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DROUGHT STRESS

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Elevated CO_2 modulates the effects of drought and heat stress on plant water relations and grain yield in wheat

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Abstract

To investigate the interactive effects of drought, heat and elevated atmospheric CO_2 concentration ([CO_2]) on plant water relations and grain yield in wheat, two wheat cultivars with different drought tolerance (Gladius and Paragon) were grown under ambient and elevated [CO_2], and were exposed to post-anthesis drought and heat stress. The stomatal conductance, plant water relation parameters, abscisic acid concentration in leaf and spike, and grain yield components were examined. Both stress treatments and elevated [CO_2] reduced the stomatal conductance, which resulted in lower leaf relative water content and leaf water potential. Drought induced a significant increase in leaf and spike abscisic acid concentrations, while elevated [CO_2] showed no effect. At maturity, post-anthesis drought and heat stress significantly decreased the grain yield by 21.3%–65.2%, while elevated [CO_2] increased the grain yield by 20.8% in wheat, which was due to the changes of grain number per spike and thousand grain weight. This study suggested that the responses of plant water status and grain yield to extreme climatic events (heat and drought) can be influenced by the atmospheric CO_2 concentration.

KEYWORDS
abscisic acid, CO_2, drought, high temperature, water status, wheat

1 | INTRODUCTION

The scenarios relevant to future climate change include not only elevated atmospheric CO_2 concentration ([CO_2]), but also extreme climatic events (ECE), such as heat waves and drought episodes. Such ECE are predicted to become more frequent and more intense along with the increased atmospheric CO_2 (Wall, 2001). Studies on interactive effects of ECE and elevated [CO_2] are gaining attention (Zinta et al., 2014, 2018). However, most of studies are focused on the effect of elevated [CO_2] on the plant responses to single ECE. Drought is often accompanied by heat waves, and such combination of heat and drought has distinct effects on plants, compared with each applied separately (Barnabas, Jager, & Feher, 2008; Dobra et al., 2010; Prasad, Staggenborg, & Ristic, 2008; Shah & Paulsen, 2003).

Wheat crops are very susceptible to the post-anthesis drought and heat events (Li, Wu, Hernandez-Espinosa, & Pena, 2013; Lu et al., 2014). Drought and heat events cause a large loss in grain yield in wheat, though often occurs in a short term (Hlavacova et al., 2018; Ihsan, El-Nakhlawy, Ismail, Fahad, & Daur, 2016; Mahrookashani, Siebert, Huging, & Ewert, 2017). Drought and heat stress both result in the inhibition of photosynthetic carbon assimilation, reduced membrane integrity, premature senescence and short grain filling process, leading to a significant yield loss (Altenbach, 2012; Dias de Oliveira et al., 2013; Prasad, Pisipati, Momcilovic, & Ristic, 2011). Stomatal conductance (g_s) is the most sensitive physiological process to drought, and abscisic acid (ABA) plays an important role in regulating stomatal aperture during soil drying (Liu, Jensen, & Andersen, 2005). Under drought, a higher stomatal resistance reduces the transpiration rate hereby sustaining plant water status (Liu et al., 2005).

However, this will reduce the cooling effect by transpiration leading to higher leaf temperature, particularly under combined drought and heat stress, hence affecting the photosynthetic capacity (Salehi-Lisar & Bakhshayeshan-Agdam, 2016). Under high temperatures, increased transpiration can hasten the stomatal closure to prevent water loss, simultaneously advancing the decline of photosynthesis (Brestic, Zivcak, Kalaji, Carpentier, & Allakhverdiev, 2012; Zivcak et al., 2013). The responses of g_s and plant water status to drought and heat stress could be affected by environmental $[CO_2]$, which is still rarely clear.

Elevated $[CO_2]$ has direct and indirect effects on plant growth and stress responses in wheat (Ainsworth & Long, 2005; And, Gonzàlezmeler, & Long, 1997; Medina, Vicente, Amador, & Araus, 2016; Varga, Vida, Varga-Laszlo, Hoffmann, & Veisz, 2017). Elevated $[CO_2]$ enhances photosynthate supply which benefits the plant vegetative growth, and it also promotes grain filling resulting in increased grain weight (O'Leary et al., 2014). The stress-mitigating effect of elevated $[CO_2]$ on responses to drought and heat stress is attributed to lower g_s, higher water use efficiency, enhanced photosynthetic enzyme activities and increased levels of defence molecules (AbdElgawad, Farfan-Vignolo, de Vos, & Asard, 2015; Meng et al., 2013; Rakic, Gajic, Lazarevic, & Stevanovic, 2015; Xu et al., 2013). In addition, elevated $[CO_2]$ reduces the impact of drought and heat stress on sugar and amino acid metabolism, but not on fatty acids (Zinta et al., 2018).

To gain a mechanistic understanding of drought and heat effects under a future climate, the changes of plant water relations should be investigated. In this study, the plant water relation parameters and grain yield were analysed in two wheat cultivars exposed to combination of heat and drought under ambient and elevated $[CO_2]$. It was hypothesized that the interactive effects of post-anthesis heat and drought on plant water relations are changed by the elevated $[CO_2]$, which is also related to the grain yield formation in wheat.

2 | MATERIALS AND METHODS

2.1 | Experimental set-up

A pot experiment was conducted from January to May 2016 in CO_2 control greenhouses in University of Copenhagen, Taastrup, Denmark. Two wheat cultivars were used, that is a drought-tolerant cultivar Gladius and a drought-susceptible cultivar Paragon. Four selected seeds were sown per pot (25 cm in height and 15.2 cm in diameter) filled with 2.4 kg peat material (Sphagnum, 32% organic matter, pH = 5.6–6.4 and EC = 0.45 ms/cm). Two seedlings were retained after thinning at the three-leaf stage. The environmental $[CO_2]$ was well controlled 24 hr a day during the whole growing season (Figure 1). From sowing, half of the plants were grown in a greenhouse cell under ambient $[CO_2]$ (A, 400 μmol/L), another half under elevated $[CO_2]$ (E, 800 μmol/L). The CO_2 enrichment was achieved by emission of pure CO_2 from a bottle tank, released in one point and distributed in the phytotrons through internal ventilation (Yan, Li, & Liu, 2017). The $[CO_2]$ in the greenhouse was monitored every 6-seconds by CO_2 Transmitter Series GMT220 (Vaisala, Helsinki, Finland) during the whole growing season. The pots were watered daily until the beginning of drought treatment. At the jointing stage, all plants were drip irrigated with nutrient solution (2 g N, 1 g P and 1.4 g K for each pot). The climatic conditions in the greenhouses were set at: 24/16°C for day/night temperature, 16 hr photoperiod, >500 μmol m^{-2} s^{-1} photosynthetic active radiation supplied by sunlight plus LED lamps.

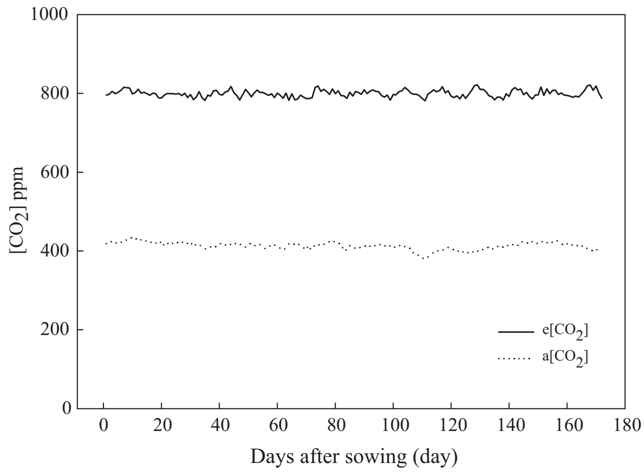

FIGURE 1 Actual atmospheric CO_2 concentration ($[CO_2]$) in the greenhouse cells designated at $a[CO_2]$ and $e[CO_2]$ during the experiment

2.2 | Treatments

The volumetric soil water content (SWC) for well-watered plants was set at 26%. Just after the anthesis stage, half of the plants in each cell were exposed to the moderate drought treatment (applied by withholding irrigation for 4 days until the SWC reached 16% and maintained for 5 days). When the SWC reached the target level in drought treatments (at the 5th day of drought treatment), half of the pots in each watering treatment were moved to the high-temperature cell for a 5-day heat treatment at 40/35°C for day/night temperature. Therefore, four treatments were established under both ambient $[CO_2]$ and elevated $[CO_2]$: C, the non-stress control; D, drought treatment; H, heat treatment; and DH, combined treatment of drought and heat. Each treatment had five pots (five replications).

2.3 | Stomatal conductance, leaf and air temperature, Chlorophyll content index

Stomatal conductance (g_s) of the flag leaf was measured at 10:00–12:00 am with a leaf porometer (Decagon Devices, Pullman, WA) just after the stress treatment. The leaf temperature and air temperature were measured with three replicates for each leaf using a portable hand-held infrared thermometer (Fluke 566, Everett, WA). The leaf cooling was the difference between leaf and air temperature. When leaf temperature was higher than air temperature, the leaf cooling was negative. The chlorophyll content index (CCI) was measured with a CCM-200 (Opti-Science, Tyngsboro, MA) on the same leaf for g_s and leaf temperature measurements.

2.4 | Water status parameters

Midday leaf water potential (Ψ_l) was measured with a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA) on the flag leaf after g_s and leaf temperature measurements. Relative water content (RWC) of the flag leaf was determined following the protocol of Jensen et al. (2000).

2.5 | Abscisic acid concentration in spike and leaf

Fresh leaf and spike samples were harvested just after stress treatment, which were ground in liquid nitrogen. The sample (40 mg) was added into 1 ml Milli-Q water and shaken overnight at 4°C to extract ABA. The extract was centrifuged at 14,000 *g* for 10 min at 4°C. The supernatant was used to determine the ABA concentration with ELISA according to the protocol of Asch (2000).

2.6 | Grain yield

At maturity, the grain yield and yield components were determined with four replicates for each treatment, including spike number per pot (SN), grain number per spike (GNPS) and thousand grain weight (TGW). The harvest index (HI) was calculated as the ratio of grain yield per plant to shoot dry weight per plant.

2.7 | Statistical analysis

All data were firstly tested for homogeneity of variance with boxplot and subjected to three-way ANOVA to assess the effects of cultivar, atmospheric [CO_2] and stress treatments, using the software of SPSS 20.0 (IBM Electronics, New York,NY). Regression analyses were used to determine the relationships between Ψ_l and RWC.

3 | RESULTS

3.1 | Stomatal conductance, leaf cooling and chlorophyll content index

The output of three-way ANOVA indicated significant effects of [CO_2] elevation and stress treatments on g_s in two wheat cultivars (Table 1). Drought stress (D) and the combination of drought and heat (DH) significantly reduced the g_s of flag leaf, compared with the non-stress control (C) (Figure 2). However, heat stress (H) increased the g_s compared with the control, except for Gladius under ambient [CO_2]. In drought-sensitive cultivar Paragon, the additive effect of D and H was found in DH treatment. In both cultivars, elevated [CO_2] significantly decreased the g_s of C and DH plants, compared with ambient [CO_2]. Interestingly, D and H plants showed higher g_s under elevated [CO_2] in Gladius, while had lower g_s under elevated [CO_2] in Paragon, compared with that under ambient [CO_2].

TABLE 1 Output of the three-way ANOVA for stomatal conductance (g_s), leaf temperature (LT), air temperature (AT), leaf cooling (LC), chlorophyll content index (CCI), leaf water potential (Ψ_l), relative water content (RWC), spike ABA concentration ($[ABA]_s$), leaf ABA concentration ($[ABA]_l$), shoot dry matter (SDM), grain yield (GY), harvest index (HI), spike number per pot (SN), grain number per spike (GNPS) and thousand grain weight (TGW) of two wheat cultivars (CV) as affected by atmospheric [CO_2] (CO_2) and drought/heat treatments (T)

Factor	g_s	LT	AT	LC	CCI	Ψ_l	RWC	$[ABA]_s$	$[ABA]_l$	SDM	GY	HI	SN	GNPS	TGW
CV	NS	NS	NS	NS	***	***	**	**	*	***	NS	***	NS	**	***
CO_2	***	NS	NS	***	NS	NS	NS	NS	NS	**	***	NS	***	**	NS
T	***	**	***	***	***	***	***	***	**	***	***	***	***	***	***
CV x CO_2	NS	NS	NS	NS	*	NS	NS	NS	**	NS	NS	NS	**	NS	**
CV x T	***	***	*	**	***	***	NS	***	NS	NS	**	**	***	NS	***
CO_2 x T	**	**	***	**	NS	**	**	NS	**	NS	NS	NS	NS	NS	NS
CV x CO_2 x T	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Notes. NS: not significant.

The data are presented in Figures 1, 2, 3, 4, 6, 7 and 8.

*, **, and ***Significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

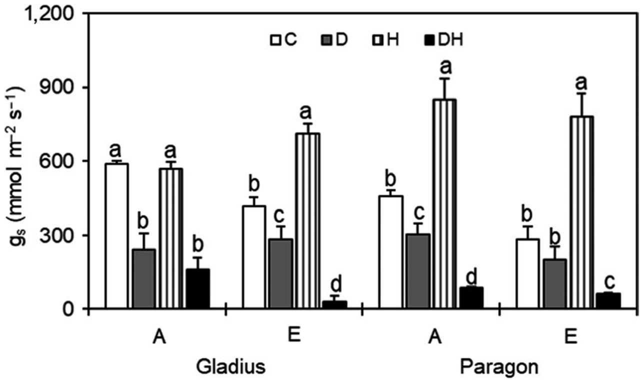

FIGURE 2 Stomatal conductance (g_s) of flag leaves in wheat (cv. Gladius and Paragon) as affected by [CO_2] (A, 400 ppm; E, 800 ppm), drought, heat and their interaction. C, the control; D, drought treatment; H, heat treatment; DH, combined treatment of drought and heat. Different letters in the same sub-group of columns indicate significant difference at p < 0.05 level

Leaf and air temperatures were significantly increased by heat stress and the combination of heat and drought stress relative to the control in both cultivars (Figure 3). The leaf temperature in Paragon was higher than that of Gladius under H and DH, indicating that the drought-tolerant cultivar Gladius showed less sensitivity to heat stress than Paragon. The elevated [CO_2] treatment had no significant effect on leaf and air temperatures under stress and non-stress conditions. The leaf cooling was positive in all non-stress control and heat treatments in both cultivars; however, it was negative in the combined treatment of heat and drought, except for Gladius under ambient [CO_2]. In addition, the leaf cooling was positive in drought-stressed plants in Paragon, whereas the negative leaf cooling was observed in Gladius under elevated [CO_2].

Results of a three-way ANOVA indicated that drought and heat stress treatments had significant effects on CCI, whereas the [CO_2] elevation did not significantly affect CCI (Table 1; Figure 4). There was a significant difference in CCI between Gladius and Paragon. Compared with the control, CCI was significantly reduced by single stress and the combination of heat and drought, except the one in Gladius under elevated [CO_2].

3.2 | Leaf water potential and relative water content

In both cultivars, the Ψ_l of flag leaf was significantly lower in all stressed plants compared with the non-stressed plants (Figure 5). Also, the lowest Ψ_l was found in DH plants, followed by D plants in the two cultivars. The Ψ_l of flag leaf was not significantly affected by [CO_2] elevation in both cultivars. The RWC in flag leaf was remarkably decreased by stress treatments, compared with the control. The lowest RWC was found in D and DH plants under both ambient and elevated [CO_2]. The DH plants had higher RWC under elevated [CO_2] than that under ambient [CO_2] in both cultivars, which might be due to the lower g_s and transpiration caused by [CO_2] elevation. A positive linear relationship between Ψ_l and RWC was found across four treatments in these two cultivars (Figure 6).

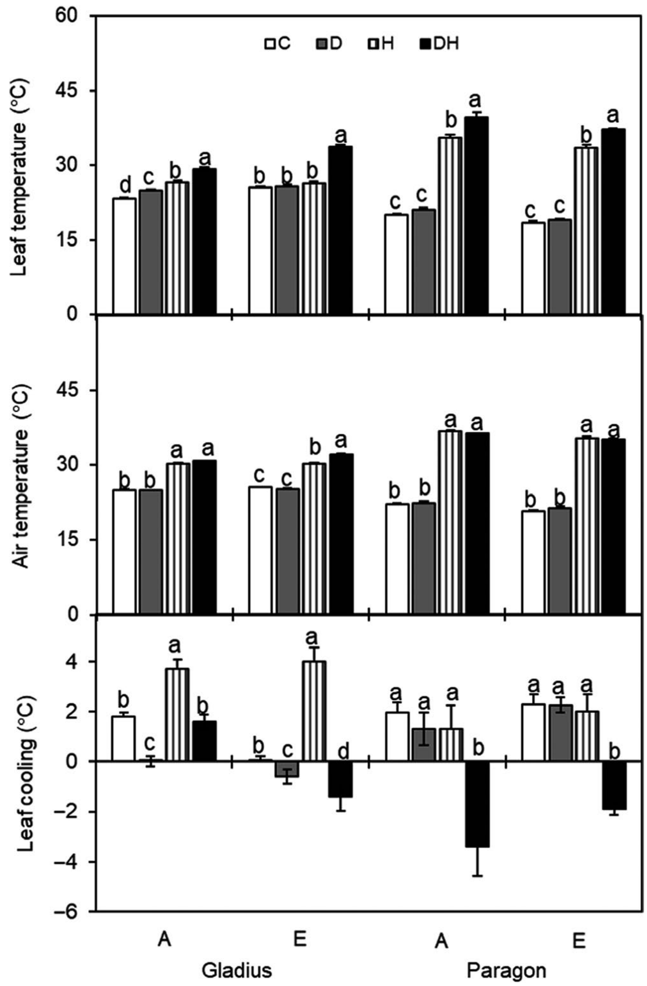

FIGURE 3 Leaf temperature, air temperature and leaf cooling of flag leaves in wheat (cv. Gladius and Paragon) as affected by [CO_2] (A, 400 ppm; E, 800 ppm), drought, heat and their interaction. C, the control; D, drought treatment; H, heat treatment; DH, combined treatment of drought and heat. Different letters in the same sub-group of columns indicate significant difference at p < 0.05 level

3.3 | Spike and leaf abscisic acid concentration

The abscisic acid (ABA) concentration in leaf was significantly enhanced by drought stress compared with the control in two cultivars under ambient [CO_2] (Figure 7). The [ABA] in spike and leaf was lower under elevated [CO_2] than that under ambient [CO_2] in Gladius; however, the opposite tendency was found in Paragon regards on spike and leaf. Exposed to combination of drought and heat stress, these two cultivars showed different responses of spike and leaf [ABA], for example. Paragon had a significant increase in spike and leaf [ABA] under DH treatment, in relation to the control, while the changes in [ABA] were slight in Gladius exposed to DH treatment.

3.4 | Grain yield

The shoot dry matter (SDM) was significantly reduced by drought, heat and their combination compared with the control, while

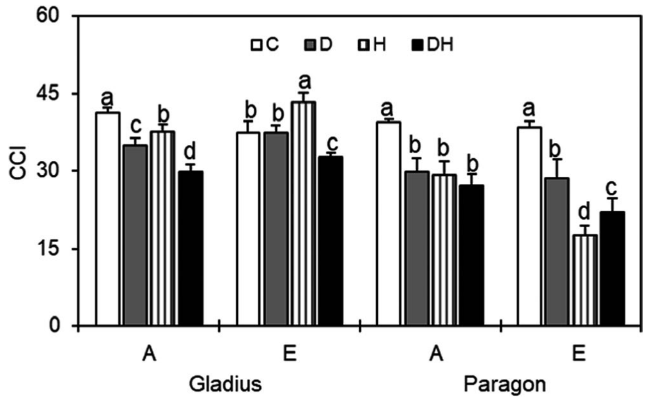

FIGURE 4 Chlorophyll content index (CCI) of flag leaves in wheat (cv. Gladius and Paragon) as affected by $[CO_2]$ (A, 400 ppm; E, 800 ppm), drought, heat and their interaction. C, the control; D, drought treatment; H, heat treatment; DH, combined treatment of drought and heat. Different letters in the same sub-group of columns indicate significant difference at $p < 0.05$ level

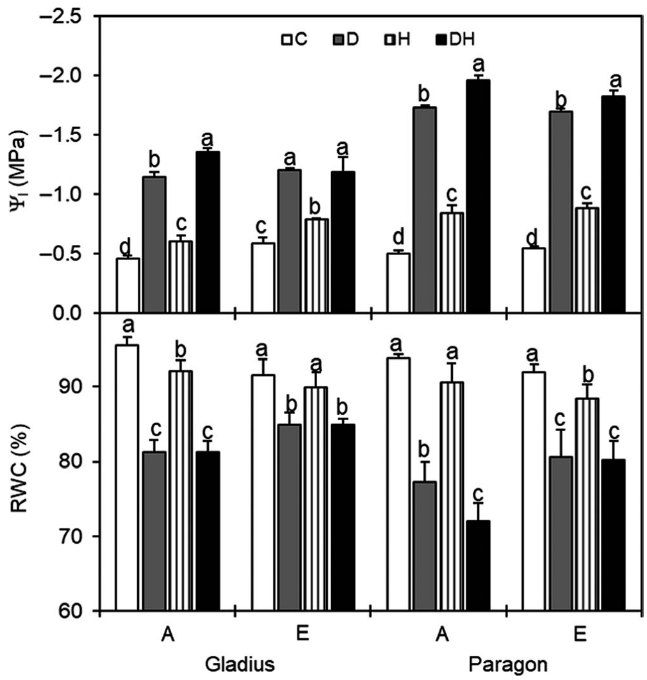

FIGURE 5 Leaf water potential (ψ_l) and relative water content (RWC) of flag leaves in wheat (cv. Gladius and Paragon) as affected by $[CO_2]$ (A, 400 ppm; E, 800 ppm), drought, heat and their interaction. C, the control; D, drought treatment; H, heat treatment; DH, combined treatment of drought and heat. Different letters in the same sub-group of columns indicate significant difference at $p < 0.05$ level

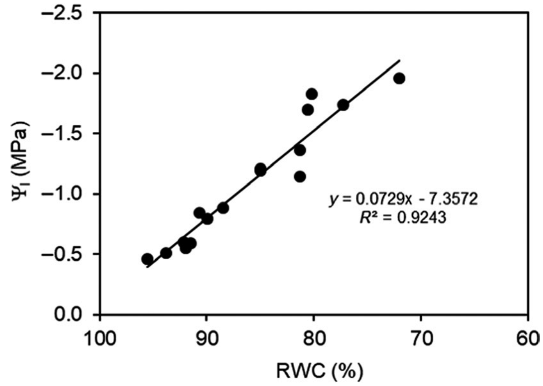

FIGURE 6 Correlation between leaf water potential (ψ_l) and relative water content (RWC) of flag leaves in wheat under various treatments

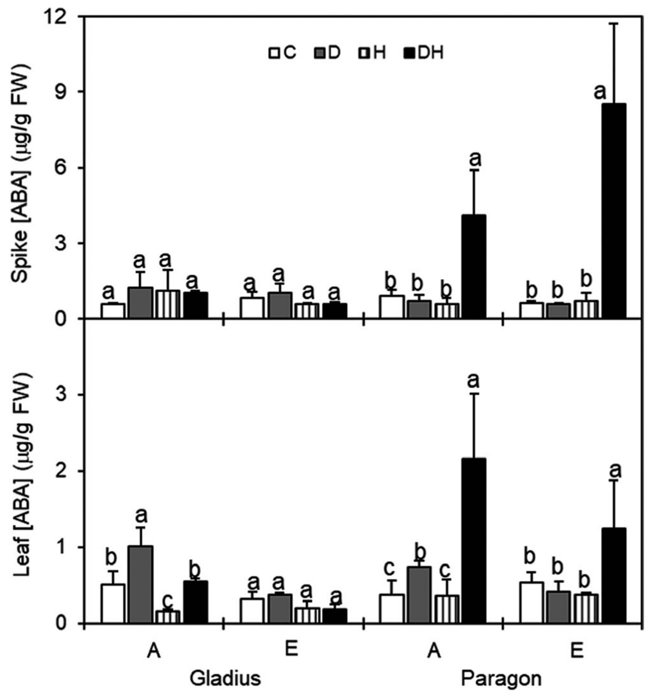

FIGURE 7 Concentration of abscisic acid (ABA) in spike and flag leaf in wheat (cv. Gladius and Paragon) as affected by $[CO_2]$ (A, 400 ppm; E, 800 ppm), drought, heat and their interaction. C, the control; D, drought treatment; H, heat treatment; DH, combined treatment of drought and heat. Different letters in the same sub-group of columns indicate significant difference at $p < 0.05$ level

increased by $[CO_2]$ elevation (Figure 8). It should be noted that DH plants had a similar SDM as D and H plants in two cultivars, indicating that the combined stress did not aggravate the reduction in SDM compared with the single stress. For both cultivars, the highest grain yield (GY) was in C treatment, followed by D and H treatment, and the lowest GY was found in DH treatment. The GY was increased by 11.9%–23.8% with $[CO_2]$ elevation under non-stress condition, while the elevated $[CO_2]$-induced increase in GY was higher under stress conditions than that under non-stress condition. The stress treatments had different effects on the harvest index (HI) in both cultivars, for example the HI was only significantly reduced by the combined stress in Gladius, while the HI was significantly decreased by single and combined treatments of drought and heat stress, compared with the control.

The output of three-way ANOVA showed that the spike number per pot (SN) was significantly affected by stress treatments

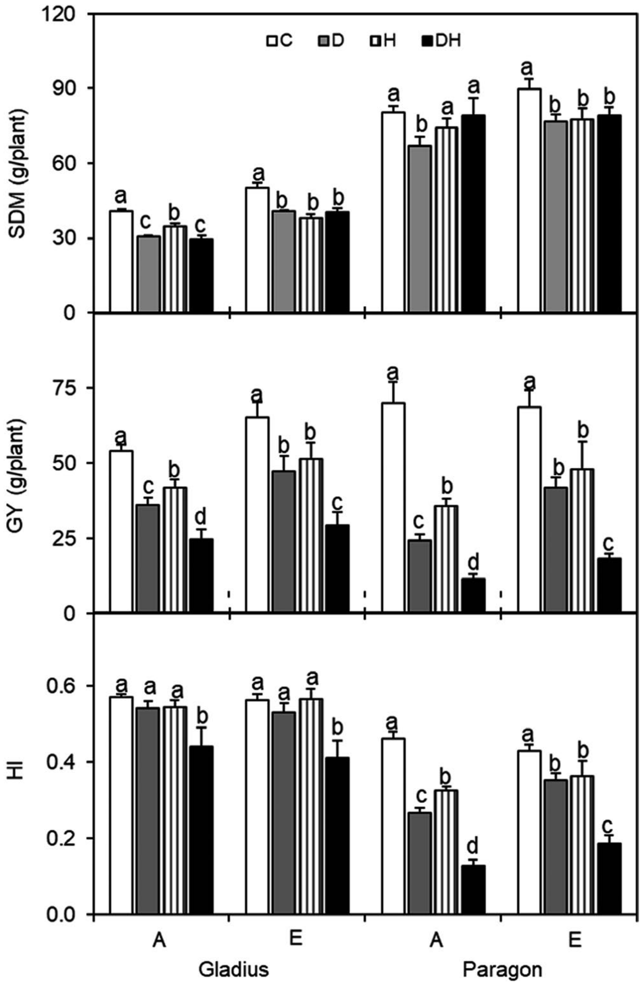

FIGURE 8 Shoot dry matter (SDM), grain yield (GY) and harvest index (HI) in wheat (cv. Gladius and Paragon) as affected by [CO_2] (A, 400 ppm; E, 800 ppm), drought, heat and their interaction. C, the control; D, drought treatment; H, heat treatment; DH, combined treatment of drought and heat. Different letters in the same sub-group of columns indicate significant difference at $p < 0.05$ level

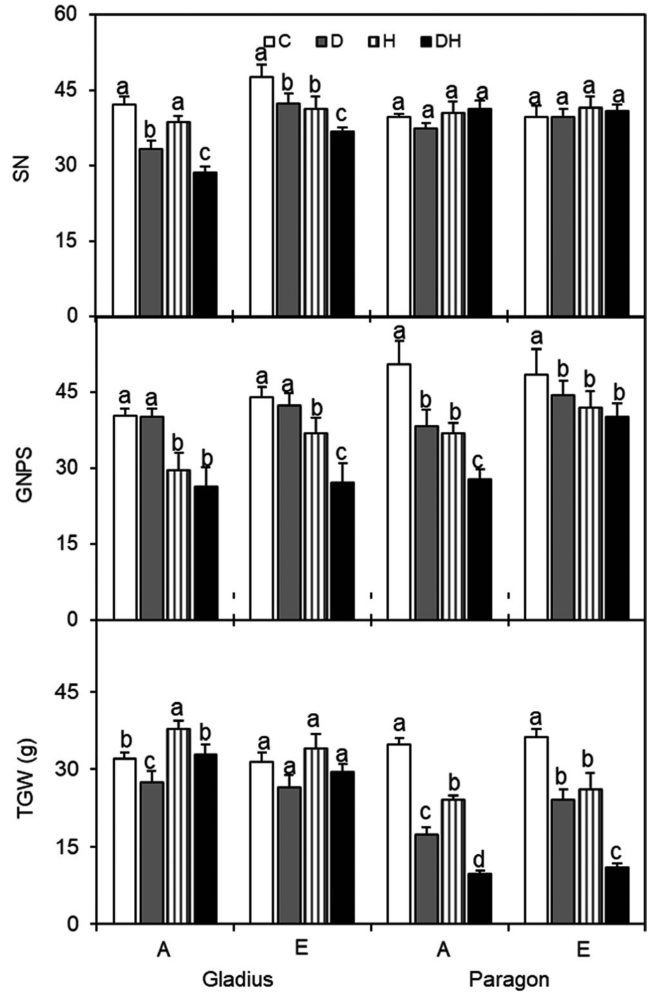

FIGURE 9 Spike number per pot (SN), grain number per spike (GNPS) and thousand grain weight (TGW) in wheat (cv. Gladius and Paragon) as affected by [CO_2] (A, 400 ppm; E, 800 ppm), drought, heat and their interaction. C, the control; D, drought treatment; H, heat treatment; DH, combined treatment of drought and heat. Different letters in the same sub-group of columns indicate significant difference at $p < 0.05$ level

and [CO_2] elevation (Table 1). The different trends in SN among four treatments were found in two cultivars (Figure 9). The SN was significantly reduced by all stress treatments compared with the non-stress control, and the lowest SN was found in DH treatment in Gladius. However, compared with the control, the SN was only reduced by drought stress in Paragon under ambient [CO_2], while it was not affected by heat and the combination of drought and heat. The GNPS was significantly reduced by D, H and DH treatments, compared with the control in Paragon. However, compared with the non-stress control in Gladius, the GNPS was decreased by H and DH, while it was not affected by D treatment. In addition, the GNPS was significantly enhanced by elevated [CO_2] in relation to the ambient [CO_2] in two cultivars, especially under stress conditions. The TGW was significantly affected by stress treatments, while it was not affected by [CO_2] elevation. Compared with the control, the TGW was decreased by 14.4% and 50.5% under drought stress in Gladius and Paragon, respectively. In addition, the lowest TGW was found in D treatment for Gladius, while the lowest one was in DH treatment for Paragon.

4 | DISCUSSION

Stomata plays an important role in regulation of plant water status and gas exchange between the interior of leaf and the exterior environment (Lawson, 2009), which is very sensitive to the environment cues, such as temperature changes and environmental [CO_2] (Dow & Bergmann, 2014; Engineer et al., 2016). It was found that g_s was lower in non-stressed plants grown under elevated [CO_2] vs. ambient [CO_2], consistent with the common conclusion about elevated [CO_2]-induced stomatal closure (Morison, 1998). Interestingly, in the drought-tolerant cultivar Gladius, the D and H plants had a slightly increased g_s under elevated [CO_2] compared with that under ambient [CO_2], while for the drought-susceptible cultivar Paragon, the g_s was reduced by [CO_2] elevation in D and H plants. This indicated that Gladius can still increase the CO_2 uptake by increasing stomatal opening when environmental [CO_2] was enhanced under stress conditions, showing that the drought-tolerant cultivar possessed a higher adaptation capacity to drought and heat stress. Also, the increased g_s in Gladius under high-temperature stress could help wheat plants to decrease the leaf temperature, hence mitigating the direct damage of heat stress on leaf (De Boeck, De Groote, & Nijs, 2012). In the present study, the negative leaf cooling was found in DH plants of Paragon, indicating that the plants had lost the capacity of cooling by stomatal regulation under drought and heat stress. However, the DH plants still had a positive leaf cooling in Gladius under ambient [CO_2], suggesting that Gladius can decrease the leaf temperature by enhancing g_s when exposed to the combination of drought and heat stress.

The drought and heat stress both depress the chlorophyll content in leaf of wheat via accelerating the degradation of chlorophyll, resulting in premature senescence (Altenbach, 2012; Dias de Oliveira et al., 2013; Prasad et al., 2011). The elevated [CO_2] effects on chlorophyll content is dose-dependent and plant species-dependent, for example a 23.6% of decrease in chlorophyll content was found in *Pigeon pea* grown under elevated [CO_2] (+150 μmol/L) (Sreeharsha, Sekhar, & Reddy, 2015), while elevated [CO_2] (+400 μmol/L) increased significantly the seasonal mean chlorophyll content in soya bean (Xu et al., 2016). Here, when exposed to drought and heat stress, the leaf chlorophyll content in Gladius was higher under elevated [CO_2] than that under ambient [CO_2], while it was contrary in Paragon. It might be also related to difference in stress tolerance of these two cultivars. The [CO_2] elevation-induced increase in chlorophyll content benefited the plant growth under stress conditions.

The drought-tolerant cultivar Gladius showed a higher Ψ_l and RWC than Paragon when exposed to drought stress. Though the Ψ_l of flag leaf was not affected by [CO_2] elevation, the RWC of drought-stressed plants (D and DH) was increased by elevated [CO_2], in relation to ambient [CO_2]. This revealed that [CO_2] elevation mitigated the drought stress on wheat by maintaining a better plant water status. The [CO_2] elevation caused lower RWC in heat-stressed plants might be due to the higher g_s. Under heat stress, the wheat plants have to compromise between plant water relation and leaf cooling. Nonetheless, when analysed across all stress and [CO_2] treatments, a positive linear relationship between RWC and Ψ_l was observed, which is also consistent with earlier studies (Liu & Stutzel, 2002; Liu et al., 2005). The previous study had well illuminated that both RWC and Ψ_l are negatively correlated to the leaf [ABA] (Li, Li, Yu, & Liu, 2017; Wang, Liu, & Jensen, 2012). The low g_s in plants grown in drying soils or under elevated [CO_2] is associated with high leaf [ABA] (Davies & Zhang, 1991; Li et al., 2017). In the present study, drought induced a significant increase in leaf and spike [ABA], while elevated [CO_2] showed no effect. Notably, in the drought-sensitive cultivar Paragon, the leaf and spike [ABA] were both significantly enhanced by the combination of drought and heat. Such large enhancement in [ABA] is adverse to keep the balance of plant water relations under stress, which indicated that the changes of leaf and spike [ABA] may be related to the genotypic variation in drought response in wheat. It was also found that genotypic variations in ABA accumulation and leaf water relations are associated with leaf desiccation tolerance under soil drying in soya bean (He et al., 2016).

The drought and heat stress after anthesis significantly affect the carbohydrate translocation to grains and depress the grain filling, causing a grain yield loss in wheat (Hlavacova et al., 2018; Liu et al., 2017; Mahrookashani et al., 2017). In the present study, post-anthesis drought and heat stress significantly reduced the grain yield in wheat, and their combination exacerbated this adverse effect. Consistent with earlier studies (Amthor, 2001; Hogy, Keck, Niehaus, Franzaring, & Fangmeier, 2010), a significant increase in grain yield was found in plants grown under elevated [CO_2], compared with that under ambient [CO_2]. It should be noted that the [CO_2] elevation-induced rise in grain yield was higher in stressed plants than non-stressed plants. A modelling study also showed that the [CO_2] elevation (+380 μmol/L) had a greater effect on grain yield if elevated [CO_2] combined with drought stress (Ewert et al., 2002). However, the other study suggested that the grain yield changes depended partly on the CO_2 level and partly on the stage where drought stress applied (Varga et al., 2017). The inconsistent trends were often found in biomass and grain yield in wheat exposed to elevated [CO_2] (Li, Kang, Zhang, & Cohen, 2003; Varga et al., 2017). For example, the elevated [CO_2] (+600 μmol/L) did not affect the grain yield but reduced the shoot biomass slightly (Varga et al., 2017). In the present study, the reduction caused by combination of drought and heat was higher in grain yield than in shoot dry matter, leading to a lower harvest index. In addition, the elevated [CO_2] increased the grain yield by a larger extent than its effect on shoot dry matter in the stressed plants in Paragon. This resulted in an increase of HI in stressed plants grown under elevated [CO_2], compared with that under ambient [CO_2]. However, in Gladius, the HI was just reduced in the combined treatment of drought and heat stress, in relation to the control. The genotypic difference in HI under stress condition can be used as an indicator for the stress tolerance screening in wheat.

The interactive effects of stress treatment and elevated [CO_2] on grain yield can be explained by the changes of grain number per spike and thousand grain weight. These two yield parameters are affected by the processes of seed setting and grain filling in wheat. In the present study, GNPS was significantly influenced by stress treatment and elevated [CO_2], while TGW was only affected by drought and heat stress. It has been reported that TGW was significantly affected by drought, elevated [CO_2] and their interaction (Varga et al., 2017). In addition, their results indicated that the TGW was reduced by elevated [CO_2] in well-watered wheat plants. This could be due to the elevated [CO_2] increased the grain number, and thus, the amount of carbohydrate translocated to each grain was lower, which had unfavourable effects on TGW. It agrees with the increase in GNPS under elevated [CO_2] in this study. In addition, the different responses of spike number in two cultivars to the stress treatments and elevated [CO_2] were found.

Though the reduction in spike number was higher in Gladius than in Paragon under drought and heat stress, the grain yield loss was lower in Gladius. This indicated that the spike number, which was related to the tiller survival rate, was sensitive to the stress treatment, but it had less effects on final yield.

In conclusion, the response of plant water relations to drought and heat stress was modulated by elevated [CO_2] in wheat. Both stress treatments and elevated [CO_2] reduced the g_s, which resulted in lower RWC and Ψ_l. However, the leaf cooling capacity was not only affected by g_s under this condition, which was related to the tolerance to drought and heat in different wheat cultivars. In addition, drought induced a significant increase in leaf and spike [ABA], while elevated [CO_2] showed no effect. At maturity, post-anthesis drought and heat stress significantly reduced, while elevated [CO_2] increased the grain yield in wheat. The interactive effects of stress treatment and elevated [CO_2] on grain yield were due to the changes of grain number per spike and thousand grain weight. This study suggested that the responses of plant water status and grain yield to extreme climatic events (heat and drought) depended on the atmospheric CO_2 concentration.

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