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REGULAR ARTICLE

Effects of elevated atmospheric CO_2 on leaf gas exchange response to progressive drought in barley and tomato plants with different endogenous ABA levels

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Abstract

Background and aims ABA plays an important role in modulating stomatal response to drought and elevated atmospheric CO_2 (e [CO_2]). This study aimed to investigate the effect of e[CO_2] on the response of leaf gas exchange and plant water relations of barley and tomato plants with different endogenous ABA levels to progressive soil drying.

Methods Barley and tomato plants were grown in ambient (a[CO_2], 400 ppm) and e[CO_2] (800 ppm) and subjected to progressive drought stress. Wild type (WT) genotypes (Steptoe barley and AC tomato) and their ABA-deficient mutants (*Az34* barley and *flacca*) were examined.

Results e[CO_2] sensitized the photosynthetic decline with soil drying. Soil-drying induced stomatal closure was affected by [CO_2] in WT genotypes, where e[CO_2] sensitized stomatal closure in barley but retarded it in tomato, whereas such effects were absent in mutants. Compared to a[CO_2], e[CO_2] maintained leaf water potential and improved turgor pressure except in the *flacca* mutant. For the WT genotypes, the stomata became less sensitive to an increase in leaf ABA concentration ($[ABA]_{leaf}$) under e[CO_2] than a[CO_2]; while for both mutants, the stomata was predominately controlled by leaf turgor and not an increase in $[ABA]_{leaf}$ during soil drying.

Conclusion Endogenous ABA level played an important role in modulating the effect of e[CO_2] on stomatal response to soil drying. These findings improve our understanding of the mechanisms of stomatal control in monocot and dicot species responding to a future drier and CO_2-enriched environment.

Keywords CO_2 · Drought · Stomata · ABA · Barley · Tomato

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Introduction

The opening and closure of a stomatal pore under different environmental conditions are controlled by the deformation and turgor of guard cells (Schroeder et al. 2001). Depolarization of the guard cell membrane

potential induces stomatal closure at elevated atmospheric CO_2 concentration ($e[CO_2]$) (Ainsworth and Rogers 2007). Besides, abscisic acid (ABA) has been suggested to play a role in inducing stomatal closure under $e[CO_2]$ (Chater et al. 2015; Tazoe and Santrucek 2015; Engineer et al. 2016). However, to date it remains largely elusive about the relative significance of chemical signal (i.e., ABA) and hydraulic signal (i.e., leaf turgor) in modulating stomatal response to $e[CO_2]$, and further investigations are needed.

It is widely accepted that decreased stomatal conductance (g_s) under drought stress is attributed to the partial stomatal closure induced by root-to-shoot chemical signaling (mainly xylem sap ABA concentration, $[ABA]_{xylem}$) at mild drought (Davies and Zhang 1991; Liu et al. 2005; Yan et al. 2017). Earlier study has revealed that ABA could be synthesized in the root and transported to leaf where triggers a decrease in stomatal aperture and causes lowered transpiration rate while maintaining plant water status during progressive soil drying (Liu et al. 2003; Wilkinson and Davies 2002). Nonetheless, a study indicated that the application of external pressure caused a short term decrease in cell volume, and induced rapid ABA biosynthesis predominantly in the leaf, not in other tissues of angiosperms (Zhang et al. 2018). Likewise, some evidence supports the dominance of foliar ABA biosynthesis during drought stress (McAdam et al. 2016), as the carotenoid precursors for ABA in leaf are most abundant (Manzi et al. 2015). A recent study also documented that ABA appears to be transported predominantly from shoot to root, but a root-derived signal triggers ABA biosynthesis in the leaf (Takahashi et al. 2018).

Soil water deficit has a stronger effect on g_s as compared to $e[CO_2]$, and a larger reduction in g_s is caused under drought associated with $e[CO_2]$ growth environment (Leakey et al. 2006). Some studies have suggested that $e[CO_2]$ could alleviate the negative effects of drought by suppressing g_s and transpiration rate, hereby maintaining a high leaf water potential (Tausz-Posch et al. 2015). However, recent evidence revealed that impaired stomatal control in response to drought stress was observed in plants grown under $e[CO_2]$ (Haworth et al. 2016). During progressive soil drying, the g_s of $e[CO_2]$ plant had a delayed response to soil water deficit as compared with that of ambient CO_2 ($a[CO_2]$) plant (Yan et al. 2017). Furthermore, the g_s reduction in $a[CO_2]$ tomato leaf was mostly induced by an increased $[ABA]_{xylem}$ at moderate soil water deficit; while the g_s was primarily regulated by leaf turgor pressure at $e[CO_2]$ (Yan et al. 2017). Similarly, Liu et al. (2019) found that $e[CO_2]$ retarded the response of leaf gas exchange to progressive soil drying, and declined g_s in $a[CO_2]$ tomato could be controlled by both leaf ABA concentration ($[ABA]_{leaf}$) and $[ABA]_{xylem}$, whereas under $e[CO_2]$, the g_s response was ABA-independent at moderate drought stress. Nevertheless, whether both chemical and hydraulic signals are involved in the g_s regulation under drought stress and $e[CO_2]$ environment still remains largely elusive.

In plant species, there are generally two morphological types of guard cell, either dumb-bell shape arranged parallel along the leaf longitudinally in monocots or kidney shape randomly distributed in dicots (Meidner and Mansfield 1968). Such difference in morphological feature of stomata could induce disparate physiological response to $e[CO_2]$ during progressive soil drying, and the underlying mechanisms on g_s regulation could be different between monocot and dicot plants (Bunce 2004).

Therefore, the objective of this study was to investigate the effects of $e[CO_2]$ on response of leaf gas exchange and plant water relations in barley and tomato plants to progressive soil drying. For each species, two genotypes differing in endogenous ABA level were examined. The *Az34* barley and *flacca* are ABA-deficient mutants and isogenic to Steptoe barley and AC tomato, respectively. Both mutants are impaired in the oxidation of ABA-aldehyde to ABA precursor and have reduced ABA concentrations (Sagi et al. 2002; Sharp et al. 2000; Walker-Simmons et al. 1989). The plants were grown in two atmospheric $[CO_2]$ (400 and 800 ppm) environments and subjected to progressive drought stress by withholding irrigation from the pots. Leaf gas exchange rates, plant water relations, and leaf ABA concentrations were determined during progressive soil drying. It was hypothesized that: 1) $e[CO_2]$ would modulate the response of leaf gas exchange and plant water relation differently in barley (monocot) and tomato (dicot) plants to progressive soil drying; and 2) ABA would be involved in mediating the stomatal response to drought stress and $e[CO_2]$ in the two species.

Materials and methods

Experimental setup

Pot experiments were conducted in climate-controlled greenhouses at Taastrup campus, University of

Copenhagen, Denmark (55°67 N, 12°30 E). The seeds of isogenic barley (*Hordeum vulgare*) Steptoe (wild type, WT) and its respective ABA-deficient mutant (*Az34* barley) were sown on 20th December 2017; and the seeds of isogenic tomato (*Solanum lycopersicum*) (WT, cv. Ailsa Craig) and its respective ABA-deficient mutant (*flacca*) were sown on 7th February 2018. The ABA-deficient mutants were unable to produce as much ABA as the WT genotype in response to soil drying (Holbrook et al. 2002; Martin-Vertedor and Dodd 2011). Both barley and tomato plants were grown in 4 L pots filled with 2.6 kg of peat material (Plugg-och Såjord-Dry matter ca.110 kg m^{-3}, organic matter >95%, pH 5.5-6.5 and EC 1.5-2.5 mS cm^{-1}). Four weeks after sowing, perlite was covered on the soil surface to minimize evaporation and fertilizers as NH_4NO_3 (2.8 g) and H_2KPO_4 (3.5 g) per pot were added together with irrigation water to avoid any nutrient deficiency.

From sowing, the plants were grown in two greenhouse cells with CO_2 concentration of 400 ppm (ambient CO_2, $a[CO_2]$) and 800 ppm (elevated CO_2, $e[CO_2]$), respectively. The desired $[CO_2]$ in the cell was sustained by pure CO_2 emission from a bottled tank, released in one point and distributed evenly by internal ventilation. The $[CO_2]$ in the cells was monitored every 6 s by a CO_2 Transmitter Series GMT220 (Vaisala Group, Helsinki, Finland). The average daily CO2 concentration ([CO2]) in each cell during experiment are shown in Fig. 1. The climate conditions in two glasshouse cells were set at: 20/16 ± 2 °C day/night air temperature, 60 ± 2% relative humidity, 16 h photoperiod and > 500 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR) supplied by sunlight plus LDE lamps. The vapour pressure deficit (VPD) in the greenhouse cells was maintained at 0.8-1 kPa.

After seedling establishment, the pots were constantly irrigated to 90% of pot holding capacity. In WT barley and *Az34* barley, the soil drying treatment started at 29th January 2018. In WT tomato and *flacca*, the soil drying treatment started at 6th March and 15th March 2018, respectively. In each cell and genotype, four plants were well irrigated as control plants, the others (20 barley and 20 tomato plants) were subjected to progressive soil drying by withholding irrigation from pots until the g_s decreased to ca. 10% of the control plants. During progressive soil drying, the drought-stressed plants were harvested five times at different soil water status; and for each genotype at each harvest, four plants were harvested.

Measurements

Soil water status

Soil water content was measured daily by weighing the pots with an Analytical Balance (Sartorius Model QA35EDE-S) at 15:30 h and expressed as the fraction of transpirable soil water (FTSW). The daily value of FTSW was estimated as ratio between transpirable soil water amount that still remained in pots and total transpirable soil water amount (TTSW). TTSW was defined as the difference of pot weight between 100% water holding capacity (i.e., 4.5 kg) and when g_s of the drought-stressed plant decreased to ca. 10% of the control plant (i.e., 2.5 kg). Then FTSW was calculated as:

$$\mathrm{FTSW} = (\mathrm{WT_n} - \mathrm{WT_f})/\mathrm{TTSW} \quad (1)$$

where WT_n is the pot weight on a given date, WT_f is pot weight at the time when g_s of drought plant was 10% of control plant (i.e. 2.5 kg). Changes of FTSW during the experimental period in each cell and genotype are presented in Fig. 2.

Leaf gas exchange measurement

During the progressive soil drying, leaf gas exchange rates, including net photosynthetic rate (A_n, μmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) were measured daily on flag leaves for barley plants and upper canopy fully expanded leaves for tomato plants between 9:00 to 12:00 h with a portable photosynthetic system (LiCor-6400XT, LI-Cor, NE, USA). Measurements were performed on one leaf per plant at 20 °C cuvette temperature and 1500 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR), and $[CO_2]$ of 400 ppm for $a[CO_2]$ and 800 ppm for $e[CO_2]$ growth environment, respectively.

Plant water relations

Midday leaf water potential (Ψ_l) was measured on flag leaves in barley and young fully expanded leaf in tomato (one leaflet per plant, four plants per genotype in each cell), respectively, using a scholander-type pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). After measuring Ψ_l, the leaf was immediately separated into two pieces, packed in aluminum foil and frozen in liquid nitrogen. The leaf

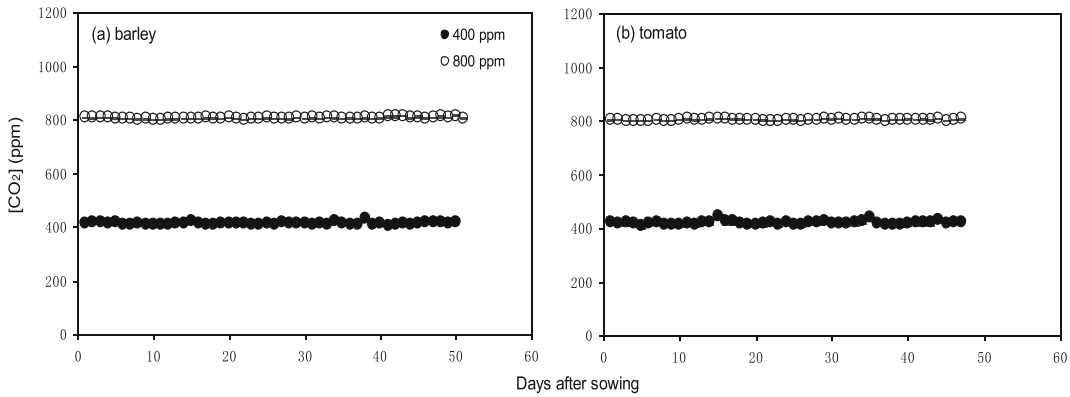

Fig. 1 The actual [CO_2] concentration in 400 and 800 ppm greenhouse cells of barley and tomato plants during the experimental period

samples were then stored at −80 °C for determination of leaf osmotic potential (Ψ_π) and leaf ABA concentration ($[ABA]_{leaf}$). Ψ_π was measured with a psychrometer (C-52 sample chamber, Wescor Crop, Logan, UT,

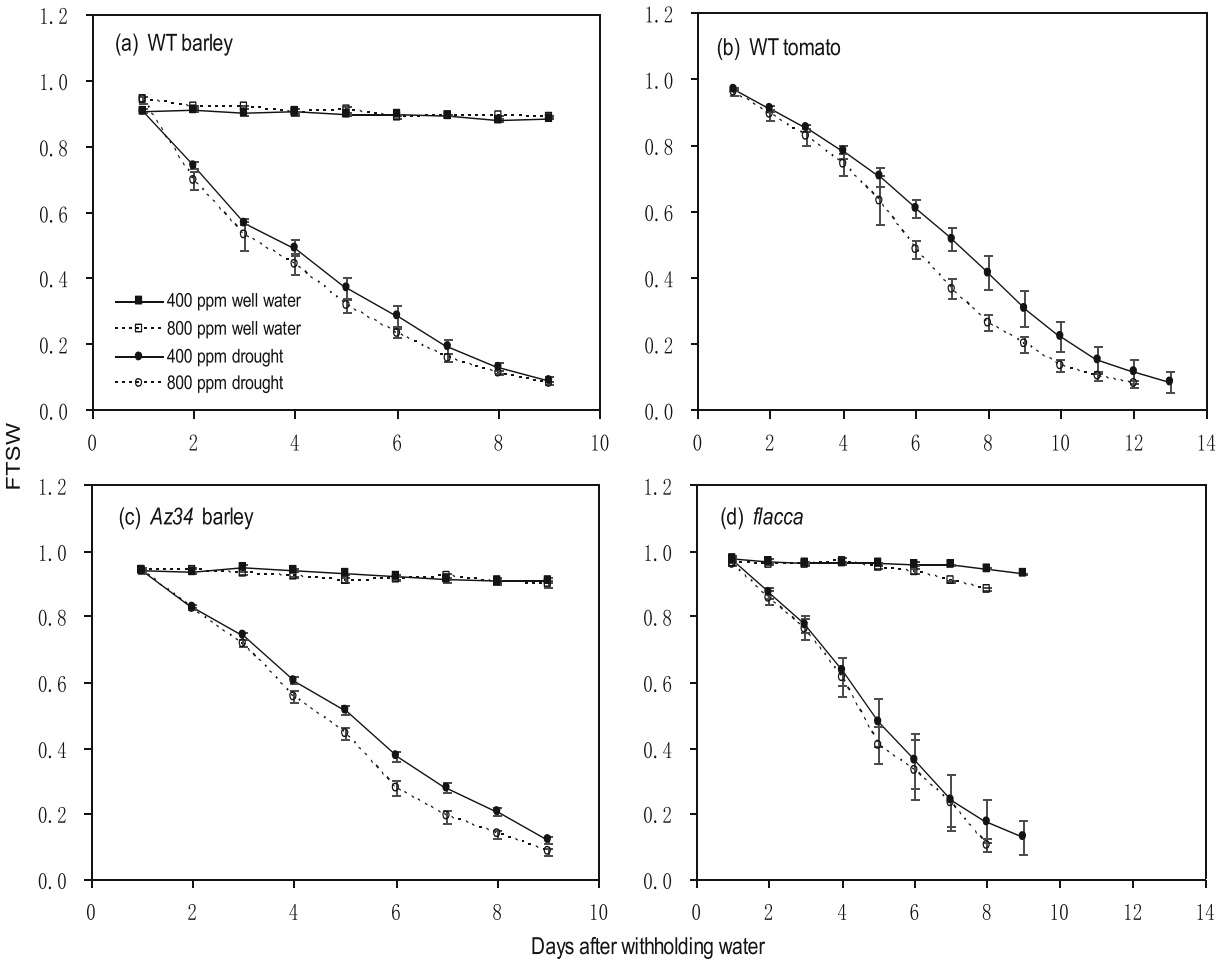

Fig. 2 Trends of fraction of transpirable soil water (FTSW) in the pots of WT barley and tomato, its ABA deficient mutant *Az34* barley and *flacca* grown under ambient (400 ppm) and elevated (800 ppm) atmospheric CO_2 concentrations during progressive soil drying. Error bars indicate standard error of the means (SE) ($n = 4$)

USA) connected to a microvoltmeter (HR-33 T, Wescor, Logan, UT, USA) at 20 ± 1 °C. Leaf turgor pressure (Ψ_p) was calculated as the difference between Ψ_l and Ψ_π.

Leaf ABA concentration

Leaf sample was grounded into fine powder, 27-33 mg per sample was weighed and added into a 1.5 ml Eppendorf tube. The ABA was extracted with 1.0 ml milli-Q water on a shaker at 4 °C over the night. The extracts were centrifuged at 14,000 *g* and 0.7 ml supernatants were collected for $[ABA]_{leaf}$ analysis. $[ABA]_{leaf}$ was determined by enzyme-linked immunosorbent assay (ELISA) using the protocol of Asch (2000).

Data analysis and statistics

The responses of A_n, g_s, Ψ_l, Ψ_π and Ψ_p to soil drying were described by a linear-plateau model (Faralli et al. 2019):

$$\text{If FTSW} > C, y = y_{initial} \quad (2)$$

$$\text{If FTSW} < C, y = y_{initial} + S \times (\text{FTSW} - C) \quad (3)$$

where y means A_n, g_s, Ψ_l, Ψ_π or Ψ_p, and $y_{initial}$ means $A_{n\ max}$, $g_{s\ max}$ or $\Psi_{l\ max}$, $\Psi_{\pi\ max}$ or $\Psi_{p\ max}$, respectively; C was the FTSW threshold at which y started to diverge from $y_{initial}$ for A_n, g_s, Ψ_l, Ψ_π or Ψ_p (denoted as C_A, C_g, C_l, C_π or C_p, respectively). The parameters y and C were estimated by PROC NLIN of PC SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2002-2012) and coefficient of determination (r^2) was calculated. Statistical comparison of each parameter obtained from the linear-plateau regression between $[CO_2]$ treatments or genotypes within each species was performed by *t*-test using MedCalc statistical software 19.0.7.

The relationships between g_s and $[ABA]_{leaf}/\Psi_l/\Psi_p$ were evaluated by linear regressions. r^2 of the regression lines were calculated and statistical difference on the slopes of regression lines between *a*$[CO_2]$ and *e*$[CO_2]$ was performed by analysis of covariance (ANCOVA, FTSW as covariate).

Results

Leaf gas exchange rates

Before imposing drought stress, the net photosynthetic rate (A_n) of WT and *Az34* barley at *e*$[CO_2]$ was 73.0 and 52.3% greater than those at *a*$[CO_2]$, respectively. In WT barley, A_n under *e*$[CO_2]$ began to decrease at a higher FTSW threshold (C_A) than that under *a*$[CO_2]$ (i.e., 0.67 vs 0.36) during the progressive soil drying (Fig. 3a; Tables 1 and 2). While in *Az34* barley, there was no notable difference in C_A between the two CO_2 treatments ($P = 0.123$) (Fig. 3b; Tables 1 and 2). The $A_{n\ max}$ was similar between WT barley and *Az34* barley under both *a*$[CO_2]$ and *e*$[CO_2]$ environment; whilst the C_A of WT barley were higher than that of *Az34* barley under *a*$[CO_2]$ (i.e., 0.36 vs 0.26) and *e*$[CO_2]$ (i.e., 0.67 vs 0.35), respectively (Fig. 3a, b; Tables 1 and 2).

Before imposing drought stress, WT and *Az34* barley grown under *e*$[CO_2]$ had 40.0 and 23.8% lower stomatal conductance (g_s) than those grown under *a*$[CO_2]$, respectively. In WT barley, g_s under *e*$[CO_2]$ started to decline at a significant higher FTSW threshold (C_g) than that under *a*$[CO_2]$ (i.e., 0.50 vs 0.37) during progressive soil drying (Fig. 3c; Tables 1 and 2). Whereas in *Az34* barley, there was no significant difference in C_g between the two CO_2 treatments ($P = 0.766$) (Fig. 3d; Tables 1 and 2). The $g_{s\ max}$ of WT barley was 16.7 and 34.4% lower than that of *Az34* barley under *a*$[CO_2]$ and *e*$[CO_2]$, respectively. While, the C_g was similar between WT barley and *Az34* barley under both *a*$[CO_2]$ and *e*$[CO_2]$ environment (Fig. 3c, d; Tables 1 and 2).

Before imposing drought stress, the $A_{n\ max}$ of WT tomato and *flacca* plants grown at *e*$[CO_2]$ were 55.1 and 19.0% greater than those grown at *a*$[CO_2]$, respectively. Compared to *flacca*, the $A_{n\ max}$ of WT tomato was 29.4 and 7.9% lower under *a*$[CO_2]$ and *e*$[CO_2]$, respectively. During progressive soil drying, C_A of WT tomato and *flacca* at *e*$[CO_2]$ were greater than that at *a*$[CO_2]$ (i.e., 0.38 vs 0.28 and 0.33 vs 0.21, respectively) (Fig. 4a, b; Tables 1 and 2). Compared to *flacca*, the C_A of WT tomato was higher at *a*$[CO_2]$ (i.e., 0.28 vs 0.21), whereas it was similar between the two genotypes at *e*$[CO_2]$ (Fig. 4a, b; Tables 1 and 2).

Before imposing drought stress, $g_{s\ max}$ of WT tomato grown under *e*$[CO_2]$ was 12.5% lower than those grown under *a*$[CO_2]$. Compared to *flacca*, the $g_{s\ max}$ of WT tomato was 60.4 and 63.1% lower under *a*$[CO_2]$ and *e*$[CO_2]$, respectively. During progressive soil drying, C_g

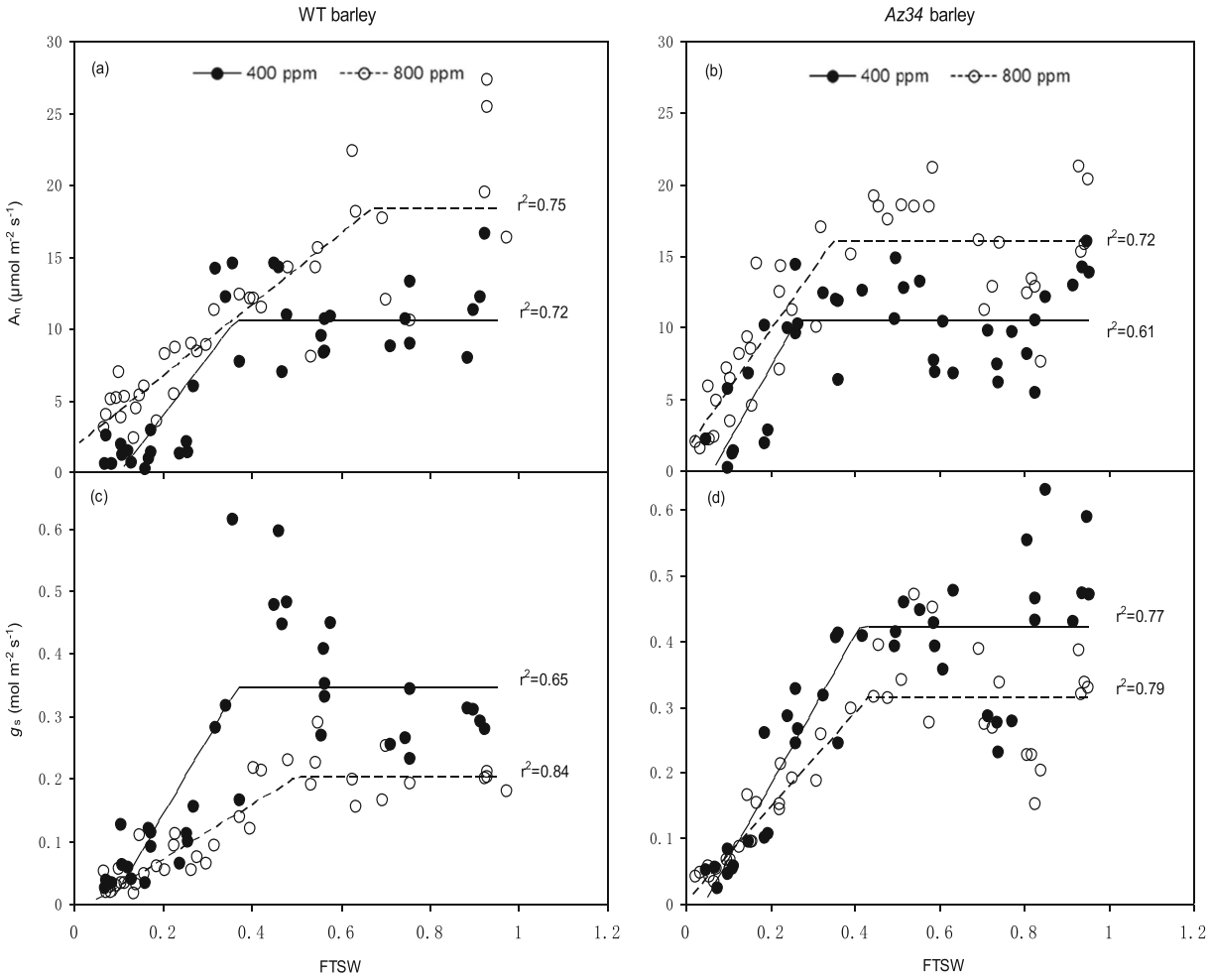

Fig. 3 Changes of net photosynthetic rate (A_n) and stomatal conductance (g_s) of WT barley ($n = 36$) and its ABA deficient mutant *Az34* barley ($n = 40$) grown under ambient (400 ppm) and elevated (800 ppm) atmospheric CO_2 concentrations during progressive soil drying. Closed circles indicate plants at 400 ppm CO_2 concentration, open circles indicate plants at 800 ppm CO_2 concentration

of WT tomato was significantly lower when grown at $e[CO_2]$ than those grown under $a[CO_2]$ (i.e., 0.51 vs 0.62) (Fig. 4c; Tables 1 and 2). While in *flacca*, there was no notable difference in $g_{s\ max}$ and C_g between the two CO_2 treatments (Fig. 4d; Tables 1 and 2). In addition, the C_g of WT tomato was higher than that of *flacca* under $a[CO_2]$ and $e[CO_2]$ (i.e., 0.62 vs 0.34 and 0.51 vs 0.29, respectively) (Fig. 4c, d; Tables 1 and 2).

Plant water relations

Before imposing drought stress, the leaf water potential (Ψ_l) was similar between the two CO_2 growth environments for both WT and *Az34* barley (Fig. 5a, b; Tables 1 and 2). In WT barley, there was no difference in C_l between the two CO_2 treatments during progressive soil drying. While in *Az34* barley, Ψ_l under $e[CO_2]$ began to decrease linearly at a lower C_l than that under $a[CO_2]$ (i.e., 0.30 vs 0.49) during progressive soil drying (Fig. 5b; Tables 1 and 2). The $\Psi_{l\ max}$ and C_l of WT barley were both similar to those of *Az34* barley at $a[CO_2]$; whilst at $e[CO_2]$, WT barley had higher $\Psi_{l\ max}$ (i.e., −0.49 vs − 0.67 MPa) and C_l (i.e., 0.41 vs 0.30) than those of *Az34* barley, respectively (Fig. 5a, b; Tables 1 and 2).

Before imposing drought stress, there was no notable difference in leaf osmotic potential (Ψ_π) of WT barley between the two CO_2 environments ($P = 0.362$) (Fig. 5c; Tables 1 and 2). While for *Az34* barley grown under $e[CO_2]$, $\Psi_{\pi\ max}$ was 0.16 MPa lower than that grown

Table 1 Results of the linear-plateau regression analyses of the responses of leaf net photosynthesis rate (A_n), stomatal conductance (g_s), leaf water potential (Ψ_l), osmotic potential (Ψ_π) and turgor pressure (Ψ_p) of WT barley and tomato, and its ABA deficient mutant (*Az34* barley and *flacca*) to the reduction in fraction of transpirable soil water (FTSW). Values are means ± SE

Genotypes	$[CO_2]$	A_n $A_{n\ max}$	C_A	g_s $g_{s\ max}$	C_g	Ψ_l $\Psi_{l\ max}$	C_l	Ψ_π $\Psi_{\pi\ max}$	C_π	Ψ_p $\Psi_{p\ max}$	C_p
WT barley	400 ppm	10.63 ± 0.67	0.36 ± 0.04	0.35 ± 0.02	0.37 ± 0.05	−0.56 ± 0.06	0.48 ± 0.05	−0.98 ± 0.05	0.45 ± 0.06	0.42 ± 0.02	0.56 ± 0.06
	800 ppm	18.39 ± 1.24	0.67 ± 0.08	0.21 ± 0.01	0.50 ± 0.05	−0.49 ± 0.05	0.41 ± 0.02	−1.04 ± 0.04	0.32 ± 0.03	0.56 ± 0.02	0.61 ± 0.05
Az34 barley	400 ppm	10.55 ± 0.58	0.26 ± 0.03	0.42 ± 0.02	0.41 ± 0.04	−0.65 ± 0.06	0.49 ± 0.06	−1.09 ± 0.05	0.48 ± 0.07	0.44 ± 0.02	0.49 ± 0.05
	800 ppm	16.07 ± 0.71	0.35 ± 0.05	0.32 ± 0.01	0.43 ± 0.06	−0.67 ± 0.05	0.30 ± 0.02	−1.25 ± 0.04	0.20 ± 0.02	0.55 ± 0.02	0.34 ± 0.03
WT tomato	400 ppm	13.91 ± 0.36	0.28 ± 0.02	0.63 ± 0.01	0.62 ± 0.02	−0.49 ± 0.03	0.34 ± 0.03	−0.76 ± 0.03	0.39 ± 0.04	0.27 ± 0.01	0.18 ± 0.01
	800 ppm	21.57 ± 0.48	0.38 ± 0.03	0.56 ± 0.02	0.51 ± 0.02	−0.44 ± 0.02	0.26 ± 0.02	−0.78 ± 0.03	0.48 ± 0.05	0.35 ± 0.01	0.26 ± 0.02
flacca	400 ppm	19.69 ± 0.47	0.21 ± 0.03	1.59 ± 0.04	0.34 ± 0.03	−0.87 ± 0.07	0.40 ± 0.05	−0.95 ± 0.07	0.42 ± 0.06	0.09 ± 0.01	0.31 ± 0.03
	800 ppm	23.43 ± 0.42	0.33 ± 0.04	1.52 ± 0.03	0.29 ± 0.02	−0.88 ± 0.06	0.37 ± 0.03	−0.97 ± 0.05	0.38 ± 0.04	0.09 ± 0.01	0.26 ± 0.02

$A_{n\ max}$, $g_{s\ max}$, $\Psi_{l\ max}$, $\Psi_{\pi\ max}$ and $\Psi_{p\ max}$, indicated the initial values of the parameters when the plants were not significantly affected by drought;

C (C_A, C_g, C_l, C_π or C_p) indicated the threshold at which the parameter (A_n, g_s, Ψ_l, Ψ_π or Ψ_p, respectively) start to decrease due to drought stress

Table 2 Output of statistical analysis of parameters derived from the linear-plateau regression of leaf net photosynthesis rate (A_n), stomatal conductance (g_s), leaf water potential (Ψ_l), osmotic potential (Ψ_π) and turgor press (Ψ_p) of WT barley and tomato, and its ABA deficient mutant (*Az34* barley and *flacca*) response to the reduction in fraction of transpirable soil water (FTSW) (see Table 1). *, ** and *** indicate significant differences of the estimated parameters between two CO_2 growth environments i.e., 400 ppm and 800 ppm CO_2 concentrations, and between wild type (WT) and ABA deficient mutant (ABA) at $P < 0.05$, $P < 0.01$ and $P < 0.001$ level, respectively; ns denotes no significant difference

Genotypes	Factor		A_n $A_{n\ max}$	C_A	g_s $g_{s\ max}$	C_g	Ψ_l $\Psi_{l\ max}$	C_l	Ψ_π $\Psi_{\pi\ max}$	C_π	Ψ_p $\Psi_{p\ max}$	C_p
Barley	WT	400 ppm	0.0001	0.001	0.0001	0.044	0.307	0.207	0.362	0.050	0.0001	0.535
		800 ppm	***	***	***	*	ns	ns	ns	*	***	ns
	ABA	400 ppm	0.0001	0.123	0.0001	0.766	0.846	0.004	0.009	0.0003	0.0005	0.013
		800 ppm	***	ns	***	ns	ns	**	**	***	***	*
	400 ppm	WT	0.897	0.003	0.013	0.498	0.292	0.925	0.149	0.707	0.557	0.458
		ABA	ns	**	*	ns	ns	ns	ns	ns	ns	ns
	800 ppm	WT	0.099	0.003	0.0001	0.337	0.009	0.002	0.0003	0.001	0.765	0.0001
		ABA	ns	**	***	ns	**	**	***	**	ns	***
Tomato	WT	400 ppm	0.0001	0.004	0.0002	0.002	0.307	0.018	0.578	0.115	0.0001	0.002
		800 ppm	***	**	***	**	ns	*	ns	ns	***	**
	ABA	400 ppm	0.0001	0.013	0.157	0.194	0.932	0.620	0.863	0.609	0.783	0.129
		800 ppm	***	*	ns	ns	ns	ns	ns	ns	ns	ns
	400 ppm	WT	0.0001	0.037	0.0001	0.0001	0.0001	0.347	0.008	0.692	0.0001	0.0003
		ABA	***	*	***	***	***	ns	**	ns	***	***
	800 ppm	WT	0.008	0.292	0.0001	0.0001	0.0001	0.004	0.005	0.080	0.0001	0.951
		ABA	**	ns	***	***	***	**	**	ns	***	ns

$A_{n\ max}$, $g_{s\ max}$, $\Psi_{l\ max}$, $\Psi_{\pi\ max}$ and $\Psi_{p\ max}$, indicated the initial values of the parameters when the plants were not significantly affected by drought;

C (C_A, C_g, C_l, C_π or C_p) indicated the threshold at which the parameter (A_n, g_s, Ψ_l, Ψ_π or Ψ_p, respectively) start to decrease due to drought stress

under $a[CO_2]$ (Fig. 5d; Tables 1 and 2). For WT and *Az34* barley, $\Psi_{\pi\ max}$ under $e[CO_2]$ started to decline at significantly lower FTSW threshold (C_π) than those under $a[CO_2]$ (i.e., 0.32 vs 0.45 and 0.20 vs 0.48, respectively) during the progressive soil drying (Fig. 5c, d; Tables 1 and 2). The $\Psi_{\pi\ max}$ and C_π of WT barley were both similar to those of *Az34* barley under $a[CO_2]$; while at $e[CO_2]$, WT barley had higher $\Psi_{\pi\ max}$ (i.e., −1.04 vs − 1.25 MPa) and C_π (i.e., 0.32 vs 0.20) than those of *Az34* barley, respectively (Fig. 5c, d; Tables 1 and 2).

Before imposing drought stress, the leaf turgor pressure ($\Psi_{p\ max}$) in both WT and *Az34* barley at $e[CO_2]$ was 33.3 and 25.0%, respectively, higher than those at $a[CO_2]$ (Fig. 5e, f; Tables 1 and 2). In WT barley, there was no significant difference in FTSW threshold (C_p) of Ψ_p between the two CO_2 treatments; while in *Az34* barley, $\Psi_{p\ max}$ under $e[CO_2]$ began to decline at a lower C_p than that under $a[CO_2]$ (i.e., 0.34 vs 0.49) during progressive soil drying (Fig. 5e, f; Tables 1 and 2). The $\Psi_{p\ max}$ and C_p of WT barley were both similar to those of *Az34* barley under $a[CO_2]$; at $e[CO_2]$, the $\Psi_{p\ max}$ was similar between WT barley and *Az34* barley, while C_p of WT barley was greater than that of *Az34* barley (i.e., 0.61 vs 0.34) (Fig. 5e, f; Tables 1 and 2).

Before imposing drought stress, the Ψ_l was similar between the two CO_2 environments in both WT tomato and *flacca* (Fig. 6a, b; Tables 1 and 2). In WT tomato, Ψ_l under $e[CO_2]$ started to decline at a lower C_l than that under $a[CO_2]$ (i.e., 0.26 vs 0.34) during progressive soil drying; whereas in *flacca*, there was no notable difference in C_l between the two CO_2 treatments ($P = 0.620$) (Fig. 6a, b; Tables 1 and 2). The $\Psi_{l\ max}$ of WT tomato was 0.38 and 0.44 MPa higher than that of *flacca* at $a[CO_2]$ and $e[CO_2]$, respectively. There was no significant difference in C_l between WT tomato and *flacca* under $a[CO_2]$ ($P = 0.347$); whereas at $e[CO_2]$, C_l of WT

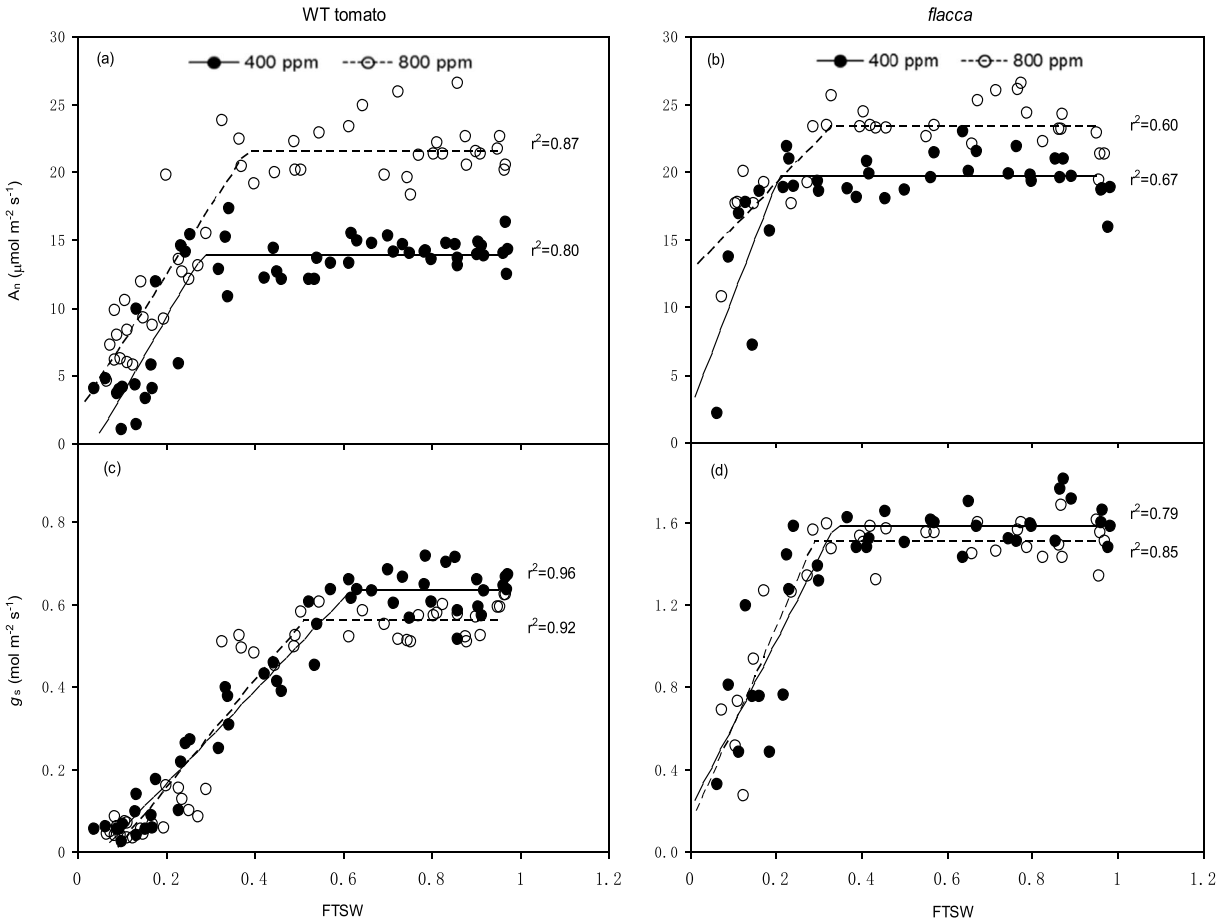

Fig. 4 Changes of net photosynthetic rate (A_n) and stomatal conductance (g_s) of WT tomato ($n = 48$) and its ABA deficient mutant *flacca* ($n = 32$) grown under ambient (400 ppm) and elevated (800 ppm) atmospheric CO_2 concentrations during progressive soil drying. The y-axis range for WT tomato g_s was from 0 to 1.0, and *flacca* g_s was from 0 to 2.0. Closed circles indicate plants at 400 ppm CO_2 concentration, open circles indicate plants at 800 ppm CO_2 concentration

tomato was lower than that of *flacca* (i.e., 0.26 vs 0.37) (Fig. 6a, b; Tables 1 and 2).

Before imposing drought stress, the Ψ_π was similar between the two CO_2 environments in both WT tomato and *flacca*. Likewise, in both WT tomato and *flacca*, FTSW threshold of Ψ_π (C_π) was similar between the two CO_2 treatments during progressive soil drying (Fig. 6c, d; Tables 1 and 2). The Ψ_π of WT tomato was 0.19 and 0.19 MPa greater than that o f *flacca* under $a[CO_2]$ and $e[CO_2]$, respectively; whilst the C_π was similar between WT tomato and *flacca* at each $[CO_2]$ treatment (Fig. 6c, d; Tables 1 and 2).

Before imposing drought stress, $\Psi_{p\ max}$ of WT tomato grown under $e[CO_2]$ had 29.6% higher than that at $a[CO_2]$. During progressive soil drying, the FTSW threshold at which $\Psi_{p\ max}$ (C_p) of WT tomato started to decline was higher at $e[CO_2]$ than at $a[CO_2]$ (i.e., 0.35 vs 0.27) (Fig. 6e; Tables 1 and 2). While in *flacca*, both $\Psi_{p\ max}$ and C_p were similar between the two CO_2 treatments (Fig. 6f; Tables 1 and 2). The $\Psi_{p\ max}$ of WT tomato was 2.0 and 2.9 times greater than that of *flacca* under $a[CO_2]$ and $e[CO_2]$, respectively. The C_p of WT tomato was lower than that of *flacca* under $a[CO_2]$ (i.e., 0.18 vs 0.31); whereas at $e[CO_2]$, there was no significant difference in C_p between WT tomato and *flacca* ($P = 0.951$) (Fig. 6e, f; Tables 1 and 2).

Leaf ABA concentration

In each CO_2 environment, leaf ABA concentration ($[ABA]_{leaf}$) increased exponentially with declining of FTSW in both WT genotypes, but not in ABA deficient mutants. (Fig. 7a, b). In WT barley, only under severe drought stress (i.e. FTSW <0.3),

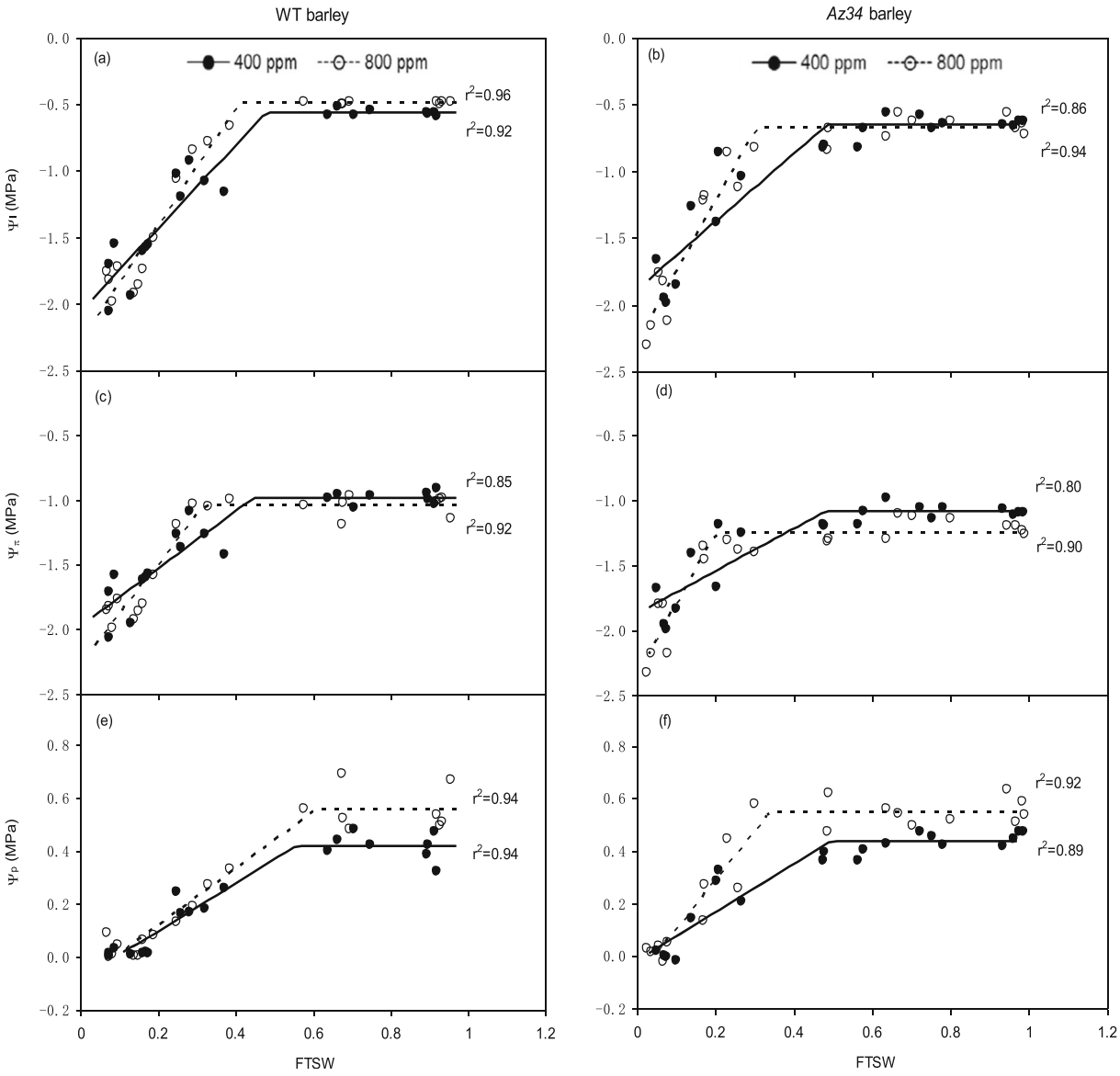

Fig. 5 Changes of leaf water potential (Ψ_l), osmotic potential (Ψ_π) and turgor pressure (Ψ_p) of WT barley ($n = 20$) and its ABA deficient mutant *Az34* barley ($n = 20$) grown under ambient (400 ppm) and elevated (800 ppm) atmospheric CO_2 concentrations during progressive soil drying. Closed circles indicate plants at 400 ppm CO_2 concentration, open circles indicate plants at 800 ppm CO_2 concentration

$[ABA]_{leaf}$ of $e[CO_2]$ plant tended to be higher than that of $a[CO_2]$ plant (Fig. 7a). While in WT tomato, $[ABA]_{leaf}$ under $e[CO_2]$ was greater compared to that under $a[CO_2]$ during the progressive soil drying ($P = 0.001$, ANCOVA) (Fig. 7b). In both ABA deficient mutants, the $[ABA]_{leaf}$ remained lower than those in the hydrated corresponding WT genotypes and were similar between the two CO_2 treatments (Fig. 7a, b).

Relationships of chemical and hydraulic signals with stomatal conductance during progressive soil drying

At moderate soil water deficits (i.e. FTSW >0.3), for both WT genotypes, g_s decreased linearly with increasing $[ABA]_{leaf}$ (Figs. 8a and 9a). The output of ANCOVA reveals that $[CO_2]$ had significant effect on the slope for the regression of g_s to

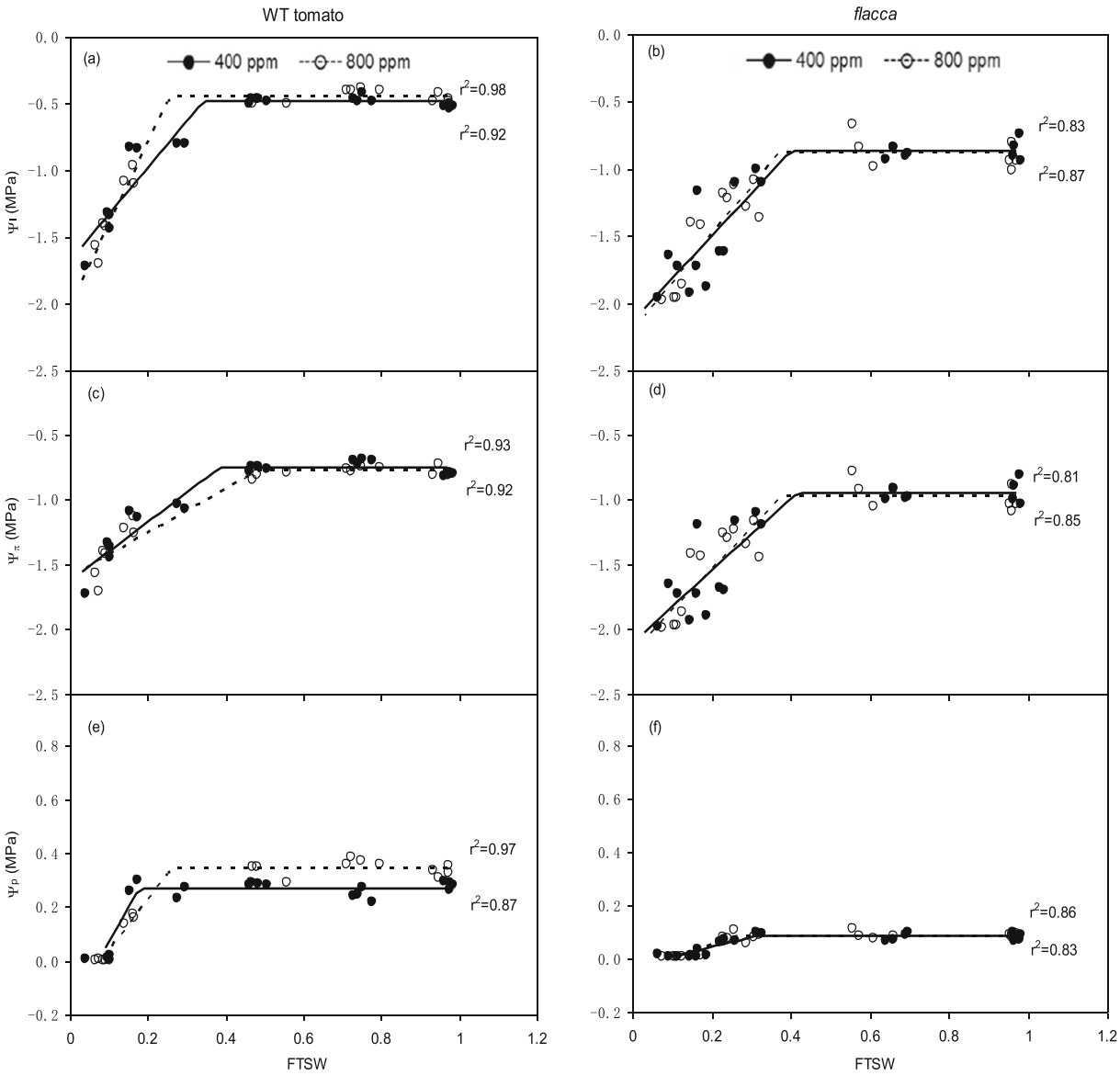

Fig. 6 Changes of leaf water potential (Ψ_l), osmotic potential (Ψ_π) and turgor pressure (Ψ_p) of WT tomato ($n = 20$) and its ABA deficient mutant *flacca* (n = 20) grown under ambient (400 ppm) and elevated (800 ppm) atmospheric CO_2 concentrations during progressive soil drying. Closed circles indicate plants at 400 ppm CO_2 concentration, open circles indicate plants at 800 ppm CO_2 concentration

$[ABA]_{leaf}$, and g_s for plants grown at $a[CO_2]$ was more sensitive to increasing $[ABA]_{leaf}$ compared to that grown at $e[CO_2]$ as g_s was initially higher under $a[CO_2]$ (Figs. 8a and 9a). However, the relationships of g_s to $[ABA]_{leaf}$ were similar for both ABA deficient mutants under the two CO_2 environments (ANCOVA output: $P = 0.58$ for *Az34* barley and $P = 0.34$ for *flacca*); Thus, only one regression line of both $[CO_2]$ treatments was made for each of the ABA deficient mutants (Figs. 8d and 9d).

The g_s decreased linearly with decreasing Ψ_l in barley and tomato plants under each $[CO_2]$ environments (Figs. 8b,e and 9b, e). The output of ANCOVA shows that the slopes of the regressions of g_s to Ψ_l were similar between the two $[CO_2]$ treatments in both barley and tomato genotypes ($P = 0.05$ and $P = 0.28$ for WT barley and *Az34*

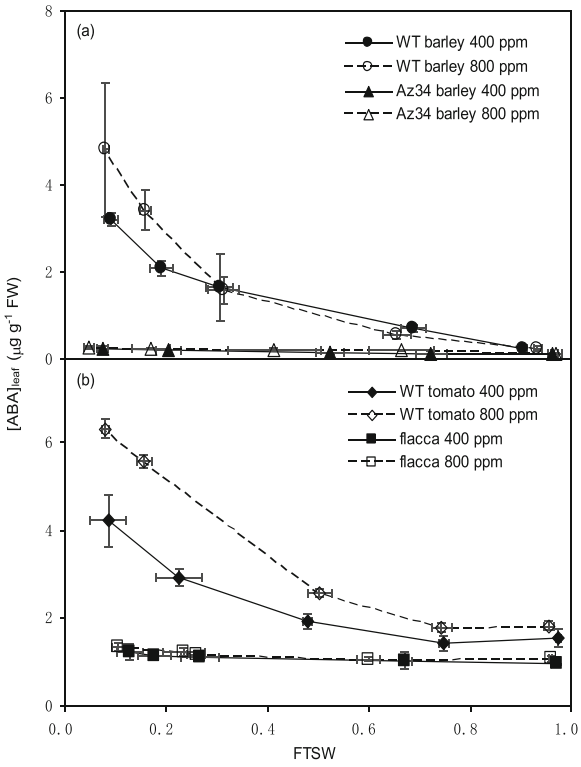

Fig. 7 Trends of leaf ABA concentration ($[ABA]_{leaf}$) of WT barley and its ABA deficient mutant *Az34* barley, WT tomato and its ABA deficient mutant *flacca* grown under ambient (400 ppm) and elevated (800 ppm) atmospheric CO_2 concentrations, respectively during progressive soil drying. Error bars indicate stand error of the means (SE) ($n = 4$)

barley, respectively, and $P = 0.79$ and $P = 0.57$ for WT tomato and *flacca*, respectively). Therefore, only one regression line of the two $[CO_2]$ treatments was made for each of the genotypes (Figs. 8b,e and 9b, e).

The g_s decreased linearly with decreasing Ψ_P in barley and tomato plants under both CO_2 environments except WT tomato grown at $a[CO_2]$ (Figs. 8c, f and 9c, f). The output of ANCOVA shows that $[CO_2]$ had significant effect on the slope of the regression lines of g_s to Ψ_P in WT barley being that g_s of $a[CO_2]$ plants was more sensitive to increasing Ψ_P than that of $e[CO_2]$. For both ABA deficient mutants, no difference in the slopes of the regression lines was found (i.e., $P = 0.07$ for *Az34* barley and $P = 0.22$ for *flacca*, respectively) (Figs. 8c, f and 9f). Therefore, only one regression line of both $[CO_2]$ treatments was made for each of the ABA deficient mutants (Figs. 8f and 9f).

Discussion

There is common consensus that $e[CO_2]$ decreases leaf g_s in angiosperms (i.e., Wei et al. 2018). Likewise, in this study except *flacca*, most of the plants grown at $e[CO_2]$ had lower $g_{s\ max}$ compared to those grown at $a[CO_2]$ (Figs. 3c, d and 4c, d; Tables 1 and 2). Besides, in accordance with previous studies (Yan et al. 2017; Liu et al. 2019), here we found that $e[CO_2]$ increased net photosynthetic rate (A_n) under well-watered or moderate drought stress, and the enhancement of $A_{n\ max}$ was observed in all plants grown at $e[CO_2]$. In addition, more pronounced increase of $A_{n\ max}$ was observed in barley as compared to tomato as $A_{n\ max}$ was lower in barley relative to tomato at $a[CO_2]$ (Figs. 3a, b and 4a, b; Tables 1 and 2). Thereby, those together lead to an improved water use efficiency at leaf scale in all plants under $e[CO_2]$ environment.

As illustrated in Fig. 7, when FTSW greater than 0.3, $[ABA]_{leaf}$ of $e[CO_2]$ WT barley plant was similar to that of $a[CO_2]$ plant, and it became higher under severe drought stress (e.g., when FTSW <0.3) (Fig. 7a). In WT tomato plant, $[ABA]_{leaf}$ under $e[CO_2]$ was generally greater than that under $a[CO_2]$ during progressive soil drying (Fig. 7b). Earlier studies have reported that $e[CO_2]$-induced stomatal closure was mediated by endogenous ABA (Chater et al. 2015; Tazoe and Santrucek 2015). In the absence of decreased leaf water status at $e[CO_2]$, the higher $[ABA]_{leaf}$ in $e[CO_2]$ plants might be resulted from stimulated root growth at $e[CO_2]$ (Wullschleger et al. 2002) as the enhanced root biomass could have stimulated root-to-shoot ABA signaling and further increasing foliar ABA concentration (Martin-Vertedor and Dodd 2011). Consistent with the finding by Li et al. (2016), here the decrease in $g_{s\ max}$ of WT tomato could be mainly ascribed to higher leaf ABA concentration under $e[CO_2]$, but the effect was absence in ABA-deficient *flacca* as the $g_{s\ max}$ was unaffected by $[CO_2]$ growth environments (Fig. 4c, d; Tables 1 and 2). Whereas, the $e[CO_2]$-induced reduction of $g_{s\ max}$ in barley was probably not related to an increase of $[ABA]_{leaf}$ and most likely ABA-independent as the $g_{s\ max}$ reduction was found in both WT genotype and ABA-deficient mutant (Fig. 3c, d; Tables 1 and 2). Thus, it is plausible that putative differences exist between barley (monocot) and tomato (dicot) plants in the response of g_s to $e[CO_2]$ environment.

In the present study, soil water status in pot was expressed as the fraction of transpirable soil water

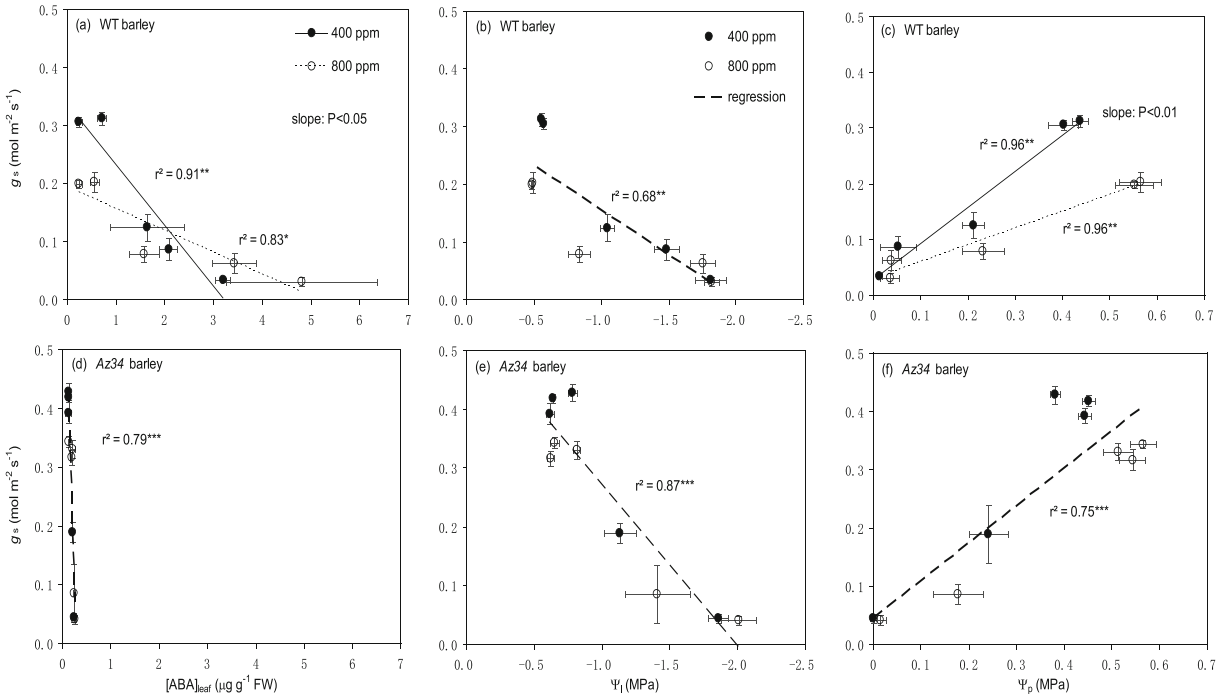

Fig. 8 Relationships between stomatal conductance (g_s) and leaf ABA concentration ($[ABA]_{leaf}$), g_s and leaf water potential (Ψ_l), g_s and turgor pressure (Ψ_p) of WT barley and its ABA deficient mutant *Az34* barley grown under ambient (400 ppm) and (800 ppm) atmospheric CO_2 concentrations during progressive soil drying. Closed circles indicate plants at 400 ppm CO_2 concentration, open circles indicate plants at 800 ppm CO_2 concentration. Error bars indicate standard error of the means (SE) ($n = 4$). *, ** and *** indicate the regression lines were statistically significantly at $P < 0.05$, $P < 0.01$ and $P < 0.001$ level, respectively (ANCOVA). Slope with P value indicates significant difference between the slopes of the regression lines for $a[CO_2]$ and $e[CO_2]$ treatments

(FTSW) and linear plateau model was used to evaluate the response of leaf gas exchange to progressive soil drying. With the progression of soil drying, $e[CO_2]$ sensitized g_s decrease in WT barley (Fig. 3c; Tables 1 and 2), while this was reverse in WT tomato where $e[CO_2]$ retarded the reduction of g_s (Fig. 3c; Tables 1 and 2), affirming our earlier findings that g_s became less sensitive to soil drying in tomato plants grown at $e[CO_2]$ than grown at $a[CO_2]$ (Yan et al. 2017; Liu et al. 2019). Furthermore, A_n of all plants grown at $e[CO_2]$ were more sensitive to soil drying than those grown at $a[CO_2]$ (Fig. 3a, b and 4a, b; Tables 1 and 2). In WT barley, the earlier reduction in A_n during soil drying could be a result of earlier decrease in g_s under $e[CO_2]$ (Kusumi et al. 2012) (Fig. 3; Tables 1 and 2). However, this was not the case for WT tomato, as g_s decreased later at $e[CO_2]$ than $a[CO_2]$ (Fig. 3c; Tables 1 and 2). Hereby, the earlier reduction in A_n of WT tomato during soil drying under $e[CO_2]$ was not due to an earlier closure of stomata, other factors could be involved. Opposite to the WT genotypes, the sensitivity of g_s to progressive soil drying for both ABA-deficient mutants was unaffected by the $[CO_2]$ growth environment (Figs. 3c, d and 4c, d; Tables 1 and 2). Therefore, it is obvious that endogenous ABA level could have been involved in modulating the g_s response to soil drying when plants grown under $e[CO_2]$.

Previous evidence has demonstrated that ABA-induced stomatal closure in tomato could increase Ψ_l, indicating the dependence of Ψ_l on leaf g_s (Chaves et al. 2016; Dodd et al. 2009). In addition, ABA-deficient mutants often had lower Ψ_l than WT genotypes as described previously for barley (Martin-Vertedor and Dodd 2011; Mulholland et al. 1996) and tomato (Fambrini et al. 1995; Jones et al. 1987; Sharp et al. 2000). In agreement with this, here the greater $g_{s\,max}$ of both ABA-deficit mutants could lead to lower $\Psi_{l\,max}$ as compared to WT genotypes except barley plant at $a[CO_2]$ (Figs. 5a, b and 6a, b; Tables 1 and 2), although the stomata was closed as Ψ_l declined in each genotype and $[CO_2]$ environment (Figs. 8b, e and 9b, e). This relationship could be resulted from the obvious decline in both g_s and Ψ_l during severe soil drying. The isohydric plants are

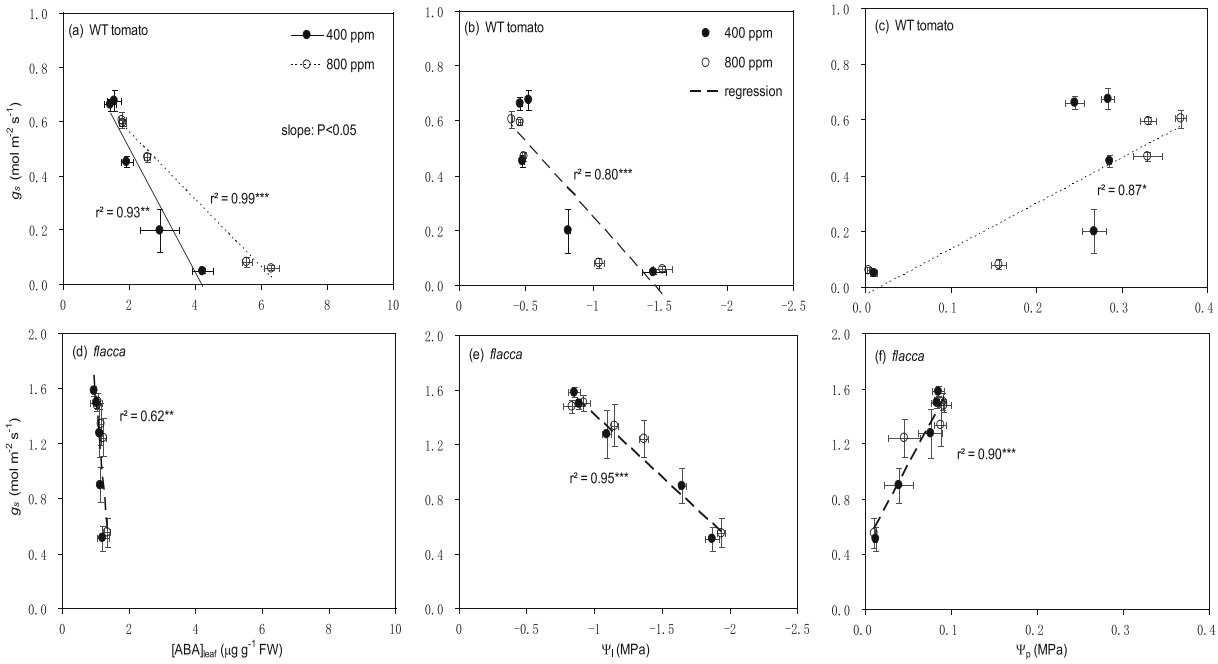

Fig. 9 Relationships between stomatal conductance (g_s) and leaf ABA concentration ($[ABA]_{leaf}$), g_s and leaf water potential (Ψ_l), g_s and turgor pressure (Ψ_p) of WT tomato and its ABA deficient mutant *flacca* grown under ambient (400 ppm) and (800 ppm) atmospheric CO_2 concentrations during progressive soil drying. Closed circles indicate plants at 400 ppm CO_2 concentration, open circles indicate plants at 800 ppm CO_2 concentration. Error bars indicate standard error of the means (SE) ($n = 4$). *, ** and *** indicate the regression lines were statistically significantly at $P < 0.05$, $P < 0.01$ and $P < 0.001$ level, respectively (ANCOVA). Slope with P values indicates significant difference between the slopes of the regression lines of $a[CO_2]$ and $e[CO_2]$ treatments

able to keep constant Ψ_l by lowering g_s in response to soil drying, whereas anisohydric plants could decrease Ψ_l while maintaining g_s (Tardieu and Simonneau 1998). In the current study, the barley and tomato plants grown under $e[CO_2]$ environment tended to delay the decline in Ψ_l during progressive soil drying as compared to those grown at $a[CO_2]$ (Figs. 5a, b and 6a, b; Tables 1 and 2). Thus, they tended towards isohydric in response to drought.

Several studies have shown that $e[CO_2]$ enhanced A_n and solutes accumulation, thereby contributing to the lower Ψ_π and higher Ψ_p, further improving leaf turgor (Mamatha et al. 2015; Yan et al. 2017). Consistent with this, in this study, compared to $a[CO_2]$ plants, the $e[CO_2]$ plants showed a tendency of lower $\Psi_{\pi\ max}$ (although only significant in *Az34* barley) and notable higher $\Psi_{p\ max}$ except *flacca*. However, it should be noted that $e[CO_2]$ delayed the Ψ_π response to progressive soil drying in barley, not in tomato, and Ψ_p response to progressive soil drying combined with $[CO_2]$ environment between barley and tomato was different (Figs. 5c–f and 6c–f; Tables 1 and 2). Moreover, in both $[CO_2]$ growth environments, the $\Psi_{p\ max}$ of *Az34* barley was similar to that of WT barley (Fig. 5e, f; Tables 1 and 2), while, the $\Psi_{p\ max}$ of *flacca* was much lower than that of WT tomato (Fig. 6e, f; Tables 1 and 2). This was probably attributed to the contrasting leaf anatomy and stomatal morphology between dicot and monocot species, indicating that hydraulic properties in response to soil drying under disparate $[CO_2]$ growth environment would be species-dependent. However, it should be notable that the linear-plateau model used in this study might have wrongly estimated the FTSW thresholds at which the leaf water relation parameters started to decline from their maximal values due to the insufficient data points. Further studies with more frequent measurements of leaf water relation characteristics during soil drying should be conducted to verify these results.

It is widely recognized that endogenous ABA level plays an important role in stomatal regulation in response to drought stress (Wilkinson and Davies 2002; Yan et al. 2017). Here, the g_s decreased linearly with the increase of $[ABA]_{leaf}$ for both WT genotypes (Figs. 8a and 9a), while such relationships between g_s and $[ABA]_{leaf}$ in both ABA-deficit mutants were not evident (Figs. 8d and 9d), implying that endogenous leaf ABA

level was involved in the regulation of stomatal aperture and this regulation was species-independent. There was little available information about the effect of $e[CO_2]$ on the sensitivity of stomata to ABA signaling when plants exposed to drying soil. Gray et al. (2016) reported that $e[CO_2]$ increased the sensitivity of soybean g_s to $[ABA]_{xylem}$ under drought stress in a multi-year study. On the contrary, Liu et al. (2019) found that ABA was less important in inducing g_s reduction at moderate drought stress under $e[CO_2]$, and Yan et al. (2017) observed that $e[CO_2]$ plants possessed lowered sensitivity of g_s to $[ABA]_{xylem}$. Similarly, in the present study, the g_s of both WT genotypes grown at $e[CO_2]$ become less sensitive to $[ABA]_{leaf}$ (Figs. 8a and 9a), implying that other signal rather than ABA was more essential for controlling g_s during mild drought stress. Yan et al. (2017) showed that the g_s of $e[CO_2]$ tomato was positively correlated with Ψ_p. In accordance with this, here the g_s of WT genotypes as well as their ABA-deficient mutants revealed positive correlations with Ψ_p under both $[CO_2]$ environments except WT tomato grown under $a[CO_2]$ (Figs. 8c, f and 9c, f). The lack of correlation between g_s and Ψ_p in WT tomato grown under $a[CO_2]$ agrees with earlier findings from the root pressurization experiments showing that soil-drying induced stomatal closure even leaf turgor was maintained (Holbrook et al. 2002), which further emphasized the significance of chemical signalling (i.e., ABA) in inducing stomatal closure. On the other hand, our results indicated that Ψ_p and not ABA could have acted as a major factor inducing stomatal closure for the ABA-deficient mutants.

Conclusions

In this experiment, $e[CO_2]$ sensitized photosynthetic decline with soil moisture deficit in most genotypes. Soil-drying induced stomatal closure was affected by $[CO_2]$ in wild type genotypes but not in ABA-deficient mutants; $e[CO_2]$ sensitized the stomata response in barely whilst delayed it in tomato. In all genotypes, $e[CO_2]$ sustained leaf water potential and caused notable higher turgor pressure except *flacca* as compared to $a[CO_2]$. In both wild type genotypes, The stomata become less sensitive to endogenous ABA at $e[CO_2]$ than $a[CO_2]$, whereas for the mutants, the stomata was predominately controlled by leaf turgor and not ABA during soil drying. These findings provide some novel insights into the mechanism of stomatal control in monocot and dicot plants response to drought stress under CO_2-enriched environment.

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