Exogenous Abscisic Acid Priming Modulates Water Relation Responses of Two Tomato Genotypes With Contrasting Endogenous Abscisic Acid Levels to Progressive Soil Drying Under Elevated CO₂

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Plants have evolved multiple strategies to survive and adapt when confronting the changing climate, including elevated CO₂ concentration (e[CO₂]) and intensified drought stress. To explore the role of abscisic acid (ABA) in modulating the response of plant water relation characteristics to progressive drought under ambient (a[CO₂], 400 ppm) and e[CO₂] (800 ppm) growth environments, two tomato (Solanum lycopersicum) genotypes, Ailsa Craig (AC) and its ABA-deficient mutant (flacca), were grown in pots, treated with or without exogenous ABA, and exposed to progressive soil drying until all plant available water in the pot was depleted. The results showed that exogenous ABA application improved leaf water potential, osmotic potential, and leaf turgor and increased leaf ABA concentrations ([ABA]leaf) in AC and flacca. In both genotypes, exogenous ABA application decreased stomatal pore aperture and stomatal conductance (gₛ), though these effects were less pronounced in e[CO₂]-grown AC and gₛ of ABA-treated flacca was gradually increased until a soil water threshold after which gₛ started to decline. In addition, ABA-treated flacca showed a partly restored stomatal drought response even when the accumulation of [ABA]leaf was vanished, implying [ABA]leaf might be not directly responsible for the decreased gₛ. During soil drying, [ABA]leaf remained higher in e[CO₂]-grown plants compared with those under a[CO₂], and a high xylem sap ABA concentration was also noticed in the ABA-treated flacca especially under e[CO₂], suggesting that e[CO₂] might exert an effect on ABA degradation and/or redistribution. Collectively, a fine-tune ABA homeostasis under combined e[CO₂] and drought stress allowed plants to optimize leaf gas exchange and plant water relations, yet more detailed research regarding ABA metabolism is still needed to fully explore the role of ABA in mediating plant physiological response to future drier and CO₂-enriched climate.

Keywords: elevated CO₂, drought stress, exogenous ABA, stomatal conductance, leaf water potential
INTRODUCTION

Elevated atmospheric carbon dioxide concentrations \( ([\text{CO}_2]) \), a major component of climate change, causes an increase in global mean surface temperature (Jia et al., 2019). Along with shifting rainfall patterns and reduced freshwater resources, more frequent occurrence of drought stress has become a major constraint on plant growth and productivity (Trenberth et al., 2014; Berg and Sheffield, 2018). It is well known that both \([\text{CO}_2] \) and drought stress influence plant water relations through the regulation of plant hormone abscisic acid (ABA), though the underlying mechanisms vary between the two environmental stimuli (Becklin et al., 2017; Li S. et al., 2020).

\([\text{CO}_2] \) has multiple physiological effects on plant water relations. Generally, \([\text{CO}_2] \) decreases stomatal conductance \( (g_s) \), thus optimizing water use efficiency (Hatfield and Dold, 2019). Meanwhile, an improved carbon assimilation rate together with an enhanced accumulation of solutes under \([\text{CO}_2] \) allow plants to achieve cell water homeostasis and maintain favorable leaf turgor through osmotic adjustment (OA) especially under abiotic stress (Wullschleger et al., 2002; Pérez-López et al., 2010). \([\text{CO}_2] \)-induced positive effects on plants tend to be more pronounced under drought (Li et al., 2017; Uddin et al., 2018). In tomato and grapevine, plants grown at \([\text{CO}_2] \) could sustain high levels of carbon assimilation rate for a longer period under drought stress due to the delayed drought effects on stomatal behavior (da Silva et al., 2017; Liu et al., 2019). Hydraulic adjustment is also one of the plants’ strategies to cope with drought stress (Comstock, 2002). Avila et al. (2020) found that coffee plants grown at \([\text{CO}_2] \) could better maintain leaf water potential \( (\Psi_{leaf}) \) and hydraulic conductance than their ambient \([\text{CO}_2] \)-counterparts under drought stress, thereby improving plant fitness. Although early studies have reported that \([\text{CO}_2] \)-induced stomatal closure could reduce plant water consumption hereby increasing the availability of water in the soil during drought (Field et al., 1995), recent studies revealed that plant grown at \([\text{CO}_2] \) might depleted soil water faster due to enlarged leaf area (Temme et al., 2018; Liu et al., 2019). Haworth et al. (2016) also indicated that the reduced effectiveness of stomatal closure at \([\text{CO}_2] \) could impair crops’ tolerance to severe drought despite of an improved water use efficiency.

Abscisic acid is involved in both the \([\text{CO}_2] \)- and drought stress-modulated plant water relation regulatory networks (Li S. et al., 2020). When plants are exposed to drought stress, rapid biosynthesis of ABA in roots is triggered, which is then transported from roots to leaves via the xylem to induce stomatal closure. The ABA-based root-to-shoot signaling has been considered the primary stomatal regulation mechanism in plants’ exposure to soil water deficits (Zhang et al., 1987; Zhang and Davies, 1990). However, root-sourced drought stress ABA-signaling theory has been challenged by reciprocal grafting studies on ABA biosynthetic mutants, revealing that leaf-sourced ABA could predominantly regulate stomatal aperture under rapid external pressure or long-term salinity/drought stress (Holbrook et al., 2002; Chen et al., 2003; Zhang et al., 2018). In addition, it is worthy of note that a significant proportion of root ABA was found to be derived from leaves and shoot (Ikegami et al., 2009; Ernst et al., 2010). These contrasting results raise questions about ABA homeostasis in plants when exposed to drought, including biosynthesis, catabolism, and transport. Furthermore, plants have evolved two different water management strategies: isohydric plants maintain a favorable \( \Psi_{leaf} \) and the integrity of the hydraulic system through an early stomatal closure, whereas anisohydric plants tend to keep stomata open for longer periods at a cost of hydraulic dysfunction (Sade et al., 2012). It has been reported that tomato plants exhibit an isohydric behavior, and this physiological trait is linked to the interaction of hydraulic and chemical (i.e., ABA) signals (Moshelion et al., 2015). Furthermore, our previous studies found that water management strategies of tomato plants could be modulated by \([\text{CO}_2] \), hydraulic signal (i.e., leaf turgor) rather than ABA predominantly controls stomatal aperture during soil drying process (Yan et al., 2017; Wei et al., 2020).

Nevertheless, ABA plays an obligatory role in various physiological responses of plants grown under the \([\text{CO}_2] \) environment, including altered stomatal behaviors (Chater et al., 2015; Liu et al., 2019), depression on hydraulic conductance (Fang et al., 2019), and subsequently reduced water loss and enhanced leaf turgor (Huang et al., 2019; Wei et al., 2020). Many reports have addressed the role of ABA in amplifying the \([\text{CO}_2] \) effects on stomatal behavior (McAdam et al., 2011; Engineer et al., 2016; Hsu et al., 2018), and increases in ABA concentration in plants grown under \([\text{CO}_2] \) have been observed in different species (Zou et al., 2007; Li B. et al., 2020). Furthermore, altered sensitivity of stomata to ABA at \([\text{CO}_2] \) has been proposed (Rasheke, 1975; Buncec, 1998). Recently, Li B. et al. (2020) found that increased ABA content at \([\text{CO}_2] \) could enhance soybean tolerance to drought stress, consistent with the findings by Gray et al. (2016) that \([\text{CO}_2] \)-grown soybean represented a stronger response to ABA under soil drying. However, these results did not show any difference in hydraulic conductance, xylem pH and \( \Psi_{leaf} \) caused by \([\text{CO}_2] \). In tomato plants, our previous studies showed that plants grown at \([\text{CO}_2] \) possessed a reduced sensitivity of stomata to leaf/xylem sap ABA during soil drying (Yan et al., 2017; Liu et al., 2019). A high xylem pH and a low hydraulic conductance had been reported in tomato plants grown under \([\text{CO}_2] \) (Fang et al., 2019), which would modulate the ABA signaling thus affecting the \( g_s \) sensitivity to drought stress.

The ABA-deficit mutant \( \text{flacca} \) has been widely used to investigate the function of ABA due to its significantly lower ABA content compared to its wild type counterpart, Ailsa Craig (AC) (Sagi et al., 2002). Due to the higher stomatal density and greater stomatal size in \( \text{flacca} \) compared to the wild type under either well-watered or drought-stressed conditions (Fang et al., 2019; Innes et al., 2021; Li and Liu, 2021), it is more vulnerable to adverse environments, including soil drought and high evaporative demand conditions. By using wild type tomato and \( \text{flacca} \) plants, Wei et al. (2020) and Li et al. (2021) found that \([\text{CO}_2] \) decreased \( g_s \), retarded stomatal drought response, and reduced hydraulic conductance in an ABA-dependent pathway.
Namely, these effects were absent or attenuated in flaca plants. Some researches about exogenous ABA application have been carried out on flaca as well as another ABA-deficit mutant sitiens (Tal et al., 1979; Sharp et al., 2000; Arocena et al., 2008), together with other treatments, to explore the abnormal phenotype caused by ABA deficit. Early studies by Tal et al. (1979) and Sharp et al. (2000) have reported that the retarded plant growth and abnormal stomatal behavior in flaca was associated with overproduction of ethylene and could be recovered by exogenous ABA application. Therefore, to investigate the role of ABA in e[CO₂]-modulated leaf gas exchange and plant water relation characteristics under drought stress, two tomato genotypes (AC and flaca) differing in the endogenous ABA concentrations were grown at two levels of [CO₂] (400 and 800 ppm), treated with or without exogenous ABA and exposed to progressive soil drying. We hypothesized that (1) exogenous ABA priming would rescue the stomatal response of flaca to e[CO₂] and soil drying and that (2) the effects of exogenous ABA on leaf gas exchange and water relations might influence e[CO₂]-modulated stomatal drought response.

**MATERIALS AND METHODS**

**Plant Materials and Growth Conditions**

Seeds of isogenic tomato cv. AC and its ABA-deficient tomato mutant (flaca) (Solanum lycopersicum) were provided by the Lancaster Environment Centre (Lancaster University, United Kingdom) and grown in climate-controlled greenhouses at the Faculty of Science, University of Copenhagen, Taastrup, Denmark. Due to the impairment in the oxidation of ABA-aldehyde to ABA, flaca had lower endogenous ABA concentrations compared to AC (Sagi et al., 2002). At the 4-leaf stage, the seedlings were transplanted to 4 L pots filled with 2.2 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). In total, 4 weeks after transplanting, perlite was used to cover the soil surface to minimize soil evaporation, and fertilizers as (NH₄)₂SO₄ (2.6 g) and H₂KPO₄ (1.5 g) per pot were added together with irrigation water to each pot to avoid nutrient deficiency.

From sowing, 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. 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 average daily [CO₂] in each cell during treatments are shown in Figure 1. The climate conditions in the two glasshouse cells were set at: 20/16 ± 2°C day/night air temperature, 60 ± 2% relative humidity (RH), 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 average temperature, RH, vapor pressure deficiency (VPD), and daily [CO₂] in the cells during the experiment are shown in Supplementary Figure 1.

**Exogenous Abscisic Acid Application and Progressive Soil Drying Treatments**

The sowing and growth of AC and flaca were conducted consecutively, and all treatments for the two genotypes were carried out separately. In each cell and genotype, 40 plants were established and constantly irrigated to 90% of the pot-holding capacity. Exogenous ABA application started 20 days after transplanting for three successive days. On each day, half of the plants were sprayed with 20 µM ABA (Sigma) twice a day on the whole plant at 10:00 and 15:00 h, respectively; the other half was sprayed with deionized water as non-ABA-treated control. All the solutions contained 0.1% (v/v) Tween-20 and 0.1% (v/v) ethanol.

In total, 1 day after finishing exogenous ABA application, four plants of each treatment (eight treatments in total) were harvested as initial control, then progressive soil drying was commenced. During soil drying, for each genotype, half of the plants were well watered to 95% of the pot’s water-holding capacity, and the remaining half was subjected to progressive soil drying by withholding irrigation from pots until all the transpirable soil water was depleted. Soil water content in the pot was expressed as the fraction of transpirable soil water (FTSW) (see below). In addition to the first harvest as initial control when FTSW was ca. 0.95, there were two destructive harvests during the soil drying period: the second was conducted when FTSW was ca. 0.50, and the last harvest was conducted at the end of soil drying when FTSW below 0.1. A total of four biological replicates (four individual plants) for each treatment were harvested. Due to the different water consumption rates between the two genotypes and the two [CO₂] levels, AC grown at a[CO₂] and e[CO₂] spent 10 and 9 days to reach the end of drought stress, respectively; flaca grown at both [CO₂] environments spent 13 days. Therefore, plants among different treatments were harvested on different days but at the same three FTSW points. The experimental treatments for AC and flaca were successively conducted, this might have caused the slightly different climatic conditions (see Supplementary Figure 1) between the two genotypes.

**Soil Water Status**

Soil water status was measured daily by weighing the pots with an Analytical Balance (Sartorius Model QA35EDE-S) at 15:30 h and expressed as FTSW. The daily value of FTSW was estimated as the ratio between the amounts of transpirable soil water remaining in the pots and the total transpirable soil water (TTSW). TTSW was defined as the difference of pot weight between full water holding capacity and when all the transpirable soil water was depleted, and calculated as:

\[
\text{TTSW} = \text{WT}_f - \text{WT}_e
\]

where WT_f is the pot weight at full water holding capacity (ca. 3.6 kg), and WT_e is the pot weight at the end of soil drying (ca. 1.8 kg).

Compared to our previous study (Wei et al., 2020), here the definition of FTSW was different which resulted in the longer duration for flaca plants to deplete the TTSW in relation to AC plants. The reason behind this was that exogenous ABA
application would affect the stomatal conductance ($g_s$) for both genotypes, thus using a common TTSW value would allow us to compare the $g_s$ response among the treatments on the same base. Therefore, for all treatments, the FTSW was calculated as follows:

$$\text{FTSW} = \frac{\text{WT}_n - \text{WT}_e}{\text{TTSW}} \quad (2)$$

where WT$_n$ is the pot weight on a given date, and WT$_e$ is pot weight at the end of soil drying.

### Stomatal Pore Aperture and Stomatal Conductance Measurements

A total of 1 day after exogenous ABA application (before soil drying), stomatal pore aperture ($S_A$, $\mu m^2$) was determined on four replicates (10 stomata for one replicates) following the method described by Yan et al. (2012). Stomata were observed under a LEITZ DMRD microscope camera system (Leica Microscope and System GmbH, D 35530, Wetzlar, Germany) equipped with a digital camera. Stomatal 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, United States, Java 1.6.0–24 (64 bit)]. Then $S_A$ was calculated as: $\pi \times W_a \times L_a / 4$.

During progressive soil drying, $g_s$ (mol m$^{-2}$ s$^{-1}$) were measured on the last youngest upper canopy fully expanded leaves between 9:00 and 12:00 h with a portable photosynthetic system (LiCor-6400XT, LI-Cor, Lincoln, NE, United States). Measurements were performed on one leaf per plant and four biological replicates for each treatment at 22°C cuvette temperature, 1500 µmol m$^{-2}$ s$^{-1}$ PAR, and [CO$_2$] of 400 ppm for a[CO$_2$] and 800 ppm for e[CO$_2$] growth environments, respectively.

### Determination of Plant Water Relations and Plant Growth

At each harvest, after gas exchange measurements, the same leaf was excised for the determination of midday leaf water potential ($\Psi_{\text{leaf}}$, MPa) with a scholander-type pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, United States) following the method described by Liu et al. (2006). Then the excised leaves were cut into two parts, frozen in liquid nitrogen, and stored at $-80^\circ$C for later determination of osmotic potential ($\Psi_{\pi}$, MPa) and leaf ABA concentration ([ABA]$_{\text{leaf}}$, µg g$^{-1}$ FW). $\Psi_{\pi}$ was measured at 20°C with a psychrometer (C-52 sample chambers, Wescor Inc., Logan, UT, United States) connected to a microvoltmeter (HR-33T, Wescor Inc., Logan, UT, United States).
The responses of $g_s$ to soil drying was described by a linear-plateau model (Faralli et al., 2019):

If FTSW $> C$: $g_s = g_{s\text{ ini}}$  
(3)

If FTSW $< C$: $g_s = g_{s\text{ ini}} + A \times (FTSW - C)$  
(4)

where $g_{s\text{ ini}}$ denotes initial $g_s$, $C$ denotes the FTSW threshold at which $y$ started to diverge from $g_{s\text{ ini}}$, and $A$ was the slope of the linear equation.

For ABA-treated *flacca* plants, before FTSW declining to the threshold, there was a significant increasing trend of $g_s$ during progressive soil drying. Therefore, when FTSW $> C$, the linear-plateau model was modified as follows:

If FTSW $> C$: $g_s = g_{s\text{ max}} - D \times (C - FTSW)$  
(5)

where $g_{s\text{ max}}$ denotes maximum $g_s$ when FTSW declined to the threshold ($C$), and $D$ was the slope of the linear equation before the threshold. $g_{s\text{ max}}$ of non-ABA-treated *flacca* was obtained from the average of the individual replicated values when FTSW reached $C$. In addition, for all *flacca* plants, $g_{\text{send}}$ indicated the $g_s$ at the end of drought treatment.

The parameters $y$, $C$, $A$, and $D$ were estimated by PROC NLIN of PC SAS 9.4 (SAS Institute Inc., Cary, NC, United States, 2002–2012) and the coefficient of determination ($r^2$) was calculated. Statistical comparison of each parameter obtained from the linear-plateau regression between treatments was performed by $t$-test using MedCalc statistical software 19.0.7.

Data were statistically analyzed using Microsoft Excel, SAS 9.4 (SAS Institute Inc., Cary, NC, United States, 2002–2012), SPSS 22.0 software (IBM SPSS Software, Armonk, NY, United States), and GraphPad Prism 9 software. Two-way analyses of variance (ANOVA) were performed to analyze the effects of [CO$_2$] and exogenous ABA ([ABA]) on plant dry weight, SA, water relation variables, and ABA concentrations in AC and *flacca*. To compare the decreasing trends of FTSW during soil drying between control treatment and exogenous ABA application, the statistical differences were analyzed by analysis of covariance (ANCOVA, days after withholding water as a covariate). One-way ANOVA (Tukey’s test) and Student’s $t$-test were conducted to determine the significant differences between treatments. Principle component analysis (PCA) of $g_s$, water relations, and leaf and xylem ABA concentrations in AC and *flacca* were performed in R version 4.0.0 (R Core Team, 2020). The relationship between $g_s$ and [ABA]$_{\text{leaf}}$, $g_{\text{s}}$, and $\Psi_p$ were evaluated by linear regression. $r^2$ of the regression lines were calculated and statistical differences on the slopes and intercepts of regression lines between treatments were performed by ANCOVA.

**RESULTS**

**Plant Growth and Soil Water Depletion**

In AC, exogenous ABA application did not have a significant influence on LDW and SDW, while e[CO$_2$] increased both LDW and SDW. In *flacca*, the ABA-treated plants had higher LDW and SDW when FTSW $= 0.50$ and 0.05 and did not respond to e[CO$_2$] except for an increased SDW at the last harvest (Table 1).

After withholding irrigation, the FTSW in the pots of drought-stressed plants decreased steadily (Figure 1). In AC, only for plants grown at e[CO$_2$], exogenous ABA slowed the soil water depletion rate during progressive soil drying. In *flacca*, at both [CO$_2$] levels, exogenous ABA had a significant influence on the rates of soil water depletion. However, from 0 to 10 days, FTSW declined slower in the ABA-treated plants compared with the non-ABA-treated plants, then it declined faster on the last 3 days.

**Stomatal Pore Aperture and Stomatal Conductance**

In both AC and *flacca*, e[CO$_2$] decreased stomatal pore aperture (SA), though it was more pronounced in AC (Figure 2). One day after ABA application (before imposing soil drying), exogenous ABA decreased SA in both genotypes, and interactions of [CO$_2$] and [ABA] were observed, indicating that the effect of e[CO$_2$] on SA was eliminated by exogenous ABA application, and the depressive effect of exogenous ABA was more pronounced in *flacca* than in AC.

In AC, during progressive soil drying, plants grown at e[CO$_2$] had significantly lower initial stomatal conductance ($g_{\text{send}}$) compared with their a[CO$_2$]-grown counterparts, and $g_s$ started to decline when FTSW dropped to an FTSW threshold ($C$). The ABA-treated plants had constantly lower $g_{s\text{ ini}}$ than the non-ABA-treated plants, and $C$ was also slightly lowered by exogenous ABA application (Figures 3A,B and Table 2).

In *flacca*, 1 day after exogenous ABA application, depression on $g_{s\text{ ini}}$ ranged from 50 to 58% at a[CO$_2$] and e[CO$_2$] environment, respectively. During soil drying, there were clear increasing trends of $g_s$ in the ABA-treated plants before FTSW reaching to the threshold, and when FTSW decreased to $C$, only the ABA-treated plants grown at a[CO$_2$] still had lower maximum $g_s$ ($g_{s\text{ max}}$) than the non-ABA-treated plants. Moreover, the FTSW thresholds were slightly advanced by exogenous ABA application. At the end of soil drying, exogenous ABA treatment still lowered $g_s$ ($g_{\text{send}}$) of plants grown at both [CO$_2$] levels, but all *flacca* plants possessed significantly higher $g_{\text{send}}$ than AC. In addition, e[CO$_2$] decreased $g_s$ in non-ABA-treated *flacca* and slightly advanced C (Figures 3C,D and Table 2).
### TABLE 1

| FTSW | [CO₂]  | [ABA] | AC     | flacca | AC     | flacca |
|------|--------|-------|--------|--------|--------|--------|
|      | 400 ppm| CON   | 3.74 ± 0.24 | 0.89 ± 0.05 | 0.92 ± 0.04 | 0.35 ± 0.02 |
|      |        | +ABA  | 3.64 ± 0.23 | 0.95 ± 0.08 | 0.90 ± 0.11 | 0.33 ± 0.02 |
| 0.95 | 800 ppm| CON   | 7.06 ± 1.22 | 1.85 ± 0.22 | 0.74 ± 0.03 | 0.25 ± 0.02 |
|      |        | +ABA  | 7.76 ± 0.80 | 2.02 ± 0.12 | 0.87 ± 0.02 | 0.25 ± 0.02 |
| [CO₂] |       |       | ***       | ***       | ns      | *       |
| [ABA] |       |       | ns        | ns        | ns      | ns      |
| [CO₂] × [ABA] | |       | ns        | ns        | ns      | ns      |
| 0.50 | 400 ppm| CON   | 9.52 ± 0.84 | 2.48 ± 0.12 | 2.67 ± 0.30 | 0.76 ± 0.05 |
|      |        | +ABA  | 10.55 ± 0.68 | 2.52 ± 0.10 | 4.15 ± 0.28 | 0.90 ± 0.06 |
| 800 ppm| CON   | 13.36 ± 1.82 | 4.12 ± 0.83 | 2.68 ± 0.09 | 0.82 ± 0.03 |
|      |        | +ABA  | 12.67 ± 1.45 | 3.14 ± 0.35 | 3.74 ± 0.67 | 1.01 ± 0.12 |
| [CO₂] |       |       | ns        | ns        | ns      | ns      |
| [ABA] |       |       | ns        | ns        | ns      | ns      |
| [CO₂] × [ABA] | |       | ns        | ns        | ns      | ns      |
| 0.05 | 400 ppm| CON   | 14.53 ± 0.66 | 4.59 ± 0.23 | 5.01 ± 0.33 | 1.49 ± 0.11 |
|      |        | +ABA  | 15.79 ± 0.82 | 4.73 ± 0.24 | 7.31 ± 0.30 | 2.04 ± 0.06 |
| 800 ppm| CON   | 18.07 ± 0.64 | 6.44 ± 0.43 | 5.04 ± 0.22 | 1.85 ± 0.11 |
|      |        | +ABA  | 18.15 ± 0.54 | 6.14 ± 0.32 | 7.93 ± 0.23 | 2.80 ± 0.17 |
| [CO₂] |       |       | ***       | ***       | ns      | ***     |
| [ABA] |       |       | ns        | ns        | ***     | ***     |
| [CO₂] × [ABA] | |       | ns        | ns        | ns      | ns      |

FTSW indicates the fraction of transpirable soil water; [CO₂] indicates [CO₂] level, [ABA] indicates without or with exogenous ABA application (expressed as CON/+ABA). * and *** indicate the significant differences between [CO₂] levels or ABA treatments at p < 0.05, p < 0.001, respectively; ns denotes no significant difference. Values are means ± standard error of the means (S.E.) (n = 3–4).

### FIGURE 2

Stomatal pore aperture (SA) of AC (A) and flacca (B) grown under ambient (400 ppm) and elevated (800 ppm) CO₂ concentrations 1 day after exogenous ABA application. CON indicates the control treatment sprayed with water, +ABA indicates exogenous ABA application. Different letters on the top of the columns indicate a significant difference between the treatments by Tukey's test at p < 0.05. ** and *** indicate the significant differences between [CO₂] and without or with exogenous ABA application ([ABA]) 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 = 40).

### Plant Water Relation Characteristics

A total of 1 day after exogenous ABA application, before imposing soil drying (i.e., FTSW = 0.95), the ABA-treated AC plants had higher leaf water potential (Ψ_leaf) and osmotic potential (Ψ₀), whereas the ABA-treated flacca plants possessed higher Ψ_leaf and turgor pressure (Ψ_p). In addition, slight interactions of [CO₂] and [ABA] were observed in Ψ_leaf and Ψ₀ of AC when FTSW = 0.95, showing that the effects of
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MODULATES EXOGENOUS ABA EFFECTS

FIGURE 3 | Changes of stomatal conductance ($g_s$) in AC (A,B) and flacca (C,D) (total samples = 32–44, $N = 3–4$) grown under ambient (400 ppm) and elevated (800 ppm) CO$_2$ concentrations during progressive soil drying. FTSW indicates the fraction of transpirable soil water. CON indicates the control treatment sprayed with water, +ABA indicates exogenous ABA application.

TABLE 2 | Output of statistical analysis of parameters derived from the linear-plateau regression of stomatal conductance ($g_s$) of AC and flacca under ambient (400 ppm) and elevated (800 ppm) CO$_2$ concentrations response to the reduction in the fraction of transpirable soil water (FTSW).

| [CO$_2$] | [ABA] | AC | flacca |
|----------|-------|-----|--------|
|          |       | C, $g_s$ ini | $g_s$ end | C, $g_s$ ini | $g_s$ end | $g_s$ max |
| 400 ppm  | CON   | 0.52 ± 0.02A | 0.57 ± 0.01aB | 0.05 ± 0.01B | 0.22 ± 0.03bB | 1.58 ± 0.03aA | 0.78 ± 0.09aA | 1.45 ± 0.02a |
|          | +ABA  | 0.41 ± 0.02A | 0.43 ± 0.01bB | 0.06 ± 0.01B | 0.31 ± 0.03abB | 1.66 ± 0.07cA | 0.23 ± 0.03bA | 1.24 ± 0.06b |
| 800 ppm  | CON   | 0.53 ± 0.05A | 0.42 ± 0.01bB | 0.05 ± 0.02B | 0.34 ± 0.04abB | 1.22 ± 0.10bA | 0.53 ± 0.07aA | 1.08 ± 0.02b |
|          | +ABA  | 0.42 ± 0.04A | 0.34 ± 0.01cB | 0.06 ± 0.01B | 0.40 ± 0.03aB | 0.55 ± 0.05cA | 0.21 ± 0.03bA | 1.04 ± 0.04b |

[CO$_2$] indicates [CO$_2$] level, [ABA] indicates without or with exogenous ABA application (expressed as CON/+ABA). The data is presented in Figure 3. Values are means ± SE. Lowercase letters indicate the significant difference among the [CO$_2$] and [ABA] treatments in each column; capital letters indicate the significant difference between AC and flacca; no letter indicates no significant difference.

C, the threshold at $g_s$ which started to decline due to drought stress; $g_s$ ini, the initial values of variables when plants were not significantly affected by drought; $g_s$ end, the final values of variables at the end of drought stress. For flacca, $g_s$ max indicates the maximum values of variables before it started to decrease.

Exogenous ABA became less significant when plants were grown at $e$[CO$_2$]. At the end of soil drying (i.e., FTSW = 0.05), all these plant water relation variables significantly decreased by drought stress, and exogenous ABA had no influence on water relations of flacca, whereas it still slightly increased $\Psi$$_{\text{leaf}}$ in AC. Moreover, $e$[CO$_2$] improved $\Psi$$_{\text{leaf}}$, $\Psi$$_{\text{x}}$, and $\Psi$$_{\text{p}}$ of AC plants compared to $a$[CO$_2$], though these effects being diminished along with soil drying (Figure 4 and Table 3).

Leaf, Xylem Sap Abscisic Acid Concentrations and Their Relationships With the Fraction of Transpirable Soil Water Threshold

In total, 1 day after exogenous ABA application, the ABA-treated AC plants possessed higher leaf ABA concentration [ABA]$_{\text{leaf}}$ than the non-ABA-treated plants, while the ABA-treated flacca had both higher [ABA]$_{\text{leaf}}$ and xylem sap ABA...
FIGURE 4 | Leaf water potential ($\psi_{\text{leaf}}$), osmotic potential ($\Psi_o$), and turgor pressure ($\Psi_t$) in AC (A–C) and flacca (D–F) grown under ambient (400 ppm) and elevated (800 ppm) CO$_2$ concentrations during progressive soil drying. CON indicates the control treatment sprayed with water, +ABA indicates exogenous ABA application. FTSW indicates the fraction of transpirable soil water. Different letters on the top of the columns indicate a significant difference between the treatments by Tukey’s test at $p < 0.05$. Error bars indicate the standard error of the means (S.E.) ($N = 3–4$).

concentration ([ABA]$_{\text{xylem}}$) compared with the non-ABA-treated controls. During soil drying, when FTSW = 0.50, there were interactions of [ABA] and [CO$_2$] on [ABA]$_{\text{leaf}}$ of both genotypes, which was relatively higher in the ABA-treated plants grown under $e$[CO$_2$] than under $a$[CO$_2$]. Moreover, at the end of soil drying (i.e., FTSW = 0.05), significantly higher [ABA]$_{\text{xylem}}$ was observed in the ABA-treated flacca than the non-ABA-treated flacca plants, accompanied by an interaction of [CO$_2$] and [ABA]. Drought stress increased both [ABA]$_{\text{leaf}}$ and [ABA]$_{\text{xylem}}$ at the end of soil drying though being less significant in flacca (Figure 5 and Table 3).

The relationships of the FTSW threshold at which $g_s$ started to decline (C) and [ABA]$_{\text{leaf}}$ at the second harvest (FTSW value was close to C) in AC and flacca are shown in Figure 6. The results showed that only in flacca, C was linearly correlated with [ABA]$_{\text{leaf}}$, whereas C of AC did not respond to increasing [ABA]$_{\text{leaf}}$. Moreover, no obvious relationship between C and [ABA]$_{\text{xylem}}$ was observed in either genotype.

Principle Component Analysis Plot of $g_s$, Water Relations, Leaf, and Xylem Abscisic Acid Concentrations of the Well-Watered Plants

Principle component analysis plot of $g_s$, water relations, leaf, and xylem ABA concentrations of well-watered AC and flacca is depicted in Figure 7. PC1 and PC2 axes explained 70.3% of cluster formation, with 49.1% attributed to PC1 and 21.2% to PC2. Overall, AC under all treatments and ABA-treated flacca were clustered toward the right side of the PCA plot, whereas all non-ABA-treated flacca were clustered to the left in the same direction as the $g_s$ vector, indicating non-ABA-treated flacca had higher $g_s$ than other plants. The clustering of the [AC, 800 ppm, +ABA] indicated a covariation between $\Psi_{\text{leaf}}$ and [ABA]$_{\text{leaf}}$ under this treatment, which was negatively associated with $g_s$. [ABA, flacca] and [AC, 800 ppm, CON] treatments were grouped along with PC2, showing a close correlation with [ABA]$_{\text{xylem}}$ and
TABLE 3 | Results of two-way ANOVA test showing the statistical significance of the effects of [CO₂] and without or with exogenous ABA application ([ABA]) on leaf water potential ($\Psi_{leaf}$), osmotic potential ($\Psi_s$), turgor pressure ($\Psi_p$), leaf and xylem sap ABA concentrations ($[ABA]_{leaf}$, $[ABA]_{xylem}$) in AC and flacca during progressive soil drying.

| Genotype | FTSW   | Factor            | $\Psi_{leaf}$ (MPa) | $\Psi_s$ (MPa) | $\Psi_p$ (MPa) | $[ABA]_{leaf}$ (µg g⁻¹ FW) | $[ABA]_{xylem}$ (pmol ml⁻¹) |
|----------|--------|-------------------|---------------------|----------------|----------------|-------------------------------|-----------------------------|
| AC       | 0.95   | [CO₂]             | ***                 | ***            | ***            | ns                           | ns                          |
|          |        | [ABA]             | *                   | *              | ns             | ns                           | ns                          |
|          |        | [CO₂] × [ABA]     | *                   | ns             | *              | ns                           | ns                          |
|          | 0.50   | [CO₂]             | ns                  | ns             | *              | ns                           | ns                          |
|          |        | [ABA]             | *                   | ns             | ns             | ns                           | ns                          |
|          |        | [CO₂] × [ABA]     | ns                  | ns             | ns             | ns                           | ns                          |
| flacca   | 0.95   | [CO₂]             | ns                  | *              | ns             | ns                           | ns                          |
|          |        | [ABA]             | ***                 | ns             | ***            | ***                          | ***                         |
|          |        | [CO₂] × [ABA]     | ns                  | ns             | ns             | ns                           | ns                          |
|          | 0.50   | [CO₂]             | ns                  | ns             | ns             | ns                           | ns                          |
|          |        | [ABA]             | ***                 | ns             | ns             | ns                           | ns                          |
|          |        | [CO₂] × [ABA]     | ns                  | ns             | *              | ns                           | ns                          |
|          | 0.05   | [CO₂]             | ns                  | ns             | ns             | ns                           | ns                          |
|          |        | [ABA]             | *                   | ns             | ns             | ns                           | ns                          |
|          |        | [CO₂] × [ABA]     | ns                  | ns             | ns             | ns                           | ns                          |

FTSW indicates the fraction of transpirable soil water.

* and ** indicate the significant differences at p < 0.05, p < 0.01, respectively.

ns denotes no significant difference.

The data is presented in Figures 4, 5.

$\Psi_p$ and no correlation with $g_s$. Furthermore, [AC, 400 ppm] was opposed by $\Psi_p$ and $[ABA]_{xylem}$, being clustered to the bottom of the plot in the same direction as $\Psi_s$ vector.

**Relationships Between Stomatal Conductance, Leaf Abscisic Acid Concentration, and Leaf Turgor**

In AC, there were linear relationships between $g_s$ and $[ABA]_{leaf}$, $g_s$ and $\Psi_s$, where $g_s$ declined linearly with increasing $[ABA]_{leaf}$ and decreasing $\Psi_s$ (Figure 8). Exogenous ABA application decreased the slope of the regression line of $g_s$ and $[ABA]_{leaf}$, while $e[CO₂]$ had no influence on it. Regarding the regression line of $g_s$ and $\Psi_s$, the slopes differed between ABA-treated and non-ABA-treated plants, whereas $e[CO₂]$ only decreased the slope in non-ABA-treated plants. In flacca, there was no significant relationship between $g_s$ and $[ABA]_{leaf}$ under either treatment, while $g_s$ decreased linearly with declining $\Psi_s$. In addition, the intercepts of these regression lines differed between [ABA] treatments due to the initial low levels of $g_s$.

**DISCUSSION**

Plant hormone ABA is a vital factor in both $e[CO₂]$- and the drought stress-mediated plant water relation regulatory network, and ABA homeostasis is essential for plants to cope with drought stress. In the present study, we investigated different effects of exogenous ABA application and $e[CO₂]$ as well as their interactions on tomato leaf gas exchange and water relation characteristics during progressive soil drying.

**Plant Growth and Stomatal Conductance as Influenced by Elevated CO₂ and Exogenous Abscisic Acid**

It is well known that $e[CO₂]$ decreases $g_s$ while stimulating carbon assimilation, thus improving plant drought tolerance (Li S. et al., 2020). In tomato plants, previous studies have shown that the plant response to $e[CO₂]$ was attenuated or absent in ABA-deficient mutant flacca, affirming the obligatory role of ABA in CO₂ signaling pathway (Fang et al., 2019; Wei et al., 2020). Consistent with this, here LDW and SDW of AC plants were improved by $e[CO₂]$ during progressive soil drying, but these stimulations were less significant in flacca. Upon exogenous ABA application, retarded plant growth of flacca was rescued under both watering conditions, while growth of AC plants did not benefit from exogenous ABA treatment (Table 1), which was consistent with previous study on tomato and confirmed the important role of ABA in maintaining the shoot development (Sharp et al., 2000; Aroca et al., 2008).

It has been reported that application of exogenous ABA inhibited water loss and reduced the rate of soil drying due to stomatal closure (Hossain et al., 2015; He et al., 2019). In the present study, in both ABA-treated AC and flacca, slower soil water depletion during progressive soil drying was recorded, which coincided with the decreases of $SA$ and $g_s$, related to the non-ABA-treated controls (Figures 1–3). However, in the drought-stressed AC plants, only those grown under $e[CO₂]$...
FIGURE 5 | Leaf and xylem sap ABA concentrations ([ABA]_{leaf, A–D}; [ABA]_{xylem, E–H}) in AC and flacca grown under ambient (400 ppm) and elevated (800 ppm) CO₂ concentrations during progressive soil drying. CON indicates the control treatment sprayed with water, +ABA indicates exogenous ABA application. FTSW indicates the fraction of transpiration soil water. *, **, and *** indicate the significant differences between ABA treatments by Student’s t-test at p < 0.05, p < 0.01, p < 0.001, respectively; ns denotes no significant difference. Error bars indicate the standard error of the means (S.E.) (N = 3–4).
possessed slower rate of soil water depletion upon exogenous ABA application compared with the non ABA-treated plants, indicating a significant interaction of e[CO$_2$] and exogenous ABA on plant water consumption when soil water was limited. Although in AC e[CO$_2$] accelerated water consumption due to stimulated plant growth, exogenous ABA application had no influence on plant growth (Figure 1 and Table 1). Therefore, the interactive effect of e[CO$_2$] and exogenous ABA on water consumption rate might be ascribed to the altered stomatal behavior, as both factors could induce stomatal closure.

It is worth noting that upon exogenous ABA treatment the depressive effect of e[CO$_2$] on SA was eliminated in both genotypes (Figure 2). Considering that e[CO$_2$] had exerted inhibiting effects on stomatal aperture, it could be assumed that exogenous ABA application overrode the effect of e[CO$_2$] on stomatal movement. However, the common view is that ABA can amplify the effects of [CO$_2$] on stomatal behavior (Engineer et al., 2016), and e[CO$_2$]-grown soybean showed a stronger response to endogenous ABA (Gray et al., 2016), though ABA and [CO$_2$] signal transductions also could be independent (Hsu et al., 2018). As our previous studies demonstrated that severe drought stress overrode the e[CO$_2$] effects, here exogenous ABA application exhibited the same influences as drought stress. Furthermore, decreases in SA and $g_s$ were more pronounced in flacca than AC (Figures 2, 3). Namely, the stomata of flacca exhibited greater sensitivity to exogenous ABA than AC due to ABA deficit. Interestingly, after the onset of soil drying, before the occurrence of stomatal closure, there were significant increasing trends of $g_s$ in the ABA-treated flacca while the ABA-treated AC possessed a constantly lower $g_s$ before the FTSW thresholds (Figure 3), indicating that the effects of exogenous ABA application lasted for a longer period in AC than in flacca. Early studies have found that the abnormal stomatal behavior in flacca was associated with the high level of ethylene resulting from an ABA deficit (Tal et al., 1979), and other chemical signals could also counteract the ABA signaling, as is the case with cytokinin (Prerostova et al., 2018), which might accelerate the degradation process of exogenous ABA in flacca plants. In addition, flacca is known to be impaired in the oxidation of ABA aldehyde to ABA (Sagi et al., 2002), vanishing effects of exogenous ABA might be ascribed to the redistribution of ABA to other plant tissues. These results suggest the necessity to explore the regulation of ABA homeostasis in the two genotypes in future studies. Despite the significantly lower $g_{sini}$ in ABA-treated flacca, the $g_{end}$ of flacca was still higher than AC under all treatments (Figure 3 and Table 2), which indicated that flacca could not fully close stomata under severe drought stress after exogenous ABA application. In another ABA-deficient mutant sitiens, a decrease in the leaf gas exchange rate by exogenous ABA (100 μM) was significant after 52 days of mild drought stress, but it still possessed a higher level of transpiration rate than wild type (Arora et al., 2008). Therefore, exogenous ABA priming before soil drying could not sufficiently induce stomatal closure under severe drought stress.

Previous studies on tomato revealed that e[CO$_2$] retarded stomatal closure during soil drying due to reduced stomatal sensitivity to ABA (Liu et al., 2019; Wei et al., 2020). However, here we did not find e[CO$_2$] delayed stomatal closure in AC, the reason behind this discrepancy is unknown, which might be ascribed to the varied experimental conditions between the different studies. By contrast, in flacca plants, exogenous ABA application sensitized the stomatal response to drought though only significant under a[CO$_2$] (Figures 3C,D and Table 2), suggesting that the stomatal closure in ABA-deficient mutant could be advanced by exogenous ABA treatment, which might contribute to water-saving under drought stress. In addition, e[CO$_2$] decreased $g_s$ in flacca despite the slight influence on SA, and these effects were absent in ABA-treated flacca. Although the retarded stomatal response to e[CO$_2$] in flacca was attributed to ABA deficit (Wei et al., 2020; Li et al., 2021), exogenous ABA application could not recover the response due to its strongly induced stomatal closure.

### Plant Water Relations as Influenced by Elevated CO$_2$ and Exogenous Abscisic Acid

Previous studies have suggested that exogenous ABA application is beneficial for plants to improve drought tolerance as a result of induced stomatal closure, enhanced water relations, and a higher OA (Du et al., 2013; He et al., 2019). In the present study, 1 day after exogenous ABA application, higher $\Psi_{leaf}$, $\Psi_{p}$, and $\Psi_{p}$ were observed compared to the non-ABA-treated plants (Figure 4), confirming the positive effects of exogenous ABA on plant water relations. In an early study with exogenous ABA application (10 μM) on flacca, the authors found that the impaired shoot growth could be restored but no improved $\Psi_{leaf}$ was observed (Sharp et al., 2000). As stimulation on expansive growth by ABA is primarily related to hydraulic control (Tardieu et al., 2015), here the improved water status in ABA-treated flacca might be responsible for the restored growth (Table 1). Moreover, the positive effects of exogenous ABA on plant water
relations were more pronounced in flacca than in AC (Figure 4), coinciding with the stomatal responses in the two genotypes. In wild-type tomato and ABA-deficient mutant sitiens, Aroca et al. (2008) found that the genotypes showed different gene regulatory patterns in response to exogenous ABA, including ABA biosynthesis-related and aquaporin-encoding genes, which might be associated with the high sensitivity to ABA in flacca plants. Surprisingly, at the end of soil drying, the improved water relations, by application of exogenous ABA, vanished in both genotypes, including $\Psi_p$ (Figure 4 and Table 3). Thereby, the decreased $g_s$ in ABA-treated flacca at the end of soil drying could not be explained by modulated water relations. In addition, OA is also considered as a vital trait for maintaining leaf turgor, and increased OA accompanied by improved leaf water relations have also been reported in exogenous ABA-treated soybean (He et al., 2019). However, here we did not find the influence of exogenous ABA on OA in both genotypes during the whole period of soil drying. Therefore, more detailed mechanisms related to the ABA-modulated plant water relations merit future studies.

The positive effects of $e[CO_2]$ on plant growth and physiology are reported to be more pronounced under drought stress which, however, depends on the severity of drought stress (Leakey et al., 2006; Gray et al., 2016). In the present study, the well-watered AC plants grown at $e[CO_2]$ had an improved $\Psi_{leaf}$, $\Psi_\pi$, and $\Psi_p$ in relation to those grown under $a[CO_2]$, but these effects were eliminated during soil drying (Figure 4 and Table 3), in line with previous findings (Wei et al., 2020). As $e[CO_2]$-grown plant already possessed improved water relations, effects of exogenous ABA on AC became less significant at $e[CO_2]$. In Figure 8, $e[CO_2]$ rendered a less sensitivity of stomata to $\Psi_\pi$, but this effect was absent in ABA-treated AC. Collectively, these results implied an offsetting effect of $[CO_2]$ and exogenous ABA on plant water relations as well as stomatal behavior.

Abscisic Acid Signal Mediates Stomatal Drought Response

In the present study, 1 day after exogenous ABA application, significant increases of $[ABA]_{leaf}$ in AC and flacca were observed, and the increase was more pronounced in flacca than in AC, though it was gradually diminished during soil drying (Figure 5 and Table 3). As oxidative degradation of ABA could occur rapidly (Zhang et al., 1995), in tomato plants, when ABA-treated plants were exposed to long-term drought (52 days), ABA contents in plants did not differ between the ABA-treated and non-ABA-treated plants despite the accumulated exogenous ABA effects on water relations still existing (Aroca et al., 2008). By contrast, here, we found that
the vanishing effect on water relations was accompanied by decreased accumulation of \([\text{ABA}]_{\text{leaf}}\) in ABA-treated \textit{flacca}. More interestingly, the interactions of \([\text{CO}_2]\) and \([\text{ABA}]\) on \([\text{ABA}]_{\text{leaf}}\) of \textit{AC} and \textit{flacca} indicated that the ABA degradation process might be disturbed under \([\text{CO}_2]\), which could explain the reduced ABA sensitivity in the drought-stressed tomato plants grown under \([\text{CO}_2]\) (Yan et al., 2017; Wei et al., 2020). In ABA-treated \textit{flacca}, an increase in \([\text{ABA}]_{\text{stem}}\) was observed especially at the end of soil drying, which was accompanied by an interaction of \([\text{CO}_2]\) and exogenous ABA (Figure 5 and Table 3), implying that \([\text{CO}_2]\) exerted an effect on ABA homeostasis. It is well documented that ABA metabolism, including biosynthesis and degradation, ABA recirculation, and exudation processes are all involved in the regulation of ABA homeostasis (Hartung et al., 2005). Moreover, redistribution of ABA within plant organs has often been reported, and translocation of ABA from leaves to roots can be intensified when roots are experiencing drought (Hartung et al., 2005; Ikegami et al., 2009; Ernst et al., 2010). Taking together, except for the ABA degradation pathway, decrease of \([\text{ABA}]_{\text{leaf}}\) in the ABA-treated \textit{flacca} could also be ascribed to the redistribution of ABA between shoot and root considering its inability of ABA synthesis in roots.

It is well known that tomato plants are likely to exhibit isohydric behaviors (Moshelion et al., 2015), while ABA-deficient mutant \textit{flacca} and \textit{sitiens} could be transpired more for longer periods during soil drying than AC (Aroca et al., 2008; Wei et al., 2020), representing a near-anisohydric characteristic. Furthermore, rapid ABA biosynthesis might facilitate isohydric behavior (Moshelion et al., 2015). In the present study, before the occurrence of stomatal closure (i.e., when FTSW = 0.50), ABA-treated \textit{flacca} grown at \([\text{CO}_2]\) possessed relatively high \([\text{ABA}]_{\text{leaf}}\) (Figure 5), which was found to be closely correlated with the FTSW threshold at which \(g_s\) started to decrease (C) (Figure 6). These results raise the possibility that anisohydric behaviors in the ABA-deficient mutant could be shifted toward near-isohydric behaviors through the combined effects of \([\text{CO}_2]\) and exogenous ABA, representing a restored stomatal drought response. The change in the water management strategies has been reported to be activated by different soil water statuses (Zhang et al., 2012), also the overexpression of a key tonoplast aquaporin (Sade et al., 2009), which could be linked to ABA.

Previous studies have shown that shoot-to-root ABA transport played a role in the regulation of water flux and induction of stress-resistant genes in roots (Ikegami et al., 2009). In the near-anisohydric grapevine cultivar Syrah, the authors found that it
showed relatively high catabolism of ABA in xylem sap, which might lower its hypersensitivity to water stress (Dayer et al., 2020). Therefore, it could be assumed that reloading of ABA into xylem sap in flacca plants might stimulate some specific metabolic process, causing feedback on stomatal movements, which might explain the low gs in ABA-treated plants at the end of soil drying but without [ABA]leaf accumulation. For example, early studies on flacca have shown that low ABA content stimulated ethylene production, which could be restored to normal levels with exogenous ABA (Tal et al., 1979; Sharp et al., 2000). As ethylene production is often increased by drought stress, it might be associated with the restored drought response in ABA-treated flacca as noticed in the present study. In addition, exogenous ABA-induced other metabolic adaptation has been reported widely, including stimulated resistant protein patterns (Zhou et al., 2014), enhanced energy storages, and activities of antioxidant enzymes (Latif, 2014; Gai et al., 2020), and these possibilities merit further studies.

Under well-watered conditions, the ABA-treated flacca plants possessed the same leaf characteristics as AC in the global PCA plots (Figure 7). However, despite the restored leaf gas exchange rates, water relation characteristics, and ABA concentrations in flacca (Figures 3–5), the PCA plot indicated that, under well-watered conditions, exogenous ABA could still not induce a correlation between gs and [ABA]leaf in flacca, but showed an interaction with Ψp and [ABA]xylem (Figure 7). Furthermore, during the whole period of soil drying, gs was not correlated with [ABA]leaf but only responded to Ψp (Figure 8). Therefore, a question arises as to how exogenous ABA application affected flacca’s stomatal movements considering the no involvement of [ABA]leaf. Pantin et al. (2013) demonstrated that ABA can induce stomatal closure through an indirect hydraulic effect on water permeability within leaf vascular tissues. In the exogenous ABA-fed near-anisohydric grapevine cultivar, the existence of indirect ABA effects on gs has also been reported, which was associated with the ABA catabolism (Dayer et al., 2020). Therefore, it could be assumed that the exogenous ABA decreased gs in flacca plants through an indirect hydraulic effect. Taking together, future research should focus on exploring the correlation of ABA metabolic process and ABA indirect hydraulic effects on stomatal behavior.

CONCLUSION

Exogenous ABA application decreased SA and gs, improved plant water relations, including Ψleaf, Ψs, and Ψp, and increased [ABA]leaf in both AC and flacca, though these two genotypes showed differential responses during soil drying, where exogenous ABA priming sensitized the gs response to soil drying in flacca. In both ABA-treated genotypes, high [ABA]leaf lasted for a longer period under e[CO₂] than a[CO₂]-grown counterparts, and e[CO₂] only lowers gs sensitivity to Ψp in non-ABA-treated AC, indicating that e[CO₂] could counteract the effects of exogenous ABA. In flacca, the effects of exogenous ABA on gs were gradually diminished during soil drying. However, ABA-treated flacca still showed a partly restored stomatal drought response at both [CO₂] conditions, and was accompanied by the recovered plant growth and increased [ABA]xylem especially under e[CO₂]. Thus, the restored drought response in the absence of accumulation of [ABA]leaf could be associated with ABA-stimulated metabolic adaptions. Although ABA-treated flacca exhibited recovered stomatal behavior, the PCA plot and regression analysis showed that [ABA]leaf was not responsible for the decreasing gs. Therefore, our results raise the possibility that exogenous ABA-induced stomatal closure in flacca could be attributed to an indirect hydraulic effect.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

Both authors conceived the concept, carried out the experiment, and have read and agreed to the published version of the manuscript. SL wrote the manuscript with support from FL. FL supervised the project.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.733658/full#supplementary-material

Supplementary Figure 1 | The average temperature, relative humidity, vapor pressure deficit (VPD), and CO₂ concentration [CO₂] in 400 and 800 ppm greenhouse cells of AC and flacca during the whole experimental period.
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