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Genetic improvement of rice resistance to blast and bakanae diseases using mutation induction

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Eleven rice mutants were selected from irradiated populations of three Egyptian cultivars (Giza 177, Sakha 101 and Sakha 104) with different doses of gamma rays (0, 150, 200, 250 and 300 Gy). These mutants were evaluated for resistance to blast (*Magnaporthe grisea*) and bakanae (*Fusarium moniliforme*) diseases under natural and artificial infection conditions in M_3 and M_4 generations. Molecular screening and genetic diversities of resistance (R) genes to blast and bakanae diseases were performed using 22 Microsatellite markers. Out of eleven mutants, nine resistant mutants were obtained from susceptible parents (Sakha 101, Sakha 104). The results of genotypic screening revealed that the blast resistant mutants have a combination of partial resistance genes, *Pi40(t), Pi20(t), Pi35(t)*; and complete resistance genes (*Pib, Pik, Pik-h, Pita, Piz*) that confer durable resistance to all blast races. In contrast, the susceptible mutants SK4151 and SK4201 showed high susceptibility to the most of tested blast races because it were not possess genes *Pik-s, Pik-h* and *Pi9* that linked to RM144, RM224 and RM541 markers, respectively. Therefore, these R genes seemed to play a significant role in resistance to the most of tested races of blast disease. For bakanae disease, RM9, a specific marker for bakanae resistance, showed one unique band for SK115 mutant (180 bp) that resistant only to isolate 10. Whereas the mutants SK4152 and SK4154, a highly resistant to both isolates, were discriminated by one band (161 bp) as well as mutant SK120 (136 bp). Hence, mutation breeding is very effective approach to develop new resistant genotypes to blast and bakanae disease in rice.

Keywords: Rice; mutation; *M. grisea*; *F. moniliforme*; SSR

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops, provides a carbohydrate source for more than half of the world's population. Also, it is currently grown on approximately 163 million hectares worldwide with production of 741 million tons (FAO, 2014). In Egypt, rice is annually grown on approximately 1.4 million feddan with production of 5.46 million tons (FAO, 2014).

Rice diseases are one of the major limiting factors of rice production worldwide. In Egypt, the major rice diseases are blast, brown spot, bakanae disease, and white tip nematode. However, rice diseases reduce rice yield by about 5 % in mildly infection. In epidemic conditions, yield losses may reach as high as 30~50 % (Hammoud and Gabr, 2014). Rice blast caused by fungus *Magnaporthe grisea*, is caused an economic loss up to 65% yield in susceptible cultivars under favorable conditions (Singh *et al.* 2015). The genetic control of blast resistance is a complex trait, and involves both major and minor genes with complementary or additive effects (Wu

et al. 2005 and Li et al. 2007). However, the breakdown of blast resistance frequently occurs due to the rapid change in the blast pathogenic races after a few years of new cultivar release (Song *et al.* 2014). In Egypt, the resistance of Sakha 101 and Sakha 104 to blast disease was broken-down in 2004 with the appearance of the specific virulent races (El-Refaee et al. 2011).Bakanae disease caused by *Fusarium moniliforme* (Sheld.), telomorph: *Gibberella fujikuroi* (Sawada), usually causes a yield loss of 10~20% and the loss could be higher than 70% at outbreak of the disease (Ou, 1985). The infected plants turn pale yellow and exhibit chlorosis and abnormal elongation, poor grain ripening such as empty panicles. In extreme cases, infected plants fall down and die (Hwang et al. 2013). There are wide variations in response of varietal resistance to bakanae under Egyptian field conditions. Both Sakha 101 and Giza 177 as pure japonica were the most abundant susceptible cultivars (El-Refaee et al. 2011).Mutation breeding has proven to be a useful method for introducing new traits that may result in crop improvement and can be used as a complementary tool in plant breeding (Babaei et al. 2010). It involves the development of new varieties that characterized with disease resistance, early maturity and better productivity of germplasm origin using chemical and physical mutagens (Shu et al. 2012). Gamma rays, physical mutagen, have been effective and are more commonly used in mutation induction than other ionizing radiations because of their availability and relatively high power of penetration (Moussa, 2011).Molecular markers are powerful tools in assessment of genetic variation, and elucidation of genetic relationships within and among species (Chakravarthi and Naravaneni, 2006), and increasing the effectiveness of selection in breeding programs. A wide range of molecular markers such as Random Amplified Polymorphic DNA (RAPD), Microsatellite or Simple Sequence Repeats (SSRs) and Amplified Fragment Length Polymorphism (AFLP) have been applied for genetic diversity studies in rice (Kumar and Bhagwat, 2012). Among these markers, Microsatellites are useful for several applications in plant genetics and breeding because of their reproducibility, multi-allelic nature, codominant inheritance, relative abundance and good genome coverage in rice (Kumar et al. 2013). The present study was conducted to improve rice resistance to blast and bakanae diseases using mutation induction, and to characterize the selected mutants at molecular level using SSR markers.

MATERIALS AND METHODS

Plant materials

Three local rice cultivars; Giza 177 (Gz177), Sakha 101 (Sk101) and Sakha 104 (Sk104); were irradiated with different doses of gamma rays (0, 150, 200, 250 and 300 Gy). The visual selection was performed in M_2 and M_3 generations based on disease resistance and distinguished morphological traits. Eleven mutants were selected; four mutants (SK115, SK120, SK125 and SK130) were obtained from Sakha 101 cultivar; and seven mutants (SK4151, SK4152, SK4153, SK4154, SK4201, SK4202 and SK4203) were obtained from Sakha 104. These mutants and its parents were used in this study as well as Giza 177 cultivar was used as highly resistant check to blast and susceptible to bakanae.

Evaluation of resistance to blast disease

Eleven mutants and its parents were tested for blast disease infection under three conditions:

Blast Nursery

All mutants and its patents were evaluated for blast resistance at seedling stage under natural infection in blast nursery in Gemmiza location for 2 years. Seedbeds were prepared during the first week of July in each season and fertilized with urea (46.5%N) at 60 kg/fed. Width of the seedbed was 1m and 10 m long, five rows of Giza171 as highly susceptible spreader were sown alternatively between five of the considered genotypes with 15 cm apart. Another five genotypes were sown followed by one row of Giza182 as a resistant check. The genotypes were left exposed to natural blast infection at seedling stage. The typical blast lesions were scored after 30-45 days from sowing according to the standard evaluation system (SES) based on a 0-9 scale as given by IRRI (1996) as follows R: Resistant (0-2); MR: Moderately resistant (3); S: Susceptible (4-6); HS: Highly susceptible (7-9).

Artificial infection

Eleven mutants and its parents were evaluated for blast resistance at seedling stage under artificial inoculation in the greenhouse using 10 races of rice blast (IB-19, IB-45, IB-54, IB-63, IC-5, IC-15, ID-13, ID-15, IG-1 and IH-1). Grains of rice genotypes were sown in plastic trays (45 x 25 x 15 cm.), each tray comprised 30 rows representing 30 genotypes in three replications.

The trays were kept in the greenhouse at 25-30ºC and fertilized with Urea 46.5% N (5 g/tray). Seedlings, about 3-4 weeks after sowing, were inoculated at 3-4 leaf stage with 100 ml of a spore suspension ($5x10^4$ spores ml^{-1} and 0.25% gelatin) using electrical spray gun. The inoculated seedlings were held in a moist chamber with at least 90% relative humidity at 25-28^oC for 24h then it were moved to the greenhouse. Blast reaction was recorded after 7 days from inoculation according to the standard evaluation system using 0-9 scale (IRRI, 1996).

Permanent field

All genotypes were tested for leaf blast reaction at adult stage under natural condition in Gemmiza location for 2 years.

Evaluation of resistance to bakanae disease

The selected mutants and its parents were artificially inoculated with the most aggressive bakanae isolates 10 and 22. One hundred grains of each rice genotypes were soaked for 24 h in spore suspension. The concentration of the inoculum suspension was adjusted using a haemocytometer to 1×10^6 spores/ml with sterile water. The inoculated grains were incubated for 48h at 28^oC±2 to enhance both germination and infection. The untreated check of each genotype was sown from healthy seeds. All rice genotypes after incubation were sown in pots of 15 cm diameter with three replications under greenhouse conditions at 30-35^oC. The infected seedlings with typical bakanae symptoms were recorded after two weeks from sowing.

Microsatellite analysis

DNA was extracted using modified CTAB method according to Dellaporta et al. (1983). Twenty five markers; 22 SSR, 2 InDel (Insertion and Deletion) and 1 SNP (Single Nucleotide Polymorphism) markers were used for PCR amplification for 11 rice mutants and its parents (Table 1). PCR reactions were conducted at a final volume of 12.5 µl, containing 6.25 µl PCR master mix (KAPA2G Fast ReadyMix PCR Kit), 1 µl genomic DNA, 1 µl for each primer and 3.25 µl dH_2O. The PCR reactions were performed in a thermal cycler (TECHNE TC-412) programmed as follows: 95°C/5 min for pre-denaturation followed by 35 cycles (95°C/30 s, appropriate annealing temperature for 30 second, 72°C/30 s) and 72°C/5 min for final extension then held at 4°C. The PCR products were separated by electrophoresis in 2% agarose gel at 100 V for 50 min in 1x TBE buffer and stained with ethidium bromide and visualized on a UV transilluminator

RESULTS AND DISCUSSION

Evaluation of resistance to blast disease

The results in Table (2) showed that Gz177 cultivar (resistant check) was highly resistant to leaf blast disease under natural infection conditions (blast nursery and permanent field) in both generations and artificial inoculation with 10 blast races. Also, all selected mutants (SK115, SK120, SK125 and SK130) that obtained from Sk101 cultivar *via* gamma radiation treatments were resistant to blast disease although its parent Sk101 cultivar showed low level of resistance (28.6 %) to blast disease at all stages and conditions in the two seasons. On other hand, all selected mutants (SK4152, SK4153, SK4154, SK4201, SK4202 and SK4203) were highly resistant to blast disease under natural and artificial infection in both generations except SK4151 and SK4201 mutants and Sk104 cultivar that were susceptible under permanent field and artificial infection conditions. Similar results were observed by Hammoud and Gabr (2014) who found that Sakha 101 and Sakha 104 were highly susceptible under greenhouse and field conditions. Hammoud *et al.* (2011) reported that Giza177 was highly resistant under blast nursery, permanent field (adult stage) and artificial condition at seedling stage with four specific races i.e., IB-63, IH-1, IB-19 and IH-1. Also, Sk104 cultivar was susceptible only to IB-45, ID-15 and IG-1 races while SK4201 mutant was susceptible to the most tested races followed by SK4151 mutant. These results are consistent with the finding of El-Refaee *et al.* (2011) who found that the resistance of Sakha 101 and Sakha 104 to blast disease was broken-down in 2004 with the appearance of the specific virulent races and became susceptible while, Giza 177 and Sakha 103 still resistant and became good sources for blast resistance. Many attempts have been made to improve disease resistance in rice through mutation breeding. Positive results, particularly for resistance to blast have been reported. Blast resistant mutant R917 was derived from the F_1 progeny irradiated by 100 Gy (Zhang et al. 2003) and the rice mutant 'Zhefu 802' has a high resistance to rice blast (Ahloowalia et al. 2004). So, mutation breeding is very effective approach to develop new resistant genotypes to blast

Table 1. Description of markers used in the study

Marker	Forward primer	Reverse primer	Chr.
RM 9	GGTGCCATTGTCGTCCTC	ACGGCCCTCATCACCTTC	1
RM 151	GGCTGCTCATCAGCTGCATGCG	TCGGCAGTGGTAGAGTTTGATCTGC	1
RM 1216	TTCCCCAATGGAACAGTGAC	AGGGTCTACCACCCGATCTC	1
t256**	GGATAGCAGAAGAACTTGAGACTG	CATGTCTTTCAACATAAGAAGTTCTC	1
RM 208	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	2
RM 211	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG	2
RM 279	CCTCTCACTCACGTGGACTCC	CCTCACCCTAGGCTTTGATATGC	2
RM 251	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC	3
RM 527	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG	6
RM 541	TATAACCGACCTCAGTGCCC	CCTTACTCCCATGCCATGAG	6
z4794*	CACGCCACCCTTCAATGGAGACT	TGAATGTGAGAGGTTGACTGTGG	6
RM 30	GGTTAGGCATCGTCACGG	TCACCTCACCACACGACACG	6
RM 587	ACGCGAACAAATTAACAGCC	CTTTGCTACCAGTAGATCCAGC	6
RM 11	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	7
RM 44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	8
RM 230	GCCAGACCGTGGATGTTC	CACCGCAGTCACTTTTCAAG	8
RM 144	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	11
RM 206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	11
k2167*	CGTGCTGTCGCCTGAATCTG	CACGAACAAGAGTGTGTCGG	11
RM 224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	11
RM 332	GCGAAGGCGAAGGTGAAG	CATGAGTGATCTCACTCACCC	11
RM 155	GAGATGGCCCCCTCCGTGATGG	TGCCCTCAATCGGCCACACCTC	12
RM 247	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG	12
RM 512	CTGCCTTTCTTACCCCCTTC	AACCCCTCGCTGGATTCTAG	12
RM 1337	GCTGAGGAGTATCCTTTCTC	ACCATAGGAAGATCATCACA	12

*: InDel markers; **: SNP marker

disease in rice.Plants defend themselves against pathogens by the activation of defense response pathways that result in coordinated defense gene expression and the subsequent containment of the pathogen (Campbell and Ronald, 2005). One feature of a host resistance reaction is hypersensitive response (HR), which is characterized by rapid plant cell death immediately at the infection site. The HR is correlated with a transient burst of active oxygen species, activation of specific defense related genes, accumulation of antimicrobial compounds, and alterations in the cell wall (Yin et al. 2000). Genetic studies have led to isolate mutants that showed an HR-like response in the absence of pathogen. These mutants are termed lesion mimics (LM) and its phenotype may occur in response to various stimuli that include alterations in light or temperature or pathogen attack (Campbell and Ronald, 2005; Yin et al. 2000). Campbell and Ronald (2005) identified three mutant lines, ebr1, ebr2 and ebr3 (enhanced blast resistance), that showed enhanced resistance to blast disease. ebr3 mutant showed a LM phenotype due to inoculation with *M. grisea*, and alterations in environmental conditions. Ono *et al*. (2001) found that the constitutively active OsRac1, a regulator of ROI production and cell death in rice, causes HR-like responses, enhances production of a phytoalexin and alters expression of defense-related genes, and involves in elicitor signaling. So, it causes resistance against a virulent race of the rice blast and bacterial blight diseases.

Table 2. Reaction of 11 rice mutants and its parents to blast disease under natural infection and artificial inoculation conditions in M_3 and M_4 generations.

Genotype	Blast Nursery		Permanent field		Artificial inoculation (Blast races)										Resistance %
	M_3	M_4	M_3	M_4	IB-19	IB-45	IB- 54	IB-63	IC-5	IC-15	ID-13	ID-15	IG-1	IH-1	
Giza 177[a]	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
Sakha 101[b]	S	S	S	S	R	S	S	S	S	S	R	MR	S	MR	28.6
SK115	R	R	R	MR	R	R	MR	R	R	R	R	R	R	R	100
SK120	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
SK130	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
SK125	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
Sakha 104[b]	R	R	S	S	R	S	R	R	R	R	R	S	S	R	64.3
SK4203	R	R	R	MR	R	R	R	R	R	R	R	MR	R	R	100
SK4201	R	R	R	MR	R	S	S	R	S	S	MR	S	S	MR	57.1
SK4152	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
SK4151	R	R	R	S	R	S	S	R	S	R	R	R	S	S	57.1
SK4154	R	R	R	R	R	R	R	R	R	R	R	R	MR	R	100
SK4153	R	R	R	R	R	R	R	MR	R	R	R	MR	MR	MR	100
SK4202	R	R	R	R	R	R	MR	R	R	R	MR	R	R	R	100

a: resistant check; b: Parent cultivars; Score of blast disease reaction (0-9), R: Resistant (0-2); MR: Moderately resistant (3); S: Susceptible (4-6); HS: Highly susceptible (7-9).

Evaluation of resistance to bakanae disease

The data in Table (3) indicated that Sk104 cultivar and 4 mutants (SK120, SK130, SK4152 and SK4154) were highly resistant against both bakanae isolates under artificial inoculation in greenhouse, while, the mutants (SK115, SK4153, SK4202 and SK4203) were exhibited resistance only to isolate 10. In contrast, Sk101 cultivar was the most susceptible cultivar and gave the highest infection percent (80%) and elongation percent (80%) with isolate 22. Also, Gz177 cultivar and 3 mutants (SK4151, SK4201and the scented mutant SK125) were susceptible to all tested isolates although, these susceptible mutants did not show any symptoms of bakanae disease under permanent field conditions. These findings are in agreement with work done by Satyavir and Singh (1998). El-Refaee *et al.* (2011) reported that Sakha 101 and Giza 177 cultivars were highly susceptible and showed high level of infection in nursery bed. While, Hammoud and Gabr (2014) found that Sakha 101 cultivar gave the highest percent and severity of bakanae infection (76.1 and 18.7 %, respectively) followed by GZ7769 (65.7 and 15.3 %, respectively) and Sakha 104 (47.6 and 13.2 %, respectively) under natural infection. On other hand, Sakha 101 gave the highest infection under artificial inoculation conditions (89.3 %) while, Sakha 104 gave the lowest infection (25.7%). Bakanae is a seed-borne as well as soil-borne disease. When seeds of rice are infected, the infected plants turn pale yellow and exhibit chlorosis and abnormal elongation, poor grain ripening such as empty panicles, stem and foot rot (Hwang et al. 2013). *F. moniliforme* produce gibberellic acid (GA) hormone that accumulate within and around rice roots during host recognition, pre-penetration morphogenesis and pathogen growth in the plants. However, high concentrations of this hormone cause abnormal internode elongation of the stem through hypertrophy of the cells in the parts of rice. In extreme cases, infected plants topple over and die

Table 3. Reaction of 11 rice mutants and its parents to bakanae disease under artificial infection in greenhouse in M_4 generations.

Genotype	Infection %		Plant height			Elongation %	
	Isolate 10	isolate 22	isolate 10	isolate 22	Healthy plant	isolate 10	isolate 22
Giza 177	30	60	16	15	11	45.5	36.4
Sakha 101	60	80	17	18	10	70.0	80.0
SK115	0	40	11	14	11	0.0	27.3
SK120	1	2	14	11	9	55.6	22.2
SK130	1	0	11	10	9	22.2	11.1
SK125	60	80	14	18	12	16.7	50.0
Sakha 104	1	1	13	12	12	8.3	0.0
SK4203	1	30	17	25	15	13.3	66.7
SK4201	40	80	14	17	11	27.3	54.5
SK4152	0	1	11	13	10	10.0	30.0
SK4151	40	80	16	18	11	45.5	63.6
SK4154	2	2	11	12	11	0.0	9.1
SK4153	1	70	12	18	11	9.1	63.6
SK4202	1	80	11	18	11	0.0	63.6

because they are no longer sturdy enough to support their own weight (Hwang et al. 2013) Studies on varietal resistance screening revealed that basmati or scented cultivars are more susceptible to bakanae disease as compared to non-scented rice cultivars (Satyavir and Singh, 1998). Rice genotypes carrying dwarf gene such as *sd1* were not only sensitive to GA3 but also susceptible to rice bakanae disease. On the other hand, all genotypes carrying *d29, sd6* or *sdq(t)* genes showed resistance to bakanae (Ma et al.2008).

SSR markers for blast disease resistance

Twenty one SSR markers and 3 specific markers were used to analyze the status of blast resistance genes in 11 mutants and its parents. Out of 24 markers, sixteen markers were linked to resistance genes (Table 4). The results of genotypic screening of the 14 rice genotypes for the presence or absence of rice blast resistance genes linked to SSR markers are shown in Table (4) and electrophoresis pattern of each SSR marker linked to blast resistant gene are shown in Fig. (1). The data revealed that genetic frequencies of the 16 major rice blast resistance genes were ranged from 100% to 28.6%. The R genes *Pib* and *Pi33* which linked to SSR markers RM208 and RM44, respectively were distributed in all genotypes. Whereas, each of R genes *Pit, Piz, Pik, Pi27(t)* and *Pi40(t)* showed 92.9 % of gene frequency in all genotypes. *Pik-h/Pik-s* gene which linked to RM224 marker showed the lowest gene frequency in all genotypes. The data in Table (4) showed that Gz177 cultivar, highly resistant to blast disease, possess two genes *Pita-2* and *Pi12*, while Sakha 101, highly susceptible, not possess these genes as well as all resistant mutants from Sakha 101 not possess *Pita-2* except mutant Sk115 therefore, the resistance of these mutants could attributed to *Pi12* gene. Previous studies showed that each of the *Pik-h* and *Pita-2* genes confers resistance to blast races IB-45, IB-54, IG-1, and IH-1; *Piz* gene confers resistance to blast races IC-17, IG-1, and IH-1 while *Pik-s* gene providing resistance to only

Table 4. Genotypic screening of 11 rice mutants and its parents for blast resistance genes linked with SSR markers.

Marker	Resistance gene	Gz177	Sk101	SK115	SK120	SK130	SK125	Sk104	SK4203	SK4201	SK4152	SK4151	SK4154	SK4153	SK4202	Gene freq.
RM44	***Pi33***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
RM144	***Pik-h, Pik-s***	1	1	1	1	1	1	1	1	1	1	0	1	1	1	92.9
RM151	***Pi27(t)***	1	1	1	0	1	1	1	1	1	1	1	1	1	1	92.9
RM155	***Pita-2***	1	0	1	0	0	0	1	1	1	0	1	1	1	1	64.3
RM206	***Pik-h***	1	1	0	1	1	0	0	1	1	1	0	1	1	1	71.4
RM208	***Pib***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
RM247	***Pita***	0	1	1	0	1	1	1	1	1	1	1	1	1	1	85.7
RM512	***Pi12***	1	0	1	1	1	1	1	1	1	1	1	1	1	0	85.7
RM527	***Pi40(t)***	1	1	0	1	1	1	1	1	1	1	1	1	1	1	92.9
RM541	***Pi9***	1	1	1	1	1	1	1	1	0	1	0	1	1	1	85.7
RM1216	***Pi35(t)***	1	1	1	1	1	1	1	1	1	1	1	0	1	0	85.7
RM1337	***Pi20(t)***	0	1	0	0	1	0	1	1	1	1	1	1	1	1	71.4
k2167	***Pik, Pik-m***	1	1	0	1	1	1	1	1	1	1	1	1	1	1	92.9
t256	***Pit***	1	1	1	1	1	1	1	1	1	1	0	1	1	1	92.9
z4794	***Piz, Piz-t***	0	1	1	0	1	1	1	1	1	1	1	1	1	1	92.9
RM 224	***Pik-h, Pik-s***	0	0	0	0	0	0	1	0	0	0	0	1	1	1	28.6
Total of resistance genes		12	13	11	10	14	12	15	15	14	14	11	15	16	14	

The rice blast resistance gene scored as the presence (1) and absence (0) of band linked to allele of specific SSR markers.

race IB-54 (Fjellstrom et al. 2004; 2006). In the present study, the results indicated that the resistant mutants have a combination of the broad-spectrum blast resistance genes (*Pi40(t), Pi20(t), Pi35(t)*), and complete resistance genes (*Pib, Pik, Pik-h, Pita, Pit, Piz*) that confer durable resistance to all tested blast races (Table 4). In contrast, the cultivar Sk101 not possess genes *Pita-2* and *Pik-h*, linked with RM155 and RM224, respectively, that confers resistance to blast races IB-45, IB-54, IG-1, and IH-1(Fjellstrom et al. 2004; 2006), therefore it was highly susceptible to the most of the blast races under artificial infection conditions. Likewise, Sk104 cultivar not possesses gene *pik-h* only that linked with RM206 marker, so it was susceptible to blast races IB-45 and IG-1. The mutants SK4151 and SK4201 not possess genes *Pik-s, Pik-h* and *Pi9* that linked with RM144, RM224 and RM541 markers, thus theses mutants showed high susceptibility to the most of tested blast races. According to results of genotypic screening, the genes *Pik-h*, *Pita-2* and *Pi9* seemed to play a significant role in resistance to the most of tested races of blast disease.

These results are in agreement with the findings of Fjellstrom *et al.* (2004); (2006); Jeung *et al.* (2007); Li *et al.* (2008); Singh *et al.* (2015). Imam *et al.* (2014) reported the genetic frequency of the nine major rice blast resistance genes *Piz, Piz-t, Pik, Pik-p, Pik-h, Pita/Pita-2, pita, Pi9* and *Pib*, ranged from 6 to 97% in the select set of rice germplasms, also found that the genes (*Pi9, Pita-2, Piz-t*) were more effective than others in reducing the infection.

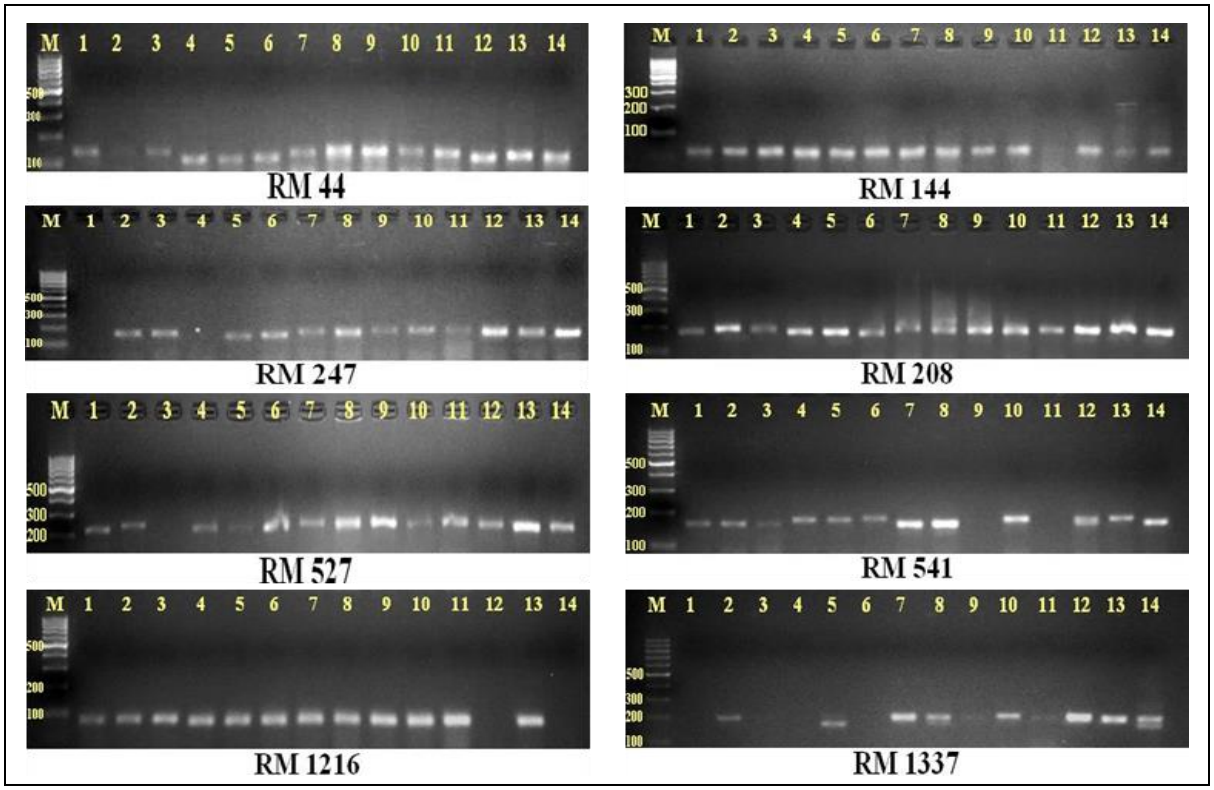

Figure 1. Microsatellite profile of 11 rice mutants obtained *via* gamma irradiation and its parents using SSR markers.

M: DNA marker (100bp); Lane 1: Giza 177 (Resistant check); 2: Sakha101 (Parent 1); 3-6: Sakha 101 mutants (SK115, SK120, SK130 and SK125); 7: Sakha 104 (Parent 2); 8-14: Sakha 104 mutants (SK4203, SK4201, SK4152, SK 4151, SK4154, SK4153 and SK4202).

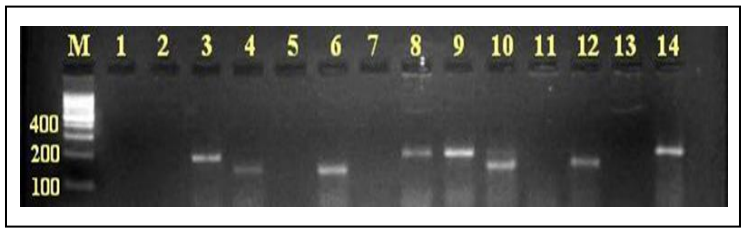

Figure 2. Microsatellite profile of 11 rice mutants obtained *via* gamma irradiation and its parents using SSR primer RM9.

M: DNA marker (100bp); Lane 1: Giza 177; 2: Sakha101 (Parent 1); 3-6: Sakha 101 mutants (SK115, SK120, SK130 and SK125); 7: Sakha 104 (Parent 2); 8-14: Sakha 104 mutants (SK4203, SK4201, SK4152, SK 4151, SK4154, SK4153 and SK4202).

The *Pi-ta* gene commonly used in rice breeding programs worldwide have originated from several traditional indica cultivars, including Tetep from Veitnam and Tadukan from the Philippines (Cho et al. 2008).

This study illustrated the utility of SSR markers to identify rice varieties likely carried the same R genes with potentially novel resistance. Rice varieties with a number of alleles in common with any specific resistance might have a similar blast R gene, and understanding the natural diversity at the specific gene is important for incorporation of specific R gene using DNA marker into rice breeding program.

SSR markers for bakanae disease resistance

In this study, SSR primer RM9 was used to analyze the status of bakanae resistance gene in 11 mutants and its parents (Fig. 2). The primer generated DNA fragments with the average size ranged from 136 to 211 bp. The results showed one positive unique band for SK115 mutant (180

bp) that resistant only to isolate 10. Whereas the mutants SK4152 and SK4154, a highly resistant to both isolates, were discriminated by one band (161 bp) as well as mutant SK120 (136 bp). Moreover, each of the resistant genotypes Sakha 104 and SK130, and susceptible genotypes SK4151 and SK4153 did not produce any bands with this marker as well as Giza 177 and Sakha 101 cultivars. Hur *et al*. (2015) found that 24 markers representing the Shingwang allele (highly resistant parent of the BC6F4 NILs) in a bakanae disease-resistant near-isogenic rice line, YR24982-9-1, were located on chromosome 1, 2, 7, 8, 10, 11, and 12. Single marker analysis using an SSR marker, RM9, showed that a major QTL is located on chromosome 1. The major QTL that designated qBK1 was mapped in 520 kb region between RM8144 and RM 11295. The identification of qBK1 and the closely linked SSR marker, RM9, could be useful for improving rice bakanae disease resistance in marker-assisted breeding.Fiyaz *et al.* (2016) identified three QTLs *qBK1.1, qBK1.2* and *qBK1.3* on the short arm of chromosome 1. The major QTL, *qBK1.2*, is a strong candidate that can be used for marker assisted introgression of bakanae resistance. While the two minor QTLs, *qBK1.1* and *qBK1.3,* can be used to increasing the resistance while developing new varieties with resistance to this disease.

CONCLUSION

Mutation breeding is a useful tool to develop new resistant genotypes to blast and bakanae disease in rice. The results exhibited that nine resistant mutants were obtained from susceptible parents (Sk101, Sk104) through mutation breeding; four mutants (SK120, SK130, SK4152 and SK4154) were highly resistant to blast and bakanae diseases at seedling and adult stages under natural infection and artificial inoculation conditions while, five mutants (SK115, SK125, SK4153, SK4202 and SK4203) were resistant to blast disease and susceptible to bakanae disease. These mutants could be used as sources of resistance genes in breeding programs. Furthermore, using DNA markers linked to the resistance gene is a powerful tool in identifying and screening these specific genes between rice genotypes.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest”

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