Localization of salt-tolerant QTL in rice germination stage under different salinity concentrations

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Abstract

Salt stress is an important abiotic stress, which has seriously affected the reproductive development of rice in many parts of the world. Therefore, it is particularly important to understand the genetic mechanism of salt tolerance in rice. In this study, we preliminarily located some quantitative trait loci (QTL) for root length, bud length, and survival percent under different salinity conditions (0, 100, 200 and 400 mM NaCl), using a population of chromosome segment substitution lines (CSSLs) constructed by Nipponbare and 9311. A total of 20 QTLs were identified, which explained the phenotypic variation of 4.7636%~37.5870%. There are 7, 5 and 8 QTLs for root length, bud length and survival percent, respectively. And *qRL1*, *qSP2*, *qSP3*, *qSP4*, *qRL5*, *qSP7*, *qSP7-1*, *qSP9*, *qRL10*, *qRL10-1*, *qRL11*, *qRL11-2*, *qBL11*, *qBL11-1* and *qSP12* were reported for the first time. Under different salinity conditions, the QTLs located are different, that is to say, the salt tolerance mechanism of rice under different salinity conditions may be different. The specific genetic mechanism remains to be further verified. Those QTLs identified in the experiment may be valuable genetic factors for improving salt tolerance of rice. These QTLs can be used to improve salt tolerance of rice by molecular markers technology, which will help to further understand the genetic mechanism of salt tolerance of rice.

Introduction

Rice is the staple grain crop in the world, feeding half of the world's population (Lai et al. 2016; Pires et al. 2015; Shi et al. 2017). Salinization of land is a serious problem in agriculture, which is one of the major abiotic stress (Kumari et al. 2018). Due to unsustainable farming methods, poor irrigation methods (He et al. 2004; Li et al. 2019), sea level rise and improper use of fertilizers (Punyawaew et al. 2016; Yang and Guo 2018), most of the land and irrigated fields in the world are affected by salt stress (Pires et al. 2015), which severely affects the growth and development of crops (Shi et al. 2017). Rice is a glycophyte with different sensitivity to salt stress at different development stages (Rao et al. 2018; Wang et al. 2011). Salt stress has a serious effect on seed germination, seedlings and reproductive development in the rice (Ganie et al. 2019). Therefore, the breeding of salt-tolerant rice varieties is an important target for breeders and also an effective method to reduce salt stress damage in rice (Lekklar et al. 2019; Shi et al. 2017).

The previous research showed that the salt tolerance of rice was controlled by many genes (Lekklar et al. 2019), and the related traits of salt tolerance were complex (Li et al. 2019). Mining and utilizing salt-tolerant genes/QTL is not only beneficial to the cultivation of salt-tolerant rice (H. et al. 2004), but also have great significance for understanding the genetic mechanism of salt tolerance in rice (He et al. 2017; Wang et al. 2012). With the development of molecular marker technology, genetic mapping has become a powerful tool to identify QTL/genes that control important complex agronomic traits (Mardani et al. 2014; Pandit et al. 2010). In rice, many QTLs of salt tolerance have been identified by genetic map, most of which were located on chromosomes 1, 2, 6 and 7, and a few are located on chromosomes 10 and 11 (Ammar et al. 2009; Zheng et al. 2015), but so far only a few salt tolerance genes have been cloned (Jahan et al. 2020). *SKC1* is the first salt-tolerant gene successfully isolated by map-based cloning, which is located on chromosome 1 (Ren et al. 2005). *SKC1* encodes a sodium transporter of HKT family (Li et al. 2017), which regulates Na^+/K^+ homeostasis under salt stress (He et al. 2004). Another salt-tolerant gene, *DST*, was obtained from salt-tolerant mutants by map-based cloning and located on chromosome 3 (Li et al. 2019). *DST* encodes a new zinc finger transcription factor, which negatively regulates the drought and salt tolerance of rice (Huang et al. 2009). *HST1* is a newly identified salt-tolerant gene, which encodes a B-type response regulatory protein OsR22. *HST1* may be involved as a transcription factor in regulating the expression of osmotic or ion transport related genes (Takagi et al. 2015). Although many QTLs for salt tolerance have been identified in rice, there are few studies on QTLs for salt tolerance under different salt concentrations (Wang et al. 2012), and the regulation mechanism of salt tolerance in rice under different salt stress is still unclear.

In this study, a set of chromosome segment substitution lines (CSSL), included 118 lines, derived from *indica* 9311 and *japonica* Nipponbare, were used to map and analyze the QTL for root length (RL), bud length (BL) and survival percent (SP) under different salinity concentrations at germination stage in rice. The aim of this study was to explore the genetic mechanism for salt stress tolerance in different salinity conditions and provide QTL for salt-tolerance rice varieties breeding by molecular-assisted selection (MAS) (Lai et al. 2016; Mardani et al. 2014).

Materials And Methods

Plant materials

A set of CSSL population, included 118 lines, derived from 9311 and Nipponbare. 9311, an *indica* variety, was used as the recipient parent; Nipponbare, an elite *japonica* variety, was used as the donor.

Stress Treatment and evaluation

Fourty filled and healthy seeds of parents and CSSLs were sterilized in 10% sodium hypochlorite solution for 15 min and then rinsed with distilled water for three times (Mardani et al. 2014; Shi et al. 2017). The seeds were soaked in distilled water for 3 days to germinate (Li et al. 2017; Wang et al. 2012). Finally, 30 uniform germinated seeds were selected and placed in a petri dish with single-layer filter paper (Li et al. 2017). In the experimental group, the seeds were treated with 100 mM, 200 mM and 400 mM sodium chloride solution. In the control group, the seeds were treated with distilled water. Each treatment has three replications (Wang et al. 2011). The treated seeds were cultured in an artificial climate chamber, maintaining a 14-hour light /10-hour dark cycle (27°C/25°C) and 80% relative humidity (Wu et al. 2020; Shi et al. 2017). The solutions were replaced everyday ensure that the concentration of sodium chloride solution and the volume of distilled water remain unchanged (Mardani et al. 2014). The RL, BL and SP of each line were measured and collected on seventh day (Basu et al. 2017).

Statistical analyses

Statistical analysis and QTL mapping of CSSL population treated with different concentrations of sodium chloride were carried out by ICIMapping.4.2 software (Wang et al. 2012). The correlation analysis between RL, BL and SP were conducted by SPSS.statistics.22 software.

Identification of QTL

Taking RL, BL and SP under different concentrations of sodium chloride treatment as indicators, QTL mapping for salt tolerance in rice germination stage was carried out on CSSLs population by ICIMapping.4.2 software, and LOD > 2.5 was selected as threshold to determine whether QTL existed (Zheng et al. 2015). QTL nomenclature refers to the method proposed by McCouch (Lai et al. 2016; Wang et al. 2011).

Results

Phenotypic variation of parents and CSSLs population

The values of RL, BL and SP of parents and CSSLs population under different salinity conditions are shown in Table 1. There is a significant difference in RL between 9311 and Nipponbare under 0 and 100 mM NaCl conditions, and the length of 9311 is significantly higher than that of Nipponbare (Fig.1); There is a significant difference in BL between 9311 and Nipponbare under 0, 100 and 400 mM NaCl conditions, and the length of 9311 is significantly lower than that of Nipponbare (Fig.1). There was no significant difference in SP under different salt conditions. RL, BL and SP of CSSLs population showed continuous frequency distribution and transgressive segregation, which were consistent with the genetic characteristics of quantitative traits (Fig.2).

Phenotypic correlation

Pearson correlation coefficients of three salt-tolerant traits RL, BL and SP under different salinity conditions are shown in Table 2. RL, BL and SP were significantly correlated at 200 and 400 mM NaCl ($p<0.01$), although there was no correlation between RL and SP at 200 mM NaCl. There is no significant correlation between RL, BL and SP under control and 100 mM NaCl, although there is a correlation between RL and BL under 100 mM NaCl ($p<0.05$).

QTL analysis

Under different salinity conditions, QTLs for three salt-tolerant traits are shown in Table 3, and the positions of these QTLs on chromosomes are shown in Fig.3.

QTLs for root length

Seven QTLs were detected for RL (Fig.3, Table 3). Under controlled condition, *qRL1* and *qRL10-1* were located on chromosome 1 and 10, with LOD values of 3.46 and 3.88, which explained 10.41% and 11.96% of phenotypic variation, respectively. Under the 100 mM NaCl condition, *qRL10* was located on chromosome 10, with LOD value of 3.11, which explained 11.43% of phenotypic variation. Under the 200 mM NaCl condition, QTLs *qRL5*, *qRL11*, *qRL11-2* were located on chromosome 5 and 10, with LOD values of 3.15, 8.52 and 4.36, which explained the phenotypic variation of 4.76%-14.34%. Under the 400 mM NaCl condition, *qRL3* was located on chromosome 3, with LOD value of 5.70, which explained 19.93% of phenotypic variation.

QTLs for bud length

Five QTLs were detected for BL (Fig.3, Table 3). Under controlled condition, *qBL8* and *qBL11* were located on chromosome 8 and 11, with LOD values of 5.13 and 2.92, which explained 15.84% and 8.63% of phenotypic variation, respectively. Under the 100 mM NaCl condition, there was no QTL detected. Under the 200 mM NaCl condition, QTLs *qBL8* and *qBL11-1* were located on chromosome 8 and 11, with LOD values of 2.56 and 3.58, which explained the phenotypic variation of 7.99% and 11.41%, respectively. Under the 400 mM NaCl condition, *qBL3* was located on chromosome 3, with LOD value of 3.43, which explained 12.51% of phenotypic variation.

QTLs for survival percent

Eight QTLs were detected for BL (Fig.3, Table 3). Under controlled condition, *qSP2*, *qSP3* and *qSP7-1* were located on chromosome 2, 3 and 7, with LOD values of 3.69, 4.31 and 15.31, which explained 7.07%, 8.46% and 37.59% of phenotypic variation, respectively. Under the 100 mM NaCl condition, *qSP12* was located on chromosome 12, with LOD value of 6.26, which explained 19.61% of phenotypic variation. Under the 200 mM NaCl condition, QTLs *qSP7* and *qSP9* were located on chromosome 7 and 9, with LOD values of 6.64 and 2.54, which explained the phenotypic variation of 22.55% and 7.95%, respectively. Under the 400 mM NaCl condition, *qSP3* and *qSP4* was located on chromosome 3 and 4, with LOD value of 5.96 and 3.50, which explained 18.38% and 10.28% of phenotypic variation.

Candidate gene analysis

Candidate genes were analyzed for QTLs with phenotypic variation over 10%. There are 310 putative genes in *qRL1*, *qSP3*, *qSP4*, *qSP7-1*, *qRL10*, *qRL11*, *qBL11-1* and *qSP12*. Among these candidate genes, homologous analysis showed that 16 genes were closely related to the previously cloned salt-tolerant genes (Lekklar et al. 2019) (Fig.4, Table 4), but these candidate genes need further verification.

Discussion

For breeders, it is a feasible way to cultivate salt-tolerant rice by aggregating salt-tolerant QTL/genes (Ganie et al. 2019). The detection of QTL is greatly promoted by using multiple related traits under different salinity stress (Wang et al. 2012). In order to reveal the genetic control of salt tolerance at rice germination stage, 20 salt-tolerant QTLs for three salt tolerance related indexes were identified on 12 chromosomes by using the newly constructed genetic map under different salinity conditions (0, 100, 200 and 400 mM NaCl). Mining these salt-tolerant QTLs can greatly promote the process of rice salt-tolerant breeding.

In QTL analysis, *qSP3* and *qBL8* were located in Chr3-bin146 and Chr8-bin428, respectively (Fig. 3, Table 3). These QTLs are highly repetitive and have been detected under two different salinity conditions. Those QTLs with large phenotypic variation can be further studied. Although *qSP3* and *qBL8* were detected at different concentrations, the other eighteen QTLs were rarely co-located under different salt concentrations, that is to say, the mechanism of salt tolerance in rice at different concentrations may be different.

qRL3 and *qBL3* controlling different traits were detected at the same position on the same chromosome, which may be related to the pleiotropy of QTLs, that is, QTLs of a certain segment on the chromosome act on multiple traits at the same time, which is common in rice (Ammar et al. 2009; H. et al. 2004). These QTL regions located in the same place are very useful for improving many traits at the same time (Lai et al. 2016).

There have been many other reports on salt tolerance QTLs before (De Leon et al. 2016; Zheng et al. 2015). In this study, twenty QTLs were detected, including seven for RL, five for BL and eight for SP (Fig. 3, Table 3). By comparing the chromosome positions of these QTLs, we found that three QTLs in this study were close to the positions of several QTLs related to salt tolerance that have been mapped. For example, *qBL8* located on chromosome 8 has the same chromosome interval as *OsCPK21*, which is involved in the positive regulation of abscisic acid and salt stress signal pathway (Asano et al. 2011). The *qSP9* on chromosome 9 has the same chromosome interval as *OsRNS4* (Zheng et al. 2014). The *qRL3* located on chromosome 3 has the same chromosome interval as *OsSUT1* and *OsJAZ9*. *OsJAZ9* is involved in regulating potassium homeostasis, affecting Na^+/K^+ homeostasis and improving salt tolerance of rice (Siahpoosh et al. 2012; Wu et al. 2015). The *qRL1*, *qSP2*, *qSP3*, *qSP4*, *qRL5*, *qSP7*, *qSP7-1*, *qSP9*, *qRL10*, *qRL10-1*, *qRL11*, *qRL11-2*, *qBL11*, *qBL11-1* and *qSP12* are different from the QTLs located before, suggesting that those QTLs may be new. These new salt-tolerant QTLs can be mapped finely by constructing secondary F_2 population, which greatly promotes the cloning of salt-tolerant genes. And the candidate genes predicted in this paper will also provide some reference for the cloning of salt-tolerant genes (Fig. 4, Table 4).

Most of the QTLs previously located are based on recombinant inbred lines or backcross inbred line population (Luo et al. 2020; Wang et al. 2012), and few of them use chromosome segment substitution lines. In this experiment, the population of CSSLs comprised of 118 line, and each line of CSSLs population is homozygous with good stability. As the background of most receptor parents in multi-generation backcross is gradually covered by recurrent parents, the interference of genetic background is eliminated, and the accuracy of QTL detection is improved (Bian et al. 2010). Therefore, CSSLs carrying QTLs detected in this experiment is an effective resource for improving salt tolerance of rice (Bian et al. 2010). These markers are closely linked with rice salt tolerance QTLs (H. et al. 2004), which will provide reference for rice salt tolerance breeding, contribute to the polymerization of rice salt tolerance QTLs, and realize high level of salt tolerance of rice.

Declarations

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Tables

Table 1 Phenotypic values of parents and CSSLs under different salt stress

Treatments	Indices	Parents		CSSLs			
		9311	Nipponbare	Min	Max	Mean	Variance
Water（control）	RL	4.86	3.43	3.47	7.12	5.69	0.48
	BL	4.37	3.84	3.41	5.45	4.60	0.19
	SP	1.00	1.00	0.92	1.00	0.99	0.00
100 mM NaCl	RL	2.27	1.54	0.61	2.12	1.30	0.11
	BL	3.09	2.69	1.45	3.70	2.83	0.13
	SP	1.00	1.00	0.88	1.00	0.99	0.00
200 mM NaCl	RL	0.89	0.76	0.18	1.37	0.49	0.03
	BL	1.57	1.60	1.06	2.41	1.75	0.08
	SP	1.00	0.99	0.70	1.00	0.95	0.00
400 mM NaCl	RL	0.00	0.00	0.00	0.24	0.04	0.00
	BL	0.45	0.75	0.00	1.11	0.24	0.05
	SP	0.94	0.94	0.00	1.00	0.13	0.02

RL root length, BL bud length, SP survival percent

Table 2 The correlation of tolerance indices under different salt stress

Traits	Water（control）			100 mM NaCl			200 mM NaCl			400 mM NaCl		
	RL	BL	SP	RL	BL	SP	RL	BL	SP	RL	BL	SP
RL	1			1			1			1		
BL	0.175	1		0.190*	1		0.370**	1		0.760**	1	
SP	-0.103	-0.013	1	-0.063	-0.138	1	-0.046	0.324**	1	0.518**	0.575**	1

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level.

RL root length, BL bud length, SP survival percent

Table 3 The QTLs for salt tolerance indices under different salt stress

Treatment	Indices	QTL	Chr	Marker	LOD	Add	PVE(%)
Water（control）	RL	*qRL1*	1	chr1-bin5	3.46	-0.56	10.41
		qRL10-1	10	Chr10-bin508	3.88	-0.65	11.96
	BL	*qBL8*	8	Chr8-bin428	5.13	-0.48	15.84
		qBL11	11	Chr11-bin544	2.92	-0.23	8.63
	SP	*qSP2*	2	Chr2-bin127	3.69	-0.01	7.07
		qSP3	3	Chr3-bin146	4.31	-0.02	8.46
		qSP7-1	7	Chr7-bin378	15.36	-0.04	37.59
100 mM NaCl	RL	*qRL10*	10	Chr10-bin501	3.11	-0.21	11.43
	SP	*qSP12*	12	Chr12-bin600	6.26	-0.05	19.61
200 mM NaCl	RL	*qRL5*	5	Chr5-bin295	3.16	0.28	4.76
		qRL11	11	Chr11-bin593	8.52	0.17	14.34
		qRL11-1	11	Chr11-bin596	4.36	-0.14	6.74
	BL	*qBL8*	8	Chr8-bin428	2.56	-0.23	7.99
		qBL11-1	11	Chr11-bin546	3.58	-0.18	11.41
	SP	*qSP7*	7	Chr7-bin370	6.64	-0.05	22.55
		qSP9	9	Chr9-bin463	2.54	-0.04	7.95
400 mM NaCl	RL	*qRL3*	3	Chr3-bin139	3.70	0.06	19.93
	BL	*qBL3*	3	Chr3-bin139	3.43	0.22	12.51
	SP	*qSP3*	3	Chr3-bin146	5.96	0.37	18.38
		qSP4	4	Chr4-bin211	3.50	0.14	10.28

RL root length, BL bud length, SP survival percent

Table 4 Candidate genes related to salt stress

QTL	Putative genes	Gene name or Annotation	Reference genes
qRL1	*LOC_Os01g03320*	BBTI2 - Bowman-Birk type bran trypsin inhibitor precursor	*OsCam1-1*
	LOC_Os01g03180	Retrotransposon protein	*OsJAZ9; OsTIFY11a*
	LOC_Os01g03210	None	*OsHKT1; 5 OsHKT2; 4*
	LOC_Os01g03452	Transposon protein	*OsHKT2; 1*
	LOC_Os01g03464	None	*OsHKT1; 4*
qSP3	*LOC_Os03g21480*	HAD superfamily phosphatase	*OsSUT1*
	LOC_Os03g21340	Retrotransposon protein	*RSS3*
qSP7-1	*LOC_Os07g35050*	OsFBX237 - F-box domain containing protein	*OsCam1-1*
	LOC_Os07g35070	OsFBX239 - F-box domain containing protein	OsHKT2; 1
qRL10	*LOC_Os10g26180*	None	*OsHAK21; qSE3*
	LOC_Os10g26030	Retrotransposon protein	*SRWD4*
	LOC_Os10g25940	Conserved hypothetical protein	*RSS2; OsPDR12*
qBL11-1	*LOC_Os11g11510*	3-5 exonuclease domain-containing protein	*OsCPK21*
	LOC_Os11g11350	Embryogenesis transmembrane protein	*OsHAK1*
qSP12	*LOC_Os12g02180*	None	*DST*

Figures

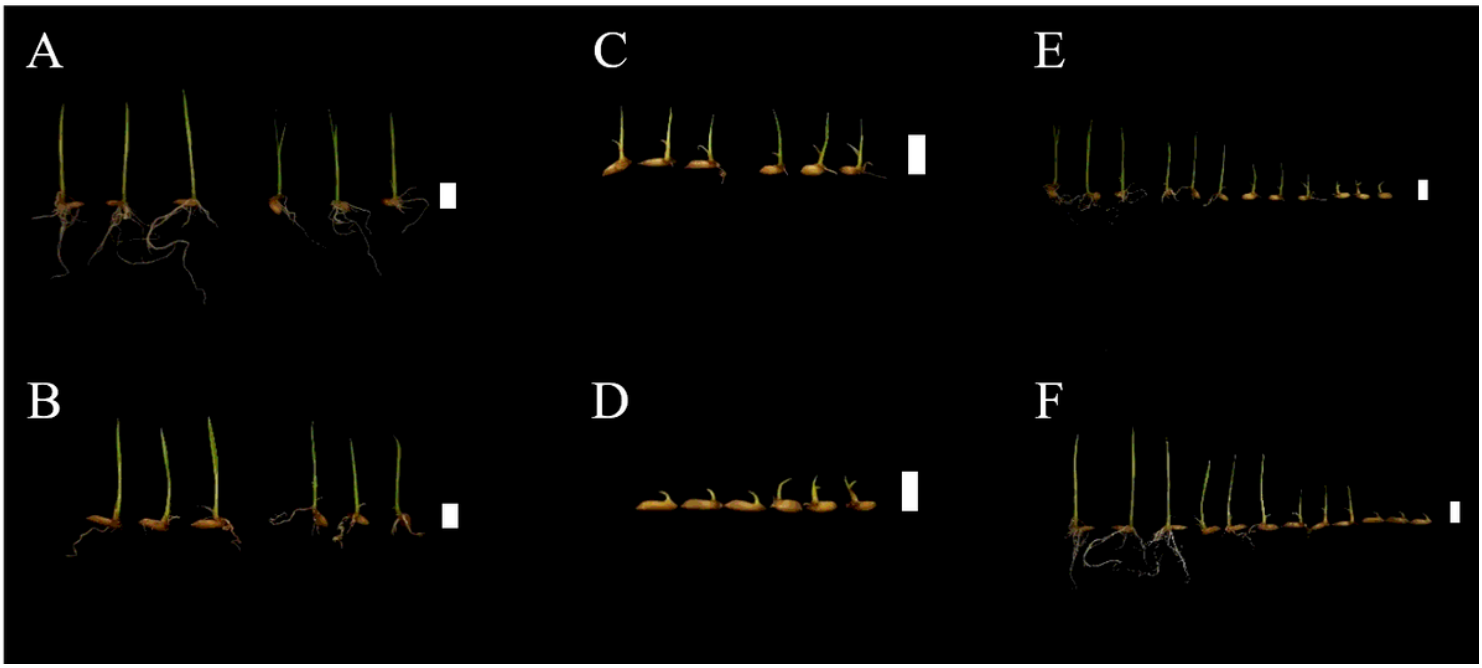

Figure 1

The situation of Nippobare and 9311 under different salt stress.

(A) The situation of 9311 (left) and Nippobare (right) under control condition; (B) The situation of 9311 and Nippobare under 100 mM NaCl; (C) The situation of 9311 and Nippobare under 200 mM NaCl; (D) The situation of 9311 and Nippobare under 400 mM NaCl; (E) The situation of Nippobare under different salt stress; (F) The situation of 9311 under different salt stres. Scale: 0.5 cm.

Figure 2

Frequency Distribution Histogram of RL, BL and SP in CSSLs population under different salt stress.

Figure 3

Position of QTL for RL, BL and SP in CSSLs population under different salt stress.

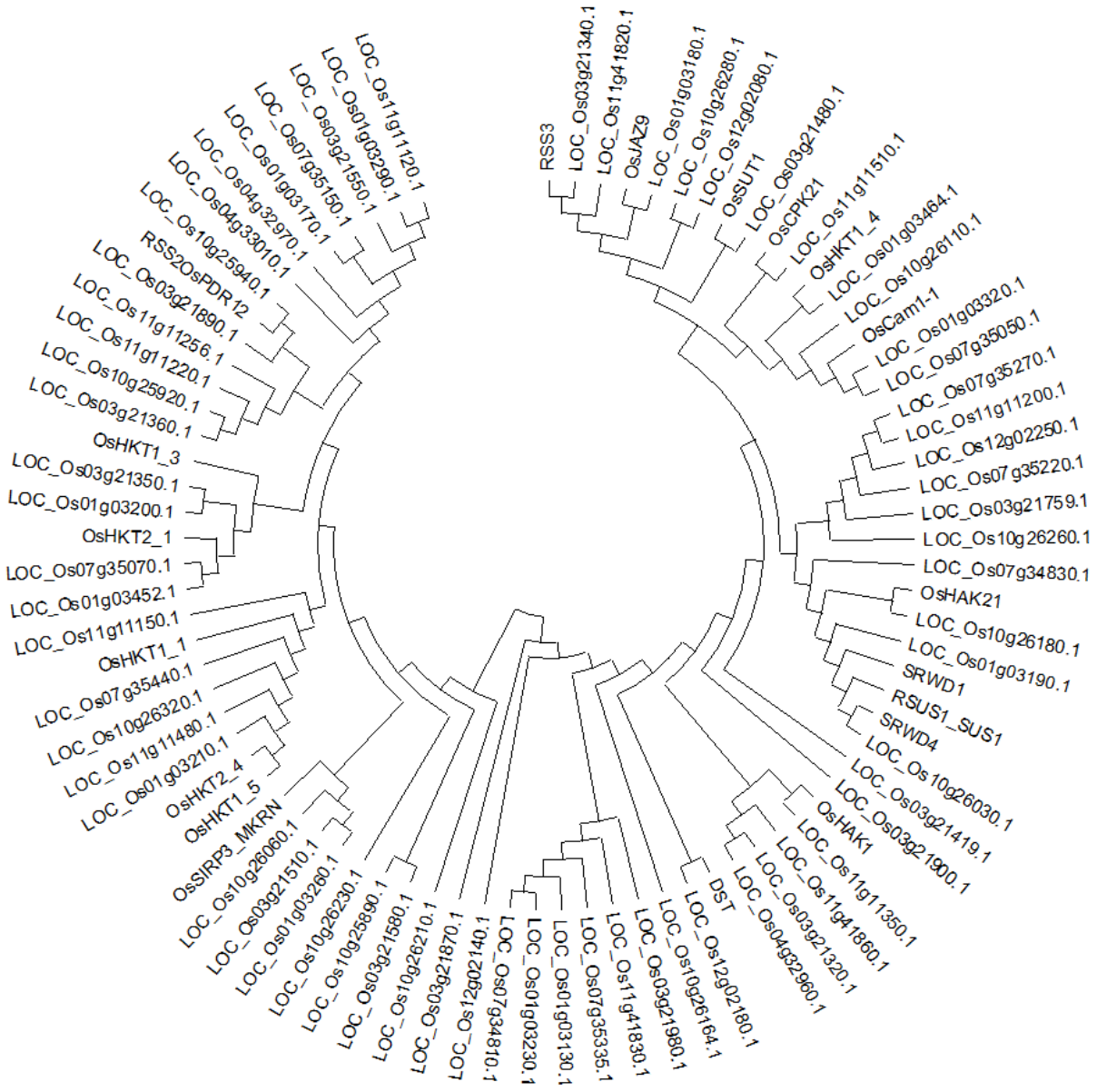

Figure 4

Evolutionary tree of candidate genes and salt tolerance related genes.