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# Using Gamma Rays for Genetic Improvement of Rice Resistance to Blast Disease

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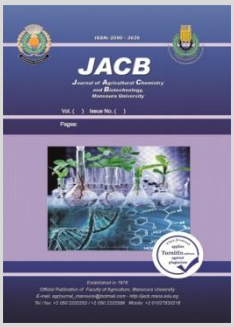


## ABSTRACT

Seeds of two blast susceptible Egyptian rice varieties; Sakha 101 and Sakha 104, were treated by four gamma rays' doses in order to establish genetic diversity and development of some desirable mutants for blast disease resistance. Sixty selected mutant lines for each variety were examined under an artificial infection for $M_3$ seedlings using two different races of *Pyricularia oryza*; ID-15 and ID-16. Results revealed that almost all Sakha 101 mutants irradiated by 400 Gy gamma rays were resistant to blast disease for both races, although their original variety was susceptible. In addition, most mutants obtained from Sakha 104 variety; which was resistant to ID-15 and susceptible to ID-16, were resistant or moderately resistant to blast disease at different gamma rays' treatments. On the bases of blast disease scoring, 16 selected mutants, as well as the two original varieties, were characterized at the molecular level using three SSR markers (RM155, RM512, and RM541) linked to rice blast resistance genes; *Pi* genes. A total of 11 polymorphic alleles (average of 3.67 alleles per primer) with sizes varied from 151 to 260 bp were amplified for the 18 studied genotypes. The appearance of the highest number of resistance alleles in the two Sakha 101 mutants SK-400-1-3 and SK1-400-1-4 irradiated by 400 Gy gamma rays; in addition, Sakha 104 mutants irradiated by 400 and 500 Gy may be supporting our findings of blast disease scoring. Thus, the three SSR markers could be useful to evaluate resistance to blast disease of rice.

***Keywords*:** Rice; Gamma rays; Mutation; Blast disease; SSR markers.

## INTRODUCTION

Diseases of rice are one of the main limiting factors of rice production around the world (Hassan *et al.*, 2017; El-Refaee *et al.*, 2020). In mild infection, rice diseases reduce about 5% of yield, while in epidemic conditions loss of yield may reach 30 to 50% (Sehly *et al.*, 2002; Hammoud and Gabr, 2014). Rice blast is the most serious fungal diseases in Egypt. It is caused by *Magnaporthe oryzae* (Elamawi and El-shafey, 2013; Hassan *et al.*, 2017). The asexual stage of this fungus is named *Pyricularia oryzae* (TeBeest *et al.*, 2012) which is the only form that is mostly present in the field (Picco *et al.*, 2001; Elamawi and El-shafey, 2013). This form can infect all aerial parts of rice plant resulting in yield losses of over 50% in susceptible cultivars under favorable conditions (Dean *et al.*, 2005; Wilson *et al.*, 2009; Singh *et al.*, 2015).

Due to the rapid change in the blast pathogenic races, the breakdown of blast resistance frequently occurs after a few years of new cultivar release (Dean *et al.*, 2005; Song *et al.*, 2014). The two varieties; Sakha 101 and Sakha 104, are Egyptian rice varieties *japonica* types characterized by high yield potential. Sakha 101 is 90 cm plant height, 140 days total growth duration and lodging resistance; in addition, it was highly resistant to rice blast. On the other hand, Sakha 104 had multiple resistance to blast and brown spot diseases as well as stem borer insects (Abd El-Azeem *et al.*, 2002 and Elmoghazy and Elshenawy, 2018). Notably, the resistance of Sakha 101 and Sakha 104 to the disease was broken-down in 2004 with the appearance of specific virulent races (El-Refae *et al.*, 2011). Accordingly, an urgent need for breeding new blast-resistant varieties.

Mutation breeding; using chemical and physical mutagens, is a powerful tool to establish genetic diversity and development of elite new varieties that are characterized by early maturity, disease resistance and better productivity (Ahloowalia *et al.*, 2004; Shu *et al.*, 2012). Among different sources of ionizing radiation, gamma rays are commonly used in mutation studies as they have shorter wavelengths, which penetrate deep into the tissue and more energy per photon (Khin, 2006; Zhu *et al.*, 2006). It is an established fact that mutagens besides causing alterations in major genes, also induce modifications at loci controlling the quantitative characters (Ramchander *et al.*, 2015).

Nowadays, traditional disease diagnoses based on pathogen morphology become not sufficient (Thierry *et al.*, 2019 and Thierry et al. 2020). Therefore, diagnosis using molecular markers is very important for disease diagnosis and subsequent management. Several *Pi* blast resistance genes demonstrated their ability in conferring resistance to many blast pathotypes. El-Refae *et al.* (2011), Hassan *et al.* (2017), El-Refaee *et al.* (2020) and yang *et al.* (2022) have reported a PCR assay based on *Pi* primer sets to analyze the presence of blast resistance genes among different rice genotypes.

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In this respect, the present study was conducted to assess the differential sensitivity of the two Egyptian rice varieties; Sakha 101 and Sakha 104, to gamma rays in order to get some of the desirable phenotypic mutants for blast disease resistance; in addition, to analysis the selected mutants at a molecular level utilizing SSR markers.

## MATERIALS AND METHODS

This study was carried out at Genetics Department Labs, Faculty of Agriculture, Kafrelsheikh University and Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt, during 2017, 2018 and 2019 summer growing seasons.

**Plant materials, irradiation treatments, and experimental design:**

In May 2017, seeds of the two local rice (*Oryza sativa* L.) varieties; Sakha 101 and Sakha 104 (obtained from rice gene bank of RRTC), were irradiated by four gamma ray doses (200, 300, 400, and 500 Gy from $Co^{60}$) at Nuclear Research Center (NRC), Egyptian Atomic Energy Authority (EAEA), Inshas, El-Sharkia, Egypt. One hundred gm dried seeds of each variety were irradiated for each treatment and the same quantity of seeds was untreated as a control. The irradiated and non-irradiated seeds were grown directly in a greenhouse to raise $M_1$ plants. After 30 days; in June 2017, the seedlings were individually transplanted to the permanent field in a randomized complete block design (RCBD) with four replicates. Each replicate; represented in a row each of 5 m long, consisted of 25 seedlings with 20×20 cm spacing. All recommended cultural practices were applied according to standard recommendations.

Seeds of the best selected three plants of each treatment were sown in the next season (May 2018) where all the yielded seeds of each plant were cultivated in a single row to arise $M_2$ generation.

Fifteen $M_2$ plants were taken randomly from each treatment (every five plants represent the progeny of the $M_1$ plant) and their seeds were harvested and kept to be grown in the next season (May 2019) with their original varieties for blast resistance evaluation experiment.

**Evaluation of rice blast resistance:**

To evaluate rice resistance to blast disease, an artificial infection was conducted using two different races; ID-15 and ID-16 (obtained from rice disease Lab), that caused by the fungus *Pyricularia oryza*. For each race, seeds of the 120 selected mutant lines in $M_2$ generations (15 mutants/ treatment/ variety) and their original varieties were sown in plastic trays (45×25×15 cm) in a single row per mutant. The trays were kept in the greenhouse of RRTC, Sakha, Kafr El-Sheikh, Egypt, at 25-30ºC and fertilized by urea 46.5% Nitrogen (5 g/tray).

Three to four weeks old seedlings (at 3-4 leaf stage) were held in a moist room (at least 90% relative humidity) at 25-28ºC for spray inoculation. Seedlings were inoculated with 100 ml of a spore suspension ($5\times10^4$ spores $ml^{-1}$ and 0.25% Gelatine) using an electrical spray gun. After 24h, inoculated seedlings were removed and grown in a greenhouse. Blast reactions were scored after 7 days from inoculation according to Standard Evaluation System, SES (IRRI, 2014). The lesions were scored from 0 to 9 scale as follows: Plants with lesion scores of 0-2 are resistant (R), 3 is moderately resistant (MR), 4-6 are susceptible (S), and 7-9 are highly susceptible (HS).

**Molecular analysis:**

Based on blast disease scoring (according to SES, IRRI 2014), a total of 16 mutant lines (two mutants/ treatment/ variety) were selected and their seeds were planted in $M_4$ generation for molecular characterization. Genomic DNA was extracted from seedling leaves of the 16 mutant lines as well as the two original varieties using CTAB method according to Murray and Thompson (1980).

Three SSR markers (introduced from SBS Genetech Co., Ltd., China) linked to rice blast resistance genes; *Pi* genes **(**Akagi *et al.*, 1996; Temnykh *et al.*, 2001; Hassan *et al.*, 2017), were screened on DNA templates. The details of the used markers and the primer sequences are presented in Table 1.

**Table 1. The used three SSR molecular markers, their primers nucleotide sequences and essential information.**

| Primer | F/R Primer 5´→3´ | CL | Linked *Pi* gene | Repeat motif | Annealing temperature | References |
|---|---|---|---|---|---|---|
| RM155 | F-GAGATGGCCCCCTCCGTGATGG<br>R-TGCCCTCAATCGGCCACACCTC | 12 | *Pita-2* | (CTT) 7 | 68 | Akagi *et al.* (1996), Hassan *et al.* (2017) |
| RM512 | F-CTGCCTTTCTTACCCCCTTC<br>R-AACCCCTCGCTGGATTCTAG | 12 | *Pi-12* | (TTTA) 5 | 60.5 | Temnykh *et al.* (2001), Hassan *et al.* (2017) |
| RM541 | F-TATAACCGACCTCAGTGCCC<br>R-CCTTACTCCCATGCCATGAG | 6 | *Pi-9* | (TC) 16 | 60.5 | Temnykh *et al.* (2001), Hassan *et al.* (2017) |

**F/R Primer: forward/reverse primer, CL: chromosomal location.**

The PCR reaction mixture was prepared by adding 1 µl genomic DNA (50 ng/µl) as a template, 6.25 µl of 2X TOPsimpleTM DyeMIX–nTaq (Enzynomics, Korea), and 1 µl of each of the forward and reverse primer (10 nmole/µl)). The final volume was then adjusted to 12.5 µl with double distilled water. The PCR amplification was carried out in a thermal cycler (TECHNE TC-412) programmed as follows: one cycle of initial denaturation at 95ºC for 5 min., followed by 35 cycles of denaturation at 95ºC for 30 Sec., annealing at 60.5-68ºC for 30 Sec., and extension at 72ºC for 30 Sec., then final extension step of 5 min at 72ºC was performed. The PCR amplified products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide, then visualized under a UV trans-illuminator, and analysed using BioDocAnalyze software (Biometra GmbH, Göttingen, Germany). The molecular size of the separated fragments was determined using a 50 bp DNA ladder (Cat-no: 300003, GeneON). Alleles number and size were recorded and polymorphic information content (PIC) value was calculated by the formula: $PIC_i = 2 fi (1 - fi)$ were $fi$ is the frequency of $i^{th}$ allele of a marker and $1 - fi$ is the frequency of null allele (Roldan-Ruiz *et al.*, 2000).

## RESULTS AND DISCUSSION

**Blast disease scoring:**

Blast disease scoring in $M_3$ generation; for Sakha 101 and Sakha 104 mutants as well as their original varieties, was illustrated in Table 2.

**Table 2. *Pyricularia oryza* reaction scores under an artificial inoculation with ID-15 and ID-16 blast races for Sakha 101 and Sakha 104 mutants as well as their original varieties.**

| Sakha 101 | | | Sakha 104 | | |
|---|---|---|---|---|---|
| Mutant line | Blast races | | Mutant line | Blast races | |
| | ID-15 | ID-16 | | ID-15 | ID-16 |
| SK1-200-1-1 | S | S | SK4-200-1-1 | R | R |
| SK1-200-1-2 | S | S | SK4-200-1-2 | R | S |
| SK1-200-1-3 | S | S | SK4-200-1-3 | R | S |
| SK1-200-1-4 | S | S | SK4-200-1-4 | R | R |
| SK1-200-1-5 | S | R | SK4-200-1-5 | R | S |
| SK1-200-2-1 | S | S | SK4-200-2-1 | R | MR |
| SK1-200-2-2 | S | S | SK4-200-2-2 | R | S |
| SK1-200-2-3 | S | S | SK4-200-2-3 | R | R |
| SK1-200-2-4 | S | S | SK4-200-2-4 | R | R |
| SK1-200-2-5 | S | S | SK4-200-2-5 | S | R |
| SK1-200-3-1 | S | S | SK4-200-3-1 | R | R |
| SK1-200-3-2 | S | S | SK4-200-3-2 | R | R |
| SK1-200-3-3 | S | S | SK4-200-3-3 | R | R |
| SK1-200-3-4 | S | S | SK4-200-3-4 | R | R |
| SK1-200-3-5 | S | S | SK4-200-3-5 | R | R |
| SK1-300-1-1 | S | S | SK4-300-1-1 | R | R |
| SK1-300-1-2 | S | S | SK4-300-1-2 | R | R |
| SK1-300-1-3 | S | S | SK4-300-1-3 | R | R |
| SK1-300-1-4 | S | S | SK4-300-1-4 | R | R |
| SK1-300-1-5 | S | S | SK4-300-1-5 | R | R |
| SK1-300-2-1 | S | S | SK4-300-2-1 | R | R |
| SK1-300-2-2 | HS | S | SK4-300-2-2 | R | S |
| SK1-300-2-3 | S | S | SK4-300-2-3 | R | R |
| SK1-300-2-4 | S | S | SK4-300-2-4 | R | S |
| SK1-300-2-5 | S | S | SK4-300-2-5 | R | R |
| SK1-300-3-1 | MR | S | SK4-300-3-1 | R | R |
| SK1-300-3-2 | S | S | SK4-300-3-2 | R | R |
| SK1-300-3-3 | S | S | SK4-300-3-3 | R | R |
| SK1-300-3-4 | S | S | SK4-300-3-4 | R | R |
| SK1-300-3-5 | S | R | SK4-300-3-5 | R | R |
| SK1-400-1-1 | R | R | SK4-400-1-1 | R | R |
| SK1-400-1-2 | R | S | SK4-400-1-2 | R | R |
| SK1-400-1-3 | R | R | SK4-400-1-3 | R | R |
| SK1-400-1-4 | R | R | SK4-400-1-4 | R | R |
| SK1-400-1-5 | MR | R | SK4-400-1-5 | R | R |
| SK1-400-2-1 | R | R | SK4-400-2-1 | R | R |
| SK1-400-2-2 | R | R | SK4-400-2-2 | MR | R |
| SK1-400-2-3 | R | R | SK4-400-2-3 | R | R |
| SK1-400-2-4 | R | R | SK4-400-2-4 | R | R |
| SK1-400-2-5 | R | R | SK4-400-2-5 | R | S |
| SK1-400-3-1 | MR | R | SK4-400-3-1 | R | R |
| SK1-400-3-2 | R | R | SK4-400-3-2 | R | R |
| SK1-400-3-3 | R | R | SK4-400-3-3 | R | S |
| SK1-400-3-4 | R | S | SK4-400-3-4 | MR | R |
| SK1-400-3-5 | R | R | SK4-400-3-5 | R | R |
| SK1-500-1-1 | S | HS | SK4-500-1-1 | R | R |
| SK1-500-1-2 | S | S | SK4-500-1-2 | R | R |
| SK1-500-1-3 | S | S | SK4-500-1-3 | R | R |
| SK1-500-1-4 | S | S | SK4-500-1-4 | R | R |
| SK1-500-1-5 | S | S | SK4-500-1-5 | MR | S |
| SK1-500-2-1 | S | MR | SK4-500-2-1 | R | R |
| SK1-500-2-2 | HS | HS | SK4-500-2-2 | R | R |
| SK1-500-2-3 | HS | HS | SK4-500-2-3 | MR | MR |
| SK1-500-2-4 | HS | HS | SK4-500-2-4 | R | S |
| SK1-500-2-5 | S | HS | SK4-500-2-5 | R | S |
| SK1-500-3-1 | S | HS | SK4-500-3-1 | R | R |
| SK1-500-3-2 | HS | HS | SK4-500-3-2 | R | R |
| SK1-500-3-3 | S | S | SK4-500-3-3 | R | R |
| SK1-500-3-4 | HS | HS | SK4-500-3-4 | R | R |
| SK1-500-3-5 | MR | S | SK4-500-3-5 | R | R |
| Original variety | S | S | Original variety | R | S |

**HS: highly susceptible, S: susceptible MR: moderately resistant and, R: resistant.**

Sixty mutant lines from different treatments of each variety were examined using two different races of *Pyricularia oryza*; ID-15 and ID-16. Data indicated that almost all Sakha 101 mutants irradiated by 400 Gy gamma rays were resistant to blast disease for both races, although their original variety was susceptible to both races. On the other hand, for Sakha 104 variety which was resistant to ID-15 and susceptible to ID-16, data showed that all mutants obtained from different gamma rays treatments were resistant or moderately resistant to blast disease, except the elven mutants SK4-200-1-2, SK4-200-1-3, SK4-200-1-5, SK4-200-2-2, SK4-300-2-2, SK4-300-2-4, SK4-400-2-5, SK4-400-3-3, SK4-500-1-5, SK4-500-2-4 and SK4-500-2-5 which were susceptible to ID-16 as their original variety. Also, the SK4-200-2-5 mutant was susceptible to ID-15 in contrast to its original variety which was found to be resistant to the mentioned race. Notably, this mutant (SK4-200-2-5) was resistant to ID-16 although it was susceptible to ID-15. This was in consistent with the findings of El-Refaee *et al.* (2011). They found that the resistance of Sakha 101 and Sakha 104 to rice blast disease was broken-down in 2004 with the appearance of new virulent races.

As mutation breeding is a very effective approach to develop modern resistant genotypes to blast disease in rice, many attempts have been made to improve blast resistance in rice using mutations (Zhang *et al*., 2003; Ahloowalia *et al.*, 2004; Hassan *et al.*, 2017; El-Refaee *et al*., 2020). The blast-resistant mutant (R917) was derived from the $F_1$ progeny treated by 100 Gy (Zhang *et al*., 2003) and the rice mutant Zhefu 802 had a high resistance to blast (Ahloowalia *et al.*, 2004). Nine resistant mutants were achieved from Sakha 101 and Sakha 104 (Hassan *et al.*, 2017). El-Refaee *et al.* (2020) found about 581 $M_2$ mutants of Sakha 104 rice variety from different irradiated populations (125 from 100 Gy, 140 from 200 Gy, 155 from 300 Gy, and 161 from 400 Gy treatments) which were resistant to blast disease.

**Molecular characterization of blast resistance:**

PCR analysis for RM155, RM512, and RM541 markers; which were documented to be linked to *Pi* genes, was performed with purified DNA samples of the 16 mutant lines; which were selected based on blast disease scoring, as well as the two original varieties. Genotypic screening of the 18 rice genotypes with the three blast resistance markers is presented in Figure 1 and Table 3.

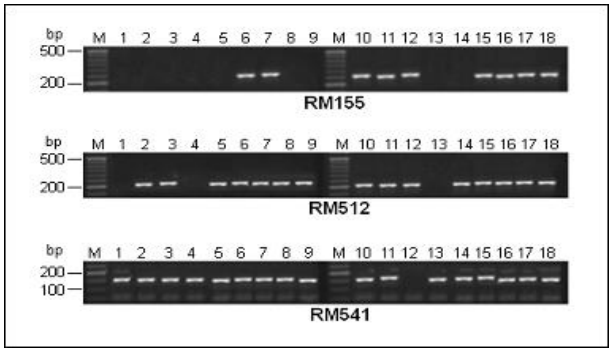


**Figure 1. Profiles of DNA amplification products generated from RM155, RM512, and RM541 markers for the 18 rice genotypes. M; 100 bp DNA ladder, lanes 1-9; Sakha 101 original variety and its mutants, and lanes 10-18: Sakha 104 original variety and its mutants.**

**Table 3. Genotypic screening of 16 rice mutants as well as their original varieties for the three SSR blast resistance markers; RM155, RM512, and RM541**.

| Primers | E.S. | P.A. | R.S. | N.A. | Size of presented alleles (bp) | | | | | | | | | | | | | | | | | | | PIC value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Sakha 101 | | | | | | | | | | Sakha 104 | | | | | | | | | |
| | | | | | Original variety | SK1-200-1-5 | SK1-200-3-4 | SK1-300-1-1 | SK1-300-3-5 | SK1-400-1-3 | SK1-400-1-4 | SK1-500-2-1 | SK1-500-3-5 | Original variety | SK4-200-1-1 | SK4-200-1-4 | SK4-300-1-3 | SK4-300-1-5 | SK4-400-1-1 | SK4-400-1-2 | SK4-500-1-1 | SK4-500-1-2 | |
| RM155 | 255 | 260 | 5 | 3 | | | | | | | + | | | | | | | | + | | | | + | + | |
| | | 257 | | | | | | | | | | | | | | | | | | | + | | | | 0.505 |
| | | 255 | | | | | | | | + | | | | + | + | | | | | | | + | | | |
| RM512 | 214 | 225 | 11 | 3 | | | | | | + | | | + | | | | | | | | + | + | + | + | |
| | | 220 | | | | | + | | + | | + | + | | | | | | + | | | | | | | 0.584 |
| | | 214 | | | | + | | | | | | | | + | + | + | | | | | | | | | |
| RM541 | 158 | 165 | 14 | 4 | | | | | | | | | | | + | | | | + | | | | | | |
| | | 160 | | | | | | | | | | | | | | | | + | | | + | | | | 0.504 |
| | | 158 | | | + | + | + | + | | + | + | + | | + | | | + | | | + | | | + | | |
| | | 151 | | | | | | | + | | | | + | | | | | | | | | | | | |
| Total | - | | - | 11 | 1 | 2 | 2 | 1 | 2 | 3 | 3 | 2 | 2 | 3 | 3 | 2 | 1 | 2 | 3 | 3 | 3 | 3 | - | | |
| Average | - | - | - | 3.67 | 2 | | | | | | | | | 2.44 | | | | | | | | | 0.531 | | |

**E.S.; Expected size, P.A.; Presented alleles, R.S.; Range of size, N.A.; Number of alleles.**

As shown in Table 3, results revealed that the three markers showed polymorphism among the 18 rice genotypes with PIC values of 0.505, 0. 584 and 0.504 for RM155, RM512, and RM541, respectively. Polymorphic information content (PIC) refers to the value of a marker depending on the number of detectable alleles and the distribution of their frequency; so, it provides an estimate of the discriminating power of the marker. Accordingly, marker RM512 that linked to resistance gene *Pi-12* was scored as the most polymorphic primer with the highest PIC (0.584). In this respect, several studies reported high PIC values for SSR primers from rice genomic sequences (Jain *et al.*, 2006; Das *et al.*, 2013).

A total of 11 DNA fragments with an average number of 3.67 alleles per primer and size varied from 151 to 260 bp were amplified among the 18 genotypes. All the three SSR primers yielded multiple PCR amplicons ranged from 3 (RM155 and RM512) to 4 (RM541) alleles including the expected product size.

For the RM155 marker, one allele with an expected size of 155 bp was observed only in four rice genotypes; Sakha 101 mutant (SK1-400-1-3) and Sakha 104 original variety as well as its two mutants (SK4-200-1-1 and SK4-400-1-2). In addition, an allele with a size of 160 bp was amplified in four genotypes; one of them was Sakha 101 mutant (SK1-400-1-4) and the other three genotypes were Sakha 104 mutants (SK4-200-1-4, SK4-500-1-1, and SK4-500-1-2). Another allele with a size of 257 bp failed to amplify in all Sakha 101 genotypes while it appeared in one mutant of Sakha 104 (SK4-400-1-1).

With respect to the RM512 marker, our results appeared a certain band with an expected size of 214 bp in Sakha 101 mutant (SK1-200-1-5) and Sakha 104 original variety as well as its two mutants (SK4-200-1-1 and SK4-200-1-4). Meanwhile, two bands were observed as different alleles with a molecular size of 220 bp (SK1-200-3-4, SK1-300-3-5, SK1-400-1-4, SK1-500-2-1, and SK4-300-1-5) and 225 bp (SK1-400-1-3, SK1-500-3-5, SK4-400-1-1, SK4-400-1-2, SK4-500-1-1, and SK4-500-1-2).

Concerning the RM541 marker, the results revealed that a band with the expected size of 158 bp was obtained in Sakha 101 original variety and all its mutants, except the two mutants SK1-300-3-5 and SK1-500-3-5. For Sakha 104 variety, the expected band was observed in four genotypes (original variety, SK4-300-1-3, SK4-400-1-2 and SK4-500-1-2). Two more bands with molecular sizes of 160 and 165 bp were also reported in Sakha 104 mutants (SK4-300-1-5 and SK4-500-1-1) and (SK4-200-1-1 and SK4-400-1-1) with allele sizes of 160 and 165 bp, respectively. Moreover, another band with a molecular size of 151 bp was obtained only in the Sakha 101 mutants (SK1-300-3-5 and SK1-500-3-5). Producing more alleles by primers than expected was reported previously by Li *et al.* (2014) and Galal and Aboulila (2018).

These findings are in constant with our results of blast disease scoring (Table 2), that almost all Sakha 101 mutants irradiated by 400 Gy were resistant to blast disease for both races, although their original variety was susceptible to both races. This result may be supported by the appearance of the highest number of resistance alleles in the two mutants SK1-400-1-3 and SK1-400-1-4, which were irradiated by 400 Gy gamma rays. As a total number of five alleles were observed in both two mutants (three alleles for each), however only one allele with molecular size of 158 bp was observed in their susceptible original variety. On the other hand; for Sakha 104 variety, which was resistant to ID-15 and susceptible to ID-16, the same result can be observed particularly for the mutants irradiated by 400 and 500 Gy. Thus, it was clear that these mutants; from each variety, carry multiple alleles with different molecular sizes which may link to one or more of rice blast resistance genes; *Pi* genes.

## CONCLUSION

Considering the results obtained in the present study, it could be concluded the potentiality of gamma rays; particularly doses of 400 and 500 Gy, in establishing genetic diversity and development of new rice genotypes resistant to blast disease. Meanwhile, our results supported the fact

that the two SSR markers; RM155 and RM512, could be useful to evaluate resistance to blast disease of rice mutants irradiated by gamma rays. This finding was in agreement with that of Liu and Wang (2016), who reported that using the host resistance gene was the most effective and economical approach to control rice blast and gene pyramiding is a promising method for providing broad-spectrum and durable resistance.

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## إستخدام أشعة جاما للتحسين الوراثي لمقاومة الأرز لمرض اللفحة

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### الملخص

تم تشعيع حبوب صنفي الأرز المصرية سخا 101 وسخا 104 القابلين للإصابة بمرض اللفحة بأربعة جرعات من أشعة جاما (200، 300، 400 و500 جراي) وذلك بهدف إستحداث تنوع وراثي وإستنباط بعض الطافرات المقاومة لمرض اللفحة. تم إختبار ستون طافر (15 لكل معاملة) من كل صنف بالعدوى الصناعية لبادرات الجيل الثالث المطفر بإستخدام سلالتين مختلفتين من فطراللفحة ID-15 وID-16. وقد أوضحت النتائج أن كل طافرات سخا 101 تقريبا والمعاملة بجرعة 400 جراي كانت مقاومة للفحة لكلا السلالتين، على الرغم من أن الصنف الأصلي كان مصاب. بالإضافة إلى أن أغلب طافرات الصنف سخا 104، والذي كان مقاوم للسلالة ID-15 ومصاب بالسلالة ID-16، كانت مقاومة أو متوسطة المقاومة لمرض اللفحة على مختلف جرعات أشعة جاما. إعتمادا على درجة الإصابة، تم إنتخاب 16 طافر (2 لكل معاملة لكل صنف) بالإضافة إلى الصنفين الأصليين لدراستهم على المستوى الجزيئي بإستخدام ثلاثة دلائل SSR (RM155, RM512, RM541) والمرتبطة بجينات مقاومة اللفحة، *Pi*. وقد تم الحصول على 11 أليل متعددة الشكل الظاهري بمتوسط 3.67 أليل لكل دليل وبأطوال تراوحت بين 150 إلى 260 زوج من القواعد، وذلك في الـ 18 تركيب وراثي المدروسة. وقد أظهر طافري سخا 101؛ SK1-400-1-3 و SK1-400-1-4 والمعاملين بجرعة 400 جراي أعلى عدد من أليلات المقاومة، بالإضافة إلى إن طافرات سخا 104 المعاملة بـ 400 و500 جراي تعضد ملاحظات درجة الإصابة بالمرض. ولذلك فالثلاثة دلائل SSR المستخدمة يمكن أن تكون مفيدة في تقييم درجة المقاومة لمرض اللفحة في طافرات الأرز المعاملة بأشعة جاما.