

SUMMARY

Identification of novel QTLs and introgression through marker-assisted backcrossing for improved salinity tolerance in rice

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Rice (*Oryza sativa* L.) is the most important food crop in the world and feeds over half of the global population. However, rice production has been decreasing due to climate change, especially salinity. Understanding the genetic basis of diverse morphological and physiological traits provides the basis for improving yield, quality, and sustainability of rice. Rice genotypes may have different stress tolerance levels depending on the growth stage of the crop. It is relatively tolerant during germination, active tillering, and towards maturity, but sensitive during early seedling stage and reproduction. Studies are needed to investigate whether traits contributing seedling stage tolerance can also contribute to reproductive stage tolerance.

The physiological bases of salinity tolerance during the early seedling stage are fairly established, key traits include salt exclusion, compartmentation of ions in structural and older tissues, vigorous growth and higher tissue tolerance. The physiological bases of salinity tolerance during the early seedling stage are fairly established, key traits include salt exclusion, compartmentation of ions in structural and older tissues, vigorous growth and higher tissue tolerance. Nonetheless, little is known about the important mechanisms associated with tolerance during reproduction. During reproductive development, tolerant genotypes tend to exclude salt from flag leaves and developing panicles. Salinity tolerance at the seedling and reproductive stages is only weakly associated hence, the discovery of contributing traits at both stages is essential for

developing resilient salt-tolerant cultivars. The general objective of this study is to identify new and novel quantitative trait loci (QTL), candidate genes and favorable haplotype, and pyramiding for the development of salt tolerant varieties.

In Chapter 2, to identify QTL associated with salt tolerance in rice, we developed an F₂ population from a cross between the salt-tolerant landrace, Kalarata, and the salt-sensitive parent, Azucena. F₃ families from this population were screened and scored for salt tolerance using International Rice Research Institute (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. Several novel QTL were identified in this study, the largest is for root sodium concentration (LOD = 11.0, R² = 25.0) on chromosome (Chr) 3, which also co-localize with a QTL for SES. Several QTL on the short arm of Chr 1 coincide with the *Saltol* locus identified before. The novel QTL identified in this study constitute future targets for molecular breeding, to combine them with other QTLs identified before, for higher tolerance and stable performance of rice varieties in salt affected soils.

In Chapter 3, 324 lines from the previously genotyped rice diversity panel 1 (RDP1) and a subset of 211 genotypes from the 3K panel were used to assess morphological and physiological traits, and map loci controlling salinity tolerance through genome-wide association studies and to identify favorable haplotype associated with salinity tolerance in rice at reproductive stage. The salinity stress treatment was 10 dS m⁻¹ in the reproductive stage experiment. Using the imputed RICE-RP dataset for RDP1 and the 3000 Rice Genome 1 Million Genome-wide association study single nucleotide polymorphism (3K RG 1M GWAS SNP) data for the 3K panel on all

chromosomes and phenotypic data were analyzed using a linear mixed-model by the R package of the Genome Association and Prediction Integrated Tool Package (GAPIT). For RDPI, significant associations were identified for relative grain yield on Chr 1, 4 and 11 and Na^+/K^+ ratio under salinity on Chr 3 and 7. For the 3K panel, significant associations were identified for relative harvest index (Chr 8), relative plant height (Chr 4), Na^+ concentration (Percent Na) under salinity (Chr 8) and Na^+/K^+ ratio under salinity (all chromosomes except Chr 7). Nine candidate loci from the 52 loci co-locating with multiple traits appeared to be the most favorable haplotype for potential donors and to check for the frequency in the elite breeding pool. Further genetic and physiological studies are needed to divulge the underlying mechanisms and genes involved in reproductive-stage salinity tolerance. Candidate genes at most significant associations are now being identified for further validation and for future use in molecular breeding programs to enhance salinity tolerance.

In Chapter 4, crosses were made between IR64-*Saltol* and IR64-*Sub1* to combine submergence and salinity tolerance. Using MABC helped in hastening the selection of recombinants with *Saltol* and *Sub1* introgressed into one genetic background. In this process, F_1 seeds were advanced to F_2 generation and 310 F_2 plants were genotyped for *Saltol* QTL located on Chr 1 and *Sub1* QTL on Chr 9 using closely linked simple sequence repeat (SSR) markers. Twenty-one F_3 progenies with homozygous alleles for both *Saltol* and *Sub1* were selected and phenotyped for salinity tolerance at 12 dS m^{-1} and submergence tolerance for 21 d. Six F_4 progenies with moderate tolerance to salinity based on SES and surviving plants from submergence screening were selected and phenotyped again for combined *Saltol* and *Sub1*. After recovery, advanced lines at F_5 stage were screened again for morpho-physiological traits under salinity. Among the 6 *Saltol-Sub1* lines, 31-3 has the lowest shoot Na^+ concentration and shoot Na^+/K^+ ratio, and has the highest

tissue tolerance, indicating that tissue tolerance can be achieved even if *Sub1* is co-introgressed and *Saltol* is not affected by the introgression. Based on SES, IR64-*Saltol* parent has the lowest SES score. IR64-*Sub1* parent has the same SES score with the 6 selected *Saltol-Sub1* lines. There was no good effect of *Saltol* on the 6 *Saltol-Sub1* lines. The introgression effect of *Saltol* was diminished by the combination of *Sub1*. Although SES is the most important indicator of salinity tolerance, it was affected by the combination of *Saltol* and *Sub1*.

In general, the knowledge of QTL 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 salinity-affected areas. The QTL detected in the Kalarata-Azucena study provides a rich source of information for molecular breeding and for identifying useful genes for salinity tolerance. Candidate genes should further be assessed for their functional roles in physiological processes that confer salt tolerance in rice and for breeding improved varieties for salt affected areas. Identification of DNA markers that are closely linked with these new QTL will be useful for pyramiding multiple QTL, to develop varieties with greater salinity tolerance for salinity-affected coastal and inland areas. Fine-mapping of selected QTLs will help identify closely linked markers for use in MABC. The actual contribution of each QTL for a phenotypic trait should be tested and confirmed in different genetic backgrounds and environment. Further studies need to identify additional polymorphic markers to fill in the large gaps in the current map to enhance the precision of QTL detection.

Compared to traditional QTL mapping, GWAS had more advantages in increasing resolution power for mapping QTL, detecting more alleles, and obtaining faster completion time. It was more frequently used to provide a promising platform for linking phenomics and genomics using diverse germplasm accessions. This strategy enables the identification of donors for

tolerance of biotic and abiotic stress in a facile and manageable approach, hence, expediting crop improvement and facilitating the development of varieties suitable for unfavorable conditions. One great advantage of the GWAS design for rice is the homozygous nature of most rice varieties, which makes it possible to employ a “genotype or sequence once and phenotype many times over” strategy, whereby once the lines are genomically characterized, the genetic data can be reused many times across different phenotypes and environments. Genes identified in this study could be considered for further analysis to investigate its possible role in physiological processes conferring salinity tolerance in rice. The promising genotypes and loci identified in this study merits further exploration of stress tolerance traits, genes, and potential salinity donor genotypes for breeding.

Rice varieties with dual tolerance to salinity and submergence stresses can sustain better in those areas which are prone to both the stresses. Genotypes tolerant to salinity and submergence identified in this study are of great value in developing the high yielding varieties tolerant to multiple abiotic stresses. The genotypes identified in this study have great potential as unique genetic resources for utilization in crop improvement programmes.