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Posted Date: 17 June 2024

doi: 10.20944/preprints202406.1091.v1

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Article

Development of Rice Lines by Pyramiding of Major Blast Resistance Genes

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Abstract. Rice blast is the most harmful disease caused by *Magnaporthe oryzae*, leading to yield loss. The aim of the work is to improve resistance of rice to blast by introducing and pyramiding genes using conventional breeding technique and modern MAS analysis. Pyramiding is the combination of several resistance genes in one genotype, in this case it improves the resistance of rice to pathogens. MAS analysis revealed that 3 out of 4 studied domestic high-yielding rice cultivars (Bakanasski, Aisaule and Aru) contain 1 blast resistance gene (*Pi-2*). It was discovered 7 pyramidal lines with the introduction of a combination of 2 blast resistance genes (*Pi-1* and *Pi-ta*) into 6 hybrids and 3 genes (*Pi-1*, *Pi-33* and *Pi-ta*) into 1 line. Phytopathological test of local cultivars and pyramidal lines of rice showed that 3 out of 7 pyramidal lines have moderate resistance: 2 lines (F₂ Bakanasski/7667 (*var. vulgaris* Koern.) and F₂ Aisaule/7664 (*var. italica* Alef.)), containing 2 resistance genes (*Pi-1* and *Pi-ta*) and 1 line – F₂ Aisaule/7689 (*var. zerafschanica* Brsch.), carrying 3 genes (*Pi-1*, *Pi-33* and *Pi-ta*), while the local cultivars Bakanasski, Aisaule and Aru are susceptible. As a result, the blast resistance of rice was improved by creating pyramidal lines. Pyramided lines can be used for germplasm exchange and in rice breeding programs to improve blast resistance in rice.

Keywords: rice; hybrid; *Magnaporthe oryzae*; resistance; Marker Assisted Selection (MAS); gene pyramiding; phytopathology

1. Introduction

Rice blast is one of the most dangerous diseases [1-2], the causative agent of which is the parasitic fungus *Magnaporthe oryzae*. The defeat of rice by pathogen in the early stages of development leads to a decrease in seed germination, sparseness of crops and death of seedlings. Blast causes the greatest harm during heading and flowering, which is associated with the formation of underdeveloped or feeble seeds, which significantly reduces grain quality [3]. Blast reduces rice production in the world, causing crop losses from 15-40% and reaching up to 80-100% in epiphytotic years [4].

Global climate change affects the evolution of pathogen biotypes, which challenges breeders and forces them to increase rice productivity by creating and introducing new cultivars resistant to changing races or isolates of *Magnaporthe oryzae* [5]. Currently, more than 100 blast resistance genes have been discovered [5, 6] [7-15]. Most of them with broad spectrum resistance to pathogen [5-6, 16-19], there are some major genes with a wide spectrum of resistance, such as: *Pi-z5* (*Pi-2*) [20], *Pi-9* (*t*) [21], *Pi-ta* [22], *Pi-1* and *Pi-33* [7-14, 23], which serve as indicators of blast resistance gene clusters, which is important when choosing parent pairs for hybridization. Most blast resistance genes are dominant, some of them are quantitative [24]. Moreover, many resistance genes are clustered on chromosomes 6, 9, 11, and 12 [25-26]. Disease resistance is controlled by one or two [27-28], three [29] or more pairs of genes [30]. Typically, rice has one or two dominant resistance genes that are effective against one race of the fungus [31].

Determination of resistance genes in genetically different rice material is important for identifying new sources of resistance to blast. DNA markers are used to identify resistance genes [26]. The most effective way to combat blast is cultivate resistant cultivars of rice. However, cultivar lost resistance after a few years due to the high variability of rice pathogens [32-33]. The selection of disease-resistant cultivars based on conventional breeding methods is complicated due to the difficulty of determining the presence of the desired allele of a particular gene. The use of molecular markers closely linked to genes that provide resistance to a pathogen greatly facilitates breeding work [34]. Therefore, the main factor in rice breeding for resistance to blast is the selection of resistance donors based on the identification of genes that control this trait in rice and the creation of new cultivars with a high level of resistance [35].

Success to combat with rice blast was achieved by introducing and pyramiding of major blast resistant genes using Marker Assisted Selection (MAS) [6-14]. Most resistance genes ensure that rice is immune only to certain races of the pathogen. It is known that such genes, when pyramided in one genotype, can provide stable resistance to the disease.

Pyramiding is the combination of several pathogen resistance genes in one genotype. Pyramiding multiple resistance genes in a single genotype is the most efficient strategy in breeding for resistance to a variable population of *Magnaporthe oryzae*. Cultivars that combine combinations of 3-5 resistance genes are more resistant, as they show an increase and expansion of the spectrum of resistance to blast. Thus, the most promising is the introduction of genes by combining (pyramiding) several blast resistance genes in one genotype [7-14, 35]. Screening cultivars for the presence of resistance genes to *Magnaporthe oryzae*, as well as introducing and pyramiding of resistance genes to the pathogen contributes to a significant reduction in the time for creation rice cultivars. Introducing resistance genes into cultivar is the preferred strategy in a rice breeding program to prevent disease.

The aim of the work is to improve resistance of rice to *Magnaporthe oryzae* based on the introducing and pyramiding of major blast resistance genes using MAS.

2. Materials and Methods

2.1. Plant Materials

The objects of research were 35 parental genotypes of local and foreign selection from the rice collection of Institute of Plant Biology and Biotechnology: 04636, 19-14, 57-14, 03-27, 04468, 04470, 04469, 95-06, 25-14, 04888, 212-05, 7653, 7662, 7663, 7664, 7666, 7667, 7668, 7679, 7683, 7684, 7686, 7689, 7690, 7695, 7698, 7701, 7702, 7712, 7824, Don 7712, Bakanasski, Fatima, Aisaule, Aru and obtained by crossing 54 hybrid lines of generation F₂ (F₂ Bakanasski/7668 (*var. subuzbekistanica* Kond.), F₂ Bakanasski / 04470 (*var. vulgaris* Koern.), F₂ Bakanasski/7653 (*var. subuzbekistanica* Kond.), F₂ Bakanasski/7653 (*var. subjanthoseros* Brsch.), F₂ Bakanasski/7684 (*var. subvulgaris* Brsch.), F₂ Bakanasski/7684 (*var. vulgaris* Koern.), F₂ 7824/Aisaule (*var. italica* Alef.), F₂ 7824/Aisaule (*var. subuzbekistanica* Kond.), F₂ Aru/04468 (*var. subvulgaris* Brsch.), F₂ 7698/Bakanasski (*var. subvulgaris* Brsch.), F₂ 7698/Bakanasski (*var. italica* Alef.), F₂ Aru/7701 (*var. vulgaris* Koern.), F₂ Bakanasski/0327 (*var. vulgaris* Koern.), F₂ Aisaule/7679 (*var. subvulgaris* Brsch.), F₂ 7698/Aisaule (*var. italica* Alef.), F₂ 7698/Aisaule (*var. suberythroseros* Kan.), F₂ Aru/7702 (*var. erythroceros* Koern.), F₂ Aisaule/7689 (*var. zerauschanica* Brsch.), F₂ Aru/0327 (*var. vulgaris* Koern.), F₂ Aru/0327 (*var. subpyrocarpa* Alef.), F₂ Bakanasski/7667 (*var. subjanthoseros* Brsch.), F₂ Bakanasski/7667 (*var. vulgaris* Koern.), F₂ Bakanasski/7701 (*var. vulgaris* Koern.), F₂ Aisaule/7664 (*var. italica* Alef.), F₂ Aisaule/7664 (*var. subvulgaris* Brsch.), F₂ Aisaule/04470 (*var. subvulgaris* Brsch.), F₂ Aisaule/04470 (*var. italica* Alef.), F₂ Aisaule/Don 7712 (*var. sundensis* Koern.), F₂ Aisaule/Don 7712 (*var. italica* Alef.), F₂ Fatima/7695 (*var. italica* Alef.), F₂ Fatima/7695 (*var. breviaristata* Vav.), F₂ Bakanasski/7683 (*var. subjanthoseros* Brsch.), F₂ 7698/Aru (*var. erythroceros* Koern.), F₂ 7698/Aru (*var. suberythroseros* Kan.), F₂ Bakanasski/Don 7712, F₂ Bakanasski/Don 7712 (*var. Desvauxii* Koern.), F₂ Aru/212-05 (*var. vulgaris* Koern.), F₂ Aru/7668 (*var. vulgaris* Koern.), F₂ Aisaule/04468 (*var. italica* Alef.), F₂ Aru/04636 (*var. vulgaris* Koern.), F₂ Bakanasski/7690 (*var. subjanthoseros* Brsch.), F₂ Bakanasski/7690 (*var. vulgaris* Koern.), F₂ Bakanasski/04468 (*var. subvulgaris* Brsch.), F₂ Bakanasski/04468 (*var. vulgaris* Koern.), F₂ Aru/7666 (*var. suberythroseros* Kan.), F₂ Fatima/7653 (*var. persica* Kan.), F₂ Fatima/7689 (*var. italica* Alef.), F₂ Aru/Don

7712 (*var. erythroceros* Koern.), F₂ Aru/Don 7712 (*var. Desvauxii* Koern.), F₂ Aru/Don 7712 (*var. vulgaris* Koern.), F₂ Aru/Don 7712 (*var. Desvauxii* Koern.) semi-awned, F₂ ♀ Aru/7683 (*var. janthoceros* Koern.), F₂ Aru/7683 (*var. vulgaris* Koern.), F₂ Aisaule/7663 (*var. italica* Alef.)).

2.2. Hybridization Method

Hybridization was carried out using pneumocastration (three-channel pneumocastrator) and the "TWEL" plant pollination method [36]. 31 genotypes were used as donors, the genome of which contains genes of resistance to rice blast (*Pi-1*, *Pi-2*, *Pi-33* and *Pi-ta*), and the recipients were highly productive zoned local cultivars, such as Bakanasski, Fatima, Aisaule and Aru.

2.3. Plant Genomic DNA Isolation

Genomic DNA isolation from two-week-old seedlings was performed according to the CTAB method [37] by CTAB reagent (PanReac, AppliChem, Germany).

2.4. Multiplex PCR Analysis

PCR was performed on a T100 amplifier (BioRad, Germany). Multiplex PCR analysis allows to determine the presence of two or more genes in the genotype. SSR (simple sequence repeat) markers were used to identify genes. The list of SSR markers (Syntol, Russia) for *Pi* complex genes is given in Table 1. The annealing temperature is the same for all primers, 65° C.

Table 1. List of tested SSR primers for *Pi* genes of the blast resistance complex.

Gene	Localization on chromosome	Name of a marker	Forward primer (5'-3') Reverse sequence (5'-3')
<i>Pi-1</i>	11	Rm 224	F - atcgatcgatcttcacgagg R - tgctataaaaggcattcgagg
		Rm144	F - tgccctggcgcaaatgtgatcc R - gctagaggagatcagatggtagtgcag
		Rm 527	F - ggctcgatctagaaaatccg R - ttgcacaggttgcatagag
<i>Pi-2</i>	6	SSR 140	F - aaggtgtgaacaagctagcaa R - ttctaggggaggggtgtgaa
		Rm 310	F - ccggcgataaaacaatgag R - gcatcggtcctaactaagg
		Rm 72	F - ccggcgataaaacaatgag R - gcatcggtcctaactaagg
<i>Pi-33</i>	8		F1 - gccgtggcttctatctttacatg R1 - atccaagtgttagggccaacattc
			F2 - ttgacactctcaaaggactgggat R2 - tcaagtcagggtgaagatgcacga
<i>Pi-ta</i>	12	Pita	

The following genotypes were used as differentiator cultivars that are carriers of resistance genes: C101-Lac (+) for *Pi-1*, *Pi-33* genes, C101-A51 (+) for *Pi-2* gene and IR 36 (+) for *Pi-ta* gene. The cultivar Flagman (-) was used as a negative control for all studied genes.

2.5. Gel Electrophoresis

Separation of amplification products with microsatellite primers was carried out by using 8% polyacrylamide gel in vertical electrophoresis chamber (Helicon, Russia) based on 1× TRIS borate electrode buffer. The gels were studied using a gel documentation system (BioRad, USA). DNA marker 100 bp (Syntol, Russia) was used in this work.

2.6. Phytopathological Screening

The test of 54 hybrid rice lines for resistance to populations *Magnaporthe oryzae* was carried out in 2023 in an artificial infectious nursery at the FSBSI "FSRC" in accordance with the methodological

guidelines [38]. The rice cultivar Pobeda 65 was used as a susceptible control, and the rice cultivar Avangard was used as a resistant control.

Rice plants were infected with a culture of the fungus *M. oryzae* in the booting phase by spraying a suspension of a conidial mixture.

The degree of plant damage (in percentage) was taken into account on the 14th day after inoculation, according to the express method for assessing rice varietal resistance to blast. The assessment was carried out taking into account the type of reaction (in points) on a ten-point scale of the International Rice Research Institute, using the designations: resistant lines - R, moderately resistant - MR and susceptible - S.

2.7. Analysis of Structural Elements of Yield of Hybrid Rice Lines

Evaluation of productivity of hybrid lines in comparison with standard cultivar Bakanasski was carried out according to biometric parameters of yield structure elements ("bushiness, pc", "plant height, cm", "panicle length, cm", "number of grains from the main panicle, pc", "weight of seeds from the main panicle, g", "weight of 1000 seeds, g" and etc.) [39]

2.8. Statistical Analyses

To characterize the productivity of hybrid lines in comparison with Bakanasski cultivar, which is the standard for Almaty region, we used such statistical parameters as mean value, standard deviation for main trait characterizing rice productivity "weight of 1000 seeds, g". Analysis of variance (one-way ANOVA) was performed to compare the mean values of the data groups. Data processing was carried out in Microsoft Excel 2021 using a data analysis package.

3. Results and Discussion

3.1. Screening of Parental Rice Genotypes for Blast Resistance Genes *Pi-1* and *Pi-2*

In the electropherogram of Figure 1 (A), the *Pi-1* gene is present in one 7668 sample (19 well) in a homozygous state. Several homozygous samples were found for the *Pi-2* gene: 03-27 (6 well), 04468 (7 well), 04470 (8 well) and 7662 (14 well).

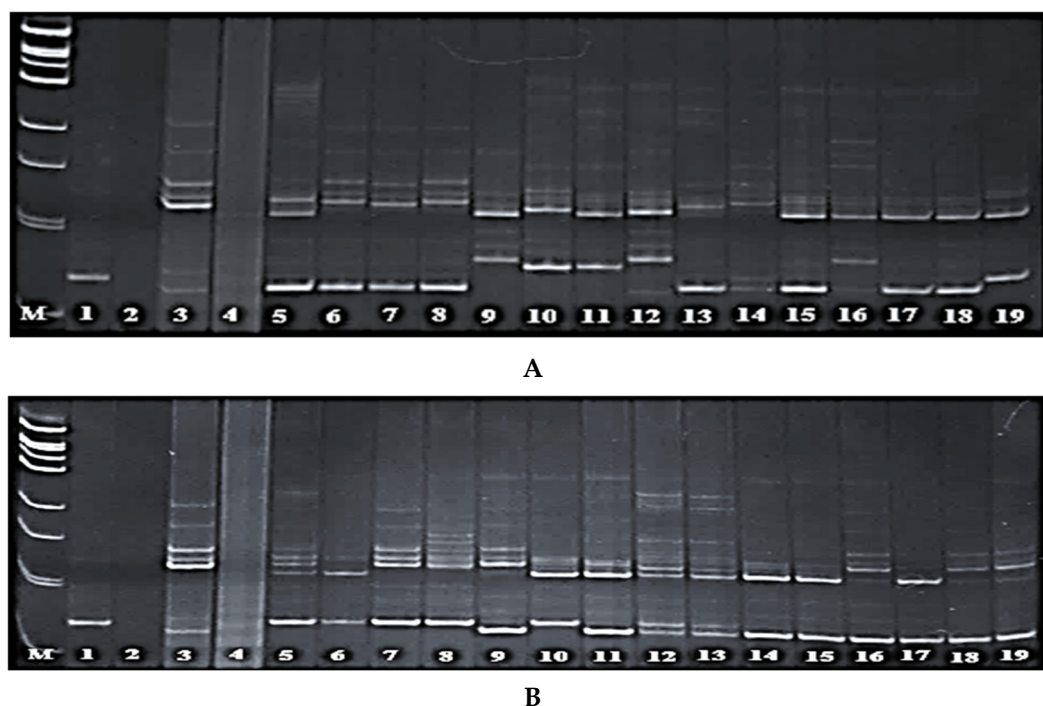


Figure 1. Electropherogram of PCR products of samples for the presence of *Pi-1* and *Pi-2* genes. (A): M – Molecular marker 100 bp, 1 – C101-Lac (+*Pi-1*), 2 – Flagman (-), 3 – C101-A51(+*Pi-2*), 4 – Flagman

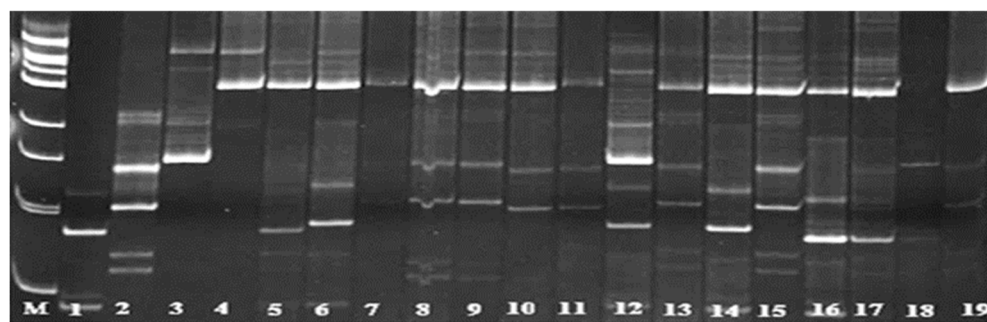
(-), 5 – 04636, 6 – 03-27, 7 – 04468, 8 – 04470, 9 – 04469, 10 – 25-14, 11 – 04888, 12 – 212-05, 13 – 7653, 14 – 7662, 15 – 7663, 16 – 7664, 17 – 7666, 18 – 7667, 19 – 7668. (B): M – Molecular marker 100bp, 1 – C101-Lac (+*Pi-1*), 2 – Flagman (-), 3 – C101-A51(+*Pi-2*), 4 – Flagman (-), 5 – 7679, 6 – 7683, 7 – 7684, 8 – 7689, 9 – 7690, 10 – 7695, 11 – 7701, 12 – 7702, 13 – 7712, 14 – 7824, 15 – Don 7712, 16 – Bakanasski, 17 – Fatima, 18 – Aisaule, 19 – Aru.

Figure 1 (B) shows that samples 7679 (5 well), 7683 (6 well), 7684 (7 well), 7689 (8 well), 7695 (10 well) turned out to be homozygous for the Rm224 and Rm144 loci, which indicates presence of a dominant rice blast resistance gene *Pi-1*, because they repeat the DNA profile of the positive control C101-Lac. Samples 7702 (12 well) and 7712 (13 well) are heterozygous for this gene.

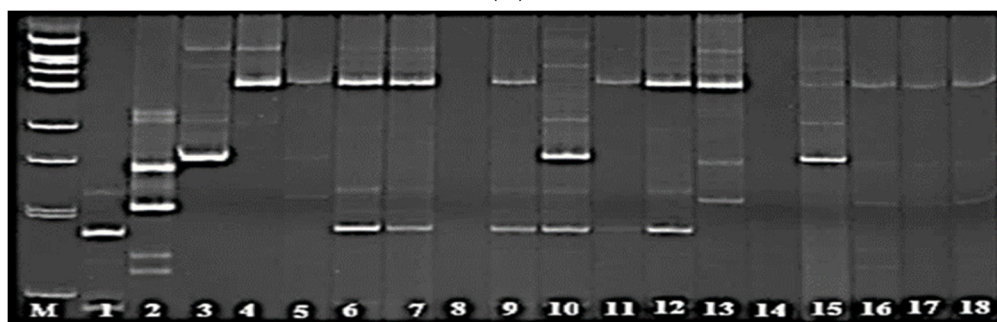
For the Rm527 and SSR140 loci, samples 7684 (7 well), 7690 (9 well), Bakanasski (16 well), Aisaule (18 well) and Aru (19 well) showed identical DNA profile similarity with the differentiator cultivar C101-A51, which indicates presence of the *Pi-2* gene. All other samples taken for PCR analysis showed the presence of recessive genes of resistance in their genotypes.

3.2. Screening of Parental Genotypes for the Presence of *Pi-33* and *Pi-ta* Genes

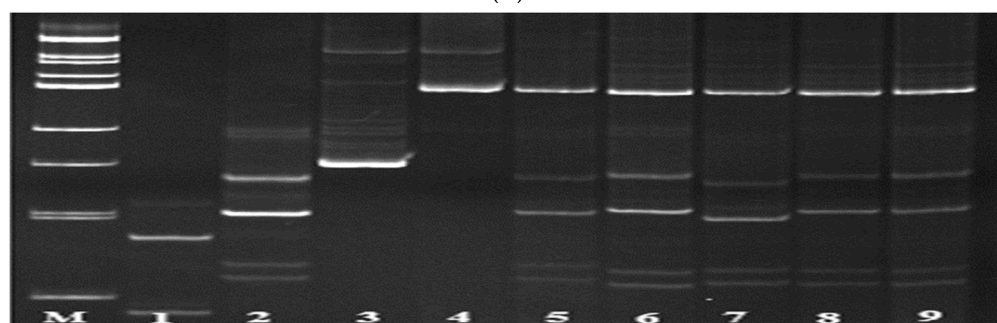
The electropherogram of Figure 2A shows that samples 7662 (16 well) and 7663 (17 well) were identified as carriers of a homozygous dominant allele of the *Pi-33* resistance gene, as these genotypes repeat the carrier profile of the C101-Lac resistance gene.



(A)



(B)



(C)

Figure 2. Electropherogram of PCR products of samples for the presence of *Pi-33* and *Pi-ta* genes. (A): M – Molecular marker 100 bp, 1 – C101-Lac (+*Pi-33*); 2 – Flagman (-); 3 – IR36 (+*Pi-ta*); 4 – Flagman (-)

); 5 – 04636; 6 – 19-14; 7 – 57-14; 8 – 03-27; 9 – 04468; 10 – 04470; 11 – 04469; 12 – 25-14; 13 – 04888; 14 – 212-05; 15 – 7653; 16 – 7662; 17 – 7663; 18 – 7664; 19 – 7666. **(B):** M – Molecular marker, 1 – C101-Lac (+*Pi-33*), 2 – Flagman (-), 3 – IR 36 (+*Pi-ta*), 4 – Flagman (-), 5 – 7667, 6 – 7668, 7 – 7679, 8 – 7683, 9 – 7684, 10 – 7686, 11 – 7689, 12 – 7690, 13 – 7695, 14 – 7698, 15 – 7701, 16 – 7702, 17 – 7712, 18 – 7824. **(C):** M – Molecular marker 100 bp, 1 – C101-Lac (+*Pi-33*), 2 – Flagman (-), 3 – IR36 (+*Pi-ta*), 4 – Flagman (-), 5 – Don 7712, 6 – Bakanasski, 7 – Fatima, 8 – Aisaule, 9 – Aru.

For the *Pi-ta* gene, a heterozygous state of the gene was found in genotypes 03-27 (8 well) and 04468 (9 well), genotypes 25-14 (12 well) and 7664 (18 well) were homozygous for this locus (Fig. 2 A), because these genotypes match the DNA of the positive control sample IR36.

According to the results (Figure 2 B), the *Pi-33* resistance gene is shown in a homozygous state in genotypes 7668 (6 well), 7679 (7 well), 7684 (9 well), 7686 (10 well), 7689 (11 well) and in 7690 (12 well) compared to C101-Lac.

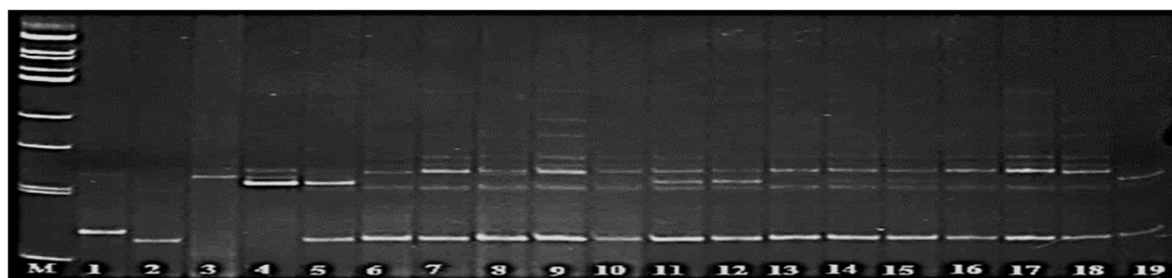
In genotypes 7686 (10 well) and 7701 (15 well), a locus was identified that co-segregates with the dominant resistance gene *Pi-ta* (Figure 2 B), and genotype 7667 (5 well) showed a heterozygous state of the *Pi-ta* gene, since repeats the bands of both IR36 and C101-Lac.

Figure 2C shows that local rice cultivars do not have *Pi-33* and *Pi-ta* resistance genes in their genotypes.

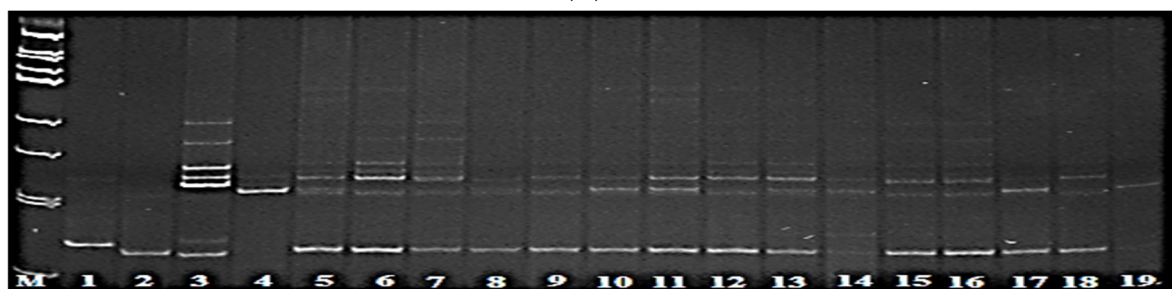
According to the data of electropherograms of 4 analyzed local released high-yielding cultivars used as maternal lines in crosses, it turned out that three cultivars Bakanasski, Aisaule and Aru contain *Pi-2* gene. It should be noted, that all four local genotypes do not contain the other three studied genes (*Pi-1*, *Pi-33* and *Pi-ta*). Consequently, we further conducted experiments to introduce and pyramid these genes into local cultivars using hybridization and MAS analysis methods to improve rice resistance to blast.

3.3. Screening of Rice Hybrids for the Presence of *Pi-1* and *Pi-2* Genes

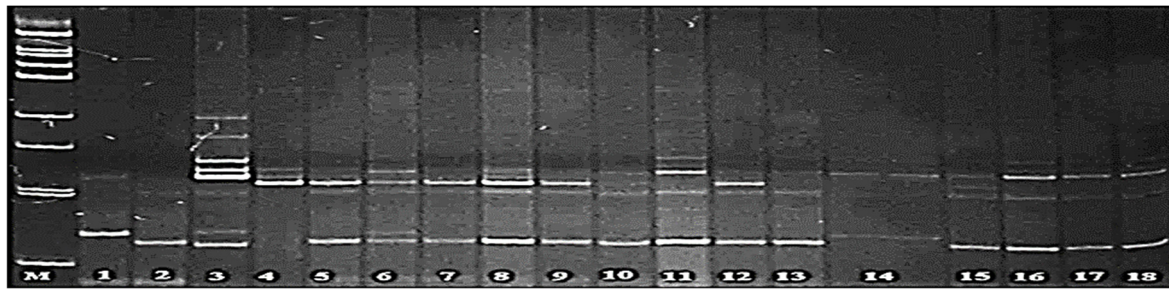
Figure 3A shows that 4 hybrids 7824/Aisaule (*var. italica* Alef.) (11 well), 7824/Aisaule (*var. subuzbekistanica* Kond.) (12 well), 7698/Bakanasski (*var. subvulgaris* Brsch.) (14 well), 7698/Bakanasski (*var. italica* Alef.) (15 well) are heterozygotes for *Pi-2*.



(A)



(B)



(C)

Figure 3. Electropherogram of PCR products of hybrids for the presence of *Pi-1* and *Pi-2* genes. **(A):** M – Molecular marker 100 bp, 1 – C101-Lac (+*Pi-1*), 2 – Flagman (-), 3 – C101-A51(+*Pi-2*), 4 – Flagman (-), 5 – Bakanasski/7668 (*var. subuzbekistanica* Kond.), 6 – Bakanasski/04470 (*var. vulgaris* Koern.), 7 – Bakanasski/7653 (*var. subuzbekistanica* Kond.), 8 – Bakanasski/7653 (*var. subjanthoseros* Brsch.), 9 – Bakanasski/7684 (*var. subvulgaris* Brsch.), 10 – Bakanasski/7684 (*var. vulgaris* Koern.), 11 – 7824/Aisaule (*var. italica* Alef.), 12 – 7824/Aisaule (*var. subuzbekistanica* Kond.), 13 – Aru/04468 (*var. subvulgaris* Brsch.), 14 – 7698/Bakanasski (*var. subvulgaris* Brsch.), 15 – 7698/Bakanasski (*var. italica* Alef.), 16 – Aru/7701 (*var. vulgaris* Koern.), 17 – Bakanasski/0327 (*var. vulgaris* Koern.), 18 – Aisaule/7679 (*var. subvulgaris* Brsch.), 19 – 7698/Aisaule (*var. italica* Alef.). **(B):** M – Molecular marker 100 bp, 1 – C101-Lac (+*Pi-1*), 2 – Flagman (-), 3 – C101-A51(+*Pi-2*), 4 – Flagman (-), 5 – 7698/Aisaule (*var. suberythroseros* Kan.), 6 – Aru/7702 (*var. erythroceros* Koern.), 7 – Aisaule/7689 (*var. zeravschanica* Brsch.), 8 – Aru/0327 (*var. vulgaris* Koern.), 9 – Aru/0327 (*var. subpyrocarpa* Alef.), 10 – Bakanasski/7667 (*var. subjanthoseros* Brsch.), 11 – Bakanasski/7667 (*var. vulgaris* Koern.), 12 – Bakanasski/7701 (*var. vulgaris* Koern.), 13 – Aisaule/7664 (*var. italica* Alef.), 14 – Aisaule/7664 (*var. subvulgaris* Brsch.), 15 – Aisaule/04470 (*var. subvulgaris* Brsch.), 16 – Aisaule/04470 (*var. italica* Alef.), 17 – Aisaule/Don 7712 (*var. sundensis* Koern.), 18 – Aisaule/Don 7712 (*var. italica* Alef.), 19 – Fatima/7695 (*var. italica* Alef.). **(C):** M – Molecular marker 100 bp, 1 – C101-Lac (+*Pi-1*), 2 – Flagman (-), 3 – C101-A51 (+*Pi-2*), 4 – Flagman (-), 5 – Fatima/7695 (*var. breviaristata* Vav.), 6 – Bakanasski/7683 (*var. subjanthoseros* Brsch.), 7 – 7698/Aru (*var. erythroceros* Koern.), 8 – 7698/Aru (*var. suberythroseros* Kan.), 9 – Bakanasski/Don 7712, 10 – Bakanasski/Don 7712 (*var. Desvauxii* Koern.), 11 – Aru/212-05 (*var. vulgaris* Koern.), 12 – Aru/7668 (*var. vulgaris* Koern.), 13 – Aisaule/04468 (*var. italica* Alef.), 14 – Aru/04636 (*var. vulgaris* Koern.), 15 – Bakanasski/7690 (*var. subjanthoseros* Brsch.), 16 – Bakanasski/7690 (*var. vulgaris* Koern.), 17 – Bakanasski/04468 (*var. subvulgaris* Brsch.), 18 – Bakanasski/04468 (*var. vulgaris* Koern.).

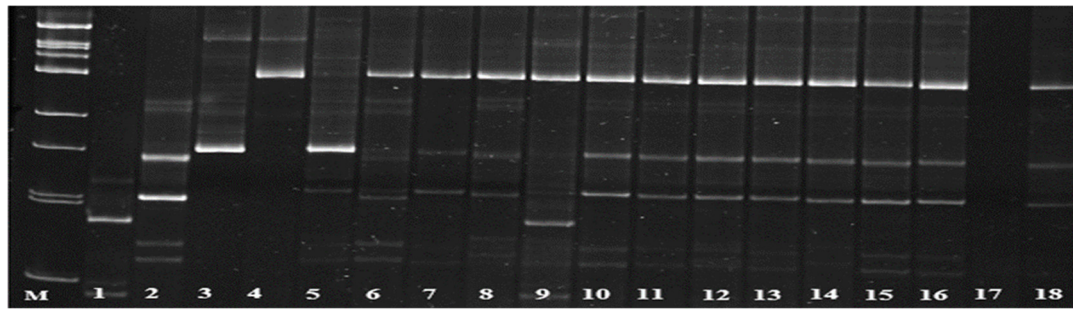
According to Figure 3B, the following 13 hybrids showed to contain *Pi-1* gene in homozygous state in their genotypes: 7698/Aisaule (*var. suberythroseros* Kan.) (5 well), Aru/7702 (*var. erythroceros* Koern.) (6 well), Aisaule/7689 (*var. zeravschanica* Brsch.) (7 well), Aru/0327 (*var. vulgaris* Koern.) (8 well), Aru/0327 (*var. subpyrocarpa* Alef.) (9 well), Bakanasski/7667 (*var. subjanthoseros* Brsch.) (10 well), Bakanasski/7667 (*var. vulgaris* Koern.) (11 well), Bakanasski/7701 (*var. vulgaris* Koern.) (12 well), Aisaule/7664 (*var. italica* Alef.) (13 well), Aisaule/04470 (*var. subvulgaris* Brsch.) (15 well), Aisaule/04470 (*var. italica* Alef.) (16 well), Aisaule/Don 7712 (*var. sundensis* Koern.) (17 well), Aisaule/Don 7712 (*var. italica* Alef.) (18 well).

The DNA profiles of the above hybrid lines are similar to those of differentiator cultivars C101-Lac and C101-A51, indicating successful transfer of blast resistance genes.

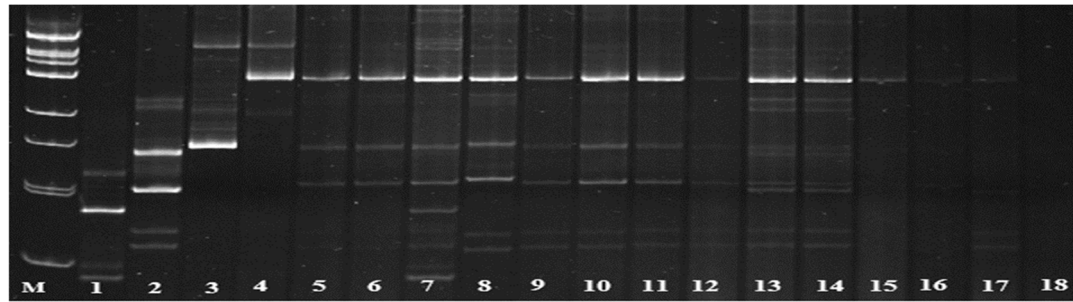
The electropherogram of Figure 3B demonstrates the heterozygous state of the hybrid Bakanasski/7683 (*var. subjanthoseros* Brsch.) for the *Pi-1*. The dominant homozygote for this gene was the hybrid line Aru/04636 (*var. vulgaris* Koern.).

3.4. Screening of Rice Hybrids for the Presence of *Pi-33* and *Pi-ta* Genes

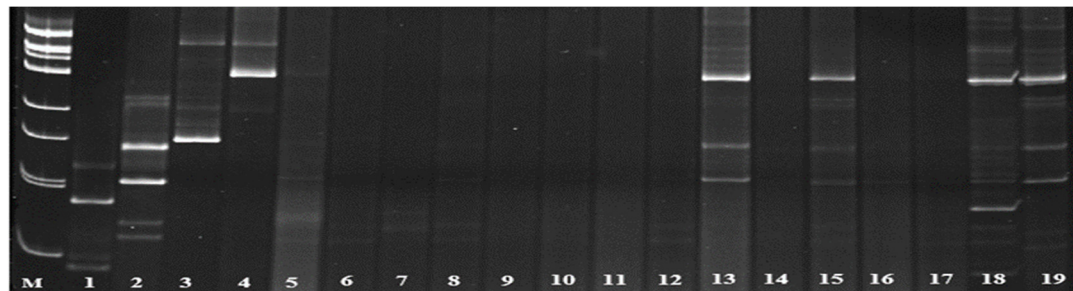
Figure 4A shows that the hybrid Bakanasski/7684 (*var. subvulgaris* Brsch.) (9 well) has the target *Pi-33* gene in the genotype in homozygous state. Line Bakanasski/7668 (*var. subuzbekistanica* Kond.) (5 well) showed the presence of a dominant *Pi-ta* resistance gene.



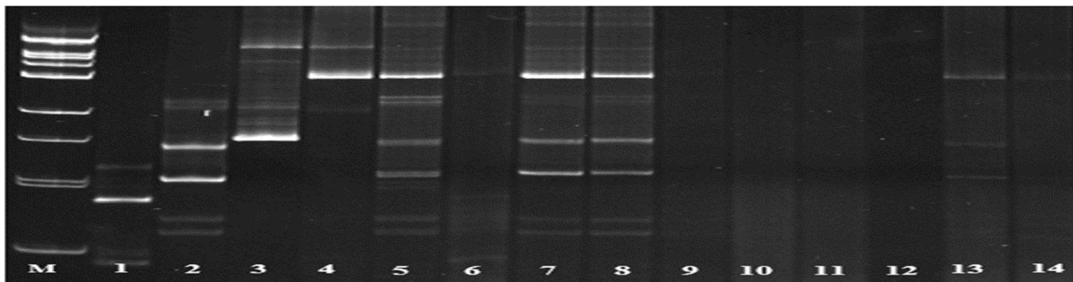
(A)



(B)



(C)



(D)

Figure 4. Electropherogram of PCR products of hybrid samples for the presence of *Pi-33* and *Pi-ta* genes. **(A):** M – Molecular marker 100 bp, 1 – C101-Lac (+*Pi-33*), 2 – Flagman (-), 3 – IR36 (+*Pi-ta*), 4 – Flagman (-), 5 – Bakanasski/7668 (*var. subuzbekistanica Kond.*), 6 – Bakanasski/04470 (*var. vulgaris Koern.*), 7 – Bakanasski/7653 (*var. subuzbekistanica Kond.*), 8 – Bakanasski/7653 (*var. subjanthoseros Brsch.*), 9 – Bakanasski/7684 (*var. subvulgaris Brsch.*), 10 – Bakanasski/7684 (*var. vulgaris Koern.*), 11 – 7824/Aisaule (*var. italica Alef.*), 12 – 7824/Aisaule (*var. subuzbekistanica Kond.*), 13 – 7698/Bakanasski (*var. subvulgaris Brsch.*), 14 – 7698/Bakanasski (*var. italica Alef.*), 15 – Aru/7701 (*var. vulgaris Koern.*), 16 – Bakanasski/0327 (*var. vulgaris Koern.*), 17 – Aisaule/7679 (*var. subvulgaris Brsch.*), 18 – 7698/Aisaule (*var. italica Alef.*). **(B):** M – Molecular marker 100 bp, 1 – C101-Lac (+*Pi-33*), 2 – Flagman (-), 3 – IR36 (+*Pi-ta*), 4 – Flagman (-), 5 – 7698/Aisaule (*var. suberythroseros Kan.*), 6 – Aru/7702 (*var. erythroseros Koern.*), 7 – Aisaule/7689 (*var. zerafschanica Brsch.*), 8 – Aru/0327 (*var. vulgaris Koern.*), 9 – Bakanasski/7667 (*var. vulgaris Koern.*), 10 – Bakanasski/7701 (*var. vulgaris Koern.*), 11 – Aisaule/7664 (*var. italica Alef.*), 12 – Aisaule/7664 (*var. subvulgaris Brsch.*), 13 – Aisaule/04470 (*var. subvulgaris Brsch.*), 14 – Aisaule/04470 (*var. italica Alef.*), 15 – Aisaule/Don 7712 (*var. sundensis Koern.*), 16 – Aisaule/Don

7712 (*var. italica Alef.*), 17 – Fatima/7695 (*var. brevistarata Vav.*), 18 – Bakanasski/7683 (*var. subjanthoseros Brsch.*). (C): M – Molecular marker 100 bp, 1 – C101-Lac (+Pi-33), 2 – Flagman (-), 3 – IR36 (+Pi-ta), 4 – Flagman (-), 5 – Aru/212-05 (*var. vulgaris Koern.*), 6 – Aru/7668 (*var. vulgaris Koern.*), 7 – Aisaule/04468 (*var. italica Alef.*), 8 – Aru/04636 (*var. vulgaris Koern.*), 9 – Bakanasski/7690 (*var. subjanthoseros Brsch.*), 10 – Bakanasski/7690 (*var. vulgaris Koern.*), 11 – Bakanasski/04468 (*var. subvulgaris Brsch.*), 12 – Bakanasski/04468 (*var. vulgaris Koern.*), 13 – Aru/7666 (*var. suberythroseros Kan.*), 14 – Fatima/7653 (*var. persica Kan.*), 15 – Aru/Don 7712 (*var. Desvauxii Koern.*) semi-awned, 16 – Aru/7683 (*var. janthoceros Koern.*), 17 – Aru/7683 (*var. vulgaris Koern.*), 18 – Aisaule/7663 (*var. italica Alef.*), 19 – Aru/0327 (*var. subpyrocarpa Alef.*). (D): M – Molecular marker 100 bp, 1 – C101-Lac (+Pi-33), 2 – Flagman (-), 3 – IR36 (+Pi-ta), 4 – Flagman (-), 5 – Bakanasski/Don 7712, 6 – Fatima/7689 (*var. italica Alef.*), 7 – Aru/04468 (*var. subvulgaris Brsch.*), 8 – Bakanasski/7667 (*var. subjanthoseros Brsch.*), 9 – Bakanasski/Don 7712 (*var. Desvauxii Koern.*), 10 – Aru/Don 7712 (*var. vulgaris Koern.*), 11 – 7698/Aru (*var. erythroceros Koern.*), 12 – 7698/Aru (*var. suberythroseros Kan.*), 13 – Aru/Don 7712 (*var. erythroceros Koern.*), 14 – Aru/Don 7712 (*var. Desvauxii Koern.*).

The electropherogram of Figure 4B shows that the hybrid line Aisaule/7689 (*var. zerafschanica Brsch.*) (7 well) is heterozygous for the *Pi-33* resistance gene. According to Figure 4B, the heterozygous state of the *Pi-ta* allele was found in 6 hybrid lines: 7698/Aisaule (*var. suberythroseros Kan.*) (5 well), Aru/7702 (*var. erythroceros Koern.*) (6 well), Aisaule/7689 (*var. zerafschanica Brsch.*) (7 well), Bakanasski/7667 (*var. vulgaris Koern.*) (9 well), Bakanasski/7701 (*var. vulgaris Koern.*) (10 well), Aisaule/7664 (*var. italica Alef.*) (11 well).

Electropherogram Figure 4C demonstrates that the line Aisaule/7663 (*var. italica Alef.*) (18 well) is heterozygous for the *Pi-33* gene. Lines Aru/7666 (*var. suberythroseros Kan.*) (13 well), Aru/Don 7712 (*var. Desvauxii Koern.*) semi-awned (15 well), Aru/0327 (*var. subpyrocarpa Alef.*) (19 well) are heterozygous for the *Pi-ta* blast resistance gene.

Electropherogram Figure 4D shows that the lines Bakanasski/Don 7712 (5 well), Aru/04468 (*var. subvulgaris Brsch.*) (7 well), Bakanasski/7667 (*var. subjanthoseros Brsch.*) (8 well) and Aru/Don 7712 (*var. erythroceros Koern.*) (13 well) have a heterozygous state of the *Pi-ta* allele in their genotypes. Consequently, on electrophoresis of PCR products of the *Pi-33* and *Pi-ta* genes, the presence of both homozygous and heterozygous lines is observed in the hybrid offspring. All other analyzed samples showed compliance of the DNA profile with the negative control cultivar Flagman, that is, they showed the presence of a recessive allele of the *Pi-33* and *Pi-ta* genes, respectively.

According to electropherograms of PCR products, 29 hybrid lines were found, the genotypes of which contain genes for resistance to blast disease. Of these, 7 hybrids are pyramidal: 6 hybrids 7698/Aisaule (*var. suberythroseros Kan.*), Aru/7702 (*var. erythroceros Koern.*), Bakanasski/7667 (*var. subjanthoseros Brsch.*), Bakanasski/7667 (*var. vulgaris Koern.*), Bakanasski/7701 (*var. vulgaris Koern.*), Aisaule/7664 (*var. italica Alef.*) have 2 resistance genes to blast *Pi-1* and *Pi-ta*. In the hybrid Aisaule/7689 (*var. zerafschanica Brsch.*) 3 genes are pyramided - *Pi-1*, *Pi-33* and *Pi-ta*. The remaining hybrids have one of the studied genes.

3.5. Phytopathological Test of Cultivars and Pyramided Lines for Resistance to Blast Disease

Phytopathological test of local cultivars used as maternal lines showed that the cultivars Bakanasski, Aisaule and Aru are susceptible, and the cultivar Fatima is moderately resistant. Test of the resistance of hybrid lines allowed us to identify hybrids with high, medium and low resistance to the phytopathogen. Among the selected 7 pyramided lines, 1 line did not grow under the conditions of an infectious nursery. In results, of the remaining 6 pyramided lines, 3 lines – Bakanasski/7667 (*var. vulgaris Koern.*) and Aisaule/7664 (*var. italica Alef.*) (carrying 2 resistance genes *Pi-1* and *Pi-ta*) and 1 line – Aisaule/7689 (*var. zerafschanica Brsch.*) (carrying 3 genes – *Pi-1*, *Pi-33* and *Pi-ta*) showed moderate resistance (MR) were developed.

3.6. Assessment of the Productivity of Pyramided Rice Lines

The evaluation of the productivity created 6 lines of rice from 7 selected lines (1 line not grown under the infectious diseases nursery) (Aru/7702 (*var. erythroceros* Koern.), Aisaule/7689 (*var. zerafschanica* Brsch.), Bakanasski/7667 (*var. vulgaris* Koern.), Bakanasski/7667 (*var. subjanthoseros* Brsch.), Bakanasski/7701 (*var. vulgaris* Koern.), Aisaule/7664 (*var. italica* Alef.)) performed by analysis of yield structure elements. The lines were evaluated based on mane productivity trait, such as “1000 grain weight, g.” Analysis of variance showed that pyramidal lines were inferior to the standard in yield characterization, since there was significant difference between the groups, p-value < 0.001 (Table 2).

Table 2. Analyses of the productivity of pyramided rice lines by One-way ANOVA.

	SS	df	MS	F	P-value	F crit
Between	367,1166333	6	61,18610556*	27,07338195	0,0000000000 00421.97	3,254124603
Within	97,18041667	43	2,26000969			

df: Degrees of Freedom. MS = Mean Square. Significance at *p-value < 0.001.

According to the characteristic of yield productivity pyramidal lines are lower in comparison with the control cultivar Bakanasski, which is highly productive for Almaty region.

4. Discussion

MAS analysis facilitates the identification of genes, their introduction and pyramiding into new lines [40, 41], that significantly speeds up rice breeding. The combination of several genes in one genotype showed the greatest effect, indicating the effectiveness of the strategy of durable rice resistance to blast [7-14, 35]. Rice lines carrying both individual resistance genes [40, 42] and lines with pyramided genes (containing combinations genes) with a broad spectrum of resistance to *Magnaporthe oryzae* were created [41, 43]. Thus, as a result of molecular screening of parental samples for the presence of genes, it was shown that the resistance of local zoned rice cultivars Bakanasski, Fatima, Aisaule and Aru, out of the four genes studied, only one gene *Pi-2* is present and only in the cultivars Bakanasski, Aisaule and Aru. The introgression of blast resistance genes from differentiating cultivars into local zoned cultivars was successful. Three pyramidal lines were identified: 2 lines F₂ Bakanasski /7667 (*var. vulgaris* Koern.) and F₂ Aisaule /7664 (*var. italica* Alef.), containing 2 resistance genes (*Pi-1* and *Pi-ta*) and 1 line F₂ Aisaule /7689 (*var. zerafschanica* Brsch.), carrying 3 gene (*Pi-1*, *Pi-33* and *Pi-ta*). The results of the phytopathological test showed that these three lines with pyramided 2 and 3 resistance genes showed moderate resistance to a mixture of fungal conidia of different races of *M. oryzae*, while 3 local cultivars (Bakanasski, Aisaule and Aru), containing 1 resistance gene (*Pi-2*) – susceptible to phytopathogen. Thus, the disease resistance of rice was improved by creating pyramidal lines.

5. Conclusions

The main idea of this work is to develop pyramided rice lines resistant to *Magnaporthe oryzae* for the breeding of local rice cultivars s adapted to the soil and climatic conditions of rice-growing regions. The use of modern MAS technologies guarantees the acceleration of rice breeding, and hence the introduction to the market of competitive rice cultivars with durable immunity to disease. The created new pyramided lines of rice that are resistant to phytopathogens will be used in rice breeding to create cultivars, which increases the yield and competitiveness of rice in the market and promotes the export of rice products. These pyramidal lines will be used as a source of resistance genes in the breeding process and for germplasm exchange.

Author Contributions: Supervision, project administration and funding acquisition, U.B.; Conceptualization and writing-original draft preparation, M.D., A.A. and D.E.; Investigation and methodology, M.D. and B.Kh.; Software, Z.Z. and M.D.; Writing review and editing, A.A. and Zh.Zh. All authors have read and agreed to the published version of the manuscript.

Funding: This research has funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan Grant AP14869300.

Data Availability Statement: All data used in this manuscript are presented in the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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