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

Kifayatullah Kakar [1,2], Tran Dang Xuan [1,*], Nguyen Van Quan [1], Imran Khan Wafa [3], Hoang-Dung Tran [4], Tran Dang Khanh [5,6] and Tran Dang Dat [7]

1 Graduate School for International Development and Cooperation, Hiroshima University, Hiroshima 739-8529, Japan; kifayatullahkakar@gmail.com (K.K.); nguyenquan26@gmail.com (N.V.Q.)
2 Faculty of Agriculture, Nangarhar University, Jalalabad City, Nangarhar 2601, Afghanistan
3 Agricultural Research Institute of Afghanistan, Ministry of Agriculture, Irrigation, and Livestock, Kabul 1001, Afghanistan; imrankhan_wafa@yahoo.com
4 Faculty of Biotechnology, Nguyen Tat Thanh University, Ward 13, District 4, Ho Chi Minh City 72820, Vietnam; thdung@ntt.edu.vn
5 Department of Genetic Engineering, Agricultural Genetics Institute, Hanoi 122300, Vietnam; tdkhanh@vaas.vn
6 Center for Expert, Vietnam National University of Agriculture, Hanoi 131000, Vietnam
7 Khai Xuan International Co. Ltd., Ha Dong District, Duong Noi Ward, LK20A-20B, Khai Xuan Building, Hanoi 152611, Vietnam; khaixuan.study@gmail.com
* Correspondence: tdxuan@hiroshima-u.ac.jp; Tel./Fax: +81-82-424-6927

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Abstract: Attempts regarding the improvement and development of novel rice with better quality and higher productivity have been increasing. Among approaches, mutation is a direct alteration on the genome and considered as one of the most beneficial routes to acquire new beneficial traits in rice. An experiment was carried out to explore the effects of N-methyl-N-nitrosourea (MNU) mutation on the antioxidant activities, phytochemical compounds, and momilactones A (MA) and B (MB) in rice. Two rice cultivars, K1 (an original cultivar DT84) and K2 (mutated DT84), were examined. Antioxidant activities, phenolic compounds, and momilactones of the rice grain, husk, and straw portions were measured and quantified. Antioxidant activities were higher in grain and straw of K2, whereas K1 showed greater antioxidant activity in rice husk. Additionally, K2 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 K1, while K2 significantly enhanced TFC in straw. Both MA and MB, two compounds obtaining antidiabetes, anticancer, antimicrobial, antigout, and antiobesity properties, were detected and quantified in grain, husk, and straw of K1 and K2 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. K2 contained higher amounts of MA and MB in straw and husk, but lower contents in grain compared with those in K1. This study illustrates that MNU mutation can improve grain quality and enhance bioactive compounds in straw, husk, and grain of rice. This approach has the potential to develop functional foods from rice, and therefore help farmers in developing countries to improve value in rice production.

Keywords: rice; MNU mutation; antioxidants; phenolics; flavonoids; momilactone A; momilactone B; antidiabetes; antigout; antiobesity; anticancer

1. Introduction

Rice (*Oryza sativa* L.), is considered one of the most dietary cereal crops which supplies essential foods worldwide, particularly producing and consuming on a large scale in Asian countries [1,2]. It provides daily calories for the majority of the world's population as well as feeds many companion animals [3]. 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 [4,5]. Asaduzzaman et al. [6] 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 [7,8]. These physicochemical properties of rice grain are largely affected by genotypic and environmental conditions [8,9]. High amylose content in rice grain leads to becoming hard, fluffy after cooking, and broken during the grain milling process [7,9]. However, protein and lipid contents play a vital performance in the nutritional value of rice grain [10]. Previously, we found a positive correlation between physicochemical properties and antioxidant activities of rice grain [9].

Additionally, bioactive compounds including antioxidants, phenolics, and momilactones play important roles in human health, pharmaceutical industries, and allelopathy [4,11,12]. Antioxidants effectively inhibit and neutralize free radical reactions by providing hydrogen or electron to the reaction chain [13]. Antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and others from synthetic sources are applied in food protection against oxidative damages [14]; 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 [5,15,16]. Plants are potential sources of natural antioxidants [17]. 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 [18].

Phenolic constituents are secondary metabolites that protect plants against pests and ultraviolet radiation as well as support plants to attract pollinating animals [18,19]. Besides, they play key roles in several activities in plants, phenolics and flavonoids are vital constituents in the human diet [12,20]. Phenolics have positive effects on human health [13,21] and are considered as the most potent natural compounds of plants with antioxidant, antimicrobial, and anti-inflammatory activities [13,22]. Additionally, phenolic compounds play a crucial role in stabilizing lipids against peroxidation as well as preventing diabetes [23] and various types of oxidizing enzymes [24]. Several factors including genetic characteristics are responsible for the variation in the content of phenolics [18].

Momilactones A (MA) and B (MB), as plant growth inhibitors, are originally isolated from rice husk [25,26]. MA and MB have been detected only in rice plants and moss (*Hypnum plumaeforme*) and belong to diterpenes groups [27,28]. Momilactones as phytoalexins evolve in the defense system of plants and increase with biotic and abiotic stresses [25,29,30]. Momilactones functioned as allelopathic potential against weeds and blast fungus [27,28,31] as well as expressed antioxidant, antitumor, antidiabetes, cytotoxic, antifungal, antibacterial, antimicrobial, and anticancer activities [27,32–34]. Quan et al. [27] 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 [35]. Some rice varieties can release momilactones, particularly MB, which can inhibit and suppress the germination of nearby weeds [36,37] and can reduce the application rate of synthetic herbicides [30].

Rice quality, nutritional value, and bioactive compounds are widely varied based on genetic backgrounds, cultivation methods, and environmental conditions [1,9,38]. Previously, we found that N-methyl-N-nitrosourea (MNU) mutation can increase growth attributes, grain yield, and the physicochemical quality of rice [39]. To date, none of the researches elucidated the effectiveness of MNU mutation on the phytochemical and momilactone contents of rice. Thus, this study aims to

explore the efficacy of MNU mutation on phytochemicals and momilactones in rice 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 experimental field of Hiroshima University in 2018 and 2019. The experiment was arranged in a randomized complete block design within three replications and two cultivars (K1; a cultivar and K2; a mutant line) as listed in Table 1. The mutant line seeds were received from Khai Xuan International Co., Ltd. for free. They were treated following a MNU mutation protocol as described in previous studies [39,40]. In short, seeds of the original cultivar (K1) 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 power tiller and leveled manually. Plots were designed at 3 m^2.

Table 1. Origin and description of the original cultivar and the mutant line.

Code	Origin	Descriptions	Status
K1	DT84	A sticky and traditional rice cultivar in the north of Vietnam with good quality	Cultivar
K2	Mutated DT84	F2 (self-pollination from the mutated DT84 F1)	Mutant line

Cultivar and mutant line were Indica subtype and provided by Khai Xuan International Co. Ltd., and Agricultural Genetic Institute, Hanoi, Vietnam.

Perfect seeds of selected cultivar and mutant line were soaked in distilled water and kept at 30 °C in a growth chamber for 48 h. 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. 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 maturity stage. The required samples of grains and straw were collected and kept at room temperature. Grain samples of each plot were threshed and dried at room temperature to obtain an 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 protein, amylose, 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, NH, 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. [41] with few changes. In brief, 100 g brown rice, 20 g straw, and 46 g husk were grinded and individually saturated in 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 h at room temperature. The filtrates were consequently evaporated to get crude extracts which finally dissolved with methanol to adjust the concentration and shored 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.

2.5. Antioxidant Assays

2.5.1. DPPH Scavenging Assay

DPPH assay was carried out based on the method explained by Quan et al. [11] 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. [12] 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 condition 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 (TPC)

The TPC was measured by the Folin-Ciocalteu method reported previously [42]. 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 standard calibration curve of gallic acid. The absorbance was recorded at 765 nm. 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 (TFC)

The TFC was determined through the aluminum chloride colorimetric method described by Tuyen et al. [12]. 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) were applied as a standard reference and the TFC were 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 (MA) and B (MB)

MA and MB were quantified based on the LC-ESI-MS system consisted of an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, CA, USA) which was equipped with 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, MA, USA). The column temperature was kept at 25 °C. The chromatography was run in a gradient model with a flow rate of 300 µL/min. The gradient of 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 [43]. 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. [27]. 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 [44].

2.9. Statistical Analysis

Data were analyzed using Minitab 16.0 software (Minitab Inc., State College, PA, 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 presented as mean ± standard error (SE).

3. Results

3.1. Antioxidant Activities

Two assays were operated to quantify the antioxidant activities in terms of DPPH and ABTS which are summarized as µg/mL in Table 2. 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 both cultivars. Generally, rice husk exhibited the greatest antioxidant activity,

followed by rice straw and grain. However, these variations were not significant among plant portions within K2 for DPPH assay. Mutant line 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, while antioxidant activity in rice husk (DPPH 503.4 μg/mL and ABTS 72.6 μg/mL) was higher in the original cultivar.

Table 2. Antioxidant activities, total phenolic and total flavonoid contents of the rice grain, husk, and straw in mutant line and original cultivar.

Samples		IC_{50} of DPPH (μg/mL)	IC_{50} of ABTS (μg/mL)	TPC (μg/g)	TFC (μg/g)
K1	Grain	2009.0 ± 181.2 b	766.5 ± 20.6 a	75.8 ± 6.3 d	1.4 ± 0.1 e
	Husk	503.4 ± 18.3 d	72.6 ± 6.9 e	462.5 ± 27.0 c	75.9 ± 1.8 c
	Straw	2372.7 ± 153.3 a	601.7 ± 27.5 b	893.3 ± 39.5 b	197.2 ± 5.3 b
K2	Grain	1267.1 ± 68.8 c	642.0 ± 10.7 b	80.3 ± 9.9 d	0.8 ± 0.1 e
	Husk	972.9 ± 69.7 c	137.6 ± 3.5 d	405.9 ± 3.0 c	54.7 ± 2.1 d
	Straw	1247.9 ± 77.4 c	371.8 ± 26.0 c	1156.6 ± 94.7 a	481.6 ± 14.8 a
BHT		28.95 ± 0.8 e	30.63 ± 0.6 f	-	-

Values are illustrated as mean ± standard errors. The similar letters in a column indicated no significant differences at the $p < 0.05$ probability level based on Tukey's multi comparison test. (-) means not measured.

3.2. Total Phenolic and Flavonoid Contents

The amounts of TPC and TFC are presented in Table 2. 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 line significantly increased the amount of TPC in the rice straw which was 1156.6 μg GAE/g DW of the sample. Besides, K2 showed a greater TPC in rice grain (80.3 μg GAE/g DW) and a lower of it in the husk (405.9 μg GAE/g DW) than the original cultivar, but the differences were not significant. TFC of rice husk was higher in K1 than those in K2, while K2 increased the amount of TFC in rice straw. The contents of total flavonoids were greater in rice grain of K1 but did not significantly differ with those in the grain of K2.

3.3. Momilactones A and B

The amounts of MA and MB as nanogram per gram (ng/g) are exhibited in Figure 1. In this study, both MA and MB were detected and quantified in rice grain, husk, and straw portions of the mutant line and original cultivar. Generally, the amount of MA was greater than MB in both K1 and K2 samples. Both MA and MB were higher in the rice straw followed by husk and grain (Figure 1A,B). Mutant line enhanced the amount of MA in straw and husk, while the quantity of MA was higher in the grain of K1. Additionally, MB was also higher in the straw of K2 but was lower in its grain, while there was no significant difference in the husk of both cultivars 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. Figures 2 and 3 displayed the EIC and MS of MA and MB of rice straw samples in the original cultivar and mutant line, which are completely in line with those of standard MA and MB peaks and retention times.

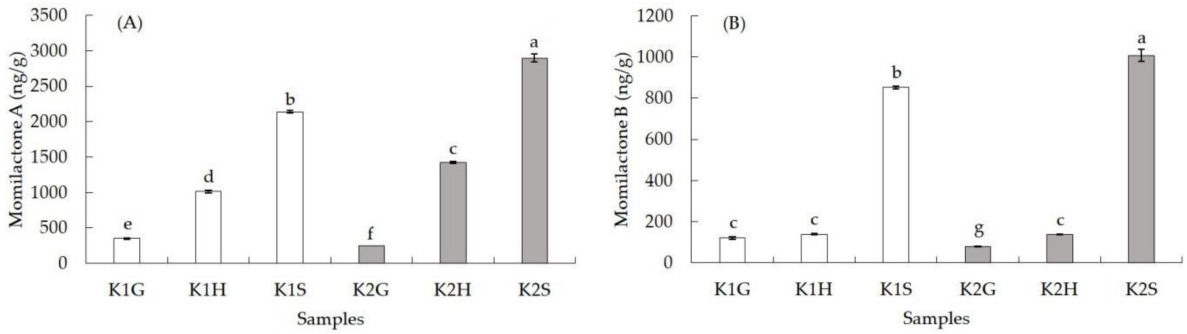

Figure 1. Contents of MA and MB in rice grain, husk, and straw of mutant line and original cultivar. (**A**): momilactone A, (**B**): momilactone B, G: grain, H: husk, S: straw.

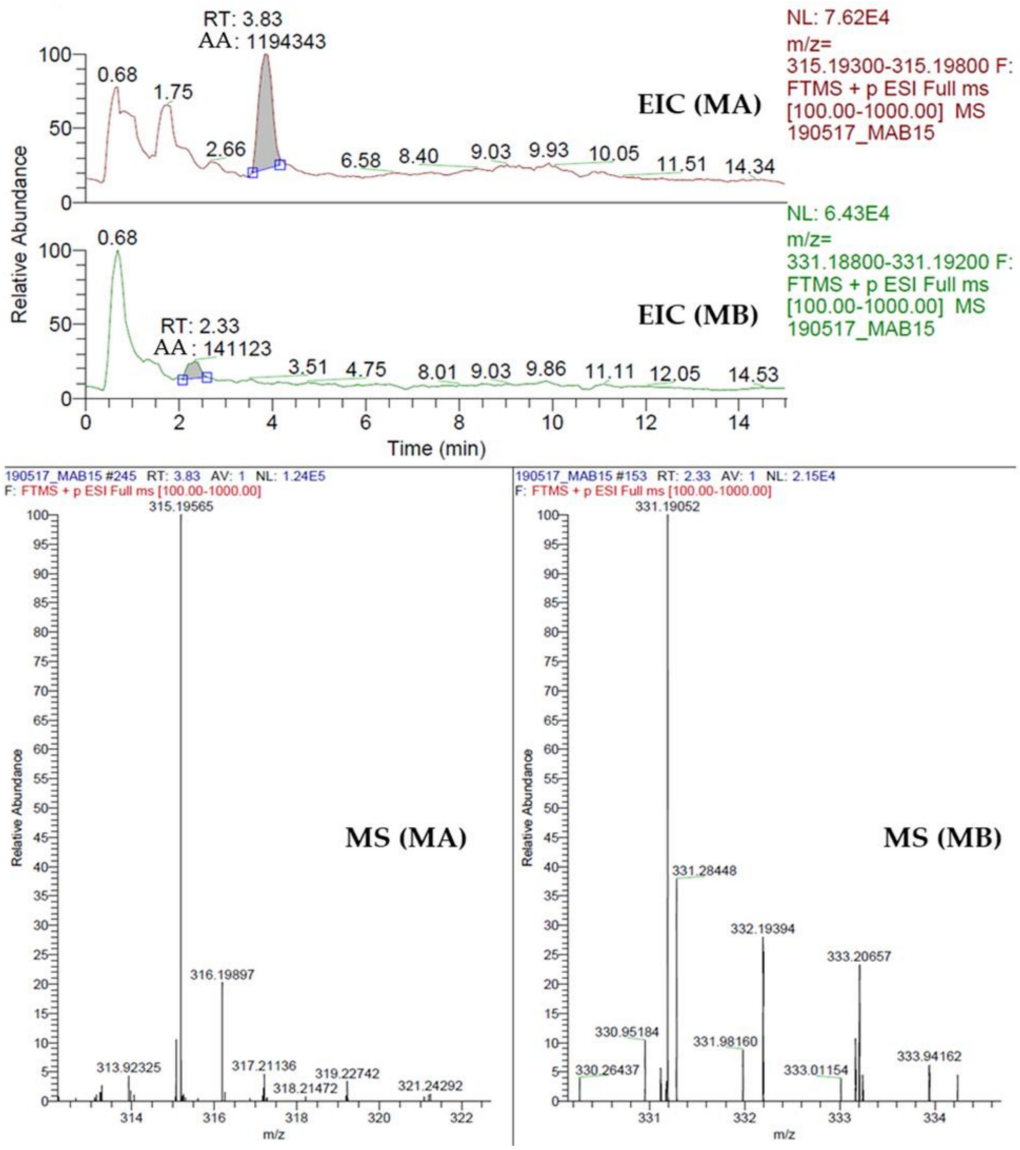

Figure 2. Extracted ion chromatograms (EIC) and mass spectra (MS) of momilactones A and B in the rice straw sample of K1.

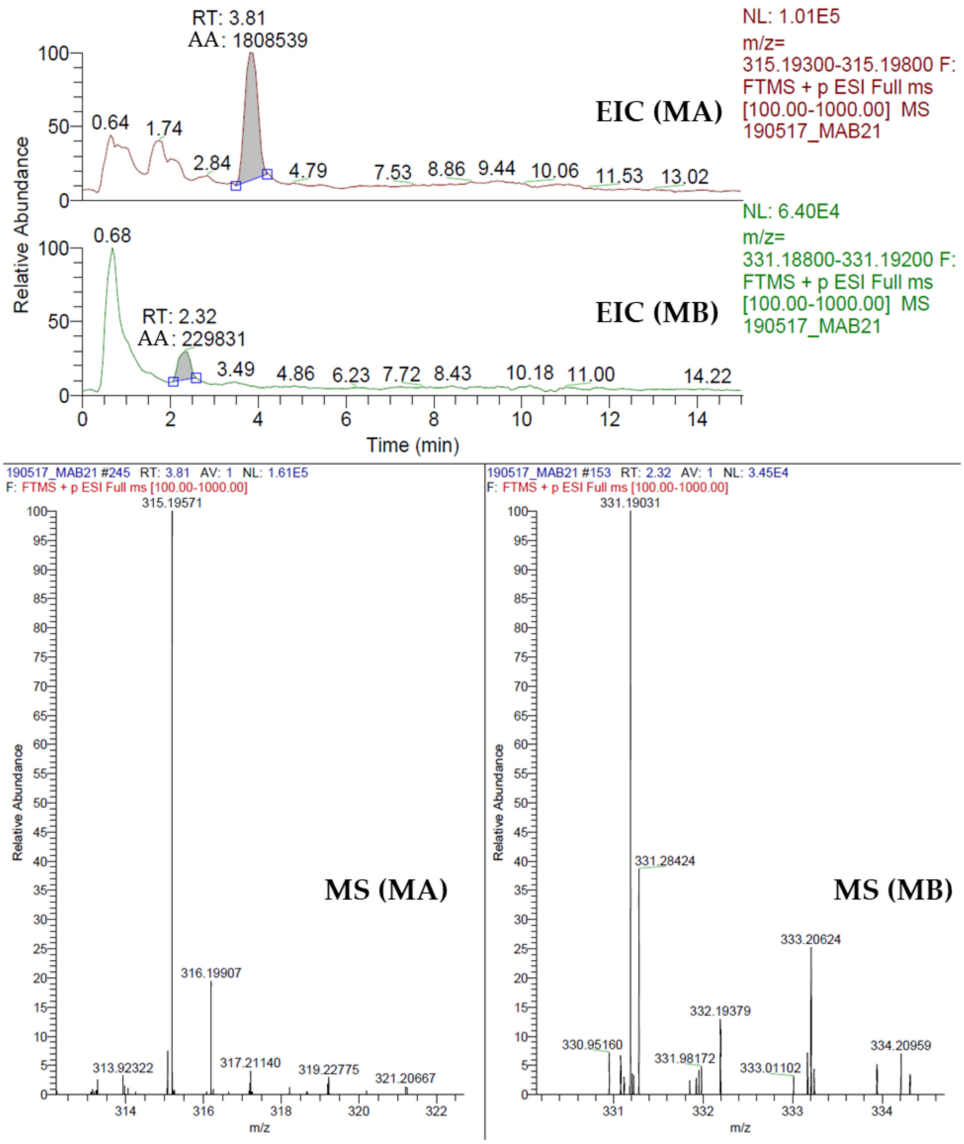

Figure 3. Extracted ion chromatograms (EIC) and mass spectra (MS) of momilactones A and B in the rice straw sample of K2.

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 3. 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. 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.

Table 3. 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 *** indicated significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

4. Discussion

Previously, we reported the importance of MNU mutation on growth attributes, yield potential, and physicochemical properties of rice. 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 [39]. 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 [7–9]. Protein and lipid contents are important elements in the nutritional value of rice grain [10]. Protein can strongly affect both 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 [45]. Zhou et al. [45] reported that high amylose and protein contents decrease rice grain acceptability in global markets.

The results of this study indicated that MNU mutation can enhance bioactive compounds and pharmaceutical value of rice plants besides improving its yield and grain quality. As rice being the main food worldwide, particularly it plays a fundamental part in social formalities and festivals in almost all Asian countries, it has meditative values too. Bioactive compounds of rice plants determine their position in the pharmaceutical and food industries [1,3–5]. 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. In this research, the DPPH and ABTS assays were conducted to evaluate the antioxidant activities of the samples. The antioxidant property is mainly characterized by phenolic contents that can effortlessly naturalize free radicals [11]. We found that K2 had higher antioxidant activity in both grain and straw than the original cultivar. The phenolic contents were also increased with the antioxidant activities. Quan et al. [11] assumed that phenolics 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 [18]. Some effects of phenolic constituents are related to the antioxidant enzymatic activities [46,47] and protein induction [48]. Phenolics and flavonoids are reported to decrease the risk of metabolic syndromes as well as type 2 diabetes [12,49] and have been used to treat several diseases including those of ovarian, breast cervical, and pancreatic [49]. It is reported that antioxidant activity and the contents of total flavonoids and total phenolics exhibited a significant positive correlation in rice grain [50,51]. These observations are in line with our findings.

Momilactones play roles in allelopathy, phytoalexins, and defense system of plants [25–30]. 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 [25–28]. Recently, some researchers reported that MA and MB increased under salinity and drought stresses in rice plants [52,53]. Xuan et al. [52] 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. [54] 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 [27]. Kim et al. [55] 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 [27,52,53]. The higher amount of MA was detected in 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 leaf as compared with other plant parts [27,52]. The secretion of momilactones increases until the flowering stage and declines thereafter [30]. Kato-Noguchi [36] 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. We found that the contents of MA and MB were higher in rice straw, followed by husk and grain in both mutant line and origin cultivar. It is found that rice seedlings release MB into the root zone of plants over their entire growing period [36]. In the current study, the seedlings of the mutant line 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 rice crop, but exerted greater biological activities compared to MA [35,54].

Rice husk, a bio-product of the rice plant which consists of a high amount of beneficial and organic compounds [56] is produced in a huge amount annually [54]. It is a valuable source of natural antioxidants and phenolic acids [57,58]. In this study, 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 [59]. 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

Demands for high productivity, preferable grain quality, and natural products of the rice plant are rising worldwide, particularly in rice-producing and consuming countries. MNU mutation displayed important roles in rice productivity, grain quality, and bioactive compounds. This approach can address and support rice farmers to improve rice-based functional foods and therefore increase value from rice production.

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