GENETIC IMPROVEMENT IN RICE FOR RESISTANCE TO SOME DISEASES USING GAMMA RAYS AND MOLECULAR TECHNIQUES

By

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2002
M.Sc. Agric. Sci. (Genetics), Fac. Agric., Cairo Univ., 2011

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ABSTRACT

Rice diseases are one of the major limiting factors of rice production worldwide specially, blast (*Magnaporthae grisea*) and bakanae or foot rot (*Fusarium moniliforme*) diseases. Therefore, this study was conducted to improve rice for resistance to blast and bakanae diseases as well as yield and yield related traits using gamma rays and Simple Sequence Repeats (SSR) technique. Where grains of three local rice cultivars Giza 177, Sakha 101 and Sakha 104 were irradiated with different doses of gamma rays (0, 150, 200, 250 and 300 Gy). Eleven rice mutants were selected and evaluated for resistance to blast and bakanae diseases under natural and artificial infection conditions in M_3 and M_4 generations. Molecular screening and genetic diversity of resistance (R) genes to blast and bakanae diseases were performed for the selected mutants and its parents using 22 SSR and 3 specific markers.

Results of both natural and artificial infection showed that out of the eleven selected mutants only nine mutants were resistant; four mutants were highly resistant to blast and bakanae diseases at seedling and adult stages under natural infection in permanent field and artificial infection in greenhouse while, five mutants were resistant to blast disease and susceptible to bakanae disease.

Results of SSR analysis revealed that all markers produced a total of 72 alleles with 13 unique bands. The genetic similarity between 11 rice mutants and its parents ranged from 5% between mutant SK4151 and Sakha 101 to 73% between mutant SK4201 and its parent Sakha 104.

Molecular screening of R genes to blast showed that blast resistant mutants have a combination of some 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 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 (180 bp) for SK115 mutant that resistant only to isolate 10. Whereas each of the mutants SK4152, SK4154 and SK120 were discriminated by one band (161, 161 and 136 bp, respectively) that were a highly resistant to both bakanae isolates 10 and 20.

Key words: Rice, gamma rays, mutation, blast, bakanae, resistance genes, SSR.

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INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food source for half of the world's population. After wheat, rice is the second largest source of calories in the human diet and provides approximately 20% of the total calories consumed worldwide (Babaei *et al.*, 2011). 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 fungal pathogen *Magnaporthe grisea*, is one of the most destructive diseases affecting rice production worldwide, which caused an economic loss up to 65% yield in susceptible cultivars under favorable conditions (Singh *et al.* 2015). The infections occur on the leaves, causing spindle-shaped lesions with a grey or white center to appear, or on the panicles, which turn white and die before grain filling stage (Selvaraj *et al*., 2011). 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).

Bakanae or foolish seedling disease caused by *Fusarium moniliforme* (Sheld.), *telomorph: Gibberella fujikuroi* (Sawada) is an important fungal disease in rice. It 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, stem and foot rot. In advanced stages of bakanae disease, infected plants fall down and die because they are no longer sturdy enough to support their own weight (Hwang *et al*., 2013).

Mutation breeding involves 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, has proven to be a useful tool for introducing new traits that may result in crop improvement also, it can be used as a complementary tool in plant breeding programs (Babaei *et al.*, 2010). The use of induced mutations over the past five decades has played a major role in improvement of crop over the world, and has led to the official release of 3222 mutant varieties from 170 different plant species (Raina *et al*., 2016).

Molecular markers are powerful tools in assessment of genetic variation and in 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), 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, simple sequence repeats (SSRs) are useful for several applications in plant genetics and breeding because of their reproducibility, multi-allelic nature, co-dominant inheritance, relative abundance and good genome coverage in rice (Kumar *et al.*, 2013). Therefore, this study was conducted to improve rice for resistance to blast and bakanae diseases as well as yield and yield related traits using gamma rays and molecular techniques. The objectives were to:

1. Estimate genetic parameters of yield and yield related traits in M_2 and M_3 bulks derived from irradiated three rice cultivars (Giza 177, Sakha 101 and Sakha 104) with different doses of gamma rays.
2. Evaluate the selected genotypes which have useful traits (*e.g.* high yield, early maturity) for resistance to blast and bakanae diseases under natural and artificial infection conditions.
3. Characterize the selected mutants at molecular level using SSR technique.

REVIEW OF LITERATURE

1. Mutation induction *via* gamma irradiation in rice

Mutation induction techniques can be utilized for crops improvement through increase the genetic diversity which enables plant breeders to select according to the desired breeding objectives (Abdul Haris *et al.,* 2013). Mutation breeding involves development of new varieties that characterized with disease resistance, early maturity and better productivity using chemical and physical mutagens (Shu *et al.*, 2012). Gamma rays 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). Also, it was employed to develop 64% of the radiation-induced mutant varieties, followed by X-rays (22%) (Ahloowalia *et al.*, 2004).

Baloch *et al.* (2006) irradiated rice variety IR6A (non-aromatic) with different gamma rays doses (150, 200, 250 and 300 Gy). The high yielding mutant IR6-25A developed from gamma rays (150 Gy). It has given consistently better paddy yield as compared to its parent, check and other promising mutants. On the basis of three years average, it gave 20% and 23% higher paddy yield than IR6 and check variety Shadab, respectively.

Uddin *et al*. (2007) identified four salt tolerant rice mutant lines by screening 400 M_2-families derived from the japonica cultivar 'Drew' after irradiation with dose 200 Gy of gamma rays from ^{137}Cs source. The selected mutant lines had significantly higher number of filled grains, lower grain sterility and higher grain yield as compared with