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Original Article

Selection of rice breeding lines for resistance to biotic and abiotic stresses

Seleção de linhas de melhoramento de arroz para resistência a estresses bióticos e abióticos

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Abstract

Rice (*Oryza sativa* L.) grown in many countries around the world with different climatic conditions and a huge number of environmental stresses, both biotic (fungi, bacteria, viruses, insects) and abiotic (cold, drought, salinity) limit rice productivity. In this regard, breeders and scientists are trying to create rice lines that are resistant to multiple stresses. The aim of this work was to screen and select cold and blast resistant rice breeding lines (RBLs) using molecular markers. Molecular screening of RBLs and parental varieties to cold tolerance was carried out using markers RM24545, RM1377, RM231 and RM569 associated with QTLs (*qPSST-3, qPSST-7, qPSST-9*). It was discovered that the presence of three QTLs characterizes the cold resistance of studied genotypes, and the absence of one of them leads to cold sensitivity. As a result, 21 cold-resistant out of the 28 studied RBLs were identified. These cold resistant 21 RBLs were further tested to blast resistance using markers Pi-ta, Pita3, Z56592, 195R-1, NMSMPi9-1, TRS26, Pikh MAS, MSM6, 9871.T7E2b, RM224 and RM1233. It was revealed that 16 RBLs from 21 studied lines contain 5-6 blast resistance genes. In accordance with the blast resistance strategy, the presence of 5 or more genes ensures the formation of stable resistance to *Magnaporthe oryzae*. Thus, 16 lines resistant to multiple stresses, such as cold and blast disease were developed. It should be noted that 6 of these selected lines are high-yielding, which is very important in rice breeding program. These RBLs can be used in breeding process as starting lines, germplasm exchange as a source of resistant genes for the development of new rice varieties resistant to multiple stress factors.

Keywords: *Oryza sativa* L., rice breeding lines, cold resistance, blast resistance.

Resumo

O arroz (*Oryza sativa* L.) é cultivado em muitos países do mundo, com diferentes condições climáticas. Um grande número de estresses ambientais, tanto bióticos (fungos, bactérias, vírus, insetos) como abióticos (frio, seca, salinidade), limita a produtividade do arroz. Neste sentido, os criadores e os cientistas tentam criar linhas de arroz resistentes a múltiplos estresses. O objetivo deste trabalho foi selecionar linhas de melhoramento de arroz resistentes ao frio e ao rebentamento (RBLs) utilizando marcadores moleculares. O rastreio molecular de RBLs e variedades parentais para tolerância ao frio foi efetuado utilizando marcadores RM24545, RM1377, RM231 e RM569 associados a QTLs (qPSST-3, qPSST-7, qPSST-9). Descobriu-se que a presença de três QTLs caracteriza a resistência ao frio dos genótipos estudados e que a ausência de um deles leva à sensibilidade ao frio. Como resultado, foram identificadas 21 RBLs resistentes ao frio das 28 RBLs estudadas. Estas 21 RBLs resistentes ao frio foram ainda testadas quanto à resistência ao rebentamento utilizando os marcadores Pi-ta, Pita3, Z56592, 195R-1, NMSMPi9-1, TRS26, Pikh MAS, MSM6, 9871.T7E2b, RM224 e RM1233. Foi revelado que 16 RBLs de 21 linhas estudadas contêm 5-6 genes de resistência ao rebentamento. De acordo com a estratégia de resistência à explosão, a presença de cinco ou mais genes assegura a formação de uma resistência estável a *Magnaporthe oryzae*. Assim, foram desenvolvidas 16 linhas resistentes a múltiplos estresses, como o frio e o míldio. É de notar que seis destas linhas selecionadas são de alto rendimento, o que é muito importante no programa de melhoramento do arroz. Estas RBLs podem ser utilizadas no processo de melhoramento como linhas de partida, intercâmbio de germoplasma como fonte de genes resistentes para o desenvolvimento de novas variedades de arroz resistentes a múltiplos fatores de stress.

Palavras-chave: *Oryza sativa* L., linhas de melhoramento de arroz, resistência ao frio, resistência à explosão.

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1. Introduction

Rice is the staple crop that is critical to the food supply of more than half of the world's population (Zhang et al., 2017). The development of new rice varieties with high yield, good quality and resistance to multiple stresses is an urgent to fulfill the demand of increasing population and a simultaneous reduction in arable land and global climate change.

If the consumption of rice worldwide in 2010 was 676 × 106 tons, then by 2035, according to scientists' forecasts, it will reach 852 × 10^6 tons. Therefore, in order to produce an additional 176 × 10^6 tons of rice, it is necessary to increase the yield (Khush, 2013) by minimizing crop losses caused by various stress factors. In regards, this requires constant renewal and expansion of the genetic base of rice breeding.

Due to global climate change the improvement of rice to multiple stresses is the best decision in increasing of the efficiency of selection processes. Creation of rice varieties resistant to cold and blast is the urgent task of the rice breeding program in Kazakhstan. Kazakhstan is the most northern rice growing region in the world. In abnormal years, during a long cold spring, up to 20-30% of rice seedlings die, which in turn negatively affects the yield. In Kazakhstan, rice cultivated in two regions: Kyzylorda (70–80 thousand ha) and Almaty region (15–20 thousand ha). Rice blast caused by *Magnaporthe oryzae* was first identified in the Kyzylorda region in the early 1930s, and the disease was not mentioned for 50-60 years. But periodic local outbreaks were noted in different years: 1998, 2006 (12 thousand ha), 2009 (4.1 thousand ha), and in 2011 (1.6 thousand ha). The selection of rice lines that are resistant to cold and blast is relevant for Kazakhstan.

Molecular marker-based technologies have facilitated the selection of resistant varieties and lines to various stresses. Achievements of marker-assisted selection (MAS) in combination with traditional breeding methods possible to select genotypes with the desired traits, which accelerates the production of significant initial lines for future breeding process. So, Suh J.P. et al. identified and successfully tested SSR markers RM569-RM231, RM1377, and RM24545 (Suh et al., 2010) for selection of cold-tolerant genotypes (Suh et al., 2013).

Among of the biotic stresses, the most destructive rice diseases that reduces yield and seed quality is blast (Devi et al., 2015; Sperotto, 2014), caused by the parasitic fungus *Magnaporthe oryzae*. Global climate change affects the evolution of pathogen biotypes, which poses challenges breeders and forces them to increase rice yields by creating and introducing new varieties resistant to changing races or isolates of the fungus *Magnaporthe oryzae*. This harmful fungal disease has a devastating impact on rice production worldwide, with crop losses of 15-40%. In the years of epiphytosis, rice seedlings die, and yield losses reach up to 80-100% (Zhang et al., 2017). Blast affects plants mainly during the growing season, the fungus *Magnaporthe oryzae* spreads by conidia, overwinters in the form of mycelium on post-harvest residues (1-3 years) and in seeds. Leaf, stem, and paniculate forms of blasts were found in rice plants (Zhang et al., 2017). Therefore, breeders and biotechnologists are trying to find effective measures to combat this disease to ensure global food security (Khush, 2013). The selection of disease-resistant rice based on classical breeding methods is time-consuming due to the difficulty of determining the presence of the desired allele of a particular gene.

The most effective way to protect rice against pathogens is to grow blast-resistant varieties created using modern molecular genetic methods. At present, more than 100 blast resistance *R* genes are known, most of them are cloned genes *Pb1, Pia, Pib, Pid2, Pid3, Pik, Pik-h/Pi54, Pik-m, Pik-p, Pish, Pit, Pita, Piz -t, Pi1, Pi2/Piz-5, Pi5, Pi9, Pi21, Pi25, Pi36, Pi37, Pi35, Pi64, Pi56, Pi63* and *PiCO39* (Akter et al., 2022; Cruz and Milach, 2004; Datta and Datta, 2006; Suh et al., 2010; Zhao et al., 2017) conferring resistance to a wide range of pathogens (Akter et al., 2022; Cruz et al., 2013; Datta and Datta, 2006; Ye et al., 2010; Zhao et al., 2017). The most promising is the introduction of genes by combining several blast resistance genes in one genotype (Akter et al., 2022; Cruz and Milach, 2004; Datta and Datta, 2006; Suh et al., 2010; Zhao et al., 2017). However, after a few years, the stability of the variety is lost due to the high variability of rice pathogens. Varieties that combine combinations of 5 or more resistance genes are highly resistant, as they show an increase and expansion of the spectrum of resistance to blast (Kostylev et al., 2017; Suh et al., 2013). Thus, the use of molecular markers allows screening of genotypes for the presence of resistance genes to *Magnaporthe oryzae*. Increasing the resistance of rice varieties allows minimizing crop losses, effectively and economically controlling diseases.

In this regard, the aim of this study is to select promising new lines of rice resistant to cold- and blast using MAS for further rice breeding program.

2. Materials and Methods

This study was carried out at the Institute of Plant Biology and Biotechnology during the periods: 2017 (cold selection) and 2023 (MAS analysis for blast). Rice breeding lines were grown in field conditions in the Balkhash district of the Almaty region (South-Eastern Kazakhstan).

2.1. Plant materials

As research objects were used 13 parent varieties (Avangard, Liman, Jinbabyeo, Lazurnyi, Marzhan, Odaebyeo, Kuban 3, Mustakillik, Altynai, Bakanasski, UzROS 7-13, Madina, Ko 293 IRRI mutant) and 28 rice breeding lines (RBLs) from F_1 hybrids (KazNIIR-5×Opytnyi, Opytnyi×KazNIIR-5, KazNIIR-5×Altynai, UzROS 7-13×Marzhan, Altynai×Opytnyi, Avangard×KazNIIR-5, Kuban 3×Opytnyi, Altynai×Kuban 3, Avangard×Opytnyi, KazNIIR-5×Liman, Liman×Jinbabyeo, Lazurnyi×KazNIIR-5, Mustakillik×Avangard, Jinbabyeo×Avangard, Opytnyi×Kuban 3, Marzhan×Liman, Marzhan×Kuban 3, Opytnyi×Marzhan, Opytnyi×Aru, Kuban 3×Liman, Kuban 3×Altynai, Kuban 3×KazNIIR-5, Opytnyi×Madina, Odaebyeo×Madina, KazNIIR-5×Kuban 3, FL 478×Todorokiwase, FL 478×Reizig, Krepysh×Ko 293 (IRRI mutant) from the collection of Institute of Plant Biology and Biotechnology (IPBB).

2.2. DNA extraction and PCR amplification

Rice leaves from 7-day-old etiolated seedlings were frozen in liquid nitrogen. Genomic DNA was extracted from frozen leaves using cetyltrimethylammonium bromide (CTAB) method (Williams and Ronald, 1994). Before use, CTAB was heated in a water bath at a temperature of 65°C. The quality and concentration of the isolated DNA was studied on a Genesys 20 spectrophotometer (Termo Spectronic, USA) and separated in a 1,0% agarose gel (Sigma-Aldrich, Agarose A4718-100G) with additional staining with ethidium bromide. PCR analysis was performed on a Thermal Cycler "T100" amplifier (Bio-Rad, USA). For PCR was used a 2 × Green Master Mix (Thermo scientific, USA). The PCR mode was selected individually for each pair of primers. PCR products were separated on a 2,0% agarose gel. A molecular marker 100 bp DNA marker (Thermo scientific, USA) was used to determine the size of PCR products. All electrophoretic gels were analyzed using the GelDoc XR gel documentation system (Bio-Rad, USA).

2.3. Molecular screening of rice breeding lines to cold resistance

Identical profiles in all studied genotypes were identified by the presence of the desired amplicons of 186 bp in size. and 175 bp and 152 bp corresponding to markers RM231, RM569 and RM24545, closely related to QTL (*qPSST-3*) and (*qPSST-9*) located on chromosomes 3 and 9, respectively. It should be noted that these QTL are present both in the standard varieties Kuban 3 and UzROS 7-13, characterized by cold resistance, and in the cold-sensitive variety Liman (negative control). Polymorphism in the spectrum of PCR products of the studied genotypes was detected using the SSR marker RM1377, closely linked with cold resistance QTL *qPSST-7*, located on the short arm of chromosome 7.

2.4. Molecular markers associated with cold resistance

PCR analysis of RBLs and parental genotypes was carried out using microsatellite markers closely linked with cold resistance QTLs: RM231 and RM569 *(qPSST-3)*, RM1377 *(qPSST-7)* and RM24545 *(qPSST-9)* (Suh et al., 2010). These markers were produced by Applied Biosystems (USA), the nucleotide sequence of which is shown below in Table 1.

In this experiment, cold-resistant standard varieties Kuban 3 and UzROS 7-13 were used as a positive control.

2.5. Molecular markers associated with blast resistance

In the study for the presence of blast resistance gene in the genotypes, 7 parental varieties and 21 RBLs pre-selected for cold were used as research objects. Among the 8 cold-resistant varieties, one genotype, Bakanasski, was removed from this experiment because there were no lines with its participation in the selected cold-resistant lines.

The molecular markers Pi-ta, Pita3, RM224, RM1233, Z56592, MSM6, 9871.T7E2b, 195R-1, NMSMPi9-1, TRS26 and Pikh MAS, closely linked with blast resistance genes (*Pi-ta*, *Pita*, *Pi-1*, *Piz-t*, *Pi-40*, *Pi-9* and *Pi-54*), were used for PCR. For multiplex PCR used two primer sets in a single reaction. The primers were synthesized by the Institute of Food Crops, Yunnan, China. The list of SSR markers and corresponding primers for the blast resistance genes of the *Pi* complex was shown in the Table 2.

As a positive control, differentiator lines and varieties carrying rice blast R genes were selected – IR BLTA-CP1 (*Pi-ta* gene), IRBLta-Zh [LT] (*Pita* gene), IRBL1-La (*Pi-1* gene), 75-1-127 (*Pi-40* gene), IR BL 9-W (*Pi-9* gene), Maratellia (*Piz-t* gene), Tetep (*Pi-54* gene) and variety Nippon bare (no R gene) was used as negative control.

The presence (1) and absence (0) of *Pi*-complex genes in studied varieties and RBLs were evaluated by visualizing amplicons of the corresponding sizes on electropherograms.

2.6. Screening rice breeding lines to a low positive temperature

Laboratory screening of RBLs to a low positive temperature (+10°C) was carried out according to the method described in the work of Cruz et al. (2010). Due to the lack of seed material, 10 RBLs were used in this experiment. 10 RBLs were used as objects of study in this experiment (Opytnyi×Kuban 3, Opytnyi×Marzhan, Kuban 3×Liman, Kuban 3×Bakanasski, Marzhan×Liman, Jinbabyeo×Avangard, UzROS 7-13×Marzhan, Krepysh×Ko 293 (IRRI mutant), FL478×Reizig, FL478×Todorokiwase) obtained by hybridization of cold-tolerant foreign and domestic rice varieties with high yield. To do this, the seeds of the RBLs were germinated in Petri dishes for

Table 1. Primer sequences and annealing temperatures of SSR markers linked with cold resistance QTLs.

Molecular markers	Nucleotide sequence of primers	Size of PCR product (bp)	Annealing T (ºC)	Localization in the chromosome
RM 24545	F-ACAGCACAGCACCCGGAAGG	152 bp	55	9
	R-GAGCAACAGGAAGGCGATAAGC			
RM 1377	F-ATTAGATACATCAGCGGGGG	145 bp	55	7
	R-GCTGCTGTACGATGTGATCC			
RM 231	F-CCAGATTATTTCCTGAGGTC	186 bp	55	3
	R-CACTTGCATAGTTCTGCATTG			
RM 569	F-GACATTCTCGCTTGCTCCTC	175 bp	55	3
	R-TGTCCCCTCTAAAACCCTCC			

Table 2. Nucleotide sequence of blast resistance-related markers.

Molecular markers	Gene	Nucleotide sequence of primers	Size of PCR product (bp)	Annealing T (ºC)	Localizationin the chromosome
Pi-ta	*Pi-ta*	F - GCTGCTTGTTCGAACAGCGCCTGC	500	57	12
		R - CAAGTCAGGTTGAAGATGCATAGC			
Pita3	*Pita*	F - AGTCGTGCGATGCGAGGACAGAAAC	861	57	12
		R - GCATTCTCCAACCCTTTTGCATGCAT			
RM224	*Pi-1*	F - ATCGATCGATCTTCACGAGG	137	56	11
		R - TGCTATAAAAGGCATTCGGG			
RM1233		F - AATAGGCCTGGAGAGAATTTCC	170	56	11
		R - CCTTATAAGCCGTCTCGATCC			
Z56592	*Piz-t*	F - GGACCCGCGTTTTCCACGTGT AA	292	57	6
		R - AGGAATCTATTGCTAAGCATGAC			
MSM6	*Pi-40*	F - TGCTGAGATAGCCGAGAAATC	256	58	6
		R - GCACCCTTTTCGCTAGAGG			
9871.T7E2b		F - CAACAAACGGGTCGACAAAGG	641	58	6
		R - CCCCCAGGTCGTGATACCTTC			
195R-1	*Pi9*	F - ATGGTCCTTTATCTTTATTG	2000	55	6
		R – TTGCTCCATCTCCTCTGTT			
NMSMPi9-1		F - CGAGAAGGACATCTGGTACG	168	55	6
		R - GAGATGCTTGGATTTAGAAGAC			
TRS26	*Pi-54*	F - GGAGAGCCAATCTGATAAGCA	266	54	11
		R - CAACAAGAGAGGCAAATTCTCA			
Pikh MAS		F - CAATCTCCAAAGTTTTCAGG	216	54	11
		R - GCTTCAATCACTGCTAGACC			

3-4 days in a thermostat at a temperature of + 28°C. Then seedlings of rice breeding lines were transferred to vessels with soil and grown to the V3-V4 stage of organogenesis in a greenhouse (Counce et al., 2000). Further, RBLs were subjected to cold treatment at +10°C for 10 days in a Low Temperature Illuminated Incubator 818 (Thermo Electron Corporation, USA) with controlled temperature and photoperiod (16/8). After 43 days of growing season from the beginning of the experiment, the survival of the lines was carried out.

2.7. Statistical analysis

The harvest was collected at the stage of full grain ripening. Statistical analysis of the elements of crop structure was carried out using the Statistica 10 program.

3. Results

Molecular screening of rice breeding lines to cold resistance presented in the Figure 1.

The desired amplicons with a length of 145 bp, corresponding to marker RM1377, in the spectrum of PCR products of 21 RBLs and 8 parental varieties (Jinbabyeo, Lazurnyi, Mustakillik, Odaebyeo, Kuban 3, Marzhan, Bakanasski and UzROS 7-13) were founded which indicates the presence of cold resistance allele in most of the created RBLs (Figure 1, Table 3).

Detection the presence or absence of DNA fragments of 145 bp in the studied genotypes using the SSR marker RM1377 made it possible to separate rice genotypes by alleles: those containing the “cold resistance” or “cold sensitivity” allele. It was found that the cold-resistant standard varieties Kuban 3 and UzROS 7-13 (positive control) contain all three QTL, while the desired DNA fragment of 145 bp, corresponding to the *qPSST-7* locus, was not observed in the cold-sensitive variety Liman (negative control). These data indicate that the presence of three loci characterizes the cold resistance of genotypes, and the absence of one of them leads to cold sensitivity.

3.1. Screening of rice breeding lines at seedling stage for cold resistance

Assessing plant resistance to low temperature stress under controlled conditions is the simplest and easiest way to identify tolerant and sensitive genotypes. There are many laboratory methods for evaluating cold-tolerant genotypes, for example, one of them is selection under controlled temperature conditions at the germination or

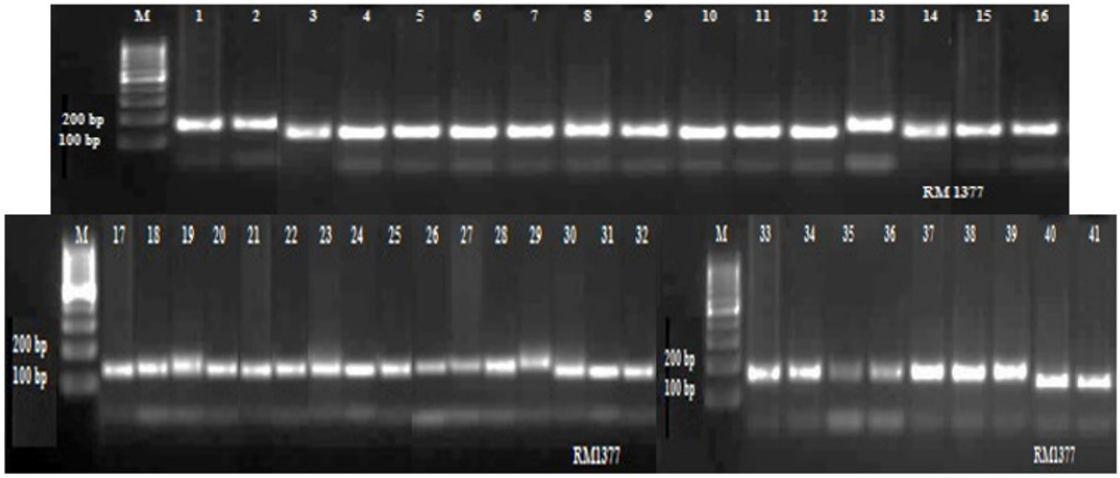

Figure 1. PCR amplification of patterns of rice breeding lines and parent genotypes using marker RM1377 closely linked with QTL *qPSST-7*. M-Marker 100 bp, 1-Avangard, 2-Liman, 3-Jinbabyeo, 4-Lazurnyi, 5-Mustakillik, 6-Avangard×KazNIIR-5, 7-Odaebyeo, 8-Opytnyi×KazNIIR-5, 9-Altynai×Opytnyi, 10-KazNIIR-5×Opytnyi, 11-KazNIIR-5×Liman, 12-KazNIIR-5×Altynai, 13-Liman×Jinbabyeo, 14-Lazurnyi×KazNIIR-5, 15-Jinbabyeo×Avangard, 16-Mustakillik×Avangard, 17-Kuban 3, 18-Marzhan, 19-Altynai, 20-Bakanasski, 21-UzROS 7-13, 22-Opytnyi×Kuban 3, 23-Opytnyi×Marzhan, 24-Kuban 3×Opytnyi, 25-Kuban 3×Liman, 26-Kuban 3×KazNIIR-5, 27-Kuban 3×Altynai, 28-Altynai×Kuban 3, 29-Marzhan×Liman, 30-Marzhan×Kuban 3, 31-KazNIIR-5×Kuban 3, 32-UzROS 7-13×Marzhan, 33-Avangard×Opytnyi, 34-Madina, 35-Ko 293 (IRRI mutant), 36-Opytnyi×Madina, 37-Opytnyi×Aru, 38-Odaebyeo×Madina, 39-Krepysh×Ko 293 (IRRI mutant), 40-FL478×Reizig, 41-FL 478×Todorokiwase.

seedling stage (Cruz and Milach, 2004). Screening of RBLs at the stage V3-V4 of organogenesis (Figure 2A-B) revealed cold-sensitive and cold-resistant lines. It was noted that most RBLs showed chlorosis after 10 days of cold treatment at +10°C. Yellowing of the leaves at varying degrees was observed, which indicates different reactions of the lines to the effect of low positive temperature (Figure 2D).

The resistance of RBLs to low positive temperatures was assessed by the number of surviving plants (Figure 3).

RBLs UzROS 7-13×Marzhan and Jinbabyeo×Avangard had average resistance, the survival rate was 26.0% and 32.0%, respectively. FL 478×Reizig and FL 478×Todorokiwase were the most sensitive to cold, with survival rates of 2.0% and 4.0%, respectively. The above-mentioned data show that the lines obtained with the participation of the cold-resistant variety Todorokiwase turned out to be cold-sensitive at the initial stages of organogenesis.

Thus, screening of rice lines at the vegetative stage to low positive temperature (+10°C) showed that 5 out of 10 studied RBLs withstand to cold treatment. Among of these, 4 RBLs (Opytnyi×Marzhan, UzROS7-13×Marzhan, Kuban 3×Liman and Jinbabyeo×Avangard) according to PCR analysis data contain all three loci of cold resistance.

3.2. Molecular screening of selected rice breeding lines to the presence of blast resistance genes

Multiplex PCR revealed genetic differences between RBLs by the presence of blast resistance genes. The presence of *Pi-ta* and *Pita* genes, located on chromosome 12, were estimated by visualization of PCR products with the linked markers Pi-ta and Pita3. It was showed that 3 out of 21 selected RBLs contain one amplicon with size 500 bp, corresponding to *Pi-ta* gene, with a frequency of 14.29%. And two amplicons 500 bp and 861 bp for both the markers were found in 17 out of 21 RBLs, which indicates the presence of two blast resistance genes *Pi-ta* and *Pita* with a genetic frequency of 80.95% and only 1 line (4.76%) did not contain both amplicons, corresponding to R genes (Figure 4A, Table 4). Among the studied 7 cold-resistant varieties, 6 contain one amplicon corresponding to the *Pi-ta* gene, and 1 variety contains both amplicons (Figure 4A, Table 4).

The presence or absence of the broad-spectrum blast R gene *Pi-1* was detected using two linked markers RM224 and RM1233. Based on these two markers, by visualizing the desired amplicon with size 137 bp, corresponding to the product of SSR marker RM224, was identified in two varieties,1 and 4 well numbers, and in 2 RBLs (with frequency 9.52%), 13 and 14 well numbers, and second 170 bp – PCR product of marker RM1233 was not founded in all of selected RBLs (0%) (Figure 4B, Table 4). The presence of DNA fragment 350 bp detected in 18 RBLs (85.71%) and in variety Nippon bare (not contain R genes) which used as a negative control. It was indicating the presence of recessive gene and heterozygosity of most of studied lines and 2 homozygous RBLs (9.52%). As well known, homozygous genotypes for a specific gene characterized by high resistance compared to heterozygous genotypes. As a result, 2 homozygous RBLs were selected for the rice breeding program (Table 4).

The PCR based amplification of the blast resistance gene *Piz-t* located on chromosome 6 was determined only in 2 studied RBLs (9.52%) by visualization of the 292 bp amplicon using SSR marker Z56592 (Table 4).

PCR amplification of blast resistant *Pi-40* gene located on chromosome 6, was revealed the presence of two

Table 3. Selection of rice breeding lines and cultivars for the presence of QTLs associated with cold resistance.

Varieties and rice breeding lines	Molecular markers			Resistance to the cold
	RM 231-RM 569	RM 1377	RM 24545	
	QTL status			
	qPSST-3	*qPSST-7*	*qPSST-9*	
	Varieties			
1	2	3	4	5
Avangard	1	0	1	C/S
Liman	1	0	1	C/S
Jinbabyeo	1	1	1	C/R
Lazurnyi	1	1	1	C/R
Mustakillik	1	1	1	C/R
Odaebyeo	1	1	1	C/R
Kuban 3	1	1	1	C/R
Marzhan	1	1	1	C/R
Bakanasski	1	1	1	C/R
UzROS 7-13	1	1	1	C/R
Altynai	1	0	1	C/S
Madina	1	0	1	C/S
Ko 293 (IRRI mutant)	1	0	1	C/S
	Rice breeding lines			
Avangard×KazNIIR-5	1	1	1	C/R
Opytnyi×KazNIIR-5	1	1	1	C/R
Altynai×Opytnyi	1	1	1	C/R
KazNIIR-5×Opytnyi	1	1	1	C/R
KazNIIR-5×Liman	1	1	1	C/R
KazNIIR-5×Altynai	1	1	1	C/R
Liman×Jinbabyeo	1	0	1	C/S
Lazurnyi×KazNIIR-5	1	1	1	C/R
Jinbabyeo×Avangard	1	1	1	C/R
Mustakillik×Avangard	1	1	1	C/R
Opytnyi×Kuban 3	1	1	1	C/R
Opytnyi×Marzhan	1	1	1	C/R
Kuban 3×Opytnyi	1	1	1	C/R
Kuban 3×Liman	1	1	1	C/R
Kuban 3×KazNIIR-5	1	1	1	C/R
Kuban 3×Altynai	1	1	1	C/R
Altynai×Kuban 3	1	1	1	C/R
Marzhan×Liman	1	0	1	C/S
Marzhan×Kuban 3	1	1	1	C/R
KazNIIR-5×Kuban 3	1	1	1	C/R
UzROS 7-13×Marzhan	1	1	1	C/R
Avangard×Opytnyi	1	0	1	C/S
Opytnyi×Madina	1	0	1	C/S
Opytnyi×Aru	1	0	1	C/S
Odaebyeo×Madina	1	0	1	C/S
Krepysh×Ko 293 (IRRI)	1	0	1	C/S
FL478×Reizig	1	1	1	C/R
FL478×Todorokiwase	1	1	1	C/R

Note: C/S – cold sensitive, C/R – cold resistant.

fragments 256 bp and 641 bp, corresponding with SSR markers MSM6 and 9871.T7E2b, in most RBLs. It was found 16 lines (76.19%) positive for both the markers (Figure 4 C, Table 4). 4 RBLs (19.05%) were contain one amplicon with size 641 bp, corresponding to SSR markers MSM6 and 1 line (4.76%) did not con-tains amplicons, corresponding to R genes (Table 4).

The presence of *Pi-9* gene, located on chromosome 6, was detected by amplifying the fragment size 168 bp, corresponding to the product of the SSR marker

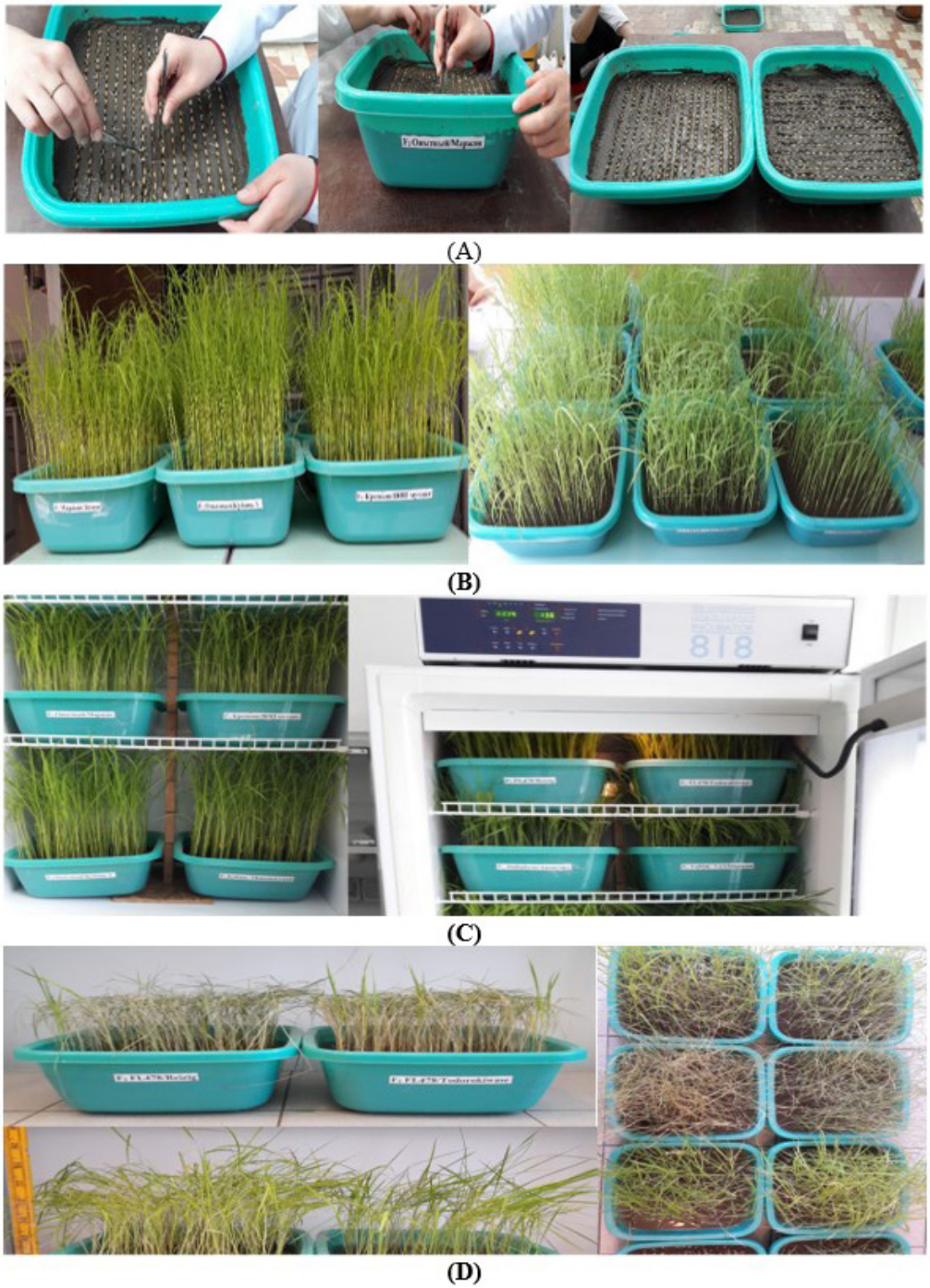

Figure 2. Laboratory screening of rice breeding lines to cold resistance: planting seeds in vegetation vessels (A); seedlings at the V3-V4 stage of organogenesis in the greenhouse (B); cold treatment of genotypes in a climate chamber (t +10°C) (C); genotypes after cold treatment (43 days of vegetation) (D).

NMSMPi9-1. The marker NMSMPi9-1 linked with *Pi-9* gene was determined the positive fragment with size 168 bp in 20 of studied RBLs (95.24%) and 1 line (4.76%) did not contain amplicons. The second marker 195R-1 did not amplify the fragment 2000 bp in all genotypes (Figure 4 D, Table 4).

PCR analysis using specific SSR primers TRS26 and Pikh MAS corresponding to *Pi-54* gene was detected the presence of 216 bp amplicon produced by the Pikh MAS primer in 13 out of 21 studied RBLs (76.19%). The SSR primers TRS26 amplify the DNA fragment with size 266 bp in 4 RBLs and desired amplicons was not detected in 1 RBLs (Figure 4E, Table 4).

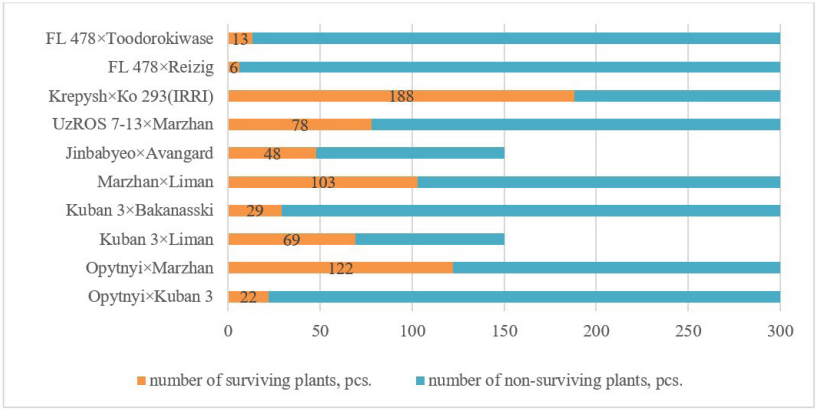

Figure 3. Assessment of cold resistance of rice breeding lines.

Table 4. Molecular screening of selected rice breeding lines and varieties to the presence of blast resistance genes.

Varieties and rice breeding lines	Molecular markers							A number of existing R genes
	Pi-ta	Pita3	RM224	Z56592	MSM6 / 9871. 7E2b	NMSMPi9-1	Pikh	
	Genes							
	Pi-ta	*Pita*	*Pi-1*	*Piz-t*	*Pi-40*	*Pi-9*	Pi-54	
	Localization in the chromosome							
	12	12	11	6	6	6	11	
	Varieties							
1	2	3	4	5	6	7	8	9
Jinbabyeo	1	0	1	0	1	1	1	5
Lazurnyi	1	0	0	0	1	1	1	4
Marzhan	1	0	0	0	1	1	0	3
Odaebyeo	1	0	1	0	1	1	0	4
Kuban 3	1	0	0	1	1	1	1	5
Mustakillik	1	1	0	0	1	1	1	5
UzROS 7-13	0	0	0	1	1	1	0	3
Rice breeding lines								
Avangard×KazNIIR-5	1	1	0	0	1	1	0	4
Opytnyi×KazNIIR-5	1	1	0	0	1	1	1	5
Altynai×Opytnyi	1	1	0	0	1	1	1	5
KazNIIR-5×Opytnyi	1	1	0	0	1	1	1	5
KazNIIR-5×Liman	1	0	0	0	1	1	1	4
KazNIIR-5×Altynai	1	0	1	0	1	1	1	5
Lazurnyi×KazNIIR-5	1	1	1	0	1	1	1	6
Jinbabyeo×Avangard	1	1	0	0	1	1	1	5
Mustakillik×Avangard	1	1	0	0	1	1	1	5
Opytnyi×Kuban 3	1	1	0	0	1	1	1	5
Opytnyi×Marzhan	0	0	0	0	0	0	0	0
Kuban 3×Opytnyi	1	1	0	0	1	1	1	5
Kuban 3×Liman	1	1	0	0	1	1	1	5
Kuban 3×KazNIIR-5	1	1	0	0	1	1	1	5
Kuban 3×Altynai	1	1	0	0	1	1	1	5
Altynai×Kuban 3	1	0	0	0	1	1	1	4
Marzhan×Kuban 3	1	1	0	1	1	1	1	6
KazNIIR-5×Kuban 3	1	1	0	0	1	1	1	5
UzROS 7-13×Marzhan	1	1	0	1	1	1	1	6
FL478×Reizig	1	1	0	0	1	1	1	5
FL478×Todorokiwase	1	1	0	0	1	1	0	4

1 - presence of gene, 0 - absence of gene.

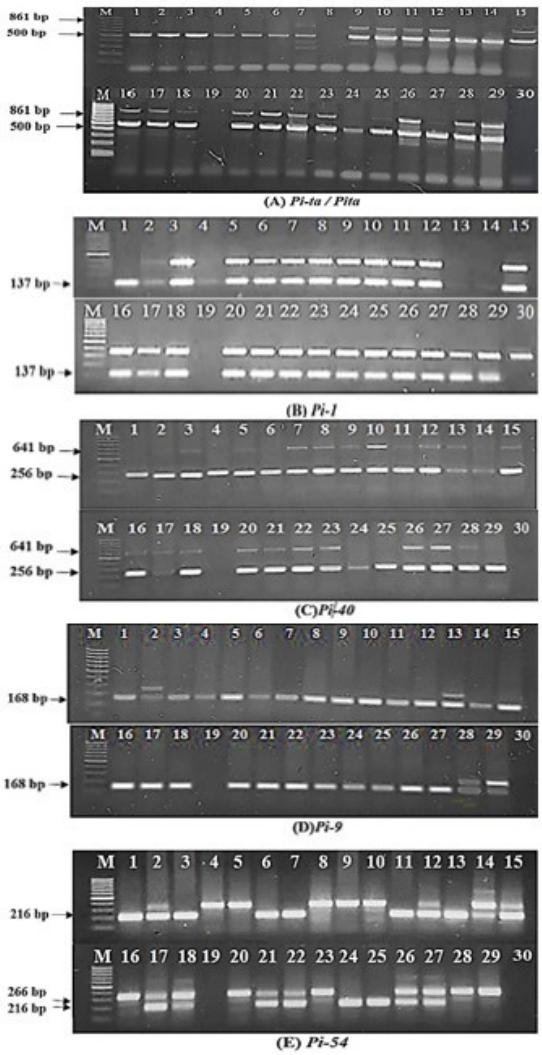

Figure 4. Screening of rice breeding lines to blast resistance by using molecular markers: (A) Pi-ta, Pita3 (corresponding to *Pi-ta* and *Pita* genes), (B) RM224 and RM1233 (*Pi-1* gene), (C) MSM6 and 9871.T7E2b (*Pi-40* gene), (D) 195R-1 and NMSMPi9-1 (*Pi-9* gene) and (E) TRS26 and Pikh MAS (*Pi-54* gene). M – 100 bp molecular marker, 1 - positive control, 2 – Jinbabyeo, 3 – Lazurnyi, 4 – Marzhan, 5 – Odaebyeo, 6 – Mustakillik, 7 – Kuban 3, 8 – UzROS 7-13, 9 - Avangard×KazNIIR-5, 10 - Opytnyi×KazNIIR-5, 11 - Altynai×Opytnyi, 12 - Kaz-NIIR-5×Opytnyi, 13 - KazNIIR-5×Liman, 14 - KazNIIR-5×Altynai, 15 - Lazurnyi×KazNIIR-5, 16 - Jinbabyeo×Avangard, 17 - Mustakillik×Avangard, 18 - Opytnyi×Kuban 3, 19 - Opytnyi×Marzhan, 20 - Kuban 3×Opytnyi, 21 - Kuban 3×Liman, 22 - Kuban 3×KazNIIR-5, 23 - Kuban 3×Altynai, 24 - Altynai×Kuban 3, 25 - Marzhan×Kuban 3, 26 - KazNIIR-5×Kuban 3, 27 - UzROS 7-13×Marzhan, 28- FL478×Reizig, 29 - FL478×Todorokiwase, 30 – Nippon bare (negative control).

3.3. Analysis of yield structure elements of rice breeding lines

Analysis of the elements of the crop structure allowed to select 7 high-yielding RBLs (Opytnyi×Marzhan, Opytnyi×KazNIIR-5, Kuban 3×KazNIIR-5, Altynai×Opytnyi, KazNIIR-5×Altynai, UzROS 7-13×Marzhan, KazNIIR-5×Kuban 3), which were identified by cold resistance. Also

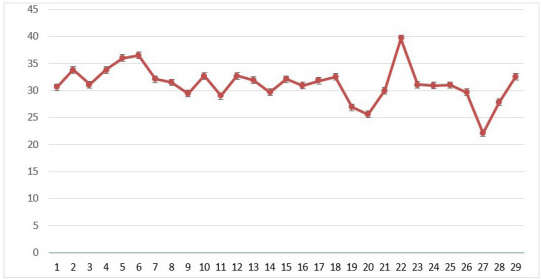

Figure 5. Analysis of RBLs for the main yield trait "Weight of 1000 seeds". 1 - Avangard×KazNIIR-5, 2 - Avangard Opytnyi, 3 – Opytnyi×Kuban 3, 4 – Opytnyi×Madina, 5 – Opytnyi×Aru, 6 – Opytnyi×Marzhan, 7 – Opytnyi×KazNIIR-5, 8 – Kuban 3×Opytnyi, 9 – Kuban 3×Liman, 10 – Kuban 3×KazNIIR-5, 11 – Kuban 3×Altynai, 12 – Altynai×Opytnyi, 13 – Altynai×Kuban 3, 14 – Marzhan×Liman, 15 – KazNIIR-5×Kuban 3, 16 – KazNIIR-5×Opytnyi, 17 – КазНИИР-5 /Лиман, 18 – KazNIIR-5×Altynai, 19 – Лиман/Jinbabyeo, 20 – Odaebyeo×Madina, 21 – Lazurnyi×KazNIIR-5, 22 – UzROS 7-13×Marzhan, 23 – Krepysh×Ко 293 (IRRI), 24 – Marzhan×Kuban 3, 25 – Jinbabyeo×Avangard, 26 – Mustakillik×Avangard, 27 – FL478×Reizig, 28 – FL478×Todorokiwase, 29 – Bakanasski.

6 RBLs of them, with the exception of one Opytnyi×Marzhan by blast resistance according to PCR results. These RBLs by the main trait of the yield "weight of 1000 grains" exceed or are at the level of the standard variety Bakanasski (for the Almaty region). Thus, the lines UzROS 7-13×Marzhan (39.6±0.6 g) and Opytnyi×Marzhan (36.5±0.6 g), which by weight of 1000 grains are 1.22 and 1.12 times higher than the standard (32.6±0.4 g) (Figure 5).

4. Discussion

Global climate change and population growth challenge plant breeders and scientists to improve the resistance of crops to ensure sustainable agricultural development and achieve zero hunger by 2030 (according to the UN) (Kim et al., 2021; Marines Marli, 2021; United Nations DESA, 2015). In this regard, research works devoted to the study of plant resistance to abiotic and biotic stresses are relevant (Akter et al., 2022; Atabayeva et al., 2022; Kissoudis et al., 2014; Zhanbyrbayev et al., 2017).

Improvement of rice varieties using marker assisted selection (MAS) is effective for sustainable rice production (Kissoudis et al., 2014). Recently, research has focused on developing rice varieties that are resistant to multiple stresses (Khush, 2013). For example, creation of rice lines resistant to BB and blast (Abhilash Kumar et al., 2016a, b; Balachiranjeevi et al., 2015).

Thus, the present study showed the results of molecular screening of RBLs for resistance to abiotic and biotic stress factors, such as cold and blast. DNA-based screening of RBLs to cold using SSR markers RM231, RM569, RM1377 and RM24545 was revealed that the presence of three QTL (*qPSST-3*, *qPSST-7* and *qPSST-9*) characterizes the cold resistance of genotypes, and the absence of one of them leads to sensitivity to cold. PCR analysis identified 21 cold-resistant RBLs. During the laboratory screening of RBLs to low temperature at the V3-V4 stage of organogenesis 5 from

10 studied RBLs were showed tolerance to cold. However, PCR results confirm that only 4 out of 5 of RBLs selected in laboratory screening contain all three loci, indicating that testing at the seedling stage does not accurately identify cold-tolerant genotypes. This indicates that laboratory screening of rice to low positive temperatures allow to determine the resistance of the tested samples, but cannot always accurately identify cold-resistant genotypes. PCR technique most accurately and quickly identifies lines containing loci and genes associated with resistance to stresses, which accelerates the selection of resistant rice lines.

Suh et al. (2010) showed that QTLs suitable for the selection of cold-resistant genotypes in combination with good yield. They noted that QTL loci located on chromosomes 3, 7 and 9 may have a common set of genes that control traits of cold tolerance and spikelet fertility. Researchers characterize the QTL data as specific and stable which facilitates the selection of high-yielding and cold-tolerant rice varieties for growing in temperate regions and highlands in the tropics. Also, Suh et al. (2013) identified a significant relationship between individual alleles and traits. In this experiment was found SSR markers were significantly associated with spikelet sterility and fertility

In a study to identify blast resistance genes in rice lines, molecular markers associated with genes *of Pi*-complex were used. Varieties containing one *R* resistance gene easy to lost their resistance when new virulent races of the fungus appear. Therefore, in the fight against the phytopathogen, it is necessary to create new rice varieties containing broad-spectrum resistance genes (Wu et al., 2007). Also, success in solving the problem of increasing rice resistance to blast and maintaining stable resistance to the constantly changing race of the fungus *Magnaporthe oryzae* can be achieved by creating varieties that have not one, but 5 or more genes in the genotype (Kostylev et al., 2017; Suh et al., 2013).

It should be noted that most of used molecular markers associated with *R* genes (*Pi-ta*, *Pita* and *Pi-1*) confer broad spectrum resistance to various *Magnaporthe oryzae* isolates. *Pita* gene is an important blast resistance gene located on the centromere region of the 12 chromosomes of rice (Bryan et al., 2000). *Pita* gene provides a wide range resistance against various blast isolates worldwide and identified in the germplasm of Indian rice. And well-known *Pi-ta* gene is found in most of the dominant rice varieties in Asia and America.

PCR analysis of selected 21 cold-tolerant RBLs for the presence of *R* genes *Pi*- complex allow to distinguish lines by the presence or absence of resistance genes to *Magnaporthe oryzae*. Significant differences between the RBls were noted, the number of resistance genes in RBLs varied from 0 to 6. According to this strategy, among the 21 studied RBLs, 16 blast-resistant ones were selected, which contain 5 or 6 blast resistance genes (Table 4). These selected lines, resistant to both cold and blast, can be used as the main candidates for obtaining domestic rice varieties resistant to multiple stresses.

Screening of RBLs for cold and blast resistance using MAS possible to quickly select resistant rice lines containing 5-6 blast resistance genes in their genotype. It is interesting to note that the analysis of the elements of crop structure help to select 6 lines high-yielding cold- and blast-resistant RBLs. High productivity of selected RBLs is very important in rice breeding.

Molecular screening has shown the effectiveness of this method in selection of rice resistant to multiple stress factors. When creating new rice varieties that are resistant to multiple stresses, it is very important to screen promising lines for the presence of genes for resistance not only to one stress, but also genes against other stress factors that are relevant in a given rice growing zone.

5. Conclusions

Identification of rice breeding lines that are resistant to multiple stresses under global climate change is relevant for obtaining a stable high yield regardless of the influence of environmental stress factors. In the present work, we identified and developed new cold and blast tolerant rice lines using MAS. These selected rice lines are valuable as donors for developing cold and blast resistant varieties, gene pyramiding and germplasm exchange to improve rice resistance to biotic and abiotic stresses.

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