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Low-energy N-ion beam biotechnology application in the induction of Thai jasmine rice mutant with improved seed storability

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ABSTRACT

Low-energy heavy-ion beam is a novel biotechnology used for mutation induction in plants. We used a low-energy N-ion beam to induce mutations in Thai jasmine rice (*Oryza sativa* L. cv. KDML 105) to improve the yield and seed quality. Seeds of BKOS6, a Thai jasmine rice mutant previously induced by ion beams, were re-bombarded with 60-kV-accelerated N-ions ($N^+ + N_2^+$) to fluences of 1–2 × 10^{16} ions/cm^2. The resulting mutant, named HyKOS21, exhibited photoperiod insensitivity, semi-dwarfness, and high yield potential. Seed storability of the mutant was studied in natural and accelerated ageing conditions and compared to that of KDML 105 and six other Thai rice varieties. In both testing conditions, HyKOS21 mutant had the highest seed storability among the tested varieties. After storage in the natural condition for 18 months, HyKOS21 had a seed germination percentage nearly two times as that of the original KDML 105. Biochemical analysis showed that the lipid peroxidation level of the mutant seeds was the lowest among those of the tested varieties. Furthermore, an expression analysis of genes encoding lipoxygenase isoenzyme (*lox1*, *lox2*, and *lox3*) revealed that the mutant lacked expression of *lox1* and *lox2* and expressed only *lox3* in seeds. These results may explain the improved seed longevity of the mutant after storage. This work provides further evidence of the modification of biological materials using a low-energy ion beam to produce rice mutants with improved yield and seed storability. The benefits of this technology, to create new varieties with improved values, could serve for local economic development.

1. Introduction

Ion beam modification of conventional solid materials has been extended to the modification of biological materials in recent decades and thus serves as a novel biotechnology applied to agriculture and biomedicine (for instance, [1,2]). Initially, high-energy ion beams with a typical energy region of MeV and higher (for instance, [3]) were used for biological modification, but later, low-energy ion beams, on an order of 10 keV, were used after similar biological effects were observed [4,5]. A low-energy ion beam provides many advantages including low damage rate, high mutation rate, and broad mutation spectrum. It has been applied as a powerful tool for the mutational breeding of many plant species [6–9]. In Thailand, low-energy N-ion beam implantation has been used as a novel source of mutagenesis for mutation induction in Thai purple rice [10], Thai jasmine rice [11], Thai fragrant rice [12], and several ornamental plants such as gerbera, rose, petunia, and chrysanthemum [13]. Many beneficial phenotypes were obtained in the plants, such as semi-dwarfness, photoperiod insensitivity, and anthocyanin accumulation in rice [11,14]; partial male sterility in *Arabidopsis* [15]; changes in flower color and shape in ornamental plants [13]; antioxidant activity improvement in rice grains [16]; and growth promotion of rice seedlings [17].

Improving rice seed quality is one of the main goals of ion beam biotechnology in mutation breeding in Thailand. Losses in seed quality occur throughout the growing season, harvesting, and storage. Grain deterioration during storage may lower the seed quality and lead to problems in the subsequent sowing [18]. Seed spoilage during storage is caused by many processes, including the peroxidation of lipids, which can be enhanced by a single oxygen atom, free radicals, metal ions, light, radiation, and lipoxygenase enzymes [19]. Lipoxygenases (LOXs)

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are enzymes involved in the initial step of lipid peroxidation that leads to the formation of hydroperoxides [20]. LOX activities have been found in rice seeds [21], and hexanal is the predominant component that causes stale off-flavors in rice seeds during storage [22]. Compared to soybean, rice seed LOX activities are low and presented mainly in embryo and bran fractions [23]. There are three forms of LOXs, namely LOX-1, LOX-2, and LOX-3, in rice seeds [24]. The studies of the effects of LOX isoenzymes on seed storability in rice and maize showed that LOX-1 and LOX-2 might play a key role in accelerating seed deterioration, thus shortening the seed lifespan. However, rice or maize varieties without LOX-1 and LOX-2 showed a slight decrease in the germination rate after seed storage in natural and accelerating ageing conditions [25,26].

By using a low-energy N-ion beam to induce the mutation of Thai jasmine rice, this study aimed to obtain mutants having high yield characteristics with improved seed storability. The results were justified by testing the seed storability in accelerated ageing and natural conditions and by analyzing the expression of the genes encoding LOX enzymes.

2. Materials and methods

2.1. Plant materials

Thai jasmine rice variety, KDML 105 (*Oryza sativa* L. cv. KDML 105), was targeted for the low-energy ion beam bombardment. After a second bombardment, KDML 105 and a selected mutant were studied in terms of seed storability and *lox* gene expression and compared to five Thai commercial rice varieties and one highland rice variety. Descriptions of these rice varieties are summarized in Table 1. All rice varieties were grown in northern Thailand and harvested during the growing season (August–December 2014).

2.2. Ion bombardment

Seeds of KDML105 were targeted for ion bombardment using the 150-kV high-current non-mass-analyzing ion implanter [27] at Chiang Mai University. Subsequently, the KDML105 mutant (BKOS6) seeds were re-bombarded to improve yield and seed quality characteristics. To break seed dormancy, BKOS6 whole seeds (seeds with the seed coat) with moisture content less than 14% (which was measured in triplicate randomly using an automatic whole grain moisture analyzer) were incubated at 49 °C for 5 days. The incubated seeds were then manually dehulled and inserted into holes in a copper sample holder for efficient heat diffusion. Each seed was placed such that the embryo faced the ion beam. Six thousand dehulled seeds were bombarded under a pressure of 10^{-3} Pa using mixed $N^{+} + N_2^{+}$ beam with a beam current of about 2 mA. N-ions were accelerated by 60 or 80 kV to ion fluences of 1×10^{16} or 2×10^{16} ions/cm^2, respectively. This ion bombardment condition was based on our experience [10–12] and literature data (for instance, [1]). For instance, in a previous work on rice mutation induction [10], we practically determined that the lethal fluence for a seedling death rate of about 60% was in the range of 1–4 $\times 10^{16}$ ions/cm^2 for a 60-kV-accelerated N-ion beam, and for a death rate of about 50%, it was 1×10^{16} ions/cm^2 for 80-kV-accelerated N-ion beam, while no possible mutation induction was observed for fluences lower than 1×10^{16} ions/cm^2 at the tested ion energy. To prevent the overheating of the rice seeds during ion bombardments, we used a beam interruption mode with a 10-s beam-on/off cycle controlled by a beam shutter and a water cooler at the target stage. Seeds that were kept only in vacuum condition but not exposed to the ion beam were used as the vacuum control. Seeds that were not subjected to vacuum and ion bombardment were used as the negative control. Because of the vacuum condition where the seeds stayed during ion bombardment, the seeds lost water. After ion bombardment, seeds were immediately soaked in sterilized water to replenish the water content in the cells and activate the physiological processes of germination. After few minutes of soaking, the water was poured out (otherwise the rice endosperm would be dissolved), and the soaked seeds were left in such moist condition overnight. The seeds were then planted the next day in an isolated culture field and grown by normal cultivation methods.

2.3. Screening and selection of rice mutants

KDML 105, the wild type of BKOS6, is photoperiod sensitive, which grows and produces grains only during the growing season (July–November, in Thailand). In an attempt to select a photoperiod-insensitive mutant, the cultivation and selection of M1 mutants in this experiment were carried out during the off-growing season (March–July); therefore, only photoperiod-insensitive mutants could flower and produce grains in this season. The ion-bombarded rice seeds were cultured and grown in a controlled field. Selection and confirmation of rice mutants were performed for generations M1 to M6. M1 plants bearing panicles were marked, and one panicle of each M1 plant was collected for M2 cultivation. In M2-M5 generations, plants of each mutant with a high tillering capacity and number of seeds per panicle were selected, and their mutational stability in photoperiod insensitivity and phenotypic variations was observed and recorded. M6 mutants were selected for seed storability testing and *lox* gene expression analysis based on their yield potential.

2.4. Storage condition

Seed storability test was carried out in both natural and accelerated ageing conditions. Approximately 100 g of whole rice seeds from each rice variety were packed in small paper bags. In the natural condition,

Table 1
Descriptions of all rice varieties used in the study.

Rice varieties	Descriptions
KDML105	Thai reputed fragrant non-sticky rice, known as Thai jasmine rice; the wild type in the study
BKOS6	Primary mutant of KDML 105, previously obtained from a low-energy N-ion beam mutation induction at an ion energy level of 60 keV and ion beam fluence of 2×10^{16} ion/cm^2 [11]
HyKOS21	Secondary mutant of KDML 105, obtained from a low-energy N-ion beam re-bombardment-induced mutation of BKOS6 at an ion energy level of 60 keV and ion beam fluence of 2×10^{16} ion/cm^2
Pathumthani 1	Thai high-yielding fragrant non-sticky rice obtained from cross-breeding
Chainat 1	Thai high-yielding non-sticky rice obtained from cross-breeding
Sanpathong 1	Thai high-yielding sticky rice obtained from cross-breeding
RD6	Thai sticky rice mutant of KDML 105 obtained from 20-krad gamma-ray irradiation mutation induction
RD10	Thai sticky rice mutant obtained from 1-krad fast-neutron irradiation mutation induction of Thai RD1 rice[1]
Highland	Thai sticky rice for highland plantation in the north of Thailand

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[1] RD1 (after the name of Rice Division) was the first officially released (in 1960s) non-glutinous, semi-dwarf, photoperiod insensitive, and high yield rice variety in Thailand, obtained by cross breeding (J. Jaroensathapornkul, The economic impact of public rice research in Thailand, Chulalongkorn J. of Economics 19(2) (2007) 111–134).