論 文 の 内 容 の 要 旨

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論文題目　Analysis of mineral value and iron biofortificaiton of Myanmar rice

(ミャンマー米の無機栄養分の分析と鉄分強化に関する研究)

Iron (Fe) is an essential element for both plant growth and human health. There are two aspects of view involved in Fe nutrition research in plants: one is Fe homeostasis in plants and the other is Fe biofortification for human health. Fe and zinc (Zn) deficiencies cause high mortality particularly in the developing countries. In Myanmar, prevalence of Iron Deficiency Anemia (IDA) in children under five years is about 75%, in adolescent school girls is about 26%, in non-pregnant women is about 45% and in pregnant women is about 71% (National Hemoglobin Survey, 2003). The main cause of these deficiencies is due to inadequate intake of Fe and Zn rich diet. Rice is the most important staple food in Myanmar. Myanmar people are one of the most rice-consumeing people in the world and their consumption is about 562 g per day per person. Therefore, it is very important to investigate the micronutrient value of rice in order to identify mineral rich rice varieties and to produce higher Fe and Zn rice varieties from present Myanmar rice varieties **(Chapter 1)**.

Fe regulation system is essential for rice to maintain Fe homeostasis in rice. In order to further understanding about Fe regulatory system by transcription factors IDEF1 and IDEF2 in rice, "**Expression analysis of transcription factors *IDEF1* and *IDEF2* in rice"** was carried out by observing **promoter activity of IDEF1 and IDEF2** spatially and temporally at seed

germination, vegetative, flowering and seed developmental stages by GUS histochemical localization method. The results showed that *IDEF1* and *IDEF2* were expressed throughout life cycle. Overlapping expression of *IDEF1* and *IDEF2* with their target genes was achieved in restricted cell types. They regulate gene expression in both Fe-sufficient and Fe-deficient roots **(Chapter 2: Aung et al. 2010).**

In order to identify mineral rich rice varieties, the research **"Analysis of mineral and nutritional value of various Myanmar rice varieties"** was carried out. Firstly, high Fe and Zn varieties were screened among 39 Myanmar rice varieties obtained from gene bank of National Institute of Agrobiological Sciences, Japan. Of these, 6 high Fe and Zn varieties were selected and grown in greenhouse. The results showed that 'Ngasein C 30-24' variety was high in Fe and Zn concentrations. Fe concentration in polished seeds of this variety was 2.9 µg/g and Zn concentration was 36.9 µg/g. In addition, 21 high yield or high quality and present popular rice varieties from Myanmar Rice Research Center (MRRC) were analyzed. Then, field cultivation was implemented in paddy field of Ishikawa Prefectural University, and the micronutrient concentrations in brown and polished seeds of selected rice varieties were examined. The results proved that the range of Fe concentrations in polished seeds were 1.5 µg/g in 'Hmawbi 4' to 2.5 µg/g in 'Thu Ka Yin' and 'Sin Nwe Yin'. Zn concentration range was 11.1 µg/g in 'Yezin Lone Thwe' to 20.4 µg/g in 'Sin Nwe Yin'. 'Sin Nwe Yin' variety was high in both Fe and Zn concentration in polished seeds. In the present study, high Fe and Zn rice varieties in Myanmar were identified, which will reduce mineral deficiency problem and be useful for further breeding and biofortification programs. From this study, 'Ngasein C 30-24' was firstly found as the unique rice variety to fulfill all of Zn demand required for human nutrition by consuming this rice alone. Without changing food custom, farmers' and consumers' choices of such nutritionally promising varieties will reduce the mineral deficiency problem for human health in Myanmar without additional cost **(Chapter 3: Aung et al. Prepared for submission-1)**.

Current indigenous and improved rice varieties cannot fulfill the required Fe content for human health yet. To address Fe deficiency problem, biofortification (i.e., the breeding of micronutrient-fortified rice) will be advantageous for Myanmar people. To achieve real transformation, criteria for varietal selection for transformation were determined. Among the criteria, **"Analysis of regeneration efficiency of various Myanmar rice varieties"** is one of the important and essential one. Callus induction and regeneration efficiency analysis were performed on various culture types with 15 Myanmar rice varieties from MRRC. After testing with various culture conditions, 13 rice varieties out of 15 were found to have the regeneration capacity but on specific media types. Moreover, callus formation and regeneration efficiency of each variety were highly influenced by types of culture trend. In addition, 2,4-dichlorophenoxy acidic acid hormone concentration specifically caused effects on callus induction on N6D culture media and subsequently on regeneration efficiency in most varieties. Finally, the best media types of trend for each variety were determined. The regeneration rate greatly varied with variety tested. 'Ayar Min', 'Hmawbi 3', 'Paw San Yin' and 'Hmawbi Kauk nyin Hmwe' were found to

have good calli induction and high regeneration rate **(Chapter 4: Aung et al. Prepared for submission-2).**

Then, **"Generation of high Fe Myanmar rice"** research was conducted. 'Paw San Yin' variety is high quality rice variety and widely consumed in Myanmar. This variety was high in Fe concentration in polished seeds (Chapter 3) and good in callus induction and regeneration (Chapter 4). As a transgene construct, HIR4 was prepared, which contained multi-genes which enhance Fe uptake from soil, Fe transportation within plant body and Fe accumulation in rice plants (Masuda et al. 2012) as follows:

1) "*Actin promoter-HvNAS1*" was used to enhance Fe and Zn transport within rice plant by over-expression of nicotianamine synthase gene HvNAS1.
2) "*SUT1pro-YSL2* and *Globulin pro-YSL2*" were used to enhance Fe flow to the endosperm by Fe (II)-nicotianamine transporter gene *OsYSL2.*
3) "*Glutelin B pro-ferritin* and *Globulin pro-ferritin*" were used to enhance Fe accumulation in grain by Fe storage protein gene *ferritin* in endosperm.

HIR4 gene construct was transformed into 'Paw San Yin' variety. Gene insertion was confirmed. Higher gene expression of introduced genes, *OsYSL2*, *HvNAS1* and *SoyferH2*, were observed in immature T_2 seeds. Then, high Fe rice was achieved with 3.4 times increase in T_2 polished seeds (**Figure 1**). This high Fe rice was 1.3 times higher also in Zn concentration than non-transgenic rice (NT). Here, high Fe Myanmar rice was successfully produced with 'Paw San Yin' variety by Fe biofortification method. With 3.4 times increase in Fe concentration, this rice may fulfill the Fe demand required for Myanmar people. This rice will help to reduce Fe deficiency anemia problem in millions of people in Myanmar and other rice eating countries (**Chapter 5: Aung et al. Prepared for submission-2**).

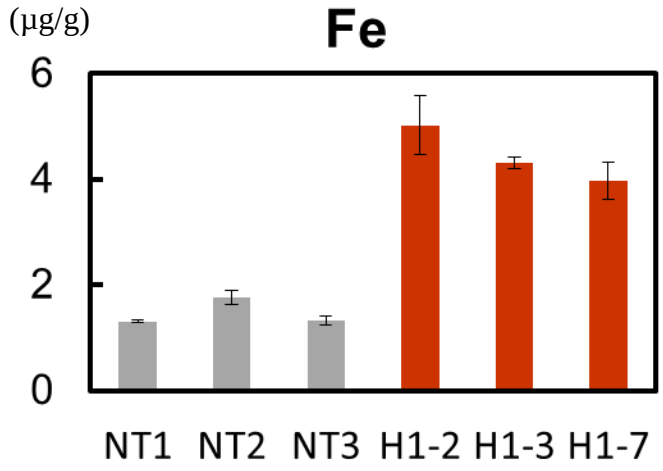

Figure 1 Fe concentration of Paw San Yin HIR4 in T_2 polished seeds.

NT1, NT2, NT3: Paw San Yin non-transgenic rice. H1-2, H1-3, H1-7: Paw San Yin HIR4 transgenic lines. The data represented the mean ± standard error of three measurements for each sample (n = 3).

Additionally, "**Generation of high Fe Myanmar rice varieties, Thai jasmine rice and Tsukinohikari**" was carried out. New HIR6 gene construct was produced by modification with combination of the follow genes into above three genes of HIR4 gene construct:

4) Genome sequence of *IDS3, which encodes mugineic acid synthase*, was inserted to enhance Fe uptake ability from soil.
5) *OsADH* was used as an enhancer sequence of protein translation.

Moreover, rice mutant gene for selection (*Acetolactate synthase*) was introduced instead of bacteria genes for public acceptance. HIR4 and/or HIR6 constructs were introduced into Myanmar rice varieties, Thai Jasmine KDML105 rice and Tsukinohikari rice and successfully transformed. **(Chapter 6-** research in progress**)**.

In conclusion, *IDEF1* and *IDEF2* are thought to be involved in Fe translocation and utilization processes throughout rice life. Aside from food security, nutritionally value-added rice culture will reduce health risk of people. In my research, the unique high Zn rice variety 'Ngasein C 30-24', which was also high in Fe concentration, was found among the presently cultivating rice varieties. Moreover, the novel high Fe rice was successfully generated with 'Paw San Yin' variety by transgenic method, which was also high in Zn content. These nutritionally promising varieties will help to millions of people from Fe deficiency problems without changing food custom and additional costs **(Chapter 7).**

Published papers:

1. May Sann Aung, Takanori Kobayashi, Yugo Ogo, Tomoko Nozoye, Hiromi Nakanishi, Takashi Yamakawa and Naoko K. Nishizawa: **The spatial expression and regulation of transcription factors IDEF1 and IDEF2 :** ***Annals of Botany***, 105, No. 7, pp. 1109-1117, March, 2010

2. Yugo Ogo, Reiko N. Itai, Takanori Kobayashi, May Sann Aung, Hiromi Nakanishi and Naoko K. Nishizawa: **OsIRO2 is responsible for iron utilization in rice and improves growth and yield in calcareous soil:** ***Plant Molecular Biology,*** 75, No. 6, pp. 593-605, February, 2011

3. Takanori Kobayashi, Reiko N. Itai, May Sann Aung, Takeshi Senoura, Hiromi Nakanishi and Naoko K. Nishizawa: **The rice transcription factor IDEF1 directly binds to iron and other divalent metals for sensing cellular iron status:** ***The Plant Journal***, 69 , No. 1, pp. 81-91, January, 2012

4. Hiroshi Masuda, Yasuhiro Ishimaru, May Sann Aung, Takanori Kobayashi, Yusuke Kakei, Michiko Takahashi, Kyoko Higuchi, Hiromi Nakanishi and Naoko K. Nishizawa: **Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition:** ***Scientific Reports,*** 2, No. 543, DOI:10.1038/srep00543, July, 2012

Papers prepared to submit:

1. May Sann Aung, Hiroshi Masuda, Yusuke Kakei, Takanori Kobayashi, Hiromi Nakanishi, Takashi Yamakawa and Naoko K. Nishizawa: **Variation in mineral value in grains of various rice varieties in Myanmar** (Papers prepared to submit -1)

2. May Sann Aung, Hiroshi Masuda, Takanori Kobayashi, Hiromi Nakanishi, Takashi Yamakawa and Naoko K. Nishizawa: **Iron biofortification of Myanmar rice** (Papers prepared to submit -2)