Jurnal Ilmiah Aplikasi Isotop dan Radiasi
*A Scientific Journal for The Applications of Isotopes and Radiation*
Vol. 5 No. 1 Juni 2009

ISSN 1907-0322

# A SINGLE RECESSIVE MUTATED GENE ($sd^{237-1}$) CONTROLING SEMI-DWARF PLANT STATURE OF RICE

Sobrizal

Center for the Application of Isotopes and Radiation Technology
National Nuclear Energy Agency, Indonesia
Jl. Lebak Bulus Raya No. 49, Kotak Pos 7002 JKSKL, Jakarta 12070
Tel. 021 7690709, Fax. 021 7691607
E-mail : sobrizal@lycos.com

Received 20 Maret 2009; accepted 13 April 2009

## ABSTRACT

**A SINGLE RECESSIVE MUTATED GENE ($sd^{237-1}$) CONTROLING SEMI-DWARF PLANT STATURE OF RICE**. Dwarfism is a valuable trait in crop breeding, because it increases lodging resistance and decreases damages due to wind and rain. During the course of this study, a semi-dwarf mutant was successfully induced through 200 Gy gamma ray irradiated KI 237 seeds. KI 237 is a pure line with high yield potency, developed through an Indica-Japonica cross of IR36 / Koshihikari. The selected semi-dwarf plant reached 60 – 62 % of plant height of original plant KI 237 at the mature stage. The length of internodes, panicle, and seed were also compared between these two plants. The retardation of the 1st (uppermost) internodes was 24 %, moreover, the retardation of panicle and seed length were only 10 % and 2 %, respectively. The elongation pattern of the internodes in this mutant was almost the same as *sd1* (Dee-geo-woo-gen), the original parent of the first release modern rice variety, but their performances were different. Based on the segregation analysis in $M_2$ and $M_3$ generation it was concluded that this mutant was controlled by a single recessive mutated gene. This gene was designated as $sd^{237-1}$. This mutant should be useful as a genetic resource for the improvement of KI 237 line through back-cross breeding as well as be developed further in breeding program directly to be a new high yielding mutant variety.

Key words : induced mutation, mutated gene, semi-dwarf, rice

## ABSTRAK

**SATU GEN RESESIF TERMUTASI ($sd^{237-1}$) YANG MENGONTROL SIFAT SEMI-PENDEK TANAMAN PADI.** Karakter semi-pendek merupakan karakter yang sangat bermanfaat pada pemuliaan tanaman, karena dengan karakter tersebut akan dapat meningkatkan ketahanan tanaman terhadap rebah serta mengurangi kerusakan akibat angin dan hujan. Pada penelitian ini mutan semi-pendek berhasil diinduksi melalui iradiasi sinar gamma dosis 200 Gy terhadap benih galur KI 237. KI 237 mempunyai potensi hasil tinggi, merupakan galur murni asal persilangan Indika – Japonika (IR 36 / Koshihikari). Mutan semi-pendek tersebut mempunyai tinggi tanaman 60 – 62 % dari tinggi tanaman asalnya KI 237. Pada penelitian ini juga terlihat perbandingan panjang antar ruas, panjang malai, dan panjang gabah kedua tanaman. Pengurangan panjang ruas I (uppermost) tanaman mutan adalah 24 %, sedangkan pengurangan panjang malai dan panjang gabahnya berturut-turut hanya 10 % dan 2 %. Pola pemanjangan antar ruas pada tanaman mutan ini hampir sama dengan pola pemanjangan antar ruas pada *sd1* (Dee-geo-woo-gen), tetua varietas modern yang pertama dilepas, tetapi penampilannya berbeda. Berdasarkan analisis segregasi pada generasi $M_2$ dan $M_3$ dapat disimpulkan bahwa mutan ini dikontrol oleh satu gen resesif. Gen termutasi tersebut diberi nama $sd^{237-1}$. Mutan ini tentu akan sangat berguna sebagai sumber genetik dalam perbaikan galur KI 237 melalui pemuliaan silang balik, dan juga dapat dikembangkan melalui program pemuliaan tanaman untuk langsung menjadi varietas mutan unggul baru.

Kata kunci : mutasi induksi, gen termutasi, semi-pendek, padi

## INTRODUCTION

The origin of agriculture led to the domestication of many plant species and to the exploitation to the natural resources. It took almost 10,000 years for food grain production to reach 1 billion tons, in 1960, and only 40 years to reach 2 billion tons, in 2000. This unprecedented increase, which has been named the "green revolution", resulted from the creation of genetically improve crop varieties, combined with the application of improve agronomic practices [1]. The main genetic improvement was reduced plant height as semi-dwarf plant type. Modern rice cultivars of semi-dwarf plant type show increased lodging resistance, number of tiller, and responsiveness to nitrogen fertilization, all of which contribute directly to increase yield.

Since the dawn of the green revolution which began in Asia with IRRI's release in 1966 of IR 8, the first modern high yielding semi-dwarf rice variety, the global rice harvest [2] as well as the average rice yield [3] have more than doubled. The IR8 variety was developed by introducing the semi-dwarf gene (*sd1*) from Chinese spontaneous mutant variety Dee geo woo gen (DGWG) to the important variety Taichung Native-1 and after a cross with the tall variety Peta from Indonesia [4, 5]. Since then, *sd1* has remained the predominant semi-dwarfing gene present in current rice varieties. This has raised a concern over the possible genetic vulnerability of rice.

The traditional mission of mutation technology, development of new and desired variation for breeding program, has recently widened [6]. Some new sources of rice semi-dwarfisms have been produced by induced mutation [7, 8]. In our research works, it was found that the semi-dwarf plants segregated in $M_2$ population derived

 ISSN 1907-0322

from irradiated KI 237 line. KI 237 is a pure line developed through Indica/Japonica crossing of IR36/Koshihikari. The present study was carried out with the objective to analyze the mutated gene controlling the semi-dwarf character of the mutant.

## MATERIALS AND METHODS

A breeding line of KI 237 is developed by crossing of Indica rice (IR36) and Japonica rice (Koshihikari). This line shows good performance with high yield potency, but susceptible to lodging because of tall plant stature [9]. To reduce the plant height, 50 gram seeds of this line were irradiated by 200 Gy of gamma ray in 2006 at Center for the Application of Isotopes and Radiation Technology, Pasar Jumat, Jakarta. Irradiated $M_1$ seeds were sown, and twenty day-old seedlings were transplanted to paddy field by planting a single seedling per hole at experimental field, Sawangan, Depok with 20 cm spacing between plants.

Five hundreds $M_1$ plants were harvested individually to obtain the $M_2$ seeds. Harvesting was conducted by collecting only one main panicle in each $M_1$ plant. $M_2$ seeds were sown, and 20 $M_2$ plants derived from each $M_1$ panicle (plant) were transplanted to develop $M_2$ lines. Observation of semi-dwarf plants was conducted in each $M_2$ lines before harvesting. The $M_2$ lines showed segregation between normal and semi dwarf plants were selected. The whole plants of these selected lines were harvested individually.

In the next growing season, 80 plants derived from each $M_2$ plant of selected $M_2$ lines were planted to develop $M_3$ lines. Observation of segregation between semi-dwarf and normal plants was conducted in each $M_3$ lines. The segregation pattern of $M_3$ lines were as in the $F_2$ lines from crossed materials. The $\chi^2$ test in segregated $M_3$ lines was performed to examine fitness of the frequencies of the semi-dwarf plants against expectation from Mendelian segregation. The formula of $\chi^2$ test as follow;

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

O = Observed value
E = Expected value

**RESULTS AND DISCUSSION**

In this experiment, all $M_1$ plants derived from irradiated seed of KI 237 grew well. No significant difference was found between $M_1$ population and the original line KI 237, except on flowering time and the degree of seed sterility. The flowering time of $M_1$ population was a litle bit late and its seed sterility was higher in comparison with the original line. These might be due to physiological damage by irradiation treatment [10, 11].

Out of 342 $M_2$ lines, each $M_2$ line consisted of 20 plants, 5 lines segregated for plant height. In this study, since the original of these lines is KI 237, the plants having the same height as KI 237 were grouped as normal plants. Among the segregated lines, the line of RKI 237-1 is segregated for 8 semi-dwarf and 12 normal plants. This line was chosen as material for further genetic study. The semi-dwarf plant reached 60 – 62 % of plant height of original plant KI 237 at the mature stage (Fig. 1A). The length of internodes, panicle, and seed between these two plants were also compared (Fig. 1B; Fig. 2; Table 1). In rice, internodes elongation starts from the bottom at the panicle initiation stage. All internodes of semi-dwarf plant were shortened in comparison with the original pant. The elongation of the upper internodes was weakly inhibited. The retardation of the 1$^{st}$ (uppermost) internodes was 24 %, moreover, the retardation of panicle and seed length was only 10 % and 2 %, respectively. The elongation pattern of the internodes in this mutant was almost the similar as *sd1* (Dee-geo-woo-gen), the original parent of the first release modern rice variety [12].

To perform the genetic analysis of this semi-dwarf character, the segregated $M_2$ line, KI 237-1, was harvested individually and 80 plants derived from each harvested $M_2$ plant were planted to generate the $M_3$ lines. Four $M_3$ lines derived from normal $M_2$ plants (RKI 237-1-2, RKI 237-1-3, RKI 237-1-8 and RKI 237-1-12) and two $M_3$ lines derived from semi-dwarf $M_2$ plants (RKI 237-1-1 and RKI 237-1-17) were selected and they were used for analysis of the mutated gene(s). It was observed that all of RKI 237-1-1 and of RKI 237-1-17 plants showed semi-dwarf stature, all of RKI 237-1-2 and RKI 237-1-8 plants showed normal stature, and the other two $M_3$ lines derived from

 ISSN 1907-0322

normal $M_2$ plants, RKI 237-1-3 and RKI 237-1-12 were segregated for 57 normal and 22 semi-dwarf and 59 normal and 21 semi-dwarf plants, respectively. The segregation ratios between normal and semi-dwarf plants in both RKI 237-1-3 and RKI 237-1-12 $M_3$ lines fitted well to Mendelian expected segregation 3 : 1 (Table 2). These results suggested that the semi-dwarf character in these lines was controlled by a single recessive gene. This gene was designated as *sd*$^{237\text{-}1}$.

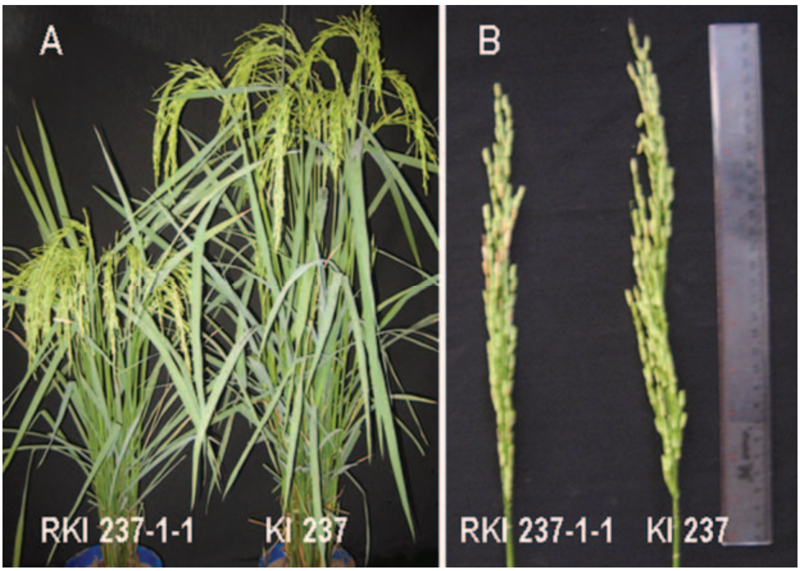


Fig. 1. Morphological characterization of semi-dwarf mutant RKI 237-1-1 and its original plant, KI 237.


Fig. 2. Comparison of culm elongation between semi-dwarf mutant RKI 237-1 and its original line KI 237.

Table 1. The length of internodes, panicles, and seeds of KI 237 and its semi-dwarf mutant.

| Line | Length of internodes, panicle, and seed (cm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | IN-I | IN-II | IN-III | IN-IV | IN-V | IN-VI | IN-VII | Panicle | Seed |
| RKI 237-1 | 0.3 | 0.5 | 1.5 | 3.0 | 7.5 | 21.5 | 34.0 | 28.0 | 10.3 |
| KI 237 | 0.7 | 1.0 | 4.5 | 10.5 | 20.0 | 28.0 | 44.5 | 31.5 | 10.5 |
| PIL (%) | 42.9 | 50.0 | 33.3 | 28.6 | 37.5 | 76.8 | 76.4 | 90.0 | 98.1 |

PIL = Percentage of internodes length of RKI 237-1 mutant over the original plant.
IN = internodes

Table 2. Segregation of normal and semi-dwarf plants in $M_3$ lines.

| $M_3$ Line | Type of $M_2$ plant | Normal | Semi-dwarf | $x^2$(3:1) |
|---|---|---|---|---|
| RKI 237-1-3 | Normal | 57 | 22 | 0.34 [ns] |
| RKI 237-1-12 | Normal | 59 | 21 | 0.34 [ns] |
| RKI 237-1-2 | Normal | 80 | 0 | - |
| RKI 237-1-8 | Normal | 80 | 0 | - |
| RKI 237-1-1 | Semi-dwarf | 0 | 80 | - |
| RKI 237-1-17 | Semi-dwarf | 0 | 80 | - |

[ns] non significant at 0.1% level.

In rice, at least 7 semi-dwarfing genes have been identified by classical genetic analysis [13], some of them, such as *sd1* on chromosome 1 and *d35* on chromosome 6 have been isolated and have been extensively analyzed to elucidate the regulatory mechanisms of plant growth and development [12, 14]. Even though the performance of rice mutants carrying $sd^{237-1}$ gene differed from that of rice mutant carrying *sd1* or *d35*, the allelic test between $sd^{237-1}$ gene and other semi-dwarfism genes are necessary to

ISSN 1907-0322

clarify whether the *sd*$^{237-1}$ is a new identified gene or not. Chromosome mapping concerning the *sd*$^{237-1}$should also be conducted in near future to locate the *sd*$^{237-1}$ gene on rice chromosome linkage map.

Semi-dwarfism is a valuable trait in crop breeding, because it increases lodging resistance and decreases damages due to wind and rain. During the green revolution, in the second half of the 20$^{th}$ century, a rice semi-dwarf variety, IR8, enabled dramatic yield increases and help to averse predicted food shortages in Asia [3]. At the same time, a dominant wheat semi-dwarf cultivar, *Rht1* as well as *Rht2*, facilitated a burst in productivity and lead to the wheat green revolution [15]. The original of *sd*$^{237-1}$ is KI 237, a pure selected line derived from Indica / Japonica cross. This line showed high yield potency, but susceptible to lodging. The *sd*$^{237-1}$ mutant improved lodging resistance without significantly changing its major characters. This mutant could be used as a genetic resource for the improvement of KI 237 line through back-cross breeding as well as be developed further in breeding program directly to be a new high yielding mutant variety.

Recent developments of gene transfer technology have enormous promise for improvement of plant productivity; however, there is a lack of available new genes which can be transferred to current high-yielding varieties. In other words, there are no genes that have been identified which can contribute to world crop production as much as *sd1* (DGWG) in rice, and *Rht1* as well as *Rht2* in wheat [6]. Since the elongation pattern of the internodes of *sd*$^{237-1}$ mutant was almost the same as *sd1*, the *sd*$^{237-1}$ mutant gene can be used with *sd1* simultaneously to avoid genetic vulnerability without reducing yield.

## CONCLUSIONS

A semi-dwarf mutant was successfully induced by gamma irradiated KI 237 seeds with dose of 200 Gy. Its semi-dwarf mutant character was controlled by a single recessive mutated gene. This gen was designated as *sd*$^{237-1}$. To clarify whether the *sd*$^{237-1}$ is a new identified gene or not, the allelic test between *sd*$^{237-1}$ and other semi-dwarfism

ISSN 1907-0322

genes are necessary. This $sd^{237-1}$ mutant should be useful as a genetic resource for the improvement of KI 237 line through back-cross breeding as well as be developed further in breeding program directly to be new high yielding mutant varieties.

**ACKNOWLEDGEMENTS**

Thanks to my colleagues at the Division of Irradiation, Electro-mechanic and Instrumentation CAIRT – NNEA for their helps in seed irradiation, and Mr. Carkum, Plant Breeding Lab. CAIRT – NNEA, for his excellent technical assistance.

**REFERENCES**

1. KHUSH, G.S., Green revolution: The way forward, *Nature Rev. Genet.*, **2**, 815-822 (2001).

2. CANTRELL, R.P. and G.P. HETTEL, Research Strategy for Rice in the 21st Century, *In:* Rice is Life: Scientific Perspectives for the 21st Century, (K. TORIYAMA, K.L. HEONG and B. HARDY, Eds.), International Rice Research Institute, 26-37 (2005).

3. KHUSH, G.S., Green revolution: Preparing for the 21st century, *Genome*, **42**, 646-655 (1999).

4. INTERNATIONAL RICE RESEARC INSTITUTE, Annual Report for 1966, 59-82 (1967).

5. HARTEN, A.M. van., Mutation Breeding, Theory and Practical Application, Cambridge University Press, 237 (1998).

6. MALUSZINSKI, M., Crop Germplasm Enhancement Through Mutation Techniques, *In:* Proceeding of the International Symposium on Rice Germplasm Evaluation and Enhancement, Stuttgart, Arkansas, USA, (J.N. RUTGER, J.F. ROBINSON and R.H. DILDAY, Eds.), 74-82 (1998).

7. HARCROVE, T.R., COFFMAN, W.R. and V.L. CABANILLA, Ancestry of improved cultivars of Asian rice, *Crop Sci.*, **20**, 721-727 (1980).

8. RUTGER, J.N., Thirty years of induction, evaluation and integration of useful mutants in rice genetics and breeding, *Plant Mutation Report*, **1** (2), 4-13 (2006).

ISSN 1907-0322

9. SOBRIZAL, CARKUM, Pemuliaan tanaman untuk mendapatkan varietas unggul nasional dan hibrida; karakterisasi sifat agronomi galur murni dari persilangan Koshihikari/IR36, Laporan Teknis, Pusat Aplikasi Teknologi Isotop dan Radiasi, Badan Tenaga Atom Nasional, Jakarta (2006).

10. SPARRAW, A.H., Type of ionizing radiation and their cytogenetic effect, mutation and plant breeding, *NAS-NRC*, 891, 55-119 (1961).

11. IAEA, Manual on Mutation Breeding, Technical Report Series No. 119, (2$^{nd}$ Ed.) IAEA, Vienna (1977).

12. ITOH, H., TATSUMI, T., SAKAMOTO, T., OTOMO, K., TOYAMASU, T., KITANO, H., ASHIKARI, M., ICHIHARA, S. and M. MATSUOKA, A rice semi-dwarf gene, Tan-Ginbozu (D35), encodes the gibberellin biosyinthesis enzyme, ent-kaurene oxidase, *Plant Moleculer Biology*, **54**, 533-547 (2004).

13. NAGATO, Y. and A. YOSHIMURA, Report of the committee on gene symbolization, nomenclature and linkage groups, *Rice Genet. Nwsl.*, **15**, 13-74 (1998).

14. SPIELMEYER, W., ELLIS, M.H. and P.M. CHANDLER, Semidwarf (*sd-1*) "Green revolution" rice, contains a defective gibberellin 20-oxidase gene, *Proc. Natl. Acad. Sci*. USA, **99**, 9043-9048 (2002).

15. EVANS, L.T., Feedings the Ten Billion. Plant and Population Growth, Cambridge University Press, Cambridge (1998).