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Co-location of quantitative trait loci for drought and salinity tolerance in rice

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ABSTRACT

Drought and salinity stresses are major constraints limiting growth and productivity of rice plants in rainfed lowland areas of the Northeast of Thailand where Thai jasmine rice cultivar Khao Dawk Mali 105 (KDML105) is planted as majority. In this study, chromosome segment substitution lines (CSSL) of the KDML105 that carry quantitative trait loci (QTL) associated with drought tolerance (DT) located on chromosomes 1, 3, 4, 8 and 9 were evaluated for salinity tolerance (ST) at seedling stage. Seven CSS lines exhibited higher ST than the recipient KDML105. Some of these CSS lines displayed higher grain yield than KDML105 when tested in rainfed lowland. Eight QTL for ST were identified on chromosomes 1, 3, 4, 7, 8, 9, 10 and 12. Of these, four are located in the same position of the DT-QTL previously reported. The co-locations of DT and ST QTL may indicate a common mechanism for tolerance. In addition, CSSLs showing good performances under both drought and salinity stresses can be used as a new source of breeding materials in Thai rainfed lowland areas.

Keywords: drought tolerance, salinity tolerance, QTL, rice, chromosome segment substitution lines

INTRODUCTION

Drought and salinity stresses are the most important constrain limiting rice growth

and productivity in the rainfed lowland areas. In Thailand, drought and salinity stresses are a major constrain in rainfed lowland areas of Northeastern region where a famous Thai jasmine rice cultivar KDML105 is planted as the major rice crop. In rainfed lowland of the Northeast, drought stress often occurs during the rainy season causing salinity stress because salt move up to the soil surface by capillary force. To increase rice production in the rainfed lowland, rice cultivar with drought and salinity tolerance must be developed and used.

Drought tolerance (DT) is a complex trait in rice. DT is reportedly controlled by many genes (Kamoshita *et al.*, 2008). Environment also strongly affects DT (Fukai and Cooper, 1995). Quantitative trait loci (QTL) associated with DT were identified and mapped to different rice chromosomes in various mapping populations (Zeng *et al*., 2006; Kamoshita *et al*., 2008). Lanceras *et al*. (2004) reported DT-QTL located on chromosomes 1, 3, 4, 8 and 9 in the CT9993 x IR62266 doubled haploid population. Co-localized DT-QTL in each chromosome generally cover a large genomic segment on rice chromosome and also co-localized with other QTL associated with morpho-physiological traits such as plant height (Fang and Wu, 2001), soluble protein content (Ishimaru *et al*., 2001) and osmotic adjustment capacity (Robin *et al*., 2003).

Salinity tolerance (ST) is also a complex trait. However traits associated with ST had shown a high heritability value (Gregorio and Senadhira, 1993). ST is reportedly controlled by many genes (Gregorio and Senadhira, 1993) and co-inherited with undesirable agronomic characters (Heu and Koh, 1991). The QTLs for ST were reported on chromosomes 1, 3, 5, 6 and 7 (Gong *et al*., 1999; Koyama *et al*., 2001; Lee *et al.*, 2007). Many genes responds to both drought and salinity stresses were identified in many plant species such as wheat (Peng *et al*., 2009) and rice (Rabbani *et al.*, 2003). More than fifty genes were activated and expressed under both drought and salinity conditions. Of these, more than half response to abscisic acid (ABA) hormone (Rabbani *et al.*, 2003).

To understand genetic mechanism for DT and ST in rice, chromosome segment substitution lines (CSSL) of the KDML105 carrying quantitative trait loci (QTL) associated with drought tolerance (DT) located on chromosomes 1, 3, 4, 8 and 9 were developed by marker assisted backcrossing (MAB) (Kanjoo *et al.*, 2007; Toojinda *et al.*, 2011). The CSSLs have been tested for agronomic performance under drought stress and irrigated conditions. Variations of agronomic traits were observed in both conditions. Some of the CSSL showed higher grain yield than the recipient parent, KDML105 under drought stress. This result demonstrated that CSSLs can be a useful material to dissect genes underlay the DT (Toojinda *et al.*, 2011). In addition, CSSLs can be used as mapping population to identify QTLs for the complex traits of interest such as

flowering dates (Kubo *et al.*, 2002; Ebitani *et al*., 2005), root trait (Suralta *et al*., 2008), grain weight (Bian *et al*., 2010) and culm length. In this study, CSSLs carrying overlapping genomic fragments on chromosomes 1, 3, 4, 8 and 9 from DT donor were evaluated for salinity tolerance. Genotypic-phenotypic association was used to identify QTL for ST in this study. CSSLs that performed well under both drought and salinity stresses were identified and will be used as a new source of breeding materials in Thai rainfed lowland areas.

MATERIALS AND METHODS

Plant materials

A set of 90 CSSLs consisted of 82 haplotypes (carrying different DT-QTL segments inherited from IR68586-F_2-CA-31 (DHL103) and IR68586-F_2-CA-143 (DHL212) was developed by marker assisted backcrossing (Kanjoo *et al.*, 2007; Toojinda *et al.*, 2011). Out of 90 CSSLs, 28, 15, 23, 20 and 10 CSS lines carry the DT-QTL located on chromosomes 1, 3, 4, 8 and 9 respectively. Genome scanning indicated that these CSSLs recovered up to 96.3% of KDML105 background genome (Toojinda *et al.*, 2011). Based on the QTL mapping experiments conducted by (Lanceras *et al.*, 2004), the chromosome segments harboring the DT QTL spanned cover 49, 14.8, 53, 60 and 30 cM respectively. QTLs for grain yield (GY) and important agronomic traits that related with drought stress (Kamoshita *et al.*, 2008) were also reportedly mapped to these regions.

Evaluation for salt tolerance

The evaluation trial was conducted in a greenhouse at Rice Gene Discovery Unit, Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom province, Thailand during April to June 2011. The experiment was conducted at young seedling stage in artificial salinity soil culture under 12 dS/m NaCl (150 mM Na^+) salinized nutrient solution. CSSLs and checks including Pokkali, CT9993, IR62266, DHL103, DHL212, IR29 and KDML105 (Table 1) were assigned following a randomized complete block design (RCBD) with 4 replications (six seedlings of each line per replication). The plant materials were directly germinated in soil tray that has 6 x12 wells containing wet soil of 1.5 in^3 (one seed per well). Two plant nutrient solutions were applied with a ratio 1:200 following the instruction from Bangsai Agricultural Center Co. Ltd. After 19 days of germination, 18.5 dS/m (150 mM Na^+) were added into the solution and the solution concentration was maintained by adding water every day to replace evapotranspirational losses. After 10, 16 and 21 days after salt treatment, salt injury was scored 1 to 9 (based on 6 plants per genotype) following the standard evaluation system for salinity tolerance at seedling stage in rice (IRRI, 2002). Salt injury score (SIS) was determined following IRRI (2002)

standard: 1 = Growth and tillering nearly normal and 9 = almost all plants dead or dying.

Table 1 Nine rice cultivars were used as a standard check in salt screening.

Cultivar names	Information
KDML105	Use as recipient background in developing of CSSLs, reported as susceptible variety to drought and salt stresses by Rice Department
CT9993	Parental variety of mapping population used for identification of QTL for drought tolerance
IR62266	Parental variety of mapping population used for identification of QTL for drought tolerance
DHL103	Donor variety used in development of CSSLs
DHL212	Donor variety used in development of CSSLs
IR29	Standard check for salt intolerance
Pokkali	Standard check for salt tolerance
FL496	Donor variety used in development of salinity tolerance in breeding program
FL530	Donor variety used in development of salinity tolerance in breeding program

Evaluation for agronomic characteristics

Seven CSSLs showing salt tolerant phenotype were evaluated for agronomic charactersistics under two different conditions, irrigated (non-stress) and rainfed (stress). The experiment was conducted at Chumphae Rice Research Center (CPA, Northeast of Thailand) in 2009. The CSSL and checks were assigned to specific plots following a randomized complete block design (RCBD) with 4 replications. Plot size was 2 x 2.50 m with each plot consisted of 10 rows with fifteen plants per row and had a planting density of 20 cm between plants (within a row), and 20 cm between rows. The field were fertilized by hand broadcasting with 120 kg/ha of urea, 75 kg/ha of P_2O_5 and 75 kg/ha of K_2O. Weed control was performed using hand weeding. For non-stress condition, all plots received sufficient water by irrigation. Field water was maintained during the tillering stage at approximately 10 cm until 10 days before harvesting. In rainfed condition, water was drained out from the field at 77 days after sowing (DAS) which is about 10 day before panicle initiation. Traits collected from CSSLs include days to flowering (DF), plant height (PH), tiller number (TN), panicle number (PN), grain yield (GY), filled grain weight (FGW), unfilled grain weight (UFGW), total grain weight (TGW), filled grain number (FGN), unfilled grain number (UFGN), total spikelet number (TSN), percent spikelet sterility (PSS) and 1,000-grain weight (1000GW). Procedures used by Lanceras *et al.* (2004) were used in collecting these traits.

DNA analysis

Rice genomic DNA was extracted from the young leaves using DNA-Trap® method as

described by Vanavichit (2007). The PCR reaction was carried out with a total volume of 10 µl containing 20 ng of genomic DNA, 0.02 µM of each primer, 0.2 mM each of dNTPs, 2.5 mM $MgCl_2$, 0.2 unit *Taq* polymerase, and 1X PCR buffer. Amplification was performed for 35 cycles (30 sec at 94°C, 30 sec at 55°C and 1 min at 72°C) followed by a final extension of 5 min at 72°C. Amplified products were separated by 4.5% denaturing polyacrylamide gel electrophoresis and were detected by the silver staining method as described by Caetano (1997) and Yi *at al*. (2009).

One hundred and thirty one SSR markers distributed throughout the 12 chromosomes of rice and showed polymorphisms between recipient parent, KDML105 and donor parents DHL103 and DHL212 were used to scan the genomic composition of the CSSLs. This information was used to estimate the proportion of donor genome remaining in the genome of each CSSL.

Data analysis

All traits were subjected to statistical analysis using the STATGRAPHIC 3.0 software programs. Analysis of variance (ANOVA) was calculated based on RCBD. Least significant difference (LSD) was determined at one and five percent probability levels to make the comparison among CSSLs and between the mean values of the CSSLs and KDML105. QTLs for salinity and drought tolerance traits (ST and DT-QTL) were constructed by single marker analysis.

RESULTS

Evaluation for salt tolerance

Figure 1 showed the SIS of the CSSLs and check varieties at 16 and 21 days after treatment. Variation was clearly observed among CSSLs after 16 days. The SIS mean value of individual CSSL ranged from 5.71 to 8.29 score with an average of 7.01 score that was lower than that of KDML105 (7.52 score). At 21 days, the tolerance lines still exhibited the low SIS score. The donor parents, DHL103 and DHL212 showed lower SIS score (6.25 and 6.58) than the recipient parent KDML105. Other check varieties Pokkali, CT9993, IR62266, FL496 and FL930 showed a tolerant phenotype (low SIS) except for IR29 that showed a susceptible phenotype (high SIS).

QTL analysis

Based on the genotypic information, individual CSSL recovered 88.50 to 100% of recipient genome (KDML105) with an average of 96.30% in the non-target regions. This information indicated that the proportion of donor alleles remaining in the non target regions is very low. The frequency of donor alleles in the non target regions of each chromosome ranged from 0.09% on chromosome 10 to 10.72% on chromosome 4, with an average of 3.70% (data not showed). Twenty six markers showed significant association with SIS (P<0.05).

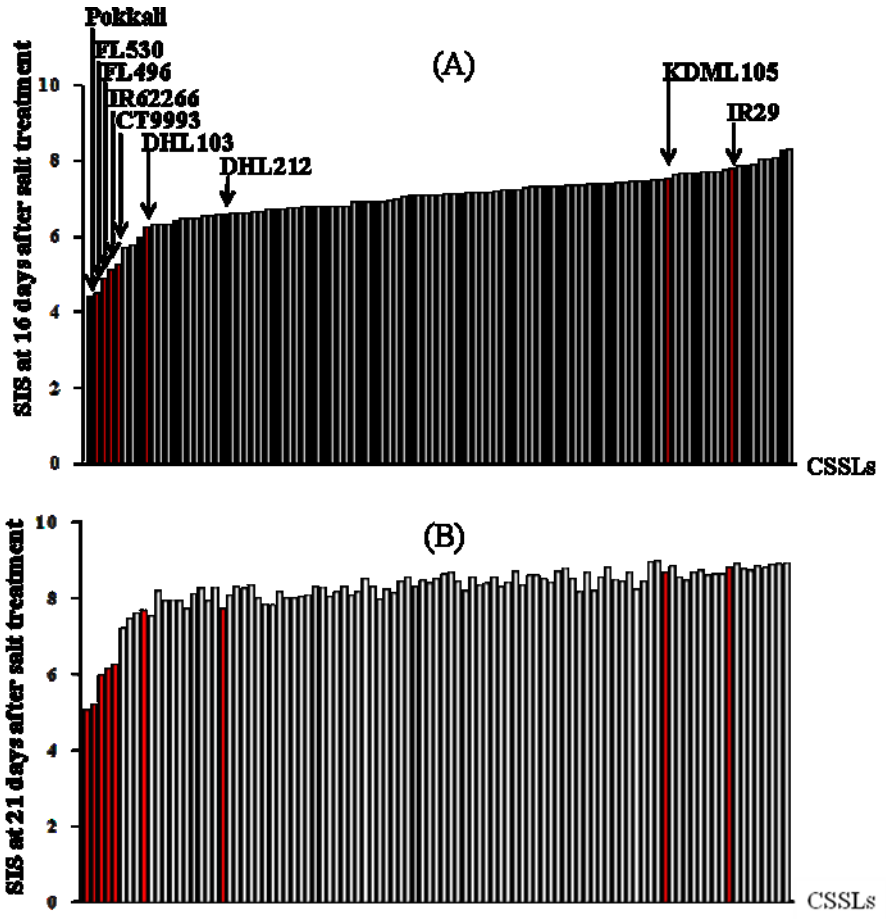

Figure 1 The SIS mean value of 90 CSSLs (gray bars) and nine standard cultivars (dark bars) after exposed to 150 mM NaCl for 16 (A) and 21 (B) days after salt treatment.

These markers were located on chromosomes 1, 3, 4, 7, 8, 9, 10 and 12. A large genomic segment on chromosome 1 (RM1003-RM5794 interval; 9.6 Mbp), was found to be associated with SIS while small genomic segments were significantly associated in the other chromosomes (Table 2).

Only seven CSSLs showed a significant difference (P<0.001) from the KDML105. The SIS of these lines (CSSL-11, -15, -16, -59, -73, -79, and -80) was lower than KDML105 but higher than CT9993 and IR62266. Table 3 showed the genotypes of seven CSSLs at the detected QTL for SIS.

Evaluation for agronomic characteristics

Agronomic performance of the CSSLs and KDML105 under irrigated and rainfed conditions was presented in Table 4. In irrigated condition, CSSLs and KDML105 were not significant differed for most of the measured traits except for DF, PH, UFGW, UFGN, TSN and PSS. CSSL-11, CSSL-15, CSSL16 and CSSL-59 have higher value of UFGN and PSS than that of KDML105 in

which they lead to lower GY (although GY of individual CSSLs was not significant different from KDML105). In rainfed condition, significant differences for DF, PH, GY, UFGW, UFGN, PSS were observed among the CSSLs and KDML105. CSSL-59, CSSL-73 and CSSL-79 exhibited higher GY than KDML105, while GY of CSSL-11, CSSL-15 and CSSL16 showed no significant difference from that of KDML105. CSSL-59 higher values of FGW, FGN and TSN than KDML105.

Table 2 Putative QTL for SIS and corresponding phenotype-genotype association analysis using single marker analysis in CSSLs.

Chr.	SSR marker	SIS (16 days)		P-value
		KDML105	DHL212 or DHL103	
1	RM302	7.18	6.75	0.0107
	RM7594	7.16	6.72	0.0337
	RM3442	7.16	6.73	0.0091
	RM1003	7.16	6.58	0.0088
	P-3	7.16	6.73	0.0069
	RM5759	7.16	6.73	0.0069
	RM1361	7.16	6.73	0.0069
	RM6827	7.16	6.73	0.0069
	RM3468	7.16	6.76	0.0108
	RM3520	7.16	6.76	0.0108
	RM529	7.18	6.7	0.0012
	RM104	7.17	6.77	0.0080
	RM5794	7.15	6.84	0.0437
	RM3482	7.16	6.60	0.0046
	RM315	7.18	6.69	0.0018
	RM8134	7.18	6.79	0.0113
3	RM6883	7.14	6.73	0.0448
	RM2421	7.16	6.69	0.0022
4	RM335	7.06	7.49	0.0161
7	RM70	7.1	6.48	0.0131
	RM234	7.1	6.48	0.0131
8	RM5353	7.15	6.74	0.0260
9	RM215	7.81	7.17	0.0152
	RM245	7.11	5.78	0.0097
10	RM304	7.11	5.78	0.0097
12	RM102	7.19	6.68	0.0052

Table 3 Genotypes of seven CSSLs expressing salinity tolerance after 16 days of salt treatment and showed SIS mean values significantly different from KDML105 by $LSD_{0.05}$ test. Homozygous donor and KDML105 alleles are shown as plus and minus symbols, respectively.

Lines		SSR loci																						
	Chr.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	7	8	10	12
	SIS	RM302	RM7594	RM3442	RM341	RM3602	RM1003	P-3	RM5759	RM1361	RM6827	RM3468	RM3520	RM529	RM104	RM5794	RM3482	RM315	RM8134	RM273	RM234	RM5353	RM304	RM102
CSSL-11	**5.71** ^{bcd}	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+
CSSL-15	**6.34** ^{cdefgh}	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+
CSSL-16	**6.32** ^{cdefg}	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+
CSSL-59	**5.78** ^{bcd}	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-	+
CSSL-73	**5.95** ^{bcde}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
CSSL-79	**6.37** ^{defghi}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-
CSSL-80	**6.30** ^{cdefg}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-
KDML105	**7.52** ^{j}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DHL103	**6.25** ^{cdef}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DHL212	**6.58** ^{defghij}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pokkali	**4.42** ^{a}	Standard variety tolerant to salinity stress																						
IR29	**7.79** ^{j}	Standard variety susceptible to salinity stress																						

Note: ^{abcdefghij} represent significant differences in SIS among CSS lines and KDML105

DISCUSSIONS

QTL locations of traits for salinity tolerance identified in the CSSL population revealed 8 chromosomal segments which include chromosomes 1, 3, 4, 7, 8, 9, 10 and 12. In chromosome 1, the QTL for SIS located near RM302 was the same chromosomal location of salt tolerance QTL that was identified by Thomson *et al*. in 2010. Moreover, Kim *et al*. (2009) found a QTL for salt tolerance near RM302 using introgression lines generated between japonica rice cultivars. It can also be observed that the QTL identified in chromosome 3 near RM2421 was the same position of QTL for Na^+/K^+ ratio and seedling salinity tolerance that were identified by Sabouri *et al*. (2009) and Lee *et al*. (2007), respectively. Similar results were obtained for QTL in chromosomes 4, 7, 9 and 10, wherein the QTL for SIS using the KDML105 CSSL population co-locates with QTL for Na+ concentration, K^+ uptake and Na^+/K^+ ratio in chromosome 4 (Koyama *et al.*, 2001) and salt tolerance in chromosomes 7, 9 and 10 (Kim *et al.*, 2009; Thomson *et al.*, 2010; Alam *et al.*, 2011). Salinity tolerance or low injury score may be related with the capacity to uptake Na+ and by the level by which the presence of these salts can be tolerated in the cell. This probably explains the co-location of QTL for SIS with other traits related to salinity tolerance. Other chromosomal locations (8 and 12) for SIS may be considered as new QTL positions which were revealed using this population and has important contribution to salinity tolerance.

Table 4 Phenotypic value of agronomic traits under irrigated and rainfed conditions of seven CSSLs showing salinity tolerance at seedling stage.

Lines	Irrigated condition												
	DF	**PH**	**TN**	**PN**	**GY**	**FGW**	**UFGW**	**TGW**	**FGN**	**UFGN**	**TSN**	**PSS**	**1000GW**
CSSL-11	96	81.6	8	8	225.4	6.8	0.9	7.7	72	28	100	28.1	23.9
CSSL-15	97	82.1	8	7	229.5	6.2	0.8	7.0	59	35	94	36.8	24.2
CSSL-16	98	80.4	7	6	223.1	7.5	0.8	8.4	67	35	101	34.6	24.1
CSSL-59	96	102.0	8	7	267.8	7.9	0.7	8.7	84	37	121	31.0	25.3
CSSL-73	95	97.1	7	7	265.9	7.8	0.5	8.3	76	16	92	17.6	24.2
CSSL-79	95	90.0	7	6	212.3	6.7	0.5	7.2	73	18	91	20.0	24.1
CSSL-80	94	94.3	7	7	248.1	5.6	0.3	5.9	81	17	97	17.5	24.6
KDML105	97	98.3	7	7	256.2	7.3	0.5	7.8	76	18	94	20.0	24.3
Mean	96	90.7	8	7	241.0	7.0	0.6	7.6	73	26	99	25.7	24.3
F-Test	*	**	ns	ns	ns	ns	**	ns	ns	***	**	**	ns
$LSD_{0.05}$	2	6.3	2	2	50.9	2.1	0.3	2.3	16	10.4	15	9.9	0.9
%CV	1.7	4.7	18.1	23.3	14.4	20.4	33.5	20.4	15.0	27.7	10.0	26.2	2.6

Lines	Rainfed condition												
	DF	**PH**	**TN**	**PN**	**GY**	**FGW**	**UFGW**	**TGW**	**FGN**	**UFGN**	**TSN**	**PSS**	**1000GW**
CSSL-11	96	84.8	7	6	171.0	7.6	1.4	9.0	73	35	108	34.8	21.8
CSSL-15	96	88.0	7	6	192.7	9.0	1.4	10.4	69	42	112	37.8	22.4
CSSL-16	96	88.0	8	7	192.4	9.2	1.8	11.1	63	47	110	42.8	22.1
CSSL-59	95	111.1	7	6	226.9	10.5	2.1	12.6	90	49	138	34.6	22.9
CSSL-73	93	109.8	8	7	253.3	11.2	0.9	12.1	91	19	110	18.3	22.3
CSSL-79	94	103.6	9	8	256.9	10.8	0.7	11.5	83	26	109	23.9	22.5
CSSL-80	94	101.3	7	5	193.4	8.4	0.8	9.1	96	26	123	21.4	22.7
KDML105	95	100.9	8	7	179.1	8.7	1.3	10.0	81	38	119	31.7	21.5
Mean	95	98.4	8	7	208.2	9.4	1.3	10.7	81	35	116	30.7	22.3
F-Test	**	***	ns	ns	*	ns	***	ns	ns	**	ns	**	ns
$LSD_{0.05}$	2	6.2	2	2	60.7	3.5	0.6	3.4	24	16	22	13.1	0.9
%CV	1.2	4.3	15.9	20.1	19.8	25.3	29.2	21.8	20.0	30.2	13.1	29.1	2.7

Note: Agronomic traits were measured from CSSLs including days to flowering (DF), Plant height (PH), tiller number (TN), panicle number (PN), grain yield (GY), filled grain weight (FGW), unfilled grain weight (UFGW), total grain weight (TGW), filled grain number (FGN), unfilled grain number (UFGN), total spikelet number (TSN), percent spikelet sterility (PSS) and 1,000-grain weight (1000GW)

The co-location of drought and salinity tolerance QTL on chromosomes 1, 3, 4, 8 and 9 may reflect the possibility of similar mechanisms in order to survive drought and salinity stresses. One obvious relationship between drought and salinity tolerance is the primary effect these stresses impose which is osmotic stress. One mechanism of ST in rice is osmotic adjustment which the plant uses by solute accumulation to prevent water loss from the cell by the osmosis process. Several solutes can be accumulated as osmoprotectant such as sugar, glycine betaine and proline and these solutes were used to balance osmotic pressure in the plant cell. In rice, osmotic adjustment for drought tolerance was mapped in chromosome 8 (Lilley *et al.*, 1996; Zhang *et al.*, 2001) which is near the chromosomal location of QTL for SIS in this study. Under salinity stress, osmotic stress tolerance is one

of the adaptations of plants to salinity. The production of osmoprotectants could be one of the mechanisms involved both drought and salinity tolerance. Rabbani *et al*. (2003) reported that the amount of expressed genes responsive to salinity and drought conditions were approximately 57 and 62 genes, respectively, and of these 56 genes show the same genes that are responsive to the hormone ABA. From this study, it is possible that one mechanism that has the salinity and drought tolerance in rice is osmotic adjustment. More studies have to be performed to determine if common mechanism for stress tolerance is present in other chromosomes wherein QTL for drought and salinity tolerance were found.

Under irrigated conditions, 7 CSS lines with salinity tolerance showed no difference in GY when compared with KDML105. Significant difference was observed between KDML105 and CSSL-73 and CSSL-79 in terms of GY under rainfed condition. Both CSSL-73 and 79 contain target segments in chromosome 8. The increase in GY in these two lines may be attributed to less UFGN and low PSS under stress. In addition, CSSLs carrying chromosome 1 segment had lower GY (not significantly different from KDML105) compared with CSSLs with chromosomes 4 and 8; due to higher PSS. High GY and low PSS are good indices of tolerance under drought and salinity stresses. Therefore, lines carrying chromosome 8 segment may be used as a good source of breeding line for rainfed lowland areas where both drought and salinity stresses often occurred. In the case of CSSLs carrying chromosomes 3 and 9, performance under drought stress may be satisfactory but may not be used under salt affected areas in rainfed lowland.

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