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RESEARCH ARTICLE

Elevated CO_2 and Nitrogen dose affect grain ionome, grain morphology and associated gene expression in wheat (*Triticum aestivum* L.)

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Abstract

The rise in atmospheric CO_2 levels impacts humankind by threatening food and nutritional security. The strong correlation between crop yield and grain weight in cereals is an essential component of yield stability. Further, improving grain protein and mineral nutrient content is a crucial breeding target for cereal crops. The study was performed to understand the interactive effects of elevated CO_2 (EC) and nitrogen (N) fertilization on grain ionome, grain yield parameters, grain morphology, and the expression of genes related to grain morphology. The changes in ionome and grain parameters were examined in response to two N levels optimal N (ON: 500 mg/pot) and high N (HN: 625 mg/pot) along with atmospheric CO_2 enrichment [ambient (CO_2) of 400 ±10 ppm and elevated (CO_2) of 700 ±10 ppm]. Grain ionome (N, K, Ca and Fe) showed a general decrease in EC-grown wheat plants. The expression of genes related to grain length (*TaGL3* and *TaGL7*) were upregulated, and those genes related to grain width (*TaGW2* and *TaGW6*) were downregulated under EC in maturing spikelet of wheat. In the case of *TaSnRK2*, the expression was promoted by EC in HN treatment. The complex regulation of source and sink-associated gene transcript abundance indicates an EC mediated alteration in N and sugar signalling in wheat.

Keywords: Grain ionome, grain yield parameters, grain morphology, gene expression, elevated CO_2, bread wheat

Introduction

Wheat is the most extensively cultivated food crop worldwide contributing to 27% of grain production. It also ranks highest as a protein source, accounting for over 20% of the protein in the human diet (FAO 2013). With a need to increase production in the coming decades, wheat cultivation, like other crops, will face significant challenges brought on by climate change. Since the inception of the industrial revolution, the levels of atmospheric CO_2 have been progressively rising from 280 ppm to present-day 418 ppm and is likely to double by 2100 (IPCC 2014). Increasing CO_2 levels enhances photosynthesis and water-use efficiency, thereby imparting a productive advantage to C_3 crops like wheat and rice (Ainsworth and Long 2005). But, the rapid increase in atmospheric CO_2 is anticipated to affect plant stoichiometry, thereby affecting the nutritional status of the crops as well as the nutritional integrity of humans. The amount of nutrients present in the grains is regulated by many factors like the bio-availability of nutrients to the plants, their uptake and translocation into grains. Enhanced rates of photosynthesis under elevated CO_2 (EC) conditions result in greater carbon release from the roots into the soil. This can affect the activity of soil microbes and/or change soil pH which can affect the availability of nutrient to the plants. The enhanced carbohydrates can cause dilution effect on nutrients there by reduces the quality (Loladze 2014). EC can also directly affect the mass-flow delivery of elements

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by closure of stomata and reduction in transpiration. Dong et al. (2018) reported that EC reduced the concentrations of protein (-9.5%), nitrogen (N) (-18.0%), magnesium (-9.2%), iron (-16.0%) and zinc (-9.4%) in vegetables. In wheat, EC increased concentrations of grain potassium (Manderscheid et al. 1995) and copper (Erbs et al. 2010) while reduced the concentrations of calcium, magnesium, iron, zinc and sulphur (Fernando et al. 2012; Högy and Fangmeier 2008). Recently we have observed a reduction in transcript abundance of genes involved in nutrient remobilization i.e., *TaNAM-B1* and *TaYSL6* under EC grown conditions in the wheat, which may be one of the possible reasons for lower grain ionome concentration. With CO_2 levels ranging from 450ppm to 800ppm, the grain yield of wheat was observed to be increased by 10-31% (Pleijel and Uddling 2011).

Grain yield is a complex trait governed by several factors like the number of productive tillers, number of grains per spike, size of the grains, and thousand seed weight. Thousand seed- weight is the most stable yield component, which is determined by the size of individual grains and other grain morphological components like length, width, and area. Under EC, plants receiving ammonical ($NH4^+$) forms of N had a greater enhancement in the growth and yield along with higher protein content as compared to those supplied with nitrate (NO_3^+) forms (Carlisle et al. 2012). In a meta-analysis conducted by Jablonski et al. (2002), a dramatic increase in the number of seeds and total seed weight was observed in 79 species distributed between wild types and crop plants grown under EC. On the other hand EC did not alter the harvest index because of increase in total plant biomass (Bunce 2017). The number of seeds and seed size, also depends on the duration to flowering. Plants grown under EC were observed to have early flowering, early senescence, and accelerated grain development (Sild et al. 1999; Springer and Ward 2007).

Advancement in functional genomics has widened the understanding of grain development and associated genes. Comparative genomics approaches aid in identifying various genes related to yield traits in wheat such as *TaSnRK2.9* (Ur Rehman et al. 2019), *TaTGW6* (Hanif et al. 2016), *TaSus1* (Hou et al. 2014), *TaSus2* (Jiang et al. 2011), and *TaGW2* (Su et al. 2011; Yang et al. 2012; Qin et al. 2014). The genes involved in the determination of grain size includes *TaGL3* (Qi et al. 2012; Yang 1 et al. 2019) and *GL7* (Wang 1 et al. 2015), both contributing to grain length; while *TaGW2* (Wang et al. 2018; Zhang et al. 2018) and *TaGW6* (Hu et al. 2016) contributing to grain width and weight. A recent study by Wang et al. (2022) showed that *TaGL3.3-5B-C* allele is associated with larger and heavier wheat grains. Though studies indicate the role of these genes in determining grain length and width, the information on how EC and N affect the expression of these genes is scarce in wheat. Hence, the present study aimed to understand the effects of EC and N dosage on 1) grain ionome in six wheat varieties in season 1 (rabi 2019-2020) 2) grain yield parameters, grain morphology, and expression of genes related to grain morphology in maturing spikelets of mega variety HD 2967 in season 2 (rabi 2020-2021). The experiments were laid out in the open-top chambers (OTC) facility of the Division of Plant Physiology, ICAR-IARI, New Delhi.

Materials and methods

Plant cultivation

Six bread wheat varieties: RAJ 3765, HD 2967, GW 322, HI 1500, B.T. Schomburgk (BTS) and Gulyas-early (GE) were grown in the open-top chambers of the Division of Plant Physiology, ICAR-IARI, New Delhi during the rabi 2019-20 cropping season.The differences in yield and N use efficiency (NUE) reported earlier (Adavi and Sathee 2021; Mahmoud et al. 2020)were used as the selection criterion for the varieties. For further study, HD 2967 variety was grown during the rabi 2020–21 cropping season. Approximately, 4 kg of the potting mixture (soil, sand, and compost in a 2:1:1 ratio) was filled into each pot with an open diameter of 20 cm (8 inches). Six seeds were sown into each pot at a depth of 5 cm, and the field capacity of the soil was maintained by regular irrigation. The number of plants was thinned to three per pot and was maintained until harvest to record various morpho-physiological and biochemical observations. A completely randomized (CRD) experimental design was followed, consisting of three replications for each treatment.

CO_2 exposures × N treatments

Twenty-four days after sowing (DAS), CO_2 enrichment was provided to well-established plants raised in open-top chambers (OTC). Half the pots were exposed to ambient CO_2 levels (400 ±10 ppm, AC) and the other half to elevated CO_2 levels (700±10 ppm, EC). The steady levels of CO_2 in the chambers were regularly monitored and maintained from sunrise to sunset using an infrared gas analyzer (IRGA) in the sensor head of a portable photosynthesis system (LI-6400 XT, LiCOR, Lincoln, Nebraska, USA). The study consisted of two levels of N: Optimum N (500 mg/pot, ON) and High N (625 mg/pot, HN). As per the standard package of practices, single superphosphate (706 mg/pot) and muriate of potash (190 mg/pot) was applied as a basal dose in all pots.

Determination of grain ionome

The impact of CO_2 and N treatments on the grain ionome was studied during *rabi* 2019-20. The nitrate content in grains was extracted and estimated as described earlier (Chopin et al. 2007). The oven-dried finely ground plant material was sieved through a 20-mesh sieve. Total N content present in grains was estimated by the micro-Kjeldahl method (Kjeldahl 1883). For estimation of phosphorous (P), potassium (K), sodium (Na), calcium (Ca), and iron (Fe) in grains, oven-dried finely ground grains were digested using di-acid (concentrated nitric acid and concentrated per chloric acid in 10:3 ratio). Upon cooling the digested mixture to room temperature, double distilled water was used to make up the volume to 50 ml and filtered using quantitative ashless filter paper (Whatman no. 42, Whatman international limited, Springfield, Kent, England). The solution was stored until further analysis. The amount of P present in the grains was estimated based on the ascorbic acid method described by Murphy and Riley (1962). The amount of K, Na, Ca, and Fe present in grains was estimated using flame photometer as described earlier (Lekshmy et al. 2013).

Assessment of yield parameters

The total number of vegetative and productive tillers was counted at physiological maturity. The ear length was measured and expressed in cm. A constant weight of spikes (ears) was achieved by drying at 60 ºC and was expressed in grams. The number of grains per ear was counted and hundred grains weight was recorded from each treatment. A total grain yield from individual plants were recorded and expressed in grams. Each parameter was taken in replications of three plants.

Expression profiling of genes determining grain length and width

The length and width of grains were measured using an aerospace digital caliper with a measuring range of 0-150mm. The measurements were recorded from 9 replications in each treatment and expressed in mm. The impact of CO_2 levels (AC and EC) and N treatments (ON and HN)on the expression of genes related to grain length (*TaGL3* and *TaGL7*), grain width (*TaGW2* and *TaGW6*), sink metabolism (*TaInv*, *TaSuSy* and *TaSnRK*) were studied in the maturing spikelets of HD 2967 at ten days after anthesis. A modified TRIzol-based method was used to isolate the total RNA from the maturing panicles (Goyal et al. 2020). The extracted RNA samples were loaded onto a 1% agarose gel (1 g of agarose in 100 ml of 1X Tris-Acetate-EDTA buffer) to confirm the integrity of RNA. The ratio of A260/A280 was measured using a thermo nanodrop 2000c spectrophotometer to quantify and check the purity of RNA. cDNA was prepared with the isolated RNA as a template using a high-capacity cDNA kit (Thermofisher, Inc). Forward and reverse primers specific to genes (Supplementary Table S1) and Power SYBR® Green Master Mix (Applied Biosystems, USA) were used to perform qRT-PCR on a real-time PCR system (The CFX96 Touch Real-Time PCR System, BIO-RAD LABORATORIES, INC., California, USA). The obtained data were normalized for each transcript using wheat *TaActin* as an internal control (Lekshmy 1 and Jha 2017). The relative fold change values are expressed using a 2-DDC^t based method (Livak and Schmittgen 2001).

Table 1. Primer sequences used in the study

Name	Primer 5'-3'
TaGW6-F	TTGACCAGAACTACTGTGACTC
TaGW6-R	CTATGCCATCGCAATGGAC
Ta GW2-A-F	CATGGGTGCTGCGGAAAGT
Ta GW2-A-R	GTCGGTTGAGCACTCTCCT
TaGL3A-F	AGGCACCTGGAAGATATG
TaGL3A-R	TAAGAGGAGGAGCAACTG
TaGL7-F	TCCTTGACACATCCTTCTACC
TaGL7-R	TGGTTTGATCTGGCTGACTTCAC
Ta SuSY2-F	GTTCGCAAATGGATCTCCCG
Ta SuSY2-R	AGTGCATGCGCAATACAATGAG
Ta CWInv-F	ATGAAGAACGCCGACGTCAA
Ta CWInv-R	CACTGCACATGAGGACGAGA
TaSNRK2-F	GACATTGACGTTGAGAGCAG
TaSNRK2-R	CAGGGATCCATCCACAGGC

Statistical analysis

Two-way analysis of variance (ANOVA) was performed using GraphPad Prism version 8 (La Jolla, California, USA) with CO2 and N as treatment effects to compute adjusted P values and level of significance. The graphs were also made using GraphPad Prism 8.

Results

Effect of EC and N treatments on biomass and yield parameters

The variation in total biomass, grain yield, plant height, number of tillers per plant, number of ears per plant, ear length, ear weight, number of spikelets per ear, 100-grain weight and grain number per ear is presented in Figs. 1-3. Plant height varied significantly based on varieties and its interaction with N treatment and CO_2 levels. In genotypes, GE, BTS and RAJ 3765, the mean value of plant height was significantly higher in EC as compared to AC. In EC-ON treatment, GW322 showed a decrease in plant height in comparison to AC-ON. In HN treatment, significant changes in plant height with respect to EC were shown by HI1500 (increase) and HD 2967/GW322 (decrease). Sidak's multiple comparisons test showed that varietal means were not significantly different in the following comparisons: RAJ 3765 vs. HI1500, RAJ 3765 vs. BTS and HD 2967 vs. GW322 (Fig. 1A).

Number of vegetative and productive tillers per plant were significantly different. With regards to EC, the number of vegetative tillers per plant showed a decrease in a few genotypes (RAJ 3765, HI1500 and GE) and an increase in the other genotypes (Fig. 1B). The effective number of ears also showed a similar trend. Genotypes HD2967, GW322 showed an increase in the number of ears in EC. Sidak's multiple comparisons test showed that varietal means of the number of tillers per plant were significantly different in the following

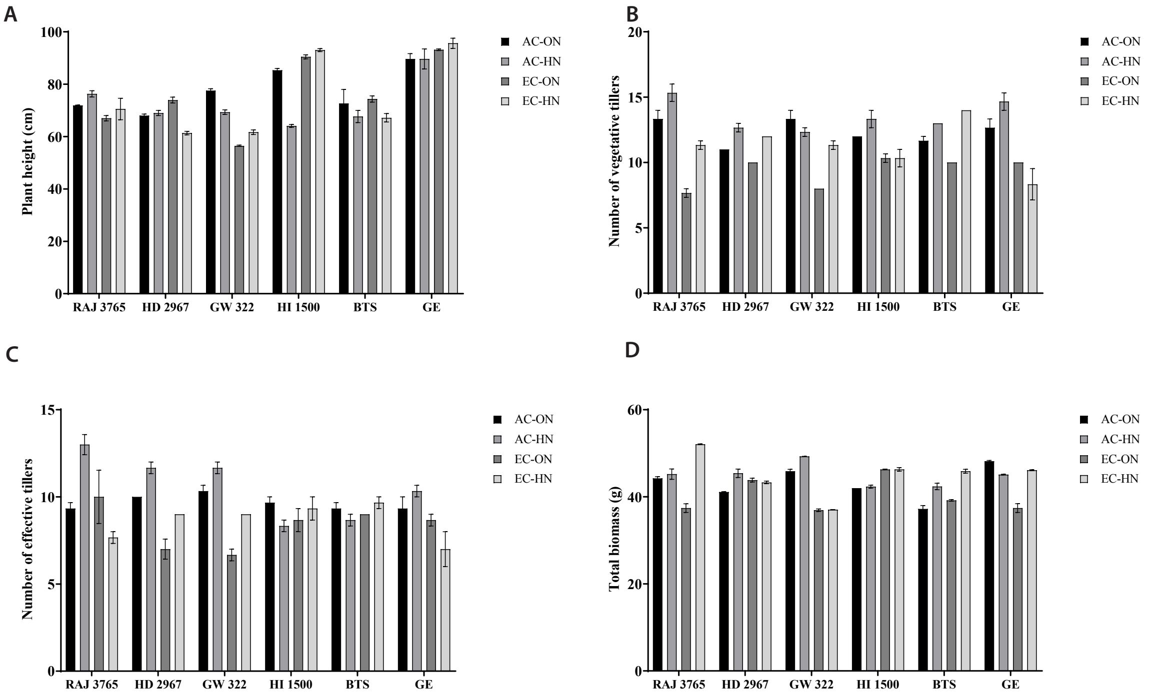

Fig. 1. Interactive effects of elevated [CO_2] and nitrogen supply on plant height (A), total biomass (B), number of tillers (C), and number of ears (D) in wheat. Wheat genotypes RAJ 3765, HD 2967, GW322, HI1500, B.T. Schomburgk (BTS) and Gluyas early (GE) receiving eitheroptimum N (ON: 500 mg/pot) or High N (HN: 625 mg/pot) grown at either ambient CO_2 (AC, 400±10 ppm) or elevated CO_2 (EC, 700±10 ppm) conditions during rabi 2019–20. Values are means (±SE) of 3 biological replicates

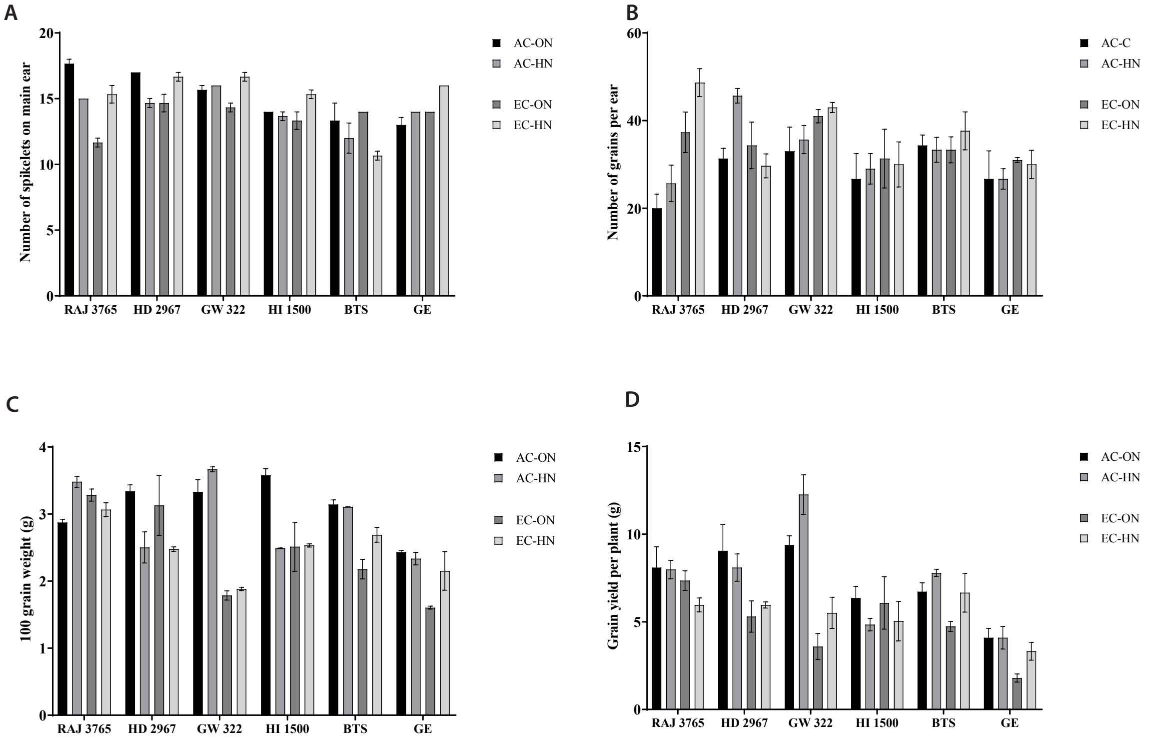

Fig. 2. Interactive effects of elevated [CO_2] and nitrogen supply on number of spikelets per ear (A), number of grains per ear (B), 100 grain weight (C) and grain yield (D) in wheat. Wheat genotypes RAJ 3765, HD 2967, GW322, HI1500, B.T. Schomburgk (BTS) and Gluyas early (GE) receiving eitheroptimum N (ON: 500 mg/pot) or High N (HN: 625 mg/pot) grown at either ambient CO_2 (AC, 400±10 ppm) or elevated CO_2 (EC, 700±10 ppm) conditions during rabi season 2019–20. Values are means (±SE) of 3 biological replicates

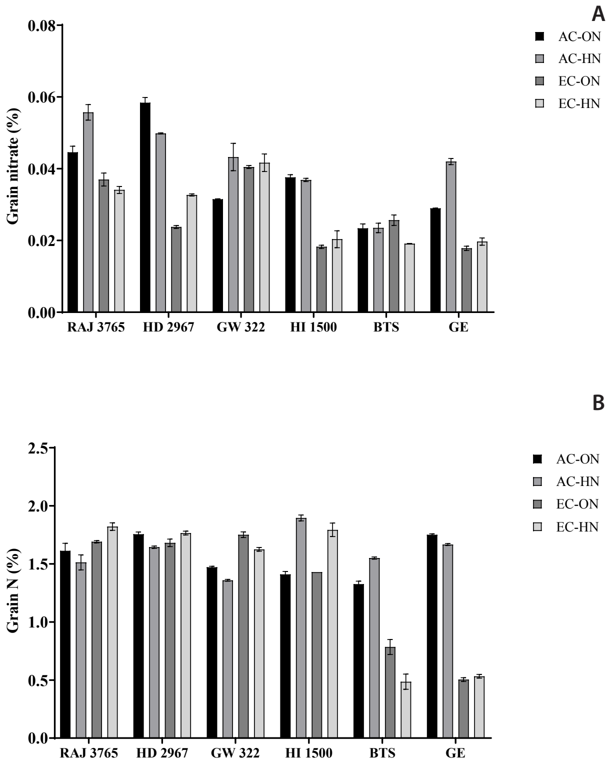

Fig. 3. Interactive effects of elevated [CO$_2$] and nitrogen supply on grain nitrate (A) and nitrogen (B) content in wheat. Wheat genotypes, RAJ 3765, HD 2967, GW322, HI1500, B.T. Schomburgk (BTS) and Gluyas Early (GE) receiving eitheroptimum N (ON: 500 mg/pot) or High N (HN: 625 mg/pot) grown at either ambient CO$_2$ (AC, 400±10 ppm) or elevated CO$_2$ (EC, 700±10 ppm) conditions during rabi season 2019–20. Values are means (±SE) of 3 biological replicates

comparisons: RAJ 3765 vs. HI1500, HD 2967 vs. HI1500, HD 2967 vs. BTS, HD 2967 vs. GE, GW322 vs HI1500 and HI1500 vs BTS. Sidak's multiple comparisons test showed that varietal means of the number of ears per plant were significantly different in the following comparisons: RAJ 3765 vs. BTS, RAJ 3765 vs. GE and HD 2967 vs. RAJ 3765 (Fig. 1C).

Total biomass accumulation (at harvest) was significantly different with variety (V), N treatments (N), CO2 levels (C), and their interactions. In genotypes GE, GW322, RAJ 3765, HI1500 and HD 2967, the mean value of biomass was significantly different between AC and EC. The average biomass was higher in the EC treatment of HI1500 and HD 2967 than that in the control treatment. RAJ 3765 showed the highest biomass accumulation amongst all varieties and treatments whereas GW322 showed a decrease in biomass in comparison to HN-AC. Sidak's multiple comparison test showed that varietal means were not significantly different in the following comparisons: RAJ 3765 vs. HI1500, HI1500 vs. GE, GW322 vs. GE, HD 2967 vs. BTS and RAJ 3765 vs. GE. There was an overall increase in total biomass in EC compared to AC (Fig. 1B).

The spikelets per ear (Fig. 2A) and grain number per ear (Fig. 2B) differed significantly in V, N, V x C and V, V x C, respectively. With respect to EC, the number of spikelets per ear showed a significant decrease in AC in all genotypes except a non-significant increase in BTS. Sidak's multiple comparisons test showed that varietal means of spikelets per ear differed in the following comparisons: HD 2967 vs. BTS, HD 2967 vs. GE, GW322 vs. HI1500, GW322 vs. BTS, GW322 vs. GE were significantly different. There was a non-significant increase in 100-grain weight (Fig. 2C) in EC compared to AC in genotypes HD 2967, GW322 and HI1500. 100-grain weight was significantly different regarding Variety and N treatment. Sidak's multiple comparisons test showed that varietal means were significantly different in the following comparisons: RAJ 3765 vs. BTS, RAJ 3765 vs. GE, HD 2967 vs. GE, GW322 vs. GE, HI1500 vs. GE and BTS vs. GE. In genotypes HD 2967 and RAJ3765, there was an increase in grain yield in EC (Fig. 2D). Grain yield was significantly different regarding V, C and interactions except for VxN and CxN. Sidak's multiple comparisons test showed that varietal means were quite different in the following comparisons: HD 2967 vs. GE, GW322 vs. BTS, GW322 vs. GE and HI1500 vs. GE. Amongst all the treatment and varieties, AC-HN plants of GW 322 recorded the highest grain yield whereas EC-ON plants of GE recorded the lowest grain yield.

EC and incremental N dosage significantly impacts grain ionome

There was a significant reduction in grain nitrate content under EC conditions (Fig. 3A). EC induced reduction in grain nitrate concentrations was prominent in varieties like GE, HD 2967, and HI 1500. Under EC, two varieties, RAJ 3765 and GW 322 showed a marked increase in grain N content whereas BTS (-55.8%) and GE showed a decrease in grain N content (Fig. 3B). EC generally increased the grain P content while reducing the grain K content (Figs. 4A-B). The highest and lowest grain P was observed in EC-HN of GE and AC-HN of RAJ 3765, respectively. The AC-ON of HD2967 (0.88%) recorded the highest grain K while the lowest was observed in EC-ON of HI 1500 (0.46%). The grain Ca content varied inconsistently (Fig. 4B). Irrespective of N treatment in HI 1500, EC enhanced the grain Ca content by 51%. Generally, EC increased the grain Fe content, prominently in RAJ 3765, HD 2967, GW 322 and HI 1500 by 76–201% (Fig. 5A). The EC-HN of HD 2967 had the highest Fe content (0.04%) while the AC-ON of HI 1500 had the lowest Fe content. Thus, EC and HN had enhanced the grain Fe content, though this difference was variety specific. There was no difference in grain Na content observed amongst treatments, but HD 2967 under EC had 30% lower grain Na (Fig. 5B).

The heatmap representing normalised values of grain nutrient content depicts an interesting pattern. For example, the N content was significantly reduced by EC in ON, while in HN treatment the reduction in N content was

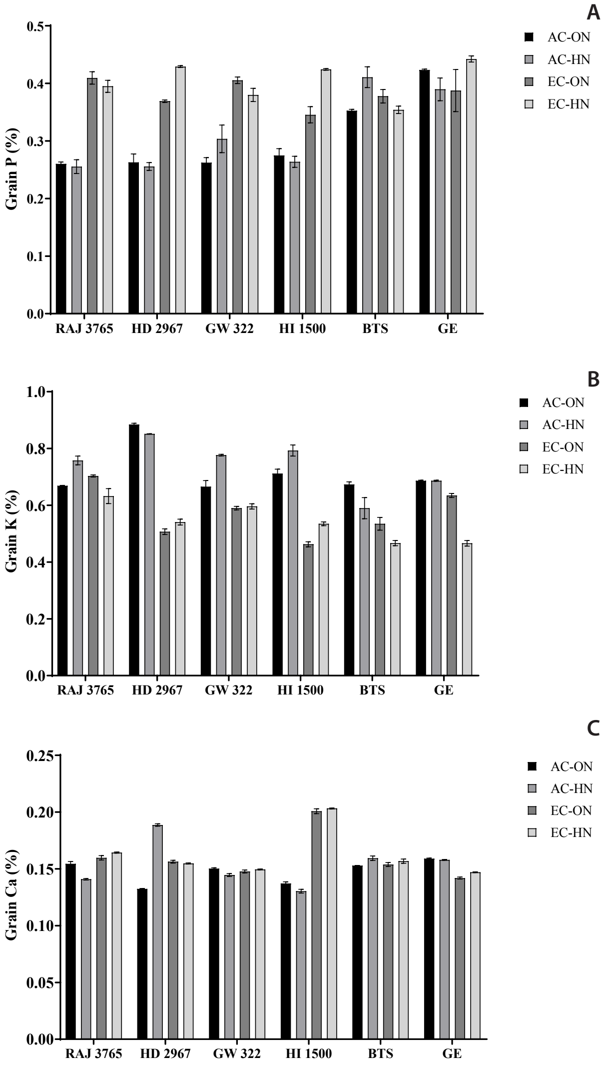

Fig. 4. **Interactive effects of elevated [CO_2] and nitrogen supply on grain phosphorus (A), potassium (B), and calcium (C) content in wheat. Wheat genotypes RAJ 3765, HD 2967, GW322, HI1500, B.T. Schomburgk (BTS) and Gluyas early (GE) receiving eitheroptimum N (ON: 500 mg/pot) or High N (HN: 625 mg/pot) grown at either ambient CO_2 (AC, 400±10 ppm) or elevated CO_2 (EC, 700±10 ppm) conditions during rabi season 2019–20. Values are means (±SE) of 3 biological replicates**

not prominent (Fig. 6). Content of Ca and K also showed a significant reduction in EC, while P and Fe content did not depict a decrease in EC. The grain N content was significantly correlated with K (positive correlation) and Fe (negatively correlated). Grain P content was also significantly correlated with K (negative correlation) and Fe (positive correlation). Potassium content was negatively correlated with Fe and P. The results indicate that N dosage and EC significantly impact grain ionome in wheat genotype HD2967, however, the genotype-specific variation in response is expected.

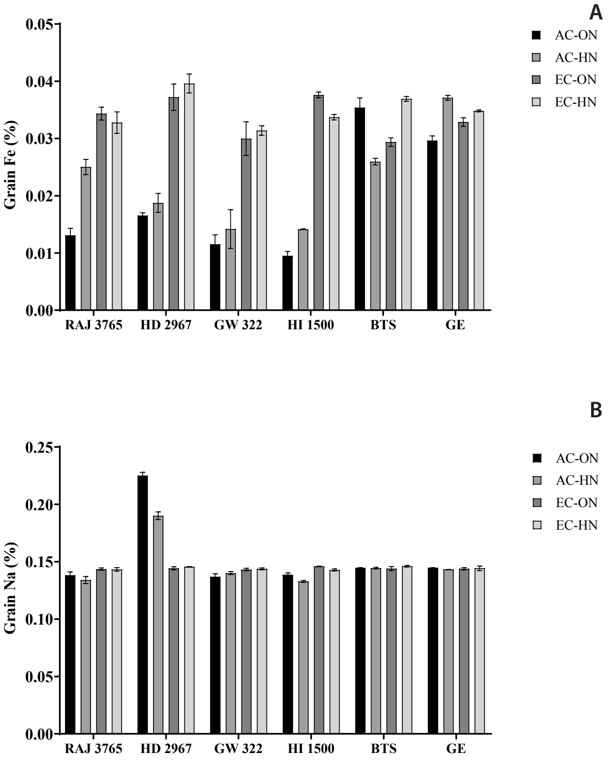

Fig. 5. **Interactive effects of elevated [CO_2] and nitrogen supply on grain iron (A) and sodium (B) content in wheat. Wheat genotypes RAJ 3765, HD 2967, GW322, HI1500, B.T. Schomburgk (BTS) and Gluyas early (GE) receiving eitheroptimum N (ON: 500 mg/pot) or High N (HN: 625 mg/pot) grown at either ambient CO_2 (AC, 400±10 ppm) or elevated CO_2 (EC, 700±10 ppm) conditions during rabi season 2019–20. Values are means (±SE) of 3 biological replicates**

Effect of EC on grain morphology and associated gene expression

The mean values of grain length and grain width were significantly higher in EC than in AC in both the N treatments. The mean value of grain length was significantly higher in EC than in AC in ON and HN treatments (Fig. 6). Impact of different N and CO_2 treatments on the expression of TaGW6, *TaGW2, TaGL3, TaGL7, TaInv, TaSuSy* and *TaSnRK* was studied in maturing spikelets of HD 2967 at ten days after anthesis (Fig. 7). The relative fold change of expression was calculated for all the treatments in comparison to AC-ON. In HD2967, EC-HN treatment recorded a generally higher expression for *TaInv* and *TaSnRK*. In the case of *TaSnRK*, the expression was promoted by EC in HN treatment. Generally, the expression of *TaGL3* and *TaGL7* were upregulated under EC. The highest expression of these genes was observed in EC-HN. There was a 1.72-fold higher expression of TaGL3 and a 3.88-fold higher expression of *TaGL7* in EC-HN while AC-HN downregulated the expression of *TaGL3* by1.07-fold. EC has reduced the expression of *TaGW2* and *TaGW6* genes with lowest EC-ON. There was a 3.03-fold lower expression of *TaGW2* in AC-HN and a 3.16-fold in EC-ON. EC had also downregulated the

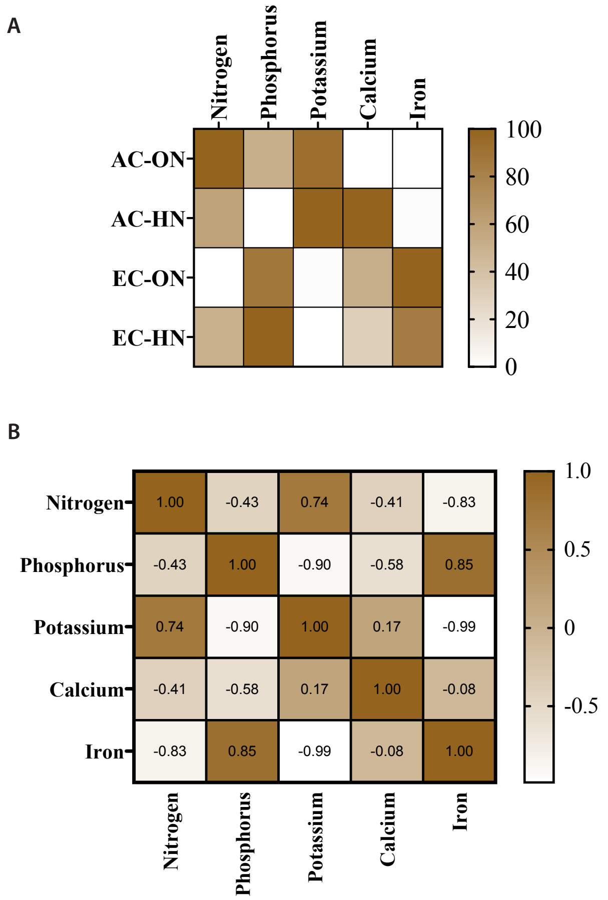

Fig. 6. Heatmaps showing the effect of elevated [CO_2] and nitrogen supply on normalised values of grain ionome (A) and Pearson correlation matrix showing relationship between content of different mineral nutrients(B)in the grains ofwheat genotypeHD 2967receiving eitheroptimum N (ON: 500 mg/pot) or High N (HN: 625 mg/pot) grown at either ambient CO_2 (AC, 400±10 ppm) or elevated CO_2 (EC, 700±10 ppm) conditions during rabi season 2019–20. Values are means (±SE) of 3 biological replicates

expression of TaGW6 by 6.77-fold in ON, and 3.48-fold in HN as compared to AC-ON.

Discussion

To feed the growing population, doubling the wheat production by 2050 seems less likely as the actual increase in yield is only 1.3% per year globally compared to an expected increase of 2.4% per year (Ray et al. 2012). Further, approximately 39% of areas under wheat cultivation have shown no increase in grain yields in the last decade (Ray et al. 2012). One of the significant impacts on wheat yields in future scenarios would be the effects of rising CO_2 levels and climate change. The impact of EC on nutritional security, causing hidden hunger is as evident as its effects on grain production. The continuous rise in CO_2 levels is expected to affect the nutritional quality of C3 crops like wheat (Myers et al. 2014). Various experiments conducted by exposing wheat crop to EC shows a 10% decline in grain protein and a 5–10% decline in essential micronutrients like iron and zinc (Loladze 1 et al. 2019; Myers et al. 2014).

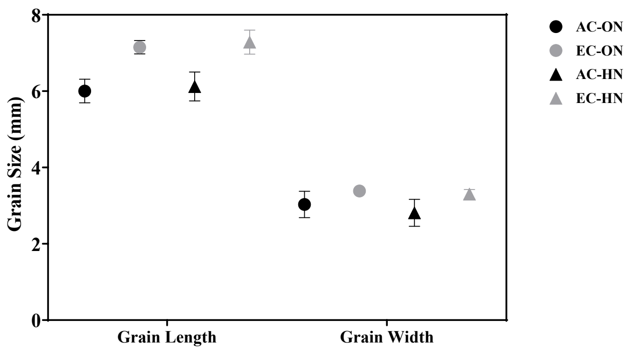

Fig. 7. Interactive effects of elevated [CO_2] and nitrogen supply on grain length and grain width in wheat genotypeHD 2967receiving eitheroptimum N (ON: 500 mg/pot) or High N (HN: 625 mg/pot) grown at either ambient CO_2 (AC, 400±10 ppm) or elevated CO_2 (EC, 700±10 ppm) conditions during rabi 2020–21. Values are means (±SE) of 9 biological replicates

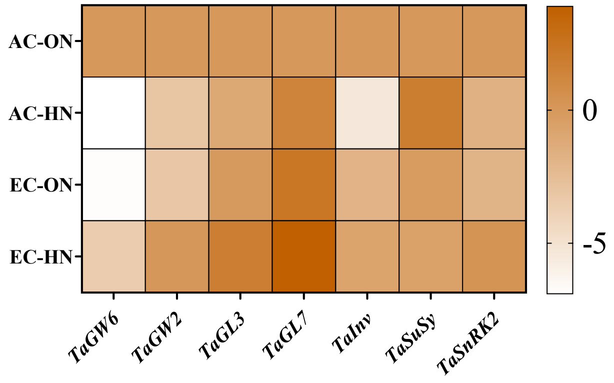

Fig. 8. Interactive effects of elevated [CO_2] and nitrogen supply on the expression of genes (*TaGL3, TaGL7, TaGW2* and *TaGW6, TaSuSy, TaSnRK2*) associated with grain size (length and width) and sink metabolism in HD 2967 genotype receiving eitheroptimum N (ON: 500 mg/pot) or High N (HN: 625 mg/pot) grown at either ambient CO_2 (AC, 400±10 ppm) or elevated CO_2 (EC, 700±10 ppm) conditions during rabi 2020–21. Values are means (±SE) of 3 biological replicates. Relative fold (Log2) expression values (with respect to AC-ON)in maturing spikelets sampled at 10 days after anthesis. TaActin was used as an internal control for the normalization of the data

The present investigation was conducted to determine the effect of reproductive stage N application on grain yield and grain ionome of six bread wheat genotypes under EC. Another aim was to understand the interactive effects of EC and N dosage on the expression of genes involved in grain filling and grain morphology in panicles of HD 2967 wheat genotype. The changes in growth, yield parameters, grain ionome, and grain N content were examined in response to two N levels (ON: 500 mg/pot and HN: 625 mg/pot) and atmospheric CO_2 enrichment in six wheat varieties.

Though the genotypic response was variable, grain ionome (N, P, K, Ca, Fe) showed a general decrease in EC-grown wheat plants. Grain protein content also showed a decreasing trend, while grain Na content showed a 1.8% increase in EC compared to AC. The global supply of nutrients is expected to decrease by 14-20% in the case of protein, Fe, and Zn relative by 2050 (Beach et al. 2019). Deprecation in Zn in rice, wheat, maize, sorghum, soybeans, and field pea is a global nutritional security issue affecting 17% of the world population (Myers et al. 2014). Similarly, deprecation in Fe availability will affect the 10–15% of the worldwide population (Smith et al. 2017) . EC significantly decreased the concentration of Ca and P in several crop plants (Myers et al. 2014). A significant decrease in wheat grain concentration of K and Ca by exposure toECwas also reported earlier (Li et al. 2019). The genotypic differences in grain ionome distribution are worth exploring, as we found significantly different genotypic responses in growth and biochemical aspects. The multi-generational and long-standing plant responses to EC are dependent on the availability of macro and micronutrients and their efficient utilization. The disparities in the magnitude of parameters among the current results and w.r.to other controlled environment studies could be attributed to the variation in the availability of nutrients. The responses of plants in hydroponics (continuous replenishment of nutrients) (Padhan et al. 2020; Lekshmy et al. 2013) and when the amount of soil available for roots to proliferate and absorb nutrients are different.

Previous reports suggest CO_2 enrichment enhances the number of tillers (Wang et al. 2013). The number of vegetative tillers increased in EC grown wheat genotype with a high N supply. This was in contrast to EC-ON treatment, where no enhancement in tiller number was observed. Broberg et al. (2019) reported that a 26% increase in wheat grain yields was mainly due to the number of grains under EC. Similar findings were reported by Högy 1 et al. (2010), where the number of grains per ear had increased due to EC, albeit with no change in the number of ears. But the findings in this study showed that EC had reduced the number of grains per ear. The number of grains was reduced by 6.89% in EC-ON and by 20% in EC-HN compared to AC-ON and AC-HN treatments, respectively. Under EC, grain yield varies considerably based on the growth conditions (Wang et al. 2013). Several studies conducted at CO_2 levels ranging from 450 ppm to 800 ppm show that the average grain yields increased in 57 experiments by 10% (Pleijel and Uddling 2011), in 59 experiments by 24% (Wang et al. 2013). Several FACE studies have also reported increasing thousand-grain weight by 3.8%–7.0%(Högy 2 and Fangmeier 2008). In the current study, the grain yields in wheat had risen due to EC and N fertilization by 6.89% to 20%, which was in agreement with the previous reports. The 100 seed weight also increased under EC, irrespective of N treatment, by 14.14% to 30.53%. The observed increase in grain yield was significantly contributed by the higher 100 seed weight as the number of productive tillers (ears) were not affected due to EC.Rising CO_2 levels will increase the rate of photosynthesis,especially in C3 crops like wheat (Ainsworth and Long 2005).Furthermore, the enhanced production of sucrose and hexoses in EC grown plants are correlated to increased spike biomass (Dreccer et al. 2014). We also observed a similar result where EC and incremental N dosage enhanced the weight of ear. But EC did not alter the ear length.

In cereals like wheat, grain size is one of the essential components of final grain weight and is contributed by grain length and width. A study conducted by Marcos-Barbero et al. (2021) showed that grain weight negatively correlated with the number of grains per ear. The size of wheat grains grown under elevated CO_2 is generally smaller(Högy 3 et al. 2009). We found that CO_2 enrichment and N dosage increased the length and width of wheat grains. Under EC, optimal and high N supply increased the grain length by about 19%. The grain width was also increased by 11–17% under optimal and increased supply of N. The grains were more prominent in size as previously reported (Högy et al. 2013).

The expression of genes related to grain length (*TaGL3* and *TaGL7*) and grain width (*TaGW2* and *TaGW6*) were studied at ten days after anthesis. Studies conducted by Yang et al. (2019) have shown that the expression levels of *TaGL3* were higher at eight days after anthesis. It was observed that the expression of *TaGL3* and *TaGL7* was upregulated by EC, which also correlated to an increase in grain length. Studies also indicate that the transcript abundance of *TaGW2* negatively influences the grain width in bread wheat (Spartz et al. 2014). *TaGW6* codes for novel indole-3-acetic acid-glucose hydrolase. In present study, the downregulation of *TaGW2* and *TaGW6* in maturing panicles under EC was also observed. The downregulation of *TaGW2* was correlated to an increase in grain width as *TaGW2* is a negative regulator of grain width.The significant impact of N doseson grain ionome is highly pertinent. Further, the information on the effect of EC and high N application on genes associated with grain morphology and sink metabolism is an important addition to the accumulating evidences on metabolic reprogramming by EC.

Authors' Contribution

Conceptualization of research (LS, VC); Designingof the experiments (LS, VC, SKJ, MP, RRK); Contribution of experimental materials (LS, VC, SKJ, MP); Execution ofexperiments and data collection (SA, DS), Analysis of data and interpretation (SA, LS); Preparation of the manuscript (SA, LS, SA).

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