（別紙様式６）
(Attached Form 6)

学位論文の要旨（論文の内容の要旨）
Summary of the Dissertation (Summary of Dissertation Contents)

論　文　題　目
Dissertation title
Improvement of Salinity Tolerance in Rice (*Oryza sativa* L.) by N-methyl-N-nitrosourea (MNU) Treatment and Exogenous Application

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Background

More than half of the world's population consumes rice (*Oryza sativa*) as a staple food and considers it as an important agricultural crop with various benefits. In the current context of the rapid increase of world population and impacts of climate change, the rice demand has increased in both productivity and quality. Therefore, the target of rice breeders is breeding and developing new cultivars which have not only high yield and good quality but also possess wide adaptability to severe environmental conditions.

Among climate change impacts, salinity is one of the most problematic hazards to rice cultivation, which adversely affects rice yield and quality. By various damages of ion toxification, nutritional disorders, osmotic imbalance, oxidative stresses, membrane disorganization, reduction of cell division and expansion, and genotoxicity, salinity intrusion significantly reduces rice growth as well as partial sterility, which ultimately results in the reduction of rice yield. Besides, different levels of salt stress can lead to the changes of quality in rice, such as amylose content, protein content, amino acid, and micro and macro mineral nutrients. Although slight salinity induces the increase of some minerals, salt conditions almost have a negative effect to the rice production. Therefore, the breeding of salt-tolerant rice cultivars is an important task to ensure food security and sustainable agriculture.

Tremendous strategies have been conducted to enhance salinity tolerance in rice. Mutation breeding is a potential technique that creates a new rice and selects the elite line through many generations. N-Methyl-N-nitrosourea (MNU) mutation is a chemical induced mutation and widely used for breeding cereal crops. MNU frequently leads to the point mutation. It is possible to alter the DNA structure of plants and cause the biological effects. MNU-induced mutants are considered as the high frequency mutation which is a valuable material for plant breeding.

In a breeding, molecular marker or DNA marker is used to identify the genotype of plant population. The molecular marker is a prominent innovation of biotechnology that helps breeders to select the target plants quickly and simply by detecting a particular gene or trait. DNA marker has been discovered for genetic analysis since 1980s and still applied for rice breeding. Among developed DNA markers, simple sequence repeat marker (SSR) is one of the powerful techniques to identify the mutant genotypes. This marker is co-dominance and useful for the detection of genetic inheritance in progeny. By the advantage compared to other markers, SSR has been achieved some achievements in mutation breeding including classification of important mutant genotypes.

Earlier studies substantiated the important functions of mineral elements in human, animal, and especially plant nutrition. Plants use minerals as structural components in carbohydrates, proteins, and organic molecules. Magnesium (Mg) is a macronutrient participated in various metabolic processes as photosynthesis, enzyme activators, and osmotic balance of plants. In the last decades, several studies reflected a correlation between

magnesium and plant growth in a number of higher plants. However, the role of magnesium in reducing salt-induced damages in rice has not been comprehensively studied.

Objectives

Considering all above mentions, to develop the tolerant rice and improve its resistance to salinity stress, this study is conducted to (1) identify the salinity tolerance of rice mutants by phenotypic measurements and SSR markers, (2) determine the inheritance pattern of salinity tolerance and beneficial phytochemicals of rice mutants, (3) shorten breeding time by the possible maternal inheritance, and (4) improve salinity tolerance of rice by application of magnesium.

Structure of dissertation

Chapter 1: General introduction

Chapter 2: Identification of salinity tolerance in rice mutants by phenotypic and simple sequence repeat analyses

Chapter 3: Maternal inheritance of salinity tolerance and beneficial phytochemicals in rice

Chapter 4: Improvement of salinity tolerance in rice by exogenous magnesium application

Chapter 5: General discussion

Materials, methods, and results

In this study, rice mutants were created by soaking rice seeds in 150 mM MNU for 3 hours, drying, and keeping in the dark for 3 months in a hermetic condition before being stored at 4 °C. The rice mutants and parents were then cultivated in Higashi-Hiroshima from 2016 to 2019 to assess their phenotypes. After that, salinity tolerance of rice samples was screened. A mutant population with prominent characteristics (TBR1/KD18) also was selected to detect their salt tolerance. From the screening, the strong tolerant and susceptible rice were chosen for experiment with exogenous magnesium.

In chapter 2, salinity tolerance of ten rice cultivars/mutant lines was identified by the combination of phenotypic measurement and genetic analysis. The phenotypic assessments were conducted followed the protocol from International Rice Research Institute. In genetic analysis, forty-two SSR markers linked to *Saltol* quantitative trait locus (QTL) (salinity tolerant) were used to identify the genotype of rice samples. The results showed that BC15 and BC15/SKLo have strong tolerance to salinity. They are valuable sources for the breeding of salinity tolerant rice. Additionally, six SSR polymorphic markers RM 237, RM 518, RM 493, RM 10748, RM 562, and RM 20224 were found to be polymorphic. These markers can be used useful for classification of salt tolerance of rice, both cultivars and mutants. The results of phenotypic measurement and genetic analysis are correlated.

Chapter 3 determines the salinity tolerance and inheritance pattern of the elite mutant rice TBR1/KD18. The F_1 generation of the cross TBR1 (female cultivar) × KD18 (male cultivar) was treated with MNU to induce the first mutant generation M_1. M_1 was then self-pollinated to obtain M_2 and M_3 populations. Control populations were F_1 and F_2. Phenotypic, chemical, and genetic analyses were conducted in the M_2, M_3, F_1, F_2, TBR1, and KD18. A total of fifty SSR markers involved in growth parameters, yield, pest resistance, and the *Saltol* QTLs were used for genetic analysis. The results showed that the salinity tolerant *Saltol* QTLs, growth parameters, yield, pest resistance are maternally inherited from the female parent TBR1 in the M_2 generation and stabilized in the M_3 generation. Besides, antioxidant activities and contents of momilactones A and B might be maternally inherited. In contrast with the literatures, this is the first study to reveal that salinity tolerance of rice can be maternally inherited (normally paternally inherited). The MNU-induced maternal inheritance provides a simple protocol to finish progeny segregation in 2-3 generations (instead of 8-10 cycles in conventional breeding) and shorten breeding time for salt tolerant rice.

Chapter 4 focuses on the promising effects of magnesium on developing salt tolerance rice. From chapter 2, BC15 (salinity tolerant) and DT84DB (salinity susceptible) were selected and used as rice materials in chapter 4. The salinization was conducted in 7 days-old seedlings with the supplement of $MgSO_4$ (0.5 mM). After treatment, physiological, antioxidant activities, and chemical properties of rice samples were investigated. The phytochemicals of rice materials including phenolic compounds as well as momilactones A and B were identified and quantified by High Performance Liquid Chromatography (HPLC) and Ultra Performance Liquid Chromatography Electrospray Ionisation Mass Spectrometry (UPLC-ESI-MS), respectively. The results showed that exogenous application of Mg partially recovers the inhibited growth and improves the antioxidant activities as well as phenolic profiles of rice seedlings under salt stress. Momilactone A was only detected in

salinity tolerant cultivar BC15 under control condition (a very low concentration that could not be measured, hence will be interpreted as undetectable). However, momilactone B were found in both tolerant and susceptible rice lines, in which, the amount of momilactone B of tolerant rice were higher than that of the susceptible one. It was also indicated that the amount of potential bioactive compound momilactone B was enhanced with Mg supplement in salt stressed rice at seedling stage. The results suggested that $MgSO_4$ is useful develop fertilizer for rice growing in saline soil. Additionally, *ρ*-coumaric acid, salicylic acid, ferulic acid, and momilactone B are involved in the tolerant ability of rice again salt stress. They can be used as promising agents to reduce salinity damages on rice production.

Key findings of the dissertation

The literatures showed that salinity tolerance of rice is nucleus inheritance (inherited from father). This study is the first to reveal a maternal inheritance in the salinity tolerance of rice. On the other hand, breeding program for salt-tolerant rice requires 8-10 years due to the complicated segregation in progenies. F_1 normally is crossed with father (backcross) and repeat in many generations to finish that segregation. The maternal inheritance induced by MNU treatment in this study helps to finish the segregation in M_2 and M_3 generations, which can shorten breeding time from 8-10 cycles to 2-3 generations. However, the mutated rice should be sequenced and compared to parental genotypes to detect their genetical changes. The mechanism of the novel maternal inheritance should be clearly identified. Besides, magnesium is effective to reduce salinity-induced damages in rice seedlings by enhancing its inhibited growth, antioxidant activities, phenolic acids, and momilactone B. The results suggest that the salinity tolerance of rice can be improved by developing fertilizer with a supplementary $MgSO_4$. However, this application needs to be further investigated in rice field.