Phenotypic Performance of Rice (*Oryza sativa* L.) Populations Induced by the MNU Mutant on the Adaptive Characteristics

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Abstract. Mutation is an impressive method to induce potent characteristics in rice breeding programs. Evaluation of the phenotypic performance in mutant populations is important to examine the effectiveness of mutation. In this study, two rice mutant populations generated from MNU (*N*-methyl-*N*-Nitrosourea)-induced mutation were used to evaluate their phenotypes. The results showed that all of varieties and mutants expressed their ability to adapt with new environmental condition via the expression of agronomic traits. Grain yield of them ranged from 6.18 to 10.70 tons/ha. In general, population BC15TB × SKLo performed their best characters. The distribution of related traits to grain yield and amylose content were also different from each population. These results indicated that mutants expressed better characters than their parents. This study provided general information on the phenotype of rice mutants and varieties in the new environment and revealed the better-adapted characteristic of rice mutants. Findings of this study confirmed the efficacy of MNU in rice breeding and promising new potential mutants for rice breeding.

Introduction

Rice is an annual plant and has enormous importance in the world due to its nutritional value [1]. Rice is recognized as the key cereal crop consumed almost exclusively by human beings, and approximately fifty percent of the world's population based on rice as their principal food [2]. Most of the world's rice (about 90%) was produced and consumed in the Asia region with 100,000 entries from twenty wild species. Among cultivated rice, *Oryza sativa* originated from Asia has the most contribution to the worldwide production [3]. Rice breeding plays an important role in increasing food production [4] for a rising global population. To date, the average of rice production is around 5.34 tons/ha [5], and it is necessary to enhance rice production for encounter with the consequent rising demands. Previous study indicated that increasing rice yield and creating superior rice to ensure food security are therefore the urgent challenges [6]. Tremendous progress has been conducted to optimal rice protocol [2], among of them, mutation is a prominent strategy for rice breeding.

As a heritable alteration in genetic material, mutation created variation allowing the organism to adjust and adapt to their environment, and this variation can be transferred from parent to offspring [7]. Mutation was considered as a practical approach, and its positive impacts were confirmed in crop improvement in a shorter time and a reduction in cost, and it is a useful tool to isolate and develop the expected variants and resistances for breeding programs [8]. The principal goal of mutation breeding is to develop new varieties possessing high yield, better nutritional quality, and resistant to biotic and abiotic stresses [9]. Considerable progress has been made by induced mutation breeding; as a result, a total of 821 rice mutant varieties have been officially released in the world. Remarkably, more than 84 percent of rice mutant varieties are generated from Asia region, in which, China, Japan, and India are considered as the top three of the world about mutants' generation [10]. These mutants have been noted as the major crop, and they were cultivated in large areas contributed to food security [11].

Physical and chemical factors are mainly mutagens to create mutagenesis in plant [6, 9, 12]. Chemical mutagens can provide a very high mutation frequency; moreover, they were very easy to use without specialized equipment [13]. Therefore, most of the mutant populations of rice, maize, barley, and sorghum were created by chemical mutagens [6]. Induced mutagenesis is admitted as a potential tool to improve crop characters within a shorter time than traditional breeding, and numerous mutant alleles are sources of genetic diversity [8]. This study was conducted to characterize each mutant's population generated by MNU-induced mutation, examine their wide-adapted ability via phenotypic assessment, and to evaluate the variation among population via the distribution of each related character to grain yield and amylose content.

Materials and Methods

Rice materials

Two populations including mutant lines (F₂ generation) and their parents (rice cultivars) were used to examine in this study. All of the cultivars are *Indica* subtype. Population 1 included DT84, M2-Bao thai, and the F₂ generation of Bao thai/DT84, and population 2 consisted of BC15TB, SKLo, and the F₂ generation of BC15TB/SKLo. Rice materials' information was listed in Table 1. Rice mutants were released from original cultivars by MNU (N-methyl-N-Nitrosourea) treatment under a novel chemical mutation, noted as "Respiration Mutation" as described in the recent study [14]. Rice seeds were treated with MNU at a low concentration in a hermetic condition in a duration of three months. After that, they were collected and stored at 5 °C in the dark until further use.

Field evaluation

Seedlings of mutants and varieties were transplanted in May and harvested in early November 2016 at a rice field affiliated to Hiroshima University, Hiroshima, Japan (85°E, 34°23'41''N 132°43'5''E, 200 m Elevation). Before cultivated at the field, all of the rice seeds were treated as described in Anh et al. [14]. Rice was soaked in NaOCl (0.1%) for 30 minutes and then washed with distilled water. These seeds were placed in beakers and kept in a plant growth chamber at 30 °C in three days. The germinated seeds were transferred into plastic trays with soil and moved to a greenhouse. After twenty days, seedlings were transplanted into the rice field. Rice was arranged as a randomized complete block design with three replications. Plant spacing was assigned as 15 × 20 cm with one seedling per hill. Standard fertilizer was applied in three times by hand broadcasting, before transplanting, during tiller formation, and during panicle initiation stage. Weeding was also controlled by hands. Standard agronomic practices were used as rice characteristics' recommendations.

Phenotypic characters assessment

Phenotypic characters were evaluated in this study including panicles number per plant (NP), panicle length (PL), 1000-grain weight (KGW), full-grain per plant (FGP), grain yield per hectare (GY), grain length (GL), grain width (GW), and grain length to width ratio (LWR), amylose content (AC), protein content (PC), and lipid content (LC). To collect data, plants were randomly chosen without border plants.

Rice grain yield measurement

Grain yield characteristics including panicles number per plant (NP), panicle length (PL), 1000-grain weight (KGW), full grains per plant (FGP), grain yield per hectare (GY) were recorded at the maturity stage. Panicle length (PL) was measured in centimeters from panicle base to the tip. The weight of 200 grains was used to calculate the weight of 1000 grains. Ten rice-grains were randomly selected and measured grain length (GL), grain width (GW), and grain length to width ratio (LWR). The value of grain length, grain width, and grain length to width ratio were recorded by millimeters. Grain yield per hectare was calculated by grain yield per square meter

Chemical quality character evaluation

Rice grains were dried by oven until getting expected moisture (14%), and then they were dehusked by an Automatic Rice Husker machine (model TR-250, Japan). After that, amylose content (AC), protein content (PC), and lipid content (LC) were measured by PGC Shizuoka Seiki PS-500 machine (version 2-12, Japan). The final values of them were the average of five times measurement.

Data analysis

The phenotypic data were analyzed by SPSS 20.0 package (SPSS Inc., Chicago, IL, USA) and Minitab version 16 software (Minitab Inc., Stage, PA, USA). The contribution of the related traits on grain yield and amylose content was estimated by regression analysis.

Table 1. List of rice materials.

P	Origins	Descriptions
1	M ₂ -Bao thai	The origin Bao Thai cultivar from China with good quality, cold tolerance, growth duration ranged from 160 – 170 days. It was cultivated in Northern Midland and Mountainous region in Vietnam
1	DT84	Traditional rice with good quality in the North of Vietnam
1	M ₂ -DT84/M ₂ -Bao thai	These materials were F ₂ generation and induced with MNU. They were originated from crossing between DT84 (female parent) and M ₂ -Bao thai (male parent).
2	SKLo	A high yield cultivar, widely cultivated in Vietnam
2	BC15TB	Rice cultivar possesses good quality, high yield, resistance to bacterial leaf blight and brown planthopper, high adaptability, growth duration varied from 130 -138 days in spring season, and 110 – 115 days in another season, widely cultivated in Vietnam
2	M ₂ -BC15TB/SKLo	These materials were F ₂ generation and treated by MNU mutagen. They were generated from crossing between BC15TB (Female parent) and SKLo (male parent).

P: population

Results

Field performance of rice mutant populations

The results of the phenotypic analysis of mutant populations were presented in Table 2. The results showed a wide variation between population 1 and 2. The average grain yield of population 1 diverged from population 2, these values ranged from 7.23 to 10.70 tons/ha. P₂ (M₂-BC15TB/SKLo, BC15TB, SKLo) gained higher yield (10.70 tons/ha) than P₁ (M₂-Bao thai, DT84, DT84/ M₂-Bao thai) 7.23 tons/ha. Panicle length ranged from 24.62 to 26.12 cm. The panicle length of P₁ was 24.62 cm, while the panicle length of P₂ was 26.12 cm. The 1000-grain weight varied from 21.78 to 22.19 gr. The higher value of 1000-weight grain was recorded in P₂ (22.19 gr), the KGW of P₁ was 21.78 gr. Both population 1 and population 2 had long grain size. Their grain length were longer than 5.50 mm, and their grain length to width ratio were greater than 2.00 mm. Their size were 5.98; 2.49 mm (P₁) and 6.19; 2.67 mm (P₂).

The results of chemical properties also showed in Table 2, the average value of amylose content of two populations ranged from 22.33 to 24.11%, in which P₂ had a higher value (24.11%) than P₁ (22.33%). Protein content value of population 1 differed from population 2, in which P₂ (5.32%) had lower value than P₁ (6.78%). The average values of LC varied from 8.66 in P₂ to 9.77% P₁.

The phenotypic expression of each population was listed in Table 3 and Table 4. These results showed that mutants had better values than their parents. In population 1, the yield of mutants was 7.84 tons/ha, while their parents' yields were 6.81 and 7.04 tons/ha. In population two, mutants' yield was 13.04 tons/ha; otherwise, their parents' yields were 9.76 and 9.32 tons/ha.

Distribution of traits to grain yield

The distribution of related traits to grain yield of two mutant populations was performed in Fig. 1. Results showed that grain yield completely linear increased with increasing full grain in both of two populations ($r = 1$). However, the contribution of a number of panicles and 1000-grain weight on grain yield was different from each population. The contribution of NP to GY ranged from 0.32 in P_1 to P_2 0.39. There was minimal relevance between KWG and GY in both populations. KWG had a very small linear relationship to GY (in P_1 $r = 0.08$; in P_2 $r = 0.05$).

Distribution of traits to amylose content

The distribution of related traits to amylose content of mutant populations was depicted in Fig. 2a and 2b. There was an inconsistent distribution among traits to AC. The contribution of GL to AC ranged from 0.26 to 0.27. There was negligible difference between P_1 and P_2 , the linear contributions of GL to AC were 0.27 in P_1 and 0.26 in P_2 . Ranged from 0.15 to 0.39, the linear contribution of GW to AC was 0.39 in P_1 and 0.15 in P_2 . The contribution of LWR to AC ranged from 0.07 to 0.43, in which, the smaller contribution of LWR to AC was found in P_2 (0.07), and the value of P_2 was 0.43. The contribution of PL to AC ranged from 0.00 to 0.09, in which, insignificant distribution of PL to AC was noted in P_2 ($r = 0.00$), and the minimal distributions in P_1 was 0.09, respectively. The contribution of PC to AC ranged from 0.00 to 0.09. No linear relationship between PC and AC ($r = 0.00$) was found in P_2 , and the low linear relationship was observed in P_1 ($r = 0.09$). The low contribution of LC to AC was considered in all populations and ranged from 0.00 to 0.14, in which no linear relationship was noted in P_2 ($r = 0.00$), and small linear was in P_1 ($r = 0.14$).

Table 2. Descriptive statistic of phenotypic traits in mutants/varieties.

Traits	P_1	P_2
NP	7.44±1.66b	8.59±1.44a
PL	24.62±1.12b	26.12±0.69a
KWG	21.78±1.36a	22.19±0.87a
GY	7.23±1.01b	10.70±0.51a
FGP	21.91±95.7b	32.43±73.3a
GL	5.98±0.24b	6.19±1.14a
GW	2.40±0.16a	2.32±0.04b
LWR	2.49±0.24b	2.67±0.02a
PC	6.78±0.89a	5.32±0.16b
AC	22.33±1.33b	24.11±0.21a
LC	9.77±1.65a	8.66±0.60b

Each value expressed Mean±Standard error; a,b,c: Different letter in the same row indicated significant differences at $P < 0.05$ level by Fisher pairwise comparisons; P_1 (Population 1): M₂-Bao thai, DT84, M₂-DT84/Bao thai, P_2 (Population 2): BC15TB, SKLo, M₂-BC15TB/SKLo; NP: number of panicle per plant; PL: panicle length; KWG: 1000-grain weight; GY: grain yield per ha; FGP: full grain per plant; GL: grain length; GW: grain width; LWR: grain length to width ratio; PC: protein content; AC: amylose content; LC: lipid content.

Table 3. Descriptive statistic of phenotypic traits in Population 1.

Traits	Bao thai	DT84	DT84/Bao thai
NP	6.50±1.80b	7.63±1.88a	8.20±1.79a
PL	25.59±1.11a	25.93±1.26a	22.33±1.22b
GL	5.97±0.19b	6.30±0.19a	5.68±0.26c
GW	2.10±0.02b	2.29±0.12b	2.17±0.01a
LWR	2.68±0.10b	2.76±0.14a	2.11±0.10c
FG	20.62±6.23a	21.34±6.16a	23.77±6.15a
KWG	19.88±2.38b	22.79±1.55b	22.67±1.46a
GY	6.81±2.06a	7.04±2.03a	7.84±2.03a
PC	5.87±1.44b	6.37±1.52a	8.09±2.12a
AC	23.19±0.83a	23.29±1.01a	20.51±1.74b
LC	8.70±3.14b	9.51±2.81a	11.10±2.24a

a, b, c: Different letter in the same row indicated the significant differences at $P < 0.05$ level by Fisher pairwise comparison. Value is the mean $\pm$ standard deviation.

Table 4. Descriptive statistic of phenotypic traits in Population 2.

Traits	BC15TB	SKLo	BC15TB/SKLo
NP	7.77±1.72b	6.53±1.53c	11.47±2.62a
PL	26.02±0.79b	24.92±1.11c	27.43±1.36a
GL	6.43±0.17a	5.96±0.20c	6.18±0.18b
GW	2.36±0.04a	2.25±0.06b	2.34±0.06a
LWR	2.73±0.09a	2.64±0.10b	2.64±0.10b
FG	29.57±8.71b	28.23±6.70b	39.60±5.20a
KWG	23.00±0.74a	20.39±1.32b	23.18±0.61a
GY	9.76±2.87b	9.32±2.12b	13.04±1.72a
PC	5.26±1.07a	5.37±1.70a	5.32±1.55a
AC	24.46±0.25a	24.17±0.5b	23.69±0.50c
LC	8.96±2.84ab	7.73±2.51b	9.30±2.74a

a, b, c: Different letter in the same row indicated the significant differences at $P < 0.05$ level by Fisher pairwise comparison. Data are expressed as the mean $\pm$ standard deviation.

Figure 1. Regression of full grain number per plant (FG), panicle number per plant (NP), and 1000-grain weight (KWG) to grain yield (GY); P₁: population 1; P₂: population 2.

Figure 2a. Regression of grain length (GL), grain width (GW), and grain length to width ratio (LWR) to amylose content (AC); P₁: population 1; P₂: population 2.

Figure 2b. Regression of panicle length (PL), protein content (PC), and lipid content (LC) to amylose content (AC); P₁: population 1; P₂: population 2.

Discussion

An indisputable position of rice has recognized in human beings' daily life because the demand for rice is always necessary and undeniable. Although climate change is currently the most formidable obstacle effecting to crop productivity [6], rice production need to be risen up to more 70% in the upcoming year to fulfil the requirement of rapid growth of global population by 2050 [1]. Remarkably, mutation is considered as a sufficient method to extend particular rice characteristics [15]. Additionally, it was documented that induced mutation had more advantages to enhance elite characters than single phenotypic variation [16]. Furthermore, an unprecedented role of mutation has been clarified both in new cultivars and gene function [17]. According to FAO/IAEA database, up to now, 1825 mutants were known as better quality and higher nutritional content [18], and they have a greater impact to the economy of their area production [17]. MNU-induced mutant population was considered as a powerful source for rice breeding [19].

To screen mutant populations, field evaluation is important because a gene can be altered by induced mutations leading to change desired characters [20]. Phenotypic performance and yield expression under field condition are valuable information because these evidences will support breeding efforts more efficient and successful [21]. Wide adaptation is one of the beneficial characters of rice varieties, rice can be able to grow over large geographical regions under the alternative of agroclimatic conditions [22]. Wide adaptation is a valuable character of rice to deal with climate change in the current situation. In this study, rice varieties and MNU mutants were presented their well-adapted ability. These populations were cultivated in the summer season from May to the early November 2016, both of them expressed their adaptive ability to the environmental condition in Hiroshima, Japan through phenotypic expression although they were *indica* varieties. Imported to Vietnam from China since 1970s, Bao thai was characterized by high quality, adaptation, and cold tolerance, while BC15TB was generated by Thai Binh seeds company possessing high productivity. These varieties' yields in Vietnam were considered from 5.5 to 10 tons/ha. In this study, the average yield of these population was acceptable, and astonishingly, mutants had a higher yield than their parents. These results confirmed that mutant lines released from varieties performed well adaptability, and they had better characteristics compared to their parents. It can be explained that mutation created allelic variations, and these alleles were able to transfer from parent to offspring and enabled organisms to become accustomed to the environment [7].

The association among phenotypic traits is a considerable feature to select desired characteristics [23, 24]. Our results found both consistent and inconsistent about the distribution of each trait to grain yield and AC in each population. In the previous study, the correlations among traits in mutants were identified [14], and the distribution of related phenotypic traits was documented in rice populations [23, 25]. The number of panicles, full grains, and grain weight are three principal elements of grain yield, in which 1000-grain weight, is a significant trait related to grain yield [26, 27]. Conversely, this study found that full grain per plant had the highest contribution to grain yield of two populations. This result was similar to our previous study [14] and suggested that full grain will decide grain yield. Grain length, grain width, and grain length to width ratio are three main factors of grain size, and they can determine grain appearance quality [27]. Former studies found that there were correlations between grain size (appearance quality) and nutritional quality [14, 23, 25]. The distribution of grain size to AC was different on each population. This result also revealed in [24, 26], and they implicated that it was difficult to demonstrate the relationship between AC and the other traits. Additionally, a correlation between grain weight and grain size was found [26]; however, the level of correlation of them has differed from independent studies. Previous research illustrated that amylose content was regulated by *Wx* gene and the other minor QTLs [26, 28] and proposed that marker-assisted selection should be applied on that process because it was difficult to classify genotypes closely by phenotypic evaluation [29, 30].

The prior studies indicated that the interaction of genotypes and environments caused the inconsistent level of correlation coefficients among phenotypic characters, and many agronomical traits such as morphological, physiological; in addition, quality characters can be sensitive from influences of environments such as climate and soil [31–33]. The environment is one of the strong

factors that effects to phenotypic performances, at many experimental conditions as diverse sites, various fertilizations, and weather conditions, elite cultivars will exhibit different phenotypes. In this study, mutants released by MNU-induced mutation expressed their good phenotype and they could be a promising source for rice breeding. MNU was documented as a high-frequency mutation agent, and it created point mutation and varied genome's organism [34, 35]. Therefore, these alterations by MNU impacted on phenotypic expression because the phenotype is a result of the interaction between genotype and environment [36]. This study evaluated both individual and group characters and supplied detailed information of mutants as well as their relatives to help us better understand on mutant characteristics, the variation of mutants, the relationships of traits in two populations, and the adaptation character of rice mutants.

Conclusions

Varieties possessed well-adapted character are a priority in rice breeding under pressure of climate changes. Furthermore, rice with high productivity is apparently needed. In this study, the wide-adapted characteristic was confirmed from rice mutants generated by MNU- mutation. This research primarily revealed valuable information of MNU mutagen on the phenotype of mutants. These findings indicate that it is possible to obtain better varieties by MNU-induced mutation as compared to their parents. Further examination as marker-assisted selection (MAS) should be conducted on these mutant population to analyse the alternations in molecular levels of mutants.

Conflict of Interest

The authors declare no conflict of interest.

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