



### Full Length Article

## Photosynthetic Parameters and Biomass Recovery of a Rice Chromosome Segment Substitution Line with a ‘KDML 105’ Genetic Background under Drought Conditions

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### Abstract

Drought is a major abiotic stress that decreases plant growth and yield. Quantitative trait loci for drought tolerance (DT-QTLs) in rice were identified and chromosome segment substitution lines (CSSLs) containing diverse DT-QTL segments in a ‘KDML105’ genetic background were generated. In this study, CSSL1 and CSSL4 were evaluated and compared with their parental lines, ‘KDML105’ and DH212, under drought conditions, which were simulated by withholding water for 12 days at the booting stage. Plants were subsequently re-watered and their biomass was determined 60 days later. The CSSL4 and DH212 flag leaves tended to maintain relatively stable gas exchange parameters compared with ‘KDML105’ and CSSL1 during the early water-withholding period. Moreover, the chlorophyll *a* and *b* contents were the same between the drought-stressed CSSL4 plants and the non-stressed plants. Interestingly, the total carotenoid content of CSSL4 increased after the drought treatment, suggesting a role for carotenoids in the non-enzymatic defense mechanism that maintains the PSII efficiency under drought conditions. For both analyzed CSSLs, the total dry mass, shoot dry mass and root dry mass increased after re-watering, particularly for CSSL4, in which the biomass increased by 20.73%, with increases in the root biomass resulting in a lower shoot:root ratio. Thus, the CSSLs increased their biomass during the recovery period likely because of the DT-QTLs from DH212. Moreover, CSSL4 plants were able to maintain photosynthetic pigment contents, which contributed to the enhanced growth during the recovery period. Therefore, CSSL4 represents a valuable genetic resource for breeding new drought-tolerant rice cultivars. © 2019 Friends Science Publishers

**Keywords:** Chromosome segment substitution line (CSSL); Chlorophyll fluorescence; Drought stress; Leaf gas exchange; Biomass

### Introduction

Drought stress is one of the major abiotic stresses that decrease crop yield and especially affects lowland rice (*Oryza sativa* L.) varieties cultivated in insufficiently irrigated areas. In Thailand, the main rice-growing regions are in the northern and northeastern parts of the country, where ‘Khoa Dok Mali 105’ (‘KDML105’) rice is commonly grown. This popular aromatic Thai rice cultivar (*i.e.*, jasmine rice) is susceptible to drought conditions (Vanavichit *et al.*, 2018). Drought stress reportedly impairs various plant growth stages at the morphological and physiological levels (Landi *et al.*, 2017). The diverse effects of this abiotic stress on susceptible plants depend on the

growth phases and developmental processes that define critical periods in the rice lifecycle (Nix and Fitzpatrick, 1969). In rice, the limited availability of water adversely affects the germination potential, early seedling growth and survival rate (Jiang and Lafitte, 2007; Swain *et al.*, 2014). It also decreases plant height (Sokoto and Muhammad, 2014), the number of tillers (Bunnag and Pongthai, 2013) and biomass production (Manickavelu *et al.*, 2006). Rice yield losses are highest when plants are exposed to drought stress during the early reproductive stage (Singh *et al.*, 2011). Moreover, drought stress can influence photosynthetic processes in various ways (Chen *et al.*, 2011; Hussain *et al.*, 2017). For example, it decreases the photosynthetic rate because of the associated limited CO<sub>2</sub> diffusion through the

carboxylation site (Flexas *et al.*, 2007; Chen *et al.*, 2011). Drought conditions can also damage the electron transport system (Valladares and Pearcy, 1997; Hura *et al.*, 2007) and disrupt photosystem II (PSII) activities (Pieters and Souki, 2005), ultimately leading to photoinhibition.

The negative effects of drought stress may be mitigated by the development of drought-tolerant rice cultivars. The results of an analysis of quantitative trait loci (QTLs) for rice grain yield and other agronomic traits influenced by water deficit stress revealed genomic regions associated with drought tolerance on chromosome 1, 3, 4, 8 and 9 (Lanceras *et al.*, 2004). These putative regions cover a considerable portion of the rice chromosomes. Therefore, to improve drought-tolerant rice varieties, chromosome segment substitution lines (CSSLs) were developed to avoid the pleiotropic effects caused by linkage drags. The CSSLs are useful genetic materials for breeding programs and may be relevant for identifying quantitative traits, functionally characterizing genes, and studying plant stress responses (Ali *et al.*, 2010; Wang *et al.*, 2018).

In this study, we compared the drought responses of two CSSLs and their parental lines, 'KDML105' and DH212, under drought conditions. Additionally, the biomass recovery of plants exposed to drought stress at the booting stage was assessed. Various photosynthetic parameters and pigment contents were analyzed to clarify the drought-tolerance mechanism. The information provided herein may be applied for selecting appropriate CSSLs for breeding of drought-tolerant rice cultivars.

## Materials and Methods

### Plant Materials

All experiments were conducted with two CSSLs (CSSL1 and CSSL4) developed by Kanjoo *et al.* (2011) and their parental lines, 'KDML105' and DH212. The DH212 rice line is a doubled haploid line generated from CT9993, which has a deep rooting system, and IR62266, which is a drought-tolerant rice line that undergoes active osmotic adjustment. Specific chromosomal segments from DH212 were introgressed into the 'KDML105' rice background (Siangliw *et al.*, 2007). The CSSLs were selected from the BC<sub>5</sub>F<sub>6</sub> population *via* marker-assisted selection.

### Growth Conditions

This study was conducted from August 2014 to January 2015 inside a greenhouse at the Tropical Vegetable Research Development Center at Kasetsart University, Kamphaeng Sean campus, in Nakhon Pathom, Thailand. The seeds of CSSLs and the parental lines ('KDML105' and DH212) were germinated for 7 days and then transplanted to plastic pots consisting of 5 kg loam soil comprising 27% sand, 48.67% silt, and 24.04% clay. After 25 and 60 days of growth, 115 mg kg<sup>-1</sup> compound fertilizer

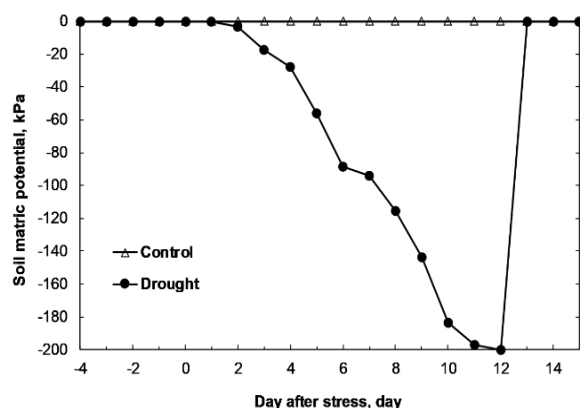
(NPK 15-15-15) was added to each pot. Rice plants were watered regularly and drought stress was imposed by withholding water when the flag leaf sheath was visibly swollen (booting stage). Drought stress was quantified based on the soil matric water potential ( $\Psi_{\text{soil}}$ ), which was measured throughout the study period with the Watermark soil moisture sensor with data logger (Spectrum Technologies Inc., U.S.A.) (Fig. 1). The drought treatment lasted for 12 days, which is how long it took for the soil matric potential to reach  $-200$  kPa. Rice leaves stop expanding when plants are grown at  $-50$  to  $-160$  kPa, which is a critical pressure potential range for rice. Moreover, when the soil water potential decreases to  $-200$  kPa or lower, rice leaves appear rolled and the percentage of dead leaves rapidly increases (Wopereis *et al.*, 1996). The microclimate inside the greenhouse was also recorded throughout the study period with the WatchDog 2475 plant growth station (Spectrum Technologies Inc., U.S.A.). The vapor pressure deficit (VPD) was maintained below 2.5 kPa to prevent it from affecting stomatal closure (Gollan *et al.*, 1985). The average photosynthetic photon flux density (PPFD) was  $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The mean daytime and nighttime temperatures were  $30.1^\circ\text{C}$  and  $24.3^\circ\text{C}$ , respectively. The average relative humidity during the day was 53.7% and at night, it was 80.2%.

### Photosynthetic Parameter Measurement

The light-saturated net photosynthetic rate ( $P_{\text{Nmax}}$ ), stomatal conductance ( $g_s$ ), intercellular CO<sub>2</sub> concentration ( $C_i$ ), transpiration rate (E), effective quantum yield of PSII photochemistry ( $\Phi_{\text{PSII}}$ ) and electron transport rate (ETR) were determined for the flag leaves on days 0, 3, 6, 9 and 12 after the treatment. All parameters were measured between 8:00 and 12:00 in the morning with the LI-6400 portable photosynthesis system (Licor Inc., Lincoln, N.E., U.S.A.) with the LI-6400-40 fluorometer chamber (Licor Inc.). The  $P_{\text{Nmax}}$  was determined under the following conditions: saturated light (as PPFD):  $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$  (with 10% blue light); air CO<sub>2</sub> concentration ( $C_a$ ):  $400 \mu\text{mol mol}^{-1}$ ; chamber block temperature:  $28^\circ\text{C}$ ; relative humidity: 70%–75%; and air VPD: 1.0–1.5 kPa (Utkhao and Yingjajaval, 2015).

Chlorophyll *a* (Chl *a*) fluorescence was determined based on the following parameters:  $\Phi_{\text{PSII}} [(F_m' - F_s)/F_m']$  (Genty *et al.*, 1989), where  $F_m'$  refers to the maximum fluorescence yield of a light-adapted leaf and  $F_s$  refers to the steady-state fluorescence yield under light; PPFD (at  $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ); and ETR ( $\Phi_{\text{PSII}} \times \text{PPFD} \times 0.5 \times 0.84$ ) (Björkman and Demming, 1987), where 0.5 represents the proportion of the excitation energy distributed to PSII and 0.84 represents the fraction of the leaf incident irradiance.

The maximum quantum yield of PSII photochemistry ( $F_v/F_m$ ) of flag leaves was determined at 4:30–5:30 (*i.e.*, predawn) with the Pocket PEA portable chlorophyll



**Fig. 1:** Soil matric potential under control and simulated drought (watering withheld) conditions in a pot experiment

fluorimeter (Hansatech Instruments, Norfolk, U.K.). Chlorophyll fluorescence was estimated based on  $F_0$  (minimum fluorescence yield of a dark-adapted leaf),  $F_m$  (maximum fluorescence yield of a dark-adapted leaf, and  $F_v$  [maximum variable fluorescence ( $F_v = F_m - F_0$ )] (Cregg *et al.*, 2004).

### Pigment Content Determination

The flag leaf was punctured to produce five leaf disks (6 mm diameter), which were then placed in 5 mL 80% acetone and incubated overnight in darkness. A 2 mL aliquot of the solution was collected to estimate the optical density at 470, 646, and 663 nm with a spectrophotometer. The Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total carotenoid contents were calculated with the equations developed by Wellburn (1994).

### Biomass Determination

Rice plants were re-watered after a 12-day drought treatment during the booting stage. They were then maintained under normal growth conditions for 60 days. The total dry mass, shoot dry mass, and root dry mass of each rice line grown under normal and drought conditions were determined. Plantlets were collected and dried in a hot air oven at 60°C for 7 days.

### Statistical Analysis

Experiments were completed according to a completely randomized design. The leaf gas exchange parameters, PSII efficiency and biomass were calculated based on the average of three replicates per treatment. Data were analyzed with the IBM S.P.S.S. Statistics 20 software. Differences in the mean values for the tested parameters under both conditions were estimated based on an analysis of variance and Duncan's multiple range test, with  $p = 0.05$  as the threshold for significance.

## Results

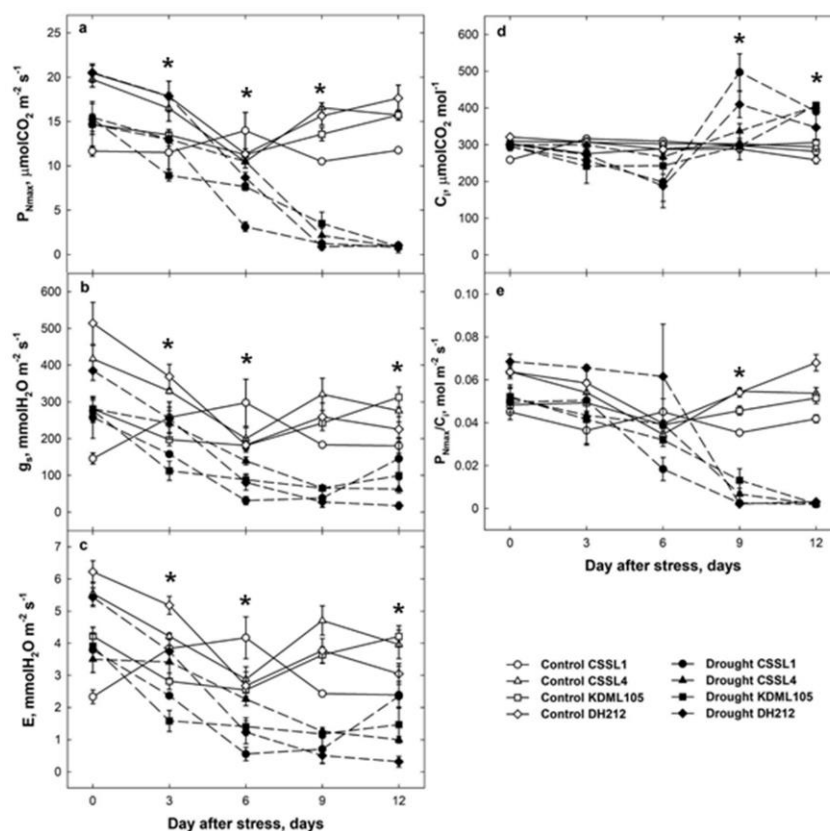
### CSSL4 Flag Leaves Maintained the Gas Exchange Traits Better than the CSSL1 Flag Leaves during the Early Stages of Drought Stress

The drought treatment during the booting stage adversely affected all photosynthetic parameters of the flag leaves of all analyzed rice lines. However, the CSSL4 flag leaves performed better than the CSSL1 flag leaves regarding the gas exchange traits. The  $P_{Nmax}$  of the non-stressed flag leaves was 11.7–20.5  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . After withholding water for 3 days ( $\Psi_{soil} = 17 \text{ kPa}$ ), the  $P_{Nmax}$  of 'KDML105' plants decreased by 33.9%. Additionally, the  $P_{Nmax}$  of 'KDML105' continued to decrease throughout the study period. The  $P_{Nmax}$  of CSSL1 decreased considerably after a 6-day exposure to drought stress ( $\Psi_{soil} = 88.5 \text{ kPa}$ ), resulting in the lowest  $P_{Nmax}$  among the tested lines. In contrast, the  $P_{Nmax}$  of CSSL4 was relatively stable after a 6-day drought treatment. After 9 days of drought conditions ( $\Psi_{soil} = 143.5 \text{ kPa}$ ), the  $P_{Nmax}$  of all lines decreased to less than 5  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . For all lines, the lowest  $P_{Nmax}$  was recorded on day 12 of the drought treatment ( $\Psi_{soil} = 200 \text{ kPa}$ ) (Fig. 2a). According to the  $g_s$ , which decreased after the plants were exposed to drought stress (Fig. 2b), the changes to the photosynthetic rate were due to stomatal closure.

Changes to  $g_s$  in response to drought stress revealed that the stomatal conductance of CSSL1, 'KDML105' and DH212 decreased immediately after initiating the drought treatment. In contrast, CSSL4 plants exhibited delayed stomatal closure ( $g_s$ , Fig. 2b) and maintained the transpiration rate ( $E$ , Fig. 2c).

During the first 6 days of the drought treatment, the  $C_i$  of the stressed flag leaves of all rice lines was similar to that of the flag leaves from non-stressed plants. After 9 days, the  $C_i$  of each line increased and the  $C_i$  of CSSL4 and 'KDML105' kept increasing until day 12 of the drought treatment. These observations indicated that the efficiency of the  $\text{CO}_2$  fixation decreased under drought conditions, and it was consistent with the decrease in  $P_{Nmax}/C_i$  under drought conditions (Fig. 2d and e). However, the  $C_i$  may have been over-estimated because of patchy stomatal closure (Brodribb, 1996).

Light reaction responses to drought stress were investigated by determining the  $\Phi_{PSII}$  and ETR (Fig. 3). During the first 6 days of drought stress, the ETR and  $\Phi_{PSII}$  of the drought-stressed plants were similar to those of the non-stressed plants. After 9 days of drought stress, the  $\Phi_{PSII}$  and ETR decreased in all lines. More severe effects were observed for CSSL1 and DH212. Additionally, the  $\Phi_{PSII}$  and ETR decreased the most in 'KDML105', with values that were 82.2% and 82.4% lower than the corresponding values for non-stressed plants, respectively (Fig. 3a and b). The  $\text{ETR}/P_{Nmax}$  represents the use of electron energy during the photosynthetic process. After 6 days of drought stress, the



**Fig. 2:** Effects of a 12-day drought treatment on the (a) light-saturated net photosynthetic rate ( $P_{Nmax}$ ), (b) stomatal conductance ( $g_s$ ), (c) transpiration rate ( $E$ ), (d) intercellular  $\text{CO}_2$  concentration ( $C_i$ ), and (e) instantaneous carboxylation efficiency ( $P_{Nmax}/C_i$ ) of control and drought-stressed rice CSSLs (CSSL1 and CSSL4) and parental lines ('KDML105' and DH212). Values are presented as the mean  $\pm$  SE ( $n = 3$ ). \*significant difference among lines under drought conditions at  $P < 0.05$

$ETR/P_{Nmax}$  increased only in CSSL1 and it continued to increase until day 12. This differed from the changes in the other three lines, in which the  $ETR/P_{Nmax}$  decreased at day 12 (Fig. 3c).

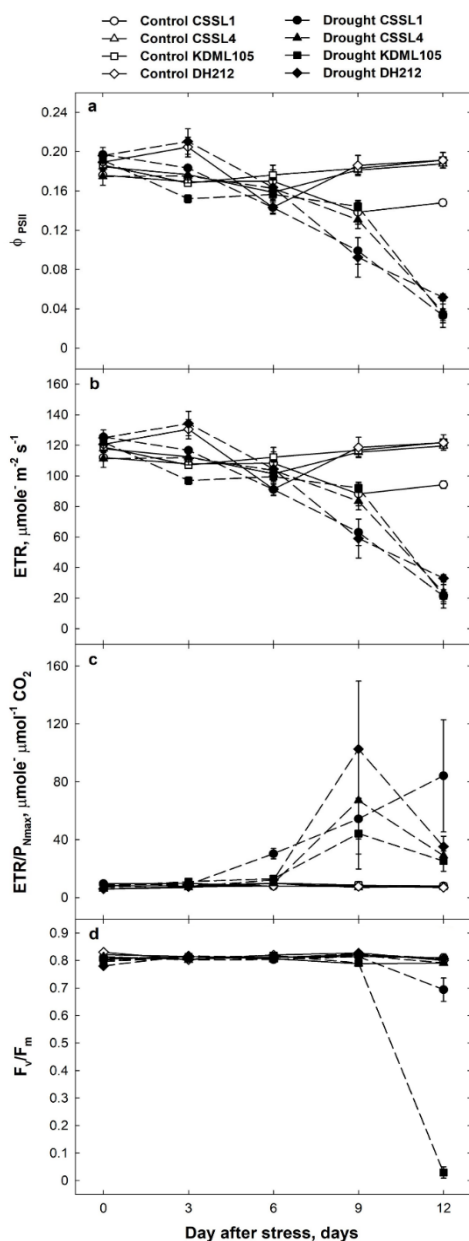
#### CSSL4 Maintained its PSII Efficiency under Drought Conditions

An analysis of PSII efficiency ( $F_v/F_m$ ) revealed that the  $F_v/F_m$  was maintained at 0.8 for the flag leaves of all rice lines after withholding water for 6 days. This  $F_v/F_m$  was the same as that of the non-stressed plants. After 9 days of drought stress, the  $F_v/F_m$  of 'KDML105' started to decrease, resulting in significant differences in the  $F_v/F_m$  among the examined lines. Moreover, after a 12-day drought treatment, the  $F_v/F_m$  decreased considerably more in 'KDML105' than in the other lines. The PSII efficiency of CSSL4 and DH212 was significantly different from that of 'KDML105'. Interestingly, the  $F_v/F_m$  of CSSL4 decreased only slightly, whereas the  $F_v/F_m$  of CSSL1 and 'KDML105' decreased to 0.69 and almost 0, respectively, after 12 days of drought stress (Fig. 3d).

#### CSSL4 Maintained the Photosynthetic Pigment Contents under Drought Conditions Better than the other Lines

The Chl *a*, Chl *b* and carotenoid contents of the flag leaves were determined after a 12-day drought treatment. The Chl *a* and Chl *b* contents of CSSL1, 'KDML105' and DH212 decreased significantly after plants were exposed to drought conditions. This was in contrast to CSSL4, which maintained Chl *a* and Chl *b* contents at normal levels (Fig. 4a and b). The chlorophyll contents decreased the most in the drought-stressed 'KDML105' leaves, with Chl *a* and *b* levels that were 91.98% and 87.30% lower, respectively, than the corresponding levels in non-stressed plants (Fig. 4a and b).

Interestingly, the total carotenoid content changes due to drought stress varied among lines. The total carotenoid content decreased significantly only in 'KDML105', whereas CSSL1 and DH212 tended to maintain the normal total carotenoid content. Moreover, the carotenoid content of CSSL4 increased after a 12-day drought treatment (Fig. 4c).



**Fig. 3:** Effects of a 12-day drought treatment on the (a) quantum efficiency of PSII ( $\Phi_{PSII}$ ), (b) electron transport rate (ETR), (c) ratio of apparent electron transport rate to CO<sub>2</sub> assimilation (ETR/ $P_{Nmax}$ ), and (d) maximum quantum efficiency of PSII ( $F_v/F_m$ ) of control and drought-stressed rice CSSLs (CSSL1 and CSSL4) and two parental lines ('KDML105' and DH212). Values are presented as the mean  $\pm$  SE ( $n = 3$ )

\*significant difference among lines under drought conditions at  $P < 0.05$

### CSSL4 Accumulated Biomass Better than the other Lines after Re-watering

The drought-treated rice plants were re-watered and grown for another 60 days. Significant decreases in shoot and root biomasses were observed for 'KDML105'. In contrast, the

root biomass of the CSSLs and DH212 increased during the post-drought recovery period (Fig. 5a and b). Moreover, shoot growth was observed in the CSSLs, but not in the parental lines, after watering was resumed (Fig. 5a) Plants were harvested to measure their biomass. Interestingly, the total dry mass increased in the CSSLs after the drought treatment ended (Fig. 5). Specifically, the total dry mass of CSSL1 and CSSL4 was 6.33% and 20.73% higher, respectively, than that of their parents (Fig. 5c). The total biomass of 'KDML105' decreased by 27.65%. Regarding the shoot:root ratio (Fig. 5d), it decreased significantly in response to drought stress only in DH212, suggesting these plants were able to divert some of their photosynthates toward root growth.

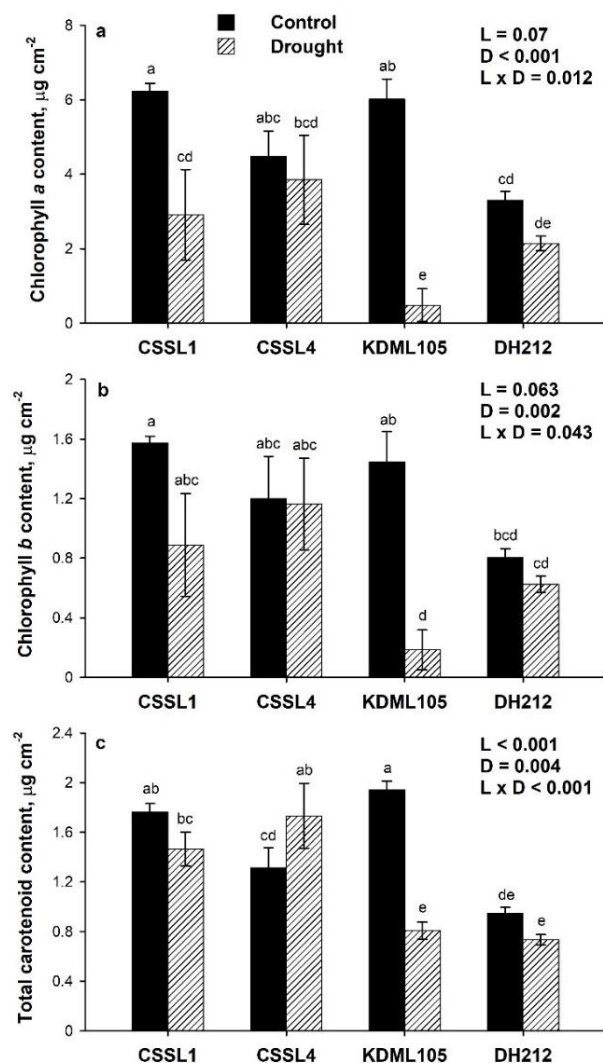
### Discussion

The  $P_{Nmax}$  of rice plants decreased when the  $g_s$  decreased in response to drought stress, whereas the  $\Phi_{PSII}$  and ETR, which represent the electron transport system, were maintained for up to 6 days after the drought treatment. These results implied that stomatal closure was the main factor limiting photosynthesis (Flexas *et al.*, 2013; Hosseinzadeh *et al.*, 2016). Stomatal closure also induced changes in E (Giuliani *et al.*, 2013; Sharma *et al.*, 2015). In contrast to the  $P_{Nmax}$ , the ETR was maintained at normal levels even if the  $g_s$  reached its minimum level. This pattern was also reported for cowpea exposed to severe drought stress. The smaller decrease in the ETR than in the  $P_{Nmax}$  reflected an increase in photorespiration (Singh and Reddy, 2011). The increase in the ETR/ $P_{Nmax}$  (Fig. 3c) also indicated that the electrons in the system were used by other metabolic processes rather than photosynthesis under stress conditions (Zivcak *et al.*, 2014). Furthermore, for all tested lines, severe drought stress decreased the instantaneous CO<sub>2</sub> fixation ( $P_{Nmax}/C_i$ ) because of stomatal closure (Fig. 2e), which ultimately led to an obvious decrease in photosynthesis (Osório *et al.*, 2011).

A substantial decrease in  $F_v/F_m$  was observed on day 12 of the drought treatment, whereas photosynthesis was inhibited at an earlier time-point (Fig. 3d). Similar phenomena were observed for karst species (Liu *et al.*, 2011), *Vitis amurensis* Rupr. (Wang *et al.*, 2012) and *Carapa surinamensis* (Oliveira and Marengo, 2019). These studies confirmed that PSII efficiency is obviously affected by severe drought conditions, but it does not represent the capacity of all photosynthetic processes (Kositsup *et al.*, 2013).

The data revealed the ability of CSSL4 to maintain the PSII efficiency under drought conditions, whereas the  $F_v/F_m$  of 'KDML105' was almost 0 after a 12-day drought treatment (Fig. 3). Moreover, the photosynthetic pigment contents of drought-stressed CSSL4 plants were similar to normally grown plants, which was in contrast to the significant decrease in the photosynthetic pigment contents of drought-treated 'KDML105' plants (Fig. 4). These results

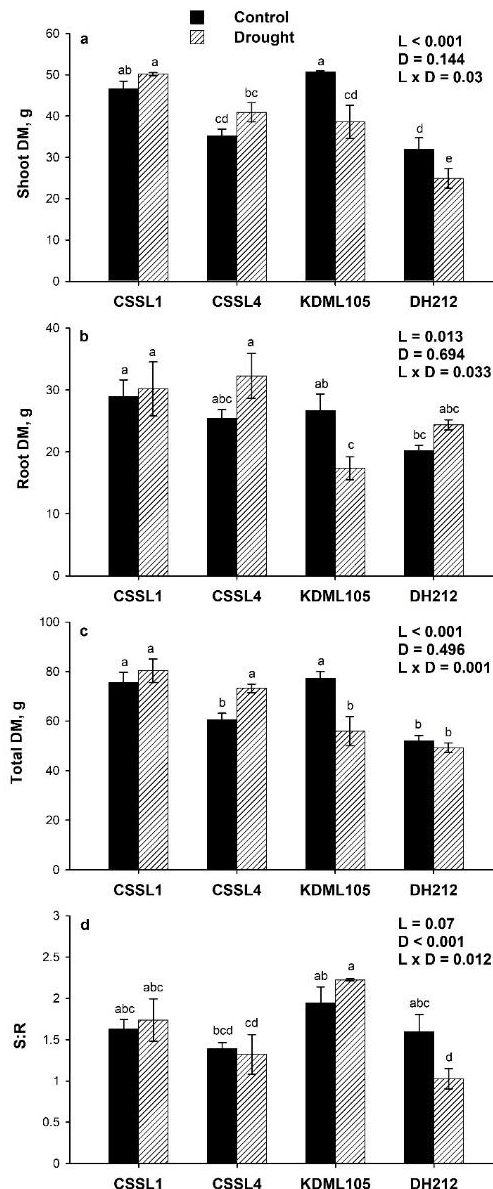




**Fig. 4:** Effects of a 12-day drought treatment on the (a) chlorophyll *a* content, (b) chlorophyll *b* content, and (c) total carotenoid content of control and drought-stressed rice CSSLs (CSSL1 and CSSL4) and parental lines ('KDML105' and DH212). Values are presented as the mean  $\pm$  SE ( $n = 3$ ). The  $p$ -values for line (L), drought treatment (D), and line  $\times$  drought treatment ( $L \times D$ ) are provided in the upper right part of each panel. Different letters above bars indicate significant differences (Duncan's multiple range test,  $P < 0.05$ )

reflected the damage to the light-harvesting complex and the limited photosynthetic activities resulting from the exposure of plants to excess energy, leading to decreased PSII efficiency (Ghotbi-Ravandi *et al.*, 2014).

Carotenoids are involved in a non-enzymatic antioxidant system that protects plants from photochemical damages by singlet oxygen ( $^1\text{O}_2$ ) (Caulet *et al.*, 2014; Ghahfarokhi *et al.*, 2015). Carotenoids can alleviate the toxicity of  $^1\text{O}_2$  species by quenching the excited triplet state of chlorophyll and minimize the loss of PSII activity (Kabiri



**Fig. 5:** Rice plant biomasses after re-watering for 60 days, including (a) shoot dry mass, (b) root dry mass, (c) total dry mass, and (d) shoot: root dry mass ratio (S:R) in control and drought-stressed rice CSSLs (CSSL1 and CSSL4) and parental lines ('KDML105' and DH212). Values are presented as the mean  $\pm$  SE ( $n = 3$ ). The  $P$ -values for line (L), drought treatment (D), and line  $\times$  drought treatment ( $L \times D$ ) are provided in the upper right part of each panel

Different letters above bars indicate significant differences (Duncan's multiple range test,  $P < 0.05$ )

*et al.*, 2014; Habibi and Ajory, 2015). The accumulation of carotenoids in CSSL4 may help to maintain the PSII efficiency of this line under drought conditions.

The biomass recovery after re-watering varied between the CSSLs, especially CSSL4, and their parents. A significant decrease in the shoot biomass was observed for

both parents, whereas re-watering slightly enhanced the shoot growth of both CSSLs (Fig. 5a). The total biomass of the drought-stressed CSSL4 plants was significantly greater than that of CSSL4 plants grown under normal conditions. In contrast, a significant decrease in biomass was detected for 'KDML105' rice (Fig. 5). These observations suggested the drought-tolerance (DT)-QTLs from DH212 in the 'KDML105' genetic background were responsible for the enhanced growth of CSSL4. Re-watering also increased root growth in CSSLs and DH212 (Fig. 5a, b). Enhanced root growth is one of the most desirable drought-tolerance traits (Naz *et al.*, 2014; Richard *et al.*, 2015) because the associated penetration of a deeper soil profile can help plants take up water (Farooq *et al.*, 2017). These results suggested that the drought-tolerance characteristics of the CSSLs were from DH212, and not 'KDML105'. Combined with 'KDML105' genes, the DT-QTLs from DH212 induced significant increases in the total biomass of CSSL4 during the re-watering recovery period.

## Conclusion

Overall, drought-treated CSSL4 plants were able to maintain photosynthetic activities and PSII efficiency as well as increase biomass production. These findings may be useful for clarifying the mechanisms underlying the adaptation to and recovery from drought stress in CSSL4. As mentioned earlier, the analyzed CSSLs contain a putative drought-tolerance genomic region from DH212. This region inserted in the CSSL4 genome may influence the photosynthetic process and growth recovery in response to drought stress. This line may be a useful genetic resource for breeders interested in improving drought tolerance of rice cultivars.

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