

Photosynthetic Performance in Improved 'KDML105' Rice (*Oryza sativa* L.) Lines Containing Drought and Salt Tolerance Genes under Drought and Salt Stress

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ABSTRACT

Rice (*Oryza sativa* L.) 'KDML105' is the most popular aromatic rice originating in Thailand. This cultivar is highly susceptible to abiotic stresses, especially drought and salt stress during the seedling stage. The objective of this study was to investigate the photosynthetic performance in response to drought and salt stress of four improved

breeding lines, specifically CSSL94 and CSSL103 (containing drought-tolerant quantitative trait loci: DT-QTLs) and RGD1 and RGD4 (containing a salt-tolerance gene, *SKC1*), with 'KDML105' (susceptible) and DH103 (tolerant to drought and salt stress) as the controls. Rice seedlings were grown for 21 days in hydroponic solutions and then exposed to salt stress (150 mM NaCl) or drought stress (20% PEG6000) for 10 days. The results indicated that when subjected to drought and salt stress, all rice lines/cultivar exhibited significant

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reductions in net photosynthesis rate (P_N), stomatal conductance (g_s), transpiration rate (E), the maximal quantum yield of PSII photochemistry (F_v/F_m), the effective quantum yield of PSII photochemistry (F_v'/F_m'), photosynthetic pigments, and SPAD readings, whereas water use efficiency (WUE) and non-photochemical quenching (NPQ) increased. Compared with 'KDML105', CSSL94, and CSSL103 were more tolerant to both drought and salinity, showing less reduction in all photosynthetic parameters. For RGD1 and RGD4, it was confirmed that these lines had a higher level of salt tolerance than 'KDML105' based on better photosynthetic performance under salt stress, demonstrating that these lines were also more tolerant to drought stress.

Keywords: Chlorophyll fluorescence, drought stress, photosynthetic performance, rice, salt stress

INTRODUCTION

Rice is the most important staple food crop for almost all countries in Asia including Thailand, and the demand for rice is ever-increasing with the growing global population (Lin et al., 2018). Rice production is associated with a whole range of influencing factors such as variety, terrain, climate, fertilization, and soil properties (Ran et al., 2018). Moreover, various types of stress, biotic, and abiotic, adversely affected the survival, growth, and performance of rice (Anami et al., 2020). Among abiotic stress factors, drought

and salinity dramatically reduced the growth and yield of rice, particularly in rainfed ecosystems (Sekar & Pal, 2012). In Thailand, approximately 76% of the total of 9.2 million ha of rice-growing areas are under rainfed conditions. The majority of the rainfed lowland areas are found in the northeast (4.8 million ha) and the north (1.4 million ha) regions. These areas are drought-prone, and rice yield is low and fluctuates between 1.5 and 2.2 t ha⁻¹ (Jongdee et al., 2006). Recently, it was estimated that 55-68% yield loss occurred in two study areas in Northeast Thailand in the drought year of 2012 (Polthane et al., 2014). Northeast Thailand is also affected by saline soils, where 18% of the agricultural land (1.84 million ha) is affected by salts to varying degrees (Arunin & Pongwichian, 2015). A study involving a total of 51 farmers' fields in this region from 2002-2005 revealed an average of 20% reduction in rice yield even under slightly saline soils having the electrical conductivity of saturated soil extract (EC_e) between 3 and 5 dS m⁻¹ (Clermont-Dauphin et al., 2010).

Photosynthesis is the fundamental metabolic process determining crop growth and yield, but it is strongly inhibited by drought and salinity (Pandey & Shukla, 2015). In rice, a strong positive relationship between photosynthesis capacity and vegetative growth (Murata, 1981) as well as grain yield (Ambavaram et al., 2014) has been reported. Therefore, more tolerant rice genotypes are better able to maintain a more efficient photosynthetic capacity

under drought (Ambavaram et al., 2014) and salt stress (Li et al., 2017). Drought and salinity negatively influence photosynthetic performance through decreased stomatal conductance, transpiration rate, photosystem II efficacy, and photosynthetic pigments (Hungsaprug et al., 2019; Netondo et al., 2004). The photosynthetic rate is also adversely affected by limited CO₂ diffusion into the chloroplast, via limitations on stomatal conductance and the mesophyll transport of CO₂, as well as alterations in leaf photochemistry and carbon metabolism (Chaves et al., 2009). These effects vary according to the intensity, duration, and frequency of stress as well as the plant species (Rötzer et al., 2012). Photosystem II is the most susceptible component of the photosynthetic machinery that carries the brunt of abiotic stress (Gururani et al., 2015). Thus, abiotic stress usually leads to photoinhibition through damage to PSII reaction centers (Nishiyama et al., 2006).

‘Khao Dawk Mali 105’ or ‘KDML105’, known in the world market as ‘Thai Hom Mali’ or ‘Thai jasmine rice’, has a unique fragrance and good eating/cooking quality. ‘KDML105’ is mostly cultivated in northeast Thailand; hence, its growth and yield are under threats from both drought and salinity stress. ‘KDML105’ is susceptible to abiotic stresses, especially drought (Kanjoo et al., 2012) and salinity (Kanawapee et al., 2012). Efforts have been made to improve rice cultivars to obtain better resistance to salinity and water deficit stresses through molecular marker-assisted

backcross breeding (MABC). Several rice lines were developed as donors for drought-tolerance quantitative trait loci (DT-QTL), including DH103 and DH212 (Lanceras et al., 2004). Subsequently, through MABC, 90 chromosome segment substitution lines (CSSLs) with a ‘KDML105’ genetic background (CSSL no. 1–90) were developed, which carried DT-QTL from chromosomes 1, 3, 4 and 9 of DH212 and chromosome 8 of DH103 (Kanjoo, 2011). These CSSLs lines were evaluated for agronomic traits in a field condition under drought stress compared with ‘KDML105’, and it was found that these improved lines showed higher grain yield than ‘KDML105’ (Kanjoo et al., 2012). Two selected CSSL lines (CSSL1 and CSSL4), which carried DT-QTL from chromosome 1 of DH212, were evaluated for physiological responses under drought stress. Only CSSL4 revealed superior tolerance over ‘KDML105’ through its ability to maintain stable net photosynthesis rates (Hungsaprug et al., 2019). However, detailed physiological drought responses of other CSSL lines introgressed with DT-QTL from other chromosomes have not been evaluated.

To improve the salt tolerance of rice, the *SKCI* gene controlling salt tolerance in rice has been identified (Gregorio et al., 2002). The *SKCI* gene functions in controlling ion homeostasis by increasing K⁺ content and lowering Na⁺/K⁺ in rice shoots, thereby increasing yields under salinity stress (Ren et al., 2005; Thomson et al., 2010). Introgression lines with the ‘KDML105’ rice

genetic background carrying QTL and *SKCI* for salt tolerance were derived by crossing 'KDML105' with salt-tolerant FL496 (IR66946-3R-196-1-1) or FL530 (IR66496-3R-230-1-1). These backcross introgression lines of 'KDML105' were proven to have higher salt tolerance than 'KDML105', as indicated by lower shoot Na^+/K^+ and higher yield under salt stress (Punyaawee et al., 2016; Vanavichit et al., 2018). However, detailed studies on the effects of drought and salt stress on physiological responses of these improved lines have not been reported. These studies are expected to provide an insight into how physiological and biochemical mechanisms are differentially enhanced by the introgressed genes, which enable higher tolerance of the improved lines compared with the sensitive parent 'KDML105'.

Photosynthetic performance under stress is one of the most important processes determining stress tolerance ability, growth, and yield. In this study, we evaluated the photosynthetic performance under drought and salt stress of four improved lines with a 'KDML105' genetic background introgressed with DT-QTL (from chromosome 8 of DH103) or the *SKCI* gene. Various photosynthetic parameters, including net photosynthesis rate, chlorophyll fluorescence, and leaf greenness index, were analyzed to clarify the drought- and salt-tolerance mechanisms. The information provided in this study may be applied for selecting appropriate

introgression lines for breeding rice cultivars with tolerance to both drought and salt stresses.

MATERIALS AND METHODS

Plant Materials and Growth Condition

Six (6) rice lines/cultivars were used in this study, including CSSL94 and CSSL103, which are CSSLs with a 'KDML105' genetic background carrying DT-QTL on chromosome 8; RGD14376 (RGD1) and RGD12150-B-21-MS3 (RGD4), which are backcross introgression lines of 'KDML105' carrying a salt tolerance gene (*SKCI*) on chromosome 1; 'KDML105', the original parental cultivar, which is susceptible to drought and salt stress; and the double-haploid line, DH103, which is known to be tolerant to both drought and salt stress (Lanceras et al., 2004; Nounjan et al., 2016). Seeds were kindly provided by the Rice Gene Discovery Unit, BIOTEC, Thailand. The experiment was conducted in the greenhouse at the Crop Station, Faculty of Agriculture, Khon Kaen University, Thailand. Seeds were soaked in 3% calcium hypochlorite for 30 min and then washed several times with distilled water. Seeds were then germinated in distilled water on filter paper in the dark at room temperature. After 3 days, germinated seeds were transferred to a plastic net placed over 15 L of Yoshida nutrient solution (Yoshida et al., 1976) in a plastic container (50 × 60 × 11 cm). In each container, 20

germinated seeds per line/cultivar were randomly arranged and allowed to grow for 21 days, during which the nutrient solution was replaced every 4 days. On day 21, the plants were separated into 3 treatment groups i.e. control, drought stress, and salt stress. For drought stress, plants were treated with Yoshida solution containing 20% polyethylene glycol 6000 (PEG6000). For salt stress, plants were fed with Yoshida solution containing 150 mM NaCl. For the control, plants continued to be fed with the Yoshida solution. The physiological traits were determined at 10 days after drought and salt treatments. The chosen concentration of PEG6000 had an osmotic potential of -0.7 MPa, corresponding to moderate water stress (Osmolovskaya et al., 2018). The 150 mM NaCl solution had the same osmotic potential as the 20% PEG6000 solution; therefore, it imposes a similar level of osmotic stress. The NaCl solution had an approximate EC value of 14.8 dS m⁻¹.

Determination of Leaf Gas Exchange Parameters

Net photosynthesis rate (P_N), stomatal conductance (g_s), transpiration rate (E) and water use efficiency (WUE) were evaluated on the youngest fully expanded leaf of a randomly selected rice plant in each replicate by using a portable infrared gas exchange analyzer (LI-6400, LI-COR, NE, USA) from 9.00 to 11.00 a.m. The net photosynthesis rate was determined under the following conditions: 30°C, CO₂

concentration at 400 ppm, and 30–70% relative humidity. The photosynthesis photon flux density was maintained at 1500 $\mu\text{mol (photon) m}^{-2} \text{s}^{-1}$.

Measurement of Chlorophyll Fluorescence Parameters

As soon as the above gas exchange measurement was completed, the photosynthetic efficiency of PSII was measured on the same leaf using a chlorophyll fluorometer (Hansatech, Kings Lynn, UK). The maximal quantum yield of PSII efficiency (F_v/F_m) was calculated from minimal fluorescence in the dark-adapted state (F_0) and maximal fluorescence in the dark-adapted state (F_m), as described by Schreiber (2004). Steady-state fluorescence in the light-adapted state (F_s) and maximal fluorescence in the light-adapted state (F_m') were determined in the light condition. The effective quantum yield of PSII efficiency (F_v'/F_m') was calculated as described by Schreiber (2004). Non-photochemical quenching (NPQ) was computed as described by Bilger and Bjorkman (1990).

Measurement of Leaf Greenness

Leaf greenness of the same youngest fully expanded leaf used for gas exchange and chlorophyll fluorescence measurements was measured at 3 positions along the leaf blade from 9.00 to 11.00 a.m. using a SPAD-502 chlorophyll meter (Minolta Corp., Ramsey, New Jersey, U.S.A.).

Determination of Photosynthetic Pigments

Chlorophyll and carotenoid contents were determined by a modified method, as outlined by Arnon (1949) and Lichtenthaler (1987), using 0.1 g of leaf tissue. The absorbance of the pigment extract was estimated at 470, 645, and 663 nm by using a spectrophotometer (Hanon, Model i3, China) with 80% acetone as a blank. The content of pigments was expressed as mg g⁻¹ tissue fresh weight. The pigment contents were calculated using the following equations:

$$\text{Total chlorophyll (TC)} = [20.2 A_{645} + 8.02 A_{663}] \times [V/(1000 \times W)]$$

$$\text{Chlorophyll } a \text{ (Chl } a) = [12.7 A_{663} - 2.69 A_{645}] \times [V/(1000 \times W)]$$

$$\text{Chlorophyll } b \text{ (Chl } b) = [22.9 A_{645} - 4.68 A_{663}] \times [V/(1000 \times W)]$$

$$\text{Carotenoid (CA)} = [1000 A_{470} - (1.82 \times \text{Chl } a) - (85.02 \times \text{Chl } b)]$$

where: V = total volume of extract (ml); W = leaf weight (g)

Experimental Design and Statistical Analysis

The experiment was laid out as 3 × 6 factorials with a completely randomized design with four replications. The data were subjected to analysis of variance. Duncan's multiple range test (DMRT) was used to compare means at a significant difference of $p \leq 0.05$ and paired-samples t-test was performed at a significant difference of $p \leq 0.05$ and 0.01. Pearson's correlation was calculated for the relationships among

photosynthesis traits under drought and salt stress conditions. All calculations and data analyses were performed using SPSS for Windows version 19. In addition, cluster analysis based on fourteen physiological parameters was analyzed using PC-ORD version 5.10.

RESULTS

Changes in Leaf Gas Exchange Parameters

Drought generally imposed stronger inhibitory effects on leaf gas exchange parameters than salt stress. Under non-stressed control conditions, RGD4 showed the highest P_N (25.87 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), whereas DH103 showed the lowest P_N (19.26 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). All improved lines (CSSL94, CSSL103, RGD1, and RGD4) and 'KDML105' showed significantly higher P_N than DH103 (Figure 1A). In contrast, under drought stress, DH103 showed the highest P_N (5.43 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; 71.8% reduction when compared with control plants), whereas 'KDML105' showed the lowest P_N (3.97 $\text{CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; 83.05% reduction when compared with control plants) (Figure 1A). For the improved lines, P_N under drought stress decreased by 79.7, 79.8, 78.8, and 79.2% for CSSL94, CSSL103, RGD1, and RGD4, respectively, when compared with control plants. Under salt stress, RGD4 showed the highest P_N (12.82 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), while 'KDML105' showed the lowest P_N (7.89 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (Figure 1A). This suggested that RGD4 had higher salt tolerance compared to CSSL94, CSSL103, RGD1, and DH103. A similar trend was

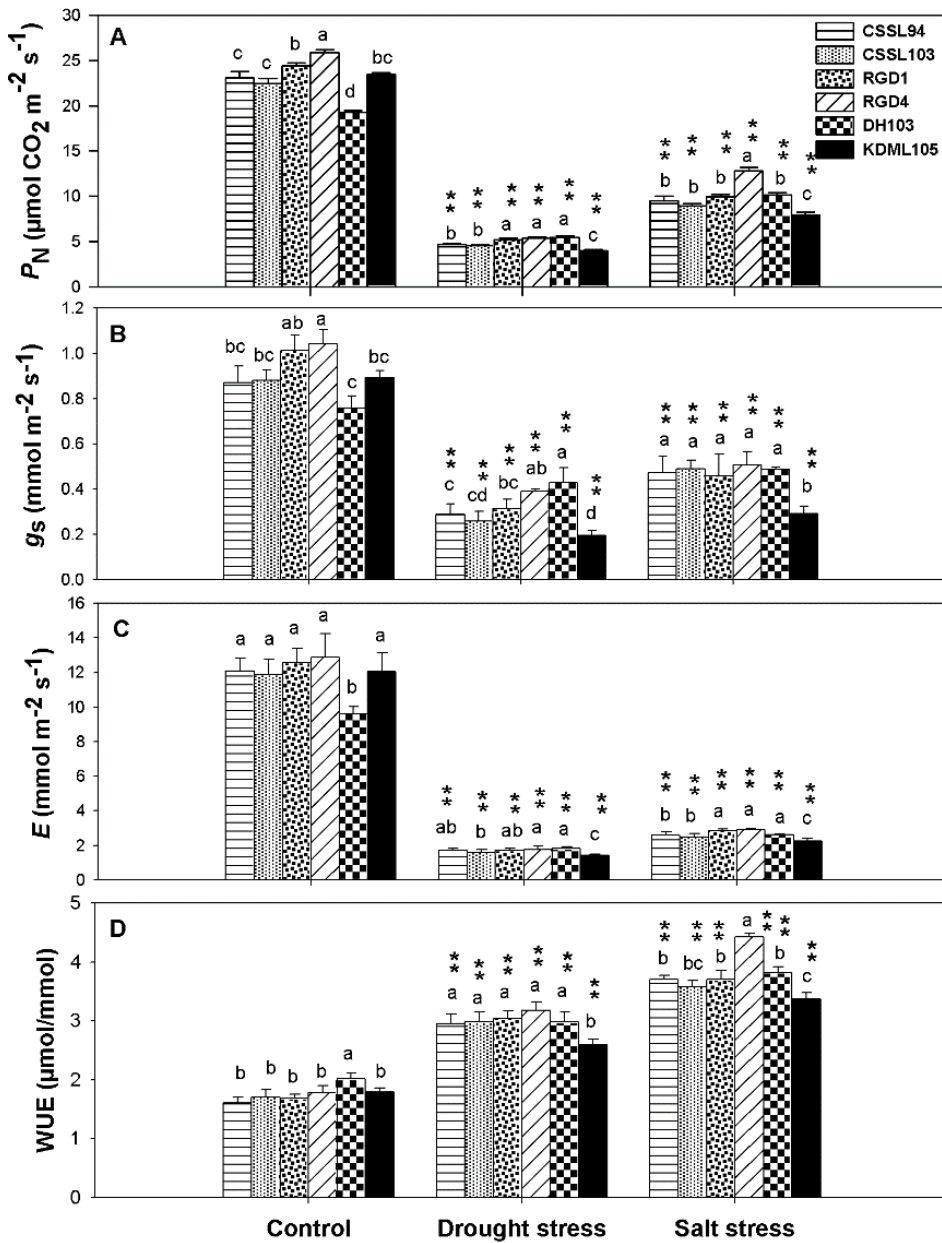


Figure 1. (A) Net photosynthesis rate (P_N), (B) stomatal conductance (g_s), (C) transpiration rate (E), and (D) water use efficiency (WUE) under control, salt, and drought stress for 10 days. Bars with different letters within each treatment group are significantly different according to DMRT ($p \leq 0.05$). The asterisk (*, **) indicates a significant difference ($p \leq 0.05$ and $p \leq 0.01$) in the mean values between control and stress (drought or salt stress) conditions of each line/cultivar

found in g_s (Figure 1B) and E (Figure 1C). Conversely, drought, and salt stress led to a large and significant increase in WUE in all rice lines/cultivars, particularly in RGD4 under salt stress (Figure 1D). Analysis of

variance showed that P_N , g_s , E , and WUE were significantly affected by treatments (T), lines/cultivar (C), and treatments and lines/cultivar interaction ($T \times C$), as shown in Table 1.

Table 1

Analysis of variance of studied parameters in six lines/cultivars of rice seedlings treated with drought treatment (20% PEG6000) and salt treatment (150 mM NaCl)

Parameter	Lines/Cultivars (C) (<i>df</i> = 5)	Treatment (T) (<i>df</i> = 2)	Lines/Cultivars × Treatment (C×T) (<i>df</i> = 10)
P_N	16.99**	2133.05**	7.97**
g_s	0.05**	2.53**	0.03**
E	2.56*	782.65**	2.51**
WUE	0.3*	24.70**	0.148*
F_0	128.39**	3376.042**	119.45**
F_m	221836.55**	77857.62**	13945.70**
F_v/F_m	0.0001**	0.03**	0.00002ns
F_v'/F_m'	0.12**	0.006**	0.01ns
NPQ	0.052**	0.055**	0.005**
SPAD	51.61**	24.23**	7.06**
TC	0.339**	1.666**	0.129**
Chl <i>a</i>	0.167**	0.637**	0.032**
Chl <i>b</i>	0.167**	0.637**	0.07**
CA	0.032**	0.268**	0.15**

Note. * and ** are means with significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively ns are means with no significant differences

Changes in Chlorophyll Fluorescence Parameters

Under drought stress, F_0 significantly increased in all lines/cultivar (except DH103) when compared to control plants. Of the lines/cultivar, ‘KDML105’ showed the highest F_0 whereas DH103 had the

lowest, and all four improved lines had intermediate values. Under salt stress, ‘KDML105’ also showed the highest F_0 , which was significantly higher than all other lines (Figure 2A). For F_m , only RGD1, RGD4, and ‘KDML105’ showed a significant reduction under drought stress;

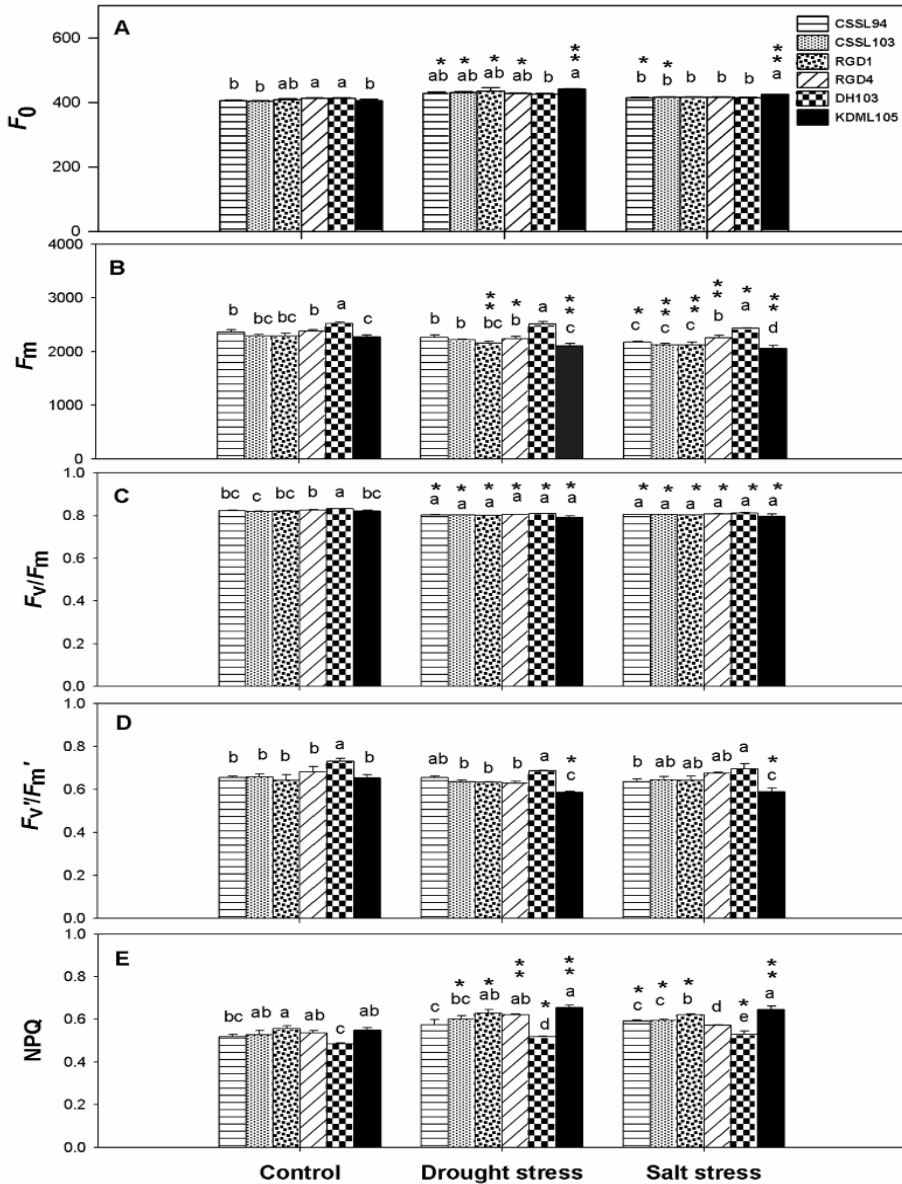


Figure 2. (A) Minimal fluorescence in the dark-adapted conditions (F_0), (B) maximal fluorescence in the dark-adapted conditions (F_m), (C) maximum quantum yield of PSII efficiency (F_v/F_m), (D) effective quantum yield of PSII efficiency (F_v'/F_m'), and (E) non-photochemical quenching (NPQ) under control, salt and drought stress for 10 days. Bars with different letters within each treatment group are significantly different according to DMRT ($p \leq 0.05$). The asterisk (*, **) indicates a significant difference ($p \leq 0.05$ and $p \leq 0.01$) in the mean values between control and stress (drought or salt stress) conditions of each line/cultivar

however, under salt stress, all six lines/cultivar showed a significant reduction in F_m (Figure 2B). Both drought and salt stress resulted in significant reductions ($p \leq 0.05$) in F_v/F_m of all rice lines/cultivar, with 'KDML105' showing the highest percent reduction (Figure 2C). However, for F_v'/F_m' , only 'KDML105' showed a significant reduction ($p \leq 0.05$) when exposed to both stress conditions (Figure 2D). Under both types of stress, compared with the controls, NPQ increased in all genotypes, with 'KDML105' showing the highest percent increase (Figure 2E). Furthermore, all improved lines (CSSL94, CSSL103, RGD1, and RGD4) showed higher F_v/F_m , F_v'/F_m' , and F_m , and lower F_0 and NPQ under drought and salt stress than 'KDML105' (Figures 2A-2D). Analysis of variance showed that differences among lines/cultivar (C), treatments (T) and treatments and lines/cultivar interaction ($T \times C$) were significant for F_0 , F_m , F_v/F_m , and F_v'/F_m' , except for F_v/F_m and F_v'/F_m' , which did not show a significant difference in ($T \times C$) (Table 1).

Changes in the Content of Photosynthetic Pigments

In general, drought had stronger effects on pigment concentrations than salt stress. Under drought stress, a significant reduction in TC was observed in CSSL94 (14.0%), CSSL103 (9.6%), RGD1 (19.5%), RGD4 (20.6%), and 'KDML105' (33.6%) when compared with control plants (Figure 3C). The tolerant line DH103 exhibited stable TC, Chl *a*, and Chl *b* contents under both types of stress (Figures 3A, 3B, and 3C). Under salt stress, only 'KDML105' showed

significant reductions in TC (19.6%), Chl *a* (12.8%), and Chl *b* (34.3%), where the only exception was CSSL103, which showed a significant reduction in Chl *b* content (18.3%). Under drought stress, all four improved introgression lines had significantly higher contents of TC, Chl *a*, and Chl *b* than 'KDML105'. Under salt stress, only RGD1 and RGD4 had significantly higher TC, Chl *a*, and Chl *b* contents than 'KDML105'. RGD4 had the highest pigment contents under salt stress (Figures 3A, 3B, and 3C). When subjected to drought stress, compared with the controls, carotenoid contents were significantly reduced in all lines/cultivar, with 'KDML105' being the most reduced (27.6%). However, under salt stress, carotenoid contents were significantly reduced in CSSL94, CSSL103, RGD1, and 'KDML105', but remained unchanged for RGD4 and DH103 (Figure 3D). RGD4 again had the highest carotenoid content under salt stress. For SPAD readings, under non-stressed conditions, DH103 showed the highest SPAD reading (37.08), whereas CSSL94, CSSL103, RGD1, RGD4 and 'KDML105' showed SPAD readings of 34.38, 33.68, 33.55, 35.20, and 34.40, respectively (Figure 3E). When subjected to drought and salt stress, leaf greenness of 'KDML105' was the most adversely affected among the six lines/cultivar. The percentage of reduction in SPAD readings under drought stress was 5.5, 1.6, 5.6, 7.0, and 15.3% in CSSL94, CSSL103, RGD1, RGD4, and 'KDML105', respectively, compared to the controls. Under salt stress,

only KDML105 showed a significant reduction ($p \leq 0.01$) in SPAD readings (decreased 9.85% compared to control), as shown in Figure 3E. Analysis of variance

showed that differences among lines/cultivar (C), treatments (T), and treatments and lines/cultivar interaction (T \times C) were significant for all pigment parameters (Table 1).

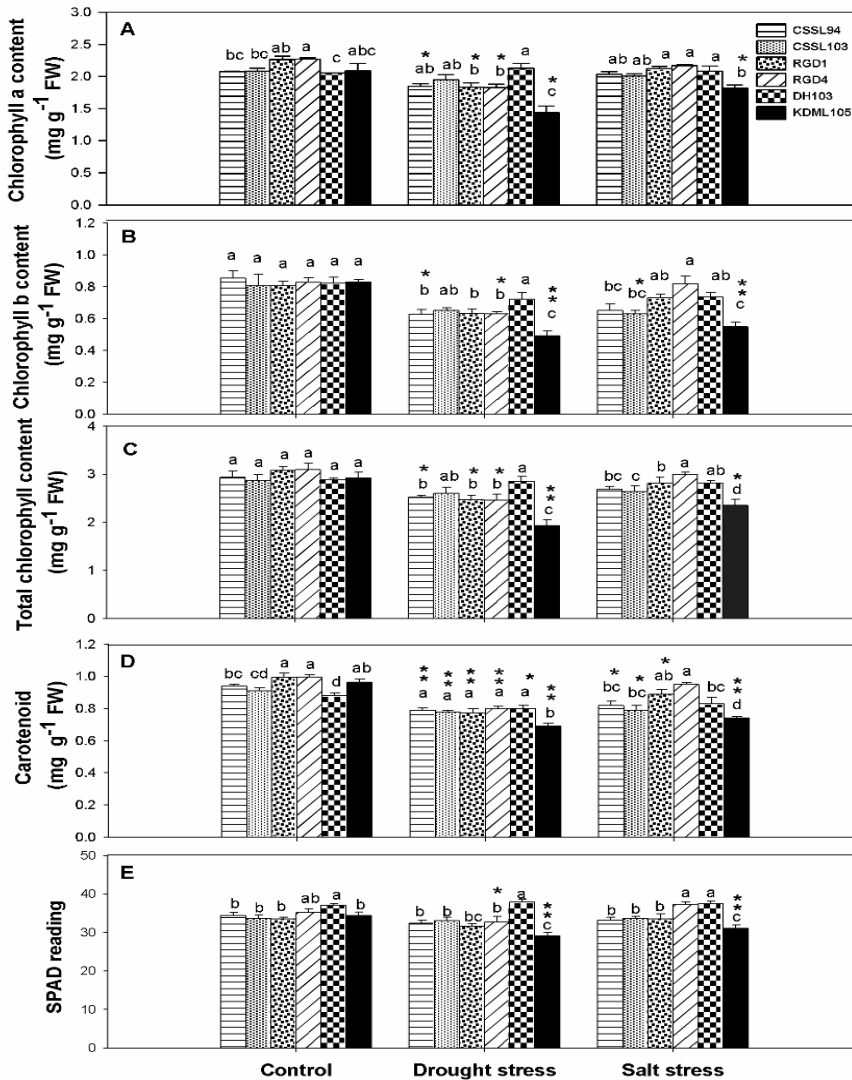


Figure 3. (A) Chlorophyll a, (B) chlorophyll b, (C) total chlorophyll, (D) carotenoid, and (E) SPAD readings under control, salt, and drought stress for 10 days. Bars with different letters within each treatment group are significantly different according to DMRT ($p \leq 0.05$). The asterisk (*, **) indicates a significant difference ($p \leq 0.05$ and $p \leq 0.01$) in the mean values between control and stress (drought or salt stress) conditions of each line/cultivar

The Correlation among Photosynthetic Traits

Relationships among fourteen photosynthetic traits (P_N , g_s , E , WUE, F_0 , F_m , F_v/F_m , F_v'/F_m' , NPQ, SPAD, TC, Chl *a*, Chl *b*, and CA) under stress conditions were determined using Pearson's correlation analysis. Under drought stress (Table 2), highly significant ($p \leq 0.01$) positive correlations were found between P_N and other leaf gas exchange parameters (g_s , E , and WUE). Furthermore, highly significant ($p \leq 0.01$) positive correlations were exhibited between P_N

and fluorescence parameters (F_m , F_v/F_m , and F_v'/F_m') and between P_N and pigment parameters (SPAD, TC, Chl *a*, and Chl *b*). In contrast, significant negative correlations were observed between P_N and F_0 ($p \leq 0.05$) as well as NPQ ($p \leq 0.01$). Similarly, under salt stress (Table 3), significant positive correlations were found between P_N and g_s , E , WUE, F_m , F_v/F_m , SPAD, TC, Chl *a*, Chl *b*, and CA (all traits; $p \leq 0.01$, except for F_v'/F_m' , and CA; $p \leq 0.05$). In contrast, negative correlations were observed between P_N and F_0 ($p \leq 0.01$) as well as NPQ.

Table 2

Pearson's correlation coefficients (*r* values) among physiological parameters in rice seedling exposed to drought stress (20% PEG6000)

Parameter	P_N	g_s	E	WUE	F_0	F_m	F_v/F_m
P_N	1						
g_s	0.787**	1					
E	0.730**	0.687**	1				
WUE	0.715**	0.488*	0.534**	1			
F_0	-0.444*	-0.482*	-0.381	-0.645**	1		
F_m	0.541**	0.610**	0.536**	0.284	-0.422*	1	
F_v/F_m	0.521**	0.366	0.680**	0.534**	-0.423*	0.553**	1
F_v'/F_m'	0.542**	0.585**	0.764**	0.408*	-0.564**	0.602**	0.594**
NPQ	-0.458**	-0.565**	-0.569**	-0.245	0.509**	-0.840**	-0.600**
SPAD	0.571**	0.671**	0.567**	0.413*	-0.464*	0.743**	0.395
TC	0.620**	0.556**	0.505**	0.414*	-0.416*	0.617**	0.506*
Chl <i>a</i>	0.549**	0.527**	0.427*	0.368	-0.441*	0.557**	0.443*
Chl <i>b</i>	0.641**	0.461*	0.589**	0.424*	-0.196	0.602**	0.539**
CA	-0.172	0.018	-0.122	0.539**	0.056	0.111	-0.245

Table 2 (Continued)

Parameter	F_v'/F_m'	NPQ	SPAD	TC	Chl <i>a</i>	Chl <i>b</i>	CA
P_N							
g_s							
E							
WUE							
F_0							
F_m							
F_v/F_m							
F_v'/F_m'	1						
NPQ	-0.745**	1					
SPAD	0.686**	-0.758**	1				
TC	0.647**	-0.501*	0.555**	1			
Chl <i>a</i>	0.600**	-0.451*	0.518**	0.978**	1		
Chl <i>b</i>	0.583**	-0.495*	0.491*	0.733**	0.575**	1	
CA	-0.060	-0.134	-0.138	0.095	0.140	-0.085	1

Note. Each value indicates the Pearson's correlation coefficient of a pair of parameters. * and ** denote correlations that are significantly different at $p \leq 0.05$ and $p \leq 0.01$, respectively

Table 3

Pearson's correlation coefficients (*r* values) among physiological parameters in rice seedling exposed to salt stress (150 mM NaCl)

Parameter	P_N	g_s	E	WUE	F_0	F_m	F_v/F_m
P_N	1						
g_s	0.576**	1					
E	0.755**	0.639**	1				
WUE	0.828**	0.436*	0.539**	1			
F_0	-0.555**	-0.710**	-0.623**	-0.441*	1		
F_m	0.585**	0.473*	0.692**	0.652**	-0.642**	1	
F_v/F_m	0.448*	0.677**	0.532**	0.386	-0.746**	0.688**	1
F_v'/F_m'	0.306	0.493*	0.576**	0.250	-0.614**	0.651**	0.693**
NPQ	-0.383	-0.639**	-0.537**	-0.371	0.714**	-0.638**	-0.769**
SPAD	0.646**	0.601**	0.726**	0.519**	-0.687**	0.852**	0.659**
TC	0.677**	0.603**	0.646**	0.611**	-0.568**	0.627**	0.689**
Chl <i>a</i>	0.533**	0.568**	0.494*	0.463*	-0.492*	0.452*	0.743**
Chl <i>b</i>	0.627**	0.416*	0.619**	0.594**	-0.457*	0.645**	0.331
CA	0.443*	0.372	0.525**	0.495**	-0.418*	0.752**	0.576**

Table 3 (Continued)

Parameter	F_v'/F_m'	NPQ	SPAD	TC	Chl <i>a</i>	Chl <i>b</i>	CA
P_N							
g_s							
E							
WUE							
F_0							
F_m							
F_v/F_m							
F_v'/F_m'	1						
NPQ	-0.663**	1					
SPAD	0.466*	-0.651**	1				
TC	0.467*	-0.635**	0.596**	1			
Chl <i>a</i>	0.478*	-0.619**	0.401	0.902**	1		
Chl <i>b</i>	0.264	-0.406*	0.656**	0.750**	0.391	1	
CA	0.593**	-0.542**	0.558**	0.521**	0.450*	0.421*	1

Note. Each value indicates the Pearson's correlation coefficient of a pair of parameters. * and ** denote correlations that are significantly different at $p \leq 0.05$ and $p \leq 0.01$, respectively

Cluster Analysis among Lines/Cultivars under Drought and Salt Stress

All six rice lines/cultivar were classified into three groups as indicated by the PC-ORD program at 75% similarity based on fourteen photosynthetic traits. Under drought stress, the dendrogram divided the rice into three groups. 'KDML105' and DH103 each formed a group individually, whereas all four improved lines were clustered into one group (Figure 4A). Under salt stress, the six rice lines/cultivar were also divided into three groups: the first group included DH103 and RGD4, the second group included 'KDML105', and the third group included CSSL94, CSSL103, and RGD1 (Figure 4B).

DISCUSSION

The ability of plants to tolerate, survive, and grow in adverse environments depends directly on the efficiency of photosynthesis. The major components limiting photosynthesis under drought and salt stress include a reduction in chlorophyll content, decrease in photochemical efficiency of PSII, limitation of CO₂ diffusion due to stomatal closure, and reduction in the activity of photosynthetic enzymes (Ashraf & Harris, 2013; Pandey & Shukla, 2015; Sudhir & Murthy, 2004). In this study, photosynthesis-related traits, including leaf greenness, photosynthetic pigments, PSII photochemical efficiency, and

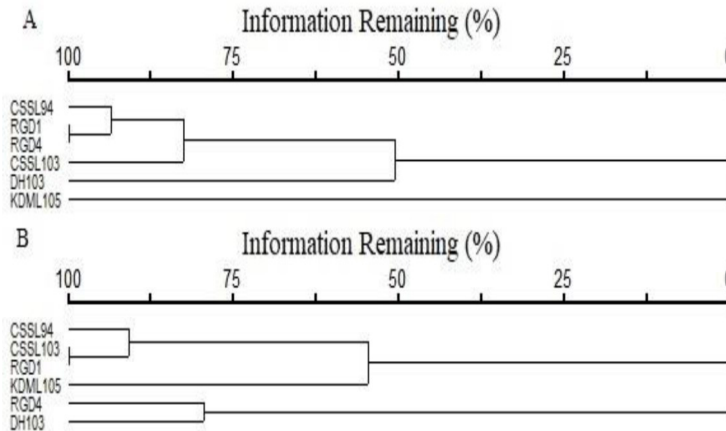


Figure 4. Dendrogram showing similarity among improved lines of rice ‘KDML105’ (CSSL94, CSSL103, RGD1, and RGD4), ‘KDML105’ and DH103 based on leaf photosynthetic parameters under (A) drought stress (20% PEG6000) and (B) salt stress (150 mM NaCl) at the seedling stage

stomatal conductance, had strong positive correlations with the net CO₂ fixation rate under stress conditions (Tables 2 and 3). This indicates the strong influences of pigment contents, energy transfer efficiency in the photosystem, and stomatal limitation on photosynthetic performance (Moradi & Ismail, 2007).

The leaf greenness values (SPAD reading) and photosynthetic pigments are indicators that can be related to photosynthetic performance. As indicated in this study, P_N was highly significantly correlated with chlorophyll contents and SPAD readings (Tables 2 and 3). A decrease in chlorophyll content under salt stress is a commonly reported phenomenon in various studies because of the adverse effects of sodium ions on the stability of the chloroplast membrane (Ashraf & Bhatti, 2000; Pongprayoon et al., 2019). A drought

stress-induced reduction in photosynthetic pigments occurred in conjunction with damage to chloroplast structure and the photosynthetic apparatus, consequently inhibiting net CO₂ assimilation (Kalefetoğlu Macar & Ekmekçi, 2009; Li et al., 2006; Wang et al., 2018). Photosynthetic pigments and SPAD readings were used as indicators of abiotic stress tolerance, where less reduction in pigment contents or SPAD readings under stress indicated a higher level of plant stress tolerance (Jinwen et al., 2009; Munns et al., 2006). For rice, under both drought (Kumari et al., 2019) and salt stress (Lee et al., 2013), more sensitive varieties showed a greater reduction in chlorophyll content than the more tolerant varieties. In the present study, drought and salt stress decreased photosynthetic pigments and SPAD readings, and these parameters were closely related (Tables 2 and 3). Moreover,

all improved lines of 'KDML105', which carried DT-QTLs (CSSL94 and CSSL103) or *SKCI* gene (RGD1 and RGD4), had higher chlorophyll, carotenoids and SPAD readings than the sensitive parent 'KDML105' under both stress conditions. Co-expression network analysis of genes located in the DT-QTL that was introgressed into CSSL94 (Nounjan et al., 2018) revealed that the gene *Os08g41990* involved in chlorophyll biosynthetic processes might play a role in maintaining chlorophyll content under salt stress.

The effects of drought and salt stress on the leaf PSII photochemical efficiency were expressed in terms of F_0 , F_m , F_v/F_m , F_v'/F_m' , and NPQ (Moradi & Ismail, 2007; Li et al., 2006). It was previously reported that drought and salt stress reduced F_m , F_v/F_m , and F_v'/F_m' and increased F_0 and NPQ (Dongsansuk et al., 2013; Mishra & Panda, 2017). A reduction in F_m , F_v/F_m , and F_v'/F_m' indicates that the thylakoid membrane and chloroplast are damaged due to photoinhibition leading to a decrease in P_N (Murata et al., 2007; Ranjbarfordoei et al., 2006). Similar results were found in this study, where drought and salt stress lowered the leaf PSII photochemical efficiency in rice seedlings of all lines/cultivar. Chlorophyll fluorescence analysis of 232 diverse rice genotypes clearly showed that less reduction in F_m , F_v/F_m , and F_v'/F_m' and less increase in F_0 and NPQ were associated with higher salt tolerance ability (Tsai et al., 2019). Under both drought and salt stress, all improved lines had significantly higher F_v'/F_m' than 'KDML105'. A reduction in

PSII photochemical efficiency was reported to be highly correlated with a reduction in net photosynthesis rate under drought (Iqbal et al., 2019), which is like the results in this study (Table 2). In contrast, F_0 and NPQ increased when plants were subjected to drought and salt treatments. An increase in F_0 was associated with photoinactivation and damage to PSII reaction centers, while NPQ indicated the photoprotective processes that removed excess excitation energy to prevent the formation of harmful free radicals (Murchie & Lawson, 2013). All improved lines (CSSL94, CSSL103, RGD1, and RGD4) showed higher F_v'/F_m' and lower NPQ than the parental line 'KDML105', indicating more efficient use of light energy in photochemical reaction and therefore less non-photochemical energy dissipation. Similar findings by Dongsansuk et al. (2013) reported that salt stress also caused a dramatic reduction in F_v'/F_m' , while NPQ was increased in 'KDML105'. In contrast, leaf PSII photochemical efficiency was unaffected by salt stress in Pokkali (salt-tolerant variety). Mishra and Panda (2017) also reported that the tolerant variety N22 showed higher F_m and F_v/F_m as well as lower F_0 and NPQ under drought stress than the susceptible variety IR64. In comparison with 'KDML105', all improved lines in this study also showed higher F_m and F_v/F_m as well as lower F_0 and NPQ under drought and salt stress.

A decline in photochemical efficiency under drought and salt stress which leads to reduced light-dependent NADPH and ATP synthesis, combined with a reduction in CO_2

uptake and diffusion due to stomatal closure, results in lower CO₂ fixation and Calvin cycle activities (Chaves et al., 2009). Hence, the P_N of rice in this study was dramatically reduced from an average (across genotypes) of 23.08 $\mu\text{mol (CO}_2\text{) m}^{-2} \text{s}^{-1}$ in the controls to 4.86 $\mu\text{mol (CO}_2\text{) m}^{-2} \text{s}^{-1}$ under drought and 9.87 $\mu\text{mol (CO}_2\text{) m}^{-2} \text{s}^{-1}$ under salt stress (Figure 1A; Tables 2 and 3). Based on the observation of Ramegowda et al. (2014), a similar level of reduction in P_N of rice at the vegetative growth stage was demonstrated, dropping from 21 $\mu\text{mol (CO}_2\text{) m}^{-2} \text{s}^{-1}$ under control conditions to approximately 6 $\mu\text{mol (CO}_2\text{) m}^{-2} \text{s}^{-1}$ under drought stress. For salt stress, Nounjan et al. (2018) found that the P_N of 'KDML105' declined from 24 $\mu\text{mol (CO}_2\text{) m}^{-2} \text{s}^{-1}$ to 4 $\mu\text{mol (CO}_2\text{) m}^{-2} \text{s}^{-1}$ when subjected to salt stress at 16.50 dS m⁻¹ for 9 days. Under slightly lower stress levels in this study (14.8 dS m⁻¹ for 10 days), P_N of 'KDML105' decreased from 23.45 to 7.90 $\mu\text{mol (CO}_2\text{) m}^{-2} \text{s}^{-1}$ (Figure 1A).

Simultaneous investigations on the effects of drought and salt stress on rice have rarely been reported, and this study demonstrated that drought imposed a stronger inhibitory effect than salt stress on photosynthesis in rice. Although the PEG and NaCl solutions had an equal osmotic potential of -0.7 MPa and were expected to induce similar levels of osmotic stress, a greater reduction in pigment contents and most photosynthetic parameters was recorded in the drought-induced PEG solutions (Figures 1A and 1B). This could be due to some intrinsic limitations of using PEG to simulate drought conditions,

such as the viscosity of the solution and absorption of PEG by roots, thus resulting in less water uptake, root damage, and leaf dysfunction (Osmolovskaya et al., 2018). Moreover, PEG solutions caused more rapid leaf rolling, compared to NaCl solution, leading to lower leaf surface area exposed to sunlight, hence lower photosynthesis rate. On the other hand, the negative effects of salt stress on photosynthesis were due to osmotic stress, which mainly induced stomatal closure as well as ion toxicity stress, which occurs as a result of excess Na⁺ accumulation in the chloroplasts (Munns & Tester, 2008). The direct influences of excess Na⁺ on photosynthesis were associated with the disruption of the proton motive force necessary for ATP production as well as its interference with CO₂ fixing enzymes (van Zelm et al., 2020).

In this study, all improved lines of 'KDML105', namely CSSL94, CSSL103, RGD1, and RGD4, showed greater values in P_N and all related gas exchange parameters (g_s , E , and WUE) than the parental cultivar 'KDML105' under both drought and salt stress. In the case of CSSL94, similar results were demonstrated by Nounjan et al. (2016), where CSSL94 had higher P_N under salinity stress compared to 'KDML105'. Co-expression network analysis of genes located in the DT-QTL introgressed into CSSL94 (Nounjan et al., 2018) revealed that the gene *Os08g41990* involved in chlorophyll biosynthetic process might play a role in maintaining chlorophyll content under salt stress. Consequently, this may lead to the higher photosynthetic

performance of CSSL94. However, photosynthesis of this line under drought stress has not been observed. In a study using a related near-isogenic line from the same CSSL population, Chutimanukul et al. (2018) reported that rice line CSSL16 (improved 'KDML105' introgressed with DT-QTL segments from chromosome 1 of DH212) showed a much lower reduction in photosynthesis under salt stress than 'KDML105'. From the co-expression analysis study, ten hub genes on the DT-QTL were found, of which six functioned in chloroplasts. One of these genes, *PsbSI*, which encodes the CP22 protein located in PSII, was proven to play a protective role through its activity in non-photochemical quenching under salt stress (Chutimanukul et al., 2018). The *SKC1* gene, also known as *OsHKT1;5* in rice, is responsible for the regulation of Na⁺ in the shoot and is more highly expressed in rice genotypes with higher salt tolerance associated with good maintenance of various physiological status, including higher photosynthetic capacity (Ueda et al., 2013). Transfer of the *HKT1;5* genes from maize into tobacco resulted in transgenic tobacco with enhanced salt tolerance (Jiang et al., 2018). The crucial role of this gene in enhancing salt tolerance was confirmed in the present study. The introgression lines (RGD1 and RGD4) carrying this gene, which was transferred from a salt-tolerant donor into the genetic background of 'KDML105', showed higher salt tolerance, as indicated by a significantly higher P_N and other

related gas exchange parameters of these lines compared to 'KDML105' under salt treatments (Figure 1). Moreover, the photosynthetic performance of these lines under drought stress was also superior to 'KDML105'. This is the first report on physiological studies, particularly in relation to photosynthesis, of these lines under salt and drought stress.

The results in this study clearly showed that introgression of DT-QTLs into the drought-sensitive 'KDML105' not only conferred drought tolerance ability to the improved lines (CSSL94 and CSSL103) but also enhanced salt resistance. Similar work by Basu et al. (2017) revealed two drought-tolerant breeding lines with superior drought and salinity tolerance based on several physiological parameters, including photosynthesis. Moreover, introgression lines (RGD1 and RGD4) of 'KDML105' carrying *SKC1* gene were more tolerant not only to salt stress but also to drought stress, as indicated by enhanced photosynthetic performance. As suggested by Nounjan et al. (2016), photosynthesis is an important target trait for the selection of breeding lines tolerant to drought and salt stress, and improving crop yield relies largely on photosynthetic performance (Zhu et al., 2010). Thus, these improved lines with high photosynthetic capacity are potentially useful as genetic resources for developing improved Thai jasmine rice lines for multiple stress tolerance via gene pyramiding.

CONCLUSION

Thai jasmine rice 'KDML105' is sensitive to drought and salinity. This study highlighted a search for improved rice breeding lines with a 'KDML105' genetic background that better tolerate drought and salinity stress based on photosynthesis-related characters, including better maintenance of pigments, PSII photochemical efficiency, and net photosynthesis rate. The improved photosynthetic performance under drought and salt stress of CSSL94, CSSL103, RGD1, and RGD4 indicated that these lines have the potential to be used in the rice breeding program and targeted for improvement of multiple stress tolerance.

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