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F_2 bulk segregant analysis reveals salt-tolerant QTLs related to the ubiquitination process

Susinya Habila[1,2], Nopphakhun Khunpolwattana[1], Teerapong Buabooch[1,3,4], Isabelle M. Henry[5], Luca Comai[5], Monnat Pongpanich[4,6], Kitiporn Plaimas[4,6], Duangjai Suriya-Arunroj[7], Meechai Siangliw[8], Waraluk Kasettranun[9], Putut Rakhmad Purnama[10] & Supachitra Chadchawan[1,4]✉

The growth and yield of rice, a major global food crop, are impaired by salt stress. This study aimed to identify quantitative trait loci (QTLs) associated with salt tolerance at the seedling stage. Bulk segregant analysis (BSA) of an F_2 population from a cross between 'Jao Khao' (salt-tolerant) and IR29 (salt-susceptible) identified four QTLs on chromosomes 2, 3, 6, and 12. Four markers linked to these QTLs were validated in an F_3 population, showing strong correlations (r = 0.7–0.8) with membrane stability, relative water content, and salt injury scores. Single nucleotide polymorphism (SNP)-by-SNP interactions across parental lines and four additional salt-tolerant and four salt-susceptible varieties revealed significant enrichment of the "plant hormone signal transduction" pathway, identifying *LOC_OS03G49990 (OsSLR1)* encoding DELLA, as a key gene. Comparative analysis of BSA-predicted QTLs with weighted gene co-expression network analysis-based key genes suggested *LOC_Os02g02830* (ubiquitin-conjugating enzyme E2 28) and *LOC_Os06g06170* (RING-type E3 ubiquitin transferase) as potential candidate genes. SNP association analysis across ten cultivars identified significant quantitative trait nucleotides on chromosomes 1, 2, 3, and 9, notably involving *OsFBOX72* (F-box-type E3 ubiquitin ligase L7). These findings highlight the role of ubiquitination and hormone signalling in salt tolerance, providing potential targets for marker-assisted breeding.

Keywords Salt tolerance, *Oryza sativa*, Quantitative trait loci, Genetic mapping, Rice breeding, Marker-assisted selection

Rice (*Oryza sativa* L.) is a vital staple consumed in almost every household globally[1]. However, rice cultivation faces multiple biotic and abiotic stresses that impair growth, development, and yield[2]. Salt stress is a major abiotic factor, particularly damaging during seedling and early reproductive stages[3]. It causes osmotic and ionic stress, disrupting metabolism[4], inducing cell death[4], increasing leaf temperature[5], inhibiting growth via stomatal closure[5], reducing germination[6], impairing seedling establishment[7], and damaging chloroplasts[8]. These effects lower photosynthetic rate[9], seed set[10], and grain yield[10], prompting intensified research into salt-tolerant cultivars.

Several studies have identified quantitative trait loci (QTLs) and putative candidate genes associated with salt tolerance in various crops using molecular markers and high-throughput sequencing[11–13]. Genome-wide association studies (GWAS) and transcriptome analysis have identified several genomic regions harboring

[1]Center of Excellence in Environment and Plant Physiology (CEEPP), Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand. [2]Department of Plant Science and Biotechnology, Faculty of Natural Science, University of Jos, Jos North 930003, Plateau State, Nigeria. [3]Center of Excellence in Molecular Crop, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand. [4]Omics Sciences Center, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand. [5]Genome Center and Department of Plant Biology, UC Davis Genome Center, UC Davis, Davis, CA 95616, USA. [6]Department of Mathematics and Computer Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand. [7]Rice Department, Ministry of Agriculture and Cooperation, Bangkok, Thailand. [8]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Phahonyothin Rd. Khlong Nueng, Khlong Luang, Pathumthani 12120, Thailand. [9]Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand. [10]Department of Biology, Faculty of Mathematics and Natural Science, State University of Surabaya, C14 Building, Campus I Ketintang, Surabaya 60231, Indonesia. ✉email: Supachitra.c@chula.ac.th

Sample ID	CMS_Sen	CMS_Tol	RWC_Sen	RWC_Tol	IR29	Jao Khao
Total read no	570,480,044	608,903,536	477,600,276	594,684,642	415,838,871	576,267,830
Clean read no	569,982,245	607,882,384	473,013,577	593,135,716	415,171,522	366,944,807
Mapped read no	562,045,869	599,954,587	465,583,693	585,413,015	409,368,252	361,909,355
Read map percentage	98.61	98.70	98.43	98.70	98.60	98.63
GC (%)	43.00	43.00	43.00	43.00	43.00	43.00
Genome coverage (×)	178.83	182.32	147.55	178.83	143.81	124.64
Average depth (×)	27.01	28.87	22.25	28.67	19.56	17.28

Table 1. Summary of NovaSeq sequencing data. CMS, cell membrane stability; RWC, relative water content; sen, sensitive; tol, tolerant.

Sample ID	Total SNPs	Failed SNPs	Passed SNPs
IR29 vs. JK	6,518,593	5,753,310	765,283
CMS_sen vs. CMS_tol	6,189,220	5,326,951	862,269
RWC_sen vs. RWC_tol	6,151,227	5,254,262	896,965

Table 2. SNPs after quality filtering.

potential candidate genes associated with traits of interest[14–21]. However, these approaches can be costly for large populations, particularly when whole-genome sequencing of each individual is required.

Bulk segregant analysis (BSA) is a cost-effective alternative that involves pooling DNA from individuals of a population with contrasting phenotypes and sequencing the bulk DNA to identify genetic markers and locate QTLs linked to the traits of interest[22]. Since its introduction in 1991[23], BSA has led to the discovery of several genomic regions containing genes of interest associated with various traits in several crops[24–34] In rice, BSA has been successfully used to identify putative markers, QTLs, and genes involved in brown planthopper pest resistance[35], basal resistance to blast disease[36], and heat tolerance[37]. Key QTLs underlying salt tolerance have also been identified employing BSA using biparental recombinant inbred lines[38] coupled with transcriptome analysis[39].

Rice plays a significant role in the economy of Thailand. Over time, Thai farmers have selected local varieties adapted to regional climates, soil conditions, and consumer preferences. The rice seed bank of Thailand preserves diverse germplasms from across the country, some of which have been evaluated for salt tolerance[17,40]. Certain varieties such as 'Jao Khao' have been identified as salt-tolerant, with comparable levels of performance to the standard salt-tolerant variety 'Pokkali'[40,41]. However, the genetic basis of 'Jao Khao's' salt tolerance remains largely uncharacterized. Identifying the underlying QTLs and candidate genes is essential for developing high-yielding, salt-tolerant rice varieties adapted to local conditions.

In the present study, we used BSA to identify putative salt-tolerant regions in an F_2 population generated from a cross between 'Jao Khao' and IR29, a salt-susceptible line. Subsequently, we developed putative markers linked to the identified QTLs and validated their association with salt-tolerance. Key genes within the identified QTLs were predicted based on the results of the single-nucleotide polymorphism (SNP)-by-SNP epistasis interactions using data from the salt-tolerant and salt-susceptible cultivars[40] and the pathway enrichment analyses. Additionally, the predicted genes within the identified QTLs were compared with previously reported key genes identified using weighted gene co-expression network analysis in indica rice[42]. Gene expression differences between 'Jao Khao' and IR29 were performed to confirm the involvement of the predicted genes in salt tolerance. This study will provide valuable insights into the genetic basis of salt tolerance in 'Jao Khao' and offers potential targets for marker-assisted breeding programs.

Results

Genomic DNA pool and sequence analysis

Cell membrane stability (CMS) and relative water content (RWC) traits assessed in a previous study[41] were used for the BSA. Whole-genome sequencing of DNA samples from the salt-susceptible (sen) and salt-tolerant (tol) pools (Supplementary Table S1) of F_2 individuals yielded more than 4×10^8 raw reads. The raw sequencing data were deposited in the National Center for Biotechnology Information (BioProject accession number PRJNA1148750). After quality filtering, the reads were mapped against the Nipponbare reference genome from the Rice Genome Annotation Project website[43], with > 98% of reads successfully aligned across all samples. Mapping percentages and sequencing depth are shown in Table 1.

BSA predicted four putative salt tolerance QTLs

Genomic regions associated with phenotypic variation in the F_2 population and the parental lines were identified based on the ΔSNP index calculated from the sen and tol pools. The total number of SNPs and InDels identified across the three pairs of comparisons was approximately 6 million. The number of SNPs after quality filtering and suitable for BSA is presented in Table 2.

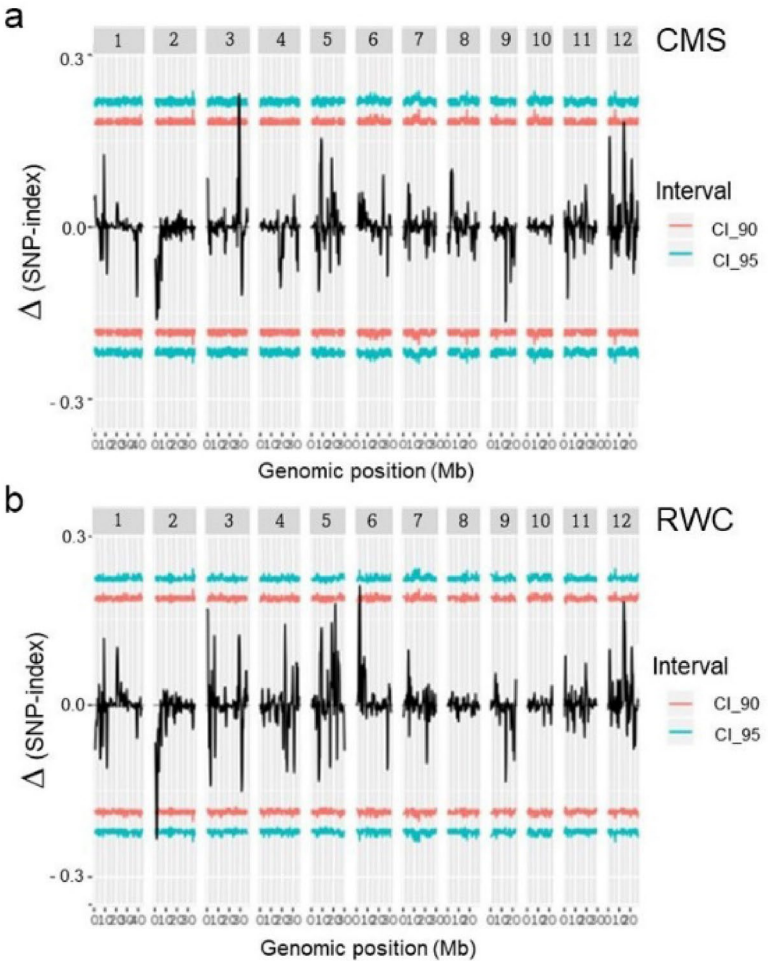

Fig. 1. Bulk segregant analysis showing ΔSNP index for CMS (**a**) and RWC (**b**) traits. The blue and red lines show the significance level. CMS, cell membrane stability; RWC, relative water content.

Trait	Chro	QTL	Start	End	Length	SNPs	Avg. SNPs (Mb)	Peak Delta SNP	Pos. Peak Delta SNP	Avg. Delta SNP
CMS	3	qCMS3.1	28,440,624	29,091,937	651,313	2454	3768	0.23182	28,732,185	0.226957
RWC	2	qRWC2.1	876,665	1,695,236	818,571	3236	3953	−0.233645	1,124,148	−0.218032
RWC	6	qRWC6.1	2,667,526	2,942,293	274,767	420	1529	0.208464	2,689,127	0.189649
RWC	12	qRWC12.1	14,383,602	14,395,028	11,426	191	16,716	0.18041	14,392,745	0.1803

Table 3. Summary of the identified QTLs associated with CMS. CMS, cell membrane stability; RWC, relative water content.

BSA of the F_2 population predicted significant loci associated with CMS and RWC (Fig. 1a, b). The DSNP index for CMS predicted only one significant QTL on chromosome 3, named as qCMS3.1 (Fig. 1a). In contrast, three significant QTLs were identified for RWC, located on chromosomes 2, 6, and 12, and named qRWC2.1, qRWC6.1, and qRWC12.1, respectively (Fig. 1b). The genomic positions of the four QTLs are summarized in Table 3, and the list of genes annotated within these QTLs is shown in Supplementary Table S2.

Phenotypic and molecular validation of identified QTLs for salt tolerance in the F_3 population

The distributions of phenotypic traits under salt stress in the F_3 population, standard evaluation score (SES), CMS, and RWC are shown in Fig. 2. After 12 days of salt stress treatment, IR29 seedlings exhibited an SES of 9, whereas both 'Jao Khao' and 'Pokkali' displayed an SES of 4 (Fig. 2a). For CMS and RWC, susceptible seedlings did not survive under 12 dS/m for 12 days; thus, these traits were scored as zero in sensitive seedlings. In contrast, salt-tolerant varieties, including 'Jao Khao' and 'Pokkali', maintained approximately 80% for both CMS and RWC (Fig. 2b).

Markers were designed within the QTL regions based on SNP differences between the parental varieties, 'Jao Khao' and IR29. These primers were tested on the parental genomes and the 'Pokkali' genome. Different

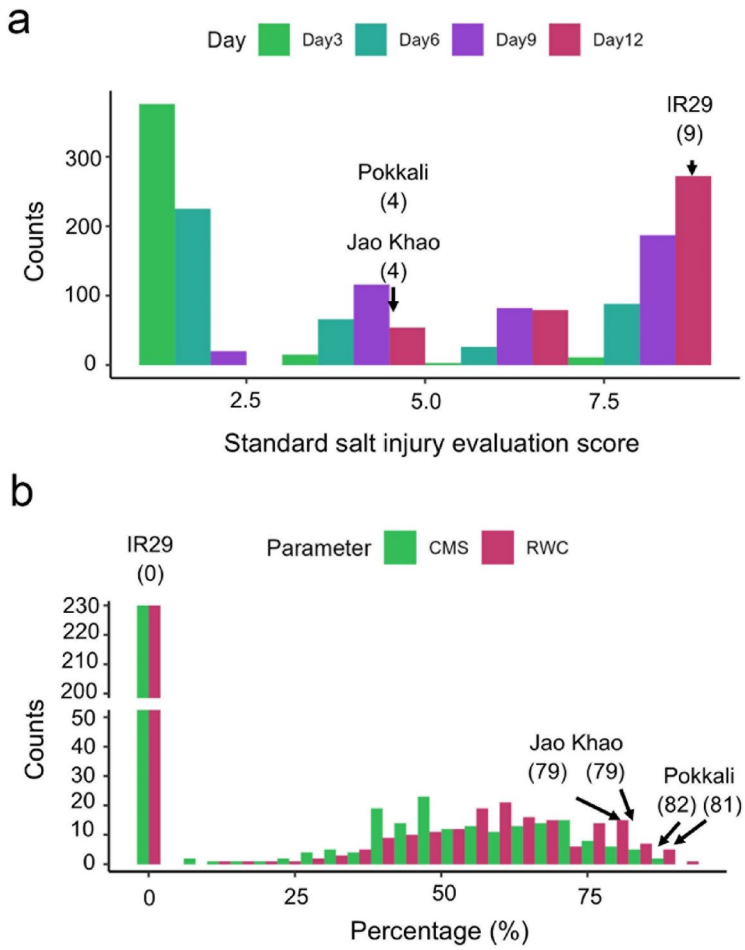

Fig. 2. Frequency distribution of three salt-responsive traits, standard salt injury evaluation score (**a**), cell membrane stability (CMS), and relative water content (RWC) (**b**) in the 402 F_3 progeny, including 'Jao Khao' and IR29 (parents), and the standard salt-tolerant variety, 'Pokkali'. Standard salt injury evaluation scores were collected on days 3, 6, 9, and 12 after salt stress treatment. The scores shown for the parents ('Jao Khao' and IR29) and 'Pokkali' were recorded on day 12 after salt stress, and CMS and RWC were collected on the same day.

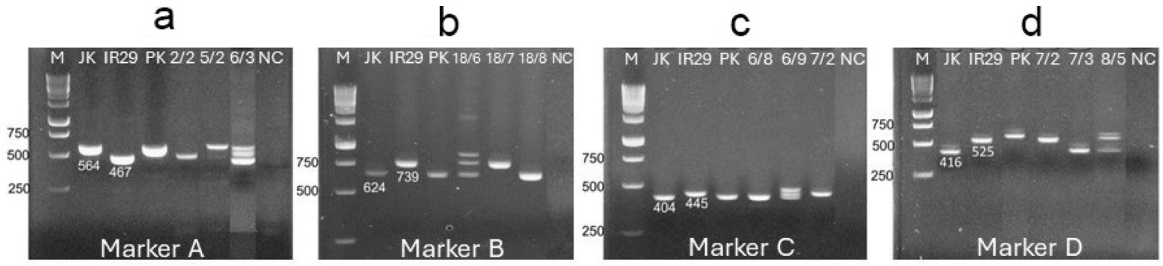

Fig. 3. Amplification patterns of markers A (**a**), B (**b**), C (**c**), and D (**d**), designed to target qCMS3.1, qRWC2.1, qRWC6.1, and qRWC12.1, respectively. NC, negative control.

sizes of amplified fragments were detected in the parental varieties. The allele from 'Jao Khao' was named allele 1, and that from IR29 was referred to as allele 2. Amplification patterns in 'Jao Khao' were similar to those in 'Pokkali' (Fig. 3a–c), except for marker D, where a larger fragment was detected in 'Pokkali' genomic DNA (Fig. 3d). Representative homozygous and heterozygous patterns from the F_3 population are shown in Fig. 3. Heterozygous detection was confirmed by the presence of heteroduplex banding[44] in samples 6/3 (Fig. 3a), 18/6 (Fig. 3b), 6/9 (Fig. 3c), and 8/5 (Fig. 3d).

The SES trait after 6 days of salt stress, and CMS and RWC traits after 12 days of stress showed a strong correlation with alleles detected by markers A–D (Fig. 4), which correspond to qCMS3.1, qRWC2.1, qRWC6.1,

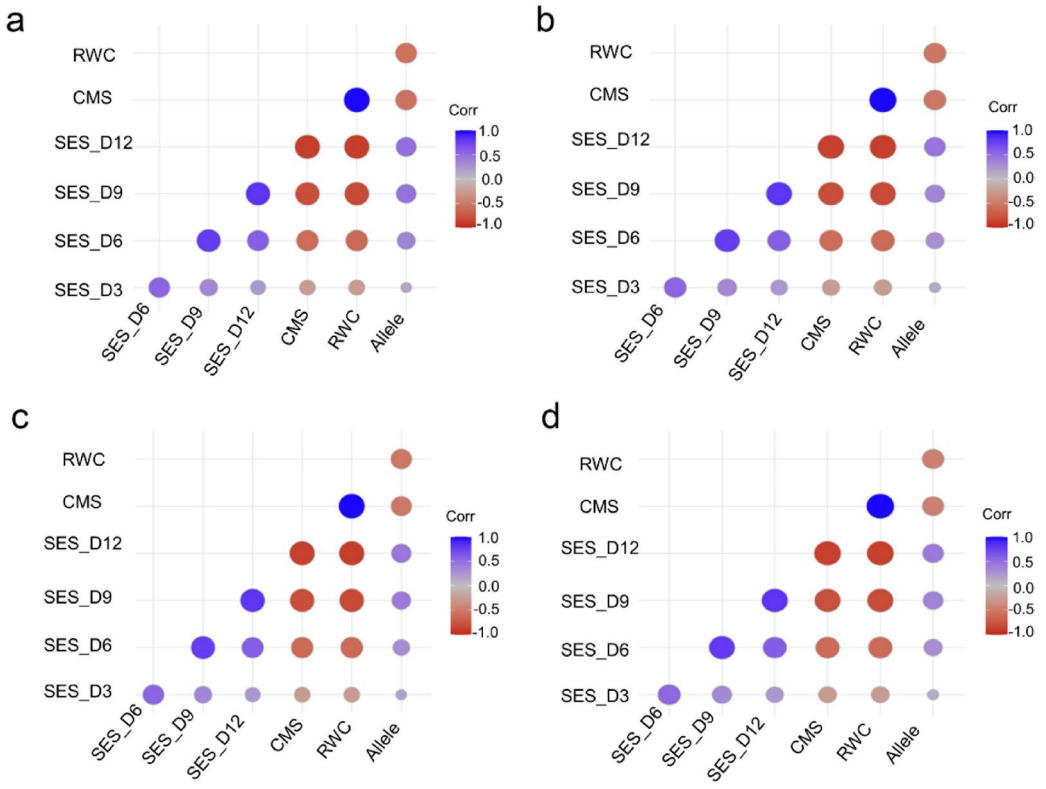

Fig. 4. Correlation plot showing the relationship between phenotypic traits and alleles for markers A (**a**), B (**b**), C (**c**), and D (**d**). The size of each circle represents the magnitude of the correlation coefficient between the marker allele and salt-responsive traits. Alleles from "Jao Khao' were coded as 1 and those from IR29 as 2. The values of the correlation coefficient are shown in Supplementary Table S3. CMS, cell membrane stability; RWC, relative water content; SES, the standard evaluation score of visual salt injury, D3–D12, days 3–12 of exposure to salt stress.

Marker	SES_D3		SES_D6		SES_D9		SES_D12		CMS (%)		RWC(%)	
	R	*p value*	R	*p value*	R	*p value*	R	*p value*	R	*p value*	R	*p value*
Marker A	0.25	2.5×10^{-1}	0.62	3.5×10^{-2}	0.74	1.4×10^{-2}	0.77	9.5×10^{-3}	0.81	5.6×10^{-3}	0.81	5.7×10^{-3}
Marker B	0.18	3.4×10^{-1}	0.50	7.5×10^{-2}	0.61	3.7×10^{-2}	0.71	1.9×10^{-2}	0.74	1.2×10^{-2}	0.74	1.3×10^{-2}
Marker C	0.25	2.5×10^{-1}	0.55	5.8×10^{-2}	0.66	2.8×10^{-2}	0.71	1.8×10^{-2}	0.76	1.1×10^{-2}	0.76	1.1×10^{-2}
Marker D	0.16	3.8×10^{-1}	0.48	8.6×10^{-2}	0.58	4.7×10^{-2}	0.64	$3.0X10^{-2}$	0.67	2.3×10^{-2}	0.69	2.2×10^{-2}

Table 4. Correlation between the markers and salt-responsive phenotypes in F_3 populations. SES was measured after stress for 3, 6, 9, and 12 days (SES_D3, SES_D6, SES_D9, and SES_D12, respectively), and CMS and RWC were measured after salt stress for 12 days. R, correlation; CMS, cell membrane stability; RWC, relative water content; SES, the standard evaluation score of visual salt injury.

and qRWC12.1, respectively. The details of correlation coefficients for each marker–trait pair are shown in Supplementary Table S3. A significantly positive correlation between all markers and the SES trait was consistently detected after 9 days of salt stress. After 12 days of salt stress, CMS and RWC showed a strong negative correlation with markers A, B, and C (Fig. 4a–c, Table 4), whereas marker D showed a negative correlation (Fig. 4d, Table 4).

The summary of correlation and significance levels between each marker and salt stress-responsive phenotypes in the F_3 population are shown in Table 4. Correlation coefficients between SES and markers increased progressively over time after stress. After 12 days of stress, markers A, B, and C were associated with SES by more than 70%, whereas marker D showed a correlation of 64%. Marker A displayed over 80% correlation with both CMS and RWC, while other markers showed correlations ranging between approximately 67–76% (Table 4).

To assess the linkage between markers and the corresponding QTLs, linkage disequilibrium (LD) analyses were performed based on GWAS data reported by Lekklar et al[17]. The LD map shows pairwise LD between markers A, B, C, and D. The SNPs within the red rectangles in the figure show the positions of these markers. Strong LD between SNPs located in block 1 (144 kb) (Fig. 5a) and block 2 (32 kb) (Fig. 5b) indicates a low recombination frequency in this genomic region, suggesting that their respective alleles are likely to be co-

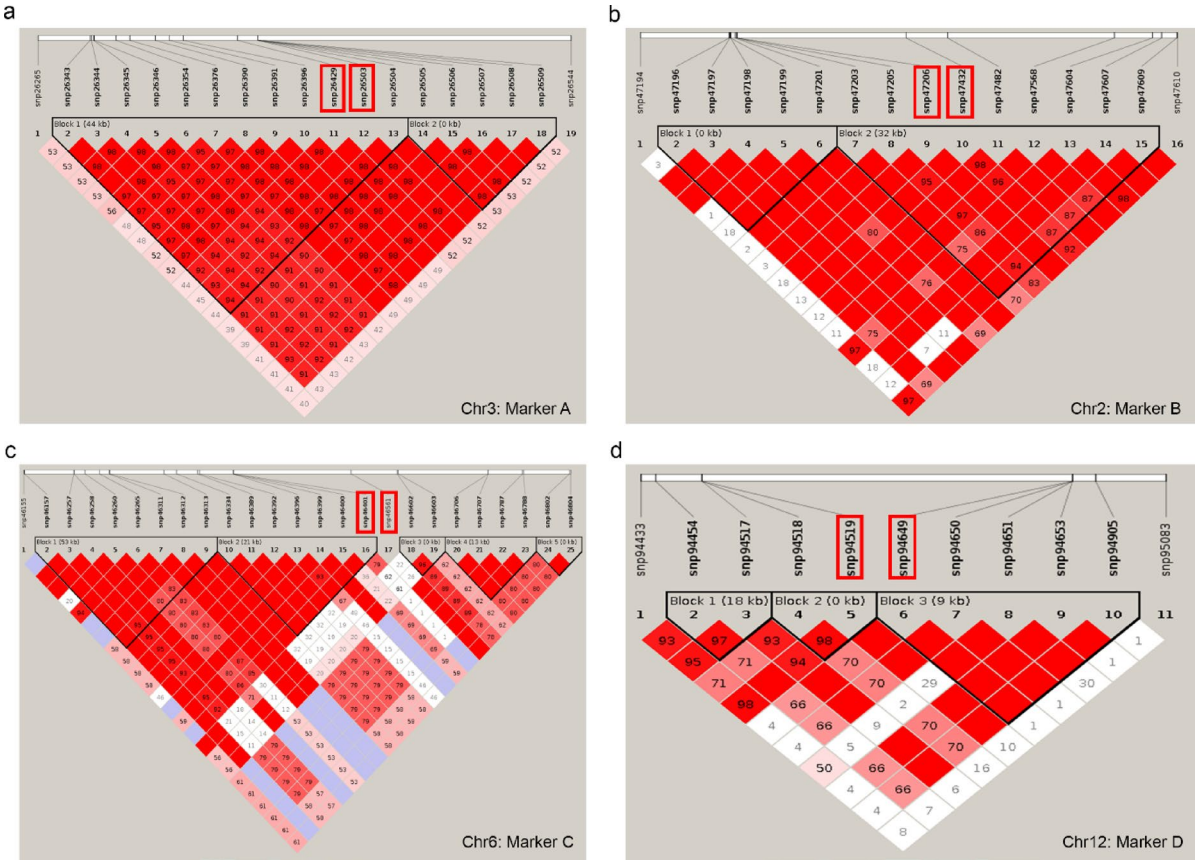

Fig. 5. Linkage disequilibrium (LD) patterns in the regions surrounding the designed markers on each chromosome: Marker A on chromosome 3 (**a**), Marker B on chromosome 2 (**b**), Marker C on chromosome 6 (**c**), and Marker D on chromosome 12 (**d**). The markers are located within the red rectangular boxes, which indicate the corresponding SNP positions used for analysis.

inherited. In contrast, no association was detected between marker C and its flanking SNPs (Fig. 5c), indicating that its alleles are not co-inherited and therefore unsuitable for marker-assisted selection (MAS). Additionally, extensive LD between SNPs in blocks 2 and 3 (9 kb) (Fig. 5d) suggests limited recombination in that region. The tight linkage of surrounding markers A, B, and D would be advantageous for MAS, particularly in salt stress, where early and accurate selection of physiological tolerance traits is crucial. Genes located within these LD blocks and their genomic positions are provided in Supplementary Table S4.

Prediction of the potential salt-tolerant genes in these QTLs

Three complementary approaches were used to predict the potential salt-tolerant genes. The first approach was to investigate epistasis using SNP-by-SNP interactions based on the information from salt-tolerant and salt-susceptible rice cultivars, reported by Habila et al[40]. The second approach was based on the comparison with hub genes identified by the weighted gene co-expression network of the indica salt-tolerant cultivar, 'Luang Pratahn', at the seedling stage[42]. The third approach, GWAS using phenotype and genotype data from 10 rice cultivars reported by Habila et al.[40], was performed to identify the potential salt-tolerant genes and possible quantitative trait nucleotide (QTN)–by–QTN interaction according to a previously described method[45].

For epistasis investigation by the first approach, SNP datasets from five salt-tolerant cultivars ('Pokkali', 'Jao Khao', 'Lai Mahk', 'Luang Pratahn', and 'Ma Gawk') and five salt-susceptible cultivars ('Mayom', 'Gam Feuang', 'KDML105', 'Plah Khaeng', and IR29) were analyzed. The epistasis analysis identified more than one million SNP-by-SNP interactions within the QTL region. Gene Ontology (GO) analysis of the most significant SNPs ($p = 0$) identified enrichment of the plant hormone signal transduction, biosynthesis of secondary metabolites, and metabolic pathways (Fig. 6). The genes identified from the SNP-by-SNP interactions showing enrichment in the plant hormone signal transduction pathway, that are involved in a range of hormone-related activities are shown in Fig. 7. Among these, two genes—*DELLA* (*LOC_Os03g49990, OsSLR1*) and *OsMAPK6* (*LOC_Os06g06090*), were colocalized in the identified QTLs.

For the second approach, we compared the genes located within qCMS3.1, qRWC2.1, qRWC6.1, and qRWC12.1 with the key genes known to be involved in salt-stress tolerance in 'Luang Pratahn'[40,42]. The analysis identified two key genes—*LOC_Os02g02830*, encoding the ubiquitin-conjugating enzyme E2 28, and *LOC_Os06g06170*, an expressed protein with no functional annotation in the reference genome—within the QTLs.

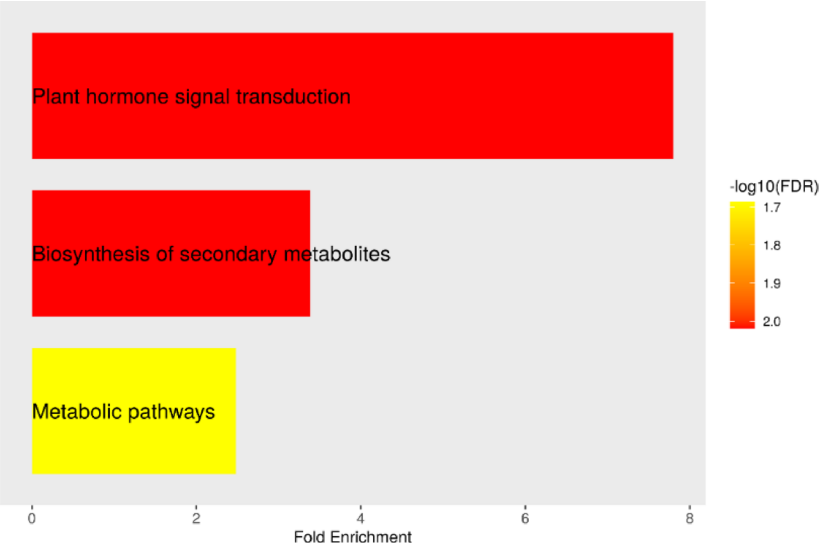

Fig. 6. Gene Ontology analysis of the SNP-by-SNP interactions with *p* values of 0 from the four QTL regions. The bar chart shows the top significant GO terms ranked by fold enrichment after analyzing all the genes. The color of the bars represents the statistical significance of the enrichment based on the false discovery rate (FDR).

Structural prediction using AlphaFold2 indicated high similarity to RING-type E3 ubiquitin transferase. These findings suggest that the ubiquitination pathway contributes to salt tolerance in 'Jao Khao' rice.

Using the third approach, we identified 21 significant SNPs across nine phenotypic traits (Fig. 8). However, no significant QTN–by–QTN interactions were detected. The list of the significant QTNs is shown in Supplementary Table S4. These QTNs were located on chromosomes 1, 2, 3, 5, and 9 (Fig. 8a–i). Among these, four QTNs were located in the genes with known functional annotations (Table 5)—*LOC_Os02g42150* (*OsWAK14*), a receptor-like protein kinase, located on chromosome 2 showed association with SES (Fig. 8b), whereas the other three including *LOC_Os01g09260* (*OsCKX1*) encoding cytokinin dehydrogenase precursor, *LOC_Os02g10700 (FBOX72*), encoding F-box-type E3 ubiquitin ligase L7, and *LOC_Os02g27000* (*OsMORC4),* encoding Mitochondria protein 4, containing ATPase-like domain located on chromosomes 1, 2, and 2, respectively, showed association with SFW (Fig. 8d).

Relative gene expression analysis of candidate genes

As the regulation of DELLA protein via ubiquitination has previously been implicated in salt tolerance[46], we selected three loci, *LOC_Os02g02830*, *LOC_Os03g49990,* and *LOC_Os06g06170*, encoding for ubiquitin-conjugating enzyme E2 28, DELLA, and RING-type E3 ubiquitin transferase, respectively, for gene expression analysis to investigate their potential role in salt tolerance. Among these, *LOC_Os03g49990* and *LOC_Os06g06170* were expressed in our samples (Fig. 9), whereas the expression of *LOC_Os02g02830* was not detected (data not shown). It is possible that this gene is not expressed in the cultivars analyzed, as it was originally predicted based on the 'Luang Pratahn' cultivar.

In IR29, expression of *LOC_Os03g49990* increased approximately two-fold at three hours post salt exposure under both normal and salt stress conditions, but the expression was comparable between the conditions throughout the 12 h stress period (Fig. 9a). In 'Jao Khao' the expression of *DELLA* was upregulated by approximately three-fold in both control and salt stress plants after 3 h of exposure to salt stress. However, its expression in salt-treated seedlings declined sharply to undetectable levels, remaining absent until 12 h. In contrast, in control conditions, expression gradually decreased to undetectable levels by the end of the experiment (Fig. 9b). In salt-treated 'Pokkali' seedlings, *DELLA* expression was lower than that in the control at 3–6 h post-stress, which decreased to undetectable levels at 9–12 h of the treatment (Fig. 9c).

The expression of *LOC_Os06g06170* declined under normal and salt stress conditions in IR29 during the experimental period (Fig. 9d). In 'Jao Khao', the expression of *LOC_Os06g06170* was maintained at 3 h after salt stress but declined dramatically in control conditions. However, at 12 h, the expression of the gene was undetectable in both salt-stressed and normally grown plants (Fig. 9e). In 'Pokkali', expression of *LOC_Os06g06170* decreased progressively in control seedlings, reaching very low levels by six hours. Under salt stress, its expression was maintained at 3 h, then decreased to match control levels by 6–12 h (Fig. 9f).

Discussion

In this study, we conducted BSA using an F_2 population derived from two parental lines with contrasting salt-stress tolerance and identified four significant QTLs— qCMS3.1, qRWC2.1, qRWC6.1, and qRWC12.1, on chromosomes 2, 3, 6, and 12, respectively—associated with salt-stress. Among these, qRWC2.1 was the largest QTL region, containing 145 genes. It overlapped with previously reported qGY2.1 trait using an F_2 population

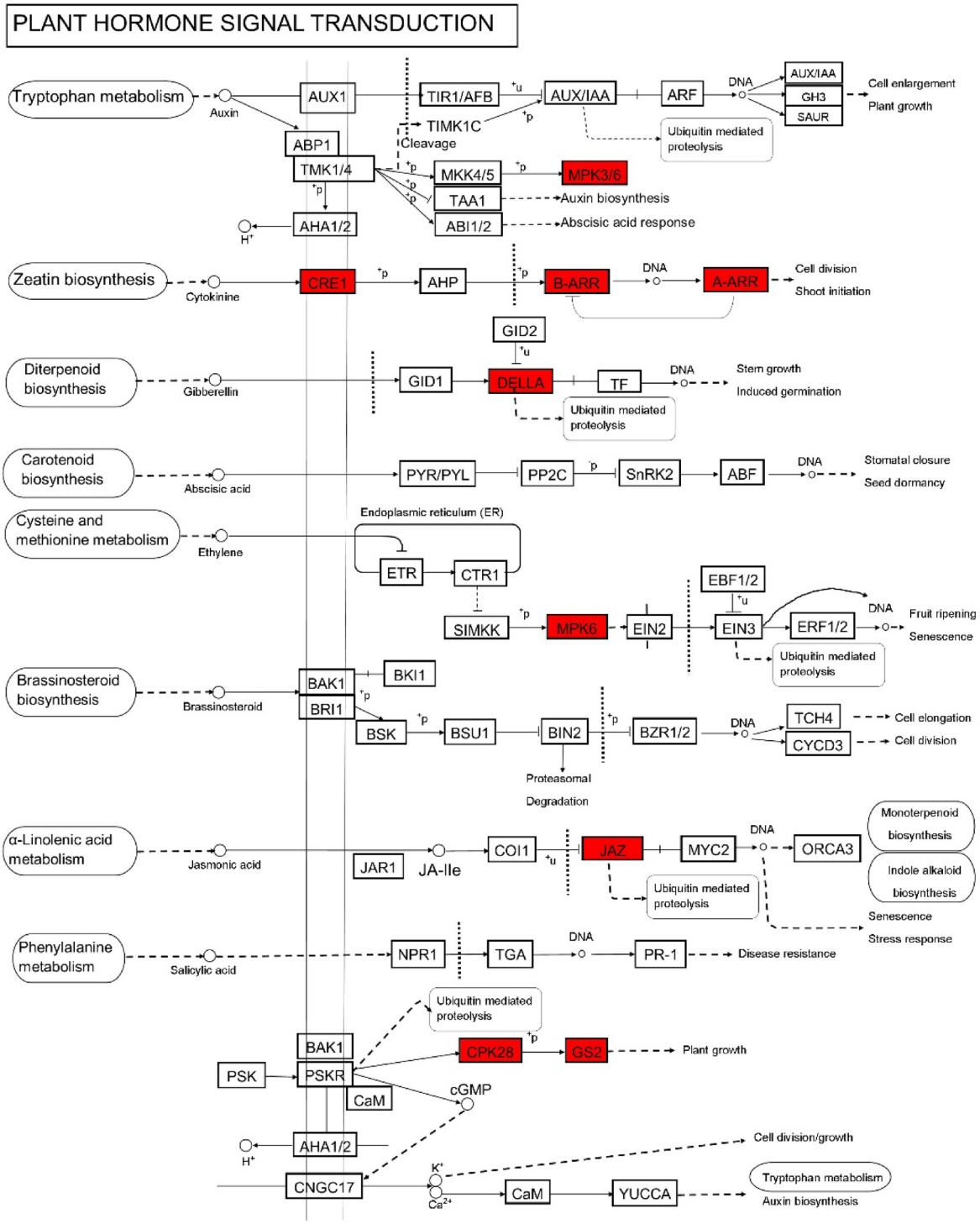

Fig. 7. Enriched KEGG pathway diagram illustrates the potential biological pathways involving the candidate genes. Genes highlighted in red represent those identified through SNP–by–SNP interactions. KEGG, Kyoto Encyclopedia of Genes and Genomes.

derived from Cheriviruppu and Pusa Basmati 1[47], and qSST2, identified using the salt toxicity scores in two inbred line populations derived from C258 × IR75862 and ZGX1 × IR75862 at the seedling stage[48]. Moreover, GWAS for salt-tolerant traits in a Thai rice population predicted eight loci in this region containing significant SNPs[17]. Weighted co-expression network analysis using time-course transcriptomes identified *LOC_Os02g02830* as a candidate gene in salt tolerance in 'Luang Pratahn' rice[42]. These data suggest that qRWC2.1 is a key genomic region influencing salt tolerance and possibly grain yield. The overlap of qRWC2.1, qSST2, and qGY2.1 supports the hypothesis that this genomic region affects both salt tolerance and grain yield. Previous studies have indicated

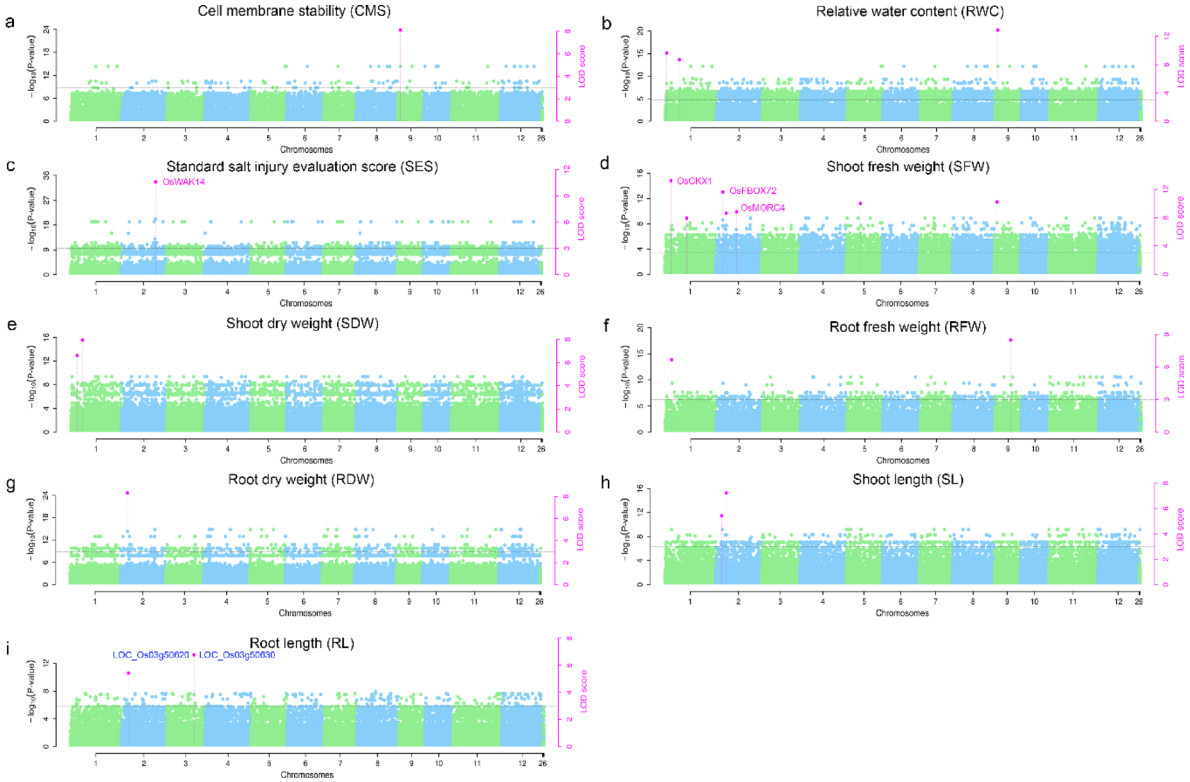

Fig. 8. Manhattan plots showing the − log (p-value) (left axis) and LOD score (right axis) from genome-wide association analysis between SNPs and phenotype traits in 10 rice cultivars reported in a previous study[40]. The traits analyzed include cell membrane stability (CMS) (**a**), relative water content (RWC) (**b**), standard salt injury evaluation score (SES) (**c**), shoot fresh weight (SFW) (**d**), shoot dry weight (SDW) (**e**), root fresh weight (RFW) (**f**), root dry weight (RDW) (**g**) and shoot length (SL) (**h**), root length (RL) (**i**). Significant QTNs with annotated gene names and functions are indicated in purple. Nearby loci within QTLs predicted by BSA are shown in blue.

Trait name	Chro	Position (bp)	Locus ID	LOD	*P* value	Gene product	Annotated function
SES	2	25,348,513	LOC_Os02g42150	10.5918	2.87E-12	OsWAK14	OsWAK receptor-like protein kinase, expressed
SFW	1	4,697,259	LOC_Os01g09260	13.2249	5.97E-14	OsCKX1	cytokinin dehydrogenase precursor, putative, expressed
SFW	2	5,631,010	LOC_Os02g10700	11.6461	2.26E-12	OsFBOX72	F-box domain and LRR-containing protein, expressed, F-box-type E3 ubiquitin ligase L7 (Oryzabase)
SFW	2	15,866,708	LOC_Os02g27000	8.8363	1.46E-09	OsMORC4	ATP-binding region, ATPase-like domain containing protein, expressed, Mitochondria protein 4

Table 5. List of the associated QTNs with salt-responsive traits located in the genes with annotated functions.

that QTLs linked to stress responses often coincide with those controlling key agronomic traits, possibly due to shared genetic pathways[49,50]. This dual function provides a strategic advantage for breeding high-performing, stress-resistant rice varieties.

LD analysis revealed different patterns for the markers A, B, C, and D. As shown in (Fig. 5a, b), SNPs in Block 1 (144 kb) and Block 2 (32 kb) exhibited strong linkage, indicating low recombination rates and a high likelihood of correlated alleles. Such expanded LD blocks are often found in regions with low recombination and may result from preserved haplotypes or selection pressure[51]. Similarly, the low recombination observed between the SNPs among Blocks 2 and 3 (9 kb) (Fig. 5d) supported their potential use in MAS.

These findings are particularly valuable for breeding salt-tolerant cultivars, where rapid and precise selection of physiological tolerance traits is essential [44]. The strong LD among markers A, B, and D implies these as promising alternatives for genotype selection of salt-resistant varieties, reducing the need for extensive phenotyping[52]. In contrast, marker C showed no substantial LD with its flanking SNPs (Fig. 5c), indicating independent segregation and rendering it unsuitable for MAS. This lack of association may be due to increased recombination rates or genetic divergence in the genomic region[53]. However, we detected a significant correlation of the marker C in the F_3 population, likely because this analysis involved progeny from only two parents, 'Jao

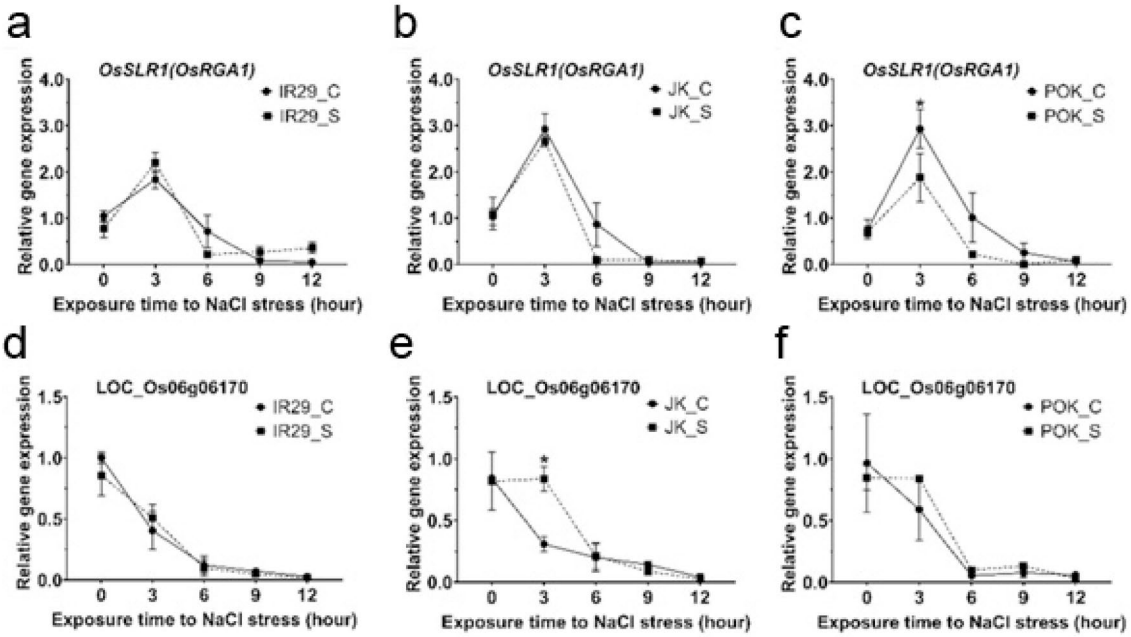

Fig. 9. Relative gene expression levels of (**a–c**) *LOC_Os03g49990* (*OsSLR1 or DELLA*) and (**d–f**) *LOC_Os06g06170* in IR29 (**a**), 'Jao Khao' (**b**), and 'Pokkali' (**c**) in 14-day-old rice leaves grown under normal (C) or salt stress conditions treated with 75 mM NaCl (S). JK, 'Jao Khao'; PK, 'Pokkali'; C, normal condition; S, salt stress condition.

Khao' and IR29, whereas the LD block was based on SNP data from more than 100 rice cultivars, suggesting that marker C is less generalized as a salt tolerance marker across diverse genetic backgrounds.

In qRWC2.1, several stress-related genes were characterized, some of which showed pleiotropic effects as they encode transcription factors or signalling molecules. OsbHLH059, encoded by *LOC_Os02g02480*, is involved in iron deficiency, as evidenced by the reduced expression of many iron deficiency-inducible genes in the OsbHLH059 knocked-out line[54]. Moreover, *OsbHLH059* is induced by wounding and methyl jasmonate, suggesting a role in herbivore defense[55]. *LOC_Os02g03294* encodes a cyclin. Many cyclins, such as OsCYCP4s, have been reported to regulate shoot growth in response to phosphate starvation[56]. The plant Rho small GTPases (Rop/Rac) system plays a role in signalling plant developmental processes and stress response. Overexpression of *OsRacB*, encoded by *LOC_Os02g02840*, is associated with increased salt tolerance in tobacco and rice[57], and is also involved in rice pollen grain germination[56,58]. The pleiotropic effects may also stem from the pre-mRNA splicing regulation. RS33, a serine/arginine (SR)-rich splicing factor, encoded by *LOC_Os02g03040*, regulates multiple stress responses, including salt stress. The *rs33* knockout mutant is more sensitive to low temperature and salt stress[59]. OsCPK4, a calcium-dependent protein kinase encoded by *LOC_Os02g03410*, plays significant roles in responses to abiotic (salt and drought) and biotic stress (blast disease)[60,61]. *OsCPK4* regulates the proteasomal turnover of *OsRLCK176*, an ortholog of receptor-like cytoplasmic kinase involved in immune signaling, demonstrating an interplay between phosphorylation and ubiquitination in immune homeostasis[62]. *LOC_Os02g02890* encodes OsCYP2, also known as *LRT2 (LATERAL ROOTLESS2)*, which is a cyclophilin protein involved in auxin signaling and lateral root development. It functions as a molecular chaperone, promoting degradation of auxin-responsive proteins, thereby influencing root architecture[63]. *OsCYP2* also confers salt tolerance via reactive oxygen species scavenging and ion homeostasis[64,65].

Based on GWAS analysis using the method of Li et al.[45], QTNs on chromosome 2 were predicted to be associated with SES, including *OsWAK14*. This locus was previously linked to disease resistance, as loss of function of *OsWAK14* increased susceptibility to blast fungus[66]. Several *WAK* genes in various plants have been reported to play roles in abiotic stress responses[67]. For example, *GbWAK5*[68] and *GhWAKL26*[69] are involved in Na^+/ K^+ homeostasis under salt stress, whereas *OsWAK112* overexpression increases sensitivity to salt stress[70]. Therefore, the role of *OsWAK14* in salt stress response warrants further investigation. QTNs associated with SFW were identified in annotated genes, *OsCKX1* on chromosome 1, and *OsFBOX72* and *OsMORC4* on chromosome 2. Homeostatic regulation of cytokinin levels is a known mechanism for abiotic stress tolerance, and overexpression of *CKX1* in a cytokinin-deficient mutant shows salt tolerance[71]. *OsFBOX72* encodes an F-box-type E3 ubiquitin ligase L7, while *OsMORC4* encodes mitochondria protein 4, containing an ATPase-like domain. While their involvement in salt tolerance has not been previously reported, the role of F-box-type E3 ubiquitin ligase L7 suggests that the ubiquitination contributes to salt stress responses in these cultivars, consistent with the findings in 'Luang Pratahn' rice cultivar[40,42]. None of these loci overlap with the qRWC2.1 detected by BSA in this study; however, further analysis is recommended.

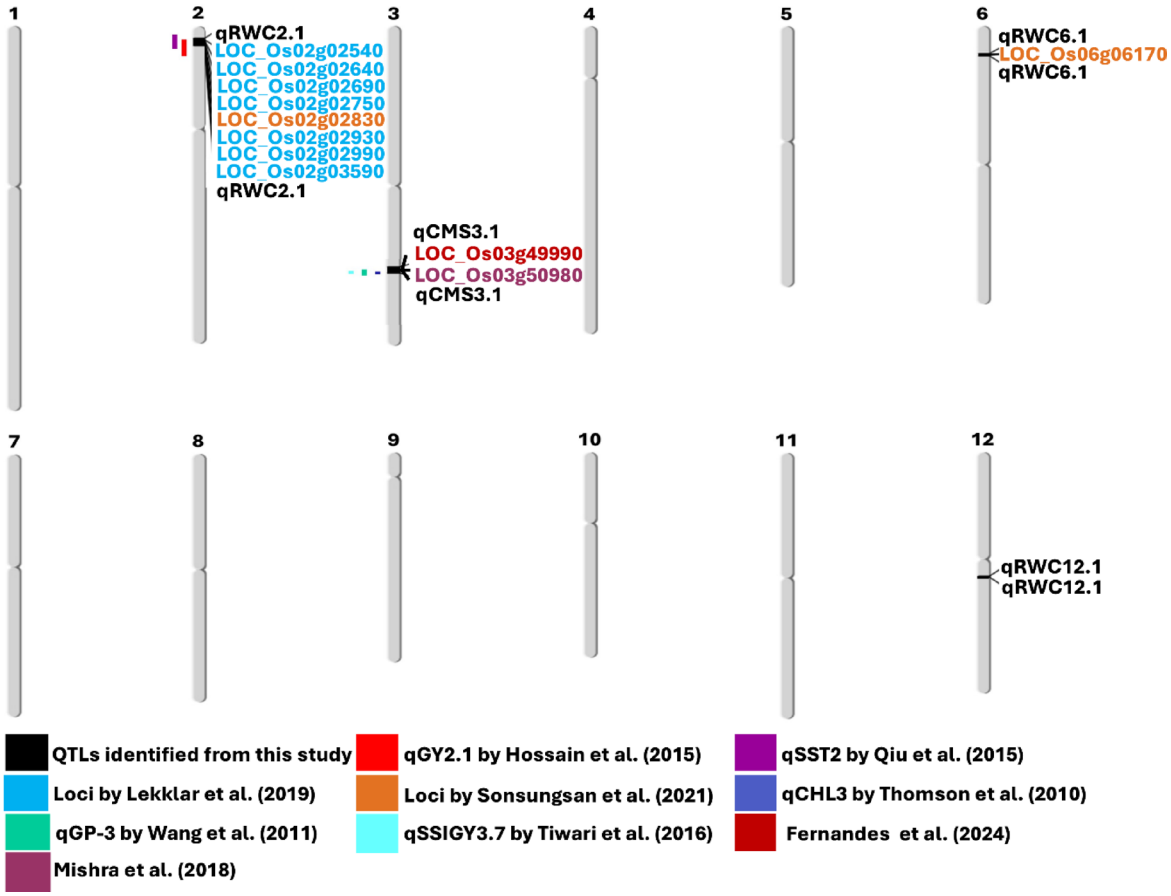

Fig. 10. Consistency of the salt-tolerant QTL with the previously reported QTLs related to salt tolerance in rice. The QTLs, qCMS3.1, qRWC2.1, qRWC6.1, and qRWC12.1 are indicated with black bands on the chromosomes, while the previously reported salt-tolerant QTLs are indicated as the lines next to the chromosomes or indication of reported loci. The boxes in front of the references, colored the same as the lines, represent the references of the QTLs.

qCMS3.1, identified for CMS, contains 92 loci and overlaps with qCHL3, identified for leaf chlorophyll content under salt stress[72], and qGP-3, identified for germination percentage after 10 days under salt stress conditions[73]. Moreover, qCMS3.1 also overlaps with qSSIGY3.7, which was responsible for salt tolerance identified using BSA of the cross between the CSR27/MI48 population using the 50 k SNP chip[38] (Fig. 10). These data suggest that this region contains important genes responsible for salt tolerance in rice by maintaining membrane stability under salt stress. SNP-by-SNP interaction analysis identified *OsSLR1* (*LOC_Os03g49990*), encoding DELLA protein, a negative regulator of plant growth negatively regulated by gibberellic acid[74], as a key gene in this region. *OsSLR1* undergoes SUMOylation under salt stress, which mitigates the negative effect of salt stress on yield, likely by modulating interactions of SLR1 with transcription factors involved in GA and abscisic acid (ABA) signaling[75].

In addition, qCMS3.1 contains the genes related to grain size, yield, and abiotic stress tolerance. *LOC_Os03g49880* encodes OsTB1 (TEOSINTE BRANCHED1), a key transcription factor that negatively regulates axillary bud outgrowth, controlling tillering and shoot branching[76]. *MP3* (*MORE PANICLES 3*), a natural allele of *OsTB1,* improves panicle number and grain yield under elevated atmospheric CO_2 levels[77]. *LOC_Os03g49900* (*LE*) encodes a C3HC4 ring finger transcription factor. The *le* mutant shows enlarged embryo size due to the increase in the size of scutella parenchyma cells[78], but it reduces the grain size, hence named as DGS1 (DECREASED GRAIN SIZE 1)[79]. DGS1 is an active E3 ubiquitin ligase that interacts with SMALL GRAIN 3 (SMG3) in the endoplasmic reticulum. DGS1 ubiquitinates the brassinosteroid receptor, BRI1, and affects its accumulation. DGS1, SMG3, and BRI1 regulate rice grain size and weight via the brassinosteroid signaling pathway[80]. Moreover, DGS1 also functions with ubiquitin conjugating enzyme *OsUBC45* as an E2–E3 pair, affecting rice yield and immunity via degradation of *OsGSK3* and *OsPIP21*, respectively[81]. *LOC_Os03g50290* encodes 14–3-3-like protein GF14F, where RNAi knockdown reduces grain length and weight[82]. GF14F overexpression increases the resistance to leaf blast and bacterial blight through the salicylic acid signaling pathway[83] and enhances osmotic stress tolerance[84] in rice. Grain development is also regulated by cytokinin signaling. *LOC_Os03g50860* encodes histidine kinase (*OsHK4*) and is highly expressed in spiklets[85,86]. *LOC_Os03g50980* encodes SIZ2 (E3 small ubiquitin-like modifier (SUMO)-protein ligase SIZ2), an E3 SUMO ligase that plays a crucial role in the SUMOylation pathway. The conjugation of SUMO proteins increased under

environmental stresses, including high and low temperature, salt, and ABA in rice plants[87]. It regulates growth, developmental responses, and yield-related traits, including Pi[88] and N homeostasis[89].

Two loci in this QTL, *LOC_Os03g50310* (*OsCOL10*) and *LOC_Os03g51030* (*PHYA*), regulate flowering time in rice. Overexpression of *OsCOL10* delays the flowering time in both short-day and long-day conditions. It functions as a flowering-time repressor downstream of Ghd7 in rice[90]. *PHYA* together with *PHYB* and *PHYC* regulate HEADING DATE 7 (*GHD7*), a floral repressor, and EARLY HEADING DATE 1, a floral inducer[91].

GWAS of the root length (RL) trait revealed a significant QTN on chromosome 3 (Fig. 8i), which colocated with the predicted QTL qCMS3.1. However, this significant QTN was not located in a functional gene, but the nearest genes were *LOC_Os03g50620* (ATP-binding protein) and *LOC_Os03g50630* (expressed protein). These genes warrant further investigation to assess their potential involvement in salt stress tolerance.

qRWC6.1 contained 48 genes, including *LOC_Os06g06170*, which has been predicted to be involved with salt tolerance in 'Luang Pratahn' by weighted co-expression network analysis using the time-course transcriptome data[42]. *LOC_Os06g06170* encodes a RING-type E3 ubiquitin transferase. Considering the co-expression network prediction indicating a role in the ubiquitination process[42], it is plausible that both DELLA-mediated regulation and ubiquitination contribute to salt tolerance in 'Jao Khao'.

Some other interesting genes identified in this QTL included *LOC_Os06g06090 and LOC_Os06g06300*. *LOC_Os06g06090* encodes mitogen-activated protein kinase (MAPK) 1, *OsMAPK1*, *OsMAPK6*, or *OsSIPK*[92]. *OsMPK1/OsMAPK6* is a key component of the MAPK signalling pathway in rice. It plays a role in ABA-induced antioxidant defense and enhances tolerance to drought, salt, and oxidative stress[93]. *LOC_Os06g06300* encodes FLOWERING LOCUS T 1 (RFT1) or FTL3, a major florigen, that induces transformation of the shoot apical meristem to the floral meristem in rice. Overexpression of this gene induced extremely early flowering in the transgenic plants[94].

qRWC12.1, a novel QTL showing association with salt tolerance, harbored several transposable elements. It showed association with both CMS and RWC traits (Fig. 1a, b). Marker D, the specific marker underlying qRWC12, showed a significant level of correlation with salt tolerance traits in the F_3 population (Table 4); however, the levels of correlation coefficient detected by marker D were lower than those detected by the other markers in this study. It is possible that the effect on this region may not be the direct function of the transposable element, but it may come from the effect of the nearest functional genes.

The relative gene expression analysis of *OsSLR1* (*DELLA*) and *LOC_Os06g06170* under salt stress provided insights into their potential role in rice salt tolerance. In IR29 and 'Jao Khao', expression of *DELLA*, which functions as a growth suppressor regulated by the GA signaling pathway[95], did not alter under salt stress; however, it decreased in 'Pokkali' rice, indicating a higher salt tolerance in 'Pokkali'. Furthermore, the degradation of the DELLA protein by ubiquitin-mediated proteasomal degradation is known to contribute to salt tolerance[96]. Additionally, we showed that the expression of *LOC_Os06g06170* (encoding RING-type E3 ubiquitin transferase) in 'Jao Khao' was stable after 3 h of salt treatment (Fig. 8e). This trend was also observed in 'Pokkali' (Fig. 8f), whereas the expression of *LOC_Os06g06170* decreased in IR29 (Fig. 8d), indicating the ability of 'Jao Khao' and 'Pokkali' to degrade DELLA protein via ubiquitination. Together, these findings suggest that the interaction between DELLA protein and RING-type E3 ubiquitin transferase helps salt-tolerant plants avoid excessive growth reduction while activating other stress tolerance mechanisms. In contrast, the decreased expression of the RING-type E3 ligase under stress in salt-sensitive plants makes them more vulnerable. Prior studies demonstrated that *SLR1* mutant lines, *slr1-d7* and *slr1-d8*, generated by the CRISPR/Cas9 system, showed a semi-dominant dwarf phenotype with altered transcription profiles. Furthermore, GO classification of the genes with differential expression in these lines showed enrichment in salt stress-associated genes, supporting the role of *SLR1* in salt tolerance[96]. Moreover, rice *NUCLEAR FACTOR-Y A3 (OsNF-YA3)*, a negative regulator of osmotic and salt stress response, physically interacts with DELLA protein. Studies have shown increased tolerance to osmotic and salt stress in *OsNF-YA3* mutant lines *ya3-1* and *ya3-2*[97]. These findings suggest that under salt stress, DELLA protein binds OsNF-YA3 and initiates ubiquitin-mediated proteasomal degradation of OsNF-YA3, leading to enhanced stress tolerance. Recently, Fernandes et al.[75] proposed that SUMOylation of SLR1 disintegrates the interaction with many transcription factors, leading to the modulations of downstream genes in GA-dependent growth and ABA-dependent salinity tolerance processes, further supporting the role of *SLR1* in salt tolerance in rice.

However, we could not detect the expression of *LOC_Os02g02830* in 'Jao Khao' via the qRT-PCR approach, which is consistent with the transcriptome data[98] showing an extremely low level of expression of this gene.

Methods

DNA extraction, DNA bulks, and library construction for high-throughput sequencing

The study involved a population of 600 F_2 progenies developed in a previous study[41] by crossing 'Jao Khao' and IR29. The population was characterized previously in terms of salt-responsive traits, which included CMS and RWC[40]. In this study, we identified the top 5% of seedlings with high and low CMS and RWC values to construct the pools. Genomic DNA was isolated from the youngest fully expanded leaf samples of 14-day-old seedlings using a Genomic DNA Mini Kit (Plant) acquired from Geneaid Biotech Ltd. (New Taipei City, Taiwan) according to the manufacturer's protocol and bulked as the tol and sen pools. The genomic DNA libraries of the two parental lines, 'Jao Khao' and IR29, and the pooled DNA samples from the two bulks were prepared using the TruSeq DNA sample prep kit (Illumina Inc., San Diego, USA). A total of paired end 150 bp sequencing reads were obtained using a NovaSeq 6000 sequencing platform (Illumina, San Diego, USA).

SNP calling and SNP index analysis for identifying salt-tolerant QTLs

Raw reads from the two parent varieties, 'Jao Khao' and IR29, and the two sets of reads representing the DNA samples of tol and sen pools for the two traits (CMS and RWC), were subjected to a quality control check using

the FASTQC software[99]. The paired-end reads were trimmed, and adapters were removed using Trimmomatic[100]. After excluding the reads with low-quality bases (quality score less than 30), the clean reads were mapped against the Nipponbare reference genome (release 7)[43] using Burrows–Wheeler Aligner (BWA version 0.7.15) software with the BWA–MEM algorithm[101]. Reads with a length of ≥ 50 bp were used for SNP calling using the Genome Analysis Tool Kit (GATK) version 4.0[102].

Genomic DNA variants, including SNPs and InDels, were subjected to BSA using the QTLseqr package[103], following the protocol described in a previous study[104]. SNPs and InDels were filtered according to the following criteria: the reference allele frequency, 0.4–0.6; total sample read depth, 100–400; read depth for each bulk, ≥ 40. SNPs with an absolute read depth difference greater than 60 between the two bulks were excluded. The minimum genotype quality score, as calculated using GATK, was 99. The SNP index value was calculated as the ratio of the number of reads containing an SNP divided by the total number of reads covering that SNP position. Change in SNP index (ΔSNP index) was calculated by subtracting the SNP index values of the sen bulks from those of the tol bulks. The average SNP and ΔSNP index distributions were estimated in each genomic interval using a sliding window approach with a 1-Mb window size and 10-kb step size[104]. The SNPs were plotted to generate ΔSNP index plots for all chromosomes and genomic regions where the ΔSNP index value was close to 1 or − 1 and passed the 95% confidence level, based on statistical simulations, were considered putative major QTLs associated with trait variation between the bulks[103,104].

Validation of the putative salt-tolerant QTLs in an F_3 population

Leaf tissues were collected from 23 randomly selected families of F_3 progeny (n = 402), which were numbered, and genomic DNA was extracted, as described in the "DNA extraction, DNA bulks, and library construction for high-throughput sequencing" above.

To validate the involvement of the identified QTLs in salt tolerance in rice, the same traits used for BSA in the F_2 population—CMS and RWC—were evaluated in F_3 progeny under salt stress conditions. Moreover, the SES of visual salt injury at the seedling stage was also assessed to confirm the effectiveness of the designed markers in determining salt tolerance, as the SES represents the salt stress-tolerant levels of the plants. F_3 progenies were initially grown in the modified WP solution[105] for 14 days and then transferred to the nutrient solution supplemented with 75 mM NaCl for 6 days to reach the salinity level of 9 dS/m. Subsequently, the salinity level was increased to 12 dS/m by changing the nutrient solution supplemented with 100 mM NaCl and culturing the seedlings for another 6 days. Data on SES were collected every 3 days after stress treatment according to the protocol of Gregorio et al.[106], whereas data on CMS and RWC were collected after 12 days of salt stress following the protocols described by Naghashzadeh et al.[107] and Sade et al.[108], respectively.

The primers targeting each predicted salt-tolerant QTL were designed by using Primer3Plus[109] and Primer-BLAST[110] software. The alleles detected by the designed primers were investigated in 'Jao Khao' and IR29 parental lines using polymerase chain reaction (PCR). The PCR amplification conditions for each primer pair are shown in Supplementary Table S5. Different alleles between the two parental lines were then used to genotype the F_3 progenies. After genotyping, the association between the detected alleles and phenotypes (SES, CMS, and RWC) was evaluated using SPSS Statistics version 22 (IBM, Armonk, NY, USA).

Linkage disequilibrium analysis

LD analysis was performed to assess genomic connectivity based on the expectation that QTNs within the same block are co-inherited during meiosis. This phenomenon can contribute to false positive associations near true QTNs in GWAS mapping. In our study, the average LD for SNPs separated by 1,000 bp was 0.76. We examined LD on chromosomes 2, 3, 6, and 12, focusing on Marker A (position 1,016,412–1,017,250); Marker B (position 28,875,234–28,875,797); and Marker C (position 2,885,160–2,885,585)—and additionally considered Marker D on chromosome 12. LD calculations were carried out using PLINK[111] with standard quality control filters, and the resulting patterns were visualized using Haploview to demonstrate the strength and decay of LD in these regions.

Candidate gene identification by using PLINK epistasis analysis

To identify the putative salt-tolerant gene(s) within the four QTLs, an epistasis analysis was performed using PLINK version 1.9[111]. SNPs from 10 Thai rice varieties, including 'Pokkali' and IR29, identified by Habila et al.[40], were used for the analysis. A case-only epistasis analysis was conducted using CMS trait values from the same set of 10 cultivars under salt stress conditions[40]. In this approach, the program assumes non-salt stress conditions and interactions between genetic variants in the population are not modeled.

A total of 5 million SNPs were filtered by an in-house bash script to remove the intergenic and intronic regions, reducing the number of SNPs included in the PLINK -epistasis analysis[111] to over 1 million. Pairwise interactions were assessed for all SNPs, and the interactions passing the Bonferroni correction thresholds were considered statistically significant. The predicted interactions were then annotated against the Rice Genome Annotation Project database (MSUv7) available at https://rice.uga.edu[43] to determine the genomic location of the SNPs. Significant SNP-by-SNP interactions located within the four identified genomic regions on chromosomes 2, 3, 6, and 12, both intra- and inter-region, were extracted.

Interactions were ranked by their *p*-values, and only those with a *p*-value of zero were retained for KEGG pathway analysis. All interactions from the four regions were combined, and the associated genes were mapped to the *Oryza sativa* database in STRING[112] and *Oryza sativa* Japonica Group genes (IRGSP-1.0) for functional annotations. KEGG pathway enrichment analysis was performed using ShinyGO 0.80 software, a graphical tool for gene set analysis in animals and plants[113]. From the enriched pathways, the key genes associated with salt tolerance were identified and considered as candidate genes.

Comparison of the genes located in the predicted QTLs with the key genes from the analysis of weighted gene co-expression network analysis in indica rice

Key genes and the mechanisms of salt stress response in indica rice proposed by Sonsungsan et al.[42] were used for filtering and comparing the genes within the four predicted QTLs. Briefly, the study[42] involved the construction of two gene co-expression networks for normal and salinity conditions based on transcriptomic data from salt-tolerant rice. Key genes were identified by comparing node centralities measures for each gene node between the two networks. Relevant modules were detected, and their key genes were proposed as crucial for salt stress tolerance. Thus, all loci within each QTL predicted by BSA in the present study were compared with the key genes identified in the relevant modules by Sonsungsan et al.[42] to identify potential candidate genes in the QTLs.

QTN identification and QTN–by–QTN interaction analysis

Phenotype and genotype data from 10 rice cultivars obtained from Habila et al.[40] were subjected to GWAS to identify the significant QTNs and QTN–by– QTN interaction analysis. GWAS was performed using the 3 Variance-component multi-locus random-SNP-effect Mixed Linear Model (IIIVmrMLM) software package[45,114]. The analysis was conducted using the Single_env method, which is designed to detect main-effect QTNs by integrating genotype and phenotype data. Genotype data (fileGen) were converted from VCF to PLINK format (BED, BIM, and FAM) using PLINK version 1.9.0. Phenotype data (filePhe) included nine traits: CMS, RWC, SES, SFW, SDW, RFW, RDW, shoot length (SL), and RL. The kinship matrix was computed internally using the IIIVmrMLM software, and Manhattan plots were automatically generated for each trait.

Additionally, the Epistasis method implemented in IIIVmrMLM was used to identify QTN-by-QTN interactions. Due to computational constraints, it was not feasible to perform epistasis analysis across all single-nucleotide variants simultaneously. Therefore, for each trait, single-nucleotide variants located within ± 100,000 bp of each significant QTN identified in the single-locus GWAS analysis were extracted and used as input for epistasis analysis. For traits with only one significant QTN, epistasis analysis was not performed.

Relative gene expression analysis

Three rice varieties, 'Jao Khao', IR29, and 'Pokkali' (the salt-tolerant standard variety), were used to investigate the expression of putative salt tolerance genes located within the identified QTLs. Rice seedlings were grown for 14 days in a WP nutrient solution[105]. For the control treatment, seedlings were transferred to fresh WP nutrient solution, whereas for the salt stress treatment, seedlings were transferred to WP nutrient solution supplemented with 75 mM NaCl. The experiment was conducted using a completely randomized design with three replicates. Leaf tissues were collected for RNA extraction at 0, 3, 6, 9, and 12 h after treatment.

Total RNA was isolated from leaf tissue using GENEzol™ reagent (Geneaid Biotech Ltd.), according to the manufacturer's instructions. All RNA samples were treated with DNase I (Invitrogen, Carlsbad, CA) and 5 µg of total RNA was incubated with an iScript cDNA Synthesis Kit to synthesize cDNA, following the manufacturer's protocol (Bio-Rad, Hercules, CA). Gene expression levels were quantified by qPCR using the Luna Universal qPCR Master Mix (New England Biolabs, Ipswich, MA, USA) and gene-specific primers listed in Supplementary Table S5. *OsEF1A* was used as the reference gene for normalization. Relative expression levels were calculated using the Pfaffl method[115], which normalizes fold change to an endogenous control and then calibrates results using suitable correction factors such as PCR efficiency. The gene expression ratio was calculated using the following formula:

$$\text{Gene expression ratio} = (E_{target})^{\Delta Ct_{target(control\text{-}sample)}} / (E_{ref})^{\Delta Ct_{reference(control\text{-}sample)}}$$

where E represents primer efficiency of the gene and Ct represents threshold cycles.

Statistical analysis

Salt-responsive traits or phenotypic data of the F_3 populations were subjected to a normal distribution curve using SPSS Statistics version 22 (IBM, Armonk, NY, USA). A Pearson pairwise correlation analysis was carried out to determine the relationships among the salt-responsive traits. The correlation coefficient plots were generated using an R 'corrplot' package[116]. Analysis of variance (ANOVA) was used to explain the effects of alleles (genotypes) on the phenotypes of the F_3 individuals using a general linear model. Mean values were compared using Duncan's multiple-range test.

Conclusion

In conclusion, BSA using F_2 population from the cross between 'Jao Khao' and IR29 rice identified four salt tolerance QTLs located on chromosomes 2, 3, 6, and 12. The markers for salt tolerance were designed and validated in an F_3 population. Moreover, we used SNP-by-SNP interaction analysis and the information from the weighted gene co-expression network to predict the important genes in these QTLs and demonstrated that *LOC_Os03g49990 (OsSLR1),* encoding DELLA, and *LOC_Os06g06170,* encoding RING-type E3 ubiquitin transferase, play key roles in salt tolerance in 'Jao Khao'.

Data availability

The data sets in this study are available and open. The datasets were submitted to NCBI with BioProject number PRJNA1148750.

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Author contributions

L.C.; I.M.H.; S.C. and T.B. provided conceptualization and project design; provision and generation of breeding populations, D.S-A provided provision and generation of breeding populations; S.H. and N.K performed methods and phenotyping; S.C.; T.B.; L.C.; M.P.; W.K.; K.P., P.R.P and S.H. worked on the database search, data analysis and data submission; S.H wrote-original draft of the manuscript; S.C. reviewed and revised the second draft; All authors reviewed and approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to S.C.

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