Summary of Doctoral Thesis

Molecular Genetic Studies on the Regulation Mechanism of Phytic Acid Content in Rice Grains

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Chapter 1

General Introduction

Micronutrient malnutrition is a serious global health issue and improving the nutritional value of major crops is paramount importance to combat micronutrient deficiencies. Rice (*Oryza sativa* L.) is one of the most important staple crops and improving the micronutrient bioavailability of rice would benefit a large population in the world.

Phytic acid (PA, *myo*-inositol 1,2,3,4,5,6-hexakis phosphate) accounts for 50%~90% of total phosphorus (P) content in cereals as a major antinutrient. Developing low PA crops by manipulating the PA content in grains has been given due attention as an effective mean of improving bioavailability. To date, several low PA (*lpa*) rice lines have been developed mainly through mutation breeding and transgenic approaches. However further, there is significant knowledge gap on how the grain PA content negatively correlate with agronomic and yield performances of the plant and the underlying regulation mechanism.

Recently, many attempts were successfully achieved in understanding the PA biosynthesis genes, mechanisms of regulation of P nutrition in rice, however, there is still very little-known at present and the molecular genetic level understanding regarding PA pathway, translocation, localization and compartmentalization functions, and pathway regulation remain to be accomplished. In addition to the issues concerning the central biosynthetic pathway of PA, most importantly, the optimal amount of PA that would not affect the normal functioning of the major metabolic pathways, such as protein or starch synthesis in the developing grain might be important.

Among the several attempts through understanding and development of successful accessions, there are only a few reports evaluating the natural variation in the PA content of rice. Exploring natural variation may help us identify novel genetic mechanisms to manipulate seed PA content in rice. Though several genetic studies have been carried out in the recent past regarding major PA biosynthesis genes, there is limited understanding on regulation mechanism of PA accumulation in rice. To date, no previous research has investigated the association of PA content and genotypes using genome-wide mapping and further genetic basis underlying the PA accumulation. Therefore, this PhD study broadly aims at understanding the molecular genetic basis underlying the regulation mechanism of PA content using natural genetic variation of rice.

Chapter 2

Literature Review

This Chapter presents a published "Literature Review" and discusses the reduction of PA content for improving the bioavailability in rice grains, focusing on genetic, physiological and environmental factors. In this review, I focused on the key determinants for PA content in rice grain primarily the genetic and environmental factors and then discuss the possible molecular methods and approaches manipulating grain PA and micronutrient distribution and the PA/micro-nutrient ratio *lpa* for manipulating the PA content to increase micronutrient bioavailability

In this review, I have focused on the key determinants for PA concentration in rice grain, mainly the genetic and environmental factors including the soil and climatic conditions affect the PA content according to the published literature and discussed the possible molecular methods and approaches for manipulating the PA content to increase micronutrient bioavailability in rice grain. In general, I explained the possible strategies toward manipulating grain PA contents, such as the use of *lpa* mutants advanced molecular genetic techniques such as transgenics and DNA markers. Further, described the possibilities concerning two key approaches, such as manipulating the distribution of grain PA and micronutrient and the PA/micronutrient ratio in the grain.

In addition, the important research findings concerning those factors until present and knowledge gaps to be understood further are presented.

This Chapter has been published in "Rice".

Ishara Perera, Saman Seneweera, Naoki Hirotsu. (2018). Manipulating the phytic acid content of rice grain toward improving micronutrient bioavailability, *Rice,* 11, 4.

Chapter 3

Identification of Low Phytic Acid and High Zn Bioavailable Rice (*Oryza sati*va L.) from 69 accessions of the World Rice Core Collection

In this Chapter, I evaluated the 69 accessions in the World Rice Core collection (WRC) for PA and Zn contents and calculated the daily absorption of Zn (TAZ) inside the human intestine. Further, I compared the contents of other minerals and heavy metals between identified low and high PA accessions. Correlations among the other morphological traits and PA contents in the same accessions to understand any association were explained in the chapter. Further, I discussed the genetic determination of Zn bioavailability with the association analysis facts of PA and Zn contents of rice grains.

The results revealed a high variation of PA and Zn contents among the WRC accessions. I have identified three low and three high PA WRC accessions for further studies. Some WRC accessions were observed having a low PA content and high Zn bioavailability and some others with a high PA content and low calculated Zn absorption. No significant differences were observed in the mineral or heavy metal contents among low and high PA lines examined suggesting that different mechanisms are controlling these traits, so that manipulating the PA content could be achieved without affecting the concentration of these elements. Genome-wide association study revealed that a chromosomal region near several significant SNPs determine the natural variation in rice PA content. Furthermore, low PA trait in rice should be an important target for increasing Zn bioavailability in rice.

This Chapter has been published in “Journal of Cereal Science”.

Ishara Perera, Ayaka Fukushima, Mako Arai, Kenta Yamada, Seiji Nagasaka, Saman Seneweera, Naoki Hirotsu. (2019). Identification of low phytic acid and high Zn bioavailable rice (*Oryza sativa* L.) from 69 accessions of the world rice core collection, Journal of Cereal Science, 85, 206-213.

Chapter 4

Genome-wide and seed proteomic studies identify important genes for phytic acid accumulation in rice

This Chapter explains a series of experiments conducted to understand the molecular genetic mechanism of PA accumulation rice grain. I have carried out an association study for PA content in WRC accessions and further investigated candidate genes responsible for the PA trait. I also investigated the most important PA accumulation stage in developing rice grains and the key PA genes expressing in selected low and high PA WRC accessions to understand the genetic basis of PA accumulation in rice grain. To further understand regulatory mechanism and the metabolic process, the protein profile of the low and high PA accessions was studied.

GWA identified significant SNPs that could be influence on determining the PA content in rice. Further, the most important PA accumulation stage in the developing grain could provide insights for future research on manipulating grain PA content in rice. GWA results identified myo-inositol 3-phosphate synthase 1 (*INO1*) as being closely localized to a significant single nucleotide polymorphism on Chromosome 3. I found high rates of PA accumulation 10 DAF, and *INO1* expression was significantly higher ($p<0.05$) in WRC 6 than that in WRC 5. Seed proteome assays also found that the expression of *INO1* was significantly higher (1.8-fold) in WRC 6. These results suggest that not only the gene itself but the regulation of expression is important for low PA trait without yield loss. However, further investigation should be needed to understand the agronomic and yield performances and storage of other nutrients in the grain, as the *INO1* gene regulates early steps in the PA pathway.

This Chapter has been prepared for submission as “Genome-wide and seed proteomic studies identify important genes for phytic acid accumulation in rice. Ishara Perera, Ayaka Fukushima, Tatsuki Akabane, Genki Horiguchi, Saman Seneweera, Naoki Hirotsu.

Chapter 5

Concluding Remarks

This chapter discusses the major conclusions derived from this series of experiments and briefly present the future directions to be researched.

Reducing the PA content of rice grains to improve nutrient bioavailability is widely accepted approach as a mean of alleviating micronutrient malnutrition. In a summary of the results, Zn absorption is affected by the PA content in rice grains, thus the reducing the PA content would be improve Zn bioavailability in rice. Further, expression regulation of *INO1* at early developmental stages is important in PA accumulation, and the findings of this project would advance the understanding on genetic regulation of PA accumulation and provide insights for research approaches toward improving bioavailability and low PA accessions in rice.