IDENTIFICATION OF THE DIVERSITY OF CULTIVATED PLANTS AND THEIR WILD RELATIVES FOR SOLVING FUNDAMENTAL AND APPLIED PROBLEMS

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Identification of fertility restorer genes *Rf3* and *Rf4* in diverse rice genotypes using SSR molecular markers

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Background. Enhancing rice yields through hybridization and the identification of fertility restorer genes is crucial for improving self-sufficiency and addressing nutritional needs. This study aims to explore the presence of these fertility restorer genes in selected rice genotypes using SSR markers.
Materials and methods. To identify the fertility restorer gene(s) in diverse rice genotypes, including local and breeding rice varieties, foreign fertility restorers, advanced mutant rice lines, and two cytoplasmic male sterile lines (JelodarA and NematA) were screened using molecular markers. Six SSR markers were employed to identify the allelic status of the major fertility restorer genes *Rf3* and *Rf4*.
Results and conclusion. Results of molecular testing indicated that the genotypes IR68061R, IR50, IR 68061-27-3-2-2-3R, IR 73014-59-2-2-2R, M9-P10-2-2-2-2-1, M9-P18-6-1-1-2-1, MILYANG 54, SUWEON 294, IR 9761-19-1, and IR46R possessed the *Rf4* gene through three markers: RM171, RM6100, and RM228. Additionally, the presence of both fertility restorer genes *Rf3* and *Rf4* in the IR67924R, IR 57301-158-1R, M9-P12-5-3-2-2, M9-P15-6-2-1, and NSIC RC 352 genotypes was confirmed with all markers. These genotypes were identified as the best fertility restorers for hybrid rice breeding. Overall, the identification and evaluation of these genotypes can facilitate improved rice production and enhance food security in developing countries.

Keywords: cytoplasmic male sterility, fertility genes, rice breeding programs, three-line hybrid

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ИДЕНТИФИКАЦИЯ ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ КУЛЬТУРНЫХ РАСТЕНИЙ И ИХ ДИКИХ РОДИЧЕЙ ДЛЯ РЕШЕНИЯ ФУНДАМЕНТАЛЬНЫХ И ПРИКЛАДНЫХ ПРОБЛЕМ

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Идентификация генов – восстановителей фертильности *Rf3* и *Rf4* в различных генотипах риса с использованием молекулярных маркеров SSR

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Актуальность. Повышение урожайности риса путем гибридизации и идентификации генов – восстановителей фертильности имеет решающее значение для повышения самообеспечения и удовлетворения потребностей в питании. Цель данного исследования – определение таких генов – восстановителей фертильности в отобранных генотипах риса с помощью SSR-маркеров.

Материалы и методы. Для идентификации генов – восстановителей фертильности в различных генотипах риса, в том числе в местных и селекционных сортах, проведен скрининг зарубежных восстановителей фертильности, усовершенствованных мутантных линий риса и двух линий с цитоплазматической мужской стерильностью (JelodarA и NematA) с использованием молекулярных маркеров. Для определения аллельного статуса основных генов – восстановителей фертильности *Rf3* и *Rf4* использовались шесть SSR-маркеров.

Результаты и заключение. Молекулярный анализ показал, что генотипы IR68061R, IR50, IR 68061-27-3-2-2-3R, IR 73014-59-2-2-2R, M9-P10-2-2-2-2-1, M9-P18-6-1-1-2-1, MILYANG 54, SUWEON 294, IR 9761-19-1 и IR46R содержали ген *Rf4*, идентифицированный тремя маркерами: RM171, RM6100 и RM228. Кроме того, наличие генов – восстановителей фертильности *Rf3* и *Rf4* у генотипов IR67924R, IR 57301-158-1R, M9-P12-5-3-2-2, M9-P15-6-2-1 и NSIC RC 352 подтверждено всеми маркерами. Эти генотипы признаны лучшими восстановителями фертильности для гибридной селекции риса. В целом идентификация и оценка этих генотипов может способствовать повышению производства риса и укреплению продовольственной безопасности в развивающихся странах.

Ключевые слова: цитоплазматическая мужская стерильность, гены фертильности, селекционные программы риса, трехлинейный гибрид

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Introduction

Climate change, global policies, and natural disasters, such as floods and droughts, have disrupted the agricultural cycle and led to increased food prices, particularly for rice, posing challenges to nutrition and food security in countries, especially those with lower incomes (El Bilali et al., 2020; Bin Rahman, Zhang, 2023). Conversely, the world's population is projected to increase by 28% by 2050 compared to 2020, accompanied by urbanization, necessitating higher food production (https://www.fao.org/faostat/en/#home). Notably, countries such as Australia (yielding over 10 t ha^{-1}), Egypt, America, Uruguay, and Peru (yielding over 8 t ha^{-1}) have achieved the highest rice yields per unit area (https://www.fao.org/faostat/en/#home). Despite a decade of increased global rice production, meeting the demand necessitates further production growth due to the rising world population trend. For instance, in recent years, Iran has imported over 1.8 million tons of rice annually, amounting to over 2 billion dollars (https://www.fao.org/faostat/en/#home). Therefore, enhancing yield per unit area is a fundamental strategy for ensuring food security and self-sufficiency of the country. China leads in employing heterosis in producing hybrids via crossing CMS and restorer lines in rice, resulting in a yield increase of over 30% compared to improved rice cultivars (Xu et al., 2023b). Recently, the area under hybrid rice cultivation in China has increased to over 16 million ha per year, accounting for 57% of total rice areas and approximately 65% of total rice production. The commercial hybrid rice yield of 7.5 tons per hectare increased production by about 2.5 million tons per year, which could feed additional 80 million people (Qian et al., 2021). Improvement in grain yield, quality, and resistance can be achieved through the utilization of heterosis (Cai et al., 2023). Various research centers in China, IRRI and India are actively engaged in breeding rice cultivars to develop hybrid seeds (Bhowmick et al., 2023; Fritsche-Neto et al., 2024; Li et al., 2024). While local cytoplasmic male sterility lines have been released in Iran (Afkhami Ghadi et al., 2015), the country has not yet achieved success regarding favorable fertility restorer lines concerning plant height, flowering period, compatibility, and restoration rate. The scarcity of suitable cultivars for fertility restoration, limited number of effective and compatible lines, and their restricted genetic base have persistently plagued hybrid rice production in the country (Afkhami Ghadi et al., 2019). The commercial production of hybrid rice using the three-line method is based on cytoplasmic genetic sterility and the fertility restorer system (*Rf*), involving three lines: CMS male sterile line (A), maintainer line (B), and restorer line (R) (Xu et al., 2023b). The availability of the rice genome map with saturated SSR markers (McCouch et al., 2002) has facilitated the precise identification of fertility restorer alleles in rice (Xu et al., 2023a). R. Jing et al. (2001) reported that the *Rf4* gene in IR24 was flanked between markers RM171 and RM228 on the long arm of chromosome 10 with a genetic distance of 3.7 and 3.4 cM, respectively. Additionally, the *Rf4* gene in the restorer line Minghui63 is flanked between markers RM258 and RM304 with a distance of 2.9 and 0 cM, respectively. The *Rf3* gene is mapped on chromosome 1 and linked with the RM1 marker with about 1.9 cM (He et al., 2002). The RM1 marker is at a distance of 5.6 cM and correlated with the *Rf3* locus on chromosome 1 (Alavi et al., 2009). A. Ahmadikhah et al. (2007) found that the IR28, Amol 1, and Amol 2 lines possessed the *Rf4* gene and were correlated with the RM171 marker on chromosome 10. J. Cai et al. (2013) identified two rice cultivars from the Indica group, including 'IR 24' and 'IR 64', with both fertility restorer genes *Rf3* and *Rf4*. The SSR markers RM258, RM490, RM151, and RM228 were reported by N. Babaeian Jelodar et al. (2013) as promising and effective for use in marker-assisted selection programs for fertility restorer lines in the CMS-WA system and in the molecular localization of the WA-type male sterility controlling gene(s) in hybrid rice. The results of molecular evaluation using microsatellite markers RM3148, RM258, and RM171 correlated with fertility restorer genes showed that rice cvs. 'Dilmani' and 'Hashemi' have both fertility restorer genes in their genome. In contrast, high-yielding rice cultivars such as 'Shiroudi', 'Tabesh', 'Fajr' and 'Shafaq' were recognized as maintainer cultivars (Kiani, 2015). S. Parveen et al. (2013) detected a number of rice lines that maintain and restore fertility by crossing 4 sterile lines and 35 advanced basmati mutant lines (induced mutation using gamma rays and EMS chemicals) through line analysis in the tester. R. Vejdan (2015) investigated fertility restorer genes in promising rice mutant lines through the evaluation of quantitative and molecular traits (SSR) and identified 6 lines, including M6-P14-1 from the mutation of cv. 'Dasht', M6-P15-2 and M6-P15-6 from the mutation of cv. 'Pazhoohesh', M6-N-14, M6-N-17 and M6-N-37 resulting from induced mutation of cv. 'Nemat', as potential fertility restorer lines. A. Afkhami Ghadi et al. (2019) evaluated the genetic capacity of local and improved rice cultivars to identify maintainer and fertility restorer lines by producing a number of hybrids and stated that the international line IR67924R with more than 80% panicle fertility, crossed with the male sterile line NedaA, is known as an effective fertility restorer line. Considering the importance of identifying fertility restorer lines for use in hybrid rice programs, the aim of this study was to investigate the presence of fertility restorer genes (*Rf3* and *Rf4*) in selected rice genotypes using SSR markers.

Materials and methods

Plant materials

The parental lines of hybrid rice consisted of two cytoplasmic male sterile lines, JelodarA and NematA (lacking a fertility restorer gene), two local and modified cultivars, four rice mutant lines from the seed bank center of the Genetics and Agricultural Biotechnology Institute of Tabarestan, and 14 foreign fertility restorer lines received from the International Rice Research Institute (IRRI) in the Philippines. Advanced mutant rice lines, specifically the ninth generation of mutants, were developed using nuclear energy from induced mutation with gamma-ray irradiation from a Cobalt-60 source at a dose of 250 Gry on cvs. 'Nemat', 'Amol 3', 'Pazhoohesh', and 'Khazar'. This process took place at the Nuclear Agriculture Research Institute and Agricultural Genetics and Biotechnology Research Institute of Tabarestan. Subsequently, the lines were further developed and modified at Sari Agricultural Sciences and Natural Resources University over several generations of selection, aiming for early maturity, suitable plant height, high yield, and optimal cooking quality. The selection process involved early generations through the multi-seed bulk method and advanced generations through the pedigree method (Table 1).

The sterility stability, genetic purity, and allogamy characteristics of the cytoplasmic sterile lines were investigated and confirmed (Afkhami Ghadi et al., 2014, 2015, 2018). Following filed preparation and seed nursery arrangement, the genotypes were sown in April. Leaf samples were collected from the young leaves of the plants when they reached the four-leaf stage to ensure minimal contamination from diseases.

Table 1. **List of local and modified rice genotypes used for identifying the restorer genes**

Таблица 1. **Список местных и селекционных генотипов риса для идентификации генов-восстановителей**

No.	Genotype name	Origin	Pedigree
1	JelodarA	IRAN	IR 58025 A // 'Jelodar'
2	NematA	IRAN	IR 58025 A // 'Nemat'
3	'Sepidrod'	IRAN	'Garm Sadri' / IR8 / 'Domsiah'
4	'Sadri'	IRAN	Local selection from a landrace
5	M9-P10-2-2-2-2-1	IRAN	Mutant of 'Nemat'
6	M9-P12-5-3-2-2	IRAN	Mutant of 'Amol 3'
7	M9-P15-6-2-1	IRAN	Mutant of 'Pazhoohesh'
8	M9-P18-6-1-1-2-1	IRAN	Mutant of 'Khazar'
9	IR68061R (R9)	IRRI	–
10	IR67924R	IRRI	–
11	IR 68061-27-3-2-2-3R	IRRI	–
12	IR 57301-158-1R	IRRI	–
13	IR 73014-59-2-2-2R	IRRI	–
14	SUWEON 294	North Korea	MILYANG 23 / MILYANG 30
15	IR 9761-19-1	IRRI	IR 30 / IR 2588-48-3 // IR 36
16	NSIC RC 352	IRRI	–
17	IR 85593-23-2-1-3-1-3-1-1-1	IRRI	–
18	IR 85593-23-2-1-3-1-2-1-1-1	IRRI	–
19	IR50	IRRI	IR2153-14-1-6 / IR28 // IR36
20	IR 56	IRRI	IR4432-53-33 / PTB 33 // IR36
21	IR46R	IRRI	IR 1416-131-5 / IR 1364-37-3-1 // IR 1366-120-3-1 / IR 1539-111
22	MILYANG 54	South Korea	MILYANG 21 / IR32 // MILYANG 23 / MILYANG 30

Note: IRRI – International Rice Research Institute (Philippines)

Примечание: IRRI – Международный исследовательский институт риса (Филиппины)

DNA extraction, PCR amplification and PCR products detection

For each genotype, equal amounts of leaves from five sample plants were combined, and then the CTAB method (Saghai-Maroof et al., 1984) was employed to extract DNA. The quantity and quality of DNA were assessed using spectrophotometry and agarose gel electrophoresis. SSR molecular markers associated with the *Rf3* and *Rf4* genes, located on chromosomes 1 and 10 of rice, were used to trace fertility restorer genes in the rice genome. PCR was performed according to the specific temperature profile of each marker, and the PCR products were subjected to 2% agarose gel electrophoresis. The gel was stained with ethidium bromide (El-Namaky et al., 2016) and photographed using a Gel Documentation device (Fig. 1).

SSR markers

To identify the allelic status of the *Rf3* and *Rf4* genes, six specific SSR markers were utilized (Table 2). Rice genotypes were screened alongside lines JelodarA and NematA (as check genotypes with recessive fertility restorer genes) for the presence or absence of fertility restorer genes. The resulting bands from each marker were scored as codes (1 for the presence and 0 for the absence of bands for each primer) and the resulting matrix was used to check the genetic status of the genotypes.

Results and discussion

Molecular testing of genotypes to identify the *Rf3* gene using the RM1 marker in rice genotypes

The results of the RM1 marker band pattern (see Fig. 1) showed distinct polymorphism between two cytoplasmic male sterile lines (JelodarA and NematA) and other genotypes. The banding pattern of the RM1 marker for the screened rice genotypes indicated that the genotypes of 'Sepidrod', IR68061R, IR50, IR67924R, IR 68061-27-3-2-2-3R, IR 57301-158-1R, IR 73014-59-2-2-2R, M9-P12-5-3-2-2, M9-P15-6-2-1, MILYANG 54, SUWEON 294, IR 56, IR46R, NSIC RC 352, IR 85593-23-2-1-3-1-3-1-1-1, and IR 85593-23-2-1-3-1-2-1-1-1 exhibited a band of 113 bp, indicating the probable presence of the *Rf3* gene. Conversely, the two cytoplasmic male sterile lines, JelodarA and NematA, along with the 'Sadri' genotypes M9-P10-2-2-2-2-1, M9-P18-6-1-1-2-1 and IR 9761-19-1, showed a band of 100 bp, indicating the absence of the *Rf3* gene.

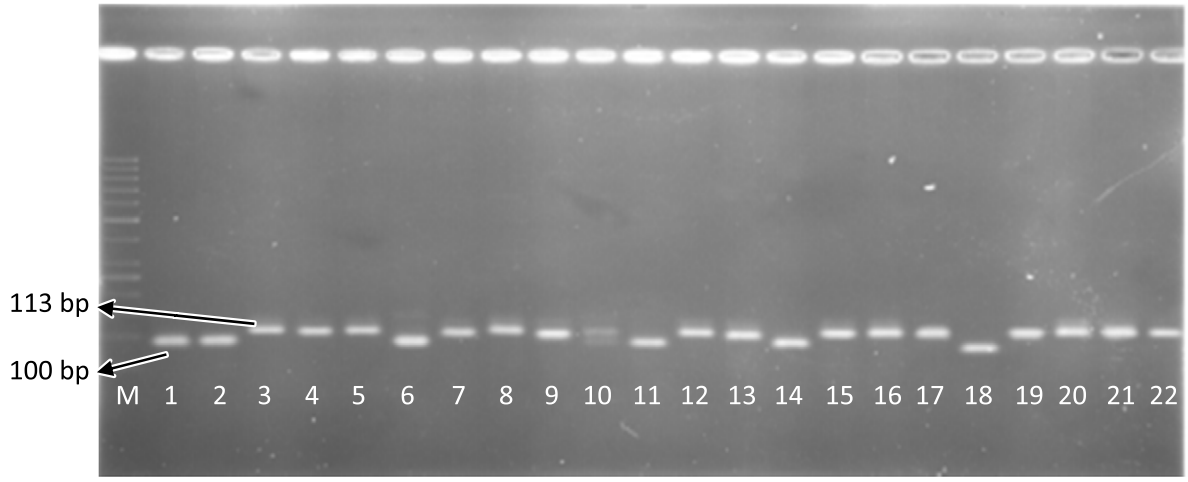

Fig. 1. Banding pattern of the RM1 marker for the screened rice genotypes (from left to right): M – 50 bp ladder marker; **1** – 'Jelodar'; **2 –** 'Nemat'; **3** – 'Sepidrod'; **4** – IR68061R; **5 –** IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

Рис. 1. Электрофоретические спектры маркера RM1 для проанализированных генотипов риса (слева направо): M – Ladder-маркер 50 пн; **1** – 'Jelodar'; **2 –** 'Nemat'; **3** – 'Sepidrod'; **4** – IR68061R; **5 –** IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

Table 2. Details of 6 molecular markers associated with the main fertility restorer genes

Таблица 2. Характеристика 6 молекулярных маркеров, связанных с основными генами – восстановителями фертильности

Marker name	Repeat motif	Annealing temperature, °C	Band size PCR	Primer_ forward	Primer_ reverse	Gene name	Ref.
RM1	(GA)26	55	113	gcgaaaacacaatgcaaaaa	gcgttggttggacctgac	Rf_3	Bazrkar et al., 2008
RM490	(CT)13	58	101	atctgcacactgcaaacacc	agcaagcagtgctttcagag	Rf_3	Eidi Kohnaki et al., 2015
RM3148	(CA)20	55	166	gactattgctcgaacactttg	ttgtctgctttggtatttgc	Rf_3	McCouch et al., 2002
RM171	(GATG)5	55	328	aacgcgaggacacgtacttac	acgagatacgtacgcctttg	Rf_4	Bazrkar et al., 2008
RM6100	(TCG)13	55	160	ttccctgcaagattctagctacacc	tgttcgtcgaccaagaactcagg	Rf_4	Alavi et al., 2009;
RM228	(CA)6(GA)36	55	154	ctggccattagtccttgg	gcttgcggctctgcttac	Rf_4	Babaeian Jelodar et al., 2013

This is in accordance with previous studies by M. Alavi et al. (2009) on mapping the *Rf3* gene location in rice using SSR markers. They reported that the RM1 primer was associated with the *Rf3* gene, and this primer, at two positions (100 and 113 bp), distinguished between CMS and fertility restorer genotypes. Furthermore, A. Sadeqi et al. (2014) utilized the RM1 primer to differentiate lines with the *Rf3* gene, demonstrating the consistency and reliability of the marker in distinguishing lines with the *Rf3* gene.

***Rf3* gene using the RM490 marker in rice genotypes**

The results of the RM490 marker band pattern (Fig. 2) revealed various band sizes, including 98 bp, 101 bp, and 103 bp. Polymorphism was observed between the two sterile cytoplasmic lines, JelodarA (101 bp) and NematA (103 bp). Therefore, the 98 bp band is likely associated with the fertility restorer gene observed in the IR67924R, IR 57301-158-1R, M9-P12-5-3-2-2, M9-P15-6-2-1 and NSIC RC 352 genotypes. The findings from S. Singh et al. (2015) also support the association of RM490 with the *Rf3* gene, which was utilized for detecting restorer lines, thereby emphasizing the significance of these markers in marker-assisted selection for fertility restoration in rice breeding programs.

***Rf3* gene using the RM3148 marker in rice genotypes**

The presence of the *Rf3* gene was investigated using the RM3148 marker in various rice genotypes. The results from the RM3148 marker band pattern (Fig. 3) revealed distinct

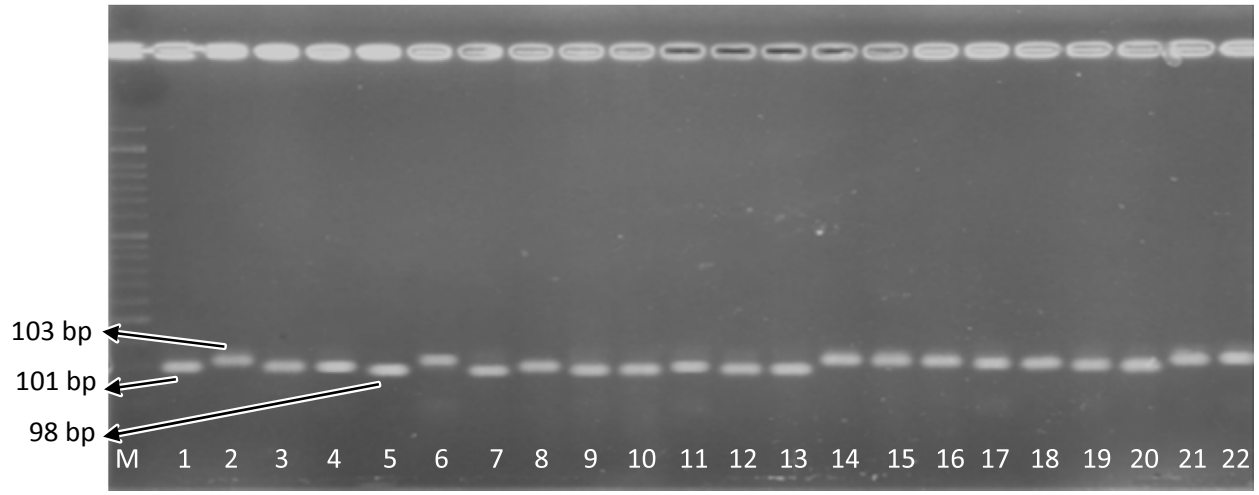

Fig. 2. Banding pattern of the RM490 marker for the screened rice genotypes (from left to right):
M – 50 bp ladder marker; **1** – JelodarA; **2** – NematA; **3** – 'Sepidrod'; **4** – IR68061R; **5** – IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

Рис. 2. Электрофоретические спектры маркера RM490 для проанализированных генотипов риса (слева направо): M – Ladder-маркер 50 пн; **1** – JelodarA; **2** – NematA; **3** – 'Sepidrod'; **4** – IR68061R; **5** – IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

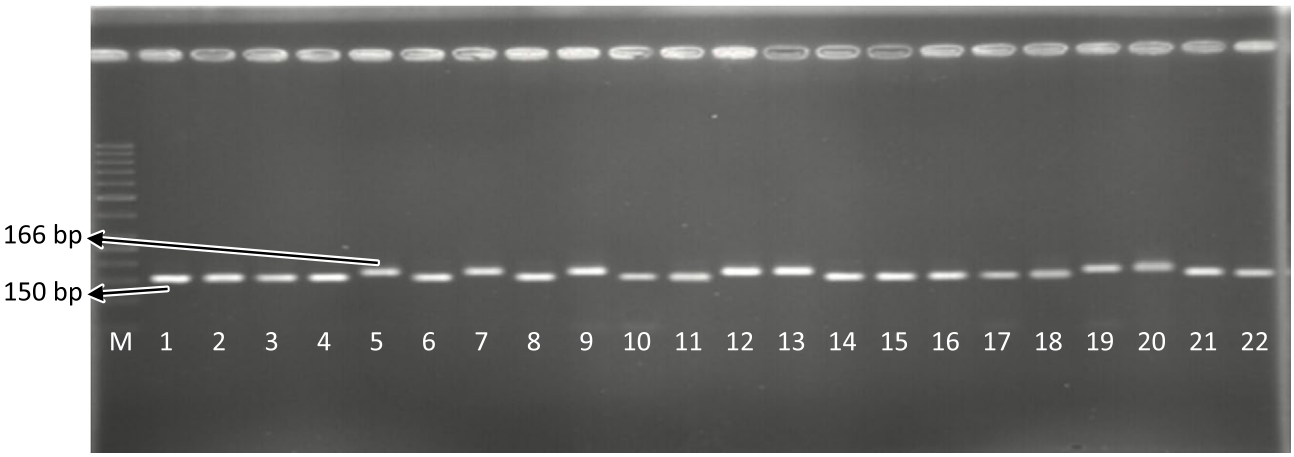

Fig. 3. Banding pattern of the RM3148 marker for the screened rice genotypes (from left to right):
M – 50 bp ladder marker; **1** – 'Jelodar'; **2** – 'Nemat'; **3** – 'Sepidrod'; **4** – IR68061R; **5** – IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

Рис. 3. Электрофоретические спектры маркера RM3148 для проанализированных генотипов риса (слева направо): M – Ladder-маркер 50 пн; **1** – 'Jelodar'; **2** – 'Nemat'; **3** – 'Sepidrod'; **4** – IR68061R; **5** – IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

polymorphism among cytoplasmic sterile lines (JelodarA and NematA) and other genotypes. The banding pattern of the RM3148 marker for the screened rice genotypes indicated that the genotypes IR50, IR67924R, IR 57301-158-1R, M9-P12-5-3-2-2, M9-P15-6-2-1, IR46R and NSIC RC 352 exhibited a 166 bp band, suggesting the likely presence of the *Rf3* gene. Conversely, the two cytoplasmic sterile lines, JelodarA and NematA, along with other genotypes, lacked the *Rf3* gene, as evidenced by the absence of the 150 bp band. Similarly, in Gh. Kiani's study (2015) on the expression validation of markers associated with the fertility restorer gene, it was reported that the primer RM3148 displayed clear polymorphism between sterile and fertility restorer parents.

***Rf4* gene using the RM171 marker in rice genotypes**

The results of the RM171 marker band pattern (Fig. 4) revealed polymorphism between the two cytoplasmic sterile lines (JelodarA and NematA) and other genotypes. The band

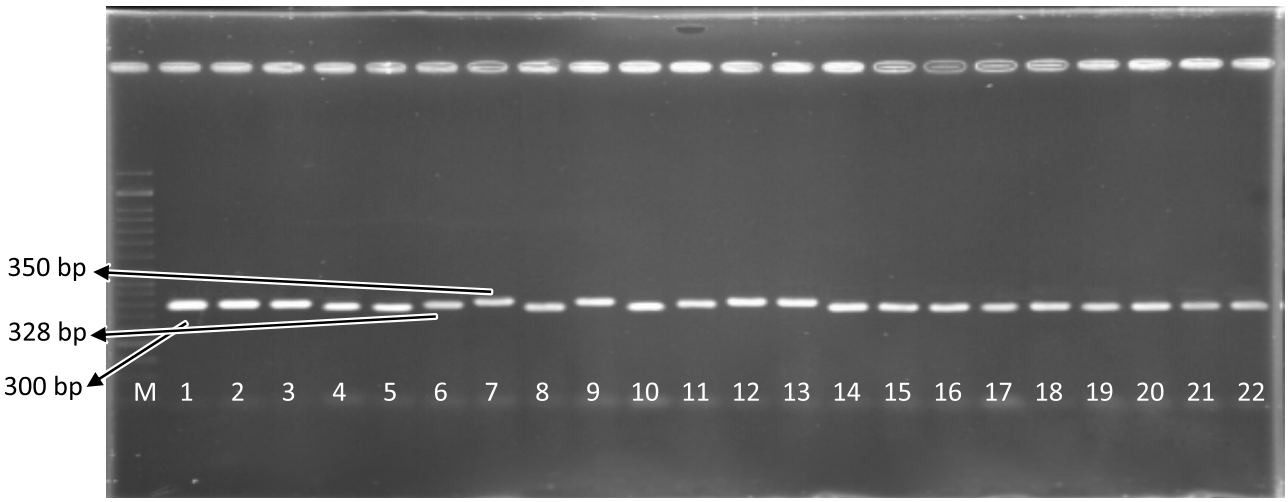

Fig. 4. Banding pattern of the RM171 marker for the screened rice genotypes (from left to right):
M – 50 bp ladder marker; **1** – JelodarA; **2** – NematA; **3** – 'Sepidrod'; **4** – IR68061R; **5** – IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

Рис. 4. Электрофоретические спектры маркера RM171 для проанализированных генотипов риса (слева направо): M – Ladder-маркер 50 пн; **1** – JelodarA; **2** – NematA; **3** – 'Sepidrod'; **4** – IR68061R; **5** – IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

pattern of the RM171 marker for the screened rice genotypes indicated that the genotypes IR68061R, IR50, IR67924R, IR 68061-27-3-2-2-3R, IR 57301-158-1R, IR 73014-59-2-2-2R, M9-P10-2-2-2-2-1, M9-P12-5-3-2-2, M9-P15-6-2-1, M9-P18-6-1-1-2-1, MILYANG 54, SUWEON 294, IR 9761-19-1, IR46R, NSIC RC 352, IR 85593-23-2-1-3-1-3-1-1-1 and IR 85593-23-2-1-3-1-2-1-1-1 exhibited bands at 328 and 350 bp, indicating the probable presence of the *Rf4* gene. However, the two cytoplasmic sterile lines, JelodarA and NematA, along with other genotypes, were found to lack the *Rf4* gene. The alignment of these findings with the study by M. Alavi et al. (2009), who mapped the location of the *Rf4* gene in rice using SSR markers, further strengthens the association of the RM171 primer with the *Rf4* gene, highlighting its potential for identifying genotypes with fertility restoration capabilities associated with *Rf4*.

***Rf4* gene using the RM6100 marker in rice genotypes**

The results of the RM6100 marker band pattern (Fig. 5), which correlated with the *Rf4* gene, showed polymorphism among cytoplasmic sterile lines (JelodarA and NematA) and other genotypes. The band pattern of the RM6100 marker for the screened rice genotypes indicated that the genotypes IR68061R, IR50, 'Sadri', IR67924R, IR 68061-27-3-2-2-3R, IR 57301-158-1R, IR 73014-59-2-2-2R, P10-3, M9-P12-5-3-2-2, M9-P15-6-2-1, M9-P18-6-1-1-2-1, MILYANG 54, SUWEON 294, IR 56, IR 9761-19-1, IR46R, NSIC RC 352, IR 85593-23-2-1-3-1-3-1-1-1 and IR 85593-23-2-1-3-1-2-1-1-1 exhibited bands at 144 and 150 bp, indicating the probable presence of the *Rf4* gene. Additionally, some genotypes identified as fertility restorers in this study, such as IR50, were also found to have both *Rf3* and *Rf4* genes (Revathi et al., 2013). Non-sterile lines with the 'Sepidrod' genotype were detected at 140 bp without the *Rf4* gene. S. Shidenur et al. (2019) identified genotypes with the *Rf4* gene through molecular evaluation of 310 advanced rice lines using the RM6100 marker. M. Alavi et al. (2009) also mapped the location of the *Rf4* gene in rice using SSR markers and indicated that the RM6100 primer was linked to the *Rf4* gene on chromosome 10 of rice. This primer in two bp positions (140 and 150) indicated CMS and fertility restorer genotypes, respectively.

Investigation of the presence of the *Rf4* gene using the RM228 marker in rice genotypes

The results of the RM228 marker band pattern (see Fig. 6), which is associated with the *Rf4* gene, revealed limited polymorphisms among cytoplasmic sterile lines (JelodarA and NematA) and other genotypes. The banding pattern of the RM228 marker for the screened rice genotypes indicated that the sterile lines JelodarA and NematA exhibited a band size of 100 bp, while the other genotypes displayed two different bands of 105 and 95 bp. Based on this band pattern, it is likely that the genotypes 'Sepidrod', IR68061R, IR50, IR67924R, IR 68061-27-3-2-2-3R, IR 57301-158-1R, IR 73014-59-2-2-2R, M9-P10-2-2-2-2-1, M9-P15-6-2-1, M9-P18-6-1-1-2-1, MILYANG 54, SUWEON 294, IR 56, IR 9761-19-1, IR46R and NSIC RC 352 with a band at 105 bp likely possess the *Rf4* gene. Conversely, 'Sadri' and M9-P12-5-3-2-2 with a band at 95 bp are likely without the *Rf4* gene, and IR 85593-23-2-1-3-1-3-1-1-1 and IR 85593-23-2-1-3-1-2-1-1-1 exhibited the same banding pattern as the male sterile lines. In the study by M. Alavi et al. (2009), the *Rf4* gene location in rice was mapped using SSR markers, and it was noted that the RM228 primer is linked to the *Rf4* gene on chromosome 10 of rice. This primer at two bp positions (110 and 120) indicated CMS and fertility restorer genotypes.

Summary of the presence of the fertility restorer genes *Rf3* and *Rf4* in rice genotypes

From the overall results of the six markers utilized in this study (see Table 3), it was determined that the presence of both *Rf3* and *Rf4* fertility restorer genes in the genotypes IR67924R, IR 57301-158-1R, M9-P12-5-3-2-2, M9-P15-6-2-1 and NSIC RC 352 was confirmed with all the markers. These genotypes were identified as the most effective fertility restorers, suitable for transferring *Rf* genes to other cultivars

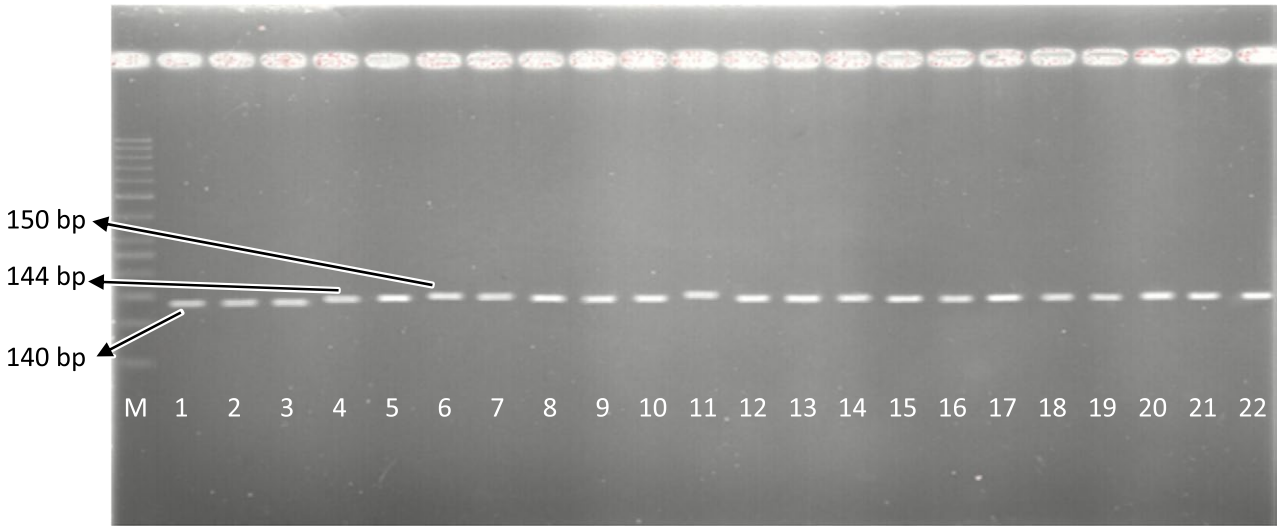

Fig. 5. Banding pattern of the RM6100 marker for the screened rice genotypes (from left to right):
M – 50 bp ladder marker; **1** – JelodarA; **2** – NematA; **3** – 'Sepidrod'; **4** – IR68061R; **5** – IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

Рис. 5. Электрофоретические спектры маркера RM6100 для проанализированных генотипов риса (слева направо): M – Ladder-маркер 50 пн; **1** – JelodarA; **2** – NematA; **3** – 'Sepidrod'; **4** – IR68061R; **5** – IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

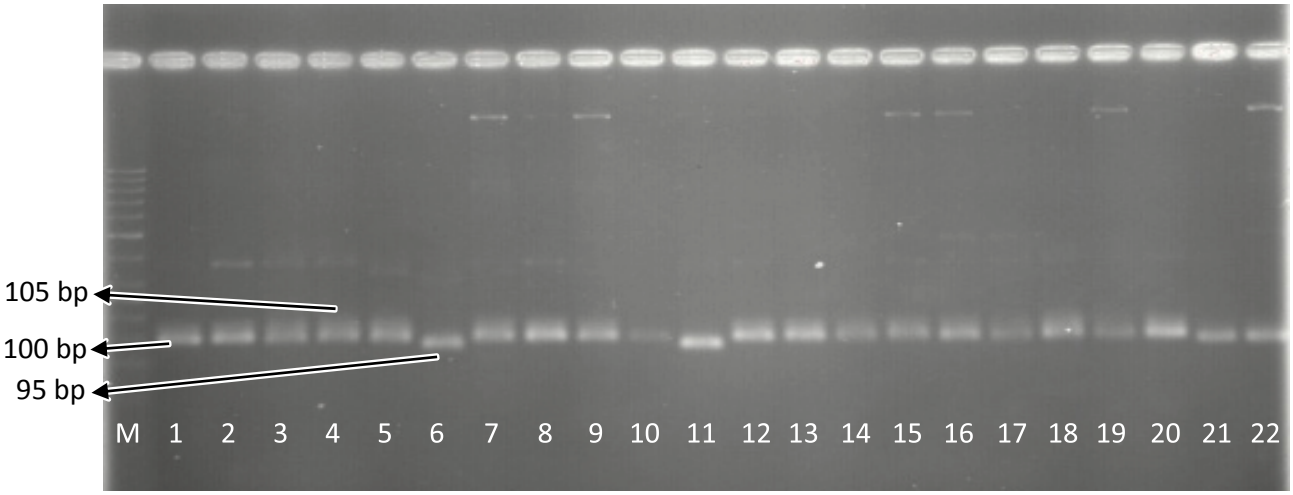

Fig. 6. Banding pattern of the RM228 marker for the screened rice genotypes (from left to right):
M – 50 bp ladder marker; **1** – JelodarA; **2** – NematA; **3** – 'Sepidrod'; **4** – IR68061R; **5** – IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

Рис. 6. Электрофоретические спектры маркера RM228 для проанализированных генотипов риса (слева направо): M – Ladder-маркер 50 пн; **1** – JelodarA; **2** – NematA; **3** – 'Sepidrod'; **4** – IR68061R; **5** – IR50; **6** – 'Sadri'; **7** – IR67924R; **8** – IR 68061-27-3-2-2-3R; **9** – IR 57301-158-1R; **10** – IR 73014-59-2-2-2R; **11** – M9-P10-2-2-2-2-1; **12** – M9-P12-5-3-2-2; **13** – M9-P15-6-2-1; **14** – M9-P18-6-1-1-2-1; **15** – MILYANG 54; **16** – SUWEON 294; **17** – IR 56; **18** – IR 9761-19-1; **19** – IR46R; **20** – NSIC RC 352; **21** – IR 85593-23-2-1-3-1-3-1-1-1; **22** – IR 85593-23-2-1-3-1-2-1-1-1

Table 3. **Status of the *Rf3* and *Rf4* fertility restorer genes in the screened genotypes**

Таблица 3. **Статус генов – восстановителей фертильности *Rf3* и *Rf4* у изученных генотипов**

Genotype name	Markers					
	RM1	RM490	RM3148	RM171	RM6100	RM228
	Rf_3			Rf_4		
JelodarA	–	–	–	–	–	–
NematA	–	–	–	–	–	–
'Sepidrod'	+	–	–	–	–	+
R68061R (R9)	+	–	–	+	+	+
IR50	+	–	+	+	+	+
'Sadri'	–	–	–	–	+	–
IR67924R	+	+	+	+	+	+
IR 68061-27-3-2-2-3R	+	-	–	+	+	+
IR 57301-158-1R	+	+	+	+	+	+
IR 73014-59-2-2-2R	+	–	-	+	+	+
M9-P10-2-2-2-2-1	–	–	-	+	+	+
M9-P12-5-3-2-2	+	+	+	+	+	–
M9-P15-6-2-1	+	+	+	+	+	+
M9-P18-6-1-1-2-1	–	–	–	+	+	+
MILYANG 54	+	–	–	+	+	+
SUWEON 294	+	–	–	+	+	+
IR 56	+	–	–	–	+	+
IR 9761-19-1	–	–	–	+	+	+
IR46R	+	–	+	+	+	+
NSIC RC 352	+	+	+	+	+	+
IR 85593-23-2-1-3-1-3-1-1-1	+	–	–	+	+	–
IR 85593-23-2-1-3-1-2-1-1-1	+	–	–	+	+	–

and for hybrid rice breeding programs. Some genotypes were found to possess a gene with one marker and not with another, possibly due to crossing over between the marker and the gene.

In a study conducted by P. Revathi et al. (2013), it was reported that the efficiency of the markers RM6100 and RM10313 in identifying fertility restorer lines correlated with the *Rf4* and *Rf3* genes, respectively, was 85% and 81%, respectively.

Genotypes IR68061R, IR50, IR 68061-27-3-2-2-3R, IR 73014-59-2-2-2R, M9-P10-2-2-2-2-1, M9-P8-7-2-1-7-1, MILYANG 54, SUWEON 294, IR 9761-19-1 and IR46R were identified as having the *Rf4* gene through all three markers: RM171, RM6100, and RM228.

The identification of genotypes with both *Rf3* and *Rf4* genes through multiple markers was reported by J. Cai et al. (2014), who found that the effect of the *Rf4* gene was slightly greater than that of the *Rf3* gene. Additionally, A. Majid et al. (2020) studied 100 rice germplasms using the RM6100 and RM3148 markers and identified 19 lines with both *Rf3* and *Rf4* genes.

Conclusion

This study successfully identified the fertility restorer genes *Rf3* and *Rf4* in a diverse range of rice genotypes using SSR molecular markers. The results highlighted several genotypes, including IR67924R, IR 57301-158-1R, M9-P12-5-3-2-2, M9-P15-6-2-1, and NSIC RC 352, as possessing both *Rf3* and *Rf4* genes, making them valuable candidates for hybrid rice breeding programs. Identifying these genes is essential for enhancing hybrid rice production and improving food security, particularly in developing countries facing agricultural challenges. The use of six specific SSR markers enabled a robust analysis of the allelic status of the targeted genes. Notably, genotypes such as IR68061R, IR50, IR 68061-27-3-2-2-3R, IR 73014-59-2-2-2R, M9-P10-2-2-2-2-1, M9-P18-6-1-1-2-1, MILYANG 54, SUWEON 294, IR 9761 1-19-1 and IR46R were confirmed to carry the *Rf4* gene, while others demonstrated varying combinations of *Rf3* and *Rf4* presence. The molecular testing results not only corroborated previous findings in the literature but also provided new insights into the genetic variability available within the screened rice germplasm. The identification of effective fertility restorers is crucial for the successful implementation of hybrid rice breeding strategies. The findings of this research can facilitate the development of new hybrid rice cultivars that leverage the benefits of heterosis, ultimately contributing to enhanced yield, quality, and resilience against climate variability. Furthermore, this study underscores the importance of molecular marker-assisted selection in advancing rice breeding ef-

forts and emphasizes the potential for these identified genotypes to meet the increasing global demand for rice as the population continues to grow. In summary, the identification of *Rf3* and *Rf4* genes in diverse rice genotypes lays the groundwork for future breeding programs aimed at improving rice production and ensuring food security, particularly in regions where rice is a staple food. The continued exploration and utilization of such genetic resources will be pivotal in addressing the challenges posed by climate change and population growth.

References / Литература

Afkhami Ghadi A., Babaeian Jelodar N., Bagheri N. Evaluation of allogamic characteristics for Iranian rice cytoplasmic male sterile lines. *Journal of Crop Breeding*. 2014;6(13):61-72. [in Persian]

Afkhami Ghadi A., Babaeian Jelodar N., Bagheri N. Evaluation of genetic distance of rice male sterile isosytoplasmic lines. *Plant Production Technology*. 2018;10(1):59-68. [in Persian] DOI: 10.22084/PPT.2018.3595

Afkhami Ghadi A., Babaeian Jelodar N., Bagheri N. Evaluation of sterility stability for some of rice cytoplasmic male sterile lines. *Journal of Crop Breeding*. 2015;7(15):14-23. [in Persian]

Afkhami Ghadi A., Khdemian R., Nematzadeh G., Jelodar Babaeian N., Bagheri N. Identification of male sterility maintainer and fertility restorer lines from Iranian landraces and improved cultivars of rice (*Oryza sativa* L.). *Seed and Plant Journal*. 2019;35(2):121-136. [in Persian] DOI: 10.22092/SPPI.2019.121907

Ahmadikhah A., Karlov G., Nematzadeh Gh., Ghasemi Bezdi K. Inheritance of the fertility restoration and genotyping of rice lines at the restoring fertility (*Rf*) loci using molecular markers. *International Journal of Plant Production*. 2007;1(1):13-21. DOI: 10.22069/IJPP.2012.521

Alavi M., Ahmadikhah A., Kamkar B., Kalateh M. Mapping *Rf3* locus in rice by SSR and CAPS markers. *International Journal of Genetics and Molecular Biology*. 2009;7:121-126. DOI: 10.5897/IJGMB

Babaeian Jelodar N., Bagheri N., Pasha P., Afkhami Ghadi A. Molecular mapping of the fertility restorer genes for WA-type CMS in hybrid rice. In: *12th Congress of Iranian Genetics Society*. Tehran: Shaid Beheshti University; 2013. p.226779. [in Persian] Available from: https://en.civilica.com/doc/226779 [accessed Mar. 04, 2025].

Bazrkar L., Ali A.J., Babaeian N.A., Ebadi A., Allahgholipour A., Kazemitabar M.K. et al. Tagging of four fertility restorer loci for wild abortive cytoplasmic male sterility system in rice (*Oryza sativa* L.) using microsatellite markers. *Euphytica*. 2008;164(3):669-677. DOI: 10.1007/s10681-008-9667-8

Bhowmick P.K., Krishnan S.G., Singh A.K., Vinod K., Bollinedi H., Ellur R.K. et al. 12. Pusa rice restorer 402 (PRR 402) (IC0637551; INGR21012), a rice (*Oryza sativa* var. *indica*) germplasm tropical japonica based NPT line, which is a restorer of WA cytoplasm possessing the restorer gene, *Rf4*, developed in the background of a popular indica rice variety Pusa 44. *Indian Journal of Plant Genetic Resources*. 2023;36(1):131-132.

Bin Rahman A.N.M.R., Zhang J. Trends in rice research: 2030 and beyond. *Food and Energy Security*. 2023;12(2):e390. DOI: 10.1002/fes3.390

Cai J., Dai Z.J., Zhu H.T., Zeng R.Z., Zhang Z.M., Ma T.F. et al. Comparative analysis of fertility restoration genes for WA, Y, and DA cytoplasmic male sterility in rice. *Biologia Plantarum*. 2014;58(2):241-246. DOI: 10.1007/s10535-013-0385-2

Cai J., Liao Q.P., Dai Z.J., Zhu H.T., Zeng R.Z., Zhang Z.M. et al. Allelic differentiations and effects of the *Rf3* and *Rf4* genes on fertility restoration in rice with wild abortive cytoplasmic male sterility. *Biologia Plantarum*. 2013;57(2):274-280. DOI: 10.1007/s10535-012-0294-9

Cai W., Li W., Duan L., Chen Y., Zhang F., Hu B. et al. Genetic analysis of novel fertility restoration genes (*qRf3* and *qRf6*) in Dongxiang wild rice using graded Pool-Seq mapping and QTL-Seq correlation analysis. *International Journal of Molecular Sciences*. 2023;24(19):14832. DOI: 10.3390/ijms241914832

Eidi Kohnaki M., Kiani G., Nematzadeh G. Morphological and molecular selection of fertility restorer gene(s) in segregating populations of rice. *Plant Productions*. 2015;38(2):89-98. [in Persian] DOI: 10.22055/ppd.2015.11322

El Bilali H., Bassolé I.H.N., Dambo L., Berjan S. Climate change and food security. *Agriculture and Forestry*. 2020;66(3):197-210. DOI: 10.17707/AgricultForest.66.3.16

El Namaky R., Sedeek S., Moukoumbi Y.D., Ortiz R., Manneh B. Microsatellite-aided screening for fertility restoration genes (*Rf*) facilitates hybrid improvement. *Rice Science*. 2016;23(3):160-164. DOI: 10.1016/j.rsci.2016.04.003

FAOSTAT. Food and Agriculture Organization of the United Nations. Food and Agriculture Data: [website]. Available from: https://www.fao.org/faostat/en/#home [accessed Mar. 15, 2025].

Fritsche-Neto R., Ali J., De Asis E.J., Allahgholipour M., Labroo M.R. Improving hybrid rice breeding programs via stochastic simulations: number of parents, number of hybrids, tester update, and genomic prediction of hybrid performance. *Theoretical and Applied Genetics*. 2024;137(1):3. DOI: 10.1007/s00122-023-04508-6

He G.H., Wang W.M., Liu G.Q., Hou L., Xiao Y.H., Tang M. et al. Mapping of two fertility-restoring genes for WA cytoplasmic male sterility in minghui63 using SSR markers. *Acta Genetica Sinica*. 2002;29(9):798-802. [in Chinese]

Jing R., Li X., Yi P., Zhu Y. Mapping fertility-restoring genes of rice WA cytoplasmic male sterility using SSLP markers. *Botanical Bulletin of Academia Sinica*. 2001;42:167-171.

Kiani Gh. Validation of SSR markers linked to restoring fertility (*Rf*) genes and genotyping of rice lines at *Rf* loci. *Journal of Agricultural Science and Technology*. 2015;17:1931-1838.

Li J., Luo X., Zhou K. Research and development of hybrid rice in China. *Plant Breeding*. 2024;143(1):96-104. DOI: 10.1111/pbr.13134

Majid A., Parray G.A., Sofi N.R., Shikari A.B., Waza S.A. Combining ability effects for various agro-morphological traits in rice under temperate conditions. *Current Journal of Applied Science and Technology*. 2020;39(38):47-58. DOI: 10.9734/cjast/2020/v39i3831095

McCouch S.R., Teytelman L., Xu Y., Lobos K.B., Clare K., Walton M. et al. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research*. 2002;9(6):199-207. DOI: 10.1093/dnares/9.6.199

Parveen S., Dhakarey R., Singh J. Identification of maintainers and restorers for WA CMS lines among Basmati mutants in rice. *Agricultural Science Digest – A Research Journal*. 2013;33(3):234-236. DOI: 10.5958/j.0976-0547.33.3.016

Qian Q., Zhang F., Xin Y. Yuan Longping and hybrid rice research. *Rice (New York)*. 2021;14(1):101. DOI: 10.1186/s12284-021-00542-4

Revathi P., Medoju P., Singh A.K., Sundaram R.M., Raju S., Senguttuve P. et al. Efficiency of molecular markers in identifying fertility restoration trait of WA-CMS system in rice. *Indian Journal of Genetics and Plant Breeding*.

2013;73(01):89-93. DOI: 10.5958/j.0019-5200.73.1.012
Sadeqi A., Ahmadi Khah A., Farsi M. Study on the effect of *Rf3* gene for fertility restoration to rice in genetic background of Neda-A line. *Agricultural Biotechnology Journal*. 2014;6(1):139-152. DOI: 10.22103/jab.2014.1297
Saghai-Maroof M.A., Soliman K.M., Jorgensen R.A., Allard R.W. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences of the United States of America*. 1984;81(24):8014-8018. DOI: 10.1073/pnas.81.24.8014
Shidenur S., Singh V.J., Vinod K.K., Krishnan S.G., Ghritlahre S.K., Bollinedi H. et al. Molecular detection of WA-CMS restorers from tropical *japonica*-derived lines, their evaluation for fertility restoration and adaptation. *Plant Breeding*. 2019;138(5):553-567. DOI: 10.1111/pbr.12701
Singh S., Verma S.K., Sahu H., Sahu P.K., Sharma D. Identification of molecular markers for fertility restoration genes in rice (*Oryza sativa* L.). *BIOINFOLET-A Quarterly Journal of Life Sciences*. 2015;12(1b):227-231.
Vejdan R. Investigation of fertility restoring genes in promising rice mutant lines through quantitative and molecular traits (SSR) [MSc thesis]. Sari: Sari Agricultural Sciences and Natural Resources University; 2015. [in Persian]
Xu Y., Yu D., Chen J., Duan M. A review of rice male sterility types and their sterility mechanisms. *Heliyon*. 2023;9(7):e01830. DOI: 10.1016/j.heliyon.2023.e18204
Xu Z., Du Y., Li X., Wang R., Zhao X., Liu Q. et al. Identification and fine mapping of a fertility restorer gene for wild abortive cytoplasmic male sterility in the elite indica rice non-restorer line 9311. *The Crop Journal*. 2023;11(3):887-894. DOI: 10.1016/j.cj.2022.11.009

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