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Molecular Genetic Studies of Heading Date Gene *OsMADS50* by using Single Segment Substitution Lines in *Oryza sativa*

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Abstract

Heading date is multifarious, polygenic character responsible for the adaptation of rice to various agro-climatic regions. W23-3-8-9-27-82 and W08-16-3-2 are Single Segment Substitution lines containing *OsMADS50* on third chromosome of rice. Segregating (F_2) populations of HJX74 × W23-3-8-9-27-82, HJX74 × W08-16-3-2 were utilized in the genetic study of *OsMDAS50* gene. From the whole genomic sequence of *OsMADS50* gene three functional markers PSM908, PSM909 and PSM910 were developed and utilized for genetic analysis. Significant Chi square values for each population and zero cM genetic distance between *OsMADS50* gene and PSM908 proved the linkage with heading date trait. Three different phenotypes of *OsMADS50* gene were identified varying in days to heading but linked with PSM908. Expression analysis of *OsMADS50*, *Hd3a*, *RFT1* revealed strongest expression in W23-3-8-9-27-82, while week expression was found in W08-16-3-2 and weakest expression was observed in HJX74, resulting in earliest heading of W23-3-8-9-27-82, followed by W08-16-3-2 and HJX74. These three different genotypes of *OsMADS50* are due to three different naturally occurring alleles of *OsMADS50* found in corresponding parental varieties, Lemont's being headed earliest followed by IR64's and least one was HJX74's.

Keywords: Rice; SSSLs; Heading date; *OsMDAS50*; Allelic variation

Introduction

Heading date or flowering time is a multifarious quantitative character controlled by dominance type of gene action and is a central event in life cycle of plant, affected by number of factors and playing a principal role in the spacious adaptability of the crop plants. Physiological and biochemical functions of many flowering-time genes have been described, and several genetic pathways to flowering have been proposed (Levy and Dean, 1998; Simpson *et al.*, 1999). When timed correctly, this trait ensures the reproductive success of plants. Diversity in heading date is the basic reason for successful cultivation of rice over different geographical regions (Khush, 1997). The inheritance pattern of this character is also mysterious due to its quantitative nature and immense influence of environment such as day length and temperature. Early heading and well adaptability assist genotypes in heat tolerance (Obari, 1990). Being a highly responsive trait to selection (Allard and Harding, 1963; Avery *et al.*, 1982) heading date is an effective and worthy trait which facilitates crop plants to cope with post-heading heat stress (Tewolde *et al.*, 2006; Ameen *et al.*, 2012).

Various populations have been used for the genetic mapping but none of them can compete the precision and accuracy of Single Segment Substitution Lines (SSSLs), because SSSLs are elite materials for gene (QTLs) mapping, cloning, evaluating as single factor and their functional analysis because of containing homozygous genetic background with its recipient except carrying a substituted segment from a donor, and can be used as permanent experimental materials in different times and areas. Secondary SSSLs and double segment pyramiding lines (DSPLs) derived from crosses of primary SSSLs are favorable in improving efficiencies of QTLs identification and epitasis effect analysis. SSSLs are influential tool for genetic mapping and dissection of multifarious quantitative traits such as heading date (He *et al.*, 2005).

Our research group has created an SSSL laboratory by using HZX74 as recipient against 24 different donors followed by marker assisted selection. All of the synthesized SSSLs encompass a genetic distance of 21674 cM, which is 14 times genomic size of rice having an average size of 19.3 cM of each segment. Mapping QTLs requires a segregating

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population for which a genetic map has been established and an accurate phenotyping of the trait. Immortal mapping populations such as single segment substitution lines are very useful because each genotype can be tested repeatedly and by applying different test systems. The later allows studying the pleiotropic effects of loci, which are suggested by collocation of QTLs for different traits.

In the last decade, two high-density molecular linkage maps of rice containing about 3,000 markers have been developed in the US and Japan, making an average density of one marker per cM (200-300 kb) (Causse *et al.*, 1994; Harushima *et al.*, 1998). The frequency of SSRs in plant genomes is estimated as one in every 6–7 kb, based on the information that can be found in public sequence databases (Cardle *et al*., 2000). Also results from screening a rice genomic library suggests that there are estimated 5700-10,000 microsatellites in rice, with the relative frequency of different repeats decreasing with increasing size of the motif. The co-dominant nature and allelic polymorphism revealed by SSR markers has provided detailed information on genetic structure (Li *et al.*, 2000; Bonnin *et al.*, 2001) and gene flow (Konuma *et al.*, 2000) in natural plant populations. Apart from this all, SSRs are not suitable for designing primers because some SSRs are located too close to the end of the flanking region to accommodate primer design, or the base composition of the flanking sequence making it unsuitable for primer designing. In our present research we have conducted the genetic studies of SSSL based F_2 segregating populations of *OsMADS50* gene along with their phenotypic characterization. Present study was designed to identify causes of variation in heading date of genetically similar SSSL, which is attributed to allelic variation. There is dire need to explore naturally occurring allelic variation in heading date trait. Utilization of these alleles in rice breeding will help in the development of geographically suitable varieties. We have studied the linkage relationship between functional markers of *OsMADS50* gene with heading date phenotype and had successfully identified three naturally occurring alleles of heading date gene *OsMADS50*.

Materials and Methods

Genetic Material and Plant Populations

SSSLs W23-3-8-9-27-82 and W08-16-3-2 contain *OsMADS50* gene at 3rd chromosome, 5.5cM genetic distance developed by crossing Lemont and IR64 as donor and HJX74 as recipient variety. W12-28-58-3-19-1 contained *Hd3a* gene located at 6th chromosome at 11.5 cM distance and is obtained from IR58025B, while W32-59-80-02-11-1-8 contained *RFT1* gene located on 6th chromosome at 11.5 cM distance and was obtained from IR66167-27-5-1-6 (Table 1). However, W32-59-80-02-11-1-8 and W12-28-58-3-19-1 were not used as individual SSSLs in population rather these were pyramided as double segment lines i.e., W32-59-80-02-11-1-8/W08-16-3-2 and W12-28-58-3-19-1/W08-16-3-2. Genes located on W32-59-80-02-11-1-8 and W12-28-58-3-19-1 did not show variation, because both parents were homozygous for these genes. Plant materials presented in (Table 1) are parental SSSL lacking variation therefore variation was generated by developing segregating population of HJX74 with W23-3-8-9-27-82, W32-59-80-02-11-1-8/W08-16-3-2 and W12-28-58-3-19-1/W08-16-3-2.

Raising Plant Population and Phenotyping

Plants were grown from Feb-June and July-Oct during early and late season of each year at the farm of South China Agricultural University. Days to heading were recorded from nursery plantation to day of 2 mm emergence of first panicle. Data was collected once every two days and at the fixed interval of time per day. Seedlings of 18-20 days were grown at plant to plant and row to row distance of 17 cm. Land preparations and all other cultural practices were accomplished according to the standard recommendations for local conditions.

Genotypic Assay

DNA was extracted according to mini-scale method (Zheng *et al.*, 1995) with minor modifications by using single piece of healthy leaf (about 2 cm long) collected after 15-20 days of transplantation in each season. PCR was conducted according to (Panaud *et al.*, 1996) with 20 µL reaction volume containing 2.0 µL of template DNA (50 ng), 1.5 µL of each primer (50 ng), 0.4 µL dNTP (0.05 mM), 2 µL of 1×PCR buffer (10 mM Tris pH 8.0, 50 mM KCl; 1.8 mM $MgCl_2$ and 0.01 mg/mL gelatin), and one unit of Taq DNA polymerase. PCR products were separated on acrylamide gel followed by silver staining as described by (Zheng *et al.*, 1995). Previously developed SSR markers (McCouch *et al.*, 2002) were used for genotyping and new functional markers were developed for removing/reducing the genetic distances (Table 2).

The JOINMAP (Stam and Van Ooijen, 1995) computer program was used to conduct Chi-square test in order to determine the fitness of observed genetic segregation ratios of alleles to the expected ratios. The MAPMAKER/Exp 3.0 (Lander *et al.*, 1987) was used to develop genetic map. Log-odds ratio (LOD) scores of 3 to 6 were examined, using the Kosambi map function (Kosambi, 1944) to determine distances, a log-odds ratio score of 4.0 was set to develop the linkage map, while MAPCHART was used for the graphical representation of the linkage map.

RNA Isolation and Expression Analysis

RNA was isolated from top leaf samples collected 45 days after sowing by using Trizole method. The RNA pellet was dissolved in the appropriate amount of nuclease-free water and stored at -80°C until use. The quality and quantity of RNA was determined using agarose gel electrophoresis and

Table 1: Molecular description of plant material used in present study

SSSL	Gene	Chr #	Gene location	Code of gene	Donor Parent
W23-3-8-9-27-82	*OsMADS50*	3	5.5	(Os03g0122600)	Lemont
W08-16-3-2	*OsMADS50*	3	5.5	(Os03g0122600)	IR64
W12-28-58-3-19-1	*Hd3a*	6	11.5	(Os06g0157700)	IR58025B
W32-59-80-02-11-1-8	*RFT1*	6	11.5	(Os06g0157500)	IR66167-27-5-1-6

Table 2: Primers and number of PCR cycles used in the Semi quantitative RT-PCR analysis and Quantitative Real time, RT-PCR

Gene	Forward primer	Reverse Primer	PCR cycles	Annealing Temp^{0}C
OsMADS50	AAAGTCGACGCTGATGGTTTG	GTTTCGACATCCATGTTGTC	24	58
Hd3a	ATGGCCGGAAGTGGCAGGGAC	ATCGATCGGGATCATCGTTAG	37	56
RFT1	GAGCAACATTTGGGCAAGA	GCTAGGCAGGTCTCAGCTT	36	57
OsACT1	TCCATCTTGGCATCTCTCAG	GTACCCGCATCAGGCATCTG	23	56
Primers Quantitative Real Time, RT-PCR analysis				
OsACT1	TGCTATGTACGTCGCCATCCAG	AATGAGTAACCACGCTCCGTCA	40	60
OsMADS50	CAGGCCAGGAATAAGCTGGAT	TTAGGATGGTTTGGTGTCATTGC	40	60

a spectrophotometer. Before cDNA synthesis, RNA samples were firstly treated by DNase to eliminate genomic DNA.

Total RNA (2 µg) was reverse transcribed using the TOYOBO cDNA synthesis kit in a 20 µL reaction volume, following the manufacturer's protocols. Synthesized cDNA were used for RT-PCR and real time PCR. *OsACT1* was used for normalizing cDNA. All RT- and real time PCR experiments were conducted three times at a point. Primers used for expression analysis are listed in (Table 2).

A 50 µL of PCR reactions included 2.5 µL of cDNA after suitable dilution, 1 µL of each gene specific primer (forward and reverse), 5 µL PCR buffer, 1 µL dNTPs and 0.3 µL Taq DNA polymerase along with 39.2µL of ddH_2O. Suitable PCR cycles for each target gene were tested by comparing PCR products from 23-37 cycles. Gene specific primers are listed in (Table 2), PCR products of 10 µL were separated on 1.2% agarose gels and visualized by UV. The expression of *OsACT1* gene was used as an internal control.

The quantification of mRNA was also conducted by real-time RT-PCR. The primers were designed in such a way that the amplicon for each gene will have almost 200 bp of size, therefore mRNA accumulation can be compared across different genes with a simple relative quantification method using SYBR Green I dye. The comparative Ct method (Livak and Schmittgen, 2001) was adapted to determine the relative enrichment of each primer region. Real-time RT-PCR master mix for 25 µL reactions was prepared as follows: 10.5 µL water, 12.5 µL of SYBR green PCR mix (Takara), 1 µL cDNA, 0.5 µL each of forward and reverse primers (0.2 µM). The thermal profile used consisted of: one cycle of denaturation (15 min at 95°C); 60 cycles of amplification and quantification (15s at 94°C; 30s at 59°C; 30s at 72°C); and one cycle of dissociation curve analysis (1 min at 95°C, 30s at 55°C and 30s at 95°C). Melting curve was observed from 55 to 97°C, reading every 0.3°C for 2 sec.

Results

By utilizing the extensive rice information from NCBI website the relationship between the physical and genetic distance around *OsMADS50* gene was analyzed. RM3202 and RM3894 are located at a distance of 260 kbp from *OsMADS50* gene on physical map, while the distance of RM3894 was found to be 2.6 cM from the linkage map.

Two new polymorphic primers RM14302 and RM14308 were inserted in between the above mentioned markers, thereby decreasing the encompassing physical distance to 151 kbp. Although it is very hard to compare the genetic distance with physical distance because of uncertainty of occurrence of genetic recombination at different locations of chromosomes, and genetic distances are directly influenced with the rate of genetic crossovers. Price (2006) proposed that in rice on an average 1 cM = 258.5 kbp and it may have 1-50 genes. Contrary to this we further dissected the 150 kbp regions with four additional markers, leaving behind 9.3 kbp between RM14302 and PSM 878, 26.6 kbp between PSM 878 and PSM881, 3.3 kbp between PSM881 and PSM882, 64.8 kbp between PSM882 and PSM883 and 47.2 kbp between PSM883 and PSM14308 (Fig. 2a). This particular region was successfully explored with maximum possible DNA markers, and segment size was consistently shortened with the attainment of complete linkage of the SSR markers located at the proximal ends of segment with the heading date, followed by development of subsequent markers in the vicinity of probable genetic location of *OsMADS50* gene. Meanwhile, three functional markers PSM908, PSM909 and PSM910 were developed inside the genetic region of *OsMADS50* genes by utilizing the information provided by Lee *et al.* (2004). Genetic region of *OsMADS50* encompassed an area of 250 kbp on 3rd chromosome. Genetic analysis of these functional markers revealed complete linkage of these markers with *OsMADS50* gene. The validity of these functional markers was confirmed by

studying these markers upon different alleles of *OsMADS50* gene. Almost similar significantly positive results were observed from all of the population studies. Linkage map showed zero cM genetic distance between *OsMADS50* gene and PSM908, which shows the complete linkage of the PSM908 and *OsMADS50*. Chi square analysis for knowing the fitness of data to Mendalian 3:1, 1:2:1 ratio revealed significant values, confirming their absolute linkage.

Study of HJX74×W23-3-8-9-27-82 F_2 Segregating Population

Populations of 234 and 150 segregating plants of above mentioned cross combination were sown in early and late season of the year 2009, respectively (Table 3). In the early cropping season 177 (heterozygous and homozygous recessive) plants were found to be early heading while 57 (homozygous dominant) plants were found to be late heading (Table 3). Chi-square analysis for segregation of PSM908 revealed χ^2 (1:2:1) = 0.97 value which is less than χ^2 0.05,2 = 5.99 showing the fitness of data to expected 1:2:1 ratio (Table 3). Linkage map of *OsMADS50* gene and PSM908 showed zero cM genetic distance between them (Fig. 2b).

In the late cropping season of year 2009, early heading plants were 112, while 38 plants were found be late heading. χ^2 analysis for segregation of PSM908 showed χ^2 (1:2:1) = 1.8 which is less than χ^2 0.05,2 = 5.99 representing fitness of 1:2:1 ratio (Table 3). W23-3-8-9-27-82 contains *OsMADS50* gene transferred from "Lemont" variety in the genetic background of HJX74. Linkage map of *OsMADS50* gene developed with PSM908 showed zero cM genetic distance (Fig. 2b). SSR markers PSM878, PSM881, PSM882 and PSM883 (Fig. 2a) along with sequence specific markers (Table 5) were developed upstream and downstream of the location of *OsMADS50* gene.

Study of HJX74×W08-16-3-2/W12-28-58-3-19-1 F_2 Segregating Population for *OsMADS50* Gene

Populations of 156 segregating plants of above mentioned population were grown in the late season of year 2009 (Table 3). Out of 156 plants 124 were found to be early heading and 32 plants were late heading regarding the phenotype. Chi-square test for segregation of PSM908 revealed significant χ^2 (1:2:1) = 2.8, because it is less than χ^2 0.05,2 = 5.99 (Table 3). This shows that observed data absolutely coincide with the expected 1:2:1 ratio. W08-16-3-2 contains *OsMADS50* gene transferred into HJX74 from IR64. SSR and functional markers such as PSM908, PSM909 and PSM910 (Table 5) were designed in the vicinity of genetic location of *OsMADS50* gene and phenotype was analyzed. Expression analysis of *OsMADS50* gene (Figs. 1a, d) out of the four heading date genes was found to be strongest as tested by Chi-square analysis and linkage maps prove that the phenotype was controlled by *OsMADS50* gene.

Study of HJX74 × W08-16-3-2/W32-59-80-02-11-1-8 F_2 Segregating Population for *OsMADS50* Gene

Population of 149 plants of above mentioned genetic composition was grown in the late season of year 2009. Out of these 149 plants, 106 plants were early heading while 43 plants were late heading. Chi-square analysis of segregation of PSM908 revealed significant χ^2 (1:2:1) = 1.2 owing to χ^2 0.05,2 =5.99 (Table 3). This shows the fitness of the data to 1:2:1 ratio. W08-16-3-2 contains *OsMADS50* gene transferred into HJX74 from IR64. Genotypic expression analysis (Figs. 1a, d) proved that the early heading phenotype was controlled by the *OsMADS50* gene.

Genotypic Variation of SSSLs

Three different phenotypic values were observed from two different SSSLs of *OsMADS50* gene and HJX74. W23-3-8-9-27-82 and W08-16-3-2 both contained different segments having *OsMADS50* gene derived from Lemont and IR64, respectively. Heading date of HJX74 × W23-3-8-9-27-82, linked with PSM908 was earlier than F2 population of HJX74 × W08-16-3-2/W12-28-58-3-19-1 and HJX74 × W08-16-3-2/W32-59-80-02-11-1-8 for earliest (homozygous recessive), late heading plant (homozygous dominant) and heterozygous plants (Table 4), which showed that there is difference between the three segments containing *OsMADS50* genes because of their different donor parents. Among these three *OsMADS50* variants, Lemont's variant headed earliest followed by IR64 and the least one was HJX74.

Expression Analysis

Semi-quantitative RT-PCR was conducted in the late season of year 2009 and 2010 to determine the responsible gene for the variation in the expression of heading date. *OsMADS50*'s expression was strongest in W23-3-8-9-27-82 with days to heading phenotype of 70.55±3.43 (Fig. 1e). Expression of *Hd3a* was found weakest in late maturing HJX74 (Fig. 1 a, d) taking 70.30 ± 2.60 days to heading and its expression was consistent with days to heading of genotype. W08-16-3-2 starts heading after 66.68±1.77 days and showed increased expression of *Hd3a* as compared to HJX74, while earliest maturing was W23-3-8-9-27-82, having 63.55±3.43 (Fig. 1e) days to flowering showed strongest expression of *Hd3a*. RFT1 nearest homologue of *Hd3a* showed mild expression in case of late maturing HJX74 and its expression increased with the reduction in days to heading of W08-16-3-2 (66.68±1.77; Figs. 1 a, d) reaching maximum level for W23-3-8-9-27-82 (63.55±3.43, Fig. 1e). It was observed that expression of each gene varied from lowest to highest level with the decrease in number of days to heading but the strongest expression *OsMADS50* remained the most strongest of all observation in case of

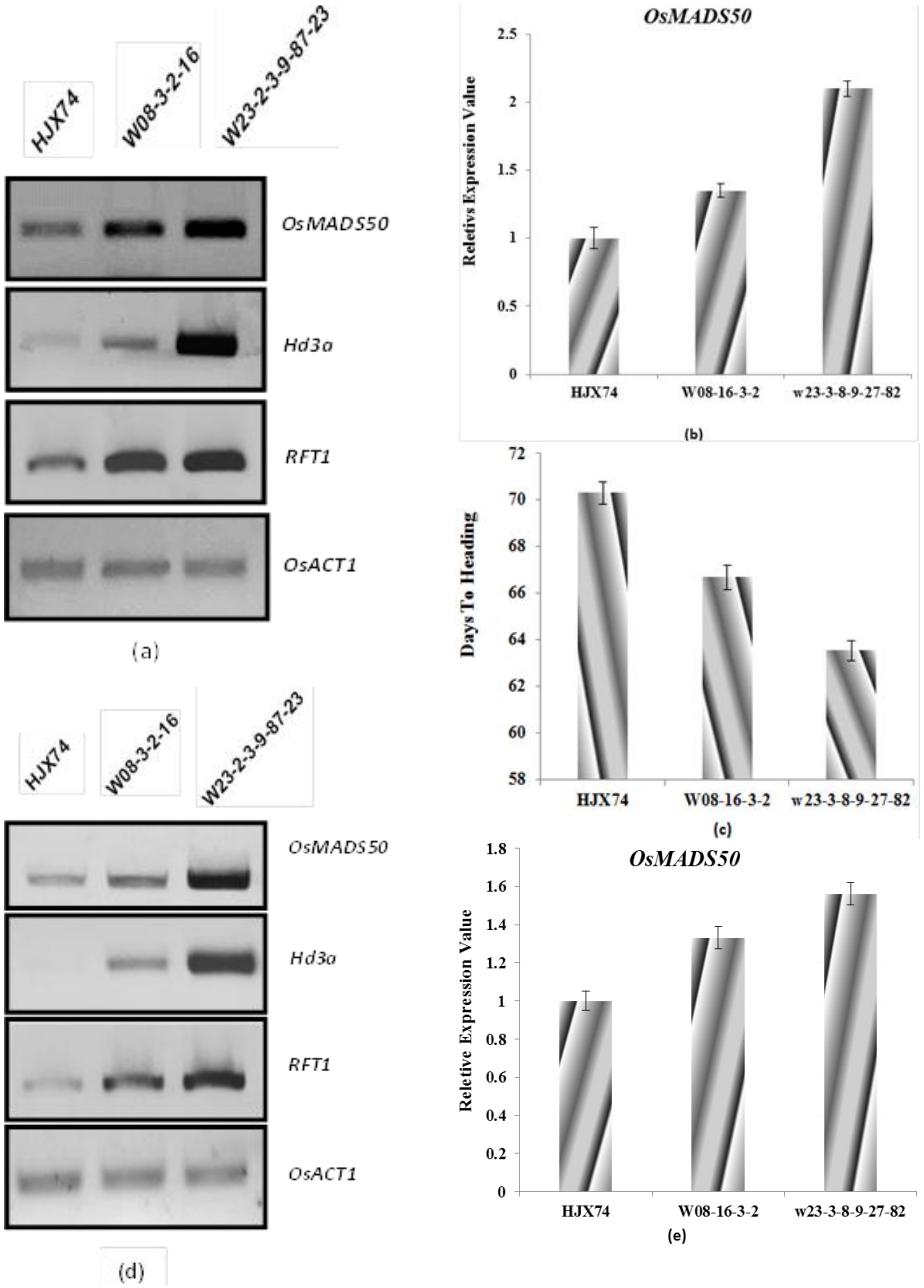
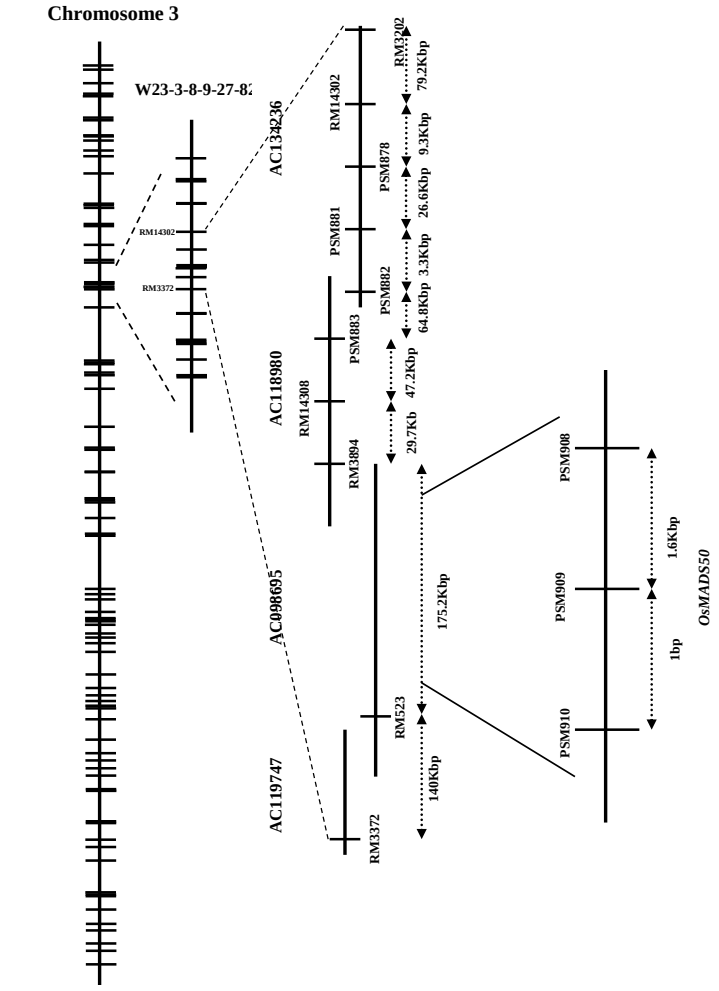


Fig. 1: (**a**) Semi-quantitative RT-PCR was performed to detect mRNA for *OsMADS50*, *Hd3a*, *RFT1* in different genotypes during 2009 late season (**b**) Real-time PCR analysis for *OsMADS50*, W23-3-8-9-27-82, W08-16-3-2 and HJX74 in late season 2009. (**c**) Days to heading of W23-3-8-9-27-82, W08-16-3-2 and HJX74 in late season 2009 and 2010 (**d**) Semi-quantitative RT-PCR was performed to detect mRNA for *OsMADS50*, *Hd3a*, *RFT1* in different genotypes during 2010 late season (**e**) Real-time PCR analysis for *OsMADS50*, W23-3-8-9-27-82, W08-16-3-2 and HJX74 in late season 2010

W23-3-8-9-27-82. Similar pattern was observed in expression of *OsMADS50* gene for W08-16-3-2 and HJX74. Therefore *OsMADS50* down regulated the expression of downstream genes *Hd3a* and *RFT1*.

Quantitative RT-PCR based expression analysis of *OsMADS50*, *Hd3a* and *RFT1* genes in late seasons of year 2009 and 2010 revealed less stronger expression for the SSSL W08-16-3-2, with 66.68±1.77 days to heading. In case of HJX74 the expression was weakest for *OsMADS50*, *Hd3a* and *RFT1* genes when studied by Quantitative RT-PCR having 70.30 ± 2.60 days to heading phenotype (Figs. 1a, d).



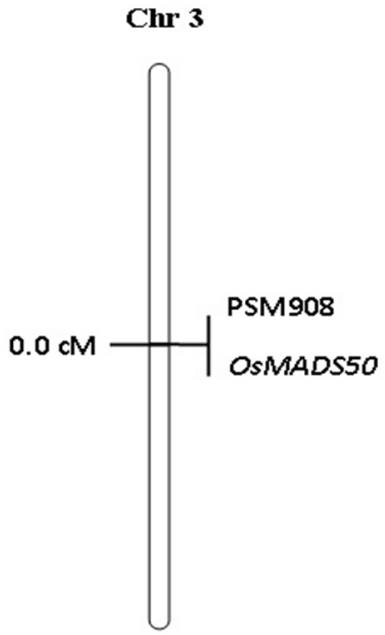


Fig. 2b: Linkage map of *OsMADS50* gene and PSM908

Fig. 2a: Physical Map showing the physical location of markers at the substituted segments containing *OsMADS50* gene on chromosome 3

Quantitative RT-PCR revealed significant Ct value of OsMDS50 gene in W23-3-8-9-27-82 as followed by W08-16-3-2 and HJZ74 in both seasons (Figs. 1b, c). Chi-square analysis as well as linkage maps showed the complete linkage of these primers with heading date and proved that early heading phenotype was controlled by *OsMADS50* gene.

Discussion

Type and size of plant populations play a vital role in the validity and accuracy of genetic mapping experiments. Among various mapping populations single segment substitution lines are particularly useful for high resolution mapping and elucidation of QTLs with minor phenotypic effects because they provide at least two-fold higher frequencies of recombination in any small chromosomal region compared to an F_2 or an F_1 backcross, and provide a permanent source of mapped individuals. Our laboratory has a huge collection of (1560) single segment substitution lines (SSSLs) developed during previous years. A draft analysis or coarse mapping of this material has been completed, but with the gradual maturity of the plant material the aim of the study become advance and fine. As for fine mapping of a major QTL fw2.2 confirming fruit weight in tomato almost, 3500 chromosomal substitution lines, which are near isogenic to the recurrent parent were used by (Alpert and Tanksley, 1996).

For coarse mapping of QTLs a distance of 10-30 cM is considered enough, which will approximately concede up to 2000 genes. Such a larger number of genes cannot be tested for candidacy so it is accepted that to identify candidate genes based on map position, the mapping must be done more precisely. This needs Mendelianizing the QTL in single substituted segment pair that is used to make a secondary population for fine mapping. For this several plants (>1000) are genotyped for markers around the target segment (Price, 2006). In the present study HJX74 × W23-

Table 3: Chi square test for segregation of marker PSM908 in F_2 populations

Population[b]	PSM908[a]				χ^2
	1	2	3	Total	(1:2:1)
1	57	124	53	234	0.97
2	38	68	44	150	1.8
3	32	88	36	156	2.8
4	43	70	36	149	1.2
Total	170	350	169	689	0.18

[a]1 = homozygous for HJX74 2= heterozygous 3= homozygous for *OsMADS50*
[b]Pop1: HJX74 × W23-3-8-9-27-82 population, 2009 early season. Pop2: HJX74 × W23-3-8-9-27-82 population, 2009 late season Pop3: HJX74 × W12-28-58-3-19-1/W08-16-3-2, 2009 late season Pop4: HJX74 × W32-59-80-02-11-1-8/W08-16-3-2 2009 late season

Table 4: Heading days of different genotypes linked with PSM908 locus in F_2 populations

	PSM908		
Population	1	2	3
1	103.47	90.28	86.67
2	72.03	61.36	57.34
3	87.25	77.13	74.78
4	88.69	78.13	76.33
Average	87.86	76.73	73.78

1 = late heading similar to HJX74, 2 = in between early and late heading, but genotype resembles early heading plants 3= early heading, similar to *OsMADS50* allele source variety (donor),
Pop1: HJX74 × W23-3-8-9-27-82 population, 2009 early season. Pop2: HJX74 × W23-3-8-9-27-82 population, 2009 late season Pop3: HJX74 × W12-28-58-3-19-1/W08-16-3-2, 2009 late season Pop4: HJX74 × W32-59-80-02-11-1-8/W08-16-3-2 during 2009 late season

Table 5: Primers specific to *OsMADS50* gene

DNA marker		Primer sequence	Temp ºC	SSR sequence	Product Size	BAC Clone Name
PSM908	F	GGGGCAACAATTAAACGGTA	59.7	AT(18)	195	OJ1126B12
	R	GGGGTCTCCCTCACTGTTTT	60.4			OJ1126B12
PSM909	F	CGCTGGTAGAAATTGGAAAAA	59.2	Att(31)	180	OJ1126B12
	R	CTGATCGCCCGATACTGTTT	60.1			OJ1126B12
PSM910	F	CGCTGGTAGAAATTGGAAAAA	59.2	TTA(31)	180	OJ1126B12
	R	CTGATCGCCCGATACTGTTT	60.1			OJ1126B12

3-8-9-27-82 F_2 population was used for linkage and phenotypic characterization of *OsMADS50* gene. RM3202 and RM3894 on chromosome 3 (McCouch *et al.*, 2002) flanking the target segment were used for the coarse studies of *OsMADS50* gene. Marker assisted selected has proven feasible for selecting traits that are labor-intensive to evaluate in field, such as heading date. Results from the phenotypic evaluation showed that the heading date of HJX74 × W23-3-8-9-27-82 F_2 plants carrying an introgressed segment of Lemont variety in the genetic back ground of HJX74 did have fewer days to heading than that of recurrent parent. Shen *et al.* (2001) proposed several reasons for phenotypic variations among NILs that carry introgressed segment from the donor parent. The first possibility is that the target QTL can be lost during successive backcrosses through double crossover between markers. In addition, the introduction of exotic genes into an elite genome can lead to a decrease of desirable agronomic characteristics due to the linkage drag of genes with undesirable effects from wild species (Monforte and Tanksley, 2000). To improve the efficiency of marker assisted selection it is important that the recombination frequency between the target gene and marker is kept as low as possible, and this can be achieved by identifying markers tightly linked to the gene (Hittalmani *et al.*, 2000). With the saturation of molecular maps, it is now possible to identify new markers closely linked to a gene of interest and also the flanking markers (Tanksley *et al.*, 1993). After decreasing the distance encompassed by the flanking markers to as much low as <1 cM, it was possible to identify the gene-linked marker.

We have developed PSM908, PSM909 and PSM910 functional markers that could be directly used in marker assisted selection program for development of early heading rice varieties and plant materials. Their independent use of phenotypic screening will allow rapid development of cultivars with improved multiple layered early heading rice varieties. The existence of multiple functional markers for each gene may also allow more latitude in the selection of closely related parents for crosses. This is a good example of generating markers for marker-assisted selection in rice using informatics to harvest data for public sites. It is important to understand that absence of the genetic distance between the DNA markers and *OsMADS50* is due to the unavailability of crossing over between *OsMADS50* gene and the marker. At present, this recombination is determined by correlating marker alleles and days to heading of varieties. Physical distance between PSM908,

PSM909 and PSM910 generally would have reflected the genetic distances estimated by recombination, but in present study due to lack of crossing over genetic distances are zero.

Quantitative trait loci detection was revolutionary gateway for sudden up-gradation in breeding history. QTLs mapping ease up the way to gene mapping. Inveterate prediction of QTLs with some confirmatory tests some time turned into exact gene mapping i.e., QTL behave as gene itself and follow the Mendelian ratio 1:2:1. On the other hand, dissection of QTL is essential if the phenotypic explanation is not significant revealing several genes with in single QTL. High resolution mapping of QTLs or genes helps in map-based cloning and characterization of QTLs or genes. The ambition of plant breeding goes on moving advance due to complex nature of biological studies. Identification and cloning of individual genes for a particular trait forecast the interactive study of multiple genes. Plant biologist suggested certain pathways for various agronomic traits to simplify their genetic improvement. Progressive improvement of these pathways continued due to availability of genetic updates for same trait. Non-allelic interaction has been discussed repeatedly due to which complex pathways have been experimentally proved and verified. Allelic interaction study is a step forward in this context, therefore making heading date study more wide but ease up its improvement as a consequence.

Several genes have been isolated and their functioning pathway has also been devised, but this is not enough because plant breeder requires much more to do in order to increase cropping intensity. Naturally occurring genetic variation is difficult to be studied therefore is the possible limiting factor for its offensive research. At moment negligible amount of research work has been reported in literature for allelic variation studies of *OsMADS50* gene. Recent progress in rice genome analysis has made it possible to analyze naturally occurring genetic variation underlying complex traits. In the present research three populations were compared for variation in the days to heading having known genotypic composition. W08-16-3-2 was converted in double segment pyramiding lines along with *RFT1* and *Hd3a* genes, in order to dilate the duration between earliest and late heading plants. F_2 population of HJX74 × W23-3-8-9-27-82 of (Lemont source *OsMADS50*) gene headed earlier than W08-16-3-2 (IR64 source *OsMADS50*). Both of the donors carried *OsMADS50* gene, but their genetic analysis revealed significant difference between the populations derives from those parents upon crossing with the common recipient. Three variants of *OsMADS50* gene were identified having Lemont allele the earliest one, HJX74 allele the latest one, while allele hosted by IR64 headed in between days to heading of two earlier mentioned alleles. Accordingly by studying 57 varieties of IR8, Asano *et al.* (2007) identified 7 naturally occurring alleles of *SD1* gene. Saito *et al.* (2009) with the help of conventional and CAPS analysis reported a new allele at *EF1* locus named as *ef1*/*ef1-h* at 10th chromosome of rice. Such types of naturally occurring allelic variation studies are extremely useful for crop improvement program and more important is the way to exploit this hidden treasure.

Once, we have found favorable alleles in donor varieties, those genes can be introduced systematically and rapidly into elite varieties in a given region, by the aid of MAS. Furthermore, by using a variety of SSSLs and DNA markers, genes derived from different donor cultivars can be introduced into elite rice cultivars (trait pyramiding). Basmati varieties having earliest heading allele will not only minimize the growth period for saving expensive inputs but it will help in the tolerance of drastic effects of heat stress. Similar observations have been reported by (Obari, 1990; Tewolde *et al*., 2006; Ameen *et al*., 2012). This strategy will help in the development of widely adaptable high yielding rice varieties.

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