

Allelopathic potential of mutant rice varieties and its relation with grain quality

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ABSTRACT

The use of allelopathic rice varieties has recently been increasing due to their safety and sustainability in weed management. This study aims to investigate the allelopathic effects of rice varieties developed through N-methyl-N-nitrosourea (MNU) mutation on the growth of lettuce and barnyard grass, as well as to establish the relationship between grain quality and the allelopathy of rice plants. Rice straw, husk, and grain samples of rice varieties K1, K2, K3 and K4 were collected and extracted with methanol. The methanol extracts were investigated for antioxidants, total phenolic content (TPC), total flavonoid content (TFC) and the presence of momilactones A (MA) and B (MB). They were subjected to high-performed liquid chromatography (HPLC) coupled with a UV detector and tested for their allelopathic potential. MA and MB were quantified using Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS). The results revealed that mutant varieties exhibited higher antioxidants and TPC in both the grain and straw. Additionally, the mutant varieties increased the presence of MA and MB in the straw and husk. The straw exhibited stronger inhibitory effects compared to the husk. The straw samples from mutant rice varieties exhibited stronger inhibition towards lettuce (*Lactuca sativa* L.) and barnyard grass (*Echinochloa crus-galli* L.). Both the straw and husk strongly inhibited seed germination, shoot length and root length in barnyardgrass compared to lettuce. The inhibition was more pronounced in root growth than shoot growth. Rice varieties with higher allelochemical contents in their straw and husk exhibited stronger allelopathic effects. These findings suggest that rice varieties developed through the MNU mutation exhibited higher allelopathic activity compared to their wild-type cultivars. Therefore, the MNU mutation shows promise for breeding allelopathic rice varieties, which can help reduce the reliance on synthetic herbicides for weed control.

Key words: Allelopathy, allelochemicals, antioxidants, barnyardgrass, *Echinochloa crus-galli*, inhibition, *Lactuca sativa*, lettuce, momilactones, mutation, phenolics, rice, weed control.

INTRODUCTION

Weeds present a significant biotic constraint to crop productivity, resulting in increased costs of crop production while simultaneously decreasing grain quality (3,16). Generally, weeds are managed through mechanical, chemical and biological methods (3). Chemical methods involve the application of herbicides, which have become problematic due to environmental pollution and the appearance of weed resistance (42). One alternative approach is the incorporation of allelopathy strategies, which are more environmentally

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friendly for weed management. Allelopathy is a biological phenomenon that can be considered a specific type of amensalism. It is the ability of a plant to directly or indirectly suppress or stimulate the growth of neighboring plants through the release of chemical compounds known as allelochemicals (7,15). Allelochemicals are significant secondary metabolites produced by plants, which are released into the environment through processes such as leaching, volatilization, root exudation and decomposition (29). These allelochemicals can have either inhibitory or stimulatory effects on weed growth (39) and several of them have been suggested for weed control (36,37).

Rice (*Oryza sativa* L.) is a major crop worldwide (18) and its allelopathic potential has attracted great attention. In recent decades, many studies have demonstrated that rice plants produce specific allelochemicals to suppress the growth of nearby weeds, particularly barnyard grass in paddy fields (25). In the context of sustainable agriculture, allelopathy is a promising tool for managing weed competitiveness in rice fields. Therefore, incorporating allelopathic potential into genotypes and breeding programs should be the focal point for breeders, aiming to enhance rice crop resistance against weeds and increase yields (14), while reducing herbicides use (28).

Several studies indicate that the allelopathic potential of rice genotypes can be utilized in sustainable weed management (2). Olofsdotter *et al.* (27) reported that allelopathy accounts for 34 % of rice competitiveness. Anuar *et al.* (4) demonstrated that straw from several rice varieties exhibited stronger inhibitory effects on the radicle and hypocotyl of barnyardgrass. Chung *et al.* (9) conducted a comparison of over a hundred rice varieties to evaluate the allelopathic potential of their husks, leaves and straw extracts on the germination and seedling establishment of barnyard grass. Their findings revealed that rice straw exhibited stronger allelopathic effects compared to husk and leaves.

Patni *et al.* (30) reported that mutant rice varieties developed by treating them with methyl salicylate exhibited higher phytotoxic effects on the growth of barnyardgrass. However, the allelopathic capacity of varieties developed through MNU mutation has not yet been investigated. Furthermore, the relationship between allelopathy and rice grain quality is also poorly understood. Therefore, this research aims to investigate the effects of MNU mutation on the germination and seeding growth of lettuce and barnyard grass, as well as to find the relation of grain quality parameters and the allelopathic potential in rice plants.

MATERIALS AND METHODS

I. Plant Materials and Chemicals

This experiment was conducted in Plant Physiology and Biochemistry Laboratory at Hiroshima University, Japan. The experiment site was located at 34.3968° N and 132.7143° E with an elevation of 213 m above sea level. The area experiences an annual rainfall of 1457.6 mm, with average maximum temperatures of 25.8 °C and average minimum temperature of 14.1 °C. The study was conducted from March 2020 to February 2021. Rice grain, husk and straw samples from four varieties (K1, K2, K3, K4) shown in Table 1 were collected from our previous research (19,20) and stored at room temperature for further analysis. Seeds of lettuce and barnyardgrass were purchased from the Japan Agriculture (JA), Higashi-Hiroshima, Hiroshima, Japan. The dormancy of barnyard grass seeds was broken by storing in a freezer at -20 °C for one week.

All reagents and standard compounds used in this experiment were analytical grade and purchased from Junsei Chemical Co., Ltd., Tokyo, Japan; Fisher Scientific Co., Hampton, New Hampshire, USA; Fujifilm Wako Pure Chemical Co., Osaka, Japan; and Kanto Chemical Co., Inc., Tokyo, Japan.

Table 1. Origin and description of the selected rice varieties.

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 in North Vietnam	Cultivar
K4	Mutated Q5	F2 (self-pollination from the mutated Q5 F1)	Mutant line

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

II. Extraction and Samples Preparation

The extraction of samples was conducted using a previously explained procedure (35). A total of 100 g of brown rice, 20 g of straw, and 46 g of husk were crushed and soaked in 100 mL, 250 mL, and 200 mL of methanol, respectively, at room temperature for one week. These extracts were then filtered and evaporated at 50 °C to obtain a concentrated methanol extract. Next, the extract was mixed in a separatory funnel with an equal volume of hexane. After 3 hours at room temperature, the methanol layer was collected and filtered. The filtered sample was again evaporated to obtain the crude extract, which was then dissolved in methanol to achieve a concentration of 10 mg/mL. The solution was stored at 4 °C for future use.

III. Antioxidants Assays

Two assays, namely DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), were conducted to measure the antioxidant activities of rice grain, husk and straw. The DPPH scavenging and ABTS cation discoloration assays were performed by the method reported previously (17). The IC₅₀ value of the sample was calculated based on the amount of the sample required to inhibit 50 % of DPPH / ABTS radical activity. Therefore, a higher DPPH or ABTS IC₅₀ value indicates a lower antioxidant activity. The IC₅₀ percentage of radical scavenging activity of the sample was calculated using the formula described below:

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

Where, A_c: Absorbance of control (MeOH), A_s: Absorbance of sample and A_b: Absorbance of blank.

IV. Determination of Total Phenolics (TPC) and Total Flavonoids (TFC) Contents

The Folin-Ciocalteu technique was used to measure TPC (31). A calibration curve was constructed using gallic acid in the concentration range of 2.5-60 µg/mL. The TPC was presented as µg gallic acid equivalent per g dry weight of the sample (µg GAE/g DW). For the TFC, the aluminum chloride colorimetric method was employed (19). A calibration curve was created using rutin solutions in the concentration range of 10-500 µg/mL. The TFC was expressed as µg rutin equivalent per g dry weight of the sample (µg RE/g DW).

V. Quantification of Momilactones A (MA) and B (MB)

LC-ESI-MS system was used to determine and quantify the amounts of MA and MB in the rice grain, husk and straw portions, following the technique previously reported by Quan et al., (32). The gradient of mobile phase was established using solvent A (0.1 % trifluoroacetic acid in water) and solvent B (0.1 % trifluoroacetic acid in acetonitrile) as follows: (i) 0-5 min, 50 % solvent A and 50 % solvent B, (ii) 5-10 min, 100 % solvent B which was maintained for 0.1 min (33). Finally, the column was equilibrated for 5 min using the initial conditions (50 % solvent A and 50 % solvent B) with a total operation time of 15.1 min. The ESI condition was kept the same as the method previously reported by Quan et al., (34). Extracted ion chromatograms (EIC) and mass spectra (MS) were used to confirm the presence of MA and MB in the rice grain, husk and straw samples by comparing them with the standard momilactones. The areas of MA and MB with the determined peaks in the EIC that matched the standard MA (RT: 3.82) and MB (RT: 2.33) were used to calculate the amount of these compounds using a linear model.

VI. Allelopathic Activities

The allelopathic assay was conducted based on the method previously reported by (35). Two test plants, lettuce and barnyard grass, were selected for allelopathic assay. Healthy seeds of lettuce and barnyardgrass were soaked in distilled water at 30 °C for 2 days for water imbibition. Prepared filter papers were placed in beakers. An aliquot 200 μ L of each extract from husk and straw (previously diluted with methanol at different concentrations) was added to the backers per filter paper and kept in the oven at 40 °C for 30 min to completely dry and to subtract the effects of methanol on the bioassay. One mL of agar medium solution (0.5 %) was added to 12-well plates, and the treated filter paper was placed in each well of the plates. Six imbibed seeds of each test plant were placed in

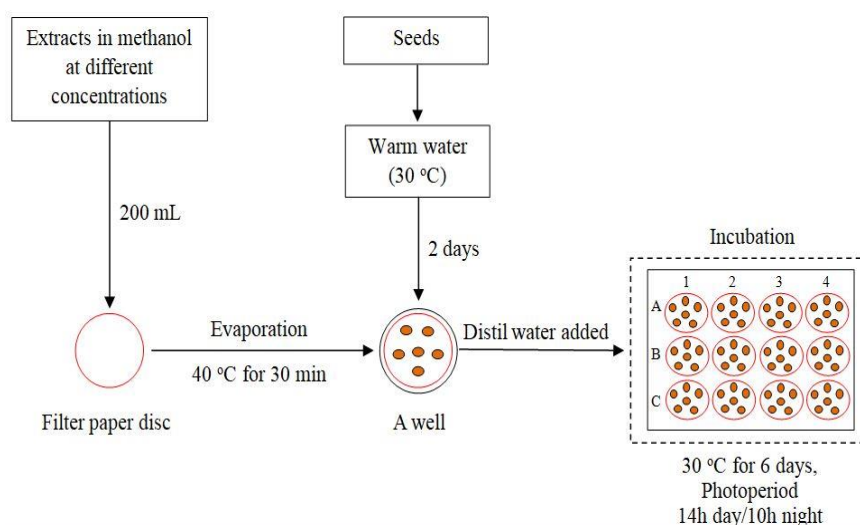


Figure 1. General experimental design of allelopathic assay (35).

each of the 12-wells of plate, and 300 μL of distilled water was added to each well before covering with wrapping paper. Additional water was added as needed. The plates were then kept in the growth chamber with a photoperiod of 14 hours day and 10 hours night at 30 °C for 6 days. Pure methanol and water were used as negative controls. Each measurement was repeated three times. A general view of allelopathic assay is shown in Figure 1.

The germination percentage, shoot length, and root lengths of lettuce and barnyard grass were recorded after 14 days. The inhibitory and stimulatory effects of the extracts on both crops were calculated based on the negative control (water). The IC_{50} value was shown as the concentration of extract that showed 50 % inhibition and was calculated by a previously described method (35). A lower IC_{50} value indicates a higher inhibitory activity on the germination and seedling growth of both lettuce and barnyard grass plants. A follow-up experiment of standard compounds, detected through HPLC, was also conducted using the same method and test plants to observe their allelopathic activities.

VII. Identification of Phenolic Compounds by HPLC

Phenolic compounds were identified using an HPLC system with a X Bridge BEH Shield RP18 (130 Å, 5 μm , 2.1 mm \times 100 mm) column (Waters Cooperation, Milford, MA, USA), following a method previously explained (31). An aliquant of 5 μL extract for each sample of rice husk and straw was gently injected into the system. Gradient elution was adjusted and run at a flow rate of 1 mL/min using the following time gradients: 5 % B (0-2 min), 5-70 % B (2-12 min), 100 % B (12-16 min) and maintained for 6 min, 100-5 % B (22-24 min) and another 10 min for equilibration. Solvent A was a 0.1 % aqueous formic acid (CH_2O_2) and solvent B was 100 % acetonitrile. The flow rate was set at 0.4 mL/min. The eluted compounds were detected at 280 nm. For several phenolic standards at different concentrations (1-100 ppm), 5 μL was injected into the HPLC system, and the retention times and areas were recorded. The peaks of the rice husk and straw extracts were identified and quantified based on the retention times and peak areas of phenolic standards.

VIII. Statistical Analysis

The data analysis was performed using Minitab 16.0 statistical software (Minitab Inc., State College, Pennsylvania, USA). One-way ANOVA was conducted to assess the significant differences among the means of extracts for phytochemicals (DPPH, ABTS, TPC, TFC, MA, and MB), inhibitory activities (germination, shoot and root inhibition), and standard compounds. Significant differences were determined at the $p < 0.05$ probability level based on Tukey's test. Principal component analysis was applied to determine the interaction among allelopathic traits and phytochemicals. The results are presented as mean \pm standard error (SE).

RESULTS AND DISCUSSION

I. Antioxidants, Phenolics, and Momilactones

Table 2 presents a summary of the antioxidant activities (DPPH and ABTS), TPC, TFC, MA, and MB in different parts of rice, including grain, husk, and straw. Overall, rice husk exhibited the highest antioxidant activity, followed by straw and grain. There was significant variation observed among the extract samples within each cultivar and mutant variety. The results indicated that mutant varieties enhanced the antioxidant activity in rice grain and straw compared to their corresponding wild-type cultivars, while the antioxidants

in husk were greater in the wild-type cultivars. The highest DPPH and ABTS values were recorded in the husk of K3 (396.2 and 57.5 $\mu\text{g/mL}$), while the lowest values were observed in K4 (1032.6 and 146.9 $\mu\text{g/mL}$). The greatest antioxidants for grain (DPPH 1267.1 and ABTS 642.0 $\mu\text{g/mL}$) and straw (DPPH 1247.9 and ABTS 371.8 $\mu\text{g/mL}$) were found in K2, while the lowest values were observed in K3 (Table 2).

The rice straw samples of each rice variety contained the highest levels of phenolics and flavonoids, followed by the husk and grain samples. The mutant rice varieties increased the amount of TPC in the rice straw (average: 1162.7 $\mu\text{g/g}$) and grain (average: 78.6 $\mu\text{g/g}$), but had lower TPC in the husk (average: 305.0 $\mu\text{g/g}$) compared to the original cultivars. The rice husk (average: 93.1 $\mu\text{g/g}$) and grain (average: 1.2 $\mu\text{g/g}$) samples of the original cultivars had higher TFC than those in the mutant varieties, while mutant varieties increased the amount of TFC in rice straw (average: 467.7 $\mu\text{g/g}$). Both the mutant varieties and original cultivars had MA and MB in their rice grain, husk, and straw samples. The content of MA was greater than MB in both types. Rice straw contained the highest levels of MA and MB, followed by the husk and grain. Mutant varieties enhanced the amount of MA and MB in straw (average: 5784.5 and 1278.2 ng/g) and husk (average: 2510.4 and 158.9 ng/g), while they were higher in the grain (average: 310.9 and 81.2 ng/g) of the original cultivars (Table 2).

Recent breeding programs have focused on rice grain quality and allelopathic potentials. Growth and yield parameters, such as plant height, strong tillers, leaf areas, and high grain yield, are closely related to the allelopathic potential of rice plants (1). Previous studies have shown that mutant rice grain samples exhibit higher contents of antioxidants and TPC, as well as lower amounts of amylose, TFC, MA, and MB (20). The current study also demonstrates that mutant varieties have higher contents of antioxidants and TPC in grain and straw samples, as well as higher TFC in husk samples. Phenolics and flavonoids are common secondary metabolites in cereal grains, and antioxidants, TPC, and TFC play important roles in human health by neutralizing hydroxyl groups, donating electrons, and stopping chain reactions (12). Therefore, MNU mutation has improved rice grain quality by enhancing the contents of antioxidants, phenolics, and flavonoids. In this study, the rice straw samples of the mutant varieties contained the highest contents of MA and MB, while only MA was higher in the rice husk compared to the original cultivars. As a result, rice straw exhibited stronger inhibitory effects on the germination and growth of both lettuce and barnyardgrass. A study that selectively removed momilactones from a complex mixture found a significant reduction in the allelopathic potential of the extracts (23,42). Therefore, rice straw and husk have the potential for weed management due to their phytotoxicity, availability, and low-cost characteristics (6,26).

II. Identification of Phenolic Compounds

Table 3 presents the identification and quantification of phenolic compounds in the rice husk and straw extracts using HPLC-UV. Ten major compounds were detected in the husk and straw samples of both wild-type cultivars and their mutant varieties. These compounds included gallic acid, *p*-hydroxybenzoic acid, syringic acid, caffeic acid, ferulic acid, vanillic acid, *p*-coumaric acid, salicylic acid, cinnamic acid, and the tricetin flavonoid. The amounts of these compounds varied between the husk and straw samples, as well as between the wild-type cultivars and their mutant varieties.

Table 2. Antioxidants, TPC, TFC, MA and MB contents of the rice grain, husk and straw in mutant lines and original cultivars

Rice Variety	Samples	IC ₅₀ of DPPH (µg/mL)	IC ₅₀ of ABTS (µg/mL)	TPC (µg GAE/g DW)	TFC (µg RE/g DW)	MA (ng/g)	MB (ng/g)
K1	Grain	1862.5 ± 181.2 a	766.5 ± 40.6 a	75.8 ± 6.3 b	1.4 ± 0.1 a	378.1 ± 23.1 a	88.6 ± 8.2 a
	Husk	503.4 ± 48.3 b	72.6 ± 6.9 b	462.5 ± 27.0 a	75.9 ± 3.8 a	1593.8 ± 175.7 c	215.9 ± 34.8 c
	Straw	1772.7 ± 153.3 a	601.7 ± 27.5 a	893.3 ± 39.5 b	197.2 ± 15.3 b	6220.9 ± 454.6 b	1311.1 ± 156.4 a
K2	Grain	1267.1 ± 168.8 b	642.0 ± 30.7 b	80.3 ± 9.9 a	0.8 ± 0.1 b	263.2 ± 21.1 c	73.2 ± 7.4 b
	Husk	972.9 ± 69.7 a	137.6 ± 13.5 a	405.9 ± 33.0 b	54.7 ± 2.1 b	2269.3 ± 180.6 b	248.3 ± 20.4 b
	Straw	1247.9 ± 177.4 b	371.8 ± 26.0 b	1156.6 ± 94.7 a	481.6 ± 44.8 a	6838.2 ± 821.4 a	1397.7 ± 173.2 a
K3	Grain	2133.4 ± 134.5 a	980.4 ± 37.6 a	59.7 ± 6.5 b	1.0 ± 0.1 a	243.7 ± 16.4 c	73.8 ± 8.3 b
	Husk	396.2 ± 40.3 b	57.5 ± 4.6 b	329.6 ± 15.4 a	110.3 ± 6.8 a	2167.9 ± 109.9 b	222.2 ± 23.1 c
	Straw	1922.2 ± 110.3 a	718.0 ± 34.0 a	428.0 ± 19.6 b	204.4 ± 16.5 b	2312.8 ± 218.1 d	821.2 ± 77.1 c
K4	Grain	1955.8 ± 119.2 b	912.7 ± 110.5 b	77.0 ± 3.8 a	0.5 ± 0.1 b	282.2 ± 15.6 b	68.8 ± 6.7 c
	Husk	1032.6 ± 102.3 a	146.9 ± 9.2 a	204.1 ± 21.9 b	64.9 ± 4.1 b	2751.5 ± 120.1 a	269.6 ± 22.2 a
	Straw	1472.1 ± 114.2 b	486.5 ± 9.2 b	1168.8 ± 123.3 a	453.9 ± 34.8 a	4730.9 ± 268.6 c	1158.7 ± 84.6 b
BHT		28.95 ± 0.8 c	30.63 ± 0.6 c	-	-	-	-

Values are illustrated as mean ± standard errors. The same letters within a column among each portion of the varieties indicate no significant differences at the p<0.05 probability level based on Tukey's multi comparison test. (-) means not measured. 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, BHT: butylated hydroxytoluene

Table 3 . Major compounds identified and qualified in the husk and straw extracts of the original cultivars and mutant lines by HPLC as µg/g dry weight.

Compounds	RT (min)	K1 Variety		K2 Variety		K3 Variety		K4 Variety	
		Husk	Straw	Husk	Straw	Husk	Straw	Husk	Straw
Gallie acid	2.14	27.4±3.2b	50.8±3.8b	38.2±3.6a	198.0±13.9a	38.2±3.6a	26.4±2.8c	24.9±2.2b	186.8±14.6a
<i>p</i> -Hydroxybenzoic acid	7.35	478.9±37.6b	259.3±11.8b	602.4±50.1a	497.6±20.5a	602.4±50.1a	105.0±6.8c	166.3±10.7d	471.3±16.2a
Syringic acid	8.12	124.2±12.6b	336.7±39.4b	168.4±13.4a	670.6±62.9a	168.4±13.4a	137.6±17.0c	63.0±9.9d	607.2±56.7a
Caffeic acid	8.33	26.0±2.6b	72.3±4.8a	34.1±4.6a	29.4±3.2c	34.1±4.6a	13.7±2.2d	4.2±0.7d	50.0±6.3b
Ferulic acid	9.58	113.0±10.4d	235.9±16.9b	176.0±9.5b	189.4±14.3c	176.0±9.5b	92.9±6.4d	129.5±9.6c	263.9±25.7a
Vanillic acid	9.61	39.3±4.8b	40.1±3.4a	65.3±10.9a	31.6±3.1b	65.3±10.9a	24.2±2.9c	17.1±2.9c	41.2±5.8a
<i>p</i> -Coumaric acid	9.70	369.8±30.8b	678.6±55.0c	571.9±57.9a	1491.8±173.8a	571.9±57.9a	404.5±88.7d	124.2±23.6d	1121.4±158.2b
Salicylic acid	10.43	997.6±74.2b	348.0±37.4c	1146.2±114.3a	1323.9±196.2a	1146.2±114.3a	266.4±10.7d	374.0±54.4d	953.2±54.9b
Cinnamic acid	11.17	20.6±3.9b	35.0±7.8c	31.2±4.5a	41.6±11.6a	31.2±4.5a	10.5±2.3d	5.5±1.2d	38.4±8.5b
Tricin	12.26	3.4±0.3b	11.0±1.3a	4.9±0.6a	12.1±1.2a	4.9±0.6a	1.3±0.1b	1.0±0.1d	3.0±0.7b

Values are illustrated as mean ± standard errors. The same letters within a row among husk or straw indicate no significant differences at the p<0.05 probability level based on Tukey's multi comparison test. RT means retention time.

In the husk samples, higher amounts of *p*-hydroxybenzoic acid, syringic acid, caffeic acid, *p*-coumaric acid, salicylic acid, cinnamic acid, and triclin were observed in the wild-type varieties compared to their mutant varieties. On the other hand, in the straw samples, the contents of gallic acid, *p*-hydroxybenzoic acid, syringic acid, *p*-coumaric acid, salicylic acid, cinnamic acid, and triclin were greater in the mutant varieties compared to their corresponding non-mutant cultivars. These differences indicated variations in phenolic compound profiles in various parts of rice and between the wild-type and mutant varieties.

Most of these identified compounds have been previously reported by Chung *et al.* (8) in rice plants. In another study, five allelochemicals, including caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and cinnamic acid, were quantified in rice straw, with caffeic and syringic acids found in lower concentrations and *p*-coumaric, ferulic, and cinnamic acids in higher concentrations compared to the results of the current study (11). It has been reported that phenolic acids with concentrations exceeding 1 mM exhibit phytotoxic activities on various plants (21).

III. Allelopathic activity of extracts and their identified constituents

Table 4 displays the inhibitory effect of rice husk and straw extracts obtained from mutant varieties and their corresponding wild type cultivars on seed germination and seedling growth of lettuce and barnyard grass.

(i). Seed germination: Rice straw demonstrated stronger inhibitory effect compared to rice husk. The highest inhibition of seed germination (GI) caused by the husk extracts was observed in the wild-type cultivar K3H, with values of 2.02 mg/mL for lettuce and 1.7 mg/mL for barnyardgrass. This was followed by K1H, K2H and K4H, respectively. However, the highest germination inhibition of straw samples was observed in K2S (a mutant variety), with values of 1.33 mg/mL for lettuce and 1.6 mg/mL for barnyard grass, followed by K4S, K1S and K3S, respectively. Overall, the mutant varieties exhibited significantly higher germination inhibition on both tested plants in straw samples, but lower inhibition was observed in husk samples. Both straw and husk samples exerted stronger germination inhibition on barnyard grass compared to the lettuce.

(ii). Seedling growth: Rice straw also exhibited greater inhibition of seedling growth compared to rice husk. The highest shoot length inhibition and root length inhibition caused by husk samples on lettuce and barnyard grass were observed in K3H, with values of 2.5 mg/mL (shoot) and 2 mg/mL (root) for lettuce as well as 2.2 mg/mL (shoot) and 1.9 mg/mL (root) for barnyard grass. However, the highest shoot and root inhibition caused by straw samples on both tested plants were observed in K2S, with values of 1.98 mg/mL (shoot) and 1.95 mg/mL (root) for lettuce, and 1.67 mg/mL (shoot) and 1.75 mg/mL (root) for barnyard grass. Both straw and husk samples exerted stronger shoot and root inhibition on barnyard grass compared to lettuce. Additionally, root length was more greatly inhibited than shoot length in both tested plants (Table 4). The results of this study demonstrate that the inhibitory potential of rice husk and straw extracts on the germination and seedling growth of both tested plants differed, with rice straw exhibiting stronger inhibition compared to rice husk. The highest germination, shoot and root inhibition were observed in the straw extract of the mutant varieties, while the highest inhibition was found in the husk extracts of the original cultivars. Previous studies have also reported stronger inhibitory effects of rice straw compared to rice husk

and leaves (24). Furthermore, both straw and husk extracts showed stronger germination, shoot and root inhibition on barnyard grass compared to the lettuce. Besides, root length was much inhibited compared to shoot length in both lettuce and barnyard grass. This is consistent with previous studies that found greater inhibition of root length compared to shoot length in barnyardgrass (6). Additionally, He *et al.* (13) reported that allelopathic potential of rice plants primarily affects the root growth of the target plant. They suggested that the stronger inhibition of root growth may be due to the direct contact of roots with the treated filter paper containing the extracted compounds. Several studies have indicated that rice landraces with phenotypic characteristics such as high tiller numbers, grain weight, and erect leaves exhibit greater allelopathic potential compared to landraces with fewer tillers and lower grain weight (25). Furthermore, Ahn *et al.* (1) demonstrated that the allelopathic activity of rice varieties is closely associated with plant height, strong tillers, high grain yield and ample leaf areas. In a previous study, we observed that the mutant varieties showed higher tiller numbers, 1000-grain weight, total grain yield, and taller plant height compared to the original cultivars (20). In the current research, we also observed that mutant varieties showed stronger allelopathic potential in straw than the original cultivars. This may be attributed to the increased plant height, tiller number and grain yield in the mutant varieties, which aligns with the findings of previous studies. Additionally, the study by Khanh *et al.* (25) showed that most Vietnamese rice varieties possessed stronger allelopathic potential on the germination and growth of barnyard grass.

Table 4. IC₅₀ inhibitory activity of rice husk and straw on the germination rate and growth parameters of selected plants.

Rice variety	Lettuce (IC ₅₀ value as µg/mL)			Barnyard grass (IC ₅₀ value as µg/mL)		
	Germination	Shoot	Root	Germination	Shoot	Root
	Husk					
K1	2110.1 ± 117.4 b	2528.1 ± 138.6 a	2185.1 ± 280.8 b	1779.4 ± 116.4 b	2456.8 ± 139.1 b	1965.1 ± 150.5 a
K2	2227.8 ± 233.1 cd	2976.4 ± 223.4 b	2464.7 ± 372.1 c	1931.9 ± 130.4 c	2829.9 ± 154.2 c	2262.1 ± 260.7 b
K3	2016.6 ± 127.6 a	2491.9 ± 125.7 a	1991.4 ± 139.2 a	1677.4 ± 124.3 a	2183.1 ± 129.7 a	1811.6 ± 129.2 a
K4	2307.4 ± 262.8 d	3053.0 ± 209.7 b	2665.2 ± 262.4 d	2053.3 ± 235.1 d	2993.8 ± 191.8 c	2594.0 ± 286.3 c
	Straw					
K1	1772.6 ± 130.4 c	2291.7 ± 139.5 b	2143.5 ± 148.7 b	1871.7 ± 129.0 b	2151.6 ± 192.9 c	2038.4 ± 234.3 b
K2	1335.4 ± 137.5 a	1978.7 ± 152.9 a	1954.2 ± 140.1 a	1574.0 ± 119.4 a	1672.6 ± 172.5 a	1756.9 ± 196.1 a
K3	1825.6 ± 190.2 c	2418.0 ± 181.5 b	2313.3 ± 225.5 c	1940.7 ± 128.4 b	2299.9 ± 249.4 d	2407.6 ± 251.8 c
K4	1557.6 ± 127.4 b	2047.8 ± 117.6 a	2076.7 ± 151.6 b	1660.6 ± 137.8 a	1934.1 ± 155.4 b	1953.0 ± 127.9 b

Values are presented as mean ± standard errors. 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.

In terms of specific compounds, *p*-hydroxybenzoic acid and salicylic acid showed the most phytotoxic effects on the germination of lettuce and barnyard grass. In contrast, *p*-coumaric acid, vanillic acid, ferulic acid, cinnamic acid, gallic acid, caffeic acid, and tricic exhibited less inhibitory effects. Gallic acid strongly inhibited the shoots of lettuce and barnyard grass, while the roots of both plant species were significantly affected by salicylic acid (Table 5). These compounds have been previously reported as rice allelochemicals and have been associated with weed suppression, along with other flavonoids, terpenoids (such as MA and MB), cytokinins, alkaloids, steroids, and tannins (5,21,23,38). Chung *et al.* (8)

Table 5. IC₅₀ value for inhibitory activity of standard compounds on the germination rate and growth parameters of selected plants.

Compounds	Lettuce (IC ₅₀ value as µg/mL)		Barnyard grass (IC ₅₀ value as µg/mL)	
	Germination	Shoot	Germination	Shoot
Gallic acid	2388.8 ± 84.8cd	2567.7 ± 68.1a	2332.6 ± 62.9d	2528.1 ± 80.0a
<i>p</i> -Hydroxybenzoic acid	1633.7 ± 78.1a	2694.6 ± 66.8abc	1766.4 ± 126.7ab	2553.2 ± 29.2ab
Syringic acid	2296.3 ± 74.1cd	2619.8 ± 81.6ab	1857.3 ± 76.4abc	2544.5 ± 25.7a
Caffeic acid	2407.4 ± 133.5cd	2956.4 ± 20.9cde	2260.2 ± 36.7d	3044.8 ± 71.3d
Ferulic acid	2074.0 ± 74.1bc	2699.7 ± 127.7abcd	2193.4 ± 14.5cd	2616.7 ± 88.3abc
Vanillic acid	1870.4 ± 66.7ab	3030.7 ± 25.9de	1819.9 ± 22.3ab	3030.7 ± 25.9d
<i>p</i> -Coumaric acid	1759.2 ± 49.0ab	3082.7 ± 39.8e	1881.5 ± 93.0abc	2930.9 ± 133.6bcd
Salicylic acid	1621.8 ± 66.3a	2638.1 ± 88.8abc	1677.9 ± 81.9a	2582.2 ± 113.2ab
Cinnamic acid	2298.3 ± 73.1cd	3090.0 ± 34.4e	2131.2 ± 96.8bcd	3100.7 ± 45.1d
Tricin	2615.6 ± 85.8d	2934.6 ± 28.9bcde	2281.2 ± 42.0d	2978.2 ± 65.8cd

Values are presented as mean ± standard errors. Different letters within a column indicate significant differences at p < 0.05 probability level based on Tukey's multi-comparison test.

reported that ferulic, *p*-coumaric, *p*-hydroxybenzoic and *m*-coumaric acids were the allelochemicals with the greater inhibitory activity on the germination, seedling growth and dry weight of barnyard grass. These compounds are suggested to play a crucial role in rice allelopathy and hold promise for the development of natural herbicides.

II. Correlation of Grain Quality with Allelopathic Activity

Figure 2 presents a biplot of the principal component analysis (PCA) illustrating the relationship between grain quality attributes and allelopathy activity of the extracts. The PCA analysis accounts for 66.5% of the total variance in the dataset. Allelopathy traits (GI, SI and RI) from straw ex.tract showed a positive correlation with the grain yield, antioxidant contents and TPC, while exhibiting a negative correlation with amylose content, TFC, MA and MB. Conversely, allelopathy traits from husk extract displayed a positive relation with amylose content, TFC, MA and MB, but were negatively related with grain yield, antioxidants and TPC. Furthermore, germination, shoot and root inhibition recorded a positive correlation to each other. Therefore, rice grains with high grain yield, antioxidant contents and TPC showed greater allelopathic potential in straw extracts, whereas grains with high amylose content, TFC, MA and MB exhibited stronger allelopathic activities in the husk extracts. The PCA analysis illustrated that rice grains with low amylose, TFC, MA, and MB exhibited greater germination, shoot inhibition, and root inhibition.

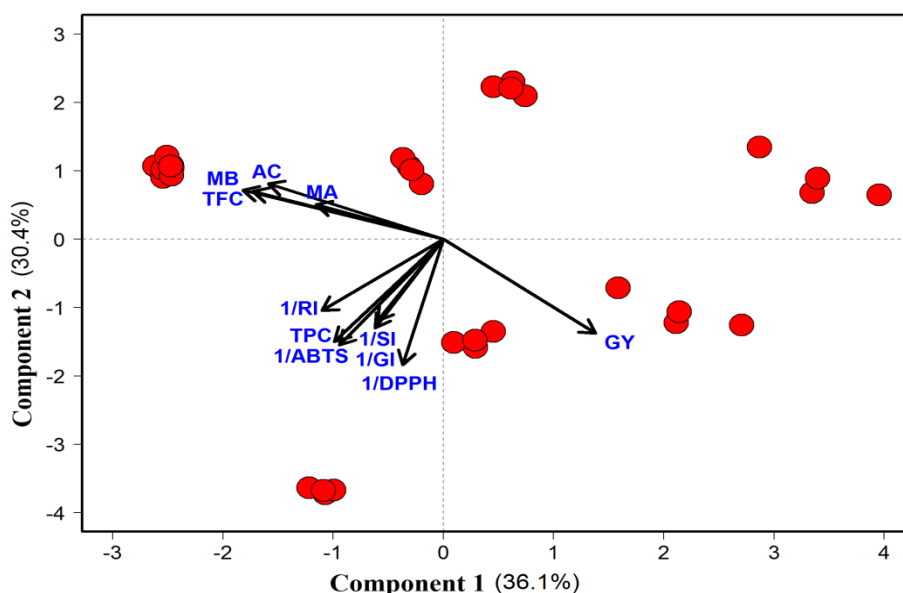


Figure 2. PCA of grain quality parameters with allelopathy traits. AC: Amylose content; GY: grain yield; 1/DPPH: 1/1,1-diphenyl-2-picrylhydrazyl; 1/ABTS: 1/2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TPC: total phenolic contents; TFC: total flavonoid contents; MA: momilactone A; MB: momilactone B; 1/GI: 1/germination inhibition; 1/SI: 1/shoot inhibition; 1/RI: 1/root inhibition.

Based on the findings, mutant varieties not only improved rice grain quality (19,20) but also enhanced allelopathic potential. Thus, MNU mutation can be utilized to improve rice grain quality by reducing weeds interference.

CONCLUSIONS

The findings of this study underscore the significant role of MNU mutation in enhancing both the grain quality attributes and allelopathic potential of rice plants. Weeds pose a major challenge to rice growth and yield, and the results demonstrate the effectiveness of mutant rice varieties in addressing this issue. Importantly, both the straw and husk by-products of these mutant varieties exhibit strong allelopathic potential, with the straw showing even greater inhibition on indicator plants compared to rice husk. This suggests that the allelochemicals present in these by-products can serve as natural herbicides. The study highlights the importance of MNU mutation in rice allelopathy and establishes a correlation between rice grain qualities and the allelopathic potential of its by-products. However, further research is needed to elucidate the genetic changes underlying the allelopathic potential induced by MNU mutation. This will contribute to a deeper understanding of the molecular mechanisms involved in rice allelopathy and facilitate the development of improved rice varieties with enhanced weed control capabilities.

AUTHOR CONTRIBUTIONS

K.K. and T.D.X. assumed the idea and wrote the manuscript. K.K. conducted the experiments and implemented measurements. T.D.X., T.D.K. R.R and K.K. revised the manuscript.

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DECLARATION

We declare that all authors of this manuscript have made substantial contributions. We did not exclude any author who substantially contributed to this manuscript. We have followed the ethical norms established by our institution.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest and approved the final version of the manuscript.

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