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An Exploratory Study on Allelic Diversity for Five Genetic Loci Associated with Floral Organ Development in Rice

Alok Priya[1], Soumya Prakash Das[1], Sayani Goswami[1], Malay Kr. Adak[2], Debal Deb[3], Narottam Dey[1*]

[1]Department of Biotechnology, Visva-Bharati, Santiniketan-731235, West Bengal, India
[2]Plant Physiology and Plant Molecular Biology Research Unit, Department of Botany, University of Kalyani, Kalyani-741235, West Bengal, India
[3]Basudha, Barrackpore, Kolkata-700120, West Bengal, India
Email: [*] narottam.dey@visva-bharati.ac.in

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Abstract

Allelic diversity for five genetic loci (*DL*, *FON*4, *OsMADS*24, *OsMADS*45 and *Spw*1) associated with floral organ development were investigated among a small heterogeneous rice population which included one wild species (*O. rufipogon* Griffiths), one indigenous less popular natural floral organ mutant (*O. sativa* var. *indica* cv. Jugal), one indigenous normal line (*O. sativa* var. *indica* cv. Bhutmoori) and one improved high yielding line (*O. sativa* var. *indica* cv. IR 36). Detailed spikelet morphology showed that var. Jugal had variable number (1 - 3) of carpels within a single spikelet which was unique and resulted in variable (1 - 3) number of kernels within a single matured spikelet (grain). The genomic DNA of each investigated line was amplified with primer sequences designed from the selected genetic loci and the derived polymorphism profiles were used for study of allelic diversity for the studied loci. The derived genetic distances among the rice lines were used for dendrogram construction. In constructed dendrogram, the mutant genotype (Jugal) showed highest similarity with the wild rice (*O. rufipogon*) instead of the rice lines. To verify this finding, the genomic DNA of each studied line was also amplified with four SSR loci, tightly linked to *saltol* QTL, mapped to rice chromosome 1. The amplified products were screened for polymorphism and another dendrogram was constructed to reveal the genetic distance among the lines for selected salt tolerance linked SSR loci. In SSR derived dendrogram, the wild rice (*O. rufipogon*) got totally separated from the all three rice genotypes though all the studied four lines showed equal sensitivity for salt sensitivity in a physiological screening experiment. From the combined experiment, it can be concluded that genetic architecture of floral organ development loci in var. Jugal may have some uniqueness which is not present in normal rice but common to *O. rufipogon*, a species which is regarded as immediate progenitor of present day modern rice (*O. sativa*). Though

[*]Corresponding author.

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this uniqueness was not confirmed by second set genetic loci associated with salt tolerance in rice, the information resulted from this experiment was preliminary and based only on allelic size (molecular weight of amplicon), which should be confirmed through sequence analysis for further analysis.

Keywords

Rice, Multiple Kernel, Floral Organ Number Mutant, Rice Microsatellite, Allelic Diversity

1. Introduction

The most important area in rice breeding is increase in quantity per unit area per unit time. As possibility for expanding cultivated rice land is very limited, future food security depends on a number of scientific strategies without utilizing more lands [1]. The two most significant ways are the development of hybrid and transgenic rice where the selected lines are utilized for possible inclusion of their beneficial traits/alleles into target genotypes. Indigenous and wild rice lines harbour a number of favourable genes that are not yet used in hybridization programmes and remain untapped in nature [2] [3]. Keeping this as an objective, a good number of indigenous rice landraces are being investigated to find out the different beneficial traits by a number of workers [4]-[9]. Of the different promising rice land races of South Bengal (an important rice growing region of Eastern India), Jugal (*O. sativa* cv. Jugal, NBPGR IC No. 567987) is an interesting and less popular indigenous line which produces one, two or even more than two kernels (seeds) per grain (spikelet) with two kernels being the majority [10] [11]. The same rice line is also available in Odisha (another rice growing state of Eastern India), where it is called as Lavkush and maintained by Central Rice Research Institute, Cuttack under the accession name JMGR. This is a unique and interesting trait, shared only by another variety, Sateen (*O. sativa* var. *indica* cv. Sateen) [11].

It is known that in normal (single-kernelled) rice, the floral meristem activity in the spikelet stops immediately after the production of single carpel (gynoecium) in the central point of apical meristems. In contrast, in Jugal, the programme for gynoecium production continues until fertilization, and further growth and development of endosperm continue thereafter, until it collectively occupies the whole space within a spikelet. Though all the endosperms within a spikelet have equal probability to develop and mature, only one or two kernels remain healthy while the rest are rudimentary and of abnormal in size and shape. This particular line was for the first time morpho-taxonomically described by Prain in 1905 from Chittagong of British India (presently in Bangladesh) and described as variety *plana* [12]. In an advance genetic study made by Pandian and Thiyagarajan, it was shown that mutipistilate trait in rice was controlled by mutant genes located on 6th chromosome [13]. Genetic analysis of another multipistilate *japonica* rice was studied by a number of Chinese and Japanese workers [14]-[17]. It has been reported that homeotic mutation in a number of genetic loci (*floral organ number*, *FON*) causes an increased number of stamens and carpels [18] [19] in rice. Rice *FON*4, an orthologous to *ArabidopsisCLV*3 caused abnormal enlargement of inflorescence meristem which ultimately developed thick culms with increased primary rachis branches and floral organs [20]. *Drooping leaf* (*DL*), a member of the *YABBY* gene family, controls carpel specification and leaf midrib formation [21] and floral meristem determinacy in rice. *DL* is an orthologue of *crabs claw* (CRC) of *Arabidopsis* and it has an antagonistic function with class B genes. The rice class B gene *superwomen*1 (*SPW*1 or OsMADS16) is involved in stamen specification [22] [23]. *SPW*1 mutant is orthologue of *ArabidopsisAP*3 for which stamens are replaced by carpels and lodicules [24] [25]. *OsMADS*24 and *OsMADS*45 are orthologue of *ArabidopsisAGL*2 and *AGL*4 [26]. These two genes function to express the development of floral organs, and act as intermediary between meristem identity and organ identity.

The objective of this present investigation was to study the allelic diversity within the selected rice lines (cultivars Jugal, Bhutmoori, IR36) and O. *rufipogon* for five genetic loci (*DL*, *FON*4, *OsMADS*24, *OsMADS*45 and *Spw*1) linked to floral organ development in rice. The functions of these loci constitute the preliminary information required for utilization of this special trait in breeding through molecular breeding.

2. Materials and Method

Plant material: A total of four rice lines were studied in this study which included one wild rice, one traditional

rice and one improved high yielding rice and one indigenous mutant line. A short description of the studied lines is presented in **Table 1**.

Seeds of the studied rice lines were disinfested with sodium hypochlorite (2%) and germinated on moist cotton kept on petriplates. Five days after germination the seedlings were transplanted to large cement tanks filled with rice field soils. The plants were maintained in natural environmental condition for further growth and development.

Morphological study of the spikelet: Young spikelets of each line were dissected under a binocular microscope and photographs were taken. Both young and mature spikelets with kernels were examined.

Study of allelic diversity for selected floral organ development loci: For study of allelic diversity five loci (*DL*, *FON*4, *OsMADS*24, *OsMADS*45 and *Spw*1) associated with floral organ development in rice were selected. The detailed information of the selected loci is presented in **Table 2** and the respective sequences for the selected genetic loci were downloaded from ensemble plant database (http://plants.ensembl.org). The primer pairs of individual loci were designed through Primer3, a free online tool to design and analyze primers for PCR amplification.

Primer sequences were subjected to BLAST analysis in NCBI database taking rice genome (IRGSP, Build 4.0) as reference to find out the possible sequence similarity in rice genome and final confirmation was done through in silico PCR targeting the respective DNA sequences using a freely available web resource (http://insilico.ehu.es). The primer sequences used to amplify these loci are given in **Table 3**.

Table 1. Detailed description of the studied rice lines.

Acc. No.	Name of the variety	Specific trait	Source
VB 9	*O. sativa* L.var.IR 36	High yielding drought sensitive lines, presently the popular lines of Bengal	Central Rice Research Institute, Cuttack, Odisha
VB 18	*O. rufipogon* Griffiths (popular name Redrice and by some tribal as Orhidhan)	Grow with rice in field as weeds, shatter readily before harvest, so that paddy field became thoroughly infested with dropped seeds, which can grow with the following crop and can remain viable for 1 - 3 years.	Collected from a low land rice field in Bankura, South Bengal
VB 156	*O. sativa* L.var. Jugal	An indigenous less popular rice lines of Bengal and Odisha having 1 - 3 kernels within a mature spikelet (grain).	Basudha farm at Panchal, Bankura, South Bengal, Central Rice Research Institute, Cuttack, Odisha
VB 162	*O. sativa* L.var. Bhut Moori	A typical drought indigenous line with medium yield, used for preparation of popped rice.	Basudha farm at Panchal, Bankura, South Bengal

Table 2. Details of the floral organ loci with reference number used in this study.

Gene name	Locus ID	Chromosomal location	Putative function
DL	OS03G0215200	3:6,041,346-6,048,357	Homeotic transformation of carpels into stamens
FON4	DQ836359	11:2,266,4996-22,665,010	Encoding a putative ortholog of Arabidopsis CLAVATA3 regulates apical meristem size in rice
*OsMADS*25	OS04G0304400	4:13,672,710-13,675,884	MADS-box transcription factor 25
*OsMADS*45	Os08g0531700	8:26,507,926-26,512,261	Similar to MADS-box transcription factor 7
*SPW*1	Os06g0712700	6:30,173,627-30,177,996	Transcription factor, Development of lodicules and stamens

Table 3. Primer sequences of the studied floral organ development loci used for PCR amplification.

Name of loci	Forward primer sequence	Reverse primer sequence
DL	5'GGGCTAGCTTGCTTGTCG3'	5'GCGCTCCATCTGCTTG3'
*FON*4	5'CTCTTCTTGTGTTTGGTGGTTG3'	5'CGCCTCATCCAGAGCAATA3'
*OsMADS*24	5'AGAACAAGATCAACAGGCAGGT3'	5'AGGCACTGCACTGTACCATACG3'
*OsMADS*45	5'TCTAGCTTGGTTGGTTGGTTG3'	5'CAACAGACACCGATAGTTTTTAAGG3'
*Spw*1	5'ACTCCTCTTCTTCCTCCTCCTC3'	5'GCAATTTATTCGTCGGCTTGT3'

The oligo sequences were synthesized from Integrated DNA Technology (IDT, USA). PCR amplification was done in a thermal cycler (M. J. Research, MC 013130) in 25 µl of reaction mixture containing 100 ng of genomic DNA, 2.5 µl of 10× Taqbuffer, 1.0 µl of 50 mM MgCl2, 0.25 µl of 2.5 mM dNTPs, 1 µl each of the forward and reverse primers (10 pmol/ µl), 0.1 µl of 5 U/µl Taq-polymerase. The thermal cycling profile for the first step was 95˚C for 5 min. For the next 35 cycles the temperature regime was 94˚C for 1 min, 1 min at annealing temperature and 72˚C for 2 min with final extension at 72˚C for 10 min. The annealing temperature of each set of PCR reaction was changed accordingly. The amplified products, obtained from the individual loci were resolved in 1.5% agarose gel and the different allelic (variation in molecular weight) forms of each individual locus were determined. All the reagents were purchased from Fermentas Life Sciences, USA.

Study of allelic diversity for selected SSR loci linked with Saltol QTL: Genetic relationship among the four studied rice genotypes was also assessed with four previously reported tightly linked SSR loci (RM10745, RM10764, RM493, RM140) linked with *Saltol* QTL mapped on rice chromosome 1 [27]-[30]. The detailed information of these markers was collected from Gramene website (http://www.gramene.org), a dedicated website for plant comparative genomics. Chromosomal position of the selected loci is presented in **Figure 1** and detailed primer sequences for these loci are given in **Table 4**.

3. Results

A magnified view of the dissected young spikelet revealed that in all the rice lines (except *O. sativa* cv. Jugal) and also in *O. rufipogon*, the reproductive unit of spikelet contains six stamens, and one carpel with bifid stigma. In the spikelets of Jugal, however, the number of carpels vary from 1 - 3 or more, of which 1 - 2 are healthy and the rest are rudimentary (**Figure 2(a)**) with six regular-sized stamens. Mature spikelets also showed single kernel in all rice genotypes and in *O. rufipogon*, whereas in Jugal, the number varies from 1 - 3 per grain with vary-

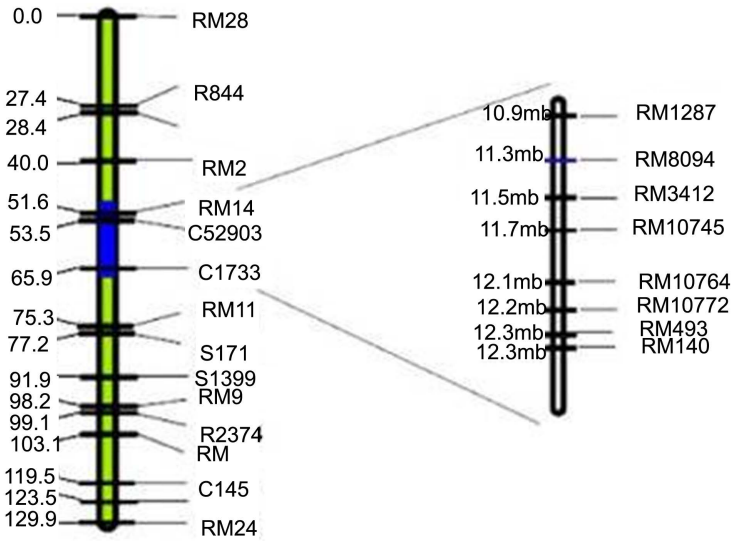

Figure 1. Location of the *Saltol* QTL on rice 1st chromosome with selected SSR loci use in this study (taken from Mohammadi-Nejad *et al*. 2008).

Table 4. Details of the used SSR used in present study.

SSR loci	Motif	Forward sequence	Reverse sequence
RM10745	(TATG)9	5'TGACGAATTGACACACCGAGTACG3'	5'ACTTCACCGTCGGCAACATGG3'
RM10764	(AT)28	5'AGATGTCGCCTGATCTTGCATCG3'	5'GATCGACCAGGTTGCATTAACAG3'
RM493	(CTT)9	5'TAGCTCCAACAGGATCGACC3'	5'GTACGTAAACGCGGAAGGTG3'
RM140	(CT)12	5'TGCCTCTTCCCTGGCTCCCCTG3'	5'GGCATGCCGAATGAAATGCATG3'

ing kernel size (**Figure 2(b)**). On germination the mature grains of Jugal produce variable number of young seedlings (**Figure 2(c)**).

Allelic diversity analysis for the selected floral organ development loci and SSR loci linked with *Saltol* QTL-DNA amplification profile generated from the selected primer pairs were used to study the allelic diversity among the selected rice genotypes for floral organ development loci. The agarose gel picture showing amplification profile for the five loci (*DL*, *FON*4, *OsMADS*24, *OsMADS*45 and *SPW*1) are presented in **Figure 3(a)** and **Figure 3(b)**. Based on the presence or absence of a specific allele among the selected genotypes, a dendrogram (**Figure 4(a)**) was constructed where Jugal showed its highest closeness with *O. rufipogon* (a wild rice species) and next to this with Bhutmoori (a traditional line) but maximum distance with IR 36 (a high yielding improved line). On the other hand, in SSR derived dendrogram (**Figure 4(b)**) all cultivated genotypes were grouped in a single cluster and the wild rice (*O. rufipogon*) got totally separated from the selected rice lines.

4. Discussions

The spikelet is the fundamental reproductive unit in cereals whose morphogenesis and development have profound influence on yield. In rice, the spikelet is a single floret, composed of a lemma and palea, which are considered as the first-whorl organs and enclose two lodicules (second whorl), six stamens (third whorl), and a carpel containing a single ovule (fourth whorl). From the detailed morphological study, it has shown that the multi-pistilate (presence of more than one pistil within a spikelet) trait is the unique genotype studied (cv. Jugal), which is a result of uncontrolled activity of reproductive meristems. In normal cultivated rice and in *O. rufipo-*

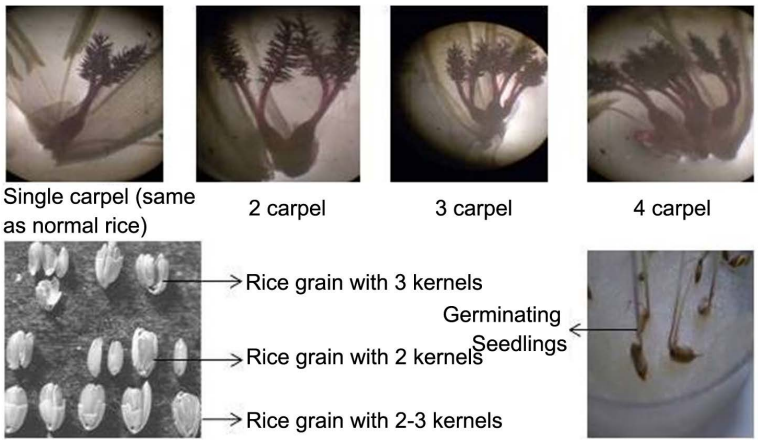

Figure 2. (a) Dissected Rice spikelet with single carpel and multiple carpels (in var. Jugal); (b) Grains with multiple kernels (Jugal); (c) Germinating rice grains with two seedlings (in var. Jugal).

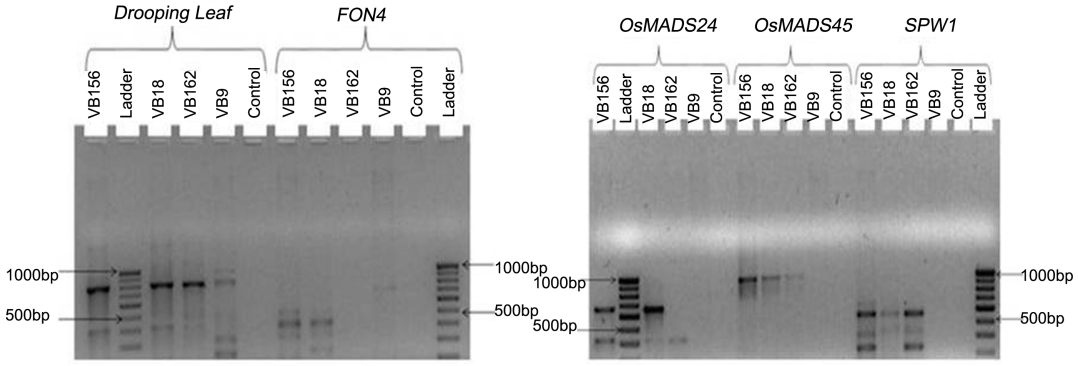

Figure 3. (a) (b) Gel picture showing amplified product the studied lines for the used floral organ development loci (*DL*, *FON*4, *OsMADS*24, *OsMADS*45 and *SPW*1) (The control lane contains only PCR master mix without genomic DNA) (VB156-Jugal, VB18-*O. rufipogon*, VB162-Bhut Moori, VB9-IR 36).

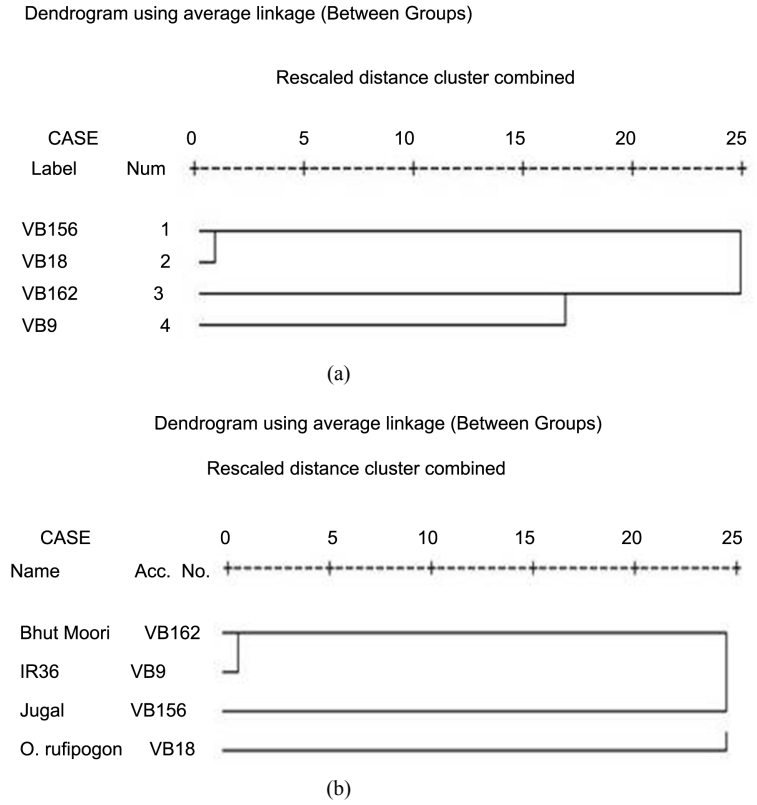

Figure 4. (a) Dendrogram derived from polymorphism screening for floral organ development loci; (b) Dendrogram derived from polymorphism screening for selected SSR loci.

gon, the meristematic activity in a spikelet stops after the production of the gynoecium, but in Jugal, the meristematic activity continues after fertilization, resulting in additional rudimentary pistil along with the mature normal one. But as the dimension is almost fixed for each spikelet, the mature kernels become reduced in size. The alternative structure (mutant form) of the rice spikelet is controlled by a number of homeotic genes, almost all of which are orthologous to a number of floral organ development loci of *Arabidopsis*.

This experiment constitutes a comparative analysis of a few selected genetic loci commonly associated with floral organ development and most importantly floral organ number in rice. For study of allelic diversity, the amplicon size for individual loci was taken as the clustering criteria where the mutant line showed uniqueness by being isolated from other lines. For further confirmation of this isolating nature, another set of molecular markers was employed which did not support the earlier clustering behavior. The prime limitation of this investigation is the use of allelic size (variation in mol. wt. of the amplified product for a locus) as the principal criterion for separation. For further confirmation, the amplified products need to be sequenced and bioinformatically analyzed. As there is no information available for this valuable mutant for its floral organ genetics, our study provides preliminary molecular information for undertaking further genomic analysis of the special trait of gynoecium replication.

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