



Phenotypic and SSR Marker-based Screening of Rice (*Oryza sativa* L.) Hybrids for Cold Tolerance

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ABSTRACT

The experiment was conducted during *rabi*, 2022–23 (December, 2022 to May, 2023) at the Regional Agricultural Research Station (RARS), Jagtial, Telangana, India to evaluate the cold tolerance of rice hybrids and parental lines under natural field conditions. During *Kharif* 2022 (July to November, 2022), four Cytoplasmic Male Sterile (CMS) lines and eight restorer lines were crossed to produce 32 rice hybrids. The experimental material comprised 32 hybrids, 12 parents, one susceptible check and one tolerant check. Seedlings were raised in a nursery during December 2022 and exposed to natural low-temperature conditions. Cold tolerance was assessed using the IRRI Standard Evaluation System (SES, 2013), along with SPAD chlorophyll measurements. The results indicated that the hybrids CMS 23A×IR 65514-5-2-19R-1, CMS 14A×IR 65483-14-1-1-4-13R, CMS 64A×IR 65514-5-2-19R-1, and CMS 64A×IR 63870-7-3-2-3-3R were tolerant to cold stress, whereas CMS 64A×JGL 29651 was moderately tolerant. Molecular confirmation of cold tolerance was carried out using 13 SSR markers, of which RM85, RM341, and RM561 exhibited clear polymorphism, while the remaining markers were monomorphic. These markers differentiated tolerant and susceptible genotypes based on specific banding patterns. The tolerant parental lines identified were CMS 64B, CMS 69B, and IR 65514-5-2-19R-1, whereas the tolerant hybrids included CMS 23A×IR 63870-7-3-2-3-3R, CMS 23A×IR 65514-5-2-19R-1, CMS 23A×JGL 27347, CMS 64A×IR 63870-7-3-2-3-3R, CMS 64A×IR 65483-14-1-1-4-13R, CMS 64A×IR 65514-5-2-19R-1, CMS 69A×JGL 21005, and CMS 69A×JGL 24444. These genotypes could serve as valuable sources of cold tolerance in future rice breeding programme.

KEYWORDS: Rice, cold stress, SSR, seedling stage, screening technique, SPAD

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1. INTRODUCTION

Rice (*Oryza sativa* L., 2n=24), a member of the Poaceae family, is the most significant food crop in the world. More than half of the world's population consumes it as their primary source of nourishment and energy. It is well known as the "Global Grain" or model crop. It makes up about 20% of the world's grain production and supplies 23% of calories (Sharma et al., 2013). There are two known cultivated species: *Oryza sativa* and *Oryza glaberrima*. Asian cultivated rice is farmed all over the world, while African cultivated rice is only found in West Africa. More than 100 million households in Asia and Africa rely on it as their main source of income and subsistence (FAO, 2004), earning it the nickname "Grain of Life" (Anonymous, 2025). A total of 535 million tonnes of rice was produced worldwide on 162 million hectares, with an average productivity of 3.3 tonnes per hectare (Food and Agriculture Organization, 2025). Rice is grown on 47.7 million hectares in India, yielding 3.04 tonnes per hectare and producing 145 metric tonnes (Anonymous, 2025). However, roughly just 40% of India's total land is used for rainfed rice, whereas 60% is used for irrigated rice. By 2050, there will be roughly 9.3 billion people on the planet. We must figure out how to produce more rice on the same amount of land in order to ensure that there is enough for everyone (Jat et al., 2022). Farmers in Telangana, as well as in India, cultivate paddy during both the *Kharif* and *Rabi* seasons. However, due to abiotic stresses, particularly due to cold injury during the *Rabi* season, paddy cultivating farmers encounter several limits, such as late crop establishment, protracted crop duration and the crop eventually being exposed to different environmental calamities (Cabello et al., 2014). As a result of these factors, farmers have lower yields and lower-quality byproducts (Zhang et al., 2014). Farmers did not get a good market price, so in order to overcome these issues, it is necessary to develop a cold-resistant, short-duration and high-yielding rice variety for the *Rabi* season (Loitongbam et al., 2017). Conventional plant breeding has achieved partial success in developing low-temperature stress-tolerant crops because of the complexity of stress tolerance traits, the low genetic variance of yield-attributing components under stress conditions and the lack of efficient selection criteria (Cruz et al., 2013). Therefore, it is imperative to find complementary strategies to conventional breeding methodologies, such as molecular marker technologies, linkage maps and advanced genomic tools to develop cold-tolerant rice cultivars (Shahzad et al., 2024). Cold tolerance in rice is a very complex and polygenic trait that is genetically controlled by multiple QTLs. Several low temperature stress tolerance QTLs have been mapped on different genomic regions distributed on all 12 rice chromosomes using various types of molecular markers such as RFLP, AFLP, SSR, STS and SNP, which has facilitated

the identification of chromosomal regions associated with tolerance of low temperature (Schläppi et al., 2023, Emon 2025 and Jo et al., 2025). To date, more than 550 QTLs have been reported for different growth stages (germination, seedling, and reproductive/booting stage) for tolerance of low temperature stress by using DNA-based molecular markers on different genetic backgrounds derived from biparental mapping populations and diverse genetic resources of rice accessions (Guo et al., 2022 and Jan et al., 2023). In the present study, the objective was to identify seedling cold-tolerant rice lines through field screening under natural cold stress conditions and to confirm their tolerance at the molecular level by validating proven SSR markers associated with cold tolerance in the material under study.

2. MATERIALS AND METHODS

The current study was carried out at the Professor Jayashankar Telangana State Agricultural University (PJTSAU), Regional Agricultural Research Station (RARS), Warangal, during *rabi*, 2022-23 (December, 2022 to May, 2023).

During *kharif*, 2022, four cytoplasmic male sterile lines and eight restorer lines were selected and crossed at the Regional Agricultural Research Station (RARS), Jagtial, to produce 32 rice hybrids. Thirty-two hybrids, 12 parents (eight testers and four lines), one susceptible check, and one tolerant check were sown on 14th December 2022 in a 10×1 m² nursery bed for cold screening during *Rabi* 2022-23 at the Regional Agricultural Research Station (RARS), Jagtial, and the seedlings were exposed to low temperature under natural field conditions for cold screening. During this period, SPAD readings were recorded, and both maximum and minimum temperatures were measured. The Standard Evaluation System for Rice (Anonymous, 2013) (Table 1) was used to score seedlings for cold tolerance before uprooting them for transplantation at 20 DAS and 30 DAS.

Table 1: Cold scoring is done by using 0-9 Standard Evaluation System (Anonymous, 2013) scale (for seedlings).

Scale	Symptom	Tolerance level
0-1	No damage to leaves, normal leaf colour	Strongly tolerant
2-3	The tips of the leaves were slightly dried, folded, and light green	Tolerant
4-5	Some seedlings were moderately folded and wilted, 30-50% seedlings dried, pale green to yellowish leaves	Moderately tolerant
6-7	Seedlings severely rolled and dried; reddish-brown leaves	Sensitive
8-9	Most seedlings are dead and dying	Highly sensitive

2.1. DNA isolation

Total genomic DNA was extracted using the standard CTAB extraction protocol (Murray et al., 1980). Leaf samples of 20–25-day-old seedlings, measuring 5–10 cm in length, were collected, cut into small pieces, and placed in 1.5 ml Eppendorf tubes along with an iron ball. A total of 200 µl of CTAB buffer was added to the contents, which were then subjected to a tissue lyser for 3 minutes. An additional 400 µl of CTAB buffer and 600 µl of chloroform were added and mixed thoroughly by gently inverting the tube approximately 100 times. The mixture was centrifuged at 13,000 rpm for 15 minutes at 4°C. Subsequently, the resulting supernatant was carefully transferred to a fresh Eppendorf tube. An equal volume of isopropanol was added to the clear supernatant to induce DNA precipitation. The mixture was then incubated at -20°C overnight. Thereafter, the solution was mixed about 10 times and centrifuged at 10,000 rpm for 10 minutes. The resulting supernatant was gently decanted without disturbing the pellet. To eliminate salts, approximately 200 µl of 70% ethanol was added to the pellet, which was then centrifuged at 7,000 rpm for 7 minutes at room temperature. The supernatant was discarded, and the pellet was air-dried until the smell of ethanol dissipated. Finally, about 100 µl of T10E1 buffer was added to dissolve the pellet.

2.2. PCR analysis

Genomic DNA extracted from 46 different rice genotypes was subjected to Polymerase Chain Reaction (PCR) amplification using 13 microsatellite markers, i.e., Simple Sequence Repeats (SSRs). To initiate the PCR reaction, 2 µl of genomic DNA was added to individual 200 µl PCR tubes. The reaction mixture comprised a total volume of 10 µl, containing the components listed in Table 2. The PCR process was carried out following the regimen outlined in Table 3. To amplify the targeted genomic DNA, stages 2 to 4 were repeated 35 times. The annealing temperature for each primer was determined by conducting PCR experiments at

temperatures ranging from 55 to 60°C.

Before loading the PCR tubes into the thermal cycler (either Applied Biosystems Veriti or Eppendorf), a brief spin was applied to the reaction mixture to ensure thorough mixing of its constituents. Following PCR amplification, the resulting products were stored at 4°C for short intervals and at -20°C for longer durations.

Table 3: PCR temperature regime

Sl. No.	Step	Temperature	Time	Cycles
1	Initial denaturation	95°C	5 minutes	1
2	Denaturation	94°C	30 seconds	35
3	Annealing	57–60°C	30 seconds	
4	Extension	72°C	1 minute	
5	Final extension	72°C	7 minutes	1
6	4°C		∞	Storage

2.3. Gel electrophoresis

To assess the PCR products, a 4% agarose gel was employed. About 4 g of agarose was mixed in 100 ml of 1×TBE solution. The mixture was heated until the agarose completely dissolved, and ethidium bromide (3 µl per 100 ml of gel) was added to the solution, which was subsequently cast in a tray with combs. DNA samples and a 100 bp ladder were loaded onto the solidified gel. The electrodes were connected to a power supply set at 85 V and 30–35 mA, with the cathode (-ve) placed at the loading side and the anode (+ve) positioned at the far end of the gel. The samples were photographed under UV light using a gel documentation system. These images were then evaluated and utilized for subsequent analysis.

3. RESULTS AND DISCUSSION

3.1. Screening of hybrids and parents for cold tolerance

After transplanting, seedlings often experienced transplant shock, which was a temporary stress that slowed their growth until they recovered and established roots in the new soil. This recovery period became longer when the plants were exposed to cold stress. Low temperatures negatively affected plant development, leading to poor germination, seedling mortality, leaf chlorosis (yellowing of leaves), stunted growth, and reduced tillering during the seedling and vegetative stages. A total of 46 genotypes (32 hybrids, 12 parents, and 2 checks) were sown at the Regional Agricultural Research Station (RARS), Jagtial, including tolerant (Tellahamsa) and susceptible (JGL 1798) checks. The seedlings were exposed to low temperatures under natural field conditions. The maximum and minimum

Table 2: PCR components, along with their concentration and volume, used for the PCR reaction

Component	Concentration	Reaction volume
Taq buffer (10X) with MgCl ₂	1X	1.0 µl
dNTP mix	2.5 mM	1.0 µl
Taq DNA polymerase	3U µl ⁻¹	0.2 µl
Forward primer	0.2 µM	0.5 µl
Reverse primer	0.2 µM	0.5 µl
Genomic DNA	50 ng µl ⁻¹	3.0 µl
Sterile distilled water	-	3.8 µl
Total	-	10 µl

temperatures were measured during the seedling growth stage. Sowing was done on 14th December 2022, and screenings were conducted at 20 and 30 days after sowing, within 5–7 days of the occurrence of the lowest temperature, to expose the seedlings to cold stress (Akter et al., 2025). A yellowing score was recorded at the point of critical low-temperature exposure. According to scoring (Anonymous,

2013), 23 of the 32 hybrids were tolerant (T), 9 hybrids were moderately tolerant (MT), and none of the entries showed highly sensitive (HS) reactions. Among the parents, 11 were tolerant (T), and one was moderately tolerant (MT) (Table 4). These results were similar to the earlier findings of Thippeswamy et al. (2015), Rasel et al. (2020), and Kanya et al. (2022).

Table 4: Scoring of parental lines, hybrids and checks evaluated for cold incidence

Sl. No.		SPAD readings		Anonymous, 2013	Cold reaction
		20 DAS	30 DAS		
Hybrids					
1.	CMS 14A×IR 63870-7-3-2-3-3R	27.4	25.8	3	T
2.	CMS 14A×IR 63877-43-2-1-3-1R	25.4	23.6	3	T
3.	CMS 14A×IR 65483-14-1-1-4-13R	26.5	24.1	3	T
4.	CMS 14A×IR 65514-5-2-19R-1	27.1	25.6	4	MT
5.	CMS 14A×JGL 21005	26.3	24.6	3	T
6.	CMS 14A×JGL 24444	26.0	24.1	3	T
7.	CMS 14A×JGL 27347	24.4	22.4	3	T
8.	CMS 14A×JGL 29651	26.9	26.2	3	T
9.	CMS 23A×IR 63870-7-3-2-3-3R	26.2	24.4	3	T
10.	CMS 23A×IR 63877-43-2-1-3-1R	24.6	21.6	4	MT
11.	CMS 23A×IR 65483-14-1-1-4-13R	27.2	25.2	4	MT
12.	CMS 23A×IR 65514-5-2-19R-1	25.0	25.2	2	T
13.	CMS 23A×JGL 21005	24.9	25.3	3	T
14.	CMS 23A×JGL 24444	25.8	24.4	3	T
15.	CMS 23A×JGL 27347	26.9	26.0	2	T
16.	CMS 23A×JGL 29651	27.2	25.5	4	MT
17.	CMS 64A×IR 63870-7-3-2-3-3R	25.9	23.8	2	T
18.	CMS 64A×IR 63877-43-2-1-3-1R	25.8	25.6	4	MT
19.	CMS 64A×IR 65483-14-1-1-4-13R	26.3	23.5	2	T
20.	CMS 64A×IR 65514-5-2-19R-1	22.2	21.4	3	T
21.	CMS 64A×JGL 21005	27.4	22.8	3	T
22.	CMS 64A×JGL 24444	24.4	21.7	4	MT
23.	CMS 64A×JGL 27347	25.5	21.7	3	T
24.	CMS 64A×JGL 29651	28.7	23.5	4	MT
25.	CMS 69A×IR 63870-7-3-2-3-3R	26.8	23.3	3	T
26.	CMS 69A×IR 63877-43-2-1-3-1R	25.7	23.8	4	MT
27.	CMS 69A×IR 65483-14-1-1-4-13R	26.7	25.8	4	MT
28.	CMS 69A×IR 65514-5-2-19R-1	25.3	21.8	3	T
29.	CMS 69A×JGL 21005	26.0	25.6	2	T
30.	CMS 69A×JGL 24444	27.6	25.9	2	T
31.	CMS 69A×JGL 27347	27.7	24.1	3	T

Table 4: Continue...

Sl. No.		SPAD readings		Anonymous, 2013	Cold reaction
		20 DAS	30 DAS		
32.	CMS 69A×JGL 29651	27.7	25.4	3	T
<u>Parents</u>					
33.	CMS 14B	23.7	21.8	3	T
34.	CMS 23B	26.3	21.7	3	T
35.	CMS 64B	25.3	27.5	2	T
35.	CMS 69B	26.6	25.6	2	T
37.	IR 63870-7-3-2-3-3R	22.8	21.4	3	T
38.	IR 63877-43-2-1-3-1R	26.5	22.2	4	MT
39.	IR 65483-14-1-1-4-13R	26.3	26.0	3	T
40.	IR 65514-5-2-19R-1	29.8	25.7	2	T
41.	JGL 21005	28.4	25.9	2	T
42.	JGL 24444	24.2	21.8	3	T
43.	JGL 27347	24.6	23.3	3	T
44.	JGL 29651	29.4	21.9	3	T
<u>Checks</u>					
45.	Tellahamsa	26.3	27.2	2	T
46.	JGL 1798	20.2	19.9	6	S
	Minimum temperature	18.9°C	12.6°C		
	Maximum temperature	30.3°C	30.7°C		

HT: Highly tolerant; T: Tolerant; MT: Moderately tolerant; S: Sensitive; HS: Highly sensitive

3.2. Molecular confirmation for cold tolerance

The main aim of this work was to validate rice genotypes for cold tolerance using previously known SSR markers associated with cold tolerance. In both the laboratory and the field, 46 rice genotypes were morphologically evaluated for cold tolerance. It was also necessary to confirm their cold stress tolerance ability at the molecular level; therefore, molecular screening of these genotypes was performed for the presence of already known cold-tolerant QTLs with available linked SSR markers, using Tellahamsa as a positive control and JGL 1798 as a susceptible control. In the present study, thirteen SSR markers were used for screening cold tolerance (Table 5). Out of the 13 markers, three markers showed clear amplification and polymorphism among the rice genotypes, namely RM85, RM341, and RM561, while eight markers showed a monomorphic banding pattern, namely RM200, RM239, RM284, RM14978, RM6651, RM14960, RM6547, and RM11239 (Amirova et al., 2024). Two markers, RM13335 and RM18776, were not amplified and were indicated by missing bands. Among the thirteen SSRs, the eight monomorphic SSR markers and the two non-amplified markers were excluded from the analysis. All three markers (RM85, RM341, and RM561) were able

to differentiate eight tolerant hybrids and three tolerant parents (Table 6) at their respective band sizes. Along with these, they also differentiated 24 susceptible hybrids and nine susceptible parents from the remaining genotypes at their respective band sizes. RM85 showed cold-tolerant genotype-specific alleles at 95 bp and susceptible genotype-specific alleles at 107 bp (Figure 1). RM341 showed cold-tolerant genotype-specific alleles at 170 bp and susceptible genotype-specific alleles at 185 bp (Figure 2). RM561 differentiated cold-tolerant genotype-specific alleles at 190 bp and susceptible genotype-specific alleles at 200 bp. The tolerant parent genotypes identified by these three markers were CMS 64B and CMS 69B (lines) and IR 65514-5-2-1-19R-1 (tester). The tolerant hybrid genotypes identified by these three markers were CMS 23A×IR 63870-7-3-2-3-3R, CMS 23A×IR 65514-5-2-1-19R-1, CMS 23A×JGL 27347, CMS 64A×IR 63870-7-3-2-3-3R, CMS 64A×IR 65483-14-1-1-4-13R, CMS 64A×IR 65514-5-2-1-19R-1, CMS 69A×JGL 21005, and CMS 69A×JGL 24444. Similar results were also reported by Thippeswamy et al. (2015), Biswas et al. (2019), Akter et al. (2022), Singh et al. (2022), and Lee et al. (2025).

The findings of the present study are consistent with earlier

Table 5: List of SSR primers used for PCR amplification in the present study				
Sl. No.	Primer	Sequence		Expected product size (bp)
1.	RM85	FP	CCAAAGATGAAACCTGGATTG	95
		RP	GCACAAGGTGAGCAGTCC	
2.	RM341	FP	CAAGAAACCTCAATCCGAGC	170
		RP	CTCCTCCCGATCCCAATC	
3.	RM561	FP	GAGCTGTTTTGGACTACGGC	190
		RP	GAGTAGCTTTCTCCCACCCC	
4.	RM 200	FP	CGCTAGGGAATTTGGATTGA	170
		RP	CGATGAGCAGGTATCGATGAGAAG	
5.	RM239	FP	TACAAAATGCTGGGTACCCC	130
		RP	ACATATGGGACCCACCTGTC	
6.	RM284	FP	ATCTCTGATACTCCATCCATCC	160
		RP	CCTGTACGTTGATCCGAAGC	
7.	RM14978	FP	TATCTGCAGGTGCGTGTAATGG	226
		RP	GCATATAGAGCGAGTAAGCGAGAGG	
8.	RM6651	FP	AAGCATCAAGTAGGCAGCACAGC	121
		RP	GGAAGGAGGGAGATGAGATGAGC	
9.	RM14960	FP	GTCAAGACCACCGCCTGGTTCC	193
		RP	CGACGAGGAGGTTATGGGTCTGG	
10.	RM6547	FP	ATGACACACCACCACCAACG	72
		RP	GGAAGCGCAGCATCAACAAAGG	
11.	RM11239	FP	GGAGTTAGTGCAAGCAATTCAGG	441
		RP	TGGTCAGGTAGACCACAGATCC	
12.	RM13335	FP	TATGCCAAGAGGAATCCTGAAGC	300
		RP	GCACTCACACTGATCTGGACAGG	
13.	RM18776	FP	CTCCAGGAGGGTACAAATTCTGC	183
		RP	CCATTGGAACATAGCAAGTGATCG	

FP: Forward primer; RP: Reverse primer

reports demonstrating that the combined use of phenotypic screening and molecular marker analysis is an effective strategy for identifying cold-tolerant rice genotypes. Leaf yellowing and the reduction in SPAD chlorophyll meter readings (SCMR) proved to be reliable indicators of seedling cold tolerance, which agrees with the observations of Thippeswamy et al. (2015) and Singh et al. (2022), who also identified leaf yellowing and chlorophyll retention as important traits for distinguishing tolerant and susceptible genotypes under low-temperature stress. The identification of tolerant genotypes with lower leaf yellowing scores and smaller reductions in SCMR further confirms the usefulness of these morpho-physiological traits for screening cold tolerance.

Among the 32 SSR markers evaluated in the present study, only RM85 exhibited a clear polymorphic banding pattern capable of differentiating cold-tolerant and susceptible genotypes. Similar findings have been reported by Thippeswamy et al. (2015), who identified RM85 as a marker associated with seedling cold tolerance. Likewise, Singh et al. (2022) reported that only 33 of the 56 SSR markers evaluated were polymorphic, with RM336, RM566 and RM1349 being the most informative for distinguishing cold tolerance. Akter et al. (2022) also observed that only a subset of the screened SSR markers effectively differentiated tolerant and susceptible genotypes, indicating that the usefulness of molecular markers varies with the genetic background of the germplasm.

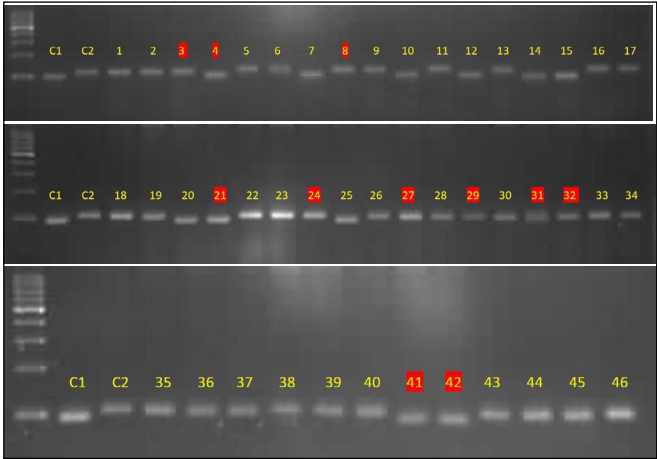


Figure 1: Representative amplification pattern with simple sequence repeat marker (SSR) RM85. The lane numbers shown on the top of the gel indicate C1-Tellahamsa (tolerant check), C2- JGL 1798 (susceptible check), while lane numbers 1-46 indicate the list of genotypes used in the present investigation and the highlighted numbers indicate the cold-tolerant lines listed in Table 6

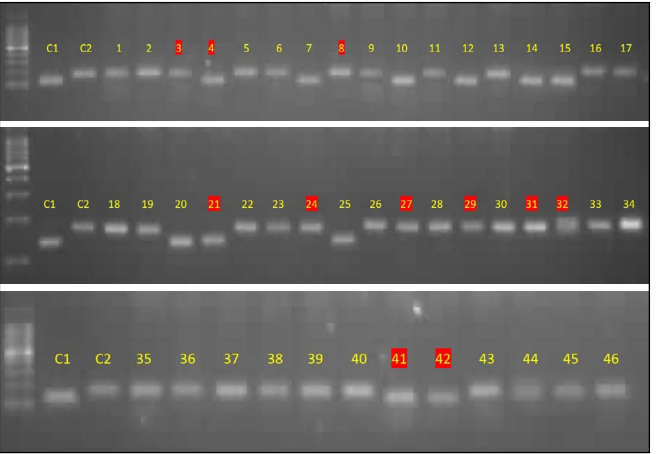


Figure 2: Representative amplification pattern with simple sequence repeat marker (SSR) RM341. The lane numbers shown on the top of the gel indicate C1-Tellahamsa (tolerant check), C2- JGL 1798 (susceptible check), while lane numbers 1-46 indicate the list of genotypes used in the present investigation and the highlighted numbers indicate the cold-tolerant lines listed in Table 6

Recent findings by Lee et al. (2025) further support the present results by demonstrating that molecular markers are valuable tools for identifying cold-tolerant rice genotypes when used in combination with phenotypic evaluation. In their study, markers developed for the *HAN1*, *COLD11* and *COLD1* genes successfully differentiated cold-tolerant and susceptible rice germplasm, but the authors also showed that combining multiple cold-tolerance alleles provided greater prediction accuracy than relying on a single marker. Their study emphasized that cold tolerance is a polygenic trait

Table 6: List of cold-tolerant parents and hybrids identified by RM85, RM341 and RM561

Sl. No.	Genotypes	Lane number
Parents		
1.	CMS 64B	3
2.	CMS 69B	4
3.	IR 65514-5-2-1-19R-1	8
Hybrids		
1.	CMS 23A×IR 63870-7-3-2-3-3R	21
2.	CMS 23A×IR 65514-5-2-1-19R-1	24
3.	CMS 23A×JGL 27347	27
4.	CMS 64A×IR 63870-7-3-2-3-3R	29
5.	CMS 64A×IR 65483-14-1-1-4-13R	31
6.	CMS 64A×IR 65514-5-2-1-19R-1	32
7.	CMS 69A × JGL 21005	41
8.	CMS 69A × JGL 24444	42

and that only informative markers can reliably discriminate tolerant genotypes across diverse genetic backgrounds. Consequently, the polymorphism observed for RM85 in the present investigation further validates its usefulness as a marker linked to seedling cold tolerance, although its association should be confirmed through co-segregation analysis using the recombinant inbred line (RIL) population under development. Such validation will facilitate its utilization in marker-assisted breeding for developing cold-tolerant rice cultivars.

4. CONCLUSION

Cold screening showed that among 12 parents, 11 were tolerant and one was moderately tolerant. Among 32 hybrids, 23 were tolerant, nine were moderately tolerant, and none were highly sensitive. Molecular screening using 13 SSR markers identified three polymorphic markers (RM85, RM341 and RM561) that differentiated tolerant and susceptible genotypes. The genotypes CMS 64B, CMS 69B, and IR 65514-5-2-1-19R-1 were identified as tolerant. The tolerant hybrids included CMS 23A×IR 63870-7-3-2-3-3R, CMS 23A×IR 65514-5-2-1-19R-1, CMS 23A×JGL 27347, CMS 64A×IR 63870-7-3-2-3-3R, CMS 64A×IR 65483-14-1-1-4-13R, CMS 64A×IR 65514-5-2-1-19R-1, CMS 69A×JGL 21005, and CMS 69A×JGL 24444. These tolerant sources could be used in breeding programmes to develop cold-tolerant cultivars.

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