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Allelic diversity analysis of *OsMADS87*, a MADS box transcription factor influencing seed size and thermo-sensitivity in rice

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Abstract

Rice (*Oryza sativa* L.) is a major staple food crop grown over hundred different countries. To feed the ever-increasing population by 2050, global food production must be doubled. Rice productivity has been stagnant due to a number of biotic and abiotic factors. Abiotic stress, on the other hand, create significant economic losses by lowering yield and quality. The quality of cereals is negatively affected by heat stress and cause loss of >10% of grain production. This warrants empowering rice crop to withstand ill effects of heat. It requires discovery of genetic factors regulating heat tolerance traits and discovery of genes and their elite alleles associated with heat tolerance traits. The current study attempted to survey the allelic variation in a putative heat tolerant gene *OsMADS87* (LOC Os03g38610) using *in silico* analysis. *OsMADS87* (LOC Os03g38610), a MADS box transcription factor influencing seed size and thermo sensitivity, contains one exon with a transcript length of 750 bps. Its allelic variation was surveyed across a set of 201 *aus* rice accessions from 3K rice genome project. Allelic diversity analysis identified 10 SNPs and 11 INDEL positions in *OsMADS87* gene sequence. Ten haplotypes (H1, H2, H3 and H4) contributed by 10 non synonymous SNPs were identified.

Keywords: *OsMADS87*, SNP, haplotype analysis, rice

1. Introduction

Rice (*Oryza sativa* L.) is the primary calorie source for more than half of the world's population. Food production is facing a challenge with an ever-increasing population and must expand to meet the demand. (Ranganathan *et al.*, 2018) [18]. Extreme weather events, such as rising CO₂ levels, rising temperatures, and drought, all have a negative impact on crop production. (Kumar *et al.*, 2018) [13]. Globally, rising temperatures and limited rainfall are wreaking havoc on agricultural crop productivity. High temperatures impede crop growth and development. High temperature can disrupt many critical plant activities, including enzyme activity, cell division, photosynthetic reactions, membrane integrity, growth, and productivity (Al-Khatib *et al.*, 1999, Mittler *et al.*, 2012) [1, 16]. Rice is more sensitive to high night-time temperatures than to high daytime temperatures. Effect of high temperatures during flowering in rice can result in spikelet sterility, reduces grain number thus, cause serious yield loss. (Ferris *et al.*, 1998; Gibson *et al.*, 1999, Jagadish *et al.*, 2008) [6, 8, 11]. Increased temperature during post-fertilization can shorten grain filling length, lower grain weight, and increase chalky grains. (Bao, 2019; Cooper *et al.*, 2006; Zhou and He, 2019) [2, 5, 20]. Short-term exposure to extremely high temperature stress may result in cellular injury, but long-term exposure to moderate to high temperature may result in harm or death. Cell damage caused by elevated temperatures may include protein aggregation and denaturation, disrupt the passage of water, ions, and organic solutes throughout the cell (Halford, 2009; Fahad *et al.*, 2015; Hu *et al.*, 2020) [9, 7, 10]. Several comparative protein identification studies resulted in identification of heat shock protein, function as molecular chaperones to maintain cellular homeostasis. The detailed analysis and identification of proteins that changed during anthesis were studied in order to better understand high-temperature-sensitive proteins in rice genotypes (Jagadish *et al.*, 2010) [12]. Transcriptome analysis studies during anthesis stage revealed several transcripts belongs to heat stress responsive transcription factor families (WRKY, HD-ZIP, ERF, and MADS) were significantly upregulated (Wang *et al.*, 2019; Liu *et al.*, 2020) [19, 14]. Recent reports on MADS box transcription factor *OsMADS87*, proven its role as regulating rice seed size under moderate to high temperature (Chen *et al.*, 2016) [4].

Understanding allelic variation in the MADS transcription factor can aid in haplotype breeding and the development of heat stress tolerant cultivars. There is an urgent need to develop heat-tolerant rice cultivars due to the world's ever-increasing population and limited resources.

2. Materials and Methods

2.1 Allelic diversity and haplotype analysis of *OsMADS87* in an *Aus* genotype

To retrieve data on a transcription factor *OsMADS87*, SNPs/INDELs variation among 201 *aus* rice lines, the SNP seek database was used. Allelic variation of *OsMADS87* (LOC_Os03g38610) was retrieved from the SNP seek database to perform haplotype analysis. SNP seek database was exploited to perform haplotype analysis for *OsMADS87* by adopting default parameters and Nipponbare was used as the reference genome. The filtered dataset was obtained from the Base SNP set with alternative allele frequency of at least 0.01, proportion of missing calls per SNP as 0.2 (Mansueto *et al.*, 2016) [15] and this was available in the SNP seek database. Allele mining was carried out by selecting only the non-synonymous SNPs and INDEL (Insertion deletion). Allelic variation data was converted into haploview fileset by gPLINK (version 1.07). (Purcell *et al.*, 2007) [17] and this haploview fileset was used to perform haplotype analysis in HaploView (version 4.1) (Barrett *et al.*, 2005) [3].

3. Results and Discussion

3.1 Allelic diversity of *OsMADS87* in *Aus*-subset

Allelic diversity analysis of *OsMADS87* revealed that the gene contains 21 genetic polymorphism (10 SNPs and 11 INDELs) among the 201 genotypes surveyed (Table 1). Ten SNPs located in the exon 1 of *OsMADS87* was found to be non-synonymous leading to Gly214Arg Ile168Val; Ser167Pro; Val151Ile; Ile119Asn; Phe98Leu; Asn96Asp; Thr74Ala; Ala53Val; Asn40Ser (Table 1). The non-synonymous mutations present in the dataset results in the modification of amino acids (Gly214Arg; Ile168Val; Ser167Pro; Val151Ile; Ile119Asn; Phe98Leu; Asn96Asp; Thr74Ala; Ala53Val; Asn40Ser) (Table 2). We identified novel variations in the first exon of the *OsMADS87* allele,

consisting of ten SNPs) at Chr03-21428526- 21429152 position. These ten non-synonymous SNPs formed four haplotypes (Fig 1). Previous studies on MADS box transcription factor *OsMADS87* had clearly indicated its role in enhanced endosperm cellularization (Chen *et al.*, 2016) [4]. The MADS box transcription factor *OsMADS87* mutant plants were less sensitive to a moderate level of heat stress in terms of seed size reduction than wild-type plants and plants that had overexpression of *OsMADS87* genes. Studies on protein profiling during anthesis had evidently specified upregulation of several protein in N22 rice variety in comparison with susceptible (Jagadish *et al.*, 2010) [12]. Our findings imply identification of allelic polymorphism in *OsMADS87* which needs further phenotypic validation. Apart from *OsMADS87* several other genes discovered so far may be suitable targets for increasing rice's thermal tolerance throughout the reproductive season.

Table 1: Non synonymous SNPs allelic variations in *OsHsp17.7* gene for the 3K RG panel- *Aus* subset

Position	Position	Alleles		Type
21428526	Exon 1	1 bp	T/T	SNP
21428527	Exon 1	1 bp	G/G	SNP
21428631	Exon 1	1 bp	C/G	SNP
21428686	Exon 1	1 bp	T/T	SNP
21428686	Exon 1	1 bp	-/-	SNP
21428769	Exon 1	1 bp	T/C	SNP
21428772	Exon 1	1 bp	A/G	SNP
21428786	Exon 1	1 bp	C/C	SNP
21428787	Exon 1	1 bp	C/C	SNP
21428788	Exon 1	1 bp	C/C	SNP
21428789	Exon 1	1 bp	T/T	SNP
21428790	Exon 1	1 bp	G/G	SNP
21428791	Exon 1	1 bp	C/C	SNP
21428792	Exon 1	1 bp	T/T	SNP
21428820	Exon 1	1 bp	C/C	SNP
21428915	Exon 1	1 bp	A/T	SNP
21428979	Exon 1	1 bp	A/G	SNP
21428985	Exon 1	1 bp	T/C	SNP
21429051	Exon 1	1 bp	T/C	SNP
21429113	Exon 1	1 bp	G-G/A	SNP
21429152	Exon 1	1 bp	T/C	SNP

Table 2: SNP Effect of allelic variation of *Aus* lines in *OsMADS87*

POSITION	Alt (ANN)	Effect (ANN)	Putative_impact (ANN)	HGV S.c (ANN)	HGV S.p (ANN)
chr03-21428631	G	Missense variant	Moderate	c.640G>C	p.Gly214Arg
chr03-21428769	C	Missense variant	Moderate	c.502A>G	p.Ile168Val
chr03-21428772	G	Missense variant	Moderate	c.499T>C	p.Ser167Pro
chr03-21428820	T	Missense variant	Moderate	c.451G>A	p.Val151Ile
chr03-21428915	T	Missense variant	Moderate	c.356T>A	p.Ile119Asn
chr03-21428979	G	Missense variant	Moderate	c.292T>C	p.Phe98Leu
chr03-21428985	C	Missense variant	Moderate	c.286A>G	p.Asn96Asp
chr03-21429051	C	Missense variant	Moderate	c.220A>G	p.Thr74Ala
chr03-21429113	A	Missense variant	Moderate	c.158C>T	p.Ala53Val
chr03-21429152	C	Missense variant	Moderate	c.119A>G	p.Asn40Ser

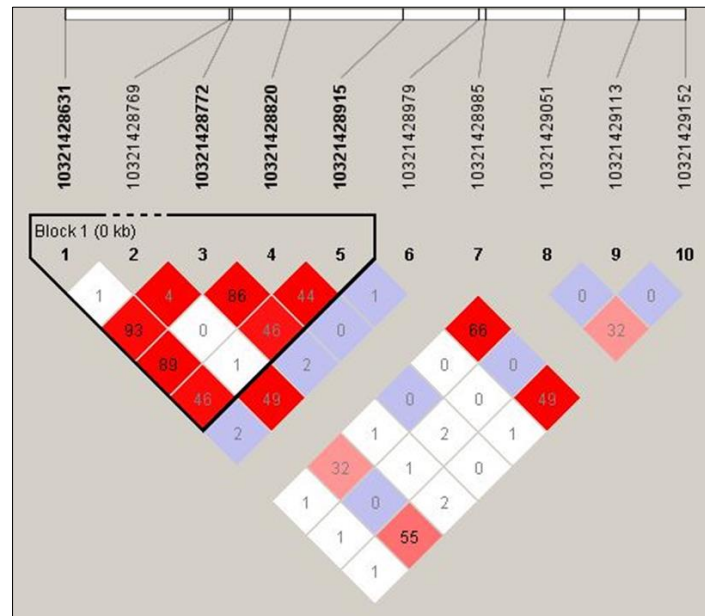


Fig 1: Linkage Disequilibrium plot of significant SNPs in *OsMADS87* using HaploView

4. Conclusion

In silico analysis of transcription factor *OsMADS87* revealed 10 non synonymous SNPs and 11 INDELS variant among 201 *aus* type rice genotypes. Four haplotype clusters were observed by haplotype analysis of *OsMADS87*. The findings of this study on *OsMADS87* allelic variation can be used on haplotype-based breeding.

5. References

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