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Genetic Diversity Core Set Development and Marker Trait Associations for Agronomic Traits in Rice Germplasm from the Northwestern Himalayas

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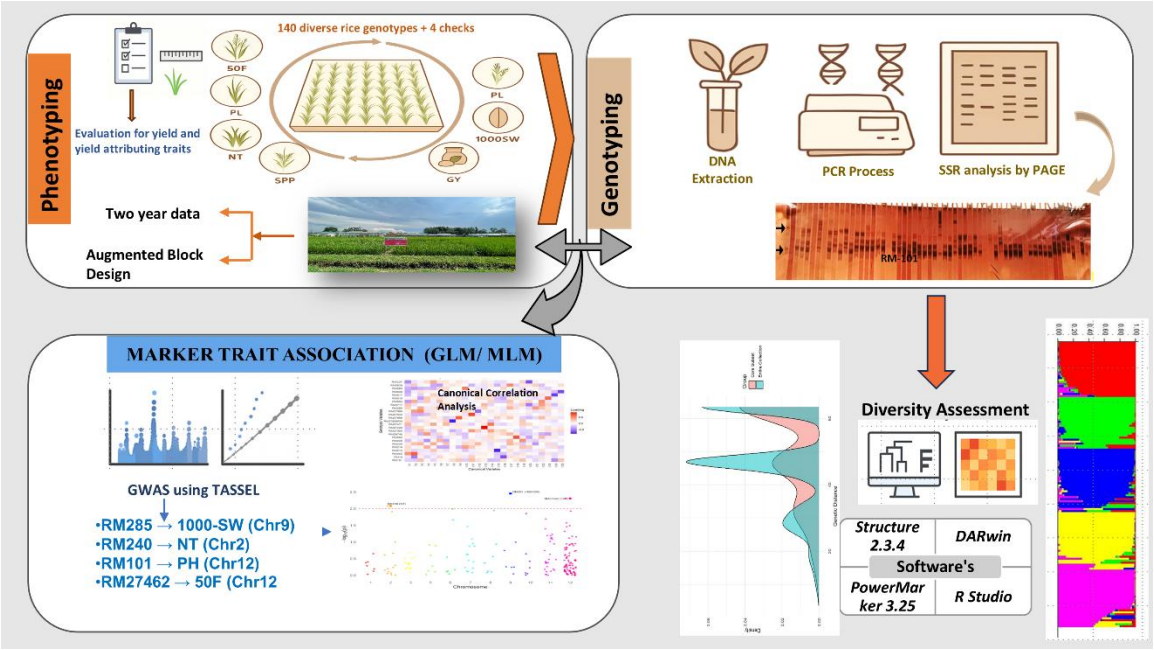
Abstract

This study investigates the genetic diversity and marker–trait relationships in temperate rice germplasm from the North-Western Himalayas. A total of 144 rice accessions (140 test genotypes and four check varieties) were evaluated for seven key agronomic traits days to 50% flowering (50F), plant height (PH), panicle length (PL), number of effective tillers (NT), spikelets per panicle (SPP), grain yield per plant (GY), and 1000-seed weight (1000SW) across two years and two locations. Genotyping using 25 simple sequence repeat (SSR) markers revealed substantial genetic variation, with high polymorphism (mean polymorphic information content, PIC = 0.6874) and considerable allelic diversity. Population structure analysis grouped the genotypes into five distinct clusters, highlighting the genetic uniqueness of traditional landraces and introduced lines. A core subset of 20 genotypes was identified, capturing broad allelic diversity while maintaining genetic divergence. Marker–trait association analysis using General Linear Model (GLM) and Mixed Linear Model (MLM) identified four significant associations ($p < 0.01$): RM285 (chromosome 9) with 1000-seed weight, RM240 (chromosome 2) with number of effective tillers, RM101 (chromosome 12) with plant height, and RM27462 (chromosome 12) with days to 50% flowering. These associations explained a moderate proportion of phenotypic variation ($R^2 = 4.1–5.6\%$), reflecting the polygenic nature of yield-related traits. The findings provide useful insights into the genetic diversity of temperate rice germplasm and identify potential markers for further validation in marker-assisted selection programs.

Keywords: Temperate rice; population structure; core subset; canonical correlation analysis; marker–trait association; QTL mapping; yield traits

Graphical Abstract



Plain Summary:

Rice breeding for the temperate regions of the North-Western Himalayas requires diverse genetic resources and reliable markers for important traits. We evaluated 144 rice accessions across two years and locations and found substantial genetic diversity, including five distinct genetic groups. A core set of 20 accessions captured much of this diversity. Four DNA markers were associated with flowering time, plant height, effective tillers, and seed weight. Although each marker explained only a small proportion of trait variation, these findings provide valuable genetic resources and promising markers for further validation and use in rice breeding programs.

1. Introduction

Rice (Oryza sativa L.) is one of the most important cereal crops, providing food for more than half of the world's population. It ranks second only to maize in global cereal production, and its cultivation and consumption are critical to food security worldwide.

Despite the success of high-yielding modern cultivars, limited genetic variability within the cultivated gene pool constrains further gains in yield potential and stress resilience. To overcome this bottleneck, plant breeders are turning to the vast genetic diversity preserved in traditional rice landraces.

To date, most studies on temperate japonica landraces have focused on descriptive inventories or limited agronomic evaluations, leaving significant gaps in our understanding of their genetic diversity, population structure, and adaptive mechanisms. Moreover, the impacts of changing temperature regimes and altered hydrological cycles on their phenology and yield stability remain poorly quantified, hindering evidence-based conservation and utilization strategies (Dar, 2019; Pandita et al., 2023). The molecular characterization of crop germplasm underpins broader genomic interventions in plant science, from genome editing and transcriptomics to microbial ecology and disease resistance, collectively advancing precision crop improvement (Ali and Xiao, 2025; Qian et al., 2024; Fu et al., 2024; He et al., 2025; Liu et al., 2025; Ali et al., 2019; Soylu et al., 2025). In this context, SSR-based diversity assessment and marker–trait association remain foundational tools for sustainable rice improvement in temperate ecosystems.

Marker–trait association studies have emerged as a powerful approach for dissecting the genetic basis of complex traits in rice, enabling the identification of candidate markers for yield components, flowering time, disease resistance, and abiotic stress tolerance (Famoso et al., 2011; Zhao et al., 2011). However, such approaches typically rely on high-density genome-wide markers to achieve adequate resolution. In contrast, SSR-based marker–trait association analysis, as employed in the present study, provides preliminary insights into genotype–phenotype relationships using cost-effective and highly polymorphic markers that are particularly suitable for moderate-scale germplasm studies. Hundreds of trait-governing genes have been cloned, and targeted genome-editing tools are now available to accelerate trait improvement (Shan et al., 2014; Zhao et al., 2014). In applied breeding, major-effect loci discovered through QTL mapping have been successfully introgressed from wild and exotic resources into elite varieties via marker-assisted selection (Collard and Mackill, 2008; Jiang, 2013). Grain yield is a quantitative trait determined by multiple components — tiller number, panicle count, seeds per panicle, and seed weight (Xing and Zhang, 2010; Devi et al., 2017; Samonte et al., 1998). Understanding the genetic architecture of these yield components in

temperate japonica rice landraces is essential for harnessing their potential in breeding climate-resilient, high-yielding cultivars.

The present study was undertaken to: (i) assess the genetic diversity and population structure of temperate rice germplasm from the North-Western Himalayas using SSR markers; (ii) develop a representative core subset capturing maximum allelic diversity; (iii) examine multivariate marker–trait relationships using canonical correlation analysis; and (iv) identify marker–trait associations for key agronomic traits across multiple environments.

2. Materials and Methods

2.1. Plant material and experimental design

The present investigation was conducted during the Kharif seasons of 2022 and 2023 at two distinct locations: the Mountain Research Centre for Field Crops (MRCFC), Khudwani (33.70°N, 75.10°E; altitude: 1590 m above sea level) and the Research Farm, Faculty of Agriculture, SKUAST-K, Wadura (34.20°N, 74.24°E; altitude: 1588 m above sea level), both located in the Kashmir region representing the niche area of temperate japonica rice. The experimental material comprised 144 rice accessions (*Oryza sativa* L.), including 140 test genotypes and four check varieties (Shalimar Rice 4, Shalimar Rice 5, SKUA-583, and Purple Rice) (Table S1). The germplasm included local rice landraces from the northwestern Himalayan region and accessions maintained in institutional germplasm collections at the Mountain Research Centre for Field Crops (MRCFC), Khudwani, under the Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K). MRCFC Khudwani served as an institutional source of rice germplasm for the study. The accessions were cultivated under experimental field conditions for phenotypic evaluation using an Augmented Block Design (ABD) consisting of seven blocks, each containing 20 entries. All genotypes, including check varieties, were included in phenotypic and molecular analyses. Spacing was maintained at 20 × 10 cm, with five tagged plants per line for morpho-physiological trait recording. Standard agronomic practices were followed throughout the growing season. Details of the traits evaluated and measurement protocols are summarized in Table 1.

2.2. DNA extraction and SSR genotyping

Genomic DNA was isolated following the cetyltrimethylammonium bromide (CTAB) method with slight modifications (Doyle and Doyle, 1987). Fresh, disease-free leaves were collected at the vegetative stage under sterile conditions and stored at −80 °C until use. DNA quality and concentration were verified using a Nanodrop spectrophotometer, and working dilutions were adjusted to 40 ng/µl with nuclease-free water.

PCR amplification was carried out using 25 SSR primer pairs selected from an initial panel of approximately 100 markers screened across a representative subset of genotypes. Only markers showing clear amplification, reproducibility, and high polymorphism (PIC > 0.5) were retained.

Additionally, the selected markers were distributed across different chromosomes to ensure broad genome coverage and included loci previously reported to be associated with agronomic traits. The primer sequences, chromosomal positions, repeat motifs, annealing temperatures, associated traits, and relevant references are listed in Table S2.

Amplification reactions were performed in a total volume of 15 µl in a Bio-Rad thermal cycler (USA). Each reaction contained: 1 µl genomic DNA (40 ng), 0.4 µl each primer (10 µM), 1.5 µl 10× PCR buffer, 0.6 µl MgCl2 (25 mM), 0.3 µl dNTP mix (10 mM), 0.2 µl Taq DNA polymerase (5 U/µl), and 10 µl PCR-grade water. The PCR program included initial denaturation at 95 °C for 3 min, followed by 32 cycles of: denaturation at 94 °C for 30 s, annealing at 52–58 °C (depending on primer; Table S2) for 45 s, and extension at 72 °C for 1 min; with a final extension at 72 °C for 7 min. Amplified products were resolved on 6% polyacrylamide gels, visualized by silver nitrate staining, and allele sizes were estimated using a 50 bp DNA ladder (Thermo Scientific) (Figure 1).

2.3. Genetic diversity analysis

Polymorphic information content (PIC), allele number, gene diversity, and observed heterozygosity were calculated using PowerMarker v3.25. Population structure was analyzed using the Bayesian clustering approach implemented in STRUCTURE version 2.3.4 (Pritchard et al., 2000). The burn-in period and Markov Chain Monte Carlo (MCMC) iterations were both set to 100,000. Three independent runs were conducted for each K value from 1 to 10, and the optimal K was determined using the Delta K (ΔK) method of Evanno et al. (2005), implemented through the STRUCTURE Harvester program (Earl and von Holdt, 2012).

2.4. Core subset selection

A representative core subset of 20 genotypes was selected from the 140 test accessions using the Core Hunter R package. The precomputed SSR-based genetic distance matrix was used as input, and the optimization criterion prioritized average pairwise genetic distance to maximize diversity.

2.5. Canonical correlation analysis

Canonical Correlation Analysis (CCA) was employed as a preliminary step to examine multivariate relationships between SSR-derived genetic distances and yield-related traits (Hotelling, 1992; Knapp, 1978; Haghshenas et al., 2019). CCA simultaneously considers two sets of variables SSR markers and phenotypic traits to identify linear combinations (canonical variates) that maximize the correlation between them. It captures multi-marker–multi-trait associations, reduces dimensionality, and accounts for multicollinearity, thereby enabling indirect selection and revealing broad patterns of genotype–phenotype covariance. CCA offered an initial framework to guide subsequent marker–trait association analysis.

2.6. Marker–trait association analysis

Marker–trait association analyses were conducted using TASSEL 5.2 software (Trait Analysis by association, Evolution and Linkage; http://www.maizegenetics.net/tassel). Two statistical models were employed: the General Linear Model (GLM), which included population structure (Q matrix) as a covariate, and the Mixed Linear Model (MLM), which incorporated both population structure (Q matrix) and kinship (K matrix) to account for relatedness and population stratification. The MLM was prioritized over the GLM as it provides superior control of false positives. Significance was assessed using p-values from each model, with $p < 0.01$ used as the primary exploratory threshold. A Bonferroni-corrected threshold ($P < 0.002$, $-\log10P \approx 2.7$) was also computed. The proportion of phenotypic variation explained by each significant marker was estimated using analysis of variance (ANOVA).

SSR markers showing significant marker–trait association hits were positioned on rice chromosomes to construct QTL maps. Physical coordinates (Mb) for SSR markers were obtained from the Os-Nipponbare-Reference-IRGSP-1.0 reference genome, MSU-RGAP Release 7 (2011; data files updated in 2024). Genetic positions were not used in the physical map; marker positions were represented exclusively by their corresponding physical coordinates on the 12 rice chromosomes. Centromere positions were based on the pseudo-centromere annotations available from rice.uga.edu, which are supported by CentO centromeric repeats and BAC-tiling/FISH evidence, rather than being estimated at a fixed percentage of chromosome length. The physical QTL map was generated in R using ggplot2, with chromosome ideograms drawn as vertical backbones scaled to their actual physical lengths (Mb). SSR marker–trait associations were overlaid at their respective physical positions, with marker size proportional to association strength ($-\log_{10}(P)$) and marker colour representing the associated trait. Marker labels were positioned using label-repelling functions to minimize overlap and improve readability. The rice reference genome and chromosome coordinates followed the IRGSP-1.0 Nipponbare assembly described by Kawahara et al. (2013).

3. Results

3.1. Phenotypic variation

Phenotypic evaluation for yield and related traits was carried out under field conditions across two years and two locations. Analysis of variance (ANOVA) revealed highly significant differences among genotypes for most traits studied, indicating the presence of substantial genetic variability exploitable in breeding programs. A summary of ANOVA results is provided in Table S3, while detailed phenotypic analyses have been published previously (Naik et al., 2025).

3.2. SSR-based genetic diversity

In total, 195 alleles were amplified by 25 SSR markers, with allele numbers per locus ranging from 4 (RM101) to 12 (RM262 and RM504), averaging 7.8. Major allele frequency ranged from 0.2153 (RM27966) to 0.7986 (RM27000), with a mean of 0.4011. Gene diversity (He) ranged from 0.3472 (RM27000) to 0.8646 (RM27966), with an overall mean of 0.7228. Polymorphic information content (PIC) values ranged from 0.3280 (RM27000) to 0.8504 (RM27966), averaging 0.6874; 22 markers were highly informative (PIC > 0.5; Botstein et al., 1980). Observed heterozygosity was comparatively low, ranging from 0.1354 (RM27966) to 0.6528 (RM27000), with a mean of 0.2770 (Table 2).

3.3. Population structure analysis

Population structure analysis, conducted using STRUCTURE v2.3.4 (Pritchard et al., 2000), assessed the genetic composition of all 144 accessions. Log-likelihood values and the ΔK statistic both peaked at K = 5 (Figure 2a), confirming five subpopulations as the most appropriate solution (Evanno et al., 2005). The population structure analysis grouped all 144 accessions into five sub-populations (Figure 2) reflecting their diverse genetic backgrounds. Many genotypes showed essentially a single colour (ancestry ≥ 80%), indicating clear cluster assignment, while others displayed two or more colours (membership fractions < 80%), indicating admixture.

3.4. Core subset selection

A core subset of 20 genotypes was selected from 140 temperate rice accessions using the Core Hunter R package, based on 25 SSR loci (Table S4). The mean pairwise genetic distance of the core set (50.60) was higher than that of the full collection (46.83), indicating greater diversity within the subset. A permutation test showed a difference of 3.77 ($p = 0.0712$). The density distribution (Figure 3) confirmed that the core subset contained more widely spaced genotypes, with a shift toward larger distances and a heavier tail at high values, supporting its representativeness.

3.5. Canonical correlation analysis

CCA between the 25 SSR marker matrix and the seven agronomic traits identified seven canonical variates, of which the first three dimensions had canonical correlations of 0.74, 0.71,

and 0.52, respectively, indicating strong linear associations between combinations of markers and traits. Analysis of canonical loadings showed that specific SSR markers and phenotypic traits contributed heavily to each axis (Figure 4). The first canonical axis was dominated by traits associated with plant productivity (grain yield, panicle length, and spikelets per panicle) together with several SSR loci known to affect yield components. The second axis was influenced mainly by plant height and days to 50% flowering versus other marker sets (Figure 4b). A scatterplot of genotype scores on the first two canonical axes (Figure 4d) revealed a clear grouping of accessions, illustrating how the combined marker–trait variation stratifies the sample. These leading canonical dimensions capture the bulk of the genotype–phenotype covariance.

3.6. Marker–trait associations

Marker–trait association (MTA) analysis using GLM and MLM in TASSEL 5.2 identified four significant associations ($p < 0.01$), summarized in Table 3. The quantile-quantile (QQ) plots (Figures 5a and 6a) showed minimal deviation from the expected distribution, with slight inflation only at the tail, indicating effective control of population structure. Manhattan plots (Figures 5b and 6b) revealed distinct association peaks corresponding to the four significant markers. All significant markers explained a moderate proportion of phenotypic variance (R^2 = 4.1–5.6%), consistent with the polygenic nature of yield-related traits.

A chromosomal map (Figure 7) was constructed for chromosomes 2, 9, and 12 using the IRGSP-1.0 Nipponbare reference genome (Kawahara et al., 2013; Khurana et al., 2005), displaying all SSR loci included in the marker–trait association panel. Five significant marker–trait associations were detected and mapped to these chromosomes. RM285 on chromosome 9 (1.0 cM; 6.8 Mb) was significantly associated with 1000 seed weight under the GLM model. RM240 on chromosome 2 (85.0 cM; 29.6 Mb) was linked to the number of effective tillers per plant (GLM). On chromosome 12, RM27462 (45.0 cM; 15.0 Mb) was significantly associated with days to 50% flowering under both GLM and MLM models, indicating a consistent signal across methods. RM101 on chromosome 12 (48.2 cM; 9.0 Mb) showed association with plant height (MLM). For each marker, chromosome number, approximate genetic position in centimorgans (cM), approximate physical position in megabases (Mb), and the major traits previously reported in the literature is given in Table 3.

4. Discussion

4.1. Genetic diversity revealed by phenotypic analysis

Significant variation was observed among genotypes for key yield-related traits, highlighting the presence of a broad genetic base crucial for effective selection in breeding programs. Similar findings have been reported in rice diversity studies across different environments, confirming that phenotypic variability provides opportunities for identifying superior genotypes (El-Agoury et al., 2024). Integrating these phenotypic traits with SSR-based molecular diversity

can enhance the identification of superior alleles and improve the precision of selection in breeding programs.

4.2. Genetic diversity patterns revealed by SSR markers

SSR-based analysis of 144 rice accessions revealed substantial polymorphism, consistent with previous diversity studies. Nachimuthu et al. (2015) reported an average of 3 alleles per SSR locus (range 2–7) with mean PIC ≈ 0.468, similar to the ~2.85–3 alleles per locus and moderate PIC (~0.44) reported by Pandita et al. (2023). In our study, the number of alleles per locus and PIC values fell within comparable ranges, indicating similar diversity levels.

Certain SSR loci proved especially informative. For instance, marker RM580 was extremely polymorphic: Wang *et al.* (2013) found RM580 amplified 16 alleles with a PIC of 0.86 in Korean rice varieties. Such high polymorphism suggests that RM580 is especially useful for differentiating genotypes. In our panel, RM580 likewise showed a large allele count and high PIC, marking it as a key diversity locus. Similarly, RM262 (an SSR linked to panicle number, a yield component) also exhibited high informativeness. In a trait-based study of basmati rice, RM262 (associated with "panicles per plant" trait) had three alleles and PIC=0.61 (Salgotra *et al.,* 2015). In our dataset RM262 likewise revealed multiple alleles and high PIC, making it valuable for capturing variation in panicle-related loci. Finally, RM27966 emerged as a marker of interest. Although less commonly reported in diversity studies, recent QTL mapping has identified RM27966 flanking a major flowering time QTL (qDFF12-1), which controls developmental timing (Kulkarni et al 2021) and indirectly influences yield potential through extended vegetative growth phases. In our study, these markers showed high polymorphism and PIC values, underscoring their informativeness and relevance for yield-associated trait analysis through linkage to key developmental and architectural components.

4.3. Population structure and genetic stratification

Population STRUCTURE analysis revealed five distinct genetic clusters (ΔK peaked at K=5), indicating substantial stratification in this germplasm set. Local landraces predominantly belonged to the green cluster, indicating high genetic uniformity with minimal admixture. Collections from other parts of India were largely assigned to the yellow cluster, with some genotypes showing admixture, suggesting historical gene flow. Advanced breeding lines exhibited significant admixture, highlighting their derivation from diverse parental sources. Similarly, released varieties displayed contributions from all clusters, reflecting efforts to combine genetic resources for improved traits. Collections from international sources primarily clustered in the red group, indicating their distinct genetic composition. This emphasized the importance of utilizing diverse genetic resources, particularly the landraces and exotic collections, in breeding programmes to enhance genetic diversity and adaptability. The identification of five subgroups is consistent with previous SSR-based surveys of Himalayan rice by Salgotra *et al.* (2015) who found K=5 among North-West Himalayan basmati

germplasm, whereas a smaller study by Pandita *et al.* (2023) reported K=3 (narrow genetic base). Likewise, Upadhyay *et al.* (2012) found that increasing K to 5 improved concordances with phylogenetic clustering in Indian rice varieties. The grouping seen here generally reflects origin and breeding history. In our study, admixture patterns indicate that while most landraces remain genetically cohesive, some exhibit mixed ancestry, perhaps due to seed exchange or breeding. The distinct clustering of local versus introduced varieties underscores the divergent gene pools present in this temperate region.

4.4. Core subset selection

Developing a representative core subset is essential for efficient germplasm management and breeding. Twenty genotypes captured the diversity of the full collection while reducing redundancy, providing a practical resource for targeted trait evaluation and long-term conservation. The higher mean genetic distance in the core subset reflects Core Hunter's effectiveness, consistent with previous reports that the tool enriches genetic diversity in subsets (Thachuk et al., 2009; Odong et al., 2013; Manrique-Carpintero et al., 2023). Although the permutation p-value (0.0712) did not reach statistical significance, the positive trend and distribution pattern indicate the selected subset preserves a broad range of genetic variation and retains unique alleles.

4.5. Canonical correlation analysis

Unlike ordinary correlation, which examines the linear relationship between only two individual variables, Canonical Correlation Analysis (CCA) simultaneously considers two sets of variables, in this case, SSR markers and yield-related traits, to identify the best possible linear combinations (canonical variates) that maximize the correlation between them. The high canonical correlations (0.74 and 0.71) demonstrate a strong multivariate link between the SSR markers and phenotypic traits, implying that genetic variation at these markers predicts key agronomic outcomes. This result is in line with the general observation that many SSR loci correlate with complex traits in rice. For instance, in our study, markers such as RM264, RM267, RM125 and RM38 previously found associated with yield (Borba *et al.,* 2010) likely have high loadings on the first axis together with yield and grain-number traits. These concordances suggest that our results have validated known marker–trait relationships in the context of temperate rice.

The canonical loadings further indicate which traits and markers are co-varying. Traits like grain yield, number of effective tillers per plant and panicle size tended to have large loadings on the first variate, paired with SSR markers that explain those phenotypes suggesting first canonical variable represents a yield-potential dimension. The second canonical variable, with somewhat lower correlation (0.52), captured additional variation (plant height, days to days to 50% flowering) and corresponding markers. These dimensions were the only ones with

correlations substantially above zero implies that the majority of predictive marker–trait covariance is concentrated in the first two axes.

Our results echo quantitative findings from association studies. In a large core collection analysis, a substantial fraction of SSRs (27%) were found to associate with at least one trait and several SSRs were linked to yield and grain quality (Borba et al., 2010). This demonstrates that SSR variation can reliably predict agronomic performance, highlighting its value for improving complex traits.

4.6. Marker–trait associations and QTL context

Marker–Trait association analysis revealed novel marker–trait associations that can be integrated with prior rice QTL studies, highlighting previously unreported loci influencing key agronomic traits. RM285 on chromosome 9 was significantly associated with 1000-seed weight, a trait not previously linked to this region. Donde et al. (2020) mapped RM285 near a plant height QTL (qPHT-9.1) and linked it to flowering time across seasons. These prior findings in indica populations suggest this region affects developmental timing and stature, while our result indicates it may also influence grain weight, possibly via pleiotropic effects or linkage with nearby yield genes. Notably, RM 285 lies within the Sub1 region on chromosome 9, previously characterized for submergence tolerance (Neeraja et al., 2007), suggesting pleiotropic effects on grain yield.

RM101 on chromosome 12 was significant for plant height in our marker–trait association, consistent with Naveenkumar et al. (2023), who reported a QTL qPh.12-1 at RM101 explaining ~6% of plant height variation. Although RM101 was not associated with flowering or tillers in our panel, it flanks a leaf-rolling QTL under drought (Prince et al., 2015) and maps to the Pi-ta region linked to blast resistance (Correll et al., 2004). The recurrence of RM101 in plant height QTL mapping suggests it tags a locus regulating growth and stress resilience.

RM240 on chromosome 2 was associated with the number of effective tillers per plant. While few studies have cited RM240 specifically, broad QTL surveys have identified multiple tillering loci on chromosome 2 (Zhao et al., 2022). Our association at RM240 (~31.4 Mb) is novel but overlaps the drought-yield QTL qDTY2.2 (flanked by RM240–RM154) (Dharmaraj et al., 2024) and deep-rooting QTLs (Kanagaraj et al., 2010), indicating it may capture a germplasm-specific tiller-QTL linked with drought-response regions.

Finally, RM27462 (chr12) was associated with days to 50% flowering. This SSR has not been previously reported with flowering time, although chromosome 12 carries known flowering/drought-related QTLs (e.g., qDTY12.1), highlighting its potential role in developmental phenology and adaptation in temperate environments.

All four SSR–trait associations reached $p < 0.01$, with modest phenotypic variance explained (R^2 ~4–5.6%), consistent with the small effects expected for complex yield-

component traits. These values align with prior studies, such as Zhao et al. (2022) and Naveenkumar et al. (2023), who reported $R^2 \approx 5–6\%$ for major QTLs. Manhattan plots for the four traits show clear peaks at RM285 (1000-seed weight, chr9), RM27462 (days to 50% flowering, chr12), RM240 (number of effective tillers, chr2), and RM101 (plant height, chr12), all surpassing the significance threshold ($-\log_{10}(p) \approx 2$). Corresponding QQ plots indicate most p-values closely follow the expected null distribution, with departures only for significant loci, suggesting low marker-trait association inflation and effective control of population structure. Given the limited number of markers, a Bonferroni-corrected threshold ($P < 0.002$) was considered; however, a less stringent threshold ($P < 0.01$) was used to identify putative associations in this exploratory analysis. While this approach increases sensitivity, it may also introduce false positives, and thus only associations supported by the mixed linear model are considered more reliable and require further validation. Despite the useful insights obtained, the present study has certain limitations. The use of a relatively small number of SSR markers provides limited genome coverage compared to high-density SNP-based approaches. SSR-based association analysis may therefore have reduced resolution and power to detect loci with small effects, and may miss important genomic regions influencing complex traits. In contrast, SNP-based genome-wide association studies offer finer mapping resolution and improved detection of marker–trait associations due to higher marker density and genome-wide coverage. Therefore, the associations identified in this study should be considered preliminary and require further validation using high-density SNP markers or sequencing-based approaches. Validation using biparental mapping populations or advanced breeding lines would also help establish their genetic effects more precisely.

Collectively, our results highlight the intertwined genetic control of stress resilience, development, and yield-related traits. RM285 and RM101 overlap regions previously linked to plant height or development, whereas RM240 and RM27462 appear as novel associations in the North-Indian temperate rice germplasm, reflecting potentially unique alleles in these landraces. The study underscores that locus historically associated with stress tolerance, disease resistance, and root architecture can also influence key agronomic traits. The identification of genetically diverse accessions and the development of a representative core set offer valuable resources for selecting parents with maximum genetic divergence, thereby enhancing recombination and heterosis. The marker–trait associations identified, particularly for key yield-related traits, highlight potential candidates for integration into marker-assisted selection strategies. Importantly, the overlap between markers associated with agronomic performance and those previously linked to stress tolerance suggests opportunities for simultaneous improvement of yield and adaptability in temperate environments.

Supplementary Material

The supplementary material for this paper is available online and comprises: Table S1 (list of 144 genotypes and check varieties); Table S2 (SSR primer information, chromosomal positions, and associated traits); Table S3 (ANOVA summary for all traits); Table S4 (core subset genotype list).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics and consent to participate

The study was conducted using cultivated rice (*Oryza sativa* L.) germplasm maintained in institutional collections at the Mountain Research Centre for Field Crops (MRCFC), Khudwani, under the Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K). The plant material was cultivated and evaluated under experimental field conditions for research purposes. Information on the rice accessions used in this study, including their identities and available source details, is provided in the supplementary data (Table S1). The use and handling of the plant material followed applicable institutional and national guidelines. No special collection permits or licences were required for the use of the institutionally maintained cultivated germplasm in this study.

Data Availability

Raw phenotypic data, SSR genotype profiles, and analysis scripts (TASSEL, R for Core Hunter) will be deposited in a public repository (Zenodo) upon acceptance to ensure reproducibility.

References

Ali, H., Muhammad, A., Sanda, N.B., Huang, Y. and Hou, Y., 2019. Pyrosequencing uncovers a shift in bacterial communities across life stages of Octodonta nipae (Coleoptera: Chrysomelidae). Frontiers in Microbiology, 10, p.466. https://doi.org/10.3389/fmicb.2019.00466

Ali, H. and Xiao, Y. (Eds.), 2025. Genome Editing for Pest Management (1st ed.). CRC Press. https://doi.org/10.1201/9781003478706

Borba, T.C.D.O., Brondani, R.P.V., Breseghello, F., Coelho, A.S.G., Mendonça, J.A., Rangel, P.H.N. and Brondani, C., 2010. Association mapping for yield and grain quality traits in rice (Oryza sativa L.). Genetics and Molecular Biology, 33, pp.515–524.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics, 32, pp.314–331.

Collard, B.C. and Mackill, D.J., 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences, 363(1491), pp.557–572. https://doi.org/10.1098/rstb.2007.2170

Correll, J., Lee, F.N., Xia, Y. and Rutger, J.N., 2004. Rice Pi-ta gene confers resistance to the major pathotypes of the rice blast fungus in the United States. Phytopathology, 94(3), pp.296–301. https://doi.org/10.1094/PHYTO.2004.94.3.296

Dar, Z.A., 2019. Genetic Resources of Rice in the Kashmir Valley. Academic Press, New Delhi.

Devi, K.R., Chandra, B.S., Lingaiah, N., Hari, Y. and Venkanna, V., 2017. Analysis of variability, correlation and path coefficient studies for yield and quality traits in rice (Oryza sativa L.). Agricultural Science Digest, 37(1), pp.1–9.

Dharmaraj, A., et al., 2024. [Chromosome 2 tiller and drought QTL study]. [Journal details as per original citation].

Donde, R., Mohapatra, S., Baksh, S.Y., Padhy, B., Mukherjee, M., Roy, S., et al., 2020. Identification of QTLs for high grain yield and component traits in new plant types of rice. PLoS One, 15(7), e0227785.

Doyle, J.J. and Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19, pp.11–15.

Earl, D.A. and von Holdt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4, pp.359–361.

El-Agoury, R.Y., El-Hashash, E.F., Abou El-Enin, M.M., Sakr, S.M., Essa, W.M., El Sherbiny, H.A. et al., 2024. Combining ability, heterosis and multivariate analysis for physiological and agronomic traits of rice genotypes under normal and water stress conditions. Agricultural Research, 13(1), pp.10–25.

Evanno, G., Regnaut, S. and Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14, pp.2611–2620.

Famoso, A.N., Zhao, K., Clark, R.T., Tung, C.W., Wright, M.H., Bustamante, C., Kochian, L.V. and McCouch, S.R., 2011. Genetic architecture of aluminum tolerance in rice (Oryza sativa) determined through genome-wide association analysis and QTL mapping. PLOS Genetics, 7(8), e1002221. https://doi.org/10.1371/journal.pgen.1002221

Fu, C., Wan, S., Yang, P., Zhao, X., Yan, Y., Jiang, S. and Ali, H., 2024. Identification of the Ilex macrocarpa anthracnose pathogen and the antifungal potential of the cell-free supernatant of Bacillus velezensis against Colletotrichum fioriniae. Frontiers in Microbiology, 15, p.1419436.

Haghshenas, H., Soltani, A., Ghanbari Malidarreh, A., Ajam Norouzi, H. and Dastan, S., 2019. Selecting the ideotype of improved rice cultivars using multiple regression and multivariate models. Archives of Agronomy and Soil Science, 66, pp.1134–1153.

He, Z., Ali, H., Wu, J., Liu, Z., Wei, X. and Zhuo, Z., 2025. Impact of climate change on the distribution of Isaria cicadae Miquel in China: predictions based on the MaxEnt model. Frontiers in Microbiology, 16, p.1509882.

Hotelling, H., 1992. Relations between two sets of variates. In: Breakthroughs in Statistics: Methodology and Distribution. Springer, New York, pp.162–190.

Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., et al., 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. Nature Genetics, 42(11), pp.961–967. https://doi.org/10.1038/ng.695

Jiang, G.-L., 2013. Molecular markers and marker-assisted breeding in plants. In: Andersen, S.B. (Ed.), Plant Breeding from Laboratories to Fields. InTech. https://doi.org/10.5772/52583

Kanagaraj, S., et al., 2010. Deep-rooting QTL qRDR-2 associated with root architecture trait in rice. Bragantia, 77(1), pp.1–14.

Kawahara, Y., De la Bastide, M., Hamilton, J.P., Kanamori, H., McCombie, W.R., Ouyang, S. et al., 2013. Improvement of the Oryza sativa Nipponbare reference genome using next generation sequence and optical map data. Rice, 6(1), p.4.

Khurana, P. and Gaikwad, K., 2005. The map-based sequence of the rice genome. Nature, 436, pp.793–800.

Knapp, T.R., 1978. Canonical correlation analysis: a general parametric significance testing system. Psychological Bulletin, 85, pp.410–416. https://doi.org/10.1037/0033-2909.85.2.410

Kulkarni, S.R., Balachandran, S.M., Ulaganathan, K., Balakrishnan, D., Prasad, A.H., Rekha, G. et al., 2021. Mapping novel QTLs for yield related traits from a popular rice hybrid KRH-2 derived doubled haploid (DH) population. 3 Biotech, 11(12), p.513.

Liu, Q., Chen, G., Zhang, J., Xu, D., Ali, H. and Wang, X., 2025. Rhizosphere and phyllosphere microbial communities of male and female plants of Morus macroura. Physiological and Molecular Plant Pathology, 136, p.102517.

Manrique-Carpintero, N.C., Berdugo-Cely, J.A., Cerón-Souza, I., Lasso-Paredes, Z., Reyes-Herrera, P.H. and Yockteng, R., 2023. Defining a diverse core collection of the Colombian Central Collection of potatoes: a tool to advance research and breeding. Frontiers in Plant Science, 14, p.1046400.

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

Nachimuthu, V.V., Muthurajan, R., Duraialaguraja, S., Sivakami, R., Pandian, B.A., Ponniah, G. et al., 2015. Analysis of population structure and genetic diversity in rice germplasm using SSR markers: an initiative towards association mapping of agronomic traits in Oryza sativa. Rice, 8, pp.1–25.

Naik, Z.A.N., Verma, A., Sudan, J., Shikari, A.B., Bhat, F.A., Wani, F.J. et al., 2025. Multivariate analysis of morphological and biochemical traits revealed some promising pigmented rice genotypes of North Western Himalayas for future breeding program. Indian Journal of Plant Genetic Resources, 38(02), pp.227–231.

Naveenkumar, R., Anandan, A., Prabhukarthikeyan, S.R., Mahender, A., Sangeetha, G., Vaish, S.S. et al., 2023. Dissecting genomic regions and underlying sheath blight resistance traits in rice (Oryza sativa L.) using a genome-wide association study. Plant Direct, 7(11), e540.

Neeraja, C.N., et al., 2007. A marker-assisted backcross approach for developing submergence-tolerant rice cultivars: the Sub1 locus confers submergence tolerance in rice. Molecular Breeding, 20(3), pp.241–256. https://doi.org/10.1007/s11032-007-9101-1

Odong, T.L., Jansen, J., Van Eeuwijk, F.A. and van Hintum, T.J., 2013. Quality of core collections for effective utilisation of genetic resources: review, discussion and interpretation. Theoretical and Applied Genetics, 126, pp.289–305.

Pandita, D., Mahajan, R., Zargar, S.M., Nehvi, F.A., Dhekale, B., Shafi, F., Shah, M.U.D., Sofi, N.R. and Husaini, A.M., 2023. Trait specific marker-based characterization and population structure analysis in rice (Oryza sativa L.) germplasm of Kashmir Himalayas. Molecular Biology Reports, 50(5), pp.4155–4163. https://doi.org/10.1007/s11033-023-08324-5

Prince, S.J., Beena, R., Gomez, S.M., Senthivel, S. and Babu, R.C., 2015. Mapping consistent rice (Oryza sativa L.) yield QTLs under drought stress in target rainfed environments. Rice, 8, pp.1–13.

Pritchard, J.K., Stephens, M. and Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics, 155, pp.945–959.

Qian, Q., Deng, X., Mureed, S., Gan, Y., Xu, D., Wang, X. and Ali, H., 2024. Integrating transcriptomics and metabolomics to analyze the defense response of Morus notabilis to mulberry ring rot disease. Frontiers in Microbiology, 15, p.1373827.

Salgotra, R.K., Gupta, B.B., Bhat, J.A. and Sharma, S., 2015. Genetic diversity and population structure of Basmati rice (Oryza sativa L.) germplasm collected from North Western Himalayas using trait linked SSR markers. PLoS One, 10(7), e0131858.

Samonte, P.B., Wilson, L.T. and McClung, A.M., 1998. Path analyses of yield and yield-related traits of fifteen diverse rice genotypes. Crop Science, 38(5), pp.1130–1136. https://doi.org/10.2135/cropsci1998.0011183X003800050004x

Shan, Q., Wang, Y., Li, J. and Gao, C., 2014. Genome editing in rice and wheat using the CRISPR/Cas system. Nature Protocols, 9(10), pp.2395–2410. https://doi.org/10.1038/nprot.2014.157

Soylu, S., Bozkurt, A., Ali, H. et al., 2025. Evaluation of resistance of bean genotypes to common bean bacterial blight disease agents and characterisation of plant defence mechanisms involved in resistance. European Journal of Plant Pathology, 173, pp.387–411. https://doi.org/10.1007/s10658-025-03066-5

Thachuk, C., Crossa, J., Franco, J., Dreisigacker, S., Warburton, M. and Davenport, G.F., 2009. Core Hunter: an algorithm for sampling genetic resources based on multiple genetic measures. BMC Bioinformatics, 10, pp.1–13.

Upadhyay, P., Neeraja, C.N., Kole, C. and Singh, V.K., 2012. Population structure and genetic diversity in popular rice varieties of India as evidenced from SSR analysis. Biochemical Genetics, 50, pp.770–783.

Wang, X.Q., Kwon, S.W. and Park, Y.J., 2013. Evaluation of genetic diversity and linkage disequilibrium in Korean-bred rice varieties using SSR markers. Electronic Journal of Biotechnology, 16(5), pp.11–11.

Xing, Y. and Zhang, Q., 2010. Genetic and molecular bases of rice yield. Annual Review of Plant Biology, 61, pp.421–442. https://doi.org/10.1146/annurev-arplant-042809-112209

Zhao, D.D., Park, J.R., Jang, Y.H., Kim, E.G., Du, X.X., Farooq, M. et al., 2022. Identification of one major QTL and a novel gene OsIAA17q5 associated with tiller number in rice using QTL analysis. Plants, 11(4), p.538.

Zhao, K., Tung, C.W., Eizenga, G.C., Wright, M.H., Ali, M.L., Price, A.H. et al., 2011. Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. Nature Communications, 2, p.467. https://doi.org/10.1038/ncomms1467

Table 1. Experimental design and trait measurement protocols.

Category	Trait	Abbreviation	Measurement Protocol
Experimental Design	Genotypes	—	140 rice genotypes + 4 check varieties in Augmented Block Design (7 blocks, 20 entries each)
	Check varieties	—	Shalimar Rice 4, Shalimar Rice 5, SKUA-583, Purple Rice
	Plant spacing	—	20 × 10 cm; five tagged plants per line (morpho-physiological traits)
Morphological Traits	Days to 50% flowering	50F	Number of days from sowing until 50% of plants in each plot had flowered
	Plant height (cm)	PH	Height from soil surface to tip of tallest tiller at maturity
	Panicle length (cm)	PL	Length from peduncle node to panicle tip
	Number of effective tillers per plant	NT	Mean count of productive tillers from 5 randomly selected plants
	Spikelets per panicle	SPP	Mean spikelet count from 5 randomly selected plants
	Grain yield per plant (g)	GY	Grain yield per plant recorded in grams at harvest
	1000-seed weight (g)	1000SW	Weight of 1000 seeds from each genotype

Table 2. Assessment of genetic diversity using 25 SSR markers in 144 temperate rice accessions.

Marker	Major Allele Frequency	No. of Alleles	Gene Diversity (He)	Observed Heterozygosity	PIC
RM216	0.6875	9	0.5025	0.4975	0.4787
RM202	0.4653	5	0.6636	0.3364	0.6078
RM27000	0.7986	8	0.3472	0.6528	0.3280
RM3717	0.4931	6	0.6606	0.3394	0.6092
RM235	0.4931	7	0.6601	0.3399	0.6104
RM26746	0.3194	8	0.7995	0.2005	0.7728
RM101	0.3681	4	0.6961	0.3039	0.6364
RM27888	0.3889	7	0.7244	0.2703	0.6782
RM27898	0.3681	8	0.7531	0.2469	0.7157
RM580	0.2292	8	0.8299	0.1701	0.8075
RM27933	0.5903	5	0.6012	0.3988	0.5644
RM27966	0.2153	11	0.8646	0.1354	0.8504
RM27428	0.4028	5	0.6922	0.3078	0.6377
RM27471	0.3819	8	0.7684	0.2316	0.7389
RM214	0.4167	7	0.7432	0.2568	0.7095
RM19	0.3125	6	0.7731	0.2269	0.7374
RM5939	0.4167	7	0.7299	0.2701	0.6912
RM262	0.3264	12	0.8065	0.1935	0.7828
RM240	0.2917	10	0.8131	0.1869	0.7887
RMS3F	0.3403	11	0.8154	0.1846	0.7959
RM285	0.3472	11	0.7997	0.2003	0.7760
RM504	0.2986	12	0.8130	0.1870	0.7893
RM516	0.3472	10	0.7646	0.2354	0.7280
RM517	0.3681	5	0.7382	0.2618	0.6943
RM539	0.3611	5	0.7094	0.2906	0.6546
Mean	0.4011	—	0.7228	0.2770	0.6874

Table 3. Chromosomal and genomic context of significant SSR markers identified in marker–trait association analysis for yield-attributing traits. R^2: proportion of phenotypic variance explained; cM: centimorgans; Mb: megabases.

Trait (Model)	Marker	Chr.	p-value	Marker R^2	Approx. Position (cM)	Approx. Position (Mb)	Major traits reported in literature	Citation
1000SW (GLM)	RM285	9	0.0035	0.0556	0.9–1.0	~6.8 (centromere-distal)	Submergence tolerance (Sub1 locus); grain yield advantage under flooding	Neeraja et al. (2007)
50F (GLM)	RM27462	12	0.0005	0.0518	~7.2	3.3–3.6 (short-arm subtelomere)	Novel small-effect flowering QTL; chromosome 12 carries known flowering/drought QTLs	Novel marker
NT (GLM)	RM240	2	0.0083	0.0433	—	~29.6 (long arm)	Deep-rooting QTL (qRDR-2); tiller number; overlaps drought-yield QTL qDTY2.2	Kanagaraj et al. (2010); Zhao et al. (2022); Dharmaraj et al. (2024)
50F (MLM)	RM27462	12	0.0089	0.0445	48.2	~8.8–9.0 (short arm)	Novel small-effect flowering QTL; chromosome 12 carries known flowering/drought QTLs	Novel marker
PH (MLM)	RM101	12	0.0087	0.0441	48.2	~8.8–9.0 (short arm)	Pi-ta blast-resistance region; plant height QTL qPh.12-1; leaf-rolling under drought	Correll et al. (2004); Naveenkumar et al. (2023); Prince et al. (2015)

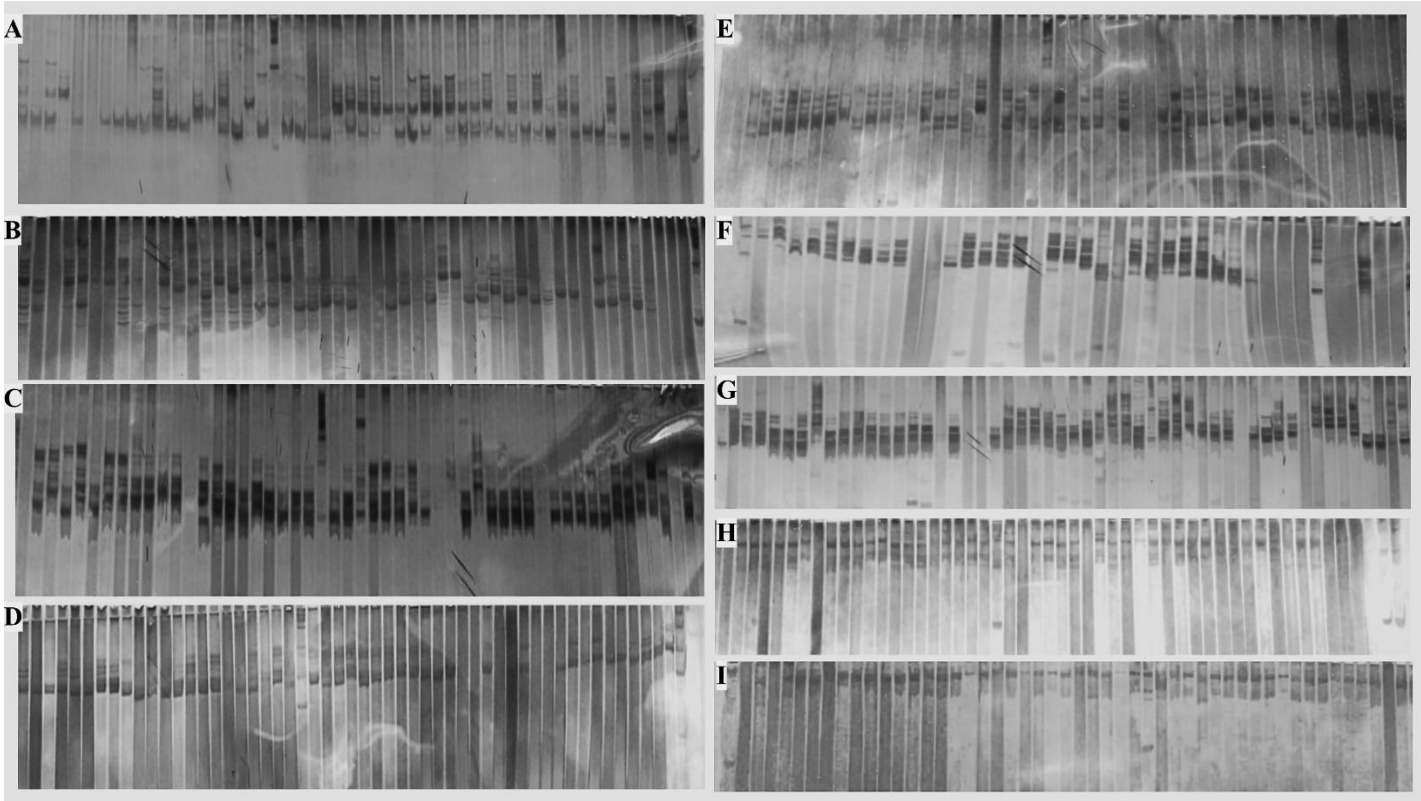

Figure 1. Polyacrylamide gel electrophoresis (PAGE) showing polymorphic banding patterns generated by SSR markers across 144 rice genotypes. The presence of multiple alleles with varying fragment sizes reflects the level of genetic polymorphism among the genotypes analysed. M: 50 bp DNA ladder (Thermo Scientific). **(A)** RM-240; **(B)** RM-285; **(C)** RM-101; **(D)** RM-27933; **(E)** RM-27462; **(F)** RM-262; **(G)** RM-3717; **(H)** RM-27428; **(I)** RM-27888. Each lane represents a single genotype. SSR markers showing multi-allelic and co-dominant variation represent high reproducibility and polymorphism

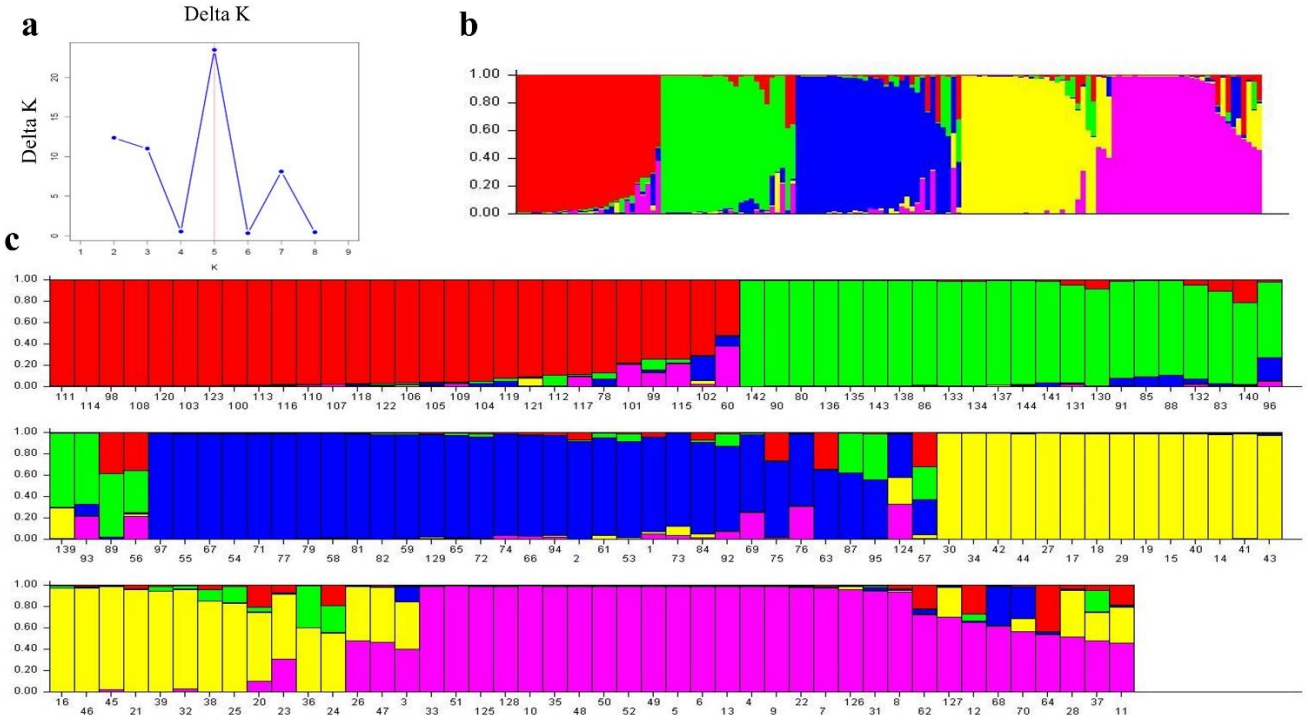

Figure 2. Population structure analysis of 144 temperate rice accessions based on 25 SSR markers. (a) ΔK plot generated using STRUCTURE Harvester indicating the optimal number of subpopulations at K = 5, based on the Evanno method. (b) Bar plot of population structure at K = 5, showing individual genotype membership proportions across five inferred subpopulations. Colors represent distinct genetic clusters. (c) Long-format bar plot displaying admixture levels and genetic relatedness among genotypes.

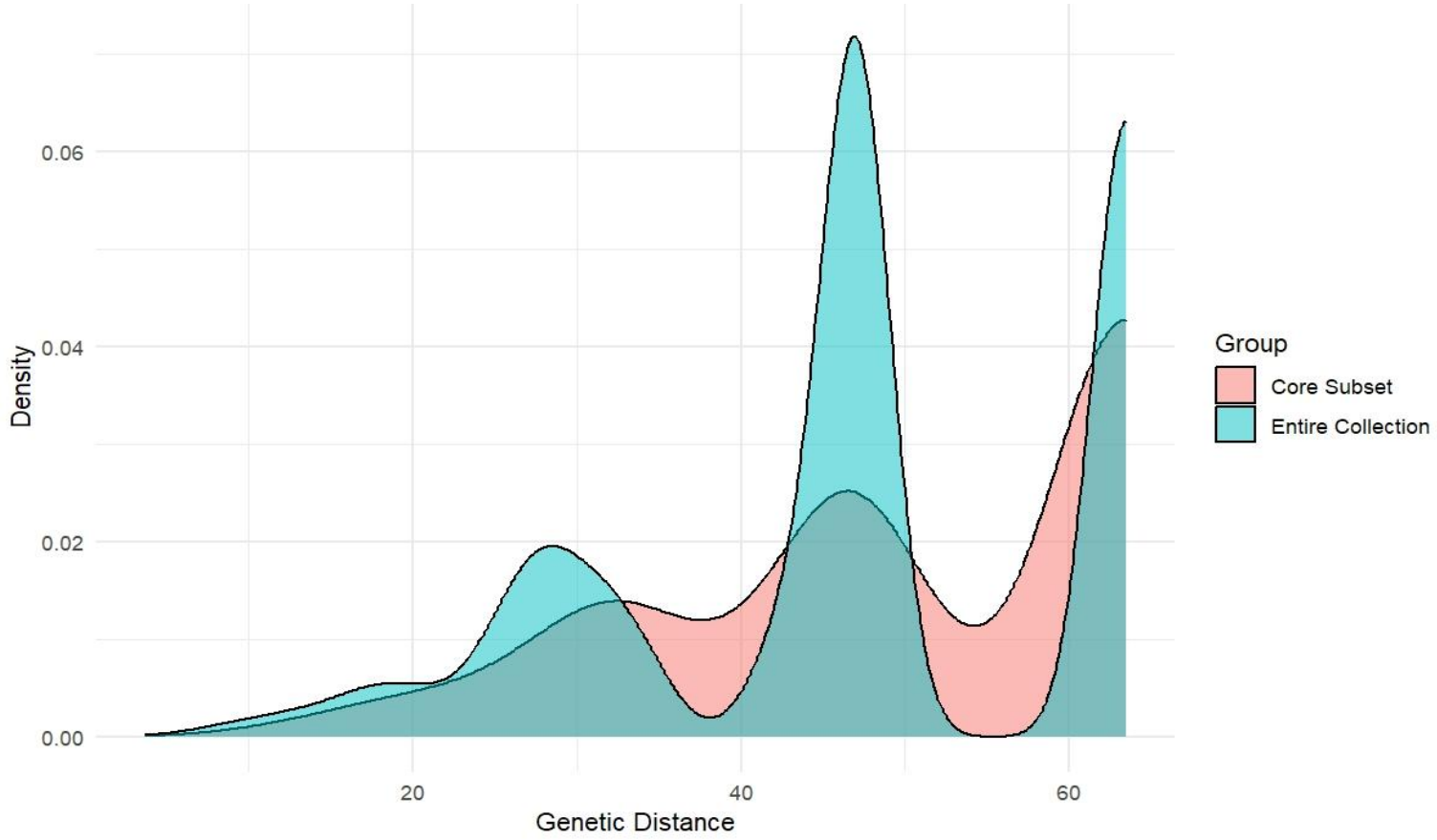

Figure 3. Density distribution of pairwise genetic distances for the entire temperate rice collection (blue, n = 140) and the selected core subset (pink, n = 20). The core subset shows a shift toward larger distances and a heavier tail at high values, confirming its representativeness of the full collection's genetic diversity.

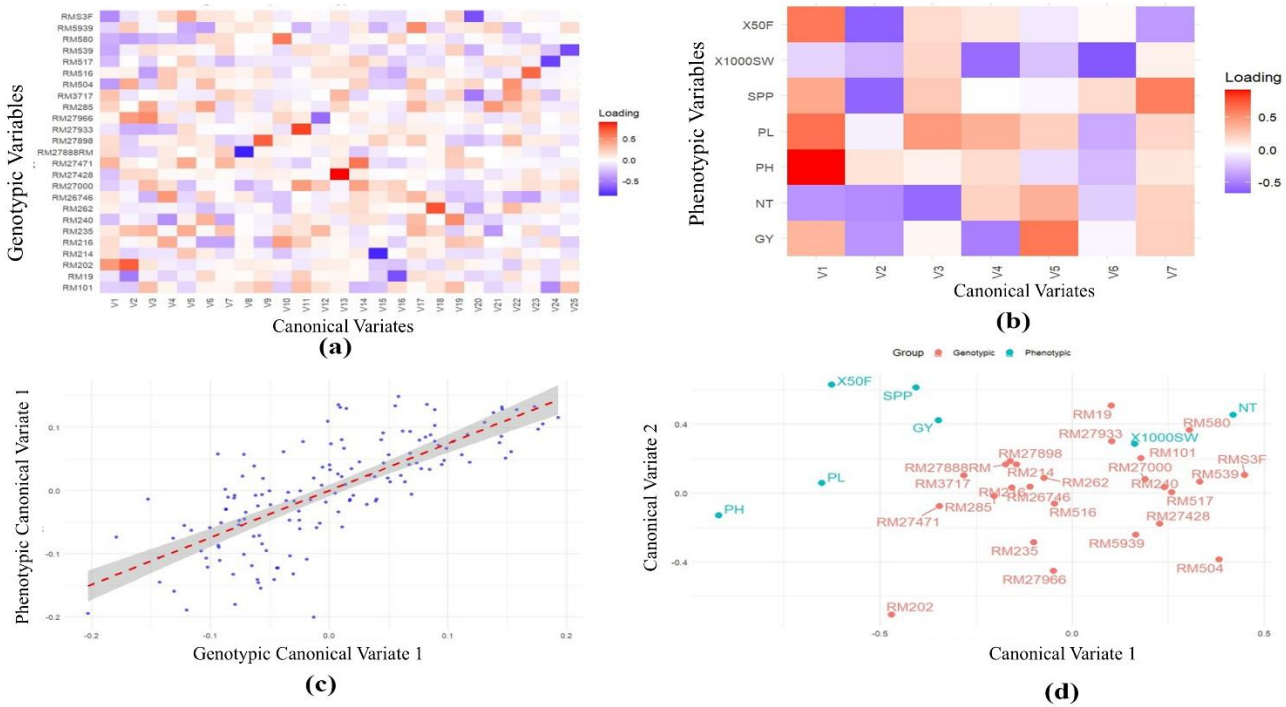

Figure 4. Canonical Correlation Analysis (CCA) between 25 SSR markers and seven agronomic traits in temperate rice. (a) Genotypic marker loadings across the first three canonical variates. (b) Phenotypic trait loadings on the first three canonical variates; trait abbreviations: 50F = days to 50% flowering; PH = plant height; PL = panicle length; NT = number of effective tillers; SPP = spikelets per panicle; GY = grain yield per plant; 1000SW = 1000-seed weight. (c) Correlation plot of the first canonical variate pair. (d) Biplot of genotype scores on the first two canonical axes showing stratification of accessions.

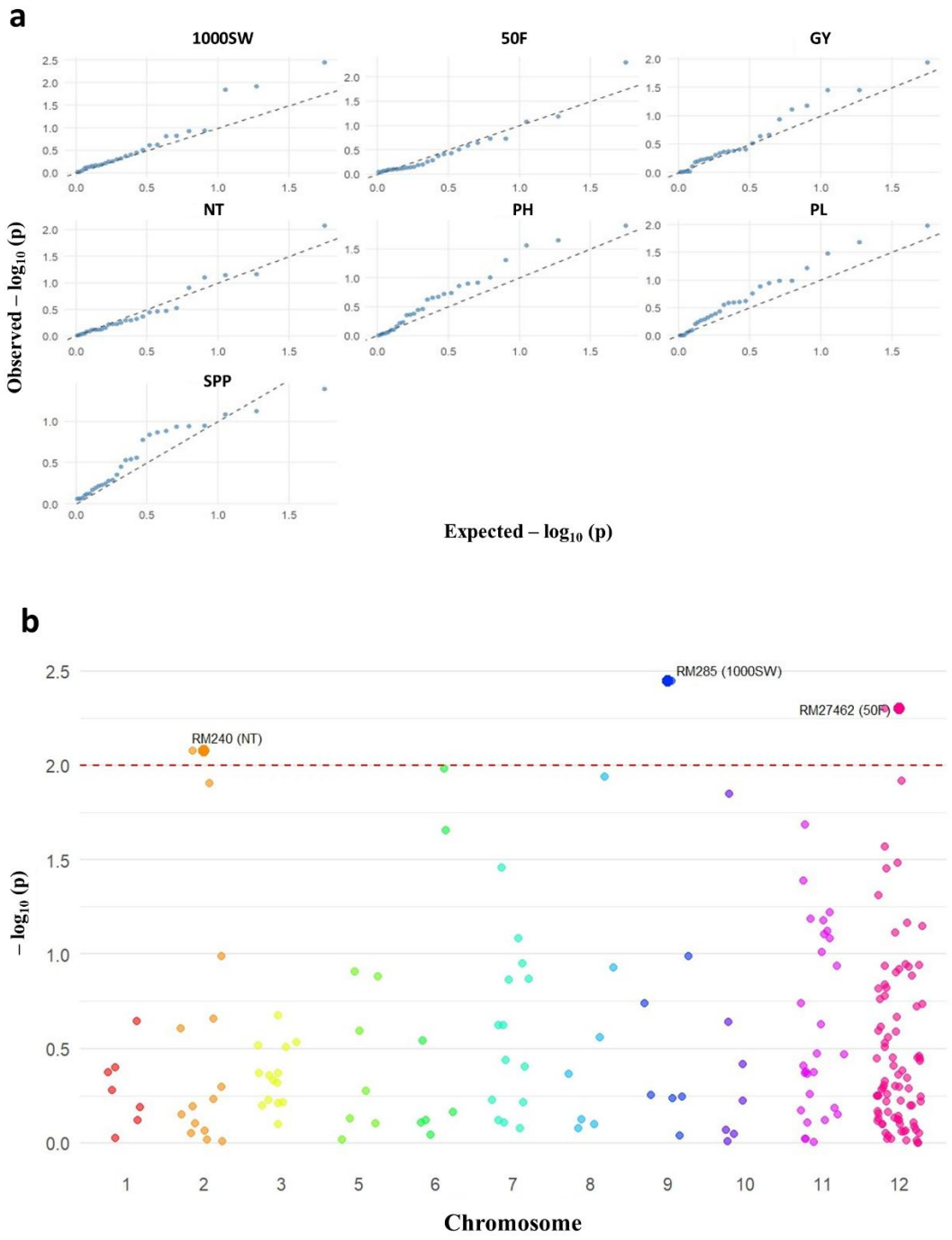

Figure 5. Marker–trait association results using the General Linear Model (GLM). (a) Quantile-quantile (QQ) plot of observed vs. expected –log10(p) values, demonstrating effective control of population structure inflation. (b) Manhattan plot displaying –log10(p) values for all 25 SSR markers across 12 rice chromosomes; the dashed horizontal line indicates the significance threshold ($p < 0.01$; –log10(p) = 2). Significantly associated markers are labelled.

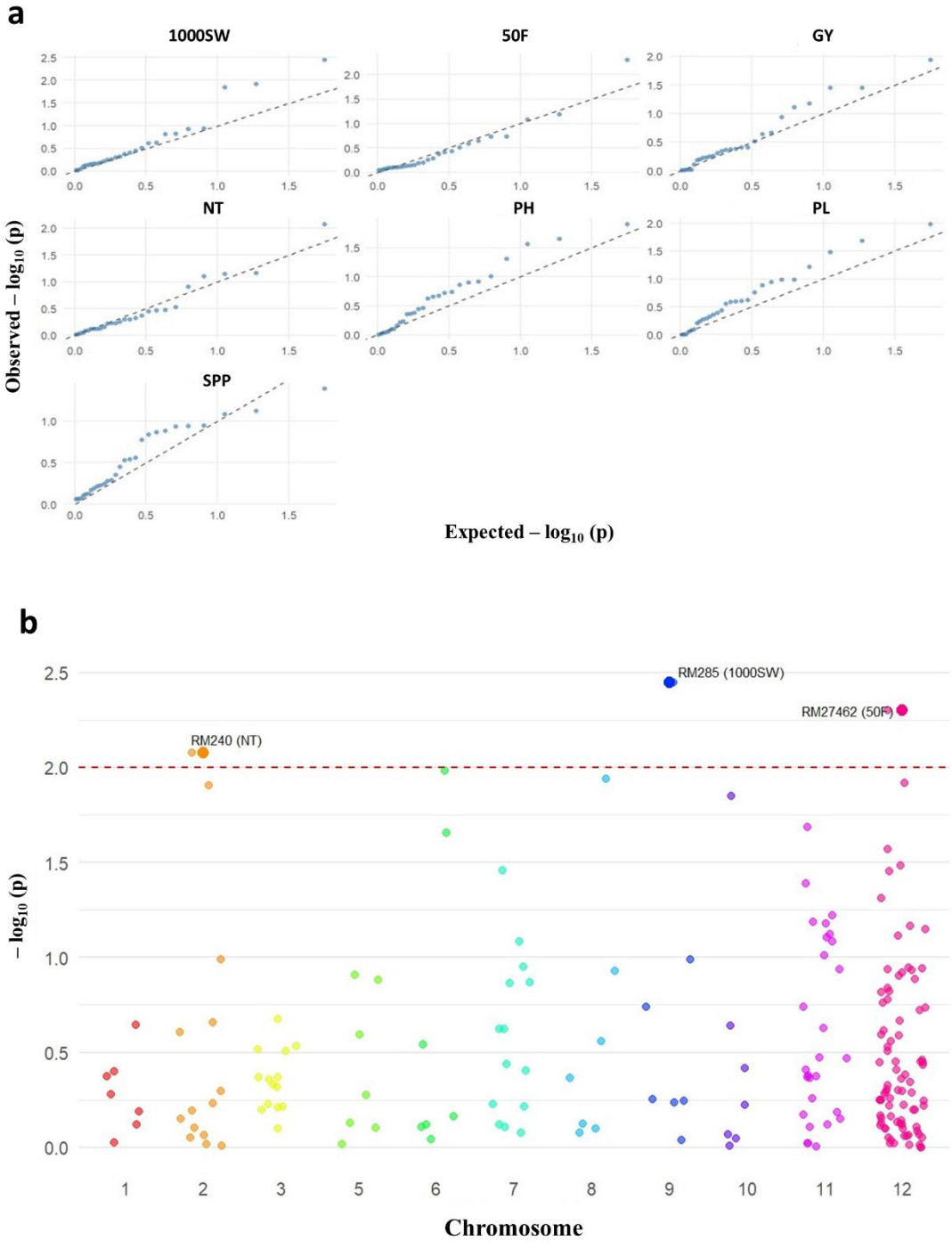

Figure 6. Marker–trait association results using the Mixed Linear Model (MLM), which accounts for both population structure (Q matrix) and kinship (K matrix). (a) Quantile-quantile (QQ) plot of observed vs. expected –log10(p) values, indicating controlled genomic inflation. (b) Manhattan plot displaying –log10(p) values across 12 rice chromosomes; the dashed horizontal line indicates the significance threshold ($p < 0.01$). MLM-significant associations are prioritized over GLM due to lower false-positive rates.

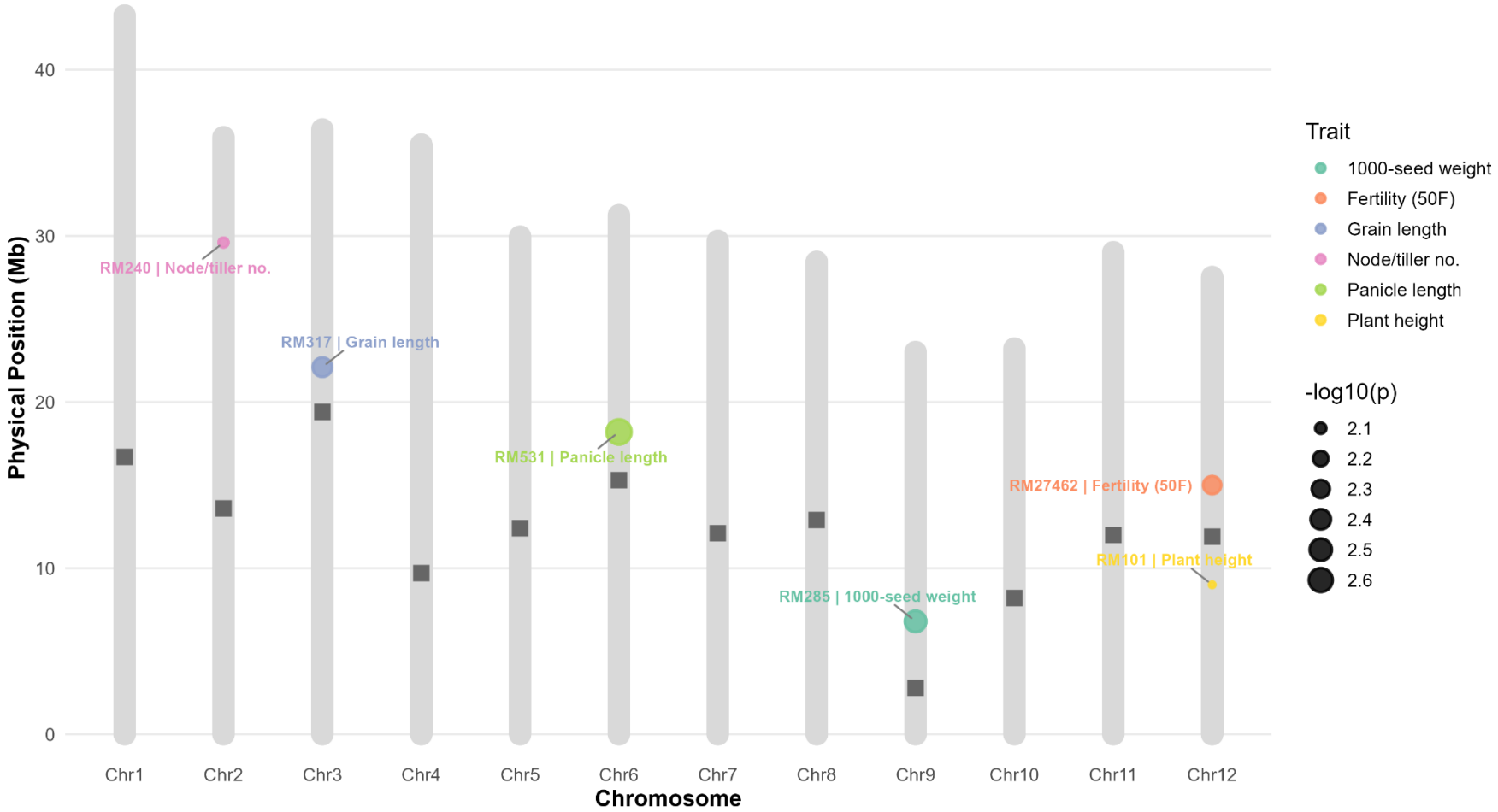

Figure 7: Physical map of rice (*Oryza sativa*) SSR marker–trait associations. The 12 rice chromosomes (Chr1–Chr12) are represented as vertical ideograms scaled according to physical chromosome length (Mb) using the Os-Nipponbare-Reference-IRGSP-1.0 reference genome and MSU-RGAP Release 7 (2011; data files updated 2024). SSR marker–trait association positions are plotted along the chromosomes, with marker size proportional to association strength (−log10(*P*)); marker colours indicate the associated trait: 1000-seed weight, fertility (50F), grain length, node/tiller number, panicle length, and plant height. Marker labels indicate the corresponding SSR marker and trait. Gray squares denote the approximate physical positions of centromeres, based on pseudo-centromere annotations supported by CentO centromeric repeats (GenBank AY101510) together with BAC-tiling/FISH evidence. The reference genome is based on the Nipponbare cultivar, as described by Kawahara *et al.* (2013).

Table S1: List of Rice Genotypes used to conduct study

No.	Genotype	Classification	Source
1	Black Rice	Introduction	China
2	GS-29	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
3	GS-45	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
4	GS-480	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
5	GS-522	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
6	GS-58	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
7	GS-613	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
8	GS-627	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
9	GS-75	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
10	GSR-32	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
11	GSR-47	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
12	IRCTN-312	Collection from outside country	International Rice Cold Tolerance Nursery, International Rice Research Institute (IRRI), Philippines

13	Kasmir zag-1	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
14	NBPGR-12	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
15	NBPGR-27	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
16	NBPGR-4	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
17	Purple Rice	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
18	SKUA-484	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
19	SKUA-524	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
20	SKUA-540-1	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
21	SKUA-557	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
22	SR-4	Released variety	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
23	Zag-V12	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
24	Zag-V4	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
25	C-9	Released variety	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
26	GS-30	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India

27	GS-455	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
28	GS-481	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
29	GS-525	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
30	GS-581	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
31	GS-616	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
32	GS-642	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
33	GS-79	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
34	GSR-37	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
35	GSR-49	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
36	Jehlum	Released variety	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
37	Kohsar	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
38	NBPGR-16	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
39	NBPGR-3	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
40	NBPGR-5	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
41	SKUA-403	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India

42	SKUA-487	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
43	SKUA-532	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
44	SKUA-544	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
45	SKUA-559	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
46	SR-5	Released variety	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
47	Zag-V13	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
48	Zag-V5	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
49	Chanab	Released variety	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
50	GS-33	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
51	GS-459	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
52	GS-484	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
53	GS-527	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
54	GS-59	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
55	GS-617	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India

56	GS-650	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
57	GS-81	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
58	GSR-40	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
59	GSR-50	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
60	K-332	Released variety	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
61	Koshihikari	Secondary selection from introduction	SKUAST-Kashmir, India (secondary selection from an introduction from Japan)
62	NBPGR-2	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
63	NBPGR-31	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
64	NBPGR-6	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
65	SKUA-404	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
66	SKUA-491	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
67	SKUA-5331-1	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
68	SKUA-546	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
69	SKUA-583	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India

70	Zag	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
71	Zag-V14	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
72	Zag-V6	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
73	GS-13	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
74	GS-37	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
75	GS-464	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
76	GS-50	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
77	GS-542	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
78	GS-596	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
79	GS-621	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
80	GS-657	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
81	GS-82	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
82	GSR-42	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
83	GSR-60	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
84	K-39	Released variety	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
85	M Budji	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India

86	NBPGR-21	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
87	NBPGR-32	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
88	NBPGR-9	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
89	SKUA-412	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
90	SKUA-495	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
91	SKUA-535	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
92	SKUA-547	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
93	SR-1	Released variety	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
94	Zag-V1	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
95	Zag-V15	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
96	Zag-V7	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
97	GS-2	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
98	GS-378	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
99	GS-474	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
100	GS-503	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India

101	GS-543	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
102	GS-6	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
103	GS-622	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
104	GS-659	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
105	GSR-19	Local Collection	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
106	GSR-43	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
107	GSR-64	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
108	K-475	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
109	NBPGR-1	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
110	NBPGR-23	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
111	NBPGR-34	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
112	Nunbeal	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
113	SKUA-415	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
114	SKUA-500	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
115	SKUA-536-1	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India

116	SKUA-548	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
117	SR-2	Released variety	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
118	Zag-V10	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
119	Zag-V2	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
120	Zag-V8	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
121	GS-22	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
122	GS-41	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
123	GS-476	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
124	GS-510	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
125	GS-550	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
126	GS-608	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
127	GS-625	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
128	GS-74	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
129	GSR-27	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
130	GSR-46	Collection from outside country	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
131	Heera	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India

132	Kamad	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
133	NBPGR-11-1	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
134	NBPGR-26	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
135	NBPGR-38	Collection from India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India
136	ORN-V1	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
137	SKUA-420	Selection from the parent material	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
138	SKUA-523	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
139	SKUA-538	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
140	SKUA-556	Advanced breeding line	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
141	SR-3	Released variety	Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), India
142	Zag-V11	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
143	Zag-V3	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India
144	Zag-V9	Local landrace	SKUAST-Kashmir germplasm collection, Jammu & Kashmir, India

Abbreviations: **GS**: Genetic Stock, **GSR:** Genetic Stock Rice, **SR:** Shalimar Rice, **SKUA:** Sher-e-Kashmir University of Agricultural, **NBPGR:** National Bureau of Plant Genetic Resources, **IRCTN:** International Rice Cold Tolerance Nursery

Table S2: List of SSR markers used in the study along with their chromosomal locations and associated details. Marker information, including chromosome number, physical position, and motif, was retrieved from the Gramene database (https://www.gramene.org)

Marker name	Marker ID	Chr.	Species	Forward primer (FD)	Reverse primer (RS)	Repeat motif	Anneal. (°C)	Source
RM-27000	(none)	11	—	GAGCGTTTGTAGGGAGTTTAAG	TTTAAACATGGGAGGTGTCAGC	—	—	—
RM-3717	24988765	11	Japonica	AGCTCTACCTTTGCTGTCGG	AACTCCCTAGACCCACCTGC	(GA)16	61	AY020392
RM-235	(none)	12	—	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC	—	55	—
RM-26746	(none)	11	—	GAAGGCCAGAACAATTCTATCAC	CCTAGGAGCGTTTGTAGGTGTGC	—	60	—
RM-101	(none)	12	—	GTGAATGGTCAAGTGACTTAGGTGGC	ACACAACATGTTCCCTCCCATGC	—	65	—
RM-44	(none)	8	—	ACGGGCAATCCGAACAACC	TCGGGAAACCCTACCCCTACC	—	55	—
RM-102	24985778	12	Japonica	AACTTTCCCACCACCACCGGCGG	AGCAGCAGCAAGCCAGCAAGCG	(GGC)7(CG)6	61	D17586
RM-27888	(none)	12	Japonica	GATCCCAGCGGTGAATTCTACTACG	TTGCATGGCTCTGTTGTTGTATGG	(AT)20	55	—
RM-27898	25007291	12	Japonica	CATCCGTACAATCCGTTTCTGG	ATAACCCGTGTATCAGGACTCAGG	(TG)12	55	—
RM-580	24986101	1	Japonica	GATGAACTCGAATTTGCATCC	CACTCCCATGTTTGGCTCC	(CTT)19	55	AP000837
RM-27933	25007320	12	Japonica	TCCTCTGTCATATGGCTGTAAACG	GGACAAGGAGGAACTATTGATTGG	(TA)15	55	—
RM-27966	25007353	12	Japonica	TCTGAGCCAACAGTAAGAGTCAGG	TGTCACCCGTAGTGTTTGTACGG	(TA)28	55	—
RM-27428	25006859	12	Japonica	GGAGCTCGATCAGGAGAATTTAAAGC	TGGAGTTTGAGTAGTACAAGCGAA	(AT)28	55	—

RM-27471	25006899	12	Japonica	CAAACGGACACGCAAGAGAGC	TTGCTTGGGTCTAAACGTGAAGG	(AT)26	55	—
RM-214	24986066	7	Indica	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA	(CT)14	55	AF344035
RM-19	24986188	12	Indica	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	(ATC)10	55	AF344019
RM-5939	24990422	12	Japonica	CACTCCACTGCTGTTGCACT	CACCTCGGTGAGGTTTCTTC	(CAA)14	55	AY022608
RM-262	(none)	2	Indica	CATTCCGTCTCGGCTCAACT	CAGAGCAAGGTGGCTTGC	(CT)16	55	Chen et al. 2004
RM-240	24086314	2	Indica	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC	(CT)21	55	AF344060
RM-S3F	24985523	9	—	—	—	—	—	—
RM285	—	9	Indica	CTGTGGGCCCAATATGTCAC	GCTGCCGACATGGAGAAAG	(GA)12	55	AF344111
RM504	24985556	3	Japonica	TCTATAATGTAGAGCCCCTC	TTTCAGGGGCTTCTACCAAC	(CA)5	55	AQS75805
RM516	24985665	5	Japonica	GTTTCCTGCATGCTTGGAAC	ATGTGATTGTATCAGGCTCG	(AT)16	55	AQ688537
RM517	24985670	3	Japonica	GGCTTACTGGCTTCGATTTG	CGTCTCCTTTGGTTAGTGCC	(CT)15	55	AQ689735
RM-3539	24985768	6	Japonica	GAGCGTCCTTGTTAAAACCG	AGTAGCGTATCACGCATCCG	(TAT)21	55	AQ861571

Table S3: ANOVA for Augmented Block Design for seven traits in 144 pigmented rice genotypes.

Source	Df	50F	PH	PL	NT	SPP	GY	1000SW
Treatment (ignoring Blocks)	143	64.04**	328.89**	7.46**	3.70**	783.41**	151.41**	18.38**
Treatment (eliminating Blocks)	143	44.46**	246.39**	6.52**	3.27ns	606.27**	125.94**	17.42**
Block (eliminating Treatments)	6	1.17ns	9.82ns	0.54ns	0.31ns	313.49ns	0.58ns	0.39ns
Block (ignoring Treatments)	6	467.86**	1976.04**	23.08**	10.51**	4535.33**	607.48**	23.16**
Checks	3	680.92**	2294.65**	57.11**	29.14**	2690.30**	433.34**	74.12**
Test entries vs. Checks	1	1.09ns	00.0**	45.87**	1.87ns	6.34ns	418.14**	421.74**
Test entries	139	51.18**	288.83**	6.12**	3.17ns	747.84**	143.40**	14.27**
Error	18	1.61	6.20	1.01	1.84	206.86	0.59	0.38

*PH = Plant Height, PL = Panicle Length, NT = Number of tillers per plant, SPP = Spikelets per Panicle, 50F = Days to 50% flowering, GY = Grain yield, 1000SW = 1000-seed weight; ** significant at p<0.01; ns = not significant.*

Table S4: Core subset of selected genotypes

S. No.	Genotype	S. No.	Genotype
1	NBPGR34	11	NBPGR2
2	IRCTN312	12	GS22
3	GS484	13	ZagV10
4	GS464	14	SKUA487
5	SKUA523	15	Heera
6	GS455	16	GSR60
7	GS480	17	SKUA548