QTL mapping and candidate gene identification in rice using a Kalarata-Azucena population under salt stress

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Abstract

Background

Salt stress is a major constraint across large rice production areas in Asia, because of the high sensitivity of modern rice varieties. To identify quantitative trait loci (QTL) associated with salt tolerance in rice, we developed an F_2 population from a cross between the salt-tolerant landrace, Kalarata, and the salt-sensitive parent, Azucena. An F_2 population was used for DNA extraction, and $F_{2:3}$ families from this population were screened in a phytotron in a saline nutrient solution at the seedling stage.

Results

After 2 weeks at an EC of 12 dS m^{-1}, the $F_{2:3}$ families were scored for salt tolerance using IRRI's *Standard evaluation system* (SES). Growth, biomass, Na^+ and K^+ concentrations in leaf tissues, and chlorophyll concentration were determined. A genetic linkage map was constructed with 151 SSRs and *InDel* markers, which cover 1463 cM with an average distance of 9.69 cM between loci. A total of 13 QTL were identified using Composite Interval Mapping for 16 traits. The short arm of chromosome 1 had the highest density of QTL associated with salt tolerance, which coincides with the *Saltol* locus; emphasizing the importance of this locus for candidate gene discovery and for use in rice breeding. Several novel QTL were identified on other chromosomes.

Conclusions

The novel QTL identified in this study constitute future targets for molecular breeding, to combine them with other QTLs identified before, for higher and stable performance of rice varieties in salt affected soils. Candidate genes for the large effect QTL on chromosome 3 were found to be involved in diverse biological processes, cellular components, and molecular functions. Several candidate genes in this locus were functionally associated with salt stress tolerance and should further be considered for genetic improvement of rice varieties.

Background

Rice is one of the most important cereal crops in the world. Among the common abiotic stresses affecting its productivity, salinity is a major constraint decreasing yields in both coastal and inland areas and both in irrigated and rainfed production ecosystems (Martinez-Atienza et al., 2007; Ismail et al., 2007; Ismail and Horie, 2017). Salt stress is worsening in coastal areas because of salt intrusion due to sea level rise caused by climate change, and in inland areas because of the build-up of salinity as a consequence of excessive use of irrigation with improper drainage, and use of poor quality irrigation water (Ismail et al. 2010). The sensitivity of rice to salt stress depends on the growth and development stages affected; rice is relatively more tolerant at germination, becomes more sensitive at the early seedling and reproductive stages, and gains relatively greater tolerance during active tillering and grain filling (Ismail et al., 2007; Walia et al., 2007).

Salt tolerance at the seedling stage is attributed mainly to low Na^+ accumulation and maintenance of low Na^+:K^+ ratio in the shoots (Lin et al., 2004; Platten et al., 2013). Therefore, Na^+ exclusion mechanisms from shoots are crucial for survival in salt-affected lands (Ismail and Horie, 2017). Quantitative trait loci (QTL) mapping has been frequently used to dissect and investigate major genes controlling salt tolerance and Na^+ exclusion mechanisms from shoots of rice using various salt tolerant genotypes (Flowers et al. 2000; Koyama et al., 2001; Lin et al., 2004; Ming-Zhe et al., 2005; Lee et al., 2007; Mohammadi-Nejad et al., 2008; Sabouri and Sabouri, 2008; Ammar et al., 2009; Pandit et al., 2010; Thomson et al., 2010; Islam et al., 2011; Cheng et al., 2012; Ghomi et al., 2013; Mohammadi et al., 2013; Hossain et al., 2015; Tiwari et al., 2016; Ismail and Horie, 2017). Among the several QTL mapped before, the availability of large effect QTL such as *Saltol* (Gregorio et al., 2002) provided opportunities to introduce these QTL into mega rice varieties or to combine them for multiple stress tolerance using marker assisted backcrossing (MABC) (Thomson et al., 2010). The *Saltol* QTL, derived from the cross of IR29 and Pokkali, is flanked by markers RM23 and RM140 (Bonilla et al., 2002). This region extends from 9.8 to 12.2 Mb on the short arm of chromosome 1 (Walia et al., 2005) and accounts for about 45% of the phenotypic variance for Na^+:K^+ ratio in rice shoots at the seedling stage. Rice chromosome 1 harbors a hotspot of QTL for shoot Na^+ content and Na^+:K^+ ratio (Negrao et al., 2011; Jing et al., 2017) where the *Saltol* QTL localises, and related QTL have been reported from various salt tolerant rice landraces such as Nona Bokra (Lin et al., 2004), Pokkali (Thomson et al., 2010; Alam et al., 2011), CSR 27 (Pandit et al., 2010), IR55178 (Koyama et al., 2001), Changbai10 (Zheng et al., 2015) and Co39 (Haq et al., 2008). On the other hand, various QTL for shoot Na^+ content, Na^+:K^+ ratio and salt tolerance at the seedling stage have been reported on different chromosomes from various rice genotypes (Koyama et al., 2001; Haq et al., 2008; Thomson et al., 2010), with novel QTL discovered from different donors. Therefore, mapping new QTL responsible for salt stress tolerance from new donors is necessary to combine them in high yielding backgrounds for high and stable salinity tolerance.

Farmers in salt affected areas still grow traditional landraces despite their long duration, poor grain quality, and low yield because they possess moderate to high tolerance of salt stress (Ismail et al. 2007; Rahman et al., 2016). Several physiological mechanisms have been reported to be associated with salt tolerance in rice, including sodium exclusion, effective sequestration of toxic salts into older leaves and roots, and generation of antioxidants (Yeo and Flowers, 1986; Ismail et al., 2007; Moradi and Ismail, 2007; Rahman et al., 2016). Aside from characterizing the physiological responses to salt stress using salt tolerant landraces, advances have been made in identifying QTL and genes controlling salinity tolerance traits (Rahman et al., 2016). The present study aimed to identify and map new major QTL associated with component traits involved in salt tolerance in rice, and to identify potential candidate genes for the promising QTLs using a new source of tolerance, Kalarata, and the salt-sensitive variety Azucena. Kalarata is an *indica* landrace grown in the brackish-water paddy fields of Western India, and was recognized for its salt tolerance at the seedling, vegetative and reproductive stages, but not during germination (Pearson et al., 1966; Makihara et al., 1999; Rahman et al., 2016). At the seedling stage, the low Na^+ accumulation in the shoots and high Na^+ accumulation in

roots of Kalarata suggests this genotype had developed salt exclusion mechanisms in roots to minimize salt transport to the shoot (Hedge and Joshi, 1974; Rahman et al., 2016). Azucena, on the other hand, is a tall low tillering, long grain aromatic upland variety from the Philippines, of *tropical* japonica origin, and is sensitive of salt stress (Hittalmani et al., 2002). The traits targeted in this study include plant growth, leaf chlorophyll concentration, visual symptoms of salt injury in seedlings, low sodium uptake and regulation of Na^+:K^+ ratio.

Results

Correlation among Physiological Traits

Significant differences in SES, shoot and root dry weights, shoot and root lengths, shoot and root K^+ and Na^+ concentrations, shoot and root Na^+:K^+ ratio, chlorophyll A and chlorophyll A + B were observed between genotypes (Table 1). Correlation coefficients among physiological traits were analyzed. SES score as a visual estimate of salt damage negatively correlated with shoot and root dry and fresh weights, shoot length, root K^+ and Na^+ concentrations, chlorophyll A, chlorophyll B and chlorophyll A + B. Shoot Na^+ concentration correlated negatively with root Na^+ concentration and shoot K^+ concentration (Table 2).

Table 1
One-way analysis of variance (ANOVA) among genotypes for different physiological traits in $F_{2:3}$ families at seedling stage under salt stress of 12 dS m^{-1} imposed for 21 days.

Trait	Sum Sq	Mean Sq	F value	Pr(> F)
SES	220.09	1.25	2.77	2.17E-11***
SDW	0.73	0.00	3.31	7.46e-15 ***
SL	12764.00	72.53	3.18	5.07e-14 ***
RDW	0.02	0.00	2.66	1.24e-10 ***
RL	1831.10	10.40	2.41	5.53e-09 ***
SFW	563.00	3.20	1.16	0.158
RFW	528.40	3.00	1.04	0.387
SKC	40.39	0.23	70.07	< 2e-16 ***
RKC	7.02	0.04	571.71	< 2e-16 ***
RNC	127.75	0.74	192.46	< 2e-16 ***
SNC	17063.00	96.95	39.78	< 2e-16 ***
SNKR	82.51	0.48	134.38	< 2e-16 ***
RNKR	451.10	2.58	240.41	< 2e-16 ***
CHLA	14.42	0.08	1.51	0.00331 **
CHLB	4.51	0.03	1.11	0.247
CHLAB	26.06	0.15	1.50	0.0041**

Data are means of two replications; *, ** and ***, significant at $P < 0.05$, 0.01 and 0.001, respectively using Fisher's least significant difference.

Table 2

Spearman correlation coefficients among different physiological traits in $F_{2:3}$ families at seedling stage un

Trait	SES	Shoot Dry Weight	Shoot Length	Root Dry Weight	Root Length	Shoot Fresh Weight	Root Fresh Weight	Shoot K^+ Concentration	Root K^+ Concentrati
Shoot Dry Weight	-0.49[a]								
Shoot Length	-0.36[a]	0.77[a]							
Root Dry Weight	-0.41[a]	0.84[a]	0.57[a]						
Root Length	0.05[ns]	0.11[ns]	0.15[b]	0.16[b]					
Shoot Fresh Weight	-0.49[a]	0.96[a]	0.74[a]	0.83[a]	0.10[ns]				
Root Fresh Weight	-0.36[a]	0.79[a]	0.52[a]	0.89[a]	0.13[ns]	0.78[a]			
Shoot K^+ Concentration	0.09[ns]	-0.11[ns]	-0.16[ns]	-0.04[ns]	-0.16[ns]	0.03[ns]	-0.12[ns]		
Root K^+Concentration	-0.33[a]	0.14[ns]	0.08[ns]	0.03[ns]	0.04[ns]	0.13[ns]	0.03[ns]	-0.24[ns]	
Root Na^+ Concentration	-0.27[a]	-0.15[b]	-0.12[ns]	-0.04[ns]	-0.03[ns]	-0.11[ns]	-0.04[ns]	0.14[ns]	-0.07[ns]
Shoot Na^+ Concentration	0.04[ns]	-0.16[b]	-0.09[ns]	-0.16[b]	-0.10[ns]	-0.17[b]	-0.11[ns]	-0.32[a]	0.01[ns]
Shoot Na^+K^+ Ratio	0.10[ns]	0.12[ns]	0.13[ns]	0.07[ns]	0.04[ns]	0.14[ns]	0.09[ns]	0.39[a]	-0.04[ns]
Root Na^+K^+ Ratio	0.01[ns]	-0.15[b]	-0.12[ns]	0.18[b]	-0.08[ns]	-0.17[b]	-0.14[ns]	-0.32[a]	-0.02[ns]
Chlorophyll a	-0.31[a]	0.44[a]	0.37[a]	0.34[a]	0.05[ns]	0.45[a]	0.26[a]	0.00[ns]	0.22[b]
Chlorophyll b	-0.33[a]	0.40[a]	0.37[a]	0.32[a]	-0.01[ns]	0.41[a]	0.25[a]	0.03[ns]	0.21[b]
Chlorophyll a and b	-0.32[a]	0.44[a]	0.39[a]	0.35[a]	0.04[ns]	0.46[a]	0.27[a]	0.00[ns]	0.22[b]

SES; Standard evaluation system score based on salt stress symptoms.

[a] and [b] indicate significance at $P < 1\%$ and 5%, respectively, ns = not significant.

QTL identification

Linkage analysis was performed with microsatellite genotyping data from the 151 SSR and *InDel* markers using QGENE version 4.3.2 (Nelson, 1997). Figure 1 shows the distribution of the 151 markers throughout the rice genome, with a total length of 1463 cM. The average interval size between markers was 9.69 cM. QTL associated with salt tolerance were identified through CIM using QGENE program.

Under salt stress, seven QTL for physiological traits were identified with LOD > 3.0. Chlorophyll b (*chlb3.1*), root K^+ concentration (*rkc3.1*) and root Na^+ concentration (*rnc3.1*) on chromosome 3, shoot K^+ concentration (*skc1.1*), shoot Na^+ concentration (*snc1.1*) and shoot Na^+:K^+ ratio (*snkr1.1*) on chromosome 1 and root K^+ concentration (*rkc11.1*) on chromosome 11 (Table 3; Figs. 1 and 2).

Table 3
Significant QTL detected in $F_{2:3}$ generation for traits related to salt tolerance from the cross of Kalarata/Azucena under salt stress condition at seedling stag
interval mapping (CIM).

TRAITS	CHROMOSOME	QTL NAME	PEAK MARKER	FLANKING MARKERS	PEAK LOD	ADDITIVE EFFECT	INCREASED EFFECT
Shoot fresh weight (SFW)	1	*sfw1.1*	RM7643	RM11570- SO1132A	4.0	0.095	K[a]
Shoot dry weight (SDW)	1	*sdw1.1*	SO1132A	RM11570- SO1140	3.3	0.016	K
Root dry weight (RDW)	1	*rdw1.1*	RM7643	RM11570- S01132A	5.2	0.0037	K
	5	*rdw5.1*	RM18161	RM18161- RM249	3.2	0.0037	K
Shoot K^+ concentration (SKC)	1	*skc1.1*	AP3206d	RM1287- RM3412a	3.9	-0.1701	A
Root K^+ concentration (RKC)	3	*rkc3.1*	SO3065	SO3072- SO3099	4.4	-0.1	A
	11	*rkc11.1*	RM26464	S11033- RM26652	3.9	0.079	K
Shoot Na^+ concentration (SNC)	1	*snc1.1*	RM10696B	RM1287- AP3206d	7.8	0.68	K
Root length (RL)	2	*rl2.1*	R2M50	RM13332- RM5404	9.1	2.3	K
Shoot Na^+/K^+ (SNKR)	1	*snkr1.1*	RM10696B	RM1287- AP3206d	9.81	0.48	K
Chlorophyll b (CHLB)	3	*chlb3.1*	SO3065	SO3072- SO3099	3.3	-0.42	A
Root Na^+ concentration (RNC)	3	*rnc3.1*	SO3065	SO3072- SO3099	11.0	-0.74	A
SES	3	*ses3.1*	SO3065	SO3072- SO3099	6.8	0.71	K

[a] K = Kalarata; A = Azucena; R^2 = Phenotypic variance explained by a particular QTL; LOD = Logarithm of Odd

Variation in growth of the seedlings was determined based on root length, root fresh and dry weights, shoot length, and shoot fresh and dry weights. QTL were detected for shoot fresh weight (*sfw1.1*), shoot dry weight (*sdw1.1*) on chromosome 1, root dry weight (*rdw1.1; rdw5.1*) on chromosome 1 and 5, and root length (*rl2.1*) on chromosome 2 (Table 3; Fig. 1). Visual symptoms using SES scores as an indicator of whole-plant salt tolerance at the seedling stage mapped on chromosome 3 as *ses3.1*, with R^2 of 17.01% (Table 3; Fig. 1).

Candidate Genes Associated with Salinity Tolerance-Related Traits

To identify putative candidate genes for salinity tolerance, we selected the region on chromosome 3 containing the major QTL associated with salinity tolerance; this locus was not previously reported. This QTL was identified for root K^+ concentration and it overlaps with the QTL for SES and root Na^+ concentration. The region within the range of flanking markers was assessed using IRRI Galaxy (Juanillas et al., 2019; http://galaxy.irri.org/). About 2,361 candidate genes were identified; most of them were involved in biological process, cellular components, and molecular functions (Supplementary Table S1). Candidate genes categorized as expressed proteins, hypothetical proteins, hypothetical conserved genes, non protein coding transcription, similar to predicted proteins, proteins of unknown functions, family proteins and uncharacterized protein families were discarded. Annotation of the candidate genes was analyzed using RiceNet2 (https://www.inetbio.org/ricenet/) and the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu). Putative candidate genes were trimmed to 555 after discarding genes disconnected from the guide genes (Supplementary Figure S1); and 34 genes that are functionally associated with salt tolerance were summarized in Table 4. Ontology analysis showed that these candidate genes were derived from many functional categories like response to abiotic stresses, metabolic processes, transporters, transcription factors, cellular component, biosynthetic processes, protein coding and photosynthesis.

Table 4
List of candidate genes that are likely associated with salinity tolerance- related traits at the QTL locus on chromosome 3.

Putative Function	MSU ID	Organism
Response to abiotic stresses		
CAMK includes calcium/calmodulin dependent protein kinases, expressed	LOC_Os03g27280	Rice
Universal stress protein domain containing protein, putative, expressed	LOC_Os03g53900	Rice
NAD dependent epimerase/dehydratase family protein, putative, expressed	LOC-Os03g32170	Rice
Ribosomal protein, putative, expressed	LOC_OS03g34040 LOC_Os03g60550	Rice
Thif family domain containing protein, putative, expressed	LOC_Os03g28330	Rice
Metabolic process	LOC_Os03g43590	Rice
Sucrose synthase, putative, expressed	LOC_Os03g48310	Rice
LSTK-1 like kinase, putative, expressed	LOC_Os03g58630	Rice
Plasma membrane ATPase, putative, expressed	LOC_Os03g40100	Rice
Thioredoxin, putative, expressed	LOC_Os03g26930	Rice
ACT domain containing protein, expressed	LOC_Os03g27210	Rice
OsSCP13-Putative serine carboxypeptidase homologue, expressed		Rice
LOL3, putative, expressed		
Transporter		
Potassium transporter, putative, expressed	LOC_Os03g37840	Rice
Potassium channel protein, putative, expressed	LOC_Os03g54100	Rice
Heavy metal-associated domain containing protein, expressed	LOC_Os03g27040	Rice
Sodium/calcium exchanger protein, putative, expressed	LOC_Os03g27960	Rice
Metal cation transporter, putative, expressed	LOC_Os03g29850	Rice
ABC transporter, ATP-binding protein, putative, expressed	LOC_Os03g32630	Arabidopsis
MATE-efflux family protein, putative, expressed	LOC_Os03g37470	Rice
Potassium transporter, putative, expressed	LOC_Os03g37830	Rice
Amino acid permease family protein, putative, expressed	LOC_Os03g37984	Rice
CorA-like magnesium transporter protein, putative, expressed	LOC_Os03g53110	Rice
Cyclic nucleotide gatedchannel 6	LOC_Os03g44440	
Transcription factor		
MYB family transcription factor, putative, expressed	LOC_Os03g51110	Rice
Calcineurin B, putative, expressed	LOC_Os03g42840	Rice
Cellular component	LOC_Os03g43650	Rice
Uncharacterized protein At4g06744 precursor, putative, expressed	LOC-Os03g56580	Rice
No apical meristem protein, putative, expressed	LOC_Os03g58260	Rice
Biosynthetic process	LOC_Os03g36540	Rice
Indole-3-glycerol phosphate lyase, chloroplast precursor, putative, expressed	LOC_Os03g60120	Rice
Magnesium-chelatase subunit chl1, chloroplast precursor, putative, expressed	LOC_Os03g43930	Rice
AP2 domain containing protein, expressed	LOC_Os03g54170	Rice
START domain containing protein, expressed		
OsMADS34-MADS-box family gene with MIKCc type-box, expressed		
Protein coding		
Brittle Culm1	Os03g0416200	Rice
Photosynthesis	LOC_Os03g39610	Rice
Chlorophyll a/b binding protein, putative, expressed		

Discussion

Salt stress in rice is complex and involves several physiological and adaptive mechanisms (Ismail et al., 2007; Ismail and Horie, 2017). Mapping and tagging of QTL is the first step for identifying genes associated with variation in an agronomically important trait such as salinity tolerance (Rahman et al., 2019). Despite the complexity of the traits associated with tolerance of stress, tolerance in most cases is controlled by relatively few QTL with large effects, and incorporation of these QTL into high-yielding varieties will potentially increase and stabilize rice yields in salt-affected areas and in areas affected by other abiotic stresses (Mackill, 2006; Ismail et al., 2007; 2013; Ismail and Horie, 2017).

Significant differences between genotypes were observed for most growth and physiological parameters (Table 1). SES scores based on visual salt-induced injury are often used for evaluating salt tolerance in rice at the seedling stage (Platten et al., 2013; Thu et al., 2017). Significant negative correlations were observed for SES score with shoot and root fresh and dry weights and shoot length. This clearly demonstrates the significance and detrimental effects of high Na^+ accumulation in plant tissues under salinity stress. Although salt tolerance evaluated by SES is attributed to low Na^+ in shoots and high Na^+ in roots in this study (Table 2), the mismatch of QTL for SES and for shoot Na^+ concentration (Table 3) indicates the complexity of the physiological mechanisms associated with salt tolerance in Kalarata. Moreover, the diversity of QTL for SES among rice varieties also suggests that salt tolerance could be controlled by multiple mechanisms, multiple genes or alleles (Ismail and Horie, 2017).

In this study we identified 13 QTL for 5 traits of the shoot and 4 traits of the roots controlling growth and physiological attributes related to salt tolerance. Within the 13 QTL, ten of them were newly mapped in this study and the other three QTL for shoot K^+ concentration, shoot Na^+ concentration and shoot Na^+:K^+ ratio identified on chromosome 1 (Table 3; Figs. 1 and 2) overlapped with QTL reported in previous studies (Koyama et al., 2001; Thomson et al., 2010). The QTL for shoot Na^+ concentration and shoot Na^+:K^+ ratio were detected in the same position on the short arm of chromosome 1, which suggests that the loci affecting Na^+ uptake also control Na^+:K^+ ratio in shoots, indicating potential functional relationships among these traits; or probability that either the same genes or tightly linked genes are involved in their control. The position of these QTL at 43.6 cM and 44 cM coincided with the well-known *Saltol* locus (Thomson et al., 2010), and, respectively accounted for 19.0 and 23.0% of the phenotypic variation (Table 3). Similar results were reported in the study of Koyama et al. (2001) where they identified QTL controlling K^+ concentration, Na^+ uptake and Na^+:K^+ ratio in this region.

Three QTL for root K^+ concentration (*rkc3.1* and *rkc11.1*) and root Na^+ concentration (*rnc3.1*) were newly identified on chromosomes 3 and 11 (Table 3; Fig. 2). Azucena contributed to the positive alleles of *rkc3.1* and *rnc3.1* and Kalarata contributed to the positive alleles of *rkc11.1*. The QTL detected for root Na^+ and K^+ concentrations were different from those detected for shoot traits. Lin et al. (2004) suggested that the genes controlling the transport of these two ions, Na^+ and K^+, between roots and shoots of rice seedlings might be different or are differentially regulated under salt stress. Koyama et al. (2001) also suggested that uptake of potassium is controlled by genes related to the structure or regulation of ion carriers and channels, while the transport of sodium in saline conditions is expected to be controlled by genes affecting root development, anatomy and architecture. Gregorio and Senadhira (1993) also observed two groups of genes involved in sodium and potassium uptake in rice; one group was envisaged to control sodium exclusion and the other to control potassium absorption. This could explain why there are different QTL for Na^+ and K^+ uptake in shoot and root. Ismail and Horie (2017) also pointed that responses to salt stress could be attributed to Na^+ efflux from roots to the rhizosphere through salt overly sensitive (SOS1) dependent Na^+ exclusion, Na^+ sequestration in vacuoles by tonoplast-localized Na^+/H^+ antiporters and Na^+ loading and unloading at the xylem mediated by some high-affinity K^+ transporter (HKT) proteins.

We identified one new QTL on chromosome 3 based on symptoms of salt injury at the whole plant level using SES scores (Table 3). The QTL for SES, with Kalarata as the source of the positive allele, overlapped with that for chlorophyll b (*chlb3.1*) in this study. A variety of QTL for visual salt-induced injury such as SES has been reported from different sets of rice crosses; e.g. chromosomes 1, 3, 4, 5 from a cross between CSR27 and MI-48 (Ammar et al., 2007), chromosomes 1, 3 from a cross between Milyang 23 and Gihobyeo (Lee et al., 2007), chromosomes 1, 4 from a cross between Hasawi and IR29 (Rahman et al., 2017), chromosomes 2, 4, 11 from GWAS study using 203 temperate japonica rice accessions (Batayeva et al., 2018), and chromosome 1, 3, 5, 12 from a cross between Capsule and BRRI dhan29 (Rahman et al., 2019). Three QTL controlling shoot fresh weight (*sfw1.1*), shoot dry weight (*sdw1.1*) and root dry weight (*rdw1.1*) were located in the same region of chromosome 1, with Kalarata as the source of positive alleles. These traits are related to seedling vigor, which is important as an avoidance mechanism under salinity (Ismail et al., 2007; Reddy et al., 2017). This result indicates the important role of this region in determining biomass and vigor of rice. The shoot fresh weight QTL identified in this study was close to the QTL detected by Haq et al. (2008) in the short arm of chromosome 1 from the cross of Co39 and Moroberekan, which also stressed the importance of this region for seedling vigor under salinity.

The QTL detected in this study should be useful for molecular breeding and for identifying useful genes for salt tolerance. Fine mapping of selected QTL will help identify closely linked markers for use in MABC (Ismail and Thomson, 2010). By developing near isogenic lines (NIL) differing in the presence of a specific QTL for each trait of interest, QTL controlling the trait could be verified more precisely and the biological functions of each QTL could be unraveled. The actual contribution of each QTL for a phenotypic trait should be tested and confirmed in different genetic backgrounds and environment. Larger populations of NILs need to be developed for fine mapping of these QTL that has large effects and agronomic value, for further use in breeding.

Candidate genes in the target region for root K^+ concentration on chromosome 3 (*rkc3.1)* were further assessed. The genes encoding expressed proteins, hypothetical proteins, hypothetical conserved genes, non protein coding transcriptions, similar to predicted proteins, protein of unknown functions, family proteins and uncharacterized protein families were eliminated. Genes that share the same functional annotations and the same GO terms in biological processes, cellular component and molecular function GO categories were listed (Table 3; Supplementary Table S1).

Five genes were identified that are associated with responses to abiotic stresses (Table 3). One gene (*LOC_Os03g27280*) predicted to encode calmodulin. CaM genes regulate plant responses to heat stress, cold stress, heavy metal stress, drought, and salt stress. High salinity and drought impose osmotic stress on plant cells that is associated with increased concentration of cytosolic Ca^{2+}, and decreased Na^+ uptake in the shoots (Zeng et al., 2015). This gene is also

similar to serine/threonine-protein kinase SAPK1. SAPK1 and SAPK2 function together to reduce Na^+ toxicity by altering Na^+ distribution between roots and shoots and enhancing Na^+ exclusion from the cell cytoplasm and its sequestration into vacuoles to improve tolerance of crop plants to salt stress (Lou et al., 2018). The universal stress protein (*LOC_Os03g53900*) is another gene involved in abiotic stress responses. One example is shown in tobacco wherein unusual expression of *SbUSP* enhances salt tolerance and increases osmotic stress resistance by removing intracellular reactive oxygen species (ROS). It then recognizes cellular level Na^+ and activates protein kinases (serine and threonine), which is involved in salt signaling (Chi et al., 2019).

Seven genes annotated in the *rkc3*.1 region were associated with metabolic processes based on GO classification (Table 3). Thioredoxin (*LOC_Os03g58630*) is a protein that plays an important role in redox regulation. One type of thioredoxins is TRX-h that is located in the cytosol and plasma membrane, and involved in the movement of plasmodesmata for cell-to-cell communication in Arabidopsis. In rice, thiredoxin takes part in C4 metabolism through PEPC-PK (Calderon et al., 2018). An example is *OsTRXh-1*, which is secreted in the extracellular region (apoplast); its knockdown causes dwarf and low-tillering phenotypes, while overexpression causes salt- and ABA-insensitive phenotypes (Zhang et al., 2011). This shows that redox regulation influences plant development under salt stress. Serine carboxypeptidase (*LOC_Os03g26930*) catalyzes the hydrolysis of the C-terminal bond in proteins and peptides and have been involved in biochemical processes including secondary metabolites (Tripathi and Sowdhamini, 2006). Among the SCPs, SCP 46 is dominantly expressed in rice developing seeds, and induced expression of ABA, and could potentially be involved in ABA signaling. Knocking down of this gene in rice affected grain size and seed germination, and inhibited sensitivity to ABA (Li et al., 2016).

Eleven genes were identified that are associated with transporter activity (Table 3). *LOC_Os03g37840* encodes a potassium transporter similar to HAK16. Expression of *OsHAK16* is downregulated in young leaves and its upregulation increases the accumulation of Na^+ in the old leaves of rice under salt stress (Wang et al., 2012), suggesting its role partition of harmful salts in older leaves to protect functional young leaves. *OsHAK1* is expressed in the epidermal and vascular cells of roots, and addition of nutrient solution with high Na^+ to low K^+ ratio decreases its K- deficiency-enhanced expression in roots and shoots in rice. Knockout of this gene also limit cell expansion resulting in stunted growth, and decreased K^+ translocation from roots to shoots. Overexpression of this gene increased K^+ concentration and K^+:Na^+ ratio in both roots and shoots (Chen et al., 2015). This high-affinity K^+ transport system plays an important role in improving tolerance of rice to salt stress. Another transporter, NCX or sodium/calcium exchanger (*LOC_Os03g27960*) plays a crucial role in Ca^{2+} homeostasis. Expression profile studies of this gene showed different responses to calcium and its chelator EGTA in the moderately stress sensitive rice variety IR64, suggesting an important role in the diverse physiological processes involving Ca^{2+} as second messenger (Singh et al., 2015). A metal cation transporter (*LOC_Os03g29850*) is similar to the genes that encode ZIP (Zinc-regulated, Iron-regulated transporter like protein). This gene is involved in the transport of metals such as Zn, Cu, Fe, Cd and Mn. OsZIP1 is a Zn uptake transporter and overexpression of this protein decreased the concentrations of Zn, Cu and Cd in rice, resulting in improved growth under high metal stress (Liu et al., 2019).

Two genes in the *rkc3*.1 region were associated with transcription factors (Table 3). MYB family transcription factor (*LOC_Os03g51110*) is similar to R2R3 type. MYB-TF is abundant in specific plants and R2R3 play vital roles in plants including responses to abiotic and biotic stresses. AtMYB20 is an example of R2R3 MYB TF. In the study of AtMYB20, *AtMYB-Ox* (overexpression lines) enhances salt tolerance over the wild type seedlings, while *AtMYB-SRDX* (dominant repression lines) seedlings were sensitive to NaCl. Salt-induced expression of ABI1, ABI2 and AtPP2CA (negative regulators of ABA signaling), decreased *ATMYB-Ox* and enhanced *AtMYB-SRDX* compared with the wild type and binding to the promoter region of ABI1 and AtPPC2A improves salt tolerance in plants (Cui et al., 2013). Calciunerin B protein (*LOC_Os03g42840*) is another transcription factor that belongs to a group of plant calcium sensors that interacts with serine/threonine kinases, CIPK proteins. In studies using Arabidopsis, CIPK9, which is a homolog of CIPK23 interacts with CBL3 to form a CBL/CIPK complex. Overexpression of CBL3 and CIPK9 leads to more sensitive phenotypes under low potassium (LK) conditions. CBL3 mutants showed similar LK tolerant phenotype as CIPK mutants in LK medium. In addition, CIPK9 and CBL3 mutants have higher K^+ content in the shoots than the wild type, resulting in better regulation of K^+ homeostasis under LK conditions (Liu et al 2013).

Apparently, most of the genes discussed above could have potential role in abiotic stress response or tolerance in rice. Genes identified using this network analysis are potential targets for molecular breeding or for engineering rice plants with improved salt tolerance (Ismail and Horie, 2017; Zhu et al., 2019). These candidate genes with putative functions likely related to salt tolerance should further be assessed for their functional roles in physiological processes that confer salt tolerance in rice and for use in breeding improved varieties for salt affected areas. Haplotype and gene expression analysis will help extract more information on these candidate genes.

Conclusions

Salinization of agricultural lands is reducing rice productivity worldwide and its effect is worsening with climate change, especially in coastal zones. The knowledge of QTLs for salinity tolerance is an important step for future plant breeding programs to help increase and sustain yield of rice and other food crops grown in salt affected areas. SES scores showed strong association with shoot and root growth, shoot length, root K^+ and Na^+ concentrations, chlorophyll concentration, and with shoot Na^+ concentration. Shoot Na^+ concentration correlated negatively with root Na^+ concentration and shoot K^+ concentration, suggesting critical roles for mechanisms involved in root-shoot translocation (Ismail and Horie, 2017). A total of 13 QTLs responsible for different physiological traits likely associated with salinity tolerance were identified; with chromosome 1 having the highest density of QTL associated with salt tolerance, and overlaps with the *Saltol* locus. QTL for root K^+ concentration (*rkc3.1*,) detected on chromosome 3 could be of much interest as a novel QTL and is likely associated with sodium sequestration in roots. This QTL does not have the same map location with previously mapped QTL. Several candidate genes with likely functional association with salinity tolerance were identified in this position, which could provide bases for further analysis to identify salt-tolerance related genes in rice. For example, *LOC_Os03g37840* mediates K^+ uptake and root-to-shoot translocation, which will affect salt exclusion and Na^+:K^+ ratio in the shoot. Identification of DNA markers that are closely linked with these new QTL for salt tolerance will be useful for MABC, to pyramid these QTL with others that have been identified before, to develop varieties with greater salt tolerance for salt affected coastal and inland areas.

Methods

Plant materials, DNA extraction, and genotyping

A total of 400 F_2 plants were developed from a cross between Kalarata and Azucena at the International Rice Research Institute (IRRI), Philippines. Two to three centimeter leaf samples collected from 14 day old greenhouse-grown F_2 plants and their parents, Kalarata and Azucena, were used to extract genomic DNA using the DNA miniprep method. Frozen tissue was crushed and 800 µl of DNA extraction buffer (100 mM Tris-HCl, 50 mM EDTA, 500 mM NaCl, 1.25% (w/v) SDS, 3.8 g per L NaBisulfite) was added to test tubes, and the tubes were vortexed and incubated at 65 °C for 20 minutes. Subsequently, a chloroform extraction was performed with 24:1 chloroform: isoamyl-alcohol solution, followed by an ethanol precipitation and resuspension in 100 µl of TE buffer. The quality and quantity of the isolated DNA were determined using a Spectrophotometer (Nanodrop, ND-1000, USA) and diluted to working concentrations of 35 ng/µl with deionized distilled water.

A parental polymorphism survey covering the whole rice genome was performed using 257 SSR and insertion-deletion markers. Out of these markers, 151 were polymorphic. Each PCR reaction was carried out in a 15 ul volume mix containing 1.5 µl 10X PCR buffer, 1 µl of 1 mM dNTPs, 1 µl of 5 µM forward and reverse primers, 0.7 µl of 5 U/µl Taq polymerase, 8.8 µl deionized distilled water and 2 µl of DNA template at 35 ng/µl concentration. PCR profiles were programmed as follows: initial denaturation of 94 ºC for 5 min, followed by 35 cycles of denaturation at 94 ºC for 30 s, annealing at 55 ºC for 30 s, extension at 72 ºC for 45 s, and a final extension step at 72 ºC for 5 min (MJ Research and G-storm thermal cyclers). PCR products were run on 6% (v/v) acrylamide gels at 100V (Dual Triple-Wide Mini-Vertical System, C.B.S. Scientific, CA, USA) followed by SYBR-Safe staining (Invitrogen, USA), gel documentation (Alpha Innotech, USA), and manual scoring of the gel pictures.

Plant Growth And Salt Treatment

Due to a high degree of sterility, only 177 lines of the 400 F_2 plants produced sufficient seeds; those 177 $F_{2:3}$ families were evaluated for salt tolerance in a phytotron with day/night temperature of 29/21 ^{o}C and relative humidity of 70%. Seeds were heat treated for 5 d in a convection oven set at 50 °C to break their dormancy, then placed in petri dishes lined with moistened filter papers and incubated at 30 °C for 48 h to germinate. Pre-germinated seeds were sown on styrofoam floats (one seed per hole) with a net bottom floated on distilled water in 11 L plastic trays, for three days, after which a culture solution (Yoshida et al., 1976) was used. At 14 d after seeding, salt stress was introduced by adding sodium chloride (NaCl) to an electrical conductivity (EC) of 6 dS m^{-1} for 3 days followed by EC of 12 dS m^{-1} until the experiment was terminated (total 21 days). The EC of the culture solution was monitored using an EC meter (HI9835, Hanna Instruments, Romania) and adjusted daily. Three replications were used, with ten individual plants per line evaluated for each replicate. IR29 (sensitive) and FL478 (highly tolerant) (Gregorio et al., 1997; Ismail et al., 2007) were used as checks. The pH of the nutrient solution was adjusted daily to 5.0, and the culture solution was replaced weekly.

Assessment Of Physiological Traits Associated With Salt Tolerance

After treatment with NaCl at 12 dS m^{-1} for 21 days, entries were scored based on visual symptoms using IRRI's Standard evaluation system (SES) scores, with ratings from 1 (highly tolerant) to 9 (highly sensitive; IRRI, 2014). The third fully expanded leaves were harvested and wrapped in aluminum foil and stored at -15 ^{0}C. Root length (RL), shoot length (SL), root fresh weight (RFW) and shoot fresh weight (SFW) were recorded. Plants were harvested, washed thoroughly with deionized water, dried at 70 ^{0}C, and their root dry weight (RDW) and shoot dry weight (SDW) were determined. To assess Na^+ and K^+ concentrations in plant tissues, shoots and roots were extracted in 10 ml of 0.1 M acetic acid in a water bath set at 90 ºC for 2 h, cooled at room temperature, and the evaporated solution replaced with deionized distilled water. Samples were then filtered and Na^+ and K^+ concentrations were measured using an atomic absorption spectrophotometer (AAS 3100, Perkin Elmer, USA). Respective root Na^+:K^+ ratio (RNKR) and shoot Na^+:K^+ ratio (SNKR) were calculated.

Measurements Of Chlorophyll Concentration In Leaves

The third fully-expanded leaves were snap-frozen in liquid nitrogen and freeze dried, then ground to a fine powder and chlorophyll was extracted using 1.0 mg dry leaf material added to 1 mL of 80% (v/v) acetone. After extraction, the chlorophyll concentration was determined using an UV/VIS-Spectrophotometer (DU 530, Beckman Counter, USA). Readings were taken at 663, 652 and 645 nanometer wavelengths and the final chlorophyll concentration (ppm) was calculated using the following formulae (Arnon, 1949):

Chlorophyll a = $12.7_{A663} - 2.7_{A645}$

Chlorophyll b = $22.9_{A645} - 4.7_{A663}$

Chlorophyll a and b = 27.8_{A652}

QTL Analysis Using Kalarata- Azucena Population

QGENE software version 4.3.2 (Nelson, 1997) was used to construct the genetic linkage map using Kosambi (1944) functions based on genotyping data of F_2 plants. Marker orders were confirmed using the published physical map from the rice database (www.gramene.org) and Cornell map. The linkage groups were

determined using command group with logarithm of odds (LOD) > 3.0; the same LOD was also used to check linkages among the SSR markers. The proportion of the total phenotypic variance explained by each QTL was calculated as R^2 value (R^2 = ratio of the sum of squares explained by the QTL to the total sum of squares). The analysis was carried out based on available information on genotype data from the genetic linkage map established for the Kalarata/Azucena cross. Composite interval mapping (CIM; Zeng, 1994) was used to examine the association between phenotypic data and marker genotype. To increase the precision of putative QTL, minimal logarithm of odd (LOD) value was analyzed empirically from 1000 permutation tests (Churchill and Doerge, 1994). This software was also used to identify the effects and origins of alleles contributed by the parents.

Statistical analysis

Statistical analysis was performed for each trait based on a randomized complete block design model with two replications using R. The Fisher's least significance (LSD) was performed at the 0.05 significance level. Correlation analysis was performed in the F_2 mapping population to dissect physiological mechanisms that are associated with salt tolerance in this population at seedling stage. Correlations among traits were calculated using Spearman correlation method. Spearman's correlation coefficient is a non-parametric measure of the strength and direction of association that exists between two variables measured on at least an ordinal scale. The test is used for either ordinal variables or for continuous data that has failed the assumptions necessary for conducting the Pearson's product-moment correlation. A high correlation means that two or more variables have a strong relationship with each other, while a weak correlation means that the variables are hardly related (Franzese and Luliano, 2019).

Analysis tools for identification of enriched gene ontology (GO) of candidate genes were acquired from the RiceNet database (Lee et al., 2015). To determine co-expression of candidate genes, network analysis were visualized using cytoscape (Shannon et al., 2003).

Abbreviations

AAS: Atomic absorption spectrophotometer; CIM: Composite interval mapping; EC: Electrical conductivity; GO: Gene ontology; LOD: Logarithm of odds; LSD: Fisher's least significance; MABC: Marker-assisted backcrossing; NIL: Near-isogenic lines; PCR: Polymerase chain reaction; QTL: Quantitative trait loci; RDW: Root dry weight; RFW: Root fresh weight; RL: Root length; SES: Standard evaluation system; SDW: Shoot dry weight; SFW: Shoot fresh weight; SL: Shoot length

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors read the manuscript.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

AMI, MJT, HVT, MDO, SM and AY conceived the idea of the study. MDO designed the experiment. HVT evaluated material under salt stress. MDO and HVT performed SSR analysis. MDO performed statistics, QTL analysis, and candidate gene analysis. MDO, AMI and SM wrote the manuscript.

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References

1. Alam R, Rahman MS, Seraj ZI, Thomson MJ, Ismail AM, Tumimbang-Raiz E, Gregorio GB (2011) Investigation of seedling-stage salinity tolerance QTLs using backcross lines derived from *Oryza sativa* L. Pokkali. Plant Breeding 130:430–437
2. Ammar MHM, Singh RK, Singh AK, Mohapatra T, Sharma R, Singh NK (2007) Mapping QTLs for salt tolerance at seedling stage in rice (*Oryza sativa* L.) In: African Crop Science Conference Proceedings 8:617–620
3. Ammar MHM, Pandit A, Singh RK, Sameena S, Chauhan MS, Singh AK, Sharma PC, Gaikwad K, Sharma TP, Mohapatra T, Singh NK (2009) Mapping of QTLs controlling Na^+, K^+ and Cl^- ion concentrations in salt tolerant indica rice variety CSR27. Plant Biochemistry Biotechnology 18:139–150
4. Arnon DI (1949) Copper enzymes in isolated chloroplasts: Polyphenoloxidase in Beta vulgaris. Plant Physiol 24:1–15
5. Batayeva D, Labaco B, Ye C, Li X, Usenbekov B, Rysbekova A, Dyuskalieva G, Vergara G, Reinke R, Leung H (2018) Genome-wide association study of seedling stage salinity tolerance in temperate japonica rice germplasm. BMC Genet 19:2. doi:10.1186/s12863-017-0590-7
6. Bonilla P, Vorak J, Mackill D, Deal DK, Gregorio G (2002) RFLP and SSLP mapping of salt tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines. Philippine Journal of Agricultural Science 85:68–76
7. 10.1007/978-3-319-75088-0_7
   Calderon A, Sevilla F, Jimenez A (2018) Redox protein thioredoxins: Function under salinity, drought and extreme temperature conditions. In: Gupta DK et al (eds). Antioxidants and Antioxidant enzymes in higher plants. Springer International Publishing: doi:10.1007/978-3-319-75088-0_7
8. Chen G, Hu Q, Luo L, Yang T, Zhang S, Hu Y, Yu L, Xu G (2015) Rice potassium transporter OsHAK1 is essential for maintaining potassium-mediated growth and functions in salt tolerance over low and high potassium concentration ranges. Plant, Cell and Environment 38:2747–2765
9. Cheng L, Wang Y, Meng L, Hu X, Cui Y, Sun Y, Zhu L, Ali J, Xu J, Li Z (2012) Identification of salt-tolerant QTLs with strong genetic background effect using two sets of reciprocal introgression lines in rice. Genome 55:45–55
10. Chi YH, Koo SS, Oh HT, Lee ES, Park JH, Phan KAT, Wi SD, Bae SB, Paeng SK, Chae HB, Kang CH, Kim MB, Kim WY, Yun DJ, Lee SY (2019) The physiological functions of universal stress proteins and their molecular mechanisms to protect plants from environmental stresses. Front Plant Sci:10. doi:10.3389/fpls.2019.00750
11. Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971
12. Cui MH, Yoo KS, Hyoung S, Nguyen HTK, Kim YY, Kim HJ, Ok SH, Yoo SD, Shin JS (2013) An Arabidopsis R2R3-MYB transcription factor, AtMYB20, negatively regulates type 2C serine/threonine protein phosphatases to enhance salt tolerance. FEBS Lett 587:1773–1778
13. Franzese M, Luliano A (2019) Correlation analysis. In: Ranganathan S, Gribskov M, Nakai K, Schonbach C (eds). Encyclopedia of bioinformatics and computational biology. Academic Press p706-721
14. Flowers TJ, Koyama ML, Flowers SA, Sudhakar C, Singh KP, Yeo AR (2000) QTL: their place in engineering tolerance of rice to salinity. J Exp Bot 1(342):99–106
15. Ghomi K, Rabiei B, Sabouri H, Sabouri A (2013) Mapping QTLs for traits related to salinity tolerance at seedling stage of rice (*Oryza sativa* L): An Agrigenomics study of an Iranian rice population. Omics 17(5):242–251
16. Gregorio GB, Senadhira D (1993) Genetic analysis of salt tolerance in rice (*O. sativa* L.). Theor Appl Genet 86:333–338
17. Gregorio GB, Senadhira D, Mendoza RD (1997) Screening rice for salinity tolerance. IRRI Discussion Paper Series No. 22:1–31
18. Gregorio GB, Senadhira D, Mendoza RD, Manigbas RD, Rozas NL, Guerta CQ (2002) Progress in breeding for salinity and associated abiotic stress in rice. Field Crops Res 76:91–101
19. Haq TU, Akhtar J, Gorham J, Steele KA, Khalid M (2008) Genetic mapping of QTLs, controlling shoot fresh and dry weight under salt stress in rice (*Oryza sativa* L.) cross between Co39 x Moroberekan. Pak J Bot 40(6):2369–2381
20. Hedge BA, Joshi GV (1974) Mineral salt absorption in saline rice variety, Kalarata. Plant Soil 41:421–424
21. Hittalmani S, Shashidhar HE, Bagadi PG, Huang N, Sidhu JS, Singh VP, Khush GS (2002) Molecular mapping of quantitative trait loci for growth, yield and yield related traits across three diverse locations in a doubled haploid rice population. Euphytica 125:207–214
22. Hossain MA, Bhattacharjee S, Armin SM, Qian P, Xin W, Li HY, Burritt DJ, Fujita M, Tran LSP (2015) Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. Front Plant Sci 6:420
23. IRRI (2014) Standard evaluation system for rice (SES), 5th edition. Los Banos (Philippines): International Rice Research Institute, p 57
24. Islam MR, Salam MA, Hassan L, Collard BCY, Singh RK, Gregorio GB (2011) QTL mapping for salinity tolerance at seedling stage in rice. Emir J Food Agric 23:137–146
25. Ismail AM, Thomson MJ (2010) Molecular breeding of rice for problem soils In: Costa de O, Varshney RK (eds). Root Genomics. doi:10.1007/978-3-540-85546-0-12
26. Ismail AM, Horie T (2017) Genomics, physiology, and molecular breeding approaches for improving salt tolerance. Annu Rev Plant Biol 68(1):405–434

27. Ismail AM, Heuer S, Thomson MJ, Wissuwa M (2007) Genetic and genomic approaches to develop rice germplasm for problem soils. Plant Mol Biol 65:547–570
28. Ismail AM, Singh US, Singh S. Dar M, Mackill DJ (2013) The contribution of submergence-tolerant (Sub1) rice varieties to food security in flood-prone areas. Field Crops Res 152:83–93
29. 10.1079/9781845936181.0154
Ismail AM, Thomson MJ, Vergara GV, Rahman MA, Singh RK, Gregorio GB, Mackill DJ (2010) Designing resilient rice varieties for coastal deltas using modern breeding tools. In: Hoanh CT, Szuster BW, Pheng KS, Ismail AM, Noble AD (eds). Tropical Deltas and coastal zones: food production, communities and environment at the land-water interface. Wallingford:CAB 154–65. doi:10.1079/9781845936181.0154
30. Jing W, Deng P, Cao C, Zhang W (2017) Fine mapping of *qSKC-1*, a major quantitative trait locus for shoot K^+ concentration, in rice seedlings grown under salt stress. Breed Sci 67:286–295
31. Juanillas V, Dereeper A, Beaume N, Droc G, Dizon J, Mendoza JR, Perdon JP, Mansueto L, Triplett L, Lang J, Zhou G, Ratharanjan K, Plale B, Haga J, Leach JA, Ruiz M, Thomson M, Alexandrov N, Larmande P, Kretzschmar, Mauleon RP (2019) Rice Galaxy: an open source for plant science. Giga Science 8:1–14
32. Kosambi DD (1944) The estimation of map distance from recombinant values. Ann Eugen 12:172–175
33. Koyama ML, Levesley A, Koebner RMD, Flowers TJ, Yeo AR (2001) Quantitative trait loci for component physiological traits determining salt tolerance in rice. Plant Physiol 125:406–422
34. Lee SY, Ahn JH, Cha YS, Yun DW, Lee MC, Ko JC, Lee KS, Eun MY (2007) Mapping QTLs related to salt tolerance of rice at seedling stage. Plant Breeding 126:43–46
35. Lee T, Oh T, Yang S, Shin J, Hwang S, Kim CY, Kim H, Shim H, Shim JE, Ronald P, Lee I (2015) RiceNet v2: an improved network prioritization server for rice genes. Nucleic Acids Res 43. doi:10.1093/nar/gkv253
36. Lin HX, Zhu MZ, Yano M, Gao JP, Liang AW, Su AW, Hu WA, Ren XH, Chao ZH DY (2004) QTLs for Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance. Theor Appl Genet 108:253–260
37. Liu LL, Ren HM, Chen LQ, Wang Y, Wu WH (2013) A protein kinase, calcineurin B-like protein-interacting protein kinase9, interacts with calcium sensor calcineurin B-like protein3 and regulates potassium homeostasis under low-potassium stress in Arabidopsis. Plant Physiol 161:266–277
38. Liu XS, Feng SJ, Zhang BQ, Wang MQ, Cao HW, Rono JK, Chen X, Yang ZM (2019) OsZIP1 functions as metal efflux transporter limiting excess zinc, copper and cadmium accumulation in rice. BMC Plant Biol 19:283. doi:10.1186/s12870-019-1899-3
39. Lou D, Wang H, Yu D (2018) The sucrose non-fermenting-1-related protein kinases SAPK1 and SAPK2 function collaboratively as positive regulators of salt stress tolerance in rice. BMC Plant Biol 18:203
40. 10.1002/9780470752708.ch14
Mackill DJ (2006) Breeding for resistance to abiotic stresses in rice: the value of quantitative trait loci. In: Lamkey KR, Lee M (eds) Plant Breeding: The Arnel R Hallauer International Symposium. Blackwell Pub, Ames, IA:201–212.doi:10.1002/9780470752708.ch14
41. Makihara D, Tsuda M, Morita M (1999) Effect of salinity on the growth and development of rice (*Oryza sativa* L.) varieties. Jpn J Trop Agr 43(4):285–294
42. Martinez-Atienza J, Jiang X, Garciadeblas B, Mendoza I, Zhu JK, Pardo JM, Quintero FJ (2007) Conservation of the salt overly sensitive pathway in rice. Plant Physiol 143:1001–1012
43. Ming-Zhe Y, Jian-Fei W, Hong-You C, Hu-Qu Z, Hong-Sheng Z (2005) Inheritance and QTL mapping of salt tolerance in rice. Rice Sci 12(1):25–32
44. Mohammadi-Nejad G, Arzani A, Rezai AM, Singh RK, Gregorio GB (2008) Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the *Saltol* QTL. Afr J Biotech 7(6):730–736
45. Mohammadi R, Mendioro MS, Diaz GQ, Gregorio GB, Singh RK (2013) Mapping quantitative trait loci associated with yield and yield components under reproductive stage salinity stress in rice (*Oryza sativa* L.). J Genet 92(3):433–443
46. Moradi F, Ismail AM (2007) Responses of photosynthesis, chlorophyll fluorescence and ros-scavenging systems to salt stress during seedling and reproductive stages in rice. Ann Bot 99:1161–1173
47. Negrao S, Courtois B, Ahmadi N, Abreu I, Saibo N, Oliveira MM (2011) Recent updates on salinity stress in rice: From physiological to molecular responses. Crit Rev Plant Sci 30(4):329–377
48. Nelson JC (1997) QGENE. Software for mapping based genomic analysis and breeding. Mol Breed 3:239–245
49. Pandit A, Rai V, Bal S, Sinha S, Kumar V, Chauhan M, Gautam RK, Singh R, Sharma PC, Singh AK, Gaikwad K, Sharma TR, Mohapatra T, Singh NK (2010) Combining QTL mapping and transcriptome profiling of bulked RILs for identification of functional polymorphism for salt tolerance genes in rice (*Oryza sativa* L.). Mol Genet Genom 284:121–136
50. Pearson GA, Ayers AD, Eberhard DL (1966) Relative salt tolerance of rice during germination and early seedling development. Soil Sci 102:1–6
51. Platten JD, Egdane JA, Ismail AM (2013) Salinity tolerance, Na^+ exclusion and allele mining of *HKT1;5* in *Oryza sativa* and *O. glaberrima*: many sources, many genes, one mechanism? BMC Plant Biol 13:32
52. Rahman MA, Thomson MJ, Shah-E-Alam M, de Ocampo M, Egdane J, Ismail AM (2016) Exploring novel genetic sources of salinity tolerance in rice through molecular and physiological characterization. Ann Bot 1–15
53. Rahman MA, Thomson MJ, de Ocampo M, Egdane JA, Salam MA, Shah-E-Alam M, Ismail AM (2019) Assessing trait contribution and mapping novel QTL for salinity tolerance using the Bangladeshi rice landrace Capsule. Rice 12:63
54. Rahman MA, Bimpong IK, Bizimana JB, Pascual ED, Arceta M, Swamy BPM, Diaw F, Rahman S, Singh RK (2017) Mapping QTLs using a novel source of salinity tolerance from Hasawi and their interaction with environments in rice. Rice 10:47

55. Reddy INBL, Kim SM, Kim BK, Yoon IS, Kwon TR (2017) Identification of rice accessions associated with K^+/Na^+ ratio and salt tolerance based on physiological and molecular responses. Rice Sci 24(6):360–364
56. Sabouri H, Sabouri A (2008) New evidence of QTLs attributed to salinity tolerance in rice. Afr J Biotechnol 7:4376–4383
57. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikoski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504
58. Singh AK, Kumar R, Tripathi AK, Gupta BK, Pareek A, Singla-Pareek SL (2015) Genome-wide investigation and expression analysis of sodium/calcium exchanger gene family in rice and Arabidopsis. Rice 8:21. doi:10.1186/s12284-015-0054-5
59. 10.1007/978-90-481-3112-9_20
Thomson MJ, Ismail AM, McCouch SR, Mackill DJ (2010) Marker assisted breeding. *In*: Pareek A, Sopory SK, Bohnert HJ, Govindjee (eds). Abiotic stress adaptation in plants: Physiological, molecular and genomic foundation. Springer Science 201:451–469. doi:10.1007/978-90-481-3112-9_20
60. Thomson MJ, de Ocampo MP, Egdane JA, Rahman MA, Sajise AG, Adorada DL, Tumimbang-Raiz E, Blumwald E, Seraj ZI, Singh RK, Gregorio GB, Ismail AM (2010) Characterizing the *SalTol* quantitative trait locus for salt tolerance in rice. Rice 3:148–160
61. Thu TTP, Yasui H, Yamakawa T (2017) Effects of salt stress on plant growth characteristics and mineral content in diverse rice genotypes. Soil Science Plant Nutrition 63:3:264–273
62. Tiwari V, Patel MK, Chaturvedi AK, Mishra A, Jha B (2016) Functional characterization of the the class glutathione-s-transferase gene (*SbGSTU*) promoter of *Salicornia brachiate* under salinity and osmotic stress. PLos One 11(2):e0148494. doi:10.1371/journal.pone.014894
63. Tripathi LP, Sowdhamini R (2006) Cross genome comparisons of serine proteases in Arabidopsis and rice. BMC Genom 7(200). doi:10.1.186/1471-2164-7-200
64. Walia H, Wilson C, Zeng L, Ismail AM, Condamine P, Close TJ (2007) Genome-wide transcriptional analysis of salinity stressed *japonica* and *indica* rice genotypes during panicle initiation stage. Plant Mol Biol 63:609–623
65. Walia H, Wilson C, Condamine P, Liu X, Ismail AM, Zeng L, Wanamaker SI, Mandal J, Xu J, Cui X, Close TJ (2005) Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. Plant Physiol 139:822–835
66. Wang H, Zhang M, Guo R, Shi D, Liu B, Lin X, Yang C (2012) Effects of salt stress on ion balance and nitrogen metabolism of old and young leaves in rice (*Oryza sativa* L.). BMC Plant Biol 12:194
67. Wang WS, Zhao XQ, Li M, Huang LY, Xu JL, Zhang F, Cui YR, Fu BY, Li ZK (2015) Complex molecular mechanisms underlying seedling salt tolerance in rice revealed by comparative transcriptome and metabolomics profiling. J Exp Bot 67(1):405–419
68. Yeo A, Flowers T (1986) Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. Australian Journal of Plant Physiology 13:161–173
69. Yoshida S, Forno D, Cock J, Gomez K (1976) Laboratory manual for physiological studies of rice, 3rd Edition. Los Banos (Philippines): International Rice Research Institute
70. Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468
71. Zeng H, Xu L, Singh A, Wang H, Du L, Poovaiah BW (2015) Involvement of calmodulin and calmodulin-like proteins in plant responses to abiotic stresses. Front Plant Sci:6. doi:10.3389/fpls.2015.00600
72. Zhang CJ, Zhao BC, Ge WN, Zhang YF, Song Y, Sun DY, Guo Y (2011) An apoplastic H-type thioredoxin is involved in the stress response through regulation of the apoplastic reactive oxygen species in rice. Plant Physiol 157:1884–1899
73. Zheng H, Wang J, Zhao H, Liu H, Sun J, Guo L, Zou D (2015) Genetic structure, linkage disequilibrium and association mapping of salt tolerance in japonica rice germplasm at the seedling stage. Mol Breeding 35:152
74. Zhu M, Xie H, Wei X, Dossa K, Yu Y, Hui S, Tang G, Zeng X, Yu Y, Hu P, Wang J (2019) WGCNA analysis of salt-responsive core transcriptome identifies novel hub genes in rice. Genes 10(9):719

Figures

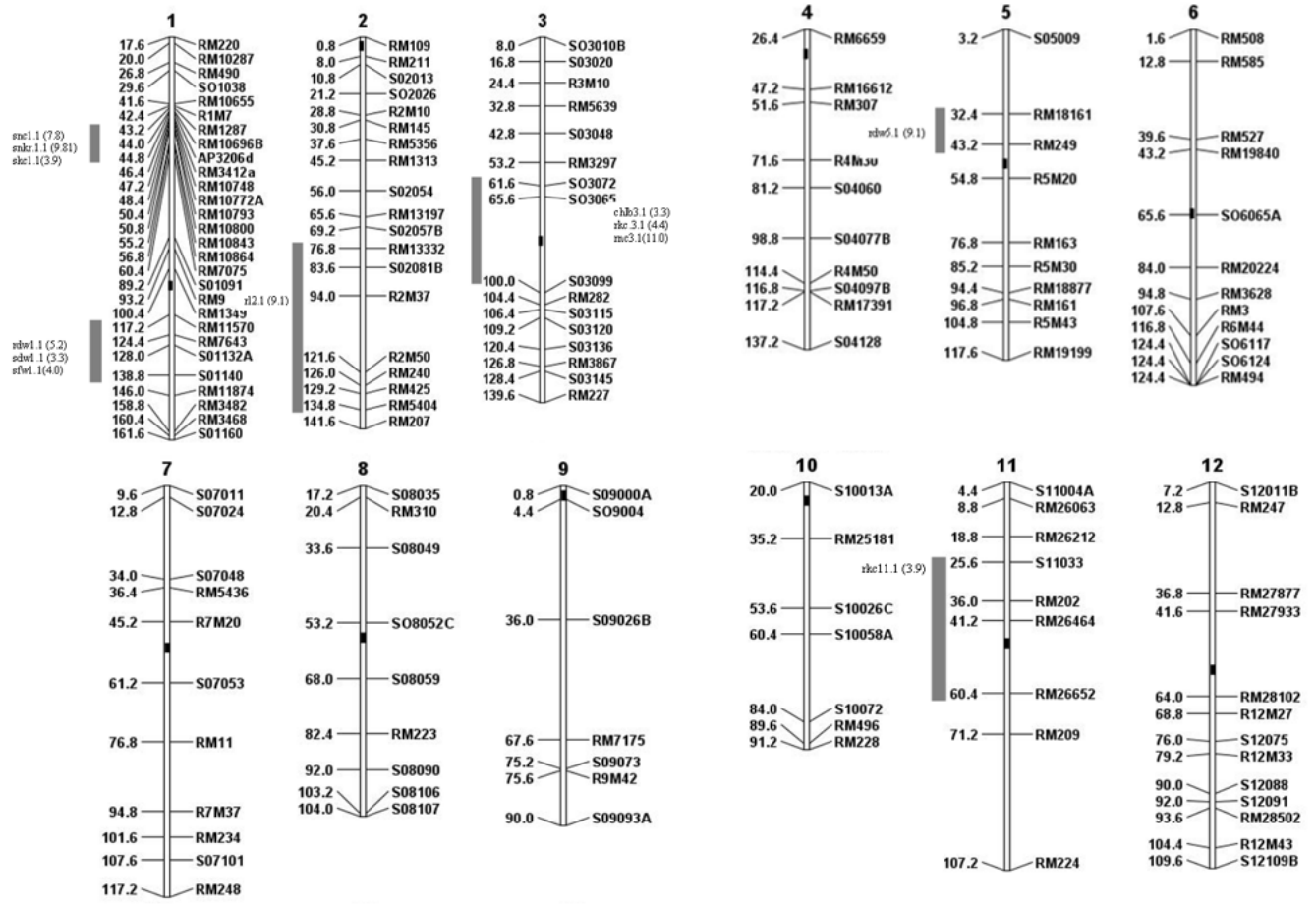

Figure 1

The genetic linkage map constructed with 151 markers across the 12 rice chromosomes, using the population derived from Kalarata/Azucena cross, with distances in cM converted from the physical map. The LOD scores are indicated in parenthesis and the labels on the right of the chromosomes reveal marker names, while the numbers on the left indicate marker positions. Black dots indicate centromere positions. Vertical bars on the left of each chromosome indicate the intervals of major QTL.

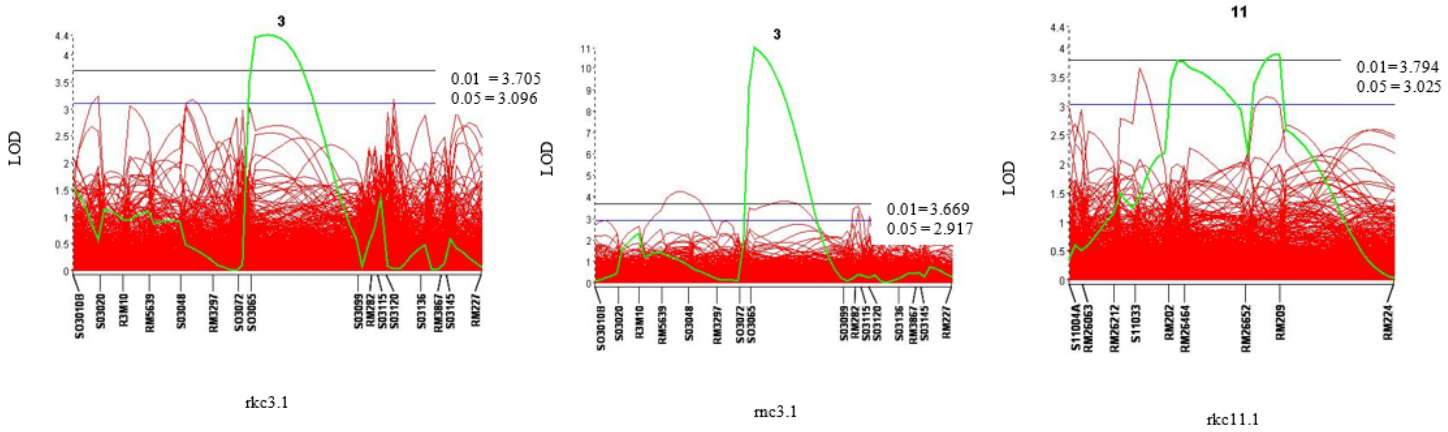

Figure 2

Significant QTL detected from Kalarata/Azucena population based on threshold LOD values at α0.05 (blue line) and α0.01 (black line) after 1000 iterations in permutation analysis

Supplementary Files

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