Applications of gamma rays irradiation and marker assisted selection for improving of bacterial leaf blight resistant rice variety, BT62.1

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Climate change creates adverse conditions for rice production in rice growing regions. For adaptation to mentioned challenges, cultivation of resistant varieties to biotic and abiotic stresses is required. Mutation technique is very effective for improving main agronomic characteristics such as yield, quality and resistance to diseases and pests. It is well-known not only in worldwide but also in Vietnam. In recent years, the marker - assisted selection (MAS) strategy have been used for selection of traits that are difficult and costly performed measurement and score. In this article, we present the works on the application of gamma ray irradiation and MAS technique for improving of Bacterial Leaf Blight (BLB) resistant rice variety BT62.1. This variety is short growth duration, high quality, easily cultivation and bacterial leaf blight resistance (carrying Xa7 and Xa21 genes), but low yield and needs to be improved. BT62.1 dry seeds were treated with 300 grey of Cobalt-60 gamma rays. Irradiated seeds were grown in experiment field of Mutation and Heterosis Division, Agriculture Genetics Institute. All M1 individuals were harvested separately for next season. From M2 to M4, mutant lines were also collected and planted in family. In M5, all mutant lines were breeded by MAS. Then they were inoculated for resistance to bacterial leaf blight and evaluated of main agronomic traits. The mutant lines, with resistant genes and good traits were kept breeding for stable and diversity analysis. The results of evaluation of genetic similarity and cluster analysis showed that genetic diversity in 40 mutant lines and original variety; Eight promising mutant lines of M5 generation retained as short duration, good quality, bacterial leaf blight resistance as original varieties but they have the higher yield than the original ones. Out of which, 2 mutant lines M24 and M33 have the highest yield (more than 7 tons/ha). These promising mutant lines are selected for further evaluation.

Keywords: mutation, gamma rays, marker, bacterial leaf blight, rice

Introduction

Rice is a major food for about 50% population all over the world. Climate change creates adverse conditions for rice production in rice growing regions. For adaptation to mentioned challenges, cultivation of resistant varieties to biotic and abiotic stresses is required.

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Mutation technique is very effective for improving main agronomic characteristics such as yield, quality, and resistance to diseases and pests (Patnaik, G.J.N. Rao., 2009). It is well-known not only in worldwide but also in Vietnam. Up to now, there are more than 90% mutant varieties created by X-rays and gamma rays. According to FAO/IAEA2012 databases, there are more than 3400 varieties induced by chemical and physical mutagens. In Vietnam, among 50 mutant varieties, of which have 32 rice varieties (Thinh D K et al., 2004; M. Q. Vinh et al.,2009). However, by conventional mutant selection method, the selection process takes a long time and unpredictable. The use of molecular markers linked to BB resistant genes to screen and evaluate the BB resistant mutants, which will speed up the process and makes it more accurate.

In recent years, the marker - assisted selection (MAS) strategy have been used for selection of traits that are difficult and costly performed measurement and score. The advantages of the MAS are: Selection can be made without phenotype data and is not being influenced by the environment and the selection is possible at the juvenile stage. Marker - assisted selection have been performed for selection bacterial leaf bligh resistant genes (Zhang.J et al., 2006). There were several reports on map construction and identification of the markers tightly linked to bacterial bligh resistance genes in rice (Porter, B. W., 2003; Zhang.Y.C et al., 2009, Xia et al., 2012),

In this article, we present the works on the application of gamma ray irradiation and marker - assisted selection for improving of Bacterial Leaf Blight (BLB) resistant rice, BT62.1.

Materials and methods

Materials

- Dried seeds of BT62.1 rice variety that carried two bacterial leaf blight resistant genes: *Xa7, Xa21*). Purpose of irradiation: improvement of yield.
- 40 rice mutant lines in M5 generation,named: M1 to M40.These mutant lines were selected from BT62.1 variety that was created by mutation method in 2013.
- 60 SSR markers on all 12 chromosomes of rice genome.
- Markers linked to bacterial blight resistance: *Xa7, Xa21:* P3 và pTA248.
- 3 isolates of *Xoo,* prevalent in the North Vietnam (*source: Vietnam National University of Agriculture)*

Methods
- *Irradiation gamma ray (Co 60): Dalat Institute of Nuclear Research, Doses: 300 Gy*
- *Total DNA extraction: Wang et al., 1993.*
- *The allelic diversity of the SSR was calculated according to the Polymorphism Information Content (PIC) was described by Anderson et al. (1993).*
- *Data analysis by using the NTSYS pc statistical package version 2.1.*
- *Phenotyping evaluation for Bacterial leaf blight resistance, described by Jica(2003).*

Results and Discussions

Seeds of irradiated varieties were grown in experiment field of Mutation and Heterosis Division, Agriculture Genetics Institute. All M1 individuals were harvested separately for next season. From M2 to M4, mutant lines were also collected and planted in family. In M5, all mutant lines were breeded by MAS. Then they were inoculated for resistance to bacterial leaf blight and evaluated of main agronomic traits. Promising lines, with resistant genes and good traits were kept breeding for stable and diversity analysis.

Genetic diversity analysis of mutant lines based on SSR markersSSR diversity

The 40 mutant lines were analyzed with 60SSR markers and 14 markers were shown polymorphic with polymorphism percent was 23% (table 1). However, this results revealed only for comparison high polymorphism between mutants and original .Because, all markers used in this experiment was high polymorphism in the previous studies. Thus, polymorphic ratio was obtained in this study normally higher than that of random other markers selection.

Table1: Survey results SSR primer pairs with rice mutant lines

Number of lines	Number primers used	Number of pairs for polymorphism	Polymorphism ratio (%)
41	60	14	23

There are 14 primers were polymorphism between BT62.1 and mutant lines. Results showed that new alleles were detected.
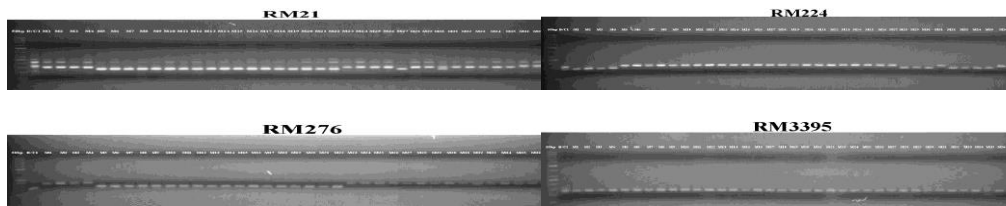
Fig. 1. Amplified products from genomic DNA of 40 rice mutants lines using RM276, RM3395, RM224 and RM21 primers.

The lanes represent, M: 50bp DNA marker, DC: BT62.1 (Origin line), M1-M40: rice mutant lines

In total of 14 SSR markers, ten markers were detected only one polymorphic alen. Four remain SSR markers were presented two polymorphic alens. In fourteen primers, four primers were located on chromosome 11 and ten primers were distributed on chromosome 1, 2, 3, 4, 6, 7, 8, 9, 10, 12.

Table 2: Allele variation, Polymorphism Information Content (PIC) for SSR loci identified in 40 rice mutant lines

No.	markers	Chromosome location	No. of allele	Frequency of the most common allele	PIC
1	RM17	12	3	65,12	0,52
2	RM21	11	3	47,62	0,61
3	RM206	11	3	56,10	0,58
4	RM224	11	3	57,14	0,57
5	RM276	6	2	53,66	0,50
6	RM3133	11	2	90,24	0,18
7	RM3395	8	2	97,62	0,05
8	RM3515	2	2	73,17	0,39
9	RM3831	7	2	55,81	0,49
10	RM5501	1	2	56,10	0,49
11	RM7000	3	2	83,72	0,27
12	RM8213	4	2	65,85	0,45
13	RM8225	6	2	69,77	0,42
14	RM25319	10	2	80,00	0,32
	Total		**32**		
	Mean		**2,28**		**0,42**

The number of alleles per locus ranged from 2 allele to 3 alleles, with a mean of 2,28 alleles

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The diversity index of mutant lines at polymorphic SSR locus showed high polymorphism in used markers in this study. PIC index ranged from 0,05 to 0,61. The marker RM3395 that was located on chromosome 8, which showed result the PIC value was 0,05 (lowest). The highest of the most common allele frequency was 97,62%. The marker RM21 that was located on chromosome 11, which showed result the PIC value was 0,61 (highest). The lowest of the most common allele frequency was 47,62%.

Cluster analysis

The genestic similarities obtained from SSR data were used to create a cluster diagram. Cluster analysis based on Dice coefficients using UPGMA grouped 40 mutant lines and original variety accessions into 3 main clusters I, II and III (fig.2). The cluster analysis repesented a significant genetic variation among the mutant lines.
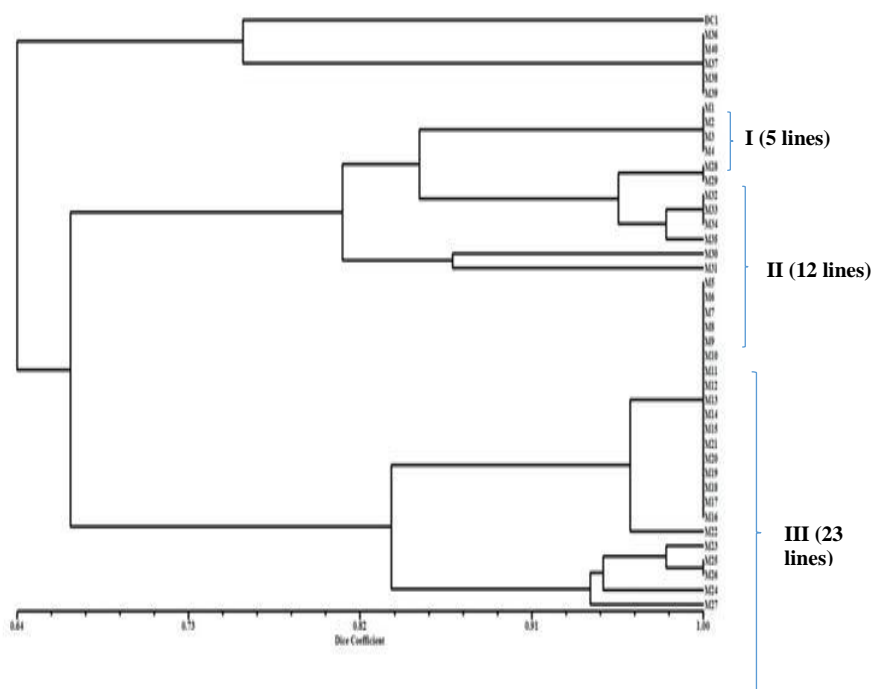
Fig.2. Dendrogram showing genetic relationship among 40 mutant lines revealed by UPGMA cluster analysis of Jaccard's similarity coefficients based on SSR markers

The genetic similarity ranged from 0,56 to 1,0 with a mean of 0,78 indicated a significant genetic variation among rice mutant lines

The evaluation of genetic similarity and cluster analysis showed genetic diversity in these mutant lines and original variety

Agronomic charaterization of the promising mutant lines

Screening 40 mutant lines based on agronomic traits in fields, mainly based on yield, 8 promising lines better than original variety were selected. Table 3 show some main characters of promising mutant lines.

Table3: Some main Agronomic traits of the promising mutant lines in M5 generation (summer season 2014)

No.	lines	Type of culm	Growth duration (days)	Plant height (cm)	Lemma color	Distribution of awns
	BT62.1 (cont.)	Erect culm	104	109,2±1,4	dark brown	Short awn
1	M3	Erect culm	107	109,2±1,5	brown	no awn
2	M9	Erect culm	103	109,2±1,4	dark brown	no awn
3	M15	Semi-Erect culm	108	110,7±1,4	dark brown	no awn
4	M24	Erect culm	104	110,4±1,9	dark brown	Short awn
5	M27	Semi-Erect culm	102	109,8±2,2	dark brown	no awn
6	M33	Erect culm	103	108,5±0,8	dark gold	no awn
7	M35	Erect culm	106	111,6±2,0	brown	no awn
8	M39	Erect culm	104	113,1±0,6	brown	Short awn

Most of promising mutant lines have keeping short growth duration, plant height, dark blue leaf color, type of culm. These promising lines can be cultivated easily, which were similar to original one. Some of major traits have been improved such as awn seed, lemma color… In eight promising lines, there were six lines evalueted with no awn seed. Further more, lemma color of M3, M33, M35 and M39 was changed.

Table 4. The grain yield components of 8 promising mutant lines (Summer season 2014)

N_o	Lines	No. of panicle /plant	No. of seeds /panicle	No. of full seed /panicle	Weight of 1000 developed grains (gr)	Potential yield (ton/ha)
	BT62.1 (Cont.)	5,1	145,5	138,2	22,5	5,62
1	M3	6,6	167,6	153,8	23,9	5,86
2	M9	6,2	202,2	181,2	23,9	6,69
3	M15	6,4	212,4	160,8	23,8	6,14
4	M24	5,6	207,7	191,0	23,5	7,1
5	M27	5,3	187,3	169,8	24,0	6,72
6	M33	5,2	217,9	198,5	23,9	7,16
7	M35	6,0	195,3	181,3	22,9	6,84
8	M39	6,0	186,7	173,0	23,8	6,57
	CV%	*0,17*	*5,91*	*6,00*	*0,05*	*2,38*
	LSD$_{0,05}$	*0,38*	*13,65*	*13,83*	*0,12*	*5,49*

With regards to the grain yield component of mutant lines in the promising mutant lines showed a significant difference compare to the control. The highest full seed per panicle were obtained at M24 and M33 lines and it showed significant difference from other lines (table 4). The potential yield of M24 and M33 lines were higher than that of original and other lines with 7,1 tons/ha and 7,16 tons/ha. These other mutant lines performed grain yield higher than original variety .

Genotyping analysis for bacterial blight resistance genes in mutant lines

Two markers linked tight to BLB genes: pTA248 for *Xa21* and P3 for *Xa7* were used for genotypic screening in mutant lines
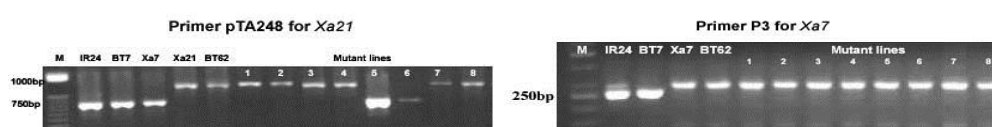
Fig. 4: PCR analysis of mutant lines using SSR markers linked to bacterial blight resistance

M: 50bp DNA ladder; IR24; BT7: Bac Thom 7; Xa7: IRBB7; Xa21: IRBB21; BT62.1: Origin line; From 1- 8: mutant lines M3, M9, M15, M24, M27, M33, M35, M39

The result indicated that the presentation of BB resistance genes was in all mutant lines. Of which, there were six mutant lines conferred both of *Xa7* and *Xa21* similar as original variety. Only *Xa7* gene was detected in M27 and M33.

Phenotyping evaluate for bacterial blight resistance in the promising mutant lines

Three prevalent isolates in the North Vietnam were inoculated for screening. Result was showed in Table 4.

Table 4: Reaction of promising mutant lines to *Xoo* isolates from the North Vietnam

No	Line	Lesion length in centimeters on mutant lines carrying genes, after inoculation with isolates of *Xoo*from the North Vietnam					
		Isolate 1		Isolate 2		Isolate 3	
		Lesion length (cm)	Reaction	Lesion length (cm)	Reaction	Lesion length (cm)	Reaction
1	IR24 (susceptible)	19,5±2,1	HS	18,7±1,5	HS	21,2±1,6	HS
2	IRBB62 (donor)	1,1±0,3	HR	1,7±0,1	HR	2,1±1,1	HR
3	M3	1,1±0.27	HR	1,5±0.29	HR	4,6±0.26	R
4	M9	1,1±0,3	HR	1,7±0,1	HR	2,1±1,1	HR
5	M15	0,9 ± 0,4	HR	2,5 ± 1,0	HR	4,7 ± 1,9	R
6	M24	0,7±0.14	HR	3,2±0.51	HR	5,6±0.3	R
7	M27	6,3±0.65	R	7,2±0,3	R	7,5±0.3	R
8	M33	7,0±0.73	R	5,2±1,8	R	7,8±3,0	R
9	M35	2,4±0,2	HR	3,6±0,5	HR	4,5±0,2	R
10	M39	3,6±0.26	HR	4,7±0,21	R	5,3±0,40	R
11	BT62.1	3,3±1.14	HR	3,0±0,31	HR	5,2±0,61	R

Fig. 5. Reaction of mutant lines to Xoo isolates in the field

The susceptible IR24 control variety was high susceptible (HS). In this experiment, all mutant lines were revealed resistance to Bacterial Leaf Blight pathogen. However, The level of resistance was different between mutant lines such as high resistance (HR) and resistance (R).

Conclusions

The evaluation of genetic similarity and cluster analysis showed genetic diversity in 40 mutant lines and original variety

Screening based on agronomic traits in fields, mainly based on yield; Testing in greenhouse and MAS in lab; From 40 mutant lines at M5 generation, 8 promising mutant lines were selected.

Main attractive characteristics of these lines are higher yield compare to origin, keeping short growth duration, easily cultivation and resistance to bacterial leaf blight. M24 and M33 mutant lines have highest grain yield (7,1 and 7,16 ton/ha)

6/8 mutant lines are carrying 2 bacterial leaf blight resistant genes: *Xa7, Xa21*and resistance to 3 Xoo isolates that were representative for the North Vietnam

Acknowledgments

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