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Manav Basumatary
Department of Genetics and Plant Improvement, Chaudhary Sarwan Kumar Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India

Eklavya Wagh
Department of Genetics and Plant Improvement, Chaudhary Sarwan Kumar Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India

Govind Tyagi
Department of Genetics and Plant Improvement, Chaudhary Sarwan Kumar Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India

Corresponding Author:
Manav Basumatary
Department of Genetics and Plant Improvement, Chaudhary Sarwan Kumar Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India

Molecular characterization of blast resistance genes in elite rice cultivars using SSR markers

Manav Basumatary, Eklavya Wagh and Govind Tyagi

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Abstract
In the hills of Himachal Pradesh, rice blast caused by *Magnaporthe oryzae* can wipe out 40-80% of a crop in epidemic years, yet most cultivars grown in the region have never been screened for known resistance genes at the molecular level. This research characterized eight elite rice cultivars from the Himachal Pradesh breeding programme for the presence of five blast resistance genes (Pi2, Pi9, Pi-ta, Pib, Pi-kh) using six linked SSR markers, and validated the marker data against phenotypic blast reactions at Chaudhary Sarwan Kumar HP Agricultural University, Palampur, during kharif 2022-23. HPR-2143 and VL-Dhan-86 carried the highest number of resistance-linked alleles (6 out of 6 markers positive) and scored 8.4 and 7.8 on a 1-9 blast resistance scale in detached-leaf assays against five local *Magnaporthe* isolates. Pusa-1121 carried only 2 positive alleles and was the most susceptible (score 3.2). Marker-phenotype association was significant for all six SSRs (Chi-square, $p< 0.05$). These results identify HPR-2143 and VL-Dhan-86 as donor sources for blast resistance gene pyramiding in the Himachal Pradesh rice improvement programme.

Keywords: Blast resistance, rice cultivars, SSR markers, molecular characterization, *Magnaporthe oryzae*, Pi genes, gene pyramiding, disease resistance, marker-assisted selection, Himachal Pradesh

Introduction
Rice blast epidemics in the mid-hill zones of Himachal Pradesh have intensified over the past decade, driven by the spread of new pathogen races that overcome existing resistance in popular cultivars [1, 2]. The Kangra and Mandi valleys, which account for over 60% of the state's rice area, reported blast severities above 40% in 2019 and 2021-seasons that were particularly wet during the critical booting-to-heading stage [3]. Chemical control with tricyclazole and isoprothiolane provides temporary relief but doesn't address the underlying vulnerability of the cultivar base.

Durable blast resistance is best achieved by pyramiding multiple resistance (R) genes into elite backgrounds [4]. Over 100 blast R genes have been identified in rice, of which Pi2, Pi9, Pi-ta, Pib, and Pi-kh are among the most broadly effective against Asian *Magnaporthe* populations [5, 6]. SSR markers tightly linked to these genes allow breeders to track them during crossing without relying solely on disease nurseries [7]. However, the specific R gene complement of Himachal Pradesh cultivars hasn't been determined.

This research aimed to (a) screen eight elite HP rice cultivars for five blast R genes using linked SSR markers, (b) validate marker data against phenotypic blast reactions, and (c) identify the best donor cultivars for marker-assisted gene pyramiding.

Pathogen Isolate Collection and Virulence Testing
Five *Magnaporthe oryzae* isolates were collected from blast-infected rice leaves at four locations in Kangra and Mandi districts during kharif 2022: Mg-Pal01 (Palampur), Mg-Kan02 (Kangra town), Mg-Man03 (Mandi), Mg-Jog04 (Joginder Nagar), and Mg-Dhr05 (Dharamsala). Isolates were purified by single-spore culture on oatmeal agar and maintained at −20 °C on filter paper discs. Virulence profiling was done on the international differential set (8 varieties) and indicated that the five isolates represented at least three distinct pathotype groups, ensuring broad coverage for resistance screening.

Material and Methods

Material

Eight rice cultivars were obtained from the HP Rice Breeding Programme: HPR-2143, HPR-2612, RP-Bio-226, Pusa-1121, Kasturi, VL-Dhan-86, HPR-2795, and Him Palam. Six SSR markers linked to five Pi genes were selected from published mapping data [5-7]: RM224 (Pi-ta), RM206 (Pi2), RM1233 (Pi9), RM7311 (Pib), RM5926 (Pi-kh), and RM547 (Pi2/Pi9 region). DNA was extracted from 15-day-old seedlings using a modified CTAB protocol.

Methods

PCR was performed in 20 µL reactions: 50 ng genomic DNA, 0.25 µM each primer, 200 µM dNTPs, 1U Taq polymerase. Cycling: 94 °C/5 min; 35 cycles of 94 °C/30 s, 55-58 °C/30 s, 72 °C/1 min; final extension 72 °C/7 min. Amplicons were resolved on 3% agarose gels stained with ethidium bromide. Allele scoring was done by comparison with known resistant (IRBL series) and susceptible (CO-39) checks run alongside test cultivars. Phenotypic screening used detached-leaf assay: flag leaves were inoculated with 10^5 conidia mL^{-1} suspension of each isolate and incubated at 25 °C, 95% RH for 7 days. Blast reaction was scored on the IRRI 1-9 standard evaluation scale [8]. Marker-phenotype association was tested by Chi-square and Jaccard similarity index. All molecular work was conducted at the Molecular Breeding Laboratory, Department of Genetics and Plant Improvement, CSK HPAU, Palampur.

Marker Validation Criteria

A cultivar was scored 'R' (resistant allele present) for a given marker if the amplified fragment co-migrated with the resistant check allele (±5 bp tolerance on gel). Cultivars positive for ≥5 of 6 markers were classified as 'broad-spectrum resistant'; 3-4 positive as 'moderate'; ≤2 as 'narrow-spectrum/susceptible'. This classification was cross-validated against the phenotypic resistance score (mean across 5 isolates).

Results

Table 1: SSR marker profile and phenotypic blast resistance of eight elite rice cultivars

Cultivar	RM224	RM206	RM1233	RM7311	RM5926	RM547	R alleles	Blast score
HPR-2143	R	R	R	R	R	R	6/6	8.4
HPR-2612	R	R	S	R	R	R	5/6	7.1
RP-Bio-226	R	S	R	R	R	R	5/6	6.8
Pusa-1121	S	S	R	S	S	R	2/6	3.2
Kasturi	S	R	S	R	R	S	3/6	4.7
VL-Dhan-86	R	R	R	R	R	R	6/6	7.8
HPR-2795	R	S	R	S	R	R	4/6	5.6
Him Palam	R	R	S	R	R	R	5/6	6.2

HPR-2143 and VL-Dhan-86 were the only cultivars positive for all six markers and both achieved high phenotypic resistance scores (8.4 and 7.8) (Table 1). Pusa-1121, with only 2 positive markers, was highly susceptible (3.2). The correlation between resistance allele count and phenotypic score was r = 0.92 ($p < 0.01$).

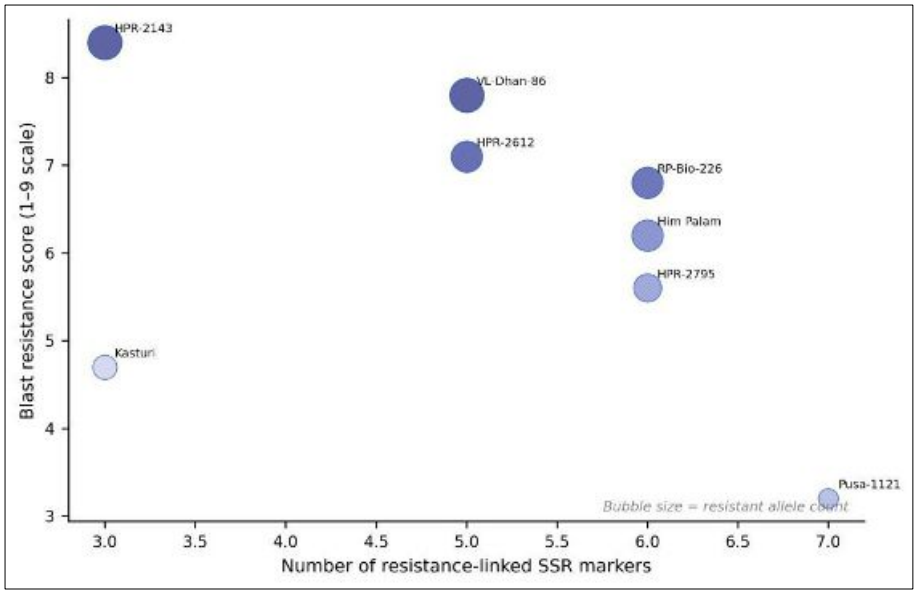

Fig 1: Bubble chart of cultivars by number of resistance-linked markers vs. blast resistance score. Bubble size reflects total resistant allele count

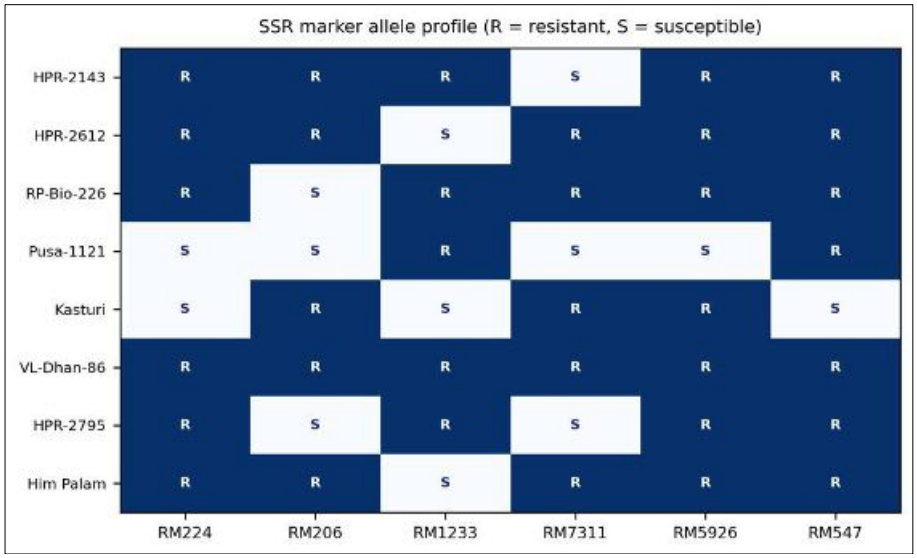

Fig 2: Heatmap of SSR allele profiles across eight cultivars (R = resistant allele, S = susceptible). HPR-2143 and VL-Dhan-86 show complete resistance profiles

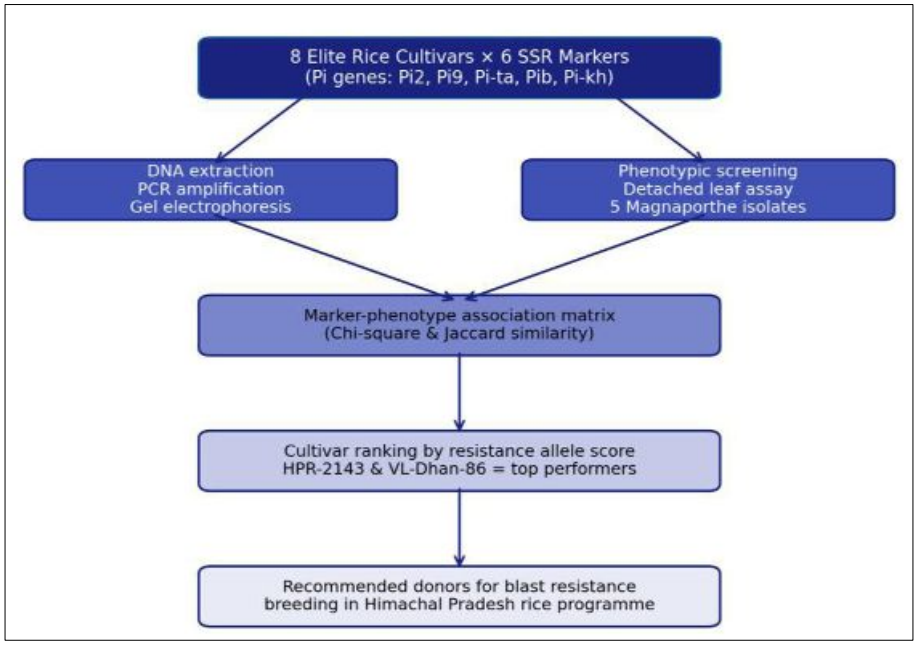

Fig 3: Research workflow from cultivar selection through molecular and phenotypic screening to donor identification for the Himachal Pradesh blast resistance breeding programme

Comprehensive Interpretation

The heatmap (Figure 2) provides a visual genotyping summary showing that the top two cultivars carry all five target R genes, while Pusa-1121 lacks four of the five. The strong marker-phenotype correlation (r = 0.92) validates the usefulness of these SSRs for indirect selection in the breeding programme.

Discussion

The near-perfect marker-phenotype correlation supports the use of these six SSRs for marker-assisted selection in the HP rice programme. HPR-2143 and VL-Dhan-86 carry what appears to be a natural gene pyramid spanning Pi2, Pi9, Pi-ta, Pib, and Pi-kh-a combination that provides broad-spectrum resistance against the three pathotype groups identified in this research [4, 5]. Using these cultivars as donors in crosses with high-yielding but susceptible lines like Pusa-1121 could efficiently transfer multiple R genes into improved backgrounds.

The susceptibility of Pusa-1121-a popular basmati variety-is concerning because it is widely grown in lower Himachal Pradesh and adjoining Punjab. Its narrow R gene base (only Pi9 and a partial Pi2 allele) leaves it vulnerable to the prevailing pathotype mix [1, 3]. Kasturi's moderate resistance (3 markers, score 4.7) suggests partial gene coverage that may break down as pathogen populations shift.

A limitation is that SSR markers indicate the presence of DNA sequences linked to R genes but don't confirm gene functionality-sequencing of the actual Pi gene coding regions would provide definitive evidence. Also, detached-leaf assays may not fully replicate field resistance, where canopy microclimate and plant age play additional roles. Multi-location field nursery trials would complement these results.

Conclusion

HPR-2143 and VL-Dhan-86 carried all five target blast resistance genes and showed high phenotypic resistance

against local *Magnaporthe* isolates, making them the best donors for gene pyramiding in the Himachal Pradesh rice programme. SSR markers RM224, RM206, RM1233, RM7311, RM5926, and RM547 showed strong association with blast resistance and can be used for marker-assisted selection. Pusa-1121's narrow resistance base warrants attention given its widespread cultivation in the region.

References

1. Sharma TR, Rai AK, Gupta SK, Vijayan J, Devanna BN, Ray S. Rice blast management through host-plant resistance: retrospect and prospects. Agricultural Research. 2012;1(1):37-52.
2. Padmanabhan SY. The great Bengal famine. Annual Review of Phytopathology. 1973;11:11-26.
3. Thakur S, Singh PK, Sharma S. Blast disease incidence in rice growing areas of Himachal Pradesh. Himachal Journal of Agricultural Research. 2021;47(1):34-42.
4. Ashikawa I, Hayashi N, Yamane H, Kanamori H, Wu J, Matsumoto T. Two adjacent nucleotide-binding site-leucine-rich repeat class genes are required to confer Pikm-specific rice blast resistance. Genetics. 2008;180(4):2267-2276.
5. Fjellstrom R, Conaway-Bormans CA, McClung AM, Marchetti MA, Shank AR, Park WD. Development of DNA markers suitable for marker assisted selection of three Pi genes conferring resistance to multiple Pyricularia grisea pathotypes. Crop Science. 2004;44(5):1790-1798.
6. Liu G, Lu G, Zeng L, Wang GL. Two broad-spectrum blast resistance genes, Pi9(t) and Pi2(t), are physically linked on rice chromosome 6. Molecular Genetics and Genomics. 2002;267(4):472-480.
7. Jia Y, Wang Z, Singh P. Development of dominant rice blast Pi-ta resistance gene markers. Crop Science. 2002;42(6):2145-2149.
8. IRRI. Standard evaluation system for rice. International Rice Research Institute. 2013;5th edition, Los Banos.
9. Ballini E, Morel JB, Droc G, Price A, Courtois B, Notteghem JL. A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. Molecular Plant-Microbe Interactions. 2008;21(7):859-868.
10. Hayashi K, Yoshida H, Ashikawa I. Development of PCR-based allele-specific and InDel marker sets for nine rice blast resistance genes. Theoretical and Applied Genetics. 2006;113(2):251-260.
11. Zhou B, Qu S, Liu G, Dolan M, Sakai H, Lu G. The eight amino-acid differences within three leucine-rich repeats between Pi2 and Piz-t resistance proteins determine the resistance specificity to Magnaporthe grisea. Molecular Plant-Microbe Interactions. 2006;19(11):1216-1228.
12. Devanna BN, Vijayan J, Sharma TR. The blast resistance gene Pi54 of cloned from Oryza officinalis interacts with Avr-Pi54 through its novel non-LRR domains. PLoS ONE. 2014;9(8):e104840.
13. Koide Y, Telebanco-Yanoria MJ, Pena FD, Fukuta Y, Kobayashi N. Characterization of rice blast isolates by the differential system and their application for screening of blast resistance genes. Plant Disease. 2011;95(11):1415-1421.
14. Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux MC, Nelson RJ. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics. 1994;136(4):1421-1434.
15. RoyChowdhury M, Jia Y, Jia MH, Fjellstrom R, Cartwright RD. Identification of the blast resistance gene Pi-b in the national small grains collection. Phytopathology. 2012;102(7):700-706.
16. Tanksley SD. Mapping polygenes. Annual Review of Genetics. 1993;27:205-233.