

SSR MARKER BASED PARENTAL POLYMORPHISM BETWEEN MTU1010 AND PHULPATTIS 72 FOR NITROGEN USE EFFICIENCY IN RICE (*ORYZA SATIVA* L.)

T. Laxmi Sravya¹, B. Krishna Veni², Brajendra³, M. S. Anantha⁴ and C. N. Neeraja^{5*}

¹Research Scholar, Agricultural College, Bapatla, ANGRAU, Andhra Pradesh

²Principal Scientist and Head, ARS, Bapatla, ANGRAU, Andhra Pradesh

³Principal Scientist, ICAR-IIRR, Hyderabad, Telangana

⁴Senior Scientist, ICAR-IIRR, Hyderabad, Telangana

⁵Principal Scientist and Head, ICAR-IIRR, Hyderabad, Telangana

*Corresponding author: C. N. Neeraja, cnneeraja@gmail.com

ABSTRACT

Detection of molecular markers with parental polymorphism is a pre-requisite for QTL mapping and further identification of genomic regions. Polymorphic studies were carried out between two parents MTU1010 and Phulpattas 72 using 1132 randomly selected SSR markers for Nitrogen use efficiency (NUE) covering the entire 12 chromosomes. Results revealed that highest percentage of polymorphism observed on 10th chromosome followed by 11th chromosome and 8th chromosome and lowest polymorphism percentage was shown in 5th chromosome with an overall 15.8% polymorphism percentage among all the markers used in this study.

KEYWORDS: Simple sequence repeats, Parental polymorphism, Rice, Nitrogen use efficiency, Recombinant inbred lines.

INTRODUCTION

Rice (*Oryza sativa* L.), a member of family poaceae, is the dietary backbone for more than half of the world's population and remains the single most important staple cereal across Asia, where the bulk of global production and consumption is concentrated (Fukagawa *et al.*, 2019). Sustainable rice production for growing population depends heavily on nitrogen (N), the mineral nutrient most limiting to crop growth and the one applied in the largest quantities out of any fertilizer input (Liu *et al.*, 2023). Yet the efficiency of the applied nitrogen is converted into grain yield remains poor in most rice growing systems. Nitrogen use efficiency (NUE) has been declining in major rice producing nations such as India and China due to overuse of fertilizer, with 40-60% of applied nitrogen lost to the environment. This inefficiency carries a heavy ecological cost, as nitrogen fertilizers are estimated to account for majority of anthropogenic reactive nitrogen release globally, contributing to air pollution, water eutrophication and greenhouse gas emissions (Chatterjee *et al.*, 2024). Improving the genetic capacity of rice to use nitrogen more efficiently is therefore both an agronomic and environmental imperative, offering a pathway to maintain yields while reducing fertilizer dependence.

Genetic dissection of NUE has revealed that this trait is governed by numerous physiological processes as of nitrogen uptake, assimilation, translocation and remobilization to grain, each under the control of multiple quantitative trait loci (QTLs) distributed across the rice genome (Kale *et al.*, 2021b; Liu *et al.*, 2022). QTL mapping enables precise dissection of complex genetic regions, facilitating targeted breeding strategies (Swamy *et al.*, 2018). Mapping populations derived from parents contrasting for NUE related traits, when genotyped with molecular have enabled to localize such QTLs and identify candidate genomic regions for use in breeding programs (Vishnukiran *et al.*, 2020; Sugasi *et al.*, 2024). Among the various marker systems available, simple sequence repeat (SSR) markers continue to be widely favoured owing to their high heritability, co dominant inheritance and abundant public availability of primer sequence validated across diverse germplasm (Kaur *et al.*, 2015; Nadeem *et al.* 2018)

A fundamental prerequisite for any marker assisted mapping is the demonstration of molecular polymorphism between the chosen parental lines. Without confirmed allelic differences at marker loci spanning the target genomic regions, neither the linkage map construction nor QTL detection is possible and the downstream goal of marker assisted selection (MAS) (Edwards and Batley., 2010; Gonzaga *et al.*, 2015; Pushpalatha Ganeshan., 2016). These markers are critical for the development of segregating populations such as F₂, backcrosses or recombinant inbred lines (RILs) and serve as anchor points in linkage analysis and QTL detection (Kale *et al.*, 2021a; Rathi *et al.*, 2021; Kayesh *et al.*, 2013). Once informative polymorphic markers are identified, they can be deployed for linkage map construction and single marker or interval mapping analysis to associate genomic regions with NUE related phenotype as physiological nitrogen use efficiency and nitrogen harvest index (Cho *et al.*, 2007).

In the present study, therefore, a parental polymorphism survey using genome wide SSR markers was carried out as the foundational step toward developing a mapping population for identifying QTLs associated with nitrogen

use efficiency in rice, with the aim of generating markers suitable for subsequent linkage mapping and marker assisted breeding for improved NUE.

MATERIAL AND METHODS

Plant Material

Two contrasting rice parents, MTU1010 and Phulpattas 72, were selected for the parental polymorphism survey in this study. MTU1010 is a high yielding, mega variety developed by ANGRAU university. The other parent Phulpattas 72 is a traditional landrace characterized by its high Nitrogen use efficiency, making it a promising donor for NUE in rice. The contrasting traits of these two parents, particularly in terms of NUE make them ideal for developing mapping population aimed at identifying QTLs linked to NUE.

Seeds of both parents were obtained from Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad. For DNA extraction, seeds were surface sterilized and germinated in petri dishes under controlled conditions. Leaf tissues were collected from 21-day old seedlings and utilized for DNA isolation.

DNA Extraction

Young leaves from 21-day old seedling of both parental lines were harvested for genomic DNA extraction. The cetyltrimethylammonium bromide (CTAB) method was employed following the protocol of Doyle and Doyle (1987). Approximately 100 mg fresh leaf were homogenized in CTAB buffer (1.5%CTAB, 0.2M Tris HCL, 0.02M EDTA, 1.4M NaCl) and kept for water bath for 40 min at 60 C. The lysate was then extracted twice with an equal volume of chloroform:isoamyl alcohol (24:10) followed by centrifugation at 12000 rpm for 10 minutes. The aqueous phase was transferred to a fresh tube and the DNA was precipitated using chilled isopropanol. After centrifugation the DNA pellet was washed with 70% ethanol, air dried and resuspended in TE buffer (10 mM Tris HCL, 1 mM EDTA, pH 8.0). The DNA concentration is quantified at OD values of 260 and 280 nm using nanodrop to check the purity and quantity of genomic DNA. All DNA samples were diluted to a working concentration of 50 ng/ul for downstream PCR amplification.

PCR Amplification

A total of 1132 simple sequence repeat (SSR) markers spanning all 12 chromosomes of rice were employed to assess parental polymorphism. These markers primarily from the RM series, were selected based on their broad genomic distribution. PCR amplification was performed in 10 ul reaction volumes comprising 2ul of genomic DNA (50ng), 1ul of 10uM primer mix, 1 ul of 2.5mM dNTPs, 3 ul of 10x PCR buffer, 0.75 ul of Taq DNA polymerase (1U) and 2.25 ul of nuclease free water. The PCR conditions included an initial denaturation at 94 C for 5 minutes, followed by 35 cycles of denaturation at 94 C for 30 seconds, annealing at 55-60 C (depending on primer melting temperature) for 30 seconds and extension at 72 C for 1 minute, with a final extension at 72 C for 10 minutes.

Electrophoresis and Visualization

PCR products were resolved on 3% agarose gels, stained with ethidium bromide and visualized under UV light. A 100 bp DNA ladder was used for size estimation. Markers showing distinct allelic bands between the two parents were recorded as polymorphic. Percentage polymorphism was calculated by the formula as below:

$$\% \text{ Polymorphism} = \frac{\text{Number of Polymorphic Markers}}{\text{Total Number of Markers Screened}} \times 100$$

Results and Discussion:

A parental polymorphism survey is crucial in QTL mapping as it ensures genetic variation for constructing a linkage map. In order to find polymorphic markers between parents, parental polymorphism studies between MTU1010 and Phulpattas 72 were carried out using 1132 simple sequence repeats (SSR) markers covering all 12 chromosomes of the rice genome. Details of polymorphic markers, % polymorphism and total number of markers are provided in Fig. 1 and Table 1. Fig 3 depicts the physical positions of polymorphic markers on each chromosome.

Results revealed that, out of 1132 markers 179 found to be polymorphic (Table 2) between parents. The percentage of polymorphic markers per chromosome ranges from 7.79% (chromosome 5) to 22.61% (chromosome 8). Notably, chromosome 8 and 10 had the highest polymorphism percentages at 22.61% and 22.50%, respectively. While chromosome 5 and 3 had the lowest at 7.79% and 13.20%.

The polymorphism rate recorded in this study is in close agreement with previous reports. The SSR marker RM495, found to be polymorphic between the parental lines in the present study, has previously been reported to be associated with important agronomic traits in rice. In a Rasi $\times$ Vibhava recombinant inbred line (RIL) mapping population screened under both nitrogen-limiting and recommended nitrogen conditions, RM495 was validated as a marker linked to total number of tillers per plant and total number of productive tillers per plant (Neeraja *et al* 2019). In the present study, the polymorphic markers RM408 and RM337 were found to lie within meta-QTL *mQTL8.1* on chromosome 8 (PVE = 30.1%), associated with leaf and root nitrogen content, while RM547 was

located within *mQTL8.2* (PVE = 14.7%), associated with leaf, grain and shoot nitrogen, grain yield and biomass (Mehrotra, 2022), supporting the relevance of these markers to nitrogen use efficiency.

Visnukiran *et al* (2020) screened 224 SSR markers between rice genotypes BPT5204 (mega variety) and PTB1 (variety with high NUE) and identified 42 polymorphic markers, through which 2 major pleiotropic QTLs were identified on chromosome 2, out of which five anchor SSR are found, three on chromosome 1 and two on chromosome 2. Similarly, cho *et al* (2007) used 228 SSR markers of which 113 markers were polymorphic.

Table 1. Parental polymorphism studies between MTU1010 and Phulpattas				
S.No	Chromosome	Total markers	Polymorphic markers	% polymorphism
1	chr1	217	37	17.05
2	chr2	99	14	14.14
3	chr3	106	14	13.2
4	chr4	83	10	12.04
5	chr5	77	6	7.79
6	chr6	97	13	13.4
7	chr7	106	15	14.15
8	chr8	84	19	22.61
9	chr9	60	9	15
10	chr10	80	18	22.5
11	chr11	40	9	22.5
12	chr12	83	13	15.66

Chromosome-wise Polymorphism Distribution

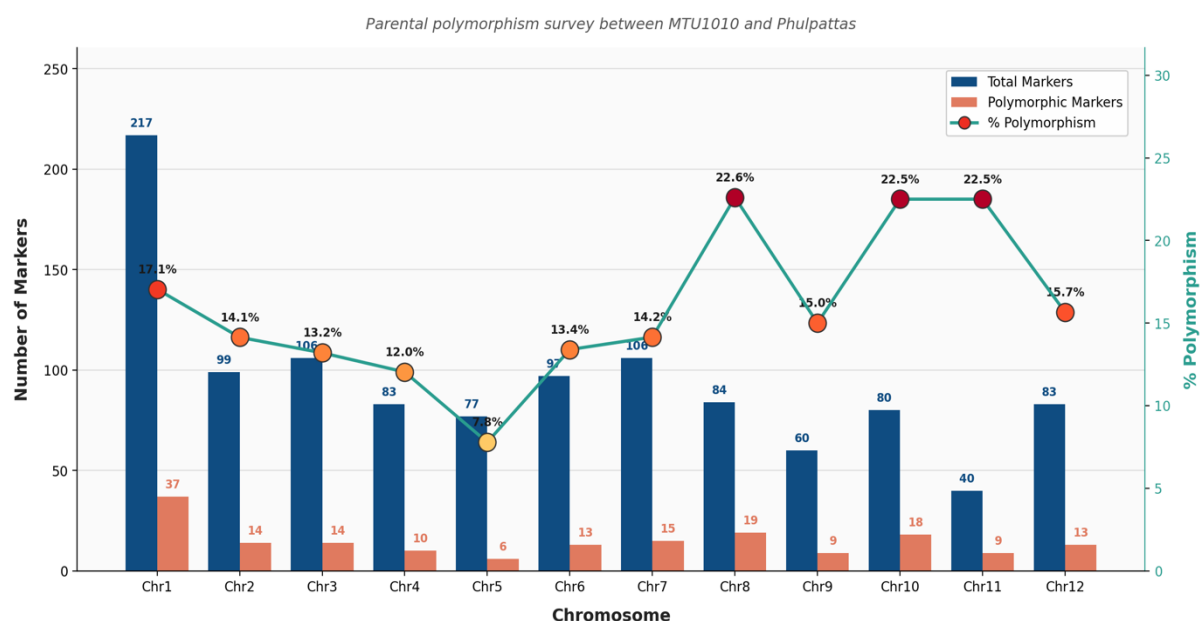


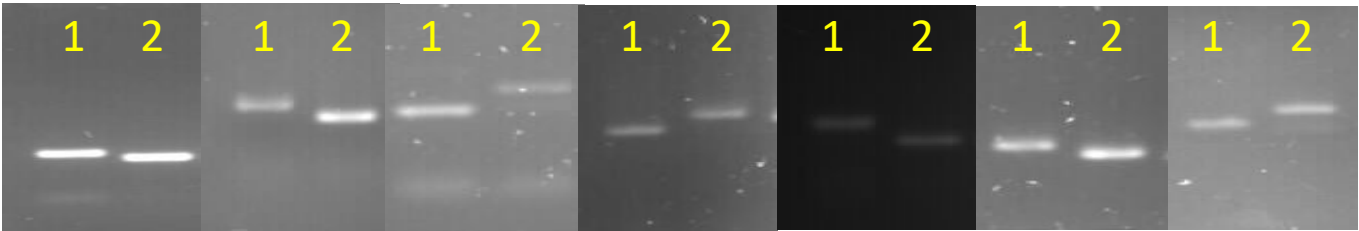
Fig.1. Frequency distribution of polymorphic markers across the chromosomes

S.No.	Marker	Chr. No.	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
1	RM495	1	ATGATGATGGACGACGACAACG	TGAATCCAAGGTGCAGAGATGG
2	RM11125	1	CCAAGAACCCTAGCTCCCTCTCC	TCGACGAGATCCTCCTCGTAAACC
3	RM466	1	CCTCACCATTGATCTCCATCACC	ACTTGCCCTCCTCTTCTCAACG
4	RM10149	1	AGAAGCCCGTGGAGTTTGTGG	AAACGAGCACTCGCGGAAGC
5	RM259	1	GAAGTGCTCCCTAAACTTGTTC	TTATGGAGGATGGATTCTGAAGG
6	RM11964	1	CTCTTCCTTTCGTCCTCTCTCC	GACCTTCAAGCCCAACTTAACC
7	RM11987	1	AACTAGCCCTAGCGACGGATGC	AGGTCATCCTCATGCCATCTACC
8	RM6740	1	GTCTTCGCAATTAAGCCATTGGAACG	GGGATAGACAAGCGCACCTAATCACC
9	RM8004	1	TCTCTACCACCGTCTTCTCTCTCC	CTGAACGCAGCCACATCTAAGC
10	RM10123	1	ACACATCCCTCATCATCATCTCC	GAATCCGATCTTGTTCTGATACCC
11	RM11635	1	TTGTCACCCTTACTAGGATCAGC	GTGTGACTCTTGATGTAAGTCAGC
12	RM10963	1	TCCAGACTGTGACGAGCAGTACC	ATAACCAGGATCTACCCACATACCC
13	RM10861	1	GATCGAGGAATATGATCCAACTCG	ATTCTGGTGTGAGCACTGATCG
14	RM10927	1	TGGATCCCCTAATCCAAATGC	GAAAGACTCCTTCCAATGTTAGGC
15	RM1387	1	GCTACCATGCACGAAACAGAGACG	ACCAAATGTGGCTGGCTGATCG
16	RM11976	1	TTGGACAGCACTTGATGTATCTGC	GAAGAGCTTTCCGAGCAACACC
17	RM10247	1	ATGCATTTTCGAGCCCATTCTAGC	CGGTTGTGGGTACGTATGCTTCG
18	RM10694	1	TTTCCCTGGTTTCAAGCTTACG	AGTACGGTACCTTGATGGTAGAAAGG
19	RM10051	1	AGTACCGTATTGCAAGAGAAGC	GGTTTGGTTCGATTTAGTCTCC
20	RM11996	1	CCGTCGTCAATATTTCTACGACACC	GGCTTAAATGCCACTGTTTCAGG
21	RM11954	1	GAAACGGAGTGAGTGCTACTGATAGG	CTGTCGCGTTCCAATTTATTAGCC
22	RM12233	1	CTTGAGTTCGAAGCGAGAAGACG	CACTTGAGCTCGAGACGTAGCC
23	RM3475	1	ATGTTGTCGAGTCGTGGTAATGC	TATTCCTCGGTGTATGGGTCTCC
24	RM529	1	TTCACCACAACGATAGAGACTTCTGG	GGGAAGAAGATGACAGAGCAAGC
25	RM6840	1	CGACTGGAAGAAGGGATCATGG	CACACTACCAAGACTCCGCTATGG
26	RM11989	1	CAGTACGGACGTCTTCGTCAGG	CCGTACGTGCGTAGCTTCACG
27	RM11994	1	GTGTTGAGTGTCAATTGGTGTATGC	CATATACTCAGCTACCCTCGTATGC
28	RM1	1	CTCCGACAGGATCTGGGACTGG	TATGACCTCATCGATCAAGGGAAGG
29	RM11988	1	CTCCGACAGGATCTGGGACTGG	TATGACCTCATCGATCAAGGGAAGG
30	RM10896	1	GCAGGCGTCGAAGTCATAGC	TATTTGCATGGCCCTGTTTGC
31	RM10074	1	GTCGCGGGTACATGACACAAGC	CGAGCTGTTACGGCAATCTCTATGG
32	RM493	1	GTACGTAAACGCGGAAGGTGACG	CGACGTACGAGATGCCGATCC
33	RM594	1	TCGAGAGAGGGAGAGTGAGAACATGG	GCCTTCGCACATAAAGGATGAACC
34	RM3362	1	ATTGCGTGGGATATGGACTATGC	CCTCTTCAATTCCCTTCCTCTGC
35	RM12276	1	GTCGACGGCTTCTCAAGATTGG	TGAGACCTCTGTGAAGGCACTCG
36	RM488	1	AACAACCAGCGTATGCGTTCTCG	CCCACGGCTTTGTAGGAAGAAGC
37	RM10655	1	AGTACCGTTGAATCCGATATGC	TGGTTGAGGTGCTGAATTGG
38	RM1342	2	ATGGCGATTGAAGCAAGAAACC	GTTGTTTGCAAGGAAGGTAGATGC
39	RM13170	2	GCCTCTTCACCCTCATCCTTCTCG	CGCCTGCCACCTTTATCTTGAACC
40	RM4355	2	GGGATGAGAGTAGAAGGCACAAGG	GCTTAATGCCTTTGATCGTTGC
41	RM5706	2	GTGGCTTGATTTCTGCAACTTCC	GCTGGTACCTACCAAGTATCATTTCCG
42	RM13884	2	CTCGTCATCGCCATCCTCATCG	AAATAGCAGCCGCCCGTTCC
43	RM12433	2	CTCTACACGCTTTGCCTGTTTGG	TGATCAGGTTGAGATTGGGTATGG
44	RM13430	2	CGCCTACATTCTTGTCTCAACC	GGAGAACTCTGCATTCTTATGAGG
45	RM12435	2	CGGAAACTCTATCAACCAATGC	TGCTACCTAGAGACATGAGTGTGG
46	RM6367	2	GCAACCACGACATCAAAGAAACC	GGAGGTTAGTGCTTCGGAGTGG
47	RM13761	2	ATCCATCCTCAGACACACATGG	GAATTCCTGATATTCTCGCGTAGC
48	RM2634	2	TCGAGGCAATTCCTAAGCTAACC	TCTGGAACCTCTTCTATACCCAAAGG
49	RM13659	2	GAACAGATTCTTGCCAATGTGC	AGCGAGAAAGAACAGGAAGTGC
50	RM7426	2	ACACACCCTGTCGGTACAATGC	CAGTGACACGTGACATGGATCG
51	RM6375	2	CGAATGGAACGACAAGAGATGC	ATAGATCGACAACGTAGATCCACAGG
52	RM16227	3	GGTACTAGAGGAAGCAGACCGATCC	GGTGCCCTAAACGAGTATTAGATTGC

53	RM5626	3	GGACGCCACCTTCCTCTTCTGC	CGGTCATAAACGCCATTAGACCAAGC
54	RM7332	3	ACACTGTACACCACACTTCAGC	CACACCAAAGGGAAATTAGG
55	RM14747	3	GACCCGGCTCATCAGGTTCTGG	TCCCGTCTCCTCGTCCTCATCC
56	RM6308	3	TCTCGACCTGGCTCTCCTCTAGC	AGTGCACGGACATGTCACTCTCG
57	RM218	3	TCAAACCAAGGTCCTTCAACTGC	TTTCTTCCACCGTCCATGTATCC
58	RM15191	3	CCTACCTGTCATTCCGTCAATCC	AAGCTGGAGCTGTGGACTACTGG
59	RM3586	3	TCTTGATTGCTGGACCACATGC	TCGAGCTAGAAGACGACACACAGC
60	RM1508	3	CGCCTGCAAGAATTAAGTGC	CTGACTGTAACTGTTCTCCAACC
61	RM15673	3	ACCAACACCAGTCCGTCGAATGC	AAGGAAGCCGAAGCGGAAGAGG
62	RM3350	3	CCCTGTTTCCACTCACATGATGC	CTCTGTGTGTTCTACCTGAGGTGTGG
63	RM6369	3	AGCTAGCTTCACCTACCTACCTCACC	ATGGTCATGTCGTTGGTTTGC
64	RM16175	3	AGCTTTGGTTTCTTGGCTTTGG	ATTAGCGTTGAACCCAAGTGTGG
65	RM1319	3	TCTTCTGTGCTTTGCAGACTTGC	CTCGCCAGTTAGCCCTTAAATCC
66	RM1359	4	CGACTTGCCAAAGGTCAACG	GATTCTACGGGCCACAAGTCC
67	RM5687	4	AGAGAAGAAGGGAAGGAGGAAGG	AGTGACTTGTGGGTGACTTGTGG
68	RM17345	4	TTTAGCTGTTTCGCACACACG	AAGGACATCCTTCATCCTCTTGC
69	RM16791	4	GGCTGCTTCAGAGCGTTTGTAGG	AAATACGCCATCAAGTTGCACACC
70	RM6909	4	TTTGGGATAAATGGGAGAGG	TCAGCTGGTTATTATGGAGAGG
71	RM16738	4	AAACGGAGTGGTCCATTAGC	GAAAGGTAGATGGGTTTGTGG
72	RM17377	4	ATATTACTTCGACGCTGGATCAGG	GTCAGTTCGTCAGGCACAACG
73	RM16649	4	CTCCCTTCATGCGTAAGCTCTCC	GCAAACAGGATCCTCCACAAAGG
74	RM16335	4	AGCACGCGTGACATGAACCTCC	GAAGAGAGGCATGTGTTGTGTTGG
75	RM5511	4	GAGTTCGTCCCTGACAAACAGAAACG	GTGAGCGAGCGAGTGAGTGAGC
76	RM17863	5	GGACGTCATGATTTGTGCTTGG	GTCATTGGCTTGCAGGATACACC
77	RM18175	5	AAAGTCACTCACACGCCGTACTCC	ACGAATGAGTCCGACCATGACC
78	RM18080	5	GCGAAACTCATCGACTGATCG	GGTGATCACATCATTGTCCATCC
79	RM3381	5	ACGAACGCGAGCTGACAGAGG	AATAGCTGCCAGCAACTGCAACG
80	RM2998	5	TCTAGATACACCGTCTCAATGG	AAAGAGGTAGCTAGGTTGAAGG
81	RM3486	5	GGAGGTCGGCACGTAGTAGAGG	GTCGGTACTATTCTGCCATCG
82	RM20046	6	AACGAGCGGCTGAGATTTATGG	ATGTACCAGCCCTTTGAGAGTGG
83	RM20623	6	GCTCGTACGGTGGGTGAATCC	GATGCGTCCCTTCTAGGTTGAAAGC
84	RM19660	6	TTTGTCCCTGCCGTACTTGC	AGCCACGTTGGGTGAAATTAGC
85	RM19666	6	CGTCAGCATCCTCTCTCGCTTTCC	CTGCGTGCTGCTCTTCCATCC
86	RM549	6	ATCCCTGAACCCAAATTCTGTCTG	CTCTTTGATCTTCCGGTGATTTCTG
87	RM19410	6	TGCTGATTGCTCACTACTTCATCC	GCGGGATACCATGGTCTAAAGG
88	RM5509	6	CCTGTCAATTGATGATCCATGC	TACCTTCCAGCAGAAAGAAGACG
89	RM19381	6	AACGGGAGATCACAGGAATTTGC	GTGTTGACTCGTCTCCATTTCG
90	RM19902	6	ACGAGTGACAGACACAATCACATGC	CACATCAAAGACTAATGCGGAAGACC
91	RM20069	6	GCGAGCGAGAGGAGAGATAGACG	CGAATTCCGGCACGAGTAATAGGG
92	RM3	6	GTCACATTCCGTTTCCCATCATTCC	CCTCACCTCACCACACGACACG
93	RM508	6	AGAAGCCGGTTCATAGTTCATGC	ACCCGTGAACCACAAAGAACG
94	RM3765	6	ACACCATGACACCAAAGGAAGG	GGATGCTTCCAATCCTCTCACC
95	RM20818	7	AGATGCAGATAGATGCATGTCACG	ACCGATCATCCACGATCCTACG
96	RM21439	7	TGATGACACTTGTCTCCTGTTGC	CCTTCGATGTGCTTTCCTTTCC
97	RM5752	7	TTGCAATTAATTGCATCTCC	GCAGATCGATTGTTAGTTC
98	RM5436	7	TGAGCTGCACAAGACAGACAAGC	ACCATTTGAACAGGATGGACTGG
99	RM3635	7	GAGAGACAGTGGAAGGGAAGACG	GTTCCCTCCCTCCTCCTAGTTCCG
100	RM3484	7	TCCGGTCGTCTCATCGTATCC	GCCCTCTTGCTCCCACATCG
101	RM20775	7	GGTGTTTCATCTTTGTTGAGTCAGG	AGGATACAGCGCACCAGATTAGC
102	RM22066	7	GGAGACAGACGGGCACAACG	CATCATCCCGTTCCTCTCTCG
103	RM21649	7	CCCTGTCTAAGTCTGTTGACG	GCCGGTTTATATTCTTGACTGG
104	RM5543	7	GGTGCTAGGTACATGGCTGACG	AGAGCAAATTCTGGGCTATCTGC
105	RM6326	7	CGATCTCTCTATCCTTCTCTTCTCC	CAGATTGTTAGCAACCCGAATCC

106	RM2381	7	GACGCCGGTTGTCCAAATTACG	CTCGCATGCAAAGATTGATCTACCC
107	RM11	7	ATCGGTGCTTGGCTGGATAGC	CCACCTTCTTCTCCTCCTCTTCC
108	RM248	7	AGAGAGCAAGTTTGAAGCGAAGC	ACCAAGAGGGTAGCCTAGCATGG
109	RM1306	7	AGTCTCAAAGGCATACAGTACACACC	GCCAATTACCTTCCCGTACATAGC
110	RM3383	8	CCTACCTCTAGCGGCTGACTGC	CCATCAATGCCGGTAGTTCCG
111	RM23518	8	TTTCGCTTCTGAGTTGGCAGAGG	GACCTGAACAACGTGCCAAACG
112	RM27966	8	CTTTGAAGGTCATTCTTGAACACC	CCGGTATTGTTATATCGCCCTTAGC
113	RM23612	8	CGATCGACCACAAGTAGAGTACG	CAATCACGTACAGGCAAGATCG
114	RM8243	8	TCTACCTTAGCTGTCTGAATTGG	CACATACCTGTTCCGTTTGATCC
115	RM408	8	AATTGCCCAACGAGCTAACTTCC	TGAGCTGTTTGTGCTCTTCTACTTCG
116	RM149	8	GGAAGCCTTTTCTCGTAACACG	GAACCTAGGCCGTGTTCTTTGC
117	RM5933	8	AGCGATTCAGAACGAATCAACG	TGCCAAAGCTACACAAATCTGACC
118	RM8264	8	TTCTACGGAATTTCTCCCTCTGG	CTAATCAATCTCTCGCGTTCTTGG
119	RM22502	8	AATCTTCACGCACCCATTCC	GCAGATACACACATTGCATACCC
120	RM22918	8	ACTCCAACGTAGTGTTCTGTGC	CCATTTGTCAAGTGTGTTGATCC
121	RM3262	8	CTGGAGATGCAGATCCTCAACC	TAGTACAACATGGGAGCCTGTGC
122	RM23096	8	GTGCAATCATGTTACATCAGC	AAATAGACTACTGGGTGCGTTCCG
123	RM3761	8	TGTGGAGTGTGGAGGCGAGAGC	AGGCACCCACCCACTGACTTCC
124	RM22415	8	ATAGCGCATCCATATCCGTGTGG	GGAAGGAGGAGGGTATCCCAAGG
125	RM547	8	TTGTCAAGATCATCCTCGTAGC	GTCATTCTGCAACCTGAGATCC
126	RM22540	8	TCTCTCTCCTCCATCGTTGAATCC	CTGTTGAGCAGGATGCACTGG
127	RM337	8	GTAGGAAAGGAAGGGCAGAG	CGATAGATAGCTAGATGTGGCC
128	RM1148	8	CATGCTCGAATCAGTGAGTAGTGG	GCTTAGCTATGCAGGAGAGAGTGG
129	RM242	9	AAACACATGCTGCTGACACTTGC	TTACTAGATTACCACGGCCAACG
130	RM23818	9	GATGATCGATTCAAGGTTCTTGG	CCTCAACTCATGGAGGCCTAACC
131	RM24260	9	GATCTCTCACCCACATGCCTAGC	TCCCATCTTGATCGATCTCTTCG
132	RM23736	9	GGCGATACCTGCCATAGTTTCC	CCGAAAGCAATCTATGAGACACC
134	RM160	9	CCCAAATCAGGAAAGTTTCTCAGC	AGTCATCCTTGGCTACCAGATGC
135	RM24197	9	ATGACGAACAGCTCGACTACATGG	AAACACGTGGGAGCTGAGTGC
136	RM3249	9	GGATCATGTCAAATCGGAGAAGG	GCTCAAGCTTCACAGATCACAGG
137	RM219	9	CGTCGGATGATGTAAAGCCT	CATATCGGCATTTCGCTG
138	RM257	9	CCGTGCAACTTAAATCCAAACAGG	GGAATCCTATATGAGCCAGTGATGG
139	RM3834	10	CAAGCCAAGTGAAACGAGAACG	CTATGCTGAGCCGGAGTAGATCG
140	RM3563	10	TGGTACGGAAAGACGAGAGATGC	TGAGGCGAGTGTCTGATAACTGC
141	RM25681	10	AGCCGCTACTTCTACTACCACAGC	TCGTCGTCTTCTGGTACATCAGG
142	RM5689	10	TTAAGTCCTGTAGTAGGTCACACC	CGATGAGTCAGATTGAAGTAGC
143	RM25066	10	GTTGTTAGGTGTAGCCGTGTAGG	GTACACCAATAACTGTGGAAGAGC
144	RM25623	10	CCACATTAAGTGAAGAGACCATGC	TTGCCCTCATCCTGTAATATGTCC
145	RM5271	10	CGAATCTTGGAACACATCAACG	GGGAGGAGTGCTGTGAGAGG
146	RM25059	10	GGGATTGGGGCCCTAAGAAGG	CCTTTGACCATGCTTTGACTGG
147	RM6179	10	GCGCCGCCGAGATGATAAGC	AGCGGCATCTCGTCCATCTCC
148	RM25292	10	TGTCCCTTTCTCCAATTCTCTCG	TGTCTTCATCTTTGGCTCGATGG
149	RM25368	10	TATAGTTAAGGGAGCCACGCAAGC	CCACCTCGTAAGAACATGGAGAACC
150	RM25491	10	GAGACTCCGCTGGAGAGTATAGATGG	CAACAAACCAACCAGCGATTAGG
151	RM216	10	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA
152	RM228	10	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC
153	RM496	10	GACATGCGAACAACGACATCC	CTATAGTTGTTGCACATGCGATCC
154	RM25664	10	CCAGCTGTTAATTAGCATGTGTGAGC	CAGCAAAGGCGTACGTATCTCG
155	RM1374	10	GCCTCGAATCAACCAAATAGTGG	GGGCTGAGACCTTGTGGTATGG
156	RM25818	10	GATGGGAATTGAAGCTGGTATCC	TCAGACCAGTCGGCAATTAGTCC
157	RM26558	11	GTTCAAATGAGGTCAGCCTATGG	AGAGTCAATGCATGCTCAAACC
158	RM3863	11	CGATTGATCCCGTGCAAGTAGG	CATGCTAGTGCATTCTGCGTAGG
159	RM2136	11	ATGTTTGAGAAAATGCAGAC	CACTAAGCTCGTTTTCAAAG

160	RM26076	11	CCTTCTCTCTCCCACATCTCTAGC	CTAGTGAGTCCCACGTGTCAACC
161	RM332	11	GAAGGCGAAGGTGAAGAAGAAGC	CCTCCCTTGATGATACCTTGG
163	RM7481	11	CTGTCTGTTCACCTTGTGTTACCC	TCGACCCAATATCTTTCTGC
164	RM27154	11	TAGTCGGGCATCTCCTCTTCC	GTTACCTCCGATGAAGGCAAGG
165	RM26643	11	CTCCCTTCTTCTGCTGAGACACC	CAAGCTACTCATTGGGCTCATCC
166	RM206	11	ATCGATCCGTATGGGTTCTAGC	GTCCATGTAGCCAATCTTATGTGG
167	RM28067	12	GAGGGAGTACTAACTTCCTAACG	ATGTGGATGTGATCTCCATAGG
168	RM28048	12	TTCAGCCGATCCATTCAATTCC	GCTATTGGCCGGAAAGTAGTTAGC
169	RM20A	12	TGTATGCACAGCTGCTCTACTCC	GCACGACCAGAAATTAACAAGG
170	RM2854	12	AGCATGAGACCCACATATCAGC	CCTATCTTAGAGTGCCATCTAGTTCC
171	RM12	12	GGAGAAAGAGAGGTGATCCTTTCC	CATGTCTTGGTGAGTGATGTTGC
172	RM28011	12	CGCTATAATAGGCCGCTATTGG	GCACTATTGCGAATGCTAAGTTCC
173	RM5479	12	CTCACCATAGCAATCTCCTGTGC	ACTTCGTTCACTTGCATCATGG
174	RM27951	12	GCGGTTCAAGAACAACATCATGG	ACACCAACCGGGACTAAAGATCG
175	RM2851	12	CAGTGCTTAAAGTGTCCATCTCC	CAGCAAGAGTAGCGACTATATGTACC
176	RM28492	12	CAGATGTGGTAAACTGGTAAGAGC	TAGCCTGGGTTCTACTTGTCC
177	RM28570	12	CGGACTCGACGAAGTAGTAGAAGAGC	ACCTCCTCCTCTCCATCCTCTCC
178	RM7018	12	TTTCTCTCCACCCGTTTCTCACG	CAAGCATGGCCAAGTCATGTGC
179	RM28574	12	TAGTTTGGTGAAGTGGCATTGG	ATAGTAGGGCAAGGATTCAGAAGAGG



RM12433 RM3484 RM21439 RM242 RM22066 RM11125 RM5436

Fig.2. Polymorphic markers between parents.
1- Phulpattas 72 and 2- MTU1010

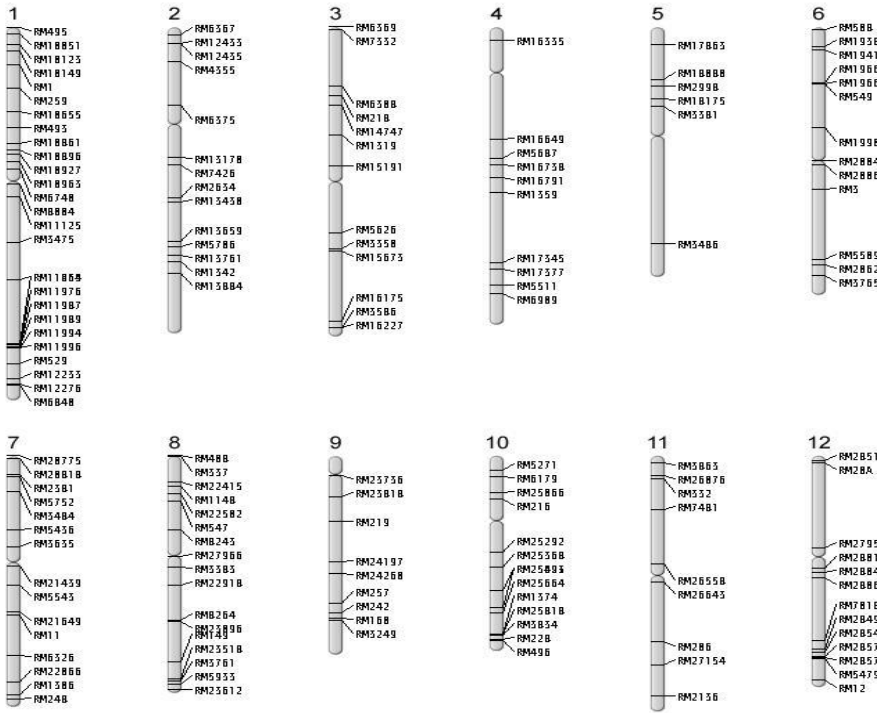


Fig.3. Physical positions of polymorphic SSR markers across 12 chromosomes.

CONCLUSION

The parental polymorphism survey identified 179 polymorphic SSR markers (15.8%) out of 1132 screened between MTU1010 and Phulpattas 72, confirming sufficient genetic divergence for linkage map construction. Polymorphism ranged from 7.79% (chromosome 5) to 22.61% (chromosome 8) and markers such as RM495, RM408, RM337 and RM547, previously linked to tiller number and nitrogen related meta-QTLs supporting the suitability of this parental combination for dissecting NUE related traits. These markers will now be used to genotype the complete RIL population, enabling construction of a high-density linkage map and identification of QTLs for nitrogen use efficiency, with potential application in marker assisted breeding of nitrogen-efficient rice varieties.

Competing Interests

Authors have declared that no competing interests exist.

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