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水稻早期生长耐盐性QTL定位及候选基因分析

QTL MAPPING AND CANDIDATE GENES OF SALINITY  
TOLERANCE AT THE EARLY STAGE IN RICE

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# 华中农业大学博士学位论文

## 水稻早期生长耐盐性QTL定位及候选基因分析

### QTL MAPPING AND CANDIDATE GENES OF SALINITY TOLERANCE AT THE EARLY STAGE IN RICE

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## Abstract

Rice (*Oryza Sativa* L.) is one of the world's staple food crops. Salinity is one of the most serious factors that affect seed germination and seedling establishment, which may result in a complete crop failure and final rice yield. To increase rice production of saline affected areas that is widely occurring worldwide, identification of quantitative trait loci (QTLs) associated with salt tolerance is an essential step for the improvement in varieties of salt-tolerant rice. The main objective of the present study is to identify QTLs related to salt tolerance at the germination and seedling stages using a rice backcross inbred line (BIL) population that was derived from a backcross of an Africa cultivated rice ACC9 as a donor and an *indica* cultivar Zhenshan 97 (ZS97) as the recurrent parent. The main results are as follows:

(1) Seven rice parental lines of several mapping populations were first evaluated for their salt tolerance at the early growth stage. Cultivar ACC9 and a wild rice accession ACC10 were more tolerance to salt stress than ZS97, which was the most sensitive one at the germination stage. The remaining four varieties (NIP, MH63, G159, and 9311) were classified as moderately tolerant ones. At the seedling stage, ZS97 was found to be the most tolerant variety, ACC10 and ACC9 were classified as the most sensitive.

(2) Using the BIL population and SNP genotypes, a molecular linkage map with 714 bin markers covered the rice genome was constructed. Using IciMapping software with inclusive composite interval method, QTL analysis identified 23 loci for germination parameters related to salt tolerance at the germination stage and 46 loci for morphological and physiological parameters at the seedling stage. Among them, 9 and 33 loci with the ACC9 alleles increased salt tolerance at the germination and seedling stages, respectively. Moreover, several major QTLs explained phenotypic variation  $\geq 10\%$  for several assessed traits were colocalized in the same or overlapping regions, indicating that the same genes or linked genes have a pleiotropic effect on these multiple traits. For example, three loci (qSH1, qSDW1 and qSHI1) colocalized in bin B01C254; five loci (qDLW2, qNa+2.2, qDLSFR2, qDLWI2 and qDLSFRI2) overlapped in bin B02C61. Some loci were identified in small bins (or regions) in the BIL population and localized within or near previously reported genes or loci associated with salt tolerance in rice.

(3) Six major loci (qGR-7d4.3, qSH1, qSFW1.1, qDLW1, qSDW1 and qSHI1) colocalized in B01C254 within a 190-kb size encompassing two known genes: the green revolution gene *sd1* and a transcriptional activator *SNAC6*, and increased rice tolerance to

high-salt stress. As qSH1 explained the highest phenotypic variation in seedling height under salt stresses, the semi-dwarf gene SD1 may be the most likely candidate for qSH1 conferring salt tolerance. The Crispr-cas9 generated sd1 mutants showed significantly higher salt tolerance than the near isogenic line (NIL-SD1NIP) that carried functional SD1 alleles from NIP. The salt tolerance was assayed by a modified standard evaluation system for some morphological traits at the seedling stage. The data of several loci with candidate genes identified for salt tolerance will be very beneficial for the improvement of variety with salt tolerance at early growth stages via marker-assisted selection in rice breeding programs.

In conclusion, salinity is one of the utmost negative abiotic stresses that affects rice production worldwide. In this study, germination parameters and seedling growth were used to evaluate salt tolerance of the BILs derived from ACC9 and ZS97. Totally, 23 and 46 QTLs were identified for the measured traits associated with salt tolerance at the germination and seedling stages, respectively. At least three loci were identified in small bins (or regions) and localized in or nearby previously reported genes associated with salt stress. Among them, the sd1 gene located within the major QTL confers salt tolerance. These data can be exploited in the genomic breeding approaches to improve rice with salt tolerance.

**Keywords:** Africa cultivated rice; salt tolerance; seedling establishment; quantitative trait loci (QTLs); backcross inbred lines (BIL).

## Abbreviations

ANOVA	Analysis of Variance
BIL	Backcross inbred line
DLSFR	Dead leaf to shoot fresh weight ratio
DLW	Dead leaf weight
EC	Electric conductivity
GI	Germination index
GII	Sensitivity index of GI
GR	Germination rate
GR-3d	Germination rate at 3d after imbibition
GR-7d	Germination rate at 7d after imbibition
GRI-3d	Sensitivity index of germination rate at 3d
GRI-7d	Sensitivity index of germination rate at 7d
GT	Mean germination time
GTI	Sensitivity index of mean germination time
K <sup>+</sup>	Potassium ion
K <sub>2</sub> O	Potassium oxide
Kg	Kilogram
Mb	Mega base pairs
M ha	Million hectare
mM	millimolar
Mts	Million tons
N	Nitrogen
Na <sup>+</sup>	Sodium ion
NaCl	Sodium chloride
NaKR	Sodium to potassium ratio
P <sub>2</sub> O <sub>5</sub>	Phosphorus pentoxide
PVE	Phenotypic variation explained
ROS	Reactive oxygen species
SDW	Shoot dry weight
SES	Modified standard evaluation system
SFW	Shoot fresh weight
SH	Seedling height

SSD	Seedling survival days
SWC	Shoot water content
ZS97	Zhenshan 97