



Full Length Article

Physiological Mechanisms of the Seedling Stage Salt Tolerance of Near Isogenic Rice Lines with the ‘KDML105’ Genetic Background

Noppawitchayaphong Khruelasan¹, Meechai Siangliw², Theerayut Toojinda², Apichat Imyim³, Teerapong Buaboocha⁴ and Supachitra Chadchawan^{1*}

¹Center of Excellence in Environment and Plant Physiology, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

²Rice Gene Discovery Unit, National Center for Genetic Engineering and Biotechnology, Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom 73140, Thailand

³Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

⁴Molecular Crop Research Unit, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

*For correspondence: s_chadchawan@hotmail.com; Supachitra.C@chula.ac.th

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Abstract

The objective of this study was to characterize the salt-tolerance mechanism of rice chromosome segment substitution lines (CSSLs) with the ‘KDML105’ genetic background. These CSSLs differed regarding the length of their drought-tolerance quantitative trait locus segment from chromosome 1 (*DT-QTL1*) of the doubled haploid donor, DH212. Twenty-nine CSSLs were screened for salt-tolerance based on the salt injury score and seedling growth traits under a severe salt stress condition (150 mM NaCl). CSSL11 and CSSL18 were identified to have the potential to be salt tolerant lines according to their appearance under 150 mM NaCl treatment, while CSSL22 had the highest shoot fresh weight and CSSL14 had the highest shoot dry weight, when compared among CSSLs. Therefore, four CSSLs (CSSL11, CSSL14, CSSL18, and CSSL22) were evaluated further regarding their shoot responses to moderate (75 mM NaCl) and severe (150 mM NaCl) salt stresses. All four CSSLs had high shoot fresh weights after an 8-day exposure to moderate stress, indicating the salt-tolerance of the above-ground tissues. Among the CSSLs, CSSL18 had the highest shoot fresh weight after a 12-day salt stress treatment. Moreover, the midday leaf water potential of CSSL18 was greater than that of the parental lines and similar to that of ‘Pokkali’ (salt-tolerant standard line). Furthermore, Na⁺/K⁺ ratios of CSSL18 and both parental lines did not show the significant difference, suggesting that *DT-QTL1* segment of DH212 does not contain the ion homeostasis trait. In conclusion, the salt-tolerance mechanism of CSSL18 due to *DT-QTL1* from DH212 is associated with osmotic adjustments rather than ion homeostasis. Finally, CSSL18 may be suitable for future attempts at identifying the salt-tolerance gene(s) and breeding new salt-tolerant rice varieties. © 2020 Friends Science Publishers

Keywords: CSSL; Ion balance; *Oryza sativa*; Osmotic adjustment; Salt-tolerance mechanism

Introduction

Salt stress is the most serious environmental stress limiting crop growth and productivity worldwide (Negrão *et al.* 2011). Salt tolerance in rice is a complex trait regulated by many genes and is strongly influenced by environmental conditions (Farooq *et al.* 2017; Lekklar *et al.* 2019; Saini *et al.* 2019). Proteome analysis in rice revealed changes in proteins involving in energy metabolism, photosynthesis, nitrogen assimilation, amino acid metabolism, and stress signalling pathways (Hussain *et al.* 2018; Fruk *et al.* 2020). Quantitative trait loci (QTLs) for salt tolerance in rice have recently been revealed and the previously identified QTL

can be dissected at a nucleotide scale by GWAS and transcriptome approaches (Jaiswal *et al.* 2019).

Near isogenic lines (NILs) of rice have been developed from chromosome segment substitution lines (CSSLs) carrying a drought-tolerance quantitative trait locus on chromosome 1 (*DT-QTL1*) between markers RM1003 and RM3362 (Kanjoo 2012). These NILs were derived from a cross between ‘KDML105’ and a doubled haploid line, DH212, as a donor. Line DH212 was developed from a cross between the drought-tolerant CT9993 and IR62266 rice lines, which have been used as a source of drought-resistance genes in rice breeding programs (Lanceras *et al.* 2004). Kanjoo *et al.* (2011) demonstrated that some NILs

performed well under salt stress condition. Therefore, they proposed that drought- and salinity-tolerance genes were located in the same genomic regions.

Rice is the most salt sensitive cereal crop (Hoang *et al.* 2016). It is more susceptible to salt stress during the seedling stage than during the tillering stage (Sahi *et al.* 2006). Salt-tolerance mechanisms involve various pathways related to metabolic and morphological adaptations (Gupta and Huang 2014; for review Reddy *et al.* 2017). High salinity induces osmotic and ionic stresses in plants. The osmotic stress is mainly caused by water deficits in plant tissues during the early phase of salt stress, whereas the ionic stress may be due to the accumulation of Na⁺ and Cl⁻ and the disturbance of the Na⁺/K⁺ ratio in plant cells, which occur during the later phase of salt stress (Munns and Tester 2008; Horie *et al.* 2012). Therefore, there are two main mechanisms for salt tolerance in rice; ion exclusion and osmotic tolerance (Munns and Tester 2008). Later, it was further classified into ion exclusion, tissue tolerance and osmotic tolerance (Roy *et al.* 2014). Ion exclusion depends on the control of Na⁺ and Cl⁻ transport in roots to prevent the accumulation of these ions in leaves. The tolerance at the tissue level involves sequestration of Na⁺ in the vacuole, the accumulation of solutes and induction of reactive oxygen species scavenging enzymes. The responses at the tissue levels will lead to the osmotic tolerance, which refers to the abilities to tolerate drought effects due to salt stress and to maintain stomatal conductance and leaf expansion.

The objective of this study was to identify a salt-tolerant CSSL population and determine whether the underlying mechanism involves an osmotic adjustment or the regulation of the Na⁺ concentration. This research omitted to investigate tissue tolerance as osmotic adjustment is inseparable from the trait of tissue tolerance (Munns *et al.* 2016). The phenotypes of 29 CSSLs under salt stress conditions were compared with those of the parental lines ('KDML105' and DH212). The most promising CSSL was then investigated regarding its salt-tolerance mechanism.

Materials and Methods

Plant materials and salt stress treatment

Rice (*Oryza sativa* L.) cultivars 'Khao Dawk Mali 105' ('KDML105') and 'Pokkali', rice lines IR29 and DH212, and 29 CSSLs were kindly provided by the Rice Gene Discovery Laboratory (RGD), Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom, Thailand. The CSSL populations included the DH212 chromosome 1 segment containing putative drought-tolerance genes between markers RM1003 and RM3362 (*DT-QTL1*) as well as the 'KDML105' genetic background (Kanjoo *et al.* 2011) (Fig. 1).

To screen for salt stress-tolerant lines, rice seeds were germinated for 5 days in 15-mL plastic cups filled with distilled water, after which the seedlings were transferred to

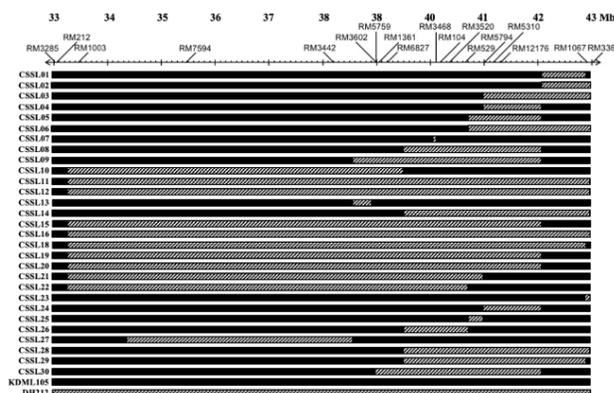


Fig. 1: The corresponding regions between markers RM1003 and RM3362 on rice chromosome 1 of DH212 and a CSSL population are represented by striped boxes. The SSR markers in the designated regions are indicated at the top

plastic trays containing clay soil, as described by Kanjoo (2012). The seedlings were grown under natural conditions from February to March 2013 in a greenhouse at RGD, Nakhon Pathom, Thailand. During the growth period, the plants were treated with Bangsai nutrient solution (Bangsai Agricultural Center, Thailand), which contained 50 g/L MgSO₄, 80 g/L KNO₃, 12.5 g/L NH₄H₂PO₄, 8.5 g/L KH₂PO₄, 0.4 g/L Mn-EDTA, 0.8 g/L micronutrients, 100 g/L Ca(NO₃)₂, and 3 g/L Fe-EDTA. Twenty days after germination, rice seedlings were treated with 150 mM NaCl. Seedlings that were not treated with NaCl were used as controls.

To elucidate the salt-tolerance mechanisms, selected salt-tolerant CSSLs were analyzed under natural conditions in March 2014 in an experiment conducted at the Faculty of Science, Chulalongkorn University, Bangkok, Thailand. The experiment was performed in a triplicate randomized complete block design with 8 genotypes factorially combined with three salt treatments. Specifically, 21-day-old seedlings were treated with 75 mM NaCl (moderate salt stress) and 150 mM NaCl (severe salt stress), after which the salt-induced physiological changes were evaluated. Seedlings grown under natural conditions with no salt treatment (0 mM NaCl) were used as controls.

Screening for salt-tolerant lines based on appearance

The appearance of control and salt-treated (150 mM NaCl) rice seedlings was analyzed with a complete randomized design involving four replicates. The salt injury score (SIS) for seedlings was recorded at 5, 7, 9, 11, 13, 15, and 21 days after initiating the salt stress treatment (six plants per genotype) according to the standard evaluation system for salinity tolerance (Table 1) (Gregorio *et al.* 1997; IRRI 2002). The data underwent an analysis of variance, and the means were compared with Duncan's multiple range test.

Table 1: Salt injury scores based on the visible symptoms during the rice seedling stage (Gregorio *et al.* 1997)

Score	Observation	Tolerance
1	Normal growth; no leaf symptoms	Highly tolerant
3	Nearly normal growth; leaf tips or a few leaves are whitish and rolled	Tolerant
5	Growth severely retarded; most leaves are rolled and few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves are dry and some plants are dying	Sensitive
9	Almost all plants are dead or dying	Highly sensitive

Screening for salt-tolerant lines based on growth traits

The growth traits of control and salt-treated (150 mM NaCl) rice seedlings were evaluated with a complete randomized design involving four replicates. Specifically, the shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW) were measured at 0, 7, and 14 days after initiating the salt stress treatment. The data underwent an analysis of variance, and the means were compared with Duncan's multiple range test.

Determination of the salt stress responses of selected CSSLs

Selected CSSLs (CSSL11, CSSL14, CSSL18, and CSSL22) as well as the parental lines ('KDML105' and DH212) and the standard checks ['Pokkali' (salt-tolerant standard) and IR29 (salt-sensitive standard)] were analyzed as follows.

Growth test: The SFW was measured at 0, 4, 8, and 12 days after initiating the salt stress treatment.

Water potential analysis: To measure the leaf water potential (Ψ_w ; LWP), the youngest fully expanded leaves were analyzed at midday (11:00–13:00) with the Plant Water Status Console (model 3005) (Soil moisture Equipment Corp., CA, USA) at 0, 4, 8, and 12 days after initiating the salt stress treatment. The analyzed leaves were then dried in a hot-air oven at 60°C for 7 days for the subsequent analyses of sodium and potassium ion contents.

Sodium and potassium ion analysis: Dried leaf samples were weighed, after which 1 ± 0.5 mg was prepared for an elemental analysis according to a modified version of the dry ashing technique described by Isaac and Johnson (1998). Samples were added to porcelain crucibles (HKT, Germany) in a cool muffle furnace (Fisher Scientific, U.S.A.) and then heated at 500°C for 2 h. After the leaf samples were cooled, they were treated with 1.0 mL of 65% HNO₃ (RCI Labscan, Thailand) and dried on a hot plate at 100–120°C. The crucibles were returned to the muffle furnace and samples were heated at 500°C for 1 h. The crucibles were removed from the muffle furnace, after which the samples were treated with 1.0 mL of 37% HCl (RCI Labscan) and then passed through a nylon filter (0.45 µm pores) (Membrane Solutions, USA). The filtrates were collected in 10-mL volumetric flasks and then diluted with ultrapure Milli-Q water for a final volume of 10 mL. The

sodium and potassium ion contents were determined with an ICP-OES system (iCAP 6500 DUO, Thermo Scientific, U.S.A.), with Na⁺ detected at 589.5 nm and K⁺ detected at 766.4 and 769.8 nm.

Statistical analysis

In the screening experiment, the data underwent the analysis of variance and the means were compared with Duncan's multiple range test. For the comparison of salt responsive traits of selected CSSLs, the 8 × 3 factorial experiment (genotype × NaCl level) was performed. The data were analyzed by Least Square Mean (LS mean) and the means were compared with Tukey's HSD (honestly significant difference) test.

Results

Screening for salt-tolerant lines in CSSL populations according to appearance

The differences in the salt tolerance of CSSL populations and 'KDML105' rice were revealed by evaluating the appearance of seedlings treated with 150 mM NaCl and determining the SIS. Twenty-nine CSSL lines, the parental lines ('KDML105', DH212), as well as 'Pokkali' and IR29 were treated with 150 mM NaCl or 0 mM NaCl (control). Under the control condition, the SIS of all lines/cultivars was 1 throughout the experiment, implying that the growing conditions of the experiment were appropriate for normal growth.

After an 11-day salt stress treatment, all lines/cultivars had severe salt-induced symptoms, including chlorosis, leaf burning, and stunted growth. The injuries were greater for 'KDML105' and the salt-sensitive standard, IR29, (SIS = 7.4) than for DH212 and 'Pokkali', the latter of which had the lowest SIS (5.7), suggesting 'Pokkali' was the most salt-tolerant line. Among rice CSSL populations, CSSL18 and CSSL11 had the lowest SIS (6.2), which was lower than that of 'KDML105' (Fig. 2).

On the basis of the SIS, some CSSL genotypes (*e.g.*, CSSL11 and CSSL18) were identified as potential salt-tolerant rice lines. These CSSLs may be useful for studying the stress-tolerance mechanisms of rice. Growth traits, such as fresh and dry weights, are important factors for accurately identifying salt-tolerant lines.

Screening for salt-tolerant lines according to growth traits

In addition to screening rice seedlings based on appearance, their salt tolerance was also evaluated according to their growth traits. The shoot and root fresh and dry weights were measured separately at 0, 7, and 14 days after initiating the salt stress treatment, and were used to represent the inhibitory effects of salt stress on CSSL rice seedling growth (Fig. 3 and 4). 'Pokkali' rice had the highest RFW,

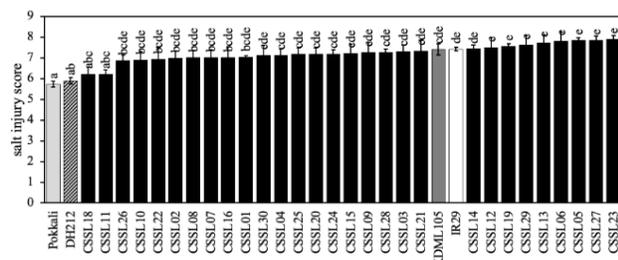


Fig. 2: Salt injury scores of rice genotypes at 11 days after initiating the severe salt stress treatment (150 mM NaCl). Data are presented as the mean of four biological replicates. Different letters represent significant differences between groups ($P < 0.05$) and error bars represent the standard error of the mean. ‘Pokkali’ (gray bar) and IR29 (white bar) rice were used as the salt-tolerant and salt-sensitive standard lines, respectively. The CSSLs (black bars) were compared with their parental lines, ‘KDML105’ (dark gray bar) and DH212 (striped bar)

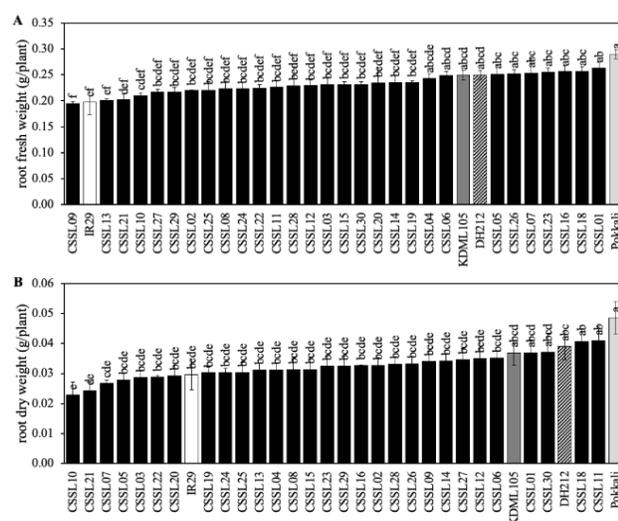


Fig. 3: Root growth traits of rice genotypes at the seedling stage after a 7-day exposure to severe salt stress (150 mM NaCl). (A) Root fresh weight. (B) Root dry weight. Data are presented as the mean of four biological replicates. Different letters represent significant differences between groups ($P < 0.05$) and error bars represent the standard error of the mean. ‘Pokkali’ (gray bar) and IR29 (white bar) rice were used as the salt-tolerant and salt-sensitive standard lines, respectively. The CSSLs (black bars) were compared with their parental lines, ‘KDML105’ (dark gray bar) and DH212 (striped bar)

RDW, SFW, and SDW under control and saline conditions, whereas IR29 had the lowest values, which were significantly different from those of the other genotypes. Line DH212, which is the donor of the stress-tolerance genes, had a higher RDW than the CSSLs at 7 days after salt stress treatment. An analysis of the CSSLs revealed differences in the growth response under salt stress conditions. For example, CSSL11 and CSSL18 had a higher RFW and RDW than both parents after a 14-day exposure to salt stress (Supplementary Table 1 and Fig. 3).

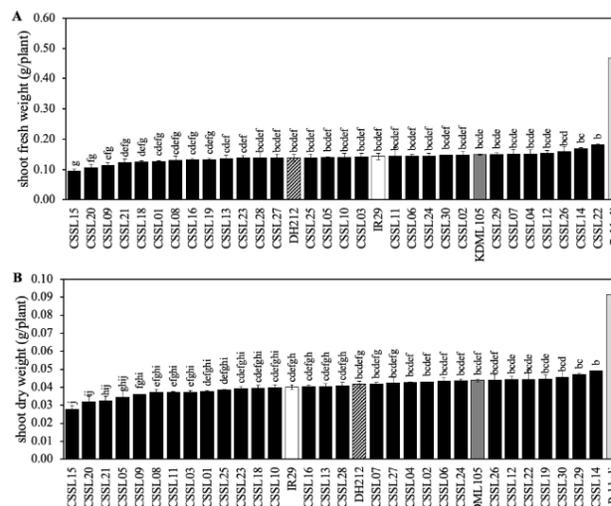


Fig. 4: Shoot growth traits of rice genotypes at the seedling stage after a 7-day exposure to severe salt stress (150 mM NaCl). (A) Shoot fresh weight. (B) Shoot dry weight. Data are presented as the mean of four biological replicates. Different letters represent significant differences between groups ($P < 0.05$) and error bars represent the standard error of the mean. ‘Pokkali’ (gray bar) and IR29 (white bar) rice were used as the salt-tolerant and salt-sensitive standard lines, respectively. The CSSLs (black bars) were compared with their parental lines, ‘KDML105’ (dark gray bar) and DH212 (striped bar)

Interestingly, CSSL11 produced the highest RDW among the CSSLs and DH212, but its RFW was lower than that of DH212. This suggested the root tissues of CSSL11 were drier than those of the other CSSLs.

After a 7-day salt stress treatment, ‘Pokkali’ seedlings had the highest SFW and SDW. Additionally, among the CSSLs, CSSL22 had the highest SFW and CSSL14 had the highest SDW (Fig. 4). In contrast, there were no significant differences in the SFW among the CSSLs after a 14-day salt stress treatment.

Some CSSLs performed well in response to salt stress

The SIS and an analysis of the quantitative growth parameters of the CSSL population indicated that CSSL11, CSSL14, CSSL18 and CSSL22 may be salt-tolerant lines. The lowest SISs were recorded for CSSL 11 and CSSL18, implying the shoots of these two lines may remain relatively healthy even under salt stress conditions (Fig. 2). Additionally, CSSL14 had the highest RFW and RDW among the CSSLs (Fig. 3), whereas CSSL22 had the highest SFW at 7 days after initiating the salt stress treatment (Fig. 4). The fact that the CSSL rice genotypes performed better than ‘KDML105’ may reflect their greater physiological adaptability to salt stress. Consequently, the physiological mechanisms responsible for the salt tolerance of these rice lines were subsequently investigated.

Determination of the physiological mechanisms underlying the salt tolerance of selected CSSLs

To investigate the salt-tolerance mechanisms, the CSSL seedlings treated with moderate salt stress (75 mM NaCl) or severe salt stress (150 mM NaCl) were compared with the parental (DH212 and 'KDML105'), salt-tolerant standard ('Pokkali'), and salt-sensitive standard (IR29) seedlings regarding shoot growth, midday LWP, and leaf Na⁺ and K⁺ contents. All traits were assessed at 0, 4, 8, and 12 days after initiating the salt stress treatment. The plants grown without NaCl treatment were used as controls.

Growth traits of selected CSSLs

Factorial in randomized complete block design (RCBD) was used to determine different responses to salt stress (75 mM and 150 mM NaCl) of 8 genotypes, which were 4 CSSLs (CSSL11, CSSL14, CSSL18 and CSSL22), parental lines ('KDML105' and DH212), standard salt tolerant line ('Pokkali') and standard salt susceptible line (IR29). Analysis of variance revealed the highly significant difference in RFW and SFW due to genotypes and NaCl level after stress. NaCl level did not affect RFW at the beginning of the experiment and no interaction (G × L) was found in RFW at that time point.

The higher of salt concentration led to the root growth inhibition in all genotypes, except in 'KDML105' that 75 mM NaCl treatment for 4 days could enhanced root growth (Table 2). For SFW, the similar pattern of negative effects on shoot growth was also found (Table 2). Under moderate (75 mM NaCl) and severe (150 mM NaCl) salt stress conditions, 'Pokkali' and IR29 seedlings produced the highest and lowest SFWs, respectively, at 12 days after initiating the salt stress treatment. All CSSLs had higher SFW than 'KDML105' after an 8-day and 12-day exposure to salt stress. Among them, CSSL18 had the highest SFW under moderate and severe salt stress conditions, which was comparable to that of the salt-tolerant 'Pokkali' rice (Table 2).

Midday leaf water potential of selected CSSLs

The LWP of rice seedlings grown under control (0 mM NaCl) or salt stress (75 and 150 mM NaCl) conditions was determined for the first fully expanded leaves at 0, 4, 8, and 12 days after initiating the salt stress treatments. Similar midday LWPs were measured for all rice genotypes under the control condition. When the seedlings were exposed to salt stress, the LWP decreased. After 4 days of moderate salt stress, IR29, the salt stress susceptible line, had the significant lower LWP than LWP of CSSLs, while DH212 had the highest LWP. After 8 to 12 day of moderate stress, LWP was decreased, but no significant difference was found among lines (Table 2).

After 8 and 12 days of the experiment, there was no interaction between genotypes and levels of NaCl stress (G × L) that affected LWP response. After a 12-day in moderate salt stress treatment, CSSL22 had the lowest LWP among CSSLs, whereas CSSL18 had the highest LWP, which was the same as that of the salt-tolerant standard ('Pokkali'). This indicated that of the tested CSSLs, CSSL18 was most able to adjust its LWP in response to salt stress. Under the severe salt stress condition (150 mM NaCl), the LWPs of all genotypes decreased over time, but there were no significant differences among lines (Table 2).

Sodium and potassium ion contents of selected CSSLs

A comparative analysis of the salt stress responses of selected CSSLs, parental lines, and standard checks was completed to determine whether an ion-balancing mechanism influences rice salt tolerance. Following the LWP measurements, the same leaf samples were dried and analyzed with an ICP-OES system to compare their sodium and potassium ion contents. Under the control condition (0 mM NaCl), there were no significant differences in the sodium/potassium content ratio of the fully expanded leaves of all rice genotypes (Table 3). Under the moderate salt stress condition, there were no significant differences in the Na⁺/K⁺ ratio among the analyzed lines (Table 3), suggesting that ion homeostasis did not influence the salt-tolerance of the tested seedlings. In response to the severe salt stress treatment, 'Pokkali' seedlings had the lowest Na⁺/K⁺ ratio after 8-day and 12-day salt stress treatments. CSSL22 showed the highest Na⁺/K⁺ ratio, which was significantly higher than Na⁺/K⁺ ratio of 'Pokkali' and CSSL18, when they were treated with severe salt stress for 8 days (Table 3). These suggested that ion homeostasis might not be the mechanism in salt stress adaptation in CSSL22. Due to the similarity in Na⁺/K⁺ ratio among most of the CSSLs and their parental lines and the tendency of higher Na⁺/K⁺ ratio than the ratio found in 'Pokkali', it is suggested that the mechanism underlying the ability of these CSSLs to maintain the ionic balance is not the same mechanism found in 'Pokkali' rice.

The salt-tolerance of CSSL18 Is likely due to osmotic adjustment

An analysis of the physiological responses of selected CSSLs revealed that CSSL18 may be the best candidate for an investigation of the mechanism regulating the salt-tolerance genes. This line performed well in terms of shoot growth and was better able to maintain the LWP compared with the other CSSLs and parental lines under salt stress conditions. Therefore, it suggests that the salt tolerance in CSSL18 obtained from *DT-QTL1* of DH212 is contributed by the osmotic adjustment mechanism.

Table 2: Effect of NaCl level on root fresh weight (RFW), shoot fresh weight (SFW), leaf water potential (LWP) of seedlings analyzed by using LS Means Differences Tukey HSD at $P < 0.05$, $Q = 3.85$. Interaction and main effects sharing the same case letter for a parameter do not differ significantly at $P < 0.05$

Varieties	NaCl level (mM NaCl)			Varieties	NaCl level (mM NaCl)			Varieties	NaCl level (mM NaCl)		
	0	75	150		0	75	150		0	75	150
<i>RFW (g/plant) 0 day</i>				<i>SFW (g/plant) 0 day</i>				<i>LWP (MPa) 0 day</i>			
CSSL11	0.50	0.36	0.37	CSSL11	0.80 ^{a-d}	0.64 ^{b-d}	0.60 ^{cd}	CSSL11	-0.23	-0.20	-0.17
CSSL14	0.52	0.43	0.37	CSSL14	1.03 ^{ab}	0.74 ^{a-d}	0.69 ^{b-d}	CSSL14	-0.22	-0.19	-0.19
CSSL18	0.51	0.56	0.52	CSSL18	0.95 ^{a-c}	0.82 ^{a-d}	0.89 ^{a-d}	CSSL18	-0.24	-0.21	-0.19
CSSL22	0.51	0.54	0.53	CSSL22	1.10 ^a	0.90 ^{a-d}	0.81 ^{a-d}	CSSL22	-0.21	-0.21	-0.21
'KDML105'	0.54	0.61	0.68	'KDML105'	0.89 ^{a-d}	0.77 ^{a-d}	0.51 ^d	'KDML105'	-0.22	-0.20	-0.19
DH212	0.52	0.61	0.36	DH212	1.01 ^{ab}	0.75 ^{a-d}	0.56 ^{cd}	DH212	-0.23	-0.23	-0.17
'Pokkali'	0.41	0.55	0.42	'Pokkali'	0.65 ^{b-d}	0.74 ^{a-d}	0.83 ^{a-d}	'Pokkali'	-0.23	-0.21	-0.21
IR29	0.38	0.46	0.42	IR29	0.66 ^{b-d}	0.74 ^{a-d}	0.65 ^{b-d}	IR29	-0.23	-0.23	-0.19
<i>RFW (g/plant) 4 day</i>				<i>SFW (g/plant) 4 day</i>				<i>LWP (MPa) 4 day</i>			
CSSL11	0.88 ^{a-d}	0.70 ^{cd}	0.57 ^{cd}	CSSL11	2.81 ^{ab}	1.65 ^{c-e}	1.19 ^e	CSSL11	-0.21 ^a	-0.29 ^{a-c}	-0.33 ^{a-d}
CSSL14	0.85 ^{a-d}	0.65 ^{cd}	0.54 ^{cd}	CSSL14	2.30 ^{a-d}	1.63 ^{c-e}	1.25 ^{de}	CSSL14	-0.23 ^{a-c}	-0.23 ^{ab}	-0.27 ^{a-c}
CSSL18	1.32 ^a	0.79 ^{b-d}	0.61 ^{cd}	CSSL18	3.30 ^a	1.92 ^{b-e}	1.21 ^e	CSSL18	-0.21 ^a	-0.29 ^{a-c}	-0.36 ^{a-d}
CSSL22	1.23 ^{ab}	0.88 ^{a-d}	0.70 ^{cd}	CSSL22	2.73 ^{ab}	1.59 ^{c-e}	1.55 ^{c-e}	CSSL22	-0.23 ^{a-c}	-0.31 ^{ab}	-0.35 ^{a-d}
'KDML105'	0.83 ^{a-d}	1.01 ^{a-c}	0.70 ^{cd}	'KDML105'	1.96 ^{b-e}	1.37 ^{de}	1.20 ^e	'KDML105'	-0.23 ^{a-c}	-0.30 ^{a-c}	-0.32 ^{a-d}
DH212	1.20 ^{ab}	0.58 ^{cd}	0.62 ^{cd}	DH212	2.43 ^{a-c}	1.82 ^{b-e}	1.20 ^e	DH212	-0.23 ^{a-c}	-0.21 ^a	-0.36 ^{a-d}
'Pokkali'	0.75 ^{bcd}	0.65 ^{cd}	0.62 ^{cd}	'Pokkali'	1.81 ^{b-e}	1.63 ^{c-e}	1.29 ^{de}	'Pokkali'	-0.23 ^{ab}	-0.32 ^{a-d}	-0.38 ^{b-d}
IR29	0.48 ^d	0.54 ^{cd}	0.56 ^{cd}	IR29	1.43 ^{c-e}	1.20 ^e	1.20 ^e	IR29	-0.22 ^{ab}	-0.48 ^d	-0.39 ^{a-d}
<i>RFW (g/plant) 8 day</i>				<i>SFW (g/plant) 8 day</i>				<i>LWP (MPa) 8 day</i>			
CSSL11	1.36	0.50	0.44	CSSL11	3.39 ^{c-e}	2.14 ^{e-i}	1.23 ^{hi}	CSSL11	-0.25	-0.25	-0.43
CSSL14	1.36	0.68	0.36	CSSL14	3.80 ^{bc}	2.31 ^{e-h}	1.38 ^{hi}	CSSL14	-0.25	-0.36	-0.33
CSSL18	2.17	0.82	0.34	CSSL18	5.10 ^a	2.08 ^{fi}	1.54 ^{hi}	CSSL18	-0.21	-0.28	-0.33
CSSL22	1.67	1.23	0.75	CSSL22	4.69 ^{ab}	2.06 ^{fi}	1.40 ^{hi}	CSSL22	-0.25	-0.32	-0.43
'KDML105'	2.00	1.18	0.80	'KDML105'	3.19 ^{c-g}	1.30 ^{hi}	1.02 ⁱ	'KDML105'	-0.24	-0.33	-0.45
DH212	1.32	0.63	0.64	DH212	3.34 ^{c-f}	2.04 ^{fi}	1.24 ^{hi}	DH212	-0.23	-0.32	-0.37
'Pokkali'	1.06	0.65	0.43	'Pokkali'	3.59 ^{b-d}	2.45 ^{d-h}	1.49 ^{hi}	'Pokkali'	-0.21	-0.35	-0.38
IR29	1.35	0.52	0.42	IR29	2.84 ^{c-g}	1.21 ^{hi}	1.02 ⁱ	IR29	-0.25	-0.41	-0.54
<i>RFW (g/plant) 12 day</i>				<i>SFW (g/plant) 12 day</i>				<i>LWP (MPa) 12 day</i>			
CSSL11	2.40 ^{c-f}	1.40 ^{e-i}	1.03 ^{fi}	CSSL11	2.40 ^{b-e}	1.29 ^e	1.20 ^e	CSSL11	-0.21	-0.46	-0.49
CSSL14	4.09 ^{ab}	1.69 ^{e-i}	0.73 ^{hi}	CSSL14	3.16 ^{a-c}	1.83 ^{c-e}	1.18 ^e	CSSL14	-0.22	-0.41	-0.41
CSSL18	3.39 ^{a-d}	1.55 ^{e-i}	0.65 ^{hi}	CSSL18	3.06 ^{a-d}	1.83 ^{c-e}	1.61 ^{c-e}	CSSL18	-0.21	-0.35	-0.42
CSSL22	4.51 ^a	1.61 ^{e-i}	0.97 ^{g-i}	CSSL22	4.51 ^a	1.65 ^{c-e}	1.34 ^e	CSSL22	-0.23	-0.57	-0.47
'KDML105'	3.62 ^{a-c}	1.38 ^{e-i}	0.90 ^{g-i}	'KDML105'	3.62 ^{ab}	1.19 ^e	0.95 ^e	'KDML105'	-0.22	-0.47	-0.52
DH212	3.58 ^{a-c}	1.70 ^{e-i}	0.77 ^{hi}	DH212	3.58 ^{ab}	1.57 ^{de}	0.87 ^e	DH212	-0.23	-0.43	-0.46
'Pokkali'	2.75 ^{b-e}	1.98 ^{e-h}	0.67 ^{hi}	'Pokkali'	2.40 ^{b-e}	2.14 ^{de}	1.70 ^{c-e}	'Pokkali'	-0.23	-0.35	-0.53
IR29	2.16 ^{d-g}	1.10 ^{fi}	0.53 ⁱ	IR29	2.16 ^{b-e}	0.98 ^e	0.90 ^e	IR29	-0.25	-0.52	-0.55

Discussion

The results on CSSL population screening were consistent with those of another study, revealing that DH212 has a lower SIS than 'KDML105' under salt stress conditions, and that CSSLs have diverse scores during the evaluation period (Kanjoo *et al.* 2011). Additionally, Leon *et al.* (2017) analyzed the growth response of rice recombinant inbred line populations under saline conditions. They discovered that the root and shoot lengths and the SDW were negatively correlated with the SIS, thereby confirming the negative effects of salt stress on plant growth. In contrast, the SIS was positively correlated with the Na^+/K^+ ratio in rice introgression line populations. However, in this study, the correlation between SIS and Na^+/K^+ ratio could not be detected, suggesting that the ionic homeostasis mechanism should not be the adaptive mechanism to salt stress in these CSSLs.

As growth changes among diverse genotypes in response to salt stress are dependent on the salt

concentration and the degree of salt tolerance (Kakar *et al.* 2019), for further analysis, two level of salt stress, the moderate stress at 75 mM NaCl and the severe stress at 150 mM NaCl were used in order to evaluate salt stress response and the mechanisms used for salt tolerance. The differential growth was detected among these lines, supporting the higher salt tolerance in CSSLs, when compared to 'KDML105'. Similar to DH212, all CSSLs had higher SFW than 'KDML105' during salt stress at 75 mM NaCl, but not RFW, suggesting that the changes in carbon partitioning in CSSLs were due to the genetic contribution from DH212. Salt-induced carbon partitioning changes were also found in other species, for example; *Pityrocarpa moniliformis* (Silva *et al.* 2019), canola (Zuo *et al.* 2019) and *Arabidopsis* (Dong *et al.* 2018). In *Arabidopsis*, the changes in the transcription of the genes in T6P/SnRK1 regulatory pathway were proposed to be partly responsible for starch metabolism and sugar export in the source leaves (Dong *et al.* 2018).

In rice and other species, the leaf water potential (LWP) is an essential physiological trait for the water-deficit

Table 3: Effect of NaCl level on Na⁺/K⁺ ratio of seedling rice genotypes. LS Means Differences Tukey HSD at $P < 0.05$, $Q = 3.85$. Interaction and main effects sharing the same case letter, for a parameter, do not differ significantly at $P < 0.05$

Varieties	NaCl level (mM NaCl)		
	0	75	150
<i>Na⁺/K⁺ ratio 0 day</i>			
CSSL11	0.16 ^{a-c}	0.11 ^{bc}	0.06 ^{bc}
CSSL14	0.11 ^{bc}	0.28 ^a	0.12 ^{bc}
CSSL18	0.08 ^{bc}	0.09 ^{bc}	0.16 ^{a-c}
CSSL22	0.10 ^{bc}	0.09 ^{bc}	0.11 ^{bc}
'KDML105'	0.16 ^{a-c}	0.13 ^{a-c}	0.14 ^{a-c}
DH212	0.19 ^{ab}	0.06 ^{bc}	0.06 ^{bc}
'Pokkali'	0.09 ^{bc}	0.12 ^{bc}	0.03 ^c
IR29	0.15 ^{a-c}	0.15 ^{a-c}	0.20 ^{ab}
<i>Na⁺/K⁺ ratio 4 day</i>			
CSSL11	0.11 ^c	0.29 ^{a-c}	0.60 ^{ab}
CSSL14	0.10 ^c	0.19 ^c	0.65 ^a
CSSL18	0.13 ^c	0.20 ^c	0.26 ^{bc}
CSSL22	0.09 ^c	0.15 ^c	0.38 ^{a-c}
'KDML105'	0.06 ^c	0.18 ^c	0.15 ^c
DH212	0.10 ^c	0.10 ^c	0.20 ^c
'Pokkali'	0.19 ^c	0.13 ^c	0.17 ^c
IR29	0.12 ^c	0.12 ^c	0.40 ^{a-c}
<i>Na⁺/K⁺ ratio 8 day</i>			
CSSL11	0.09 ^{cd}	0.17 ^{a-d}	0.13 ^{a-d}
CSSL14	0.08 ^{cd}	0.15 ^{a-d}	0.13 ^{a-d}
CSSL18	0.08 ^{cd}	0.16 ^{a-d}	0.11 ^{b-d}
CSSL22	0.09 ^{cd}	0.16 ^{a-d}	0.33 ^a
'KDML105'	0.08 ^{cd}	0.18 ^{a-d}	0.16 ^{a-d}
DH212	0.02 ^d	0.27 ^{a-c}	0.29 ^{ab}
'Pokkali'	0.06 ^d	0.11 ^{b-d}	0.08 ^{cd}
IR29	0.05 ^d	0.29 ^{ab}	0.13 ^{a-d}
<i>Na⁺/K⁺ ratio 12 day</i>			
CSSL11	0.03	0.17	0.34
CSSL14	0.05	0.19	0.22
CSSL18	0.06	0.25	0.22
CSSL22	0.06	0.19	0.39
'KDML105'	0.06	0.20	0.33
DH212	0.03	0.15	0.36
'Pokkali'	0.06	0.09	0.12
IR29	0.04	0.22	0.18

tolerance under stress conditions (Jongdee *et al.* 2002; Wang *et al.* 2019; Huang *et al.* 2019). After 12 days of salt stress, CSSL14 and CSSL18 had higher LWP than 'KDML105', which was similar to what detected in DH212 and 'Pokkali', suggesting that CSSL14 and CSSL18 contained the gene responsible for LWP adjustment under salt stress condition, presumably from DH212. Accordingly, our LWP data suggested that CSSL18 was more tolerant to salt stress than the other analyzed CSSLs. Recently, transcriptomic comparison between CSSL18 and 'KDML105' under salt stress condition has revealed the potential genes responsible for salt tolerance to be located on chromosome 1, *OsiRO2* and *OsiMSR2*. *OsiRO2* is a putative bHLH transcription factor, while *OsiMSR2* encodes *OsiCML31*, which plays a role in calcium signaling process (Khruuasan *et al.* 2019).

Conclusion

Twenty-nine near isogenic rice lines were developed from

CSSLs carrying the drought-tolerance QTL (*DT-QTL1*) on chromosome 1 between RM1003 and RM3362. These lines exhibited diverse salt stress responses at the seedling stage, with CSSL18 exhibiting the highest salt-tolerance under moderate and severe salt stress conditions. This line maintained a higher midday LWP compared with 'KDML105', suggesting the role of *DT-QTL1* region from DH212, but its Na⁺/K⁺ ratio was lower than DH212, suggesting no contribution of *DT-QTL1* region from DH212 for ionic homeostasis. The data presented herein indicate that the drought-tolerance QTL, *DT-QTL1*, regulates salt-tolerance phenotypes in rice mainly with osmotic adjustment mechanism.

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References

- Dong S, J Zhang, DM Beckles (2018). A pivotal role for starch in the reconfiguration of ¹⁴C-partitioning and allocation in *Arabidopsis thaliana* under short-term abiotic stress. *Sci Rep* 8; Article 9314
- Farooq M, N Gogoi, M Hussain, S Barthakur, S Paul, N Bharadwaj, HM Migdadi, SS Alghamdi, KHM Siddique (2017) Effects, tolerance mechanisms and management of salt stress in grain legumes. *Plant Physiol Biochem* 118:199–217.
- Frukh A, TO Siddiqi, MIR Khan, A Ahmad (2020). Modulation in growth, biochemical attributes and proteome profile of rice cultivars under salt stress. *Plant Physiol Biochem* 146:55–70
- Gregorio GB, S Dharmawansa, RD Mendoza (1997). Screening rice for salinity tolerance. In: *IRRI Discussion Paper Series No. 22*, pp: 2–23. International Rice Research Institute, Makati, Manila, Philippines
- Gupta B, B Huang (2014). Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *Intl J Plant Genomics* 2014:1–19
- Hoang TML, TN Tran, TKT Nguyen, B Williams, P Wurm, S Bellairs, S Mundree (2016). Improvement of salinity stress tolerance in rice: Challenges and opportunities. *Agronomy* 6; Article 6040054
- Horie T, I Karahara, M Katsuhara (2012). Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. *Rice* 5; Article 11
- Huang X, L Wang, J Ma (2019). Physiological and biochemical changes of *Populus euphratica* seedlings under saline irrigation stresses. *Fresen Environ Bull* 28:6485–6497
- Hussain M, S Ahmad, S Hussain, R Lal, S Ul-Allah, A Nawaz (2018). Rice in saline soils: physiology, biochemistry, genetics, and management. *Adv Agron* 148:231–287
- IRRI (2002). *Standard Evaluation System for Rice*. International Rice Research Institute, Los Baños, Laguna, Philippines
- Isaac RA, WC Johnson (1998). Elemental determination by inductively coupled plasma atomic emission spectrophotometry. In: *Handbook of Reference Methods for Plant Analysis*, pp:165–170. Kalra YP (Ed.). CRC Press, Boca Raton, Florida, USA

- Jaiswal S, RK Gautam, RK Singh, SL Krishnamurthy, S Ali, K Sakthivel, MA Iqbal, A Rai, D Kumar (2019). Harmonizing technological advances in phenomics and genomics for enhanced salt tolerance in rice from a practical perspective. *Rice* 12; Article 89
- Jongdee B, S Fukai, M Cooper (2002). Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. *Field Crops Res* 76:153–163
- Kakar N, SH Jumaa, ED Redoña, ML Warburton, KR Reddy (2019). Evaluating rice for salinity using pot-culture provides a systematic tolerance assessment at the seedling stage. *Rice* 12; Article 57
- Kanjoo V (2012). Development of chromosome segment substitution lines related to drought tolerance in rice (*Oryza sativa* L.). *Ph.D. Thesis*. Faculty of Agriculture, Kasetsart University, Bangkok, Thailand
- Kanjoo V, S Jearakongman, K Punyawaew, JL Siangliw, M Siangliw, A Vanavichit, T Toojinda (2011). Co-location of quantitative trait loci for drought and salinity tolerance in rice. *Thai J Genet* 4:126–138
- Khruuasan N, P Chutimanukul, K Plaimas, T Buaboocha, M Siangliw, T Toojinda, L Comai, S Chadchawan (2019). Comparison between the transcriptomes of 'KDML105' rice and a salt-tolerant chromosome segment substitution line. *Genes* 10; Article pii: E742
- Lanceras JC, G Pantuwan, B Jongdee, T Toojinda (2004). Quantitative trait loci associated with drought tolerance at reproductive stage in rice. *Plant Physiol* 135:384–399
- Lekklar C, M Pongpanich, D Suriya-arunroj, A Chinpongpanich, AH Tsai, L Comai, S Chadchawan, T Buaboocha (2019). Genome-wide association study for salinity tolerance at the flowering stage in a panel of rice accessions from Thailand. *BMC Genom* 20:76
- Leon, TBD, S Linscombe, PK Subudhi (2017). Identification and validation of QTLs for seedling salinity tolerance in introgression lines of a salt tolerant rice landrace 'Pokkali'. *PLoS One* 12; Article e0175361
- Munns R, M Tester (2008). Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59:651–681
- Munns R, RA James, M Gilliam, TJ Flowers, TD Colmer (2016). Tissue tolerance: An essential but elusive trait for salt-tolerant crops. *Funct Plant Biol* 43:1103–1113
- Negrão S, B Courtois, N Ahmadi, I Abreu, N Saibo, MM Oliveira (2011). Recent updates on salinity stress in rice: from physiological to molecular responses. *Crit Rev Plant Sci* 30:329–377
- Reddy INBL, B Kim, I Yoon, K Kim, T Kwon (2017). Salt tolerance in rice: Focus on mechanisms and approaches. *Rice Sci* 24:123–144
- Roy SJ, S Negrão, M Tester (2014). Salt resistant crop plants. *Curr Opin Biotechnol* 26:115–124
- Sahi C, A Singh, K Kumar, E Blumwald, A Grover (2006). Salt stress response in rice: genetics, molecular biology, and comparative genomics. *Funct Integr Genomics* 6:263–284
- Saini P, D Kamboj, RC Yadav, NR Yadav (2019). SRAPs and EST-SSRs provide useful molecular diversity for targeting drought and salinity tolerance in Indian mustard. *Mol Biol Rep* 46:1213–1225
- Silva HAD, DFAD Oliveira, AP Avelino, CECD Macêdo, T Barros-Galvão, EL Voigt (2019). Salt stress differentially regulates mobilisation of carbon and nitrogen reserves during seedling establishment of *Pityrocarpa moniliformis*. *Plant Biol* 21:1110–1118
- Wang Z, H Li, X Li, C Xin, J Si, S Li, Y Li, X Zheng, H Li, X Wei, Z Zhang, L Kong, F Wang (2019). Nano-ZnO priming induces salt tolerance by promoting photosynthetic carbon assimilation in wheat. *Arch Agron Soil Sci* 2019:1–15
- Zuo Q, J Liu, J Shan, J Zhou, L Wang, G Yang, S Leng, H Liu (2019). Carbon and nitrogen assimilation and partitioning in canola (*Brassica napus* L.) in saline environment. *Commun Soil Sci Plant Anal* 50:1700–1709