主 論 文 の 要 旨

論 文 題 目　Identification of novel QTLs and introgression through marker-assisted backcrossing for improved salinity tolerance in rice
（イネの耐塩性向上のための新規量的形質遺伝子型の同定とマーカーを利用した戻し交雑）

氏　　　名　DE OCAMPO Marjorie Punzalan

論 文 内 容 の 要 旨

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. Studies are needed to investigate whether traits contributing seedling stage tolerance can also contribute to reproductive stage tolerance. 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 F_2 population from a cross between the salt-tolerant landrace, Kalarata, and the salt-sensitive parent, Azucena. F_3 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, R^2 = 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 RDP1, 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. 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.