Improvement of RD 15 rice lines by molecular markers assisted backcrossing for photoperiod insensitive, semi-dwarf and glutinous rice

Trung Vo Van[1*], Varaporn Sangtong[2], Adisak Joomwong[1] and Saengtong Pongjaroenkit[2]

Abstract

RD 15 rice is a non-glutinous rice variety with good cooking quality. However, the plant is tall and photoperiod sensitive which carries homozygous Hd1Hd1Sd1Sd1WxWx genotypes. With the aid of functional markers, RD 15 rice was successfully improved by introgressions of the recessive *hd1, sd1* and *wx* genes from improved RD 6, the rice variety which is originally photoperiod insensitive, semi-dwarf and glutinous, containing homozygous recessive hd1hd1sd1sd1wxwx genotypes. Molecular marker assisted backcrossing was applied in two backcrosses and one selfing to select four best RD 15 lines that carry homozygous hd1hd1Sd1Sd1WxWx, hd1hd1Sd1Sd1wxwx, hd1hd1sd1sd1WxWx and hd1hd1sd1sd1wxwx genotypes. These lines were then tested for yield trial and checked for photoperiod response under long-day length of 14 hours per day. The results showed that four best backcrossing lines of RD 15 were flowering while the original RD 15 was not flowering under long-day length. Nevertheless yield and other most important traits were not significantly different with the original RD 15.

Keywords: Rice, yield, yield components, marker assisted backcrossing, hd1, sd1 and wx genes

Introduction

Rice (*Oryza sativa* L.) is a short-day plant. Long-day condition can prevent or considerably delay its flowering time. Photoperiod sensitive rice can grow only in rainy season. When growing in dry season (light over 12 hours/day), its flowering time is delayed but non-photoperiod sensitive rice can grow in all seasons of the year (Swaminathan, 1985). Studies on quantitative trait loci (QTLs) showed that photoperiod sensitive rice was found to be controlled by six loci: Hd1, Hd2, Hd3a, Hd3b, Hd5 and Hd6; with Hd1 as the major gene (Yano et al., 2000). The Hd1 allele is a photosensitive functional allele and works in long-day lengths by delaying inflorescence while hd1 allele is a non-photosensitive non-functional allele and works in short-day lengths by promoting inflorescence. Besides, plant height is one of the most important traits of rice cultivar in relation to the falling down or resistance effect of environmental conditions such as wind and rain. Tall plants are easy to be down or break when they are exposed to wind and rain thus reducing 25% of grain yield (Monna, et al., 2002), while short plants can resist wind and rain. Plant height in rice is found to be controlled by the gene Sd1/sd1 with Sd1 gene for tallness and sd1 gene for semi-dwarfness (Khush, 2001). On the other hand, presence of endosperm amylose is one of the most important traits of rice cultivar that determine qualities of rice grain. Rice endosperm was found to be controlled by Wx/wx gene with glutinous phenotypes controlled by single recessive gene, wx and non-glutinous phenotypes controlled by dominant gene, Wx (Mikami et al., 1999). One of the developments in rice biotechnology was the advent of molecular markers. In rice breeding, molecular markers assisted backcrossing (MAB) was applied widely much recently (Collard and Mackill, 2007). The use of MAB will help rice breeders to save time, efficiency and accuracy in selecting complex traits. RD 15 is a non-glutinous rice variety with good cooking qualities. However, the plant is tall and photoperiod sensitive hence it can only planted during the wet season and can be easily blown down. That is why the aim of this research is to use molecular markers assisted backcrossing to improve RD 15 rice lines for photoperiod insensitive, semi-dwarf and glutinous rice.

[1] Division of Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290
[2] Division of Genetics, Faculty of Science, Maejo University, Chiang Mai 50290
*Corresponding author: htrung83@gmail.com

Materials and Methods

Materials

RD 15 rice variety is photoperiod sensitive, tall and non-glutinous with Hd1Hd1Sd1Sd1WxWx genotypes. Improved RD 6 rice line is non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotypes. This was selected in Maejo University as derived from Taichung 65 which is a non-functional non-photoperiod sensitive, *hd1,* gene and semi-dwarf, *sd1*, gene from RD 1 rice. Three markers were used: *hd1* marker specific for *Hd1/hd1* gene; *sd1* marker specific for *Sd1/sd1* gene; and *wx* marker specific for *Wx/wx* gene.

Molecular marker assisted backcrossing (MAB)

Improved RD 6 rice (hd1hd1sd1sd1wxwx genotypes) was used as the donor parent to cross with RD 15 rice (Hd1Hd1Sd1Sd1WxWx genotypes) as the recurrent parent to generate F_1 generation (Hd1hd1Sd1sd1Wxwx genotypes). F_1 plants were then backcrossed to RD 15 to generate BC_1F_1 seeds (with eight genotypes) and with *hd1, sd1* and *wx* markers were then used to select only one heterozygous Hd1hd1Sd1sd1Wxwx genotype, and later backcrossed again with RD 15 to generate BC_2F_1 seeds (with eight genotypes); and using *hd1, sd1* and *wx* markers to select only one heterozygous Hd1hd1Sd1sd1Wxwx genotype. BC_2F_1 plants were later self-pollinated to generate BC_2F_2 seeds. A total of 2,000 BC_2F_2 seeds were then grown in the field to select four best lines with corresponding phenotypes and genotypes, such as RD 15 line (non-photoperiod sensitive, tall and non-glutinous with hd1hd1Sd1Sd1WxWx genotypes), RD 15 line (non-photoperiod sensitive, tall and glutinous with hd1hd1Sd1Sd1wxwx genotypes), RD 15 line (non-photoperiod sensitive, short and non-glutinous with hd1hd1sd1sd1WxWx genotypes) and RD 15 line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotypes). These four best lines of RD 15 were then tested for photoperiod response by growing them in greenhouse under light exposure for 14 hours per day and were later checked for yield and other traits by yield trial.

Yield trial

This experiment was conducted in Maejo University, Chiang Mai 50290 during the rainy season 2010 and using the Randomized Complete Block Design (RCBD) in three replications with 10 treatments. Using improved RD 6 line, Chainat 80, Sanpatong 1 and RD 10 as the standard rice. Each plot consisted of four rows with one seedling per hill, 21 seedlings per row and had a planting density 25 cm between plants and rows. In the field, data were recorded for grain yield (kg/rai), age to 50% flowering (days), plant height (cm), number of tillers per hill (tillers), number of panicles per hill (panicles), number of seeds per panicle (seeds), fertility (%), weigh of 1,000 seeds (g) and grain appearance including the calculation of the analysis of variance (ANOVA).

Results

Analysis of variance (ANOVA) for yield related traits is presented in Table 1 showing that the four RD 5 lines which were selected for homozygous recessive hd1hd1 genotype by using *hd1* marker derived from improved RD 6 line exhibited flowering in long-day condition (14 hours/day). This indicated their non-photoperiod sensitivity as compared to the original RD 15 containing non-flowering homozygous dominant Hd1Hd1 genotype. Grain yield of four best lines were not different with the original RD 15. Two lines which contained homozygous recessive sd1sd1 genotype were short (89 cm), while other two lines which contained homozygous dominant Sd1Sd1 genotype were tall as RD 15. Age to 50% of two tall lines was earlier than two short lines and original RD 15 and showed highly significantly different. The short lines had more tillers and panicles per hill than the tall lines. Number of seeds per panicle, fertility and weight of 1,000 seeds of the four RD 15 lines were not significantly different with original RD 15. The lines that contained homozygous dominant WxWx genotype were non-glutinous as showed by a translucent endosperm grain appearance, while the lines that contained homozygous recessive wxwx genotype were glutinous as showed by a chalky endosperm grain appearance.

Table 1 Mean comparison for grain yield and some agronomic traits between the original RD 15 and the selected four best lines of RD 15 lines grown in the rainy season of 2010

Lines/varieties	Grain yield (kg/rai)	Plant height (cm)	Age to 50% flowering (day)	Number of tiller/hill (tiller)	No. of panicle/ hill (panicle)	Number of seed/ panicle (seed)	Fertility (%)	Weight of 1,000 seeds (g)	Grain appearance	Flowering time under light 14 hours/day
RD 15 (photo., tall and non-glu.)	772 c	140 b	97 c	14 d	13 c	144 abcd	83	25.0 cd	Translucence	-
RD15 line (non-photo., tall and non-glu.)	841 abc	138 b	93 d	15 cd	14 bc	158 a	81	25.7 c	Translucence	115
RD15 line (non-photo., tall and glu.)	728 c	139 b	93 d	14 d	12 c	135 bcd	83	23.7 d	Chalkiness	113
RD15 line (non-photo., short and non-glu.)	802 bc	89 e	96 c	21 a	19 a	125 d	78	26.4 c	Translucence	108
RD15 line (non-photo., short and glu.)	807 abc	89 e	96 c	19 b	16 b	133 bcd	81	25.1 cd	Chalkiness	111
Improved RD 6 line (non-photo., short and glu.)	836 abc	103 d	104 a	18 b	14 bc	149 abc	79	29.2 b	Chalkiness	118
Improved RD 6 line (non-photo., tall and glu.)	812 abc	154 a	96 c	13 d	12 c	148 abc	80	28.4 b	Chalkiness	-
Chainat 80 (non-photo., short and non-glu.)	932 ab	116 c	96 c	17 bc	15 b	151 ab	79	29.2 b	Translucence	100
Sanpatong 1 (non-photo., short and glu.)	935 a	118 c	105 a	18 b	15 b	151 ab	81	31.5 a	Chalkiness	-
RD 10 (non-photo., short and glu.)	821 abc	112 c	100 b	15 cd	13 c	129 cd	82	32.8 a	Chalkiness	-
Mean	829	120	98	16	14	142	81	28	-	-
F-test	*	**	**	**	**	**	ns	**	-	-
CV (%)	8.17	2.45	0.75	5.69	6.14	5.62	3.84	2.12	-	-

*, ** Significant difference at 5% and 1% probability level, respectively; ns = non-significant difference; photo. = photoperiod sensitive; glu. = glutinous. Within column, means followed by the same letter are not significantly different based on the DMRT procedure.

Discussion

Photoperiod sensitivity, plant height and endosperms characteristics are three of the most important traits for rice cultivar. The results in this study clearly showed that all of four RD 15 lines had agronomic characteristics that were similar to the original RD 15 in most of the important traits. Also, results from this experiment showed that when rice was grown under light exposure for 14 hours per day, flowering time of photoperiod sensitive rice was delayed, while non-photoperiod sensitive rice showed normal flowering. This result was quite similar to Nishida *et al.* (2004) who presented that long-day condition suppression of rice flowering became apparent when the photoperiod was longer than 13 hours per day. On the other hand, this experiment showed that the two lines of RD 15 which were selected for homozygous recessive sd1sd1 genotype were short with same plant height of 89 cm, in conformity with Negrao *et al.* (2010) who reported that plant height differed significantly between the breeding lines and is controlled by recessive *sd1* gene. Besides, glutinous rice endosperm is known to be controlled by recessive waxy gene, *wx*. Further results of this experiment indicated that rice plants were controlled by *wx* gene that showed a chalky endosperm grain appearance, a glutinous trait, thus in agreement with Wanchanan *et al.* (2003) who reported that rice tropical glutinous was controlled by recessive *wx* gene.

Conclusion

Molecular markers assistsed backcrossing was successfully used to improve RD 15 to produce non-photoperiod sensitive, semi-dwarf and glutinous rice. The selected four best lines of RD 15 were non-photoperiod sensitive with hd1hd1 genotype, while two lines of RD 15 were selected for short plant with sd1sd1 genotypes and two lines of RD 15 were selected for glutinous trait with wxwx genotype. Results showed that agronomic characteristics of selected four best lines of RD 15 were not significantly different with the original RD 15 rice in almost of important traits such as grain yield, seeds per panicle, fertility and weight of 1,000 seeds.

Acknowledgements

The authors are grateful to AST project - Ministry of Agriculture and Rural Development of Vietnam for its financial support to this research which was conducted in Maejo University, Chiang Mai 50290, Thailand.

Literature cited

Collard, B.C.Y. and D.J. Mackill. 2007. Marker assisted selection: An approach for precision plant breeding in the twenty-first century. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 17: 1-16.

Khush, G.S. 2001. Green revolution: the way forward. Nature 2: 815-821.

Mikami, I., M. Aikawa, H. Hirano and Y. Sano. 1999. Altered tissue-specific expression at the *Wx* gene of the opaque mutants in rice. Euphytica 105: 91-97.

Monna, L., N. Kitazawa, R. Yoshino, J. Suzuki, H. Masuda, Y. Maehara, M. Tanji, M. Sato, S. Nasu and Y. Minobe. 2002. Positional cloning of rice semidwarfing gene, *sd1*: rice "Green revolution gene" encodes a mutant enzyme involved in Gibberellin synthesis. DNA Research 9: 11-17.

Negrao, S., J. Palaniappan, J. Maroco, T. Lourenco, D. Mackill and M.M. Oliveira. 2010. Bridging *sd1* molecular knowledge with recent breeding strategies for the improvement of traditional rice varieties-a *japonica* case-study. African Journal of Biotechnology 9(15): 2192-2200.

Nishida, H., H. Inoue, Y. Okumoto and T. Tanisaka. 2004. A novel gene *fg1-h* conferring an extremely long basis vegetative growth period in rice. Crop Sci. 42: 348-354.

Swaminathan, M. S. 1985. The Flowering response of the rice plant to photoperiod. A review of the literature. 4th ed. The international rice research institute. Philippines.

Wanchana, S., T. Toojinda, S. Tragoonrung and A. Vanavichit. 2003. Duplicated coding sequence in the waxy allele of tropical glutinous rice (*Oryza sativa* L.). Plant Sci. 165: 1193-1199.

Yano, M., Y. Katayose, M. Ashikari, U. Yamanouchi, L. Monna, T. Fuse, T. Baba, K. Yamamoto, Y. Umehara, Y. Ngamura and T. Sasaki. 2000. *Hd1,* a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. Plant Cell 12: 2473-2484.