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Research Article

Haplotype analysis of *OsMATL*, a pollen specific phospholipase gene responsible for haploid induction

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Background: Double haploid breeding is a modern plant breeding approach that enables the rapid development of fully homozygous lines within a single generation. In rice, the creation of efficient haploid inducer lines requires the identification of novel allelic variants of the *OsMATL* gene across diverse germplasm. To achieve this, *OsMATL* sequences from rice accessions in the 3K panel were analyzed to detect unique SNPs that could potentially contribute to enhance haploid induction efficiency and support hybrid rice breeding programs.

Methods: Sequence retrieval from SNP seek database (https://snp-seek.irri.org), allelic diversity and haplotype analysis.

Results: *OsMATL* has 30 variations according to allelic diversity analysis. Three haplotypes (H1, H2 and H3) were identified based on three non-synonymous SNPs for *MATL* gene.

Conclusion: Established haplotype analysis for the *MATL* gene for the 783 lines belongs to 3K Rice genome panel.

Keywords: *rice, OsMATL gene, allelic variation, haplotype analysis, haploid induction, hybrid rice breeding*

Introduction

The rice production has been increased by development of improved short duration varieties and hybrids. However, the rice production is affected by many factors including genetic parameters and environmental factors. Hence, the several traits including stress tolerance, improvement of yield, quality and nutrient efficiency are governed by genetic interaction of multiple genes and environmental factors (Colasuonno et al., 2021). The targeted improvement of desirable characters through identification of novel alleles for is essential for crop improvement programmes. Previously, mutation breeding and marker-assisted selections were widely employed to broaden the genetic base for crop improvement (Ahmar et al., 2020). However, conventional breeding approaches typically require six to seven generations to isolate elite homozygous lines, making the process labour intensive and time-consuming (Kyum et al., 2021). The recent advanced methods *viz.*, GWAS, CRISPR technology, double haploid breeding, speed breeding and improvement in next generation sequencing has paved way for identification of superior haplotypes/novel alleles for targeted improvement of important traits in shorter time period (Singh et al., 2019). The utilization of double haploid breeding can produce homozygous lines with desirable traits in F_2 generation.

The *MATL* gene in rice is a pollen-specific receptor-like kinase (RLK) that plays a crucial role in fertilization. *MATL* gene encodes a leucine-rich repeat receptor-like kinase (LRR-RLK) that is primarily expressed in the male gametophyte, particularly in the pollen tube. *MATL* is essential for pollen tube growth, signalling, and successful fertilization of ovule. Mutations or disruptions in the *MATL* gene lead to pollen tube dysfunction, resulting in male sterility. Therefore, *MATL* is considered an important gene for reproductive success and seed formation in rice. Due to its important role in controlling male fertility, it has potential applications in hybrid rice breeding, where controlled male sterility is valuable for efficient hybrid seed production. The genetic mechanism underlying haploid induction has been clarified, with two key genes identified in maize. The first is *MTL/ZmPLA1/NLD*, which acts as the primary trigger for haploid formation (Gilles et al., 2017; Liu et al., 2020), and the second is DMP, which contributes to improving haploid induction efficiency (Zhong et al., 2022).

Haploid inducer lines exhibiting a haploid induction rate greater than 6% are typically employed for the development of doubled haploid populations. Notably, *MTL* is evolutionarily conserved among cereal crops, and disruption of its homolog *OsMATL* in rice has been shown to generate 2%-6% haploid plants (Yao et al., 2018; Wang et al., 2019), thereby offering significant potential for advancing hybrid seed production and rapid breeding in rice. The investigation of allelic diversity in diverse rice accessions identifies superior haplotypes for identification of haploid inducer lines with novel alleles in rice. Using publically available sequence data from the IRRI 3K rice genome sequencing project, the present study was aimed to examine the allelic diversity of *OsMATL* (LOC_OS03g27610) gene across a diverse set of 783 rice accessions.

Materials and Methods

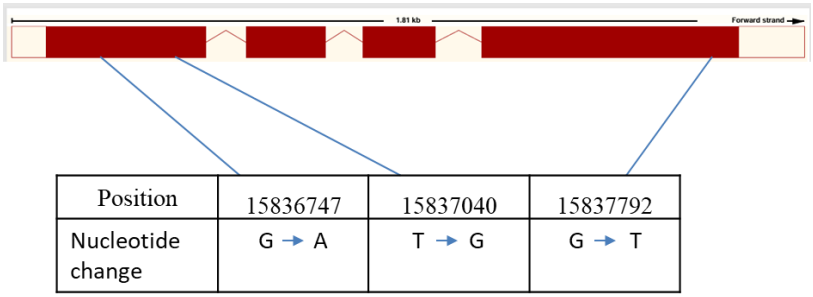
Allelic diversity and haplotype analysis of *OsMATL*

A diverse set of 783 lines from 3K RG panel were used for the current study. Allelic variation of *OsMATL* was retrieved from SNP seek database (https://snp-seek.irri.org) to perform haplotype analysis. The 3K filtered SNP dataset present in the SNP seek database was utilized for haplotype analysis. The 3K filtered SNP dataset was achieved by adopting criteria *viz*., alternative allele frequency at least 0.01 and 0.2 missing calls per SNP. Allele mining was performing by selecting only the non-synonymous SNPs. This data was further converted into haploview file set using gPLINK (version 1.07) (Purcell et al., 2007). The generated haploview file set was used for performing haplotype analysis using HaploView (version 4.1) and the significant SNPs were chosen with the cut off value of 0.001 (Barrett et al., 2005).

Results

Allelic diversity of *OsMATL* in subset of 3K RG panel

OsMATL gene contains 30 variations (includes 3 INDELs and 27 SNPs) but only three SNPs were non-synonymous in the 783 lines used. Out of three non-synonymous SNPs, two were located in the exon 1 and one at exon 4 of *OsMATL* (Figure 1).



Position	15836747	15837040	15837792
Nucleotide change	G → A	T → G	G → T

Figure 1. Three significant SNPs and their SNP changes (Solid blocks - exons, lines in between solid blocks – introns)

Haplotype analysis of 783 genotypes and diversity between haplotypes

Among the three significant non-synonymous SNP's found in *OsMATL* gene, three haplotypes namely H1, H2, H3 were formed which comprises 593, 110 and 53 genotypes respectively based on three SNP's (15836747, 15837040 and

15837792). The LD plot (Figure 2) showed the correlation of three SNPs involved in haplotype grouping and the correlation coefficient.

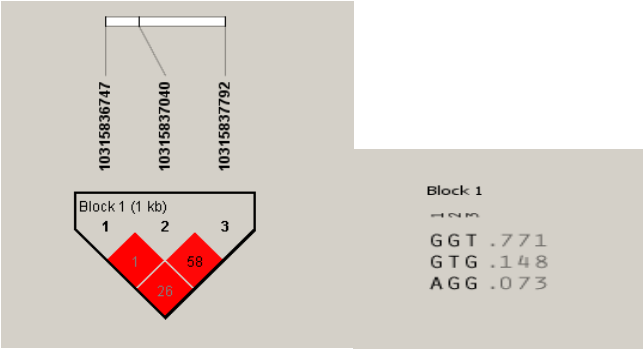


Figure 2. Linkage Disequilibrium (LD) plot of significant SNPs in *OsMATL* using HaploView (numbers in the block indicates LD r^2)

Discussion

Doubled haploid breeding relies on the generation of haploids either through natural occurrence or artificial induction and subsequent chromosome doubling of haploid lines to produce completely homozygous lines within two generations. The development of haploid inducer lines with increased haploid induction rates linked with reliable molecular markers is essential for an efficient doubled haploid breeding pipeline (Chen et al., 2023). The lines possessing specific SNPs causing truncation of protein leads to disruptions in normal pollen development due to absence of specific phospholipases (Kelliher et al., 2017). Analysis of allelic variations of *OsMATL* gene revealed presence of 30 variations (includes 3 INDELs and 27 SNPs) but only three SNPs were non-synonymous among the rice accessions used. In maize, Dutta et al. (2024) reported the presence of 103 polymorphic sites comprising 38 indels and 65 SNPs through sequence analysis of the *MTL* gene. Three non-synonymous SNPs were detected in the first and fourth exons, and these variants can be further evaluated for their potential impact on protein function. In the current study, three haplogroups were formed based on three nonsynonymous SNPs comprising 593, 110 and 53 genotypes. Using ten gene-based markers, 40 distinct haplotypes across 48 genetically diverse inbred lines was identified (Dutta et al., 2024). The identification of superior haplogroups were used for development of superior genetic stocks of haploid inducer lines in hybrid rice breeding.

Conclusion

OsMATL with 30 allelic variations in 3K RG subset, only three were non-synonymous. Among the three non-synonymous SNPs, two SNPs were present in first and one at the fourth exon. Based on non-synonymous SNPs, three haplotype groups were identified by performing haplotype analysis. The identified 30 allelic variations are need to be validated in all these 783 lines by haplo-pheno analysis. The novel SNPs identified in the study can be validated for the function of the protein to identify haploid inducer lines. The identification of novel variations in *OsMATL* gene can provide strategy for deploying haplotype-based breeding to develop elite rice varieties which suits future food demands.

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Author contributions

RM: Conceptualized the study and manuscript revision. VRR, SAR and SM: Analysed the data and drafted the manuscript.

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Conflict of interest

The authors declare no conflict of interest. The manuscript has not been submitted for publication in any other journal.

Ethics approval

Not applicable.

AI tool usage declaration

The authors declare that no AI and associated tools are used for writing scientific content in the article.

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