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CANDIDATE GENE-TARGETED TRAP MARKER ANALYSIS REVEALS GENETIC DIVERSITY AMONG RICE (ORYZA SATIVA L.) GERMPLASM FOR IRON BIOFORTIFICATION

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Abstract:
Iron deficiency remains one of the most prevalent micronutrient disorders worldwide, particularly in populations that rely heavily on rice as a staple food. Developing rice cultivars with enhanced grain iron content requires a comprehensive understanding of the genetic diversity present in iron-related genes. In the present study, Target Region Amplification Polymorphism (TRAP) markers derived from candidate iron transporter genes were employed to evaluate the genetic diversity of 30 rice (Oryza sativa L.) genotypes. Thirty gene-specific primers targeting iron transport and homeostasis-related loci were paired with two arbitrary primers (ME02 and ME05) to generate polymorphic DNA profiles. The primer combinations produced a total of 703 scorable amplification products ranging from 50 to 800 bp, of which 654 (93.0%) were polymorphic and 49 (7.0%) were monomorphic, demonstrating a high level of genetic variability among the evaluated germplasm. The polymorphic information content (PIC) values ranged from 0.09 to 0.25, with an average of 0.22, indicating moderate to high discriminatory power of the candidate gene-based TRAP markers. Cluster analysis based on UPGMA grouped the genotypes into two major clusters, while Bangara kovi and Jeerige sanna formed distinct solitary branches, reflecting their unique genetic constitution. The observed genetic relationships indicate substantial diversity within the studied germplasm and demonstrate the effectiveness of candidate gene-targeted TRAP markers in detecting functional polymorphisms associated with iron transporter genes. These findings provide valuable information for marker-assisted breeding, association mapping, and the development of iron-biofortified rice cultivars.

KEYWORDS: Target Region Amplification Polymorphism (TRAP); candidate genes; iron transporter genes; genetic diversity; Oryza sativa; iron biofortification; molecular markers; marker-assisted breeding.

INTRODUCTION

Rice (Oryza sativa L.) is the primary staple food for more than half of the global population and serves as a major source of daily caloric intake, particularly in Asia and sub-Saharan Africa. Despite its importance in food security, polished rice grains contain relatively low concentrations of bioavailable iron (Fe), making populations dependent on rice highly vulnerable to iron deficiency anemia and associated health disorders, including impaired cognitive development, reduced immunity, and decreased work productivity (1–3). Therefore, increasing the iron concentration of rice grains has become a major objective in crop improvement programs.

Biofortification, the genetic enhancement of micronutrient content in staple crops through plant breeding and biotechnology, has emerged as a sustainable and cost-effective strategy to alleviate hidden hunger (3–5). Unlike food fortification and mineral supplementation, biofortification offers a long-term solution by developing nutrient-rich cultivars that can be cultivated and consumed without additional economic burden. The success of biofortification depends largely on the availability of genetically diverse germplasm and a comprehensive understanding of the molecular mechanisms regulating micronutrient uptake, transport, and accumulation in grains (4,5).

Iron homeostasis in rice is controlled by a coordinated network of genes involved in metal uptake, transport, chelation, sequestration, and remobilization. Members of the Natural Resistance-Associated Macrophage Protein (NRAMP), Yellow Stripe-Like (YSL), Metal Tolerance Protein (MTP), Zinc/Iron-Regulated Transporter (ZIP), Nicotianamine Synthase (NAS), and Adenine Phosphoribosyl Transferase (APRT) gene families have been reported to play central roles in iron acquisition and translocation within the plant (5–8). Natural allelic variation within these functional genes provides valuable genetic resources for identifying superior donor lines and accelerating marker-assisted breeding for iron biofortification.

Molecular markers have become indispensable tools for evaluating genetic diversity, identifying trait-associated loci, and supporting modern crop improvement programs. Conventional marker systems such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Sequence-Related Amplified Polymorphism (SRAP), Simple Sequence Repeats (SSR), and Single Nucleotide Polymorphisms (SNPs) have been widely used for genetic characterization in rice (9–14). However, many of these marker systems detect polymorphisms randomly throughout the genome and may not adequately represent functional variation within genes governing economically important traits.

Target Region Amplification Polymorphism (TRAP) is a PCR-based, gene-targeted marker system that combines a fixed primer designed from a candidate gene sequence with an arbitrary primer to amplify genomic regions adjacent to functional loci (15). Because TRAP markers are directly associated with genes of known biological function, they provide greater opportunities for detecting functionally relevant polymorphisms than anonymous molecular markers. The technique is highly reproducible, technically simple, cost-effective, and capable of generating numerous polymorphic loci in a single PCR reaction (15,16).

The TRAP marker system has been successfully employed for genetic diversity analysis, cultivar identification, linkage mapping, and quantitative trait locus (QTL) analysis in several crop species, including lettuce, wheat, sugarcane, and rice (16–19). More recently, SSR- and SNP-based studies have identified genomic regions associated with grain iron and zinc accumulation and have facilitated marker-assisted breeding for nutritional improvement in rice (6,20–22). Nevertheless, relatively limited information is available regarding the application of candidate gene-based TRAP markers specifically targeting iron transporter genes for assessing genetic diversity in rice germplasm.

Therefore, the present study employed candidate gene-based TRAP markers developed from iron transporter-related genes to evaluate the genetic diversity among thirty rice genotypes. The study further assessed the effectiveness of TRAP markers in detecting functional polymorphisms associated with iron transport and explored the genetic relationships among diverse rice germplasm. The information generated is expected to facilitate germplasm characterization, parental selection, association mapping, and marker-assisted breeding aimed at developing iron-biofortified rice cultivars with enhanced nutritional quality.

MATERIALS AND METHODS

4.1 Plant material

Thirty genetically diverse rice (Oryza sativa L.) genotypes, including released cultivars and indigenous landraces, were used in the present investigation. The experimental materials consisted of Am65, Azucena, BI33, BJ21, Devamallige, Burma Black, IR20, Jeerige Sanna, Alur Sanna, Amrutha, Antrasali, Bangara Kovi, Bangaru Sanna, BI43, Bidar Local-1, Bile Kalavi, Champakali, Chippiga, Dambersali, Doddamullare, Doddiga, Farm Valya, Gandhasali, Gopal Doddiga, Gujarat Buddha, Holesali Chippiga, Honasu, Honnekattu, HY 258-1, and J-192. These genotypes were selected to represent broad genetic variability for evaluating diversity associated with iron transporter genes.

Table 1. Primer sequences used in the present study

Sl. No.	Primer Name	Sequence (5′–3′)
1	OsYSL4d	5′-TCTAGAACGGTCACACAGAAAA-3′
2	OsMTP1d	5′-TGCCATGTGACAATCACTCA-3′
3	OsNRAMP5g	5′-GATCATTACGTATGTCGTTGTATCTC-3′
4	APRT1a	5′-CTCTCTCCCGCTTTTTGCTT-3′
5	OsNRAMP5c	5′-GCTTTGCTGATCGGGATTAGTT-3′
6	OsYSL4e	5′-TATGCATGCGGTGGATGA-3′
7	OsMTP1b	5′-GGGGACCTTCTTGTTGTTGG-3′
8	OsNRAMP5g	5′-GATCATTACGTATGTCGTTGTATCTC-3′
9	GrmM1-1	5′-GTCGTCATGATCTGGGACT-3′
10	OsNRAMP1c	5′-GGGTTCTCATTGCTGGCTCT-3′
11	OsNRAMP1c	5′-GGGTTCTCATTGCTGGCTCT-3′
12	OsNRAMP5f	5′-GATTAGCAAAATGTCACTGACTAGC-3′
13	OsMTP1d	5′-TGCCATGTGACAATCACTCA-3′
14	OsYSL1a	5′-TAAACCAAAGATGGCAGACC-3′
15	OsNRAMP5d	5′-TAATTTCGGGCTCCAGTACC-3′
16	OsYSL4c	5′-TATTAGTGAGGGCGATCCAC-3′
17	OsYSL4e	5′-TATGCATGCGGTGGATGA-3′
18	OsZIP8a	5′-ATGAGGACGAACACCACCAC-3′
19	OsYSL4a	5′-GCAAACACTACCCCAAAAGC-3′
20	APRT1c	5′-GGAAGTTTCCTTTTGGCTGT-3′
21	APRT1b	5′-GTTGTGAAATATCAGGTGTTGAAGC-3′
22	OsNAS3a	5′-TCACCAGTTGGAGCTAATCG-3′
23	OsYSL4f	5′-CACCTCTACATCCCGAGCAT-3′
24	APRT1a	5′-CTCTCTCCCGCTTTTTGCTT-3′
25	OsYSL4b	5′-AGCTCTGCGGGTGAACATAC-3′
26	OsMTP1a	5′-TCTCTCCTCCCCATCTCCAA-3′
27	OsNRAMP1b	5′-TTGTACGTCTTTGAGACTTTGACTG-3′
28	OsNRAMP5c	5′-GCTTTGCTGATCGGGATTAGTT-3′
29	OsMTP1a	5′-TCTCTCCTCCCCATCTCCAA-3′
30	OsYSL4b	5′-AGCTCTGCGGGTGAACATAC-3′

Arbitrary primers

Sl no.	Arbitrary primer	Sequence
1	ME05	5′-TGAGTCCAAACCGGAAG-3′
2	ME02	5′-TGAGTCCAAACCGGAGC-3′

Some gene-specific primers were intentionally paired with different arbitrary primers (ME02 and ME05) to generate multiple TRAP marker combinations.

4.2 Primer designing

Expressed sequence tag (EST) sequences for Fe-transporter genes were retrieved from a rice genomic database, and forward gene-specific primers were designed using a primer-design database.

To determine the spatial and temporal expression pattern of these genes across tissue libraries, the gene sequences were analyzed in silico for co-localized ESTs (http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer). The locus ID of each gene was used as a query to identify ESTs mapped to that gene; ESTs corresponding to a given tissue library indicated the putative site of expression, expressed as transcripts per million (TPM), an indirect measure of relative gene expression (27). Two arbitrary primers, ME05 and ME02, were used in the study.

4.3 Genomic DNA isolation

Genomic DNA was extracted from fresh leaves of 21-day-old seedlings using the cetyltrimethylammonium bromide (CTAB) method with minor modifications (25). DNA quality was assessed by electrophoresis on 0.8% agarose gel, and DNA concentration and purity were determined spectrophotometrically. DNA samples were diluted to approximately 25 ng/μL^{-1} and stored at −20°C until PCR amplification.

4.4 TRAP-PCR amplification

TRAP analysis was performed according to the method described by (18). Each PCR reaction (10 μL) contained 25 ng genomic DNA, 1× PCR buffer (10 mM Tris-HCl, pH 8.4, 50 mM KCl), 2.5 mM $MgCl_2$, 0.05 mM each dNTP, 5 pmol each of the fixed and arbitrary primers, and 1 U Taq DNA polymerase. Amplification was carried out in an Eppendorf Nexus Gradient Thermal Cycler with an initial denaturation at 95°C for 5 min, followed by five cycles of 94°C for 45 s, 35°C for 45 s, and 72°C for 1 min. Subsequently, this was followed by 35 amplification cycles at 94°C for 45 s, 52°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 7 min.

4.5 Electrophoresis and marker scoring

Amplified products were separated on 6% polyacrylamide gels at 150 V for approximately 1.5–2 h and visualized using a rapid silver staining procedure (26,27). Only clear and reproducible bands ranging from 50 to 800 bp were considered for analysis. Amplified fragments were scored as present (1) or absent (0), and a binary data matrix was generated for subsequent genetic diversity analyses.

4.6. Statistical analysis

The binary marker matrix was used to estimate genetic diversity among the rice genotypes. Major allele frequency, observed allele number, gene diversity, and polymorphic information content (PIC) were calculated using PowerMarker version 3.25 (28). Pairwise genetic similarity coefficients were estimated using Dice's similarity coefficient (29), and an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram was generated using NTSYS-pc version 2.21 (30). Principal Coordinate Analysis (PCoA) was performed using GenAlEx version 6.5 to further evaluate genetic relationships among the rice genotypes (31).

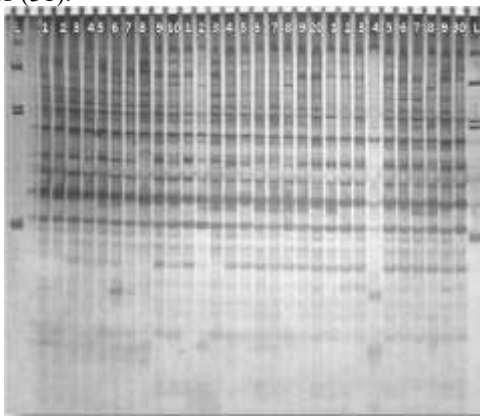

Fig. 1. TRAP marker banding pattern generated by the primer combination 1 Osnrmpc and ME02.

5. RESULTS AND DISCUSSION

5.1 TRAP marker polymorphism and amplification efficiency

The candidate gene-based Target Region Amplification Polymorphism (TRAP) markers successfully amplified genomic regions associated with iron transporter genes across all 30 rice genotypes. Generated a total of 703 clear and reproducible DNA fragments ranging from 50 to 800 bp. Among these, 654 fragments (93.03%) were polymorphic, whereas only 49 fragments (6.97%) were monomorphic, demonstrating a high level of genetic variability within the evaluated germplasm (Table 4). The number of amplified fragments per primer combination ranged from 14 to 30, with an average of approximately 23 fragments.

The high proportion of polymorphic loci indicates that TRAP markers designed from candidate iron transporter genes effectively captured functional genetic variation among rice genotypes. Because the fixed primers were anchored to genes involved in iron uptake and transport, the amplified polymorphisms are likely to represent biologically relevant allelic variation rather than randomly distributed genomic polymorphisms. Similar levels of polymorphism have been reported in TRAP-based diversity studies conducted in rice, sugarcane, lettuce, and wheat, confirming the efficiency and reproducibility of this marker system for functional diversity analysis (15–18,26).

The present findings further demonstrate that gene-targeted TRAP markers provide greater specificity than anonymous marker systems such as RAPD and AFLP because amplification is directed toward functionally important genomic regions. Consequently, these markers may provide greater value for identifying useful alleles associated with iron accumulation and nutritional improvement.

5.2 Marker informativeness

The informativeness of each primer combination was evaluated using polymorphic information content (PIC). PIC values varied from 0.09 to 0.25, with an average value of 0.22 (Table 4). The highest PIC values (0.25) were observed for the primer combinations Osnramp5c/ME05, Osysl4a/ME02, and Osmtp1a/ME02, whereas Osysl4b/ME05 exhibited the lowest PIC value (0.09).

PIC values between 0.20 and 0.30 indicate moderate levels of marker informativeness for dominant marker systems. The relatively high PIC values obtained for several candidate gene-derived primers suggest that substantial allelic diversity exists within iron transporter loci among the studied rice germplasm. Functional genes involved in iron homeostasis are often subjected to natural selection during domestication and environmental adaptation, which may contribute to the observed genetic variation. Similar PIC values have been reported for TRAP markers in rice and sugarcane, indicating that candidate gene-based TRAP markers are suitable for genetic diversity studies and marker-assisted breeding (15,17).

5.3 Genetic diversity among rice germplasm

Genetic similarity analysis based on Dice's coefficient revealed considerable genetic diversity among the thirty rice genotypes. Pairwise similarity coefficients ranged from moderate to high, indicating the presence of both closely related and genetically distinct accessions (Table 2). The highest similarity (0.90) was observed between AM65 and Azucena, suggesting that these cultivars possess comparable genetic backgrounds. Likewise, BI43 and Bidar Local-1 exhibited high genetic similarity (0.79), while Honnekattu and HY258-1 shared a similarity coefficient of 0.89.

In contrast, several traditional landraces displayed lower similarity values, indicating the existence of unique allelic combinations that could serve as valuable genetic resources for future breeding programmes. The observed diversity reflects differences in geographical origin, breeding history, and adaptation to local agro-climatic conditions. Indigenous landraces generally retain greater genetic variability than modern cultivars because they have experienced relatively limited selection pressure during crop improvement. Such diverse germplasm represents an important reservoir of favourable alleles for biofortification breeding.

5.4 Cluster analysis

The UPGMA dendrogram constructed from TRAP marker data grouped the thirty rice genotypes into two major clusters (Figure 2). Most improved cultivars and local landraces were distributed within these principal clusters according to their genetic similarity. However, Bangara Kovi and Jeerige Sanna formed two independent solitary branches, clearly indicating their distinct genetic backgrounds.

The clustering pattern largely reflected pedigree relationships and genetic divergence among the evaluated genotypes. The separation of Bangara Kovi and Jeerige Sanna suggests that these traditional cultivars possess unique alleles within iron transporter genes that were absent or less frequent in the remaining germplasm. Such genetically distinct accessions are particularly valuable for broadening the genetic base of breeding populations and may serve as potential donor parents for improving grain iron concentration.

Comparable clustering patterns have been reported in previous TRAP-based diversity analyses in rice and other cereal crops, where candidate gene-derived markers effectively differentiated genetically diverse accessions according to functional genomic variation (17–19).

5.5 Principal coordinate analysis

Three-dimensional principal coordinate analysis (PCoA) further confirmed the clustering pattern observed in the UPGMA dendrogram (Figure 3). The majority of rice genotypes formed two major groups with clear spatial separation, while Bangara Kovi and Jeerige Sanna remained isolated from the principal clusters.

The close agreement between cluster analysis and PCoA indicates the robustness of the TRAP marker data for evaluating genetic relationships among rice germplasm. Multivariate analyses are particularly valuable because they provide graphical visualization of genetic distances without assumptions regarding hierarchical relationships. The consistency between both analytical approaches demonstrates the reliability of the generated marker data and supports the observed patterns of genetic diversity.

5.6 Significance of candidate gene-based TRAP markers

The present investigation demonstrates that candidate gene-based TRAP markers represent an efficient tool for assessing functional genetic diversity associated with iron transporter genes in rice. Unlike anonymous molecular markers, TRAP markers amplify genomic regions directly linked to biologically important candidate genes, thereby increasing the probability of detecting polymorphisms associated with target traits.

The high level of polymorphism, moderate marker informativeness, and clear discrimination among rice genotypes indicate that TRAP markers can effectively support germplasm characterization, parental selection, and marker-assisted breeding programmes. Furthermore, the identified genetically diverse accessions constitute valuable genetic resources for future association mapping, quantitative trait locus validation, and genomic studies aimed at improving grain iron

concentration. Integration of candidate gene-based molecular markers with conventional breeding and modern genomic approaches is expected to accelerate the development of nutritionally enhanced rice cultivars capable of contributing to sustainable iron biofortification and global nutritional security.

6. CONCLUSION

Candidate gene-targeted TRAP markers effectively detected functional genetic diversity among thirty rice genotypes and revealed high polymorphism associated with iron transporter genes. The genetically distinct accessions identified in this study, particularly Bangara Kovi and Jeerige Sanna, represent valuable donor parents for future biofortification programmes. These findings demonstrate the usefulness of candidate gene-based TRAP markers for germplasm characterization, marker-assisted breeding, and the development of nutritionally enhanced rice cultivars.

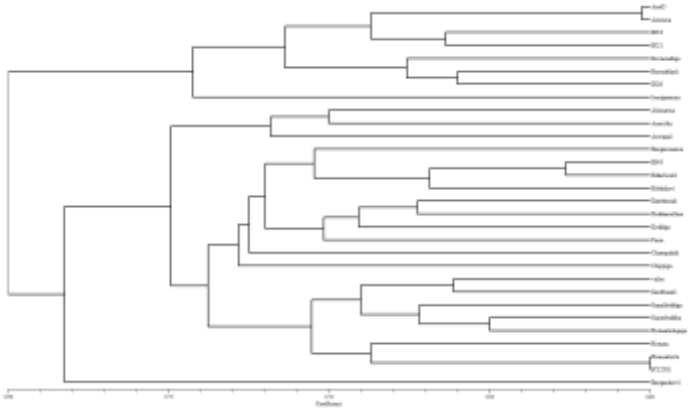

Fig. 2. UPGMA cluster dendrogram showing genetic relationships and diversity among 30 rice accessions based on TRAP marker polymorphisms.

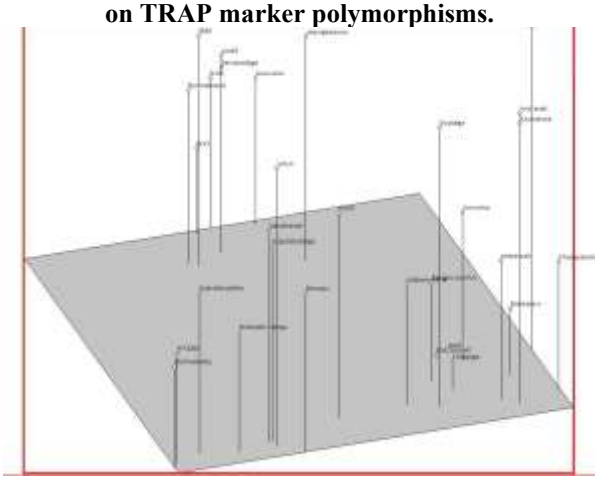

Fig. 3. Three-dimensional (3D) plot showing genetic relationships and diversity among 30 rice accessions based on TRAP marker data

Table 2. Correlation matrix of genetic distances derived from the complement of pairwise similarity coefficients among 30 rice genotypes (genotype numbers correspond to the rice genotypes listed in Materials and Methods) (Section 2.1).

Geno.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2	1.00																													
3	0.90	1.00																												
4	0.81	0.82	1.00																											
5	0.79	0.80	0.83	1.00																										
6	0.77	0.76	0.79	0.79	1.00																									
7	0.77	0.77	0.81	0.80	0.83	1.00																								

8	0.76	0.75	0.76	0.78	0.81	0.84	1.00																						
9	0.74	0.73	0.73	0.76	0.73	0.75	0.77	1.00																					
10	0.65	0.66	0.65	0.64	0.68	0.69	0.68	0.68	1.00																				
11	0.68	0.70	0.70	0.69	0.71	0.72	0.71	0.70	0.79	1.00																			
12	0.65	0.64	0.63	0.64	0.64	0.66	0.64	0.64	0.77	0.77	1.00																		
13	0.66	0.67	0.62	0.65	0.65	0.65	0.65	0.67	0.69	0.70	0.74	1.00																	
14	0.67	0.68	0.70	0.68	0.70	0.70	0.69	0.68	0.74	0.75	0.74	0.71	1.00																
15	0.70	0.71	0.71	0.72	0.73	0.73	0.71	0.71	0.73	0.78	0.71	0.70	0.79	1.00															
16	0.71	0.73	0.76	0.73	0.73	0.74	0.73	0.72	0.74	0.81	0.73	0.71	0.80	0.87	1.00														
17	0.68	0.70	0.68	0.69	0.68	0.70	0.69	0.70	0.74	0.76	0.75	0.72	0.77	0.82	0.84	1.00													
18	0.65	0.65	0.65	0.65	0.66	0.65	0.65	0.64	0.74	0.74	0.76	0.73	0.74	0.76	0.78	0.79	1.00												
19	0.67	0.68	0.67	0.66	0.67	0.68	0.65	0.64	0.71	0.73	0.72	0.68	0.74	0.75	0.78	0.77	0.75	1.00											
20	0.68	0.69	0.66	0.67	0.68	0.69	0.68	0.68	0.75	0.79	0.76	0.72	0.79	0.77	0.80	0.80	0.79	0.79	1.00										
21	0.69	0.70	0.71	0.68	0.70	0.72	0.70	0.69	0.74	0.77	0.75	0.72	0.77	0.79	0.81	0.80	0.76	0.78	0.82	1.00									
22	0.66	0.67	0.64	0.64	0.69	0.67	0.68	0.66	0.72	0.74	0.76	0.71	0.72	0.73	0.74	0.75	0.73	0.71	0.81	0.80	1.00								
23	0.68	0.69	0.68	0.67	0.72	0.71	0.71	0.67	0.72	0.75	0.75	0.72	0.76	0.75	0.78	0.76	0.74	0.77	0.77	0.80	0.80	1.00							
24	0.66	0.64	0.65	0.66	0.67	0.69	0.69	0.66	0.72	0.71	0.74	0.69	0.74	0.71	0.74	0.74	0.71	0.70	0.76	0.77	0.76	0.82	1.00						
25	0.67	0.67	0.68	0.68	0.67	0.70	0.69	0.68	0.70	0.73	0.74	0.70	0.76	0.74	0.77	0.77	0.72	0.72	0.76	0.78	0.75	0.78	0.83	1.00					
26	0.68	0.67	0.67	0.67	0.68	0.71	0.70	0.66	0.72	0.74	0.73	0.68	0.76	0.74	0.76	0.74	0.72	0.71	0.78	0.78	0.77	0.77	0.78	0.80	1.00				
27	0.67	0.66	0.68	0.67	0.70	0.73	0.71	0.67	0.70	0.74	0.74	0.66	0.76	0.74	0.77	0.74	0.72	0.72	0.77	0.80	0.75	0.78	0.81	0.83	0.83	1.00			
28	0.67	0.67	0.68	0.66	0.68	0.69	0.69	0.67	0.69	0.74	0.73	0.67	0.76	0.74	0.79	0.75	0.71	0.74	0.76	0.79	0.75	0.78	0.79	0.82	0.81	0.85	1.00		
29	0.65	0.64	0.65	0.63	0.65	0.67	0.66	0.64	0.71	0.73	0.73	0.69	0.71	0.74	0.74	0.73	0.75	0.73	0.75	0.76	0.73	0.77	0.76	0.78	0.77	0.77	0.78	1.00	
30	0.65	0.64	0.66	0.65	0.66	0.69	0.67	0.64	0.69	0.73	0.71	0.65	0.72	0.74	0.74	0.72	0.71	0.74	0.73	0.76	0.70	0.77	0.76	0.76	0.77	0.82	0.80	0.82	1.00
31	0.67	0.66	0.67	0.67	0.68	0.71	0.69	0.65	0.69	0.75	0.72	0.66	0.73	0.74	0.77	0.74	0.72	0.75	0.75	0.77	0.72	0.79	0.78	0.79	0.80	0.84	0.81	0.80	0.90

Table 3. TRAP primer combinations (fixed candidate-gene primer × arbitrary primer) used for genotyping

Sl. No.	Primer Combination	Gene-specific Primer Sequence (5′–3′)	Arbitrary Primer Sequence (5′–3′)
1	OsYSL4d + ME05	TCTAGAACGGTCACACAGAAAA	TGAGTCCAAACCGGAAG
2	OsMTP1d + ME05	TGCCATGTGACAATCACTCA	TGAGTCCAAACCGGAAG

3	OsNRAMP5g + ME05	GATCATTACGTATGTCGTTGTATCTC	TGAGTCCAAACCGGAAG
4	APRT1a + ME02	CTCTCTCCCGCTTTTTGCTT	TGAGTCCAAACCGGAGC
5	OsNRAMP5c + ME05	GCTTTGCTGATCGGGATTAGTT	TGAGTCCAAACCGGAAG
6	OsYSL4e + ME05	TATGCATGCGGTGGATGA	TGAGTCCAAACCGGAAG
7	OsMTP1b + ME05	GGGGACCTTCTTGTTGTTGG	TGAGTCCAAACCGGAAG
8	OsNRAMP5g + ME02	GATCATTACGTATGTCGTTGTATCTC	TGAGTCCAAACCGGAGC
9	GrmM1-1 + ME02	GTCGTCATGATCTGGGACT	TGAGTCCAAACCGGAGC
10	OsNRAMP1c + ME02	GGGTTCTCATTGCTGGCTCT	TGAGTCCAAACCGGAGC
11	OsNRAMP1c + ME05	GGGTTCTCATTGCTGGCTCT	TGAGTCCAAACCGGAAG
12	OsNRAMP5f + ME02	GATTAGCAAAATGTCACTGACTAGC	TGAGTCCAAACCGGAGC
13	OsMTP1d + ME05	TGCCATGTGACAATCACTCA	TGAGTCCAAACCGGAAG
14	OsYSL1a + ME02	TAAACCAAAGATGGCAGACC	TGAGTCCAAACCGGAGC
15	OsNRAMP5d + ME02	TAATTTCGGGCTCCAGTACC	TGAGTCCAAACCGGAGC
16	OsYSL4c + ME02	TATTAGTGAGGGCGATCCAC	TGAGTCCAAACCGGAGC
17	OsYSL4e + ME02	TATGCATGCGGTGGATGA	TGAGTCCAAACCGGAGC
18	OsZIP8a + ME02	ATGAGGACGAACACCACCAC	TGAGTCCAAACCGGAGC
19	OsYSL4a + ME02	GCAAACACTACCCCAAAAGC	TGAGTCCAAACCGGAGC
20	APRT1c + ME02	GGAAGTTTCCTTTTGGCTGT	TGAGTCCAAACCGGAGC
21	APRT1b + ME02	GTTGTGAAATATCAGGTGTTGAAGC	TGAGTCCAAACCGGAGC
22	OsNAS3a + ME02	TCACCAGTTGGAGCTAATCG	TGAGTCCAAACCGGAGC
23	OsYSL4f + ME05	CACCTCTACATCCCGAGCAT	TGAGTCCAAACCGGAAG
24	APRT1a + ME05	CTCTCTCCCGCTTTTTGCTT	TGAGTCCAAACCGGAAG
25	OsYSL4b + ME02	AGCTCTGCGGGTGAACATAC	TGAGTCCAAACCGGAGC
26	OsMTP1a + ME02	TCTCTCCTCCCCATCTCCAA	TGAGTCCAAACCGGAGC
27	OsNRAMP1b + ME02	TTGTACGTCTTTGAGACTTTGACTG	TGAGTCCAAACCGGAGC
28	OsNRAMP5c + ME02	GCTTTGCTGATCGGGATTAGTT	TGAGTCCAAACCGGAGC
29	OsMTP1a + ME05	TCTCTCCTCCCCATCTCCAA	TGAGTCCAAACCGGAAG
30	OsYSL4b + ME05	AGCTCTGCGGGTGAACATAC	TGAGTCCAAACCGGAAG

Table 4. Marker in formativeness statistics — major allele frequency, allele number, gene diversity, and polymorphic information content (PIC) — for the 30 TRAP markers.

Marker	Major Allele Frequency	Allele No	Gene Diversity	PIC
M01	0.78	1.83	0.30	0.24
M02	0.84	1.75	0.22	0.18
M03	0.88	1.93	0.18	0.16
M04	0.83	1.96	0.25	0.21
M05	0.78	2.00	0.30	0.25
M06	0.81	1.85	0.25	0.20
M07	0.79	1.93	0.29	0.23
M08	0.79	1.90	0.30	0.24
M09	0.83	1.92	0.25	0.21
M10	0.79	1.96	0.28	0.23
M11	0.80	2.00	0.29	0.24
M12	0.82	1.86	0.26	0.21
M13	0.79	2.00	0.29	0.24
M14	0.81	2.00	0.27	0.23
M15	0.79	2.00	0.28	0.23
M16	0.79	1.92	0.28	0.22
M17	0.82	1.88	0.25	0.21
M18	0.83	1.97	0.25	0.21
M19	0.77	2.00	0.31	0.25
M20	0.79	2.00	0.30	0.24
M21	0.79	2.00	0.29	0.24

M22	0.81	1.93	0.25	0.21
M23	0.78	2.00	0.30	0.24
M24	0.86	1.80	0.21	0.17
M25	0.81	1.81	0.26	0.21
M26	0.81	2.00	0.26	0.22
M27	0.79	2.00	0.31	0.25
M28	0.83	2.00	0.25	0.21
M29	0.80	1.89	0.28	0.23
M30	0.94	1.67	0.10	0.09

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