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Letter to the Editor

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The DNA Topoisomerase VI–B Subunit OsMTOPVIB Is Essential for Meiotic Recombination Initiation in Rice

Dear Editor,

In flowering plants, the life cycle alternates between diploid sporophyte and haploid gametophyte generations. Development of the male organ and germ cells is essential for successful plant fertility and crop yield. Male reproduction is a highly orchestrated process controlled by various intrinsic components. Although significant advances have been made in understanding male gametophyte formation, the molecular mechanisms controlling this process are still largely unknown (Zhang and Liang, 2016).

To identify male sterility genes in rice, we have constructed a mutant library by treating the rice cultivar 9522 (*Oryza sativa* ssp. *japonica*) with gamma ray radiation. This has led to the identification of two allelic male sterile mutants designated *Oryza sativa meiotic topoisomerase VIB-like* (*osmtopVIB-1* and *osmtopVIB-2*). The mutant plants exhibited normal vegetative growth but complete sterility during reproductive development. Iodine potassium iodide solution (I2-KI) staining showed that almost all the pollen grains were empty and shrunken (Figure 1A). Moreover, when mutant flowers were pollinated with wild-type pollen, no seeds set, indicating that mega-gametogenesis was also affected in the mutant. Semi-thin sections were prepared to clarify the cause of sterility in *osmtopVIB* mutants. No obvious phenotype was observed in *osmtopVIB* mutants until the tetrad stage. Tetrads of abnormal size and shape were found in the anther locule of *osmtopVIB* mutants (Supplemental Figure 1), suggesting that *osmtopVIB* mutants may be defective during meiosis.

To confirm that OsMTOPVIB is required for normal meiosis, chromosome behavior was investigated in pollen mother cells of both wild-type and *osmtopVIB* mutants. In the wild-type, chromosomes began to condense and become visible as thin threads at leptotene. At zygotene, the chromosomes continued to condense and synapsis initiation sites formed, bringing the homologs into close apposition. At pachytene, synapsis concluded with the formation of the synaptonemal complex (SC). Twelve bivalents appeared at diakinesis, which then aligned along the equatorial plate at metaphase I (Figure 1B). The homologous chromosomes then separated and were pulled toward the opposite poles by the spindle from anaphase I to telophase I. During the second meiotic division, sister chromatids of each chromosome segregated and tetrads formed at the end of meiosis II (Supplemental Figure 2A–2C). In *osmtopVIB-1* meiocytes, chromosome morphogenesis was similar to the wild-type from leptotene to zygotene. However, homologous chromosomes did not pair with each other and no synapsed homologs were observed. Due to the lack of homologous pairing and synapsis, 24 univalents were observed at diakinesis (Figure 1B). At metaphase I, these univalents could not align on the equatorial plate, and thus segregated randomly to opposite poles during anaphase I. As a result, abnormal tetrads with several micronuclei were generated after the second meiotic division (Supplemental Figure 2D–2F). The chromosome behavior in *osmtopVIB-2* meiocytes was similar to that in *osmtopVIB-1* (Supplemental Figure 3C–3H). These results indicated that *osmtopVIB* mutants were defective in homologous pairing and synapsis.

To investigate whether SC formation is affected in *osmtopVIB-1*, we checked the localization of PAIR2, PAIR3, and ZEP1, which are SC components (Nonomura et al., 2006; Wang et al., 2010, 2011). OsREC8, a conserved meiosis-specific component of the cohesion complex, can be used as a marker for meiotic chromosomes (Shao et al., 2011). In *osmtopVIB-1*, OsREC8 localization was indistinguishable from wild-type and therefore used as a maker for chromosome behavior in rice meiosis. In *osmtopVIB-1* meiocytes, PAIR2 and PAIR3 loaded normally onto the chromosomes and colocalized with OsREC8, which is consistent with the wild-type (Supplemental Figure 4). In the wild-type, ZEP1 appeared as punctate foci on leptotene chromosomes and then formed continuous linear signals along the entire chromosomes at pachytene (Figure 1C). However, no foci or linear ZEP1 signals were observed in *osmtopVIB-1* meiocytes from leptotene to pachytene, indicating that the central element is absent in the mutant (Figure 1C). Overall, these results suggest that although axial elements install normally, SC formation is severely disrupted in *osmtopVIB-1*.

Rice DMC1 mediates single-strand invasion during meiotic recombination and is essential for recombination-dependent synapsis (Wang et al., 2016). MER3 is required for the formation of interference-sensitive COs, the major pathway in rice (Wang et al., 2009). Thus, DMC1 and MER3 were used as markers to monitor the process of meiotic recombination. We found that punctate foci of DMC1 and MER3 were observed in the wild-type meiocytes at zygotene. By contrast, no DMC1 and MER3 foci were observed in the *osmtopVIB-1*, suggesting that OsMTOPVIB is required for inter-homologous chromosome recombination during meiosis (Figure 1C).

In most organisms, including rice, meiotic recombination and synapsis depend on the formation of double-strand breaks (DSBs). To investigate whether abolishment of recombination in *osmtopVIB-1* is caused by defects in DSB formation, we performed dual immunolocalization of OsREC8 and γH2AX. The histone H2A variant, H2AX, becomes rapidly phosphorylated to

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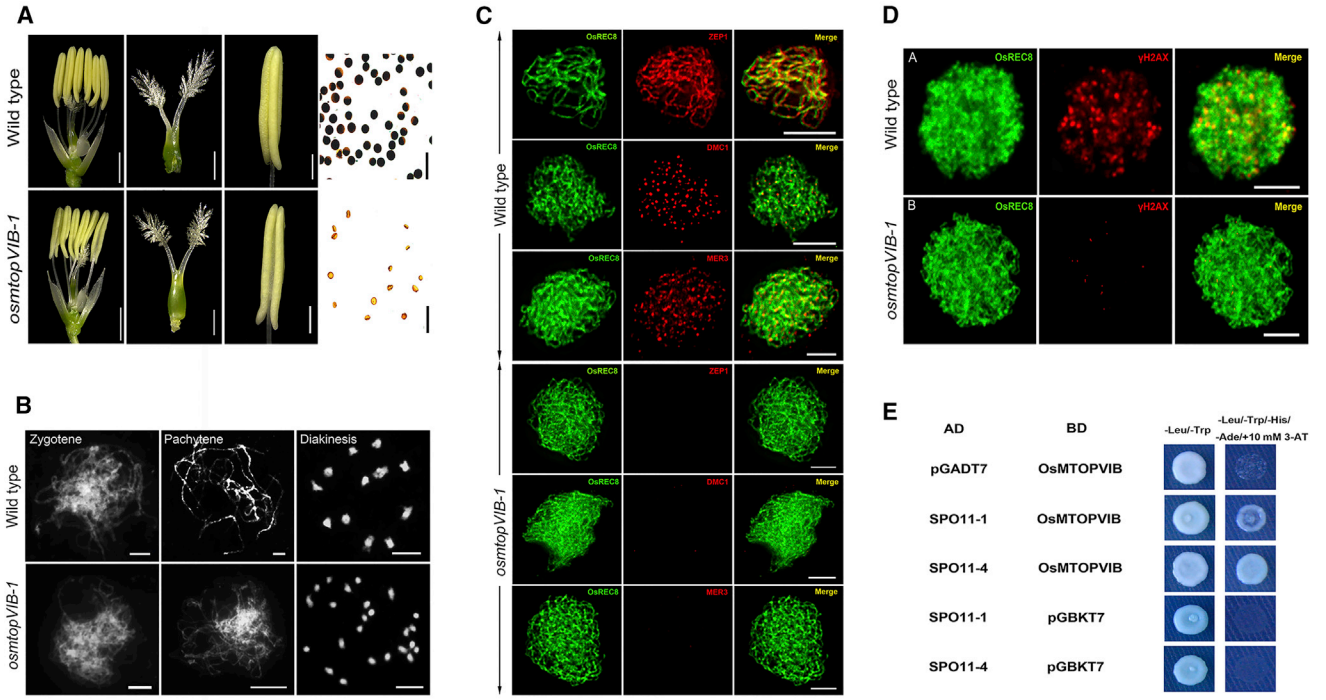

Figure 1. OsMTOPVIB Is Required for Meiotic DSB Formation.

(A) Phenotypic characterization of wild-type and *osmtopVIB-1*. From left to right are spikelets after moving the lemma and pelea (scale bar, 500 μm), pistils (scale bar, 200 μm), anthers (scale bar, 200 μm) and I2-KI staining of the pollen grains within the anther of wild-type and *osmtopVIB-1*(scale bar, 100 μm).
(B) Chromosome behaviors of male meiocytes of wild-type and *osmtopVIB-1* at meiotic prophase I. Scale bar, 5 μm.
(C) Dual immunolocalization of REC8 and γH2Ax in wild-type and *osmtopVIB-1* male meiocytes. Scale bar, 5 μm.
(D) Dual immunolocalization of ZEP1, DMC1, and MER3 with REC8 in wild-type and *osmtopVIB-1* male meiocytes. Scale bar, 5 μm. REC8 signals (green) were used to indicate the meiotic chromosome axes in **(C)** and **(D)**.
(E) Yeast two-hybrid assay to detect interactions of OsMTOPVIB with SPO11-1 and SPO11-4, respectively. Empty vector pGADT7 and pGBKT7 were used as controls. The interactions were verified by the growth of yeast strains on the -Leu-Trp-His-Ade selection medium containing 10 mM 3AT.

γH2AX when meiotic DSBs are formed. Therefore, γH2AX foci have been considered as suitable markers for DSB sites. We detected numerous γH2AX signals during zygotene in wild-type. However, no γH2AX signals were observed in *osmtopVIB-1*, which indicated that OsMTOPVIB is essential for DSB formation in rice (Figure 1D).

To identify the mutated gene, a map-based cloning approach was used. The mutated gene was mapped to chromosome 6 between markers FM603 and FM606, defining a 56-kb region (Supplemental Figure 5). Sequence analysis revealed that one base (T) deletion (*osmtopVIB-1*) and a base (C) deletion (*osmtopVIB-2*) in the second exon of LOC_Os06g49450 (http://www.gramene.org/) caused a frameshift and premature translational termination (Supplemental Figure 5).

OsMTOPVIB encodes a protein of 487 amino acids in length. OsMTOPVIB shares 39% sequence identity with the *Arabidopsis* ortholog MTOPVIB as well as showing homology with topoisomerase VIB from other flowering plants. The OsMTOPVIB protein sequence has no obvious similarity with known functional domains. However, HHpred analysis suggested that this protein shares structural homology with the two archaeal topo VIB proteins with known crystal structures. The OsMTOPVIB contains four characteristic domains of the TOPVIB protein family, including the GHKL domain, the small domain (SmD), the transducer domain, and the C-terminal domain (Supplemental Figure 6). TOPVIB is one of the two subunits of the archaeal type VI topoisomerase, which catalyzes a DNA topology change through generating DSBs during DNA replication, transcription, and recombination. Topo VI is a heterotetramer composed of two TopVIA and TopVIB subunits each. TopVIA is responsible for DNA cleavage and Top VIB is involved in ATP binding and hydrolysis. The evolutionarily conserved DSB protein SPO11 is the eukaryotic ortholog of the archaeal TopVIA (Bergerat et al., 1997).

To define the spatial and temporal distribution of OsMTOPVIB in meiosis, dual immunolocalization was conducted using OsMTOPVIB and OsREC8 antibodies. In the wild-type, OsMTOPVIB first appeared as punctate foci at leptotene. Then the number of OsMTOPVIB foci accumulated and reached a peak at late zygotene to early pachytene. The OsMTOPVIB foci started to diminish during pachytene, and only a few residual foci were observed at late pachytene. After late pachytene, OsMTOPVIB signals were no longer detectable (Supplemental Figure 7). In wild-type meiocytes, γH2AX foci were mainly detected at zygotene. The OsMTOPVIB foci can be detected at pachytene, indicating that it may have other functions besides DSB formation during meiotic prophase I. The OsMTOPVIB signals only partially overlapped with the axis signals, which may represent a dynamic distribution of DSBs, and that pre-DSB sites originate in the chromosome loops before moving to the axes.

These results suggest that OsMTOPVIB specifically functions during early prophase I.

Most organisms contain one SPO11; however, higher plants generally possess more than one SPO11 homolog. For example, three and five SPO11 homologs exist in the *Arabidopsis* and rice genome, respectively (An et al., 2011). It is currently unclear which of the SPO11 homologs are required for meiotic DSB formation in rice. Previous studies indicate that the archaeal topoVIA associates with topoVIB to form a topo VI-like complex. A similar interaction of OsMTOPVIB with OsSPO11(s) involved in DSB formation may also occur in rice. A yeast two-hybrid assay revealed that OsMTOPVIB is able to interact with OsSPO11-1 and OsSPO11-4, but not with OsSPO11-2 and OsSPO11-3 (Figure 1E and Supplemental Figure 8). This result suggests that OsSPO11-1, OsSPO11-4, and OsMTOPVIB may associate together to form a heterotetrameric structure similar to that of the archaeal Topo VI. In addition, previous work has demonstrated that OsSPO11-1 is essential for homologous chromosome pairing and CO formation, while OsSPO11-4 possesses double-strand DNA cleavage activity *in vitro* (An et al., 2011). Whether OsSPO11-1/4 are capable of interacting with OsMTOPVIB *in planta* and are required for DSB formation needs further investigation.

In summary, we have identified an ortholog of archaeal TOPVIB in rice and demonstrated that OsMTOPVIB is essential for meiotic recombination initiation. It is well known that meiotic DSBs are catalyzed by SPO11, the eukaryotic equivalent of TOPVIA (Keeney et al., 1997). However, due to sequence divergence, whether TopVIB is also required for meiotic DSB formation has long been a mystery until very recently when the orthologs of TopVIB were identified as the partner of SPO11 in *Arabidopsis* and mice (Robert et al., 2016; Vrielynck et al., 2016). Our study proved the conserved function of TopVIB proteins in the model monocot rice, highlighting the evolutionary importance of TopVIB in meiotic DSB formation.

SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

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AUTHOR CONTRIBUTIONS

W.L. and D.Z. designed the experiments. M.F. carried out most of the experiments. C.W., F.X., and M.C assisted in gene cloning and immunolocalization analysis. J.D.H. assisted in analyzing the data. M.F., J.D.H., and W.L. wrote the article.

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Ming Fu[1], Chong Wang[1], Feiyang Xue[1], James Higgins[2], Mingjiao Chen[1], Dabing Zhang[1,3] and Wanqi Liang[1,]*

[1]Joint International Research Laboratory of Metabolic & Developmental Sciences, Shanghai Jiao Tong University-University of Adelaide Joint Centre for Agriculture and Health, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 20040, China
[2]Deparment of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK
[3]School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Urrbrae, SA 5064, Australia
***Correspondence: Wanqi Liang (wqliang@sjtu.edu.cn)**
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