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Research article

Unearthing novel QTLs for enhancing rice straw quality through genome-wide association mapping

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Abstract

The potential of rice straw as an energy source for ruminants is substantial and depends on improving its cell wall composition through genetic enhancement. This study mapped quantitative trait loci (QTLs) for key straw quality traits, including dry matter, ash, nitrogen, lignin, silica, cellulose, hemicellulose, and digestibilityusing 133 rice lines genotyped with 133 SSR markers. A total of 37 significant marker-trait associations were detected, with eight markers influencing multiple traits and three linked to all traits except silica. Candidate genes included OsSND2, OsMYB55/OsPL and PGIP for cellulose content, OsNADH-GOGAT2 for nitrogen content and OsPG1 for hemicellulose content. These loci, genes and associated markers offer valuable tools for breeding dual-purpose rice varieties that combine high grain yield with superior, more digestible straw.

Keywords: Association mapping, Biofuel, Biomass, Paddy straw, QTL, Rice

Introduction

India contributes 84% (826 Mt) of the world's total rice residues, 980 Mt (Goswami *et al*., 2019). The crop residues, mainly from rice and wheat, are crucial for meeting livestock nutritional needs in India. Fodder availability varies seasonally, with more abundant supply during the wet season and scarcity during the dry season and rice serves as essential dry fodder during lean periods, providing a cost-effective option when high-quality roughages are scarce (Kaur *et al*., 2022). Crop residues, particularly straw, are widely utilized as a major source of roughage to meet the dry matter requirements of ruminant livestock. Straw with higher digestibility is nutritionally superior, as it improves voluntary intake, rumen fermentation efficiency, and overall animal productivity (Subudhi *et al*., 2020). Therefore, it is imperative to systematically examine key cellular and structural characteristics of rice straw and exploit this information for the development of high-digestibility straw rice varieties. Achieving this objective requires a thorough understanding of the genetic basis underlying straw quality traits, which will enable their effective incorporation into rice varietal improvement. Identification of genomic regions governing key straw quality traits enables the development of rice varieties with superior straw quality for diverse applications, including high-value animal feed, biofuel production, and industrial uses, while simultaneously maintaining high grain yield. Recent advances in modern molecular tools have facilitated the precise mapping of genomic regions controlling complex quantitative traits such as straw quality (Nayak *et al*., 2022). Genome-Wide Association Studies (GWAS) offer a heuristic approach of mapping genomic regions using naturally diverse populations (Beena *et al*., 2021; Sah *et al*., 2022). This method leverages natural recombination to identify gene complexes for traits like straw quality through genome-wide DNA marker scanning. GWAS can simultaneously identify multiple traits influencing straw quality. Previous studies, such as Nguyen *et al*. (2020), have

highlighted its effectiveness over bi-parental mapping, identifying QTLs for lignin content and digestibility in rice straw. Thus, the present experiment was aimed to (a) evaluate the variation in rice lines for straw quality traits, (b) identify genomic regions for cell wall component traits, and (c) identify candidate genes that regulate these traits. The findings will have significant implications for understanding the genetic basis of straw quality traits and improving them. Additionally, these findings will directly impact the use and improvement of rice straw for various purposes, such as fodder, industry, etc.

Materials and Methods

Genetic material and phenotyping: The experiment consists of 133 rice genotypes (improved varieties, traditional landraces of indica, japonica, and aus types, and advanced breeding lines) maintained through panicle progeny rows (Sahu *et al*., 2020). Seeds were sown in a nursery bed, irrigated regularly, and transplanted into the main field after 30 days in a randomized block design with three replicatesin 2019. Post-harvest, straw samples were air-dried for two days, then oven-dried at 60°C for two days. Straw quality components analyzed included dry matter (DM), ash content (AC), nitrogen (N), lignin (Li), silica (Si), *in-vitro* organic matter digestibility (IVOMD), cellulose (Cl), hemicellulose (HC), and digestibility (Di). The evaluation, conducted at the International Livestock Research Institute, Hyderabad, used Near Infrared Spectroscopy (NIRS) calibrated by the FOSS Forage Analyzer 6500 with WinISI II software, following methods from Subudhi *et al*. (2020).

Genotyping: Genomic DNA was extracted from all 133 genotypes at 23-day-old rice seedlings using the Doyle and Doyle (1987) protocol. Genotyping was performed with 133 microsatellite markers (SSR markers) distributed across all 12 rice chromosomes. PCR amplification was carried out and the products were separated on a 3% agarose gel stained with ethidium bromide. The separated amplicons were visualized, along with a 50 bp DNA ladder, under a gel documentation system as per the method followed by Sah *et al*. (2023).

Statistical analysis: Descriptive statistics and ANOVA were performed on phenotypic data using Windostat 7.5 software. Principal component analysis was executed using the R package '*factoextra*' (Kassambara, 2017) in R to analyze the data. To understand the interaction between the morphological traits and straw quality, Pearson correlation was executed using the R package '*corrplot*' (Wei and Simko, 2021) in R version 3.6.3. The amplicon was scored on the basis ofit presence and absence; the same data was used to estimate gene diversity, major allele frequency, polymorphic information content (PIC), and heterozygosity for each SSR locus using Power Marker software version 3.25. An unrooted unweighted neighbor-joining tree was constructed with the NEI dissimilarity index using Darwin 6.0 software. Molecular variance analysis (AMOVA) was performed with GenAlex version 6.502. Population structure was analyzed using the STRUCTURE software version 2.3.4. The number of subpopulations was determined using the ΔK method from Evanno *et al*. (2005) and results were processed with Structure Harvester. GWAS was using the general linear model (GLM) and mixed linear model (MLM) in TASSEL version 5.2.63. Markers with p-values greater than 0.05 were considered significantly associated with the phenotype. Linkage disequilibrium (LD) was plotted using r^2 values between markers. Significant markers were aligned to the IRGSP 1.0 genome via The Rice Annotation Project to identify genes linked to straw quality traits. The regions 200kb on either side of each linked marker were designated as QTL regions and searched for associated genes.

Results and Discussion

Phenotypic variation of association panel: The phenotypic variation among 133 rice genotypes was assessed using principal component analysis (PCA) and descriptive statistics based on eight straw quality traits. The PCA biplot revealed that the first two principal components together explained a substantial proportion of the total phenotypic variation, with Dim1 (PC1) accounting for 71.1% and Dim2 (PC2) for 17.3%, collectively capturing the major diversity present in the association panel. PC1 was predominantly influenced by hemicellulose with a strong positive loading, while ash and silica showed negative loadings, reflecting an inverse relationship between structural carbohydrates and mineral components of straw. Cellulose exhibited a strong positive association with PC2, indicating its independent contribution to variation in straw composition. In contrast, lignin, nitrogen, dry matter, and digestibility were positioned close to the origin, suggesting relatively smaller contributions to the variation captured by the first two components. The opposing orientation of cellulose and ash vectors further indicates a negative correlation between these components, whereas the proximity of digestibility to dry matter and lignin suggests moderate associations among these traits. Similar PCA- and biplot-based multivariate approaches have been widely applied for multi-trait evaluation and genotype selection in crop improvement studies by Yan and Frégeau-Reid (2018), highlighting the robustness of biplot analysis for interpreting complex trait relationships.

The descriptive statistics of the straw quality traits were recorded (Table 1). The dry matter ranged from 92.84 to 94.45%, with an average of 93.29%, while the ash

Table 1. Descriptive statistics of phenotypes studied for straw quality

Trait	Mean	Phenotypic variance	Standard error	Range		Skewness	Kurtosis	Shapiro-Wilks' p'
				min	max			
DM	93.29	0.07	0.02	92.84	94.45	1.39	4.13	0.04
Ash	21.93	2.65	0.14	18.78	27.67	0.58	0.42	0.25
N content	1.27	0.04	1.27	0.73	1.83	0.06	0.23	0.71
Lignin	4.84	0.18	0.03	3.59	5.61	-0.37	0.65	0.21
Silica	14.15	0.52	0.06	12.45	16.43	0.07	0.27	0.22
Cellulose	46.60	2.21	0.13	42.82	52.26	0.92	2.40	0.09
Hemicellulose	13.21	5.14	0.19	5.22	18.10	-0.64	0.90	0.03
Digestibility	44.38	0.63	0.07	41.75	46.39	-0.15	0.64	0.28

content ranged from 18.78 to 27.67%, with an average of 21.93%. Straw nitrogen ranged from 0.73 to 1.83%, with an average of 1.27%. The average lignin content of straw was 4.84%, which ranged between 3.59 and 5.61%, while the silica content of straw ranged from 12.45 to 52.26%, with a mean of 14.16%. The cellulose content ranged from 42.83 to 52.26%, with a mean of 46.60%. The average hemicellulose content of straw was 13.21%, which ranged from 5.22 to 18.10%, while the digestibility of straw ranged from 41.75 to 46.39%, with a mean of 44.38%. Further, the third-degree statistic-skewness estimated for all the traits was found to be negligible, and the fourth-degree statistic-kurtosis estimated was also found platykurtic for all the traitsindicating the role of polygenes in controlling these traits (Robson, 1956; Mohanty *et al*., 2025) except for dry matter, which was leptokurtic, where most genotypes are clustered around a central value with fewer extreme deviations. Similar patterns of phenotypic variation in grain, yield, and germination traits have been reported earlier (Nayak *et al*., 2022; Sah *et al*., 2022; Behera *et al*., 2025).

Frequency distribution plots with overlaid normal curves were used to visualize the distribution patterns of phenotypic observations for straw quality traits. Shapiro-Wilk's test indicated that most traits followed a normal distribution. However, dry matter, cellulose, and hemicellulose contents exhibited slight deviations from normality, which were non-significant at the 5% level but significant at the 1% level (Shapiro and Wilk, 1965).

Pearson's correlation analysis was conducted to assess the strength and direction of linear relationships among eight straw quality traits. In this analysis, the values in the upper panels represent Pearson's correlation coefficients (r), and asterisks indicate the level of statistical significance ($p \leq 0.05$*, $p \leq 0.01$**, and $p \leq 0.001$***). Several significant correlations were observed among the traits. Dry matter showed a significant positive correlation with lignin (r= 0.199*) and cellulose content (r = 0.292***), while it was significantly negatively correlated with straw nitrogen (r= −0.420***) and silica content (r= −0.258**). Ash content exhibited significant correlations with most component traits, showing strong positive associations with silica (r= 0.677***), nitrogen (r= 0.297***), lignin (r= 0.300***), and cellulose (r= 0.249***), but a strong negative correlation with hemicellulose content (r= −0.880***) and digestibility (r= −0.245**). Straw nitrogen content showed a strong positive correlation with silica (r = 0.452***), and a significant positive association with digestibility (r = 0.178*), whereas it was strongly negatively correlated with cellulose content (r = −0.513***). Lignin content exhibited a significant positive correlation with cellulose (r= 0.284***), but showed strong negative correlations with hemicellulose (r= −0.439***) and digestibility (r= −0.502***). Silica content was strongly negatively correlated with hemicellulose (r= −0.588***) and digestibility (r = −0.254**). Cellulose content also showed a strong negative correlation with hemicellulose (r= −0.476***), while its association with digestibility was non-significant. The observed correlations suggest that improvement in one trait can influence others. For example, high levels of silica, lignin, and cellulose negatively impact straw digestibility, while lignin also negatively affects hemicellulose content. Similar results were observed in wheat by Joshi *et al*. (2019). In rice straw, high levels of lignin negatively impact digestibility (Nguyen *et al*., 2020). An ideal straw composition for bioethanol synthesis and fodder includes high hemicellulose content, low to medium lignin levels, and low to medium silica concentrations. The analysis of our panel revealed significant associations between these traits, suggesting that they can be improved simultaneously. Straw with high cellulose and hemicellulose content but low silica levels also serves as an excellent substrate for mushroom growth. Our study highlights the potential for multi-trait improvement of straw quality traits, including digestibility.

Genotypic information and population stratification: During the research, 133 microsatellite primers were employed to amplify the individuals within

the association panel. The robustness of these markers in genotyping was evaluated by calculating allele frequency and gene diversity. The major allele frequency for these markers varied from 0.27 to 1, averaging 0.64 alleles. Conversely, gene diversity spanned from 0 to 0.82, with an average of 0.44. The polymorphic information content (PIC) value of these SSR primers was utilized to determine the informativeness of the marker system, with a PIC greater than 0.5 deemed high. The PIC values for these markers ranged from 0 to 0.79, with an average of 0.38. Out of all the primers tested, 44 showed high PIC (>0.5) values when amplified. These findings were consistent with the research conducted by Norton *et al*. (2018).

The primer amplicon data were utilized for analyzing the population structure, which is essential for conducting GWAS analysis, on a group of 133 individuals from the association panel. Initially, population stratification was performed by categorizing individuals through the construction of a neighbor-joining tree using NEI coefficients. This resulted in two major clusters having an almost equal number of individuals (Fig 1A). Further, the Structure analysis ΔK=2, indicating two subpopulations within the association panel (Fig 1B-C). According to the cluster analysis and kinship coefficient, the association panel consisted of two distinct subpopulations, which aligns with the results reported by (Nguyen *et al*., 2020; Sah *et al*., 2022). Molecular variance (AMOVA) estimated between and among subpopulations explained 95% of molecular variance among individuals and 4% variation among populations (Fig 1D), indicating a considerable level of genetic diversity within the sampled population and some level of genetic differentiation between these groups. Similar findings have been previously documented by Mohanty *et al*. (2025).

GWAS and LD analysis: Genome-wide association analysis of straw quality traits was conducted using both generalized linear (GLM) and mixed linear (MLM) models. A total of 37 significant marker–trait associations (MTAs) were identified at p <0.05, distributed across 10 chromosomes. The Q-Q plots showed close agreement between observed and expected p-values, indicating effective control of false positives (Fig 1E). Linkage disequilibrium analysis using TASSEL revealed rapid LD decay, supporting the precision of the detected MTAs (Fig 1F).

GWAS identified significant MTAs for all straw quality traits, with most traits associated with multiple SSR markers under both models, except lignin content. Lignin showed a single association with marker RM334, explaining 6.9% of phenotypic variation, detected only under the MLM model. Straw dry matter content was associated with four SSR markers (RM3825, RM5784, RM400, and RM7443) located on chromosomes 1, 5, 6, and 11, explaining 5.2–7.3% of the variation. Among these, RM5784 was consistently detected under both GLM and MLM, indicating a stable association. Straw ash content was associated with five SSR markers located on chromosomes 1, 3, 5, and 10. Three markers (RM3482, RM22, and RM251) were detected under both models, and RM146 and RM147 exhibited model-specific associations, explaining 5.1–10.7% of the phenotypic variation. Straw nitrogen content was associated with six SSR markers located on chromosomes 4, 5, 6, 7, and 9. Four markers (RM551, RM159, RM336, and RM3823) were detected under both models, whereas RM5784 and RM6917 showed model-specific associations, explaining 5.2–10.0% of the phenotypic variation. Straw silica content was associated with seven SSR markers located on chromosomes 3, 5, 7, and 12. Six markers (RM251, RM426, RM569, RM13, RM336, and RM101) were detected under both models, while RM164 showed a model-specific association, explaining 5.2–12.6% of the phenotypic variation. Straw cellulose content was associated with seven SSR markers located on chromosomes 3, 5, 6, and 11. Five markers (RM5626, RM159, RM334, RM2615, and RM1233) were detected under both models, whereas RM13 and RM5784 exhibited model-specific associations, explaining 5.8–15.8% of the phenotypic variation. Straw hemicellulose content was associated with four SSR markers located on chromosomes 1 and 3. All markers (RM11229, RM3482, RM22, and RM251) were detected under both models, explaining 5.5–9.5% of the phenotypic variation. Straw digestibility was associated with three SSR markers located on chromosomes 1, 4, and 5. All markers (RM3482, RM5709, and RM334) were detected under both models, explaining 6.0–17.8% of the phenotypic variation.

Out of the 37 significant marker-trait associations, eight markers were associated with more than one straw quality trait (Table 2). The SSR, RM5784, located on chromosome 5 was associated with dry matter, nitrogen, and cellulose content, while marker RM3482 on chromosome 1 was associated with ash content, hemicelluloses, and straw digestibility. Similarly, RM251 on chromosome 3 was associated with ash, silica, and hemicellulose content, while RM22 on chromosome 3 was associated with ash and hemicellulose content. The SSR, RM159, present on chromosome 5 was found to be associated with nitrogen content and cellulose content, while marker RM336 on chromosome 7 established associations with nitrogen and silica content. Two markers on chromosome 5, RM334 associated with lignin, cellulose, and digestibility, and marker RM13 associated with straw silica and cellulose content, were found to be particularly useful in breeding rice for the improvement of straw quality traits. These markers can be used for the surrogate selection of more than one trait for straw quality improvement.

Overall, the 37 MTAs corresponded to 37 putative QTLs, of which 26 were consistently identified under both GLM and MLM approaches. While most QTLs had a minor

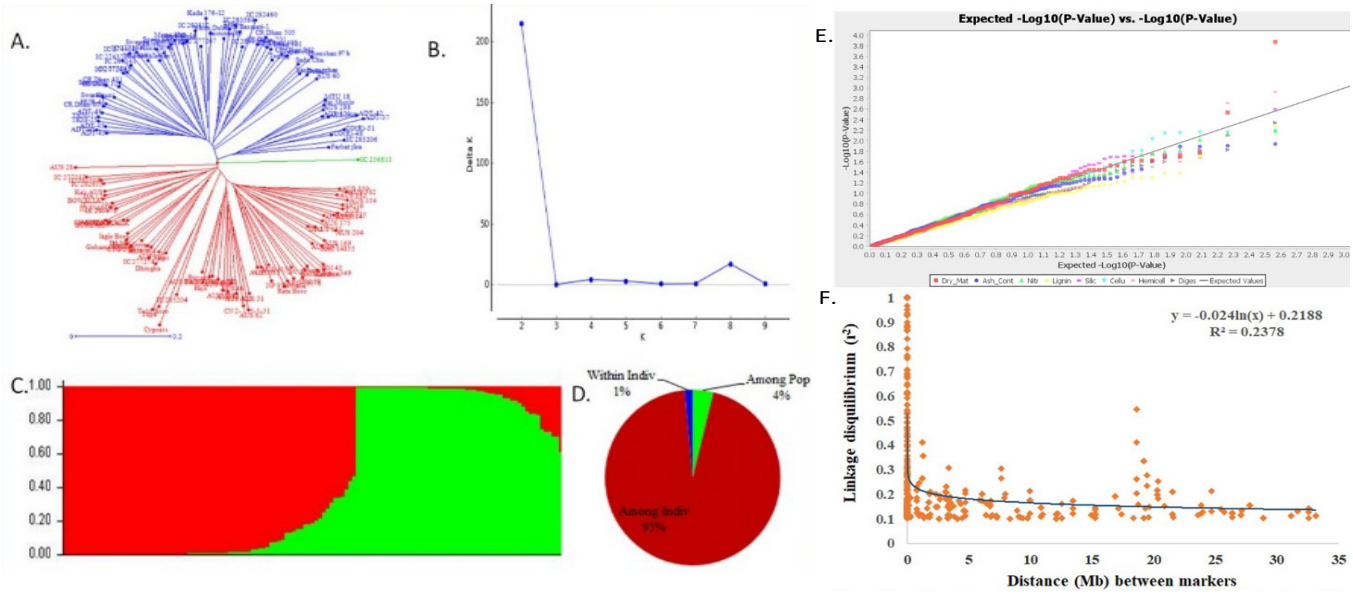

Fig 1. Presents various analyses related to the genetic architecture for straw quality traits in rice- A. shows a Neighbor-Joining Tree of rice genotypes, illustrating their genetic relationships; B. displays Delta K values derived from STRUCTURE analysis, indicating the optimal number of clusters based on mean log-likelihood probabilities; C. inferred clusters (K) ranged from 1 to 9; D. depicts the results of the Bayesian model-based clustering method (STRUCTURE), providing insights into the population structure of the rice lines; E. presents the Analysis of Molecular Variance (AMOVA), highlighting the percentage of molecular variance among and within populations; E. a Q-Q plot, explaining the p-value distribution and association between straw quality traits; F. shows the linkage disequilibrium (LD) between markers, illustrating the genetic linkage across the rice genome

Table 2. Markers significantly associated with multiple traits

Sl. No	Marker	Position	Chromosome	Traits associated
1	RM5784	110.51	5	Dry Matter, nitrogen and cellulose content
2	RM3482	158.76	1	Ash content, hemicelluloses and digestibility
3	RM22	6	3	Ash and hemicelluloses content
4	RM251	40.7	3	Ash, silica and hemicelluloses content
5	RM159	1.79	5	Nitrogen and cellulose content
6	RM336	87.06	7	Nitrogen and silica content
7	RM334	112.61	5	Lignin, cellulose and digestibility
8	RM13	12.44	5	Silica and cellulose content

effect, a few were categorized as major QTLs. They explained phenotypic variation, which ranged from 5.1 to 17.8%. These major QTLs can be used in breeding programs after validation in an independent population. Co-localized QTLs for different traits were also identified, indicating the possibility of using associated markers for the simultaneous improvement of multiple traits. For instance, co-localized QTLs were identified for dry matter content, straw nitrogen content, and cellulose content in straw on chromosome 5. These three traits were positively associated and confirmed by phenotypic correlation. Similarly, one QTL for ash content on chromosome 1 and two QTLs on chromosome 3 were co-localised with hemicelluloses and digestibility, and hemicelluloses and silica content, respectively; the phenotypic correlation between these traits was also found to be significant. Hence, simultaneous improvement of these traits can be highly rewarding, and similar results have been reported by Joshi *et al*. (2019) in wheat. Co-localised QTL for lignin, cellulose, and digestibility was identified on chromosome 5; indicating improvement of any one of these traits may indirectly improve another. Similar results for lignin and digestibility were reported by Hu *et al*. (2018). In general, the co-localized QTL identified can be effective for the simultaneous improvement of more than one trait through a marker-aided breeding program.

Prediction of potential candidate genes: To get a deeper insight into how the genes participate in straw quality traits, we performed GO (gene annotation) and enrichment analysis (http://bis.zju.edu.cn/ricenetdb/) on the genes significantly associated with multiple traits separately (Fig 1E-F; Table 2). Results revealed that the sequence-specific site of that chromosome was mostly enriched with biological processes like metabolic pathways, molecular mechanisms, and subcellular function. Based on GWAS analysis eight multi-traits associated markers (RM5784, RM3482, RM22, RM251, RM159, RM336, RM334, RM13) were subjected to analysis. These markers have not been reported for straw traits. The genes in the 200 kb vicinity of the associated markers were retrieved from the Rice Annotation Project database (https://rapdb.dna.affrc.go.jp/viewer/gbrowse/irgsp1/, accessed on 18 August 2022) based on their putative function. Upon screening of the loci in the proposed region, known abiotic and biotic stresses, morphological and seed-characterized genes were identified. However, those genes were also involved in the various cellular and biological functions related to straw traits, which were not reported. Annotation, GO enrichment analysis, and trait ontology (TO) were performed for all the significant markers. The marker information was searched through the RiceNetDB and Oryzabase databases and 3 associated markers, RM5784, RM22 and RM334, linked to the straw traits were also linked to some well-characterized genes reported for biotic stress and morphological traits, which were searched for functional characterization.

The SSR marker RM5784, located on chromosome 5, was significantly associated with dry matter, nitrogen, and cellulose content of rice straw. Gene enrichment analysis within the RM5784 genomic region identified 71 significantly enriched genes, of which 21 were related to morphological traits and biotic and abiotic stress tolerance in rice. Among these, the well-characterized gene OsSND2 (*LOC_Os05g48850*), encoding a NAC family transcription factor, was identified as a key candidate underlying the observed associations. OsSND2 plays a crucial role in secondary cell wall biosynthesis and acts as a positive regulator of cellulose accumulation. Functional studies have shown that knockout of OsSND2 results in reduced cellulose content and downregulation of secondary cell wall-related genes, supporting its role in determining straw quality traits (Ye et al., 2018). Another putative gene, OsNADH-GOGAT2 (*LOC_Os05g48200*), encoding NADH-glutamate synthase 2, was also linked to marker RM5784 and showed a strong association with nitrogen content in rice straw. OsNADH-GOGAT2 plays a central role in nitrogen metabolism, particularly in the remobilization of nitrogen from senescing tissues. This gene encodes a key enzyme of the glutamine–glutamate cycle, which is essential for nitrogen assimilation and redistribution in plants. Previous studies have demonstrated that OsNADH-GOGAT2 significantly contributes to nitrogen use efficiency and nitrogen remobilization during plant development (Chao *et al*., 2015), supporting its involvement in regulating straw nitrogen content observed in this study. A putative gene, OsMYB55 (also known as OsPL; *LOC_Os05g48010*), encoding an R2R3-MYB transcription factor, was identified in association with cellulose and dry matter content in rice straw. OsMYB55 is known to regulate multiple aspects of plant growth and development, including stress responses, secondary metabolism, and cell wall biosynthesis, particularly through modulation of the lignin biosynthesis pathway, which is important for cell wall reinforcement and the overall quality of rice straw. Overexpression of OsMYB55 can increase dry matter content in rice straw (Miyamoto *et al*., 2020). The marker RM22, located on chromosome 3, was associated with ash content and hemicellulose content of rice straw. Gene enrichment analysis around RM22 identified 91 genes involved in diverse biological pathways, of which 24 genes were related to morphological traits and biotic and abiotic stress responses. Among these, the well-characterized gene OsPG1 (*LOC_Os03g03350*), encoding a polygalacturonase involved in pectin degradation and regulation of intercellular adhesion, is associated with hemicellulose content of straw, which is involved in the breakdown of pectin, a major component of plant cell walls. Mutations in OsPG1 altered cell wall structure and composition and enhanced resistance to the bacterial blight pathogen (Cao *et al*., 2021).

The marker RM334, located on chromosome 5, was associated with nitrogen and cellulose content of rice straw. Gene enrichment analysis of the RM334 region identified 162 significantly enriched genes, including 13 involved in disease resistance and seed morphological traits. Among these, the polygalacturonase-inhibiting protein (PGIP) genes, including PGIP1 (*LOC_Os05g01380*), PGIP2 (*LOC_Os05g01370*), PGIP3 (*LOC_Os05g01430*), and PGIP4 (*LOC_Os05g01444*), These genes are related to the defense response against fungal polygalacturonase enzymes and indirectly contribute to maintaining the integrity and structure of the cell wall, which includes cellulose. The presence of PGIP proteins can impact the cellulose content in rice straw by preventing the breakdown of pectin, which is a component of the cell wall matrix.

Conclusion

Understanding the complex biochemical, physical and genetic variation in rice straw is essential for improving its quality. Genome-wide association analysis with SSR markers identified 37 significant marker–trait associations for straw quality traits, including three markers consistently associated with all traits except silica, suggesting pleiotropic effects. Putativecandidate

genes such as OsSND2, OsMYB55/OsPL and PGIP for cellulose, OsNADH-GOGAT2 for nitrogen and OsPG1 for hemicellulose highlight key components of the genetic architecture of straw quality, providing promising targets for simultaneous improvement of multiple traits through marker-assisted breeding after validation.

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