete successive growth stages (e.g. tillering by 38% and flag leaf emergence by 34%, Table 1), plants grown under PC environment inevitably received less cumulative radiation. Receiving less cumulative radiation particularly at the vegetative stage not only reduces number of spikes but also results in reduction of photosynthetic energy harvest. Obviously, even the enhanced RuBisCO carboxylation under elevated atmospheric CO_2 (Leakey et al. 2009) could not compensate the severe loss in grain yield (Fig. 4 a) mainly due to accelerated growth and development (Table 1, Fig. 1 a, Fig. 2) and reduced number of spikes per plant under the PC treatment (Fig. 4 b).

Zn is an important micronutrient involved in different physiochemical processes such as enzyme activities, DNA replication and transcription (Broadley et al. 2012) and is very important for improving the productivity of wheat in Zn deficient soils (Alloway 2008; Cakmak 2008). In this study grain yield under adequate N supply was highly dependent on the level of Zn supply in both AC and PC environments. Adequate Zn supply increased grain yield by 52 and 50% in AC and PC conditions respectively (Fig. 4 a). In contrast to N where grain yield was improved through increasing number of spikes per plant, the yield gain by Zn was due to increased main spike length, main spike grain yield and number of grains per spike (Fig. 5). Recently, Ma et al. (2017) found that the increase in grain yield by Zn is linked to significant increase in number of grains per spike or thousand-kernel weight, whereas spikes per plant were unaffected. The effect of adequate Zn supply on number of grains per spike and grain yield per spike can be associated with the role of Zn on pollen fertility. In different crop species Zn deficiency was shown to severely reduce pollen and anther viability (Brennan 1992; Ma et al. 2017) which could be improved by Zn amendment resulting in higher grain yields.

Similar to grain yield, straw yield was significantly increased by adequate N and Zn supply whereas PC treatment counteracted on the straw yield gain (Fig. 4 c). In this study, low N treatment dominated straw yield and rendered climate and Zn treatments as ineffective (Fig. 4 c). Like grain yield, increase in straw yield by N can also be assigned to increased leaf area index, canopy photosynthesis and tillers per plant (Cassman et al. 1992; Dreccer et al. 2000). Straw yield was deteriorated by PC treatment irrespective of N or Zn most likely due to the same factors explained above for the grain yield (i.e. accelerated growth, reduced number of tillers, inefficient harvesting of cumulative radiation energy) (Fig. 4; Table 1).

Harvest index was unaffected by most of the treatments, however adequate N significantly increased harvest index as compared to low N treatments alone as well as it significantly interacted with Zn application resulting in harvest index improvement in plants adequately fertilized with N and Zn as compared to inadequately fertilized plants (Fig. 4 d). The increment in harvest index by adequate N supply found here is in line with previous studies (Jan et al. 2013) in which the effect was traced back to increased photosynthate allocation to grains.

Grain Zn concentration was increased not only by adequate Zn supply but also adequate N as well as PC treatments. Enhancement of Zn uptake and translocation by adequate N was demonstrated in young wheat plants cultured in nutrient solution (Erenoglu et al. 2011; Nie et al. 2017). When Zn supply was adequately high, both soil and foliar N applications were shown to significantly improve grain Zn concentration (Kutman et al. 2010, 2011). Increase in Zn uptake and grain deposition by ample N supply is attributed to induction and expression of transporter proteins involving in xylem loading and unloading of Zn as well as production of nitrogenous compounds (e.g. nicotianamine and deoxymugineic acid) facilitating Zn transport (Cakmak et al. 2010).

Previously, elevated CO_2 alone was reported to decrease grain Zn concentration and the effect was traced back to dilution of Zn in the grain due to higher yields and reduced Zn uptake (Högy and Fangmeier 2008; Myers et al. 2014; Asif et al. 2017). Here we studied the combined effect of elevated CO_2 and increased temperature to simulate the predicted climate scenario (IPCC 2013) and found no decrease, but a non-significant increase in grain Zn concentration of adequate N and Zn plants. The variation in grain Zn concentration was highly influenced by grain yield as judged by the stability of grain Zn yield in response to PC. Unlike previous reports on the effect of elevated CO_2 alone (Myers et al. 2014; Högy et al. 2009), results presented here do not indicate a deterioration of grain nutritional quality under PC conditions (elevated CO_2 and increased temperature). This further indicates that the negative effect of elevated CO_2 on grain nutritional quality is a consequence of enhanced grain yield (i.e. dilution), rather than a climatic effect. Apparently, global climate change involves both promoting (i.e. elevating atmospheric CO_2 levels) (Wang et al. 2013) and suppressing (i.e. rising global temperatures) (Asseng et al. 2015) components acting simultaneously on physiology and productivity of the wheat crop. Therefore, a climate change-induced nutritional quality deterioration is most likely to occur only in areas of the world where the effect of elevated CO_2 would be greater than that of temperature rise on grain yield, such as high latitudes.

As expected, grain protein concentration increased with adequate N supply. However, in contrast to the effect of adequate N on grain Zn concentration, grain protein concentration was decreased with adequate Zn supply. In fact, increased grain yield by Zn resulted in dilution of grain protein ruling out a correlation between grain Zn and protein concentration (Fig. 7 a). In line with Zn, grain protein concentration also remained unaffected by the PC treatment, negating the concept of deterioration of grain nutritional quality with climate change (Myers et al. 2014; Asif et al. 2017). However, PC treatment significantly reduced grain protein yield (Fig. 7 b) as a function of decreased grain yield (Fig. 4 a).

Conclusion

Results of this study conclude that the predicted climate with elevated CO_2 and raised temperature levels can threaten global food security by reducing the growth and yield performance of wheat, and the effect would be greater in high input production systems receiving ample fertilization with macro- and micronutrients such as N and Zn. We also conclude that wheat growth cycle is significantly reduced under predicted climate scenario, particularly shortening the time to complete vegetative growth stages as well as time to reach full maturity. Whereas adequate N supply increased grain yield mainly through increasing the number of spikes per plant, adequate Zn application influenced grain yield through increased spike length, number of grains and grain yield per spike. Adequate Zn improved grain quality by increasing grain Zn concentration, while adequate N enhanced grain protein along with an additive effect on grain Zn, implying the importance of N nutrition on grain nutritional quality. Sustaining a higher number of spikes per plant along with adequate nutrition with N and Zn are key factors to benefit from elevating atmospheric CO_2 levels while minimize adverse effects of rising temperatures on wheat yield and quality.

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