3. Indonesia

Improvement of Yield and Grain Quality of Rice through a Wide Cross and Mutation Breeding

Sobrizal

Center for the Application of Isotopes and Radiation
National Nuclear Energy Agency

3.1 Abstract

To meet the Indonesian domestic demand for both rice grain quality and quantity, high variability of pure lines derived from a cross of Indica rice variety, IR36 and Japonica rice variety, Koshihikari was constructed. Among these lines, KI237 line was treated by gamma ray irradiation to remove its undesirable characters. KI237 shows good performance with high yield potency and red phericarp, but susceptible to lodging because of tall plant stature. To reduce the plant height, 50g seeds of this line were irradiated by 200Gy of gamma ray at Center for the Application of Isotopes and Radiation Technology, Pasar Jumat, Jakarta, Indonesia. Selections and purifications were conducted in M_2 and M_3 generation, finally, one dwarf and four semi-dwarf M_3 mutant lines were selected. Based on the segregation analysis in M_2 and M_3 generation, it was concluded that the semi-dwarf of RKI237-1-17 mutant line was controlled by single recessive mutated gene. For breeding purpose, based on their agronomical performances ten semi-dwarf lines were selected and subjected to yield and other character evaluations. The results of multi-location yield trials showed that the highest average yield of multi-locations yield trials in five locations was 7.39t/ha for RKI199 and followed by 7.24t/ha for RKI241, while average yield of chiherang, a national leading variety is only 6.56 ton/ha. Out of ten lines, three lines are still with brown phericarp and the phericarp color of other lines change to white. These ten lines have various amylose contents, ranging from 13.41% to 20.83%. Various amylose contents and phericarp colors would be useful as genetic resources for improvement of rice grain quality to serve the wide preference variability of Indonesian consumers. To fulfill the requirement of variety release in Indonesia, other multi-location yield trials as well as other examinations such as pests, diseases and other grain quality examinations should be conducted.

Key words: yield, grain quality, intra-specific cross, mutation

3.2 Introduction

Rice is a steeple food of majority Indonesian population. In the mid-1980s Indonesia firstly achieved self-sufficiency for rice, however the growth of rice production has slowed down since the 1990s (BAPENAS, 2002). The decline in rice production is frequently attributed to loss of rice fields caused by conversion to non-agricultural uses, and to the declining productivity. To fulfill the domestic demand in which the annual population growth is still more than 1.3%, Indonesian rice production should be increased. This can be achieved by introducing the high yielding varieties to the farmers.

Recently, the demand for superior grain quality increased for national markets in Indonesia. Rice consumers in Indonesia exhibit wide preference variability and consumption patterns because of archipelagic nature of the country (Toquero 1991). Consumers in Medan, Padang and Makassar, for example, prefer non-sticky cooked rice, while consumers in Java prefer sticky and soft-cooked rice. Nowadays, consumers have become more discriminating in terms of rice quality due to higher incomes and better life quality. Indonesian rice consumers are now willing to pay higher price for better quality desired. These imply requirement of technology generation for better quality.

The primary components of rice grain quality include milling efficiency, appearance, cooking and edibility characteristics, and nutritional quality (Li et al. 2004). Generally, the milling efficiency is determined by head rice yield; appearance is determined by grain length, grain width, width-length ratio, and translucency of endosperm; cooking and edibility characteristics are determined by amylose content of endosperm, gelatinization temperature, and aroma; nutritional quality is determined by protein, oil, phytic acid, and micro nutrient contents.

IR36 is an Indica improved rice variety, tolerance to various pests and diseases, and adapted well in almost all Indonesian rice growing areas. Recently, IR36 rice variety is almost not grown in Indonesian because of its poor in cooking and edibility characteristics. Whereas, Koshihikari is a very popular Japonica variety and known having good cooking and edibility with low amylose content, but it cannot grow well in Indonesia due to photoperiod sensitivity. By using these unique germplasms as well as mutations ten rice promising lines with high yield and grain quality have been constructed. The objective of this research is to develop high yielding and grain quality of rice varieties.

3.3 Materials and Methods

To increase the genetic variability of breeding materials, IR36 and Koshihikari were cross, and the F_1 plants obtained from this cross were self-fertilized up to F_5 generation. F_5 lines were developed from 100 F_2 plants by pedigree method with 10 plants per line for each generation, and finally the size of F_5 population became 100,000 plants. Selection for agronomical and other major characters was performed in F_5 populations. Selected plants were purified up to F_7 or F_8 generations, then, selected pure lines were designated as KI #. KI lines are used as basic materials in further breeding program.

Among these lines, the seeds of KI237 line were treated by gamma ray irradiation. This line shows good performance with high yield potency and red pericarp, but susceptible to lodging because of tall plant stature. To reduce the plant height, 50g seeds of this line were irradiated by 200Gy of gamma ray 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 20cm spacing between plants.

Five hundred 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 plant were transplanted to develop M_2 lines. Observations of semi-dwarf plants were conducted in each M_2 lines before harvesting. The M_2 lines showed segregation between normal and semi dwarf plants was 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. Observations of segregation between semi-dwarf and normal plants were conducted in each M_3 lines. The χ^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.

Selected semi-dwarf plants were purified up to M_5 generations, then, selected pure lines were designated as RKI #. RKI lines were subjected to yield trials, amylase content, and other examinations. Breeding scheme of plant materials used in this study can be shown in Fig. 1.

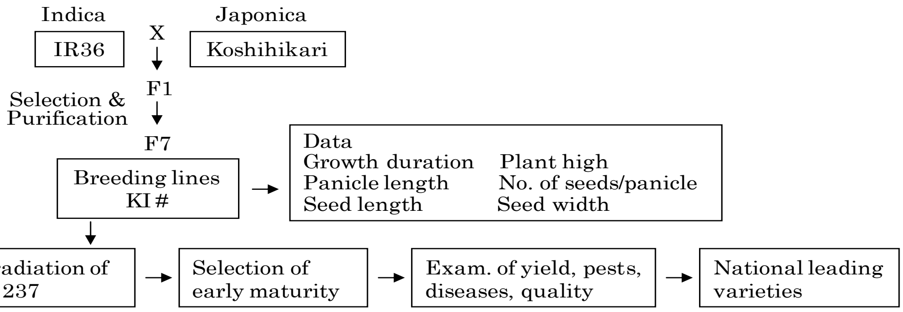

Figure 1. Breeding scheme of plant materials used in this study.

3.4 Results and Discussion

In this study, all the F_1 plants derived from cross of IR36/Koshihikari grew vigorously but their pollens as well as seeds were semi-sterile. The fertilities were improved gradually from early to the next generations. Selection was conducted in F_5 population with emphasis on agronomical characters, seed fertilities, and pest and disease tolerances in the field. Of the 100,000 F_5 plants, 568 plants were selected and they were purified up to F_7 or F_8 generation to construct 568 fixed lines. Selected pure lines were designated as KI #.

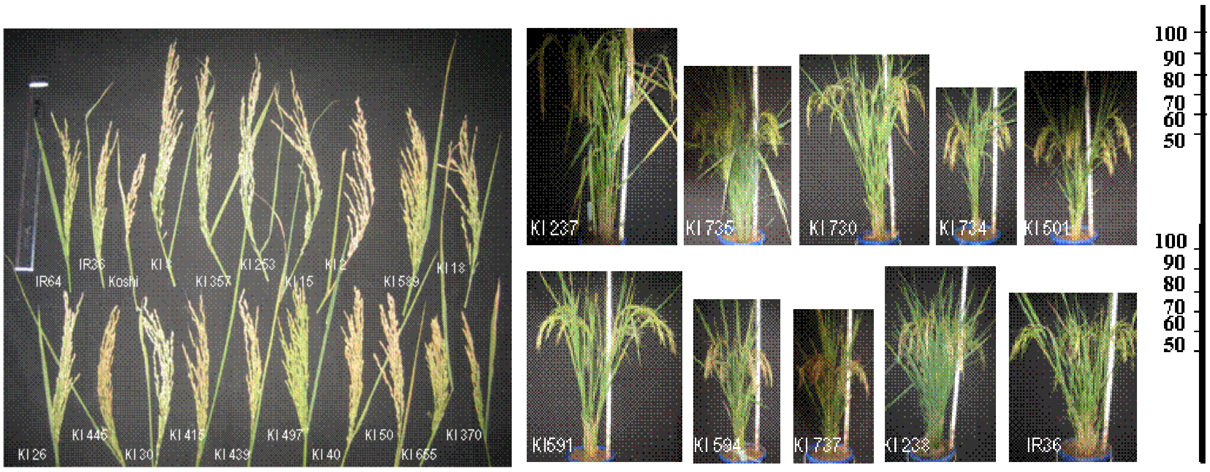

Fig. 2. Variability of selected pure lines derived from a cross of Koshihikari and IR36.

Figure 2 shows varying features of KI lines. Variations were shown in many characters such as plant height, growth duration, number of tillers, flag life, panicle length, number of seeds per panicle, seed length, seed width, and others. For rice breeding program, some of these lines showing good performance were directly subjected to yield trials and several others were irradiated by gamma ray to induce mutations for amelioration of one or two undesirable characters, or crossed each other to gather some desirable characters in one individual.

Among these KI lines, the KI237 was selected to improve by mutation breeding because this line showed good yield potency but so tool that susceptible to lodging. To reduce the plant height of KI237, its seeds were irradiated by 0.2kGy gamma ray. As much as 500 M_1 plants were harvested individually to obtain M_2 seeds. Selection for dwarf and semi-dwarf were conducted in M_2 lines. 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 KI237, the plants having the same height as RKI237 were grouped as normal plants. Among the segregated lines, the line of RKI237-1 segregated for 8 semi-dwarf and 12 normal plants. The semi-dwarf plant reached 60 – 62% of plant height of original plant KI237 at the mature stage (Fig. 3A). It was also compared the length of internodes, panicle, and seed between these two plants (Fig. 3B; Fig. 4; 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 same as *sd1* (Dee-geo-woo-gen), the original parent of the first release modern rice variety (Itoh *et al*. 2004).

To perform the genetic analysis of this semi-dwarf character, the segregated M_2 line of RKI 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 use them for analysis of the mutated gene(s). It was observed that all M_3 plants derived from RKI 237-1-1 and of RKI 237-1-17 M_2 plants showed semi-dwarf stature, all M_3 plants derived from RKI 237-1-2 and of RKI 237-1-8 M_2 plants showed normal stature, and the other two M_3 lines derived from normal M_2 plants, RKI 237-1-3 and RKI 237-1-12 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 tentatively designated as $sd^{237\text{-}1}$.

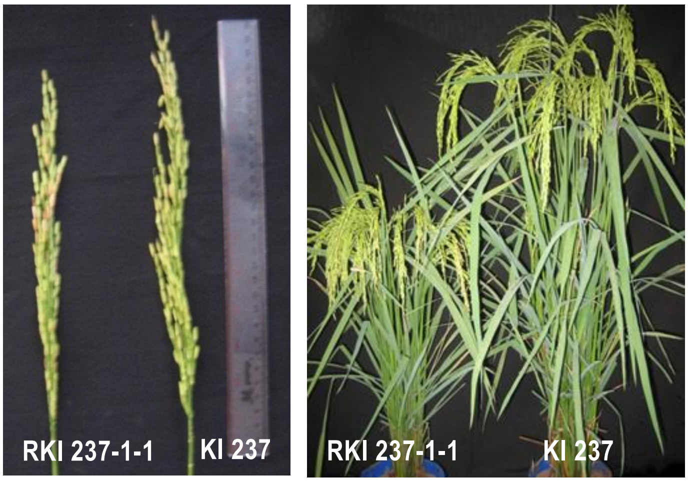

Fig. 3. Morphological characterization of semi-dwarf mutant RKI237-1-1 and its original plant, KI237.

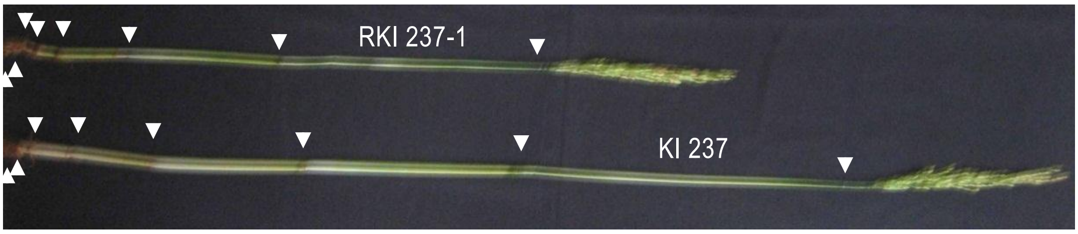

Fig. 4. Comparison of culm elongation between semi-dwarf mutant RKI237-1 and its original line KI237.

Table 1. The length of internodes, panicles, and seeds of KI237 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	31,0	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

In rice, At least 7 semi-dwarfing genes have been identified by classical genetic analysis (Nagato and Yoshimura 1998), 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 (Spielmeyer et al. 2002; Itoh et al. 2004). Even though the performance of rice mutants carrying $sd^{237\text{-}1}$ gene differed from that of rice mutant carrying *sd1* or *d35*, the allelic test between $sd^{237\text{-}1}$ gene and other semi-dwarfism genes are necessary to clarify whether the $sd^{237\text{-}1}$ is a new identified gene or not. Chromosome mapping concerning the $sd^{237\text{-}1}$should also be conducted in near future to locate the $sd^{237\text{-}1}$ gene on rice chromosome linkage map.

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

M_3 line	Type of F_2 plant	Normal	Semi-dwarf	χ^2 (3:1)
RKI 237-1-3	Normal	57	22	0.34 ns
RKI 237-1-12	Normal	59	21	0.08 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.

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 20th century, a rice semi-dwarf variety, IR8, enabled dramatic yield increases and help to avert predicted food shortages in Asia (Khush 2001). 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 (Evans, 1998). The original of $sd^{237\text{-}1}$ is KI237, a pure selected line derived from Indica / Japonica cross. This line showed high yield potency, but susceptible to lodging. The $sd^{237\text{-}1}$ mutant improved lodging resistance without significantly changing its major characters. This mutant could be used as a genetic resource for the improvement of KI237 line through back-cross breeding as well as be developed further in breeding program directly to be new high yielding mutant varieties.

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 (Maluszinki 1998). Since the elongation pattern of the internodes of $sd^{237\text{-}1}$ mutant was almost the same as *sd1*, the $sd^{237\text{-}1}$ mutant gene can be used with *sd1* simultaneously to avoid genetic vulnerability without reducing yield.

To evaluate the yield performances, ten selected semi-dwarf pure lines were subjected to yield trials in five locations. The highest average yield in five locations is 7.39t/ha for RKI199 and followed by 7.24t/ha for RKI241, whereas the average yield of Ciherang, a national leading variety is only 6.56t/ha (Table 3). The lowest average yield is 6.54t/ha for KI237 line, an original line of mutant lines. Multi-location yield trials will be continued in next growing season to reach at least 16 locations as a requirement of variety release in Indonesia.

Table 3. Multi-locations yield trials in Pusakanegara, Landak, Purbalingga, Pariaman and Banyuwangi.

Line	Yield (ton/ha) *)					
	Pskngr	Lndk	Prblg	Prmn	Bywgi	Average
RKI-124	6.80 abc	4.95 a	6.80 d	7.30 ab	8.85 a	6.94
RKI-130	6.14 c	5.15 a	7.40 c	7.25 a	8.85 a	6.96
RKI-131	6.92 ab	4.64 a	6.45 de	6.75 abc	8.68 a	6.69
RKI-198	7.15 a	5.52 a	6.10 ef	7.20 ab	9.21 a	7.04
RKI-199	7.12 a	5.60 a	7.85 b	7.15 a	9.21 a	7.39
RKI-211	6.63 abc	7.77 a	6.02 f	6.63 dc	9.55 a	7.32
RKI-237	6.18 c	5.52 a	7.75 bc	6.80 abc	8.85 a	7.02
RKI-240	5.40 d	6.35 a	7.95 b	6.43 d	9.03 a	7.03
RKI-241	6.94 ab	5.54 a	8.75 a	6.97 abc	7.99 a	7.24
RKI-245	6.39 bc	5.44 a	7.75 bc	7.50 a	8.51 a	7.11
KI-237	4.51 e	6.27 a	6.50 de	7.00 abc	8.42 a	6.54
Ciherang	6.52 abc	4.54 a	5.95 f	7.10 ab	8.69 a	6.56

*) The numbers followed by different characters are significantly different for 5% level.

The amylosa content of tested RKI lines are varied largely, ranging from 13.41% to 20.83%. The amylose content of rice is recognized as one of the most important determinants of eating and cooking quality (Bao et al. 2002). The lines having various content of amylose should be useful as genetic resources for improvement of grain quality to meet the wide preference variability of consumers. The amylosa contents of RKI199 and RKI241 are 17.16% and 15.27%, respectively (Table 4). The color of phericarp is white for RKI199 and brown for RKI241 (Fig 5). If the yield of RKI199 and RKI241 is stable in next multi-location yield trials these lines can be recommended to release as new leading varieties in Indonesia. To fulfill the requirement of variety release in Indonesia these data should also be completed with other examinations such as pests, diseases and other grain quality examinations.

Line	Amylose Content (%)	Endosperm color
RKI-124	20.83	White
RKI-130	19.89	White
RKI-131	20.69	White
RKI-198	17.35	White
RKI-199	17.16	White
RKI-211	14.17	Brown
RKI-237	14.25	White
RKI-240	13.41	White
RKI-241	15.27	Brown
RKI-245	16.71	Brown
KI-237	24.36	Brown
Koshihikari	16.90	White
Ciherang	18,00	White

Tabel 4. Amylose contents and phericarp color of selected RKI lines.

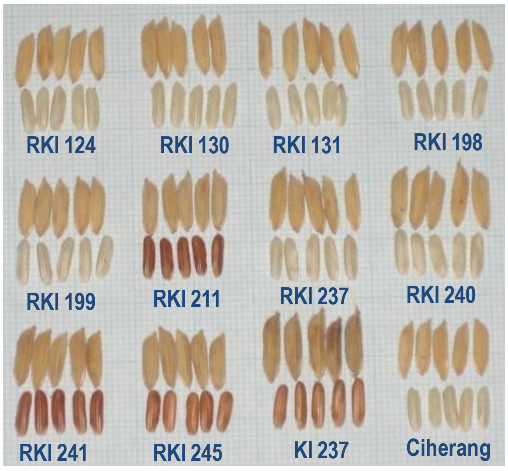

Fig. 5. Grain rice performances of selected lines

3.5 Conclusion

Based on the results it can be concluded as follow;

1) Large genetic variation was observed in fixed lines derived from a wide cross of IR36 and Koshihikari. These lines can be used as basic materials in breeding program.
2) Semi-dwarf character of RKI237-1 line was control by a single mutated gene, this gene was tentatively designated as sd^{237-1}.
3) Out of ten selected lines, three lines are still with brown phericarp and the phericarp color of other lines change to white.
4) These lines have various amylose contents, ranging from 13.41% to 20.83%.
5) The highest average yield was 7.39t/ha for RKI199 and followed by 7.24t/ha RKI241.
6) To fulfill the requirement of variety release in Indonesia, other multi-location yield trials and other examinations such as pests, diseases and other grain quality examinations should be conducted.

3.6 Acknowledgement

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

3.7 References

Bao, J.S., Sun, M., Corke, H. 2002. Analysis of genetic behavior of some starch properties in indica rice (*Oryza sativa* L.): thermal properties, gel texture, swelling volume. Theor. Appl. Genet. 104:404-413.

BAPENAS/DEPARTEMAN PERTANIAN/USAID/DAI FOOD POLICY ADVISORY TEAM. 2002. Indonesian food policy program. Available at www.macrofoodpolicy.com

Evans, L. T. 1998. Fidding the ten billion. Plant and Population Growth. Cambridge University Press. Cambridge.

Itoh, H., Tatsumi, T., Sakamoto, T., Otomo, K., Toyomasu, T., Kitano, H., Ashikari, M., Ichihara, S. and M. Matsuoka. 2004. A rice semi-dwarf gene, Tan-Ginbozu (D35) encodes the gibberellin biosyinthesis enzyme, ent-kaurene oxidase. Plant Moleculer Biology 54: 533-547.

Khush, G. S. 2001. Green revolution: the way forward. Nature Rev. Genet. 2: 815-822.

Li, J., Xiao, J., Grandilo, S., Jiang, L., Wan, Y., Deng, Q., Yuan, L., McCouch, S.R. 2004. QTL detection for rice grain quality traits using an interspecific backcross population derived from cultivated Asian (*O. sativa*) and African (*O. glaberima* S.) rice. Genome 47:697-704.

Maluszinski, M. 1998. Crop germplasm enhancement through mutation techniques. In Rutger, J. N., Robinson, J. F. and R. H. Dilday (eds). Proceeding of the International Symposium on Rice Germplasm Evaluation and Enhancement. Stuttgart, Arkansas, USA, p. 74-82.

Nagato, Y. and A. Yoshimura, 1998. Report of the committee on gene symbolization, nomenclature and linkage groups. Rice Genet. Nwsl. 15: 13-

Spielmeyer, W., Ellis, M. H. and P. M. Chandler. 2002. Semidwarf (sd-1) “green revolution” rice, contains a defective gibberellin 20-oxidase gene. Proc. Natl. Acad. Sci. USA 99: 9043-9048.

Taquero, Z.F. 1991. Consumer demand for rice grain quality in rice grain marketing and quality issues. Selected paper from the International Research Conference. 27-31 August 1990, Seoul, Korea. IRRI, Manila. p. 37-46.