
Title: Improving drought tolerance by altering the photosynthetic rate and stomatal aperture via green light in tomato (*Solanum lycopersicum* L.) seedlings under drought conditions

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**Improving drought tolerance by altering the photosynthetic rate and stomatal aperture via green light in tomato (*Solanum lycopersicum* L.) seedlings under drought conditions**

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**Highlights:**

- Adding green light enhanced the drought tolerance of tomato by inducing an early decrease in stomatal aperture.
- Green light supplementation improved mesophyll conductance, which maintained higher photosynthetic capability under drought stress.
- A clear induction of the expression of the drought stress-associated gene *SlAREB1* occurred under green light exposure, and green light induced the downregulation of the *SlHA1*, *2* and *4* genes, which are related to stomatal opening.

**ABSTRACT**

The regulation of stomatal aperture is one of the most important strategies for plants to tolerate drought. Green light has been shown to reverse some effects of red light and/or blue light on plant growth and development and can enhance plant defense against biotic and/or abiotic stress by triggering the expression of specific genes. However, the effects of green light on plant drought tolerance are still unknown. To elucidate the effects of green light on plant drought tolerance, tomato (*Solanum lycopersicum* L.) seedlings were treated with short-term drought stress and were concomitantly exposed to red and blue light-emitting diodes (LEDs) supplemented with or without green light in an environment-controlled growth chamber. The results show that adding green light induced significant decreases in stomatal conductance ($g_s$), which increased the intrinsic and instantaneous water-use efficiency, concomitantly enhanced mesophyll conductance ($g_m$) and maintained relatively high photosynthetic capability under short-term drought stress. Moreover, green light supplementation alleviated stomatal opening and not only involved the downregulation of the *SlHA1*, *2* and *4* during

stomatal opening but also resulted from a *SlAREB1*-activated signaling pathway, which led to drought tolerance.

***Keywords:*** Green light, Stomatal aperture, Water use efficiency, Drought, Tomato

## 1. Introduction

Drought is a critical threat to plant growth and severely deceases agricultural production (Somerville and Briscoe 2001). For instance, drought causes 7.0%-8.1% of cereal yield losses worldwide every year (Lesk et al., 2016). With frequent and unpredictable drought, food security is likely to become further aggravated in the future (Somerville and Briscoe 2001). Although traditional and marker-assisted breeding have been widely used to enhance crop drought tolerance, the complexity of drought tolerance mechanisms at the physiological and genetic levels has limited the utilization of genetic engineering approaches (Mittler and Blumwald 2010). Therefore, this limitation has emphasized the urgent need to develop adaptive agricultural strategies to guarantee future food security.

Greenhouse cultivation, also known as controlled environmental agriculture or protected agriculture, is the most popular way to produce horticultural crops with high production (Sabir and Singh 2013). Worldwide, approximately 115 countries commercially produce vegetables in glasshouses, and the total estimated greenhouse vegetable production area was 473,466 hectares in 2016 (Hickman 2016; Sabir and Singh 2013). Greenhouse cultivation has been an important part of agriculture in terms of food security. Although greenhouses can create a suitable environment for plant growth and development, crops in greenhouses are sometimes inevitably affected by fluctuations in the internal environment (Gruda 2005). In nature, plants usually face short-term water shortages or slowly developing water deficits. Adjustments to stomatal aperture or early stomatal closure is one of the most important strategies for plants to tolerate drought stress (Fischer and Turner 1978). Under short-term drought stress, plants can increase their water-use efficiency (WUE) by reducing stomatal aperture and, hence, their transpiration rate, thereby minimizing the potential loss of yield under water deficit (Martin-StPaul et al., 2017). However, in some crop species, a relatively low yield is usually correlated with a decrease in photosynthesis as a consequence of stomatal closure caused by severe or long-term drought stress (Mafakheri et al., 2010; Serraj and Sinclair 2002). Moreover, drought-induced stomatal closure can restrict nutritional substance anabolism and accumulation in fruits, which can impair the formation of desirable flavors (Sánchez-Rodríguez et al., 2010). Therefore, improving plant WUE and stabilizing photosynthesis via stomatal regulation are important for increasing plant drought tolerance and

minimizing drought-induced yield losses.

In plants, plasma membrane $H^+$-ATPases belong to the superfamily of P-type ATPases, and their activity plays a vital role in stomatal opening (Hashimoto-Sugimoto et al., 2013; Kinoshita and Shimazaki 1999; Merlot et al., 2007; Zhao et al., 2000). Plasma membrane $H^+$-ATPases are encoded by multiple genes (hereafter referred to as member of the HA family). In tomato, there are at least 8 (*SlHA1-8*) genes in tomato plants encoding different plasma membrane $H^+$-ATPase isoforms (Liu et al., 2016). The transcripts of *SlHA1*, *2* and *4* are widely present in all tissues, while those of *SlHA3*, *5*, *6* and *7* are almostalways expressed only in the flowers (Ewing and Bennett 1994; Ferrol et al., 2002; Liu et al., 2016). Previous studies have shown differences in the regulation of HA members in response to various abiotic stresses (e.g., nutritional deficiency and salt stress) (Sibole et al., 2005; Zeng et al., 2012). Moreover, activating plasma membrane $H^+$-ATPases can prevent abscisic acid (ABA)-mediated stomatal closure (Merlot et al., 2007). Under drought stress, ABA-responsive element-binding proteins (AREBs), specifically the ABA-dependent transcription factor in plants encoded by *AREB1-2*, play an important role in regulating gene expression in response to some stresses (Uno et al., 2000). In addition, overexpression of *SlAREB1-2* can increase tomato plant drought tolerance (Hichri et al., 2016; Orellana et al., 2010).

Light is not only the driving force but also the important transduction signal that regulates plant growth and development by triggering gene expression (Kami et al., 2010). Compared with those of light intensity and light duration, the effects of light spectra on plant growth and development are more complex. It is well known that red and blue light compose the most efficient light spectra for photosynthesis. However, other light spectra, such as that composing UV light and green light, have been proven to profoundly affect plant growth (Folta and Maruhnich 2007; Zhang et al., 2011). For instance, green light can enhance plant defenses to resist biotic and/or abiotic stress by triggering the expression of specific genes (Nagendran and Lee 2015; Zhang and Folta 2012). In our previous studies, we demonstrated that green light supplementation showed positive effects on maintaining photosynthetic capability by upregulating photosynthesis-related gene expression (Bian et al., 2018a; Bian et al., 2018b). Other studies have reported that blue light can stimulate stomatal opening by activating plasma membrane $H^+$-ATPases (Kinoshita and Shimazaki 1999; Yamauchi et al., 2016), while green light reverses blue light-induced stomatal opening (Frechilla et al., 2000). However, the mechanism by which green light regulates stomatal behavior under short-term drought stress is still

unclear. We hypothesize that green light may affect drought tolerance via the control of stomatal aperture resulting from the expression of *SlAREB1-2* and *SlHA1*, *2* and *4*.

Therefore, the aims of this study were to investigate whether green light had a positive effect on tomato drought tolerance during short-term drought stress and to verify whether this effect concerning stomatal regulation is related to the expression of *SlHA1*, *2* and *4* and *SlAREB1-2*. From the results of this study, we hope to characterize the potential function of green light on plant drought tolerance.

## 2. Materials and methods

### *2.1. Plant growth conditions*

Tomato (*Solanum lycopersicum* L. cv. Ailsa Craig; wild type) seeds were sown in rock wool cubes (3 × 3 × 4 $cm^3$) and germinated under white LED light (Heliospectra RX30, Sweden) in an environmentally controlled growth chamber. The light intensity, day/night temperature, air humidity, $CO_2$ level and photoperiod were 150 μmol $m^{-2}$ $s^{-1}$, 25/20 °C, 65%, 400 μmol $mol^{-1}$ and 16 h, respectively. Half-strength Hoagland solution was added from the bottom to supply nutrition for seedlings every other day.

### *2.2. Drought treatment and light conditions*

At the end of the dark period, at 28 days after germination, healthy and similarly sized plants with five true leaves were transplanted into rock wool media (7.5 × 7.5 × 6.5 $cm^3$). Before the plants were transplanted, the rock wool media were watered with half-strength Hoagland solution until they reached full water-holding capacity. The plants were randomly grown under two watering regimes: (1) well watered (90±5% water-holding capacity) and (2) drought stressed (nonwatered until the plants showed severe drought stress symptoms–obvious turgor loss and wilting). During the study, the irrigation strategy was performed according to the methods of Wang et al. (2013), and the light treatment was delivered by red (peak at 660 nm), blue (peak at 450 nm) and green (peak at 530 nm) LED light. Three different combinations of light treatments together with drought or well-watered conditions were used in this study. In the first treatment, plants were exposed to 100 μmol $m^{-2}$ $s^{-1}$ red light and 100 μmol $m^{-2}$ $s^{-1}$ blue light and were grown under drought conditions (RB-drought). The second treatment consisted of RB-drought supplemented with 50 μmol $m^{-2}$ $s^{-1}$ green light (RBG-drought). The light spectral composition of the third drought treatment included 100 μmol $m^{-2}$ $s^{-1}$ red light, 50 μmol $m^{-2}$ $s^{-1}$ blue light and 50 μmol $m^{-2}$ $s^{-1}$ green light (RbG-drought). Well-watered plants exposed to 100 μmol $m^{-2}$ $s^{-1}$ red light and 100 μmol $m^{-2}$ $s^{-1}$ blue light were used as controls (RB-water). The detailed information of these treatments is summarized in Table S1.

*2.3. Leaf area and dry weight measurements*

After 9 days of drought treatment, eight plants were randomly selected from each treatment for measuring plant height, leaf area and shoot dry weight (DW). The leaf area was measured using an LI-3000C leaf area meter (LI-COR, Lincoln, NE, USA). The petioles, stems and leaves were separated from the shoots of these plants and dried in an oven at 80 °C for 72 h before DW determination. The leaf mass per area (LMA) was calculated according to the method of Feng et al. (2008).

*2.4. Gas exchange measurements*

The net photosynthetic rate ($A_{net}$) and chlorophyll fluorescence of the third fully expanded leaves from the top were simultaneously measured before (day 0) and after (days 2, 4, 6 and 9) treatment using a portable photosynthesis system (LI-6800F, LI-COR, Inc., Lincoln, NE). The rapid response of $A_{net}$ to irradiance corresponded to the following light intensities: 0, 30, 50, 100, 200, 500, 800 and 1200 μmol $m^{-2}$ $s^{-1}$) (LI-6800F, LI-COR, Inc., Lincoln, NE); the A-Ci curve was conducted according to the method of Trouwborst et al. (2011). The temperature, $CO_2$ level, and air flow in the leaf chamber were set at 25 °C, 400 μmol $mol^{-1}$ and 500 μmol $s^{-1}$, respectively. The actinic light in the leaf chamber was supplied by a red/blue light source (10% blue, 90% red). A nonrectangular hyperbola according to the methods of Thorney (1976) was used to fit the rapid light response curve data by the nonlinear fitting procedure NLIN in SigmaPlot software (version 12.3, Systat Software Inc., San Jose, CA, USA) to calculate dark respiration ($R_d$) and the maximum gross photosynthetic rate ($A_{max}$):

$$A_{net} = \frac{\alpha \cdot PPF + A_{max} - \sqrt{(\alpha \cdot PPF + A_{max})^2 - 4\theta \cdot PPF \cdot A_{max}}}{2 \cdot \theta} - R_d$$

where α is the light-limited quantum efficiency and $\theta$ is the scaling constant for the curve.

The fluorescence data calculated from the rapid light response curve at growth light levels (200 or 250 μmol $m^{-2}$ $s^{-1}$) were used to calculate the following parameters (Baker 2008; Baker et al., 2007): photosystem II (PSII) quantum efficiency ($\Phi_{PSII}$) and the electron transport rate (ETR). The photosynthetic data obtained at the growth light levels were considered the photosynthetic characteristics. The maximum quantum efficiency in the dark ($F_v/F_m$) was monitored at the start of each rapid light response curve after the leaves were dark adapted in the leaf chamber for 30 min. A modified version of the Farquhar, von Caemmerer and Berry (FvCB) model (Farquhar et al., 1980) was used to fit the A-Ci data to estimate the potential rate of electron transport under saturating light ($J_{max}$), the maximum velocity of Rubisco for carboxylation ($V_{cmax}$) and mesophyll conductance ($g_m$), as described

by Sharkey (2016) and Trouwborst et al. (2011). The intrinsic water-use efficiency ($A_{net}/g_s$) was calculated as the ratio of $A_{net}$ to stomatal conductance ($g_s$), while the instantaneous water-use efficiency ($A_{net}/E$) was determined as the ratio of $A_{net}$ to the transpiration rate ($E$) (Medrano et al., 2015). The total diffusion conductance ($g_t$) was calculated as $g_t = g_s g_m/(g_t + g_m)$ (Centritto et al., 2009).

*2.5. Stomatal aperture determination*

The method of Zeng et al. (2008) was used to determine the length and width of the stomata. Fully expanded leaves at a similar position (one leaf per plant, six plants per treatment) were sampled and immediately treated with transparent nail polish to obtain slides of the leaf epidermal fingerprints. The slides were analyzed by optical microscopy (D71, Olympus Inc., Tokyo, Japan) combined with Motic Images Plus 2.0 software. Ten images taken of different parts of each leaf were analyzed. The stomatal aperture was subsequently calculated as the ratio of stomatal width to length.

*2.6. Relative water content determination*

The relative water content (RWC) of plant leaves before (day 0) and after (days 2, 4, 6 and 9) treatment was separately measured (Pan et al., 2012). Three plants were randomly selected from each treatment. Whole tomato leaves were detached and weighed to obtain their fresh weight (FW). These leaves were soaked in distilled water at room temperature for 24 h. The leaves were then weighed to obtain their turgid weight (TW) and subsequently dried in an oven at 80 °C for 48 h to a constant weight (dry weight, DW). The RWC was then calculated via the following formula:

RWC (%) = (FW − DW) / (TW − DW) × 100

*2.7. Water loss determination*

The method of Leung et al. (1997) was used to determine the water loss of the leaves of tomato plants under drought stress and different light treatments. Tomato leaves were detached and placed under 160 μmol $m^{-2}$ $s^{-1}$ fluorescent white light. The temperature and relative humidity were maintained at 25 °C and 45%, respectively. The leaves were weighed every 30 min, and the total time was 270 min. The water loss was expressed as the percentage of initial FW, and each determination involved three replications.

*2.8. Lipid peroxidation determination*

Lipid peroxidation was expressed as equivalents of malondialdehyde (MDA). The MDA concentration was spectrophotometrically determined using a thiobarbituric acid (TBA) test according to the methods

of Velikova et al. (2000). The absorbance monitored at 532 and 600 nm was used to calculate the MDA concentration against the blank prepared by replacing the sample with extraction medium. The concentration of MDA-TAB complex was then calculated from the extinction coefficient 155 $mM^{-1}$ $cm^{-1}$.

*2.9. Total RNA extraction and real-time qRT-PCR*

To elucidate the effects of green light on drought tolerance and stomatal aperture-related gene expression, the second youngest and fully expanded leaves were sampled at different time points (days 0, 2, 4, 6 and 9) from each treatment. Total RNA was extracted using an RNeasy Plant Mini RNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To avoid any contamination of genomic DNA, the extracted total RNA was treated with 50 μL of RNase-free DNase I (Sigma-Aldrich, Poole, UK) at 37 °C for 15 min before the reverse-transcription reaction in accordance with the manufacturer's instructions. The concentration and purity (260/280 ratio) were determined via a Nanodrop 2000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) before and after DNase I treatment. Prior to cDNA synthesis, the integrity and quality of the total RNA were visually checked by 1% agarose gels stained with ethidium bromide. RNA (1000 ng) was used for cDNA synthesis in a 20 μL reaction using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Epsom, UK). qPCR was used to quantify the relative transcription levels using a CFX Connect™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The qPCR was carried out in a volume of 20 μL containing 2 μL of cDNA sample (100 ng), 10 μL of SsoFast™ EvaGreen® Supermix (Bio-Rad) and gene-specific primer mix at 0.2 μM. The primers for the HA and AREB1-2 genes described by Liu et al. (2016) and Orellana et al. (2010), respectively, were used to assay the relative transcripts of each target gene by normalizing the transcription levels to those of the tomato constitutive Actin gene (Chen et al., 2014). The thermocycling conditions of the real-time qPCR were as follows: 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 5 s; and then a melting curve (65−95 °C). The qRT-PCR experiment was performed in triplicate via three separate RNA extracts from nine plants.

*2.10. Statistical analyses*

All the data were subjected to one-way ANOVA using SAS software (version 8.1, SAS Institute, Cary, NC), and significant differences between the means were assessed by Duncan's multiple range test at $P < 0.05$. The light response and $CO_2$ response curve fitting were performed using SigmaPlot software (version 12.3, Systat Software Inc., San Jose, CA, USA).

## 3. Results

### *3.1. Green light promotes tomato plant growth under short-term drought stress*

The growth of tomato plants under short-term drought stress is summarized in Table 1. Short-term drought significantly reduced plant growth, and the drought-induced decrease in plant growth was also affected by light spectral composition. Compared with the well-watered control (RB-water), RB-drought led to significant decreases in plant DW, plant height and leaf area. However, green light supplementation alleviated the drought-induced decrease in plant growth, as shown by the higher values of DW, leaf area, LMA and plant height under RBG-drought and RbG-drought than under RB-drought. The shoot DW under RbG-drought was higher than that under RBG-drought, but no significant differences between RBG-drought and RbG-drought were observed in the other above mentioned parameters.

### *3.2. Green light alleviates drought stress by maintaining a high photosynthetic rate*

Short-term drought led to a decrease in photosynthetic capability beginning on day 4, but green light supplementation mitigated the decline in photosynthetic capability (Fig. S1 and Fig. S2). Compared with the well-watered control (RB-water), RB-drought led to markedly decreasing trends in the $A_{net}$ and $g_s$. Adding green light slowed the decreasing tendency of $A_{net}$ caused by drought stress but led to a severe decrease in $g_s$, as shown by the higher $A_{net}$ and concomitantly lower $g_s$ under RBG-drought and RbG-drought between day 6 and day 9 (Fig. 1A and B). The $g_m$ under RB-drought was lower than that under RB-water between day 4 and day 9. Moreover, the $g_m$ for RBG-drought and RbG-drought showed an increasing trend, and the levels were higher than those for RB-water between day 0 and day 4. From day 6, the $g_m$ under RBG-drought and RbG-drought also decreased, but the values were higher than those under RB-drought between day 6 and day 9 (Fig. 1C). Relative to the values in the RB-water treatment, drought led to an increase in stomatal limit value (*Ls*), and the highest value was observed under RBG-drought between day 6 and day 9; however, there was no significant difference between RbG-drought and RB-drought (Fig. 1D). After pooling together the data collected from the different light treatments, the $A_{net}$ was hyperbolically correlated with $g_m$ (Fig. 2A), while positive linear relationships were detected between $A_{net}$ and both $g_s$ and $g_t$ (Fig. 2B and C).

After a period of 6 days of drought treatment, the $F_v/F_m$ of plant leaves significantly decreased. In the drought treatments, the lowest $F_v/F_m$ was detected under RB-drought, followed by RbG-drought and

RBG-drought (Table 2). On day 6, the $\Phi_{PSII}$ and ETR under RB-drought calculated at the growth light intensity were lower than those under RB-water; however, these parameters under RBG-drought and RbG-drought were comparable to those under RB-water (Table 2). Drought for 6 days led to lower examined fitting parameters than those of plants under well-watered conditions. However, adding green light mitigated this negative effect caused by drought, as shown by the higher $A_{max}$, $R_d$, $V_{max}$ and $J_{max}$ under both RBG-drought and RbG-drought than under RB-drought. It is worth noting that these fitting parameters (except $R_d$) under RbG-drought were significantly higher than those under RBG-drought (Table 2).

*3.3. Green light increases plant water-use efficiency under short-term drought*

Compared with well-watered conditions, drought led to a significant decrease in the relative water content of tomato leaves, but green light supplementation alleviated the drought-induced decrease in relative water content, as shown by the higher relative water content under both RBG-drought and RbG-drought than under RB-drought from day 4 to day 9 (Fig. 3A). The water loss under drought stress was lower than that under well-watered conditions. Under drought stress, the water loss was the highest under RB-drought, followed by RBG-drought, and then RbG-drought (Fig. 3B). The intrinsic and instantaneous WUE of tomato leaves under drought stress showed increasing trends after day 4. Moreover, the intrinsic and instantaneous WUE under RBG-drought and RbG-drought were significantly higher than those under RB-drought from day 4 to day 9. However, no significant differences in these parameters were detected (except intrinsic WUE on day 6) between RBG-drought and RbG-drought (Fig. 3C and D).

*3.4. Green light induced early stomatal closure and mitigated lipid peroxidation caused by drought*

The stomatal aperture of leaves under RBG-drought and RbG-drought decreased beginning on day 2, and the values were significantly lower than those under RB-drought. Compared with that under RB-water, the stomatal aperture under RB-drought was also reduced, but a significant difference in stomatal aperture between RB-water and RB-drought was detected only on day 9 (Fig. 4). The degree of lipid peroxidation of the drought-treated leaves is presented as the MDA content. Drought led to substantial increases in MDA content between day 4 and day 9, but green light could alleviate the lipid peroxidation caused by drought, as shown by the relatively low MDA content under RBG-drought and RbG-drought. Notably, the MDA content under RbB-drought was lower than that under RBG-drought during the

period between day 4 and day 9 (Fig. 5).

*3.5. Relative expression of SlAREB1-2* and *SlHA1, 2* and *4* under short-term drought

The expression of genes involved in the plasma membrane $H^+$-ATPase and AREB1-2 was investigated (Fig. 6). During 9 days of drought conditions, the transcript levels of *SlHA1, 2* and *4* in the leaves under the different light treatments showed similar expression patterns but were relatively lower than those of the well-watered controls (Fig. 6A-C). Compared with the RB-drought conditions, the addition of green light led to much lower expression levels for all studied *SlHA*s, and the onset of this downregulation occurred earlier than that under RB-drought, as shown by the transcript levels under RBG-drought and RbG-drought (Fig. 6A-C). The expression of *SlAREB1* was upregulated under RBG-drought and RbG-drought after 4 days of drought, while the gene expression level under RB-drought remained steady and was comparable to that under RB-water before day 6. The enhanced expression of *SlAREB1* under RB-drought was detected only on day 9, with *SlAREB1* upregulated by 1.7-fold on day 9 (Fig. 6D). With respect to the expression of *SlAREB2*, no significant difference was detected between the drought-stressed and well-watered plants during the study (Fig. S3).

*3.6. Correlations between both water-use efficiency and stomatal aperture and the relative expression of SlAREB1 and SlHA1, 2 and 4*

After pooling together the gene expression and WUE data, we found that the instantaneous and intrinsic WUE were negatively correlated with the transcripts of *SlHA1* and *SlHA4* (Fig. 7A and C). However, the instantaneous and intrinsic WUE were both positively correlated with the expression of *SlAREB1* (Fig. 7D). Under the different light spectra and drought conditions, the stomatal aperture was positively correlated with the expression of *SlHA1* and *SlHA4* (Fig. 8A and C). However, there was no clear relationship between WUE and the expression of *SlHA2* (Fig. 7B) or between stomatal aperture and the expression of *SlHA2* (Fig. 8B).

## 4. Discussion

This work extends our previous works on the effects of green light on nitrate reduction (Bian et al., 2016; Bian et al., 2018a) as well as crop growth and quality (Bian et al., 2018b). Here, we have demonstrated the positive effects of green light on enhancing the drought tolerance of tomato plants during controlled environmental cultivation.

For plants, one of the most important physiological responses to drought stress is photosynthesis inhibition, which in turn leads to the yield loss (Perez-Martin et al., 2014). In this study, adding green

light mitigated the negative effects of drought on plant growth (Table 1). This finding implies that green light can be applied to maintain relatively high photosynthetic capabilities under drought stress (Fig. 1A and Fig. S1). Under severe stress, the significant decline in both the ETR and $F_v/F_m$, two important fluorescence parameters, reflects the occurrence of photoinhibition or the downregulation of photosynthesis (Cechin et al., 2006). The ability to maintain photosynthetic machinery function under drought stress is considered to be of major importance in drought tolerance (Zlatev and Lidon 2012). In the present study, plants under green light supplementation that maintained their ETR and $F_v/F_m$ values were significantly less affected by drought stress than the other plants, indicating that green light enhanced plant drought tolerance in terms of photosynthetic activity. These results confirm our previous findings that green light has a positive effect on maintaining relatively high photosynthetic capability under abiotic stress (Bian et al., 2018b). Under most drought stress conditions, the decrease in photosynthesis is mainly caused by both stomatal closure and reduced $g_m$, which limits $CO_2$ diffusion from the atmosphere to the site of carboxylation (Flexas et al., 2008). In the present study, the hyperbolic relationship between the $A_{net}$ and $g_m$ (Fig. 2A) confirms the important role of the internal diffusion of $CO_2$ in promoting plant photosynthesis (Pallozzi et al., 2013; Terashima et al., 2011). Previous studies have shown that $g_m$ is regulated by light spectra and that the decrease in photosynthesis after blue light exposure is attributed to blue light-induced significant decreases in $g_m$ (Wang and Folta 2013). However, green light can reverse the effects of red and blue light on plant growth and development (Folta and Maruhnich 2007; Wang and Folta 2013). In this study, the positive effects of green light on the $A_{net}$ under drought stress could be partly explained by the relatively high $g_m$ after the addition of green light (Fig. 1C), which accelerates $CO_2$ from the intercellular spaces to the active site of Rubisco inside the chloroplasts to promote photosynthesis under drought stress (Pallozzi et al., 2013; Terashima et al., 2011).

Plant drought tolerance is defined as the ability to overcome low tissue water potential under water deficit (Chaves et al., 2003). In nature, plants have developed several different drought resistance strategies, such as drought avoidance and drought tolerance, to resist dehydration stress (Hsieh et al., 2010). The reduction in water loss through stomata is one of the most important drought resistance strategies to improve water-use efficiency and drought tolerance (Hsieh et al., 2010; Kim et al., 2012). In this study, the relatively high RWC after green light supplementation (Fig. 3A) can be explained by the reduced water loss in plant leaves (Fig. 3B) as a consequence of the green light-induced early

stomatal aperture reduction (Fig. 4). Together with the relative high intrinsic and instantaneous WUE under RBG-drought and RbG-drought (Fig. 3C and D), these results suggest that green light has a positive effect on enhancing plant drought resistance by increasing WUE via stomatal regulation. This view is also supported by the study of Sun et al. (2014), who found that light-induced stomatal responses are a necessary step toward improving the drought tolerance of crops.

It is well known that stomatal aperture is regulated by light spectra (Inoue and Kinoshita 2017; Wang et al., 2014). Blue light is one of the most important environment signal that regulates stomatal opening in plant leaves in the natural environment (Inoue et al., 2008; Mott et al., 2008). Exposure to equal doses of blue light and green light results in an approximately 50% reversal of blue light-induced stomatal opening (Talbott et al., 2002). However, the blue light-induced stomatal opening requires red light as a background, because of its synergistic effect on the blue light response in guard cells (Inoue et al., 2008; Shinmazaki et al., 2007). Previous studies have shown that red light also plays an important role in inducing the aperture of stomata via red/far red light absorbing phytochromes (Chen et al., 2012; Mao et al., 2005; Wang et al., 2010) and the red light-induced stomatal opening is red light intensity-dependent (Kinoshita et al., 2001; Shinmazaki et al., 2007). In addition, the red light-induced stomatal opening is not only just an indirect response but also results from a phyactiviated signaling pathway independently of photosynthesis (Chen et al., 2012; Talbott et al., 2003). To minimize the effects of red light on stomatal opening, two different green light supplemental strategies were used without changing the red light dose in these light treatments: (1) maintaining equal fluence rates of blue light and green light without changing the total light intensity (RbG-drought) and (2) adding green light without changing the ratio and intensities of the red light and blue light (RBG-drought). Similarly green light supplementation strategies have been reported in the study of green light inducing shade avoidance symptoms in plants (Zhang et al., 2011). Compared with RB-drought, the higher drought tolerance, water using efficiency and lower stomatal aperture under RbG-drought could demonstrate the positive effects of green light supplementation and the ratio of blue to green on enhancing tomato drought tolerance without changing the condition of red light intensity (100 μmol $m^{-2}$ $s^{-1}$) and total light intensity (200 μmol $m^{-2}$ $s^{-1}$). The enhanced drought tolerance and other related parameters of plant under RBG-drought could further verify the effect of green light on regulating plant drought tolerance without changing red and blue light condition (red and blue light intensity: 200 μmol $m^{-2}$ $s^{-1}$) by comparing with RB-drought. In this study, adding green light to red light and blue light led to significant decreases in

stomatal aperture under short-term drought stress, and this phenomenon was dependent on the blue-to-green ratio, as shown by the difference in stomatal aperture between RBG-drought and RbG-drought (Fig. 4). Similar results of green light-reversed stomatal opening were also reported in various plant species by Talbott et al. (2002).

In plants, distinct mechanisms involves in the regulation of stomatal aperture in response to red and blue light (Shinmazaki et al., 2007). Blue light acts as a signal to induce stomatal opening via phot1 and phot2 (Kinoshita et al., 2001). The blue light-induced stomatal opening does not require high blue light intensity, when blue light is added to the background of red light. For instance, at the background of red light, only 5 μmol $m^{-2}$ $s^{-1}$ blue light can induce fully opening of stomata in wild-type of *Arabidopsis* (Kinoshita et al., 2001). The weak blue light (around 5 μmol $m^{-2}$ $s^{-1}$ or less) superimposed on the red light induces stomatal aperture has been show in a number of $C_3$ and $C_4$ plants, such as wheat and sugarcane (Kinoshita et al., 2001; Shinmazaki et al., 2007). Although green light supplementation led to blue light fluent decrease (from 100 μmol $m^{-2}$ $s^{-1}$ to 50 μmol $m^{-2}$ $s^{-1}$) in RbG-drought, the rest of blue light fluent was still efficient to induce stomatal opening in the background of red light (Zeiger et al., 1982). Therefore, green light supplementation played the dominant role causing the most drastic changes in stomatal aperture under RbG-drought.

In plants, the PM $H^+$-ATPase belongs to a family of P-type ATPases that encode HA genes, and the expression of *HA*s plays a dominant role in mediating blue light-induced stomatal opening (Inoue and Kinoshita 2017). The downregulation of HA gene expression leads to decreased stomatal aperture or a closed-stoma phenotype (Osakabe et al., 2016; Yamauchi et al., 2016). In the present study, the green light-induced downregulation of *SlHA1*, *2* and *4* (Fig. 6) at the early drought stage and the significant negative correlations between stomatal aperture and the expression of *SlHA1* and *4* (Fig. 8) indicate that, under short-term drought stress, green light can mediate stomatal opening to enhance plant drought tolerance by regulating the expression of members of the HA gene family.

A previous study showed that the expression of *AREB1* increases multiple stress tolerance responses in transgenic *Arabidopsis* (Fujita et al., 2005) and that overexpression of *SlAREB1* enhances the drought tolerance of tomato (Hsieh et al., 2010). Therefore, the relatively high drought tolerance after green light supplementation, as assessed by physiological parameters such as the relatively high relative water content (RWC) (Fig. 3) and chlorophyll fluorescence (Table 2) and the relatively low lipoperoxidation (Fig. 4), could be a consequence of the green light-induced upregulation of *SlAREB1* expression. Adding

green light significantly upregulated *SLAREB1* gene expression but had little effect on the transcription of *SlAREB2* under short-term drought stress (Fig. S2). These results confirmed that *SlAREB1* plays a dominant role in the response to green light-regulated drought tolerance.

In this study, green light supplementation induced an increase in $g_m$, in addition to stomatal regulation. Green light may be involved in the regulation of $g_m$ by regulating aquaporins (AQPs) and carbonic anhydrase (CA) to enhance plant drought tolerance (Perez-Martin et al., 2014). Therefore, given the importance of green light on plant growth and development, future investigation via transcriptomics and metabolomics not only will facilitate our understanding of the molecular mechanism of the green light signal transduction pathway in plants but also will provide important information for developing adaptive agricultural strategies to increase the yield of crops.

## 5. Conclusion

The evidence in this paper supports the views that (1) adding green light results in positive effects on enhancing tomato plant drought tolerance and (2) adding green light leads to an early decrease in stomatal aperture to enhance water-use efficiency by downregulating *SlHA1*, *2* and *4* expression and by inducing *SlAREB1* expression to enhance tomato drought tolerance. Moreover, green light supplementation facilitated $g_m$ to mitigate the negative effects of drought stress on the photosynthetic capability. In the future, transcriptomic data will contribute to our knowledge of the molecular mechanism by which light spectra regulate photosynthetic capability and water-use efficiency.

## Author contributions

Z.B. and C.L. conceived the original research plan and designed the experiment, and Z.B., Y.W. and X.Z. performed the experiments and analyzed the data. The manuscript was written by Z.B. and was reviewed by C.L.

## CRediT Author Statement

Zhong-Hua Bian, Chun-Gui Lu: Conceptualization;
Zhong-Hua Bian, Xiao-Yan Zhang, Yu Wang: Formal analysis;
Zhong-Hua Bian, Yu Wang: Data curation;
Zhong-Hua Bian, Chun-Gui Lu: Funding acquisition;
Zhong-Hua Bian, Chun-Gui Lu, Xiao-Yang Zhang: Methodology;
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Zhong-Hua Bian, Yu Wang, Chun-Gui Lu, Xiao-Yang Zhang: Investigation;
Zhong-Hua Bian, Yu Wang: Resources;
Zhong-Hua Bian, Yu Wang: Writing-original draft;
Zhong-Hua Bian, Chun-Gui Lu, Xiao-Yang Zhang: Writing-review & editing.

## Competing interests

The authors declare that they have no conflicts of interest.

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## Reference

Baker, N.R., 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59, 89–113.

Baker, N.R., Harbinson, J., Kramer, D.M., 2007. Determining the limitations and regulation of photosynthetic energy transduction in leaves. Plant Cell Environ. 30, 1107–1125.

Bian, Z.H., Cheng, R.F., Yang, Q.C., Wang, J., Lu, C.G., 2016. Continuous light from red, blue, and green light-emitting diodes reduces nitrate content and enhances phyto-chemical concentrations and antioxidant capacity in lettuce. J. Am. Soc. Hortic. Sci.141, 186–195.

Bian Z.H, Cheng, R.F., Wang, Y., Yang Q.C., Lu C.G., 2018a. Effect of green light on nitrate reduction and edible quality of hydroponically grown lettuce (*Lactuca sativa* L.) under short-term continuous light from red and blue light-emitting diodes. Environ. Exp. Bot. 153, 63–71.

Bian, Z.H., Yang, Q.C., Li T., Cheng, R.F., Barnett Y., Lu C.G., 2018b. Study of the beneficial effects of green light on lettuce grown under short‐term continuous red and blue light-emitting diodes. Physiol. Plant. 164, 226–240.

Cechin, I., Rossi, S.C., Oliveira, V.C., Fumis, T.F., 2006. Photosynthetic responses and proline content of mature and young leaves of sunflower plants under water deficit. Photosynthetica 44, 143.

Centritto, M., Lauteri, M., Monteverdi, M.C., Serraj, R., 2009. Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. J.Exp. Bot. 60, 2325–2339.

Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to drought—from genes to the whole plant. Funct. Plant Biol. 30, 239–264.

Chen, A., Chen, X., Wang, H., Liao, D., Gu, M., Qu, H., Sun, S., Xu, G., 2014. Genome-wide investigation and expression analysis suggest diverse roles and genetic redundancy of Pht1 family genes in response to Pi deficiency in tomato. BMC Plant Biol. 14, 61. doi: 10.1186/1471-2229-14–61.

Chen, C., Xiao, Y.G., Li, X., Ni, M., 2012. Light-regulated stomatal aperture inArabidopsis. Mol. Plant 5: 566–

572.

Ewing, N.N., Bennett, A.B., 1994. Assessment of the number and expression of P-type $H^+$-ATPase genes in tomato. Plant Physiol. 106, 547–557.

Farquhar, G.V., Caemmerer, S.V., Berry, J.A., 1980. A biochemical model of photosynthetic $CO_2$ assimilation in leaves of $C_3$ species. Planta 149, 78–90.

Feng, Y.L., Fu, G.L., Zheng, Y.L., 2008. Specific leaf area relates to the differences in leaf construction cost, photosynthesis, nitrogen allocation, and use efficiencies between invasive and noninvasive alien congeners. Planta 228, 383–390.

Ferrol, N., Pozo, M.J., Antelo, M., Azcón‐Aguilar, C., 2002. Arbuscular mycorrhizal symbiosis regulates plasma membrane $H^+$-ATPase gene expression in tomato plants. J. Exp. Bot. 53, 1683–1687.

Fischer, R., Turner, N.C., 1978. Plant productivity in the arid and semiarid zones. Annu. Rev. Plant Physiol. 29, 277–317.

Flexas, J., Ribas‐Carbo, M., DIAZ‐ESPEJO, A., GalmES, J., Medrano, H., 2008. Mesophyll conductance to $CO_2$: current knowledge and future prospects. Plant Cell Environ. 31, 602–621.

Folta, K.M., Maruhnich, S.A., 2007. Green light: a signal to slow down or stop. J. Exp. Bot. 58, 3099–3111.

Frechilla, S., Talbott, L.D., Bogomolni, R.A., Zeiger, E., 2000. Reversal of blue light-stimulated stomatal opening by green light. Plant Cell Physiol. 41, 171–176.

Fujita, Y., Fujita, M., Satoh, R., Maruyama, K., Parvez, M.M., Seki, M., Hiratsu, K., Ohme-Takagi, M., Shinozaki, K., Yamaguchi-Shinozaki, K., 2005. *AREB1* is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in Arabidopsis. The Plant Cell 17, 3470–3488.

Gruda, N., 2005. Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption. Crit. Rev. Plant Sci. 24, 227–247.

Hashimoto-Sugimoto, M., Higaki, T., Yaeno, T., Nagami, A., Irie, M., Fujimi, M., Miyamoto, M., Akita, K., Negi, J., Shirasu, K., 2013. A Munc13-like protein in Arabidopsis mediates $H^+$-ATPase translocation that is essential for stomatal responses. Nat. Commun. 4, 2215.

Hichri, I., Muhovski, Y., Clippe, A., Žižková, E., Dobrev, P.I., Motyka, V., Lutts, S., 2016. *SlDREB2*, a tomato dehydration‐responsive element‐binding 2 transcription factor, mediates salt stress tolerance in tomato and Arabidopsis. Plant Cell Environ. 39, 62–79.

Hickman, G., 2016. International greenhouse vegetable production-statistics. Cuesta Roble Greenhouse Consultants, Mariposa, CA..

Hsieh, T.H., Li, C.W., Su, R.C., Cheng, C.P., Tsai, Y.C., Chan, M.T., 2010. A tomato bZIP transcription factor, *SlAREB*, is involved in water deficit and salt stress response. Planta 231, 1459–1473.

Inoue, S.I., Kinoshita, T., 2017. Blue light regulation of stomatal opening and the plasma membrane $H^+$-ATPase.

Plant Physiol. 174, 531–538.

Inoue, S.I., Kinoshita, T., Matsumoto, M., Nakayama, K.I., Doi, M., Shimazaki, K.I., 2008. Blue light-induced autophosphorylation of phototropin is a primary step for signaling. P. Natl. Acad. Sci. 105, 5626–5631.

Kami, C., Lorrain, S., Hornitschek, P., Fankhauser, C., 2010. Light-regulated plant growth and development. Current topics in developmental biology. Elsevier, pp. 29–66.

Kim, Y.M., Han, Y.J., Hwang, O.J., Lee, S.S., Shin, A.Y., Kim, S.Y., Kim, J.I., 2012. Overexpression of Arabidopsis translationally controlled tumor protein gene *AtTCTP* enhances drought tolerance with rapid ABA-induced stomatal closure. Mol. Cells, 33, 617–626.

Kinoshita, T., Shimazaki, K.I., 1999. Blue light activates the plasma membrane $H^+$ - ATPase by phosphorylation of the C - terminus in stomatal guard cells. The EMBO J. 18, 5548–5558.

Kinoshita, T., Doi, M., Suetsugu, N., Kagawa, T., Wada, M., and Shimazaki, K., 2001. Phot1 and phot2 mediate blue light regulation of stomatal opening. Nature. 414, 656–660.

Lesk, C., Rowhani, P., Ramankutty, N., 2016. Influence of extreme weather disasters on global crop production. Nature 529, 84–87.

Leung, J., Merlot, S., Giraudat, J., 1997. The Arabidopsis *ABSCISIC ACID-INSENSITIVE2* (*ABI2*) and *ABI1* genes encode homologous protein phosphatases 2C involved in abscisic acid signal transduction. The Plant cell 9, 759–771.

Liu, J.L., Liu J.J, Chen A.Q., Ji, M.J., Chen, J.D., Yang, X.F., Gu, M., Qu, H.Y, Xu, G.H., 2016. Analysis of tomato plasma membrane $H^+$-ATPase gene family suggests a mycorrhiza-mediated regulatory mechanism conserved in diverse plant species. Mycorrhiza 26, 645–656.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P., Sohrabi, Y., 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust. J. Crop Sci. 4, 580–585.

Mao, J., Zhang, Y.C., Sang, Y., Li, Q.H., Yang, H.Q., 2005. Fromthe cover: A role for Arabidopsis cryptochromes and COP1 in the regulation of stomatal opening. Proc. Natl Acad. Sci. U S A. 102, 12270–12275.

Martin - StPaul, N., Delzon, S., Cochard, H., 2017. Plant resistance to drought depends on timely stomatal closure. Ecol. Lett. 20, 1437–1447.

Medrano, H., Tomás, M., Martorell, S., Flexas, J., Hernández, E., Rosselló, J., Pou, A., Escalona, J.M., Bota, J., 2015. From leaf to whole-plant water use efficiency (WUE) in complex canopies: limitations of leaf WUE as a selection target. The Crop J. 3, 220–228.

Merlot, S., Leonhardt, N., Fenzi, F., Valon, C., Costa, M., Piette, L., Vavasseur, A., Genty, B., Boivin, K., Müller, A., 2007. Constitutive activation of a plasma membrane $H^+$ - ATPase prevents abscisic acid - mediated stomatal closure. EMBO J. 26, 3216–3226.

Mittler, R., Blumwald, E., 2010. Genetic engineering for modern agriculture: challenges and perspectives. Ann. Rev. Plant Biol. 61, 443–462.

Mott, K.A., Sibbernsen, E.D., Shope, J.C., 2008. The role of the mesophyll in stomatal responses to light and $CO_2$. Plant Cell Environ. 31, 1299–1306.

Nagendran, R., Lee, Y.H., 2015. Green and red light reduces the disease severity by Pseudomonas cichorii JBC1 in tomato plants via upregulation of defense-related gene expression. Phytopathology 105, 412–418.

Orellana, S., Yanez, M., Espinoza, A., Verdugo, I., Gonzalez, E., RUIZ‐LARA, S., Casaretto, J.A., 2010. The transcription factor *SlAREB1* confers drought, salt stress tolerance and regulates biotic and abiotic stress‐related genes in tomato. Plant Cell Environ. 33, 2191–2208.

Osakabe, Y., Watanabe, T., Sugano, S.S., Ueta, R., Ishihara, R., Shinozaki, K., Osakabe, K., 2016. Optimization of CRISPR/Cas9 genome editing to modify abiotic stress responses in plants. Sci. Rep. 6, 26685.

Pallozzi, E., Tsonev, T., Marino, G., Copolovici, L., Niinemets, Ü., Loreto, F., Centritto, M., 2013. Isoprenoid emissions, photosynthesis and mesophyll diffusion conductance in response to blue light. Environ. Exp. Bot. 95, 50–58.

Pan, Y., Seymour, G.B., Lu, C.G., Hu, Z.L., Chen, X.Q., Chen, G.P., 2012. An ethylene response factor (ERF5) promoting adaptation to drought and salt tolerance in tomato. Plant Cell Rep. 31, 349–360.

Perez-Martin, A., Michelazzo, C., Torres-Ruiz, J.M., Flexas, J., Fernández, J.E., Sebastiani, L., Diaz-Espejo, A., 2014. Regulation of photosynthesis and stomatal and mesophyll conductance under water stress and recovery in olive trees: correlation with gene expression of carbonic anhydrase and aquaporins. J. Exp. Bot. 65, 3143–3156.

Sánchez-Rodríguez, E., Rubio-Wilhelmi, M.M., Cervilla, L.M., Blasco, B., Rios, J.J., Rosales, M.A., Romero, L., Ruiz, J.M., 2010. Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant Sci. 178, 30–40.

Sabir, N., Singh, B., 2013. Protected cultivation of vegetables in global arena: A review. Indian J. Agr. Sci. 83, 123–135.

Serraj, R., Sinclair, T., 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ. 25, 333–341.

Sharkey, T.D., 2016. What gas exchange data can tell us about photosynthesis. Plant Cell Environ. 39, 1161–1163.

Shimazaki, K., Doi, M., Assmann, S.M., Kinoshita, T., 2007. Light regulation of stomatal movement. Annu. Rev. Plant Biol. 58, 219–247.

Sibole, J.V., Cabot, C., Michalke, W., Poschenrieder, C., Barceló, J., 2005. Relationship between expression of the PM $H^+$-ATPase, growth and ion partitioning in the leaves of salt-treated Medicago species. Planta 221, 557–566.

Somerville, C., Briscoe, J., 2001. Genetic engineering and water. Science 292, 2217.

Sun, Z., Jin, X., Albert, R., Assmann, S.M., 2014. Multi-level modeling of light-induced stomatal opening offers new insights into its regulation by drought. PLoS computational biology 10: e1003930.

Talbott, L.D., Nikolova, G., Ortiz, A., Shmayevich, I., Zeiger, E., 2002. Green light reversal of blue‐light‐

stimulated stomatal opening is found in a diversity of plant species. Am. J. Bot. 89, 366–368.

Talbott, L.D., Shmayevich, I.J., Chung, Y., Hammad, J.W., Zeiger, E., 2003. Blue light and phytochrome-mediated stomatal opening in the npq1 and phot1 phot2 mutants of Arabidopsis.Plant Physiol. 133, 1522–1529.

Terashima, I., Hanba, Y.T., Tholen, D., Niinemets, Ü., 2011. Leaf functional anatomy in relation to photosynthesis. Plant Physiol. 155, 108–116.

Thornley, J.H.M.,1976. Mathematical Models in Plant Physiology: A Quantitative Approach to Problems in Plant and Crop Physiology. Academic Press, New York.

Trouwborst, G., Hogewoning, S.W., Harbinson, J., van Ieperen, W., 2011. Photosynthetic acclimation in relation to nitrogen allocation in cucumber leaves in response to changes in irradiance. Physiol. Plant. 142, 157–169.

Uno, Y., Furihata, T., Abe, H., Yoshida, R., Shinozaki, K., Yamaguchi-Shinozaki, K., 2000. Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. P. Natl. Acad. Sci. 97, 11632–11637.

Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 151, 59–66.

Wang, F.F., Lian, H.L., Kang, C.Y., Yang, H.Q., 2010. Phytochrome B is involved in mediating red light-induced stomatal opening in Arabidopsis thaliana. Mol. Plant 3, 246–259.

Wang, P., Sun, X., Li, C., Wei, Z., Liang, D., Ma, F.W., 2013. Long‐term exogenous application of melatonin delays drought‐induced leaf senescence in apple. J. Pineal Res. 54, 292–302.

Wang, Y., Folta, K.M., 2013. Contributions of green light to plant growth and development. Am. J. Bot. 100, 70–78.

Wang, Y., Noguchi. K., Ono, N., Inoue, S.I., Terashima, I., Kinoshita, T., 2014. Overexpression of plasma membrane $H^+$-ATPase in guard cells promotes light-induced stomatal opening and enhances plant growth. P. Natl. Acad. Sci. 111, 533–538.

Yamauchi, S., Takemiya, A., Sakamoto, T., Kurata, T., Tsutsumi, T., Kinoshita, T., Shimazaki, K.I., 2016. The plasma membrane $H^+$-ATPase *AHA1* plays a major role in stomatal opening in response to blue light. Plant Physiol. 171, 2731–2743.

Zeng, B., Wang, Q.Y., Tang, C.M., 2008. Anatomic analysis on heterosis in three transgenic *bt* pest-resistant hybrid cotton (*G. hirsutum* L.). Acta Agron. Sinica. 34, 496–505.

Zeng, H., Liu, G., Kinoshita, T., Zhang, R., Zhu, Y., Shen, Q., Xu, G., 2012. Stimulation of phosphorus uptake by ammonium nutrition involves plasma membrane $H^+$-ATPase in rice roots. Plant soil. 357, 205–214.

Zeiger, E.,Field, C., 1982. Photocontrol of the functional coupling between photosynthesis and stomatal conductance in the intact leaf. Blue light and PAR-dependent photosystems in guard cells. Plant Physiol. 70, 370–375.

Zhang, T., Folta, K.M., 2012. Green light signaling and adaptive response. Plant Signal. Behav. 7, 75–78.

Zhang, T., Maruhnich, S.A., Folta, K.M., 2011. Green light induces shade avoidance symptoms. Plant Physiol. 157, 1528–1536.

Zhao, R., Dielen, V., Kinet, J.M., Boutry, M., 2000. Cosuppression of a plasma membrane $H^+$-ATPase isoform impairs sucrose translocation, stomatal opening, plant growth, and male fertility. Plant Cell 12, 535–546.

Zlatev, Z., Lidon, F.C., 2012. An overview on drought induced changes in plant growth, water relationsand photosynthesis. Emir. J. Food Agri. 24, 57–72.

**Tables**

Table 1. Effects of light quality on tomato shoot dry weight (DW), leaf area and leaf mass per area (LMA) under drought conditions.

| | DW (g) | | | | Leaf area ($cm^2$) | LMA ($g\ m^{-2}$) | Plant height (cm) |
|---|---|---|---|---|---|---|---|
| | Stems | petioles | leaves | Shoots | | | |
| RB-water | 1.18±0.13 a | 0.25±0.03 a | 0.45±0.08 a | 1.88±0.21 a | 395.38±31.57 a | 21.59±1.06 c | 29.04±1.82 a |
| RB-drought | 0.52±0.06 c | 0.14±0.02 c | 0.31±0.04 b | 0.87±0.10 d | 200.31±20.40 c | 22.46±0.45 bc | 21.24±0.56 c |
| RbG-drought | 0.74±0.05 c | 0.19±0.04 b | 0.47±0.05 a | 1.39±0.12 b | 241.75±18.08 b | 23.21±0.87 ab | 24.33±1.62 b |
| RBG-control | 0.58±0.06 c | 0.15±0.03 bc | 0.40±0.07 a | 1.13±0.09 c | 221.84±34.39 bc | 23.84±0.39 a | 25.45±1.92 b |

R, red LED light; B, blue LED light; G and g, green LED light. The shoot DW is the sum of the DW of the stems, petioles and leaves. The data are means ± SEs (n = 8). The different letters in the same column indicate significant differences among treatments at $P < 0.05$.

Table 2. Effects of green light on the photosynthetic parameters of tomato leaves after 6 days of drought stress.

| | RB-water | RB-drought | RBG-drought | RbG-drought |
|---|---|---|---|---|
| *Measured parameters* | | | | |
| $F_v/F_m$ | 0.81±0.002 a | 0.76±0.01 d | 0.78±0.009 c | 0.80±0.005 b |
| $\Phi_{PSII}$ | 0.67±0.02 a | 0.60±0.003 b | 0.67±0.02 a | 0.65±0.01 a |
| ETR | 56.16±1.45 a | 51.40±1.26 b | 56.00±1.87 a | 54.76±0.92 a |
| *Fitting parameters* | | | | |
| $A_{max}$ | 18.29±1.63 a | 12.81±0.99 c | 11.74±1.98 c | 15.48±0.59 b |
| $R_d$ | 1.43±0.16 a | 0.95±0.11 b | 1.02±0.18 b | 1.05±0.12 b |
| $V_{max}$ | 43.80±0.62 a | 37.46±0.98 c | 39.44±0.74 b | 44.18±0.80 a |
| $J_{max}$ | 38.04±0.26 a | 36.29±0.54 d | 37.75±0.47 b | 38.79±0.29 a |

The $\Phi_{PSII}$ and ETR were calculated at the growth light intensity (200 or 250 μmol $m^{-2}$ $s^{-1}$) of each light treatment. The data are means ± SEs (n = 4). The different letters in a row indicate significant differences among the treatments at $P < 0.05$.

**Figures**

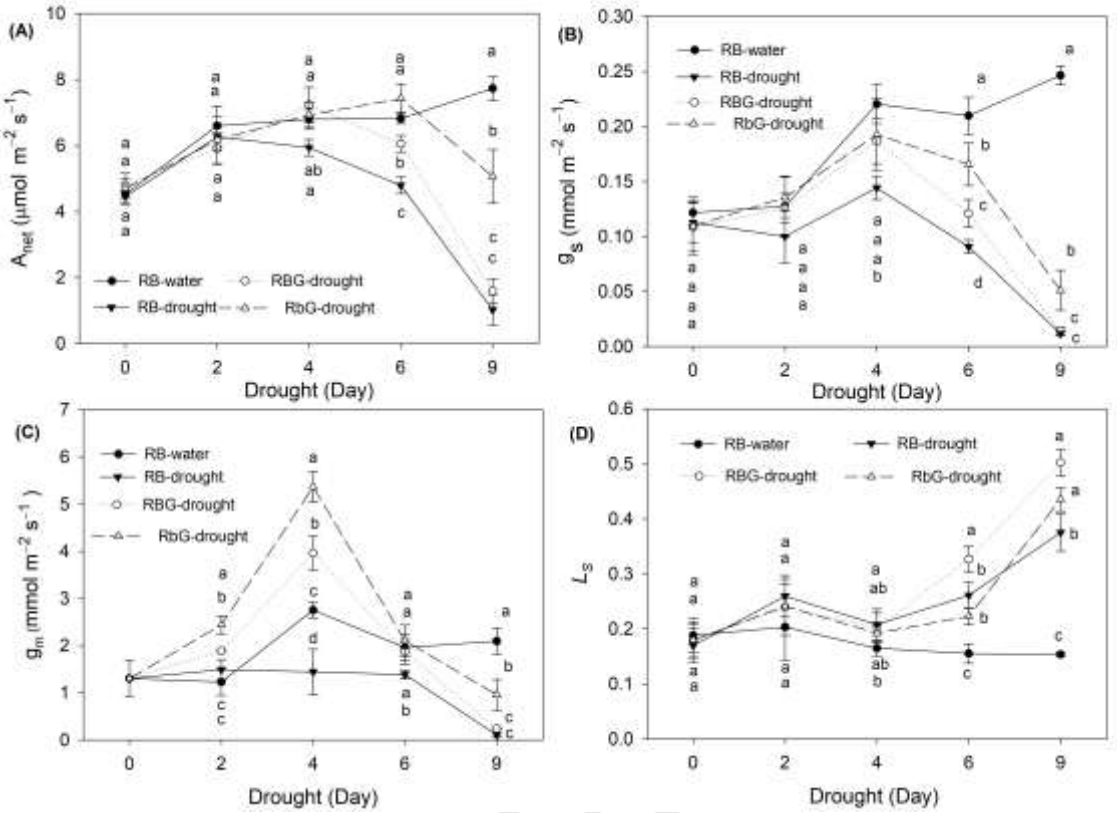


**Fig. 1. Effects of green light on gas exchange parameters and mesophyll conductance of fully expanded tomato leaves under short-term drought stress.** (A) Net photosynthetic rate ($A_{net}$), (B) stomatal conductance ($g_s$), (C) mesophyll conductance ($g_m$) and (D) stomatal limitation (*Ls*) at the growth light intensity. The error bars indicate the SEs (n = 4). The different letters at the same time point indicate significant differences among the treatments at $P < 0.05$.

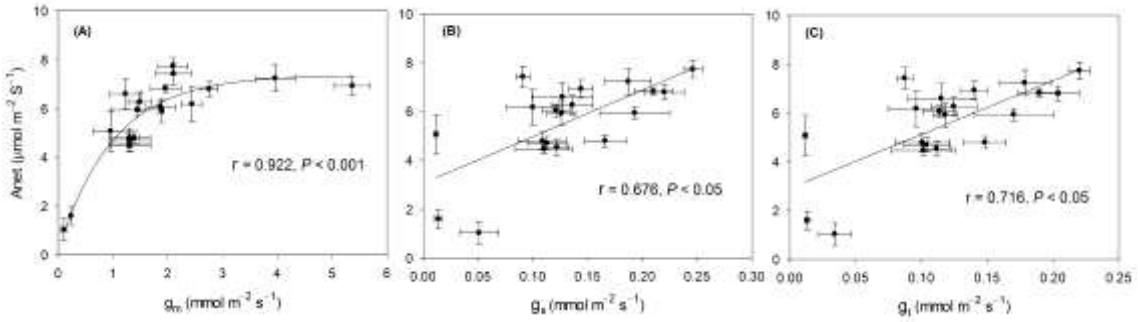


**Fig. 2. Influence of diffusion conductance on the net photosynthetic rate ($A_{net}$) in response to exposure to different light spectra under drought conditions.** (A) The relationship between mesophyll conductance ($g_m$) and $A_{net}$. (B) The relationship between stomatal conductance ($g_s$) and $A_{net}$. (C) Correlation analysis between total diffusion conductance ($g_t$) and the $A_{net}$. The error bars through the data points represent ± SEs (n = 4).

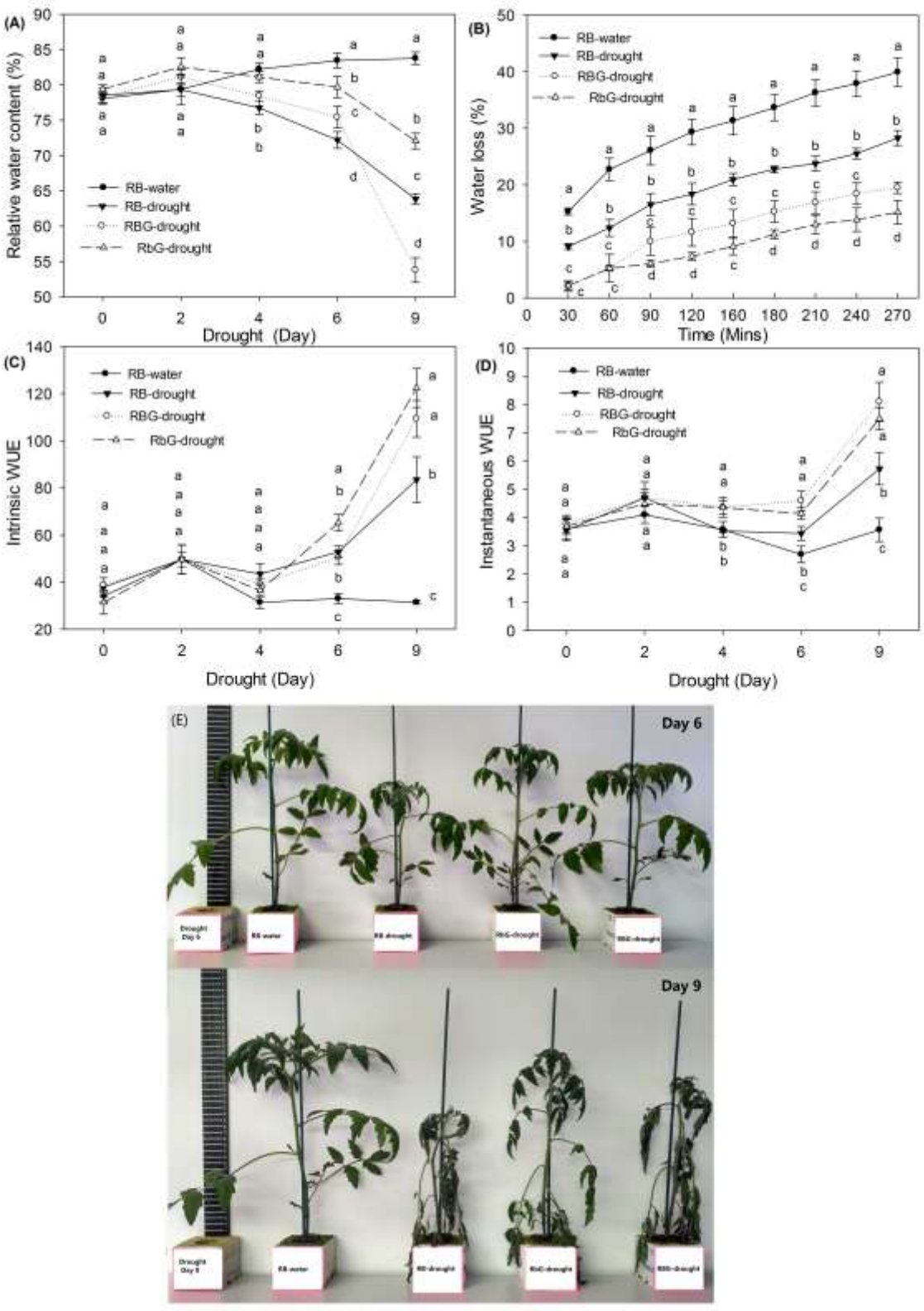


**Fig. 3. Effects of green light on water status and water use-efficiency (WUE) of tomato seedlings under short-term drought stress.** (A) The relative water content, (B) water loss, (C) intrinsic water use efficiency (WUE) and (D) instantaneous WUE under short-term drought conditions. (E) The

phenotypes of plants after 6 and 9 days of drought. The error bars indicate the SEs (n = 4). The different letters at the same time point indicate significant differences among the treatments at $P < 0.05$.

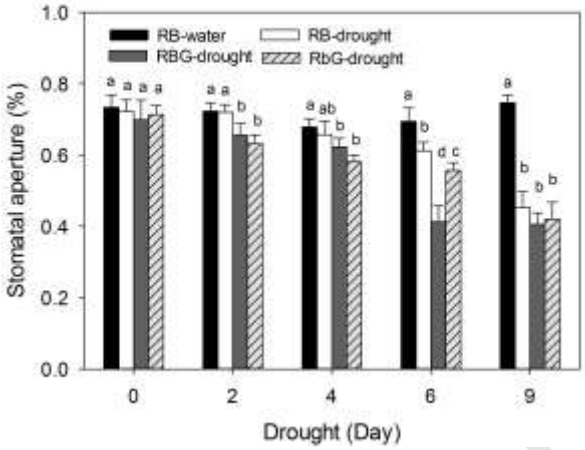


**Fig. 4. The malondialdehyde (MDA) contents in tomato leaves exposed to different light spectra under short-term drought stress.** The error bars indicate the SEs (n = 4). The different letters at the same time point indicate significant differences among the treatments at $P < 0.05$.

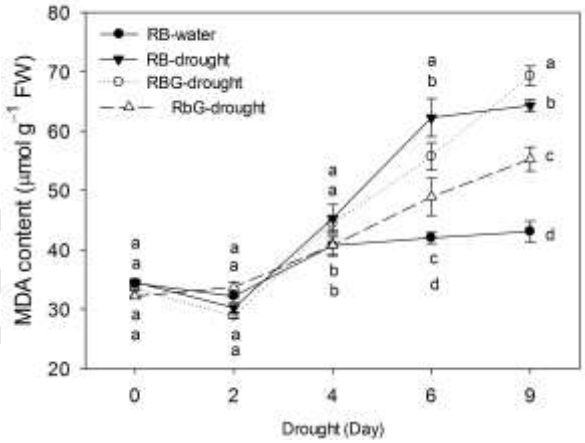


**Fig. 5. Effects of green light on stomatal aperture under short-term drought stress.** The error bars indicate the SEs (n = 10-15). The different letters at the same time point indicate significant differences among the treatments at $P < 0.05$.

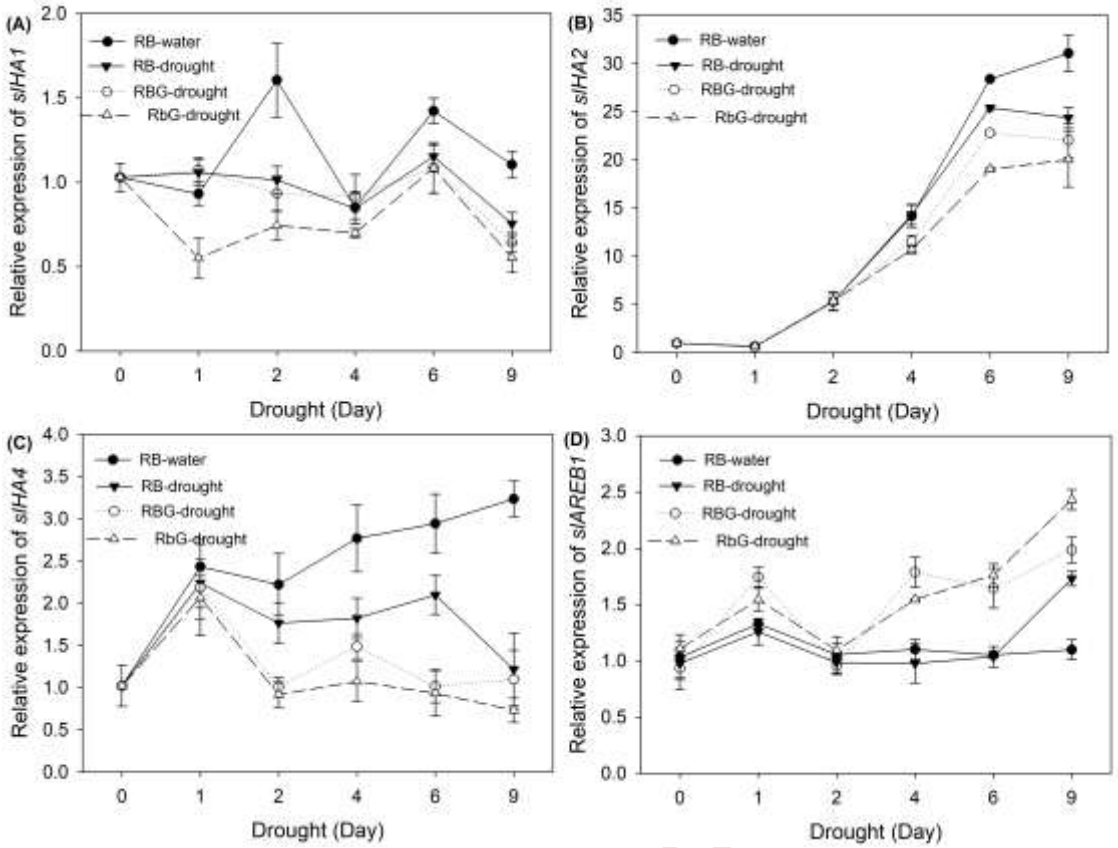


**Fig. 6. Influence of green light on expression profiling of stomatal opening and drought tolerance related genes in tomato leaves under short-term of drought stress.** (A-C) The expression profiling of stomatal opening-related genes: *SlHA1*, *SlHA2* and *SlHA4*. (D) The expression profiling of drought tolerance related gene—*SlAREB1*. Total RNA was isolated from samples collected at different time points and converted to cDNA before being subjected to real-time qRT-PCR. The error bars indicate the SEs (n = 3).

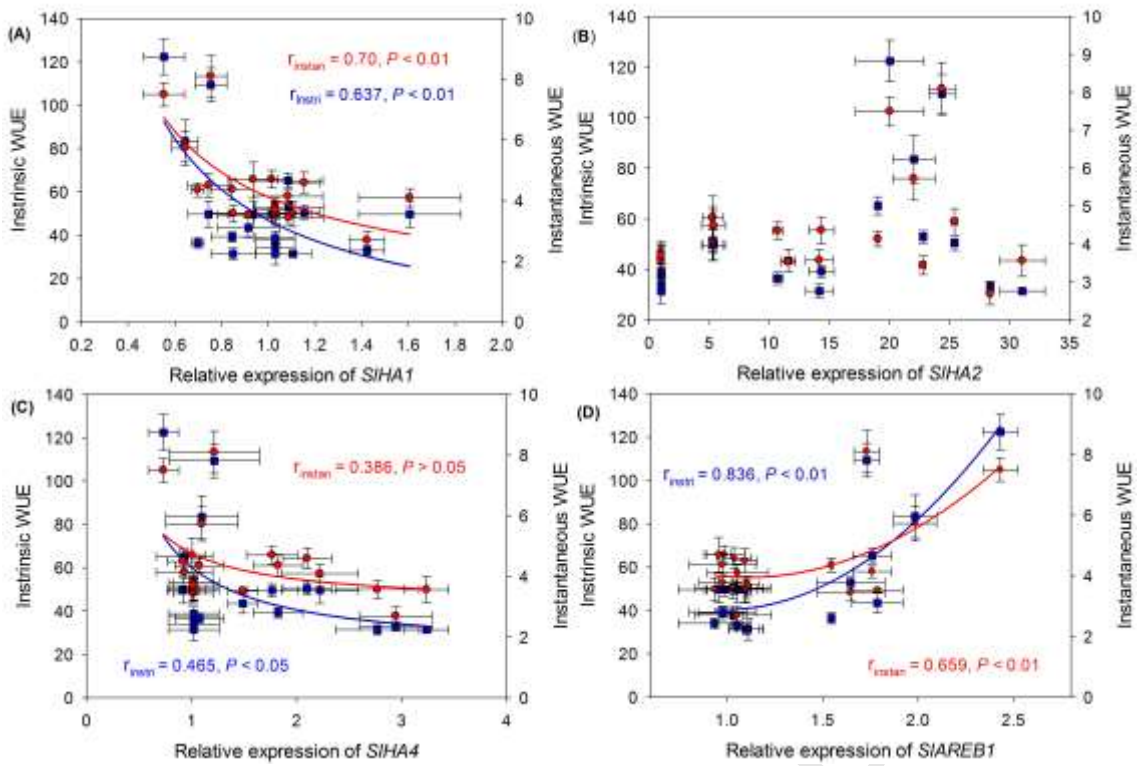


**Fig. 7. Influence of the expression of stomatal opening and drought tolerance related genes on water-use efficiency (WUE) under short-term drought stress.** (A-C) Correlation analysis revealing the links between WUE and the relative expression of *SlHA1*, *2* and *4*. (D) Correlation analysis between WUE and the expression of *SlAREB1*. The error bars indicate the SEs (n = 3).

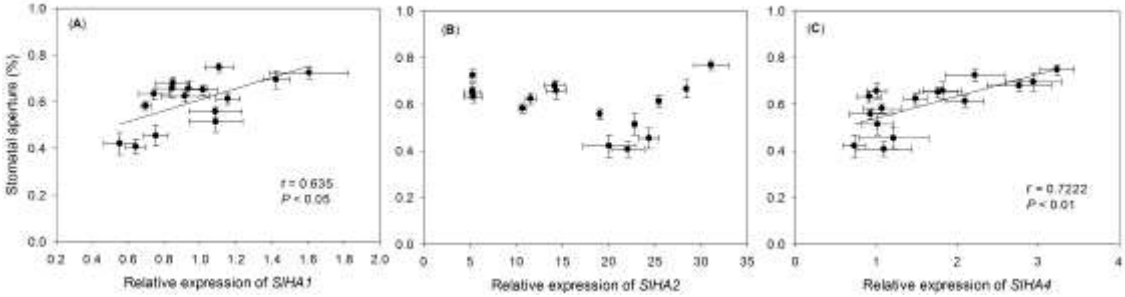


**Fig. 8. Influence of the expression of stomatal aperture and drought tolerance related genes on stomatal aperture under short-term drought stress.** (A-C) The relationship between stomatal aperture and the relative expression of *SlHA1*, *2* and *4*. (D) The relationship between stomatal aperture and the relative expression of *SlAREB1*. The error bars indicate the SEs (n = 3).