## 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

Effects of MNU Mutation on Productivity, Grain Quality and Allelopathic Potential of Rice

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A better and comprehensive understanding of the factors that influence and contribute to the overall growth and yield performances as well as grain quality of rice will lead the way for the foundation and development of new breeding strategies to enhance rice productivity and improve grain quality. This is urgent need to meet the increasing world demand for high yield and quality rice grain while providing developing countries extra opportunities for generating higher export outcomes. Recently, numerous genetic, metabolomic, and phenomic developments have enhanced our understanding related to the productivity and quality traits of rice plant. Challenges in breeding novel rice varieties that have a high yielding potential, preferable grain quality, and are adaptable to environmental conditions have been the main and major constraint factor of rice production in several developing countries. The development and releasing of new rice varieties with a high yield, adaptability, acceptable grain quality, and resistance to biotic and abiotic factors is urgently needed, but this procedure is not ease and commonly needs 8-10 years or more due to the great recombination in phenotypic and genotypic attributes as well as hard works and vast expenditures. Therefore, the lack of novel rice varieties has resulted unsustainable rice production and quality improvement in many developing countries. In recent decades, mutation technology, a direct alteration on the genome and considered as one of the most beneficial routes to acquire new beneficial traits has been applied more to increase rice productivity and achieve novel products that are not commonly found in nature through the improvement and development of new rice varieties with better quality and higher productivity. Thus, experiments were conducted in the research field and laboratory of the plant physiology and biochemistry, the graduate school for international development and cooperation, Hiroshima University to examine the effects of an N-methyl-N-nitrosourea (MNU) mutation on the growth and yield performances, physicochemical properties, phytochemical contents, biological activities, and allelopathic potential of rice. The mutant lines have already treated following a MNU mutation protocol. In short, seeds of the original cultivars were treated for three hours with 150 mM MNU, dried, and kept in hermetic conditions for three months. Then, the required seeds were possessed and stored in the darkness at 5 °C for further application. The mutated F1 was self-pollinated to yield the mutated F2 population. Perfect seeds of six rice cultivars/mutant lines (K1, K3, and K5), along with their mutant lines (K2, K4, and K6), were submerged in distal water and sown in nursery boxes, and the established seedlings were transplanted to an open field at 25-days-old seedling stage. 15 cm  $\times$  20 cm spaces were respectively considered as a plating density between plants and rows. Weeds were manually controlled at the maximum tillering and heading stages. Standard fertilizer (14-10-13; JA-ZEN CHU Co., Hiroshima, Japan) at 130 g per plot was applied at early tillering and milking stages. Ten hills per plot were randomly selected to evaluate growth parameters, yield, and components. Plants were harvested at maturity stage. The required samples of grain and straw were collected and kept at room temperature. Grain samples of each plot were threshed and dried at room temperature to obtain 18% moisture content. The grains were then de-husked by an automatic rice husker machine (model TR-250, Kett Electric Laboratory, Tokyo, Japan) and the husks were also collected for further evaluation. Physicochemical attributes, including protein, amylose, and lipid contents, as well as taste score were measured by a quality tester device. Rice grain, husk, and straw samples were extracted and the antioxidant activities including DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assays,

phenolic and flavonoid compounds, and momilactones A (MA) and B (MB) of the rice grain, husk, and straw portions using ultra performance liquid chromatography (UPLC) were measured and quantified. Additionally, allelopathic potential of rice husk and straw on the germination and seedling growth of lettuce and barnyard grass as well as the correlation of improved rice grain with the allelopathy activity of rice plant were evaluated. The possible involvement of phenolic acids in allelopathic effects from rice straw and husk extracts using high performance liquid chromatography (HPLC) were investigated. The results showed that plant length, tiller number, and panicle length were higher in mutant lines than those of their cultivars. Furthermore, mutant lines took longer to reach heading and maturity stage. The highest panicle number, spikelet number, repined ratio, 1000 grain weight, 1000 brown rice weight, and grain yield were obtained in mutant lines, as compared to cultivars. The greatest grain yield was obtained in the K6 mutant line (11.9 t/ha), while the lowest was recorded in the K1 cultivar (7.7 t/ha). Lower amylose, protein, and lipid contents were observed in mutant lines compared to those in cultivars. The taste score, which increased from 67.7 to 82.3, was found to be correlated with lower amylose, protein, and lipid contents. Higher taste score, grain length, and grain length to width ratio were recorded in mutant lines. The mutation approach increased the grain length but decreased the grain width of tested varieties. Rice grains produced by K1 were white in color and recorded to be a more sticky rice grain producer cultivar which was change to a brown and non-sticky rice grain cultivar (K2) after treating with MNU mutation, while the others were brown in color. Antioxidant activities were higher in grain and straw of mutant lines, whereas original cultivars showed greater antioxidant activity in rice husk. The antioxidant activity of husk was higher followed by straw and grain. Additionally, mutant lines displayed higher total phenolic contents (TPC) in grain and straw as well as lower of it in the husk, but these variations significantly differed only in the straw portion. An increase in total flavonoid contents (TFC) was observed in the husk of origin cultivar, while mutant lines significantly enhanced TFC in straw. Both MA and MB, two compounds obtaining antidiabetes, anticancer, antimicrobial, antigout, and antiobesity properties, were detected and quantified in grain, husk, and straw of origin cultivars and mutant lines samples. Generally, the contents of MA were higher than MB in all tested portions of rice crop. MA and MB were higher in straw followed by those in husk and grain, respectively. Mutant lines contained higher amounts of MA and MB in straw and husk, but lower contents in grain compared with those in origin cultivars. Grain yield showed a significantly positive relation with DPPH, and ABTS, whereas it showed a negative correlation with amylose content, TFC, MA, and MB. Grain yield did not have correlation with protein and lipid contents, and TPC. Amylose content displayed a positive interaction with TFC, MA, and MB, but showed a negative relationship with taste score, grain yield, DPPH, and ABTS. The current researches demonstrated that the inhibitory and suppressive potential of the rice husk and straw on the germination and seedling growth of both tested plants were differed. Of them, the rice straw exerted stronger germination and seedling growth inhibition compared to the rice husk. The highest germination as well as shoot and root lengths inhibitions of husk samples on the lettuce and barnyard grass were recorded in the origin cultivars; however, the highest germination and shoot and root lengths inhibitions of straw samples on lettuce and barnyard grass were observed in the mutant lines. Mutant lines significantly showed higher germination inhibition on both tested plants in straw samples but exhibited lower in husk samples. Both straw and husk samples exerted stronger germination inhibition on barnyard grass compared to the lettuce plant. Additionally, root length was much inhibited compared to shoot length in both lettuce and barnyard grass. Ten major phenolic and flavonoid compounds were detected in both husk and straw samples of the origin cultivars and their mutant lines. In the husk samples, the amounts of p-hydroxybenzoic acid, syringic acid, caffeic acid, p-coumaric acid, salicylic acid, cinnamic acid, and tricin were higher in the origin cultivars than mutant lines. In the straw samples, the contents of gallic acid, p-hydroxybenzoic acid, syringic acid, p-coumaric acid, salicylic acid, and tricin were greater in the mutant lines compared to the origin cultivars. These experiments highlight and suggest the importance of MNU mutation in terms of rice yield enhancement and grain quality improvement through increasing values of bioactive compounds in straw, husk, and grain of rice. Besides, they provide comprehensive information related to the crucial roles of MNU mutation on rice allelopathy and weed management. Therefore, the MNU mutation approach has the potential to develop rice varieties associated with high productivity and allelopathic potential as well as enhance functional foods from rice, consequently help farmers in developing countries to improve value in rice production. Additionally, new research strategies and breeding programs should undertaken to increase awareness and techniques regarding the MNU mutation and to advance our understanding of the molecular mechanisms and genomic studies of rice varieties released through the MNU mutation.

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