CHAPTER V

CONCLUSION

1. Determination of chitosan effects on protein profiles of rice seedlings after chitosan application

Proteomic analysis of 'LPT123' rice seedlings treated with foliar application of chitosan at 40 mg/L revealed discovery of 352 proteins in leaves. Among them, there were significant changes of 105 proteins mainly in metabolic process and signaling. Chitosan activated oxidative burst and ROS scavenger to balance ROS homeostasis. It also enhanced key enzymes in glycolysis and Calvin cycle as well as photosynthetic components. In addition, two receptors involving in plant growth, PSKR and BRL1 were induced.

2. Identification and characterization of some chitosan-responsive genes in *Arabidopsis*

2.1 Transcriptomic analysis of chitosan-responsive genes in *Arabidopsis*

Application of chitosan at 80 mg/L in *Arabidopsis* significantly retarded seedlings growth both shoot and root development. Transcript profiling revealed significant change of 1,557 genes mainly in metabolic process and stress responses. High dose chitosan inhibited plant growth by disturbing several growth-related genes and receptors. Besides, high dose chitosan adversely influenced on photosynthesis by disturbing photosystem I and II, iron homeostasis and promoting chlorophyll breakdown. Chitosan was recognized as PAMPs (pathogen-associated molecular patterns) to activate defense and stress responses. High dose chitosan triggered pattern recognition receptors (PRRs), pathogen-related (PR) genes, transcription factors, hormone signaling, and abiotic stress responsive genes. Abscisic acid (ABA) and jasmonic acid (JA) appear to play a prominent role in high dose chitosan response.

2.2 EMS-mutagenesis *Arabidopsis*

There were 350 mutants that showed the chitosan resistant phenotypes such as larger in plant size or longer roots compared to wild-type plants. After multiple confirmation tests, there were only 5 candidate mutants that consistently resisted to high dose chitosan. Finally, only two of five mutants seemed to possess recessive single gene mutation, which were 106A and 161A mutant lines. The T-DNA insertion mutants carrying putative mutated gene were ordered from ABRC stock center and were subjected to characterize in high dose chitosan treatment. The ABRC mutant carrying insertion in *TOM3* or *OPT4* gene showed the best chitosan-resistant phenotypes.

3. Identification of ortholog gene(s) involving in chitosan responses in rice

There were 9 orthologous genes from transcriptomic data and none of them from mutagenesis analysis. *Sugar transport protein 14* (*STP14*) appears to be the best orthologous gene with the highest identity score and the same direction of gene expression.

Future prospects

Concentration of chitosan is one of the key factors to be a switch of chitosan-elicited responses varying from plant growth induction to retardation. From omic studies, it is interesting to investigate the role of hydrogen peroxide and abscisic acid in chitosan-elicited response as well as the activity of chloroplast, which seems to be one of chitosan target organelles. Besides, chloroplast is also a bank of energy for plant growth. The increase of chitosan-elicited chloroplast activity might lead to the enhancement of plant growth. From chitosan mutant analysis, single gene mutation might not enough for clearly chitosan-insensitive responses. It is interesting to study the multiple gene mutation by crossing putative mutants to produce double gene or triple gene mutants.