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Original Research Article

Comparisons and selection of rice mutants with high iron and zinc contents in their polished grains that were mutated from the *indica* type cultivar IR64

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ABSTRACT

Developing rice varieties biofortified with iron (Fe) and zinc (Zn) is an important strategy to alleviate nutritional deficiencies in developing countries, where polished rice is consumed as the staple food. In this study, the contents of several macro- and micro-minerals in polished rice grains of cultivar IR64 and its 254 sodium azide-induced mutants (M8 generation) were assessed. The results indicated that the contents of potassium, phosphorus, calcium, magnesium, iron, manganese, copper, and zinc varied among the tested mutants. The polished rice grains of mutants M-IR-75 and M-IR-58 accumulated more Fe (28.10 and 27.26 mg kg^{-1}, respectively) than cultivar IR64 (3.90 mg kg^{-1}). Mutant M-IR-75 also produced higher yield (average of 8.65 ton ha^{-1} over two crop seasons) than cultivar IR64 (average of 7.27 ton ha^{-1}). Mutants M-IR-180, M-IR-49 and M-IR-175 contained more Zn (26.58, 28.95 and 26.16 mg kg^{-1}, respectively) than cultivar IR64 (16.00 mg kg^{-1}), but only mutant M-IR-180 showed a grain yield comparable to cultivar IR64. Thus, the mutant M-IR-75 can be recommended to rice growers to produce Fe-rich rice grains. Additionally, the high-Fe (M-IR-75 and M-IR-58) and high-Zn (M-IR-180, M-IR-49 and M-IR-175) mutants can be used as genetic resources for rice improvement programs.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereals worldwide and serves as a staple food for more than half of the world's human population. However, in spite of its great importance, rice on its own is not considered a balanced diet choice, because the majority of rice is consumed in the form of polished grains, from which the bran layer and germ (the main storage sites for essential minerals such as iron (Fe) and zinc (Zn)) are removed during the milling process (Hoa and Lan, 2004). Therefore, in developing countries, where polished rice is consumed as a staple food, anemia due to Fe deficiency is the most widespread nutritional disorder (Yip, 2002; Hotz et al., 2008; White and Broadley, 2009).

To alleviate Fe deficiency, application of Fe containing fertilizers has been used to increase the Fe content in edible parts of staple food crops. Nevertheless, this approach is not unambiguous in increasing the Fe content in rice grains (Zhang et al., 2008; Chandel et al., 2010). Fortification of Fe has also been used to restore the Fe content in polished rice grains (Fresco, 2005; Moretti et al., 2005; Prom-u-thai et al., 2008). However, Fe fortification affects the quality and textural properties of cooked rice grains and causes negative reactions from consumers (Prom-u-thai et al., 2009). Moreover, fortification is an expensive technique; therefore, it is generally not affordable to the people suffering from anemia induced by Fe deficiency in the developing world. Genetic modification is an effective strategy to increase the Fe content in rice grains (Brinch-Pedersen et al., 2007; Zhu et al., 2007; Ye et al., 2008; Waters and Sankaran, 2011); but the transgenic rice may not be acceptable to all the consumers. Thus, producing Fe-rich rice varieties through conventional breeding methods appears to be a manageable and non-controversial solution (Gregorio, 2002; Prom-u-thai et al., 2007; Zhang et al., 2008; Frank et al., 2009).

Chemical-induced (e.g. sodium azide (NaN_3) and ethyl methanesulphonate) mutagenesis has been used to create genetic variability and improve some agronomic traits (Tai, 2007; Jeng et al., 2011). Therefore, it is worthwhile to use this technique to increase genetic diversity in some of important rice varieties and, subsequently, increase the iron content in their polished grains. The *indica* type cultivar IR64 is a widely accepted rice variety with high yield, short growth duration and good-tasting qualities in

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many countries (Fujita et al., 2009). The wide adaptability of IR64 also makes it an ideal genotype to develop a mutation pool for selection of mutants with desirable characteristics. Recently, a mutation pool of IR64 varieties induced with NaN_3 has been created by Agricultural Research Institute of Taiwan (Wufeng, Taichung, Taiwan, ROC). In this study, attempts were made to compare the contents of several minerals in polished rice grains of IR64 and its NaN_3-induced mutant population. The grain yields of several selected mutants were also assessed and compared with their wild type cultivar. Knowledge of these variations should provide useful insights on the potential value of these mutants to breeding programs of the Fe-rich rice.

2. Materials and methods

2.1. Mutagenesis

The rice (*O. sativa* L.) grains of cultivar IR64 were soaked in distilled water for 24 h initially, and then were soaked in a phosphate buffer (pH 3.0) for 4 h. These rice grains were then divided into four groups and further soaked in 1, 2, 5 and 10 mM of NaN_3 solution (dissolved in the same phosphate buffer) for 24 h, respectively. Afterwards, the treated grains were rinsed with de-ionized water and then planted in nursery trays. The calculated rates of seedling emergence of those rice grains that had been subjected to various NaN_3 treatments were generally above 76% (Table 1). Through randomized selection 500 three-leaf-stage seedlings from each treatment group were transplanted in the experimental farm field of Taiwan Agricultural Research Institute (Wufeng, Taichung). Only the M1 generation plants with morphological characteristics distinctly different from the wild type IR64 were marked and collected (only one panicle was harvested from each plant) to produce the M2 generation plants. The produced M2 plants were advanced to the M8 generation through the single-seed-descent method (two generations were grown per year) (Grafius, 1965). A total of 254 mutants of the M8 generation were grown and maintained in the field for quantitative analyses.

2.2. Agronomic practices

The grains of cultivar IR64 and its 254 NaN_3-induced mutants were grown on the experimental farm of Taiwan Agricultural Research Institute (Wufeng, Taichung) in the spring crop season of 2010. The experiment was designed as a complete randomized block design with four replicates. Grains were sown in the nursery plots, where the seedlings grew to the three-leaf stage. The grown seedlings were then transplanted to the experimental plots on February 6, 2010. Three seedlings per hill were planted with a spacing of 30 cm × 15 cm in each experimental plot of 3 m × 1 m. Each plot received a basal application of fertilizer before transplanting (24 kg N ha^{-1}, 36 kg P_2O_5 ha^{-1} and 24 kg K_2O ha^{-1}, respectively), and three top-dressings of fertilizer on the 20th day (6 kg N ha^{-1}, 9 kg P_2O_5 ha^{-1} and 6 kg K_2O ha^{-1}, respectively), the 40th day (9 kg N ha^{-1}, 13.5 kg P_2O_5 ha^{-1} and 9 kg K_2O ha^{-1}, respectively) and the 60th day (9 kg N ha^{-1}, 13.5 kg P_2O_5 ha^{-1} and 9 kg K_2O ha^{-1}, respectively) after transplanting. All the rice grains from twenty panicles randomly sampled and hand-harvested from each plot were refrigerated at −4 °C until they were used for the chemical analyses.

2.3. Minerals determination

Prior to chemical analyses, in separated batches, all the rice grains of IR64 and its 254 NaN_3-induced mutants were mechanically de-hulled with a Satake THU-35 de-husker (Satake Corp., Hiroshima, Japan). The resulting brown rice grains were then milled with a commercial bench-top miller (Kett Electrical Laboratory, Tokyo, Japan). The polished rice grains were sieved through a 0.84-mm sieve (E.H. Sargent & Co., Chicago, Ill., USA), and stored at −20 °C.

The polished rice grains (the moisture contents were kept at 140 g kg^{-1}) of IR64 and its mutants were ground in separated batches. The ground samples were digested using the method of AOAC (1990) with minor modifications. Briefly, a portion of each ground sample lot (1 g ground rice) was weighed and placed in a digestion vessel, to which 10 mL of $HClO_4$:HNO_3 (1:4) were added, and kept overnight for pre-digestion. All pre-digested samples were then mixed well by swirling and placed onto a hot plate in separated batches. The temperature of each sample batch was gradually increased to 225 °C for digestion. All the digested samples were then used for the quantification of K, P, Ca, Mg, Fe, Mn, Cu and Zn by using an inductively coupled plasma atomic emission spectrometer (JY50P, Jobin-Yvon, France). All the chemical reagents used were of analytical grade.

2.4. Grain yield determination

In order to support measurements of grain yield, some of the mutants selected from the 2010 spring crop (based on measurements of Fe and Zn contents) were grown on the experimental farm using the same agronomy practices but in larger test plots (2 m × 4 m) in the autumn crop season of 2010 and the spring crop season of 2011. The experiment was also designed as a complete randomized block design with four replicates. The middle four rows (2 m in length) of each plot were hand-harvested. The grain mass of those hand-harvested samples was weighed, and the moisture content of each sample was also measured. Yields were expressed at 140 g kg^{-1} moisture.

2.5. Statistical analysis

The experiment was set up following a randomized complete block design with four replicates. Values were expressed as means ± standard deviation (SD), and means were separated using a least significant difference (LSD) test. Correlation analysis was also used to characterize the relationships between the examined minerals. Principal coordinate analyses of mineral contents were performed by using NTSYS-PC software version 1.80 (Exeter Software, New York, USA).

Table 1
Percentages of emerged normal seedlings from rice grains of cultivar IR64 that were subjected to various NaN_3 treatments.

Mutagen	Treated rice grains	No. of emerged normal seedlings	Seedling emergence rate (%)
1 mM NaN_3	1000	985	98.5
2 mM NaN_3	1000	965	96.7
5 mM NaN_3	1500	1196	79.7
10 mM NaN_3	1500	1148	76.5