

ABA signaling in regulation of mesophyll conductance in tomato during progressive soil drying

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Abstract

Objectives of this study were to clarify the involvement of root to shoot ABA in regulation of leaf stomatal conductance (g_s) and mesophyll conductance (g_m) during progressive soil drought, and to investigate its impact on leaf photosynthesis and intrinsic water use efficiency (WUE_i) in pot-planted tomato. A fast-stomatal closure was related to decrease of Ψ_{leaf} in the early stage of soil drought, whereas g_m kept unchanged until ABA_{xylem} synchronously increased at threshold of relative soil water content (RSWC) decreasing from 51.43 to 44.69%. This out-of-step response between g_s and g_m resulted in an increase of g_m/g_s ratio and potentially improved WUE_i during RSWC decreased from 51.43 to 40.16%. Meanwhile, g_s and g_m declined logarithmically with increasing xylem sap ABA concentration ($p < 0.01$). The sensitivity of g_m response to root to shoot ABA signaling, even though less than g_s , played a key role in regulation of CO₂ diffusion into the chloroplast as soil drying proceeded. g_s mainly limited leaf photosynthesis with RSWC in range of 51.43 to 40.16%, and relative contribution of g_m limitation exceed that of g_s limitation as soil further dried. These results provided new understanding of ABA in the regulation of g_m and WUE_i in response to drought stress.

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Abstract: Objectives of this study were to clarify the involvement of root to shoot ABA in regulation of leaf stomatal conductance (g_s) and mesophyll conductance (g_m) during progressive soil drought, and to investigate its impact on leaf photosynthesis and intrinsic water use efficiency (WUE_i) in pot-planted tomato. A fast-stomatal closure was related to decrease of Ψ_{leaf} in the early stage of soil drought, whereas g_m kept unchanged until ABA_{xylem} synchronously increased at threshold of relative soil water content (RSWC) decreasing from 51.43 to 44.69%. This out-of-step response between g_s and g_m resulted in an increase of g_m/g_s ratio and potentially improved WUE_i during RSWC decreased from 51.43 to 40.16%. Meanwhile, g_s and g_m declined logarithmically with increasing xylem sap ABA concentration ($p < 0.01$). The sensitivity of g_m response to root to shoot ABA signaling, even though less than g_s , played a key role in regulation of CO₂ diffusion into the chloroplast as soil drying proceeded. g_s mainly limited leaf photosynthesis with RSWC in range of 51.43 to 40.16%, and relative contribution of g_m limitation exceed that of g_s limitation as soil further

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Key words : stomatal conductance; mesophyll conductance; water use efficiency; ABA signaling

1. Introduction

Water scarcity, as one of major environmental stress factors, could strongly disturb the balance between H_2O efflux via stomata and CO_2 diffusion from air to the chloroplast stroma, and simultaneously limit plant productivity. Stomatal closure driven by hydraulic and chemical signals, mainly leaf water potential and abscisic acid (ABA), has been recognized as an early response to soil drought (Dodd et al. 2005; Jacobsen et al. 2008; Tombesi et al. 2015). Similar to stomatal conductance (g_s), there was increasing evidence that mesophyll conductance (g_m) could be the important limiting factor in the regulation of plant photosynthetic capacity response to environmental stresses, since g_m controlled the drawdown of CO_2 from sub-stomatal cavities to chloroplasts (Flexasa et al. 2002; Niinemets et al. 2009a; Qiu & Katul 2019). Many studies have suggested that g_m decrease accompanied usually by a decline of chloroplastic CO_2 concentration (C_c) during progressive soil drying in many species, such as grapevines (Flexasa et al. 2002; Ferrio et al. 2012), cotton (Han et al. 2016) and rice (Wang et al. 2018), but the mechanisms for this decrease were still unknown in tomato as a sensitive water-stressed vegetable in the world. Besides the impact of leaf anatomical characteristics on g_m (Niinemets et al. 2009b; Muir et al. 2014), recent study in leaf hydraulic signals suggested that the vulnerability of leaf hydraulic conductance contributed to the decrease of g_m and g_s in response to soil drought (Wang et al. 2018). While Th  roux-Rancourt et al (2014) considered that g_m was less sensitive to drought stress than g_s or hydraulic signals mediated by ABA, due to the unresponsive action of the bulk of mesophyll cells to exogenous ABA application (Shatil-Cohen et al. 2011). In line with this, Vrabl et al (2009) reported that 20 μM application of ABA could not affect g_m and the rate of photosynthesis, while induced a reduction in g_s . On the contrary, several studies also showed exogenous ABA application could result in a decrease in g_m in soybean and tobacco (Flexas et al., 2006) and in four woody species (Sorrentino et al., 2016). Similarly, a recent study revealed that there was a negative relationship between ABA concentration and g_m under drought condition, by means of ABA-deficient mutant (Mizokami et al. 2015). Nonetheless, the threshold of ABA signaling in regulation of g_m response to drought was still unclear.

Despite all of the negative response of leaf gas exchange to drought stress, many studies have confirmed that leaf intrinsic water use efficiency (WUE_i), termed as ratio of net photosynthetic rate to stomatal conductance, improved by decreasing g_s under mild drought stress (Liu et al. 2005; Xue et al. 2016). It was also worth to be noted that drought-induced closure of stomatal aperture could simultaneously in turn reduce assimilation rate and yield, higher WUE_i and A_n seemed to be only achieved with improving g_m (Flexas et al. 2013, 2016). Correlations between WUE_i and the ratio of g_m/g_s was indeed observed based on the Fick's law of diffusion, as higher g_m/g_s contributed to higher A_n and WUE_i under drought condition (Flexas et al. 2013; Cano et al. 2013; Han et al. 2016;). However, the relations between g_m/g_s and ABA signaling during the progressive soil drying were largely unknown. Therefore, revealing the exact role of g_m or g_m/g_s in improving A_n and WUE_i in response to progressive drought was necessary to improve plant water productivity for solving food shortage problem all over the world.

Moreover, it was important to address the impact of water stress on photosynthetic capacity, which was commonly quantified as: stomatal conductance (g_s), mesophyll conductance (g_m) and photosynthetic biochemistry (including the carboxylation capacity or the electron transport rate) (Cano, et al. 2013; Tosens et al. 2016; Wang et al. 2018). Since a quantitative limitation analysis proposed by Grassi and Magnani (2005) has been applied to estimate the contributions of each factor to photosynthesis, several researches shown that g_s and g_m were the main limitations to maximum photosynthesis under drought condition (Tosens et al., 2016; Wang et al., 2018). Therefore, we examined the applicability of this method to determine the main constraint in tomato plants under progressive soil drought condition.

In this study, the photosynthetic diffusive components (g_s and g_m) and possibly related to leaf water potential and root to shoot ABA signaling were examined in tomato seedlings during progressive soil drying. The

objectives of this study were (i) to reveal especially ABA signaling that might be involved in the control of mesophyll conductance and leaf intrinsic water use efficiency, as well as determining the threshold of mesophyll conductance response to ABA signaling during progressive soil drying; and (ii) to quantitatively analyze the relative contribution of each limitation and determine the main constraint to photosynthesis during progressive soil drying.

2. Materials and methods

2.1 Plant material and water treatments

Seeds of tomato (*Solanum lycopersicum* L., cv. Helan108) were sown on the trays with nursery substrate. When three true leaves emerged, tomato seedlings were transplanted into 0.53 L pots filled with 6.5 kg air-dried soil with sandy loam texture. The field water capacity (θ_{FC}) was 22% (g g^{-1}), the wilting point was 6.8% (g g^{-1}). After finishing transplanting, all pots were irrigated to 85% θ_{FC} with Hoagland solution (5 mM KNO_3 , 5 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 1 mM KH_2PO_4 , and 1 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 ml l^{-1} micronutrients, pH=6.0). Seedlings were cultivated in an environment-controlled chamber (day/night air temperature 28/18 °C, 50% relative humidity, 12 hours photoperiod at 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD) supplied by LED lamps from 7:00 to 19:00). During 1-13 days after transplanting (DAT), water was applied sufficiently with same volume of Hoagland solution and supplied distilled water to all pots. All pots were weighted daily at 8:00 a.m. to calculate daily irrigation quota according to water balance formula.

Soil water content in the pot was expressed as relative soil water content (RSWC), the ratio between the current soil moisture (θ_C) and the field water capacity. For well-watered treatment, RSWC was maintained within the range of 70-82% during the experiment. For drought-stressed treatment, in the first circle of soil progressive drying, the RSWC decreased from 85% to 40% from DAT 14 to 26. At DAT 27, both the well-watered and drought treatments were irrigated to 82% θ_{FC} with same volume of Hoagland solution and additional distilled water. After that, the drought-stressed tomato seedlings suffered second circle of drought stress from DAT 28 to 33. During the second progressive soil drying, the relevant experimental indexed were measured and collected for the two treatments daily.

2.2 Leaf gas exchange and chlorophyll fluorescence measurements

The light and CO_2 response curves were measured both on the latest and fully expanded leaves between 9:00-15:00 in a red-blue chamber with a LI-6400 portable photosynthesis system (LiCor Inc., Lincoln, NE, USA). For the CO_2 response curve, the leaf was consecutively exposed to different CO_2 levels: 400, 300, 200, 150, 100, 50, 400, 400, 600, 800, 1000, 1200, 1400, 1600 $\mu\text{mol mol}^{-1}$ under 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. For the light response curve, the CO_2 was kept as constant of 400 $\mu\text{mol mol}^{-1}$ and PPFD decreased gradually in the order of 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 150, 100, 50, 20, 10, 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Each sampled leaf was acclimated for 30-minutue in environment with 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$. Leaf gas-exchange data was logged before starting A_n/C_i curve measurement. Intrinsic water use efficiency (WUE_i) was calculated as the ratio of net photosynthetic rate divided by stomatal conductance:

$$\text{WUE}_i = \frac{A_n}{g_s} \quad (1)$$

Leaf chlorophyll fluorescence parameters were measured by the fluorescence probe (MINI PAM-2000; Walz, Effeltrich, Germany) on the same leaves as the measurement of response curves. The photochemical efficiency of photosystem II ($\Phi_{II\Sigma II}$) was determined as follows:

$$\Phi_{\text{PSII}} = \frac{(F_m' - F_s)}{F_m'} \quad (2)$$

where F_m' and F_s were maximal fluorescence and steady-state fluorescence during a light-saturating pulse by the multiphase flash protocol (Genty et al. 1989), respectively. The electron transport rate (J_f) was then calculated as:

$$J_f = \Phi_{\text{PSII}} \times \text{PPFD} \times \alpha \times \beta(3)$$

where PPFD was maintained at $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$ on both the well-watered and water-stressed leaves. α was the leaf absorptance and β reflected the partitioning of absorbed quanta between photosystems II and I. α and β were considered to be 0.84 and 0.5, respectively (Laisk et al. 1996; Flexas et al. 2002).

Based on the variable J method described by Harley (1992), g_m was calculated as following:

$$g_m = \frac{A_n}{C_i - \frac{G^* (J_f + 8 (A_n + R_d))}{J_f - 4 (A_n + R_d)}} \quad (4)$$

where C_i was intercellular CO_2 concentration ($\mu\text{mol m}^{-2} \text{s}^{-1}$); G^* was chloroplast CO_2 compensation point ($\mu\text{mol m}^{-2} \text{s}^{-1}$); R_d was day respiration ($\mu\text{mol m}^{-2}\text{s}^{-1}$), calculated by the Photosynthesis Assistant of Ye (2007) according to light curve and changed as soil progressive drying from DAT 28 to 33.

Γ^* was a leaf temperature dependent parameter, and could be estimated as:

$$\Gamma^* = \exp \left(c - \frac{H_a}{RT_K} \right) \quad (5)$$

where c was the scaling constant (dimensionless), H_a was the energies of activation (KJ mol^{-1}), and R was the molar gas constant ($\text{KJ K}^{-1} \text{mol}^{-1}$). According to the study of Hermida-Carrera et al (2016), the value of these three parameters at 25°C was 12.7, 23.2, 8.314 in our experiment, respectively. T_k was the leaf absolute assay temperature (K), which was recorded by the LI-6400 and corrected to Kelvin temperature.

2.3 Photosynthetic limitation analysis

Based on the Fickian diffusion of CO_2 from leaf surface to the chloroplasts on steady-state conditions, photosynthesis limitation to light-saturated photosynthesis could be defined as the stomatal (l_s), mesophyll (l_m) and biochemical characteristics (l_b). And the relative contribution of three components ($l_s + l_m + l_b = 1$) was calculated in terms of the method described by Grassi & Magnani (2005). Light-saturated photosynthesis model (Farquhar et al. 1980) was used as follow:

$$A = \frac{V_{c\max}(C_c - \Gamma^*)}{C_c + K_c(1 + \frac{O}{K_o})} + R_d \quad (6)$$

$$C_c = \frac{\Gamma^*(J_f + 8(A + R_d))}{(J_f - 4(A + R_d))} \quad (7)$$

$$l_s = \frac{\frac{g_t}{g_{sc}} \frac{\partial A}{\partial C_c}}{g_t + \frac{\partial A}{\partial C_c}} \quad (8)$$

$$l_m = \frac{\frac{g_t}{g_m} \frac{\partial A}{\partial C_c}}{g_t + \frac{\partial A}{\partial C_c}} \quad (9)$$

$$l_b = \frac{g_t}{g_t + \frac{\partial A}{\partial C_c}} \quad (10)$$

where, K_c and K_o were the Michaelis–Menten constants for CO_2 and O_2 , and $V_{c\max}$ was the maximum carboxylation capacity, all parameters were temperature dependent and calculated with the equation 5. The specific value of these three parameters at 25°C was obtained from Sharkey et al., 2007. O is the O_2 concentration in air ($=210 \text{ mmol mol}^{-1}$). And g_t was the total conductance, which was calculated as:

$$g_t = \frac{1}{\frac{1}{g_s} + \frac{1}{g_m}} \quad (11)$$

2.4 Leaf water potential measurement and xylem sap collection

Leaves measured for gas exchange and chlorophyll fluorescence were also sampled for determination of leaf water potential by the WP4C Dewpoint Potentiometer (Meter, USA). 5 repetitions were taken from each treatment. Meanwhile, the de-topped shoot was put into the pressure chamber (Model 3115, Soil Moisture Equipment, CA, USA), increased gradually pressure until sap solution outflowed from the cut surface. After the first 1-2 drops was discarded, nearly 2 ml of sap was collected into centrifuge tube frozen in liquid nitrogen and then stored at -80°C for ABA analysis.

2.5 ABA determination

The concentration of ABA was determined following the procedure of You et al (2006) with little modification. 2 ml of extracted xylem sap sample was placed into a microcentrifuge tube containing 5 ml extraction buffer composed of isopropanol/hydrochloric acid and 8 μl 1 $\mu\text{g}\cdot\text{ml}^{-1}$ deuterated internal standard solution. After 10 ml dichloromethane was added, sap samples were shaken at 4 °C for 30 min and then centrifuged at 13,000 rpm for 5 min at 4 °C. The supernatant was carefully removed and the lower organic phase was dried by N_2 under shading condition and dissolved in 400 μl methanol (containing 0.1% methane acid) then filtered with a 0.22 mm filter membrane. The purified sample was then injected into high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), fitted with a POROSHELL120 SB-C18 (Agilent Technologies) column (2.1 mm \times 150 mm; 2.7 mm), at 30 °C. The solvent gradient used was 100% A (99.9% methanol: 0.1% CHOOH) to 100% B (99.9% H_2O : 0.1% CHOOH) over 15 min. The injection volume was 2 μl . MS conditions were as follows: the spray voltage was 4500 V; the pressure of the air curtain, nebulizer, and aux gas were 15, 65, and 70 psi, respectively; and the atomizing temperature was 400 °C. The concentration of ABA was calculated with reference to peak area of deuterated internal standard.

2.6 Statistical analysis

Statistically differences were analyzed by one-way variance (ANOVA) using SPSS Statistic Software. The relationship between pairs of variables was assessed by means of Pearson correlation (r) at $p < 0.05$ and $p < 0.01$ level. All graphics and regression with maximum likelihood (r^2) were performed with OriginPro 2017 (USA).

3. Results

3.1 Soil water status at the day after transferring

Dynamic change of RSWC in the pots during the two progressive soil drying cycles was shown in Fig.1. RSWC of the well-watered pots were maintained above 70%-82%, indicating no water stress occurred during the experiment. By withholding irrigation from DAT 28 to 33 in second drying cycle, RSWC in drought treatment gradually decreased from 80 to 37.27%. There was a significant difference in water status between the drought-stressed and well-watered seedlings from DAT 29 to 33.

3.2 Leaf water potential (Ψ_{leaf}) and $\xi\psi\lambda\epsilon\mu$ sap ABA concentration ($\text{ABA}_{\xi\psi\lambda\epsilon\mu}$)

In well-watered treatment, soil water content varied between 16% and 19% at DAT 28 to 33, and Ψ_{leaf} maintained with an average of -0.82 MPa. Ψ_{leaf} of the drought-stressed tomato decreased gradually as drought proceeded, and finally a significant decline of Ψ_{leaf} in drought-stressed tomato seedlings occurred at DAT 29 (Fig.2a). However, the ABA concentration of xylem sap kept little changed until RSWC decreased from 51.43 to 44.69% during DAT from 30 to 31 (Fig.2b and Fig.1). As soil further dried, the $\text{ABA}_{\text{xylem}}$ increased exponentially with increasing drought intensity and resulted in a significant difference between the drought and control treatment. In addition, it increased sharply to 97.86 $\text{ng}\cdot\text{ml}^{-1}$ at the end of our experiment.

3.3 Δρουγητ εφφεστ ον T_{leaf} , Γ^* , C_c and P_d

Parameters of T_{leaf} , Γ^* , C_c and R_d were closely related to the estimation of g_m . To determine accurately g_m , we analyzed the effect of drought on four parameters daily at the DAY from 28 to 33. The relative value of these four parameters was calculated as ratio between drought and well-watered treatment. The results showed that, compared to the well-watered treatment, the relative R_d decreased as RSWC decreased, and a significant difference was observed at RSWC=44.68% at the DAT 31 (Fig.3 and Fig.1). Drought stress had a more pronounced effect on T_{leaf} , Γ^* and C_c , and resulted in a significant increase in T_{leaf} and Γ^* , but decline in C_c at RSWC=51.43%.

3.4 Hydraulic and chemical signaling in regulation of leaf gas exchange and mesophyll conductance (g_m)

Sensitivity of g_s and g_m response to leaf water potential and ABA signaling were present in Fig.4. g_s , g_m and g_t showed remarkably hyperbolic regression response to Ψ_{leaf} ($r^2=0.55$, $r^2=0.58$, $r^2=0.67$, respectively), while

g_s declined logarithmically with increasing ABA concentration in root to shoot xylem sap with good correlation ($r^2=0.71$), as well as same ways of g_m and g_t response to ABA_{xylem} ($r^2=0.68$, $r^2=0.81$, respectively) (Fig.4 e-l). The results of ANOVA indicated that g_s and Tr instantly decreased in parallel with decreasing Ψ_{leaf} at onset of soil drought. Whereas, A_n , g_m , g_t remained unchanged until ABA_{xylem} significantly increased from 2.03 to 9.708 ng·ml⁻¹. The conductance of stomata and mesophyll decreased markedly along with the further increase in ABA_{xylem} at DAT 31 to 33. The results indicated that g_s response quickly to hydraulic signal in mild drought, whereas g_m was more pronounced sensitive to root-shoot ABA signaling as drought farther proceeded during RSWC decreased from 44.69 to 37.27%.

3.5 WUE_i response to ratio of stomatal conductance and mesophyll conductance

WUE_i increased with increase of g_m/g_s during RSWC decreased from 62.55 to 40.16%, accompanied by decrease of Ψ_{leaf} from -0.948 to -1.33 MPa, but decreased gradually with decrease of g_m/g_s after Ψ_{leaf} was less than -1.632 (Fig.5a). WUE_i responded to ABA_{xylem} changed in the similar way as WUE_i response to Ψ_{leaf} : WUE_i increased in conjunction with increasing g_m/g_s as ABA_{xylem} rose from 2.13 to 31.23 ng·ml⁻¹ at same soil water condition, and then decreased due to the decline of g_m/g_s as soil drought proceeded. In addition, the relationship between WUE_i and g_m/g_s was tightly represented by a logarithmic function ($r^2=0.771$).

3.6 Quantitative analysis of photosynthetic limitation response to soil drying

Relative contribution of all limiting factors (stomatal conductance, mesophyll conductance and photosynthetic biochemistry) to photosynthetic capacity was shown in Fig.5. Photosynthetic biochemistry (l_b) was found to be the main factor in well-watered plants with a good fitting ($r^2=0.88$). As soil drying, the relative contribution of stomatal conductance (l_s) limitation increased up to 50.43% while seedlings were suffered mild soil drought at $\Psi_{leaf} = -1.05$ MPa. As soil drought proceeded, the relative contribution of mesophyll conductance limitation (l_m) increased up to 42.03% when Ψ_{leaf} decreased from -1.33 to -1.63 MPa. As a result, the relative resistance of stomatal conductance and photosynthetic biochemistry correspondingly decreased to 36.92% and 21.07% at RSWC =37.23% (Fig.2a).

4. Discussion

Drought-induced stomata movement was closely related to hydraulic conductance, which has been extensively studied (Ripullone et al. 2007; Rodriguez-Dominguez et al. 2016). We found that stomatal aperture initially decreased concomitantly with decrease of leaf water potential at early stage of drought-stress (Fig.3a), which was consistent with the common consensus that stomatal closure acted as an early response to soil drought (Martin-Stpaul et al. 2017). Here, the increase of ABA was statistically insignificant when relative soil water decreased from 74.76 to 62.55%, i.e., from 0.34 to 0.45 ng ml⁻¹, which implied that root to shoot ABA signaling might not play an essential role in inducing stomatal closure in tomato under mild soil drought condition (Fig.2a, Fig.4c and 4d). Consistent with this, Huber et al. (2019) compared three signals of regulation in stomatal behavior (chemical, hydraulic and electrical) and reported that hydraulic signals were most passively linked to the stomata aperture due to the delayed increase in leaf ABA occurred after the onset of close of stomata. However, as soil drought proceeded, stomatal aperture decreased concurrently with an exponential increase in ABA_{xylem} , presenting a close relationship between g_s and ABA_{xylem} ($r=-0.548$, $p<0.01$). Therefore, it could be concluded that stomatal closure was primarily regulated by leaf hydraulic signal at the early stage of soil drought and maintained by root to shoot ABA signaling as relative soil water dropped from 51.43 to 37.27% (Tombesi et al. 2015). The ability of mesophyll conductance (g_m) decreasing the concentration of CO₂ in the chloroplast stroma has been stressed in these years (Flexas et al. 2002; Flexas et al. 2008; Cano et al. 2013). It should be firstly mentioned that the four parameters involved in g_m estimation (T_{leaf} , Γ^* , C_c and R_d) were markedly affected by soil drought and changed as soil drought proceeded (Fig.3). Here in my study, to get precise estimation of g_m , R_d was calculated from the light response curve based on the Photosynthesis Assistant of Ye (2007) and modified according to soil water status during DAT from 28 to 33. Compared to the response of g_s , g_m in the drought-stressed seedlings kept little change in the beginning of soil drought until a significant increase in ABA_{xylem} occurred (Fig.4g

and 4h), indicating that g_m was less sensitive to mild drought stress than g_s . This delayed response of g_m to drought was in accordance with the results reported by Theroux-Rancourt et al. (2014), who suggested that the threshold of regulation in mesophyll aquaporins was expected to appear under more severe soil drought stress, e.g. a 51.43-44.68% decrease in θ_{FC} in our study. It was important to note that the concurrence between ABA_{xylem} and g_m was not a mere coincidence. Moreover, it was found that the change of g_m was tightly related to ABA_{xylem} ($r=-0.643$, $p < 0.01$). In line with this, Mizokami et al (2015) concluded that g_m in ABA-deficient mutant (*abal*) did not decrease under drought condition as compared to the wild type of *Nicotiana plumbaginifolia*. Therefore, root to shoot ABA signaling seemed to be a candidate for actively controlling the conductance of mesophyll as relative soil water decreased from 51.43 to 37.27%. There was no doubt that, as reported in many studies (Ferrio et al. 2012; Theroux-Rancourt et al. 2014; Wang et al., 2018), g_m decreased as Ψ_{leaf} decreased with a good relationship ($r=0.760$, $p < 0.01$) at the later stage of soil drought, indicating that the decrease in g_m might be partly due to the impact of changes in leaf hydraulics. The reason of g_m unaffected by decreasing Ψ_{leaf} at the early stage of soil drought might result from the hydraulic compartmentalization of mesophyll cells from the transpiration stream, meaning cells could be buffered against short-term changes in leaf water status (Zwieniecki et al. 2007; Theroux-Rancourt et al. 2014). We did not investigate the specific interaction between ABA signaling and hydraulic conductance in regulation of mesophyll conductance in this experiment, but dual ways of ABA in controlling stomatal movement: a direct biochemical effect on guard cells and an indirect effect on leaf hydraulic conductance have been confirmed by Pantin et al (2013). Considering the coordinated role between hydraulic and chemical signals (Pantin et al. 2013; Mizokami et al. 2015; Tombesi et al. 2015), therefore, we concluded that ABA in xylem could be one signaling responsible for regulation mesophyll conductance at the severe soil drought, e.g. RASC <51.43% in the present study.

As a result of unaffected g_m and g_t , A_n did not decrease until RSWC reached to 44.68%. Though A_n significantly decreased during RSWC reduction from 51.43% to 40.16%, WUE_i increased with decrease of soil moisture (Fig.5). Our data was in accordance with the report of Liu et al. (2005), who thought that mild soil water shortage could improve water use efficiency. In this study, this drought-induced WUE_i increase might be explained by the improving g_m/g_s due to the more positive correlation between WUE_i and g_m/g_s ($r=0.771$, $p < 0.01$), compared to the correlation between WUE_i and g_s or g_m ($r=-0.759$ and $r=-0.439$, respectively) (Fig.5). Therefore, it was clear that, the less sensitivity to soil drought in g_m than that in g_s improved g_m/g_s , and thereby enhanced WUE_i . This explanation was supported by the results of the important role of g_m or g_m/g_s in improving WUE_i and A_n simultaneously (Flexas et al. 2013; Han et al. 2016). However, it was important to note that, such improving behavior in g_m/g_s could be beneficial for maintaining water status under short-term drought during RSWC reduction from 51.43% to 40.16%, but difficult for a long-term and under the further increasing intensity of soil drought. This was due to the improved WUE_i at leaf scale may not always result in an improvement of WUE at the whole plant scale resulted from remarkable reduction of crop yield under serious soil drought (Flexas et al. 2015). Nonetheless, drought-induced CO_2 drawdown from leaf surface to the site of carboxylation could in turn prevented water loss, which increased chances of survival under prolonged severe drought stress (Yan et al. 2017).

In order to accurately understand the effects of progressive soil drought on photosynthetic capacity, we quantitatively analyzed contribution of each limiting factor according to the methods proposed by Grassi and Magnani (2005). In our study, the relative contribution of g_s could account for about 41.99% of the net photosynthesis reduction (Fig.6), indicating that stomatal resistance may be the main limiting factor during RSWC decrease from 62.55 to 40.16% (Fig. 2a). Our result was closely coincident with Xue et al (2016) who reported that stomatal conductance mainly dominated the rate of assimilation in response to drought stress. However, it should be noted that, with increasing drought intensity, the contribution of g_m increased rapidly and nearly equalled to that of g_s when RSWC reached to 37.27%. In addition, although small difference in the main limitation was observed as compared to the study of Wang et al (2018) might due to the different plant species and soil water status, generally speaking, diffusive limitations to CO_2 were the major constraints to photosynthesis under drought condition.

In conclusion, our results confirmed the important role of root to shoot ABA in regulation of mesophyll

conductance and identified the threshold of ABA signaling, i.e. relative soil water content decreasing from 51.43 to 44.68% in the present study. Compared to g_s , g_m was less sensitive to mild soil drought until a concurrent marked increasing ABA in xylem occurred, which potentially enhanced the ratio of g_m and g_s , and thereby improved WUE_i during RSWC reduced from 51.43 to 40.16%. The decrease in g_s and g_m was the main constraints to photosynthesis under progressively soil drought condition.

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Author contributions

AD and YG planned and designed the experiments; SL and JL performed the experiments; SL analyzed the data and wrote the manuscript. AD, YG and HL revised the manuscript. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Declarations of interest: none.

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Figures

Fig.1. Dynamic change of relative soil water content in well-watered and drought-stressed treatments after transplanting.

Fig.2. Leaf water potential (n=6) and xylem sap ABA concentration (n=3) in response to progressive soil drying. Bars indicated stand error. ** indicated significant difference at $p < 0.01$ level and ns indicated no significant difference between drought and well-watered treatment (one-way variance analysis).

Fig.3. Dynamic change of relative leaf temperature (T_{leaf}), chloroplast CO₂ compensation point (Γ^*), chloroplastic CO₂ concentration (C_c) and day respiration (R_d) as relative soil water content decreased. The letter (ns and **) was shown in chronological order as soil drying at the DAT from 28 to 33 (n=6).

Fig.4. Response of physiological parameters to leaf water potential (Ψ_{leaf}) and ABA concentration (ABA_{xylem}) was shown by bubble diagram: A_n , Tr , g_s to Ψ_{leaf} (a, b) and to ABA_{xylem} (c, d); A_n , Tr , g_m to Ψ_{leaf} (e, f) and to ABA_{xylem} (g, h); A_n , Tr , g_t to Ψ_{leaf} (i, j) and to ABA_{xylem} (k, l). X coordinate axis indicated Ψ_{leaf} and ABA, respectively. Y₁ coordinate axis indicated the conductance of CO₂ (g_s , g_m , g_t).

Y_2 coordinate axis indicated A_n and T_r , respectively. The size of bubble indicated the rate of A_n and T_r . Correlations between X and Y_1 , Y_1 and Y_2 were presented (** $p < 0.01$).

Fig.5. Relationship between WUE_i and g_m/g_s in response to (a) leaf water potential and (b) xylem sap ABA concentration was shown by bubble diagram. X coordinate axis indicated Ψ_{leaf} and ABA, respectively. Y_1 coordinate axis indicated the g_m/g_s . Y_2 coordinate axis indicated WUE_i . The size of bubble indicated the value of WUE_i . Correlations between X and Y_1 , Y_1 and Y_2 were presented (** $p < 0.01$).

Fig.6. Effect of leaf water potential (Ψ_{leaf}) on the relative contribution of the photosynthesis capacity limiting factors: stomatal conductance (l_s), mesophyll conductance (l_m) and photosynthetic biochemistry (l_b).

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