

Molecular screening for the presence of *Magnaporthe oryzae* resistance Pi-b gene in rice hybrids

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Abstract. Blast is a rice disease that affects all above-ground parts of the plant, such as nodes, stems, panicles, leaves, etc. Damage to these plant organs negatively affects the developmental stage, that is, the growing season of the plant. As a result, it is the reason for the formation of puny seeds and decreased productivity. The creation of lines and varieties resistant to blast disease is an urgent problem for all regions involved in rice growing. The reason is the rapid adaptation of the pathogen to environmental conditions, as well as to the immune defense of plants. This research includes results of crossing domestic highly productive zoned varieties with donor varieties of blast resistance genes. Three lines have been identified that carry the blast resistance gene Pi b in their genotype. To assess the productivity of the resulting lines, the elements of the crop structure were assessed and the preservation of productivity by the new lines was determined.

1 Introduction

One of the most popular and extensively grown grains in the world, particularly in Asia and Africa, is rice. On the other hand, some illnesses cause biotic stress to rice, which leads to notable reductions in production and quality [1]. Blast is a harmful disease of rice that causes enormous damage every year in many areas where rice is cultivated. According to scientific data and the economic damage caused by this disease, it ranks first among fungal pathogens. There are three main types of the disease: leaf, nodular and panicle forms. The causative agent of blast disease is the imperfect fungus *Magnaporthe oryzae* [2]. Fungal spores can overwinter in plant residues, such as mycelium in seeds, rice stubble and straw. According to FAO, the demand for rice is growing every year; as of the current month, the world's rice consumption is 522.2 million tons. For comparison, this figure in 2019 was 501.0 million tons [3]. Protecting rice from diseases that affect productivity is now a major concern.

The loss of potential rice yield from blast disease in normal years reaches up to 25%, and in epiphytotic years up to 60% or even more. The degree of harmfulness of the disease is high and manifested in a decrease in seed germination, death of seedlings, loss of individual

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plants during the growing season, formation of a smaller amount of grain in spikelets, formation of underdeveloped or weak seeds.

Development conditions. Blast can develop at temperatures from +8°C to +45°C. For the germination of its conidia, high air humidity is required - 88-100%. During long wet periods with air temperatures around +26-28°C, rapid mass spread of the disease is observed, which causes the death of rice varieties susceptible to the disease. Symptoms manifest in different ways. The weather can affect illness and the nature of the manifestation of rice blast and the resistance of the variety. In wet and warm weather conditions, susceptible varieties often show all forms of damage. In this case, in plants of resistant varieties, one organ is usually affected and weak sporulation of the pathogen appears [4].

In the leaf form on plants, symptoms appear in the form of brownish-gray spots with a light center: oval or diamond-shaped light brown spots appear on the blades and in the leaf sheaths, which gradually increase and reach 3-4 cm in length and 0.5 cm in width.

The nodal form is characterized by the appearance of black-brown spots on the nodes and stems. At first they are small, then quickly increase and cover the entire node. The affected node turns black, becomes soaked and becomes covered with a dirty gray coating.

The panicle form is observed with severe damage to the main and lateral axes of the panicle, glumes and caryopses. The panicle axes, namely the upper stem region under the main panicle axis, darken, become softened, and gray mycelium is found on the inside. The lower glumes region darkens. In such cases, the grains are formed puny with a discolored shell or not formed at all. With late development of the panicle form, the grain may have an outwardly healthy appearance, but often contains an infection [5].



Fig. 1. Types of rice blast disease [6]

Numerous strategies, such as using healthy seed material, deep plowing to destroy plant residues, balanced crop fertilization with limited nitrogen input, crop rotation and weed control in crops, along irrigation canals, ditches, and use of chemicals are used to counteract blast disease. But none of these methods can guarantee a solution susceptible to the problem and the implementation of resistance genes in genotype varieties using modern methods (Marker-assisted selection) is required to achieve a longer-lasting effect.

2 Materials and Methods

The work used domestic highly productive zoned rice varieties from the collection of the Institute of Plant Biology and Biotechnology, as well as resistance gene donor varieties provided by colleagues from the Federal State Scientific Center for Rice (Krasnodar, Russia).

2.1 Plant materials

The following combinations of rice genotypes were selected for the crossbreeding study: ♀ Fatima x ♂ 7690 (var. breviaristata Vav), ♀ Aisaule x ♂ 7663 (var. italica Alef), ♀ Fatima x ♂ 7824 (var. Breviaristata Vav), ♀ Aisaule x ♂ 7667 (var. italica Alef), ♀ Aisaule x ♂ 7668 (var. italica Alef), ♀ Aisaule x ♂ 7684 (var. italica Alef), ♀ Fatima x ♂ 7702 (var. mutica Vav), ♀ Aru x ♂ 7653 (var. italica Alef) were selected for investigation.

2.2 Conventional methods of selection

Hybrid combinations were derived from traditional breeding methods: pneumo castration and “TVELL” experience [7], these methods used for the removal of anthers using special means and pollination of domestic varieties by anthers of donor varieties. Crossing reaches the flowering phase of rice.

2.3 DNA extraction and PCR analysis

Genomic DNA was isolated using the CTAB method according to Doyle [8]. Amplification was carried out on amplifier Thermal Cycler "T100" (Bio-Rad, USA). Rice hybrid lines were tested for the presence of the broad-spectrum resistance gene Pi-b [9]. To identify the gene Pi-b, the molecular marker Pib was used, the nucleotide sequence of which is presented in Table 1.

Table 1. Sequence of nucleotides of the marker used for the Pi-b gene

Gene	Name of marker	Nucleotide sequence 5’ – 3’
Pi-b	Pib	F4 – cat caa cga agt cca gct ca
		R5 – ccg cgc tat ctt gta cat tc
		R6 – ctc agc ata tgt ggc agc tc

2.4 Crop structure analysis

To investigate the yield structure elements by assessing the various morphological traits of hybrids and parental cultivars. Components of yield structure were assessed after post-full maturation.

3 Results and Discussion

As a result of crossing, 8 rice hybrid lines were obtained: ♀ Fatima x ♂ 7690 (var. breviaristata Vav), ♀ Aisaule x ♂ 7663 (var. italica Alef), ♀ Fatima x ♂ 7824 (var. Breviaristata Vav), ♀ Aisaule x ♂ 7667 (var. italica Alef), ♀ Aisaule x ♂ 7668 (var. italica Alef), ♀ Aisaule x ♂ 7684 (var. italica Alef), ♀ Fatima x ♂ 7702 (var. italica Alef), ♀ Aru x ♂ 7653 (var. subvulgaris Brsch). These hybrid lines were assessed for the presence of blast resistance gene Pi-b. Consequently, hybrid lines containing this gene were found, suggesting that the blast resistance gene was effectively transferred into genotypes that were vulnerable. Figure 2 shows the PCR analysis findings.

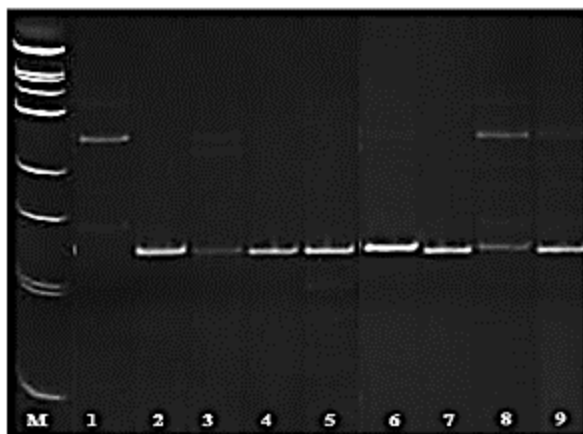


Fig. 2. Electropherogram of PCR of Pi-b gene products in rice hybrids. Note: M – molecular marker, 1 – BL1 (positive control), 2 – ♀ Fatima x ♂ 7690 (var. breviaristata Vav), 3 – ♀ Aisaule x ♂ 7663 (var. italica Alef), 4 – ♀ Fatima x ♂ 7824 (var. Breviaristata Vav), 5 – ♀ Aisaule x ♂ 7667 (var. italica Alef), 6 – ♀ Aisaule x ♂ 7668 (var. italica Alef), 7 – ♀ Aisaule x ♂ 7684 (var. italica Alef), 8 – ♀ Fatima x ♂ 7702 (var. italica Alef), 9 – ♀ Aru x ♂ 7653 (var. subvulgaris Brsch)

The electropherogram shows that samples No.6 ♀ Aisaule x ♂ 7668 (var. italica Alef), No.8 ♀ Fatima x ♂ 7702 (var. mutica Vav) and No.9 ♀ Aru x ♂ 7653 (var. italica Alef) show in their genotypes the presence of a gene Pi-b. This facilitates an evaluation of the effectiveness of the molecular screening method in identifying the mentioned gene. It makes it possible to use these lines in further studies. The resistance gene Pi-b is able to provide broad-spectrum resistance, especially against races of the fungus pathogen *M. oryzae* from Southeast Asia [10]. The first rice blast resistance gene to be cloned is called Pi-b. In 1999, Wang et al. isolated it from the Malaysian rice variety Engkatek [11].

Analysis of plant productivity elements demonstrates significant genetic diversity of hybrid lines, which forms the foundation of breeding procedures. "The number of grains from the main panicle" is among the primary elements of rice productivity. On average, this trait in the studied parent lines ranged from 51.5 ± 11.08 to 110.75 ± 19.8 pcs. For breeding purposes, the samples with more than 100 grains per panicle are the most desirable, which was observed in 6 hybrid lines. F3 Aisaule x 7667 (var. Italica Alef), F3 Aisaule x 7684 (var. Italica Alef), F3 Aru x 7653 (var. italica Alef.) had the highest indicator for this feature- 164.0 ± 24.9 pcs., 163.0 ± 14.1 pcs. and 163.5 ± 7.8 pcs., respectively. Therefore, it can be inferred that hybrid lines exhibit a notable advantage over their parental counterparts in this characteristic.

The "grain weight from 1 panicle" correlates with the productivity of the plant, and as a result, its yield. The studied lines formed a different mass of grain from the panicle: the highest value among the parent forms was 2.5 ± 0.7 g, while the mass of grain from the panicle in hybrid forms reached up to 3.5 ± 1.4 g. The values of this trait in hybrid forms also exceed those of their parents.

The parameter known as "grain weight per plant" serves as a crucial metric in the assessment of plant performance, playing an essential role in estimating its potential yield contribution. Researchers can determine the average size and weight of grains by measuring the weight of grains per plant. This information helps breeders choose varieties that will produce more grain with better quality. In the parental lines, this parameter ranges from 2.6 to 8.5 grams. Among the hybrid lines, the highest grain weight per plant is observed in F3 Aisaule x 7684 (var. italica Alef) is 11.2 ± 1.25 , while the lowest is recorded in F3 Aisaule x 7663 (var. italica Alef) is 5.2 ± 0.38 . It may be concluded that the hybrid lines demonstrate a slight excess in this trait.

An important characteristic of rice varieties is the nature of the grain. The trait "mass of 1000 seeds" is little exposed to the negative influence of weather. Value of this trait in hybrid lines varied from 21.7 to 33.0 g, which turned out to be 1.0g less than that of the parent forms, whose values ranged from 21.9 to 34.2 g.

Among the studied lines, an increase in the empty-grains was observed particularly in the combinations involving F3 Fatima x 7702 (var. mutica Vav), F3 Aisaule x 7667 (var. Italica Alef), F3 Aisaule x 7684 (var. Italica Alef), and F3 Aru x 7653 (var. italica Alef).

4 Conclusion

In conclusion, 8 hybrid lines of the F3 generation were examined for the presence of the Pi-b resistance gene, and an analysis among the parameters of the yield structure of the line data and parental forms was carried out for comparison. From the molecular analysis carried out, three ♀ Aisaule x ♂ 7668 (var. italica Alef), ♀ Fatima x ♂ 7702 (var. mutica Vav) and ♀ Aru x ♂ 7653 (var. italica Alef) hybrid lines carrying the gene sustainability. This finding underscores the effectiveness of transferring the blast resistance gene into vulnerable genotypes, paving the way for potential utilization of these lines in further studies.

Also, this study highlights the significant genetic diversity of crop productivity and its components, which underpins breeding processes to improve agricultural performance. Hybrid lines show promising advantages over parental lines, especially in terms of traits critical for improving yield, such as number of grains per plant, weight of grains per plant, weight of grains per plant.

According to the parameter "The number of grains from the main panicle", 6 hybrid lines distinguished themselves with high performance, of which the highest indicators belonged to F3 Aisaule x 7667 (var. Italica Alef), F3 Aisaule x 7684 (var. Italica Alef), F3 Aru x 7653 (var. italica Alef.) lines.

The yield of cereal is a very important trait. The hybrid line F3 Aisaule x 7684 (var. italica Alef.) distinguished itself for "grain weight per plant" trait.

Studying grain weight per rice plant can help to improve the understanding of factors affecting yield formation and optimize cultivation practices to increase productivity and tolerance to stress conditions.

In the future, it is planned to use these lines to pyramid other blast resistance genes in order to obtain highly resistant domestic varieties.

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