CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world that provides a staple food for over half of the global population. Rice offers various benefits from having the smallest genome (estimated 389 million base pairs) among the major cereals, dense genetic maps and known complete genome sequence. The given information of a genomic sequence database promotes rice to be an excellent monocot model organism for molecular biology. World rice production may increase by 30% over the next 10 years to supply the population and economic development (International Rice Genome Sequencing, 2005).

2549742163

Rice is an annual grass with round, hollow and jointed stem; long and flat leaf blades joined to the leaf sheaths with collars. In general, the growth stage of rice plant is divided into three stages: vegetative stage (germination to panicle initiation), reproductive stage (panicle initiation to heading) and ripening stage (heading to mature grain) (Moldenhauer and Slaton, 2001). Rice plant height is an important agronomic trait correlated with rice yield. The height slightly increases in the vegetative development stage but rapidly lengthen in the reproductive stage before heading. The average rice yield nearly reached the maximum capacity under the present production technology. Technical efficiency of rice production could be increased by improvement of soil quality and application of bio-fertilizer, pest and disease control substances, and plant growth enhancer.

Chitosan is a modified natural polysaccharide produced by the partial N-deacetylation of chitin, a major component of crustacean shells such as crabs, lobsters, and shrimps. Chitosan is also found in some microorganisms and fungi (Chenite *et al.*, 2001). In recent decades, chitosan and its derivatives have raised greater scientific interest in life science and technologies. The number of publication in term of chitosan or chitosan derivatives has been getting an increasing trend due to their properties. Chitosan has numerous and unique physiological and biological properties, leading to a great potential in a sustainable agriculture and food safety application. The application of chitosan in agriculture consisted of seed coating and direct spraying on plants (Zeng and Shi, 2009). In agriculture, chitosan has been applied on seeds, leaves,

and fruits, and used as fertilizers with the controlled agrochemical release to increase plant growth and yield, stimulate the plant immunity, and protect plants against microorganisms (Abdelbasset *et al.*, 2010).

Oligomeric chitosan has been reported to be effective on plant growth and plant defense against pathogen (Nge *et al.*, 2006; Falcon-Rodriguez *et al.*, 2009). In our lab, oligomeric chitosan with deacethylation percentage of 80 (O80) has been used to improve growth of many plants. For instance, in rice, O80 at the concentration of 40 mg/L significantly enhanced 'LPT123' rice growth at the vegetative stage and also enhanced photosynthetic pigment content in rice under drought stress (Pongprayoon *et al.*, 2013). In orchids, O80 at the concentration of 10 and 20 mg/L was found to be the most appropriate chitosan type and concentration to induce protocom-like body formation of hybrid *Dendrobium* orchids (Pornpienpakdee *et al.*, 2010). Additionally, foliar application of O80 at the concentration of 1, 10, 50, and 100 mg/L could induce early flowering and increase the inflorescence accumulative number of orchids (Limpanavech *et al.*, 2008).

25497421 63

Nowadays, utilization of oligomeric chitosan to enhance plant growth has been spread out; however, the question about chitosan mechanism in plant growth is still not elucidated. There are many researches reported the chitosan response focusing on plant defense mechanism, as it was shown to be able to activate the defense responses in plants (Agrawal *et al.*, 2002; Lizama-Uc *et al.*, 2007; Abdelbasset *et al.*, 2010).

Proteomics is a large-scale protein study at a particular condition including protein abundances information, protein modifications and variations, along with their interactions and co-expression networks to understand the cellular responses. The proteomic approaches of crop plant have been increased within the last decade with the improvement of its techniques to be better accuracy and efficiency (Komatsu *et al.*, 2013). Two-dimensional electrophoresis (2-DE) is the prominent technique for protein separation. However, it has a limitation in protein characteristics including high or low molecular masses, extreme pI, highly hydrophobic protein, and low abundance proteins. These characters are not easy to deal with a standard 2-DE (Park *et al.*, 2006). An alternative technique is one-dimensional electrophoresis (1-DE) with high-throughput liquid chromatography coupled with mass spectrometry (LC-MS/MS) to

directly analyze the protein abundances. Although, 1-DE has a best coverage and can be reproducible, its resolution capacity is lower than 2-DE due to the complexity of protein in the same protein band in gel. To improve the resolution of 1-DE, gel is serially cut into the very small pieces corresponding to the range of known molecular weight of protein ladder. The cut gel has to label the slice number or position in the gel to harvest the correct mass spectrometric information. The replacement of 2-DE with 1-DE could increase a number of identified protein pool especially membrane protein (Petushkova and Lisitsa, 2012). Due to the elicitor property of chitosan, it might interact with the molecule on plasma membrane surface to trigger a signal transduction. This technique has a potential to improve the understanding of chitosan responses.

In the deep understanding of molecular basis of oligomeric chitosan response particular in plant growth and development, identification of chitosan-responsive genes should be investigated. The first previous study of transcriptional change responding to oligomeric chitosan revealed that chitosan elicited the plant immune system in oilseed rape (*Brassica napus*). This defense response connected with jasmonic acid/ethylene-mediated signaling pathway (Yin *et al.*, 2006). In addition, it was also found the production of hydrogen peroxide (H_2O_2) and nitric oxide (NO) in epidermal cells after oligomeric chitosan treatment (Li *et al.*, 2009).

Transcriptome is whole transcripts in a cell or organisms at the specific developmental stage or condition. Transcriptome reveals the functional elements, constituent of cells and tissues leading to understand cellular responses at the stage of interest (Wang *et al.*, 2009). Recently, high-throughput sequencing has been developed to quantify and map transcriptomes. RNA sequencing (RNA-seq) is a PCR-based and powerful tool for transcriptomic analysis to measure the level of whole transcript expression including the low copy and novel genes. RNA-seq could be replaced microarray with more accuracy, highly reproducible, low background noise, and required small amount of RNA sample (Li *et al.*, 2010). The advantage of RNA-seq could enable the discovery of unprecedented overview of the chitosan-responsive transcriptome.

2549742163

Forward genetics has been widely used to study the gene function and molecular events of particular biological process. It can be used to identify the sequence variation responsible for the phenotype of interest. Initially, this method starts with generating random mutation in an organism and is followed by subsequence breeding and isolating individuals with aberrant phenotypes (Page and Grossniklaus, 2002). To identify the mutated gene, fast-forward genetics has become a technique for rapid identification. This method is a combination of bulk-segregation analysis and next-generation sequencing. It has been used in well-defined genomic sequence plant including *Arabidopsis* (Mokry *et al.*, 2011; Schneeberger and Weigel, 2011). *Arabidopsis thaliana* is a plant model for molecular biology as its small genome size and rapid life cycle (Meinke *et al.*, 1998). For the best of my knowledge, genetic screening for chitosan-responsive gene has not been performed. This might be the first study to isolate chitosan insensitive/reduced-sensitive mutants for further investigation of chitosan responding mechanism in plants.

25497421 63

The general aim of this study was to investigate the potential of cellular components responsible for chitosan elicitation particular in plant growth and development via omics and genetic approaches. This study purposed the cellular proteome response of rice seedlings treated with oligomeric chitosan. An identification of chitosan-responsive gene in *Arabidopsis*, chitosan-responsive transcriptome and the genetic screen of chitosan-reduced-sensitive mutants were elucidated. The results from two model organisms were compared for better understanding in chitosan response at the molecular level.

The objectives of this study are:

1. To investigate protein profiles of rice seedlings after chitosan application.
2. To identify and characterize some chitosan-responsive genes in *Arabidopsis*.
3. To identify ortholog gene(s) involving in chitosan responses in rice.