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Genetic Variability Analysis In Rice Mutant Lines From Gamma Rays Radiation Using Agromorphological And Ssr Markers

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In this work, genitic variability of rice mutant lines from gamma rays radiation was surveyed based on agromorphological and SSR markers. Seed of original variety HC62.2 with low yield was irradiated by gamma rays (Cobal 60) for improvement. Fourteen mutant lines maintaining good chacracteritics and having better productions were selected and analyzed. Twenty-six agromorphological traits (maturity, plant height, flag leaf angle, awning, yield...) were evaluated and then data were transformed into the binary system. Thirty-one SSR markers, located on twelve chromosomes, were considered for genetic diversity analyses in order to estimate the extent of diversity generated by gamma rays radiation in rice. The similarity between genotypes was obtained based on Dice's Coefficient. The UPGMA defined three main clusters. Results indicated that polymorphism based on SSR markers is not far diffirent from variation based on agromorphological traits. On the other hand, gamma rays radiation was effective not only to improve yielding but also to create variation materials for rice breeding.

Keywords: genetic variability analysis, agromorphological, SSR marker, mutation, gamma rays.

Introduction

Mutation breeding could be considered especially successful to obtain new features while maintaining interested chacracteritics and to broad the genetic in genome of cultivar. So it has been used as the sole technique for the improvement of special rice type such as: Basmati rice in India and Pakistan, Tamthom rice in Vietnam…. Mutation techniques have proven not only useful for improving agronomic traits: yield, plant height, growth duration… but also for enhancing resistance to biotic stress and tolerance to abiotic stress (Wang, L. Q. 1991). Morever, mutation induction has become an important tool in gene discovery and functional genomics studies. Recently, more and more mutant lines are being generated and analyzed worldwide.

Knowledge regarding the amount of genetic variation in mutant lines and genetic relationships between genotypes are important considerations

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for assessing effective of mutation factor in breeding programs. In the past, the characterization of germplasm diversity was carried out by means of morphological and biochemical markers which, in many cases, did not have the resolution power for revealing polymorphisms in genetic analyses and/or for differentiating between closely related genotypes. Advances in plant genetics and molecular biology have led to the development of many types of molecular markers which can be used to characterize germplasm. Different types of DNA markers are available nowadays, each method differing in principle, application, type and amount of polymorphism detected, and cost and requirement. SSRs are an excellent molecular marker system for many types of genetic analyses, including linkage mapping, germplasm surveys, and phylogenetic studies (Alba Alvarez *et al*., 2000). They have been used for characterizing genetic diversity in several crop species including sorghum, maize, cotton, wheat and rice (Herrera T. G. et al., 2008). All results showed that SSR markers are efficient in detecting genetic polymorphisms and discriminating among genotypes (Alvarez A. *et al.*, 2007, Giarrocco L.E. *et al.*, 2007).

The objective of this study was combining agromorphological and SSR markers to estimate the genetic variability between 14 rice mutant lines from HC62.2 and to distinguish the difference between DNA and agromorphological variations.

Materials and methods

Rice genotypes: the group of promising mutant lines obtained from HC62.2 is presented in Table 1. All of them are early maturity, short height and high resistance to BLB.

Table 1. Main agromorphological traits of mutant lines and their control variety (spring season)

Lines	Mat.	PH	TA	LS	PE	W	Yield	Ldg.	BLB	LB	PB
Control (HC62.2)	1	5	9	9	3	2,24	5,1	1	1	3	0
L1	1	5	7	9	3	2,16	5,6	1	1	3	0
L2	1	5	7	9	3	2,25	6,2	1	1	3	0
L3	1	5	7	9	3	2,22	5,8	1	1	3	0
L4	1	5	7	9	3	2,21	5,6	1	1	2	0
L5	1	5	7	9	3	2,21	7,3	1	1	3	0
L6	1	5	7	9	3	2,29	6,4	1	1	3	0
L7	1	5	7	9	3	2,22	6,0	1	1	3	0
L8	1	5	7	9	3	2,27	7,1	1	1	3	0
L9	1	5	7	9	3	2,25	5,9	1	1	3	0
L10	1	5	7	9	3	2,24	6,3	1	1	3	0
L11	1	5	7	9	3	2,24	5,6	1	1	2	0
L12	1	5	7	9	3	2,23	5,7	1	1	2	0
L13	1	5	9	7	3	2,06	5,2	1	1	2	0
L14	1	5	7	9	3	2,21	5,5	1	1	2	1

Maturity (1,3,5 IRRI scales); PH: Plant height (1,5,9 IRRI scales); TA: Tilling ability (1,3,5,7,9 IRRI scales); LS: Leaf senescence (1,5,9 IRRI scales); PE: Panicle exsertion (1,3,5,7,9 IRRI scales); W(g): 100-grain weight; Ldg: Lodging resistance (1,3,5 IRRI scales); BLB: Bacterial leaf blight resistance (0,1,3,5,7,9 IRRI scales); LB: Leaf blast resistance (0,1,2,3,4,5,6,7,8,9 IRRI scales); PB: Panicle blast resistance (0,1,3,5,7,9 IRRI scales)

Twenty-six agromorphological traits were assessed under field conditions by Standard Evaluation System (IRRI, 2002) (Table 2)

Table 2. Agromorphological traits using genetic variability analysis

No.	Traits	Scale	No.	Traits	Scale
1	Maturity	1,3,5	14	Leaf blade color	1,2,3,4,5,6,7
2	Plant height	1,5,9	15	Seed coat color	1,2,3,4,5,6,7
3	Tilling ability	1,3,5,7,9	16	Scent (aroma)	0,1,2
4	Panicle exsertion	1,3,5,7,9	17	Panicle thresh ability	1,3,5,7,9
5	Number of full seed*	1,3,5,7,9	18	Awning	0,1,5,7,9
6	100 grain weight*	1,3,5	19	Leaf blade pubescent	1,2,3
7	Yield*	1,2,3,4,5,6,7,8,9	20	Panicle axis	1,2
8	Flowering duration*	0,1,2	21	Panicle type	1,2,3
9	Flag leaf angle	1,3,5,7	22	Stigma color	1,2,3,4,5
10	Culm angle	1,3,5,7,9	23	BLB resistance	0,1,3,5,7,9
11	Leaf angle	1,5,9	24	Leaf blast resistance	0,1,2,3,4,5,6,7,8,9
12	Husk color	1,2,3,4,5,6,7	25	Panicle blast resistance	0,1,3,5,7,9
13	Leaf senescence	1,5,9	26	Lodging resistance	1,3,5

(*: traits assessed by modified scales)

The detail information (name, sequence, location) of thirty-one SSR markers used in this study was showed in Table 3.

Table 3. List and infomation of thirty-one SSR markers used in this study

No.	Name	Forwad	Reverse	Chr.
1	RM495	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	1
2	RM6840	TACCAAGACTCCGCTATGGC	GAAGAAGGGATCATGGATCG	1
3	RM240	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC	2
4	RM262	CATTCCGTCTCGGCTCAACT	CAGAGCAAGGTGGCTTGC	2
5	RM324	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	2
6	RM8208	GCCCAAACTACACTCTCTTG	GTAAATGCCTGAGTGCCTAC	2
7	RM1347	AACAAATTAAACTGCCAAG	GTCTTATCATCAGAACTGGA	2
8	RM7000	CCCTTCTTTTCAACTGAATA	TTGTAACAATGAACTCGTTC	3
9	RM3317A	CCTGACAGAAGAATGGTACACC	TGTGGCTTCTCGTTGAGTTG	4
10	RM3524	CGGAGCTGGTCTAGCCATC	GTCTCCGTCTTCCTCACTCG	4
11	RM8213	AGCCCAGTGATACAAAGATG	GCGAGGAGATACCAAGAAAG	4
12	RM267	TGCAGACATAGAGAAGGAAGTG	AGCAACAGCACAACTTGATG	5
13	RM3476	GATTCTCGTCGTAATCAAGA	ATCCACGGTTAAGATAAATG	5
14	RM6313	ATCCAGATCCACTTTGACCG	GGAGGACTTCTACCATCCTTG	5
15	RM162	GCCAGCAAAACCAGGGATCCGG	CAAGGTCTTGTGCGGCTTGCGG	6
16	RM508	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC	6
17	RM510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC	6
18	RM3138	TTGACAAGAGATCAAGGCGG	GTGAATGTTGAGCTGCATGG	6
19	RM11	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	7
20	RM3831	CTCCACGTTCTCCGACGAG	GCGGCAACTCCTACATATCC	7
21	RM1134	ACACCCAACTTTTCTCACGC	AGCTAGGGTTTCGATCTCCC	7
22	RM330	CAATGAAGTGGATCTCGGAG	CATCAATCAGCGAAGGTCC	8
23	RM3153A	CACAAAGTTTCAAATATAGC	GATCTCATGATAGTCACTCA	8
24	RM3395	ACCTCATGTCCAGGTGGAAG	AGATTAGTGCCATGGCAAGG	8
25	RM1328	CCATGAGTGACATCAAAAGG	CCATGAGTGACATCAAAAGG	9
26	RM258	TGCTGTATGTAGCTCGCACC	TGGCCTTTAAAGCTGTCGC	10
27	RM21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	11
28	RM3133	TCAATAGACACACGGGCATG	CGATTTTGCTCACTGCACAG	11
29	RM552	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGTCC	11
30	RM5704	TTTCAGTGCATGTCTTCG	GATTGTATGCATGGTTCAAAG	11
31	RM17	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA	12

(http://www.gramene.org)

Diversity analysis:

Data of agromorphological and SSR markers were transformed into binary system. Cluster analysis using Unweighed Pair Group Method with Arithmetic Average (UPGMA) were performed on the similarity based on agromorphological and SSR markers employing SAHN program of NTSYS-pc package 2.1 (Mohammadi S. A. and Prasanna B.M., 2003; Rohlf F. J. 1997).

Variations of agromorphological and SSR were calculated according to the Polymorphism Information Content (PIC): Anderson et al., 1993.

DNA extraction: Wang et al., 1993.

PCR technique: by Veriti 96well Thermal cycler: Total reaction volume: 15 µl (5µl ADN, 0.15µM primer, 0.2 mM dNTPs, 1X PCR buffer, 2.5mM $MgCl_2$ and 0.25 unit Taq). PCR proceeding: 95^0C - 7 minutes; 35 cycles (94^0C - 15 seconds, 55^0C - 30 seconds, 72^0C - 1 minutes; 72^0C - 5 minutes; stored in 4^0C). PCR amplified products were electrophoresis onto agarose gel 2%.

Field assessment: by Standard Evaluation System, IRRI, 2002 .

Results

Survey results based on agromophological and SSR markers

The number of monomorphic and polymorphic bands/scales and percentage of genotypes identified for each marker types system appears in Table 4. Eight SSR markers: RM495, RM324, RM7000, RM3524, RM3831, RM1134, RM21 and RM17 resulted polymorphic, respectively in Figure 1.

Table 4. Survey results with rice studied samples based on agromophological and SSR markers

Type of marker	Total surveyed	No. of polymorphism markers	Polymorphism percentage (%)	List of polymorphism markers
SSR	31	8	25,8	RM495, RM324, RM7000, RM3524, RM3831, RM1134, RM21, RM17
Agromorphological	26	14	53,8	Culm angle, Flag leaf angle, Leaf angle, Awning, Panicle type, Husk color, Seed coat color, Scent (aroma), Leaf senescence, Tilling ability, Number of full seed, Yield, Leaf blast resistance, Panicle blast resistance

While fourteen agromorphological markers showed polymorphic are: Culm angle, Flag leaf angle, Leaf angle, Awning, Panicle type, Husk color, Seed coat color, Scent (aroma), Leaf senescence, Tilling ability, Number of full seed, Yield, Leaf blast resistance, Panicle blast resistance. Polymorphism percentage of SSR markers (25.8%) is lower than that of agromophological markers (53.8%).

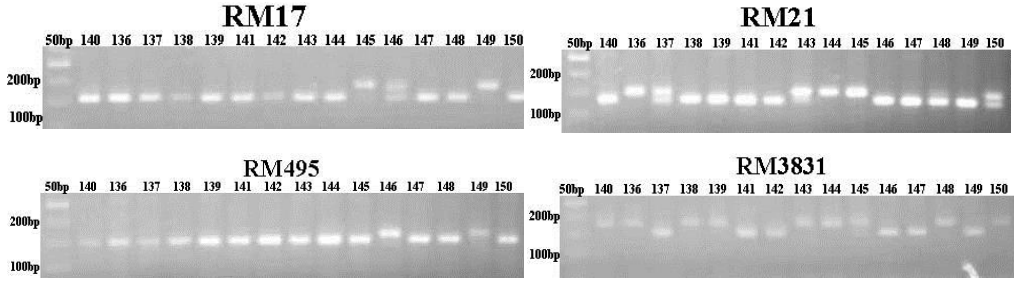

Figure 1. Amplified products from genomic DNA of studied samples using RM17, RM21, RM495 and RM3831 primers.
(50bp ladder, 140: control, 136: L1, 137: L2, 138: L3, 139: L4, 141:L5, 142: L6, 143: L7, 144: L8, 145: L9, 146: L10, 147: L11, 148: L13, 149: L13, 150: L14)

SSR markers analysis

Index of polymorphism SSR markers: location, number of allele, frequency of the most common allele and PIC were showed detail in Table 5.

Table 5. Allele variation, Polymorphism Information Content (PIC) for SSR loci identified in rice studied samples

No.	Markers	Chromosome location	No. of allele	Frequency of the most common allele	PIC
1	RM495	1	2	86.67	0.23
2	**RM324**	**2**	**2**	**43.75**	**0.65**
3	RM7000	3	2	70.59	0.42
4	**RM3524**	**4**	**2**	**93.75**	**0.12**
5	RM3831	7	2	60.00	0.48
6	RM1134	7	2	52.38	0.50
7	RM21	11	2	64.71	0.46
8	RM17	12	2	81.25	0.30
	Total		**16**		
	Mean		**2**	**69.14**	**0.40**
	Min			**43.75**	**0.12**
	Max			**93.75**	**0.65**

In total of eight polymorphic SSR markers, all of them were detected two alleles. In that, two markers were located on chromosome 7 and six ones were distributed on chromosome 1, 2, 3, 4, 11, 12. This result indicated that, based on thirty-one SSR markers, variations among mutant lines and

their control were only detected on chromosome 1, 2, 3, 4, 7, 11, 12; not on remain chromosomes.

Frequency of the most common allele and PIC are inverse index. Data of the most common allele frequency presented from 43,75% to 93,75% (mean at 69,14%). PIC index of mutant lines at polymorphic SSR locus ranged from 0,12 to 0,65 (mean at 0,4). The marker RM3524 located on chromosome 4, showed the PIC value was 0,12 (lowest) and the highest of the most common allele frequency was 93,75%. The marker RM324 located on chromosome 2, constructed the PIC value was 0,65 (the highest) and the lowest of the most common allele frequency was 43,75%. It could be suggested that mutant lines including variation allele based on RM324 on chromosome 2 were the most in polymorphic markers.

Agromorphological traits analysis

Index of agromorphological variation: number of variation scale for each polymorphic trait, the most variation scale, frequency of the most common scale and variation index were presented in Table 6.

Table 6. Agromorphological variation mesuared in rice studied samples

Traits	**No. of variation scale**	**The most variation scale**	**Frequency of the most common scale (%)**	**Variation Index of scale**
Tilling ability	2	7	86.67	0.23
Number of full seed	2	3	86.67	0.23
Yield (tons/ha)	**4**	**5**	**40.00**	**0.69**
Flag leaf angle	2	3	86.67	0.23
Leaf angle	2	3	80.00	0.32
Husk color	2	1	80.00	0.32
Culm angle	2	3	80.00	0.32
Leaf senescence	**2**	**9**	**93.33**	**0.12**
Seed coat color	2	1	73.33	0.39
Scent (aroma)	2	1	86.67	0.23
Awning	2	0	73.33	0.39
Panicle type	2	2	86.67	0.23
Leaf blast resistance	2	3	73.33	0.39
Panicle blast resistance	2	0	86.67	0.23
Total	**30**			
Mean	**2.14**		**79.52**	**0.31**
Min			**40.00**	**0.12**
Max			**93.33**	**0.69**

In fourteen polymorphic traits, thirteen were presented two scales and only one yield showed 4 scales. Total variation scales conducted were thirty, with mean at 2,14.

Data of the most variation scale construced to the most common feature in studied samples. It means that almost mutant lines have phenotype with: tilling ability at 7 scale; number of full seed at 3 scale; yield at 5 scale, flag leaf angle at 3 scale…..

The analysis of frequency of the most common scale and variation index base on yield showed at 40,00% (the lowest frequency) and 0,69 (the highest). Inside out, results based on leaf senescence trait were assessed at 93,33% (the highest frequency) and 0,12 (the lowest). It could be indicated that there were the most variability about production and the least about leaf senescence selected in this study.

Cluster analysis

The genestic similarity obtained from agromorphological and SSR data were used to create a cluster diagram. Cluster analysis based on Dice coefficients using UPGMA grouped 14 mutant lines and original variety accessions into 3 main clusters I, II, III at 0,64 value, respectively in Figure 2.

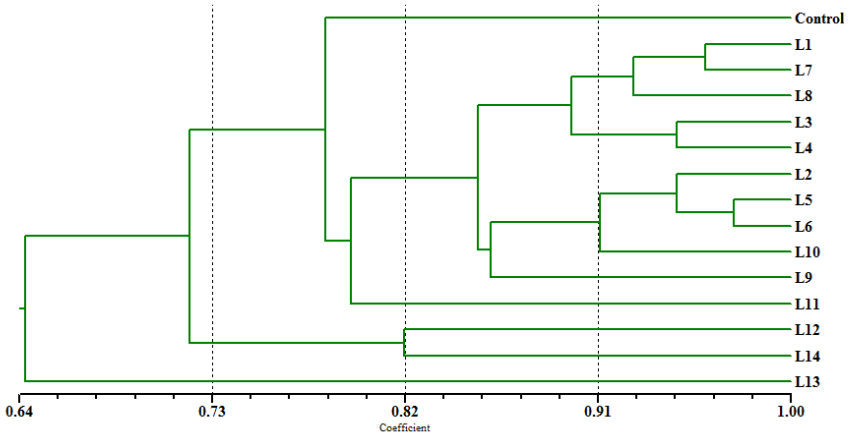

Figure 2. The UPGMA showing genetic relationship among rice studied samples revealed by UPGMA cluster analysis of Dice's coefficients based on agromorphological and SSR markers

Group I: incuding control and eleven mutant lines L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11.

Group II: including L12 and L14

Group III: including L13.

The groups formed in the UPGMA were repesented that the mutant L13 has the farthest distance from control and other lines.

Morever, the genetic similarity based on matrix analysis ranged from 0,56 to 0,97 with mean at 0,79 indicated a significant genetic variation

among rice mutant lines.

Discussion

SSR marker analysis:

Thirty one SSR markers used in this study were highly informative and polymorphic as evident from its PIC mean value of 0,4. It was seen that PIC values were relatively higher for markers: RM 324 (0,65), RM1134 (0,5) and RM3831 (0,48) having GA and AC repeats which is because of the fact that these repeats are highly variable and polymorphic in nature.

Agromorphological traits analysis:

Mutation techniques have more useful for improving agronomic traits (quantitive traits) such as: yield, plant height, growth duration…..than for inducing quanlity traits such as: resistance and tolerance. The analysis of variation index base on yielding trait were highly polymorphic as evident from its value of 0,69. It could be indicated that mutation breeding is very effective for improving production.

UPGMA clustering:

The UPGMA cluster analysis showed that all mutant lines of rice variety HC62.2 could be distinguished based on the information generated by 26 agromorphological and 31 SSR markers. The genetic similarity value ranged from 0,56 to 0,97 with mean at 0,79 suggested that variabilities among fourteen mutant lines were at mediate level.

All our results indicated that mutation breeding by gamma rays irradiation was effective not only to improve yielding but also to create variation materials for rice breeding.

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