Application of Mutation Breeding of Submergence tolerance Rice for Sustainable Agriculture, in Thailand

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Abstract

The world is facing a food and energy crisis of unprecedented proportions and also climate change, especially the devastating flood in Thailand, which affected at least 25 of the country's 77 provinces and affected 9 million citizens in 2011. Normally in plant genetics and breeding research, mutation is a source of variation. Spontaneous mutation rate is very low, induced mutation have contributed to the discovery and identification of gene function following the completion of genome sequencing project in rice. Therefore, radiation can cause genetics change in living organism to increase significantly. The aims of this project are to improve rice variety with enhanced to tolerance to flood and/or wetland conditions with good grain quality. RD31, a non-glutinous photoperiod–insensitive rice variety was irradiated with 0.44 kGy electron beam due to its several preferable agricultural traits such as high yield and moderately resistance to brown planthopper, bacterial leaf blight, brown spot disease and dirty panicle disease. M_1 plants were planted in dry season 2014. Five hundred panicles were harvested from the main tiller of each M_1 plant. M_2 plants were grown as 1 panicle/row for 500 rows or 10,000 plants in total. M_3 lines were screened for submergence and 317 lines survived with the score of 5-7. M_4 were screened for blast resistance. The result showed that all mutant lines were highly resistance to blast. Some of these lines were screened for submergence and found that 91 submergence tolerant mutant lines were identified. Grain quality were tested and revealed that 16 mutant lines showed 23.22-24.44 % amylose content which were lower than RD31 the original (27.52%). Physical property of all these mutant showed clear chalkiness of endosperm. M_5 mutant lines were grown for observation yield trials and also physical and chemical properties were examined. M_6 mutant lines were grown for intrastation yield trial which were screened for submergence tolerant during 2018. Further work will be carried out before released the new rice variety.

Keywords: Rice, Electron Beam, Flood Tolerance

Introduction

Rice is the principle staple crop of Asia and any deterioration of rice production systems through climate change would seriously impact food security in the world. Climate change has many facets, including changes in long-term trends in temperature and rainfall regimes. The impact of these changing conditions on agriculture are already being seen. Although climate change is a global phenomenon. It will manifest itself as locally variable impacts. Modern rice varieties are not adapted to these conditions and their yield is severely reduced because of high mortality, suppressed tillering ability, reduced panicle size, and high sterility. These are probably the reasons why farmers in affected areas still rely on low-yielding local landraces.

In the past, conventional breeding was used to develop new varieties, but those varieties left breeders unsatisfied still. Mutation breeding can be used to obtain suitable rice varieties which can lead to sustainable agriculture. Therefore, rice can be further improved. The rice improvement by mutation breeding through ion beam and gamma rays irradiation is very useful for creating genetic diversity. The irradiated rice population and its subsequent generations serve as the source of genetic variation for selection or screening according to our objectives. The aims of this project are to improve rice varieties with enhanced to tolerance to flood and/or wetland conditions.

Materials and Methods

Two hundred gram of RD31 recommended rice variety was irradiated with 440 gray electron-beam in 2014. M_1 plants were grown by broadcasting. Five hundred panicles from each main clum were collected as M_2 seeds. M_2 plants were planted as 1 panicle/row for 500 rows or 10,000 plants in total. Agricultural traits, including plant height, number of panicle per hill and maturity of these M_2 plants were observed. Seeds from six plants were collected as M_3 seeds. M_3 plants were screened for submergence tolerance (Appendix 1) and M_4 plants were screened for blast resistance (Appendix 2). The remaining were screened for submergence tolerance, and were examined for grain quality. M_5–M_6 plants were planted for yield trial observation.

Results

RD31, a non-glutinous and photoperiod–insensitive rice cultivar, is one of the certified cultivars for irrigated areas in the central plain of Thailand. It has a relatively high yield and moderate resistance to brown plant hopper, bacterial leaf blight, brown spot disease and dirty panicle disease with a good grain quality.

M_1 plants were planted by broadcasting in dry season 2014. Five hundred panicles were harvested from the main tiller of each M_1 plant to obtain M_2 seeds. M_2 plants were grown as 1 panicle/row for 500 rows or 10,000 plants in total in dry season 2014. (Figure 1). Plant height, number of panicle per hill and maturity of these M_2 plants were observed and recorded (Table 1).

Figure 1. Electron beam irradiated M_2 plants of RD31.

Table 1. Plant height of RD31 and M_2 populations.

Plant groups	Plant height (cm)	
	Height (cm)	No. of plants
RD31	121	20
M_2 of RD31		
-Dwarf	40-54	-
-Semidwarf	55-100	2
-Intermediate-tall	101-120	279
-Tall	121-155	218

The plant height of RD31 was 121 cm. It was classified according to Loresto and Chang (1978) as tall class. Loresto and Chang (1978) used the following criteria to classified plant height in the F_2 populations : 1) tall (121-155 cm), 2) intermediate-tall (101-120 cm), 3) semi-dwarf (55-100 cm), and dwarf (40-54 cm). While Fernandez et al., classified dwarfs into four categories : 1) tall (more than 130 cm), 2) intermediate (110-130 cm), 3) semi-dwarf (80-110 cm), and dwarf (40-80 cm). Upon electron-beam irradiation, the height of M_2 plants ranged from 99-143 cm (Table1 and Figure 2), covering all three height classes, including semi-dwarf, intermediate-tall and tall. The observed frequency were 2, 279 and 218, respectively.

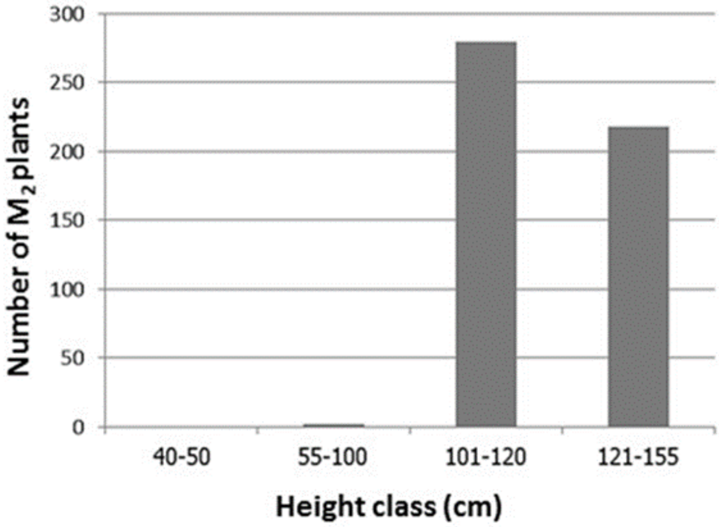

Figure 2. Distribution of M_2 plant height according to the classification of Loresto and Chang, (1978).

Panicle number of each hill was counted as it was a key component of the yield. Yield components refer to the structures of the rice plant that directly translate into yield. The relationship among growth duration, plant stature and grain yield was well explained more than 1,400 years ago (Liu, 1986). Yield components is important for fixing up the character, which plays an ultimate role in influencing the yield. Many reports identified as yield of crops depend on yield components including, the number of panicles per given area, the number of spikelets (potential) grain per panicle, the 1,000 grain weight, and the percent of fill grains per panicle. Upon electron-beam irradiation, panicle number of each hill was counted. Yield components were determined during each rice cycles, first at the vegetative phase (tiller number), second at the reproductive phase (number of fertile tillers and size of panicle sinks) and finally at the grain filling phase (spikelet filling rate). The results showed that M_2 population provided as 5-9 panicle/hill in 176 plants and 10-15 panicle/hill in 323 plants (Table 2).

Table 2. Panicle/hill of RD31 and M_2 plants.

Plants	Panicle/hill	
	Number of panicles	Number of plants
RD31		10
M_2 of RD31	<5	0
	5-9	176
	10-15	323

Normally, maturity of RD31 depends on planting method which is 118 days for transplanting and 111 days for boardcasting. The M_2 plants were grown by transplanting and maturity dates were varying. Approximately 500 M_2 plants with shortening maturity period were selected. Three hundred and thirty-three of them had 110-115 days for maturity; 166 plants with 116-120 days; one plant with 121 days (Table 3). Shortened maturity period was the preferable trait to cope with climate change. The central plain of Thailand frequently encounters a water shortage during the dry season which is follow by a heavy flood during the rainy season. Cultivars with a shortened maturity period are highly desirable because harvesting can be done early before the flooding period. Therefore, two crop will be possible per year before and after the flooding period.

Table 3. Days to maturity of RD31 and M_2 plants.

Plant groups	Maturity (days)	
	Days to maturity	Number of plants
RD31	123	20
M_2 of RD31		
	110-115	333
	116-120	166
	121	1

About 3,000 M_3 lines were screened for submergence (Appendix 1). The plants were submerged under a flash flood condition for 14 days. Only 317 lines survived the flood with the score of 5-7, which were selected for planting in the following season (Table 4, Figure3 and 4).

Table 4. Number of survived M_3 plants with score.

Score	Number of plants
1	-
3	-
5	95
7	222
9	2,680

Figure 3. Submergence tolerant mutants lines from field screening.

Figure 4. Protection of 317 mutant lines from bird damage by nets.

M_4 were screened for blast resistance (Appendex 2). The result showed that all mutant lines were highly resistance to blast (Table 5). Some of these lines were screened for submergence tolerance and found that 91 submergence tolerant mutant lines were identified. Among the biotic factor disease is the most important factor which results in crop losses of $5 billion every year (Asghar et al., 2007). Rice blast caused by *Pyricularia oryzae* Cavara, is one of the most destructive and wide spread diseases (Jin et al., 2000). This disease has caused significant yield losses in many rice growing countries e.g. 75% losses of grains in India (Padmanabhan, 1965), 50% in Philippines (Awoder and Esuruoso, 1975) and 40% loss in Neigeria (Ou, 1985). In Brazil rice blast is considered to be one of the major yield constrains in both irrigated and upland ecosystems (Prabhu and Morais, 1986). However, the use of resistant cultivar is the most economical and environment friendly method for the management of rice blast.

Table 5. Number of M_4 lines which were screened for blast resistance.

Score	Site 1 (PCR)	Site 2 (KHS)
0	305	317
1	11	-
2	1	-
7	-	-
9	-	-

Grain quality were tested and revealed that 16 mutant lines showed 23.22-24.44 % amylose content which were lower than RD31 the original (27.52%) (Table 6). Physical property of all these mutant showed clear chalkiness of endosperm (Table 7).

Table 6. M_4 lines which were examined for percentage of amylose content.

% amylose content	Classification	No. of plants
10-19	Low amylose	-
20-25	Intermediate amylose	16
26-34	High amylose	301

Table 7. Physical property of M_4 lines, chalkiness.

Score	Description	No. of plants
0	None	-
1	Small (less than 10%)	158
2	Medium (11-20%)	147
3	Medium (11-20%)	149
4	Large (more than 20%)	-
5	Large (more than 20%)	-

M_5 mutant lines were grown for observation yield trials during dry season 2017 and physical and chemical properties were examined as shown in Table 8.

M_6 mutant lines were grown for intrastation yield trial during wet season 2018 which were screened blast resistance and screened for submergence tolerant during 30th July 2018 and de-submerge on 13th August 2018 as shown in Table 9.

Further work such as interstation yield trials, farmers field yield trials and adoption from the farmers, nitrogen used efficiency, physical and chemical properties, screening for blast resistant and screening for submergence tolerant will be carried out for more information before released the new rice variety.

Table 8. Observation yield trials and physical and chemical properties of mutant lines during dry season 2017.

No.	Source #16 WS	Identity	Mat. (days)	Ht. (cm)	Grain yield/ kg/rai ave.	Grain size Sh.	Brown rice size Sh.	Clk.	Amy. (%)	Gel Con.		Gel temp. (^oC)	AlkD		El. Ratio
1	3	RD31-B-390-3-B-	113	112	608	4.05	3.23	1.88	28.23	38	H	74.5-79	3	H	1.46
2	5	RD31-B-483-6-B-	113	123	406	3.93	3.22	1.28	27.90	38	H	74.5-79	3	H	1.48
3	6	RD31-B-389-3-B-	110	121	546	4.21	3.32	0.87	27.65	38	H	74.5-79	3	H	1.57
4	8	RD31-B-479-1-B-	111	124	319	4.08	3.40	1.59	27.25	38	H	74.5-79	3	H	1.58
5	9	RD31-B-393-2-B-	113	126	478	3.91	3.28	1.39	27.58	38	H	74.5-79	3	H	1.54
6	13	RD31-B-472-6-B-	113	113	350	3.94	3.22	2.06	28.13	38	H	74.5-79	3	H	1.54
7	14	RD31-B-415-4-B-	112	123	98	3.95	3.22	1.87	28.16	38	H	74.5-79	3	H	1.47
8	43	RD31-B-388-5-B-	113	118	487	3.94	3.21	1.38	27.68	38	H	74.5-79	3	H	1.57
9	44	RD31-B-298-5-B-	112	124	354	4.03	3.40	2.16	27.87	39	H	74.5-79	3	H	1.65
10	46	RD31-B-395-4-B-	113	115	132	4.16	3.26	1.94	27.38	38	H	74.5-79	3	H	1.64
11	47	RD31-B-297-2-B-	112	113	113	4.19	3.27	2.03	28.23	38	H	74.5-79	3	H	1.61
12	49	RD31-B-331-5-B-	112	121	418	3.92	3.41	1.45	29.09	38	H	74.5-79	3	H	1.58
13	50	RD31-B-368-6-B-	112	128	234	3.90	3.25	1.40	28.00	39	H	74.5-79	3	H	1.62
14	71	RD31-B-368-4-B-	111	116	138	3.93	3.27	0.77	27.58	38	H	74.5-79	3	H	1.63
15	74	RD31-B-369-5-B-	113	120	150	4.06	3.33	1.96	27.87	38	H	74.5-79	3	H	1.62
16	75	RD31-B-385-3-B-	113	127	395	4.21	3.41	1.56	27.84	38	H	74.5-79	3	H	1.55
17	76	RD31-B-312-6-B-	113	123	290	4.11	3.26	1.00	27.90	38	H	74.5-79	3	H	1.53
18	77	RD31-B-449-1-B-	113	115	212	4.04	3.26	1.80	28.29	38	H	74.5-79	3	H	1.58
19	88	RD31-B-290-6-B-	112	121	525	4.02	3.28	1.94	27.55	38	H	74.5-79	3	H	1.55
20	96	RD31-B-276-4-B-	113	123	488	3.98	3.41	2.11	27.87	38	H	74.5-79	3	H	1.65
21	123	RD31-B-314-4-B-	114	122	551	4.16	3.29	0.91	28.06	38	H	74.5-79	3	H	1.61
22	134	RD31-B-277-5-B-	114	120	437	4.01	3.35	1.88	28.03	38	H	74.5-79	3	H	1.49
ck.		RD31	118	125	420	4.09	3.48	0.74	28.13	38	H	74.5-79	3	H	1.68
ck.		RD49	111	110	409	4.30	3.63	0.63	27.06	38	H	70-74	6	I/L	1.67

Table 9. Intra station yield trial of M6 mutant lines.

No.	Source	Identity	Ht. (cm)	% recov	Mat.	Ht. (cm)	Grain yield/ kg/rai	BI'PCR
	#16 WS		before Sub		(days)	Mat.	ave.	2018
1	3	RD31-B-390-3-B-B-B	70	80	132	129	760	R
2	5	RD31-B-483-6-B-B-B	69	70	133	130	619	R
3	6	RD31-B-389-3-B-B-B	73	70	131	134	615	R
4	8	RD31-B-479-1-B-B-B	67	80	132	133	813	R
5	9	RD31-B-393-2-B-B-B	73	70	133	134	650	R
6	13	RD31-B-472-6-B-B-B	72	70	132	131	698	R
7	14	RD31-B-415-4-B-B-B	71	70	131	129	679	R
8	43	RD31-B-388-5-B-B-B	71	80	132	131	854	R
9	44	RD31-B-298-5-B-B-B	69	65	132	134	662	R
10	46	RD31-B-395-4-B-B-B	68	70	132	130	692	R
11	47	RD31-B-297-2-B-B-B	71	65	132	128	514	R
12	49	RD31-B-331-5-B-B-B	70	65	133	131	522	R
13	50	RD31-B-368-6-B-B-B	71	80	134	130	865	R
14	71	RD31-B-368-4-B-B-B	72	70	133	129	623	R
15	74	RD31-B-369-5-B-B-B	70	70	134	132	632	R
16	75	RD31-B-385-3-B-B-B	68	70	133	131	589	R
17	76	RD31-B-312-6-B-B-B	72	70	134	132	535	R
18	77	RD31-B-449-1-B-B-B	71	70	134	129	661	R
19	88	RD31-B-290-6-B-B-B	72	70	134	131	673	R
20	96	RD31-B-276-4-B-B-B	70	80	134	133	711	R
21	123	RD31-B-314-4-B-B-B	71	65	130	129	522	R
22	134	RD31-B-277-5-B-B-B	71	65	131	129	488	R
ck.		RD31	73	30	134	133	151	R
ck.		RD49	74	20	135	127	79	R

Disscussion

IRRI released the first high-yielding modern rice cultivar, the IR8, for the tropical irrigated lowlands in 1966 (Chandler, 1969). Since then, IRRI has developed many high-yielding rice varieties such as IR36, IR60 and IR72 (Peng and Khush, 2003). New plant type (NPT) was developed at IRRI in the late 1980s. The NPT was designed based on the results of simulation modeling. The proposed NPT has a low tilling, few unproductive tillers, 200-250 grains per panicle, a plant height of 90-100 cm, thick and sturdy clumps, dark green and erect leaves, vigorous root system with 100-130 days for maturity (Peng *et al.,* 1994)

Under flooding condition, the oxygen and carbon dioxide level are reduced. Photosysthesis and cellular respiration are also affected. Xu and Mackill (1996) reported a major gene that control the ability of rice to tolerate submergence condition, which was named *Sub-1.* Then, several studies have shown that plants with *Sub-1* gene could survive under a fully submerged condition for two weeks. Quantitative trait locus (QTL) analysis revealed that submergence tolerance is controlled by a single major QTL on chromosome 9 along with a number of other minor QTLs (Xu and Mackill. 1996; Nandi *et al.,*1997; Toojinda *et al.,*2003).

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Appendix 1. Screening for submergence tolerance.

Seeds were germinated and subsequently planted in the row in the experimental field for 75 cm long and 25 cm wide. FR13A variety was used as tolerant check, IR42 variety as susceptible check and the original parental variety were planted after every tenth row for comparison.

Fourteen days after germination, water level was raised to 10 cm. In the 30th days, the water level was suddenly increased to 150 cm and maintained for 14 days as a flash flood condition. All were observed once a week for their performance under flooding condition. After 14 days of flooding or susceptible check died, the water was drained out, to allow the remaining plants recover for 14 days. Then, visual scoring for recovering ability was carried out according to Standard Evaluation System for Rice (IRRI, 1996)(Table 1a).

Table 1a. Standard Evaluation System for Rice, scoring submergence tolerance.

Index	Description	Scale
1	Minor visible symptom of injury	100
3	Some visible symptom of injury	95-99
5	Moderate injury	75-94
7	Sever injury	50-54
9	Partial to complete death	0-49

Appendix 2. Screening for blast resistant.

Seeds of each mutant lines were soaked and after two days sprouted seeds of each lines were sown in a single row on a raised bed of the disease nursery plot. The length of each row sown was 50 cm and after every two test rows/lines there was a row of a susceptible of KDML 105 The row were kept 10 cm apart. The disease nursery was also bordered around with two lines of susceptible. The nursery was watered on 5th day of transplanting and the chopped leaves were spread over the entries of the nursery when the seedling were 10 days old. At three week stage, the test entries of the nursery were also sprayed with already prepared inoculum of *P. oryzae* as described by Khan et al ., (2001) by adjusting the spore suspension to 1x106 spores/ml the nursery was continuously sprayed with tap water to maintain the humidity. Normal agronomic practice were followed and data of disease were recorded three weeks after spray inoculation by using rating scale of IRRI (1996).

Appendix 2b. 0-9 grade disease rating scale used for screening for blast nursery.

Grade	Disease severity	Host response
0	No lesion observed	Highly Resistant
1	Small brown specks of pin point size	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesion are mostly found on the lower leaves	Moderately Resistant
3	Lesion type same as in 2, but significant number of lesions on the upper leaves	Moderately Resistant
4	Typical susceptible blast lesions; 3 mm or longer infecting less than 4% of leaf area.	Moderately Susceptible
5	Typical susceptible blast lesions of 3 mm or longer infecting 4- 10% of the leaf area.	Moderately Susceptible
6	Typical susceptible blast lesions of 3 mm or longer infecting 11-25% of the leaf area.	Susceptible
7	Typical susceptible blast lesions of 3 mm or longer infecting 26-50% of the leaf area.	Susceptible
8	Typical susceptible blast lesions of 3 mm or longer infecting 51-75% of the leaf area many leaves are dead	Highly Susceptible
9	Typical susceptible blast lesion of 3 mm or longer infecting more than 75% leaf area affected	Highly Susceptible

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