

Elevated CO₂ and high endogenous ABA level alleviate PEG-induced short-term osmotic stress in tomato plants

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ABSTRACT

Elevated CO₂ concentration ($e[\text{CO}_2]$) alleviates the impact of drought stress on plants where abscisic acid (ABA) is involved. To explore the mechanisms by which tomato plants respond to short-term osmotic stress, *Solanum lycopersicum* cv. Ailsa Craig (AC), a transgenic line overproducing ABA (sp5), and an ABA-deficient mutant (*flacca*) were hydroponically grown under ambient CO₂ (400 ppm) and $e[\text{CO}_2]$ (800 ppm) and then exposed to 10% or 15% (w/v) polyethylene glycol (PEG) 6000 for 24 h before transferring to PEG-free nutrient solution for 24 h. Under non-stress condition, $e[\text{CO}_2]$ decreased root hydraulic conductance (K_{root}), which was overridden by high endogenous ABA in sp5 through increasing specific leaf area and root branching intensity. Basically, $e[\text{CO}_2]$ improved stress resistance through enhanced water status. PEG stress decreased stomatal conductance and osmotic potential in AC but these effects were less pronounced in sp5, which exhibited a stronger osmotic adjustment (OA) and improved plant fitness. A greater flexibility of hydraulic system and a reduced sensitivity of K_{root} to ABA might confer sp5 a great ability to recover from PEG stress. On the contrary, high stomatal density, size and pore aperture of *flacca* rendered plants suffering severe stress. Moreover, the premise that PEG stress could mimic soil water deficit was the sufficient achievement of OA. Our results indicate that $e[\text{CO}_2]$ and high endogenous ABA level could improve osmotic stress resistance in tomato plants via osmotic and hydraulic adjustments.

1. Introduction

Intensified incidence of drought threatens crop growth and productivity (McDowell et al., 2008; Trenberth et al., 2014). At the same time, elevated atmospheric carbon dioxide concentration ($e[\text{CO}_2]$) is a major component and driver of climate change that can alleviate the adverse effects of drought stress on plants (Becklin et al., 2017; Jia et al., 2019). In order to cope with multiple environmental perturbations, plants have evolved diverse physiological mechanisms and many of those involve the abscisic acid (ABA)-based signalling (Domec et al., 2017; Li et al., 2020), particularly when plants exposure to drought stress.

The stomata control leaf gas exchange through a fine-tuned regulation of stomatal aperture in response to environmental stimuli (Bergmann and Sack, 2007). Both drought stress and $e[\text{CO}_2]$ induce stomatal closure, thus decreasing stomatal conductance (g_s ; Li et al., 2020). Besides fast modulation of stomatal pore aperture (SA), the stomatal morphological traits, including stomatal size (SS) and stomatal density

(SD), also respond to fluctuation in environmental conditions, representing a longer-term plant response (Royer, 2001; Woodward et al., 2002). SD is reported to be negatively correlated with SS, together determining the maximal g_s in plants (Franks and Beerling, 2009). It has been suggested that plants with low SD exhibited better performance when exposed to $e[\text{CO}_2]$ and water-scarce environments (Doheny-Adams et al., 2012). Furthermore, under drought stress, long-distance signals derived from the roots, mainly xylem-borne ABA, induce stomatal closure, thus decreasing g_s (Zhang et al., 2006). Although in angiosperms stomatal closure is likely to be predominantly controlled by an ABA signal rather than hydraulic signal (Brodribb and McAdam, 2011), it has been reported that they may coexist to regulate stomatal behaviour. For example, in sunflowers and grapevine, a hydraulic signal (leaf turgor) was responsible for the onset of stomatal closure, and foliar ABA only significantly increased after stomata have been fully closed and prevented the recovery of stomatal aperture during re-watering (Huber et al., 2019; Tombesi et al., 2015). Interestingly, in addition to reducing g_s , $e[\text{CO}_2]$ was found to decrease leaf and root hydraulic

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Abbreviations

$[ABA]_{\text{leaf}}$	leaf abscisic acid concentration	RD	root diameter
$[ABA]_{\text{xylem}}$	xylem sap abscisic acid concentration	RDW	root dry weight
$a[\text{CO}_2]$	ambient CO_2 concentration	RL	root length
$e[\text{CO}_2]$	elevated CO_2 concentration	RTD	root tissue density
g_s	stomatal conductance	RWC	relative water content
K_{root}	root hydraulic conductance	SA	stomatal pore aperture
LA	leaf area	SD	stomatal density
OA	osmotic adjustment	SDW	shoot dry weight
PCA	principle component analysis	SLA	specific leaf area
PEG	polyethylene glycol	SRL	specific root length
RBI	root branching intensity	SS	stomatal size
		Ψ_{leaf}	leaf water potential
		Ψ_{π}	osmotic potential

conductance (K_{leaf} and K_{root} , respectively) in tomato plants and this was at least partly mediated by ABA (Fang et al., 2019; L. Li et al., 2021).

Under well-watered conditions, the general view is that $e[\text{CO}_2]$ enhances water use efficiency through inducing partial stomatal closure while stimulating net photosynthesis (Li et al., 2020). These positive effects of $e[\text{CO}_2]$ are believed to be more pronounced when plants experience abiotic stress, thus improving stress resistance. Recently, a study on tomato showed that $e[\text{CO}_2]$ -grown plants exhibited a retarded stomatal closure compared to those grown under ambient $[\text{CO}_2]$ during progressive soil drying (Liu et al., 2019). Similar results have been found in grapevines, where $e[\text{CO}_2]$ delayed drought effects on plant photosynthesis and Rubisco activity through reducing g_s and SD (da Silva et al., 2017). Nevertheless, whether plants can benefit from the altered stomatal drought response when grown under $e[\text{CO}_2]$ environment is still unclear, as severe or prolonged drought might eliminate the $e[\text{CO}_2]$ -induced positive effects due to disturbed plant water balance (Temme et al., 2018; Wei et al., 2020). Therefore, further researches are needed to clarify $e[\text{CO}_2]$ effects under different intensities of drought stress.

$e[\text{CO}_2]$ -induced stomatal closure reduces plant water consumption. Meanwhile, it has been proposed that altered root system architecture under $e[\text{CO}_2]$ might confer plants a better ability for water uptake and transport (Day et al., 1996). Based on a meta-analysis, it is suggested that $e[\text{CO}_2]$ promoted root development and root system expansion, including increased root length, diameter and biomass, which contributed to belowground C sequestration (Nie et al., 2013). However, whether plants can benefit from the root system alteration in terms of soil water uptake when exposure to soil water deficits is still controversial. In wheat plants, $e[\text{CO}_2]$ stimulated root length in both upper and deeper soil layers, and the latter was closely associated with improved water use efficiency and plant growth (Uddin et al., 2018). By contrast, there was evidence that under $e[\text{CO}_2]$ longer primary root but more branched lateral root could render the root system less efficient to absorb water (Pritchard and Rogers, 2000). In addition, the correlations between root morphology and K_{root} are still controversial. An early study on different rice genotypes showed that root anatomy was not the predominant factor affecting K_{root} (Matsuo et al., 2009), but recently Canales et al. (2021) found that increases in total root length and the thinnest root length improved K_{root} . Therefore, further researches on the coordination between root morphology and hydraulic adjustment of plant grown under $e[\text{CO}_2]$ are needed.

At the whole plant level, the water potential gradient between leaf and root, the leaf and root hydraulic conductivity as well as the root system architectures together determine water transport from roots to shoots in plants (Moshelion et al., 2015). At the cellular level, osmotic gradients and the elasticity of the cell wall determine the turgor pressure, which in turn drives cell expansion and maintains the upright structure of whole plants; turgor pressure of guard cells also mediates the opening and closing of stomata (Haswell and Verslues, 2015). Under

drought stress, the initial perception of water deficit in plants triggers downstream osmotic stress signaling and induces a cascade of regulatory processes, including the accumulation of compatible solutes such as proline and the biosynthesis of ABA (Haswell and Verslues, 2015; Wilsson et al., 2014).

High molecular weight polyethylene glycol (PEG) treatment has been widely used to mimic plant water deficit via osmotic effects (Verslues et al., 2006), it provides a suitable method to induce osmotic stress when investigating the interactions between osmotic stress and other factors such as $e[\text{CO}_2]$. Given that high-molecular weight PEG (6000 or above) cannot penetrate the cell wall and causes cytorrhysis (shrinking of both protoplast and cell wall) similarly to soil water deficit (Verslues et al., 2006), it is regarded as an acceptable mimic of osmotic stress in hydroponic systems with minimal toxic effects on plants. In various studies, PEG exposure could induce decreases in leaf gas exchange rates and plant water potentials as well as the great antioxidative response, which could be used for screening of tomato cultivars with improved drought tolerance (Aazami et al., 2010; Aydin et al., 2014; Zgallaï et al., 2005). Under osmotic stress, initial stomatal closure and root-to-shoot signaling can occur within 1 h (Dubois and Inzé, 2020), and these fast responses can prevent plants from severe desiccation damage (Takahashi and Shinozaki, 2019).

High PEG concentration in the surrounding medium acts as an osmoticum to cause outward movement of water in plants cells, and triggers osmotic adjustment (OA) when stress becomes severe and therefore categorized as osmotic stress (Dubois and Inzé, 2020). High level of OA was associated with increased solute accumulation, and could be an indicator of higher drought resistance (Jensen et al., 1996). Interestingly, carbohydrate and solute accumulation under $e[\text{CO}_2]$ also can lead to an enhanced OA, thus lowering osmotic potential (Fang et al., 2019; Yan et al., 2017); however others reported that $e[\text{CO}_2]$ -induced OA is minimal and could not be considered to be directly beneficial effect of plant against drought (Robredo et al., 2007; Wullschlegel et al., 2002).

Therefore, the present study aimed to investigate the effects of PEG-induced short-term osmotic stress on stomatal conductance and water relations of tomato plants exposure to $e[\text{CO}_2]$. To explore the role of ABA in these regulations, tomato cv. Alisa Craig (AC) was compared to the isogenic transgenic line known to overproduce ABA (sp5) and the near-isogenic ABA-deficient mutant *flacca*. We hypothesized that tomato plants differing in endogenous ABA levels possessed different responses to $e[\text{CO}_2]$ in terms of stomatal, leaf and root morphological traits, and these diversities would influence plant osmotic stress resistance when exposed to PEG stress and recovery.

2. Materials and methods

2.1. Plant materials and growth conditions

The experiment was conducted using isogenic tomato (*Solanum lycopersicum*) cv. Ailsa Craig (AC) and two homozygous true-breeding lines: the transgenic line sp5 that overexpresses the rate-limiting ABA biosynthetic gene *LeNCED1*, provided by A. Thompson (Cranfield University, UK); an ABA-deficient tomato mutant (*flacca*), provided by the Lancaster Environment Centre (Lancaster University, UK). The *flacca* mutant has lower endogenous ABA concentrations compared to AC due to the impairment in the oxidation of ABA-aldehyde to ABA (Sagi et al., 2002); the sp5 line harbors a construct with the *LeNCED1* transgene driven by the Gelvin Superpromoter and has a higher ABA concentration (Thompson et al., 2007, 2000). As ABA overproduction increased seed dormancy, the protocol for seed germination and establishment of sp5 developed by Thompson et al. (2007) was used to ensure all three genotypes reached the 4-leaf stage when transplanted into the hydroponic system. Briefly, seeds of sp5 were placed on filter paper soaked in distilled water plus 0.1 mg L⁻¹ norflurazon (an inhibitor of phytoene desaturase, Sigma) for 24 h, then washed thoroughly in distilled water to remove the residual norflurazon. All seeds were firstly germinated onto filter paper soaked in distilled water and kept in the dark at 22 °C until radicle emergence, then sown in pots (10-cm diameter by 10-cm length) containing 4:1 (volume: volume) peat (Plugg-och Såjord-Dry matter ca 0.110 kg m⁻³, organic matter >95%, pH 5.5–6.5 and EC 1.5–2.5 mS cm⁻¹): sand. At the 4-leaf stage, after washing the roots carefully, all the seedlings were transplanted to a hydroponic system and grown in 1-L containers filled with half-strength Hoagland's nutrient solution (Sigma). The nutrient solution was continuously aerated using an air compressor and changed every five days. The pH of the nutrition solution was monitored daily and adjusted to 6.0 with 0.1 M HCl or 0.1 M NaOH.

From germination, the plants were grown in two greenhouse cells with CO₂ concentrations of 400 ppm (ambient CO₂, a[CO₂]) and 800 ppm (elevated CO₂, e[CO₂]), respectively. During the experiment, the actual daily average [CO₂] was 432.6 and 798.9 ppm, respectively. The [CO₂] in the cells was sustained by pure CO₂ emission from a bottle tank and distributed evenly by the internal ventilation system. The [CO₂] in the cells was monitored every 6 s by a CO₂ Transmitter Series GMT220 (Vaisala Group, Helsinki, Finland). The climate conditions in the two glasshouse cells were set at: 20/16 ± 2 °C day/night air temperature, 60 ± 2% relative humidity, 16 h photoperiod and > 300 μmol m⁻² s⁻¹ photosynthetically active radiation (PAR) supplied by sunlight plus LED lamps (Philips GreenPower LED toplighting, Denmark). The vapor pressure deficit in the greenhouse cells ranged from 0.8 to 1.0 kPa.

2.2. Polyethylene glycol (PEG) 6000 treatments

After two weeks of growing in the hydroponic system, through application of polyethylene glycol (PEG) 6000 solution to the roots, plants for all three genotypes under the two [CO₂] levels were exposed to 10% or 15% PEG for 0, 2, 4 and 24 h (expressed as P0, 2, 4, 24, respectively) to mimic short-term osmotic stress, then transferred back to PEG-free nutrient solution for 2, 4 and 24 h as short-term recovery (expressed as R2, 4, 24). PEG stress and recovery started around 8:30 h. According to the equation developed by Michel and Kaufmann (1973), the osmotic stress levels in the hydroponic solution were -0.16 and -0.31 MPa for 10% and 15% PEG stress, respectively. Each treatment included four plant replications.

2.3. Determination of stomatal, leaf and root traits

One day prior to commencing PEG stress, stomatal morphological traits, including stomatal density (SD, no. mm⁻²), stomatal size (SS, μm⁻²) and stomatal pore aperture (SA, μm⁻²) were determined on four

replicates following the method described by Yan et al. (2012). Stomatal morphology were observed under a LEITZ DMRD microscope camera system (Leica Microscope and System GmbH, D 35530, Wetzlar, Germany) equipped with a digital camera. SD, stomatal size parameters including guard cell length (W_s), guard cell pair width (L_s), pore aperture length (W_a) and pore aperture width (L_a) were measured by ImageJ software (Version 1.51k, Wayne Rasband, National Institutes of Health, USA, Java 1.6.0–24 (64 bit)). SA and SS were then calculated using the following equations as described by Doheny-Adams et al. (2012):

$$SA = \frac{\pi \times w_a \times L_a}{4} \quad (1)$$

$$SS = \frac{\pi \times W_s \times L_s}{4} - SA \quad (2)$$

For each treatment, plants on four replicates were harvested, then shoot (leaf plus stem) dry weight (SDW, g), root dry weight (RDW, g) and root: shoot ratio were determined, and total leaf area (LA, cm²) was measured by a leaf area meter (LICOR 3100). Specific leaf area (SLA, cm² g⁻¹) was calculated as the ratio of LA to leaf dry weight. Fresh root samples were photo-scanned and the images were analyzed by the software WinRHIZO Pro (Version 2009c, 32-bit) to determine root length (RL, m), root diameter (RD, mm), root volume and the number of root tips. Specific root length (SRL, m g⁻¹) was calculated as: RL divided by corresponding RDW. Root tissue density (RTD, g cm⁻³) was calculated as RDW divided by corresponding root volume. Root branching intensity (RBI, tips cm⁻¹) was calculated as the number of root tips divided by corresponding RL.

2.4. Stomatal conductance measurements

At each sampling time point (P0, 2, 4, 24; R2, 4, 24), stomatal conductance (g_s, mol m⁻² s⁻¹) was measured on the upper canopy fully expanded leaves with a portable photosynthetic system (LiCor-6400XT, Li-Cor, NE, USA). Measurements were performed at 22 °C cuvette temperature, 1500 μmol m⁻² s⁻¹ PAR, and [CO₂] of 400 ppm for a[CO₂] and 800 ppm for e[CO₂] growth environments, respectively.

2.5. Determination of plant water relations

At each sampling time point, a leaf was excised for the determination of midday leaf water potential (Ψ_{leaf}, MPa) with a Scholander-type pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) following the method described by Liu et al. (2006). After that, fresh weight (FW) of the excised leaves was obtained and turgid weight (TW) was recorded following a re-hydration period in distilled water for 2 h; then dry weight (DW) was measured after oven-drying at 75 °C for 48 h. Relative water content (RWC) was calculated as: [FW - DW]/(TW - DW). Fresh leaves were frozen in liquid nitrogen and stored at -80 °C for later determination of osmotic potential (Ψ_π, MPa) and leaf ABA concentration ([ABA]_{leaf}, μg g⁻¹ FW). Ψ_π was measured with a psychrometer (C-52 sample chambers, Wescor Inc., Logan, UT, USA) connected to a microvoltmeter (HR-33 T, Wescor, Logan, UT, USA) at 22 ± 1 °C. Ψ_π at full turgor was then determined as Ψ_π × RWC. Osmotic adjustment (OA, MPa) was calculated as the difference in Ψ_π at full turgor between well-watered and PEG-stressed plants, and value less than 0.01 was considered as no OA.

2.6. Determination of root hydraulic conductance and ABA concentrations

PEG-stressed and recovered plants were harvested three times for determination of root hydraulic conductance (K_{root}) and xylem sap ABA concentration ([ABA]_{xylem}, pmol ml⁻¹) at P0, P24 and R24, respectively. The whole pots were put into a scholander-type pressure chamber following the procedure described by Liu et al. (2006). Briefly, the

chamber was sealed and only the above-soil part of the plants was left out. The stem was cut with a scalpel at approximate 10 cm above the soil surface. After a good seal was obtained, the pressure of the chamber was increased to 0.4 MPa and kept constant. The xylem sap appeared from the cutting surface was collected to Eppendorf tube by a pipette for 1 min and weighted, then stored at -80°C for determination of $[\text{ABA}]_{\text{xylem}}$. K_{root} was calculated as the rate of sap exudation (J , g min^{-1}) divided by the applied pressure and then normalized by RDW ($\text{g min}^{-1} \text{MPa}^{-1} \text{g}^{-1}$). Before the experiment, for all genotypes, J was measured under different applied pressures (0.1–0.7 MPa), and we found that when pressure ranged between 0.2 and 0.5 MPa, there was stable linear relationship between the J and applied pressure. Therefore, 0.4 MPa was used for measurement.

$[\text{ABA}]_{\text{leaf}}$ and $[\text{ABA}]_{\text{xylem}}$ were determined by Enzyme-linked immunosorbent assay following the protocol of Asch (2000).

2.7. Statistical analyses

Data were statistically analyzed using Microsoft Excel, SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2002–2012), SPSS 22.0 software (IBM SPSS Software, New York, USA) and GraphPad Prism 9 software. Two-way analyses of variance (ANOVA) were performed to compare the differences between $[\text{CO}_2]$ and genotypes. One way ANOVA (Tukey's test) were conducted to determine the significant differences between genotypes or treatments. Analysis of covariance (ANCOVA, time of treatment as covariate) was conducted to determine the significant differences between $[\text{CO}_2]$ and PEG stress. Principle component analysis (PCA) of leaf and root traits in three genotypes were performed in R version 4.0.0 (R Core Team, 2020). Independent from the PCA, a multiple regression analysis of K_{root} against leaf and root traits was performed to determine if there were common drivers for the K_{root} response.

3. Results

3.1. Stomatal morphological traits of well-watered plants

Compared with AC and sp5 plants, *flacca* had significantly higher SD , SS and SA , while there was no difference for these parameters between AC and sp5. $e[\text{CO}_2]$ decreased SA in all genotypes, whereas it had no influence on SD and SS (Fig. 1).

3.2. Leaf and root traits and their relationship with root hydraulic conductance

Table 1 shows the leaf and root traits and K_{root} in the three well-watered tomato genotypes grown under $a[\text{CO}_2]$ and $e[\text{CO}_2]$ environments. Compared with AC, *flacca* had particularly lower LA and SDW, whereas sp5 had significantly higher SLA. Except RDW, root:shoot ratio and RD, other root traits significantly differed between the three genotypes: AC had greater RL and SRL, and lower RTD; sp5 possessed the highest RBI and K_{root} ; *flacca* had lowest RBI and K_{root} . $e[\text{CO}_2]$ increased LA, SDW, RDW, RL and RBI in all genotypes, and decreased SLA except in *flacca*. Moreover, $e[\text{CO}_2]$ only significantly decreased K_{root} in AC.

PCA plots of leaf and root traits and K_{root} of AC, sp5 and *flacca* are depicted in Fig. 2. PC1 and PC2 axes explained 63.7% of cluster formation, with 42.7% attributed to PC1 and 21.0% to PC2. Overall, the three genotypes were distributed separately: sp5 was clustered to the left top of the PCA plot, with the same direction as the root: shoot ratio, RTD, SLA, K_{root} and RBI vectors; AC was clustered to the right of the plot, with the same direction as RDW, SDW, RL, RD and SRL vectors; *flacca* showed weak association with leaf/ root traits, though some traits (SRL and RD) were overlapped with AC.

As K_{root} was obviously associated with several leaf and root traits in the PCA plot, multiple regression analysis for K_{root} responsiveness to leaf and root traits was performed. Pearson's correlation test confirmed significant positive correlations of K_{root} with SLA, RTD and RBI, and

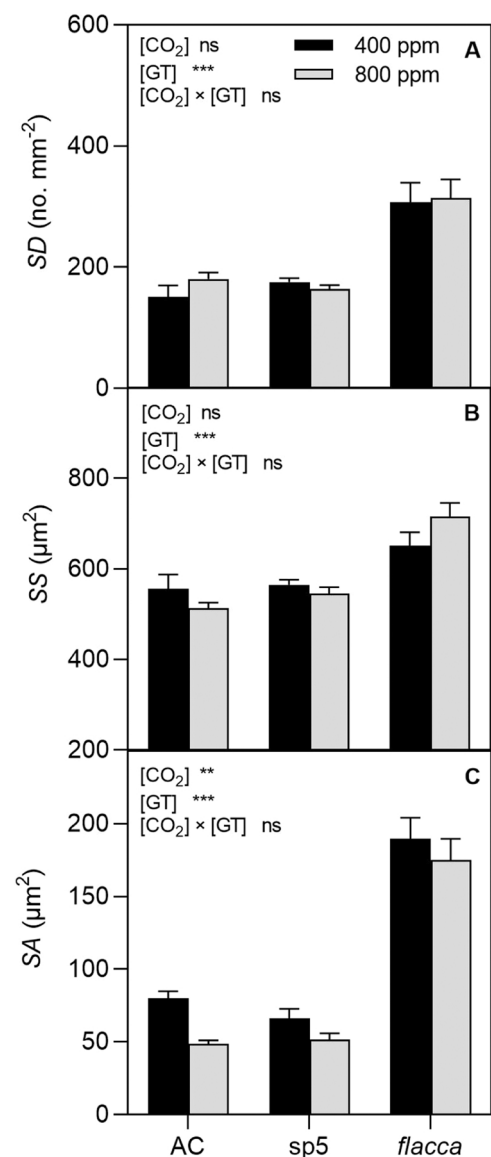


Fig. 1. Stomatal density (SD) (A), stomatal size (SS) (B) and stomatal pore aperture (SA) (C) of well-watered AC, sp5 and *flacca* grown under ambient (400 ppm) and elevated (800 ppm) CO_2 concentrations. **, and *** indicate the significant differences between $[\text{CO}_2]$ levels or genotypes (GT) in two-way ANOVA at $P < 0.01$, $P < 0.001$, respectively; ns denotes no significant difference. Error bars indicate the standard error of the means (S.E.) ($N = 4$).

negative correlation of K_{root} with RL (Table S1), and Fig. 3 shows scatter plots summarizing the relationships between K_{root} and these four leaf and root traits.

3.3. Plant wilting phenotype and stomatal conductance

Fig. 4 shows the plant wilting phenotypes of AC, sp5 and *flacca* grown under $a[\text{CO}_2]$ and $e[\text{CO}_2]$ when exposed to 10% or 15% PEG stress and recovery. Under well-watered condition, there was no difference in plant phenotype between the two $[\text{CO}_2]$ levels in all three genotypes, and *flacca* exhibited significant retarded plant growth accompanied by curled leaves compared with AC and sp5. When exposed to PEG stress, all plants showed visible signs of wilting especially under 15% PEG, though the symptom was less severe in sp5 and the most severe in *flacca* compared to AC. Only in sp5, the leaf wilting phenotype was mostly restored after 24-h recovery under the two $[\text{CO}_2]$ levels. Moreover, in all genotypes, $e[\text{CO}_2]$ could alleviate the wilting

Table 1
Output of two-way analysis of variance (ANOVA) and means \pm standard error (S.E.) (N = 4) of leaf area (LA), shoot dry weight (SDW), root dry weight (RDW), root shoot, root length (RL), specific root length (SRL), root diameter (RD), root tissue density (RTD), root branching intensity (RBI) and root hydraulic conductance (K_{root}) of well-watered AC, sp5 and *flacca* grown under ambient (400 ppm) and elevated (800 ppm) CO₂ concentrations. *, **, and *** indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$ level, respectively; ns denotes no significant difference.

	LA (cm ²)	SLA (cm ² g ⁻¹)	SDW (g)	RDW (g)	Root:shoot	RL (m)	SRL (m g ⁻¹)	RD (mm)	RTD (g cm ⁻³)	RBI (tips cm ⁻¹)	K_{root} (g min ⁻¹ MPa ⁻¹ g ⁻¹)	
AC	400 ppm	980.06 \pm 46.74ab	260.35 \pm 13.58b	4.91 \pm 0.32b	0.49 \pm 0.01	0.23 \pm 0.01	72.08 \pm 4.14b	146.77 \pm 6.64	0.45 \pm 0.01	0.043 \pm 0.003	1.53 \pm 0.12	0.77 \pm 0.03b
	800 ppm	1076.64 \pm 52.00a	217.46 \pm 6.76c	6.45 \pm 0.41a	0.58 \pm 0.06	0.23 \pm 0.01	88.54 \pm 2.58a	157.64 \pm 17.56	0.46 \pm 0.01	0.039 \pm 0.005c	1.73 \pm 0.15b	0.47 \pm 0.04c
sp5	400 ppm	848.64 \pm 21.63bd	330.46 \pm 21.55a	3.48 \pm 0.24c	0.44 \pm 0.02	0.25 \pm 0.01	52.07 \pm 2.03c	119.11 \pm 7.27	0.44 \pm 0.01	0.056 \pm 0.004a	1.87 \pm 0.12ab	1.27 \pm 0.14a
	800 ppm	952.87 \pm 40.50ab	265.18 \pm 14.15b	4.79 \pm 0.26b	0.56 \pm 0.05	0.24 \pm 0.02	61.93 \pm 2.56	112.78 \pm 6.71	0.44 \pm 0.01	0.059 \pm 0.001a	2.22 \pm 0.11a	1.16 \pm 0.10a
<i>flacca</i>	400 ppm	516.54 \pm 27.28d	248.36 \pm 9.71	2.80 \pm 0.17c	0.42 \pm 0.03	0.25 \pm 0.01	51.96 \pm 1.64c	127.41 \pm 10.67	0.44 \pm 0.02	0.053 \pm 0.001ab	1.18 \pm 0.12c	0.55 \pm 0.01c
	800 ppm	691.39 \pm 49.76 cd	234.02 \pm 3.89	3.82 \pm 0.29	0.55 \pm 0.05	0.23 \pm 0.01	63.15 \pm 4.76	115.54 \pm 7.91	0.46 \pm 0.02	0.054 \pm 0.002ab	1.51 \pm 0.10	0.52 \pm 0.06c
[CO ₂]	**	***	***	**	ns	ns	ns	ns	ns	***	**	*
[GT]	***	***	***	ns	ns	ns	**	ns	***	***	***	***
[CO ₂] \times [GT]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Values are means \pm standard error of the means (S.E.) (N = 4); *, **, and *** indicate significance levels at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively; ns denotes no significant difference.

symptoms especially under 15% PEG stress and recovery.

Table 2 shows the average g_s of the three genotypes. Under well-watered condition (P0), only *flacca* exhibited higher g_s than the other two genotypes; under PEG stress and recovery, sp5 showed significantly higher g_s than AC and *flacca* still had highest g_s . In AC, g_s strongly decreased within the first 2 h and was maintained at constant low levels during the whole period of treatments (Fig. 5A, B). In sp5 and *flacca*, 15% PEG stress rapidly and strongly decreased g_s , while 10% PEG had mild influence on g_s especially in *flacca*, where g_s showed a recovery increase (Fig. 5C-F). Furthermore, $e[CO_2]$ had overall depressive effect on g_s of *flacca* when exposed to 10% PEG and during recovery (Fig. 5E).

3.4. Plant water relation characteristics

Ψ_{leaf} of the three genotypes under PEG stress and recovery is shown in Fig. S1. In both AC and sp5, Ψ_{leaf} gradually increased when exposed to PEG, and Ψ_{leaf} of 10% PEG-stressed sp5 could be recovered to initial levels. In addition, Ψ_{leaf} of *flacca* did not significantly respond to PEG. In Fig. S1D, under PEG stress, a drop of xylem sap immediately appeared on the cut surface of the petiole after leaf excision in all of the three genotypes, even though the leaf showed severe wilting symptoms.

Under well-watered condition, there was no difference in Ψ_{π} between the three genotypes; under PEG stress and recovery, sp5 had slight lower average Ψ_{π} than AC, whereas *flacca* possessed higher Ψ_{π} (Table 2). In Fig. 6, Ψ_{π} exhibited decreasing trends in AC and *flacca* along with PEG stress and recovery, while it was not significantly altered in sp5 under the two levels of PEG stress. RWC showed the similar response with Ψ_{π} , where decreased RWC in sp5 could be restored during recovery period. Moreover, $e[CO_2]$ improved RWC of the three stressed genotypes and it was more pronounced under 15% PEG stress (Fig. S2).

AC plants achieved OA until recovery period, while sp5 and *flacca* possessed OA after 2-h PEG stress, though OA in *flacca* was evident only during recovery. In sp5, the 15% PEG-stressed plants only showed significant OA within the first 4-h stress (Table S2).

3.5. Leaf ABA concentration and its relationship with stomatal conductance

Under well-watered condition, compared to AC, sp5 had slightly higher $[ABA]_{leaf}$, while *flacca* had significantly lower $[ABA]_{leaf}$; under PEG stress and recovery, sp5 possessed dramatic lower $[ABA]_{leaf}$ than AC, and in *flacca* it was remained at a low level (Table 2). In Fig. 7, $[ABA]_{leaf}$ showed increasing trends in AC plants but in sp5 and *flacca* it was only significantly increased under 15% PEG stress. Moreover, $[ABA]_{leaf}$ of AC exhibited large fluctuation during recovery period, and $[ABA]_{leaf}$ of 15% PEG-stressed sp5 reached a peak at R2 and then decreased. In Fig. 8, although g_s decreased along with increasing $[ABA]_{leaf}$ in AC and sp5, there was no clear relationship between g_s and $[ABA]_{leaf}$ across the three genotypes under all treatments.

3.6. Xylem sap ABA concentration, root hydraulic conductance and their relationships

In most of the plants except 10% PEG-stressed *flacca*, $[ABA]_{xylem}$ increased at P24 and then decreased at R24, and 15% PEG stress had a stronger effect than did 10% PEG (Fig. 9A–C). In addition, in AC and sp5, increase in $[ABA]_{xylem}$ was less significant when plants were grown under $e[CO_2]$ compare to those under $a[CO_2]$, and this effect caused by $e[CO_2]$ was also observed in $[ABA]_{leaf}$ of 10% PEG-stressed sp5 (Fig. 7C). Similarly to $[ABA]_{leaf}$, sp5 possessed a general lower $[ABA]_{xylem}$ than AC during the whole treatments (Fig. 9D). Furthermore, K_{root} decreased at P24 and then increased at R24 in the three genotypes. Compared with AC and *flacca*, fluctuation of K_{root} in sp5 during PEG stress and recovery was more dramatic, and sp5 exhibited a general higher average K_{root} than other two genotypes (Fig. 9E–H).

Relationships between K_{root} and $[ABA]_{xylem}$ is shown in Fig. 10.

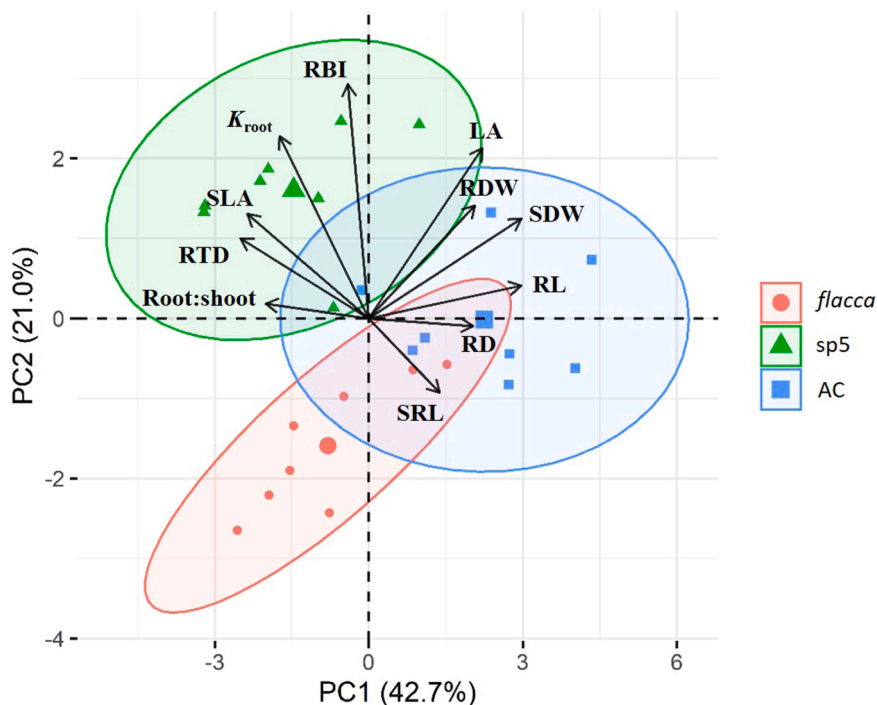


Fig. 2. Principal component analysis of leaf area (LA), specific leaf area (SLA), shoot dry weight (SDW), root dry weight (RDW), root: shoot, root length (RL), specific root length (SRL), root diameter (RD), root tissue density (RTD), root branching intensity (RBI) and root hydraulic conductance (K_{root}) in well-watered AC, sp5 and *flacca*. The contributions of each PCA axis (PC1 and PC2) are indicated on the graph.

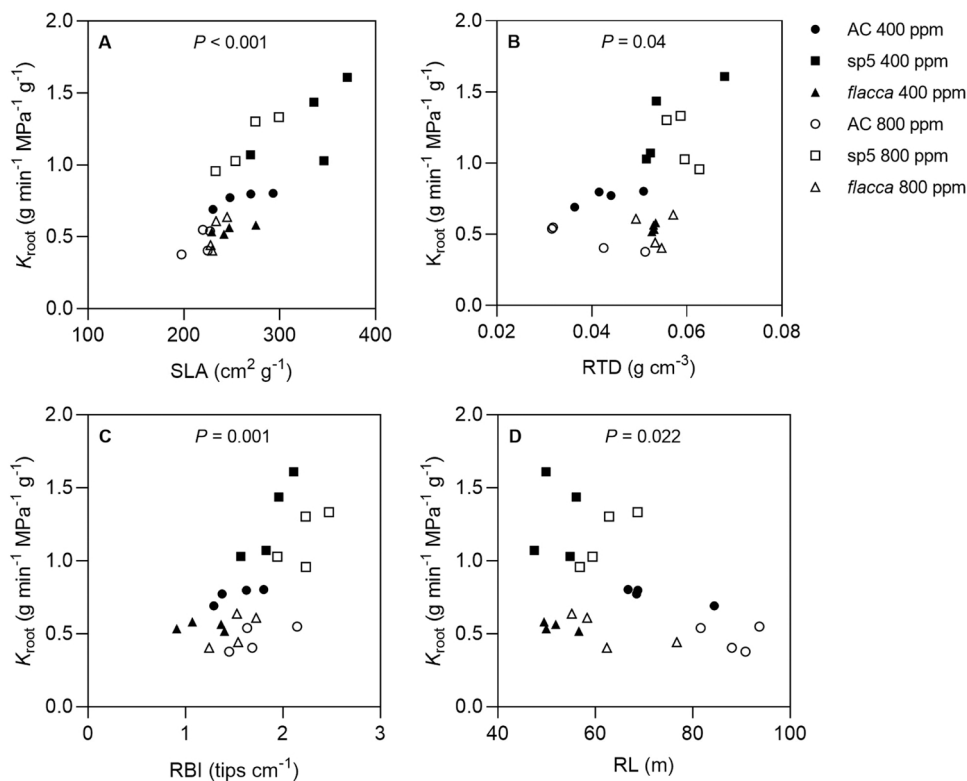


Fig. 3. Scatter plots for root hydraulic conductance (K_{root}) and four root traits which had significant correlations with K_{root} confirmed by multiple regression analysis (Table S1), including specific leaf area (SLA) (A), root tissue density (RTD) (B), root branching intensity (RBI) (C) and root length (RL) (D) in well-watered AC, sp5 and *flacca* grown under ambient (400 ppm) and elevated (800 ppm) CO₂ concentrations. *P* values were derived from multiple regression analysis.

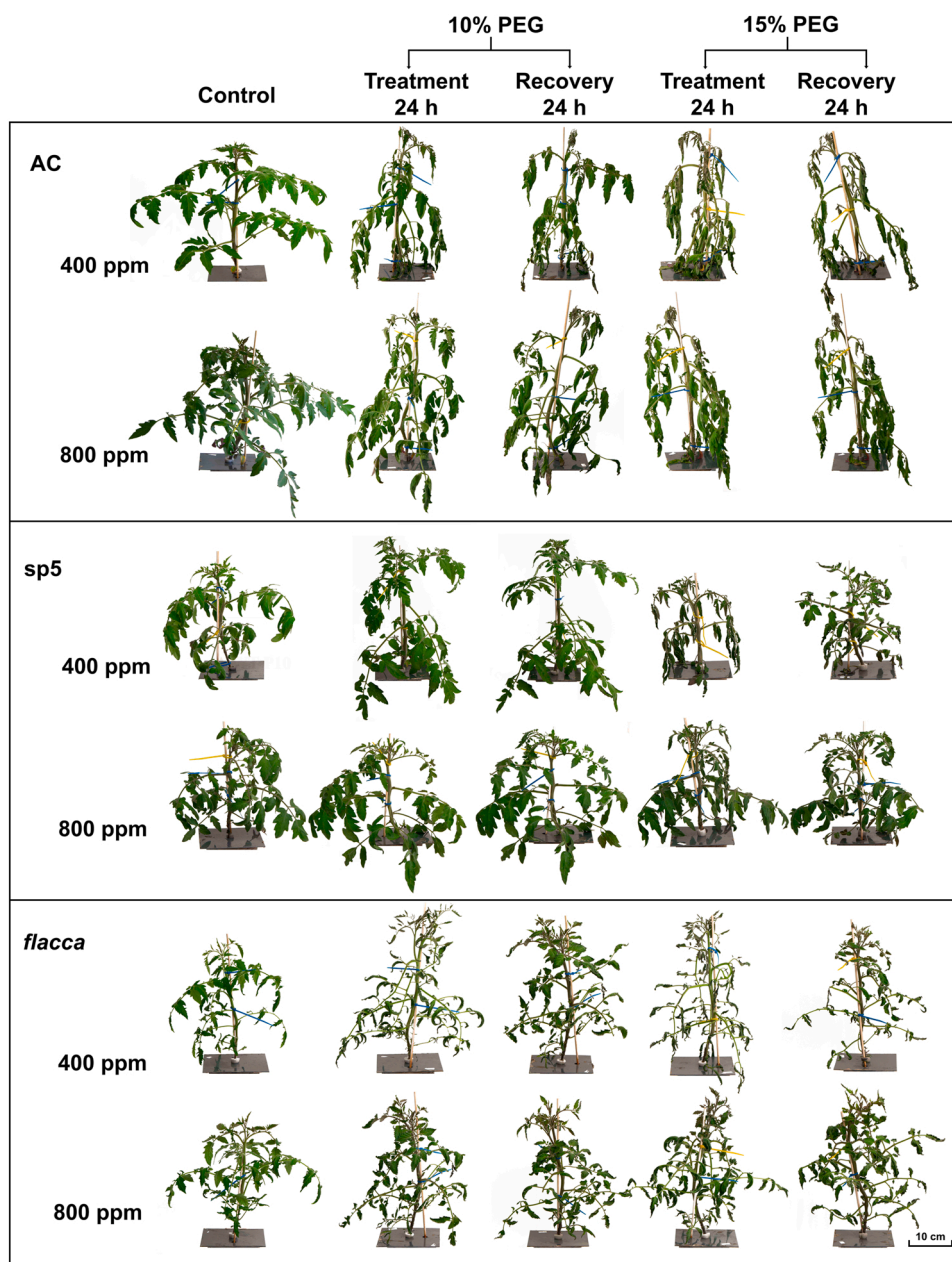


Fig. 4. Representative images of AC, sp5 and *flacca* grown under ambient (400 ppm) and elevated (800 ppm) CO₂ concentrations when exposed to 10% or 15% PEG stress and recovery. Small plant size and leaf curling were shown in *flacca*; different leaf wilting symptoms were shown between genotypes and [CO₂] levels.

Table 2

Output of one-way ANOVA and means \pm standard error of the means (S.E.) of stomatal conductance (g_s), leaf osmotic potential (Ψ_π) and leaf ABA concentration ($[ABA]_{\text{leaf}}$) of AC, sp5 and *flacca* under well-watered condition ($N = 8$) and under PEG stress and recovery ($N = 72-96$). Different letters indicate significant difference between genotypes by Tukey's test at $P < 0.05$; no letters indicates there was no significant difference.

	Well-watered condition			PEG stress and recovery		
	AC	sp5	<i>flacca</i>	AC	sp5	<i>flacca</i>
g_s (mol m ⁻² s ⁻¹)	0.77 \pm 0.02b	0.71 \pm 0.02b	1.17 \pm 0.02a	0.04 \pm 0.01c	0.12 \pm 0.01b	0.78 \pm 0.04a
Ψ_π (MPa)	-0.70 \pm 0.03	-0.71 \pm 0.01	-0.73 \pm 0.02	-0.97 \pm 0.04ab	-0.92 \pm 0.02b	-1.02 \pm 0.01a
$[ABA]_{\text{leaf}}$ ($\mu\text{g g}^{-1}$ FW)	590.10 \pm 97.41ab	794.82 \pm 98.16a	315.40 \pm 47.35b	2323.89 \pm 161.13a	1380.39 \pm 66.75b	580.89 \pm 23.81c

Under well-watered condition, K_{root} was positively correlated with $[ABA]_{\text{xylem}}$ in the three genotypes under two [CO₂] levels (Fig. 10A). Under PEG stress and recovery, K_{root} decreased along with increasing $[ABA]_{\text{xylem}}$ in all genotypes, but only in AC there was a slight linear relationship between these two variables (Fig. 10B-D).

4. Discussion

$e[\text{CO}_2]$ is believed to enhance plant drought resistance through regulation of stomatal behaviour where ABA is involved (Li et al., 2020). In the present study, we investigated the effects of $e[\text{CO}_2]$ and PEG stress

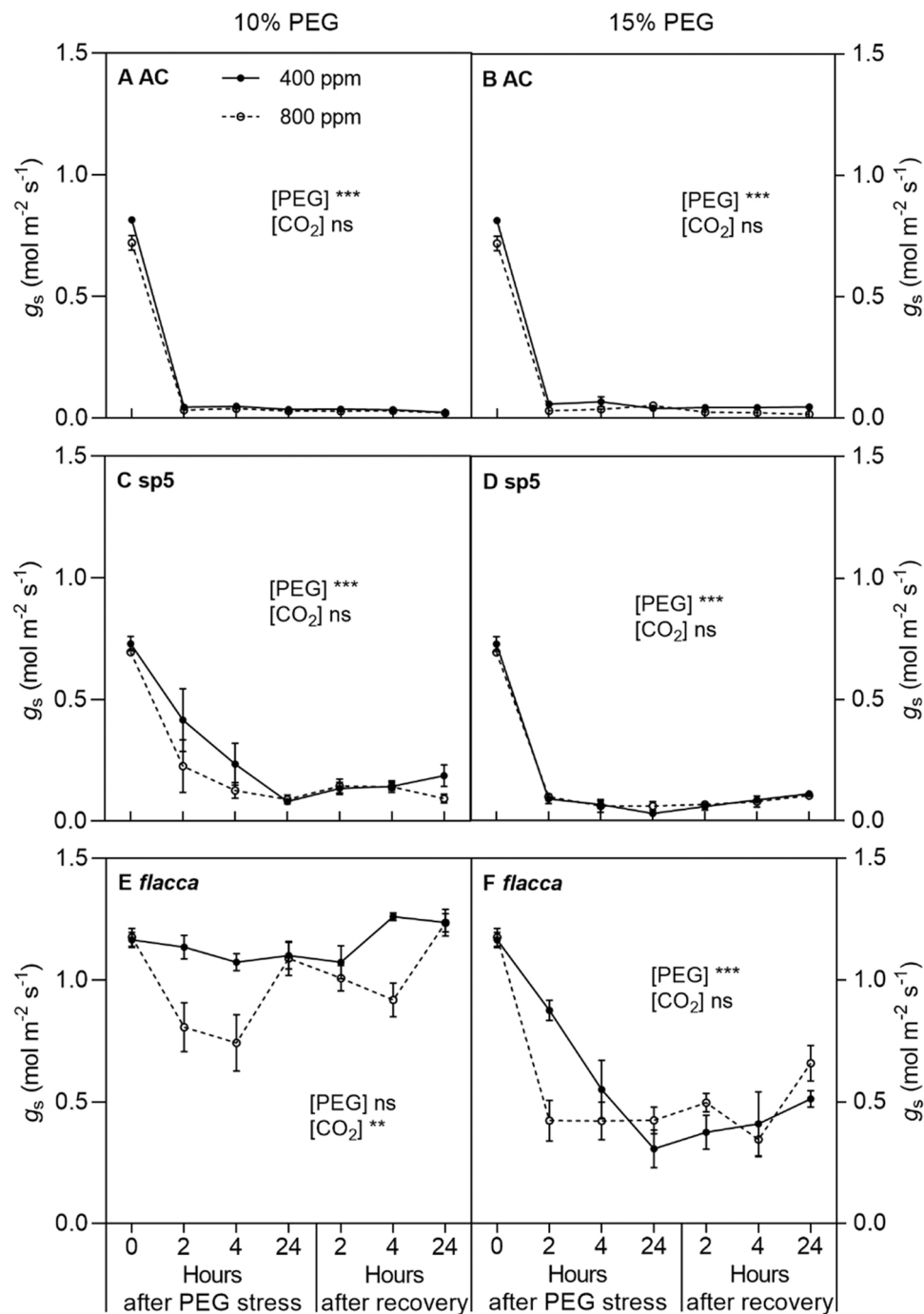


Fig. 5. Stomatal conductance (g_s) of AC (A,B), sp5 (C,D) and *flacca* (E,F) grown under ambient (400 ppm) and elevated (800 ppm) CO_2 concentrations exposed to 10% or 15% PEG stress and recovery. **, and *** indicate the significant difference among [CO₂] and PEG stress in ANCOVA (time of PEG treatments as covariate) at $P < 0.01$, $P < 0.001$, respectively; ns denotes no significant difference. Error bars indicate standard error of the means (S.E.) (N = 3–4).

on physiological processes of tomato genotypes differing in endogenous ABA levels. Our objective was to explore the role of ABA in mediating these responses.

4.1. Stomatal, leaf and root morphological traits as influenced by $e[\text{CO}_2]$ and endogenous ABA

Stomatal features, including SA , SD , and SS , are crucial for controlling water balance of plants in response to environmental stimulus (Franks and Beerling, 2009). The common view is that $e[\text{CO}_2]$ induces stomatal closure and decreases g_s (Hatfield and Dold, 2019), and plants

possess diverse stomatal control strategies under $e[\text{CO}_2]$ (Haworth et al., 2015). In angiosperm species, particularly crops, plants tend to exhibit no SD response to $e[\text{CO}_2]$, namely only decreased SA is responsible for decline in g_s . Consistent with this, here in AC, sp5 and *flacca*, $e[\text{CO}_2]$ only decreased SA and had no influence on SD and SS (Fig. 1). Furthermore, *flacca* plants possessed significant higher SD , SS and SA than other two genotypes, which coincided with our previous studies (Fang et al., 2019). It has been reported that smaller stomata can open and close more rapidly (Hetherington and Woodward, 2003), and lower SD also provides plants a better adaptive ability (Doheny-Adams et al., 2012). Therefore, compared to *flacca*, AC and sp5 might perform better when

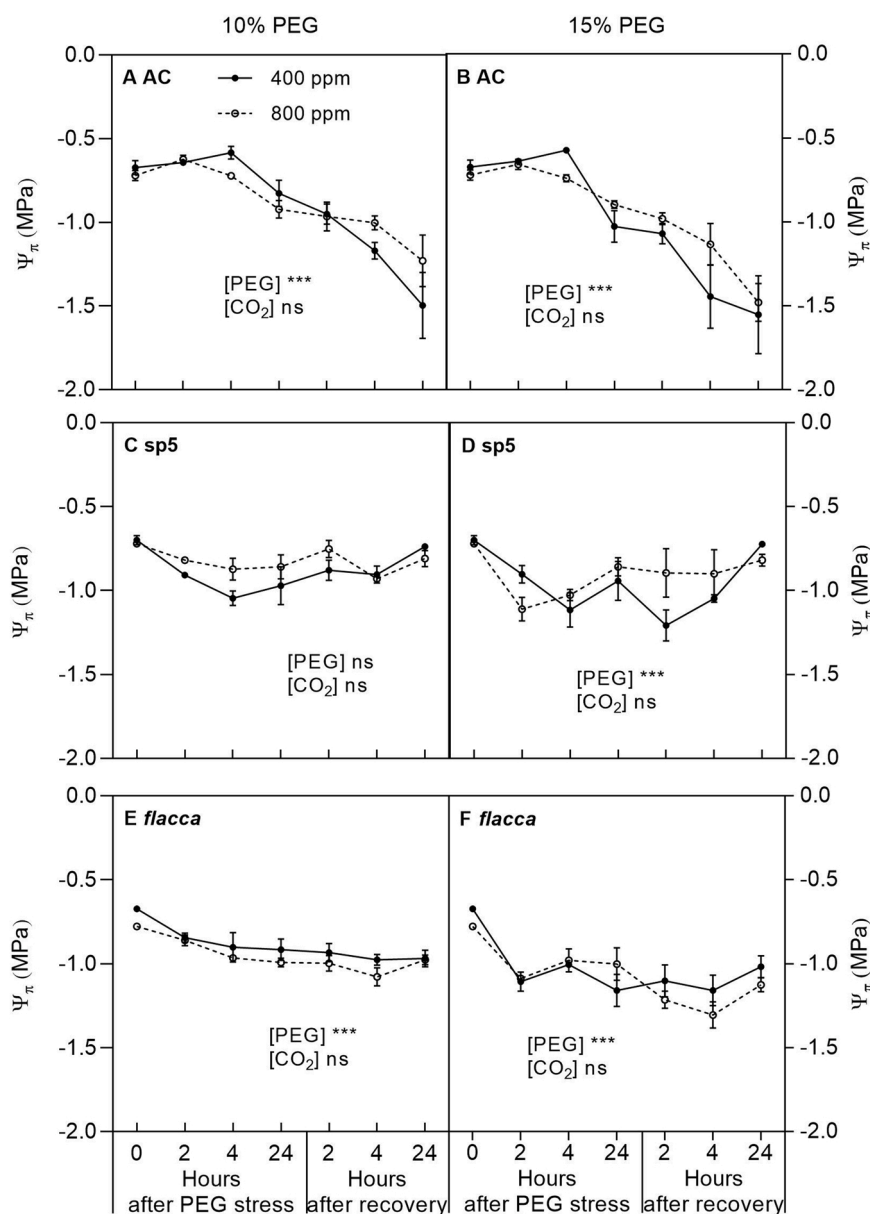


Fig. 6. Leaf osmotic potential (Ψ_{π}) of AC (A,B), sp5 (C,D) and *flacca* (E,F) grown under ambient (400 ppm) and elevated (800 ppm) CO_2 concentrations exposed to 10% or 15% PEG stress and recovery. *** indicate the significant difference among $[\text{CO}_2]$ and PEG stress in ANCOVA (time of PEG treatments as covariate) at $P < 0.001$; ns denotes no significant difference. Error bars indicate standard error of the means (S.E.). (N = 3–4).

exposed to adverse environmental conditions due to better stomatal functionality. It also should be noted that there was no difference between AC and sp5 on stomatal traits, indicating that slight high endogenous ABA level might have minor influence on stomatal features when water supply was sufficient.

Due to the stimulation on carbon gain, improved plant growth under $e[\text{CO}_2]$ has been widely reported in different crops (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007). In the present study, for all genotypes, LA and plant dry weight were greater for plants grown under $e[\text{CO}_2]$ compared to $a[\text{CO}_2]$ (Table 1). Moreover, $e[\text{CO}_2]$ also decreased SLA in AC and sp5, consistent with previous studies on tomato and other species (Wang et al., 2020; Wei et al., 2021), indicating that the thickness of leaves was enhanced by $e[\text{CO}_2]$, and which was associated with the improved carbon accumulation (Gielen et al., 2001). Furthermore, regarding to root morphological traits, $e[\text{CO}_2]$ increased RL and RBI in all genotypes, but had no effect on RD, SRL and RTD. Previously root branching patterns were found to act as the leading root traits to affect

both aboveground (e.g., indirectly related to SLA) and belowground resource uptake (Liese et al., 2017), and development of the lateral roots was involved in the regulation of the plant sensitivity to drought stress in *Arabidopsis* (Rosales et al., 2019). Temperate woody and herbaceous species grown under $e[\text{CO}_2]$ tended to construct thicker roots (e.g., increasing RD, decreasing SRL and RTD), representing an increase in root absorption capacity (Wang et al., 2020), whereas we observed that the root system of tomato benefited from $e[\text{CO}_2]$ through an increase in root branching that would help in water acquisition. In a free air carbon enrichment experiment, an increase in the number of roots was also observed in plants grown under $e[\text{CO}_2]$, and the authors proposed that climate change might lead to more allocation of carbon into roots relative to aboveground tissues (Arndal et al., 2018).

In *Arabidopsis*, application of a low concentration of exogenous ABA promoted root development (i.e., enhanced root length and biomass) and improved root conductivity (Rosales et al., 2019). Although increased K_{root} by endogenous or exogenous ABA manipulation has been

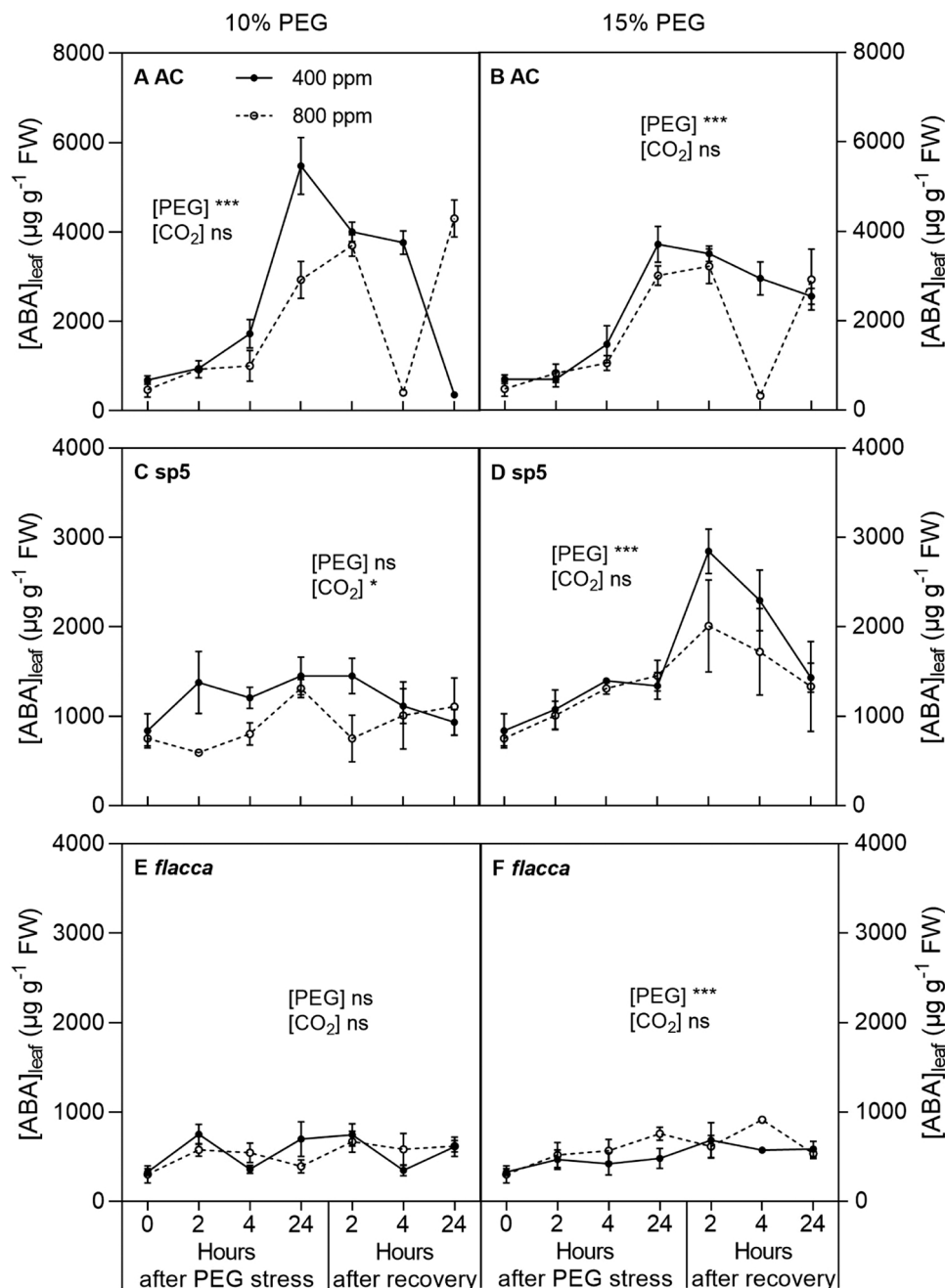


Fig. 7. Leaf ABA concentrations ($[ABA]_{\text{leaf}}$) of AC (A,B), sp5 (C,D) and flacca (E,F) grown under ambient (400 ppm) and elevated (800 ppm) CO_2 concentrations exposed to 10% or 15% PEG stress and recovery. * and *** indicate the significant difference among $[\text{CO}_2]$ and PEG stress in ANCOVA (time of PEG treatment as covariate) at $P < 0.05$, $P < 0.001$, respectively; ns denotes no significant difference. Error bars indicate standard error of the means (S.E.) ($N = 3-4$).

widely reported (Beaudette et al., 2007; Thompson et al., 2007; Veselov et al., 2018), yet little is known about whether the relationships between K_{root} and root traits are regulated by endogenous ABA level. In the present study, sp5 possessed significant higher RBI, SLA and K_{root} than other genotypes, also higher RTD and lower RL than AC (Table 1). Moreover, the PCA plot and multiple regression analysis revealed that those four variables were closely associated with K_{root} (Figs. 2 and 3; Table S1), particularly in sp5. Therefore, it could be assumed that the relatively higher level of endogenous ABA of sp5 would improve K_{root} through regulation of specific leaf and root traits. A similar coordination between SLA and root branching has also been found in temperate trees, revealing an acquisitive strategy for water and nutrient uptake (Liese et al., 2017). However, an opposite effect by $e[\text{CO}_2]$ was observed here: it increased RBI but declined K_{root} only in AC (Table 1). Our previous

studies found that in tomato plants $e[\text{CO}_2]$ decreased K_{root} through downregulation of aquaporins, and ABA was required in these response (Fang et al., 2019). Considering that exogenous ABA could also upregulate aquaporin activities thus enhancing K_{root} (Hose et al., 2000; Parent et al., 2009), high endogenous ABA level might override the depressive effect of $e[\text{CO}_2]$ on K_{root} , which could be one of the reasons that K_{root} of sp5 did not respond to $e[\text{CO}_2]$ (Table 1).

4.2. Plant phenotype and stomatal conductance as influenced by PEG-induced osmotic stress

Although it has been documented that only relatively high level of osmotic stress ($> 20\%$ PEG6000) induced short-term transcriptome response as well as severe dehydration response (Dubois and Inzé,

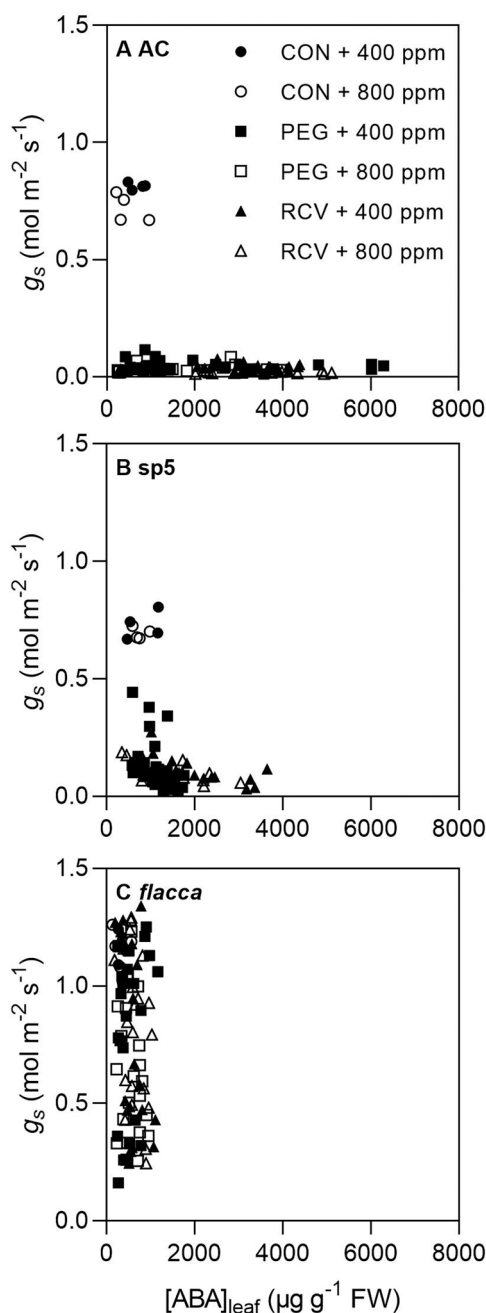


Fig. 8. Relationship between stomatal conductance (g_s) and leaf ABA concentrations ($[ABA]_{\text{leaf}}$) in AC (A), sp5 (B) and *flacca* (C) grown under ambient (400 ppm) and elevated (800 ppm) CO_2 concentrations exposed to 10% or 15% PEG stress and recovery. CON indicates controlled plants before PEG stress, PEG indicates plants under PEG stress, RCV indicates plants under recovery.

2020), in barley and rice 10–15% PEG stress could cause dramatic decreases on leaf gas exchange rates within 3 h upon treatment (L. Li et al., 2021; Teng et al., 2014). By contrast, maize plants showed moderate reduction on gas exchange rates when exposed to 10% PEG (Qiao et al., 2020). These results indicated that different species possess different sensitivities to PEG-induced osmotic stress. In the present study, severe declines in g_s of AC were observed upon 2 h of both 10% and 15% PEG exposure, along with visible signs of leaf wilting (Figs. 4 and 5), indicating that for tomato plants these two levels of PEG stress caused severe osmotic stress. Although in AC there was no difference in g_s between the two PEG levels during the whole treatment period, 15% PEG-stressed plants exhibited more severe wilting symptoms under both $[\text{CO}_2]$

levels (Fig. 4).

It is believed that plants perform better when grown under $e[\text{CO}_2]$ due to altered stomatal behaviour especially under drought stress (Li et al., 2020). Although previous studies indicated that severe drought stress diminished the positive effects of $e[\text{CO}_2]$ (Temme et al., 2018; Wei et al., 2020), here in all tomato genotypes, the leaf wilting was less severe in $e[\text{CO}_2]$ -grown plants than those grown under $a[\text{CO}_2]$, especially when treated with 15% PEG (Fig. 4). These results indicate that, to some extent, $e[\text{CO}_2]$ could alleviate the PEG-induced short-term severe osmotic stress. As no effect of $e[\text{CO}_2]$ on g_s was observed except in 10% PEG-stressed *flacca*, plants might benefit from $e[\text{CO}_2]$ through enhanced water relations but not altered stomatal behaviour. Furthermore, sp5 showed less reduction in g_s than AC, accompanied by an improved plant fitness particularly under $e[\text{CO}_2]$, whereas g_s of *flacca* were also less affected by PEG stress but accompanied by more severe leaf wilting compared to AC and sp5 (Figs. 4 and 5; Table 2). Generally, sp5 possessed a dampened response to osmotic stress which was probably due to its high initial endogenous ABA level leading to its greater ability of pre-adaptation to stress, while *flacca* could not close its stomata under osmotic stress, and had very low K_{root} , which might be associated with its low endogenous ABA, resulting in significant loss of leaf turgor (Fig. 4).

4.3. Plant water relations as affected by PEG-induced osmotic stress

PEG-induced osmotic stress is thought to be similar to drought stress (Verslues et al., 2006). In the short-term response, plants tend to close stomata to avoid lowering Ψ_{leaf} and RWC. When the stress is severe and plants is not able to maintain water balance, lowering Ψ_{leaf} becomes important to maintain the driving force for root water uptake (Ludlow et al., 1983). In the present study, PEG stress conversely increased Ψ_{leaf} especially in AC (Fig. S1), which was contrary to the widely accepted view in many reports (L. Li et al., 2021; Qiao et al., 2020; Zgallai et al., 2005). In addition, Fig. S1D shows the quickly formed xylem sap drop from the cut surface after leaf excision in all PEG-stressed plants, affirming the relatively high hydrostatic pressure in the xylem of the stressed plants. As PEG acts as an osmoticum to induce osmotic stress, the prerequisite that PEG can be used to mimic soil water deficit is that it triggers the same subsequent protection responses as soil water deficit, including osmotic adjustment, stomatal closure and decreasing Ψ_{leaf} . In the present study, the possible explanation of the observed bleeding of xylem sap upon PEG treatments accompanied by a decreased g_s of tomato plants could be that PEG stress caused substantial outward movement of water from cells to apoplasts and xylem before the commencement of physiological protection process, causing a fast leaf desiccation thus inducing stomatal closure. Although adding 5–20% PEG to the growth medium has been reported to cause a decrease of Ψ_{leaf} in tomato plants (Bressan et al., 1981), it could be assumed that the tomato cultivar used here was particularly sensitive to osmotic stress, thus PEG stress was not comparable with soil water deficit (at least in AC and *flacca*) and Ψ_{leaf} could not be used as an indicator of plant water status.

Under osmotic stress, plants accumulate more solute hence lowering Ψ_{π} to prevent water loss, contributing to the maintenance of leaf turgor (Verslues et al., 2006), and which has often been observed in plants exposed to PEG stress (Heyser and Nabors, 1981; Zgallai et al., 2005). Here, decreases in Ψ_{π} were found in all tomato genotypes, though they performed differently under different treatments (Fig. 6; Table 2). Decrease of Ψ_{π} was less pronounced in sp5 than in other two genotypes especially under 10% PEG stress, and only in sp5 the decreased Ψ_{π} could be restored during recovery, corresponding to the recovered leaf phenotype and RWC (Fig. 4 and S2). These results confirmed that sp5 had a dampened response to osmotic stress, and small fluctuation in Ψ_{π} could be indicator of improved stress resistance. In addition, during the whole treatment period, $e[\text{CO}_2]$ improved RWC in all genotypes especially for plants grown under 15% PEG stress (Fig. S2), which coincided

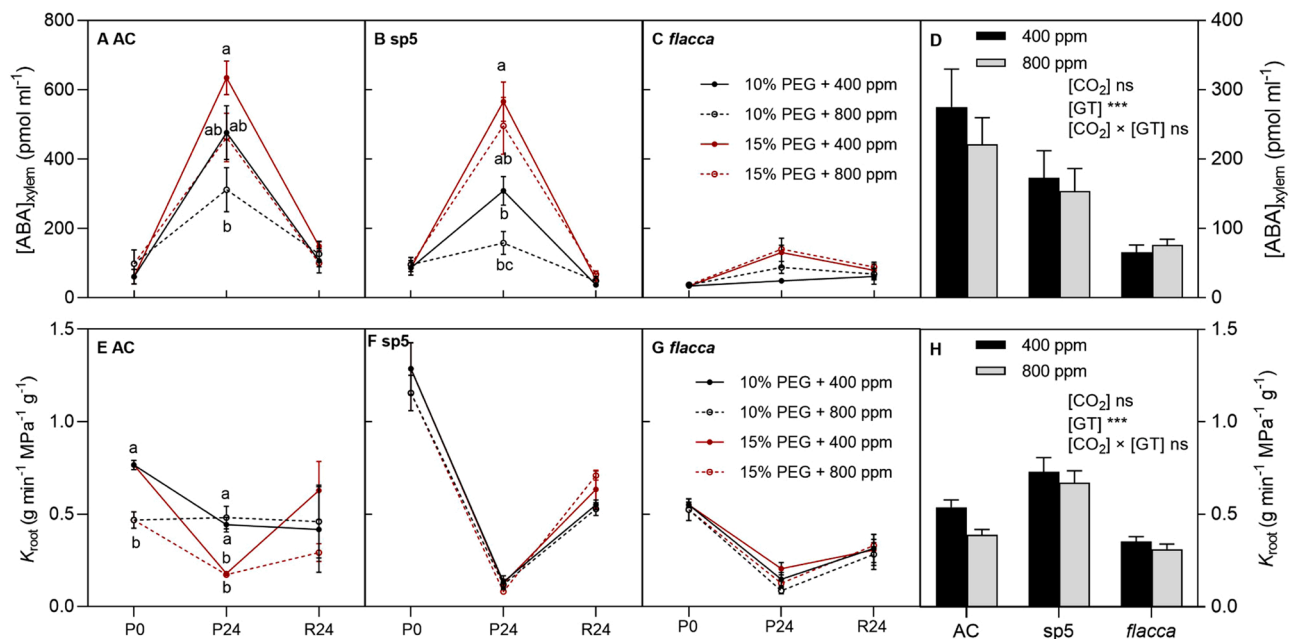


Fig. 9. Xylem sap ABA concentration ($[ABA]_{\text{xylem}}$) (A-C) and root hydraulic conductance (K_{root}) (E-G) of AC, sp5 and *flacca* grown under ambient (400 ppm) and elevated (800 ppm) CO_2 concentrations exposed to 10% or 15% PEG stress and recovery. P0 and P24 indicate 0 and 24 h after PEG stress, R24 indicate 24 h after recovery. Different letters indicate significant difference between treatments at a certain time in a certain genotype by Tukey's test at $P < 0.05$; no letters indicates there was no significant difference. Error bars indicate standard error of the means (S.E.) ($N = 4$). D and H show means of $[ABA]_{\text{xylem}}$ and K_{root} in the three genotypes during the three harvests. *** indicate the significant differences between $[\text{CO}_2]$ levels or genotypes (GT) in two-way ANOVA at $P < 0.001$; ns denotes no significant difference. Error bars indicate the standard error of the means (S.E.) ($N = 18-24$).

with the improved plant fitness under $e[\text{CO}_2]$ (Fig. 4), confirming that $e[\text{CO}_2]$ alleviated the PEG-induced severe osmotic stress through enhanced plant water relations.

It is worth noting that, in AC decreased Ψ_{π} by PEG stress became significant after 24 h of exposure, and was more pronounced during recovery, despite the early stomatal closure. A slight increase of Ψ_{π} by short-term 10% PEG stress (within 10 h) has also been reported in maize (Qiao et al., 2020). In addition, the function of solute accumulation under drought stress is associated with OA (Zhang et al., 1999). In the present study, AC plants did not achieve OA until recovery period, while sp5 and *flacca* showed significant OA earlier during the treatment (Table S2). Great ability of OA under osmotic stress is associated with high stress resilience, which can maintain turgor pressure at a low Ψ_{leaf} (Zurita-Silva et al., 2015). These results indicate that the improved osmotic stress resistance in sp5 might be ascribed to its more robust and earlier triggered OA, thus enhancing plant fitness. Furthermore, bulk modulus of elasticity of the cell walls (ϵ), representing the flexibility of plant tissues, also plays an important role in maintaining water balance and responds to osmotic stress (Sassi et al., 2010; Touchette et al., 2014). Increased ϵ could allow plants to maintain RWC without solute accumulation but with loss of turgor, thought to be a strategy of plants with more rigid and thicker cell walls to avoid dehydration even in the absence of OA (Sassi et al., 2010; Verslues et al., 2006), which could be the case for AC (lower SLA than sp5) at the early stage of PEG stress (Fig. 6 and S2), though we could not get the value of ϵ here due to lack of leaf turgor data. In *Spartina alterniflora*, increase of ϵ was associated with plants' susceptibility to water stress and indicated a loss of the capacity to re-establish leaf turgor (Hessini et al., 2009), which is consistent with our study, where AC plants could not be recovered from PEG stress (Fig. 3). Collectively, changes in ϵ and achievement of OA might provide plants' different strategies to cope with osmotic stress, depending on leaf traits, which merits further investigations.

4.4. ABA concentrations act as an indicator of drought resistance

Due to overexpress of the rate-limiting ABA biosynthetic gene *LeNCED1*, sp5 accumulates more ABA than wild type (Thompson et al., 2007), while ABA-deficient mutant *flacca* possessed significant lower ABA concentrations (Sagi et al., 2002), which is the case in the present study under well-watered condition (Fig. 7; Table 2). However, upon PEG treatments, AC plants had overall higher $[ABA]_{\text{leaf}}$ than both sp5 and *flacca* especially during the recovery period. Given the less severe of leaf wilting and more favorable water status in sp5 (Figs. 4 and 6), it could be assumed that sp5 suffered less severe osmotic stress than AC, thus exhibiting a dampened response and accumulating less $[ABA]_{\text{leaf}}$ under PEG stress, which could be a proxy of improved osmotic stress resistance. In a study conducted on another transgenic line over-expressing the *LeNCED1* gene (sp12), upregulation of genes involved in ABA metabolism in sp12 was observed under control conditions but not under salinity (Martínez-Andújar et al., 2020), which might be associated with an enhanced salinity tolerance similarly to our study. Furthermore, although previous studies on tomato plants revealed that $e[\text{CO}_2]$ -grown plants accumulated more $[ABA]_{\text{leaf}}$ and $[ABA]_{\text{xylem}}$ than those grown under $a[\text{CO}_2]$ (S. Li et al., 2021; Wei et al., 2020), here $e[\text{CO}_2]$ had a slight depressive effect on $[ABA]_{\text{leaf}}$ of sp5 (Fig. 7C), and in both AC and sp5 $e[\text{CO}_2]$ -grown plants accumulated less $[ABA]_{\text{xylem}}$ especially under 10% PEG stress (Fig. 9A, B). These results indicated that, in the present study, lower $[ABA]$ in sp5 and $e[\text{CO}_2]$ -grown plants were ascribed to their improved drought resistance, which might be associated with greater OA and altered metabolic processes (Ahmed et al., 2020; He et al., 2019; Teng et al., 2014); whereas dramatic low $[ABA]$ in *flacca* resulted in impaired stomatal functionality and imbalance of water control. In addition, it should be noted that $e[\text{CO}_2]$ -grown AC exhibited significantly lower $[ABA]_{\text{leaf}}$ after 4 h of recovery, then increased again, which was consistent with the changes in RWC (Fig. 7 and S2). The possible explanation could be that $e[\text{CO}_2]$ contributed to the recovery of functional integrity of the hydraulic system, but severely damaged roots by PEG had lost the ability for water uptake.

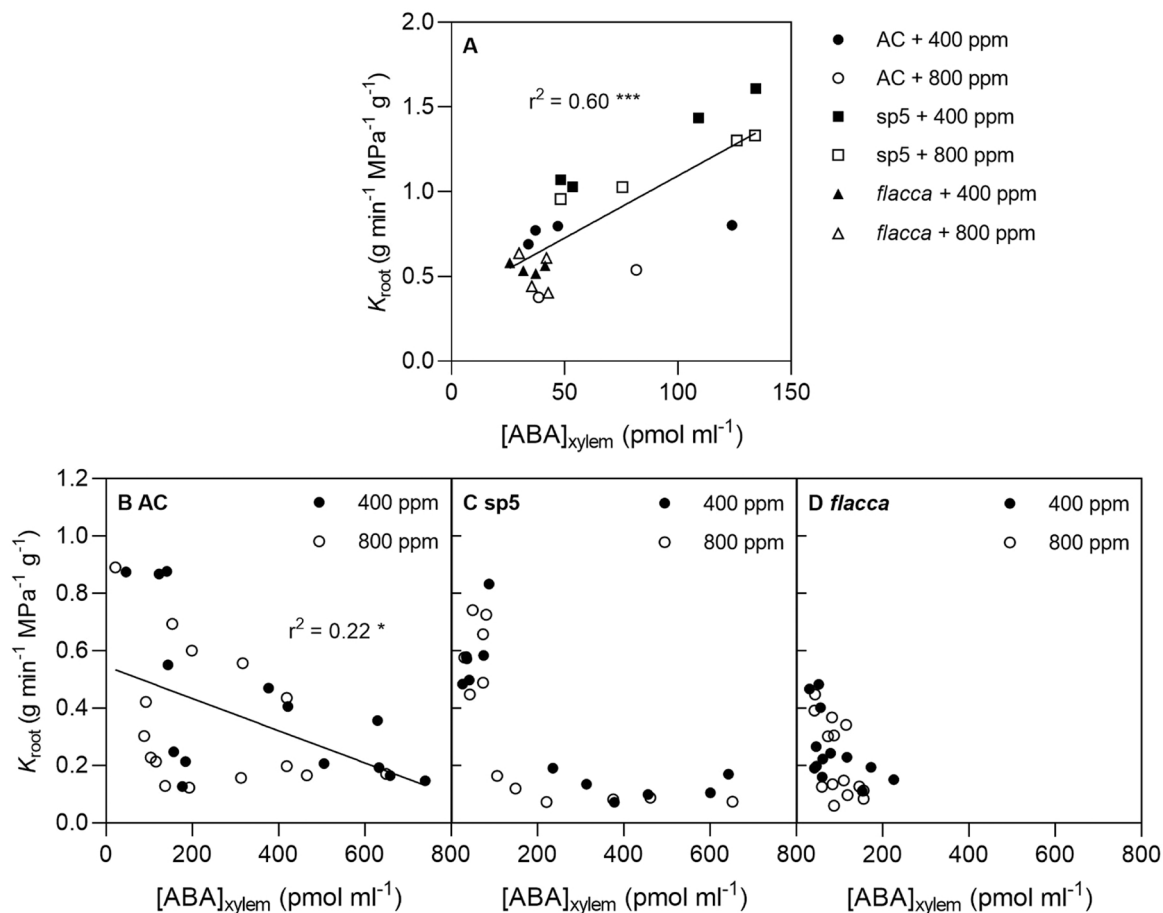


Fig. 10. A, relationship between root hydraulic conductance (K_{root}) and xylem sap ABA concentration ($[ABA]_{xylem}$) in well-watered AC, sp5 and *flacca* grown under ambient (400 ppm) and elevated (800 ppm) CO₂ concentrations. *** indicate significant difference of the regression line at $P < 0.001$. B-D, relationships between K_{root} and $[ABA]_{xylem}$ in AC, sp5 and *flacca* exposed to PEG stress and recovery. Only a regression line in B indicates that only in AC there was linear relationship between K_{root} and $[ABA]_{xylem}$.

It has been well established that stomatal closure in angiosperms is predominantly controlled by ABA (Brodrribb and McAdam, 2011), though hydraulic signaling also exists to induce stomatal closure in some species (Huber et al., 2019; Tombesi et al., 2015). In the present study, in all genotypes, $[ABA]_{leaf}$ significantly increased only after stomata being fully closed (Fig. 8), indicating that g_s in the short-term osmotic stress was not primarily regulated by ABA signal, which coincided with a study on grapevine (Tombesi et al., 2015) but was contrary to our previous study with plants subjected to long-term progressive soil drying (Wei et al., 2020). Therefore, tomato plants might exhibit different stomatal regulatory mechanisms under long-term vs. short-term drought stress, and a hydraulic signal (i.e., leaf turgor) rather than ABA induced initial stomatal closure during short-term PEG-induced osmotic stress.

Previous studies have demonstrated that $e[CO_2]$ decreased K_{root} in AC but not in *flacca* (Fang et al., 2019; S. Li et al., 2021), and sp5 with overproduction of ABA possessed higher root hydraulic conductivity than wild type (Thompson et al., 2007), which coincided with the results here (Fig. 9). Under PEG stress and recovery, K_{root} decreased and increased again in all three genotypes, but there was a much greater fluctuation in sp5 compared with other genotypes. A previous study with sp12 demonstrated that its hydraulic system was more vulnerable to embolism and exhibited a higher risk of hydraulic failure than wild type (Lamarque et al., 2019). However, in the present study, only sp5 could be recovered from osmotic stress despite of maintained stomatal closure (Figs. 4 and 5). Therefore, the high flexibility of hydraulic system in sp5 might conferred plants a better ability to coordinate the root water uptake with leaf water loss, thus contributing to plant hydraulic

homeostasis. Furthermore, positive relationship between K_{root} and $[ABA]_{xylem}$ was observed under well-watered condition (Fig. 10 A), confirming that high endogenous ABA levels enhance K_{root} (Beaudette et al., 2007). Nevertheless, under stressed condition, K_{root} decreased along with increasing $[ABA]_{xylem}$ in all three genotypes (Fig. 9B-D). It is worthy to note that K_{root} of sp5 was less sensitive to $[ABA]_{xylem}$, which was also an indicator of pre-adaption and conferred plants an improved drought resistance.

5. Conclusion

In tomato plants, both endogenous ABA levels and $e[CO_2]$ regulated stomatal, leaf and root morphological traits. sp5 with overproduction of ABA possessed higher SLA, higher RBI and K_{root} than AC, while ABA-deficient *flacca* had greater SD, SS and SA, accompanied by retarded plant growth and lower K_{root} . The global PCA showed that K_{root} was closely associated with SLA and RBI, and the depressive effect of $e[CO_2]$ on K_{root} could be overridden by high endogenous ABA level. When exposed to short-term PEG-induced osmotic stress and recovery, the three genotypes grown under two $[CO_2]$ levels exhibited different responses. Under both 10% and 15% PEG stress, AC and sp5 quickly closed stomata, accompanied by gradual decreases in Ψ_{π} and increases in $[ABA]_{leaf}$. Compared with AC, sp5 showed smaller fluctuations in Ψ_{π} and $[ABA]_{leaf}$, and a more robust and earlier OA response during treatments, accompanied by less severe leaf wilting symptoms. In *flacca*, low endogenous ABA caused abnormal stomatal development and low K_{root} , thus could not fully closed stomata and was more vulnerable to osmotic

stress. During recovery period, only in sp5 Ψ_{π} and RWC could be recovered, coinciding with restored plant phenotype. The improved stress resistance in sp5 also could be associated with the greater flexibility of hydraulic system and a reduced sensitivity of K_{root} to $[\text{ABA}]_{\text{xylem}}$ due to the preexisting effects of high endogenous ABA, representing a better coordinated plant water loss and uptake traits. Similarly, $e[\text{CO}_2]$ also improved stress resistance through enhanced water relations. Our results also provided evidences that whether PEG stress could mimic soil water deficits was depending on the achievement of OA in plants.

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CRediT authorship contribution statement

SL and FL conceived the concept and carried out the experiment. AT designed the protocol of seed germination. XW and XL helped to conduct the experiment. SL wrote the manuscript with support from FL and AT. FL supervised the project. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All data supporting the findings of this study are available within the paper and within its supplementary data published online.

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Author contributions

SL and FL conceived the concept and carried out the experiment. AT designed the protocol of seed germination. XW and XL helped to conduct the experiment. SL wrote the manuscript with support from FL and AT. FL supervised the project. All authors have read and agreed to the published version of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.envexpbot.2021.104763](https://doi.org/10.1016/j.envexpbot.2021.104763).

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