1 **Drought stress delays photosynthetic induction and accelerates photoinhibition of**
2 **photosystem I under fluctuating light**

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4 Hu Sun^{1,2}, Qi Shi^{1,2}, Ning-Yu Liu^{1,2}, Shi-Bao Zhang¹, Wei Huang¹

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6 ¹ Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

7 ² University of Chinese Academy of Sciences, Beijing 100049, China

8

9 **Corresponding authors:**

10 Shi-Bao Zhang: sbzhang@mail.kib.ac.cn

11 Wei Huang: huangwei@mail.kib.ac.cn

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16

17 **Running head:**

18 Photosynthesis under drought and fluctuating light

19

20 Abstract

21 Fluctuating light (FL) and drought stress usually occur concomitantly. However, whether
 22 drought stress affects photosynthetic performance under FL remains unknown. Here, we
 23 measured gas exchange, chlorophyll fluorescence, and P700 redox state under FL in
 24 drought-stressed tomato (*Solanum lycopersicum*) seedlings. Drought stress significantly
 25 affected stomatal opening and mesophyll conductance after transition from low to high light
 26 and thus delayed photosynthetic induction under FL. Therefore, drought stress exacerbated
 27 the loss of carbon gain under FL. Furthermore, restriction of CO₂ fixation under drought
 28 stress aggravated the over-reduction of photosystem I (PSI) upon transition from low to high
 29 light. The resulting stronger FL-induced PSI photoinhibition significantly suppressed linear
 30 electron flow and PSI photoprotection. These results indicated that drought stress not only
 31 affected gas exchange under FL but also accelerated FL-induced photoinhibition of PSI.
 32 Furthermore, drought stress enhanced relative cyclic electron flow in FL, which partially
 33 compensated for restricted CO₂ fixation and thus favored PSI photoprotection under FL.
 34 Therefore, drought stress has large effects on photosynthetic dark and light reactions under
 35 FL.

36

37 **Keywords:** drought, fluctuating light, photoinhibition, photosynthesis, stomatal conductance,
 38 tomato.

39

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43

44 Conflict of Interest:

45 The authors declare no conflict of interest.

46

47 Introduction

48 During diurnal photosynthesis under natural field conditions, leaves usually experience
 49 dynamic changes in light intensity on timescales from milliseconds to hours, owing to the
 50 variation of the solar angle, cloud movement, wind-induced leaf fluttering, and shading from
 51 overlapping leaves and neighboring plants (Percy 1990; Slattery, Walker, Weber & Ort
 52 2018). In addition to fluctuating light (FL), plants often experience suboptimal conditions,
 53 such as drought. Many studies have examined the effect of drought stress on photosynthesis
 54 under stable light intensity (Oukarroum, Schansker & Strasser 2009; Galmés *et al.* 2013;
 55 Zivcak *et al.* 2013). However, drought stress is rarely studied under FL conditions (Grieco *et al.*
 56 2020; Qu *et al.* 2020). Under FL, plants may exhibit different photosynthetic performance
 57 when also experiencing drought stress compared with sufficient water conditions. To
 58 understand how plants perform under a combination of FL and drought stress in the field, it is
 59 important to explore the photosynthetic physiology of drought-stressed plants under FL.

60 After transition from low to high light, the net CO₂ assimilation rate (A_N) gradually
 61 increases, which is termed “photosynthetic induction”. The rate of photosynthetic induction
 62 significantly affects carbon gain and thus affects plant biomass when grown under FL (Adachi
 63 *et al.* 2019; Kimura, Hashimoto-Sugimoto, Iba, Terashima & Yamori 2020; Yamori, Kusumi,
 64 Iba & Terashima 2020). Therefore, promoting the rate of photosynthetic induction is a
 65 potential target for improving crop yield (De Souza, Wang, Orr, Carmo-Silva & Long 2020;
 66 Acevedo-Siaca *et al.* 2020). Under high light, A_N is determined by CO₂ diffusional
 67 conductance and biochemical factors (Grassi & Magnani 2005). CO₂ diffusional conductance
 68 refers to CO₂ diffusion from the atmosphere to the chloroplast stroma, including stomatal
 69 conductance (g_s) and mesophyll conductance (g_m) (Flexas *et al.* 2014). Biochemical factors
 70 include the activation of electron transport, Calvin–Benson cycle enzymes, especially
 71 Rubisco, and sucrose synthesis (Sakoda, Yamori, Groszmann & Evans 2021). A recent study
 72 revealed that the induction of A_N under FL was mainly affected by g_s rather than g_m in
 73 *Arabidopsis thaliana* and tobacco (*Nicotiana tabacum*) plants in the absence of water stress
 74 (Sakoda *et al.* 2021). Increasing the stomatal opening significantly enhanced the induction of
 75 A_N and plant biomass under FL (Kimura *et al.* 2020; Yamori *et al.* 2020). Drought stress

usually reduces g_s and g_m under constant high light, leading to decreased chloroplast CO_2 concentration (C_c) (Warren, Livingston & Turpin 2004). Under such conditions, CO_2 fixation in chloroplast carboxylation sites is restricted by the lack of CO_2 . Therefore, the relative limitations of g_s and g_m imposed on A_N are enhanced under drought stress. However, it is unclear whether drought stress affects the induction responses of g_s , g_m , and A_N under FL. Characterizing the dynamics of g_s and g_m and how they limit A_N under drought and FL is crucial for understanding the physiological mechanisms regulating carbon gain by plants under suboptimal field conditions.

In addition to photosynthetic dark reactions, FL affects photosynthetic light reactions. Upon a sudden increase in light intensity, electron flow from photosystem II (PSII) to photosystem I (PSI) rapidly increases (Huang, Yang & Zhang 2019a; Sun, Zhang, Liu & Huang 2020b). Meanwhile, CO_2 fixation is not fully activated (Tanaka, Adachi & Yamori 2019), generating an imbalance between the production of excited states and the consumption of reducing power (Gerotto *et al.* 2016; Tan, Huang, Zhang, Zhang & Huang 2021). The resulting PSI over-reduction increases the production of reactive oxygen species (ROS) in PSI. Consequently, oxidative damage to PSI occurs because the ROS produced within thylakoid membranes cannot be immediately scavenged by antioxidant systems (Takagi, Takumi, Hashiguchi, Sejima & Miyake 2016). Therefore, FL can cause selective photoinhibition of PSI in many angiosperms (Yamori, Makino & Shikanai 2016; Yamamoto & Shikanai 2019; Huang, Yang & Zhang 2019b). PSI damage suppresses photosynthetic electron flow, photoprotection, and CO_2 assimilation (Sejima, Takagi, Fukayama, Makino & Miyake 2014; Zivcak, Brestic, Kunderlikova, Sytar & Allakhverdiev 2015; Shimakawa & Miyake 2019), first impairing plant growth and even causing plant death (Suorsa *et al.* 2012; Yamori *et al.* 2016). The PSI redox state under FL is significantly affected by the electron sink downstream of PSI (Wada *et al.* 2018; Tazoe *et al.* 2020; Sun, Yang & Huang 2020a). However, little is known about the effects of drought stress on the PSI redox state and PSI photoinhibition under FL.

Plants have evolved several alternative electron flows to protect PSI against photoinhibition under FL (Allahverdiyeva, Suorsa, Tikkanen & Aro 2015; Armbruster, Correa Galvis, Kunz & Strand 2017; Shikanai & Yamamoto 2017). Cyclic electron flow (CEF)

around PSI is used by angiosperms to fine-tune photosynthetic apparatus under FL (Suorsa *et al.* 2012; Yamamoto & Shikanai 2019). After transition from low to high light, CEF activity usually increases to allow fast formation of the trans-thylakoid proton gradient (ΔpH), which alleviates the PSI over-reduction at donor and acceptor sides (Kono, Noguchi & Terashima 2014; Yang, Ding & Huang 2019a). Once a sufficient ΔpH forms, CEF activity decreases to steady-state levels to prevent over-acidification of the thylakoid lumen. Therefore, the dynamic regulation of CEF activity under FL is crucial for balancing photoprotection and photosynthesis (Alboresi, Storti & Morosinotto 2019). Previous studies have documented that CEF activity under FL is largely affected by the redox state of PSI (Yang *et al.* 2019a; Tan *et al.* 2021). In particular, CEF activity increases with moderate PSI over-reduction but maintains at a low level when PSI over-reduction is missing. However, the effects of drought stress on the dynamic regulation of CEF activity under FL is largely unknown.

The aim of this study was to investigate whether and how drought stress affects photosynthetic light and dark reactions under FL. With this knowledge it may be possible to understand how drought stress interacts with FL to affect photosynthetic physiology and plant growth. Tomato was used in this study, as it is a C3 model species with intermediate leaf photosynthetic capacity and an important vegetable crop worldwide. To address the above question, tomato plants were grown under full sun light with sufficient or deficient water. We then measured the rapid changes in gas exchange, chlorophyll fluorescence, and P700 signals under FL.

126

127 **Materials and methods**

128 **Plant materials and growth conditions**

Tomato (*Solanum lycopersicum* Miller cv. Hupishizi) plants were grown in a greenhouse under 40% full sunlight. Day/night air temperatures were approximately 30/20°C, relative humidity was approximately 60-70%, and maximum light intensity was approximately 800 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The plants were grown in 19-cm diameter plastic pots with humus soil, and the initial soil N content was 2.1 mg/g. Plants were fertilized with 0.15 g N/plant every two days using Peters Professional's water solution (N:P:K = 15:4.8:24.1) and watered every day. After cultivation for one and a half months, plants were watered using running water with

136 400g/pot (CK) or 200g/pot (drought) for 1 week, and then mature leaves were used for
137 photosynthetic measurements.

138

139 **Chlorophyll content measurement in vivo**

140 A handheld chlorophyll meter (SPAD-502 Plus; Minolta, Tokyo, Japan) was used to
141 non-destructively measure the relative content of chlorophyll per unit leaf area.

142

143 **Gas exchange and chlorophyll fluorescence measurements**

144 An open gas exchange system (LI-6400XT; Li-Cor Biosciences, Lincoln, NE, USA) was
145 used to simultaneously measure gas exchange and chlorophyll fluorescence. After
146 photosynthetic induction at 1500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and 400 $\mu\text{mol mol}^{-1} \text{ CO}_2$
147 concentration for 20 min, the net CO_2 assimilation rate and g_s reached steady-state values.
148 Then, the light intensity was changed to 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for 5 min to conduct low
149 light adaptation. Afterward, the light intensity was changed back to 1500 $\mu\text{mol photons m}^{-2}$
150 s^{-1} to measure the photosynthetic induction phase. After adequate photosynthetic induction,
151 the response of CO_2 assimilation rate to incident intercellular CO_2 concentration (A/C_i) curves
152 were measured by decreasing the CO_2 concentration to a lower limit of 50 $\mu\text{mol mol}^{-1}$ and
153 then increasing stepwise to an upper limit of 1500 $\mu\text{mol mol}^{-1}$. For each CO_2 concentration,
154 photosynthetic measurement was completed in 3 min. Using the A/C_i curves, the maximum
155 rates of RuBP regeneration (J_{max}) and carboxylation (V_{cmax}) were calculated (Long &
156 Bernacchi 2003).

157 The quantum yield of PSII photochemistry was calculated as $\Phi_{\text{PSII}} = (F_m' - F_s)/F_m'$ (Genty,
158 Briantais & Baker 1989), where F_m' and F_s represent the maximum and steady-state
159 fluorescence after light adaptation, respectively (Baker 2004). The total electron transport rate
160 through PSII (J_{PSII}) was calculated as follows (Krall & Edwards 1992):

$$J_{\text{PSII}} = \Phi_{\text{PSII}} \times \text{PPFD} \times L_{\text{abs}} \times 0.5$$

161 where PPFD is the photosynthetic photon flux density and leaf absorbance (L_{abs}) is assumed
162 to be 0.84. We applied the constant of 0.5 based on the assumption that photons were equally
163 distributed between PSI and PSII.

164

165 Estimation of mesophyll conductance and chloroplast CO₂ concentration

166 Mesophyll conductance was calculated according to the following equation (Harley,
167 Loreto, Di Marco & Sharkey 1992):

$$g_m = \frac{A_N}{C_i - \Gamma^* (J_{PSII} + 8(A_N + R_d)) / (J_{PSII} - 4(A_N + R_d))}$$

168 where A_N represents the net rate of CO₂ assimilation; C_i is the intercellular CO₂ concentration;
169 Γ^* is the CO₂ compensation point in the absence of daytime respiration (Yamori, Noguchi,
170 Hikosaka & Terashima 2010b; von Caemmerer & Evans 2015), and we used a typical value
171 of 40 $\mu\text{mol mol}^{-1}$ in our current study (Xiong, Douthe & Flexas 2018). Respiration rate in the
172 dark (R_d) was considered to be half of the dark-adapted mitochondrial respiration rate as
173 measured after 10 min of dark adaptation (Carricú *et al.* 2015).

174 Based on the estimated g_m , we then calculated the chloroplast CO₂ concentration (C_c)
175 according to the following equation (Long & Bernacchi 2003; Warren & Dreyer 2006):

$$C_c = C_i - \frac{A_N}{g_m}$$

176 To identify the rate-limiting step of CO₂ assimilation, we subsequently estimated C_{trans}
177 (the chloroplast CO₂ concentration at which the limitation to A_N transitioned from RuBP
178 carboxylation to RuBP regeneration) (Yamori, Evans & Von Caemmerer 2010a; Yamori,
179 Nagai & Makino 2011):

$$C_{trans} = \frac{K_c(1 + O/K_o)J_{max}/4V_{cmax} - 2\Gamma^*}{1 - J_{max}/4V_{cmax}}$$

180 where K_c ($\mu\text{mol mol}^{-1}$) and K_o (mmol mol^{-1}) are assumed to be 407 $\mu\text{mol mol}^{-1}$ and 277 mmol
181 mol^{-1} at 25°C, respectively (Long and Bernacchi 2003); O was assumed to be 210 mmol mol^{-1}
182 (Farquhar *et al.* 1980). The rate-limiting step for CO₂ assimilation was analyzed by comparing
183 the values of C_c and C_{trans} . A_N tends to be limited by RuBP carboxylation when C_c is lower
184 than C_{trans} and tends to be limited by RuBP regeneration when C_c is higher than C_{trans} .

185

186 Quantitative limitation analysis of A_N

187 Relative photosynthetic limitations were assessed as follows (Grassi & Magnani 2005):

$$L_s = \frac{g_{tot}/g_s \times \partial A_N / \partial C_c}{g_{tot} + \partial A_N / \partial C_c}$$

$$L_{mc} = \frac{g_{tot}/g_m \times \partial A_N / \partial C_c}{g_{tot} + \partial A_N / \partial C_c}$$

$$L_b = \frac{g_{\text{tot}}}{g_{\text{tot}} + \partial A_N / \partial C_c}$$

188 where L_s , L_{mc} , and L_b represent the relative limitations of stomatal conductance, mesophyll
189 conductance, and biochemical capacity, respectively, in setting the observed value of A_N . g_{tot}
190 is the total conductance of CO_2 between the leaf surface and sites of RuBP carboxylation
191 (calculated as $1/g_{\text{tot}} = 1/g_s + 1/g_m$).

192

193 **P700 and chlorophyll fluorescence measurements**

194 PSI and PSII parameters were measured at 25°C under atmospheric CO_2 condition using
195 a Dual-PAM 100 measuring system (Heinz Walz, Effeltrich, Germany). Light from a 635-nm
196 light-emitting diode array equipped in Dual-PAM 100 was used as actinic light for
197 illumination. After dark adaptation for at least 15 min, a saturating pulse (20,000 μmol
198 photons $\text{m}^{-2} \text{s}^{-1}$, 300 ms) was used to measure the maximum fluorescence intensity (F_m) and
199 the maximum photo-oxidizable P700 (P_m). Subsequently, leaves were exposed to 1455 μmol
200 photons $\text{m}^{-2} \text{s}^{-1}$ for 5 min to activate photosynthetic electron sinks and then illuminated at
201 fluctuating light alternating between low light (59 μmol photons $\text{m}^{-2} \text{s}^{-1}$, 2 min) and high light
202 (1455 μmol photons $\text{m}^{-2} \text{s}^{-1}$, 1 min). During this fluctuating light treatment, PSI and PSII
203 parameters were recorded simultaneously. After the fluctuating light treatment, P_m was
204 measured after a 15-min dark adaptation.

205 The chlorophyll fluorescence parameters were calculated as follows: $Y(\text{II}) = (F_m' - F_s) /$
206 F_m' ; $\text{NPQ} = (F_m - F_m') / F_m'$; $Y(\text{NO}) = F_s / F_m$. $Y(\text{II})$ is the quantum yield of PSII
207 photochemistry; NPQ, non-photochemical quenching in PSII; $Y(\text{NO})$, the quantum yield of
208 non-regulatory energy dissipation in PSII. F_m and F_m' are the maximum fluorescence intensity
209 after dark and light acclimation, respectively. F_s is the light-adapted steady-state fluorescence.

210 PSI photosynthetic parameters were measured by a Dual PAM-100 based on P700 signal
211 (difference of intensities of 830 and 875 nm pulse-modulated measuring light reaching the
212 photodetector). The P700⁺ signals (P) may vary between a minimal (P700 fully reduced) and
213 a maximal level (P700 fully oxidized). The maximum level (P_m) was determined with
214 application of a saturating pulse (20,000 μmol photons $\text{m}^{-2} \text{s}^{-1}$ and 300 ms) after
215 pre-illumination with far-red light, and P_m was used to estimate the PSI activity. P_m' was

determined similarly to P_m but with actinic light instead of far-red light. PSI parameters were calculated as follows: $Y(I) = (P_m' - P)/P_m$; $Y(ND) = P/P_m$; $Y(NA) = (P_m - P_m')/P_m$. $Y(I)$ is the quantum yield of PSI photochemistry; $Y(ND)$, the quantum yield of PSI non-photochemical energy dissipation due to donor side limitation; $Y(NA)$, the quantum yield of PSI non-photochemical energy dissipation due to acceptor side limitation. The photosynthetic electron transport rate was calculated as $ETRI$ (or ETR_{II}) = $PPFD \times Y(I)$ [or $Y(II)$] $\times 0.84 \times 0.5$, light absorption is assumed to be 0.84 of incident irradiance, and 0.5 is the fraction of absorbed light reaching PSI or PSII. The relative CEF value was measured by the ratio of $Y(I)$ to $Y(II)$ (Grieco *et al.* 2020).

225

226 Statistical analysis

One-way ANOVA and t-tests were used to determine whether significant differences existed between different treatments ($\alpha = 0.05$). The software SigmaPlot 10.0 was used for graphing and fitting.

230

231 Results

232 Effects of drought stress on gas exchange under FL

After water deficit treatment for one week, the maximum quantum yield of PSII after dark adaptation was maintained at approximately 0.84 (data not shown), indicating that PSII activity was not significantly photoinhibited under drought stress. At low light intensity of $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, the steady-state net rate of CO_2 assimilation (A_N) was slightly affected by drought stress. However, drought stress significantly affected steady-state A_N , stomatal conductance (g_s), and mesophyll conductance (g_m) under high light. Furthermore, the photosynthetic induction after transition from low to high light was largely affected by drought stress (Fig. 1A). After transition from 100 to $1500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for 1 min, A_N rapidly increased to $17.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in control (CK) plants but only increased to $4.0 \mu\text{mol m}^{-2} \text{ s}^{-1}$ under drought (Fig. 1A). The time required for A_N to reach 80% of the maximum value was approximately 2 min in CK plants but 10 min under drought stress (Fig. 1B). Drought stress decreased g_s under low and high light compared to CK levels (Fig. 1C). Furthermore, the g_s level at low light was greatly reduced under drought stress compared with

the maximum g_s under high light, whereas there was less relative difference in g_s between low and high light in CK (Fig. 1D). Drought stress also lowered g_m under high light compared to CK (Fig. 1E). After transition from 100 to 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the time taken for g_m to reach the maximum value was approximately 3 min in CK plants but 11 min under drought stress (Fig. 1F). Therefore, drought stress delayed the induction responses of g_s , g_m , and A_N under FL.

Due to lower levels of CO_2 diffusional conductance, the intercellular CO_2 concentration (C_i) and chloroplast CO_2 concentration (C_c) under high light largely decreased under drought stress compared to CK (Fig. 2A and B). The values of C_c during photosynthetic induction were always higher than C_{trans} (the chloroplast CO_2 concentration at which the limitation to A_N transitioned from RuBP carboxylation to RuBP regeneration) in CK plants but were always lower than C_{trans} under drought stress, indicating that A_N tended to be limited by RuBP regeneration in CK plants and by RuBP carboxylation under drought stress. Therefore, the rate-limiting step for A_N during photosynthetic induction was altered by drought stress. The change in A_N during photosynthetic induction was tightly and positively correlated to C_c (Fig. 2C), indicating CO_2 fixation under FL was largely restricted by C_c . Quantitative analysis of photosynthetic limitations revealed stomatal limitation (L_s) and mesophyll conductance limitation (L_{mc}) were enhanced under drought stress (Fig. 3A and B). Concomitantly, biochemistry limitation (L_b) decreased under drought stress (Fig. 3C). Therefore, the major limitation of photosynthesis under FL also changed under drought stress.

Effects of drought stress on light reactions under FL

During photosynthetic induction, the quantum yield of PSI photochemistry ($Y(\text{I})$) under high-light phases decreased under drought stress (Fig. 4A), whereas the PSI donor side limitation ($Y(\text{ND})$) decreased under drought stress (Fig. 4B), leading to the higher PSI acceptor side limitation ($Y(\text{NA})$) in plants exposed to drought stress (Fig. 4C). Therefore, drought stress induced stronger PSI over-reduction under FL. Furthermore, under sufficient water supply, the PSI over-reduction under FL was mainly observed after transitioning from low to high light for 10 s (Fig. 4C). By comparison, plants also displayed severe PSI over-reduction after this light transition for 30 s under drought stress (Fig. 4C). Therefore,

276 drought stress prolonged the time course of PSI over-reduction under FL.

277 Similar to Y(I), drought stress decreased the value of Y(II) at high light during
278 photosynthetic induction (Fig. 5A). The induction response of NPQ was not affected by
279 drought stress (Fig. 5B), leading to small changes in the quantum yield of non-regulatory
280 energy dissipation in PSII (Y(NO)) between CK and drought-stressed plants (Fig. 5C).
281 Drought stress had minimal effects on electron transport rates through PSI and PSII (ETRI
282 and ETRII) at low light (Fig. 6A and B). However, the relative values of ETRI and ETRII
283 under high light significantly decreased after drought treatment (Fig. 6A and B), which was
284 consistent with the decreased A_N under drought stress. The relative CEF value under high
285 light, measured as the ratio of Y(I) to Y(II), was higher in drought-stressed plants compared to
286 CK plants (Fig. 6C). Furthermore, CEF could not be fully activated within the first 10 s under
287 drought stress, suggesting that drought stress induced a delayed activation of CEF under FL.
288 Therefore, the changing model of CEF under FL was altered by drought stress.

289 After FL treatment, the decrease in P_m was significantly higher under drought stress (Fig.
290 7A), indicating that drought stress significantly accelerated PSI photoinhibition under FL. The
291 greater FL-induced PSI photoinhibition under drought stress was largely caused by the
292 stronger PSI over-reduction within the first 30 s after transition from low to high light (Fig.
293 7B). Furthermore, a tight inverse relationship was found between the extent of FL-induced
294 PSI photoinhibition and the maximum CO₂ assimilation rate (Fig. 7C), suggesting that the
295 restriction of CO₂ fixation under drought stress accelerated FL-induced PSI photoinhibition.
296 After the FL treatment, ETRII under high light significantly decreased in drought-stressed
297 plants (Fig. 8). Concomitantly, Y(ND) significantly decreased and Y(NA) significantly
298 increased (Fig. 8). These results indicated that the greater FL-induced PSI photoinhibition
299 under drought stress significantly affected linear electron flow and PSI photoprotection under
300 high light.

301

302 Discussion

303 In nature, FL usually occurs concomitantly with drought stress. However, the effects of
304 drought stress on photosynthetic performances under FL are little known. We here for the first
305 time examined the effects of drought stress on photosynthetic induction and PSI

306 photoinhibition under FL in tomato.

307

308 **Drought stress delayed photosynthetic induction under FL**

309 The response of A_N to a rapid change of light intensity plays an important role in
 310 determining plant biomass under FL (Violet-Chabrand, Matthews, Simkin, Raines & Lawson
 311 2017; Slattery *et al.* 2018; Kimura *et al.* 2020; Yamori *et al.* 2020). Drought stress usually
 312 decreases steady-state g_s , g_m , and A_N under constant high light (Warren *et al.* 2004; Huang *et al.*
 313 2013; Zivcak *et al.* 2013), but the effects of drought stress on the induction response of g_s ,
 314 g_m , and A_N under FL are not well known. Similar to previous studies, we found the maximum
 315 g_s , g_m , and A_N under constant high light significantly declined in drought-stressed tomato
 316 plants (Fig. 1). Moreover, drought stress largely affected the induction responses of g_s and g_m
 317 after transition from low to high light and consequently delayed the induction response of A_N .
 318 A_N in CK plants reached 80% of the maximum value approximately 2 min after transition
 319 from 100 to 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 1). Such photosynthetic induction required 10
 320 min in drought-stressed plants. Therefore, drought stress caused a larger loss of carbon gain
 321 upon transition from low to high light in tomato. Similar to observations under drought stress,
 322 tomato plants display relatively lower g_s under salt stress, and the decrease in g_s under salt
 323 stress reduced plant biomass when grown under FL (Zhang, Kaiser, Marcelis, Yang & Li
 324 2020). However, the underlying mechanisms of this response are not well known. Our present
 325 study provided a possible explanation for why salt stress reduces plant biomass in tomato
 326 plants grown under FL.

327 Recent studies indicate stomatal opening under FL could be affected by stomatal density
 328 and area (Zhang *et al.* 2020; Sakoda *et al.* 2020). In the present study, the drought treatment
 329 just lasted for 1 week, and we used mature leaves developed prior to the treatment period for
 330 photosynthetic measurements. Therefore, the slower stomatal opening under drought stress
 331 was independent of stomatal density and area and was likely caused by other factors such as
 332 abscisic acid and gene regulation. Abscisic acid is upregulated under drought stress, which
 333 could induce stomatal closure (Ramachandra & Viswanatha 2004; Harb, Krishnan,
 334 Ambavaram & Pereira 2010; Kaiser, Morales, Harbinson, Heuvelink & Marcelis 2020).
 335 Furthermore, the expression of *slow anion channel-associated 1 (slac1)*, *open stomata 1*

336 (*ost1*), and *proton ATPase translocation control 1 (PATROL1)* play important roles in
337 stomatal opening under FL (Kimura *et al.* 2020; Yamori *et al.* 2020). Drought stress might
338 influence the expression of these target genes and thus affect the stomatal opening under FL.

339 Photosynthesis can be limited by g_s , g_m , and biochemical factors, but the relative
340 photosynthetic limitations largely vary among species (Grassi & Magnani 2005; Carriqui *et al.*
341 2015; Xiong *et al.* 2018). We found that drought stress increased the diffusional limitation of
342 A_N under FL, and the major limiting factor of photosynthesis was altered by drought stress
343 (Fig. 3). In *Arabidopsis thaliana* and tobacco, CO_2 diffusional limitation was the major
344 limiting factor of photosynthesis in the initial 10 min after transition from dark to light
345 (Sakoda *et al.* 2021). By comparison, L_s , L_{mc} and L_b changed little after the transition from
346 low to high light in the CK plants (Fig. 3). Therefore, the dynamic changes in relative
347 limitations of A_N after the transition from low to high light differed from that after the
348 transition from dark to high light. Due to decreased g_s and g_m under drought stress, C_c was
349 lower in high-light phases under FL, and therefore photosynthesis under FL was strongly
350 restricted by C_c (Fig. 2). Furthermore, after transition from low to high light, the C_c values in
351 the CK plants were always higher than C_{trans} (Fig. 2), indicating that A_N was mainly limited by
352 RuBP regeneration. By contrast, the C_c values under FL were always lower than C_{trans} under
353 drought stress (Fig. 2), and hence A_N tended to be mainly limited by RuBP carboxylation.
354 Therefore, the rate-limiting step for A_N during photosynthetic induction was influenced by
355 drought stress.

356

357 **Drought stress accelerated PSI photoinhibition under FL**

358 A sudden increase in light intensity causes a rapid increase in PSII electron flow to PSI,
359 whereas the full activation of CO_2 fixation requires more time (Lawson & Blatt 2014; Yamori
360 *et al.* 2016). Therefore, the excited states in PSI cannot be immediately consumed by primary
361 metabolism, leading to the accumulation of reducing power in PSI. The resulting PSI
362 over-reduction promotes ROS formation in PSI and causes PSI photoinhibition (Yamori *et al.*
363 2016; Huang *et al.* 2019a). Under drought stress, lower g_s restricted CO_2 fixation and thus
364 decreased the rate of NADPH production. Because the pool of NADPH is relatively small,
365 drought stress will increase the NADPH/NADP⁺ ratio when exposed to a sudden increase in

illumination. Under such conditions, electron flow from PSI to NADP⁺ would be limited by the lack of NADP⁺, suppressing the oxidation of PSI under FL (Grieco *et al.* 2020). Therefore, drought stress is hypothesized to accelerate PSI over-reduction under FL. Consistently, we found that PSI over-reduction under FL was aggravated under drought stress (Fig. 4). After a sudden increase in irradiance, tomato plants showed a transient PSI over-reduction in CK plants, indicating that the electrons transported to PSI could not be immediately consumed by downstream sinks. In angiosperms, outflows of electrons from PSI include two pathways: linear electron flow (LEF) (PSI to NADP⁺) and the water-water cycle mediated by the Mehler reaction (Ilík *et al.* 2017; Shikanai & Yamamoto 2017). Recent studies have indicated that the water-water cycle can rapidly consume the excess reducing power in PSI and thus prevents a transient PSI over-reduction under FL (Huang *et al.* 2019b; Sun *et al.* 2020a). However, a transient PSI over-reduction under FL was clearly observed (Fig. 4C), indicating that the water-water cycle is negligible in tomato leaves. Therefore, the transient PSI over-reduction under FL is attributed to the limitation of LEF. Under drought stress, the restriction of CO₂ fixation caused LEF to be further restricted by the lack of NADP⁺ and thus increased PSI over-reduction upon the transition from low to high light.

Once PSI is over-reduced under high light, the donation of electrons from PSI electron carriers to O₂ accelerates, aggravating the production of ROS within PSI (Takagi *et al.* 2016). Moreover, ROS produced within thylakoid membranes cannot be immediately scavenged by antioxidant systems (Takagi *et al.* 2016). Therefore, PSI over-reduction under high light easily causes PSI photoinhibition (Yamamoto & Shikanai 2019; Tan *et al.* 2021). Consistently, the stronger PSI over-reduction in FL accelerated PSI photoinhibition under drought stress (Fig. 7). Furthermore, the stronger PSI photoinhibition under drought stress led to decreased rETRII and Y(ND) and increased Y(NA), suggesting that light use efficiency and photoprotection were significantly affected by the PSI photoinhibition (Fig. 8). After photodamage, the recovery of PSI activity is a slow process that requires several days (Zhang & Scheller 2004; Zivcak *et al.* 2015). During the recovery period, the lower CO₂ assimilation rate impairs starch accumulation and plant growth (Lima-Melo, Gollan, Tikkanen, Silveira & Aro 2019). Therefore, when grown under FL, drought stress would decrease plant biomass partially owing to stronger PSI photoinhibition. In contrast to PSI, the PSII excitation pressure

under FL was slightly affected by drought stress (Fig. 5), which was consistent with previous studies reporting that PSII is tolerant to FL (Yamamoto & Shikanai 2019).

To protect PSI against photoinhibition under FL, angiosperms mainly use CEF to fine-tune the PSI redox state through regulation of ΔpH (Armbruster *et al.* 2017). Under low light, upon a low CEF activation, a relatively low ΔpH is formed to facilitate electron flow through Cyt b6/f and thus to maximize light use efficiency (Tikkanen & Aro 2014). Under high light, CEF is highly activated to generate a high ΔpH , which slows down the oxidation of plastoquinone and thus down-regulates the rate of electron transport toward PSI (Shikanai & Yamamoto 2017). When transitioning from low to high light, the full acidification of the thylakoid lumen requires dozens of seconds (Huang *et al.* 2019b; Yang, Zhang & Huang 2019b). Therefore, plants cannot generate sufficient ΔpH within the first seconds after an abrupt increase in irradiance. To avoid uncontrolled PSI over-reduction under FL, CEF rapidly activates to aid the formation of ΔpH (Kono *et al.* 2014; Yang *et al.* 2019a). In the present study, we found relative CEF activity in high-light phases of FL increased under drought stress (Fig. 6), suggesting that the relative contribution of CEF to ΔpH formation was enhanced in drought-stressed plants. Such up-regulation of CEF activity partially compensated for the restriction of CO₂ fixation and thus favored PSI photoprotection under FL.

Conclusions

We established that drought stress largely affects g_s and g_m after the transition from low to high light and thus delays photosynthetic induction under FL. Furthermore, restriction of CO₂ assimilation under drought stress accelerates PSI over-reduction under FL, which increases the susceptibility of PSI to photoinhibition. Therefore, drought stress strongly affects the photosynthetic dark and light reactions under FL. These findings provide insight into photosynthetic physiology under drought and FL.

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612

613 **Figure legends**

614 **Figure 1.** The effects of drought stress on photosynthetic induction after transition from low
615 to high light. Time course of net CO₂ assimilation (A_N ; A and B), stomatal conductance (g_s ; C
616 and D) and mesophyll conductance (g_m ; E and F) after transition from 100 to 1500 μmol
617 photons $\text{m}^{-2} \text{s}^{-1}$. Before this measurement, leaves were adapted to low irradiance (100 μmol
618 photons $\text{m}^{-2} \text{s}^{-1}$) for 5 min. A_N , g_s and g_m were normalized against the maximum values after
619 photosynthetic induction for 30 min. Values are means $\pm$ SE of five independent experiments
620 ($n = 5$).

621
622 **Figure 2.** Effects of drought stress on the time course of intercellular CO₂ concentration (C_i ;
623 A) and chloroplast CO₂ concentration (C_c ; B), and the relationship between C_c and A_N after
624 transition from low to high light. Red and grey dotted lines represent the values of C_{trans} (the
625 chloroplast CO₂ concentration at which the limitation to A_N transitioned from RuBP
626 carboxylation to RuBP regeneration) in CK and drought stressed plants, respectively. The
627 experimental design was the same as described in Figure 1. Values are means $\pm$ SE of five
628 independent experiments ($n = 5$).

629
630 **Figure 3.** Effects of drought stress on the quantitative analysis of stomatal limitation (L_s),
631 mesophyll conductance limitation (L_{mc}) and biochemical limitation (L_b) after transition from
632 low to high light. The experimental design was the same as described in Figure 1. Values are
633 means $\pm$ SE of five independent experiments ($n = 5$).

634
635 **Figure 4.** Effects of drought stress on photosystem I parameter under fluctuating light
636 alternating between 59 μmol photons $\text{m}^{-2} \text{s}^{-1}$ (2 min) and 1455 μmol photons $\text{m}^{-2} \text{s}^{-1}$ (1 min).
637 $Y(I)$, the quantum yield of PSI photochemistry; $Y(ND)$, the quantum yield of PSI
638 non-photochemical energy dissipation due to donor side limitation; $Y(NA)$, the quantum yield
639 of PSI non-photochemical energy dissipation due to acceptor side limitation. Values are
640 means $\pm$ SE of five independent experiments ($n = 5$).

641

642

643 **Figure 5.** Effects of drought stress on photosystem II parameter under fluctuating light
644 alternating between 59 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (2 min) and 1455 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (1 min).
645 Y(II), the quantum yield of PSII photochemistry; NPQ, non-photochemical quenching in PSII;
646 Y(NO), the quantum yield of non-regulatory energy dissipation in PSII. Values are means $\pm$
647 SE of five independent experiments (n = 5).

648

649 **Figure 6.** Effects of drought stress on photosynthetic electron transport rate under fluctuating
650 light alternating between 59 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (2 min) and 1455 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (1
651 min). rETRI, the relative electron transport rate through PSI; rETRII, the relative electron
652 transport rate through PSII; Relative CEF/LEF, the relative cyclic and linear electron flow
653 ratio. Values are means $\pm$ SE of five independent experiments (n = 5).

654

655 **Figure 7.** Effects of drought stress on PSI photoinhibition after fluctuating light treatment. (A)
656 The extent of PSI photoinhibition was measured by the decrease in Pm; (B) The relationship
657 between PSI over-reduction within the first 30 s after transition from low to high light
658 [average Y(NA)_{30s}] and PSI photoinhibition; (C) The relationship between the maximum CO₂
659 assimilation rate during photosynthetic induction (A_{max}) and PSI photoinhibition. Values are
660 means $\pm$ SE of five independent experiments (n = 5). Asterisk indicates a significant different
661 between the CK-plants and drought-stressed plants.

662

663 **Figure 8.** Effects of drought stress on values of rETR, Y(ND) and Y(NA) 1455 $\mu\text{mol photons}$
664 $\text{m}^{-2} \text{ s}^{-1}$ after fluctuating light treatment. Values are means $\pm$ SE of five independent
665 experiments (n = 5). Asterisk indicates a significant different between the CK-plants and
666 drought-stressed plants.

667

Figure 1. The effects of drought stress on photosynthetic induction after transition from low to high light. Time course of net CO₂ assimilation (A_N ; A and B), stomatal conductance (g_s ; C and D) and mesophyll conductance (g_m ; E and F) after transition from 100 to 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Before this measurement, leaves were adapted to low irradiance (100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 5 min. A_N , g_s and g_m were normalized against the maximum values after photosynthetic induction for 30 min. Values are means $\pm$ SE of five independent experiments ($n = 5$).

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Figure 3. Effects of drought stress on the quantitative analysis of stomatal limitation (L_s), mesophyll conductance limitation (L_{mc}) and biochemical limitation (L_b) after transition from low to high light. The experimental design was the same as described in Figure 1. Values are means $\pm$ SE of five independent experiments ($n = 5$).

Figure 4. Effects of drought stress on photosystem I parameter under fluctuating light alternating between $59 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (2 min) and $1455 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (1 min). Y(I), the quantum yield of PSI photochemistry; Y(ND), the quantum yield of PSI non-photochemical energy dissipation due to donor side limitation; Y(NA), the quantum yield of PSI non-photochemical energy dissipation due to acceptor side limitation. Values are means $\pm$ SE of five independent experiments (n = 5).

Figure 5. Effects of drought stress on photosystem II parameter under fluctuating light alternating between $59 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (2 min) and $1455 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (1 min). Y(II), the quantum yield of PSII photochemistry; NPQ, non-photochemical quenching in PSII; Y(NO), the quantum yield of non-regulatory energy dissipation in PSII. Values are means $\pm$ SE of five independent experiments (n = 5).

Figure 6. Effects of drought stress on photosynthetic electron transport rate under fluctuating light alternating between $59 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (2 min) and $1455 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (1 min). rETRI, the relative electron transport rate through PSI; rETRII, the relative electron transport rate through PSII; Relative CEF/LEF, the relative cyclic and linear electron flow ratio. Values are means $\pm$ SE of five independent experiments ($n = 5$).

Figure 7. Effects of drought stress on PSI photoinhibition after fluctuating light treatment. (A) The extent of PSI photoinhibition was measured by the decrease in Pm; (B) The relationship between PSI over-reduction within the first 30 s after transition from low to high light [average Y(NA)_{30s}] and PSI photoinhibition; (C) The relationship between the maximum CO₂ assimilation rate during photosynthetic induction (A_{max}) and PSI photoinhibition. Values are means $\pm$ SE of five independent experiments (n = 5). Asterisk indicates a significant different between the CK-plants and drought-stressed plants.

Figure 8. Effects of drought stress on values of rETR, Y(ND) and Y(NA) 1455 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ after fluctuating light treatment. Values are means $\pm$ SE of five independent experiments ($n = 5$). Asterisk indicates a significant different between the CK-plants and drought-stressed plants.