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การทดสอบความทนแล้งและทนเค็มในระยะต้นกล้าของสายพันธุ์ข้าวที่ปรับปรุงจากข้าวขาวดอกมะลิ 105 ที่มี QTL ทนแล้งและยีนทนเค็ม *SKC1*

Evaluation of Drought and Salinity Tolerance at Seedling Stage of Introgression Lines of ‘KDML105’ Rice Carrying Drought Tolerance QTL and *SKC1* Salt Tolerance Gene

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อคัดเลือกสายพันธุ์ข้าวที่สามารถทนได้ทั้งความแล้งและความเค็มในระยะต้นกล้า จากสายพันธุ์ข้าวจำนวน 22 สายพันธุ์ที่ได้รับการปรับปรุงด้วยวิธีผสมกลับโดยการถ่ายทอด QTL ทนแล้งบนโครโมโซมแท่งที่ 8 (DT-QTL8) และยีนทนเค็ม (*SKC1*) เข้าสู่ข้าวพันธุ์ขาวดอกมะลิ 105 (‘KDML105’) ขั้นตอนแรกชักนำให้เกิดความเครียดแล้งแก่ต้นกล้าข้าว 22 สายพันธุ์ อายุ 14 วัน ที่ปลูกในสารละลายไฮโดรโพนิกส์ โดยการเติมโพลีเอทิลีนไกลคอล 20% ลงใน

สารละลาย จากนั้นทำการประเมินความทนแล้งจากลักษณะการม้วนใบ การแห้งตายของใบ และการลดลงของมวลชีวภาพแห้ง พบสายพันธุ์ข้าว 10 สายพันธุ์ที่แสดงร้อยละการลดลงของมวลชีวภาพแห้งต่ำกว่า ‘KDML105’ หลังจากได้รับความเครียดแล้ง 28 วัน ได้แก่ L13 L7 L24 L3 L14 L17 L8 L4 L6 และ L16 ต่อจากนั้นทำการประเมินความทนเค็มของต้นกล้าข้าว 10 สายพันธุ์ดังกล่าว โดยปลูกข้าวในสารละลายไฮโดรโพนิกส์จนมีอายุ 14 วัน แล้วให้ความเครียดเค็มโดยการให้เกลือโซเดียมคลอไรด์ 150 มิลลิโมลาร์ จากการประเมินความทนเค็มหลังจากต้นข้าวได้รับความเครียดเค็ม 21 วัน พบว่าข้าวทั้ง 10 สายพันธุ์มีคะแนนความเสียหายจากเกลือ อัตราส่วน Na^+/K^+ และร้อยละการลดลงของมวลชีวภาพต่ำกว่า ‘KDML105’ สายพันธุ์ที่ทนทานสูงสุด L13 มีมวลชีวภาพแห้งลดลงร้อยละ 62.58 และ 47.71 ภายใต้ความเครียดแล้งและเครียดเค็มตามลำดับ เปรียบเทียบกับ ‘KDML105’ ที่มีการลดลงร้อยละ 77.35 และ 83.64 การศึกษาในขั้นตอนต่อไปควรมีการทดสอบความทนเค็มและแล้งของสายพันธุ์ปรับปรุงเหล่านี้ในสภาพไร่นา สายพันธุ์เหล่านี้เป็นแหล่งพันธุกรรมที่ดีสำหรับการปรับปรุงพันธุ์ข้าว ‘KDML105’ ให้ทนทานต่อสภาพเครียดหลายประเภทต่อไป

ABSTRACT

The salinity and drought tolerance levels were evaluated in twenty-two backcross improved rice lines carrying drought tolerance quantitative trait loci on chromosome 8 (DT-QTL8) and the salt tolerance *SKC1* gene in the genetic background of the Thai elite rice cultivar ‘KDML105’. Firstly, drought stress was imposed on seedlings of the 22 rice lines grown in hydroponic culture by adding 20% polyethylene glycol 6000 (PEG6000), and drought tolerance was evaluated by leaf rolling, leaf drying, and biomass reduction. Ten rice lines that exhibited lower decrement in dry biomass than ‘KDML105’ after 28 days of drought stress, namely L13, L7, L24, L3, L14, L17, L8, L4, L6, and L16, were then selected for evaluation of salt tolerance in hydroponic culture in the presence of 150 mM NaCl. After 21 days of salt stress, all ten rice lines exhibited lower salt injury scores, lower Na^+/K^+ ratios, and lower biomass reductions than ‘KDML105’. The most tolerant line, L13, exhibited 62.58% and 47.71% reduction in dry biomass under drought and salt stress, respectively, compared with 77.35% and 83.64% reduction in ‘KDML105’. These improved lines may be tested for enhanced production in field conditions and serve as potentially good genetic resources for further improvement of ‘KDML105’ rice to tolerate multiple stresses.

คำสำคัญ: การม้วนใบ การแห้งตายของใบ ความเสียหายจากเกลือ อัตราส่วน Na^+/K^+
Keywords: Leaf rolling, Leaf drying, Salt injury, Na^+/K^+ ratio

INTRODUCTION

Rice (*Oryza sativa* L. spp. *indica*) is a staple food crop for more than 3 billion Asia’s population and accounting for 35-75% of the daily calories consumed (Khush, 2005). Thailand is in the top five rice exporting countries of the globe. Among several exported rice genotypes, Khao Dawk Mali 105 (‘KDML105’) is the most well-known and popular due to its good cooking quality and distinctive aroma. The best quality ‘KDML105’ is produced in the northeast of Thailand (Vanavichit et al., 2018), but the yield per area is much lower than that grown in other geographical areas (Office of Agricultural Economics, 2021). A majority of agricultural areas in northeastern Thailand confronts saline soil and drought (Arunin and Pongwichian, 2015; Polthanee et al., 2014) resulting from low precipitation quantity and excess accumulation of salt ions especially Na^+ and Cl^- (Ghosh et al., 2016). These conditions cause salt and drought stress in rice leading to loss of grain yield in rainfed lowland conditions.

Drought stress is a major abiotic stress factor that causes complex physiological and biochemical changes in plants (Zhu, 2002). The plant's first response to water deficit is stomata closure to protect the transpiration water loss, resulting in the restriction of CO_2 diffusion from the atmosphere into leaves and the reduction in net photosynthesis rates (Osakabe et al., 2014). Under water limited conditions, less water is absorbed, transpiration is diminished, leaf relative water content (RWC) is reduced leading to restriction to cell division and expansion, and a decline in leaf area (Hussain et al., 2018). Salt stress is another abiotic factor causing osmotic and ionic stress that suppress plant growth (Munns and Tester, 2008). In salt-affected soils, increased amounts of salt ions lead to the reduced water potential of the external soil solution surrounding roots which imposes osmotic stress on the root cells leading to less water absorption (Parida and Das, 2005). The ionic stress occurs when the plants absorb and accumulate Na^+ and Cl^- ions to a toxic level in the cells (Yang and Guo, 2018). Due to similar atomic size and properties, high Na^+ concentration obstructs K^+ absorption, resulting in disruptions of biochemical processes because K^+ is an essential element which performs many critical roles in plant cells such as acting as cofactor for various enzymes, regulating stomatal movement, and involving in attaching tRNA to ribosomes in protein synthesis (Shrivastava and Kumar, 2015). Meanwhile, over accumulation of Cl^- interrupts chlorophyll production which subsequently causes leaf chlorosis and leaf burns (Tavakkoli et al., 2011). High concentrations of salts also restrain uptake of other essential plant nutrients like phosphorus (P), nitrogen (N), and calcium (Ca^{2+}) leading to metabolic disturbances and growth inhibition (Munns and Tester, 2008; Parida and Das, 2005). Ultimately, the alteration of various processes under both drought and salt stress leads to growth retardation and yield loss in crop.

'KDML105' is generally known to be susceptible to abiotic (salt, drought, flooding, and heat) and biotic (blast, bacterial leaf blight, and brown planthopper) stress (Vanavichit et al., 2018). Breeding efforts have been successfully carried out to improve stress tolerance in 'KDML105'. To develop the drought-tolerant 'KDML105' rice lines, DH103 (IR68586-F2-CA-31) was applied as a donor for drought-tolerance quantitative trait loci (DT-QTL) on chromosome 8 (DT-QTL8). QTL located in this region was reported to be associated with dry weight, percent spikelet sterility, panicle number and osmotic adjustment (Siangliw et al., 2007). The drought tolerant line DH103 was crossed with 'KDML105' until the chromosome segment substitution lines (CSSLs) were obtained through molecular marker assisted backcrossing (MAB) (Kanjoo, 2011). The validation of agronomic traits in improved lines and 'KDML105' found that these CSSLs produced higher grain yield than 'KDML105' under drought and irrigated conditions (Kanjoo et al., 2012). The *SKC1* gene serves in maintaining K^+ homeostasis by Na^+ unloading from the shoot xylem and increasing K^+ concentration, as a result, shoot Na^+/K^+ ratio is lowered leading to higher yields under salt stress (Thomson et al., 2010). The *SKC1* gene is located on chromosome 1 in the salt-tolerant Nona Broka variety, and it is later recognized to be localized within the *Saltol* locus identified in the salt-tolerant indica variety, Pokkali (Ismail and Horie, 2017). In the breeding program aimed at improving salt tolerance of ‘KDML105’, introgression lines harboring the *SKC1* gene were produced from the cross between ‘KDML105’ and the salt-tolerant line FL530 (IR66946-3R-230-1-1) as *SKC1* donor. These introgression lines were more tolerant to salt stress than ‘KDML105’, with a lower shoot Na^+/K^+ ratio and higher yield under salt stress (Punyawaew et al., 2016). After almost two decades of research in marker assisted breeding (MAB), Vanavichit et al. (2018) has produced a new generation of 'KDML105', designated HM84, by integrating abiotic and biotic stress resistance genes which conferred greater tolerance to flooding, diseases and insect outbreak. However, pyramiding more than one abiotic stress tolerance genes into ‘KDML105’ has not been reported. In order to produce ‘KDML105’ rice lines carrying both DT-QTL8 and *SKC1*, a molecular-assisted backcross breeding scheme was recently developed from a cross between the recipient line CSSL-103 (an improved line of ‘KDML105’ carrying DT-QTL8) with RGD4 (RGD12150-B-21-MS3; an improved ‘KDML105’ line harboring *SKC1*) as the donor parent (Pamuta, 2021). In this study, a subset of the $BC_1F_{2:3}$ population derived from the cross CSSL103 x RGD4 were evaluated for the level of salt and drought tolerance based on physiological traits in seedlings grown in hydroponic solutions to identify the improved rice lines tolerant to both abiotic stress conditions.

MATERIAL AND METHODOLOGY

1.1 Plant materials, growth condition and evaluation of drought tolerance scores

A total of 22 $BC_1F_{2:3}$ improved ‘KDML105’ rice lines (L1, L3-L19, L22-L25) which were derived from the cross between CSSL103 and RGD4 were assessed for the tolerance to drought stress at the seedling stage. The female parent, CSSL103 (an improved line of ‘KDML105’ carrying DT-QTL8), and the male parent, RGD4 (an improved ‘KDML105’ line harboring *SKC1* gene) were also included in the experiment. The check varieties for salinity tolerance (Pokkali, original donor of *SKC1*), drought tolerance (DH103, original donor of DT-QTL8), saline and drought susceptible cultivar (‘KDML105’) were also included for comparison.

The seeds were germinated on filter paper in petri dishes holding distilled water. Three days later, germinated seeds were transferred to holes drilled Styrofoam sheets floated in a plastic tray (50 x 60 x 11 cm) filled with water. Three days after transplanting, water supply was replaced with 15 L of half-strength Yoshida nutrient solution (Yoshida et al., 1976). Then, the nutrient solutions were adjusted to full strength three days later. When the seedlings were 14 days old, they were imposed with drought stress by adding 10% polyethylene glycol 6000 (PEG6000) to the nutrient solutions. Three days later, the solution was changed to the final concentration of 20% PEG6000 (to create the water potential of -0.7 MPa). After that, the nutrient solutions were renewed every 5 days. The control set continued to be fed with standard nutrient solutions, also renewed every 5 days. The experiment was conducted during July to September 2021 in the greenhouse at the Field

Crop Research Station, Faculty of Agriculture, Khon Kaen University. The daily mean temperature ranged from 22.8 – 37.4 °C, daily mean humidity from 60.6 - 96.1%, and daily mean light intensity was 693 µmol photon m^{-2} s^{-1}.

Drought tolerance was evaluated from the symptoms of leaf rolling and drying. Leaf rolling scores were evaluated 3, 6, and 9 days, and leaf drying scores were 8, 16, and 24 days after exposure to 20% PEG. Scoring for leaf rolling and drying (Table 1; Fig. 1) was performed according to IRRI (2013). Mean scores were obtained from two seedlings of each line/variety in each replication.

Table 1 Evaluation scores for drought tolerance at seedling stage according to IRRI (2013)

Score	Observation	
	Leaf rolling	**Leaf drying**
0	Leaves healthy	No symptoms
1	Leaves start to fold (shallow)	Slight tip drying
3	Leaves folding (deep V-shape)	Tip drying extended up to 1/4 length in most leaves
5	Leaves fully cupped (U-shape)	1/4 to 1/2 of all leaves dried
7	Leaf margins touching (0-shape)	More than 2/3 of all leaves fully dried
9	Leaves tightly rolled	All plants apparently dead

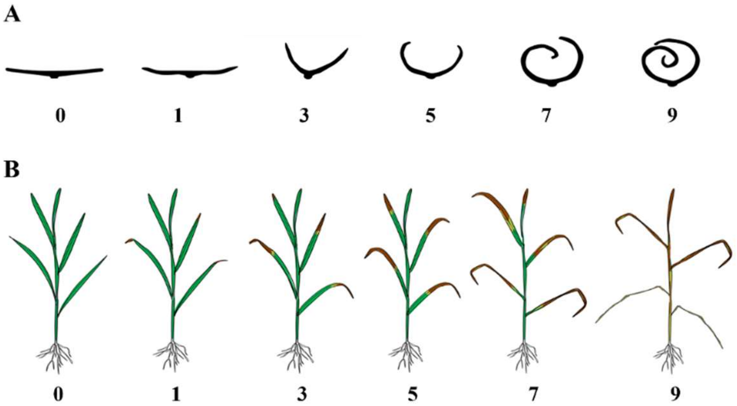

Figure 1 Diagrammatic representation of leaf rolling (A), and leaf drying scores (B).

1.2 Biomass reduction

After the seedlings were exposed to PEG-induced drought stress for 28 days, they were harvested for determination of growth parameters. The seedlings each line/variety were immediately weighed to get the fresh weight. For the estimation of dry weight, seedlings were dried in a forced air oven for 3 days at 80 °C. The reduction percentage of dry biomass compared to the control plants (RB) was calculated according to the following formula, where RB is the reduction percentage of biomass, CB is the control biomass and SB is the stressed biomass.

$$RB\ (\%) = [(CB-SB)/CB] \times 100$$

The reduction percentage of dry biomass was employed as major criterion for selecting improved ‘KDML105’ rice lines that best tolerated drought stress.

1.3 Plant materials, growth condition, evaluation of salt tolerance scores, and growth parameters

Salt tolerance was evaluated from 10 selected improved $BC_1F_{2:3}$ 'KDML105' rice lines having the lowest reduction in dry biomass under drought stress. The procedure for growing plants was the same as described in 1.1, except that salt stress was imposed by adding 75 mM NaCl to the nutrient solution when the seedlings were 14 days old. After 3 days, concentration of NaCl was increased to 150 mM (the electrical conductivity of 15 dS m^{-1}). The salinized solution was renewed every 5 days. The control set continued to be fed with standard nutrient solutions, also renewed every 5 days. Salt tolerance levels were estimated from salt injury symptoms observed 5, 10 and 15 days after stress exposure to 150 mM NaCl using the modified standard evaluation system, as shown in Table 2 (Gregorio et al., 1997). The mean salt injury scores (SIS) were calculated from two seedlings of each line/variety in each replication. After the seedlings were grown in nutrient solutions supplemented with NaCl for 21 days, fresh and dry weights were determined as described in 1.1.

1.4 Determination of Na^+ and K^+ content

The Na^+ and K^+ contents of seedlings were determined after the plants were oven-dried for 3 days, tissue samples of each rice line/variety were ground in a mortar with liquid nitrogen. About 0.2 g of the tissue sample was digested with 10 ml of nitric acid at 300 °C, 5 ml of perchloric acid at 200 °C and 20 ml of 6 M HCl. The Na^+ and K^+ contents were analyzed by an atomic absorption spectrometer (Corning, Model GBC932AAA, England).

1.5 Statistical analysis

The experiments were performed as a randomized complete block design (RCBD) with three replications. The data were analyzed through SPSS ver. 23 statistical software, and comparison of means were conducted using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ or $p \leq 0.01$ significance level.

Table 2 The modified standard evaluation system of salt injury at seedling stage (Gregorio et al., 1997)

Salt Injury Score (SIS)	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or a few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dried; some plants are dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

RESULTS

1.1 Growth performance in rice lines/varieties under drought stress

PEG-induced drought stress induced huge reduction in fresh and dry weights of all tested rice lines/varieties (Table 3). The reduction in fresh biomass was lowest (82.75%) in L7 followed by L13, DH103, L3 and L24 with the diminution of 83.00%, 83.69%, 84.82% and 85.56%, respectively when compared to control plants (Table 3). While the highest reduction of 92.48%, 92.46%, 92.30%, 92.03% and 91.29% were found in L22, L10, L5, Pokkali and L23, respectively. For ‘KDML105’, there was a reduction in fresh biomass of 89.93%. For dry biomass, the highest reduction in dry biomass was observed in Pokkali (85.53%) and the lowest in DH103 (53.63%). ‘KDML105’ suffered a dry biomass reduction of 77.35%, while the parental lines CSSL103 and RGD4 showed 76.60% and 73.76% reduction, respectively. Among the 22 improved lines tested, 11 exhibited a lower dry biomass reduction than ‘KDML105’, varying from 62.58% (L13) to 77.08% (L12). The two most drought tolerant improved lines with respect to low dry biomass reduction were L13 and L7 which exhibited only 62.58% and 64.08% reduction, respectively.

1.2 Evaluation scores for drought tolerance in rice lines/varieties

Three days after drought stress, all rice lines/varieties exhibited significant variation in leaf rolling scores. The rice lines/varieties DH103, L13, L5, L9 and CSSL103 had minimal scores in the range of 0.00-1.33, but the maximal scores were noted in lines/varieties Pokkali, L10, L6, RGD4, L16 and L15 (scores in the range of 5.00-3.00) (Fig. 2A). Leaf rolling scores increased in all rice lines/varieties after drought imposition for 6 days. The lowest increase was found in lines/varieties DH103, L7, L12, L13 and CSSL103 with a score in range of 4.33-5.00, whereas lines/varieties Pokkali, ‘KDML105’, RGD4, L25, L24, L23, L19, L15, L9 and L1 possessed the highest increase with score range of 8.33-6.33 (Fig. 2B). However, no significant differences in leaf rolling scores were observed among all rice lines/varieties after 9 days of dehydration, except for rice variety DH103 which showed the maximum level of tolerance with the significantly different score of 5.0. Among the improved lines L6, L3, L4, L13, L17 and L23 were relatively more tolerant having scores similar to the DT-QTL8 donor parent CSSL103 (scores in the range of 7.00-7.67). In contrast, the minimum levels of tolerance were recorded in rice lines/varieties Pokkali, ‘KDML105’, L10, L11 and L22 with the score of 9.0 (Fig. 2C). Pokkali is the only rice variety showing significant differences in leaf drying score of 2.33 after drought imposition for 8 days (Fig. 2D). Subsequently, leaf drying symptoms in all rice lines/varieties became more severe 16 days after drought stress. Lines/varieties DH103, L8, L17, CSSL103 and L3 manifested the lowest drying symptoms at the score range of 3.00-5.00 and the highest drying symptoms appeared in lines/varieties Pokkali, L22, L15, L11 and L9 showing scores in range of 7.00-5.67 (Fig. 2E). Twenty-four days after drought stress, the rice lines/varieties DH103, CSSL103, L8 and L16 exhibited the highest tolerance against water deficit with leaf drying scores in range of 5.00-6.33. On the other hand, the most susceptible lines/varieties were Pokkali, L23, L19, L12, L10 and L4 with leaf drying scores in the range of 9.00-7.33 which is greater than the score of susceptible check ‘KDML105’ (score = 7) (Fig. 2F).

Table 3 Reduction in the fresh and dry biomass of improved ‘KDML105’ rice lines and check varieties under drought stress.

Line/variety	Fresh biomass (g/plant)			Dry biomass (g/plant)		
	Control	**Drought**	**% Reduction**	**Control**	**Drought**	**% Reduction**
L1	16.88cde	1.65a-f	89.80	3.33c-g	0.67b-f	78.67
L3	12.68a-d	1.79b-f	84.82	2.48a-f	0.70b-f	70.07
L4	14.82a-d	1.73a-f	87.20	2.99b-f	0.71c-f	73.73
L5	21.06def	1.62a-f	92.30	3.89d-g	0.65b-f	83.32
L6	15.94a-e	1.74a-f	88.34	3.09b-f	0.70b-f	75.57
L7	11.21abc	1.61a-f	82.75	2.07abc	0.63a-f	64.08
L8	14.42a-d	1.66a-f	87.52	2.82a-f	0.68b-f	73.56
L9	11.56abc	1.31abc	88.71	2.25a-d	0.51abc	77.51
L10	23.23ef	1.73a-f	92.03	4.14fg	0.68b-f	82.06
L11	20.61def	2.17f	87.78	3.95efg	0.75ef	78.54
L12	11.31abc	1.43a-d	87.37	2.29a-e	0.53a-e	77.08
L13	8.44ab	1.37abc	83.00	1.59ab	0.56a-e	62.58
L14	16.96cde	2.06ef	87.39	3.14b-f	0.82f	73.03
L15	18.65cde	1.80b-f	89.67	4.10fg	0.69b-f	82.52
L16	16.70b-e	1.62a-f	88.45	3.14b-f	0.63a-f	76.95
L17	12.06abc	1.53a-e	87.37	2.32a-e	0.62a-f	73.39
L18	14.81a-d	1.43a-d	90.24	2.91a-f	0.55a-e	81.32
L19	17.80cde	1.84b-f	89.66	3.63c-g	0.69b-f	80.94
L22	16.58b-e	1.45a-d	91.29	3.02b-f	0.59a-e	79.13
L23	15.30a-e	1.15a	92.48	2.86a-f	0.52a-d	81.77
L24	13.28a-d	1.89c-f	85.56	2.52a-f	0.75def	69.51
L25	20.92def	1.84b-f	89.55	4.14fg	0.74def	78.48
Pokkali	26.89f	1.98def	92.46	4.93fg	0.69b-f	85.53
DH103	8.10a	1.14a	83.69	1.31a	0.43a	53.63
CSSL103	10.76abc	1.28ab	88.07	2.09abc	0.48ab	76.60
RGD4	11.92abc	1.57a-f	86.51	2.61a-f	0.65b-f	73.76
KDML105	12.74a-d	1.24ab	89.93	2.34a-e	0.51abc	77.35
Mean	**15.39**	**1.62**	**88.29**	**2.96**	**0.63**	**75.58**
F test	**	**	ns	**	**	ns

The data exhibited are means from 3 replications. Different letters in each column represent statistical difference at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and non-significant (ns) using Duncan's Multiple Range Test (DMRT)

1.3 Growth performance in rice lines/varieties under salt stress

Ten improved ‘KDMl105’ rice lines that represented better drought tolerance than ‘KDML105’ based on lower percentage reduction in biomass, were evaluated for salt tolerance in nutrient solution containing 150 mM NaCl. The least reduction in fresh biomass of 54.83%, 55.24% and 58.27% were noticed in rice lines/varieties L24, L13 and DH103, respectively (Table 4). On the other hand, the highest reduction in fresh biomass was recorded in ‘KDML105’ (83.79%), followed by CSSL103 (80.74%) and Pokkali (75.50%). After drying, the rice lines/varieties L13, L24 and DH103 had the lowest reduction in percentage of dry biomass (47.71%, 51.37% and 52.84%, respectively). These improved rice lines (L13 and L24) displayed higher level of salt tolerance than the *SKC1* donor parent (RGD4; 58.90% reduction in dry weight). However, ‘KDML105’ exhibited the highest reduction in dry biomass of 83.64%, followed by CSSL103 (79.98%) and Pokkali (75.55%), respectively (Table 4).

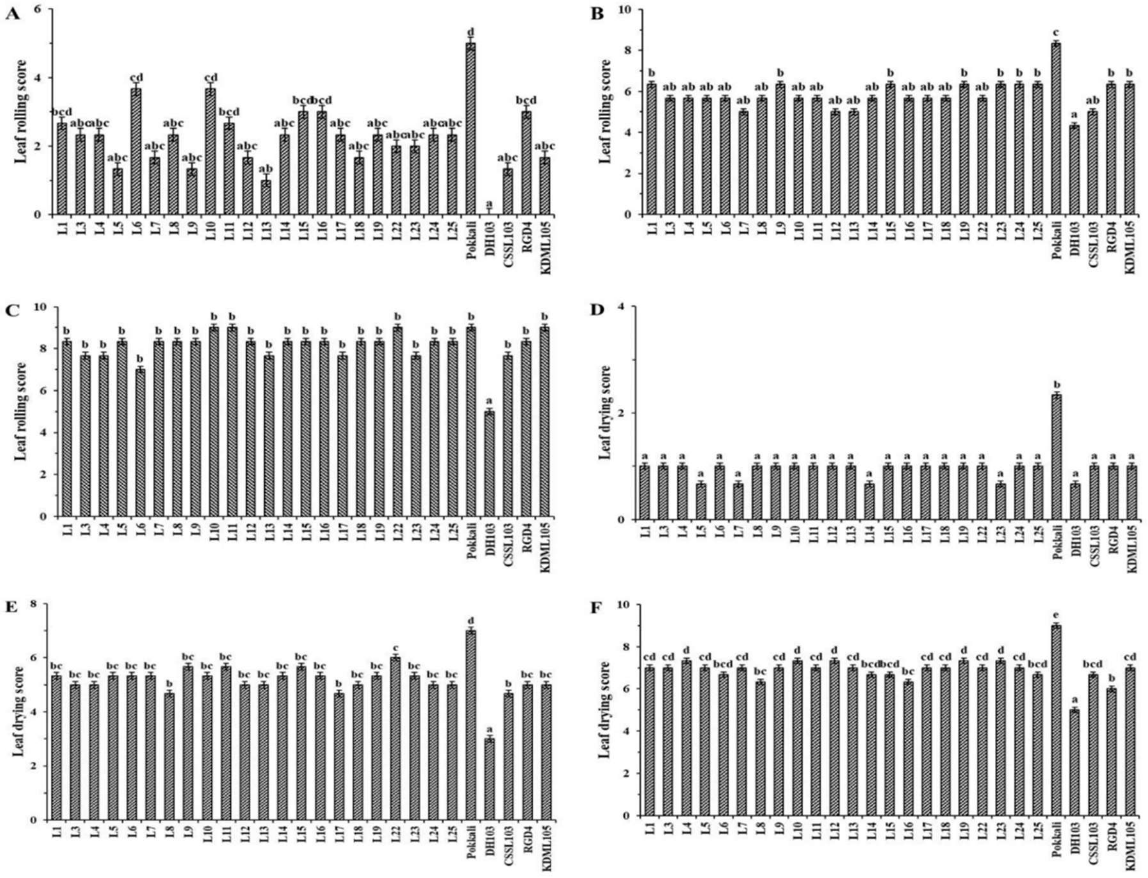

Figure 2 Leaf rolling scores of rice lines/varieties under 3 (A), 6 (B) and 9 (C) days drought stress and leaf drying scores of rice lines/varieties after drought stress for 8 (D), 16 (E) and 24 (F) days.

1.4 Evaluation scores for salt tolerance in rice lines/varieties

Salt toxicity degree in all rice varieties was determined as SIS at 5, 10 and 15 days after adding 150 mM NaCl into nutrient solutions. After 5 days of salt imposition, no significant differences in SIS were recorded among rice lines/varieties (Fig. 3A). Ten days after salt stress, the lowest SIS was found in rice lines/varieties Pokkali and RGD4 (scores of 3.00 and 4.00, respectively). Most of the improved rice lines had an injury score of 4.67 (L3, L4, L6, L7, L8, L13 and L17), whereas the higher injury scores of 6.33, 5.33 and 5.33 were noted in ‘KDML105’, CSSL103 and DH103, respectively (Fig. 3B). After 15 days, salt stress resulted in the slightest injury in Pokkali, L24 and RGD4 with the SIS of 5.00, 5.33 and 5.33, respectively. In contrast, ‘KDML105’, DH103 and CSSL103 were among the most injured from salt toxicity showing the scores of 8.67, 8.33 and 8.00, respectively. It is noted that, all ten BC_1F_2 improved ‘KDML105’ lines had significantly lower SIS than ‘KDML105’. (Fig. 2C).

Table 4 Reduction in the fresh and dry biomass of improved ‘KDML105’ rice lines and check varieties under salt stress.

Line/variety	Fresh biomass (g/plant)			Dry biomass (g/plant)		
	Control	**Salt**	**% Reduction**	**Control**	**Salt**	**% Reduction**
L3	9.33abc	3.18abc	65.48	1.97abc	0.76b-f	61.34
L4	10.55abc	3.87cd	63.50	2.40bc	0.92def	61.93
L6	10.55abc	3.23abc	68.83	2.33abc	0.78b-f	66.51
L7	10.46abc	2.70abc	72.99	2.23abc	0.64a-e	70.39
L8	9.93abc	3.02abc	70.31	2.17abc	0.74b-e	66.62
L13	7.42ab	3.08abc	55.24	1.55ab	0.75b-e	47.71
L14	13.54c	4.23cd	65.03	2.93c	1.02ef	61.37
L16	10.47abc	3.37bc	67.86	2.30abc	0.81c-f	65.10
L17	8.92abc	2.23ab	74.05	1.95abc	0.56a-d	69.81
L24	9.18abc	4.11cd	54.83	2.07abc	0.98ef	51.37
Pokkali	21.85d	5.25d	75.50	4.70d	1.13f	75.55
DH103	6.44a	1.87ab	58.27	1.36a	0.40ab	52.84
CSSL103	11.18bc	2.09ab	80.74	2.42bc	0.46abc	79.98
RGD4	8.93abc	3.17abc	63.16	2.26abc	0.84def	59.80
KDML105	10.62abc	1.68a	83.79	2.34abc	0.37a	83.64
Mean	**10.62**	**3.14**	**67.97**	**2.33**	**0.74**	**64.93**
F test	**	**	ns	**	**	ns

The data exhibited are means from 3 replications. Different letters in each column represent statistical difference at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and non-significant (ns) using Duncan's Multiple Range Test (DMRT)

1.5 Na^+ and K^+ content in rice lines/varieties

The contents of Na^+ and K^+, and Na^+/K^+ ratio in rice shoots 21 days after salt stress are displayed in Fig. 4. The drought tolerant variety DH103 showed the highest Na^+ content (4.86%) followed by ‘KDML105’ (4.09%) and L6 (3.71%) whereas the least contents were found in RGD4 (the *SKC1* donor), Pokkali (salt-tolerant check) and L24 (the improved line) with the values of 2.23%, 2.40% and 2.65%, respectively (Fig. 4A). The increased Na^+ concentration impeded K^+ uptake, resulting in high Na^+/K^+ ratios in all rice lines/varieties. The lowest Na^+/K^+ ratio was noticed in Pokkali, RGD4 and L24 (1.25, 1.38 and 1.54, respectively). In contrast, the salt sensitive lines/varieties had relatively high Na^+/K^+ ratios. The highest Na^+/K^+ ratio of 4.03 was recorded in the drought tolerant check (DH103) followed by the salt-sensitive parent 'KDML105' with the ratio of 3.84. (Fig. 4B). All ten improved lines had significantly lower Na^+/K^+ ratios than the susceptible parent, 'KDML105'. For analysis of relationship between shoot Na^+/K^+ ratio with dry biomass and SIS parameters, the results indicate that Na^+/K^+ ratio had significantly negative correlation with dry biomass (r^2 = 0.5036) (Fig. 4C), but it showed significantly positive correlation with SIS (r^2 = 0.7538) (Fig. 4D).

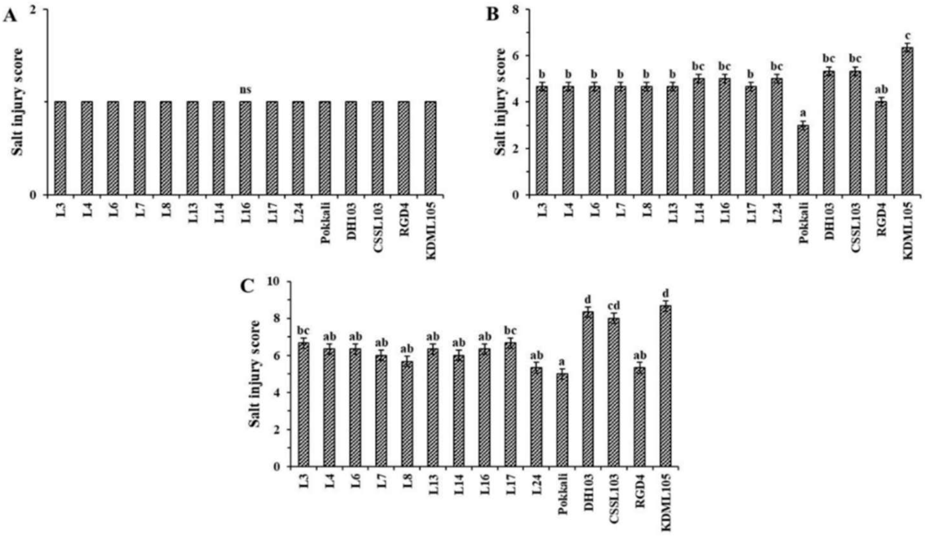

Figure 3 Salt injury scores (SIS) of rice lines/varieties at 5 (A), 10 (B) and 15 (C) days after salt imposition.

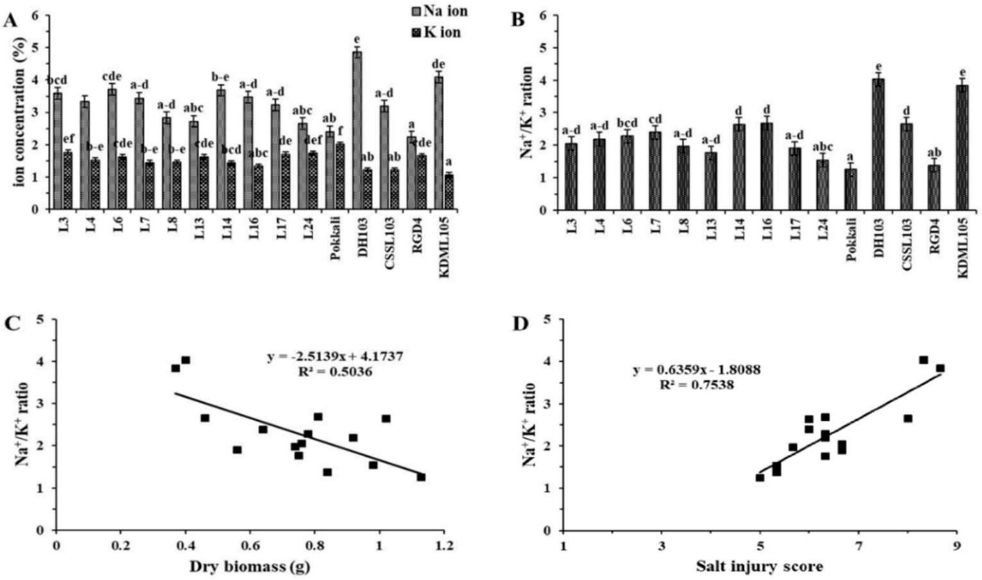

Figure 4 Na^+, K^+ contents (A), Na^+/K^+ ratio (B) of each rice shoot lines/varieties after 21 days salt treatment and correlation between Na^+/K^+ ratio with dry biomass (C) and salt injury scores (D).

DISCUSSION

Rice grown under rainfed lowland and upland conditions in the northeast Thailand are prone to drought at varying stages of development (Polthanee et al., 2014). Rice develops three main strategies namely, drought escape, drought avoidance and drought tolerance, to resist and survive under drought conditions (Fukai and Cooper, 1995). Leaf rolling and partial drying are some of the shoot mechanisms to avoid drought stress through reducing transpiration, hence maintain good water status and turgor during stress periods (Manickavelu et al., 2006). However, too much rolling and drying also reduces photosynthetic surfaces. Therefore, slow leaf rolling/drying during mild stress and moderate leaf rolling/drying at severe stress are ideal traits which allow optimal balance between photosynthetic assimilation and maintenance of plant water status (Zou et al., 2011). Leaf rolling and drying have classically been used as selection criteria for drought resistance in rice (Courtois et al., 2000). In this study, the leaves of the drought-tolerant check DH103 displayed the slowest and the slightest leaf rolling and drying and displayed the lowest percent reduction in dry biomass. Among the 22 tested $BC_1F_{2:3}$ lines, it was found that L3, L4, L13 and L17 displayed relatively low leaf rolling (9 d after stress) and drying (16 d after stress) scores similar to the female parent CSSL103 which carried DT-QTL8 from DH103 (Figs. 2C and 2E). Moreover, growth of these lines was superior to CSSL103 showing lower percentage reduction in dry biomass (Table 1). Lines L7 and L24 also showed lower percent reduction in biomass even though their leaf rolling, or drying scores were higher than CSSL103. These lines might have employed other strategies to maintain growth under drought such as osmotic adjustment or dehydration tolerance (Chandra Babu et al., 2001). The results in this study confirmed that DT-QTL8 conferred drought tolerance to the introgression lines as previously reported in which the biomass reduction was used as one of the most important parameters for evaluation of drought tolerance (Siangliw et al., 2007; Kanjoo et al., 2012; Pamuta, 2021).

Rice production in the northeast Thailand is also restrained by salinity which affected 1.84 million ha (11.5 million rai), accounting for 18% of agricultural land in this area (Pongwichian, 2016). Salinity is significant abiotic stress limiting crop growth and productivity because of the excessive absorption of salt ions, particularly Na^+ and Cl^- (Yang and Guo, 2018). The plant's ability to uptake soil water is blocked by high salt concentration, leading to an interruption in plant growth due to inhibition of cell division and expansion. Reducing dry biomass is a reliable criterion for selecting salt-tolerant genotypes (Ashraf et al., 1999). In this study, the 10 tested $BC_1F_{2:3}$ lines showed dry biomass reduction in the range of 47.71% (L13) to 70.39% (L7) which were lower than the 83.64% reduction found in ‘KDML105’ (Table 4). Moreover, L13 and L24 showed lower percent reduction in dry biomass than the male parent, RGD4 (59.80%). These results were in line with the report of Pamuta et al. (2022) that, under salt stress, shoot dry weight of ‘KDML105’ was reduced by 36% while that of two improved lines introgressed with the *SKC1* gene, showed only 12 and 16% reduction (for the lines RGD4 and RGD1, respectively). The adverse effects of NaCl on tissue injury and growth inhibition are mainly related to the toxic effects of Na^+ on cell metabolism and the adverse effects of Na^+ due to its interference with the uptake of K^+ (Munns and Tester, 2008; Parida and Das, 2005). Salt-tolerant genotypes possess greater ability for early signaling, more efficient Na^+ exclusion and compartmentalization at both cellular and whole plant level, hence efficiently maintain Na^+/K^+ homeostasis (Van Zelm, et al., 2020). Therefore, Na^+/K^+ ratio has been classically used as an indicator for screening salt tolerance ability in rice in several reports (Gregorio et al., 1997; Pamuta et al., 2022; Kanawapee et al., 2011). In this study, low Na^+/K^+ ratios were related to both low SIS (R^2 = 0.7538, Fig. 4D) and high biomass (R^2 = 0.5036, Fig. 4C). All 10 tested $BC_1F_{2:3}$ lines showed significantly lower SIS (Fig. 3) and Na^+/K^+ ratios (Fig. 4B) than 'KDML105'. The lines L13 and L24, particularly, showed similar SIS and Na^+/K^+ ratios as those of the male donor, RGD4. Previous reports also showed that the backcross introgression lines carrying *Saltol* QTL or *SKC1* gene showed higher salt tolerance ability (lower SIS, lower Na^+/K^+, and lower growth inhibition) than the parent lines (Punyawaew et al., 2016; Pamuta et al., 2022; Thanasilungura et al., 2020).

The process of selection performed in this study i.e., primary screening for drought followed by that for salt tolerance, satisfactorily fulfilled the objectives of identifying lines with dual tolerance. The unselected 12 lines from the drought screening were undesirable due to their higher biomass reduction than KDML105. If the screening steps were conducted in reverse, these 12 lines will finally be rejected even though some would possibly be tolerant of salt stress. The 10 selected lines viz., L13, L7, L24, L3, L14, L17, L8, L4, L6 and L16 were more tolerant than 'KDML105' under both drought and salt stress due to the introgression of both DT-QTL and *SKC1*. Particularly, L13 and L24 were the best preforming lines based on percentage biomass reduction. Therefore, the introgressed DT-QTL8 and *SKC1* gene effectively conferred drought and salt tolerance to 'KDML105'. However, stress tolerance ability of these lines should be further evaluated under the field conditions. Nevertheless, these two lines may be used as good genetic resources for further improvement of Thai elite rice 'KDML105' for multiple stress tolerance.

CONCLUSION

A set of 22 $BC_1F_{2:3}$ improved 'KDML105' rice lines harboring DT-QTL8 and *SKC1* gene were screened for drought tolerance, and ten lines which exhibited lower biomass reduction than 'KDML105' were subsequently evaluated for salt tolerance. All ten lines exhibited higher level of salt tolerance than 'KDML105' based on SIS, Na^+/K^+ ratio, and biomass reduction. Therefore, ten improved $BC_1F_{2:3}$ lines tolerant to both abiotic stress conditions have been identified. Performance under field conditions of these ten lines should be further investigated and the best performing lines can be further improved to obtain 'KDML105' lines with multiple resistance to abiotic stresses.

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