RESEARCH PAPER

Bivalent Formation 1, a plant-conserved gene, encodes an OmpH/coiled-coil motif-containing protein required for meiotic recombination in rice

Lian Zhou1,2,3, Jingluan Han1,2,3, Yuanling Chen1,2,3, Yingxiang Wang4,* and Yao-Guang Liu1,2,3,*

1 State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, 510642 Guangzhou, China
2 Key Laboratory of Plant Functional Genomics and Biotechnology of Guangdong Provincial Higher Education Institutions, 510642 Guangzhou, China
3 College of Life Sciences, South China Agricultural University, 510642 Guangzhou, China
4 State Key Laboratory of Genetic Engineering, Institute of Plant Biology, School of Life Sciences, Fudan University, 200438 Shanghai, China

* Correspondence: ygliu@scau.edu.cn and yx_wang@fudan.edu.cn

Received 31 October 2016; Editorial decision 20 February 2017; Accepted 6 March 2017

Editor: Zoe Wilson, University of Nottingham

Abstract

Meiosis is essential for eukaryotic sexual reproduction and plant fertility. In comparison with over 80 meiotic genes identified in Arabidopsis, there are only ~30 meiotic genes characterized in rice (Oryza sativa L.). Many genes involved in the regulation of meiotic progression remain to be determined. In this study, we identified a sterile rice mutant and cloned a new meiotic gene, OsBVF1 (Bivalent Formation 1) by map-based cloning. Molecular genetics and cytological approaches were carried out to address the function of OsBVF1 in meiosis. Phylogenetic analyses were used to study the evolution of OsBVF1 and its homologs in plant species. Here we showed that the bvf1 male meiocytes were defective in formation of meiotic double strand break, thereby resulting in a failure of bivalent formation in diakinesis and unequal chromosome segregation in anaphase I. The causal gene, OsBVF1, encodes a unique OmpH/coiled-coil motif-containing protein and its homologs are highly conserved in the plant kingdom and seem to be a single-copy gene in the majority of plant species. Our study demonstrates that OsBVF1 is a novel plant-conserved factor involved in meiotic recombination in rice, providing a new insight into understanding of meiotic progression regulation.

Key words: Bivalent, coiled-coil motif, double strand break formation, meiosis, OmpH domain, rice.

Introduction

Meiosis is a specialized form of cell division that halves the chromosome number of diploid cells in producing haploid cells; it is highly conserved for sexual reproduction in most eukaryotes (Gerton and Hawley, 2005; Ramesh et al., 2005). It comprises two rounds of cell division, meiosis I and meiosis II, and each round can be divided into four stages: prophase, metaphase, anaphase, and telophase. Prophase I is a relatively long phase taking up 85–95% of the total time of meiosis, and has been further divided into five stages: leptotene, zygotene, pachytene, diplotene, and diakinesis (Wang et al., 2014b). Homologous chromosome (homolog) interaction is the crucial event during meiotic prophase I, including pairing, synapsis, recombination, and segregation. Proper interaction not only ensures the subsequently accurate segregation between...
homologs, but also redistributes the genetic alleles among the progeny, which has a great impact in biological diversity.

In the last three decades, molecular genetic studies have identified many genes involved in different meiotic processes in a variety of model species, such as *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Caenorhabditis elegans*, and several higher plants (Zickler and Kleckner, 1998, 2015, 2016; Osman et al., 2011; Ma et al., 2014; Mercier et al., 2015). In the dicot model plant Arabidopsis, so far, more than 80 meiosis-related genes have been identified (Osman et al., 2011; Wang et al., 2014b; Mercier et al., 2015). By contrast, only ~30 meiotic genes in the monocot model plant rice (*Oryza sativa* L.) have been cloned and functionally studied (Luo et al., 2014). For example, *OsMEL12* and *OsAML* are required for the initial meiotic events and their mutations cause the failure of meiotic entrance or arrest at an early stage (Nonomura et al., 2007; Che et al., 2011; Nonomura et al., 2011). It has been well studied that meiotic recombination is initiated by the programmed formation of double strand breaks (DSBs) catalysed by SPO11, which is an evolutionarily conserved type II topoisomerase in eukaryotes (Keeney et al., 1997; Grelon et al., 2001; Stacey et al., 2006; Yu et al., 2010; An et al., 2011). In rice, two SPO11 homologs, OsSPO11-1 and OsSPO11-4, were identified as being required for DSB formation (Nonomura et al., 2004a; Yu et al., 2010; An et al., 2011). In addition, in yeast, there are at least eight genes involved in this process (Keeney, 2008). In Arabidopsis, AtPRD1/2, AtDFO, AtPCH2, and MTOPVIB were required for DSB formation (De Muyt et al., 2007; De Muyt et al., 2009; Zhang et al., 2012; Lambing et al., 2015; Vrielynck et al., 2016). By contrast, only OsPAIR1, OsCRC1, OsSDS, and OsMTOPVIB were characterized as being DSB formation related in rice (Nonomura et al., 2004a; Miao et al., 2013; Wu et al., 2015; Fu et al., 2016; Xue et al., 2016). It seems that divergence of regulation of meiotic progression exists between rice and Arabidopsis.

After DSB formation, further resection of a single end produces 3' end overhang, which is protected by replication protein A (RPAs) proteins (Iftode et al., 1999). Three RPA proteins were discovered to have a role in meiotic recombination in rice (Chang et al., 2009; Li et al., 2013). Further single end invasion is facilitated by RecA homologs; several rice RecA members were identified, such as OsDMC1, OsRAD51, OsRAD51C, and OsXRCC3 (Ding et al., 2001; Deng and Wang, 2007; Rajanikant et al., 2008; Tang et al., 2014; Zhang et al., 2015), suggesting that this process is conserved. As a consequence, repair of DSBs yields crossovers (COs) or noncrossovers (NCOs). Most organisms have two types of COs, of which the interference-sensitive CO (class I) depends on ZMM proteins, while the interference-insensitive CO (class II) is MUS81 dependent (Hollingsworth and Brill, 2004). In rice, several ZMM proteins such as OsMSH4, OsMSH5, OsMER3, OsHEI10, and OsZIP4 are involved in the class I CO pathway (Wang et al., 2009; Shen et al., 2012; Wang et al., 2012a; Luo et al., 2013; Zhang et al., 2014), but the MUS81 homolog has not yet been characterized. In addition, several proteins required for meiotic chromosome segregation have been isolated in rice, such as OsSGO1 (Wang et al., 2011b), OsREC8 (Shao et al., 2011), and OsBRK1 (Wang et al., 2012b).

The synaptonemal complex (SC) forms between homologous chromosomes and is important for the maturation of some recombination intermediates by stabilizing the paired chromosomes (Page and Hawley, 2004; Zickler and Kleckner, 2015, 2016). The SC is a tripartite structure consisting of two parallel lateral elements and a central element. The rice PAIR2 and PAIR3 are axial elements, while OsCSC1 and OsZEP1, the homolog of ZIP1 in *Saccharomyces cerevisiae* and ZYP1 in Arabidopsis, are central elements of the SC (Sym et al., 1993; Wang et al., 2010; Wang et al., 2011a; Higgins et al., 2005; Nonomura et al., 2007; Yuan et al., 2009; Miao et al., 2013). Interestingly, unlike other species, partial loss of function of the rice ZEP1 has a distinct role in increase of COs (Wang et al., 2010; Wang et al., 2015), suggesting that different plant species may have the specific factors controlling meiosis.

In this study, we identified a sterile rice mutant with meiotic defects and isolated a gene (named *Bivalent Formation 1*, *OsBF1*) by map-based cloning that encodes a conserved protein with a putative coiled-coil motif and an outer membrane protein H (OmpH) motif. In the *bf1* mutant, meiotic DSB formation failed to be detected, thereby resulting in the failure of synapsis. At diakinesis, unlike the wild type (WT) that formed 12 bivalents, *bf1* produced 24 univalents and had improper chromosome segregation in both anaphase I and II. Further analysis showed that installation of the central element, OsZEP1, of the SC was also defective. Taken together, our results reveal a new protein that is required for meiotic DSB formation and the subsequent synopsis and recombination in rice.

### Materials and methods

#### Experimental materials

The *bf1* mutant was identified from the *japonica* cv Nipponbare (Nip) mutant library induced by 60Co γ-ray radiation in our laboratory. The mapping populations were constructed by crossing the heterozygote (*BF1/bf1*) with * indica* cv Huanghuazhan (HHZ), and backcrossed with HHZ. All the materials were planted in fields in Guangzhou from spring to autumn (two growth seasons). For the recombinant screening, germinated seeds were planted in 96-well plates, and 3-week-old seedlings were used for high-throughput DNA preparation as described previously (Wang et al., 2013). Detected recombinant plants were planted in field or buckets.

#### Observation of pollen viability

Spikelets with mature pollen at the heading stage were collected and fixed in 70% ethanol. Then pollen grains were disseceted out of anthers in 1% L–KI solution. The stained pollen grains were firstly observed under a microscope (Olympus CX31), and then pictures were taken under an Axio Observer Z1 fluorescence microscope (Zeiss, Oberkochen, Germany).

#### Observation of meiotic chromosome morphology

Young panicles (4–8 cm in length) of both WT and *bf1* mutant were collected and fixed in Carnoy’s solution (ethanol:glacial acetic acid (v:v) 3:1) at room temperature in less than 24 h (Cheng, 2013).
The fixed panicles were washed with 70% ethanol three to five times until the glacial acetic acid faded and then stored in it at 4 ℃. Pollen mother cells (PMCs) undergoing meiosis was squashed in water or phosphate-buffered saline (PBS). The slides with PMCs were then moved to a hot block at 45 ℃, mixing the cells with a few drops of 65% glacial acetic acid and heating for 1 min. Before the drop dried, previously frozen Carnoy’s solution was added to the center of the drop to separate the cells (Wang et al., 2014a). After the liquid dried, 4,6-diamidino-2-phenylindole (DAPI) in anti-fade solution (Vector Laboratories, Burlingame, CA, USA) was added to the slide and covered up for observation. Chromosome images were captured under the Axio Observer Z1 fluorescence microscope.

Expression vector construction

Total RNA from spikelets of WT rice were extracted. Total RNA (1 μg) was reverse transcribed by using M-MLV Reverse Transcriptase (Promega, Madison, WI, USA) with Oligo-T (18) as primer, the products of which were taken as the template used afterwards. The ORF sequence of Os05g0251400 was amplified by primers pOX-BVF1-F/R (see Supplementary Table S1 at JXB online) and ligated into a binary vector so as the ORF was under the control of the ubiquitin promoter. The green fluorescent protein (GFP) fusion vectors were constructed with the Ω-PCR procedure (Chen et al., 2013) with primers GFP-BVF1/BVF1-GFP. The fluorescence images were captured using an LSM 7 DUO Confocal Microscope (Zeiss).

Rice transformation and genotyping

By Agrobacterium (stain EHA105)-mediated transformation, the vector constructs were transferred into callus induced from seeds of heterozygous mutant plants. Positive transformants were screened by PCR amplification with HPT primers and vector-specific primer pOX-T (see Supplementary Table S1 at JXB online), respectively. The endogenous genotypes of the transformants were identified by a semi-nested PCR with specific primers BVF1(F)/(R)/(R2) (Supplementary Table S1).

Immunostaining assays

The methods of material fixation and slide preparation are given in Cheng (2013). After removing the coverslip, the slides were marked by a stain circle pen and incubated in washing buffer I (1×PBS with 1% (v/v) Triton X-100) for an hour at room temperature. Then slides were incubated with the primary antibodies, including anti-γH2AX (raised in rabbit; Miao et al., 2013), OsREC8 (raised in both rabbit and mouse; Shao et al., 2011), OsMER11 (raised in mouse; Ji et al., 2013), OsCOM1 (raised in mouse; Ji et al., 2012), OsDMC1 (raised in mouse; Wang et al., 2016), OsMER3 (raised in mouse; Wang et al., 2009), OsPAIR2 (raised in mouse; Wang et al., 2009) or anti-OsZEP1 (raised in mouse; Wang et al., 2010) antibody solution (diluted 1:200 in blocking buffer: 1×PBS, 0.1% (v/v) Tween 20, 5% BSA), at 4 ℃ overnight. After three rounds of washing in washing buffer II (1×PBS with 0.1% (v/v) Tween 20), Alexa Fluor 488-conjugated goat anti-mouse secondary antibody or Alexa Fluor 555-conjugated donkey anti-rabbit secondary antibody (Beiyotime, Shanghai, China) was added to the slides. The chromosomes were counterstained with DAPI (10 mg mL⁻¹) in an anti-fade solution (Vector Laboratories).

Results

Identification and characterization of a sterile rice mutant

We created a mutant library of a japonica cultivar Nipponbare by 60Co γ-ray radiation. By screening the mutant library, we obtained a sterile mutant, named bivalent formation 1 (bvf1) according to our later observation that the mutated causal gene affects bivalent formation in meiosis. The mutant had as normal vegetative growth as the WT plants, but with no seed setting at the reproductive growth stage (Fig. 1A, D). Further characterization showed that the mutant exhibited smaller anthers and completely sterile pollen grains (Fig. 1B, C). When the mutant plants were pollinated with WT pollen grains, no seed was produced, suggesting that the female gametes were also sterile. The segregation of fertile (104) to sterile (34) individuals in the progeny of self-fertilized mutant heterozygotes fitted the 3:1 ratio (Supplementary Table S2), indicating that a single recessive gene is responsible for the male and female sterile phenotypes.

Meiosis is defective in pollen mother cells of bvf1

It is known that defective mutation of many meiotic genes causes male and female sterility in both human and plants (Székvélygi and Nicolas, 2010; Luo et al., 2014). To explore the possibility for the sterility in bvf1, we observed the meiotic chromosome behavior of pollen mother cells (meiocytes)
using chromosome spreads stained with DAPI at different meiotic stages in both WT and bvf1. As shown in Fig. 2, in WT, at leptotene, the chromosomes began to condense and displayed a thread-like feature under microscopy (Fig. 2A). At zygotene, the homologous chromosomes aligned together and began to pair with each other (Fig. 2B). At pachytene, the homologs were stabilized by the synaptonemal complex (SC) and displayed thick thread-like chromosomes (Fig. 2C). At diakinesis, following the disassembly of the SC, the 12 pairs of homologs (also called bivalents) physically associated by chiasma and sister chromatid cohesion were observed (Fig. 2D). At metaphase I, all bivalents were aligned at the equatorial plate pulled by spindles (Fig. 2E), thereby resulting in the subsequent segregation to the opposite poles (Fig. 2F). Finally, the two dyads simultaneously underwent meiotic II cell division and formed the tetrad microspores (Fig. 2G, H).

Compared with the WT chromosome morphology, no obvious difference was observed in bvf1 from leptotene to zygotene (Fig. 2I, J). At pachytene, unlike WT with fully synapsed homologs, the bvf1 chromosomes condensed and aligned together, but did not show thick chromosomes \( (n=82\) meiocytes) (Fig. 2K), suggesting a defect in synapsis. From diplotene to diakinesis, in contrast to the WT that formed 12 bivalents, bvf1 had 24 univalents \( (n=110\) meiocytes) (Fig. 2L), suggesting a failure of crossover formation. Due to the recombination defect, the 24 univalents were not well aligned at the equatorial plate at metaphase I \( (n=167\) meiocytes) (Fig. 2M), and showed an uneven segregation to the two poles at anaphase I. Moreover, 70.9% bvf1 meiocytes at anaphase I \( (n=79)\) had lagging chromosomes (Fig. 2N). At meiosis II, due to the unequal segregation of chromosomes, the bvf1 meiocytes produced abnormal tetrads with uneven chromosome numbers and micronuclei (Fig. 2O, P). The failure of bivalent formation and the aberrant chromosome segregation provides an explanation for the complete sterility in bvf1. Together, these results indicate that OsBVF1 is required for normal bivalent formation in rice meiosis.

**Map-based cloning of OsBVF1**

To isolate the gene conferring the mutant phenotype, we crossed the heterozygous BVF1/bvf1 plants (male and female fertile) with an indica rice variety, HHZ. The F1 plants were further backcrossed with HHZ. By linkage analysis using 10 sterile F2 plants and a set of polymorphic markers covering the whole genome, a region on the short arm of chromosome...
The role of OsBVF1 in rice meiotic recombination

5 was found to link with bvfl. Then we used a total of 775 F₂ and BC₁F₂ plants and a number of molecular markers on this region (Supplementary Table S3) to primarily map the locus on a region of ca 2821 kb (Fig. 3A). Through further screening of new recombinants in the segregated F₁ and F₄ populations with the markers 507966 and 510787, OsBVF1 (Osbvfl) was further delimited to an 84-kb region between two markers, 509167 and 509251 (Fig. 3A), a region that includes seven annotated genes. Then we amplified these genes by PCR for subsequent sequencing (Fig. 3A). A single base deletion in the third exon of the gene Os05g0251400 was detected in bvfl (Fig. 3B), which caused a frame-shift and a premature stop codon (Fig. 3B). Because no other mutations in the other genes within the 84-kb region were found, we considered Os05g0251400 as the candidate gene for OsBVF1.

To verify the function of Os05g0251400, we constructed a binary vector (pOX-BVF1) with the whole 1115-bp open reading frame sequence (AK103883) of Os05g0251400 driven by the maize ubiquitin promoter. This construct was used to transform calli induced from immature seeds of the heterozygous BVF1/bvf1 plants. By genotyping of the endogenous Os05g0251400 in the transgenic-positive transgenic (T₀) plants with the mutation site-specific primer set 1400-T (Supplementary Table S1), four out of 17 T₀ plants were found to have homozygous Osbvfl, and they all showed recovered fertility and normal seed-setting (Fig. 3C and Supplementary Fig. S1). In the T₁ generation of these four plants, the

---

**Fig. 3.** Map-based cloning of the gene for bvfl. (A) The Osbvfl locus was primarily mapped to a 2821-kb region on chromosome 5 using 775 segregation plants (F₂ and BC₁F₂) of the BVF1/bvf1×HHZ (indica cv) cross, then further delimited to an 84-kb region by analysis of eight recombinant plants selected from the F₃ and F₄ populations. Seven genes have been predicted in this mapped region. (B) Sequencing analysis detected a single base (‘A’ nucleotide) deletion in the position +292 (+1752 including the introns) of the ORF of Os05g0251400. (C) The pollen (left) and grain-filled spikelet (right) fertilities of the transgenic plants (T₀) with homozygous Osbvfl were restored by transformation with a binary construct expressing the ORF of Os05g0251400. Bars, 50 μm for pollen and 20 cm for plants.
segregants with and without the transgenes co-segregated with the fertile and sterile phenotypes (Supplementary Table S4 and Supplementary Fig. S1). Therefore, we conclude that the single-base deletion in Os05g0251400 is responsible for this sterile mutation of the target gene.

Sequence analysis (www.ncbi.nlm.nih.gov/, last accessed 13 March 2017) showed that OsBVF1 encodes a hypothetical protein of 286 amino acids (aa) (protein Accession No.: NP_001055029) with a putative conserved OmpH (outer membrane protein H) domain from the 62nd to 152nd aa, and this protein is unique in the rice genome (Fig. 4A). The mutation in the bvfl allele produces a truncated protein of 99 aa. By running the ‘COILS’ program (http://www.ch.embnet.org/software/COILS_form.html, last accessed 13 March 2017) using OsBVF1 as query, it is predicted that OsBVF1 also can form two coiled-coil motifs in the central region (54–81 aa and 90–124 aa) (Supplementary Fig. S2), which partially overlaps with the OmpH domain (Fig. 4A and Supplementary Fig. S3). Thus, OsBVF1 encodes a new protein with a unique OmpH domain coupling with the coiled-coil motif in rice.

OsBVF1 is indispensable for meiotic DSB formation

Meiotic recombination is initiated from the programmed DSB formation (Keeney et al., 1997). The formation of DSBs triggers the phosphorylation of the histone variant H2AX (γ-H2AX), which specifically marks DSBs and facilitates post-replication DNA repair (Dickey et al., 2009). To detect whether DSBs are formed in bvfl, we used immunofluorescence to examine the distribution of phosphorylated γ-H2AX with an anti-γH2AX antibody generated using the sequence from rice (Miao et al., 2013). To mark the chromosomes, we used OsREC8, a homolog of Arabidopsis meiotic specific cohesin SY1 (Cai et al., 2003), which has a linear distribution pattern on chromosomes during early prophase I (Shao et al., 2011). As shown in Fig. 5, WT zygote meiocytes showed dot-like signals of γH2AX (Fig. 5A), while no signals were detected in bvfl (Fig. 5B), indicating that BVF1 is indispensable for rice meiotic DSB formation.

Following the DSB formation, the DSB ends are further processed by the MRX complex (Mre11/Rad50/Xrs2) and COM1/SAE2 (Mimitou and Symington, 2009). The rice OsMRE11 and OsCOM1 homologs have also been reported to participate in meiotic DSB repair (Ji et al., 2012, 2013). We further examined the localization of OsMRE11 and OsCOM1.

Fig. 4. Structure of the OsBVF1 protein, the OsBVF1 expression patterns, and the protein subcellular localization. (A) The structure of OsBVF1 with an OmpH domain and two coiled-coil motifs. (B) Expression patterns (mean with standard deviation of three biological replicates) of OsBVF1 (WT) and Osbvfl in developmental spikelets. The spikelets of 2–3 mm in length were at the PMC to meiosis stages. Actin 1 mRNA was used as the internal control. (C) The constructs expressing OsBVF1–GFP and a nuclear-localized fusion protein, GHD–mCherry, were co-transferred into rice protoplasts. Bars, 10 μm.
OsBVF1 is dispensable for axial element installation, but required for the central element installation of SC

After the progression of meiotic recombination, the SC, a proteinaceous structure including lateral and central elements formed between homologs, is important for the stabilization of recombination intermediates and facilitates subsequent homolog recombination (Zickler and Kleckner, 1999). The rice axial element (AE) protein OsPAIR2 is the homolog of yeast HOP1 and Arabidopsis AtASY1 (Nonomura et al., 2007). We examined the localization patterns of OsPAIR2 in WT and bvfl meiocytes and found a normal linear pattern overlapping with zygotene chromosomes between WT and mutant (Fig. 6A, B), implying that the assembly of AEs is probably unaffected in the mutant.

To investigate whether the installation of SC occurs in bvfl, we examined the localization of rice OsZEPI (Wang et al., 2010), a homolog of Arabidopsis AtZYP1, the central element of the SC (Higgins et al., 2005) in WT and mutant. The immunostaining signals for OsZEPI at pachytene showed linear signals along with the entire chromosomes in WT meiocytes (Fig. 6C). By contrast, no such immunostaining signals were observed in the bvfl meiocytes (Fig. 6D). Thus, we conclude that OsBVF1 is required for the installation of the SC in rice, probably by an indirect effect due to lack of DSB formation in the mutant.

OsBVF1 and its homologs are highly conserved in the plant kingdom

By protein homology search in NCBI (www.ncbi.nlm.nih.gov, last accessed 13 March 2017) and other databases, we found a number of homologous proteins of OsBVF1 in different plant species. The homolog from wild rice, Oryza brachyantha, is 283 aa in length and shares the highest identity (89%) with OsBVF1. The homologs of other monocot plants have high levels of sequence identities to OsBVF1, such as Brachypodium distachyon (76.9%), Sorghum bicolor (75.1%), Setaria italic (80.2%), Zea mays (74.4%), Hordeum vulgare (73.1%), and Triticum aestivum (76.9%) (Supplementary Fig. S5). In contrast, the homologs from eudicots have relatively low levels of identities, such as Arabidopsis thaliana (41.5%), Brassica rapa (39.9%), Carica papaya (31.82%), and Glycine max (37.63%). Homologs of OsBVF1 also were found in the streptophyta plant Klebsormidium flaccidum (13.1%) and the alga Coccomyxa bellipsoidea (14.2%). Moreover, nearly all the OsBVF1 homologs in the examined species can form one
to two coiled-coil motifs except those from Selaginella moellendorffii, Coccomyxa subellipsoidea and Klebsormidium flaccidum (Supplementary Fig. S5).

According to the amino acid similarity, we built a phylogenetic tree among 23 representative plant species (Fig. 7). The proteins were divided into four groups among eudicots, monocots, pteridophytes and streptophyta/algae. The data suggest that OsBVF1 and the homologs are plant-conserved and they should be derived from a common ancestor. It is notable that, except for Populus trichocarpa, all the examined species have only a single copy of BVF1 or its homologs (Fig. 7), suggesting that the homologous genes did not expand during the evolution of plants.

Discussion

Identification of a new meiotic gene in rice

Most of the reported meiotic genes in plants are comparatively conserved from yeast to higher eukaryotes (Osman et al., 2011; Luo et al., 2014; Mercier et al., 2015; Zickler and Kleckner, 2016). According to homology alignment in terms of sequence identity or similarity, previous studies have identified several meiotic genes in rice, such as OsDMCI (Ding et al., 2001), OsSPO11-4 (An et al., 2011) and OsRAD21-4 (Zhang et al., 2006). Compared with yeast or fruit fly, plants with larger genome sizes are supposed to have more complicated meiotic regulation. As previously reported, some meiotic genes, such as OsAMI (Che et al., 2011)/AtSWI1 (Mercier et al., 2001), OsMOF1 (He et al., 2016) and OsPAIR1 (Nonomura et al., 2004a)/AtPRD3 (De Muyt et al., 2009), were found to be plant specific, and the rice meiotic genes OsMEL1 (Nonomura et al., 2007) and OsMEL2 (Nonomura et al., 2011) have no homologs in other plant species. Therefore, meiotic control may vary somewhat among different species, even in plants. Thus, identification of more meiotic genes is necessary to expand our knowledge of meiosis. In this study, through mutant screening and map-based cloning, we identified OsBVF1 from rice. Both sequence alignment and functional characterization support the fact that OsBVF1 is a novel meiotic gene that encodes a coiled-coil motif- and OmpH domain-containing protein.

The role of OsBVF1 in meiotic recombination

In this study, we provided several lines of evidence to support a role of OsBVF1 in rice meiosis. First, the rice OsBVF1 was required for fertility, and mutation of OsBVF1 caused male and female sterility; second, chromosome morphology analysis showed that bvfl was defective in formation of well-synapsed chromosomes and only produced univalents, suggesting a failure of synopsis and crossover formation; third, the meiotic recombination defect in bvfl is likely caused by failure of DSB formation, which is supported by the observation of the disappearance of the marker for localization of DSB and other proteins required for meiotic recombination;
The role of OsBVF1 in rice meiotic recombination

fourth, the undetectable OsZEP1 signal in bvf1 suggests a role in SC formation, but the failure of the SC is likely a consequence of the initial defect in DSB formation (as reviewed in Gray and Cohen, 2016).

Sequence analysis showed that OsBVF1 has an OmpH domain and two coiled-coil motifs. It is reported that OmpH-containing proteins may play roles as protein folding catalysts or as chaperones in extracytoplasmic compartments (Missiakas et al., 1996). In addition, the coiled-coil motifs play an important role in mediating subunit oligomerization in many proteins (Lupas, 1996; Mason and Arndt, 2004). Among the identified meiotic proteins, the central element of the SC shares a coiled-coil motif in the central region with one globular domain at each end, as with OsZEP1 in rice (Wang et al., 2010), ZYP1 in maize (Golubovskaya et al., 2011) and Arabidopsis (Higgins et al., 2005), and Zip1 in budding yeast (Sym et al., 1993; Page and Hawley, 2004). The coiled-coil motifs of the proteins form ladder-like or hinge-like parallel structures in the central region of the SC. Besides, some other meiotic proteins also contain the coiled-coil motifs, including OsPAIR1, OsPAIR3, OsAM1, OsSGO1, and OsHEI10 in rice (Nonomura et al., 2004a; Che et al., 2011; Wang et al., 2011a, 2012a; Wang et al., 2011b), DSY2 in maize (Lee et al., 2015) and RED1 in budding yeast (Smith and Roeder, 1997), providing evidence that the coiled-coil motif is one of the important domains among proteins required for meiosis.

Since our yeast two-hybrid assay did not detect any interaction between OsBVF1 and OsPAIR2/3 (Nonomura et al., 2007; Yuan et al., 2009; Wang et al., 2011a), OsZEP1 (Wang et al., 2010) and OsCRC1 (Miao et al., 2013), it is likely that OsBVF1 may not directly participate in SC assembly in rice.

The function of OsBVF1 and its homologs might be highly conserved in plants

Research into evolutionary biology has indicated that between 55 and 75 million years ago, plants had their genomes duplicated so as to increase the chance of survival (Lohaus and Van de Peer, 2016). Comparison of plant species showed that at least several million years ago, many monocot lineages, including wild rice, had already experienced two distinct paleopolyploidies (Jiao et al., 2014). Lots of genomic hints can verify this notion, for example, the highly conserved meiotic genes such as OsDMC1 (Ding et al., 2001; Metkar et al., 2004), OsRAD51 (Rajanikant et al., 2008) and OsPAIR2 (Nonomura et al., 2004b) all have two copies in the rice genome, with one of the copies silenced like OsPAIR2 or both functionally reserved. Interestingly, in the case of OsBVF1 and its homologs (orthologs), from algae to monocots and eudicots, only a single copy of the gene was reserved in all the genomes (except for P. trichocarpa) during plant genome evolution, suggesting that these orthologous genes may...
be important for plant sexual reproduction. Therefore, we infer that OsBVF1 and its homologs in other plants may be highly conserved, with a primary role in recombination. Both the present results and previous findings indicate that some plant-specific genes, including OsBVF1, have evolved in the regulation of plant-specific meiosis.

**Supplementary data**

Supplementary data are available at JXB online.

*Fig. S1.* Genetic and phenotypic analyses of the *OsBVF1*-transgenic plants.

*Fig. S2.* Coiled-coil motif prediction of OsBVF1 and OsPAIR3 based on the web-tool COILS.

*Fig. S3.* Comparison of OmpH and coiled-coil motif sequences of OsBVF1 and OsPAIR3.

*Fig. S4.* Expression pattern of *OsBVF1* according to the Rice Expression Profile Database.

*Fig. S5.* Sequence alignment of OsBVF1 and its homologous proteins.

Table S1. Primers used in the study.

Table S2. Segregation of fertile and sterile plants in *bvf1* M1 lines.

Table S3. Segregation of fertile and sterile plants in *bvf1* mapping populations.

Table S4. Sequences of OsBVF1 homologs of some plant species that are not available at GenBank.

**Acknowledgements**

We greatly appreciate the kind gifts of γH2AX, OsREC8, OsMER11, OsCOM1, OsDMC1, OsMER3, OsPAIR2 and OsZEP1 antibodies and technical assistance from Prof. Zhukuan Cheng at Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing (CHINA). We also thank Dr. Liangsheng Zhang (Fujian Agriculture and Forestry University) for the phylogenetic tree construction. This work is supported by The Ministry of Science and Technology of China (Grant No. 2012AA10A303) for Y.C. and the National Natural Science Foundation of China (31370347 and 31570314) and Fudan University for the phylogenetic tree construction. This work is supported by The Ministry of Science and Technology of China (Grant No. 2012AA10A303) for Y.C. and the National Natural Science Foundation of China (31370347 and 31570314) and Fudan University for the phylogenetic tree construction.

**References**

An XJ, Deng ZY, Wang T. 2011. OsSpoo11-4, a rice homologue of the archaeal TopVIA protein, mediates double-strand DNA cleavage and interacts with OsTopVIB. PLoS ONE 6, e20327.

Cai X, Dong F, Edelmann RE, Makaroff CA. 2003. The Arabidopsis SYM1 cohesin protein is required for sister chromatid arm cohesion and homologous chromosome pairing. Journal of Cell Biology 166, 2999–3007.

Chang Y, Gong L, Yuan W, Li X, Chen G, Li X, Zhang Q, Wu C. 2009. Replication protein A (RPA1α) is required for meiotic and somatic DNA repair but is dispensable for DNA replication and homologous recombination in rice. Plant Physiology 151, 2162–2173.

Che L, Tang D, Wang K, Wang M, Zhu K, Yu H, Gu M, Cheng Z. 2011. OsAM1 is required for leptotene-zygotene transition in rice. Cell Research 21, 654–665.

Chen L, Wang F, Wang X, Liu YG. 2013. Robust one-tube Ω-PCR strategy accelerates precise sequence modification of plasmids for functional genomics. Plant & Cell Physiology 54, 634–642.

Cheng Z. 2013. Analyzing meiotic chromosomes in rice. Methods in Molecular Biology 990, 125–134.

De Muyt A, Pereira L, Vezon D, et al. 2009. A high throughput genetic screen identifies new early meiotic recombination functions in Arabidopsis thaliana. PLoS Genetics 5, e1000654.

De Muyt A, Vezon D, Gendrot G, Gallois JL, Stevens R, Grelon M. 2007. AIPRD1 is required for meiotic double strand break formation in Arabidopsis thaliana. The EMBO Journal 26, 4126–4137.

Deng ZY, Wang T. 2007. OsDMC1 is required for homologous pairing in Oryza sativa. Plant Molecular Biology 65, 31–42.

Dickey JS, Redon CE, Nakamura AJ, Baird BJ, Sedelnikova OA, Bonner WM. 2009. H2AX: functional roles and potential applications. Chromosoma 118, 683–692.

Ding Z, Wang T, Chong K, Bai S. 2001. Isolation and characterization of OsDMC1, the rice homologue of the yeast DMC1 gene essential for meiosis. Sexual Plant Reproduction 13, 285–288.

Fu M, Wang C, Xue F, Higgins J, Chen M, Zhang D, Liang W. 2016. The DNA topoisomerase VI-B subunit OsMTOPVIB is essential for meiotic recombination initiation in rice. Molecular Plant 9, 1539–1541.

Gerton JL, Hawley RS. 2005. Homologous chromosome interactions in meiosis: diversity amidst conservation. Nature Reviews. Genetics 6, 477–487.

Golubovskaya IN, Wang CJ, Timofejeva L, Cande WZ. 2011. Maize meiotic mutants with improper or non-homologous synopsis due to problems in pairing or synaptonemal complex formation. Journal of Experimental Botany 62, 1533–1544.

Gray S, Cohen PE. 2016. Control of meiotic crossovers: from double-strand break formation to designation. Annual Review of Genetics 50, 175–210.

Grelon M, Vezon D, Gendrot G, Pelletier G. 2001. AtSPO11-1 is necessary for efficient meiotic recombination in plants. The EMBO Journal 20, 589–600.

He Y, Wang C, Higgins JD, Yu J, Zong J, Lu P, Zhang D, Liang W. 2016. MEIOTIC F-BOX is essential for male meiotic DNA double-strand break repair in rice. The Plant Cell 28, 1879–1893.

Higgins JD, Sanchez-Moran E, Armstrong SJ, Jones GH, Franklin FC. 2005. The Arabidopsis synaptonemal complex protein ZYP1 is required for chromosome synopsis and normal fidelity of crossing over. Genes & Development 19, 2488–2500.

Hollingsworth NM, Brill SJ. 2004. The Mus81 solution to resolution: generating meiotic crossovers without Holliday junctions. Genes & Development 18, 117–125.

Iftode C, Daniey Y, Borowiec JA. 1999. Replication protein A (RPA): the eukaryotic SSB. Critical Reviews in Biochemistry and Molecular Biology 34, 141–180.

Ji J, Tang D, Wang K, Wang M, Che L, Li M, Cheng Z. 2012. The role of OsCOM1 in homologous chromosome synopsis and recombination in rice meiosis. The Plant Journal 72, 18–30.

Ji J, Tang D, Wang M, Li Y, Zhang L, Wang K, Li M, Cheng Z. 2013. MRE11 is required for homologous synopsis and DSB processing in rice meiosis. Chromosoma 122, 363–376.

Jiao Y, Li J, Tang H, Paterson AH. 2014. Integrated syntenic and phylogenomic analyses reveal an ancient genome duplication in monocots. The Plant Cell 26, 2792–2802.

Keeney S. 2008. Spoo1 and the formation of DNA double-strand breaks in meiosis. Genome Dynamics and Stability 2, 81–123.

Keeney S, Giroux CN, Kleckner N. 1997. Meiosis-specific DNA double-strand breaks are catalyzed by Spoo1, a member of a widely conserved protein family. Cell 88, 375–384.

Lambing C, Osman K, Nuntasookorn K, et al. 2015. Arabidopsis PCH2 mediates meiotic chromosomal remodelling and maturation of crossovers. PLoS Genetics 11, e1005372.

Lee DH, Kao YH, Ku JC, Lin CY, Meeley R, Jan YS, Wang CJ. 2015. The axial element protein DESYNAPTIC2 mediates meiotic double-strand break formation and synaptonemal complex assembly in maize. The Plant Cell 27, 2516–2529.

Li X, Chang Y, Xin X, Zhu C, Li X, Higgins JD, Wu C. 2013. Replication protein A2c coupled with replication protein A1c regulates crossover formation during meiosis in rice. The Plant Cell 25, 3885–3899.

Lohaus R, Van de Peer Y. 2016. Of dupes and dinos: evolution at the K/Pg boundary. Current Opinion in Plant Biology 30, 62–69.
Luo Q, Li Y, Shen Y, Cheng Z. 2014. Ten years of gene discovery for meiotic event control in rice. Journal of Genetics and Genomics 41, 125–137.

Luo Q, Tang D, Wang M, Luo W, Zhang L, Qin B, Shen Y, Wang K, Li Y, Cheng Z. 2013. The role of OsMSH5 in crossover formation during rice meiosis. Molecular Plant 6, 729–742.

Ludas A. 1996. Coiled coils: new structures and new functions. Trends in Biochemical Sciences 21, 375–382.

Ma H, Cooke HJ, Shi Q. 2014. Meiosis: recent progress and new opportunities. Journal of Genetics and Genomics 41, 83–85.

Maquat LE. 2004. Nonsense-mediated mRNA decay: splicing, translation and miRNA dynamics. Nature Reviews. Molecular Cell Biology 5, 89–99.

Mason JM, Arndt KM. 2004. Coiled coil domains: stability, specificity, and biological implications. ChemBioChem 5, 170–176.

Mercier R, Mézard C, Jenczewski E, Macaisne N, Grelen M. 2015. The molecular biology of meiosis in plants. Annual Review of Plant Biology 66, 297–327.

Mercier R, Vezon D, Bullier E, Motamayor JC, Sellier A, Lefèvre F, Pelletier G, Horlow C. 2001. SWITCH (SWI1): a novel protein required for the establishment of sister chromatid cohesion and for bivalent formation at meiosis. Genes & Development 15, 1859–1871.

Metkar SS, Sainis JK, Mahajan SK. 2004. Cloning and characterization of the DMC1 gene in Oryza sativa. Current Science 87, 353–357.

Miao C, Tang D, Zhang H, Wang M, Li Y, Tang S, Yu H, Gu M, Cheng Z. 2013. Central region component1, a novel synaptonemal complex component, is essential for meiotic recombination initiation in rice. The Plant Cell 25, 2998–3009.

Mimitou EP, Simyntom L. 2009. DNA end resection: many nucleases make light work. DNA Repair 8, 983–995.

Missiakas D, Betton JM, Raina S. 1996. New components of protein folding in extracytoplasmic compartments of Escherichia coli: FkpA and Skp/OmpH. Molecular Microbiology 21, 871–884.

Nonomura K, Iwatsuki S, Nakano M, Takashima K, Komeda N, Fukuchi S, Miyazaki S, Miyao A, Hirochika H, Kurata N. 2011. A novel RNA-recognition-motif protein is required for premeiotic G1/S-phase transition in rice (Oryza sativa L.). PLoS Genetics 7, e1001265.

Nonomura K, Morohoshi A, Nakano M, Iwatsuki S, Miyao A, Hirochika H, Kurata N. 2007. A germ cell specific gene of the ARGONAUTE family is essential for the progression of premeiotic mitosis and meiosis during sporogenesis in rice. The Plant Cell 19, 2583–2594.

Nonomura K, Nakano M, Fukuda T, Iwatsuki S, Miyao A, Hirochika H, Kurata N. 2004a. The novel gene HOMOLOGOUS PAIRING ABERRATION IN RICE MEIOSIS1 of rice encodes a putative coiled-coil protein required for homologous chromosome pairing in meiosis. The Plant Cell 16, 1008–1020.

Nonomura KI, Nakano M, Murata K, Miyoshi K, Iwatsuki S, Miyao A, Hirochika H, Kurata N. 2004b. An insertional mutation in the rice PAIR2 gene, the ortholog of Arabidopsis ASY1, results in a defect in homologous chromosome pairing during meiosis. Molecular Genetics and Genomics 271, 121–129.

Osman K, Higgins JD, Sanchez-Moran E, Armstrong SJ, Franklin FC. 2011. Pathways to meiotic recombination in Arabidopsis thaliana. The New Phytologist 190, 523–544.

Page SL, Hawley RS. 2004. The genetics and molecular biology of the synaptonemal complex. Annual Review of Cell and Developmental Biology 20, 525–558.

Rajanikant C, Melzer M, Rao BJ, Sainis JK. 2008. Homologous recombination properties of OsRad51, a recombinase from rice. Plant Molecular Biology 68, 479–491.

Ramesh MA, Malik SB, Logsdon JM Jr. 2005. A phylogenomic inventory of meiotic genes; evidence for sex in Giardia and an early eukaryotic origin of meiosis. Current Biology 15, 185–191.

Shao T, Tang D, Wang K, Wang M, Che L, Qin B, Yu H, Li M, Gu M, Cheng Z. 2011. OsREC8 is essential for chromatid cohesion and metaphase I monopolar orientation in rice meiosis. Plant Physiology 156, 1396–1398.

Shen Y, Tang D, Wang K, Wang M, Huang J, Luo W, Luo Q, Hong L, Li M, Cheng Z. 2012. ZIP4 in homologous chromosome synopsis and crossover formation in rice meiosis. Journal of Cell Science 125, 2581–2591.

Smith AV, Roeder GS. 1997. The yeast Rad1 protein localizes to the cores of meiotic chromosomes. The Journal of Cell Biology 136, 957–967.

Stacey NJ, Kuromori T, Azumi Y, Roberts G, Breuer C, Wada T, Maxwell A, Roberts K, Sugimoto-Shirasu K. 2006. Arabidopsis SPO11-2 functions with SPO11-1 in meiotic recombination. The Plant Journal 48, 206–216.

Sym M, Engebret JA, Roeder GS. 1993. ZIP1 is a synaptonemal complex protein required for meiotic chromosome synopsis. Cell 72, 365–378.

Székelygő L, Nicolas A. 2010. From meiosis to postmeiotic events: homologous recombination is obligatory but flexible. The FEBBS Journal 277, 571–589.

Tang D, Miao C, Li Y, Wang H, Liu X, Yu H, Cheng Z. 2014. OsRAD51C is essential for double-strand break repair in rice meiosis. Frontiers in Plant Science 5, 167.

Vrielynck N, Chambon A, Vezon D, Pereira L, Chelysheva L, De Myt A, Mézard C, Mayer C, Grelen M. 2016. A DNA topoisomerase VI-like complex initiates meiotic recombination. Science 351, 939–943.

Wang H, Hu Q, Tang D, Liu X, Du G, Shen Y, Li Y, Cheng Z. 2016. OsDMC1 is not required for homologous pairing in rice meiosis. Plant Physiology 171, 230–241.

Wang HN, Chu ZZ, Ma XL, Li RQ, Liu YG. 2013. A high through-put protocol of plant genomic DNA preparation for PCR. Acta Agronomica Sinica 39, 1200–1205.

Wang K, Tang D, Wang M, et al. 2009. MER1 is required for normal meiotic crossover formation, but not for presynaptic alignment in rice. Journal of Cell Science 122, 2055–2063.

Wang K, Wang C, Liu Q, Liu W, Fu Y. 2015. Increasing the genetic recombination frequency by partial loss of function of the synaptonemal complex in rice. Molecular Plant 8, 1295–1298.

Wang K, Wang M, Tang D, Shen Y, Miao C, Hu Q, Lu T, Cheng Z. 2012a. The role of rice HEI10 in the formation of meiotic crossovers. PLoS Genetics 8, e1002809.

Wang K, Wang M, Tang D, Shen Y, Qin B, Li M, Cheng Z. 2011. PAR3, an axis-associated protein, is essential for the recruitment of recombination elements onto meiotic chromosomes in rice. Molecular Biology of the Cell 22, 12–19.

Wang M, Tang D, Luo Q, Jin Y, Shen Y, Wang K, Cheng Z. 2012b. BRK1, a Bub1-related kinase, is essential for generating proper tension between homologous kinetochores at metaphase I of rice meiosis. The Plant Cell 24, 4961–4973.

Wang M, Tang D, Wang K, Shen Y, Qin B, Miao C, Li M, Cheng Z. 2011b. OsSGO1 maintains synaptonemal complex stabilization in addition to protecting centromeric cohesion during rice meiosis. The Plant Journal 67, 583–594.

Wang M, Wang K, Tang D, Wei C, Li M, Shen Y, Chi Z, Gu M, Cheng Z. 2010. The central element protein ZEP1 of the synaptonemal complex regulates the number of crossovers during meiosis in rice. The Plant Cell 22, 417–430.

Wang Y, Cheng Z, Lu P, Timofejeva L, Ma H. 2014a. Molecular cell biology of male meiotic chromosomes and isolation of male meiocytes in Arabidopsis thaliana. Methods in Molecular Biology 1110, 217–230.

Wang Y, Cheng Z, Ma H. 2014b. Meiosis: interactions between homologous chromosomes. In: Assmann S, Liu B. eds. Cell Biology. New York: Springer, 1–34.

Wu Z, Ji J, Tang D, Wang H, Shen Y, Shi W, Li Y, Tan X, Cheng Z, Luo Q. 2015. OsSDS is essential for DSB formation in rice meiosis. Frontiers in Plant Science 6, 21.

Xue Z, Li Y, Zhang L, et al. 2016. OsMTOPVIB promotes meiotic DNA double-strand break formation in rice. Molecular Plant 9, 1535–1538.

Yu H, Wang M, Tang D, Wang K, Chen F, Gong Z, Gu M, Cheng Z. 2010. OsSPO1-1 is essential for both homologous chromosome pairing and crossover formation in rice. Chromosoma 119, 623–636.

Yuan W, Li X, Chang Y, Wen R, Chen G, Zhang Q, Wu C. 2009. Mutation of the rice gene PAIR3 results in lack of bivalent formation in rice. The Plant Journal 59, 303–315.

Zhang B, Wang M, Tang D, Li Y, Xu M, Gu M, Cheng Z, Yu H. 2015. XRCC3 is essential for proper double-strand break repair and homologous recombination in rice meiosis. Journal of Experimental Botany 66, 5713–5725.
Zhang C, Song Y, Cheng ZH, Wang YX, Zhu J, Ma H, Xu L, Yang ZN. 2012. The Arabidopsis thaliana DSB formation (AtDFO) gene is required for meiotic double-strand break formation. The Plant Journal 72, 271–281.

Zhang L, Tang D, Luo Q, Chen X, Wang H, Li Y, Cheng Z. 2014. Crossover formation during rice meiosis relies on interaction of OsMSH4 and OsMSH5. Genetics 198, 1447–1456.

Zhang L, Tao J, Wang S, Chong K, Wang T. 2006. The rice OsRad21-4, an orthologue of yeast Rec8 protein, is required for efficient meiosis. Plant Molecular Biology 60, 533–554.

Zickler D, Kleckner N. 1998. The leptotene-zygotene transition of meiosis. Annual Review of Genetics 32, 619–697.

Zickler D, Kleckner N. 1999. Meiotic chromosomes: integrating structure and function. Annual Review of Genetics 33, 603–754.

Zickler D, Kleckner N. 2015. Recombination, pairing, and synapsis of homologs during meiosis. Cold Spring Harbor Perspectives in Biology 7, a016626.

Zickler D, Kleckner N. 2016. A few of our favorite things: Pairing, the bouquet, crossover interference and evolution of meiosis. Seminars in Cell & Developmental Biology 54, 135–148.