Summary of Doctoral Thesis

Enrollment year: 2018, April
UGAS Specialty: Bio-production Science
Name DINH THI LAM

Title	**Studies on wild genetic resources to improve phosphorus deficiency tolerance in rice (*Oryza sativa* L.)**

Phosphorous (P) deficiency is a key constraint for rice cultivation. Problematic soils like acid sulfate soil occupying 44% of total land in Mekong Delta Vietnam are known as P deficiency. It causes severe damage to rice cultivation in the area. In order to widen gene pools for rice improvement for the tolerance to phosphorus deficiency, genetic diversity among wild rice *Oryza rufipogon* along the Mekong River in Vietnam was evaluated. Genetic mechanism involving to the above tolerance was also studied.

In order to characterize genetic variation, DNA markers were developed in cytoplast and nuclear genome. Those markers were well applied to others crop studies such as Australia's wild rice and citrus.

Results:

Unique genetic resources were found for chloroplast and mitochondrial genomes. The most interested resources were detected in upstream areas where the highest diversity was found (*He*=0.659). Some of the resources carried significant re-arrangements around mitochondrial gene *orf153* involving to male sterility. In additions, several novel chloroplast types were detected by INDELs markers. Unique maternal lineage was detected in downstream population due to vegetative propagation inferred from the genotypes. High diversity in wild rice populations in Mekong Delta will give us a prospecting gene pool for rice improvement. In fact, one elite variety named AS996, which has tolerance to acid sulfate soil in the Mekong Delta has been established by exploiting these wild rice resources. The methods to develop INDEL markers to trace maternal lineages of genetic resources were applied to Citrus resources and Australian wild rice. Valuable germplasms were detected for future breeding program.

AS996 is an improved variety crossed between mega variety IR64 and a particular wild rice *O. rufipogon* inhabited in the upstream area of the Mekong Delta. The wild rice conferred the acid sulfate soils tolerance into IR64 genetic background. The tolerance was due to ability of efficiently utilize phosphorus to generate seeds and sustain yields under phosphorus deficient condition. Hydroponic system was adopted to analyze the genetic segregation. Both F2 and backcrossing populations suggested that the tolerance is regulated by a single dominant gene from the wild rice parental line, which is not a gene, *PSTOL1* reported previously. It suggested that a novel gene introgressed from the wild rice involves to the tolerance would be expected. In order to detect the gene, molecular tools have been established. Genome composition in AS996 was compared with IR64 using Next Generation Sequencing (NGS). Possible introgressed chromosomal segments were identified by SNPs detection. Higher SNPs were detected in several regions along genome. Particularly, relatively higher number of SNPs was found in chromosome 4, indicating various wild introgressed segments. Molecular markers were also established to apply for linkage analysis. Mapping candidate gene is still undergoing. ICP-AES analysis was applied to evaluate P allocations in different organs between IR64 and AS996 grown under different P conditions. Reasonable allocation of P in physiological reproduction process facilitate enough seed production under P deficient condition in AS996. It resulted in relatively high seed fertility (79.1%) under P deficient condition compared to that under normal condition in AS996. IR64 showed 19.5% seed fertility under P deficient condition compared to that under sufficient P condition. The most significant difference in P allocation in AS996 compared to IR64 was detected in grains. AS996 did not show differences in P content in grain under either field condition and relatively low P concentration was kept in the grains, whereas IR64 carried high P content in grains under P sufficient condition. Under P sufficient condition, IR64 required around 9.53 mg. g^{-1} phosphorus accumulation in grain, while AS996 required only around 3.66 mg. g^{-1}. Under phosphorus deficiency condition, 5.17 mg. g^{-1} of phosphorus was measured in AS996 grain, versus 4.32 mg. g^{-1} in IR64. It seems the delay of heading and invested nutrients to vegetative organs in IR64 was the efforts of sufficient P acquisition. Thus,

IR64 failed to allocate P during grain filling under P depleted condition. Seed could not be matured and the appearance was poor in IR64. The reduction of P content in AS996 suggested that the low P requirement to bear seeds in AS996 is one of desirable trait cultivated in acid sulfate soil along the Mekong river. The genetic mechanism for the tolerance can also work to other delta areas where acid sulfate soil generated. In order to know affected genes by P deficient condition, RNA-seq analysis was performed. Transcriptions were compared between AS996 and IR64 grown under either condition, P sufficient or P deficient with hydroponic culture. It detected a co-ordinated expression of 184 genes in roots and 276 genes in shoot involving to various physiological processes such as Phosphate transporter, Potassium transporter, C-glycosylflavone biosynthesis Glycerol-3-phosphate acyltransferase, anther development, pollen formation, and so on. The gene pathway would be clear after candidate gene is detected. To saturate alleles of the tolerant gene for P deficiency, mutagenesis of AS996 was established by Sodium-azide as a mutagen. Mutation screening identified high mutagenized efficiency in the treated population. Although AS996 has long grains, three independent round kernel mutant lines were detected such as twin, round shape kernel and dwarf mutants. In addition, one large grain mutant and a novel mutation representing narrow hulls and leaves also were created. Some of mutants would be used as new resources for rice breeding program in Vietnam and also for development biological researches. The mutagenesis can open the way to saturated mutagenized in the candidate tolerant gene.

Through these researches, genetic mechanism involving to tolerance to P deficiency was identified and genes regulated by the candidate gene were also detected by RNA-seq analysis. The information would be used to improve varieties which are tolerant to phosphorus deficiency as well as acid sulfate soil.