Doctoral Dissertation

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

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

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Table of Contents

Efficacy of *N*-methyl-*N*-nitrosourea (MNU) Mutation on Enhancing the Yield and Quality of Rice …...……………………………………..……………..…….... 10

List of Figures

List of Tables

List of Appendixes

List of Abbreviations

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AC	Amylose content
ANOVA	Analysis of variance
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
EIC	Extracted ion chromatograms
EMS	Ethyl methanesulfonate
G	Grain
g	Gram
GAE	Gallic acid equivalent
GI	Germination inhibition
GL	Grain length
GW	Grain weight
GWD	Grain width
GY	Grain yield
H	Husk
H_3NO	Hydroxylamine
ha	Hectare
HF	Hydrogen fluoride
HPLC	High-performance liquid chromatography
IC_{50}	Inhibition concentration (50%)

IRRI	International Rice Research Institute
K1	DT84 rice cultivar
K2	Mutated DT84
K3	Q5 rice cultivar
K4	Mutated Q5
K5	Cho dao rice caltivar
K6	Mutated Cho dao
LC	Lipid content
MA	Momilactone A
MB	Momilactone B
MeOH	Methanol
min	Minutes
mL	Milliliter
µg	Micro gram
µL	Micro liter
mM	Millimole
mm	Millimeter
MMS	Methyl methanesulfonate
MNU	*N*-methyl-*N*-nitrosourea
MS	Mass spectra
ng	Nanogram
nm	Nanometer
OH	Hydroxyl radical
PC	Protein content
PC1	Principal component 1

PC2	Principal component 2
PCA	Principal component analysis
PL	Plant length
PN	Panicle number
RE	Rutin equivalent
RI	Root inhibition
RNA	Ribonucleic acid
RR	Ripening ratio
RT	Retention time
S	Straw
SA	Sodium azide
SD	Standard deviation
SE	Standard error
SI	Shoot inhibition
SN	Spikelet number
t	Ton
T-DNA	Transfer-Deoxyribonucleic acid
TFC	Total flavonoid content
TN	Tiller number
TPC	Total phenolic content
TS	Taste score
UPLC	Ultra-high performance liquid chromatography

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Yours sincerely,

Kifayatullah Kakar
(Afghanistan)

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Dedication

This dissertation is dedicated to my sweet parents for their usual prays, supports, and encouragement, particularly during my doctoral studies. The dissertation is also dedicated to all my countrymen who have suffered and lost their lovely family members during several decades of war in Afghanistan. Finally, to those, without whom none of my success would be possible.

Summary of Dissertation

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 an 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 the 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 release of new rice varieties with a high yield, adaptability, acceptable grain quality, and resistance to biotic and abiotic factors are urgently needed, but this procedure is not easy 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 in unsustainable rice production and quality improvement in many developing countries. In the recent decades, mutation technology, a direct alteration on the genome, is considered as one of the most beneficial routes. Mutations have been applied more to acquire new beneficial traits, 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 grain 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 seeds of mutant lines were received from Khai Xuan International Co., Ltd. and Agricultural Genetics Institute, Vietnam. The mutant lines have already been treated following an 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. The experimental field was puddled by a power tiller and leveled manually. The plots were designed at 3 m^2. Perfect seeds of six rice cultivars/mutant lines including K1, K3, and K5, along with their mutant lines consisting of 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 the 25-days-old seedling stage. The 15 cm × 20 cm spaces were respectively considered as a plating density between plants and rows. Weeds were manually controlled by hand 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 the 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 grain 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 contents, 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, the allelopathic potential of rice husk and straw on the germination and seedling growth of lettuce and barnyard grass as well as the correlation of quality rice grain with the allelopathic activity of the 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 time to reach the heading and maturity stage. The highest panicle number, spikelet number, repined grain ratio, 1000 grain weight, 1000 brown rice weight, and grain yield were obtained in the mutant lines as compared to their original 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 changed to a brown and non-sticky rice grain cultivar (K2) after treating with the MNU mutation, while the others were brown in color. In addition, there were significant positive correlations among grain yield with tiller number per hill, panicle length, panicle number per hill, spikelet number per panicle, repined grain ratio, 1000 grain weight, and taste score. However, a significant negative correlation was found between grain yield and amylose content. Furthermore, grain yield did not show any significant correlation with protein content, lipid content, grain length, and grain width in these cultivars and mutant lines. The correlation between grain yield with its components, amylose content, and taste score exhibited a linear relation but a nonlinear correlation was observed with protein and lipid contents. Antioxidant activities were higher in grain and straw of mutant lines, whereas original cultivars showed greater antioxidant activity in the 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 the origin cultivar, while mutant lines significantly enhanced TFC in straw. Both MA and MB, two compounds obtaining anti-diabetes, anti-cancer, anti-microbial, anti-gout, and anti-obesity properties, were detected and quantified in grain, husk, and straw of the 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 correlate 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 principal component analysis (PCA) showed that PC1 accounts for 44.5 % of the total variation around the PCA chart, while PC2 exhibited 34.3%, respectively. The current research demonstrated that the inhibitory and suppressive potential of the rice husk and straw on the germination and seedling growth of both tested plants 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 length inhibitions of husk samples on the lettuce and barnyard grass, were recorded in the origin cultivars; however, the highest germination, shoot, and root length 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. In addition, root length was much inhibited compared to shoot length in both lettuce and barnyard grass. Ten major phenolic and flavonoid compounds including gallic acid, *p*-hydroxybenzoic acid, syringic acid, caffeic acid, ferulic acid, vanillic acid, *p*-coumaric acid, salicylic acid, cinnamic acid, and tricin were detected in both husk and straw samples of the origin cultivars and their mutant lines. However, the quantity of each compound differed between husk and straw samples as well as among all six cultivars/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 be undertaken to increase the 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.

Chapter 1
General Introduction

Abstract

Rice is among the most cultivated and consumable cereal which provides food for more than half of the world's population. Demands for rice production and quality improvement are raised due to the increase in the world's population and customer's preferences. The development of new adaptable and improved rice varieties with high yielding potential and acceptable grain quality is needed to meet the demands. High yielding capacity, resistance to pests and diseases, tolerance to lodging and salinity, adaptability to environmental conditions, market needs, resistance to biotic and abiotic stresses, and better grain quality are mainly considered as the most crucial and important factors in rice breeding programs. The most implacable and important methods of breeding in rice crops are pedigree, bulk selection, crossing, doubled haploid, male sterility, hybridization, mutation, molecular marker, and genetic manipulation. Among the above methods, the mutation is an effective and impressive method that applies in many crops, particularly in rice. It plays a crucial and notable role in the development and release of desired rice varieties. The mutation may happen naturally due to sudden changes and alterations of base pairs in a DNA sequence of a chromosome or conduct artificially as induction. MNU mutagens have been widely applied in several plants and the results showed that this approach affected a range of physiological processes in plants which lead the way for genetic variability. Recently, MNU mutation is one of the most applied mutagens in rice breeding programs. The mutant rice lines produced by treating with the MNU mutation showed an influence on the biosynthesis in rice endosperm, particularly the two components of starch including amylose and amylopectin. The use of MNU mutation may helps to promote rice responses against biotic and abiotic stresses. As the results, this study found that antioxidant activity, phenolic, and flavonoid contents were also increased in rice grains with MNU treatment. Some studies clearly illustrated the efficiency and potential application of MNU mutation in rice improvement and genomic programs. On the other hand, allelopathy is a phenomenon of interference between plants through releasing chemical compounds into the environment known as allelochemicals. Therefore, a comprehensive investigation is required to evaluate the effects of MNU mutation on growth attributes, yield capacity, biological activities, and allelopathic potential of the rice plant. Such efforts will not only increase rice yield and quality aspects but also find out the relationship of allelopathic potential with rice grain quality parameters and biological activities.

1. Methods of Rice Improvement

Rice (*Oryza sativa* L.) is the second most cultivated and consumable cereal crop and the staple food for more than half of the world's population. It provides the main source of calories for the people in Asia, Africa, and Latin America (Peng et al., 2008). The increasing world's population raised the demand for foods and agriculture products; particularly rice cultivation and production (Kakar et al., 2019b). Rice production should be increased in the future in order to fulfill raising demands. The development of new adaptable and improved rice cultivars with high yielding potential and acceptable grain quality is needed to meet this demand and secure food shortage (Collard et al., 2017).

Improvements in the yield potential and grain quality preference of rice are the major strategies to fulfill the requirement of world rice production. In rice breeding program, high yielding potential, resistance to diseases and pests, tolerance to lodging and salinity, adaptable to environmental conditions, market needs, resistance to biotic and abiotic stresses, and good grain quality are mainly considered as the most crucial and important factors that influence breeding procedures; thus, these essential characters are desired to be obtained as much as possible in the breeding programs (Shih-Cheng and Loung Ping, 1980). However, these characters may not achieve in a single breeding period and need a long-term strategy.

Genetically modified crops (GM crops) refer to the plants in which their DNA has been changed through genetic engineering methods. In most cases, the DNA of the microorganism is transferred to plants to introduce a new character to the targeted plant which does not occur naturally in the species (Hails, 2000). On the other hand, mutation breeding is a genetic transformation process by which the genetic of a plant can be modified without the transformation of foreign DNA (Holme et al., 2019). Plants developed by mutagenesis also consider GM plants and their purpose is to bring variation and increase genetic diversity. Genetically modification techniques in both plants and animals have been approved for application in research activities. Currently, there are no commercial GM plants; thus, for food safety, GM plants and their products must be attested by a food safety authority (Holst-Jensen, 2009). In Japan, GM crops are now permitted as well as many countries did, including Vietnam, however several countries such as the United States have allowed the grown and consumption of the GM crops such as maize.

The purposes of rice breeding programs are not only to improve crop productivity but also to achieve good and preferred grain quality attributes through alterations in genetic and heredity using biotechnologies (Sharma and Singh, 2013). Diverse and tremendous progress and efforts have been conducted in rice breeding programs to obtain remarkable and novel achievements (Abacar et al., 2016). As a result, several new rice varieties have been developed and released with high yield potential and good grain quality performance. However, many years needed to develop and release a new variety and this situation lead the way more challengeable and difficult for breeders to respond to the demands and desires of rice consumers as quickly as possible (Tanaka et al., 2016). Therefore, it is apparently important to have several options to shorten and reduce the breeding period as well as respond easily and quickly.

There are numerous breeding approaches and strategies to develop and release new varieties of crops, particularly rice. The approaches are conducting to increase yield potential as well as to improve grain quality. Among the most implacable and important methods and approaches of breeding; pedigree, bulk selection, crossing, ideotype, doubled haploid, male sterility, hybridization, mutation, molecular marker, and genetic manipulation are to be mentioned (Breseghello and Coelho, 2013; Khan et al., 2015). Each of the mentioned above breeding methods has strengths and weaknesses. Therefore, breeders consider several aspects to enhance effectiveness and improve utilization of natural resources. Commonly, breeding programs take a long time (about 8-10 years or more) to develop and release a new variety. Recently, mutation breeding attracted the attention of rice breeders due to its efficiency, effectiveness, and time-saving properties (Wei et al., 2013).

2. Mutation Technology

Since the rice plant domestication at thousand years ago, mass selection was applied in the rice breeding program and has brought a significant change in its genetic variability (Jain, 2010). Releasing new adaptable and improved rice varieties becomes the main challenge for breeders, thus developing strategies to enhance the genetic variability has attracted the attention of several researchers. Several breeding approaches were conducted to achieve the goals, among the advanced breeding programs, understanding mutations and their applications have led the way for developing in the elucidation of a genetic, physiological, and biochemical base of rice traits. Variability creating by mutations has been grown and considered as the most important tools to improve rice productivity (Viana et al., 2019; Yu et al., 2002).

The mutation is an effective and impressive method that applies in many crops, particularly in rice. It plays a crucial and notable role in the development and release of desired rice varieties. The mutation may happen naturally due to sudden changes and alterations of base pairs in deoxyribonucleic acid (DNA) sequence of a chromosome (Sharma and Singh, 2013) or conduct artificially. These alterations and changes can be transferred from one generation to the coming generations; thus, the mutation is the preliminary source of genetic modifications and variations. As the main source of germplasm, genetic diversity is a natural source for rice breeding to meet current food demands (Reig-Valiente et al., 2016). The high level of genetic alterations and variations in a population lead the way for more valuable resources that are used to enlarge the genetic base in breeding programs (Nachimuthu et al., 2015). In contrast to the other breeding approaches such as transgenic breeding and recombinant breeding, mutation is currently the most effective approach and tool of sexual reproductive crops (Oladosu et al., 2016).

Mutation technology is considered as a practical and applicable approach. Its positive impacts such as reduction of cost and shortening of time are confirmed in several crop improvement studies; thus, it is a useful method to isolate and develop the expected variants and resistances for breeding programs (Oladosu et al., 2016). The principal and basic goal of mutation breeding are to develop and release new adaptable varieties with high yielding potential, better nutritional quality, and resistance to biotic and abiotic stresses (Shu et al., 2012). In recent years, a large number of mutant crop varieties have been successful released with acceptable results.

Considerable progress in rice improvement has been made by the induced mutation breeding approach; as a result, more than 821 mutant rice varieties have been officially released worldwide. Remarkably, approximately 84% of mutant rice varieties are developed and released from Asian countries, among them, China, Japan, and India are accounted as the top three countries of the world regarding mutant’s generation (Suprasanna et al., 2015). These mutant rice varieties have been reported as the main source of the rice crop and are cultivated in large areas which contributed to food security worldwide, particularly in Asian countries (Suprasanna et al., 2015).

As mentioned previously, mutation is the main source of genetic variability and modification. Mutation may occur naturally due to the sudden changes in the chromosome or artificially induced by mutagens (Oladosu et al., 2016; Wei et al., 2013). Induced mutation by physical or chemical agents has been reported as an impressive approach to effectively enhance food production in several crops including rice, sunflower, wheat, and many other field crops (Ahloowalia et al., 2004; Riaz and Gul., 2015).

Rice has been a major and popular target of mutagenesis due to its position in providing food for humanity and is the world's leading food crop with diploid inheritance and self-pollinated characteristics (Viana et al., 2019). The advantage of applying chemical agents in rice mutation breeding is the possibility to improve one or two desirable characters while avoiding undesirable changes (Jeng et al., 2011). There are three main agents for mutagenesis in rice including biological agents such as retrotransposons, transposons, and T-DNA; chemical agents such as nitrous acid, aminopurine, and alkylating agents and azides; and physical agents such as X-rays, ultraviolet light, and ionizing radiations (Serrat et al., 2014).

3. Type of Mutations

As mentioned, mutation can occur naturally or artificially. Artificial mutation conducts through physical, chemical, and biological agents, thus various types of mutagens are available to induce mutations in plants. These mutagens are classified by physical, chemical, and biological which are subgroups as follow (Krishnan et al., 2009):

1. Physical mutation is conducting through physical agents that are including of radiation such as X-rays, γ-rays, gamma rays, cosmic rays, and ultraviolet light. These rays ionize cell water and release hydroxyl (OH) free radicals. The released hydroxyl radical oxidizes the phosphodiester bond of DNA. Additionally, ultraviolet light causes the formation of thymine dimer and change DNA replication.
2. Chemical mutation is carried out by chemical agents including acridine orange, ethidium bromide, proflavin, nitrous acid, ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), hydrogen fluoride (HF), sodium azide (SA), *N*-methyl-*N*-nitrosourea (MNU), hydroxylamine (H_3NO) and nitrosamides. These chemical agents intercalate between two base pairs of DNA and alter the morphology and replication of DNA.
3. Biological mutation is induced by biological agents including transposons of DNA (T-DNA), insertion sequence, transposable elements, RNA insertion, mutator gene, and bacteriophage. These sequences move from one site of DNA to another site and cause alteration in the DNA strand which is known as the jumping gene.

The associated challenges or problems of the biological mutation particularly insertion mutants are to include a large number of additional mutations (Suzuki et al., 2008). Transformation of callus mostly induces several tissue cultures which produced point mutations and deletion mutations. Transgenic is another problem of T-DNA lines. Therefore, many countries severely restricted the cultivation and utilization of transgenic materials in the field and limited the utilization of these resources. In contrast, induced mutant lines developed through chemical mutagenesis are easy to generate and handle under field conditions. The chemical mutagens such as EMS, MMS, HF, SA, MNU, and H_3NO are the most frequently used chemicals in plants (Parry et al., 2009). Among them, MNU mutation widely applies in rice breeding programs.

4. MNU Mutation

Chemical mutagens especially MNU have been applied to lead genetic screens in several organisms (Guenet, 2004). MNU is a compound that induces single nucleotide alteration through alkylation of particular nucleotides, causing high density and randomly distributed mutations. Thus, a small population of individuals relatively can provide an allelic series that includes numerous modifications with diverse effects on genetic function, or splice site alterations that results in truncation of the gene product (Sega, 1984; Vogel and Natarajan, 1995). Several mutants induced with chemical agents have been carried out in mutation breeding program of plants and allocated for genetic studies. Among the chemical mutagens, MNU mutation showed biological influences on cells through the transformation of a methyl group to the oxygen and nitrogen atoms of the nucleotide bases (Xuan et al., 2019).

MNU mutation is one of the most applied mutagens in rice breeding programs (Kakar et al., 2019e). MNU mutation illustrates high reactivity in the DNA molecule with oxygen atoms and is a mono-functional alkylating agent that initiates with the addition of alkyl groups to nitrogenous bases, mainly guanine and cytosine. The generation of *O6*-alkylguanine induced by MNU treatment mismatches with thymine during DNA replication and leads the way for guanine/cytosine to adenine/thymine transitions (Satoh et al., 2010). MNU mutagens have been widely applied in several plants and the results showed that the approach affected a range of physiological processes in plants which lead the way for genetic variability.

Satoh et al. (2008) reported that the application of MNU mutation resulted to modify genes in rice crop, especially the PHO1 gene. This gene is considered to play an important role in the biosynthesis of starches through the formation of a functional protein-protein complex associated with carbohydrate metabolizing enzymes. The mutant rice lines produced by treating with MNU mutation showed an influence on the biosynthesis in rice endosperm, particularly the two components of starch including amylose and amylopectin. Findings using MNU also help to understand rice responses against biotic and abiotic stresses.

Additionally, MNU mutation leads the way for a better understanding of the embryogenic processes in rice. MNU mutation undoubtedly focuses on the most important agronomical parameters which are grain yield potential and grain quality attributes (Ikeda et al., 2005). Research found that rice grain oil content was influenced by MNU mutation, as mutant lines were observed to increase triacylglycerol content in rice grain (Sakata et al., 2016). Furthermore, antioxidant activity, phenolic, and flavonoid contents were also increased in rice grains with MNU treatment (Kim et al., 2018). The studies clearly illustrated the efficiency and potential application of MNU mutation in rice improvement and genomic programs. Another crucial and unique property of MNU mutation is that its action is highly associated with DNA replication (Satoh et al., 2010). Figure 1 shows the chemical structure of the MNU compound.

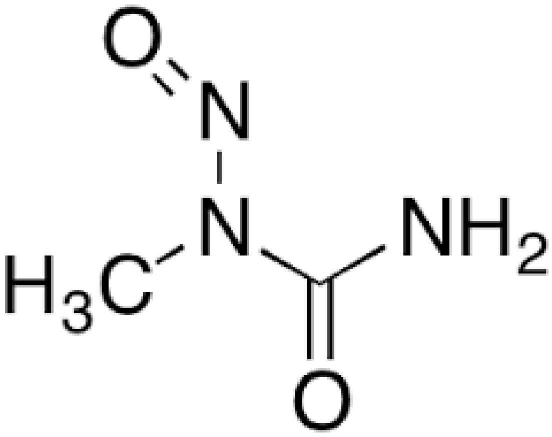


Figure 1. Chemical structure of MNU mutagenesis

4.1. MNU Mutation and Rice Productivity

Mutation technology can be applied to bring alterations in genetic organs and is used as a tool for genetic studies and variability. The analysis of mutant rice varieties through phenotype screening can pave the way for a better understanding of genotype and gene function (Lo et al., 2016). Induced mutations have been documented as an effective method to improve rice production. The rice varieties developed by mutations can assist to define a pathway or can directly use in agriculture (Sudianto et al., 2013). The main goal of mutations is to develop new rice varieties associated with high yielding potential, better nutritional quality, and resistance to biotic and abiotic stresses (Anh et al., 2019).

Previous studies have addressed the effects and significant role of mutants and pointed that induced mutation showed intensive impacts on rice production, especially in Asian countries (Maluszyski et al., 2001). Many types of induced mutation, particularly the MNU mutation focus on grain yield enhancement and grain quality improvement traits, which surely are the most important agronomic traits in rice production (Kakar et al., 2019e; Shehata et al., 2009). Many characters of rice crop have been improved through mutations, resulted in releasing varieties with high yield potential, acceptable grain quality, resistance to diseases, pests, and drought as well as early maturity character (Minn et al., 2008), dwarf type (Ananda, 2005), and being slightly aromatic (Greene et al., 2003; Sharma and Singh, 2013). Mutations showed influences on rice growth traits such as plant height, harvest index, 1000 grain weight, fertile tillers, leaf area index, and dry matter production (Babaei et al., 2010; Elayaraja et al., 2004).

Xuan et al. (2019) documented that MNU mutation generated a novel and useful maternal inheritance in rice on important agronomic characters including plant height, pests and diseases resistance, as well as grain physicochemical properties and suggested further elaboration and research on the subsequent generations ensure the stability of the maternal inheritance. In a study conducted by Anh et al. (2019), they observed that the average yield of rice varieties developed through MNU mutation was acceptable and was higher than their parents as well as expressed better phenotypes. The above results confirmed that rice varieties released by MNU mutation showed well adaptability and had better growth and yield performances than their parents. These phenotypic variations explained that MNU mutation created allelic alteration in the chromosome, thus these alleles might be able to transfer from parent to the coming generations and will enable the coming generation to become accustomed to the environmental factors (Anh et al., 2019).

Based on the previous studies and results, it is cleared that the application of mutation has been undertaken well but few experiments related to MNU mutation have been conducted on rice adaptation, growth, and yield performances. Therefore, researches are required to elucidate the effects of MNU mutation on rice growth and yield performances as well as the correlation of grain yield with its components, growth traits, physicochemical properties, and biological activities should be understood well to ensure food security and point out promising sources for rice breeding.

4.2. MNU Mutation and Rice Grain Quality

Grain quality is a complex character of rice that is highly influenced by its physical and chemical attributes, thus its improvement has become a very important consideration for most the breeders around the world. Rice grain quality comprises milling, appearance, cooking, eating, and nutritional quality (da Luz et al., 2020). Several aspects of rice grain quality traits have been improved by artificial mutations which have increased the level of acceptance among consumers as well as breeders. Many preferred and quality improved rice varieties have been released through physical and chemical mutagenesis in several countries (Ashida et al., 2009).

The application of different mutation methods provides encouraging results on rice grain quality worldwide, especially for Asian countries. Among the quality attributes which have been already improved through mutation breeding can be list as increase or decrease in amylose, protein, and lipid contents, improvement in starch resistance, and decline in the toxic elements and compounds such as arsenic, cadmium, and phytic acid which are anti-nutritional compounds (da Luz et al., 2020; Fitzgerald et al., 2009).

The utilization of mutations did not bring improvement in rice grain quality only for human consumption but also for industrial application. Important outcomes were achieved from mutation treatment on a rice cultivar (Suweon 542). This mutant rice cultivar with the potential application on rice flour production shows floury grains associated with small and less degraded starch particles (Mo et al., 2013). Phenolic and flavonoid constituents as well as antioxidant activity and biological activities in rice seeds were also increased with mutation breeding (Kim et al., 2018).

Recently, a study reported that rice grain oil content was influenced by the application of MNU mutation and mentioned that the content of triacylglycerol was increased in mutant lines (Sakata et al., 2016). The study clearly illustrates the effectiveness of MNU mutation on rice grain quality and pointed out its potential application in rice functional foods and genomic studies. Therefore, a comprehensive understanding of MNU mutation application on rice grain physicochemical properties, phytochemicals, and momilactone contents are required to produce quality and prefer rice grain as well as to increase natural product value of rice grain and its by-product in the pharmaceutical industries.

4.3. MNU Mutation and Rice Allelopathy

Allelopathy is a phenomenon, indicates the effect and interference of one plant on another by releasing chemical constituents via aboveground and underground pathways into the environment which are called allelochemicals (Weih et al., 2008). These effects might be either growth-promoting or inhibiting and are depending upon the released allelochemicals and targeted plants. Allelopathy plays an important role in many cropping systems, including multiple cropping, crop rotation, mixed cropping, cover cropping, and no-till cropping systems (Ben et al., 2001). The main goal of allelopathy application is to suppress weeds in agro-ecosystems as a natural means based on a friendly environmental approach without the help of synthetic herbicides and pesticides which resulting autotoxicity in crop production (Ma et al., 2014). Thus, it has attracted the consideration of researchers for a long time (Putnam and Weston, 1986).

Plants release thousands of compounds against pests and neighboring plants for self-defense (Fukuta et al., 2007). Rice allelopathy mostly depends upon allelochemicals especially the presence of phenolics, momilactone A, and momilactone B in rice plant (Toyomasu et al., 2008). Phenolics are the main source of plant allelochemicals in the ecosystem and have a key role in allelopathy. On the other hand, momilactones, as plant growth inhibitors were firstly isolated from rice husks (Kato et al., 1973). They suppress the growth of several weeds including barnyard grass (*Echinochloa crus-galli* L.) which is one of the most noxious weeds in rice fields (Kong, 2008; Rao et al., 2007). Studies reported that momilactone B showed significantly greater growth-inhibitory than momilactone A (Chung et al., 2005; Takahashi et al., 1976).

The application of mutations, particularly MNU mutation has been well studied on rice growth, yield potential, grain physicochemical properties but phytochemicals, momilactones, and other biological activities of rice grain and its by-products have been reported in very few articles. However, understanding the allelopathic potential of MNU mutation developed rice varieties and the correlation of allelopathy with improved rice varieties have not yet undertaken. Therefore, it is important to evaluate the effect of MNU mutation on the allelopathic potential of rice and to find out the relation of allelopathy with rice grain quality parameters.

5. Research Objectives

The main goal of these experiments was to utilize the efficacy of MNU mutation on the yield potential, physicochemical properties, biological activities, and allelopathic potential of rice grain and its by-products (straw and husk). The specific objectives to achieve the main goal are listed as below:

1. To evaluate the effects of MNU mutation on growth parameters, yield potential, and physicochemical properties of the rice plant.
2. To illustrate the relationship between grain yield with its components, growth parameters, and grain physicochemical properties.
3. To examine the utilization of MNU mutation on phytochemical contents and momilactone A and B of the rice grain, husk, and straw.
4. To find out the correlation between grain yield with phytochemical and momilactone contents of rice grain.

5. To investigate the allelopathic activities of mutant rice cultivars on the germination and growth performance of barnyard grass (*E. crus-galli*) and lettuce (*Lactuca sativa* L.).
6. To find out the relationship between allelopathic activity and grain quality of rice grain and its by-products.

6. Scientific Contribution of the Research

Based on the scientific consideration and responsibility, two scientific papers were published in impact factor journals that became the backbone of this dissertation which are as follows:

1. Kakar, K., Xuan, T.D., Quan, N.V., Wafa, I.K., Tran, H.D., Khanh, T.D., and Dat, T.D. 2019. Efficacy of *N*-methyl-*N*-nitrosourea (MNU) Mutation on Enhancing the Yield and Quality of Rice. Agriculture, 9(10), 212 (Chapter 2).
2. Kakar, K., Xuan, T.D., Quan, N.V., Wafa, I.K., Tran, H.D., Khanh, T.D., and Dat, T.D. 2019. Efficacy of *N*-methyl-*N*-nitrosourea Mutation on Physicochemical Properties, Phytochemicals, and Momilactones A and B in Rice. Sustainability, 11(23), 6862 (Chapter 3).

7. Structure of Thesis Dissertation

This thesis dissertation is prepared into five Chapters; each Chapter has its own materials and sections which are as below:

1. General Introduction (Chapter 1)
2. Efficacy of *N*-methyl-*N*-nitrosourea (MNU) Mutation on Enhancing the Yield and Grain Quality of Rice (Chapter 2)
3. Efficacy of *N*-methyl-*N*-nitrosourea Mutation on Physicochemical Properties, Phytochemicals, and Momilactones A and B in Rice (Chapter 3)
4. Evaluation of MNU Mutation on the Relation between Allelopathic Potential and Grain Quality of Rice Plant (Chapter 4)
5. General Discussion and Conclusion (Chapter 5)

Chapter 2

Efficacy of *N*-methyl-*N*-nitrosourea (MNU) Mutation on Enhancing the Yield and Grain Quality of Rice

Abstract

Rice production should enhance to meet the requirements of the increasing population worldwide. Several approaches and techniques including selection, genetic modification, hybridization, and mutation have been implemented to increase rice production to fulfill the increasing demands. Among the approaches, mutation technology is one of the appropriate methods to enhance rice production and improve grain quality aspects. It has been widely applied in recent decades to obtain desirable and novel characteristics that are not commonly found in nature. An experiment was carried out to investigate the influence of MNU mutation on the growth attributes, yield potential, and physicochemical properties of rice. Seeds of six rice cultivars (K1: DT84, K3: Q5, and K5: Cho dao), along with their mutated F2 generation (K2: mutated DT84, K4: mutated Q5, and K6: mutated Cho dao), were put in a backer with distal water for pre-germination. The pre-sprouted seeds were then sown in nursery boxes with commercial soil, and the established seedlings of 25-days-old were transplanted to an open field. Ten hills per plot were randomly selected to measure growth parameters, grain yield, and its components. Physicochemical properties, consisting of amylose, protein, and lipid contents, as well as taste score, were recorded by a grain quality tester device. The results revealed that plant length, tiller number, and panicle length were higher in mutated generations (mutant lines) than those of their cultivars. Furthermore, mutated generations took a longer time to reach the heading and maturity stage, except for K5 and K6 which were not statistically significant. The highest panicle number, spikelet number, ripened grain ratio, 1000 grain weight, 1000 brown rice weight, and grain yield were obtained in mutated generations, as compared to their original cultivars. The greatest grain yield was achieved in the K6 mutant line (11.9 t/ha), while the lowest was recorded in the K1 cultivar (7.7 t/ha). The lower contents of amylose, protein, and lipid were observed in mutant lines compared to those in their cultivars. The taste score, which was varied from 67.7 to 82.3, was recorded to be associated with the lower contents of amylose, protein, and lipid. The mutated generations showed longer grain length but shorter grain width compared to their original cultivars. Rice grains of K1 were sticky in contrast to other cultivars. Additionally, grain yield exhibited positive correlations with panicle number, spikelet number, ripened grain ratio, and 1000 grain weight. In addition, grain yield had negative relations with amylose, protein, and lipid contents but showed a positive correlation with taste score. This experiment highlights and suggests the importance of the MNU mutation method in terms of rice production, yield potential, and grain quality improvement.

1. Introduction

Rice is among the most leading cereal crops which provide the major source of calories and carbohydrates for more than half of the world's population (Kakar et al., 2019a; Wang et al., 2013). The increasing world's population has raised demands for agricultural production, particularly rice cultivation and production, especially in Asian countries where rice is consumed as a main part of the daily diet (Anh et al., 2019; Kakar et al., 2019a). To meet increasing demand, various methods and techniques of genetic manipulation have been applied to optimize rice production, such as mass selection, hybridization, mutation, and genetic modification (Breseghello and Coelho, 2013).

Mutation technology has been used to achieve novel attributes that are rarely observed in nature (Sikora et al., 2011). Several types of the mutation have been applied to increase crop productivity and food quality. The utilization of gamma rays has recently raised attention as a speedy method to improve the qualitative and quantitative properties of several crops (Bentota, 2006). El-Degwy (2013) stated that gamma irradiation increased heading time, plant length, and panicle number per plant, but it decreased total grain yield compared to those of non-mutagenic rice plants. Hence, the induced mutation is a promising tool for creating new genotypes, changing major genes, and governing the quantitative character (Shadakshari et al., 2001).

Among known methods of mutation, *N*-methyl-*N*-nitrosourea (MNU) mutation is a significant approach that effectively enhances and improves food production in many crops, especially in rice (Ahloowalia et al., 2004: Riaz and Gul, 2015). Recently, Xuan et al. (2018) documented that rice mutant lines developed by MNU mutation exhibited high yield, good grain quality, and were a potential source for breeding new rice cultivars. The application of mutant rice cultivars is spreading and becoming more prevalent in rice-producing countries. China has been increasingly using this technology for over 15 years (Fang et al., 2004). As a result, hundreds of new rice varieties have been released worldwide, especially by China, Japan, and India (Anh et al., 2019).

On the other hand, genotypes play crucial and dominant roles in crop production and improvement. Genotype can influence crop productivity in different aspects such as yield potentials, resistance to pests and diseases, and adaptation under various environmental conditions (Hussain et al., 2014). Along the period of civilization and green revelation, numerous rice varieties have been selected or released through different methods to increasing productivity (Singh et al., 2000). Manipulation of genetic resources is much important towards fulfilling the increasing demands of food for the escalating world's population. Rice varieties with high resistance rates against biotic and abiotic stresses by using modern and applicable technologies can enhance rice yields and improve grain quality to satisfy growers and consumers (Khush, 2005).

Each variety has its own morphological and physiological characteristics that contribute towards the growth, yield, and grain quality performances (Yang et al., 2007). Adequate fertilization, improved irrigation system, and management of pests and diseases are the key elements of yield enhancement. Proper and optimal utilization of these resources largely depends upon the genotype of a particular crop which has a decisive role towards the final production and quality. Genetic and environmental factors are the main sources of alteration in the genotypes which consequently influence the phenotype of a crop and can be visible through growth, yield, and grain quality characteristics (Alam et al., 2009). During the past several decades, huge numbers of rice varieties were released worldwide through mutation methods, of which, induced mutations have been the most implemented and frequently method for directly developed mutant varieties (Ahloowalia et al., 2004).

Overall, the developed mutant rice varieties do not have ideal growth performances or quality and are not as adaptable (Prasanna and Jain, 2017). Hence, more attention is required to evaluate the growth performance, yield potential, and physicochemical properties of mutant rice varieties based on growing regions to increase productivity and improve desired grain qualities. The screening process is an essential approach to find suitable and adaptable environmental conditions for the developed mutant lines (Lo et al., 2016). In the screening process, phenotypic evaluation is a crucial factor to determine the effectiveness of mutation technology. Very few studies have evaluated the utilization of the MNU mutation on rice crop (Anh et al., 2018 and 2019; Xuan et al., 2019). Thus, this research examines the effects of MNU mutation on the growth, yield, and physicochemical properties of rice.

2. Materials and Methods

2.1. Experimental Site and Design

This experiment was conducted in the experimental paddy field and the Laboratory of Plant Physiology and Biochemistry, the Graduate School for International Development and Cooperation affiliated to Hiroshima University, Higashi-Hiroshima City, Japan. It was carried out during the rice-growing season from May to October 2018 in irrigated conditions. The field cultivation was arranged in a randomized complete block design with three replications and six cultivars/mutant lines. Mutant lines (F2 generations) were the self-pollination F1 generation of the mutated K1, K3, and K5 original cultivars, which were obtained from the treatment of original cultivars with MNU mutation following a protocol described previously (Anh et al., 2018; Xuan et al., 2019). Briefly, seeds of the original cultivars were treated with 150 mM MNU for three hours, dried, and kept in hermetic conditions for three months. Afterward, the seeds were collected and stored at 5°C in the darkness for further application. The mutated F1 generation was self-pollinated to yield the mutated F2 population. The experimental field was puddled by a power tiller, leveled manually, and each plot was designed at 3 m^2. The details of rice cultivars and mutant lines including their origin, description, and status are illustrated in Table 1.

Table 1. Origin and description of the selected rice cultivars and mutant lines

Code	Origin	Descriptions	Status
K1	DT84	A traditional sticky rice with good quality in the north of Vietnam	Cultivar
K2	Mutated DT84	F2 (self-pollination from the mutated DT84 F1)	Mutant line
K3	Q5	A commercial rice cultivar with good quality in the north of Vietnam	Cultivar
K4	Mutated Q5	F2 (self-pollination from the mutated Q5 F1)	Mutant line
K5	Cho dao	A traditional rice with slim long grain and good scent in the south of Vietnam	Cultivar
K6	Mutated Cho dao	F2 (self-pollination from the mutated Cho dao F1)	Mutant line

Cultivars and mutant lines were subtype *Indica* and provided by Khai Xuan International Co. Ltd., and Agricultural Genetics Institute, Hanoi, Vietnam

2.2. Plant Materials and Seedling Management

Perfect seeds of the selected cultivars and mutant lines were soaked in distilled water and managed in a growth chamber (Biotron NC system, Nippon Medical & Chemical Instrument, Co. Ltd., Osaka, Japan) for 48 hours at 30 °C with a photoperiod of 14 hours day and 10 hours night. The pre-germinated seeds were sown in nursery boxes (30 × 60 cm) in commercial soil (N.P.K. contents at 0.8, 1.0, and 1.3 g/kg, JA-ZEN CHU Co., Hiroshima, Japan). The nursery boxes were placed and managed in a greenhouse for 25 days. The seedlings (25-days-old) with 3.5 plant age in leaf number were transplanted to the prepared rice field. The distances between plants and rows were 15 cm and 20 cm, respectively. Every hill contained one seedling. Weeds were manually controlled at maximum tillering and heading stages by hands. Standard fertilizer (N.P.K; 14-10-13) at 130 g per plot was applied at early tillering and panicle initiation stages. Water was kept normally based on environmental conditions and plant requirements. Plants were harvested at the maturity stage and the required data and samples were recorded or collected at the field and laboratory. Figure 2 shows the general platform of seedling management from germination to harvesting stage.

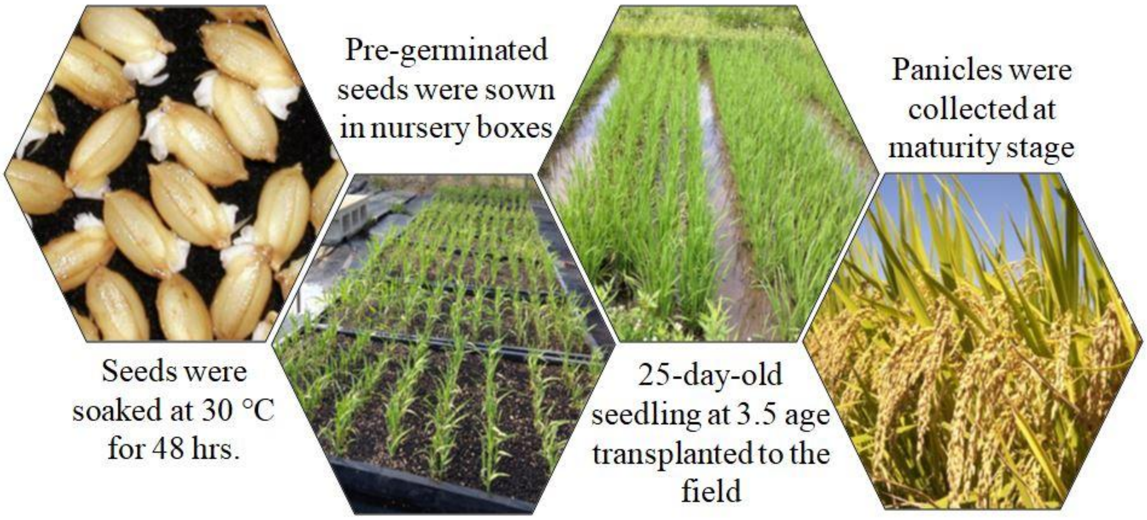


Figure 2. Description of nursery establishment and field management

2.3. Measurements of Growth and Yield Attributes

Ten hills per plot were randomly chosen to evaluate the growth parameters as well as yield and its components. Growth parameters consisted of plant length, tiller number, days to heading and maturity, and panicle length were recorded at the maturity stage in the field. Plant length was measured from the surface of the soil to the tip of the flag leaf with a ruler. Tillers were counted by hand, and panicle length was measured by a sample ruler. Panicles were counted in the field, and spikelet numbers were summed per each panicle. Collected grains of each plot were threshed by hands and dried at room temperature to obtain 18% moisture content. The ripened grain ratio was calculated based on the formula shown below after the separation of filled and unfilled grains by salt solution (NaCl in the water at a degree of 1.06 hydrometer). The 1000 grain weight was recorded in triplicate with a digital balance. The grain yield was determined with the below formula. The grains were then dehusked using an automatic rice husker machine (model TR-250, Kett Electric Laboratory, Tokyo, Japan), and the brown rice grains were used for further procedures.

$$\text{Ripened grain ratio (\%)} = (\text{filled grains}/\text{total grains}) \times 100$$

$$\text{Grain yield (g/hill)} = \text{Panicle number per hill} \times \text{Spikelet number per panicle} \\ \times \text{Ripened grain ratio} \times \text{1000 grain weight},$$

2.4. Measurements of Physicochemical Properties

Physicochemical properties, including protein, amylose, and lipid contents, as well as taste score were measured by a quality tester machine (PGC Shizuoka Seiki PS-500 machine, version 2-12, Shizuoka Seiki Co. Ltd., Shizuoka, Japan) using 100 g brown rice grains with three replications. Ten perfect brown rice grains were selected from each cultivar and mutant line to record grain length, grain width, and grain length-to-width ratio using a vernier caliper as well as to observe their appearance.

2.5. Statistical Analysis

Data were analyzed by Minitab 16.0 statistical software (Minitab Inc. State College, PA, USA). T-test was conducted to express the differences between cultivars and their mutant lines, followed by Tukey's multi-comparison test. Significant differences were defined at $p<0.05$ probability level. Pearson correlation was conducted to indicate the relationship between grain yield with growth parameters and physicochemical properties.

3. Results

3.1. Growth Attributes

Growth parameters in terms of plant length, tiller number per hill, panicle length, and days to heading and maturity are summarized in Table 2. Plant length of mutant lines was not significantly higher than those of the original cultivars except for K6 which was significantly higher than its origin cultivar K5. The tiller number per hill was also significantly higher in the mutant lines; however, the difference was not significant between K3 and K4. Additionally, the panicle length was significantly higher in both mutant lines K2 (26.4 cm) and K4 (29.2 cm) in comparison with their origins K1 (23.1 cm) and K3 (26.3 cm), respectively, while it was not significantly different between K5 (26.1 cm) and K6 (26.9 cm). Days to heading and maturity were significantly different ($p<0.05$) between mutant lines (K2 and K4) and their origin cultivars (K1 and K3); it means that mutant lines took a longer time to reach the heading and maturity stages. However, the variation was not significantly different between K5 and K6. Plant length and tiller number per hill ranged from 108.5 to 117.7 cm and 11.1 to 13.7, respectively, with the highest value in K6 for plant length and K2 for tiller number. Panicle length was within the interval of 23.1 to 29.2 cm, and the longest one was obtained in K4. Based on the heading and maturity behaviors, K4 and K3 were the late and early maturity varieties, respectively (Table 2).

Table 2. Description of growth parameters between cultivars and mutant lines

Code	Plant length (cm)	Tiller number per hill	Days to heading	Days to maturity	Panicle length (cm)
K1	113.4 ± 3.6 a	12.1 ± 0.4 b	106.3 ± 0.6 b	136.7 ± 1.1 b	23.1 ± 0.5 b
K2	114.1 ± 5.6 a	13.7 ± 0.4 a	107.6 ± 0.7 a	138.2 ± 0.7 a	26.4 ± 0.3 a
K3	108.5 ± 3.1 a	11.1 ± 1.1 a	104.0 ± 2.6 b	132.7 ± 2.3 b	26.3 ± 0.2 b
K4	109.5 ± 2.2 a	12.5 ± 1.3 a	122.3 ± 1.1 a	151.0 ± 1.0 a	29.2 ± 0.6 a
K5	115.5 ± 0.4 b	12.7 ± 0.1 b	106.7 ± 0.9 a	138.7 ± 1.2 a	26.1 ± 0.6 a
K6	117.7 ± 0.3 a	13.6 ± 0.4 a	107.0 ± 1.9 a	138.3 ± 1.7 a	26.9 ± 0.3 a

Values are presented as means ± standard deviations. Different letters within a column indicate significant differences at $p<0.05$ probability level between the original cultivar and its mutant line

3.2. Grain Yield and Its Components

Grain yield and its components are illustrated in Table 3. There were significant differences ($p<0.05$) in panicle number per hill, spikelet number per panicle, 1000 grain weight, 1000 brown rice grain weight, 1000 husk weight, and grain yield among mutant lines and their origin cultivars. Panicle number per hill significantly differed between mutant lines and their cultivars. The highest panicle number per hill was recorded in K6 and the lowest was in K3. Spikelet number per panicle was higher in K4 and lower in K1, while the difference was not significantly different between K5 and K6. The ripened grain ratio was not statistically different among tested varieties. The 1000 grain weight and 1000 brown rice grain weight were greater in mutant lines than in their origin cultivars, while these differences were not significant between K5 and K6. Furthermore, 1000 husk weight was decreased in the mutant lines and was increased in the origin cultivars, however, this statistical difference was observed only between K1 and K2. Additionally, greater grain yield was obtained in the mutant lines rather than in their origin cultivars (Table 3).

Panicle number per hill, spikelet number per panicle, and ripened grain ratio, respectively, ranged from 9.3 to 12.5, 127.7 to 148.6, and 81.0% to 90.0%. The 1000 grain weight, 1000 brown rice grain weight, and 1000 husk weight were ranged from 22.0 to 24.1 g, 16.9 to 21.3 g, and 2.6 to 5.5 g, respectively. The greater grain yield (11.9 t/ha) was obtained in the mutant line K6, following by K4 (11.6 t/ha), K2 (10.8 t/ha), K5 (9.2 t/ha), K3 (7.9 t/ha), and the lowest grain yield (7.7 t/ha) was recorded in K1 which is a cultivar (Table 3).

Table 3. Description of grain yield and its components between cultivars and mutant lines

Code	Panicle number per hill	Spikelet number per panicle	Ripened grain ratio (%)	1000 grain weight (g)	1000 brown rice grain weight (g)	1000 husk weight (g)	Grain yield (t/ha)
K1	9.7 ± 0.4 b	127.7 ± 8.8 b	83.4 ± 5.6 a	22.5 ± 0.1 b	19.2 ± 0.3 b	3.3 ± 0.1 a	7.7 ± 0.5 b
K2	10.6 ± 0.1 a	142.0 ± 6.1 a	90.0 ± 1.5 a	23.9 ± 0.4 a	21.3 ± 0.2 a	2.6 ± 0.2 b	10.8 ± 0.2 a
K3	9.3 ± 0.9 b	135.3 ± 5.2 b	86.2 ± 1.2 a	22.0 ± 0.6 b	18.0 ± 0.4 b	4.0 ± 1.0 a	7.9 ± 0.6 b
K4	11.1 ± 1.3 a	148.6 ± 3.1 a	87.3 ± 3.6 a	24.1 ± 0.4 a	21.1 ± 0.3 a	3.0 ± 0.5 a	11.6 ± 0.2 a
K5	10.4 ± 0.2 b	145.6 ± 2.9 a	81.0 ± 3.1 a	22.5 ± 0.4 a	16.9 ± 0.4 a	5.5 ± 0.8 a	9.2 ± 0.4 b
K6	12.5 ± 0.3 a	146.7 ± 0.7 a	85.5 ± 1.3 a	22.8 ± 0.1 a	17.1 ± 0.3 a	5.1 ± 0.8 a	11.9 ± 0.4 a

Values are presented as means ± standard deviations. Different letters within a column indicate significant differences at $p<0.05$ probability level between mutant line and its origin cultivar

3.3. Physicochemical Properties and Appearance

Physicochemical properties, including amylose, protein, and lipid contents as well as taste score, grain length, grain width, and grain length to width ratio are presented in Table 4. A significant difference ($p<0.05$) between the origin cultivars and mutant lines was observed in terms of amylose content which was higher in the origin cultivars than their mutant lines. Protein content was lower in the mutant lines; however, the difference was not statistically significant. Lipid content was also lower in the mutant lines but the statistical difference was only observed between K5 and K6. Taste score, grain length, and grain length to width ratio significantly differed between the mutant lines and origin cultivars; however, grain width was not significantly different. Higher taste score, grain length, and grain length to width ratio were recorded in the mutant lines (Table 4).

Amylose, protein, and lipid contents ranged from 21.4 to 23.3%, 5.4 to 7.0%, and 7.0 to 10.7%, respectively. The lower amylose, protein, and lipid contents were recorded in the mutant lines rather than in their cultivars, but the protein and lipid contents were not statistically significant, except for the lipid content of K5 and K6. Taste score extended from 67.7 to 82.3 (as reference), which was enhanced with the decrease in amylose, protein, and lipid contents. Higher and lower amylose was observed in K1 and K4, while higher and lower protein content was recorded in K1 and K6. Additionally, K1 (an origin cultivar) showed the highest lipid content which was 10.7%, while K6 (a mutant line) exhibited the lowest lipid content at 7.0% (Table 4).

Grain length, grain width, and grain length to width ratio ranged from 5.8 to 7.1 mm, 2.1 to 2.5 mm, and 2.5 to 3.3 mm, respectively. Mutant line (K4) displayed an increase in grain length as compared to its cultivar (K3). Furthermore, K4 was recorded as the longest grain producer variety compared to other cultivars and mutant lines; however, K1 was observed as the thickest grain variety. Figure 3 shows the physical appearance of the cultivars and mutant lines. Rice grains produced by K1 were white in color and recorded to be a much sticky rice grain producer cultivar which was changed to a brown and non-sticky rice grain cultivar (K2) after treating with MNU compound, while the others were brown in color (Figure 3).

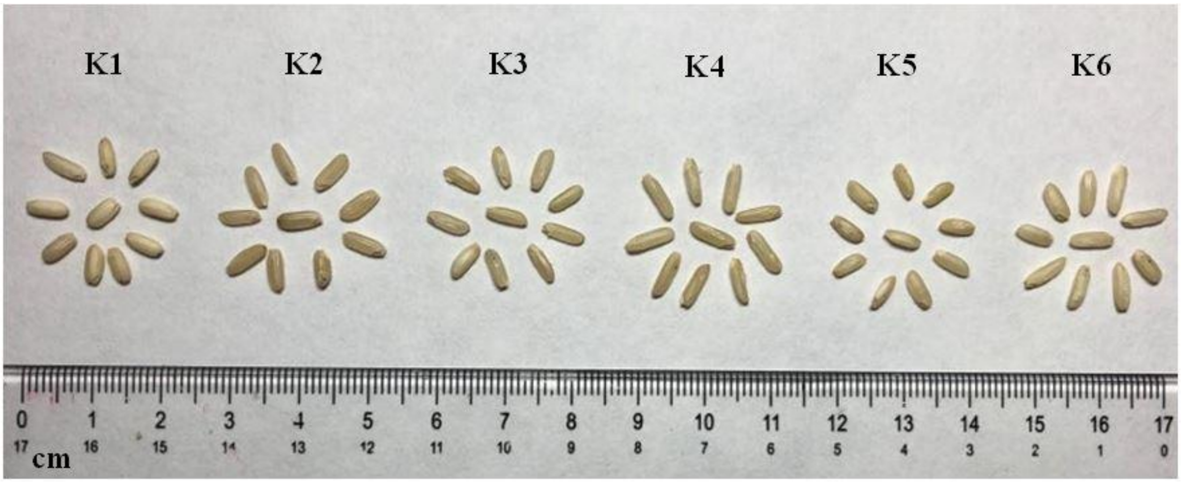


Figure 3. The appearance quality of cultivars and mutant lines

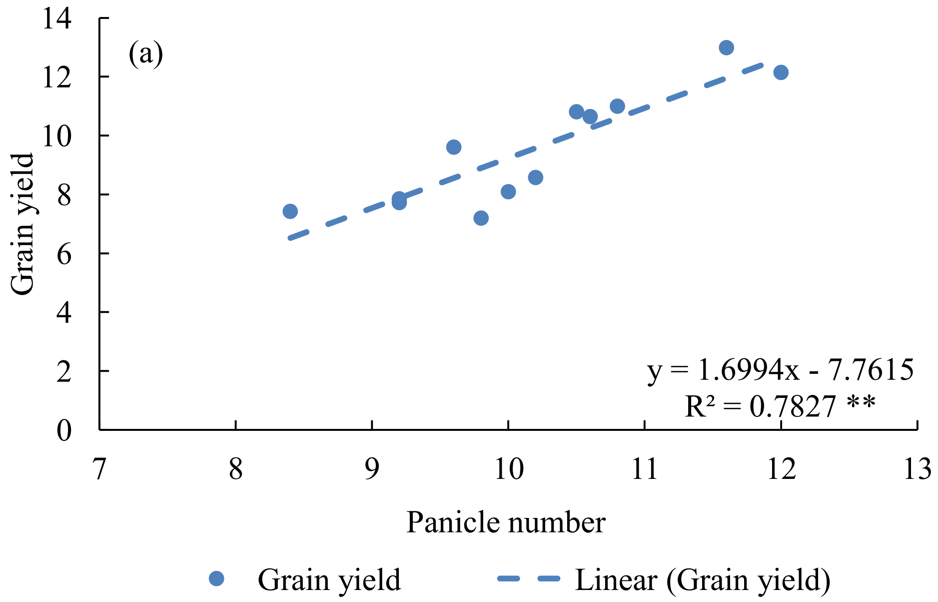
Table 4. Description of physicochemical properties of rice grain between cultivars and mutant lines

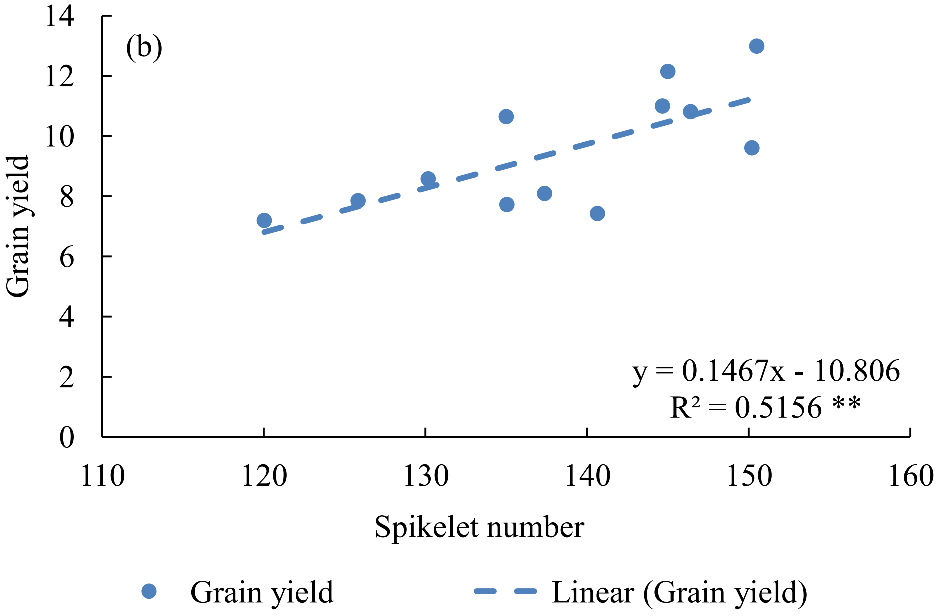
Code	Amylose (%)	Protein (%)	Lipid (%)	Taste score (as reference)	Grain length (mm)	Grain width (mm)	Grain length to width ratio
K1	23.2 ± 0.1 a	7.0 ± 0.4 a	10.7 ± 0.6 a	68.0 ± 0.0 b	6.2 ± 0.3 a	2.5 ± 0.1 a	2.5 ± 0.0 a
K2	22.5 ± 0.1 b	6.6 ± 0.4 a	10.3 ± 0.6 a	73.7 ± 3.0 a	6.3 ± 0.1 a	2.4 ± 0.1 a	2.6 ± 0.1 a
K3	23.3 ± 0.0 a	6.9 ± 0.1 a	8.3 ± 0.6 a	67.7 ± 1.1 b	6.2 ± 0.1 b	2.2 ± 0.2 a	2.8 ± 0.4 b
K4	21.4 ± 0.4 b	6.6 ± 0.3 a	7.7 ± 1.1 a	70.0 ± 1.0 a	7.1 ± 0.4 a	2.1 ± 0.0 a	3.3 ± 0.2 a
K5	23.0 ± 0.1 a	5.8 ± 0.3 a	9.3 ± 0.5 a	76.7 ± 0.4 b	5.8 ± 0.1 b	2.2 ± 0.1 a	2.6 ± 0.2 b
K6	22.4 ± 0.1 b	5.4 ± 0.2 a	7. 0 ± 0.1 b	82.3 ± 1.2 a	6.3 ± 0.2 a	2.1 ± 0.0 a	3.0 ± 0.1 a

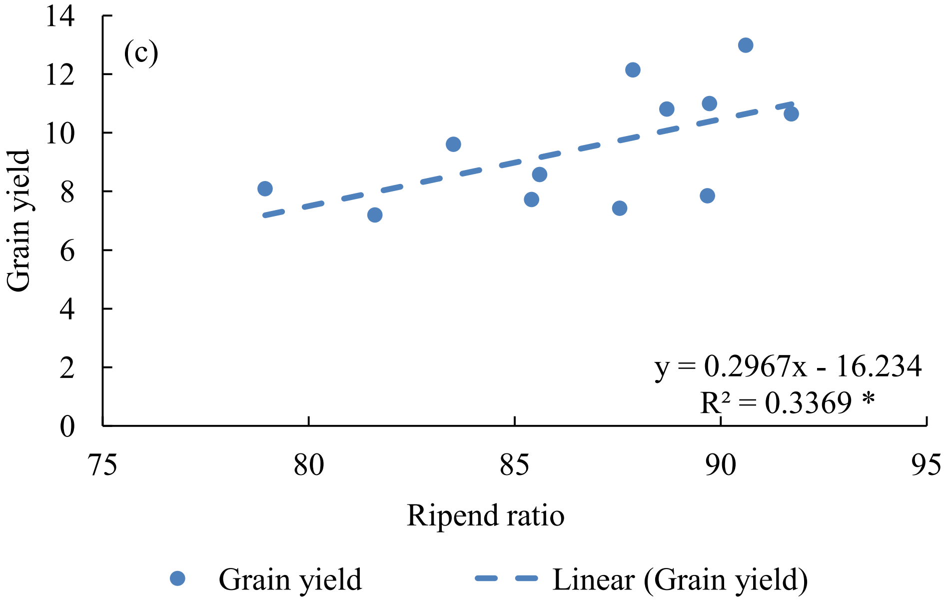
Values are presented as means ± standard deviations. Different letters within a column indicate significant differences at $p<0.05$ probability level between mutant line and its origin cultivar

3.4. Relationship of Grain Yield with Other Parameters

The correlation coefficient of grain yield with growth parameters, yield components, and physicochemical properties are illustrated in Table 5. There were significantly positive correlations among grain yield with tiller number per hill, panicle length, panicle number per hill, spikelet number per panicle, ripened grain ratio, 1000 grain weight, and taste score. However, a significant negative correlation was found between grain yield and amylose content. Furthermore, grain yield did not show any significant correlation with protein content, lipid content, grain length, and grain width in these cultivars and mutant lines. In addition, tiller number showed a positive relationship with panicle length, ripened grain ratio, 1000 grain weight, grain yield, and taste score; however, it did not exhibit correlation with the remaining parameters. Amylose content illustrated a negative correlation with panicle length, panicle number per hill, spikelet number per panicle, 1000 grain weight, grain yield, and grain length. However, it did not show any relationship with the remaining parameters. The correlation between grain yield with its components (panicle number, spikelet number, ripened grain ratio, and 1000 grain weight) is demonstrated in Figure 4. The results show that there was a linear and positive relationship between grain yield and its components ($r = 0.885$, $p = 0.001$; $r = 0.718$, $p = 0.009$; $r = 0.580$, $p = 0.048$; and $r = 0.890$, $p = 0.001$), respectively. The results of correlation between grain yield with amylose content and taste score also exhibited a linear relation ($r = -0.836$, $p = 0.001$; and $r = 0.641$, $p = 0.025$) but a nonlinear correlation with protein and lipid contents ($r = -0.381$, $p = 0.222$; and $r = -0.170$, $p = 0.597$) which are presented in Figure 5.




(b)
Grain yield
14
12
10
8
6
4
2
0
110
120
130
140
150
160
Spikelet number
y = 0.1467x - 10.806
R² = 0.5156 **
Grain yield
Linear (Grain yield)


(c)
Grain yield
14
12
10
8
6
4
2
0
75
80
85
90
95
Ripend ratio
y = 0.2967x - 16.234
R² = 0.3369 *
Grain yield
Linear (Grain yield)

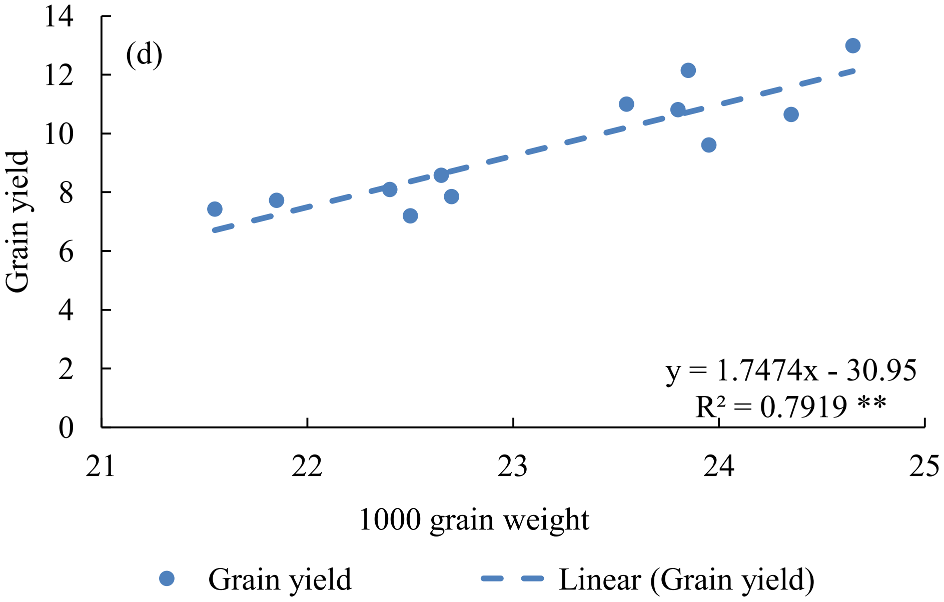
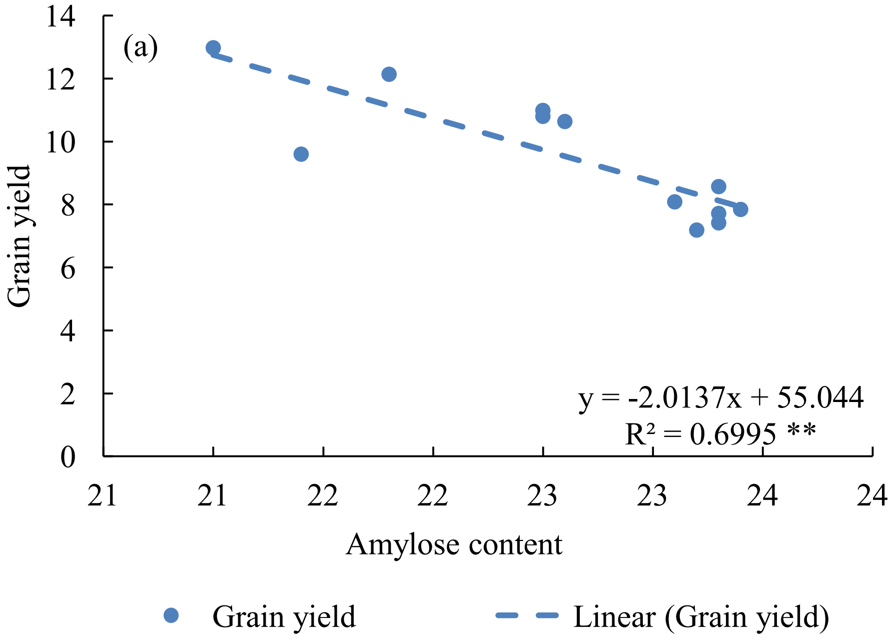
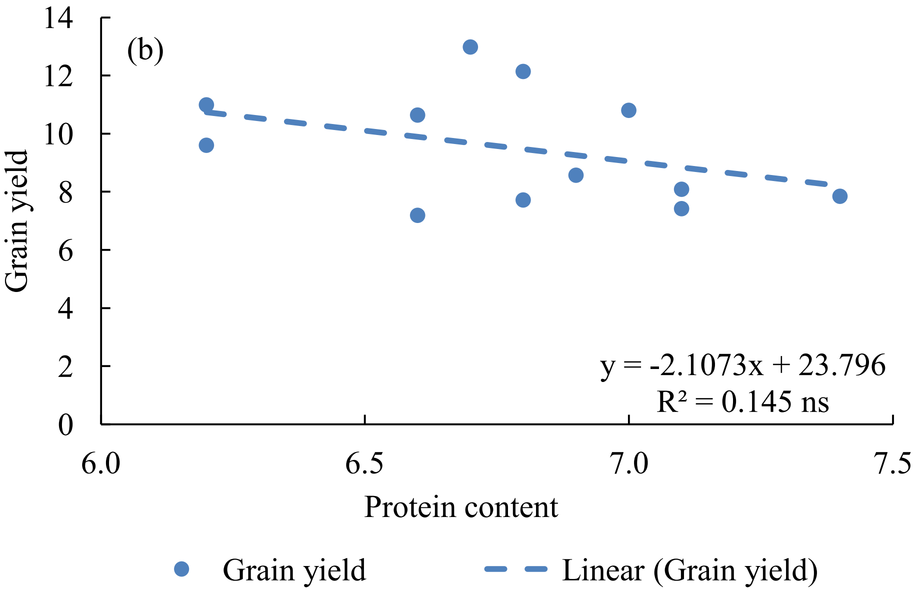
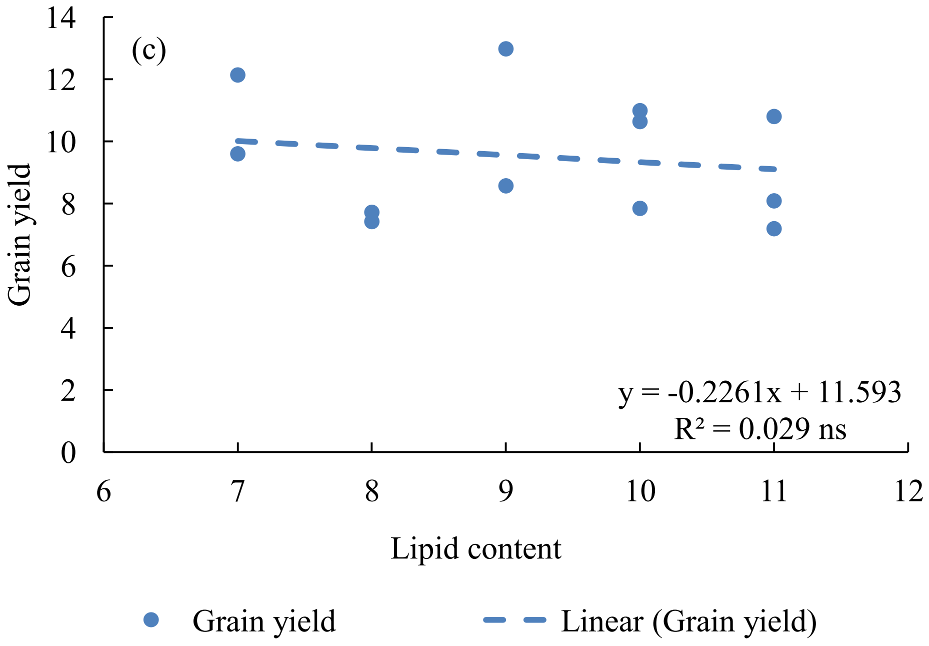


Figure 4. The correlation of grain yield with panicle number, spikelet number, ripened ratio, and 1000 grain weight. (a), Panicle number; (b), Spikelet number; (c), Ripened ratio; (d), 1000 grain weight. * and ** indicate significant differences at p<0.05 and p<0.01, respectively




14
12
10
8
6
4
2
0
(b)
Grain yield
y = -2.1073x + 23.796
R² = 0.145 ns
6.0
6.5
7.0
7.5
Protein content
Grain yield
Linear (Grain yield)


14
12
10
8
6
4
2
0
(c)
Grain yield
y = -0.2261x + 11.593
R² = 0.029 ns
6
7
8
9
10
11
12
Lipid content
Grain yield
Linear (Grain yield)

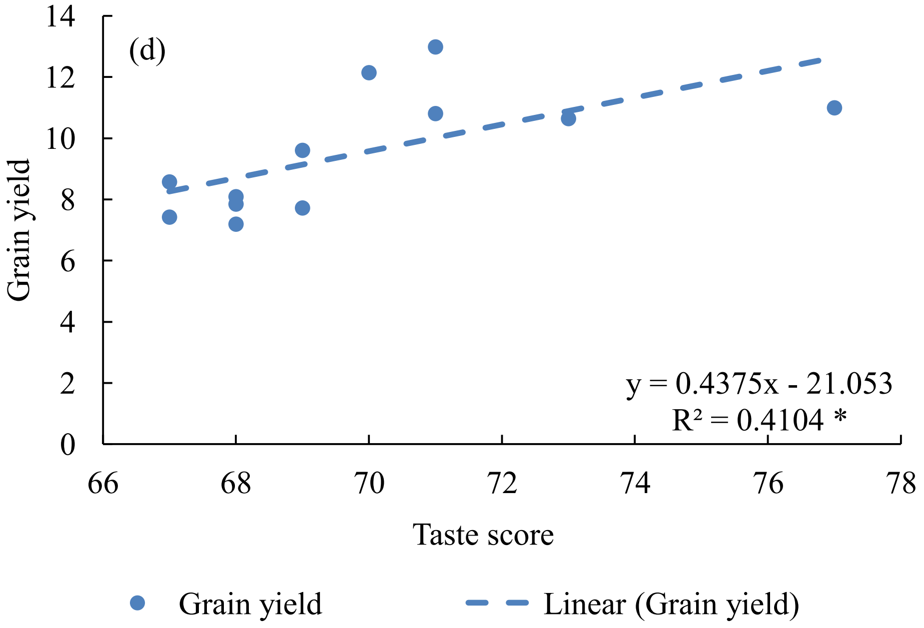


Figure 5. The correlation of grain yield with amylose, protein, and lipid contents, and taste score. (a) Amylose content; (b) protein content; (c) lipid content; and (d) taste score. * and ** indicate significant differences at $p<0.05$ and $p<0.01$, respectively. ns means not significant

Table 5. The correlation coefficient of grain yield with growth parameters, yield components, and physicochemical properties between cultivars and mutant lines

	TN	**PL**	**PN**	**SN**	**RR**	**GW**	**GY**	**AC**	**PC**	**LC**	**TS**	**GL**
PL	0.047											
PN	0.726 **	0.437										
SN	0.178	0.810 ***	0.422									
RR	0.559 *	0.306	0.328	0.280								
GW	0.658 *	0.574 *	0.779 **	0.554	0.500							
GY	0.685 **	0.671 **	0.885 ***	0.718 **	0.580 *	0.890 ***						
AC	−0.330	−0.807 ***	−0.664 **	−0.786 **	−0.274	−0.833 ***	−0.836 ***					
PC	−0.242	−0.553 *	−0.320	−0.373	−0.018	−0.476	−0.381	0.534				
LC	0.322	−0.734 **	−0.004	−0.433	−0.101	−0.027	−0.170	0.393	0.232			
TS	0.803 **	0.260	0.528	0.415	0.549	0.597 *	0.641 *	−0.379	−0.595 *	0.180		
GL	−0.003	0.707 **	0.360	0.587 *	0.115	0.520	0.497	−0.723 **	−0.294	−0.693 **	0.013	
GWD	0.357	−0.686 **	−0.282	−0.307	−0.013	−0.256	−0.302	0.444	0.195	0.574 *	0.298	−0.478

*, **, and *** indicate significant differences at $p<0.05$, $p<0.01$, and $p<0.001$ probability levels. TN (tiller number), PL (panicle length), PN (panicle number), SN (spikelet number), RR (ripened ratio), GW (1000 grain weight), GY (grain yield), AC (amylose content), PC (protein content), LC (lipid content), TS (taste score), GL (grain length), and GWD (grain width)

4. Discussion

Genotypic properties play a dominant role in crop production. Manipulation of genetic sources is an option toward meeting rising food demand (Hussain et al., 2014). Mutation technology modifies the genotypic mechanism of plants through the alteration of selected genes (Xuan et al., 2019). Such changes cause variation in phenotypic performances and productivity of crops (Anh et al., 2018; Xuan et al., 2019). Variations in rice grain yield and its quality are largely depending upon genetic factors and environmental conditions (Singh et al., 2003). In this study, MNU mutation showed a wide variation in the growth, yield, and physicochemical properties of rice.

Growth attributes, including plant length, tiller number, and panicle length, were varied among cultivars and mutant lines. Plant length largely depends on the length of internodes (Ashrafuzzaman et al., 2009) and the application of nitrogenous fertilizer (Sultana and Ali, 2014), which varies based on genetic differences between varieties and environmental conditions (Kanegana and Kargbo, 2011). On the other hand, increased plant length is often associated with stress responses such as light and shading (Feng et al., 2019); therefore, further observations on the expressions of genes and gene clusters in the determination of the plant length should be conducted. Simultaneously, experiments with other environmental stresses should be carried out to confirm responsive levels between mutant lines and the corresponding cultivars. Besides, tiller number is the most important factor for panicle formation and development in rice plants (Badshah et al., 2014) and is a major determinant of grain yield (Champagne, 2004). Laza et al. (2004) reported that rice varieties with long and intermediate panicle sizes produced greater grain yields than the varieties with small panicles. Thus, mutant lines produced high yields compared to the original cultivars.

An increase or decrease in the vegetative period of rice plants can affect yield and its components. Kasim et al. (2018) found that mutations can reduce plant age by shortening the plant heading and maturity times. On the contrary, the present study showed that mutant lines K2 and K4 matured later than the original cultivars K1 and K3, whereas there was no change between K5 and K6. The results suggested that the mutation by MNU might elongate the maturity period of normal rice rather than sticky rice, but grain yields of both rice types increased. Previous studies also reported that late-maturity varieties contribute to higher biomass production and accumulation of photosynthates (Xuan et al., 2019). As vegetative growth of mutant lines was favorable and had extensive root visibility, this situation might lead mutant lines to take advantage of soil nutrients and applied fertilizers, consequently postponing heading and maturity stages, as reported by Ali et al. (2015), who stated that excessive nutrients tend to delay heading and maturity stages. On the other hand, shortened vegetative growth in the selected cultivars may lead to decrease spikelet number per panicle, ripened grain ratio, 1000 grain weight, and grain yield. Similar results were reported by Vergara et al. (1964). These influences might be determined by the genetic backgrounds of varieties and their responses to various environmental conditions.

Yield components are the main factors affecting grain yield (Abacar et al., 2016). Duan et al. (2015) stated that panicle number, spikelet number, ripened grain ratio, and 1000 grain weight are the most important traits and key enhancers of yield. In the present study, panicle number per hill, spikelet number per panicle, ripened grain ratio, and 1000 grain weight were higher in mutant lines, which consequently resulted in increased grain yield. These results are in line with previous studies (Duan et al., 2015). Anh et al. (2019) explained that full-grain weight is the key decider for grain yield; however, the results of this study showed that all the yield components were responsible for grain yield production in both cultivars and mutant lines. Grain yield has a linear correlation with its components (Abacar et al., 2016). I also found a linear correlation between yield and its components, which is in line with the previous study (Abacar et al., 2016; Duan et al., 2015).

Rice grain quality is a complex trait and determined by several physical, chemical, and nutritional characteristics; thus, a specific consumer class might have special preferences (Kakar et al., 2019b; Noori et al., 2018). Rice grain physical quality traits encompass appearance, translucence, grain size, shape, whiteness, chalkiness, and cooking properties. The importance of each trait is differed according to the cultural differences and local cuisine. The mentioned above traits, as well as chemical properties, are major elements influencing market value (Champagne et al., 1999). The appearance, size, and shape quality of milled rice is an important aspect to the consumers, producers, and millers. Thus, they are the main considerable parameters in rice breeding programs. Based on the grain shape classification by Jennings et al. (1979), two types of grain were observed in this study. The K4, a mutant line with slender grain producer properties, and K1, K2, K3, K5, and K6 medium shape grain producer cultivars and mutant lines.

Rice grain appearance, in terms of cooking and eating quality, mainly depends upon cultural differences and consumer preferences (Noori et al., 2018; Qiao et al., 2011). Physical traits including grain length, grain width, and grain length to width ratio are considerable elements of appearance (Anh et al., 2019; Kakar et al., 2019e). Noori et al. (2017) reported that grain length and width mostly depend on the genotype of a variety. In this study, the MNU mutation increased the grain length. It is reported that the grain appearance quality is associated with grain nutritional quality (Anh et al., 2019; Xu et al., 2004). Table 6 gives comprehensive and useful information regarding the classification of grain length and shape for milled rice.

Table 6. Classification of grain length and shape of milled rice

Category	Grain length (mm)	Grain shape	Grain length to width ratio
Extra-long	More than 7.50	Slender	More than 3.01
Long	6.61 to 7.50	Medium	2.1 to 3.0
Medium	5.51 to 6.60	Bold	1.1 to 2.0
Short	5.0 to 5.50	Round	Less than 1.1
Extra short	Less than 5.0	-	-

Source: Ali et al., 2014; Jennings et al., 1979

Furthermore, standards and preference for the evaluation of grain length and shape of rice grain may enormously vary among countries and market requirements. Short and medium grain is almost preferable for Japonica rice consumers, while consumers from Asian countries mostly accept rice grain with a medium to long or some extra-long grain size. In the American continent, the long or extra-long grain is mainly preferred for the Japonica rice varieties grown areas where low temperature requires. The grain length is more important and variable than grain width, thickness, and shape; although a slender to medium rice grain width usually has the highest demand in markets (Cuevas et al., 2016; Nadvornikova et al., 2018).

On the other hand, chemical properties of rice grain including amylose, protein, and lipid contents are the main elements of the physicochemical quality of rice grain and should be considered as a crucial factor in rice breeding strategies (Kakar et al., 2019b and 2019c; Kaur et al., 2016). These quality traits are largely affected by genotypic and environmental conditions (Noori et al., 2018). The results of this research indicated that MNU mutation decreased the percentage of amylose content in the rice grain. Low amylose content in rice grain increases softness and stickiness, while higher amounts cause rice grain to become hard and easily breakable during the grain milling process (Kakar et al., 2019b and 2019c; Juliano and Villareal, 1993). It has been shown that high amylose, protein, and lipid contents decrease grain taste score; thus, the selected cultivars exhibited lower taste scores, and mutant lines showed a higher taste score. Protein content did not differ significantly between origin cultivars and mutant lines; however, lipid content was significantly varied between K5 and K6 but did not differ in other populations. It is reported that protein and lipid contents are major indicators of the nutritional value of rice grain (Abacar et al., 2016; Singh et al., 2003). Zhou et al. (2002) mentioned that rice grains with low amylose and protein contents are preferred in Japanese markets, while vice-versa in the tropical Asian countries.

Amylose is the most important and crucial predictor of rice grain quality in terms of cooking, eating, and sensory quality. Philpot et al. (2006) stated that rice grain is categorized in different groups according to the content of amylose which is waxy grain (0–2%), very low amylose grain (3–9%), low amylose grain (10–19%), intermediate amylose grain (20–25%), and high amylose grain (more than 25%). In addition, protein content in rice grain is divided into two types consists of functional protein (~10%) and seed storage protein (~90%). The seed storage protein is further classified into four groups based on the solubility-linked physical properties which are albumins, globulins, prolamins, and glutelins. Among them, glutelin is considered to be the most abundant once, which accounts for 60–80% of the total seed storage protein (Kusaba et al., 2003; Saito et al., 2012).

Rice farmers and producers are looking for high yield and improved grain quality, while consumers pay close attention to desirable grain quality attributes and nutritional value. Rice consumer prefers grain with a clear endosperm and good appearance as well as grain length based on their interest. All rice grain production parameters including growth attributes, yield potential, and grain physicochemical properties are important factors due to their influence on farmer's income, market requirement, grain milling process, and consumer's acceptability (Ishizuki et al., 2013). The results of this experiment proved that MNU mutation technology can lead the way for better growth, increase grain yield potential, and produce quality rice grains. Hence, the MNU mutation approach may be used to support the production of high-quality rice grain as well as help developing countries to increase their productivity by releasing new cultivars within a short period of time.

5. Conclusions

The cultivated area under paddy rice production has not increased since several years; whereas, rice demand is rising worldwide. Therefore, enhancement of rice yield per unit area is required. High yields and stable performances of new rice varieties under different environmental conditions are key factors for sustainable rice production. Determination of the interaction between genotypic and environmental traits is an effective and stable approach to rice productivity. Released varieties by MNU mutation showed improved growth parameters, yield potential, and physicochemical properties from the F2 generation compared to their parental cultivars. The tiller number per hill and panicle length was significantly higher in the mutant lines. The greatest grain yield was recorded in the mutant lines as well as mutant lines improved rice grain physicochemical properties by a reduction in the content of amylose, protein, and lipid. Mutant lines displayed an increase in grain length as compared to their cultivars. Besides, significantly positive correlations were observed among grain yield with tiller number per hill, panicle length, panicle number per hill, spikelet number per panicle, ripened grain ratio, 1000 grain weight, and taste score. Further rice varieties should be treated with the MNU mutation and the screening process should be conducted to evaluate the phenotypic performance of newly developed mutant rice varieties for better adaptability, higher yield, and improve grain quality.

Chapter 3

Efficacy of *N*-methyl-*N*-nitrosourea Mutation on Physicochemical Properties, Phytochemicals, and Momilactones A and B in Rice

Abstract

Rice is not only a primary source of carbohydrate, but also a good alternative candidate for the exploration of natural antioxidants and numerous medicinal properties which can lead to developing rice-based functional foods, preservatives, cosmetics, and pharmaceutical products. Rice production and grain quality aspects should improve to fulfill the requirement of its consumers worldwide. Attempts regarding the improvement and development of novel rice with better grain quality and higher productivity have been increasing. Among approaches, mutation, a direct alteration on the genome, is considered as one of the most beneficial routes to acquire new beneficial traits in rice. By this study, an experiment was conducted to investigate the effects of MNU mutation on the physicochemical properties, antioxidant activities, phytochemical compounds, and momilactones A (MA) and B (MB) in rice. Six rice varieties including original cultivars (K1, K3, and K5), and their mutant lines (K2, K4, and K6), were examined. Physicochemical properties (the content of amylose, protein, and lipid), antioxidant activities (DPPH and ABTS), total phenolic contents, total flavonoid contents, and momilactones A and B of the rice grain, husk, and straw portions were measured and quantified. Antioxidant activities in terms of DPPH and ABTS were higher in the husk followed by straw and grain. Mutant lines increased the antioxidant activities in grain and straw but decreased in the husk of the rice plant. The contents of DPPH and ABTS in the BHT standard were higher compared to the DPPH and ABTS of the rice grain, husk, and straw. Moreover, total phenolic contents and total flavonoid contents were higher in the straw following by husk and grain, respectively. Mutant lines enhanced the amount of TPC and TFC in the rice straw and declined in the husk. TPC in grain was increased in the grain of mutant lines but the amount of TFC in grain was higher in the original cultivars. Both MA and MB, two compounds obtaining anti-diabetes, anti-cancer, anti-microbial, anti-gout, anti-aging, and anti-obesity properties, were detected and quantified in grain, husk, and straw of original cultivars and mutant lines samples. Generally, the content of MA was 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 the original cultivars. This study illustrates that MNU mutation can improve grain quality and enhance bioactive compounds in straw, husk, and grain of rice. This approach is the potential to develop functional foods from rice and therefore help farmers in developing countries to improve value from rice production.

1. Introduction

Rice is considered as one of the most dietary cereal crops which supplies essential foods worldwide, particularly producing and consuming on a large scale in Asian countries, which account for 80% of rice production of the world (Kakar et al., 2019d; Rohman et al., 2014). It provides daily calories for the majority of the world's population as well as feeds many companion animals (Ryan, 2011). The majority of rice consumers constantly involve rice in their diet which provides approximately 2808 calories per person per day (Bhat and Riar, 2016; Jiang et al., 2012). Rice is not only a primary source of carbohydrate, but also a good alternative candidate for the exploration of natural antioxidants and numerous medicinal properties which can lead to developing rice-based functional foods, preservatives, cosmetics, and pharmaceutical products (Bhat and Riar, 2015; Chaudhari et al., 2018). Additionally, Asaduzzaman et al. (2013) stated that rice could be used as functional foods and natural phytochemical ingredients.

On the other hand, rice grain quality is a complex character influenced by its physical and chemical compositions, especially the amount of amylose, protein, and lipid contents (Noori et al., 2018; Kakar et al., 2019c). These physicochemical properties of rice grain are largely affected by genotypic and environmental conditions (Noori et al., 2018; Kakar et al., 2019b). High amylose content in rice grain leads to becoming hard, fluffy after cooking, and broken during the grain milling process (Kakar et al., 2019b; 2019c). However, protein and lipid contents play a vital performance in the nutritional value of rice grain (Abacar et al., 2016). Previously, a positive correlation between physicochemical properties and antioxidant activities of rice grain was found (Kakar et al., 2019c).

Additionally, bioactive compounds including antioxidants, phenolics, and momilactones play important roles in human health, pharmaceutical industries, and allelopathy (Chaudhari et al., 2018; Quan et al., 2019e; Tuyen et al., 2017). Antioxidants effectively inhibit and neutralize free radical reactions by providing hydrogen or electron to the reaction chain (Tuyen et al., 2016). Antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and others from synthetic sources are applied in food protection against oxidative damages (Liu et al., 2011); however, concerns regarding the side effects of them have been increased among scientists and nutritionists. Therefore, the exploration of economical and physiological justified natural antioxidants rather than synthetic resources is of interest (Bhat and Riar, 2015; Razali et al., 1997; Soong and Barlow, 2004). Plants are potential sources of natural antioxidants (Chandra et al., 2014). Of them, rice is among the most produced and consumed cereal crops worldwide with a crucial character in the relationship between diet and human health (Walter and Marchesan, 2011).

Phenolic constituents are secondary metabolites that protect plants against pests and ultraviolet radiation as well as support plants to attract pollinating animals (Parr and Bolwell, 2000; Walter and Marchesan, 2011). Besides, they play key roles in several activities in plants, phenolics and flavonoids are vital constituents in the human diet (Stalikas, 2007; Tuyen et al., 2017). Phenolics have positive effects on human health (Saeed and Khan, 2012; Tuyen et al., 2016) and are considered as the most potent natural compounds of plants with antioxidant, anti-microbial, and anti-inflammatory activities (Hyun et al., 2016; Tuyen et al., 2016). Additionally, phenolic compounds play a crucial role in stabilizing lipids against peroxidation as well as preventing diabetes (Yawadio and Tanimori, 2007) and various types of oxidizing enzymes (Wojdylo et al., 2007). Several factors including genetic characteristics are responsible for the variation in the content of phenolics (Walter and Marchesan, 2011).

Momilactones A and B, as plant growth inhibitors, are originally isolated from rice husk (Ahmad et al., 2019; Lee et al., 1999). MA and MB have been detected only in rice plants and moss (*Hypnum plumaeforme*) and belong to diterpenes groups (Kato-Noguchi, 2004). Momilactones as phytoalexins evolve in the defense system of plants and increase with biotic and abiotic stresses (Cartwright et al., 1981; Kato-Noguchi et al., 2010). Momilactones functioned as allelopathic potential against weeds and blast fungus (Kato-Noguchi, 2011; Toyomasu et al., 2008) as well as expressed antioxidant, antitumor, antidiabetes, cytotoxic, antifungal, antibacterial, antimicrobial, and anticancer activities (Joung et al., 2008; Minh et al., 2018). Quan et al. (2019b) stated that MA and MB are inhibitors of two key enzymes (α-amylase and α-glucosidase) which are associated with diabetes. It is reported that MB effectively controls ketosis related to low blood sugar levels (Kang et al., 2017). Some rice varieties can release momilactones, particularly MB, which can inhibit and suppress the germination of nearby weeds (Kato-Noguchi, 2004; Kato-Noguchi and Ino, 2003) and can reduce the application rate of synthetic herbicides (Kato-Noguchi et al., 2010).

Grain quality, nutritional value, and bioactive compounds of rice grain and its by-products are widely varied based on genetic backgrounds, cultivation methods, and environmental conditions (Kakar et al., 2019a; Rayee et al., 2019; Rohman et al., 2014). Previously, we found that MNU mutation increased growth attributes, grain yield, and the physicochemical properties of rice grain (Kakar et al., 2019e). To date, none of the research elucidated the effectiveness of MNU mutation on the phytochemical and momilactone contents of rice grain and its by-products. Thus, this study aims to explore the efficacy of MNU mutation on phytochemicals and momilactones in rice crop in order to support farmers to produce rich medicinal and pharmaceutical value from rice production.

2. Materials and Methods

2.1. Experimental Design and Plant Materials

An experiment was conducted at the Laboratory of Plant Physiology and Biochemistry, Hiroshima University during 2018 and 2019. The experiment was arranged in a randomized complete block design within three replications and six cultivars (K1, K3, and K5; the origin cultivars as well as K2, K4, and K6, their mutant lines) as listed in Table 1 (Chapter 2). The seeds of mutant lines and cultivars were received from Khai Xuan International Co., Ltd., and Agricultural Genetics Institute, Vietnam. The mutant lines have already treated following an MNU mutation protocol as described in previous studies (Anh et al., 2018; Kakar et al., 2019e). In short, seeds of the original cultivars (K1, K3, and K5) were treated for three hours with 150 mM MNU, dried, and kept in hermetic conditions for three months. Then, the required seeds were stored in the darkness at 5 °C for further application. The mutated F1 was self-pollinated to yield the mutated F2. The experimental field was puddled by a power tiller and leveled manually. The plots were designed at 3 m^2.

Perfect seeds of the selected cultivars and mutant lines were soaked in distilled water and kept at 30 °C in a growth chamber for 48 hours. The pre-sprouted seeds were then broadcasted in plastic nursery boxes on the commercial soil. The 25-day-old seedling with 3.5 leaf growth age was transplanted to the prepared rice field as one seedling per hill. Subsequently, 15 cm × 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. Plants were harvested at the 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.

2.2. Measurement of Physicochemical Properties

Physicochemical properties of rice grain including amylose, protein, and lipid contents, and taste score were evaluated by a grain quality tester machine (PGC Shizuoka Seiki PS-500 machine, version 2-12, Shizuoka Seiki Co., Ltd., Shizuoka, Japan) using 100 g brown rice grains with three replications. The data were used to evaluate the correlation of physicochemical properties with phytochemicals and momilactones in rice grain.

2.3. Standards and Reagents

All standard compounds and reagents including gallic acid (GE), rutin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS), butylated hydroxytoluene (BHT), potassium persulfate ($K_2S_2O_8$), Folin-Ciocalteu's reagent (FC), sodium carbonate (Na_2CO_3), aluminum chloride hexahydrate ($AlCl_3$ $6H_2O$), hydrochloric acid (HCl), sodium hypochlorite (NaOCl), sodium hydroxide (NaOH), and sodium acetate ($C_2H_3NaO_2$) were analytical grade. The solvents for extraction and isolation as well as acetonitrile were acquired from Junsei Chemical Co., Ltd., Tokyo, Japan and Fisher Scientific Co., Hampton, New Hampshire, USA. Chemicals for antioxidant assays were purchased from Fujifilm Wako Pure Chemical Corporation, Osaka, Japan. The remaining chemicals were procured from Kanto Chemical Co., Inc., Tokyo, Japan.

2.4. Extraction and Samples Preparation

The extractions of grain, husk, and straw samples were conducted through the technique reported by Quan et al. (2019d) with few changes. In brief, 100 g brown rice, 20 g straw, and 46 g husk were grinded and individually saturated by 100, 250, and 200 mL methanol for one week at room temperature. The extractions were then filtered and evaporated at 50 °C to obtain methanol extracts. Afterward, the extracts were mixed proportionately in a separatory funnel with an adequate volume of hexane. The methanol layers were collected and filtered after 3 hours at room temperature. The filtrates were consequently evaporated to get crude extracts which finally dissolved with methanol to adjust the concentration and stored for further analysis at 4 °C. Ahead of grinding, the straw samples were treated with 1% sodium hypochlorite and washed with water to remove infections. Figure 6 shows a general platform of the extraction method and sample preparation.

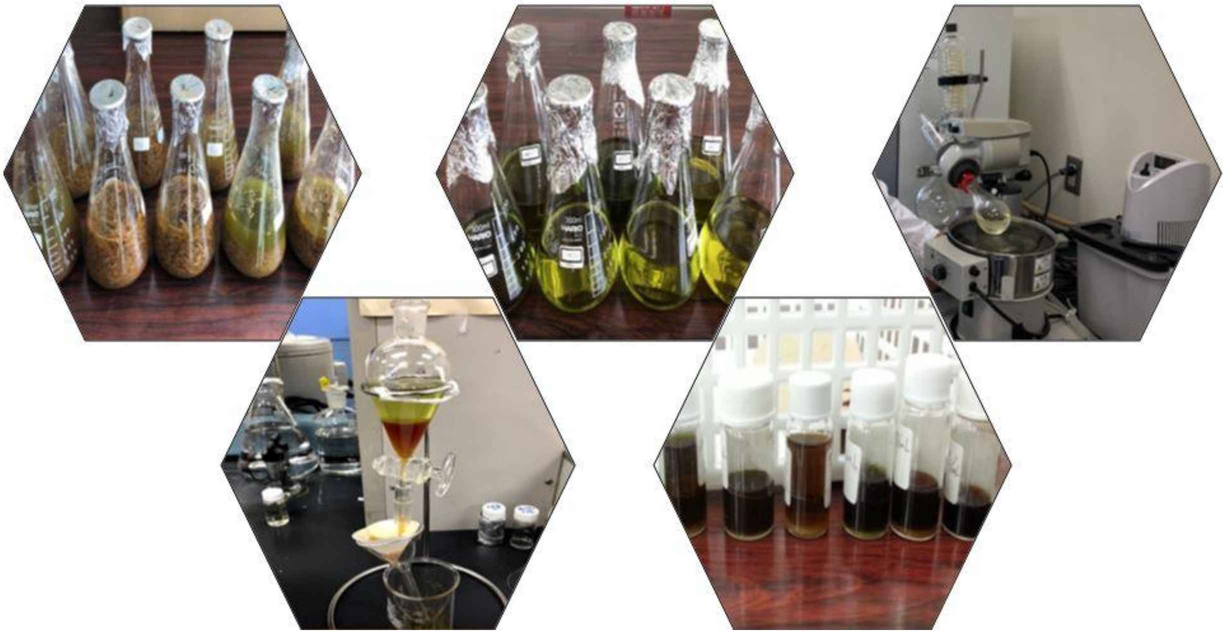


Figure 6. Description of the extraction method of the samples

2.5. Antioxidant Assays

2.5.1. DPPH Scavenging Assay

DPPH assay was carried out based on the method explained by Quan et al. (2019e) with few adjustments. Shortly, 50 µL of the sample extract was added with 50 µL of DPPH solution (500 µM in methanol) and 100 µL of 0.1 M acetate buffer (pH 5.5). The combination was then incubated for 20 min in darkness at room temperature. The absorbance was recorded at 517 nm using a microplate reader (Multiskan^{TM} Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan). Butylated hydroxytoluene (BHT) at various concentrations (10, 20, and 50 µg/mL) was used as a standard, whereas pure methanol was used as a negative control. The IC_{50} value was calculated and presented as the amount of the sample needed to scavenge 50% of DPPH radical. Thus, the lower IC_{50} value implied the higher antioxidant activity. The IC_{50} percentage scavenging activity of the tested sample was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = (A_c - (A_s - A_b)/A_c) \times 100$$

where A_c is the absorbance of the control (MeOH), A_s is the absorbance of the sample, and A_b is the absorbance of blank (without DPPH).

2.5.2. ABTS Cation Discoloration Assay

ABTS radical assay was conducted following the technique elucidated by Tuyen et al. (2017) with minor adjustment. Briefly, ABTS cation solution was obtained by mixing ABTS (7 mM) solution with 2.45 mM potassium persulfate (1:1 *v*/*v*). The mixture was then incubated for 16 h at room temperature in darkness, and the working solution was made by adding methanol until an absorbance of 0.70 ± 0.05 at 734 nm was achieved. Hereafter, 40 µL of sample and 180 µL of the working solution were blended in each well of a microplate and maintained for 20 min in dark conditions at room temperature. The absorbance was recorded at 734 nm through the microplate reader. BHT was used as a standard while methanol was selected as a control. The percentage discoloration activity was measured as the following formula:

$$\text{ABTS radical discoloration activity (\%)} = (A_c - A_s)/A_c \times 100$$

where A_c is the absorbance of the control (MeOH), and A_s is the absorbance of the sample. The IC_{50} value was obtained by the similar method described above.

2.6. Determination of Total Phenolic Contents

The TPC was measured by the Folin-Ciocalteu method reported previously (Quan et al., 2016). An aliquot of 20 µL of the plant extract was mixed with 100 µL of Folin-Ciocalteu's reagent (10%) and 80 µL of sodium carbonate solution (0.7 mM *w*/*v*, Na_2CO_3), respectively. The obtained mixture was incubated for 30 min at room temperature in darkness. Gallic acid at different concentrations (2.5–60 µg/mL) was used as a standard and the TPC was evaluated by the standard calibration curve of gallic acid. The absorbance was recorded at 765 nm wavelength. The TPC was expressed as µg gallic acid equivalent per g dry weight of the sample (µg GAE/g DW).

2.7. Determination of Total Flavonoid Contents

The TFC was determined through the aluminum chloride colorimetric method described by Tuyen et al. (2017). An amount of 50 µL sample was mixed and incubated with 50 µL aluminum chloride (2% in methanol, *w*/*v*) in darkness for 15 min at room temperature. The absorbance of the mixture was read at 430 nm wavelength using the microplate reader. Rutin at different concentrations (10–500 µg/mL) was applied as a standard reference and the amount of TFC was indicated as µg rutin equivalent per g dry weight of the sample (µg RE/g DW) by standard curve.

2.8. Quantification of Momilactone A and B

MA and MB were quantified based on the LC-ESI-MS system consisted of an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, California, USA) which was equipped with an electrospray ionization (ESI) source. The LC was conducted by injecting 3.0 µL of a sample (at different concentrations) into the Acquity UPLC® BEH C18 (1.7 µm, 50 × 2.1 mm i.d.) column (Waters Cooperation, Milford, Massachusetts, USA). The column temperature was kept at 25 ^{o}C. The chromatography was run in a gradient model with a flow rate of 300 µL/min. The gradient of the mobile phase was established as follows: 50% solvent A (0.1% trifluoroacetic acid in water) and 50% solvent B (0.1% trifluoroacetic acid in acetonitrile) over 0–5 min, then increased to 100% B over 5–10 min which was upheld for 0.1 min (Quan et al., 2019a). Finally, the column was equilibrated by the initial condition for 5 min. The total operation time was 15.1 min. The ESI condition was set up in a similar index to the manner explained by Quan et al. (2019c). The availability and presence of MA and MB in the sample were confirmed through a comparison of extracted ion chromatograms (EIC) and mass spectra (MS) with those of standard momilactones. The areas of identified peaks that matched with standard MA (RT: 3.82) and MB (RT: 2.33) in the EIC were used to calculate the quantity of such compounds over a linear model. All buffer components were acquired from Sigma-Aldrich, St. Louis, Missouri, USA, as well as pure MA and MB, were isolated and validated previously by our laboratory (Quan et al., 2019a).

2.9. Statistical Analysis

Data were analyzed using Minitab 16.0 software (Minitab Inc., State College, Pennsylvania, USA). One-way analysis of variance (ANOVA) was conducted to express the differences among samples, followed by Tukey's multi comparison test at the $p<0.05$ probability level. Pearson correlation was carried out to indicate the interaction between physicochemical properties and biological contents. Data are presented as means ± standard errors (SE).

3. Results

3.1. Antioxidant Activities

Two assays were carried out to quantify the antioxidant activities in terms of DPPH and ABTS which are summarized as µg/mL in Table 7. The lower value indicated a higher antioxidant activity. Variations were observed in terms of IC_{50} inhibition of DPPH and ABTS radical scavenging activities among the samples of all mutant lines and origin cultivars. Generally, rice husk exhibited the greatest antioxidant activity, followed by rice straw and grain. These variations were also significant among plant portions within each cultivar and mutant line. Same differences were found for the origin cultivars (K1, K3, and K5) and mutant lines (K2, K4, and K6); therefore, K1 and K2 are compared to each other. The results showed that mutant line (K2) increased the antioxidant activity in grain (DPPH 1267.1 µg/mL and ABTS 642.0 µg/mL) and straw (DPPH 1247.9 µg/mL and ABTS 371.8 µg/mL) compared to the original cultivar (K1), while antioxidant activity in rice husk (DPPH 503.4 µg/mL and ABTS 72.6 µg/mL) was higher in the original cultivar.

The IC_{50} value of the antioxidant activity in rice husk was at DPPH 503.4 µg/mL and ABTS 72.6 µg/mL for K1 and DPPH 972.9 µg/mL and ABTS 137.6 µg/mL for K2, followed by straw at DPPH 1772.7 µg/mL and ABTS 601.7 µg/mL for K1 and DPPH 1247.9 µg/mL and ABTS 371.8 µg/mL for K2 and grain at DPPH 1862.5 µg/mL and ABTS 766.5 µg/mL for K1 and DPPH 1267.1 µg/mL and ABTS 642.0 µg/mL for K2, respectively. The greatest

DPPH and ABTS were recorded in the husk of K3 and the lowest were observed for K4. Additionally, the greatest DPPH and ABTS for rice straw were recorded in K2 and the lowest were observed for K5 as well as the greatest DPPH and ABTS for rice grain were recorded in K2 and the lowest were observed for K5, respectively.

3.2. Total Phenolic and Flavonoid Contents

The amounts of TPC and TFC are also presented in Table 7. They are illustrated as µg GAE and µg RE per g dry weight of the sample, respectively. Generally, TPC and TFC were higher in straw followed by husk and grain. The mutant lines significantly increased the amount of TPC in the rice straw portion which was 1156.6 µg GAE/g DW for K2, 1168.8 µg GAE/g DW for K4, and 714.9 µg GAE/g DW for K6, respectively. Besides, mutant lines showed greater TPC in rice grain (80.3 µg GAE/g DW for K2), (77.0 µg GAE/g DW for K4), and (85.9 µg GAE/g DW for K6); however, they had lower TPC in the husk sample (405.9 µg GAE/g DW for K2), (204.1 µg GAE/g DW for K4), and (231.0 µg GAE/g DW for K6) than the original cultivars. The differences among sample portions were also significant for TPC. The highest and lowest amounts of TPC were recorded in the straw portion of K4 and the grain portion of K5. TFC of rice husk was higher in the origin cultivars than those in mutant lines, while mutant lines increased the amount of TFC in rice straw. The contents of total flavonoids were greater in rice grain of the origin cultivars and were lower in the grain portion of mutant lines.

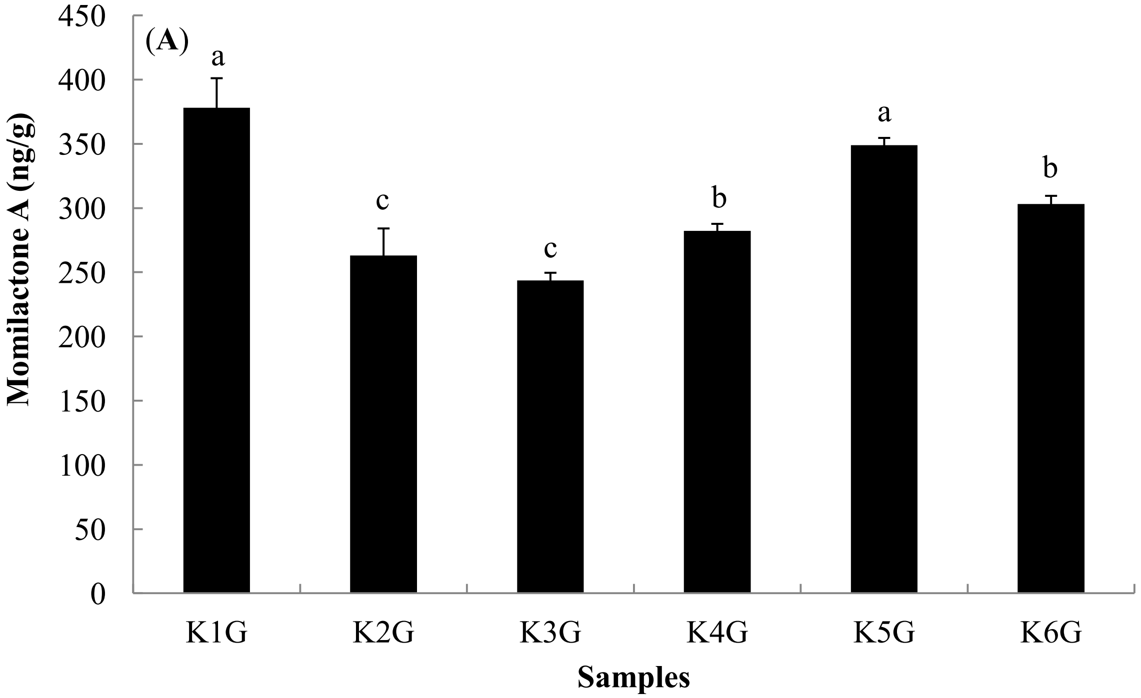
Table 7. Antioxidant activities, total phenolic and total flavonoid contents of the rice grain, husk, and straw in mutant lines and original cultivars

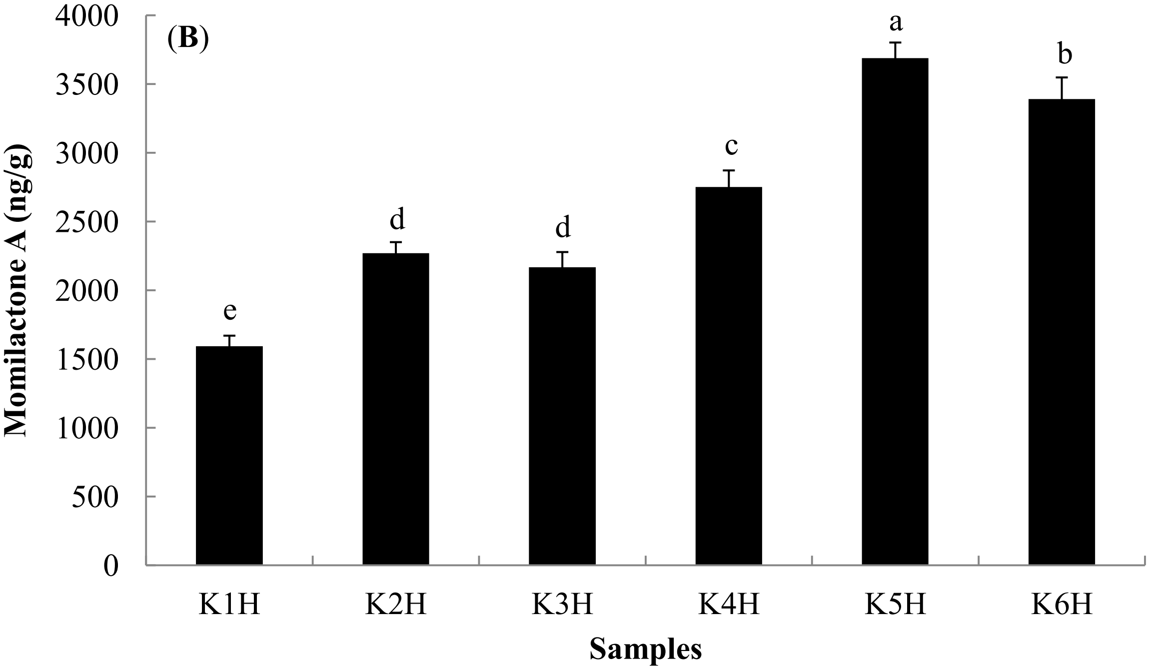
Samples		IC_{50} of DPPH (µg/mL)	IC_{50} of ABTS (µg/mL)	TPC (µg GAE/g DW)	TFC (µg RE/g DW)
K1	Grain	1862.5 ± 181.2 a	766.5 ± 20.6 a	75.8 ± 6.3 b	1.4 ± 0.1 a
	Husk	503.4 ± 18.3 b	72.6 ± 6.9 b	462.5 ± 27.0 a	75.9 ± 1.8 a
	Straw	1772.7 ± 153.3 a	601.7 ± 27.5 a	893.3 ± 39.5 b	197.2 ± 5.3 b
K2	Grain	1267.1 ± 68.8 b	642.0 ± 10.7 b	80.3 ± 9.9 a	0.8 ± 0.1 b
	Husk	972.9 ± 69.7 a	137.6 ± 3.5 a	405.9 ± 3.0 b	54.7 ± 2.1 b
	Straw	1247.9 ± 77.4 b	371.8 ± 26.0 b	1156.6 ± 94.7 a	481.6 ± 14.8 a
K3	Grain	2133.4 ± 34.5 a	980.4 ± 7.6 a	59.7 ± 0.5 b	1.0 ± 0.0 a
	Husk	396.2 ± 10.3 b	57.5 ± 1.6 b	329.6 ± 5.4 a	110.3 ± 0.8 a
	Straw	1922.2 ± 10.3 a	718.0 ± 4.0 a	428.0 ± 9.6 b	204.4 ± 1.5 b
K4	Grain	1955.8 ± 19.2 b	912.7 ± 11.5 b	77.0 ± 3.8 a	0.5 ± 0.1 b
	Husk	1032.6 ± 12.3 a	146.9 ± 9.2 a	204.1 ± 1.9 b	64.9 ± 2.1 b
	Straw	1472.1 ± 14.2 b	486.5 ± 9.2 b	1168.8 ± 23.3 a	453.9 ± 4.8 a
K5	Grain	2254.9 ± 17.2 a	1013.5 ± 30.3 a	49.7 ± 1.9 b	0.7 ± 0.2 a
	Husk	626.5 ± 14.8 b	85.8 ± 1.6 b	261.2 ± 4.6 a	108.1 ± 0.8 a
	Straw	1995.5 ± 16.7 a	853.7 ± 7.3 a	410.3 ± 6.8 b	131.3 ± 3.3 b
K6	Grain	1889.6 ± 17.2 b	805.5 ± 10.7 b	85.9 ± 3.4 a	0.4 ± 0.1 b
	Husk	982.4 ± 11.0 a	137.5 ± 3.5 a	231.0 ± 4.9 b	52.2 ± 5.1 b
	Straw	1812.8 ± 19.4 b	677.3 ± 16.3 b	714.9 ± 6.4 a	299.4 ± 6.7 a
BHT		28.95 ± 0.8 c	30.63 ± 0.6 c	-	-

Values are illustrated as means ± standard errors. The same letters within a column between each portion of the original cultivar and its mutant line indicate no significant differences at the $p<0.05$ probability level based on Tukey's multi comparison test. (-) means not measured

3.3. Momilactones A and B

The amounts of MA and MB in rice grain, husk, and straw portion of the mutant lines and origin cultivars as nanogram per gram (ng/g) are exhibited in Figures 7 and 8. Identical results were found for mutant lines and origin cultivars; therefore, the results for the contents of MA and MB are only explained for the origin cultivar (K1) and mutant line (K2) as a representative. In this study, both MA and MB were detected and quantified in rice grain, husk, and straw portions of the mutant lines and original cultivars. Generally, the amount of MA was greater than MB in both origin cultivars and mutant lines samples. Both MA and MB were higher in the rice straw followed by husk and grain. Mutant lines enhanced the amount of MA in straw and husk, while the quantity of MA was higher in the grain of the origin cultivars. Additionally, MB was also higher in the straw of mutant lines but was lower in their grain, while there was no significant difference in the husk of both cultivars and mutant lines for the content of MB. Furthermore, a positive and certain mass range to confirm the presence of MA and MB in all portions of both cultivars was conducted which showed two major peaks, retention times, and fragmentation patterns of the samples. Figure 9 shows the chemical structure of MA and MB. Additionally, Figures 10 and 11 displayed the EIC and MS of MA and MB of rice straw samples in the original cultivar (K1) and mutant line (K2), which are completely in line with those of standard MA and MB peaks and retention times. The content of MA was higher in the grain sample of K1 followed by K5, K6, K4, K2, and K3, while it was greater in the husk sample of K5 following by K6, K4, K, K3, and K1. Besides, the straw sample of K2 had a high content of MA followed by K1, K4, K6, K3, and K5. Additionally, the content of MB was higher in the grain sample of K1 followed by K3, K2, K4, K5, and K6, whereas it was greater in the husk sample of K5 following by K4, K6, K2, K3, and K1. The straw sample of K2 had greater content of MB followed by K1, K4, K3, and K6. In addition, MB was not detected in the straw sample of K5.





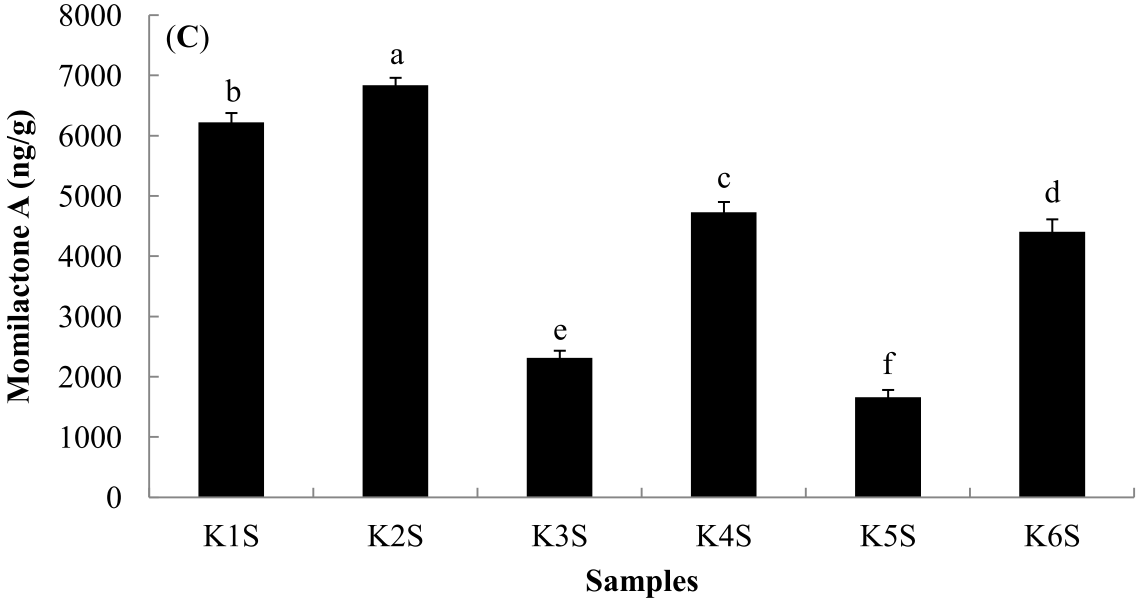
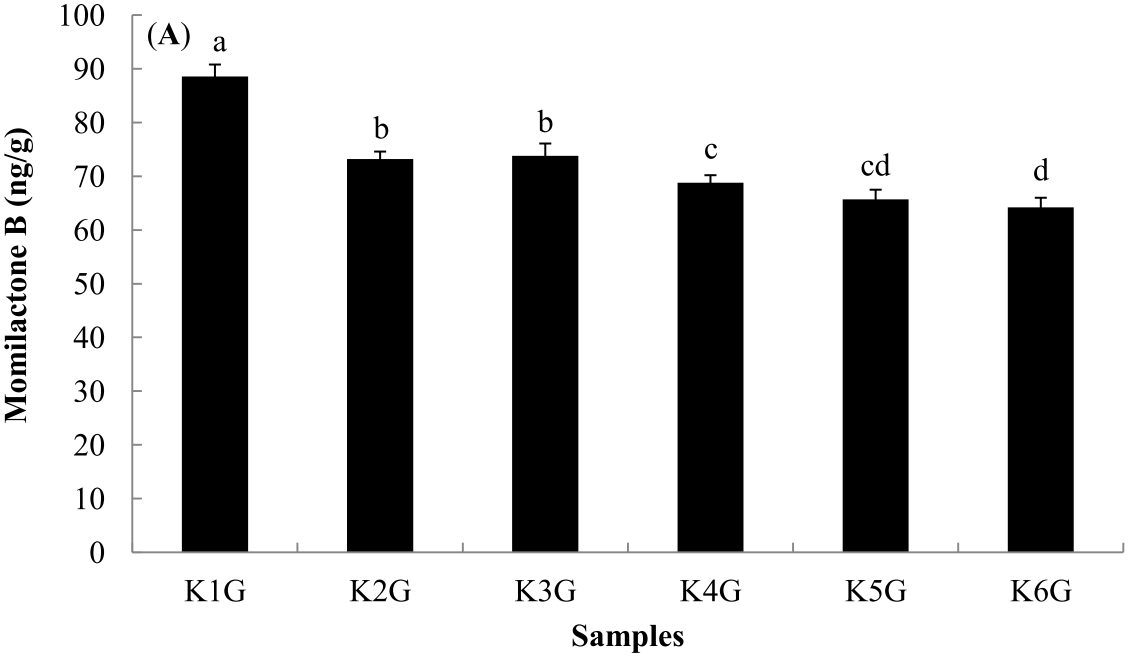
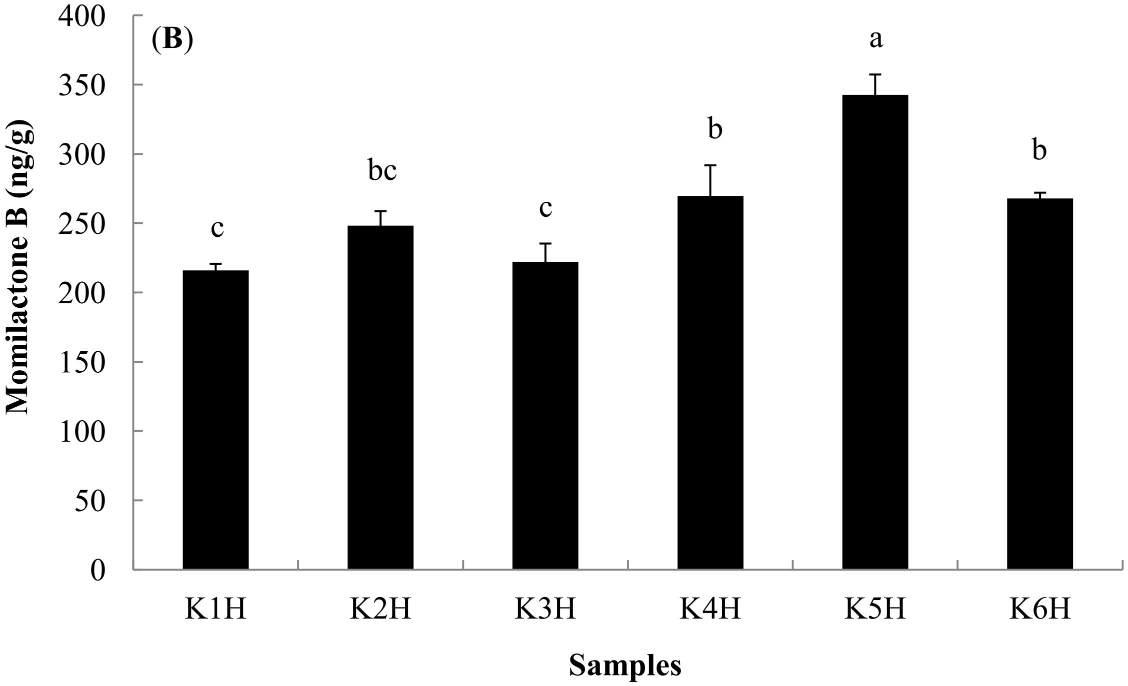


Figure 7. Contents of MA in rice grain, husk, and straw of the original cultivars and mutant lines. (**A**): grain samples, (**B**): husk samples, (**C**): straw samples. Different letters indicate significant differences at $p<0.05$ probability level among samples of mutant lines and their origin cultivars. G, grain; H, husk; S straw


(A)
Momilactone B (ng/g)
100
90
80
70
60
50
40
30
20
10
0
a
b
b
c
cd
d
K1G
K2G
K3G
K4G
K5G
K6G
Samples


(B)
Momilactone B (ng/g)
400
350
300
250
200
150
100
50
0
c
bc
c
b
a
b
K1H
K2H
K3H
K4H
K5H
K6H
Samples

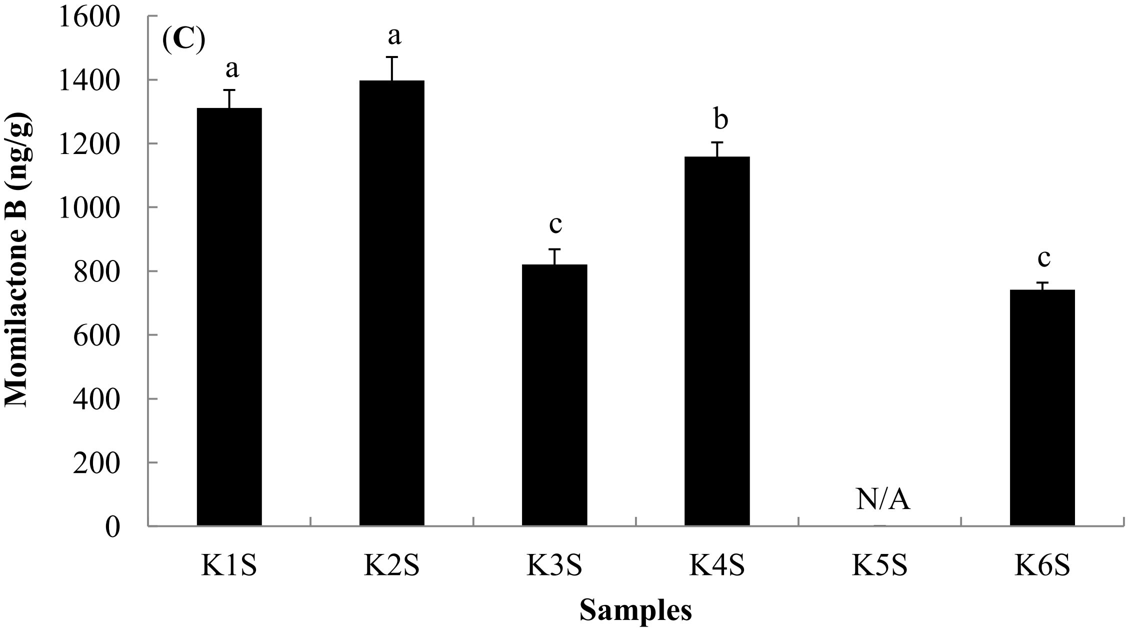


Figure 8. Contents of MB in rice grain, husk, and straw of the original cultivars and mutant lines. (**A**): grain samples, (**B**): husk samples, (**C**): straw samples. Different letters indicate significant differences at p<0.05 probability level among samples of mutant lines and their origin cultivars. G, grain; H, husk; S straw. N/A, not detected

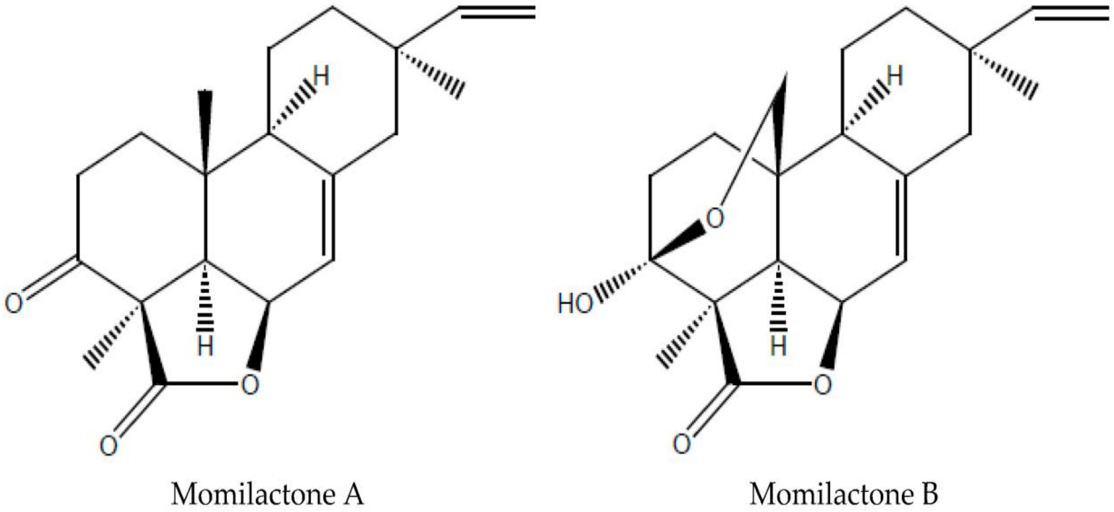


Figure 9. Chemical structure of momilactones A and B
Source: Minh et al. (2018a)

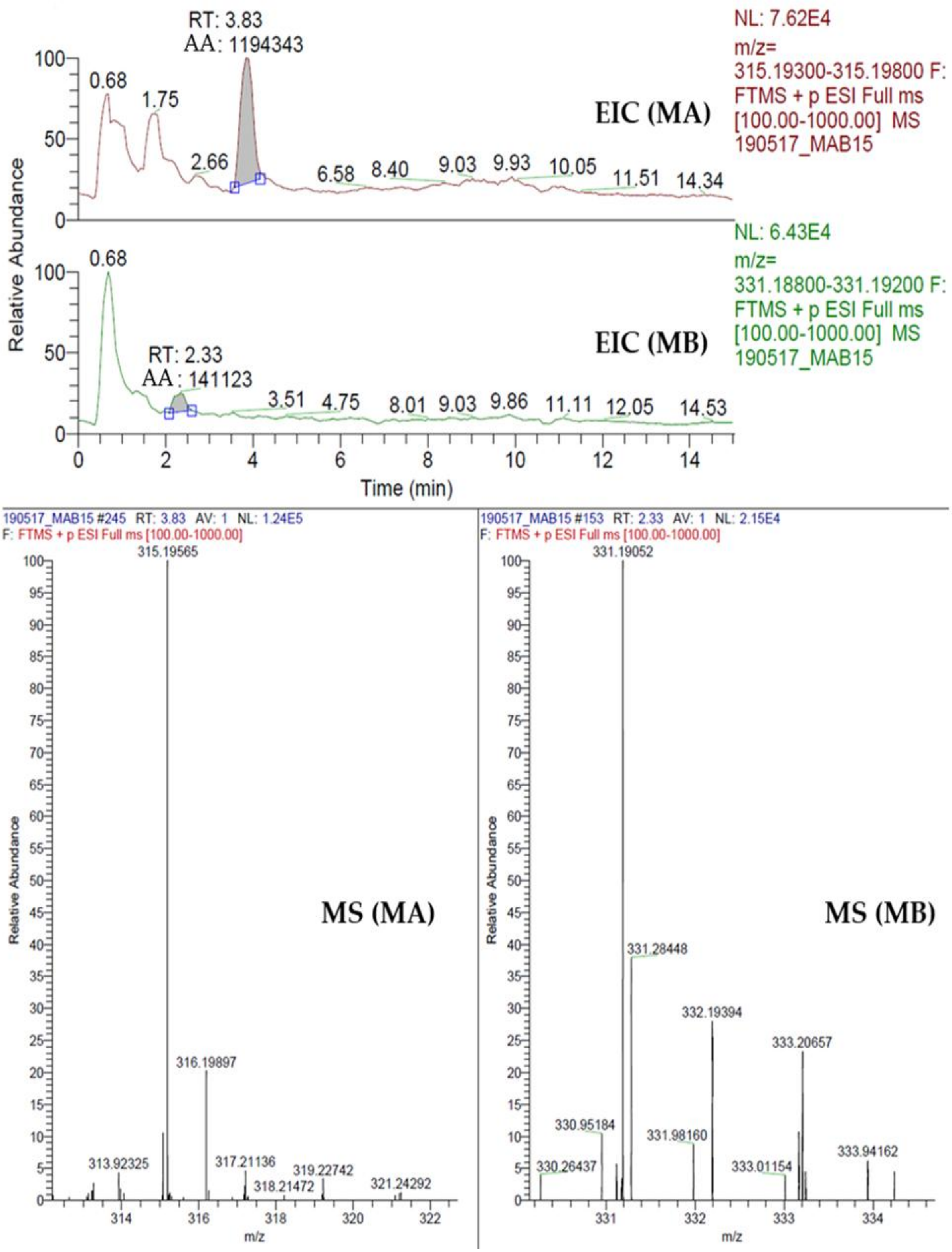


Figure 10. Extracted ion chromatograms (EIC) and mass spectra (MS) of momilactones A and B in the rice straw sample of K1

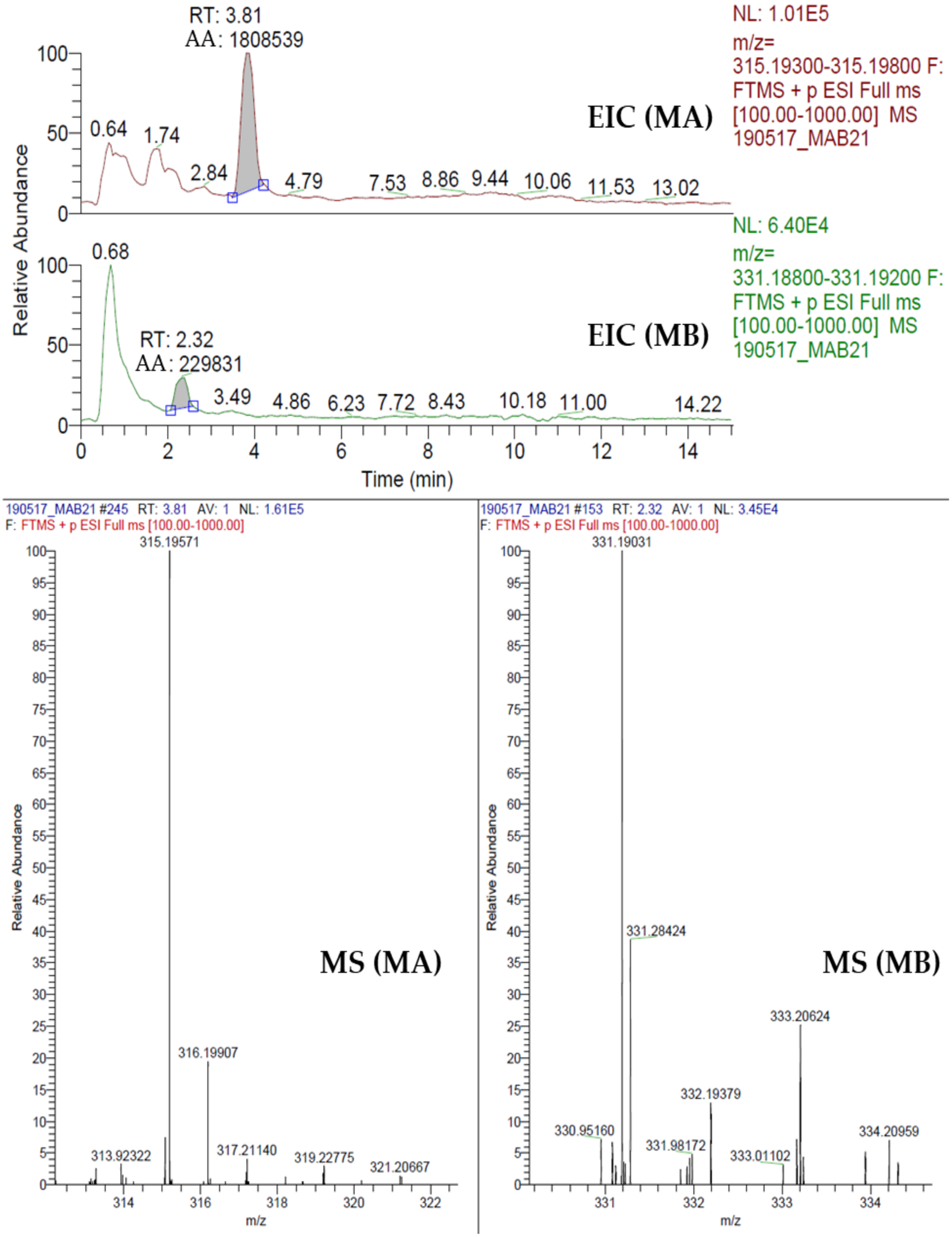


Figure 11. Extracted ion chromatograms (EIC) and mass spectra (MS) of momilactones A and B in the rice straw sample of K2

3.4. Correlation of Biological Contents of Rice Grain

The correlation of grain yield and physicochemical properties with phytochemical contents and momilactones of the rice grain is summarized in Table 8. Grain yield showed a significantly positive relation with taste score, DPPH, and ABTS, whereas it showed a negative correlation with amylose content, TFC, MA, and MB. Grain yield did not correlate 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. Antioxidant activities exhibited a significantly positive correlation with taste score and grain yield, but a negative relation with TFC, MA, and MB. TFC displayed a significantly positive interaction with amylose and a negative relation with taste score, grain yield, DPPH, and ABTS. Momilactones A and B displayed a significantly positive correlation with amylose content and TFC but showed a negative relation with taste score, grain yield, DPPH, and ABTS. Momilactones A and B showed a strong positive interaction with each other.

3.5. Principal Component Analysis

The principal component analysis (PCA) of grain yield with phytochemical properties and momilactones is presented in Figure 12. The PCA showed that the first principal component (PC1) accounts for 48.2% of the total variation around the PCA chart, while the second principal component (PC2) exhibited 32.7%, respectively. The PCA showed that GY and TS have a closer relationship, while DPPH, ABTS, and TPC have similar relations. Additionally, LC, PC, AC, TFC, MA, and MB are correlated to each other.

Table 8. The correlation coefficient of grain yield and physicochemical properties with phytochemical contents and momilactones of rice grain

	AC	PC	LC	TS	GY	1/DPPH	1/ABTS	TPC	TFC	MA
PC	0.610									
LC	0.138	0.212								
TS	−0.829 *	−0.772	−0.549							
GY	−0.958 **	−0.481	−0.359	0.858 *						
1/DPPH	−0.992 ***	−0.549	−0.122	0.798 *	0.961 **					
1/ABTS	−0.970 ***	−0.560	−0.302	0.830 *	0.972 ***	0.957 **				
TPC	−0.204	0.306	0.151	−0.139	0.238	0.308	0.209			
TFC	0.913 **	0.634	0.466	−0.915 **	−0.949 **	−0.919 **	−0.929 **	−0.259		
MA	0.946 **	0.478	0.371	−0.832 *	−0.989 ***	−0.958 **	−0.972 ***	−0.344	0.967 **	
MB	0.921 **	0.414	0.407	−0.814 *	−0.987 ***	−0.938 **	−0.956 **	−0.360	0.954 **	0.996 ***

AC, amylose content; PC, protein content; LC, lipid content; TS, taste score; GY, grain yield; 1/DPPH, 1/1,1-diphenyl-2-picrylhydrazyl; 1/ABTS, 1/2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TPC, total phenolic contents; TFC, total flavonoid contents; MA, momilactone A; MB, momilactone B. *, **, and *** indicate significant differences at $p<0.05$, $p<0.01$, and $p<0.001$, respectively

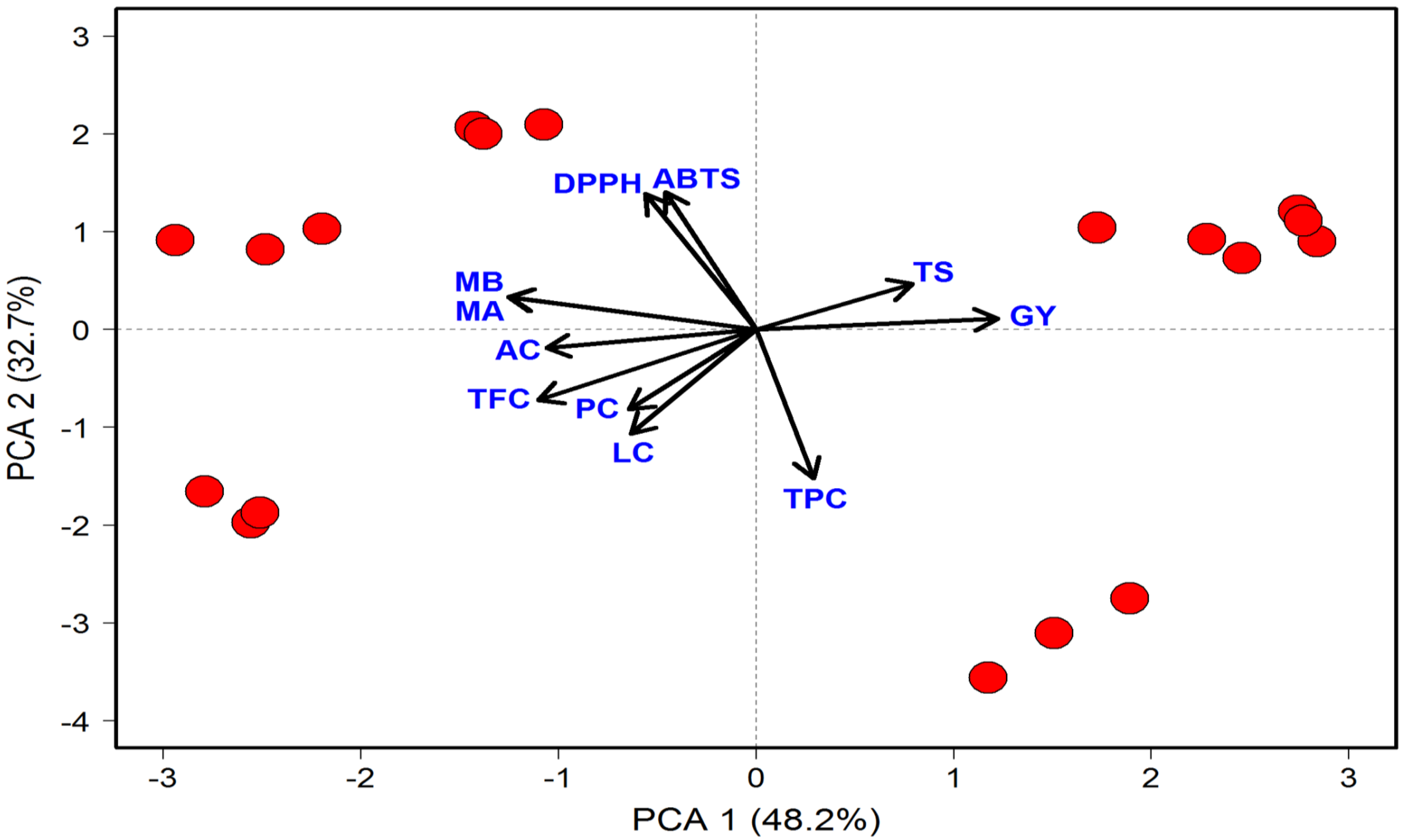


Figure 12. Principal component analysis of grain yield with physicochemical properties and biological activities. AC, amylose content; PC, protein content; LC, lipid content; TS, taste score; GY, grain yield; DPPH, 1/1,1-diphenyl-2-picrylhydrazyl; ABTS, 1/2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TPC, total phenolic contents; TFC, total flavonoid contents; MA, momilactone A; MB, momilactone B

4. Discussion

Previously, the importance of MNU mutation on growth attributes, yield potential, and physicochemical properties of rice has been reported. The MNU mutation enhanced rice productivity by improving grain yield and its components as well as the physicochemical properties of rice grain by a reduction in the percentages of amylose, protein, and lipid contents (Kakar et al., 2019c). Low amylose content in rice grain leads to exhibit softness and stickiness. However, high content of it causes rice grain to become hard, fracture during the milling process, and produce more broken grains (Kakar et al., 2019b; 2019c; Noori et al., 2018). Protein and lipid contents are important elements in the nutritional value of rice grain (Abacar et al., 2016). Protein can strongly affect both the eating and cooking quality of rice grain, as well as lipid content, which has an important and influential performance on viscoelastic properties and amylose structure (Zhou et al., 2002). It is reported that high amylose and protein contents decrease rice grain acceptability in global markets (Zhou et al., 2002).

The results of this experiment indicated that MNU mutation can enhance bioactive compounds and pharmaceutical value of rice plants besides improving their yield and grain quality. As rice is the main and staple food worldwide, particularly it plays a fundamental part in social formalities and festivals in almost all Asian countries, it has meditative values too (Rohman et al., 2014). Bioactive compounds of rice plant determine its position in the pharmaceutical and food industries (Bhat and Riar, 2015; Chaudhari et al., 2018; Ryan, 2011). Thus, the improvement of antioxidant activities, phenolic contents, and momilactones are unique aspects. The antioxidant activities of plants can be measured by applying several techniques and methods to measure the speedy screening of substances (Nunes et al., 2012). Free radicals play an important role in several pathological aspects not only in humans but also in food preservation. Antioxidants neutralize free radicals and save humans from various diseases and health problems. They act by scavenging the reactive oxygen species (Umamaheswari and Chatterjee, 2008).

In this research, the DPPH and ABTS assays were conducted to evaluate the antioxidant activities of the samples. The electron provision capacity of natural products can be evaluated through DPPH scavenging activity which shows purple color (Nunes et al., 2012). This method works by scavenging of the DPPH by the addition of a radical or antioxidant that decolorizes the DPPH solution. The degree or concentration of changed color is proportional to the capacity and potency of the antioxidants. A large reduction in the absorbance of the reaction mixture of the under-tested compound denotes significant free radical scavenging activity (Krishnaiah et al., 2011). On the other hand, ABTS radical discoloration assay is an approach that generates a blue to green color through the reaction of ABTS and potassium persulfate. The ABTS radical cation is a result of ABTS oxidation with potassium persulfate, its reduction in the presence of hydrogen releasing antioxidants is recorded at 745 nm by a spectrophotometer. The antioxidant property is mainly characterized by phenolic contents that can effortlessly naturalize free radicals (Quan et al., 2019e). It was found that mutant lines had higher antioxidant activity in both grain and straw than the original cultivars. The phenolic contents were also increased with the antioxidant activities. Quan et al. (2019e) assumed that phenolic compounds might not be the only contributors toward the antioxidant properties.

Several reports have elucidated the important roles of phenolic compounds in human health from various aspects and different sources (Walter and Marchesan, 2011). Some effects of phenolic constituents are related to the antioxidant enzymatic activities (Chiang et al., 2006; Shao et al., 2014) and protein induction (Chung et al., 2006). Phenolics and flavonoids are reported to decrease the risk of metabolic syndromes as well as type 2 diabetes (Tuyen et al., 2017; Ravishankar et al., 2013) and have been used to treat several diseases including those of ovarian, breast cervical, and pancreatic (Ravishankar et al., 2013). Studies elucidated that phenolic compounds can be absorbed by intestinal tract walls and are beneficial to human health not only due to their potential antioxidant activity but also to promote the anti-inflammation capacity of human beings (Kumar and Goel, 2019). It is reported that antioxidant activity and the contents of total flavonoids and total phenolics exhibited a significant positive correlation in rice grain (Goffman et al., 2004; Zhang et al., 2006). These observations are in line with the findings of this research.

It was reported that phenolic compounds distribution can change during the storage period. A reduction in the amount of bound phenolic acids during the storage period was observed in both brown and white rice grains; this reduction was greater in high temperature (37 ºC) and was lower at 4 ºC (Zhou et al., 2004). Compared to bound phenolics, the concentration and content of free phenolic acids were significantly increased in white rice during the storage; the reason might be due to the releasing of enzymatic or non-enzymatic activities of the bound phenolic acids. The phenolic compounds are largely correlated with the rice grain pericarp; thus, the milling procedure decreases the content and concentration of phenolic compounds in the white and polished grains (Shen et al., 2009). There are wide variations in the content of total phenolic compounds in the rice grain, among the rice accessions, white rice grain exhibits lower total phenolic content, while red and black rice grain shows the higher contents (Walter and Marchesan, 2011). Harukaze et al. (1999) reported that phenolic acids can affect the flavor properties of flours, grains, and oilseeds.

Momilactones play roles in allelopathy, phytoalexins, and the defense system of plants (Ahmad et al., 2019; Kato-Noguchi et al., 2010). In the current experiment, the amount of MA was greater in all parts of the rice plant than MB, which is matched with previous studies (Ahmad et al., 2019; Kato-Noguchi, 2011; Quan et al., 2019b). Recently, some researchers reported that MA and MB increased under salinity and drought stresses in rice plants (Quan and Xuan, 2018). Xuan et al. (2016) documented that MA and MB can be applied as biological indicators to decrease abiotic stresses in rice production and demonstrated that momilactones have strongly interacted with drought and salinity stresses rather than the weed tolerance in rice. Minh et al. (2018) stated that the quantity of MB was lower in the husk and other parts of rice but exhibited higher biological activities than MA. Both MA and MB are antidiabetic chemicals; thus, rice grain and other parts of the rice plant might be useful to exploit for antidiabetic treatments (Quan et al., 2019b). Kim et al. (2007) mentioned that MB has cytotoxic and antitumor activity against human colon cancer cells as well as suggested that

MB is a potential candidate for novel treatments to decrease death cells caused by colon cancer in humans.

Some researchers stated that the contents and presences of momilactones are varied among rice plant parts, genotypes, and growing stages (Quan et al., 2019b; Quan and Xuan, 2018; Xuan et al., 2016). A higher amount of MA was detected in rice grain, husk, and root compared to MB, but the content was lower in leaf. The highest MA quantity was in the husk, whereas the maximum MB content was found in the leaf as compared to other plant parts (Quan et al., 2019c; Xuan et al., 2016). The secretion of momilactones increases until the flowering stage and declines thereafter (Kato-Noguchi, 2010). Kato-Noguchi and Ino (2005) mentioned that the content of MB was greater in the shoot of rice seedlings than in root and pointed out that MB may perform a vital role in rice weed management and allelopathy. In this experiment, the contents of MA and MB were higher in the rice straw, followed by husk and grain in both mutant lines and origin cultivars. It is found that rice seedlings release MB into the root zone of plants over their entire growing period (Kato-Noguchi and Ino, 2003). In the current study, the seedlings of the mutant lines were grown healthy and produced a high yield with preferred quality. This may be due to the greater availability and presence of MA and MB in the rhizosphere of these crops which might be suppressed and inhibited the growth of surrounding weeds. The content of MB was lower than MA in all parts of the rice crop but exerted greater biological activities compared to MA (Kang et al., 2017; Minh et al., 2018a).

Rice husk, a by-product of the rice plant which consists of a high amount of beneficial and organic compounds (Kalapathy et al., 2003) is produced in a huge amount annually (Minh et al., 2018). Rice husk is not only a protective cover for its grain but also is a valuable source of natural antioxidants and phenolic acids (Butsat and Siriamornpun, 2010; Tan and Norhaizan, 2017). In this study, the antioxidants, TPC, TFC, MA, and MB contents of rice husk were higher than their grain, which can consider as a valuable source of natural bioactive substances. Additionally, rice straw exhibited greater amounts of TPC, TFC, MA, and MB than husk and grain, which is not only important for the production of natural products but also fortification of companion animals (Fardet et al., 2008). Both rice husk and straw are produced on large scales in the rice-growing areas. They are used as organic amendments to improve soil quality and in some areas as fuel related component. However, both of them are high-value by-products and can be used as natural sources for antioxidants, herbicides, and other essential compounds. The findings of this research display the important roles of MNU mutation in the production of rice-based functional foods and pharmaceutical industries as well as its key performances in the improvement of bioactive compounds in the rice plant.

5. Conclusions

Rice as an essential and staple food for human beings has been widely consumed and grown around the world, particularly, in Asian countries. It provides the main source of calories for more than half of the world's population. As the world population is increasing and climate change are undeniable and challenging agricultural sector; therefore, demands for high yield and better grain quality of rice have become a critical issue. Thus, breeding new adaptable rice varieties with high yielding potential and improved grain quality is an unprecedented challenge and urgent need. Additionally, demands for high productivity, preferable grain quality, and natural products of the rice plant are rising worldwide, particularly in rice-producing and consuming countries. In the current study, MNU mutation increased antioxidant activity in terms of DPPH and ABTS as well as TPC of rice grain and straw. Furthermore, MNU mutation enhanced TFC in the straw portion of the rice plant. Both MA and MB were detected in the samples of mutant lines. Both MA and MB were greater in the straw and husk portions of the mutant lines compared to the origin cultivars. In conclusion, MNU mutation displayed important roles in rice productivity, grain quality, and bioactive compounds. Thus, this approach can address and support rice farmers to improve rice-based functional foods and therefore increase value from rice production.

Chapter 4

Evaluation of MNU Mutation on the Relation between Allelopathic Potential and Grain Quality of Rice Plant

Abstract

Allelopathy is a biological approach and considered as the ability of a plant to suppress or stimulate the growth of nearby plants by the production of chemical compounds called allelochemicals. The application of this approach is recently rising due to its safety, low cost, and sustainability in weed management. Research has been conducted on rice with respect to its allelopathy potential as a part of the breeding strategy for sustainable weed management such as breeding and releasing allelopathic rice varieties. The allelopathic potential of rice varieties released by mutation technology is somewhat studied but the allelopathic activities of varieties released through MNU mutation are not yet investigated. Additionally, the relationship between the allelopathy and the rice grain quality is not well elucidated. Therefore, the aims of this study are to investigate the effects of selected mutant and origin rice cultivars on lettuce and barnyard grass seed germination and seedlings growth as well as to evaluate the possible involvement of phenolic acids in allelopathic effects from rice husk and straw extracts and to find out the relationship of grain quality with allelopathic potential in the rice plant. This experiment was conducted in the Laboratory of Plant Physiology and Biochemistry, Hiroshima University. Rice plant straw, husk, and grain samples were collected and kept at room temperature for further procedures. The extraction of samples was carried out and antioxidant activity, TPC, TFC, MA, and MB contents were measured. High-performed liquid chromatography (HPLC) and allelopathy assay were conducted. The results revealed that the inhibitory and suppressive capacity of the rice husk and straw on the germination of both tested plants differed. Of them, the rice straw exerted stronger inhibition compared to the rice husk. The highest germination inhibition of husk samples on the lettuce and barnyard grass was recorded in the origin cultivars; however, the highest germination inhibition of straw samples on lettuce and barnyard grass was 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 lettuce. In addition, the inhibitory and suppressive capacities of the rice husk and straw on the shoot and root lengths of both tested plants also differed. The rice straw exerted stronger inhibition compared to the rice husk. The highest shoot and root length inhibitions of husk samples on lettuce and barnyard grass were recorded in the origin cultivars; however, the highest shoot and root length inhibition of straw samples on both tested plants was observed in the mutant lines. Generally, mutant lines significantly showed higher shoot and root length inhibitions on both tested plants in straw samples but exhibited lower in husk samples. Both straw and husk samples exerted stronger shoot and root length inhibitions on barnyard grass compared to lettuce. Additionally, root length was much inhibited compared to shoot length in both lettuce and barnyard grass. Rice

variety with high phytochemical and momilactone contents displayed stronger allelopathy activities. Besides, ten major phenolic and flavonoid compounds including gallic acid, *p*-hydroxybenzoic acid, syringic acid, caffeic acid, ferulic acid, vanillic acid, *p*-coumaric acid, salicylic acid, cinnamic acid, and tricin were detected by HPLC in both rice 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. This research suggests that rice varieties release through the MNU mutation exerted higher allelopathy activities than their origin cultivars. Thus, this approach may be a good candidate for breeding allelopathy rice varieties to reduce the application of synthetic herbicides.

1. Introduction

Weeds are considered as one of the major biotic constraints to crop productivity and grain quality as well as the main causes of economic burden in agriculture production (Hong et al., 2004). To increase agriculture productivity, crop losses by the competition of weeds should be minimized. Weeds compete with crops for growth factors including nutrients, sunlight, water, and space and hinder cultivation practices, as well as they do not only decrease crop yields but also have negative effects on grain quality (Antralina et al., 2015). Additionally, they are alternate hosts for several pests and pathogens of the field crops (Baucom and Holt, 2009). Weeds are yearly responsible for 10% crop yield reduction and financial losses in agricultural production worldwide (Gharde et al., 2018). Rice yield reduction by weeds is estimated at 35% to 74% in the United States (Scavo and Mauromicale, 2020).

Weed management is a big challenge for farmers. Generally, weeds are managed through mechanical, chemical, and biological methods (Antralina et al., 2015). Chemical method is implemented with the application of herbicides which becoming increasingly problematic due to herbicide resistance and environmental pollution. Therefore, new methods should consider managing weeds and preventing environmental pollution. The suggestion that plants release specific natural products (allelochemicals) to suppress the germination and growth of their neighbor plants was observed a century ago and the term was named ‘allelopathy’ (Xu et al., 2012). Among the available approaches, allelopathy can be a tool to give greater sustainability to the agriculture production system through integration with the existing agronomic solutions.

Allelopathy is a biological phenomenon, a particular form of amensalism, simply understood as the ability of a plant to directly or indirectly suppress (as a harmful aspect) or stimulate (as a beneficial aspect) growth of nearby other plants in the environment by the production of chemical compounds called allelochemicals (Cheng and Cheng, 2015; Rice, 1984). In another way, allelopathy is the studying of chemical interactions and interferences on the growth of nearby plants mediated by bioactive secondary metabolites (referred at as allelochemicals), which produce and release from one plant on its neighbors (Khanh et al., 2018a). Allelopathic activities of the donor plants help them to protect themselves from insects, microorganisms, viruses, pathogens, and predators. Such activities can inhibit or stimulate the growth of neighboring plants or their seeds (Einhelling, 1996; Seigler, 1996).

Allelochemicals such as alkaloids, flavonoids, glucosinolates, hydroxamicacids, phenols, salicylates, and terpenoids are major plant secondary metabolites that are released to the environment through leaching, volatilization, root exudation, and decomposition procedure (Patni et al., 2018). Allelochemicals are natural survival compounds and have been released by a plant to decrease the competition of other neighboring plants as well as used in agriculture practices for weed control and crop protection since ancient times (Cheng and Cheng, 2015). Recently, it has been documented that plant secondary metabolites associated allelopathic potentials showed either inhibitory or stimulatory effects on the weeds (Vyvyan, 2002). These constituents were suggested to be beneficial for biological approaches of weeds and pathogens control (Rowshan et al., 2014; Scognamiglio et al., 2012).

Plants release these secondary metabolites by root exudation or aboveground pathway into nature in order to protect themselves and reduce the influence of certain abiotic and abiotic stresses. Numerous plant species were found out to secrete and release several constituents (allelochemicals) into the root zone called as rhizosphere to change the physical and chemical properties of the soil rhizosphere, which consequently affect the soil microbial community, fungi, and other neighboring plants (Hawes et al., 2000; McCully, 1999). The released allelochemicals are in a passive mechanism in the environment until they reach other plants or pathogens. The recipient plant or pathogen will promote or suppress when the degradation of these compounds began. In the last decades, several studies have demonstrated that rice plants produce specific allelochemicals to manage and suppress the growth of nearby weeds, particularly barnyard grass in paddy fields (Khanh et al., 2018b).

Attentions have been globally increased on plant allelopathic studies since the evidence of allelopathy potential which is applied as an important biological approach to inhibit and reduce weed growth and interference on crops and consequently increased crop yields is raising (Berendji et al., 2008). In the concept of sustainable agriculture, allelopathy is a promising approach to manage weed competitiveness; thus, exploration of incorporating allelopathic potential into genotypes and breeding programs should be a focal point for breeders to enhance crop resistance and increase yields (He et al., 2004). The competitiveness of crops with weeds caused by genetically allelopathic potential will reduce the cost and burden of conventional herbicides application; therefore, decreasing agrochemical inputs on agricultural fields (Parvez et al., 2003). Rice is considered to be one of the most important cereal crops worldwide and its allelopathic potential has attracted great attention, as the allelopathic capacity of the same genotypes has been evaluated on several noxious paddy weeds and other crops (Dilday et al., 1989; Patni et al., 2019).

Rice growth and yield are significantly influence and reduced due to weeds interferences each season associated with the highest losses of yield (Fujii, 2001). Farmers apply huge amounts of synthetic herbicides to control and reduce the interference of weeds. Nevertheless, the adverse impacts of commercial herbicides applied on the environment and their economic consideration makes it possible to diversify options and stimulate alternative methods for weed management in the agro-ecosystems (Putnam and Tang, 1986; Weston, 1996). To date, the exploitation and evaluation of plant's allelopathic capacity in agricultural practice as an approach of weed control have been observed in weed reduction, pathogen prevention, and soil enrichment (Kohli et al., 1998). Among the plants, the allelopathy activity of the rice plant has extensively studied and is mostly depends upon genetic backgrounds (Khanh et al., 2007).

Research has been conducted on rice with respect to its allelopathy potential as a part of the breeding strategy for sustainable weed management such as developing and releasing allelopathic rice varieties (Olofsdotter et al., 2002; Takeuchi et al., 2001). Studies reported that a total of 1800 weed species have interaction and competition with rice plant, of them, barnyard grass species (*Echinochloa* spp.) is the dominant weed and has been demonstrated as a strong competitor with rice in respect to rice production areas worldwide (Rao et al., 2007). Based on the phenotypic evaluation and breeding programs, a vast number of rice varieties with allelopathic potential were observed and released to inhibit the growth of several weed species and other plants when these rice varieties were cultivated together with the plants or weeds under field or laboratory conditions (Kato-Noguchi 2004). Additionally, Chung et al. (2003) compared more than a hundred rice varieties in terms of the allelopathic potential of their husks, leaves, and straw on germination and seedling establishment of barnyard grass.

Field and laboratory experiment screening programs in several countries have elucidated that allelopathic rice varieties release allelochemicals into the environment. Among the allelochemicals, phenolic acids are recorded as putative and imperative compounds that are mostly investigated as they have been found in a wide range of plants and soils (Dalton, 1999). Phenolic acids have been also detected in the soil of paddy fields, rice roots exudates, and decomposed rice residues (Kim and Kim, 2000; Seal et al., 2004). However, some studies revealed that the amounts of phenolic acids found in paddy soils are not sufficient to exhibit phytotoxic effects, leading to doubts about their relevance to rice allelopathy (Tanaka et al., 1990). In support of this view, phenolic acids usually are present in paddy soils at concentrations less than 5 mg/kg, which is below the threshold for phytotoxic effects, and additionally, allelopathic rice varieties do not release significantly greater amounts of phenolic acids than non-allelopathic rice varieties (Olofsdotter et al., 2002b).

A study was conducted by Patni et al. (2019) on rice mutant varieties treated with methyl salicylate (MeSA) and demonstrated that mutant varieties showed higher phytotoxic effects on the growth of barnyard grass. The allelopathic potential of rice varieties released by mutation technology is somewhat studied but the allelopathic activities of varieties released through MNU mutation are not yet investigated. Additionally, the relationship between the allelopathy and the rice grain quality is not well elucidated. Therefore, the aims of this study are to investigate the effects of selected mutant lines and origin rice cultivars on barnyard grass and lettuce germination and seedlings growth as well as to evaluate the possible involvement of phenolic acids in allelopathic effects from rice straw and husk extracts and to find out the relationship of grain quality with allelopathic potential in the rice plant.

2. Materials and Methods

2.1. *Plant Materials and Experimental Design*

This experiment was conducted in the Laboratory of Plant Physiology and Biochemistry affiliated to the Graduate School for International Development and Cooperation, Hiroshima University, Higashi-Hiroshima City, Japan. The research was carried out during the rice-growing season from May to October 2018 with a randomized complete block design with three replications and six cultivars and mutant lines, as shown in Table 1. The field experiment was conducted as described previously in Chapters 2 and 3. Afterward, plants were harvested at the maturity stage as well as the samples of rice straw, husk, and grain were collected and kept at room temperature for further procedures.

2.2. *Reagents and Standards*

All reagents and standard compounds incorporating 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS), butylated hydroxytoluene (BHT), gallic acid (GE), Folin-Ciocalteu's reagent (FC), sodium carbonate (Na_2CO_3), aluminum chloride hexahydrate ($AlCl_3$ $6H_2O$), rutin, potassium persulfate ($K_2S_2O_8$), hydrochloric acid (HCl), sodium hypochlorite (NaOCl), sodium hydroxide (NaOH), syringic acid ($C_9H_{10}O_5$), *p*-coumaric acid ($C_9H_8O_3$), *p*-hydroxybenzoic acid ($C_7H_6O_3$), ferulic acid ($C_{10}H_{10}O_4$), vanillic acid ($C_8H_8O_4$), caffeic acid ($C_9H_8O_4$), salicylic acid ($C_7H_6O_3$), gallic acid ($C_7H_6O_5$), cinnamic acid ($C_9H_8O_2$), and tricin ($C_{17}H_{14}O_7$) were analytical grades. The extraction and isolation solvents, as well as acetonitrile, were purchased from Junsei Chemical Co., Ltd., Tokyo, Japan and Fisher Scientific Co., Hampton, New Hampshire, USA. All Chemical materials for antioxidant scavenging assays were acquired from Fujifilm Wako Pure Chemical Corporation, Osaka, Japan. The remaining chemical constituents were bought from Kanto Chemical Co., Inc., Tokyo, Japan.

2.3. *Extraction and Samples Preparation*

The extraction of samples was carried out with the procedure explained previously (Kakar et al., 2019a). Shortly, an amount of 100 g brown rice, 20 g straw, and 46 g husk were pounded and steeped in quantities of 100, 250, and 200 mL methanol at room temperature for one week. Ahead of evaporation at 50 °C to get methanol extract, the obtained sample was filtered twice. Afterward, the extract was added to a separatory funnel and the same volume of hexane was combined to the funnel. Two layers (methanol and hexane) were created; the methanol layer was compiled and filtered at room temperature after 3 hours. The filtrated sample was then evaporated to achieve crude extract which consequently dissolved in methanol to justify the concentration of sample and stock at 4 °C for future uses.

2.4. *Antioxidant Assays*

Two assays (DPPH and ABTS) were conducted to measure the antioxidant activities of the rice grain, husk, and straw. The DPPH scavenging assay was measured by the method reported previously (Quan et al., 2019e) with some changes. In summary, an amount of 50 µL of the sample was added in a well of microplate followed with 50 µL of DPPH solution (500 µM in methanol) and 100 µL of 0.1 M acetate buffer (pH 5.5). The mixture was kept at room temperature and dark place to incubate for 20 min. The mixture was then inserted into the microplate reader and the absorbance of the sample was measured at 517 nm. 10, 20, and 50 µg/mL concentrations of BHT were applied as standards as well as pure methanol was utilized as a negative control. The IC_{50} of the sample was calculated based on the amount of the sample required to inhibit 50% of DPPH radical activity. Thus, the higher DPPH IC_{50} value means the lower antioxidant activity. The IC_{50} percentage radical scavenging activity of the sample was calculated based on the formula described in Chapter 3.

The ABTS cation discoloration assay was carried out through the technique reported previously (Kakar et al., 2019a). Shortly, the ABTS cation solution was prepared by combining 2.45 mM potassium persulfate with ABTS (7 mM) solution (1:1 *v*/*v*) in a dark covered tube. The obtained solution was then placed in darkness at room temperature and incubated for 16 hours. The working solution was achieved by diluting the obtained solution in methanol until the absorbance of the solution came to 0.70 ± 0.05 at 734 nm. Then, an amount of 40 µL of the extracted sample and 180 µL of the working solution were dropped in each well of a microplate and kept for 20 min in darkness at room temperature. The absorbance of the mixture was measured at 734 nm through the microplate reader. BHT was applied as a standard reference, whereas methanol was chosen as a control. The percentage of radical discoloration activity was computed through the formula reported in Chapter 3.

2.5. *Determination of TPC and TFC*

The Folin-Ciocalteu technique was selected to measure TPC as reported previously (Quan 2016; Kakar et al., 2019a). Each well of a microplate was filled out by 20 µL of the sample extract, 100 µL of Folin-Ciocalteu's reagent (10%), and 80 µL of sodium carbonate solution (0.7 mM *w*/*v*, Na_2CO_3), respectively. The prepared mixture was then placed in darkness at room temperature and incubated for 30 min as well as the absorbance of the samples was measured at 765 nm. A range of concentrations (2.5–60 µg/mL) from gallic acid was applied as a standard reference and the TPC was calculated by standard calibration curve of gallic acid. The TPC was presented as µg gallic acid equivalent per g dry weight of the sample (µg GAE/g DW).

The aluminum chloride colorimetric method was applied to evaluate the amount of TFC through the technique described previously (Kakar et al., 2019a). In brief, 50 µL of the extracted sample was added in a well of a microplate, followed by 50 µL of aluminum chloride (2% in methanol, *w*/*v*). The mixture was then kept in a dark place at room temperature for 15 min and the absorbance was recorded at 430 nm by the microplate reader. A range of concentrations (10–500 µg/mL) from rutin was applied as a standard reference to evaluate the amount of TFC in samples. Finally, TFC was calculated as µg rutin equivalent per g dry weight of the sample (µg RE/g DW) by standard curve calibration of rutin.

2.6. *Quantification of Momilactones A and B*

LC-ESI-MS system was used to determine and quantify the amounts of MA and MB in rice seedlings, following the technique reported by Quan et al. (2019a). The system contained an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, California, USA) and was facilitated by an electrospray ionization (ESI) source. The LC was carried out by injecting different concentrations of a sample at 3.0 µL into the column (Waters Cooperation, Milford, Massachusetts, USA) of the Acquity UPLC® BEH C18 (1.7 µm, 50 × 2.1 mm i.d.). The temperature of the column was adjusted at 25 °C and the chromatography was run with a flow rate of 300 µL per min in a gradient model. The gradient of the mobile phase was established based on solvent A (0.1% trifluoroacetic acid in water) and solvent B (0.1% trifluoroacetic acid in acetonitrile) as follows: (1) 0-5 min, 50% solvent A and 50% solvent B, (2) 5-10 min, 100% solvent B which was upheld for 0.1 min (Quan 2019c). Finally, the column was equilibrated for 5 min by the initial condition (50% solvent A and 50% solvent B) with a total operation time of 15.1 min. The ESI condition was kept in the same index as the method reported previously by Quan et al. (2019b). Extracted ion chromatograms (EIC) and mass spectra (MS) were applied to confirm the presence and availability of MA and MB in the rice grain, husk, and straw samples by comparison of them with the standard momilactones. The areas of MA and MB with the determined peaks in the EIC that matched with the standard MA (RT: 3.82) and MB (RT: 2.33) were used to calculate the amount of such compounds over a linear model. All buffer components used in the UPLC measurements were purchased from Sigma-Aldrich, St. Louis, Missouri, USA, however, isolated and validated pure MA and MB were provided by our laboratory previously (Quan 2019a).

2.7. *Allelopathy Activities*

The allelopathy assay was conducted based on the method previously reported by Quan et al. (2019d). Two plants including lettuce and barnyard grass were selected for allelopathy assay. Healthy seeds of lettuce and barnyard grass were soaked in distilled water at 30 °C for 2 days for water absorption and imbibition. Prepared filter papers (20 mm diameter) were placed in backers and an aliquot 200 µL of each sample extraction of husk and straw that was previously diluted with methanol at different concentration was added to the backers per filter paper and kept into the oven at 40 °C for 30 min to be completely dry as well as to subtract the effects of methanol on bioassay. An amount of 1 mL agar medium solution (0.5%) was added to 12-well plates and the treated filter paper was put on each well of the plates. The imbibed six seeds of the two tested plants were placed on each 12-well plate and cover with wrapping paper. The plates were then kept in the growth chamber with a photoperiod of 14 hour day and 10 hour night at 30 °C for 6 days. Pure methanol and water were used as a negative control. Each measurement was repeated three times. A general view of allelopathy assay is illustrated in Figure 13.

The germination percentage, as well as shoot and root lengths of lettuce and barnyard grass, were recorded. The inhibitory and stimulatory effects of the extracts on both crops were calculated based on the negative control (water). The IC_{50} value illustrated as the concentration that showed 50% inhibition and was calculated by a previously described method of Quan et al. (2019d). Each well of the plate that contained six seeds of lettuce or barnyard grass received an amount of 300 µL of distilled water prior to cover by wrapping paper and water was added based on the requirement. The lower IC_{50} value exhibited the higher inhibitory activity on the germination and seedling growth of both lettuce and barnyard grass plants.

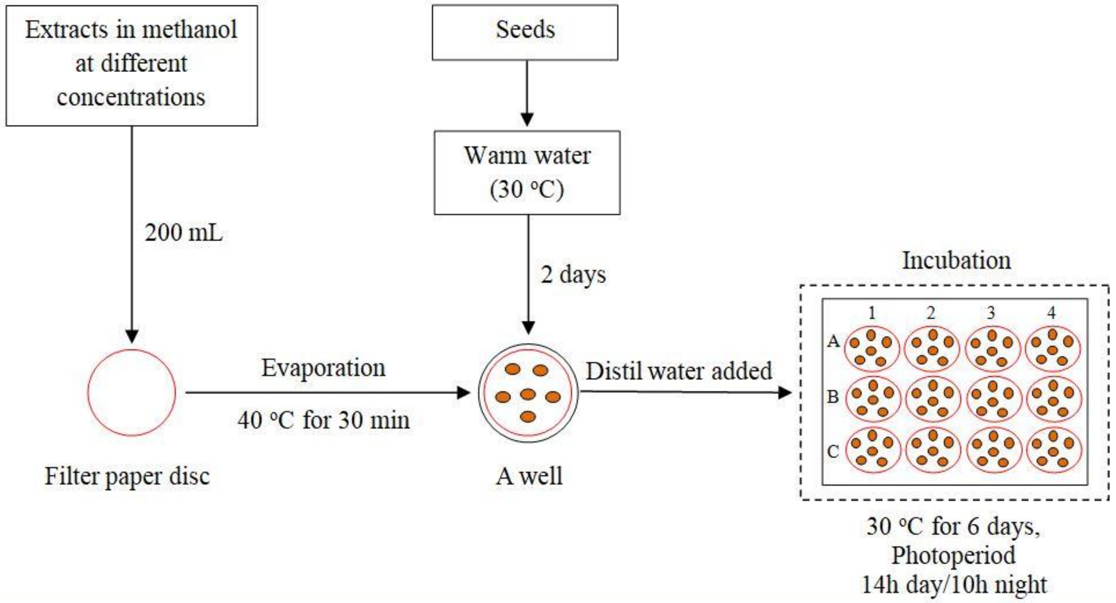


Figure 13. General experimental design of the allelopathic assay (Quan et al., 2019d)

2.8. *Identification of Phenolic Compounds by HPLC*

Phenolic compounds were identified by a high-performed liquid chromatography (HPLC) system following a method previously explained by Quan et al. (2016). An HPLC system (JASCO, Tokyo, Japan) composed of an LC-Net II/ADC, UV-2075 Plus, quaternary pump detectors, and an X Bridge BEH Shield RP18 (130 Å, 5 µm, 2.1 mm × 100 mm) column (Waters Cooperation, Milford, MA, USA) was applied for the identification and separation of phenolic compounds. The column temperature was kept at 25 ^{o}C. All samples were filtered through a 0.45 µm filter membrane. An aliquant of 5 µL extract for each sample of rice husk and straw was injected gently into the system. Gradient elution was adjusted and run with a 1 mL/min flow-rate using the following time gradients: 5% B (0–2 min), 5-70% B (2–12 min), 100% B (12–16 min) and maintain for 6 min, 100-5% B (22–24 min), and other 10 min for equilibration. Solvent A was 0.1% aqueous formic acid (CH_2O_2) and solvent B was 100% acetonitrile. The flow rate was at 4 mL/min. The wavelength of ultraviolet absorption of the defector was at 280 nm. An amount of 5 µL for several phenolic standards at different concentrations (1–100 ppm) was injected into the HPLC system and the retention times and areas were collected. The peaks of the rice husk and straw extracts were identified and calculated based on the retention times and peak areas of phenolic standards.

2.9. *Statistical Analysis*

Minitab 16.0 statistical software (Minitab Inc., State College, Pennsylvania, USA) was used to analyze the data. T-test and one-way ANOVA was carried out to evaluate the significant differences between the extracts of the mutant lines and their origin cultivars. Significant differences were defined at the $p<0.05$ probability level. Pearson correlation, as well as PCA, was subjected to determine the interaction among germination and seedling growth parameters with phytochemicals and momilactones. Data are presented as means ± standard errors (SE).

3. Results

3.1. *Inhibitory Activity of Extracts*

The inhibitory effect of the rice husk and straw extracts of all mutant lines and origin cultivars on the germination of lettuce and barnyard grass are illustrated in Figures 14 and 15, and Table 9. It was observed that the inhibitory and suppressive capacity of rice husk and straw on the germination of both tested plants differed. Of which, rice straw exerted stronger inhibition compared to rice husk. The highest germination inhibition of husk samples on lettuce and barnyard grass was recorded in K3H (an origin cultivar) which was 2016.6 µg/mL for lettuce and 1677.4 µg/mL for barnyard grass, followed by K1H, K5H, K2H, K6H, and K4H, accordingly. However, the highest germination inhibition of straw samples on both tested plants was observed in K2S (a mutant line) which was 1335.4 µg/mL for lettuce and 1574.0 for barnyard grass, followed by K4S, K6S, K1S, K3S, and K5S, respectively. Generally, 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 lettuce.

The inhibitory effect of the rice husk and straw extracts on the shoot and root lengths of lettuce and barnyard grass are presented in Figures 16 and 17, and Table 9. It was recorded that the inhibitory and suppressive capacity of rice husk and straw on the shoot and root lengths of both tested plants also differed. Of which, rice straw exerted stronger inhibition compared to rice husk. The highest shoot and root length inhibitions of husk samples on lettuce and barnyard grass were recorded in K3H (an origin cultivar) which was 2491.9 µg/mL (shoot) and 1991.4 µg/mL (root) for lettuce as well as 2183.1 µg/mL (shoot) and 1811.6 µg/mL (root) for barnyard grass, followed by K1H, K5H, K2H, K6H, and K4H, accordingly. However, the highest shoot and root length inhibitions of straw samples on both tested plants were observed in K2S (a mutant line) which was 1978.7 µg/mL (shoot) and 1954.2 µg/mL (root) for lettuce as well as 1672.6 µg/mL (shoot) and 1756.9 µg/mL (root) for barnyard grass, followed by K4S, K6S, K1S, K3S, and K5S, respectively. Generally, mutant lines significantly showed higher shoot and root lengths inhibitions on both tested plants in straw samples but exhibited lower in husk samples. Both straw and husk samples exerted stronger shoot and root lengths inhibition on barnyard grass compared to lettuce. Additionally, root length was much inhibited compared to the shoot length in both lettuce and barnyard grass plants.

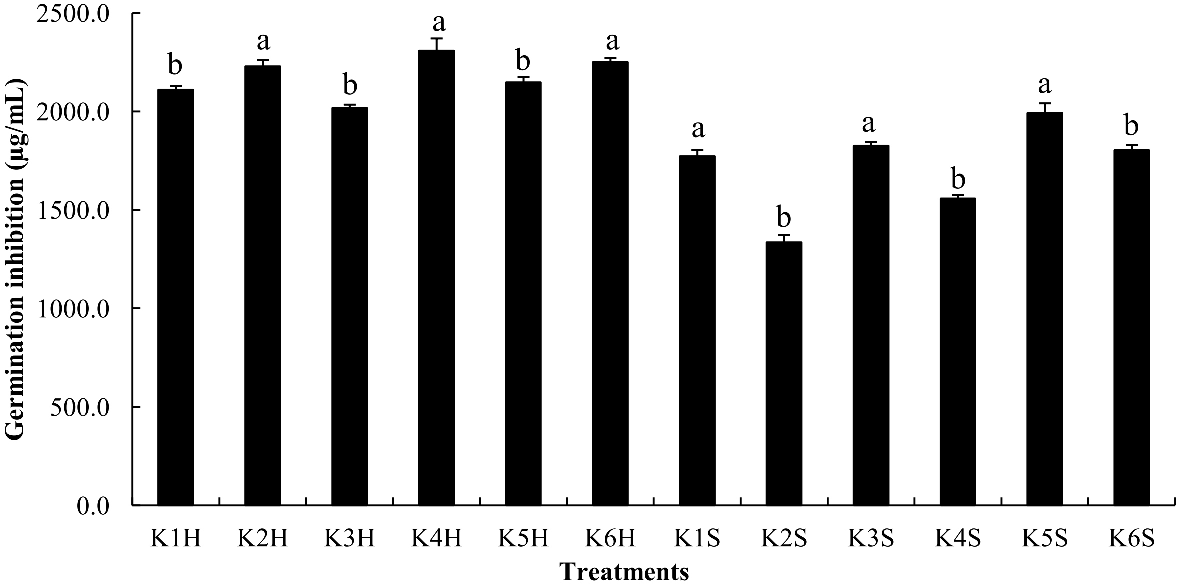


Figure 14. The IC_{50} value for inhibitory activity of rice husk and straw on the germination of lettuce. The same letters in bars of the original cultivar and its mutant line indicated no significant differences at the $p<0.05$ probability level based on the t-test

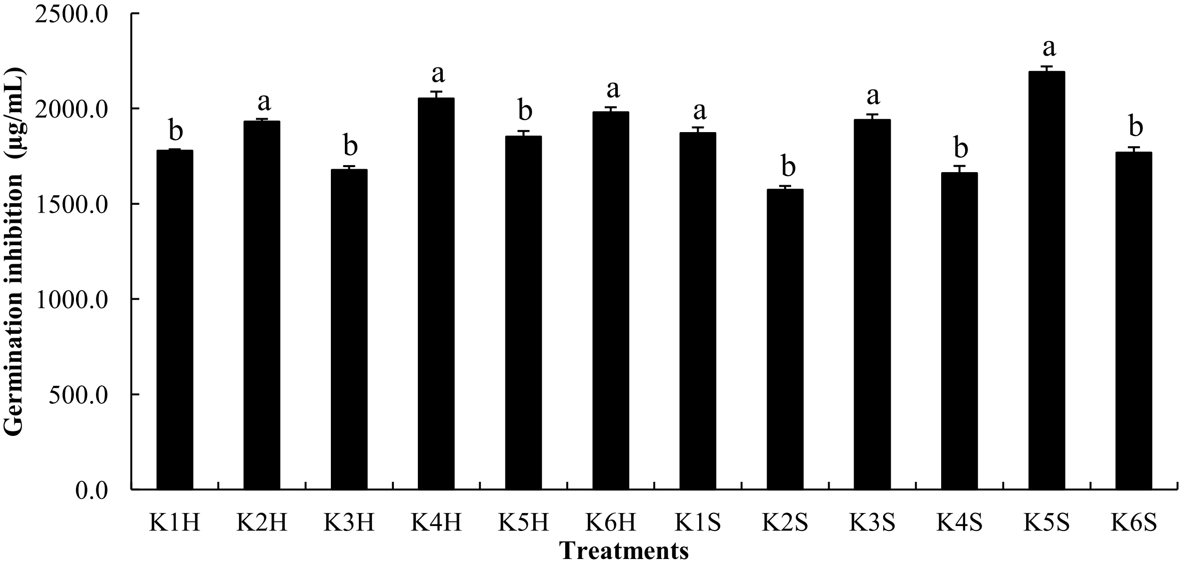


Figure 15. The IC_{50} value for inhibitory activity of rice husk and straw on the germination of barnyard grass. The same letter in bars of the original cultivar and its mutant line indicated no significant differences at the $p<0.05$ level based on the t-test

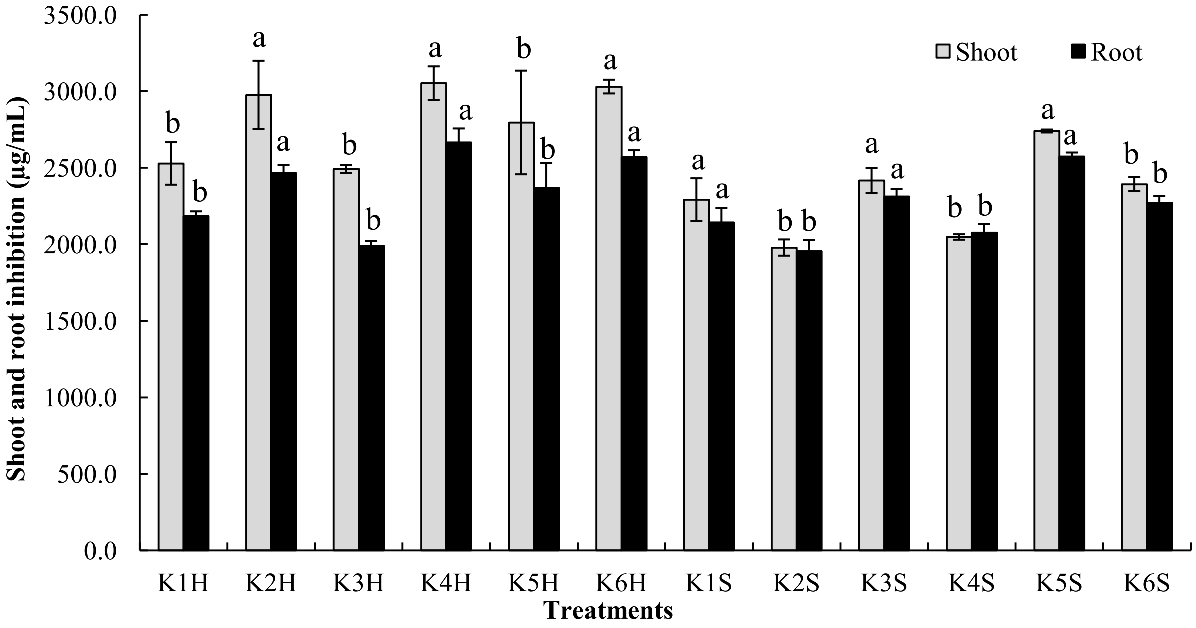


Table 16. The IC_{50} value for inhibitory activity of rice husk and straw on shoot and root length of lettuce. The same letter in bars within each portion of the original cultivar and its mutant line indicated no significant differences at the $p<0.05$ level based on the t-test

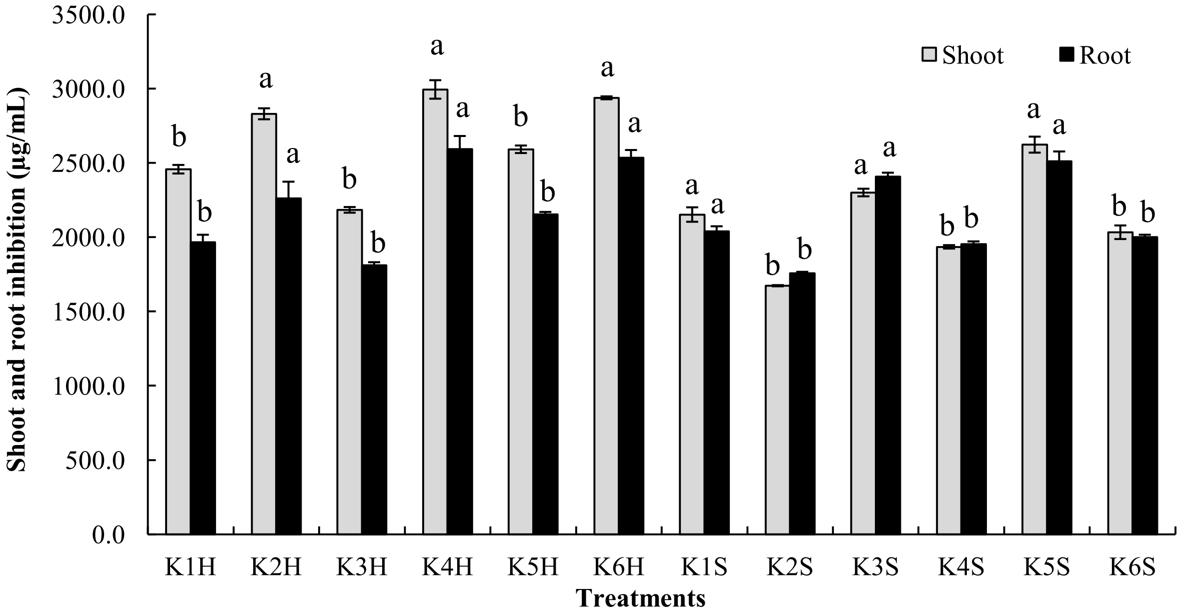


Table 17. The IC_{50} value for inhibitory activity of rice husk and straw on shoot and root length of barnyard grass. The same letter in bars within each portion of the original cultivar and its mutant line indicated no significant differences at the $p<0.05$ level based on the t-test

Table 9. The IC$_{50}$ value for inhibitory activity of rice husk and straw on the germination rate and growth parameters of selected plants

Treatment	**Lettuce (IC$_{50}$ value as µg/mL)**			**Barnyard grass (IC$_{50}$ value as µg/mL)**		
	Germination	**Shoot**	**Root**	**Germination**	**Shoot**	**Root**
K1H	2110.1 ± 17.4 b	2528.1 ± 138.6 a	2185.1 ± 28.8 b	1779.4 ± 16.4 b	2456.8 ± 30.1 b	1965.1 ± 50.5 a
K2H	2227.8 ± 33.1 cd	2976.4 ± 223.4 ab	2464.7 ± 37.1 d	1931.9 ± 13.4 d	2829.9 ± 54.2 c	2262.1 ± 60.7 b
K3H	2016.6 ± 17.6 a	2491.9 ±125.7 a	1991.4 ± 19.2 a	1677.4 ± 20.3 a	2183.1 ± 29.7 a	1811.6 ± 29.2 a
K4H	2307.4 ± 62.8 d	3053.0 ± 109.7 b	2665.2 ± 62.4 f	2053.3 ± 35.1 e	2993.8 ± 91.8 c	2594.0 ± 86.3 c
K5H	2147.7 ± 27.2 bc	2796.1 ± 138.8 ab	2368.8 ± 25.4 c	1852.4 ± 29.4 b	2591.1 ± 61.5 b	2153.5 ± 25.7 b
K6H	2249.8 ± 20.1 d	3030.7 ± 144.9 b	2569.8 ± 19.8 e	1980.1 ± 26.3 d	2937.4 ± 44.7 c	2535.7 ± 50.6 c
K1S	1772.6 ± 30.4 c	2291.7 ± 139.5 b	2143.5 ± 48.7 b	1871.7 ± 29.0 bc	2151.6 ± 92.9 cd	2038.4 ± 34.3 b
K2S	1335.4 ± 37.5 a	1978.7 ± 152.9 a	1954.2 ± 40.1 a	1574.0 ± 19.4 a	1672.6 ± 72.5 a	1756.9 ± 19.1 a
K3S	1825.6 ± 19.2 c	2418.0 ± 181.5 b	2313.3 ± 25.5 c	1940.7 ± 28.4 cd	2299.9 ± 49.4 d	2407.6 ± 25.8 c
K4S	1557.6 ± 17.4 b	2047.8 ± 117.6 a	2076.7 ± 11.6 b	1660.6 ± 37.8 ab	1934.1 ± 55.4 b	1953.0 ± 27.9 b
K5S	1990.7 ± 50.3 d	2740.3 ± 190.7 c	2573.1 ± 53.8 d	2192.6 ± 28.1 d	2622.3 ± 26.6 e	2510.2 ± 66.4 d
K6S	1803.8 ± 24.4 c	2392.4 ± 145.9 b	2271.0 ± 45.7 c	1768.8 ± 27.7 abc	2032.5 ± 45.2 bc	2000.4 ± 25.3 b

Values are presented as means ± standard deviations. Different letters within a column among husk (H) or straw (S) samples indicate significant differences at $p<0.05$ probability level based on Tukey's multi-comparison test

3.2. *Identification of Phenolic Compounds*

Rice husk and straw extracts were evaluated by the HPLC system and based on the peak comparison of several standards with the peaks from husk and straw extracts, phenolic compounds were identified and quantified which are listed in Tables 10 and 11. Ten major phenolic and flavonoid compounds including gallic acid, *p*-hydroxybenzoic acid, syringic acid, caffeic acid, ferulic acid, vanillic acid, *p*-coumaric acid, salicylic acid, cinnamic acid, and tricin were detected in both husk and straw samples of the origin cultivars and their mutant lines. However, the quantity of each compound was differed between husk and straw samples as well as among all six cultivars/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 (Table 10). 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 (Table 11).

3.3. *Correlation of Grain Quality with Allelopathic Activity*

The correlation coefficient among grain quality attributes with allelopathy activity of husk extracts is summarized in Table 12. Grain yield showed a significantly positive correlation with germination, shoot, and root inhibition. It recorded a negative relation with amylose content, TFC, MA, and MB. Amylose content exhibited a positive relationship with TFC, MA, and MB; whereas, it showed a negative relation with germination, and shoot and root inhibition. Besides, TFC, MA, and MB demonstrated significantly negative correlations with germination, and shoot and root inhibition. Germination inhibition displayed a strongly positive relationship with shoot and root length inhibition. Additionally, shoot and root length inhibitions recorded a positive correlation to each other.

Furthermore, the correlation coefficient among grain quality attributes with allelopathy activity of straw extractions is illustrated in Table 13. Grain yield did not show any correlation with germination, shoot, and root inhibition but it recorded a negative relation with amylose content, TFC, MA, and MB. Amylose content exhibited a positive relationship with TFC, MA, MB, germination, and shoot inhibition but did not display any relation with root inhibition. Besides, DPPH and ABTS showed a positive relation with MA, MB, germination, shoot, and root inhibition. The TPC also exhibited a negative correlation with germination, shoot, and root inhibition. MA and MB demonstrated a significantly positive relation with germination, and shoot and root inhibition. Germination inhibition displayed a strongly positive relationship with the shoot and root length inhibition in straw extracts too. In addition, shoot and root length inhibitions recorded a positive correlation to each other in the straw sample as they showed in husk extracts.

Table 10. Major phenolic compounds identified and qualified in the husk extracts of the original cultivars and their mutant lines by HPLC as µg/g dry weight

No.	Phenolic acids	Retention time (min)	Samples					
			K1H	K2H	K3H	K4H	K5H	K6H
1	Gallic acid	2.14	27.4±3.2b	34.3±4.1a	38.2±3.6a	24.9±2.2bc	26.1±3.4b	20.5±2.7c
2	*p*-Hydroxybenzoic acid	7.35	478.9±37.6b	292.7±26.8d	602.4±50.1a	166.3±10.7f	427.3±31.7c	266.7±14.3e
3	Syringic acid	8.12	124.2±12.6b	82.7±14.1c	168.4±13.4a	63.0±9.9d	112.3±11.7b	88.7±8.3c
4	Caffeic acid	8.33	26.0±2.6b	16.5±1.4c	34.1±4.6a	4.2±0.7d	24.3±3.2b	15.3±2.4c
5	Ferulic acid	9.58	113.0±10.4d	236.7±39.8a	176.0±9.5bc	129.5±9.6cd	111.8±7.6d	214.3±10.4ab
6	Vanillic acid	9.61	39.3±4.8bc	55.4±7.7ab	65.3±10.9a	17.1±2.9d	34.3±7.8c	25.1±4.3cd
7	*p*-Coumaric acid	9.70	369.8±30.8b	236.3±21.3c	571.9±57.9a	124.2±23.6d	356.7±38.4b	222.1±29.1c
8	Salicylic acid	10.43	997.6±74.2b	591.4±45.9d	1146.2±114.3a	374.0±54.4e	1136.5±151.5a	792.7±59.7c
9	Cinnamic acid	11.17	20.6±3.9b	12.0±1.8c	31.2±4.5a	5.5±1.2d	16.6±2.9b	11.5±1.7c
10	Tricin	12.26	3.4±0.3b	2.1±0.4c	4.9±0.6a	1.0±0.1d	3.4±0.3b	2.9±0.2bc

Values are illustrated as means ± standard deviations. The same letters within a row indicate no significant differences at the p<0.05 probability level based on Tukey's multi comparison test

Table 11. Major phenolic compounds identified and qualified in the straw extracts of the original cultivars and their mutant lines by HPLC as µg/g dry weight

No.	Phenolic acids	Retention time (min)	Samples					
			K1S	K2S	K3S	K4S	K5S	K6S
1	Gallic acid	2.14	50.8±3.8b	198.0±13.9a	26.4±2.8c	186.8±14.6a	21.1±3.3c	64.7±5.7b
2	*p*-Hydroxybenzoic acid	7.35	259.3±11.8b	497.6±20.5a	105.0±6.8d	471.3±16.2a	64.4±7.5e	181.9±12.8c
3	Syringic acid	8.12	336.7±39.4b	670.6±62.9a	137.6±17.0cd	607.2±56.7a	82.9±5.3d	225.5±31.3c
4	Caffeic acid	8.33	72.3±4.8a	29.4±3.2c	13.7±2.2d	50.0±6.3b	10.7±1.2de	7.1±1.4e
5	Ferulic acid	9.58	235.9±16.9b	189.4±14.3c	92.9±6.4d	263.9±25.7a	58.8±5.1e	104.6±7.4d
6	Vanillic acid	9.61	40.1±3.4a	31.6±3.1b	24.2±2.9c	41.2±5.8a	3.1±0.3e	12.2±2.6d
7	*p*-Coumaric acid	9.70	678.6±55.0c	1491.8±173.8a	404.5±88.7d	1121.4±158.2b	297.1±27.3d	409.4±26.6d
8	Salicylic acid	10.43	148.0±37.4d	1323.9±196.2a	66.4±10.7d	953.2±54.9b	71.7±11.9d	303.5±25.8c
9	Cinnamic acid	11.17	35.0±7.8c	41.6±11.6a	10.5±2.3d	38.4±8.5b	7.0±1.2e	13.3±2.9d
10	Tricin	12.26	11.0±1.3a	12.1±1.2a	1.3±0.1b	3.0±0.7b	2.7±0.4b	3.3±0.8b

Values are illustrated as means ± standard deviations. The same letters within a row indicate no significant differences at the $p<0.05$ probability level based on Tukey's multi comparison test

Table 12. The correlation coefficient of grain quality traits with allelopathy parameters of husk extractions

	GY	**AC**	**DPPH**	**ABTS**	**TPC**	**TFC**	**MA**	**MB**	**GI**	**SI**
AC	-0.770**									
DPPH	-0.325	0.191								
ABTS	-0.245	0.081	0.920**							
TPC	0.129	-0.023	-0.939**	-0.968**						
TFC	-0.849**	0.847**	-0.051	-0.178	0.277					
MA	-0.869**	0.791**	0.603**	0.500*	-0.401	0.720**				
MB	-0.860**	0.778**	0.599**	0.493*	-0.395	0.709**	0.996**			
GI	0.820**	-0.877**	-0.369	-0.326	0.231	-0.761**	-0.843**	-0.834**		
SI	0.799**	-0.684**	-0.299	-0.222	0.141	-0.733**	-0.738**	-0.715**	0.845**	
RI	0.842**	-0.864**	-0.291	-0.237	0.143	-0.826**	-0.832**	-0.822**	0.960**	0.806**

GY, grain yield; AC, amylose content; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TPC, total phenolic contents; TFC, total flavonoid contents; MA, momilactone A; MB, momilactone B; GI, germination inhibition; SI, shoot inhibition; RI, root inhibition. * and ** indicate significant differences at $p<0.05$ and $p<0.01$, respectively

Table 13. The correlation coefficient of grain quality traits with allelopathy parameters of straw extractions

	GY	**AC**	**DPPH**	**ABTS**	**TPC**	**TFC**	**MA**	**MB**	**GI**	**SI**
AC	-0.770**									
DPPH	-0.325	0.191								
ABTS	-0.245	0.082	0.920**							
TPC	0.129	-0.023	-0.939**	-0.968**						
TFC	-0.839**	0.847**	-0.051	-0.178	0.277					
MA	-0.869**	0.791**	0.603**	0.500*	-0.401	0.720**				
MB	-0.860**	0.778**	0.599**	0.493*	-0.395	0.709**	0.996**			
GI	-0.418	0.480*	0.882**	0.713**	-0.769**	0.177	0.691**	0.679**		
SI	-0.355	0.516*	0.754**	0.668**	-0.702**	0.172	0.647**	0.629**	0.914**	
RI	-0.294	0.398	0.819**	0.777**	-0.812**	0.026	0.603**	0.592**	0.916**	0.950**

GY, grain yield; AC, amylose content; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TPC, total phenolic contents; TFC, total flavonoid contents; MA, momilactone A; MB, momilactone B; GI, germination inhibition; SI, shoot inhibition; RI, root inhibition. * and ** indicate significant differences at $p<0.05$ and $p<0.01$, respectively

4. Discussion

Allelopathy is a biological approach that can suppress weed growth and reduce the interference of weeds on crop yield. Research related to the allelopathy potential of rice plant has been raised worldwide due to the increasing evidence of weed inhibition and yield improvement (Anuar et al., 2015; Berendji et al., 2008). Recently, several allelopathic potential rice varieties were identified and released to suppress weeds of the paddy fields and increase yields but the allelopathic potential of rice varieties released through MNU mutation has not yet been studied. Additionally, rice by-products including husk and straw are producing on a large scale throughout the world. These by-products are used in numerous aspects but less is applied for allelopathy purposes due to farmer's less knowledge. In the current research, rice husk and straw extracts were evaluated for the allelopathy activity on the germination and growth of lettuce and barnyard grass.

The reason that lettuce and barnyard grass was selected in this research is that lettuce is among the speedy germinating and growing crop. Its root and shoot measurement is conducting easily and accurately. On the other hand, barnyard grass is among the most dangerous, toxic, herbicide-resistant, and problematic weeds in agricultural land, particularly in paddy fields worldwide. In paddy fields, different species of barnyard grass competes with rice crop for growth and environmental factors including sunlight, water absorption, nutrition uptake, space, and other resources (Khanh et al., 2018a). One barnyard grass that emerged 40 cm from a rice crop can reduce the rice yield by 27% (Stauber et al., 1991). This weed can also bring negative alterations in the biomass, yield, and quality properties of rice grain including grain size, chalkiness, and gel consistency due to the influence and interference in canopy light transmission in rice plant (Baig et al., 2005; Yamori et al., 2016; Zhang et al., 2009 and 2017b).

The results of this study revealed that the inhibitory and suppressive capacity of the rice husk and straw extractions on the germination of both tested plants differed. Of them, the rice straw exerted stronger inhibition compared to the rice husk. The highest germination inhibition of husk samples on the lettuce and barnyard grass was recorded in the origin cultivars; however, the highest germination inhibition of straw samples on lettuce and barnyard grass was observed in the mutant lines. It is reported that the rice straw showed stronger inhibition effects compared to the rice husk and leaves, as well as the sticky rice varieties possessed the greatest allelopathic potential in contrast to the non-sticky rice varieties (Khanh et al., 2009).

Furthermore, 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 lettuce. Khanh et al. (2007) reported that the Japonica rice varieties exhibited higher allelopathic potential compared to the Indica or Japonica–Indica rice varieties. Besides, Asghari and Musavi (2002) researched 10 Iranian rice varieties to evaluate their allelopathic potential on two plants including barnyard grass and umbrella sedge (*Cyperus difformis* L.); they observed significant reduction and inhibition in the germination and seedling growth of these weeds by some of the rice varieties.

It is clearly observed in this study that the inhibitory and suppressive capacities of the rice husk and straw on the shoot and root lengths of both tested plants differed. The rice straw exerted stronger inhibition compared to the rice husk. The highest shoot and root length inhibitions of husk samples on lettuce and barnyard grass were recorded in the origin cultivars; however, the highest shoot and root lengths inhibition of straw samples on both tested plants was observed in the mutant lines. Generally, mutant lines significantly showed higher shoot and root length inhibition on both tested plants in straw samples but exhibited lower in husk samples. Both straw and husk samples exerted stronger shoot and root length inhibition on barnyard grass compared to lettuce. Additionally, root length was much inhibited compared to shoot length in both lettuce and barnyard grass. It was reported in previous studies that the inhibition of root length of barnyard grass was greater compared to the shoot length (Olofsdotter and Navarez, 1996) which is in line with the results achieved in this study. Additionally, He et al. (2012) stated that the allelopathic potential of the rice plant can greatly inhibit the root growth of the tested plant rather than the shoot growth. They further explained the reason for this interaction and noted that the greater inhibition and suppression of root growth may be due to the negative influence from the intimate contact of roots with the treated filter paper from extracted compounds.

Recently, clear and logical evidence has been recorded in plants that they can release several allelochemicals (phytotoxic compounds) into the environment to suppress nearby weeds and other plants. For example, numerous allelochemicals from rice plant including cytokinins, MA, MB, and phenolic acids were found and determined by releasing to the soil and environment during rice growth periods which have been responsible for weed suppressing (Kato-Noguchi & Ino, 2005). Therefore, the screening approach and evaluation of root exudates, straw, and husk portions should be conducted to explain and observe the allelopathic potential of the rice plant. Such a method is a better way to evaluate the actual allelopathy potential and capacity of rice extracts rather than the application of organic solvent extracts of rice residues. To date, thousands of rice landraces have been evaluated and assessed for their allelopathic capacity worldwide through either different screening methods or isolating allelochemicals compounds from rice by-products, residues, and root exudates (Berendji et al., 2008; Khanh et al., 2013).

In this study, the mutant lines recorded high tiller number, 1000-grain weight, total grain yield, and increased plant length compared to the origin cultivars as well as they showed stronger allelopathic potential than the origin cultivars. Some studies reported that phenotype characteristics of rice landraces such as high tiller capacity, grain weight, and erect leaves have exhibited greater allelopathic potential compared to the rice landraces with low tiller and grain weight characters (Khanh et al., 2013). Furthermore, Ahn et al. (2005) reported and suggested that the allelopathic potential of rice varieties is directly associated with high plant height, strong tillers, high grain yield, and sufficient leaf areas, which are in line with the results of the current experiment. The findings and results of Khanh et al. (2013) have shown that almost all Vietnamese rice varieties possessed stronger allelopathic potential on the germination and growth of barnyard grass.

Generally, rice husk exhibited the greatest antioxidant activity than rice straw. These variations were also significant among plant portions within each cultivar and mutant line. Additionally, TPC and TFC were higher in the straw followed by the husk. The mutant lines significantly increased the amount of TPC in the rice straw portion. Furthermore, both MA and MB were detected and quantified in rice husk and straw portions of the mutant lines and original cultivars. The amount of MA was greater than MB in both origin cultivars and mutant lines samples. Both MA and MB were higher in the rice straw compared to the husk. Studies reported that allelopathic potential is largely dependent upon the amount of MA and MB as well as different phenolic compounds (Chung et al., 2002; Kato-Noguchi and Ino, 2005).

The important groups of allelochemicals involve in rice allelopathic activity consists of steroids, alkaloids, flavonoids, terpenoids, tannins, and phenolic compounds (Areco et al., 2014). These compounds work through diverse mechanisms such as volatile compounds emission to the air, leaching, or root exudation to the soil. These allelochemicals suppress plant germination and growth as well as can be employed successfully against pathogens, weed reduction, and improving crop productivity (Xuan et al., 2005). Chung et al. (2002) reported that ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and *m*-coumaric acid were the main active allelochemicals and lead the way for the greatest suppression and inhibitory activity on barnyard grass seed germination, seedling growth, and seedling total dry weight. They further recommended that the mentioned above allelochemicals may be a crucial and key factor in the rice allelopathy against barnyard grass weed; thus, it plays a vital role in establishing and developing promised natural herbicides.

In this research, ten major phenolic and flavonoid compounds including gallic acid, *p*-hydroxybenzoic acid, syringic acid, caffeic acid, ferulic acid, vanillic acid, *p*-coumaric acid, salicylic acid, cinnamic acid, and tricin were detected in both husk and straw samples of the origin cultivars and their mutant lines. However, the quantity of each compound was differed between husk and straw samples as well as among all six cultivars/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. It can be proposed that *p*-hydroxybenzoic acid, *p*-coumaric acid, syringic acid, and salicylic acid may play the main role in the allelopathic potential of rice husk and straw samples.

The bio-herbicides derived and developed from allelochemicals may be a promising approach for the biological control of weeds and pests (Lin et al., 2006). Momilactones A and B identified in rice plant at higher than 1 and 10 μM concentrations, respectively inhibited and suppressed the growth of barnyard grass which is one of the most noxious and problematic weeds of the paddy fields (Kato-Noguchi et al., 2008; Kong, 2008; Rao et al., 2007). In the current study, it is found that mutant lines recorded high MA and MB in the rice straw and only MA in the rice husk compared to the origin cultivars. Thus, rice straw showed stronger inhibitory effects on germination and seeding growth of both lettuce and barnyard grass. It is demonstrated that rice plants release phytotoxic levels of momilactones into the rhizosphere from their roots during their entire growth period. Therefore, momilactones are the main element in rice allelopathy (Kato-Noguchi et al., 2008; Kato-Noguchi and Ota, 2013).

Genetic evaluations and studies have revealed that momilactones play important roles in rice allelopathy and weed inhibition. In a study, when momilactones were selectively removed from a complex mixture, it was observed that the allelopathic potential of extracts from rice root exudates was significantly reduced. It means and demonstrates the importance of momilactones which serve as allelochemicals and reflects the presence and availability of momilactones biosynthetic gene cluster in the rice genome (Kato-Noguchi and Ota, 2013; Xu et al., 2012). Kato-Noguchi et al. (2010) evaluated the allelopathic potential effectiveness of momilactones on lettuce, barnyard grass, and some rice varieties. They reported that momilactones A and B were more effective and play stronger inhibitory potential on the growth of barnyard grass compared to those rice cultivars. These results demonstrate and suggest that the allelopathy potential and toxicities of momilactone A and B may be much less on rice plant compared to those to other plant species (Kato-Noguchi and Ota, 2013). Thus, rice straw and husk are potential for weed management due to their phytotoxicity potential, availability, and low-cost characteristics (Munzuroglu and Geckil, 2002; Wang et al., 2001; Yulianto and Xuan, 2018).

5. Conclusion

Weeds are considered as one of the major biotic constraints to crop productivity and grain quality, and the main causes of economic burden in agriculture production. Rice growth and yield are significantly influence and reduced due to weeds interferences each season associated with the highest losses of yield. Allelopathy is the studying of chemical interactions and interferences on the growth of nearby plants mediated by bioactive secondary metabolites (allelochemicals), which produce and release from one plant on neighbors. The results revealed that the inhibitory and suppressive capacity of rice husk and straw on the germination and seedling growth of both tested plants differed. Rice straw exerted stronger inhibition compared to rice husk. The highest germination and seedling growth inhibition of husk samples on lettuce and barnyard grass were recorded in the original cultivars as well as the highest germination and seedling growth inhibition of straw samples on both plants were observed in the mutant lines. Generally, mutant lines significantly showed higher germination and seedling growth 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 lettuce. Mutant lines showed a higher content of gallic acid, *p*-hydroxybenzoic acid, syringic acid, *p*-coumaric acid, salicylic acid, and tricin compared to the original cultivars. This study explores the importance of MNU mutation on rice allelopathy and weeds management as well as exhibits the relationship between rice grain qualities with the allelopathic potential of its by-products.

Chapter 5
General Discussions and Conclusions

1. Rice Productivity

The impact of the green revolution is diminishing due to increasing demands of crop production and food commodities, especially for rice. The total area under rice cultivation and production are the same; however, the world population is becoming manifold. The available options to fulfill the demands are to enhance rice grain yield per unit land area through developing and releasing high yielding rice varieties and to bring more lands under agriculture cultivation and practices (IRRI, 1993; Yuan, 1994) which consequently can increase production. Developing rice varieties which have resistance against biotic and abiotic stresses through the application of conventional and modern biotechnology can enhance rice yields to meet world requirement (Khush, 2005). The released varieties have different morphological and physiological characteristics that contribute towards yield (Ashrafuzzaman et al., 2009; Yang et al., 2007). Therefore, morphological, physiological, and physicochemical properties of different rice varieties should evaluate to point out adaptable cultivars based on the cultivation region and yield requirement.

Speedy urbanization and the issue of climate change threatened rice cultivation and production which resulted in losing farmlands, decreasing food production, and affecting negatively the availability of grain for consumption, (Bergman and Xu, 2003; Khush, 2005; Rosegrant and Cline, 2003; Shi et al., 2008). All of the mentioned above and other factors, directly or indirectly, affect both the quantitative and the qualitative production traits of rice that is available for consumption. The rice scientific communities and researchers should constructively engage and work harder in solving the current and future challenges of rice cultivation, production, food security, distribution, and quality aspects. Therefore, the development and release of new genotypes with desirable characteristics and attributes must take place to improve rice production and grain quality.

Genotype performances and characters of a crop have an important and decisive role towards the utilization of several resources including agricultural inputs and finally production of economic yield. Manipulation and utilization of diverse genetic resources have contributed much towards fulfilling the increasing demands and needs of food and other living materials for the ever-rising world population (Hussain et al., 2014). Among the available approaches, artificially induced mutations are proposed and expected to play crucial and important roles in establishing new bio-resources for genomic studies and science of rice plant; such mutations have the power to enable the comparison mechanism between the original type and the mutant lines based on a genetically uniform background (Till et al., 2007). Therefore, mutagenesis is among the most effective and applied methods to achieve novel quantitative and qualitative improvements in the rice plant as well as other field crops through the best utilization of bio-resources.

Mutant populations, developed by different mutation methods, are imperative and indispensable genetic resources for functional genomics study in rice as well as in other organisms. Kyushu University in Japan maintains and reports more than ten thousand mutant lines along with their morphological, physiological, and biochemical characteristics (Satoh et al., 2010). The Kyushu University library of MNU induced mutants will be a principal functional genomics resource in the characterization and identification of mutation population in any rice gene and will complement other available mutant gene resources (Suzuki et al., 2008). Additionally, other countries including China and India also have a high record in releasing and utilization of mutation technology. However, few rice mutant populations have been released and developed that have suitable and desirable attributes.

The fundamental purpose of the rice breeding program is to develop and release improved and productive varieties; however, the other traits such as resistance to pest and disease, morphology, and others are secondary elements and are to support the main purpose. The success in developing improved rice varieties is directly proportional to the ability of the breeder to accurately identify research priorities and to correctly orient his goals and activities (Jennings et al., 1979). The greatest single factor facilitating rice improvement is the extraordinary varietal diversity found within *Oryza sativa* and its close relatives. Wide variability is the cornerstone of successful varietal improvement programs. Additionally, around 35,000 rice varietal accessions are maintained at the International Rice Research Institute (IRRI) and further collecting continues in geographical areas of special interest. Thus, a catalog of field observations and laboratory descriptions of the rice accessions is available to all rice workers worldwide. However, few programs can maintain even a small percentage of the collected varieties due to the enormous difficulties involved in the procedures. Fortunately, IRRI can provide seeds with specific characters to breeders throughout the world based on the catalog and request (Jennings et al., 1979; McKenzie et al., 1994).

Recently, mutation technology has been applied more in recent decades to achieve novel products that are not commonly found in nature. Thus, the breeder can apply various methods, particularly, the MNU mutation on the available accessions from IRRI to developed new and improved rice varieties. Based on the results achieved in Chapter 2, it is concluded that MNU mutation brought alteration in growth parameters, yield and its component, and physicochemical properties of rice grain. The results showed that plant length, tiller number, and panicle length were higher in the mutant lines than those of their cultivars. Furthermore, mutant lines took longer time to reach the heading and maturity stage. The highest panicle number, spikelet number, ripened grain ratio, 1000 grain weight, 1000 brown rice weight, and grain yield were obtained in mutant lines, as compared to the original 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).

It is assumed that MNU mutation is a promising approach that can increase rice productivity and improve rice grain physicochemical properties. Additionally, Grain yield exhibited positive correlations with panicle number, spikelet number, ripened grain ratio, and 1000 grain weight. In addition, grain yield had negative relations with amylose, protein, and lipid contents but showed a positive correlation with taste score. Huong et al. (2020) reported that the M2 and M3 generation of rice varieties treated with the MNU mutation showed greater salinity tolerance. Therefore, new rice varieties should be treated with MNU mutation to develop novel rice based on cultivation region and cultivation purpose as well as all growth parameters and agronomical practices should consider in the breeding program to increase rice productivity and grain quality. Further research should also conduct to screen the phenotype and genotype performances of newly developed rice varieties for better adaptability and high yield.

2. Rice Grain Quality

Grain quality is a complex and multi-factorial character of rice which is determined by its physical, chemical, and nutritional evaluations. The physical traits are consisting of appearance, shape, size, chalkiness, and perfectness. However, chemical and nutritional aspects are including the content of amylose, protein, and lipid as well as other elements that have an influential part in cooking and eating quality (Juliano et al., 1992). After the milling, rice grains are separating into two categories which are the whole and the broken grains. The first category is the percentage and presence of head rice (unbroken grain) and the second is the percentage or total amount of rice grains (the sum total of unbroken and broken rice grains) which are based on the initial weight of rough rice. The presence of the head rice yield is more important and critical than the total rice yield as it differed considerably due to several factors (Alcantara et al., 1996).

Moreover, rice grain length, width, and length-to-width ratio are important and considerable elements of appearance quality (Anh et al., 2019; Kakar et al., 2019c). It is reported that grain length and width mostly depend on the genotypic character and agronomical practices (Noori et al., 2017). Additionally, it is stated that rice grain appearance quality is associated with grain nutritional quality (Anh et al., 2019; Xu et al., 2004). Grain length and width is differed based on region and consumer preferences. For example, the short and medium grain is acceptable and preferable for Japonica rice consumers, while consumers from tropical Asian countries mostly prefer rice grain with a medium to long or some extra long grain size. The grain length is more important and variable than grain width, thickness, and shape; although a slender to medium rice grain width usually has the highest demand in markets (Cuevas et al., 2016; Nadvornikova et al., 2018).

On the other hand, rice grain endosperm is composed of starch (amylose and amylopectin), protein, and lipids. Amylose is highly available in non-glutinous rice varieties and is the linear fraction of starch; however, amylopectin is the branched fraction of starch and provides up the remaining of the starch (Kakar et al., 2019b). Both the contents of amylose and amylopectin have the main influence on the performance of cooked rice. It is reported that amylose negatively correlated with taste score for cohesiveness, color, and tenderness of the cooked rice. Williams et al. (1958) classified rice varieties into four groups based on the amylose content, (1) waxy rice (which has 1 to 2% amylose), (2) low amylose rice (which has 8 to 20% amylose), (3) intermediate amylose rice (which has 21 to 25% amylose), and (4) high amylose rice (which has more than 25% amylose).

The high amylose rice varieties are also known as non-waxy or non-glutinous rice grains which have amylose content ranged from 8 to 37% but the majority of them used in the human diet are ranged from 3 to 32% (Syahariza et al., 2013). These varieties account for the most cultivated and consumptive rice grain of the world's rice which are commonly consumed in tropical and south Asian countries. Rice varieties with high amylose contents are single grain, non-sticky, and not glossy after cooking as well as they readily split if overcooked (Gao et al., 2014). Waxy rice or glutinous is a variety with high amylopectin and low amylose and is the staple food in some Asian countries. This kind of rice grain is mostly used to prepare rice cakes, sweets, desserts, puffed rice, and parboiled rice flakes due to its lower water absorption and volume expansion during the cooking period. Glutinous rice is becoming very sticky, moist, and glossy after cooking. The glutinous attributes of this rice type are controlled by a single recessive gene but characteristics of waxy rice can be managed and modify through alteration in its genes.

Rice grain is classified into three sub-types (Japonica, Indica, and Javanica) based on the origin and amylose content. The Japonica rice varieties have high amylopectin and low amylose content as well as they are sticky and glossy after cooking. On the other hand, Indica rice varieties are high in amylose and vary mainly due to the content of amylose based on consumer preference and regional differences. Indonesia and the Philippines are the intermediate amylose content acceptable and referable consumers, it is probably due to the softness of cool rice grain after cooking. Studies reported that high amylose rice varieties are largely cultivated and produce in Asian countries but some countries prefer intermediate or low amylose, while consumers from Latin American are generally preferred intermediate types. The world's major markets mainly preferred intermediate amylose rice grains (Syahariza et al., 2013).

Rice is rich in carbohydrates and protein, and nutritionally superior to other several foods containing carbohydrates. The milled rice grain contains 7% protein while it accounts for 8% in the brown grain; although, protein content is largely differed and depended upon varietal difference, cultural practices, and environmental conditions. Rice grain protein exceptionally has numerous and balanced amino acids. For example, lysine content in rice grain protein is average from 3.8 to 4.0%. It is well documented that rice grain protein content is greatly influenced by the application of fertilizers, particularly the amount of nitrogenous fertilizer (Kakar et al., 2019b). Improved and high yielding rice varieties increased the content of protein compared to local and traditional varieties when treated with little or no nitrogen fertilizer application. However, improved cultural practices and abundant nitrogen fertilizer application increased both grain yield and protein content of the new high yield rice varieties. These results demonstrate that the new improved and high-yielding varieties enhance protein consumption in the human diet (Martre et al., 2006; Shewry, 2007).

Hence, attempts regarding the improvement and development of novel rice with better grain quality and higher productivity have been increasing. Among available approaches, mutation is a direct alteration on the genome and is considered as one of the most beneficial routes to acquire new beneficial traits in rice. Mutation technology, especially, MNU mutation has been applied more in recent decades to achieve novel products that are not commonly found in nature. The results achieved in Chapters 2 and 3 showed that lower amylose, protein, and lipid contents were observed in mutant lines compared to those in the origin cultivars. Additionally, the taste score, which increased from 67.7 to 82.3, was found to be correlated with lower amylose, protein, and lipid contents. Furthermore, a linear correlation was observed between the grain yields with amylose content and taste score.

The mentioned above results highlight and suggest the importance of MNU mutation in terms of rice yield improvement with preferable grain quality. Thus, MNU mutation should consider as one of the promising rice breeding approaches due to its numerous improvement in rice grain physicochemical properties. This approach is a time-saving method and can be easily applicable in all developed, developing, and underdeveloped countries. As MNU mutation enhanced rice productivity and grain quality parameters, thus, it can be applied to ensure food security and achieve desirable grain quality.

Cooking and eating quality of rice is also an important trait in consumer's preferences and market value. Grain elongation, water absorption, gel consistency, alkaline digestion, softness, smell, taste, cooking time, pasting temperature are some important traits in cooking and eating quality (Hori et al., 2016; Tan et al., 1999). In the current study, I only focus on the physicochemical properties and appearance quality of the rice grain from the original cultivars and mutant lines. Thus, further research should be conducted to evaluate the cooking and eating quality of rice grains developed by MNU mutation technology.

3. Rice Plant Biological Activity

Rice provides the major source of calorie intake for many people of the world and about 60% of the world population involved rice in their daily diet. Rice whole grain which also called as brown rice is the unpolished type that consisted of bran, embryo, and endosperm as compared to the polished rice grain (Imam et al., 2012). Whole rice grains have high nutritional value; therefore, the majority of health-promoting characteristics have been related to the consumption of brown rice grain. As a result, high attention and consideration have been devoted by the scientific community and researchers towards the identification, quantification, and isolation of bioactive constituents from brown rice grains (Halliwell, 2007). In the recent decade, progresses and contributions in the quantitative and qualitative determination of several bioactive compounds including phenolic acids, flavonoids, momilactones and others in the rice plant have been highlighted to provide natural antioxidants and other health beneficiaries. Thus, special consideration has been paid to the most recent approaches and strategies for the identification and extraction of the important and target compounds from rice plant along with the analytical methods implicated for the identification, separation, quantification, and isolation of phenolic and other major compounds (Ciulu et al., 2018; Halliwell, 2007).

Antioxidants reduce the level of free radicals in the human body and maintain the homeostatic balance as well as improve the human defense system for the proper functioning of the body. When the reactive oxygen species are highly overwhelming in the human body, they inhibit and surpass the activity of the antioxidant defense system making a situation known as oxidative stress (Saeed et al., 2012). This condition and imbalance between antioxidant and oxidative stress lead the way for certain disorders and diseases including diabetes, cardiovascular, atherosclerosis, cancer, etc (Sultan, 2014). The possible way and remedy for these diseases and conditions are the supplementing of the antioxidant defense system. Recently, plants have attracted considerable interest in managing oxidative stress and its related diseases due to their ethno-pharmacological properties applies in preventing diseases as well as their richness in important phytochemicals which associated antioxidant properties (Vanessa Fiorentino et al., 2013). Therefore, phenolics from plant sources are increasingly being applied in the food industry to improve the nutritional value and quality of processed foods (Kahkonen et al., 1999).

As mentioned before, plants are potential sources of natural antioxidants, of them, antioxidants and other important compounds from the rice plant are protective against various chronic diseases (Ames et al., 1993; Vinson et al., 1995). The protective effects of these natural compounds have been related to several constituents such as carotenoids, vitamins, and phenolic acids (Paganga et al., 1999). Many researchers have focused and documented the biological activities of phenolics and demonstrated their antioxidant potential and free radical scavenging activity as potent therapeutic agents (Kahkonen et al., 1999; Sugihara et al., 1999). The antioxidant capacity of phenolics is highly dependent upon their redox reaction properties, which permits them to act as hydrogen donors, reducing agents, and singlet oxygen quenchers (Chandra et al., 2014; Rice-Evans et al., 1996; Stephane et al., 2012).

The majority of antioxidants available in markets are synthetic, thus concerns related to their instability and carcinogenic activity are rising (Goufo et al., 2014). On the other hand, natural origin antioxidants have attracted great and considerable attention from scientists and consumers (Ramalakshmi et al., 2008). Among the major constituents, phenolics are important and highly associated with antioxidant activity. There are many varieties of foods such as fruits, vegetables, cereals, and others that have these compounds (Dimitrios, 2006; Holden et al., 2005). In addition, concentrations and types of phenolics are largely varied due to the genetic background, processing method, and environmental conditions (Kris-Etherton et al., 2002). Thus, the uptake concentration of phenolics differs highly among the diets, relating to the quantity and type of consumed food. Benzoic acid and cinnamic acid's derivatives are the two subgroups of phenolic acids (Balasundram et al., 2006).

Phenolic compounds may possess their antioxidant activity in various pathways. Firstly, they may scavenge some oxidative reactive species directly through hydroxyl, superoxide, peroxyl, and other radicals which are acting as chain-breaking antioxidants (Halliwell, 2007). Secondly, they may inhibit lipid per-oxidation by recycling other antioxidants. Some phenolic compounds may prevent the formation of free radicals through the bind pro-oxidant metals including iron and copper or maintain simultaneously their potential to scavenge free radicals (Moran et al., 1997; Kris-Etherton et al., 2002). Furthermore, antioxidant enzyme activity and antioxidant protein synthesis induction can greatly affect and increase the effectiveness of some phenolics (Chiang et al., 2006; Chung et al., 2006).

Plant phenolic constituents are classified into several categories, major among them are the flavonoids which have possessed potential antioxidant activities (Nunes et al., 2012). Flavonoids are naturally occurring compounds in plants and are considered to exhibit positive effects and roles on human health and pharmaceutical industries. Researchers have reported a large range of activities from flavonoid derivatives such as anti-viral, anti-bacterial, anti-allergic, anti-inflammatory, and anti-cancer activities (Di Carlo et al., 1999; Montoro et al., 2005). Other studies have stated the effective scavenging activities of several oxidizing molecules of flavonoids including various free radicals and singlet oxygen implicated in many human diseases (Bravo, 1998).

Flavonoid compounds in plants are formed by 15 carbons, three-carbon chain link, and organize the two aromatic rings (Ross and Kasum, 2002). Flavonoids can be subdivided into several classes. The anthocyanidins are the most famous pigments in plants that are responsible for the majority of the colors such as red, purple, pink, and blue. These pigments are acting for the attraction of the animals to lead the way for pollination and seed dispersal. Tannins on the other hand are another group of flavonoids with defense characteristics of plants and are divided into hydrolysable and condensed tannins (Kong et al., 2003). Tricin is among the flavonoids that reported as anti-skin aging agent (Quan et al., 2019a)

Momilactones A and B are important constituents in the defense system and pathway of the rice plant. Previous studies discovered that momilactones A and B from rice grain and bran have potential diabetic and obesity inhibitory activities as well as their biological activities including allelopathic, anti-skin aging, anti-bacterial, anti-fungal, and anti-cancer were also documented from work of literature (Quan et al., 2019a). MA and MB, as plant growth inhibitors, were firstly isolated from rice husks, while latterly reported as phytoalexins to fungal rice blast disease from rice leaves and straw. The function of MA and MB is extensively documented as they are among the potential growth inhibitors identified in the rice plant (Kato-Noguchi and Ota 2013). Due to their high price, MA and MB cannot be applied to develop new herbicides; however, they can be useful to use in pharmaceutical industries as well as natural products.

In the current studies, it is observed that the antioxidant activities were higher in grain and straw of mutant lines, whereas origin cultivars showed greater antioxidant activity in the rice husk. Additionally, mutant lines displayed higher 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 TFC was observed in the husk of origin cultivars, while mutant lines significantly enhanced TFC in straw. Both MA and MB, two compounds obtaining anti-diabetes, anti-cancer, anti-microbial, anti-gout, and anti-obesity properties, were detected and quantified in grain, husk, and straw of origin cultivar and mutant line 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.

Besides, grain yield showed a significantly positive relation with taste score, DPPH, and ABTS, whereas it showed a negative correlation with amylose content, TFC, MA, and MB.

Grain yield did not correlate with PC, LC, 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. Antioxidant activities exhibited a significantly positive correlation with taste score and grain yield, but a negative relation with TFC, MA, and MB. TFC displayed a significantly positive interaction with amylose and a negative relation with taste score, grain yield, DPPH, and ABTS. Momilactones A and B displayed a significantly positive correlation with amylose content and TFC but showed a negative relation with taste score, grain yield, DPPH, and ABTS. MA and MB showed a strong positive interaction with each other.

These studies illustrate that MNU mutation can improve grain quality and enhance bioactive compounds in straw, husk, and grain of the rice plant. Thus, this technology has the potential to develop functional foods from rice, and therefore help farmers in developing countries to improve value in rice production. Both rice husk and straw are produced on large scales in the rice-growing areas. They are used as organic amendments to improve soil quality and in some areas as fuel-related components. However, both of them are high-value by-products and can be used as natural sources for antioxidants, herbicides, and other essential compounds. In addition, it has been proven recently by scientists that physical exercise and proper nutrition management are more crucial in the prevention of diseases (Dietz et al., 2016). Thus, the development and application of medicinal cereals is an upcoming approach in drug improvement studies related to diets from natural products.

4. Rice Plant Allelopathy

The application of natural compounds is much friendly environmental compared to the most synthetic herbicides used for weed management (Duke et al., 2000). In several cases, the natural constituents are highly active and prevent weeds and pathogens at a molecular target concentration (Streibig et al., 1999). Plant secondary metabolites including phenolic compounds and momilactones have not only been evaluated by ecologists and pharmacologists due to their health benefits but also extensively investigated by phytochemists for their complex biological activities and weed inhibition. Numerous secondary metabolites identified in plants and other organisms have been considered to be potential allelochemicals and play crucial and important roles in the interaction networks between living communities (Li et al., 2010).

Several studies reported that phenolic compounds and momilactones play a major role in weed management and allelopathy (Ranagalage et al., 2015), but due to complex activities, it is not clearly cited that which compound has the high allelopathic potential as well as the actual modes of improved rice interaction with the allelopathy activity is remained unclear (Zhang et al., 2018). This research provides new insights into the allelopathic potential of rice straw and husk extracts on the inhibition of lettuce and barnyard grass germination and seedling growth as well as how the rice grain quality attributes have interactions to inhibit the growth of both tested plants through allelochemical pathways.

As mentioned previously, barnyard grass is among the dangerous and worse rice weed worldwide. Due to its excellent ecological adaptation and superior biology, it causes the problem in about 61 countries for over 36 crop species (Baig et al., 2005; Yamori et al., 2016). The strong allelopathic potential of barnyard grass leads the way for the wide application of it in the rice allelopathy research as an indicator (Dilday et al., 2001; Khanh et al., 2018b). Barnyard grass competes with the rice plant for light, space, water, and nutrition (Chin, 2001; Malik et al., 2003). One barnyard grass plant distanced 40 cm from a rice plant can significantly decrease tiller number and consequently reduce the rice yield by 27% (Mennan et al., 2012; Stauber et al., 1991). The global reduction of rice yield because of the competition from barnyard grass is estimated at around 35% (Oerke and Dehne, 2004). Some studies showed that barnyard grass is not only competing with the rice plant and removing nutrients from the soil but also hosting several pathogens (Allen et al., 1995; Holm et al., 1977).

The current research demonstrated that the inhibitory and suppressive potential of the rice husk and straw on the germination and seedling growth of both tested plants 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 length inhibitions of husk samples on the lettuce and barnyard grass, were recorded in the origin cultivars; however, the highest germination, shoot, and root length 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.

The allelopathic activities of rice husk and straw from the original cultivars and their mutant lines were evaluated on the germination inhibition and seedling growth of lettuce and barnyard grass in the current experiment under laboratory conditions. The on-farm allelopathic activity of rice varieties developed by MNU mutation is still unclear. Therefore, several field research is needed to carry out to evaluate the allelopathic potential of the MNU mutation developed rice varieties on the germination inhibition and seedling growth of several weeds particularly barnyard grass under open field conditions.

Ten major phenolic and flavonoid compounds including gallic acid, *p*-hydroxybenzoic acid, syringic acid, caffeic acid, ferulic acid, vanillic acid, *p*-coumaric acid, salicylic acid, cinnamic acid, and tricin were detected in both husk and straw samples of the origin cultivars and their mutant lines. However, the quantity of each compound was differed between husk and straw samples as well as among all six cultivars/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. Research should be conducted from the pure standards of the mentioned above compounds to confirm whether *p*-hydroxybenzoic acid, *p*-coumaric acid, syringic acid, and salicylic acid may play the main role in the allelopathic potential of rice husk and straw samples or not.

Phenolic compounds, as well as momilactone A and B, may have a potential role in the establishment and development of novel plant control agents due to their selective suppression and inhibitory characteristics for different weed species. More importantly, identification, quantification, and isolation of phenolic compounds and momilactones as allelochemicals in rice plant provides important tools and molecular markers for breeding or biotechnological engineering programs to direct and increase the allelopathic potential of this critical staple food crop. Besides, rice straw and husk are potential sources for weed management due to their phytotoxicity potential, availability, and low-cost characteristics. Additionally, MNU mutation exhibited novel attributes and potential allelopathic activities compared to the original cultivars. Thus, it can be a good and promising approach to improve the allelopathy potential of the rice plant and therefore reduce the application rate of herbicides in the paddy field.

Further research and investigations should be conducted on DNA sequencing and genetic modification, adaptation, pests, and diseases resistance of the rice varieties developed through the MNU mutation approach based on the cultivation region. As the developed mutant rice varieties are also considered as genetically modified plants; thus, permission from an authorized organization for food safety and security is needed for producing grains and by-products for marketing purposes as well as food industries.

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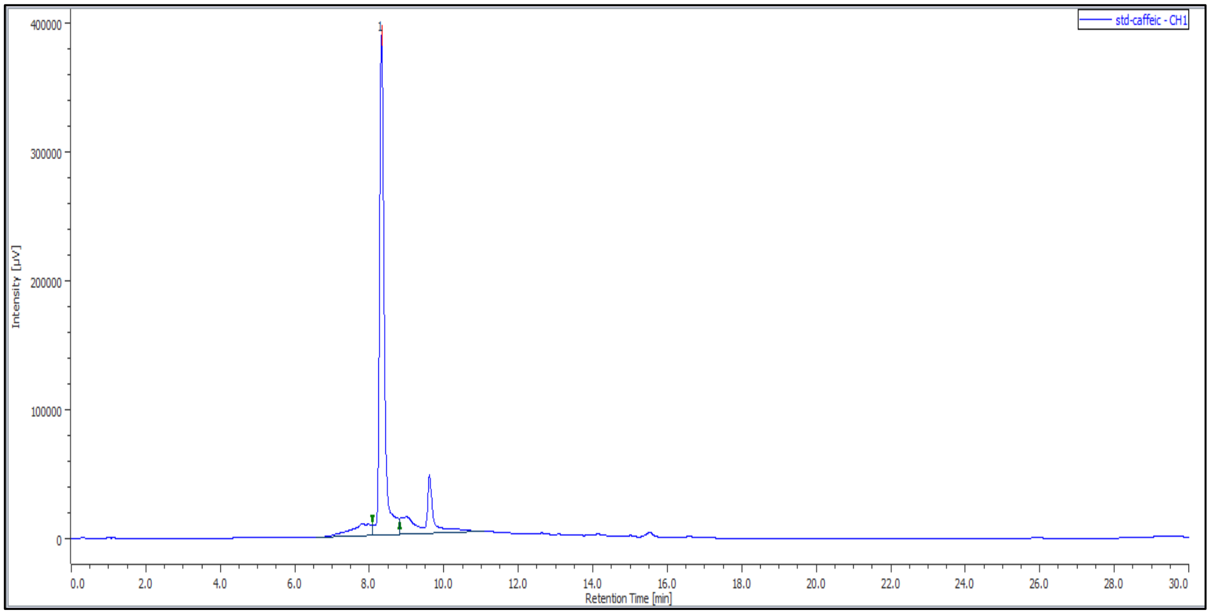
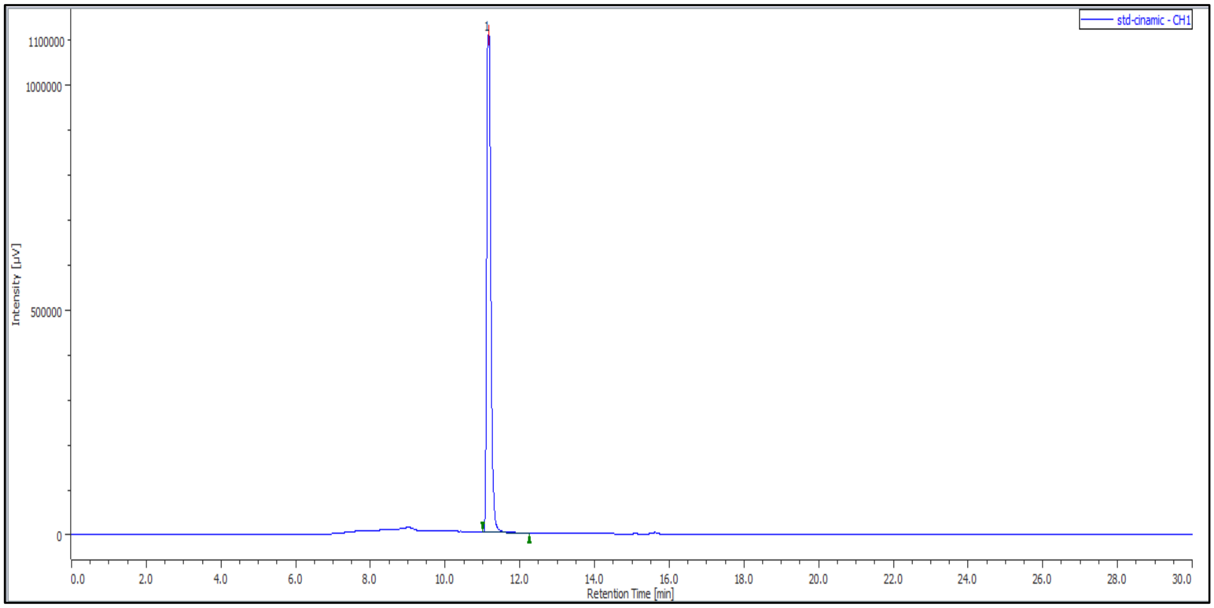
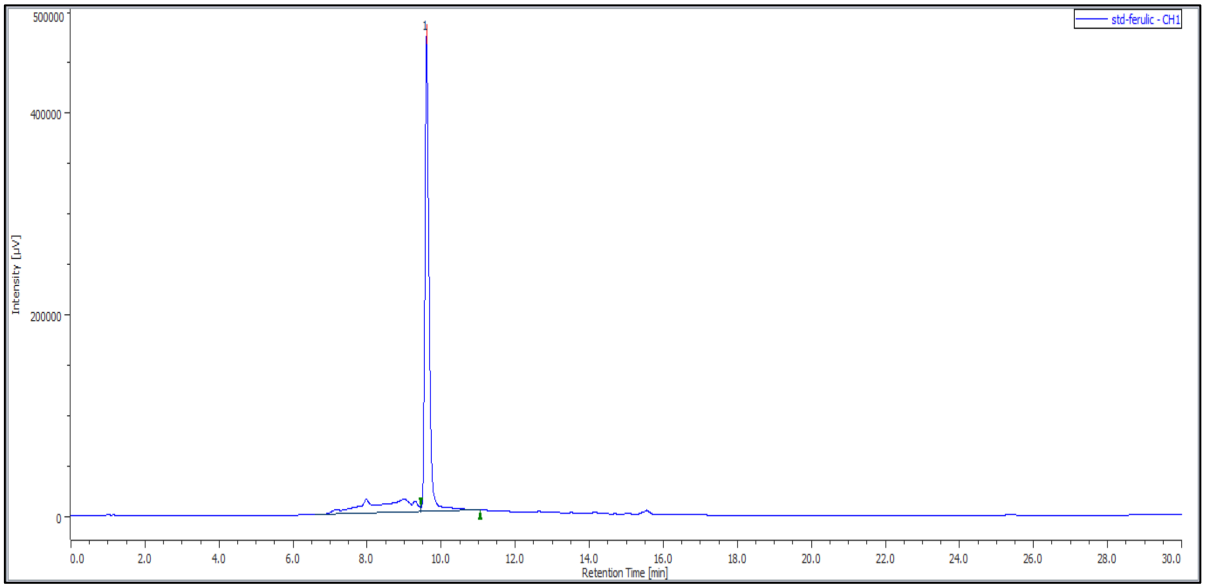
Appendixes



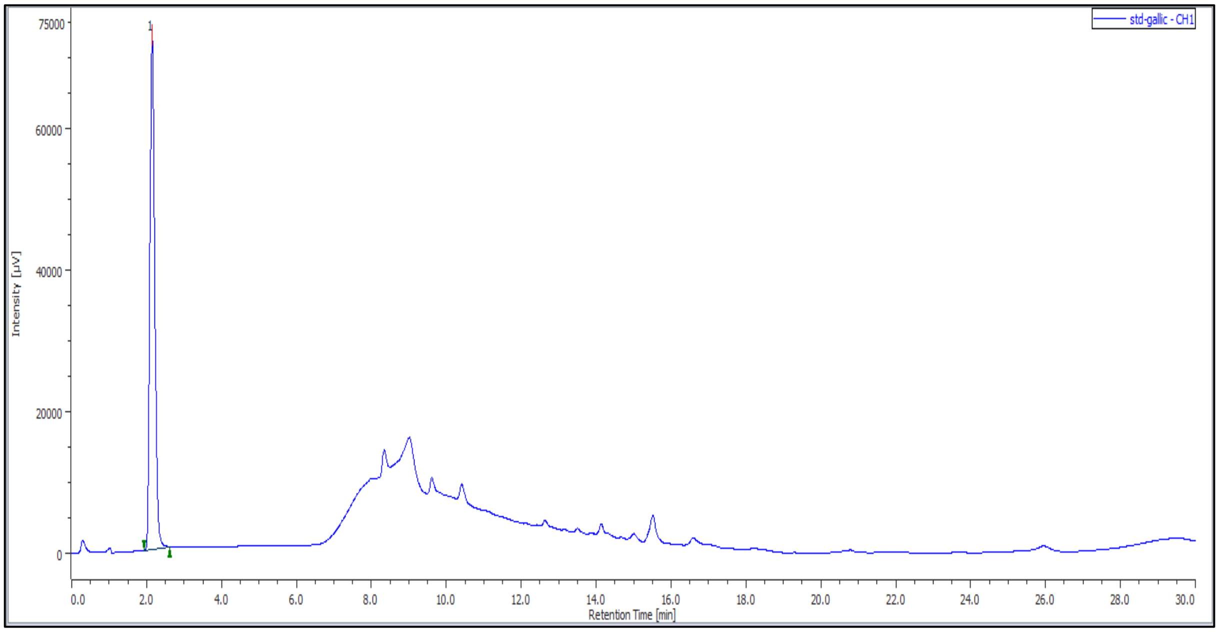
Appendix 1. Chromatograph of caffeic acid standard at 100 ppm concentration by HPLC



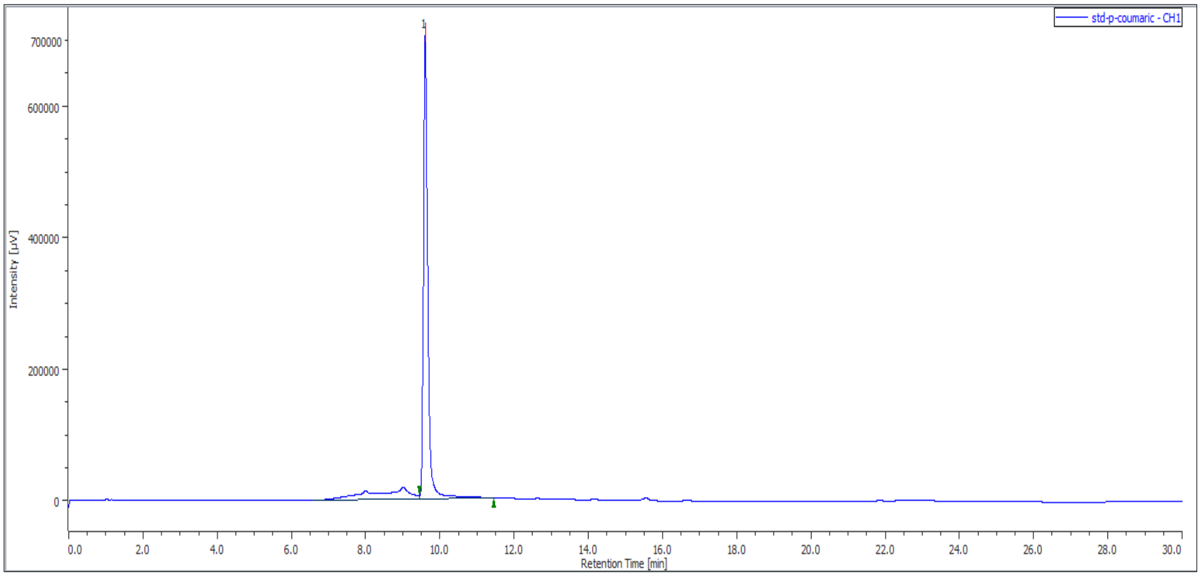
Appendix 2. Chromatograph of cinnamic acid standard at 100 ppm concentration by HPLC



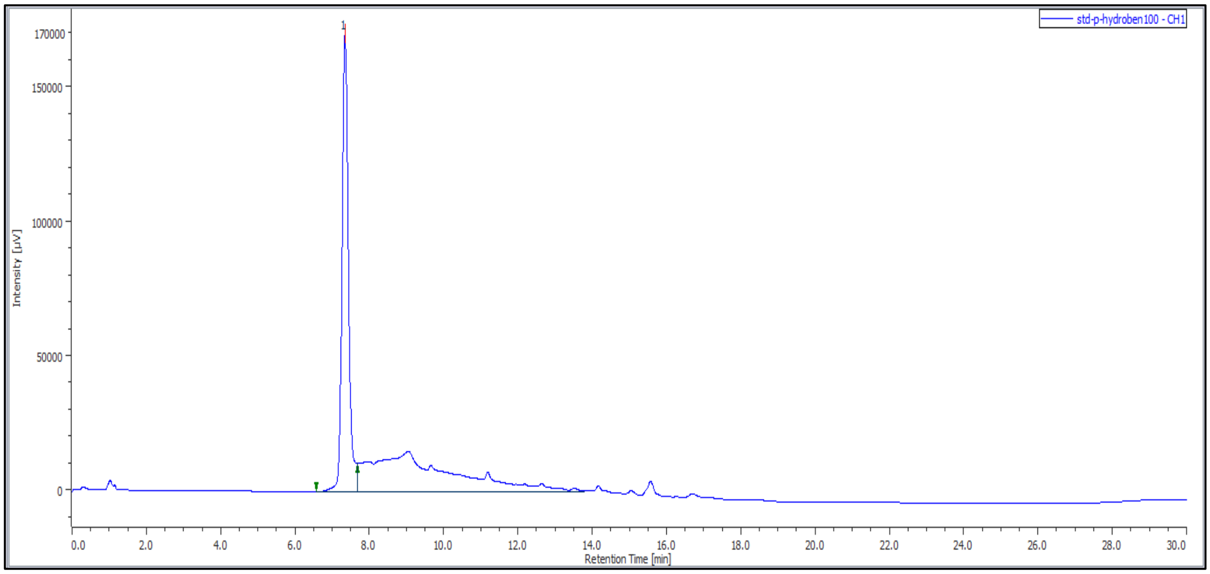
Appendix 3. Chromatograph of ferulic acid standard at 100 ppm concentration by HPLC



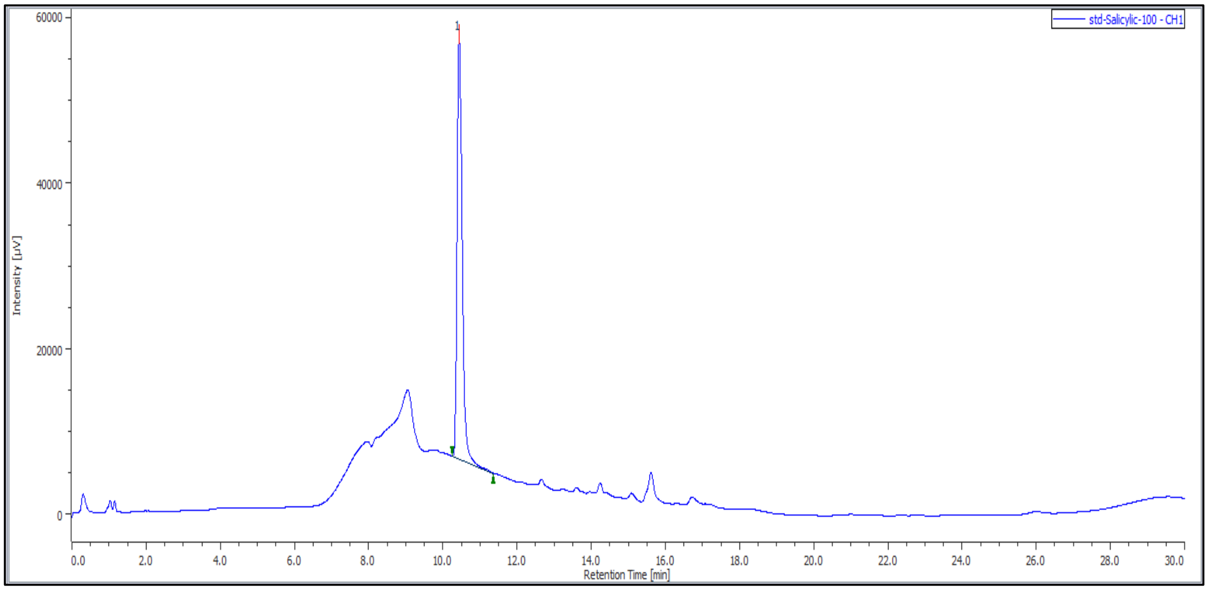
Appendix 4. Chromatograph of gallic acid standard at 100 ppm concentration by HPLC



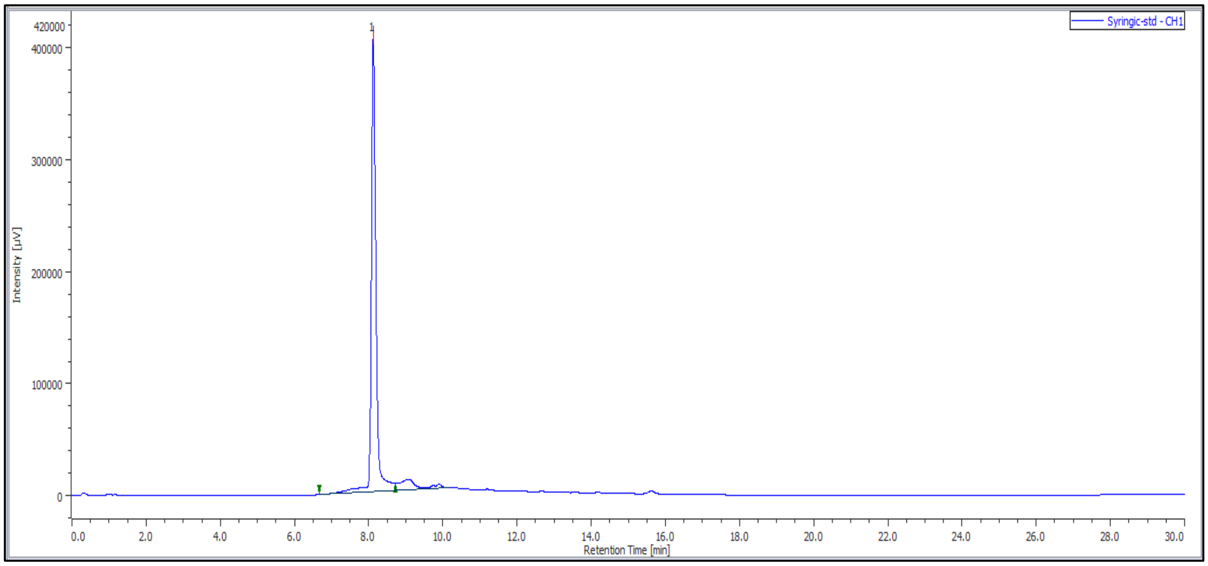
Appendix 5. Chromatograph of *p*-coumaric acid standard at 100 ppm concentration by HPLC



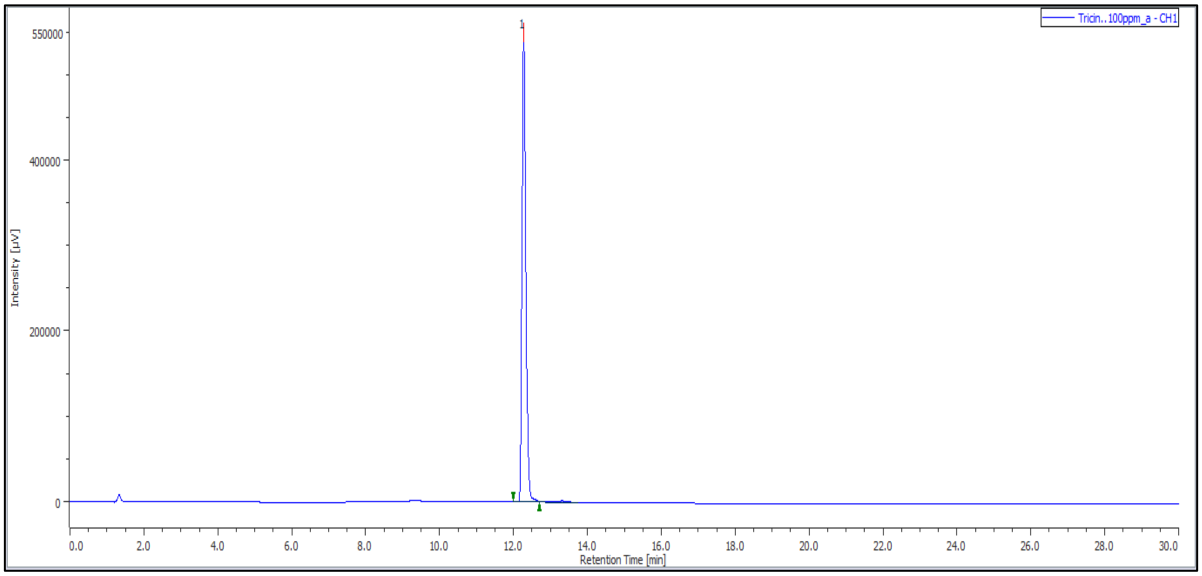
Appendix 6. Chromatograph of *p*-hydroxybenzoic acid standard at 100 ppm concentration by HPLC



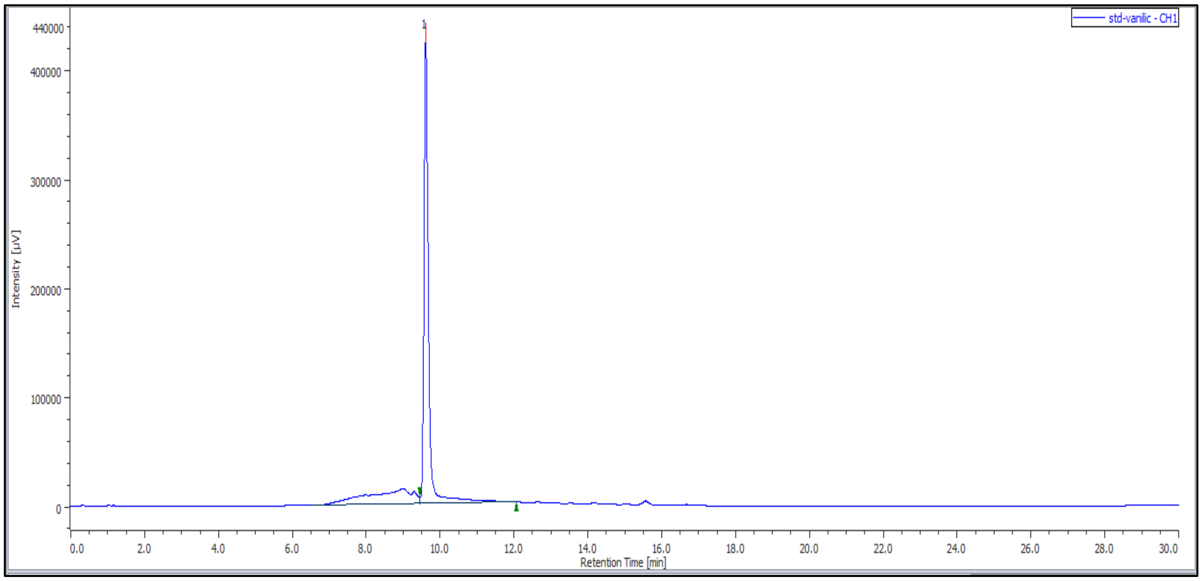
Appendix 7. Chromatograph of salicylic acid standard at 100 ppm concentration by HPLC



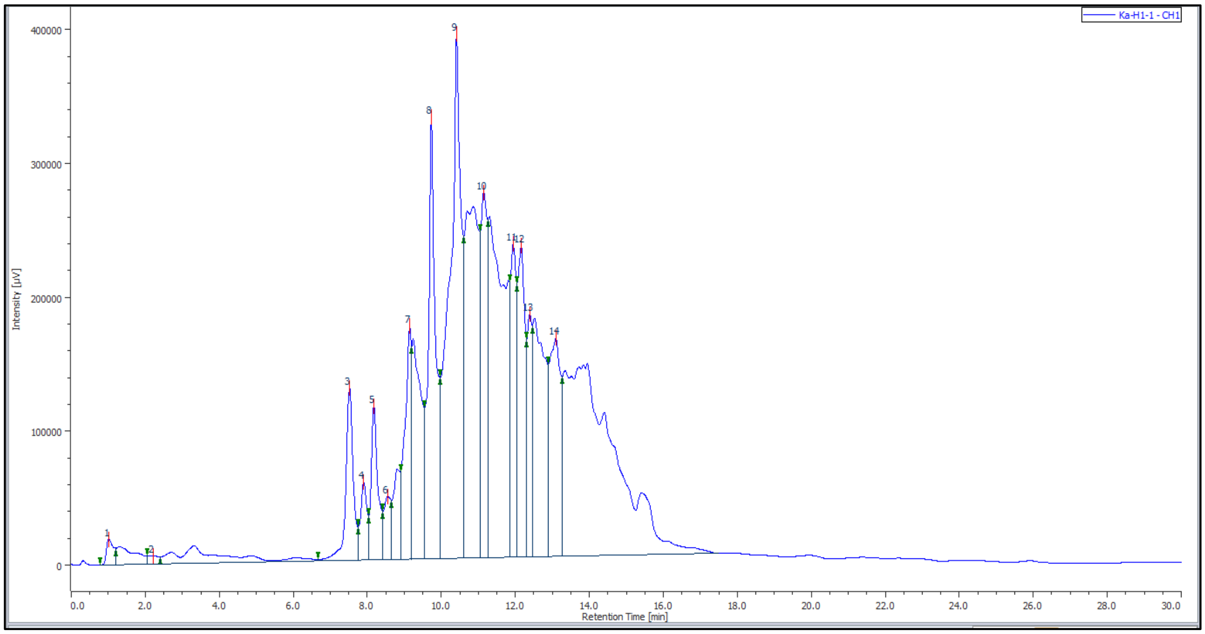
Appendix 8. Chromatograph of syringic acid standard at 100 ppm concentration by HPLC



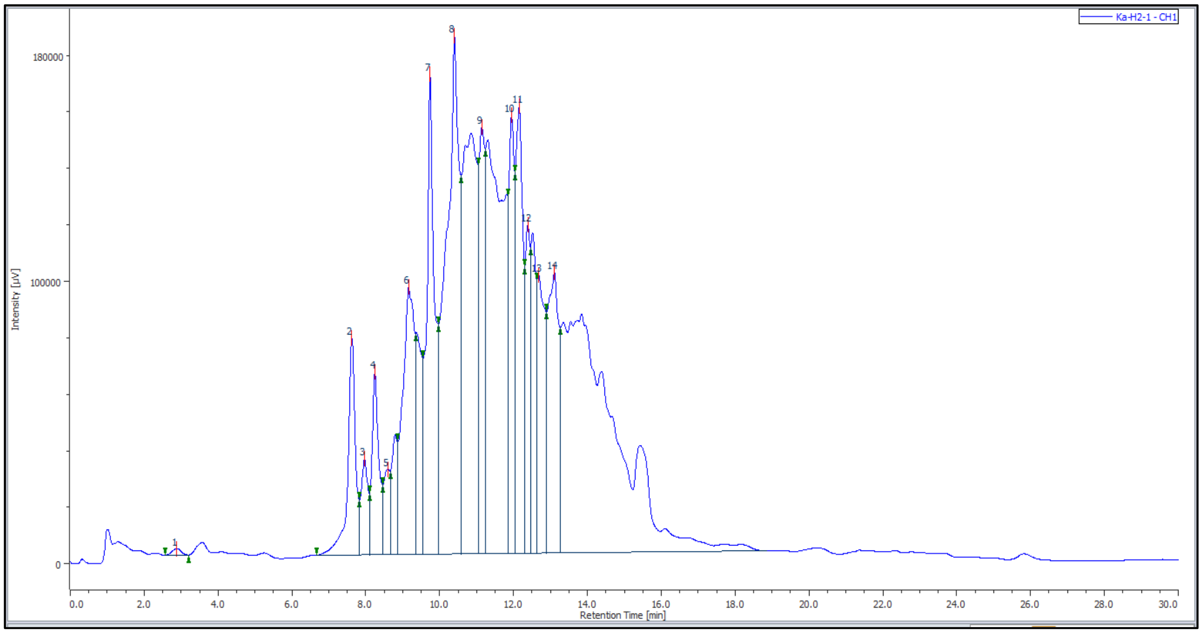
Appendix 9. Chromatograph of tricin standard at 100 ppm concentration by HPLC



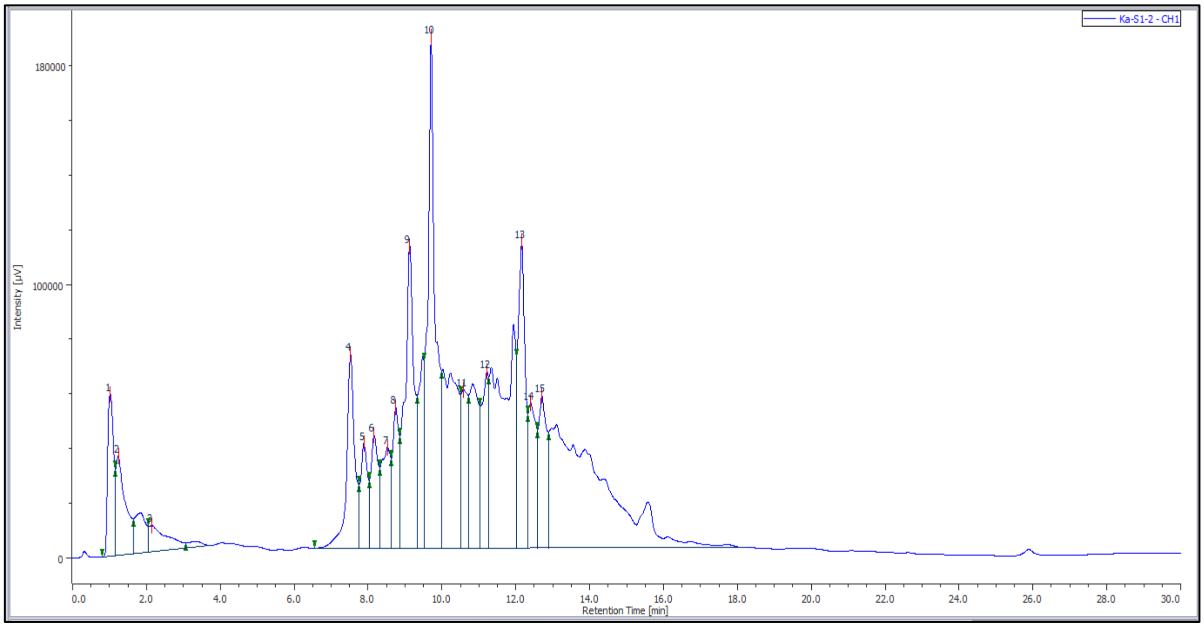
Appendix 10. Chromatograph of vanillic acid standard at 100 ppm concentration by HPLC



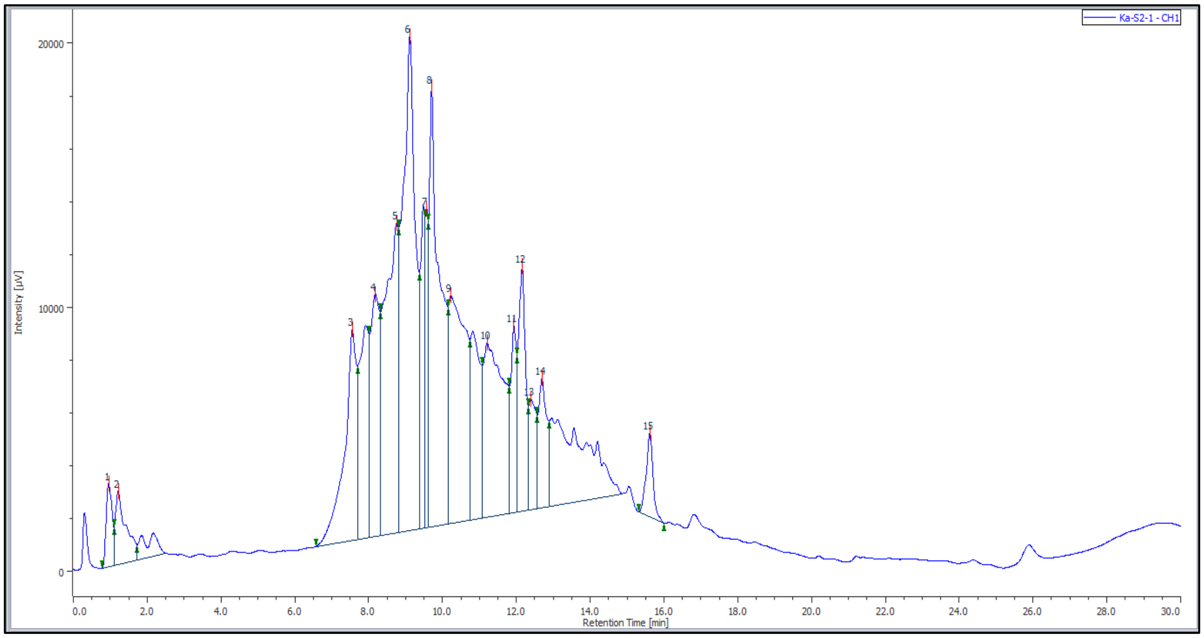
Appendix 11. Chromatograph of husk sample of K1 at 10000 ppm concentration by HPLC



Appendix 12. Chromatograph of husk sample of K2 at 10000 ppm concentration by HPLC



Appendix 13. Chromatograph of straw sample of K1 at 10000 ppm concentration by HPLC



Appendix 14. Chromatograph of straw sample of K2 at 10000 ppm concentration by HPLC